

Genetic management for increased tolerance to aluminium and iron toxicities in rice—A review

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Several tolerant donors were identified from rice germplasms and significant strides were made in their genetic improvement through conventional breeding during the last 40 years in Indian subcontinent. In majority of the cases the physiological mechanism governing tolerance towards Fe and Al toxicities were unzipped. It is also worth mentioning that progress in breeding largely depends upon the efficiency and effectiveness of the screening technique to identify the true tolerants, availability of the tolerant gene(s) in the germplasms and selection of desirable lines from the segregating population. With increase in Fe-toxicity level, plant height, number of ear bearing tillers, panicle length and grain yield were found decreased in rice; whereas, vegetative growth period got extended with increased spikelet sterility. Toxic concentrations of Al generally inhibit root growth, restrict water intake and nutrients uptake that lead to poor growth and low yield *per se*. *In vitro* screening for developing tolerant genotypes is relatively a straightforward and amply demonstrative method. Toxicity tolerance was also found transferable to a desirable plant type. Further, marker aided selection (MAS) would facilitate reliable selection for abiotic stress tolerant genotypes. Quantitative trait loci (QTL) for toxicity tolerance towards Fe and Al were identified and expected to help in developing tolerant, high yielding varieties through MAS. The present review deals with the possible regulatory mechanisms of Fe and Al tolerance and improvement of rice through identification of the tolerant lines with the help of efficient molecular markers *en route* biotechnological approaches.

Keywords: Biotechnological tools, Fe and Al toxicities, rice, stress tolerance mechanisms

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Introduction

Rice is a widely grown crop the world over. It constitutes the staple food of about two-third of the global population. About 90% of the rice is cultivated and consumed in its homeland—Asia¹. Now, any increase in area to meet the increasing demand for rice must have to come from marginal lands and problem soils. Globally, 138 m ha of available land is constrained with abiotic stresses. Of which 23 m ha is considered to have potential for rice cultivation. However, they are often found to be handicapped owing to the presence of multiple nutrient stresses. In addition, deficiency or toxicity of nutrients affects the soil quality. The nutrient toxicity is largely observed for aluminium, iron, boron, hydrogen sulphide and manganese. Such problem soils may be brought under rice cultivation through development and cultivation of tolerant varieties, which would be less expensive, eco-

compatible and more permanent than more expensive transient soil reclamation by physico-chemical means. This article reviews genetic management of problem soils with special reference to modulation for improved Al and Fe toxicity tolerances in rice.

Iron Toxicity

Overview

Iron (Fe) toxicity creates a range of nutrient disorders and deficiencies of P, K, Ca, Mg, Mn and Zn in plants. These elements were reported to play decisive roles in manifesting toxicity symptoms in rice²⁻⁵. Excessive absorption and translocation of Fe in the rice plants led to the toxicity, which has been a major yield-limiting factor in wetland rice⁶. Fe toxicity has been reported as one of the major soil constraints of lowland acid soils, inland valley swamps, coastal swamps and irrigated lowlands in ultisols and oxisols. Fe is also abundantly found in heavy soils⁷. In the humid forests and moist Savanna zones of Africa, interflow of ferrous ion occurs from upper slopes⁸. More than 50% lowland rice is being affected with Fe

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toxicity in Sierra Leone, Liberia, Guinea, Nigeria, Ivory Coast, and Senegal. It is also reported in Sri Lanka, Vietnam, Malaysia, India (especially in Kerala, Orissa, West Bengal and Andaman Islands), Indonesia (Kalimantan and Sumatra), Philippines, Brazil, Colombia, and Madagascar^{9,10}. Preponderant influence of native non-applied elements, like Fe, Ca, Mn and Zn in laterite and organic 'kole' soils, and Ca, S and Mn in alkali black soils, result in excessive absorption and accumulation of Fe that severely changes the cation-anion balance. This facilitated substantial chlorophyll degradation that led to low yield in rice, especially in west coast of India¹¹. Young acid sulphate soils in the coastal areas also show rampant symptoms of Fe toxicity¹². The Island swamps and low lying valleys in Andaman and Nicobar Islands constitute a vast area constrained with Fe toxicity, which is seriously limiting the rice production¹³.

Mechanism of Fe-toxicity Tolerance

Fe toxicity in rice is caused by its accumulation in the leaves in toxic concentrations. Further, it is associated with high concentration of ferrous ion (Fe^{2+}) in the soil solution, mobilized *in situ*. In the recent past, Fe toxicity has emerged as a serious nutrient disorder of lowland rice. Although, it would not be eliminated entirely by improving the nutritional status of the rice growing lands; however, application of other nutrients, especially, N, P, K, and Zn, was found to reduce Fe toxicity and increased the yield of lowland rice cultivars considerably^{14,15}.

Fe chlorosis often persists in upland rice¹⁶. However, rice cultivars display differential tolerance towards Fe toxicity. In general, upland cultivars are more sensitive to Fe toxicity than lowland cultivars¹⁷. Fe and Al concentrations were found to be higher at shallow submergence depths in the same field. Therefore, farmers are generally advised to use a shallow submergence depth and preferably deep-rooted cultivars¹⁸. The Fe-tolerant rice cultivars absorb less Fe from root to leaves, indicating the governance of a physiological avoidance mechanism at any given concentration of Fe in the leaves. Fe-tolerant cultivars display superior performance under Fe-toxic condition partly due to avoidance and partly due to Fe tolerance. Tolerance of rice plants to higher concentration of ferrous ion (Fe^{2+}) is governed by age, nutritional status of the plant and chemical environment of the root¹⁹. The chemosphere surrounding the root is greatly influenced by the oxidative power of the root system. Rice and other plants generally absorb Fe as

Fe^{2+} and convert to Fe^{3+} and thus reduce the Fe intake. Enzymatic oxidation of Fe was found to be the principal mechanism for higher oxidation activity of rice roots²⁰. Glycolic acid pathway present in the rice root system was reported to be chiefly responsible for root oxidation power in addition to catalase and peroxidase enzymes²¹. *Oryza rufipogon* and *O. sativa* cv. TRC 229-F41 with higher glycolic acid oxidase and leaf nitrate reductase activities were found to be more tolerant to the adverse effects of higher concentration of Fe^{2+} .

Application of Fe down regulated the glycolic acid oxidase activities both in root and shoot tissues irrespective of genotypes²². This might be attributed to the fact that with the increased level of Fe, amount of available Fe increased under submergence²³. The increased level of Fe (Fe^{2+}) enhanced the excessive intake by the rice root, which might disturb some of the normal metabolic processes including the disruption of glycolic acid pathway. Upon addition of Fe to the growth media, leaf nitrate reductase activity was found decreased²². The higher Fe uptake of plant reduces the protein synthesis in leaf²⁴ and subsequently inhibits or suppresses the normal nitrate reductase activity. The *in vitro* nitrate reductase activity was found to be lower at panicle initiation stage than active tillering stage²⁵.

The Fe-tolerant lines exude less sap than those from Fe-susceptible lines²⁶. Fe-content in the leaves of the least affected plants was found in the region of 680 ppm in comparison to 560-580 ppm in most susceptible plants. Fe concentration in soil increases when submerged soil is reduced. Chelates of Fe(III) and occasionally of Fe(II) are found to be dominant forms of soluble Fe in the soil. As a rule Fe(II) is the favourite species taken up by the plants. Therefore, Fe(III) must be reduced at the root surface before transported into the cytosol²⁷.

Genotypic Difference in Fe-toxicity Tolerance

Rice plants show symptoms of bronzing due to high concentration of dissolved Fe^{2+} in the soil solution around the root zone. With increase in Fe toxicity, plant height, number of earbearing tillers per plant, panicle length and grain yield get decreased; whereas, vegetative period gets extended with increased spikelet sterility²⁸. The rice cultivars, viz. Pokkali, IR50, IR2151-190-3 and IR2153-96-1²⁹⁻³¹ were found to be the source of tolerance to Fe toxicity. Two different genes were reported to govern Fe-tolerance in rice. Suakokos harbours a dominant

gene, while Gossi 27 possesses a recessive gene for imparting tolerance to Fe-toxicity³².

Aluminum toxicity

Status and Effects on Productivity

Aluminum (Al) affects about 40-70% of the world's arable land, which has the potential for food crop and biomass production³³. Crop productivity in acid soil is restricted owing to prevalence of multiple abiotic stresses. Al-toxicity impairs productivity in soils having low pH (below 5.0). Free Al ions are solubilized at low pH. However, other factors, such as Mn toxicity and deficiency of P, Ca and Mg, also interplay in the manifestation of Al toxicity. It is a fact that plant species or cultivars within the same species greatly differ in their tolerance pattern to Al stress³⁴. In recent years, considerable research has been done in understanding physiological³⁵⁻³⁸, molecular³⁹⁻⁴¹ and genetic⁴² mechanisms governing Al tolerance. Al tolerance is manifested at cellular level too⁴³.

Site of Al Accumulation

The primary site of Al accumulation and toxicity target in plants was reported to be the root meristem, which consists of actively dividing cells^{44,45}. At cellular level, Al is mainly localized in the cell wall, although some are also found in the nucleus. It is evident that mass signal spreads throughout the intercellular area and is not intense in the cell wall as evident through secondary ion mass spectrometry involving $^{27}\text{Al}^+$ ⁴⁶. An increase in the rigidity of the actin network⁴⁷ and the inhibition of phospholipase C⁴⁸ were proposed as possible inter cellular Al-target sites that might be involved in root growth.

Mechanism of Action

Toxic concentrations of free Al ions are generally found in acid soils with pH 5.0 and below. A reduction in the amount of available P at low pH in the soil increases the Al toxicity intensely. A severe inhibition of root growth is the direct major effect of Al on plants⁴⁹ with no appearance of clearly diagnosable symptoms in the plant tips⁵⁰. Thus, the meristematic tip and root caps are considered to be the most likely or most easily recognizable sites of primary effects of Al^{51-54} or the sites at which a "signal" may be induced that brings a response in remote regions⁴⁵. Inhibition in root elongation is generally observed because Al^{3+} is a toxic ionic species with high binding ability in cellular components of roots and usually shows marginal translocation to the upper parts of the plants. A

wide range of results such as less uptake of some cations^{55,56}, reduced root growth, nutrient deficiency symptoms⁵⁷ and low yield⁵⁴ responses were amply recorded in different plant species/genotypes under Al stressed environments^{58,59}. Al also acts directly on the cell particularly in inducing lesion on the cell membrane possibly by interacting with membrane associated proteins⁶⁰ and on the whole plants by impairing intake of essential ions and water. Its presence also leads to deficiencies in P, Ca, Mg and Fe. Al interacts strongly with proteins, polynucleotides, lipids and glycosides⁶¹⁻⁶⁴.

Variation in genetic make up, such as involvement of a major dominant gene or two major genes with modifiers⁶⁵, for Al tolerance was observed in different crops. Most plants accumulate Al ; 0.2 mg g⁻¹ dry wt. They may also contain Al more than 10 times of this level without displaying any Al-injury. Tea plants are found to be typical Al accumulators. The Al content in their old leaves could reach as high as 30 mg, although young leaves contained only about 0.6 mg g⁻¹ dry wt⁶⁶. Some trees in the tropical rain forests, such as *Richeria grandis*, were reported to accumulate Al more than 1 mg g⁻¹ in their leaves⁶⁷. Hydrangea (*Hydrangea macrophylla*) is also a well-known Al accumulator and the relationship between Al and blue colouration of its sepals was thoroughly investigated^{50,68,69}. Total accumulation of intercellular Al in tobacco root tip was as high as 210 nmol g⁻¹ fresh wt after 4 hrs⁴⁶.

Al generally enters into the symplasm of the root cells very quickly within 1-2 hrs of initiation of exposure⁷⁰ and Al phytotoxicity might be the result of a symplastic Al interaction⁷¹⁻⁷³. Although concentration of free Al^{3+} was reported to be decreased to less than 10^{-10} M at pH 7.0 due to formation of insoluble $\text{Al}(\text{OH})_3$, such low concentrations are potentially phytotoxic because of the strong affinity of Al for oxygen donor compounds such as inorganic phosphate, ATP, RNA, DNA, protein, carboxylic acids and phospholipids⁷⁴. Therefore, internal detoxification mechanisms are required for building tolerance to high Al in Al-accumulating plants.

Whole root responses to Al toxicity showed discernible effect within the first few hours of uptake of Ca^{2+} ^{75,76}, K^+ ⁷⁷ and NH_4^+ ^{78,79}. However, comparison of direct effects on Al-induced nutrient uptake among developmental regions of a root is scanty. In response to 5 μM Al exposure for 24 hrs, translocation of $^{45}\text{Ca}^{2+}$ to the shoot of wheat, subsequent to absorption

in 0-5 mm root tip, was decreased by 75% but the figure was only 20% in case of mature root region (5 cm behind the tip)⁸⁰. An immediate inhibition of K^+ uptake in Al-sensitive wheat was demonstrated in a zone 2 cm behind the root apex⁸¹. Uptake of $^{15}NO_3^-$ was found inhibited by 2 hrs exposure to Al in four regions of soybean roots, as well as in the whole roots⁸².

The sensitivity of tobacco (*Nicotiana tabacum* L.) cells to Al changes was found largely depended upon the phase of growth^{72,83,84}. Cells at the logarithmic phase were found sensitive to Al, whereas at stationary phase they showed resistance. After ~ 10 hrs of exposure, cells started accumulating Al in the logarithmic-phase. Positive correlation existed between the extent of growth inhibition and the amount of Al accumulated in the cells⁷⁰. Since, the cells in stationary-phase did not take up Al, it appears that accumulation of Al is a prerequisite for the manifestation of toxicity⁸³. The localization of Al in tobacco cells was estimated by staining with haematoxylin, which indicated the presence of Al over the entire cell surface and in the nucleus⁷⁰. Al was either observed in the entire cell or found to be tightly bounded to the plasma membrane or some minor parts of the cell wall⁸³.

Several potential mechanisms of tolerance for intake of Al in the symplasm were advocated^{35,85}. These include chelation of the metal in the cytosol, compartmentalization in the vacuoles, involvement of Al-binding proteins, elevation of Al-tolerant enzyme activities etc. However, experimental data supporting these hypotheses have yet to be obtained. Exudation of malate was suggested as an important mechanism governing Al tolerance in wheat⁸⁶. Malate exudation was correlated with Al tolerance in the whole root^{87,88} and in mature root regions even though exudation per unit length was much greater for 4 mm root tips. It was proposed that organic acid exudation acts in maintaining low Al^{3+} in the rhizosphere, thereby leading to Al exclusion from the root cells³⁵. Concurrently, malate might remove Al from either the apoplast or symplast, possibly accounting for Al efflux⁴⁴. Proposed biochemical mechanisms governing Al toxicity tolerance, such as the effects on calmodulin⁸⁹ or on phospholipase⁹⁰ might be altering the metabolism in mature cells as well as at the tip, if Al were present intercellularly throughout the root. On the other hand, the proposed mechanisms for biochemical mediated Al phytotoxicity assumes that Al must enter cells and should remain somewhat active within the cells⁴⁶.

Silicon (Si) concentrations are generally found to be much higher in monocot than dicot plants. Conversely, high mineral cation-anion uptake ratio is being observed in dicots. When large amount of anionic Si participate in cation-anion balance to add the excess of anion uptake, equivalent of hydroxyl ion (OH^-) should be expelled from roots, which can increase rhizosphere pH and decrease the uptake of Al and Fe.

Plants that are able to maintain sizeable concentrations of Al within their tissues with concurrent maintenance of adequate P levels must have endowed with a mechanism whereby Al is prevented from precipitating with P at physiological pHs. The plants that chelate Al by organic acids are generally prevented from interfering P metabolism. Organic acids in plants may be responsible for maintaining high foliar concentrations of Al. Resistance for Al toxicity in calcifuges results from a binding or chelating mechanism, which also has an affinity for Fe, since many of the calcifuges were found to be highly susceptible to Fe chlorosis when grown in absence of Al. The ability of certain plant species to tolerate Al in their tissues might also result because of the presence of Al tolerant enzymes. The majority of plants growing in acid environments do not accumulate large concentration of Al in their foliage and majority of the Al they take up remains inside or in the roots. Thus, Al tolerance in these species must result from a mechanism that restricts the entry of Al into cell metabolism within the root. Under *in vitro* culture condition, quantum of Al present in selected and non-selected calli and plants substantiated the concept of different mechanisms being involved in governing Al resistance at both cellular and *in planta* levels.

Variation in Al-toxicity Tolerance across Crops

Extensive field and laboratory experiments indicated that rice genotypes show sizeable variability for Al toxicity tolerance⁹¹⁻⁹⁴. Despite the problems encountered in adapting culture media for *in vitro* selection for Al-resistance^{43,95,96}, Al-resistant cells were obtained in several species, e.g. alfalfa^{97,98}, carrot⁹⁹, soybean¹⁰⁰, tomato^{101,102}, tobacco¹⁰³ or *Nicotiana plumbaginifolia*^{94,104,105}, sorghum¹⁰⁶ and rice¹⁰⁷⁻¹⁰⁹.

Biotechnological Intervention in Modulating Tolerance Levels

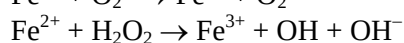
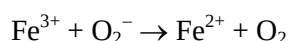
In vitro Screening

In vitro screening to develop tolerant genotype is relatively a straight forward method and demonstrated

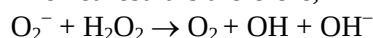
by many workers^{13,107,108}. Selection imposed to cells under *in vitro* culture acts directly on cellular phenotypes. Consequently, the trait of interest must have a well-defined cellular manifestation, which must express in cultured cells. Many agronomical important characters do not meet this requirement and are too complex residing at the levels of many functions. In fact, if the sought cellular phenotype is known, its isolation is possible by adoption of appropriate selection strategy. Comprehensive understanding of the conditions in which the crops deem to be grown is essential to simulate appropriate *in vitro* selection pressure. However, the selected tolerant calli from *in vitro* condition need not necessarily produce tolerant lines. Reaction norms towards different stresses were found to be diverse at cellular and *in planta* levels.

Induction of calli or callus culture in growth medium, supplemented with excessive Fe/Al, followed by plantlet regeneration was reported to induce metal toxicity tolerance^{13,108}, which might be heritable. Successful inheritance of tolerance was observed in tissue culture derived plants for Al resistance^{97,107,110}. Plant cell cultures are known to undergo genetic instability. Although, the mechanism subtending such instability in many cases were partly elucidated in exploitation of somaclonal variation¹¹¹ and callus selection for prospective production of lines tolerant to environmental stresses^{94,110,112,113}. Van Sint Jan *et al*¹⁰⁷ also exploited somaclonal variability (SV) and generated toxicity tolerant plantlets by applying selection pressure both at callus and plantlet regeneration levels.

Both Fe and Al in the culture medium reduce the plantlet regeneration and it was found to be inversely proportional to the increment of metal concentrations. More Fe was found to create necrosis and metal toxicity that renders total loss of plantlet regeneration at higher concentrations^{107,109}. Probably at low pH (~5.0), abundance of Fe cation causes toxicity at cellular level by intervening dedifferentiation of calli into plantlets. Furthermore, during aerobic mitochondrial respiration reactive oxygen species (ROS) are formed. The most toxic ROS is OH⁻, which is often formed when O₂⁻ and H₂O₂ are exposed to metal Fe and Cu.



The net result is therefore,



Reaction of ROS with lipid leads to highly damag-

ing reaction — lipid peroxidation. Singlet oxygen reacts with unsaturated fatty acid and forms lipid hydroperoxides that break down to several products of lipid peroxidation. Lipid peroxidation causes degeneration of cellular membranes¹¹⁴ and affects cellular functions that leads to cell death.

It is necessary to mention that *in vitro* screening for Al tolerant variants at cellular level is obviously a difficult task, which requires standardization and adoption of efficient techniques/methodologies to exclude artifacts⁹⁶. Regeneration of Al tolerant rice plants was amply reported by many^{107-109,115}. *In vitro* screening for Al tolerance may, therefore, be an alternative route to tailor Al toxicity tolerance in important field crops like rice and possibly could become complementary to genotype improvement by classical breeding methods. Of course, further test must be needed to make new plant lines available for large-scale cultivation for agricultural purpose. Field evaluation on acid soil is essentially required over years across locations for final recommendation. On the other hand, progeny testing and backcrossing would be required to establish new resistant lines and restoration of all essential parental characters in an improved variety.

F₁ Anther Culture

Doubled-haploids increase the selection efficiency. Thus, it would be easier to develop quickly superior varieties involving F₁ hybrids. The anther culture strategy also enables greater use of minor genes for resistance. The cumulative effect of which may provide more desirable resistance than provided by the major genes¹¹⁶. Rice lines have been developed through anther culture for saline soils¹¹⁷. Thus, F₁ anther culture may be utilized in development of metal toxicity tolerant rice lines.

Marker Assisted Selection

Quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) like approaches offer precision and efficient indirect selection of complex characters. Identification and integration of stress tolerant QTLs into high yielding varieties (HYVs) would increase rice production in metal toxic soils. MAS can assist gene pyramiding to achieve multiple tolerances also. Molecular markers linked to Fe and Al toxicities were mapped recently^{118,119}. Refined mapping using near isogenic lines for the development of polymerase chain reaction (PCR)-based MAS are now possible and being pursued. Rapid and reliable techniques

Table 1—Donor germplasms harbouring Fe and Al tolerance in rice

Genotype**Fe-tolerant**Pokkali, IR50, IR2151-190-3, IR2153-96-1^{29,30,31}Chakau Amubi, Burdwan local, SheetaBhog, Ramsail, Ngoba, Black Jonga, Meetmuri, Digio, Sendurjoba, Nania, Chipti, Chinadhan, Sirsoria, Thaothabi, Laiphoe, Phouren, Kalma, Framdhan, Charaimukhi²⁸Suakokos, Gossi 27³²C73, CK4, TOX3027-43-1-E3-1-1-1, TOX3050-46-E3-3-3-3, TOX3118-6-E2-3-3¹²⁵Azucena¹¹⁸*Oryza rufipogon*, *O. sativa* cv. TRC-229-F-41²²IR8192-200, IR9764-45, Kautic Putin, Mashuri¹²⁶BR5331-93-2-7-2, BR5881-93-2-8-3, IFR53647-3B-9-1, and Madhukar¹²⁷**Al-tolerant**BW196, Bhura Rata, Co37, Basmati 370¹²⁸Azucena, IRAT104, Morobekeran, Siyam Kuning, Gudabang Putih, Siyam, Lemo, Khao Daeng, Siyamhalus, Bjm-12, Ketan, Seribu Gantang, Bayer Raden, Rati, Padi Kanji³⁴IR43, Agulh Arroz, Vermelho, IAC3, IRAT109, Dinorado¹²⁶

were developed at International Rice Research Institute, Philippines to detect tolerance to Fe and Al toxicities. Wu *et al*¹¹⁸ mapped the QTLs by using a doubled-haploid (DH) population, which revealed that Fe toxicity tolerance as measured by the leaf-bronzing index (LBI) was associated with two loci of chromosome 1. The first locus was also found associated with Fe-induced enzyme glutathione-S-reductase involved in ascorbate-specific H₂O₂ scavenging system. Molecular markers associated with root length were also mapped in rice¹¹⁹. F₈ recombinant inbred line of IR1552/Azucena population was developed to map the genes for Al toxicity tolerance using related root length (RRL) as a parameter.

Transgenic Development

At molecular level, identification of tolerant genes to Fe and Al toxicities remains scanty. However, development of transgenic tobacco plants, tolerant to metal toxicity, was reported by Pan *et al*¹²⁰. Mexican scientists have also developed transgenic rice conferring tolerance to high concentrations of Al¹²¹, which could have immense prospects in genetic management of Al-toxicity.

Conclusion

Besides salinity, Fe and Al toxicity pose serious threat to rice productivity in the problem soils. At low concentrations metal ions, viz. Fe, acts as a growth stimulator or micronutrient¹²²⁻¹²⁴ but at higher concentrations, they are highly toxic to crop plants. Both Fe and Al are found abundantly in different parts of the world, causing huge losses in rice production. To overcome the problem, development of tolerant varie-

ties of rice may be achieved stepwise by unzipping the mechanism of toxicity tolerance in plants and subsequently development of toxicity tolerant rice cultivars through judicious use of conventional and non-conventional breeding methodologies. In conventional breeding, evaluation of available genotypes tolerant to Al and Fe toxicities is a prerequisite to embark upon. A dossier of tolerant genotypes, required for recombination breeding, presented in Table 1¹²⁵⁻¹²⁸ shows diverse genotypes, including both traditional cultivars and modern HYVs. Al tolerance is reported to be a complex multigenic character. In breeding for increased Al toxicity tolerance, selection can be made in early generations and pedigree method is expected to be suitable³⁴. Toxicity tolerance in induced *in vitro* mutants might have separate mechanism and would be worthy to use as *de novo* source of variation. *In vitro* culture and exploitation of SV deem to be useful in developing genotypes tolerant to Al and Fe toxicities. Furthermore, this can be combined for progeny testing and backcrossing so as to introgress new tolerant gene/s in well-adopted background. Biotechnological approaches may not be able to substitute conventional breeding; however, would be supplementary in manipulating such complex characters in future.

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