Organic acids of crop plants in aluminium detoxification

Dharmendra Singh[1,*] and S. K. Chauhan[2]

[1]Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012, India
[2]Division of Horticulture, Indian Council of Agricultural Research, KAB-II, Pusa Campus, New Delhi 110 012, India

Phytotoxicity of aluminium is one of the most serious problems in limiting plant growth in acidic soils. A number of plant species exhibit inheritable aluminium tolerance by the secretion of organic acids which is highly specific to aluminium stress and localized to the root apices. Organic acids have been considered to play an important role in the detoxification of aluminium, both externally and internally. Some plants detoxify aluminium by the secretion of organic acids from the roots. Other plant species that accumulate aluminium in their leaves, detoxify aluminium internally by forming complexes with organic acids. The kind of organic acids and the secretion pattern depends on plant species. This review summarizes current understanding of the mechanism and regulation of the secretion of organic acids from roots under aluminium stress. The advantages that plants get from the presence of organic acids in the rhizosphere are described and the biotechnological approaches to increase the secretion of organic acids are highlighted.

Keywords: Aluminium phytotoxicity, crop plants, detoxification, organic acids.

PHYTOTOXICITY of aluminium ion (Al^{3+}) is a serious problem limiting crop production in acidic soils in many parts of the India. This is particularly important in the northeastern region, where more than 90% of the soils are acidic, creating the potential for Al toxicity in surface and subsurface layers[1]. The initial response to Al toxicity is inhibition of root elongation by destroying the root apex[2], resulting in inefficient uptake of water and nutrients. Several strategies have been adopted to manage acid soils. The primary method has been the application of large amount of lime to raise soil pH. However, liming is not a remedy for sub-soil acidity and it is not always economically feasible. An alternative to liming is to select and breed aluminium-tolerant cultivars on the acid soil. Several studies provided strong evidence that Al-tolerant genotypes have developed strategies to adapt to Al toxicity, and one of these strategies involves the efflux of organic acids[3,4]. The Al-dependent stimulation of organic acid efflux from roots has now been reported in many species, and this response has been associated with an increase in Al resistance. The anions of organic acids secreted by the roots are thought to chelate the toxic Al cations, and thus prevent them from interacting with the root apices. However, some researchers observed that the secretion of organic acids is not the only mechanisms for aluminium resistance in plants. The understanding of the mechanisms and regulation of organic anion secretion from the roots under aluminium stress are not yet fully understood. The biochemical, physiological and genetic bases of the mechanisms can produce and export organic acids to the root apoplast and rhizosphere. Such aspects are beginning to be understood and will be the main focus of this review.

*For correspondence. (e-mail: dharmendrapbg@rediffmail.com)

Strategies for detoxification of Al

Crop species and cultivars exhibit wide genetic variability, both within and between species, in their response to Al resistance of plants, suggesting that Al-tolerant species or cultivars possess mechanisms for detoxifying Al. There have been two strategies for the detoxification of Al by plants cells[5,6]. One is the exclusion of Al from the root tips and the other is tolerance to Al that absorbs the plant cells[6]. The main difference between these two strategies is the site of Al detoxification: apoplasm (external) and symplasm (internal). Thus organic acids play an important role in the external and internal neutralization of Al.

Two patterns of secretion of organic acids have been proposed in terms of time required in plants (Figure 1)[3]: The release of organic acids is quick in pattern I, whereas it is delayed for several hours in pattern II after the addition of Al in nutrient solution. In the first pattern, there is no discernable delay between the moment of Al addition and the onset of organic acid efflux. Activation of an anion channel located on the plasma membrane by Al is a possible mechanism responsible for quick secretion[7–9]. This quick response suggests that the necessary metabolic machinery is constitutively expressed in the root cells and that organic anion efflux is simple triggered by Al^{3+} and induction of a novel protein is not required[10]. In pattern I, as observed in wheat[9,10], buckwheat[8,11] and barley[12], the secretion of organic acids is rapidly activated (15–30 min) after exposure of the plants to Al solution and the rate of release remains constant with time. In Al-tolerant

genotype of wheat, Al-stimulated secretion of malate from both intact roots and excised root apices was observed within 20 min exposure to Al[13]. Similarly, Osawa and Matsumoto[10] showed that malate efflux started 5 min after the addition of Al in wheat. The efflux rate in this pattern is the same at any time after the exposure to Al. In the second pattern, organic acid efflux is delayed for several hours after exposure to Al[14–16] and protein induction is required[17]. These induced proteins could be involved in organic acid metabolism or in the transport of organic acid anions. Such secretion pattern was observed in *Cassia tora*[18], rye[15], triticale[19] and rice bean[16]. For example, in *C. tora* maximal efflux of citrate occurs after 4 h of exposure to Al[18]; rice bean roots released citrate to alleviate Al toxicity and the efflux was delayed by at least 3 h (ref. 16), and in rye citrate and malate efflux increased steadily during a 10-h period[15]. In Al-resistant cultivars of maize, a considerable lag phase before the maximal citrate efflux was observed[19,20]. Al-induced secretion of malate and citrate was found to significantly increase after 6 and 12 h respectively, in triticale line[21]. At sufficient concentrations, these organic acids can form complexes with Al ions, prevent the Al ions from binding to the fixed negative sites of the cell wall and plasma membrane, and confer Al tolerance to plants to maintain the normal functions of the cell wall and plasma membrane[19].

External exclusion for Al tolerance

Secretion of organic acids from roots under Al stress has been identified as the most important mechanism avoiding Al toxicity[20] as result of the formation of Al chelates with organic acids[22,23]. Among organic acids, citrate, oxalate, malate and acetate have been identified as important Al-chelating ligands secreted by the roots[21,24,25]. These organic acids have different capabilities for detoxifying Al in the soils. Citrate has the maximum ability followed by malate and oxalate to alleviate Al toxicity. Secretion of organic acids tends to be localized to the root apex and is associated with the level of Al resistance and the presence of genes conferring Al resistance[19,26]. The organic acid exudation from different crops is summarized in Table 1.

Citrate

Citrate is produced in the mitochondria through the tricarboxylic acid (TCA) or Krebs cycle, and citrate carrier, an intrinsic protein of the inner mitochondrial membrane, plays a vital role in exporting citrate out of the mitochondria (Figure 2). Citrate is one of several organic acids exuded by plants, and correlation between organic acid exudation and Al tolerance was initially detected by Miyasaka *et al.*[27] in snapbean and found to play an important role in many other plants[21,28]. The Al-tolerant

Table 1. Organic acid exudation from different plant roots

Organic acid produced	Crop plants
Citrate	Barley[12], carrot[64], citrus[61], *Hydrangea*[45], maize[19], pea[66], oat[67], pineapple[68], rape[67], radish[67], rice[17], rice bean[16], rye[15], snapbean[27], soybean[69], tobacco[56], eucalyptus[65], triticale[21].
Malate	*Arabidopsis*[49], eucalyptus[65], oat[67], pineapple[68], rape[67], radish[67], rye[15], sunflower[70], triticale[21]
Oxalate	Buckwheat[29], spinach[71]

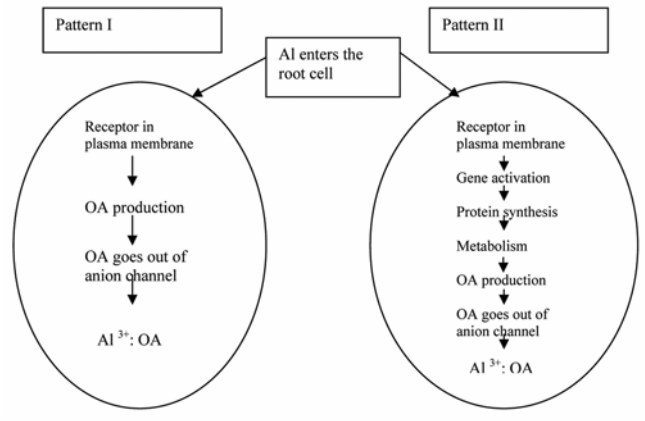

Figure 1. Two patterns of aluminium-induced secretion of organic acid (OA) anions in roots. Pattern I: There is no discernible delay between the addition of Al and the onset of secretion. Al activates the anion channel present in the plasma membrane resulting in specific release of organic acid anions to roots. Pattern II: Organic acid secretion is delayed for several hours after Al exposure. Induction of genes for release and/or biosynthesis of organic acid anions may be involved.

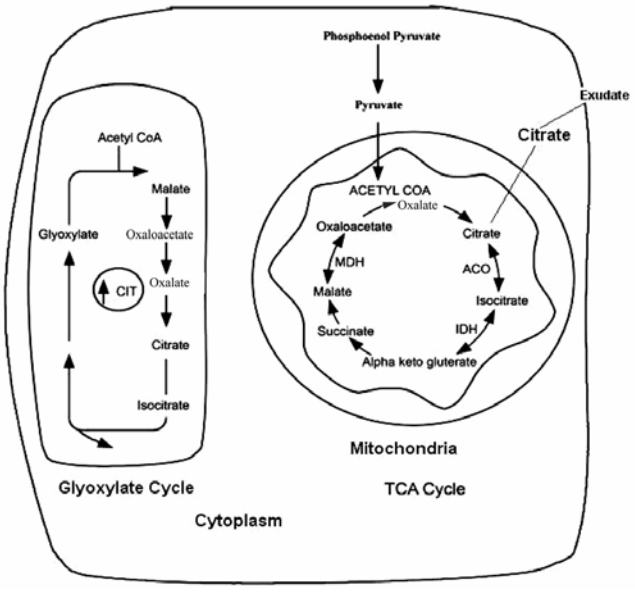

Figure 2. Metabolic map of tricarboxylic acid and glyoxylate cycles.

snapbean cultivar secreted 10-fold more citrate than the Al-sensitive cultivar. Similar observations of 3.5–7-fold citrate release were made from the roots of Al-tolerant than Al-sensitive maize with Al exposure[19]. Ma *et al.*[18] observed that Al exposure resulted in 2.5–3.0 times more citrate secretion from roots of the Al-tolerant shrub *Cassia tora* than the Al-sensitive *Cassia occidentalis*. Ma *et al.*[17] observed that citrate was detected in the presence of Al in secretion from *japonica* rice cultivar Koshihikari and *indica* rice cultivar Kasalath, while no citrate was detected in the absence of Al. The amount of citrate secreted was low ranging from 0.74 to 1.33 μmol g^{-1} root day^{-1} for Koshihikari and from 0.68 to 1.08 μmol g^{-1} root day^{-1} for Kasalath at various Al concentrations.

Oxalate

Organic acid is dominant in buckwheat leaves. Secretion of oxalate from the roots indicates that external Al-detoxification mechanisms contribute to the high Al resistance seen in buckwheat. One of the mechanisms responsible for this high resistance in buckwheat is proposed to be rapid and specific secretion of oxalate by the roots[8,29]. The secretion of oxalate can prevent Al^{3+} from entering the roots. Oxalate was secreted in the region 0–10 mm from the root tip of buckwheat[29]. It was observed that in buckwheat, the secretion of oxalate occurred within 30 min after the exposure to Al. Zhang *et al.*[8] found that buckwheat had higher resistance to Al compared with an Al-tolerant cultivar of wheat, cv. Atlas 66.

Malate

Removal of aluminium from the roots results in a rapid decline in malate secretion to non-Al level, indicating responsive Al and malate-secreting mechanisms. The differences in the degree of tolerance to aluminium depend on transport of malate out of the apical root cells via Al-activated malate-permeable channel[30]. Three possibilities have been proposed on the activation of the anion channel[7]: (i) Al interacts directly with the channel protein to trigger opening; (ii) Al interacts with a specific receptor on the membrane surface or with the membrane itself to initiate secondary messenger cascade, which then activates the channel; (iii) Al enters the cytoplasm and activates the channel directly or indirectly via secondary messengers.

The role of malate in Al^{3+} tolerance was first reported by Kitagawa *et al.*[31] in wheat. Later, Christiansen-Weniger *et al.*[32] found that an Al-tolerant wheat cultivar excreted more malate from its roots than a sensitive cultivar. However, the most convincing findings came from near-isogenic wheat lines[9]. It was found that Al^{3+} stimulated up to 10-fold greater efflux of malate from the roots of the Al-tolerant line than from the roots of the Al-sensitive line. Malate was mostly exuded from the terminal 3 mm of the root, which is the part of the root most susceptible to Al toxicity[2]. Salazar *et al.*[33] observed that Al-tolerant genotypes secreted about 10-fold higher malate and about 3–5 fold higher succinate than Al-sensitive seedlings over 24 h exposure to 50 μM Al. Malate efflux has been confirmed for other wheat cultivars differing in Al tolerance[34–36]. Li *et al.*[15] found that alteration in the metabolism of organic acid was involved in the Al-induced secretion of organic acids in rye but only activation of an anion channel seems to be responsible for the rapid secretion of malate in wheat.

Internal detoxification of Al by organic acids

In internal tolerance mechanisms, absorbed Al is detoxified by the organic acids in the cytosol. About 100 plant species accumulate Al in their stem and leaves without showing symptoms of Al toxicity[37]. The formation of a non-toxic Al complex with organic acids or other chelators, and sequestering these complexes in the vacuoles play an important role in internal detoxification of Al in Al-accumulating plants[38]. Foy[39] defines Al accumulator plants as those with more than 1000 mg kg^{-1} of Al in the leaves. Al tolerance is associated with Al accumulation in plant shoot as seen in *Arnica montana*, *Deschampsia flexuosa* L.[40], *Melastona malabathricum*[41] and *Camellia sinensis*[42]. These species are well-known Al accumulators and collect a large amount of Al in the leaves. This suggests that Al accumulator plants detoxified the internal Al^{3+} by forming Al organic complexes. *M. malabathricum* L. is a woody plant that accumulates more than 10,000 mg kg^{-1} Al, in its leaves in the form of monomeric Al and Al-oxalate complexes[41]. In tea (*Camellia sinensis* L.) plants, Al is taken up and stored in the central vacuole as complexes with organic acids[42]. High Al tolerance in buckwheat has been associated with internal[43] and external[8] detoxification mechanisms by the formation of oxalate complexes non-phytotoxic with Al. Buckwheat accumulates (1500 mg kg^{-1}) high levels of Al in the leaves without showing any symptoms of toxicity[11] and most of this Al is complexed with oxalate[29]. Most of the Al in both roots and leaves of buckwheat was complexed with oxalate in a 1 : 3 Al–oxalate complex[29]. Later, it was observed that the Al being transported to the shoot in the xylem sap was complexed with citrate and not oxalate[43]. These results suggest that Al undergoes a ligand exchange from oxalate to citrate when it is transported into the xylem, and is exchanged back with oxalate when in the leaves. Leaf compartmental analysis showed that 80% of Al in buckwheat leaves was stored in vacuoles as a 1 : 3 Al–oxalate complex[44]. *Hydrangea macrophylla* can also accumulate more than 3000 mg kg^{-1} Al dry weight in its leaves[45] and Al is complex with citrate at 1 : 1 ratio. These findings indicate that Al accumulating

species detoxify the internal Al by forming aluminium organic complexes.

Exogenous application of organic acids to reduce Al toxicity

Organic acids play an important role in both internal and external aluminium detoxification. Meriga *et al.*[46] observed that Al treatment reduced the root and shoot lengths of the control seedlings (without citrate) in rice-tolerant cultivar by 26% and 21% respectively, and those of sensitive cultivars by 51% and 23% respectively. However, corresponding seedlings grown in citrate-supplemented solution exhibited better root and shoot growth particularly at a citrate concentration of above 100 μM. At 200 μM citrate concentration, the root and shoot lengths of tolerant and sensitive cultivars improved by 60% over their respective controls. Li *et al.*[47] reported that exogenous organic acids such as citrate at 1 : 1 ratio with Al can completely detoxify Al, but the ratio of organic acid : Al that resulted in the same degree of Al detoxification for oxalate and malate was 2 : 1 and 8 : 1 respectively. These findings suggest that citrate is a better chelator of aluminium toxicity than oxalate and malate.

Molecular-assisted biotechnology to enhance secretion of organic acids

Several genes encoding organic acid production and chelation of aluminium in the rhizosphere are available. These discoveries will open up new avenues in the understanding of physiological and molecular mechanisms. The most commonly documented mechanisms of Al tolerance are the Al chelating ligands such as citrate and malate from the root tips and subsequent formation of non-toxic Al complexes in the apoplast or rhizosphere. The four cloned Al tolerance genes all encode organic anion transporters involved in this tolerance mechanism. The first two plant Al tolerance genes isolated were those encoding an Al-activated malate transporter in wheat and *Arabidopsis*[48,49] and subsequently two Al-activated citrate transporters belonging to the multidrug and toxic compound extrusion (MATE) family of membrane transporters were encoded by Al tolerance gene in barley and sorghum[50,51]. In wheat, the *TaALMT1* gene was located on the 4DL chromosome arm and was completely sequenced[52]. In barley, the expression of *ALMT1* significantly increased the flow of Al-activated malate, and Al tolerance[53]. Ma *et al.*[21] found that release of organic acid is linked to the genes on the short arm of chromosome 3R in triticale. Ma *et al.*[54] observed that tightly linked marker Bmag 353 explained 51.3% of phenotypic variance for citrate secretion in barley. Further, Furukawa *et al.*[50] identified a gene (*HvAACT1*) responsible for the Al-activated citrate secretion by fine-mapping combined with microarray analysis in barley.

Genetic engineering technology for the release and synthesis of organic acids

Identification and isolation of genes that specify Al tolerance are prerequisites for direct engineering of crop plants to increase crop production in acidic soils. The production of transgenic plants with an increased capacity to produce and/or excrete organic acids that chelate and detoxify Al in the rhizosphere, is an appealing strategy to produce Al-tolerant plants. Genetically engineered plants that overexpress genes involved in the biosynthesis and transport of organic acids and Al toxicity events at the cell level have been produced. Plant breeders can take advantage of genetic engineering, by which useful genes are made available in any species. Researchers have manipulated the biosynthesis capacity of cells which produce and accumulate higher amounts of organic acids. As a result, this will change the root exudation profile and Al resistance of a genotype. Table 2 summarizes the attempts to obtain transgenic plants with higher Al resistance by enhancing organic acid exudation.

Overproduction of citrate appears to be an important strategy to produce Al-tolerant transgenic plants. The production of citrate by the condensation of acetyl CoA and oxaloacetate is the first step in the TCA cycle and is catalysed by the enzyme citrate synthase (Figure 2). The most well-known example of successful achievement in this direction is the work of de la Fuente *et al.*[55]. They introduced a *Pseudomonas aurginosa CSb* gene into tobacco and papaya. As a result, the transgenic plants showed enhanced citrate efflux and greater Al tolerance than non-transformant lines. The transformed lines of tobacco expressing *CSb* had up to 10-fold greater internal citrate in their root tissues, whereas in papaya citrate level in the roots was only 2–3 fold. Increased production of citrate was shown to result in Al tolerance in both the species. However, an attempt to repeat the work using the same transgenic lines as those of de la Fuente *et al.*[55] and tobacco transgenics expressing the *P. aeruginosa CS* gene to 100-fold greater levels, has shown neither increased citrate concentration in the roots nor increased citrate efflux. Thus no improvement of Al tolerance was achieved[56].

Tolerance to Al was also conferred on other crops, viz. rice[57] and carrot cells[58] by overexpression of the *CSb* gene, and on alfalfa by overexpression of the malate dehydrogense gene[59]. These promising findings strengthen the possibility of engineering plants with superior resistance to aluminium toxic acid soils.

The strategy of overexpressing enzymes involved in organic acid metabolism has proven to be effective to enhance organic acid exudation and to increase Al resistance with other transgenic plants. The overexpression of a mitochondrial *CSb* gene from *Arabidopsis* into carrot cells resulted in higher CSb activity and higher secretion of citrate compared with wild-type cells[58]. Koyama *et al.*[60]

Table 2. Aluminium tolerance in transgenic plants expressing genes involved in organic acid synthesis and release

Gene	Gene product	Source plant	Target	Aluminium-resistance
CSb	Citrate synthase	*Pseudomonas aeruginosa*	Tobacco, papaya[55]	Increases
CSb	Citrate synthase	*P. aeruginosa*	Tobacco, Alfalfa[56]	Does not change
neMDH	Malate dehydrogenase	Alfalfa	Alfalfa[59]	Increases
PEPC	Phosphoenopyruvate carboxylase	Alfalfa	Alfalfa[59]	Does not change
At-mtCS	Citrate synthase	*Arabidopsis*	Oilseeds[72]	Does not change
ALMT1	Malate channel	Wheat	Barley[53]	Increases

introduced a mitochondrial *CSb* gene isolated from *Daucus carota* into *Arabidopsis thaliana* causing the same effect in transgenic plants. The transformants showed up to three fold increase in citrate synthase activity and 1.6-fold increase in citrate secretion compared with controls.

Deng *et al.*[61] observed that exposure to Al triggered the exudation of citrate from the yuzu root (*Citrus junos* Sieb. ex Tanaka). Al also elicited an increase in citrate content and increased the expression level of mitochondrial citrate synthase (*CjCS*) gene and enzyme activity in yuzu. The *CjCS* gene was cloned from yuzu and overexpressed in *Nicotiana benthamiana* through *Agrobacterium tumefaciens*. The transgenic *Nicotiana* plants showed increased levels of citrate in roots compared to wild-type plants. The exudation of citrate from the roots of transgenic plants significantly increased when exposed to Al. Increased expression level of the *CjCS* gene and enhanced enzyme activity were observed in transgenic plants compared with wild-type plants. The results with transgenic plants suggest that overexpression of mitochondrial citrate synthase can be a useful tool to achieve Al tolerance.

In cultured tobacco, the functions of the BnALMT1 and BnALMT2 (*Brassica napus* Al-activated malate transporter) proteins were studied by heterologous expression. Such transfection system showed an enhanced capacity for malate efflux but not citrate efflux when exposed to Al. Transgenic tobacco cells grow significantly better than control cells. This indicated that expression of BnALMT1 and BnALMT2 increased the resistance of these plant cells to Al stress[62]. Thus use of citrate synthase gene may prove to be an effective strategy for the production of Al-tolerant crop species without undesirable effects on other agronomic traits.

Other approaches aim to increase organic acid exudation by overexpressing phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH)[63]. Overexpression of plant genes for such enzymes (PEPC, MDH) enhanced organic acid synthesis and secretion and greater Al tolerance in alfalfa[59]. Selected transgenic plants with a 1.6-fold increase in MDH specific activity showed a 4.2-fold increase in citrate, oxalate, malate, succinate and acetate in root tissues compared to the control untransformed line. A plant line containing the *PEPC* transgene with a two fold increase in PEPC activity had increased amounts of malate compared to the control. Plants expressing *MDH* or *PEPC* transgene showed enhanced root elongation compared with the control untransformed line in solution culture assay[59]. In alfalfa, overexpression of MDH resulted in enhanced organic acid synthesis and secretion and greater Al resistance[59]. Begum *et al.*[57] reported that PEPC transgenic rice was more tolerant to Al than the wild type, because root tips of transgenic rice accumulated less Al than those of the wild type. Al-induced oxalate exudation from the roots occurred at increased rates in the transgenic line. Overexpresssion of C4-PEPC drastically increased PEPC activity in the leaves of transgenic rice, and resulted in enhanced Al tolerance in transgenic rice causing higher organic acid concentration in the leaves and roots.

Conclusion

There is evidence that some plant species secrete organic acids to protect their root tips from Al^{3+} toxicity in acidic soil. Thus organic acids such citrate, oxalate and malate play an important role as a response to aluminium stress. Al-tolerant species secrete more organic acids than sensitive plants. However, research on organic acids has also resulted in many questions that remain unanswered. For example, why do species release different organic acids as a response to Al stress? How much organic acid secretion is sufficient to detoxify Al? How does Al exposure activate or induce the secretion of specific organic acids out of root cells? Are there any other defence mechanisms for Al resistance? We are just releasing internal detoxification of Al with organic acids and its sequestration as Al-oragnic complexes in the vacuole in tolerating Al toxicity. It is also likely that other additional resistance mechanisms exist.

The cloning of genes whose products increase the synthesis of organic acids and their transport across the plasma membrane of roots are needed to enhance conventional breeding approaches for improving Al tolerance. Several genes are expressed in Al-tolerant genotypes of wheat, soybean and *Arabidopsis*. These genes are good candidates to be studied in other crops to find mechanisms of Al tolerance in plants. Citrate synthase gene has

been identified, and there are many yet to be discovered before targeted genetic modifications can be effectively designed. The molecular aspects underlying Al resistance mechanisms via Al-induced expression of some other enzymes catalysing organic acid synthesis and metabolisms need to be studied.

1. Sharma, U. C. and Singh, R. P., Acid soils of India: their distribution, management and future strategies for higher productivity. *Fert. News*, 2002, **47**, 45–52.
2. Ryan, P. R., Ditomaso, J. M. and Kochian, L. V., Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. *J. Exp. Bot*., 1993, **44**, 437–446.
3. Ma, J. F., Role of organic acids in detoxification of aluminium in higher plants. *Plant Cell Physiol*., 2000, **41**, 383–390.
4. Ryan, P. R., Delhaize, E. and Jones, D. L., Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 2001, **52**, 527–560.
5. Taylor, G. J., Current view of the aluminium stress response: the physiological basis of tolerance. *Curr. Top. Plant Biochem. Physiol*., 1991, **10**, 57–93.
6. Kochian, L. V., Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 1995, **46**, 273–260.
7. Delhaize, E. and Ryan, P. R., Aluminium toxicity and tolerance in plants. *Plant Physiol*., 1995, **107**, 315–321.
8. Zhang, S., Ma, J. F. and Matsumoto, H., High aluminium resistance in buckwheat: I. Al induced specific secretion of oxalic acid from root tip tips. *Plant Physiol*., 1998, **117**, 745–751.
9. Delhaize, E., Ryan, P. R. and Randall, P. J., Aluminium tolerance in wheat (*Triticum aestivum* L.): Part II. Aluminium-stimulated excretion of malic acid from root apices. *Plant Physiol*., 1993, **103**, 695–702.
10. Osawa, H. and Matsumoto, H., Possible involvement of protein phosphorylation in aluminium responsive malate efflux from wheat root apex. *Plant Physiol*., 2001, **126**, 411–420.
11. Zhang, S., Ma, J. F. and Matsumoto, H., High aluminium resistance in buck wheat: II. Oxalic acid detoxifies aluminium internally. *Plant Physiol*., 1998, **117**, 753–759.
12. Zhao, Z., Ma, J. F., Sato, K. and Takeda, K., Differential aluminium resistance and citrate secretion in barley (*Hordeum vulgare* L.). *Planta*, 2003, **217**, 794–800.
13. Ryan, P. R., Delhaize, E. and Randall, P. J., Characterization of Al-stimulated efflux of malate from the apices of Al tolerant wheat roots. *Planta*, 1995, **196**, 103–110.
14. Ma, J. F., Ryan, P. and Delhaize, E., Aluminium resistance in plants and the complexing role of organic acids. *Trends Plant Sci*., 2001, **6**, 273–278.
15. Li, X. F., Ma, J. F. and Matsumoto, H., Pattern of Al induced secretion of organic acids differs between rye and wheat. *Plant Physiol*., 2000, **123**, 1537–1543.
16. Yang, L. J., Zhang, L., Ya, L. Y., You, F. J., Wu, P. and Shao, Z. J., Citrate transporters play a critical role in aluminium stimulated citrate efflux in rice bean (*Vigna umbellata*) roots. *Ann. Bot.*, 2006, **97**, 579–584.
17. Ma, J. F., Shen, R., Zhao, Z., Wissuwa, M., Takeuchi, Y., Ebitani, T. and Yano, M., Response of rice to Al stress and identification of quantitative trait loci for aluminium tolerance. *Plant Cell Physiol*., 2002, **43**, 652–659.
18. Ma, J. F., Zheng, S. J. and Matsumoto, H., Specific secretion of citric acid induced by Al stress in *Cassia tora* L. *Plant Cell Physiol*., 1997, **38**, 1019–1025.
19. Pellet, D. M., Grunes, D. L. and Kochian, L. V., Organic acid exudation as aluminium tolerance mechanisms in maize (*Z. mays* L.). *Planta*, 1995, **196**, 788–795.
20. Ma, J. F., Role of organic acids in detoxification of aluminium in higher plants. *Plant Cell Physiol*., 2000, **41**, 383–390.
21. Ma, J. F., Taketa, S. and Yang, Z. M., Aluminium tolerance genes on the short arm of chromosomes 3 R are linked to organic acid release in triticale. *Plant Physiol*., 2000, **122**, 687–694.
22. Hue, N. V., Croddock, G. R. and Adams, F., Effect of organic acids on aluminium toxicity in subsoil. *Soil Sci. Soc. Am. J*., 1986, **50**, 28–34.
23. Lu, H. M., Watnabe, T., Shinano, T. and Tadano, T., Comparison of aluminium tolerance and phosphate absorption between rape (*Brassica napus* L.) and tomato (*Lycopersicon esculentum* Mill) in relation to organic acid exudation. *Soil Sci. Plant Nutr*., 1999, **45**, 897–907.
24. Ma, Z. and Miyasaka, S. C., Oxalate exudation by taro in response to Al. *Plant Physiol*., 1998, **118**, 861–865.
25. Matsumoto, H., Cell biology of aluminium toxicity and tolerance in higher plants. *Int. Rev. Cytol*., 2000, **200**, 1–46.
26. Zheng, S., Ma, J. F. and Matsumoto, H., Continuous secretion of organic acids is related to aluminium in the apical cells of wheat roots. *Plant Physiol*., 1988, **125**, 1459–1472.
27. Miyasaka, S. C., Buta, J. G., Howell, R. K. and Foy, C. D., Mechanism of Al tolerance in snapbeans. Root exudation of citric acid. *Plant Physiol*., 1991, **96**, 737–743.
28. Yang, Z. M., Sivaguru, M., Horst, W. J. and Matsumoto, H., Aluminium tolerance is achieved by exudation of citric acid from roots of soybean (*Glycine max* L. Merr.). *Physiol. Plant*., 2000, **110**, 72–77.
29. Ma, J. F., Zheng, S. J., Matsumoto, H. and Hiradate, S., Detoxifying aluminium with buckwheat. *Nature*, 1997, **390**, 569–570.
30. Zhang, W. H., Ryan, P. R. and Tyerman, S. D., Malate-permeable channels and cation channels activated by Al in the apical cells of wheat roots. *Plant Physiol*., 2001, **125**, 1459–1471.
31. Kitagawa, T., Morishita, T., Tachibana, Y., Namai, H. and Ohta, Y., Differential aluminium tolerance in wheat and secretion of organic acids. *Jpn. J. Soil Sci. Plant Nutr*., 1986, **57**, 352–358.
32. Christiansen-Weniger, C., Groneman, A. F. and Vanveen, J. A., Associative N_2 fixation and root exudation of organic acids from wheat cultivars of different aluminium tolerance. *Plant Soil*, 1992, **139**, 167–174.
33. Salazar, F. S., Pandey, S., Narro, L., Peres, J. C., Cebalos, H., Parentoni, S. N. and Filho, A. F. C., Diallel analysis of acid tolerant and intolerant tropical maize populations. *Crop Sci*., 1997, **37**, 1457–1462.
34. Pellet, D. M., Papernik, L. A. and Kochian, L. V., Multiple aluminium resistance mechanisms in wheat: roles of root apical phosphatate and malate exudation. *Plant Physiol*., 1996, **112**, 591–597.
35. De Andrade, L., Ikeda, M. and Ishizuka, J., Excretion and metabolism of malic acid produced by dark fixation in the roots in relation to aluminium tolerance of wheat. In *Plant Nutrition for Sustainable Food Production and Environment* (eds Ando, T. *et al*.), Kluwer, Dordrecht, 1997, pp. 445–446.
36. Basu, U., Godbold, D. and Taylor, G. J., Aluminium resistance in *Triticum aestivum* associated with enhanced exudation of malate. *J. Plant Physiol*., 1994, **144**, 747–753.
37. Barcelo, J. and Poschenrieder, C., Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environ. Exp. Bot*., 2002, **48**, 75–92.
38. Ma, J. F., Physiological mechanisms of Al tolerance in higher plants. *Soil Sci. Plant Nutr*., 2006, **51**, 609–612.
39. Foy, C. D., Physiological effects of hydrogen, aluminium and manganese toxicities in acid soils. In *Soil Acidity and Liming* (ed. Adams F.), Madison, WI, 1984, 2nd edn, pp. 57–97.
40. Pegtel, D. M., Effect of ionic Al in culture solutions on the growth of *Arnica montana* L. and *Deschampsia flexuosa* (L.). *Plant Soil*, 1987, **102**, 85–92.

41. Watnabe, T., Osaki, M., Yoshihara, T. and Tadano, T., Distribution and chemical speciation of aluminium in the Al accumulator plant, *Melastoma malabatharicum* L. *Plant Soil*, 1998, **201**, 165–173.
42. Matsumoto, H., Hirasawa, E., Morimura, S. and Takahashi, E., Localization of absorbed aluminium in tea leaves. *Plant Cell Physiol*., 1976, **17**, 627–631.
43. Ma, J. F. and Hiradate, S., Form of aluminium for uptake and translocation in buckwheat (*Fagopyrum esculentum* Moench). *Planta*, 2000, **211**, 355–360.
44. Shen, R., Ma, J., Kyo, M. and Iwashita, T., Compartmentation of aluminium in leaves of an Al-accumulator, *Fagopyrum esculentum* Moench. *Planta*, 2002, **215**, 394–398.
45. Ma, J. F., Hiradate, S., Nomoto, K., Iwashita, T. and Matsumoto, H., Internal detoxification mechanism of Al in *Hydrangea*: identification of Al form in the leaves. *Plant Physiol*., 1997, **113**, 1033–1039.
46. Meriga, B., Reddy, B. K., Jageswar, G., Reddy, L. A. and Kishor, P. B., Alleviating effect of citrate on Al toxicity of rice seedlings. *Curr. Sci*., 2003, **85**, 383–385.
47. Li, Y. Y., Zhang, Y. J., Zhou, Y., Yang, J. L. and Zhang, S. J., Protecting cell walls from binding aluminium by organic acids contribute to Al resistance. *J. Integr. Plant Biol.*, 2009, **51**, 574–580.
48. Sasaki, T. *et al.*, A wheat gene encoding an aluminium activated malate transporter. *Plant J*., 2004, **37**, 645–653.
49. Hoekenga, O. A. *et al.*, AtALMT1, which encodes a malatae transporter is identified as one of several genes critical for aluminium tolerance in Arabidopsis. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 9738–9743.
50. Furukawa, J. *et al.*, An aluminium-activated citrate transporter in barley. *Plant Cell Physiol*., 2007, **48**, 1081–1091.
51. Magalhaes, J. V., Liu, J. and Guimaraes, C. T., A gene in the multidrug and toxic compound extrusion (MATE) family members confers aluminium tolerance in sorghum. *Nature Genet*., 2007, **39**, 1156–1161.
52. Raman, H. *et al.*, Molecular characterization and mapping of ALMT1, the aluminium tolerance gene of bread wheat (*Triticum aestivum* L.). *Genome*, 2005, **48**, 781–791.
53. Delhaize, E., Ryan, P. R., Hebb, D. M., Yamamoto, Y., Sasaki, T. and Matsumoto, H., engineering high level aluminium tolerance in barley with the ALMT1 gene. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 15249–15254.
54. Ma, J. F., Nagao, S., Sato, K., Ito, H., Furukawa, J. and Takeda, K., Molecular mapping of a gene responsible for Al-activated secretion of citrate in barley. *J. Exp. Bot*., 2004, **55**, 1335–1341.
55. de la Fuente, J. M., Ramirej-Rodriguez, V., Cabrera-Ponce, J. and Herrera-Estrella, L., Aluminium tolerance in transgenic plants by alteration of citrate synthesis. *Science*, 1997, **276**, 1566–1568.
56. Delhaize, E., Hebb, D. M. and Ryan, P. P., Expression of a *Psedomonas aeruginosa* citrate synthese gene in tobacco is not associated with either enhanced citrate accumulation or efflux. *Plant Physiol*., 2001, **125**, 2059–2067.
57. Begum, H. H., Osaki, M., Watanabe, T., Shinano, T. and Islam Md, T., Mechanisms of aluminium resistance in phosphoenolpyruvate carboxylase (PEPC) transgenic rice. In Paper presented in the International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 9–12 February 2005.
58. Koyama, H., Takita, E., Kawamura, A., Hara, T. and Shibata, D., Over-expression of mitochondrial citrate synthase in *Arabidopsis thaliana* improves the growth of carrot cells in Al-phosphate medium. *Plant Cell Physiol*., 1999, **40**, 482–488.
59. Tesfaye, M., Temple, S. J., Allan, D. L., Vance, C. P. and Samac, D. A., Over-expression of malate dehydrogenase in transgenic alfalfa enhances organic acid synthesis and confers tolerance to aluminium. *Plant Physiol*., 2001, **127**, 1836–1844.
60. Koyama, H., Kawamura, A., Kihara, T., Hara, T., Takita, E. and Shibata, D., Overexpression of mitochondrial citrate synthase in *Arabidopsis thaliana* improved growth on a phosphorus-limited soil. *Plant Cell Physiol*., 2000, **41**, 1030–1037.
61. Deng, W., Luo, K., Li, Z., Yang, Y., Hu, N. and Wu, Y., Over-expression of *Citrus junos* mitochondrial citrate synthase gene in *Nicotiana benthamiana* confer Al tolerance. *Planta*, 2009, **230**, 355–365.
62. Ligaba, A., Katsuhara, M., Ryan, P. R., Shibasaka, M. and Matsumoto, H., The Bn*ALMT1* and Bn*ALMT2* genes from rape encoded aluminium-activated malate transporters that enhance the aluminium resistance of plant cells. *Plant Physiol*., 2006, **112**, 1289–1300.
63. Raghothama, K. G., Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 1999, **50**, 665–693.
64. Takashi, K., Katsunori, S., Hideki, O., Kunihiko, O. and Masahiko, S., Al tolerance of carrot (*Daucus carota* L.) S1 lines from selected cell cultures and locational variation of exuding citric acid from their roots. *Jpn. J. Soil Sci. Plant Nutr*., 2001, **72**, 49–55.
65. Silva, I. R. *et al.*, Response of eucalyptus species to Al: the possible involvement of low molecular weight organic acid in the aluminium tolerance mechanisms. *Tree Physiol.*, 2004, **24**, 1267–1277.
66. Kobayashi, Y., Yamamoto, Y. and Matsumoto, H., Studies on the mechanisms of aluminium tolerance in pea (*Pisum sativum* L.) using cultivar 'Alsaka' and Al-sensitive cultivar 'Hyogo'. *Soil Sci. Plant Nutr*., 2004, **50**, 197–204.
67. Zheng, S., Ma, J. F. and Matsumoto, H., Continuous secretion of organic acids is related to Al resistance during relatively long-term exposure to Al stress. *Physiol. Plant*., 1998, **103**, 209–214.
68. Le-Van, H. and Masuda, T., Physiological and biochemical studies on aluminum tolerance in pineapple. *Aust. J. Soil Res*., 2004, **42**, 699–707.
69. You, J. F., Hou, N. N., Xu, M. Y., Zhang, H. M. and Yang, Z. M., Citrate transporter play an important role in regulating Al-induced citrate secretion in *Glycine max*. *Biol. Plant*., 2010, **54**, 766–768.
70. Saber, N., Abdel-Moneim, A. and Barakat, S., Role of organic acids in sunflower tolerance to heavy metal. *Biol. Plant Prague*, 1999, **42**, 65–73.
71. Yang, J. L., Zheng, S. J., He, Y. F. and Matsumoto, H., Aluminium resistance requires resistance to acid stress: a case study with spinach that exudates oxalate rapidly when exposed to Al stress. *J. Exp. Bot*., 2005, **56**, 1197–1203.
72. Anoop, V. M., Basu, U., McCammon, M. T., McAlister-Henn, L. and Taylor, G. J., Modulation of citrate metabolism alters aluminium tolerance in yeast and transgenic canola overexpressing a mitochondrial citrate synthase. *Plant Physiol*., 2003, **132**, 2205–2217.

Received 23 March 2010; revised accepted 8 February 2011