Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance

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Abstract

Aluminum (Al) toxicity in acid soils is a significant limitation to crop production worldwide, as approximately 50% of the world's potentially arable soil is acidic. Because acid soils are such an important constraint to agriculture, understanding the mechanisms and genes conferring resistance to Al toxicity has been a focus of intense research interest in the decade since the last article on crop acid soil tolerance was published in this journal. An impressive amount of progress has been made during that time that has greatly increased our understanding of the diversity of Al resistance genes and mechanisms, how resistance gene expression is regulated and triggered by Al and Al-induced signals, and how the proteins encoded by these genes function and are regulated. This review examines the state of our understanding of the physiological, genetic, and molecular bases for crop Al tolerance, looking at the novel Al resistance genes and mechanisms that have been identified over the past ten years. Additionally, it examines how the integration of molecular and genetic analyses of crop Al resistance is starting to be exploited for the improvement of crop plants grown on acid soils via both molecular-assisted breeding and biotechnology approaches.

Contents

INTRODUCTION

Aluminum (Al) toxicity is a primary factor reducing crop yields on acidic soils. At pH values below 5, Al^{3+} ions are dissolved from clay minerals and are quite toxic to plant roots, inhibiting root growth and function. This in turn leads to reduced crop yields caused by drought and mineral deficiencies. As much as 40–50% of the world's potentially arable lands are acidic (127, 129), and approximately 60% of acid soils are located in the tropics and subtropics. Because many developing countries are located where the majority of acid soils reside, Al toxicity limits crop production in those parts of the world where food security is most tenuous. Al toxicity is exceeded only by drought among the abiotic limitations to crop production (127). Because Al is such a reactive element, there are many mechanisms of Al toxicity that involve its interaction with the root cell wall, plasma membrane, and symplasm. For a summary of potential mechanisms of Al toxicity, we refer readers to the Overview of Aluminum Toxicity section in the previous *Annual Review of Plant Biology* article on this topic (49).

For more than 50 years, plant breeders have exploited the significant genetic diversity in Al resistance present in many crop species to effectively breed for increased crop Al resistance, especially in tropical breeding programs. Because of the agronomic importance of acid soils, there has also been considerable investigation since the last *Annual Review of Plant Biology* article on acid soil resistance (49) into the molecular biology and physiology of crop Al resistance with the goal of identifying Al resistance genes and their associated mechanisms. Hence, there has been

considerable progress and a wealth of published material regarding advances in the molecular, genetic, and physiological bases for crop Al resistance.

In this review, we focus on some of the most significant advances in this field over the past ten years (for other fairly recent reviews on the topic, see 19, 63, 105, 112). Furthermore, as the field of crop Al resistance research has matured, researchers have begun to translate our fundamental understanding of the function and interplay of Al resistance genes and their networks in order to facilitate additional improvements in Al resistance via molecular-assisted breeding and biotechnology approaches, which can be used to build on the progress already made by previous crop breeding programs.

Al tolerance: an Al resistance mechanism in which Al^{3+} ions are sequestered and detoxified in subcellular compartments and/or translocated away from the root tip

Al exclusion: an Al resistance mechanism based on exudation of Al-chelating organic compounds (e.g., organic acids or phenolics) into the rhizosphere, preventing toxic Al species from entering root cells

PLANT ALUMINUM RESISTANCE AND TOLERANCE STRATEGIES

Prior to the identification and characterization of the different physiological mechanisms plants use to deal with Al toxicity, the terms Al tolerance and Al resistance were often used interchangeably. In this review, we use the term Al resistance to refer to the ability of a plant to maintain reasonable growth and yield on acidic, Al-toxic soils and/or Al-toxic nutrient solutions. Two main types of Al resistance mechanisms have been documented: (*a*) Al exclusion mechanisms, which aim at preventing Al from entering the root apex (both apoplasm and symplasm), and (*b*) Al tolerance mechanisms, in which Al enters the plant and is detoxified and sequestered. This section focuses on the molecular physiology of Al resistance mechanisms that have been identified and/or proposed, providing an overview of the different mechanisms described along the pathway Al^{3+} encounters as it moves from the rhizosphere into the root, and in some cases into the shoot.

Aluminum Exclusion Mechanisms

Al resistance via Al exclusion from the root apex, the primary site of Al toxicity, involves the regulated release of organic compounds from the root tip. In this section, we focus on the release of organic acids as the most widely described Al exclusion mechanism; in the subsequent section, we then describe recent work suggesting that release of phenolic compounds may also play a role in this mechanism.

Aluminum exclusion via root organic acid exudation. By far the most well-characterized Al exclusion mechanism is AL-dependent root exudation of organic acid (OA) anions into the rhizosphere, where they chelate Al^{3+} ions, forming nontoxic compounds that do not enter the root. Pioneering physiological studies on snap bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) Al resistance during the early 1990s demonstrated a correlation between increased Al resistance and increasing levels of Al-dependent exudation of Al-chelating OAs (citrate and malate in snap bean and wheat roots, respectively) (18, 21, 82). This led to numerous studies in a range of monocot and dicot species that demonstrated similar correlations between variation in Al resistance and the AL-dependent exudation of malate, citrate, and (in some species) oxalate from roots (see table 1 in Reference 49). The primary differences between plant species that employ this Al resistance mechanism are (*a*) the identity of the OAs released (malate, citrate, or oxalate), (*b*) the magnitude of the OA exudation, and (*c*) the time course for AL-dependent OA exudation. Although many plant species release one type of OA, it is not unusual to find plant species that release more than one OA compound in response to Al stress [e.g., malate and citrate in wheat and *Arabidopsis* (60, 109)], suggesting that multiple release mechanisms or transporters may be operating in tandem in these species.

Electrophysiological studies of root Al resistance mechanisms in the late 1990s and early 2000s provided the first insights into the molecular mechanisms mediating OA transport, by

demonstrating the existence of specific plasma membrane anion-permeable channels that were activated by extracellular Al^{3+} in protoplasts isolated from root tips of Al-resistant wheat (111, 144) and maize (*Zea mays*) (50, 97, 98). More recently, the first genes underlying Al resistance mechanisms were cloned, and it was not surprising that they encoded malate and citrate transporters in the roots of wheat, sorghum (*Sorghum bicolor*), and barley (*Hordeum vulgare*) (29, 71, 114). These and subsequent studies established that the members of two families of membrane transporters—the Al-activated malate transporter (ALMT) family of anion channel transporters and the multidrug and toxic compound extrusion (MATE) family of OA/H^+ antiport transporters—are responsible for plasma membrane malate and citrate efflux, respectively, from plant root cells into the rhizosphere in response to toxic Al^{3+} ions. We discuss these and other membrane transporters involved in Al resistance in more detail below (see The Role of Membrane Transporters in Aluminum Resistance). **Figure 1** depicts a model of Al exclusion based on ALMT- and MATE-mediated OA efflux.

Aluminum exclusion via release of compounds other than organic acids. Roots release a wide range of organic compounds, and some of those compounds, such as phenolics, have the capacity to chelate Al^{3+} ions in the rhizosphere. Phenolic compounds are less potent chelators of Al^{3+} than OAs are (4), but the electrophilic nature of oxygen atoms in −OH groups of phenolic rings imparts a reasonable capacity for Al^{3+} chelation. Kidd et al. (46) determined that, in response to Al, the roots of an AL-resistant maize genotype exude the phenolic compounds catechol, catechin, and quercetin, leading them to speculate that root phenolic exudation may play a role in maize root Al exclusion along with citrate release. There have been no additional published reports of root exudation of phenolics as an Al resistance mechanism, although the same research group found that, in response to Al stress, the roots of an Al-resistant maize variety had significantly higher internal levels of caffeic acid, catechol, and catechin than an Al-sensitive variety, suggesting that an internal Al tolerance mechanism works in concert with root tip Al exclusion mediated by citrate exudation (124).

Several other studies have also suggested a role for root phenolics in internal Al tolerance. Tahara et al. (122) found that, in response to Al stress, the roots of highly Al-resistant camphor (*Cinnamomum camphora*) and *Eucalyptus camaldulensis* trees synthesize oenothein B, a dimeric hydrolyzable tannin containing several adjacent phenolic hydroxyl groups, which they showed was an effective Al-binding ligand. The higher levels of oenothein B content in roots were positively correlated with the degree of Al resistance displayed among five tree species. Another report on camphor trees proposed a unique phenolic-mediated root Al exclusion mechanism (89). In this study, the authors identified a specialized outer cell layer of the root that, in contrast to less Al-resistant species, accumulates significant levels of the flavonoid proanthocyanidin. They proposed that this cell layer, which is adjacent to the outer layer of root epidermis cells, continually grows and gradually detaches from the root, providing protection against Al^{3+} accumulation in the root apex. In light of these recent publications, the role of phenolic compounds in Al exclusion and internal Al detoxification mechanisms is likely to garner additional attention.

Aluminum Tolerance Mechanisms

Much of our knowledge of the mechanisms plants employ to tolerate and detoxify Al^{3+} comes from studies of more Al-resistant plant species, especially rice (*Oryza sativa*) and Al accumulators such as buckwheat (*Fagopyrum esculentum*) and hydrangea (*Hydrangea macrophylla*). Research into the physiology and molecular biology/genetics of rice Al resistance and tolerance has been quite illuminating, as this highly resistant cereal species employs multiple Al resistance genes and mechanisms, many of which involve Al tolerance within the root. To date, Al tolerance

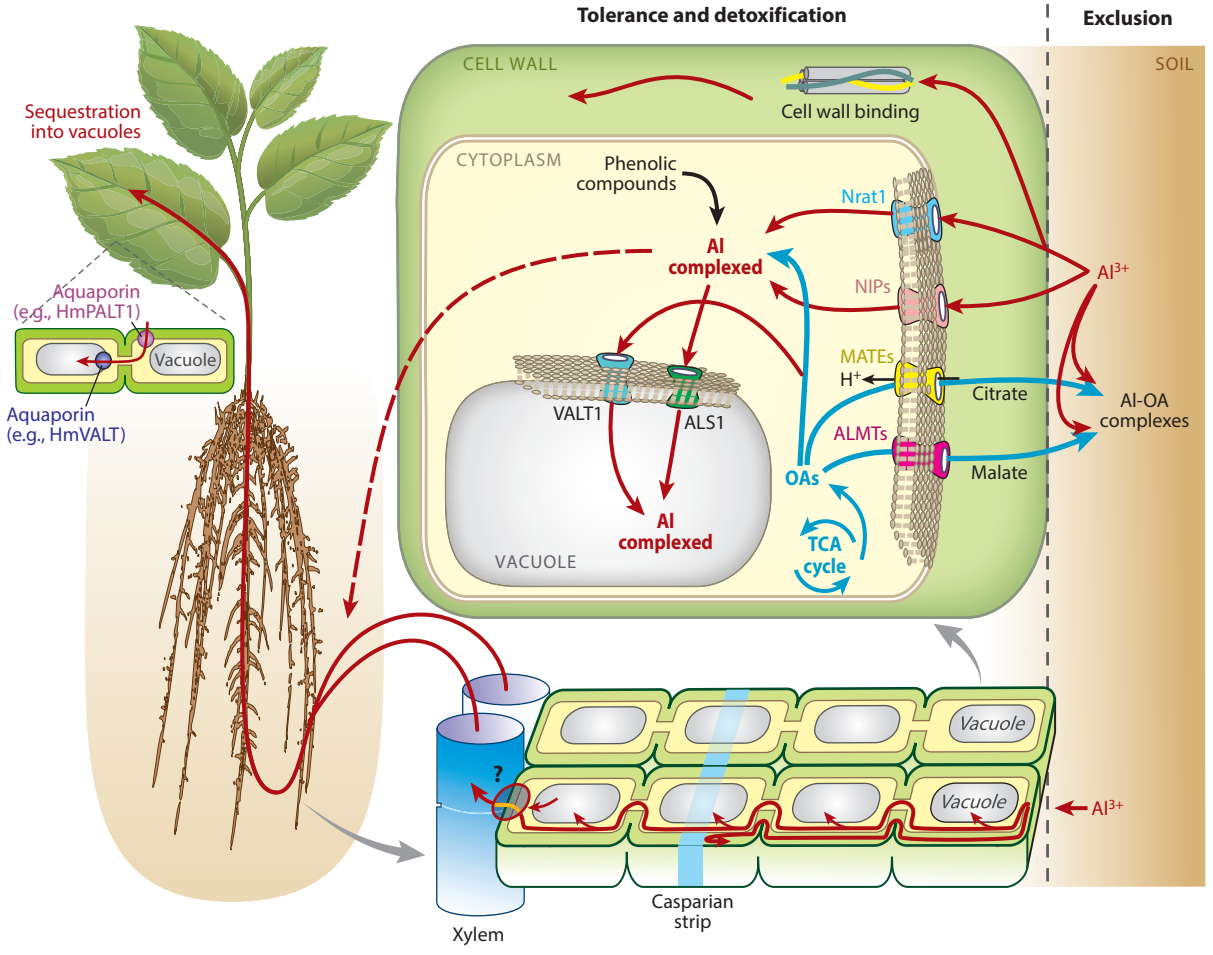


Figure 1

General model illustrating mechanisms of Al resistance (Al exclusion and Al tolerance/detoxification) that plants employ as Al^{3+} is taken up from the soil solution by the root and some of the absorbed Al is translocated to and stored in the shoot. In the upper right, Al exclusion and internal Al detoxification (tolerance) mechanisms in the root are based on the chelation of Al^{3+}, primarily by OA anions. Root Al exclusion mechanisms involve the root exudation of OA anions via Al-activated anion channels (ALMTs) or carriers (MATEs) across the plasma membrane. The internal Al detoxification mechanisms involve Al^{3+} influx across the root cell plasma membrane (via Nramp and/or NIP Al transporters) into the cytosol, where the Al is chelated with OA anions and (in some species) phenolic compounds. Finally, the Al (which could be either free Al^{3+} ions or an Al-chelate complex) is transported into the vacuole, possibly via an ALS1-type ABC or VALT aquaporin transporter. The red arrows denote Al fluxes into and within the cell, and the blue arrows denote fluxes of OA anions. Another mechanism of Al tolerance involves plant-mediated modification of cell wall components that alter the binding of Al^{3+} within the cell wall. Either Al^{3+} or Al-chelate complexes can be loaded into the xylem by an unknown mechanism and transported to the shoot as an Al-OA complex. In Al accumulator plant species, the Al-OA complex is translocated to the shoot, where it is sequestered primarily in the leaf vacuoles. In the Al accumulator *Hydrangea macrophylla*, this translocation occurs through transport processes involving aquaporins and permeases such as HmPALT1 and HmVALT (85, 86). Abbreviations: ABC, ATP-binding cassette; Al, aluminum; ALMT, Al-activated malate transporter; ALS1, Al-sensitive 1; MATE, multidrug and toxic compound extrusion; NIP, nodulin 26–like intrinsic protein; Nramp, natural resistance-associated macrophage protein; Nrat1, Nramp Al transporter 1; OA, organic acid; PALT1, plasma membrane Al transporter 1; TCA, tricarboxylic acid; VALT, vacuolar Al transporter.

mechanisms appear to involve either detoxification mechanisms related to modification of the properties of the root cell wall, where the majority of root Al is located [between 85% and 99.9% (63)], or the uptake and subsequent sequestration and/or translocation of Al once it enters the plant. **Figure 1** summarizes Al exclusion and Al tolerance mechanisms in plants.

Xyloglucan endotransglucosylase/hydrolases (XTHs): a class of cell wall enzymes involved in cell expansion that catalyze the endotransglucosylation of two xyloglucan polysaccharides, breaking and then re-forming the xyloglucan polymer

Cell wall modification. For Al^{3+} ions that are able to traverse the OA barrier in the rhizosphere, the root cell wall constitutes the next site of Al interaction with the plant. The primary root cell wall is a complex and heterogeneous structure consisting of cellulose microfibrils embedded in a matrix of pectins and hemicelluloses (for a review, see 115). Al^{3+} ions can interact both electrostatically with the negatively charged carboxyl groups in wall pectins and via adsorption to uncharged hemicellulose polymers. Al interactions in the cell wall have been suggested to be involved in Al toxicity through the modification of cell wall properties (specifically reduction of wall plasticity/elasticity), intoxication of important cell wall enzymes such as expansins, or increases in soluble cell wall Al^{3+} in equilibrium with cell wall–bound Al. Changes in the structural properties of cell wall carbohydrates are mediated by the activity of a complex network of cell wall–modifying enzymes, most noticeably expansins, endo-β-1,4-glucanases, xyloglucan endotransglucosylase/hydrolases (XTHs), and pectin methylesterases. Al^{3+} binding to pectins is due to its high affinity for the pectins' negatively charged carboxylic groups (11), while the cell wall's negative charge is determined by the degree of pectin methylation, which results from the activity of the pectin methylesterases. The involvement of pectin methylesterases in Al resistance was demonstrated by recent work showing that differential Al resistance in maize, buckwheat, and rice genotypes is correlated with differences in the degree of pectin methylation in the root apex cell wall (24, 137, 138). Al-resistant cultivars exhibit greater levels of methylated pectin, and under Al stress the resistant cultivars have lower pectin methylesterase activity. This results in a lower level of net negative charge within the cell wall, which reduces the accumulation and binding of Al^{3+} ions in the wall. Additionally, overexpression of pectin methylesterases in roots of potato (*Solanum tuberosum*) and rice resulted in higher Al content in the root tip cell wall and increased Al sensitivity (117, 140).

Yang et al. (138) recently showed that hemicelluloses in *Arabidopsis* are as important as pectins in binding Al and thus possibly play a role in Al toxicity and resistance. Furthermore, Zhu et al. (147) found that Al stress inhibits the *Arabidopsis* cell wall enzyme xyloglucan endotransglucosylase (XET), which cleaves and rejoins hemicellulosic xyloglucan polymers during cell expansion. This study showed that the gene *XTH31* is a strong candidate for the gene encoding the XET/XEH enzyme impacted by Al stress. Characterization of an *xth31* mutant resulted in an increase in Al resistance. Through this and subsequent studies in *Arabidopsis* (148, 149), the authors presented an interesting model whereby Al toxicity involves xyloglucan metabolism in the cell wall, and strategies that reduce the production of cleaved xyloglucans in the cell wall may increase Al resistance.

Progress in understanding Al tolerance mechanisms and the underlying genes has come from recent research on rice Al tolerance, as the high level of rice Al resistance has been shown to involve multiple genes underlying different Al tolerance mechanisms that operate in conjunction with a root Al exclusion mechanism mediated by the MATE citrate transporter OsFRDL4. Root OA exudation appears to play a more minor role in rice Al resistance compared with the other cereals, and therefore Al tolerance genes and mechanisms are more important to rice (19, 25). A unique protein complex involved in rice Al tolerance was identified from the map-based cloning of an Al-sensitive mutation, which identified an ATP-binding cassette (ABC) transporter complex encoded by *sensitive to Al rhizotoxicity 1* (*STAR1*) and *STAR2* (37). This ABC transporter appears to mediate the efflux of UDP-glucose into the cell wall, which could possibly alter the cell wall

composition, leading to a reduction in Al-binding capacity; we describe it in more detail below (see The Role of Membrane Transporters in Aluminum Resistance).

The role of aluminum transporters in aluminum tolerance mechanisms. One of the more surprising revelations in recent years has been the role of Al transporters operating at the plasma membrane and tonoplast in plant Al tolerance mechanisms. Given that it is widely assumed that symplastic Al is highly phytotoxic, an Al detoxification mechanism based on Al transport into plant cells seems intuitively contradictory. However, especially in crop species such as rice, where the roots tolerate high levels of Al in the cell wall, one possible way to reduce levels of Al that will disrupt cell wall functions involved in root growth is to move the Al from the wall into the root cytoplasm and then transport and sequester it in the vacuole. This is apparently the role that a unique plasma membrane protein, natural resistance-associated macrophage protein (Nramp) Al transporter 1 (OsNrat1), plays in rice Al tolerance. As described in more detail below (see The Role of Membrane Transporters in Aluminum Resistance), this protein transports Al^{3+} into the root cytoplasm, where it possibly functions in concert with the vacuolar ABC transporter *O. sativa* Al-sensitive 1 (OsALS1) to remove Al from the cell wall and sequester it in the root cell vacuole (37, 54, 131–133).

Aluminum accumulators and their aluminum tolerance mechanisms. A few plant species, including hydrangea, buckwheat, *Melastoma malabathricum*, and tea (*Camellia sinensis*), are capable of translocating and accumulating Al in the shoots to concentrations above 1,000 mg/kg, sometimes exceeding 3,000 mg/kg. Physiological characterizations of Al accumulation in the shoots of hydrangea and buckwheat have been published (64, 65, 67, 69, 146). These studies have revealed several features of this unique Al tolerance syndrome, including that (*a*) Al accumulates in the root and leaf symplasm as nontoxic Al-citrate in hydrangea and Al-oxalate in buckwheat (65, 69, 118); (*b*) in hydrangea, Al accumulates in the sepals as a blue-colored complex of Al, delphinidin-3-glucoside, and 3-caffeolylquinic acid (65); and (*c*) in buckwheat, Al in the xylem stream is complexed with citrate rather than oxalate (64), indicating that Al undergoes a ligand exchange from oxalate to citrate when it is loaded into the xylem, and is then exchanged back onto oxalate when transported into the leaves (see **Figure 1**). More recently, Negishi et al. (85, 86) identified two plasma membrane and tonoplast Al transporters involved in hydrangea Al accumulation, *H. macrophylla* plasma membrane Al transporter 1 (HmPALT1) and vacuolar Al transporter (HmVALT). These transporters are members of the aquaporin family and are highly expressed specifically in sepal cells.

THE ROLE OF MEMBRANE TRANSPORTERS IN ALUMINUM RESISTANCE

Given the extensive published information on root tip Al exclusion based on root OA efflux for many plant species, starting with the seminal studies on wheat malate efflux (18, 21), it is not surprising that the first Al resistance genes identified were those that encode malate and citrate efflux transporters from the ALMT and MATE families of membrane transporters, respectively. However, as our understanding of other Al resistance mechanisms and associated genes has expanded, it has become clear that other membrane transporters also play roles in Al resistance. These include Al^{3+} transporters from the Nramp, ABC, and aquaporin families of membrane transporters and ABC transporters that are involved in the efflux of UDP-glucose out of the cell, which presumably alters cell wall composition or function with regard to apoplastic Al^{3+}

(35, 36, 52–54, 85, 86, 131, 132). In this section, we look at the functions of these different transporters and their roles in plant Al resistance.

ALMT Malate Transporters

Electrophysiological (patch-clamp) studies in protoplasts isolated from root tips of Al-resistant wheat (111, 144) and maize (50, 97, 98) provided the first indication of the existence of plasma membrane malate and citrate anion efflux transporter channels regulated by extracellular Al^{3+}. The correlation between the properties of these anion transporters and the Al-induced malate release responses reported in intact roots suggested that these anion channels were underlying the Al-activated root OA release. This was verified by the cloning of these transporters, beginning with the identification of *TaALMT1*, the gene encoding the wheat malate efflux channel (114).

TaALMT1, underlying a major wheat Al resistance locus (Alt_{BH}), was the first Al resistance gene to be identified. Functional characterization of TaALMT1 expressed in *Xenopus* oocytes and tobacco (*Nicotiana tabacum*) cells demonstrated that *TaALMT1* encodes a transporter that mediates the passive efflux of malate down its electrochemical gradient (94, 114, 143). Most importantly, although it is functional in the absence of extracellular Al^{3+}, the transporter's basal activity is enhanced by extracellular Al^{3+}. The remarkable similarities between the functional features of the TaALMT1 transporter expressed heterologously, the ion channel conductances described in earlier patch-clamp studies, and the Al-activated OA exudation responses in intact roots of Al-resistant genotypes and transgenic plants ectopically expressing TaALMT1 verified its role as an Al resistance protein. Accordingly, expression of TaALMT1 in transgenic rice, wheat, and barley plants as well as tobacco suspension cells resulted in enhanced Al-activated malate efflux and increased Al resistance (20, 93).

Subsequently, *TaALMT1* orthologs encoding Al^{3+}-enhanced malate transporters involved in Al resistance were identified in rape (*Brassica napus*) and *Arabidopsis* (34, 57). TaALMT1 is the founding member of this anion channel family unique to plants (i.e., the Viridiplantae clade). Although the ALMT family is named after its founding member's role in Al resistance, it has soon become clear that not all members of the family are involved in this trait. Other root-localized plasma membrane ALMTs have been identified in maize (ZmALMT1 and ZmALMT2) and barley (HvALMT1) (31, 59, 95) that lack the Al^{3+}-enhanced transport, and ZmALMT1 transports inorganic anions rather than malate. These observations and an increasing number of publications indicate that the in planta roles of ALMTs extend beyond Al resistance and include mineral nutrition, ion homeostasis, turgor regulation, and guard cell function (16, 17, 31, 51, 55, 79, 80, 113).

ALMTs share a high degree of secondary structural similarity, consisting of a highly conserved N-terminus region containing six transmembrane domains (TMDs) followed by a more variable long hydrophilic C terminus that may contain one or two more TMDs (23). As they are permeable to inorganic anions and (in most cases) malate, members of the ALMT family share similarities in transport, including the identity of the transported species. Al^{3+} enhancement of transport activity appears to be the key determinant for the small subgroup of root-localized ALMTs involved in Al resistance. Functional studies have indicated that the C terminus plays an important role in Al^{3+} enhancement (28, 56); however, there is still a lack of consensus regarding the topology of the transporter, especially whether the C terminus is inside or outside the cell (compare Reference 83 with References 78 and 84). Recently, the integration of structure-function analyses of a structurally altered TaALMT1 protein and phylogenetic analyses of the ALMT family showed that the shorter N-terminus domain is also involved in Al^{3+} enhancement, indicating that both N- and C-terminal regions of the protein jointly contribute to the Al response phenotype (56). In

addition to regulation of TaALMT1 (and the AtALMT1 homolog) transport activity by extracellular Al^{3+}, the use of broad-spectrum protein kinase and phosphatase inhibitors suggests that these transport proteins are posttranslationally regulated via reversible protein phosphorylation (47, 90). Electrophysiological studies in *Xenopus* oocytes indicated that protein kinase inhibitors caused a rapid and strong inhibition of TaALMT-meditated transport, whereas the protein kinase activator PMA enhanced TaALMT1-mediated anion efflux (58).

MATE Citrate Transporters

Members of the plasma membrane–localized MATE family of transporters are responsible for Al resistance based on root citrate exudation in response to Al stress. MATEs were first identified as Al resistance genes from the map-based cloning of the major Al resistance loci in sorghum (71) and barley (29). Subsequently, MATE homologs were shown to be root citrate transporters and Al resistance proteins in *Arabidopsis* (AtMATE1) (60), maize (ZmMATE1) (74), rice bean (*Vigna umbellata*) (VuMATE1) (139), and rice (OsFRD1) (142). As ALMTs and MATEs both confer Al resistance via root OA release, this appears to be a striking example of functional coevolution of Al resistance by two transporters that are structurally and functionally quite different.

Much more is known about the transport properties of ALMTs than MATEs. Functionally, from electrophysiological and ^{14}C efflux studies in oocytes, SbMATE as well as ZmMATE1 and VuMATE1 mediate citrate efflux by a citrate/H^+ (and possibly Na^+) antiport mechanism (71, 74, 77, 139). Hence, although the ALMT malate transporters function as anion channels, the MATE citrate transporters are carriers that mediate citrate efflux coupled to H^+ influx. It is puzzling that an antiport mechanism usually employed to mediate active substrate efflux coupled to passive H^+ influx is used to facilitate the strongly thermodynamically passive transport of the $citrate^{2-}$ anion out of root epidermal cells. Possibly this coupling confers an additional degree of regulation to a process that is based on the release of a valuable plant carbon commodity to the rhizosphere. We discuss this issue in more detail in the next section.

Carbon Use Efficiency and Root Organic Acid Efflux

The OAs malate and citrate, which are used by many plant species for Al resistance based on root Al exclusion, are key intermediates in the tricarboxylic acid cycle, a key metabolic hub for all organisms (5). This Al resistance mechanism is therefore likely to be a significant carbon cost for the plant, and therefore it is not surprising that it is highly regulated, both spatially and in response to Al. It is well documented that Al-activated root malate and citrate exudation is spatially localized to the root tips, which reduces the carbon cost because only the root apex needs to be protected from damage due to Al toxicity (108, 120, 121). The lengths to which plants will go to spatially regulate this process was not realized until recently. Sivaguru et al. (121) used laser capture microdissection coupled to a high-sensitivity quantitative real-time polymerase chain reaction (qRT-PCR) assay to show that Al-induced *SbMATE* gene expression was specifically localized to the epidermal and outer cortical cell layers of the sorghum root distal transition zone (DTZ, the root region between cell division and cell elongation), which they demonstrated was also the site of greatest Al-induced damage. Another layer of regulation comes from the many reports that root Al exposure not only induces expression of the *ALMT* and *MATE* genes in many species but also posttranslationally activates the OA transport proteins to release OA anions. The findings in Reference 121 suggest that the root releases malate and citrate only when there are sufficient levels of Al^{3+} ions in the rhizosphere to require root protection from Al toxicity. This is important because acid soils are usually quite heterogeneous spatially with regard to pH and Al saturation (128), and acid soil pH can vary by more than 1 unit over relatively small distances.

Finally, another aspect of carbon-efficient Al resistance based on root OA exudation involves the identity of the released OA anion. Citrate is a much stronger chelator for Al^{3+} than malate (21, 62, 106, 107) and is approximately eightfold more effective than malate in ameliorating Al toxicity (21, 107, 145). Therefore, Al resistance based on citrate exudation may be a more carbon-efficient process. Using transgenic *Arabidopsis* overexpressing *AtALMT1* and *AtMATE*, Liu et al. (60) found that root citrate and malate exudation can impose a measurable cost on root growth. In the absence of Al^{3+}, roots of these transgenic *Arabidopsis* lines had rates of malate and citrate release that were two to ten times those of wild-type plants, and root growth was inhibited 20–30% compared with wild-type plants. These findings suggest that plants go to significant lengths to release a minimum but sufficient amount of OA anions to detoxify the Al^{3+} in the rhizosphere in order to limit the carbon cost of this Al resistance mechanism.

Haplotype: a set of genetic loci that tend to be inherited together; they can range in size from multiple single-nucleotide polymorphisms to groups of genes

Aluminum Transporters: Nramps

Functional characterization of rice genes regulated in an Al-inducible manner by the rice C_2H_2 zinc-finger transcription factor ART1 (Al resistance transcription factor 1) has identified a number of novel Al resistance genes that are involved in diverse resistance mechanisms (132, 135). These include the plasma membrane–localized Nramp Al^{3+} uptake transporter OsNrat1, which may operate in concert with a vacuolar ABC transporter, OsALS1, to remove Al from the cell wall and sequester it in the root cell vacuole (35, 132). Rice, unlike sorghum, wheat, and barley, deals with toxic Al primarily by tolerating the Al in the root. Because as much as 90% of the Al^{3+} resides in the cell wall (63), it appears that Nrat1 may confer Al tolerance by lowering the level of toxic Al in the wall via this novel and somewhat unexpected mechanism.

Nrat1 belongs to the Nramp family but does not share much functional similarity with other Nramps. The OsNrat1 transporter specifically transports Al^{3+} but not divalent metal ions such as Fe^{2+}, Mn^{2+}, and Cd^{2+} transported by other plant Nramps (132). *Nrat1* expression is rapidly upregulated by Al but not by low pH or other similar metals, such as cadmium and lanthanum, and the expression is localized to the plasma membrane of all root cells except epidermal cells. A genome-wide association study (GWAS) analysis of rice Al tolerance identified an *Nrat1* haplotype that is unique to the *aus* rice subpopulation and explained 40% of the phenotypic variation for Al tolerance within this subpopulation (26). DNA sequence variation in the *Nrat1* coding and regulatory regions was associated with changes in *Nrat1* expression and Nrat1 Al transport properties, such that the tolerant *Nrat1* allele was more highly expressed and transported more Al than the sensitive allele (54). In this same study, it was surprising that expression of both the tolerant and sensitive *Nrat1* alleles in transgenic *Arabidopsis* increased Al resistance and root Al uptake, with the tolerant allele conferring greater resistance. Another genetic diversity analysis of rice *Nrat1* also identified tolerant and sensitive alleles associated with differences in expression but not transport (131). Taken together, these studies indicate that Nrat1 serves an important Al resistance function in rice by lowering Al levels in the root cell wall via transport into and sequestration within the root cell. It is intriguing that rice Nrat1 enhances Al resistance in both rice, which does not depend very much on root Al exclusion, and *Arabidopsis*, which does. Hence, Nrat1 and its orthologs may be useful tools for enhancing Al tolerance in a wide range of plant species.

Aluminum Transporters: ABC Transporters

ABC transporters are encoded by one of the largest gene families in organisms ranging from bacteria to humans (33). ABC transporters are ATP-driven pumps and contain two TMDs, which constitute the membrane-spanning pore, and two cytosolic nucleotide-binding domains (NBDs)

(75). The TMD and NBD subunits can be encoded by a single gene (full-size ABC) or by two genes, each encoding one TMD and one NBD (half-size ABC). In plants, ABC transporters are involved in pathogen responses, surface lipid deposition, seed phytate accumulation, and hormone transport (44).

In rice, *OsSTAR1* and *OsSTAR2* encode the NBD and TMD domains, respectively; *OsSTAR1* was identified via map-based cloning of the Al-sensitive locus *ALS1* (37). The OsSTAR1/OsSTAR2 protein complex is localized to endomembrane vesicles in the root, and when expressed in oocytes, it functions as a UDP-glucose transporter. These UDP-glucose-containing vesicles fuse to the plasma membrane and release UDP-glucose into the root cell wall (37). It has been speculated that the transported UDP-glucose could be used for cell wall modification, limiting Al accumulation and reducing Al toxicity.

In *Arabidopsis*, *AtALS3*, which was identified via map-based cloning of the *als3* Al-sensitive mutation, encodes a plasma membrane–localized ABC transporter that only constitutes the TMD and lacks the NBD (53). *AtALS3* is expressed primarily in the root cortex, the leaf hydathodes, and the phloem throughout the plant. Although the NBD domain partner and transport substrate of AtALS3 have not been identified, Larsen et al. (53) have speculatively suggested AtALS3 is an Al transporter and is responsible for redistributing Al in the plant away from the sensitive root apex. In addition, Huang et al. (36) have suggested that *AtSTAR1*, which encodes the ATP-binding domain of an ABC transporter homolog of OsSTAR1, may be the functional partner of ALS3. *Atstar1* mutants are hypersensitive to Al and also flower early, a phenotype similar to that of the *Atals* mutants, but the transport function of AtSTAR1 has not been determined.

Another interesting ABC transporter that appears to be involved in rice Al resistance is OsALS1, a half-size ABC transporter that comprises TMDs and ATP-binding domains. *OsALS1* expression is rapidly upregulated by Al in roots (35). The OsALS1 protein is localized to the tonoplast membrane, and may be an Al transporter, based on *OsALS1* knockouts in rice and expression in yeast. Huang et al. (35) speculated that OsALS1 functions in tandem with the plasma membrane Al uptake transporter OsNrat1 to move Al from the cell wall into the cell and then sequester it in the root vacuole.

Aluminum Transporters: Aquaporins

Most plant species sequester the majority of the Al they encounter in the root. As described above, a small subset of plant species can transport and store a significant amount of Al in the shoot. These Al accumulators include hydrangea, buckwheat, and tea, which compared with other plants can accumulate more than ten times as much Al in the shoot with no phytotoxicity (65). In hydrangea, the color of the sepals changes from red to blue when grown on acid soils owing to the accumulation of Al (38) and the formation of the blue Al:delphinidin-3-*O*-glucoside complex.

Negishi et al. (85, 86) recently used a functional genomic approach with hydrangea sepal tissue to identify two aquaporin genes, *HmPALT1* and *HmVALT*, that encode sepal plasma membrane and vacuolar Al uptake transporters. HmVALT is a member of the tonoplast intrinsic protein (TIP) subfamily of tonoplast-localized aquaporins, whereas HmPALT1 belongs to the nodulin 26–like intrinsic protein (NIP) subfamily. Aquaporins are generally believed to transport nonionic substrates, so the forms of Al transported by HmPALT1 and HmVALT are in question. In this study, HmPALT expression in yeast conferred Al sensitivity with increased Al accumulation, whereas HmVALT expression conferred Al resistance with increased Al accumulation; these results are consistent with these proteins functioning as plasma membrane and tonoplast Al transporters, respectively. The authors suggested that HmVALT may transport the neutral Al species $Al(OH)_3$ (gibbsite) from the pH 7 cytoplasm into the vacuole, although the low solubility

of gibbsite at pH 7 raises the question of whether there are high enough gibbsite concentrations in the cytoplasm to support transport into the vacuole. Also, which Al species HmPALT transports is not clear. This is a very interesting issue that will require more investigation.

HOW DOES THE PLANT SENSE ALUMINUM TO REGULATE ALUMINUM RESISTANCE GENES AND PROTEINS?

It is clear from the recent literature that Al resistance involves Al regulation of both gene expression and protein function. Al induces the expression of a number of Al resistance genes associated with different mechanisms, including the rice Al resistance genes *OsSTAR1/2*, *OsNrat1*, *OsFRDL4*, and *OsALS1* (37, 132, 142); the *MATE* genes *SbMATE*, *ZmMATE*, *VuMATE*, and *AtMATE* (60, 71, 74, 139); and the *ALMT* gene *AtALMT1* (34, 47). Al also appears to regulate the function of both MATE and ALMT OA transport proteins (28, 56, 94). In some cases, such as the function of the wheat TaALMT1 protein, Al interacts directly with the transporter protein. But it is also very likely that Al regulation involves indirect interactions that influence gene expression and protein function, for example, via the binding of Al^{3+} ions to an unknown plasma membrane Al sensor (see **Figure 2**).

In addition, we now know that Al^{3+} ions enter plant cells via plasma membrane transporters, such as the Nramp OsNrat1 in rice and the aquaporin HmPALT1 in hydrangea (85, 132), and can interact with intracellular molecules, including the transcription factors ART1 and sensitive to proton rhizotoxicity 1 (STOP1) (39, 135) (**Figure 2**). Because Al stress triggers a number of changes to cellular homeostatic processes, these changes likely play a role in signaling or regulation of Al resistance mechanisms. This might include Al-induced changes in cytosolic Ca^{2+}, pH, or

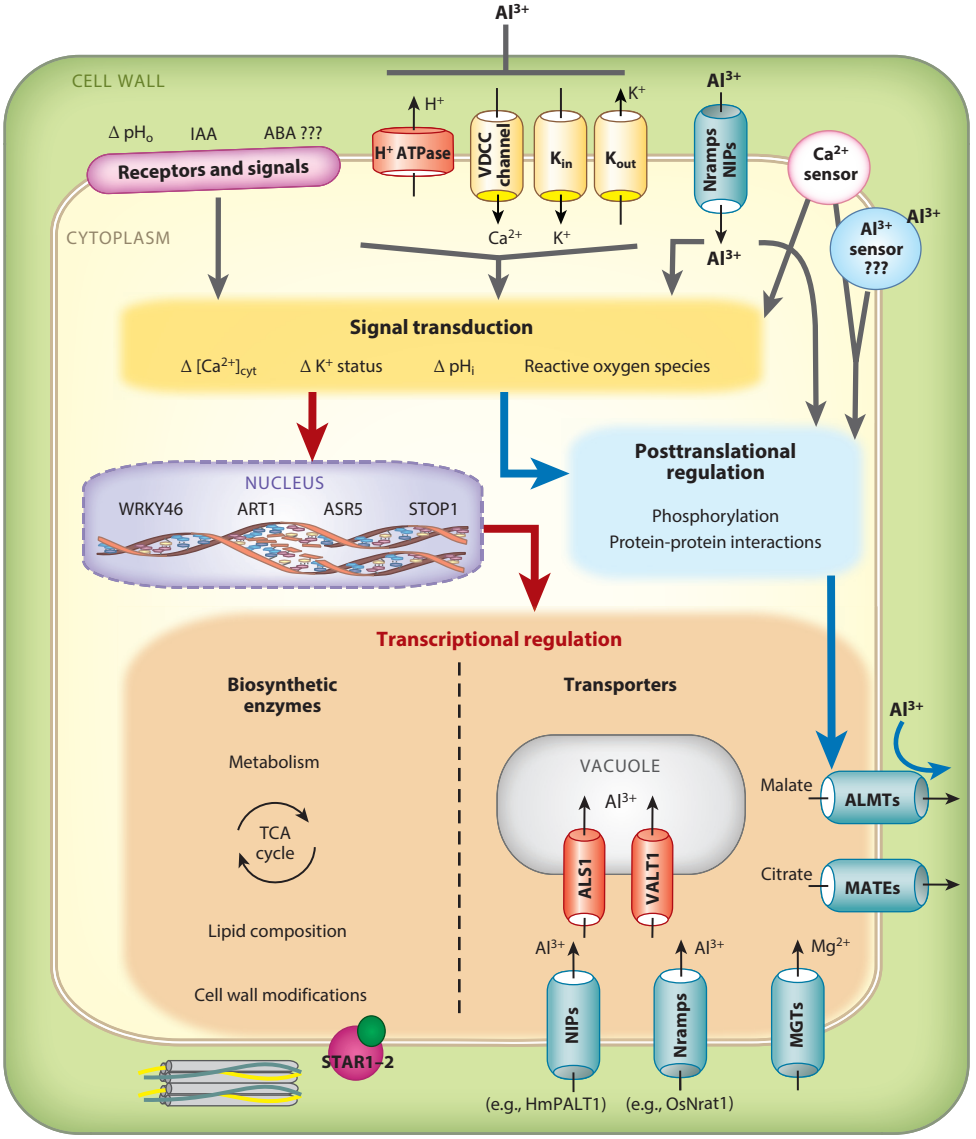
Figure 2

Erratum

General model illustrating putative Al-mediated signaling pathways involved in the perception and triggering of Al stress and resistance responses in roots. The model is both based on experimental evidence from the literature and somewhat speculative, owing to the lack of evidence for Al receptors and/or sensors and limited understanding of pathways linking Al perception to the responses resulting in Al resistance. In this model, the perception of Al stress (denoted by *gray arrows*) can involve direct Al^{3+} interaction with one or more putative plasma membrane Al receptors (*upper right corner of model*), Al perception due to increases in cytoplasmic Al levels that are the product of the plasma membrane Al^{3+} influx via Al transporters (e.g., Nramps and NIPs), and Al-induced inhibition of membrane transport and/or involvement of plasma membrane signal transducers [e.g., hormones (*upper left*) and Ca^{2+} sensors (*upper right*)]. These changes underlie putative signal transduction cascades that indirectly link Al to the activation of processes leading to Al resistance via two major pathways. The first pathway is Al-induced transcriptional activation (*left side*) of biosynthetic enzymes and membrane transporters underlying Al exclusion and Al tolerance mechanisms (*red arrows*). The coupling between Al perception and the regulation of expression of these genes involves homologs of the *Arabidopsis* and rice transcription factors WRKY46, ART1, ASR5, and STOP1. The second pathway is posttranslational Al activation and/or regulation at the level of protein function (*blue arrows*). The latter involves Al activation of the organic acid efflux transporters that occurs either directly, via binding of Al to the transport protein (i.e., an ALMT), or indirectly, possibly via processes such as protein phosphorylation and dephosphorylation and/or other protein-protein interactions modulating transporter activity. Abbreviations: ABA, abscisic acid; Al, aluminum; ALMT, Al-activated malate transporter; ALS1, Al-sensitive 1; ART1, Al resistance transcription factor 1; ASR5, abscisic acid, stress, and ripening 5; HmPALT1, *Hydrangea macrophylla* plasma membrane Al transporter 1; IAA, indole-3-acetic acid; MATE, multidrug and toxic compound extrusion; MGT, magnesium transporter; NIP, nodulin 26–like intrinsic protein; Nramp, natural resistance-associated macrophage protein; OsNrat1, *Oryza sativa* Nramp Al transporter 1; STAR1–2, sensitive to Al rhizotoxicity 1–2; STOP1, sensitive to proton rhizotoxicity 1; TCA, tricarboxylic acid; VALT, vacuolar Al transporter; VDCC, voltage-dependent calcium channel.

K^+, which in turn could trigger additional downstream components in an Al signaling pathway. It is well known that Al stress causes changes in cytosolic Ca^{2+} activity (42, 43, 101, 102), which has been suggested to play a role in Al toxicity. But it is also possible that Al-mediated alterations in cytosolic Ca^{2+} participate in signaling processes that trigger increased Al resistance.

Al stress has also been shown to lead to increased levels of reactive oxygen species (ROS) in plant roots (41, 136). This has generally been hypothesized to be part of the Al toxicity syndrome, but ROS can also be important participants in plant signaling (3). Sivaguru et al. (121) speculated that Al-induced ROS generation could be a cellular signal involved in increased sorghum Al resistance that is unique in this cereal species because of the long Al exposures needed to fully induce sorghum Al tolerance (4–6 days). Magalhaes et al. (71) had shown previously that this slow induction of



Al resistance was due to an Al-induced increase in *SbMATE* gene expression, resulting in higher rates of root citrate exudation over the same 4–6-day period. In the study by Sivaguru et al. (121), Al-induced ROS production, cell damage, callose production (a measure of Al toxicity), and Al accumulation were microscopically imaged in root apices of Al-tolerant and Al-sensitive sorghum near-isogenic lines exposed to Al for 1–4 days. They found that the greatest cell damage and ROS generation caused by Al stress were located specifically in the root DTZ, a region 1–3 mm behind the root tip where the transition from cell division to cell elongation occurs. Previously, Sivaguru & Horst (120) had shown that this region is the primary site of Al toxicity in maize. Laser capture microdissection coupled to high-sensitivity qRT-PCR and immunolocalization showed that Al-induced *SbMATE* gene and protein expression were specifically localized to the epidermal and outer cortical cell layers of this same DTZ in the Al-resistant near-isogenic line, and the time courses for Al-induced *SbMATE* expression and onset of recovery of roots from Al-induced damage precisely coincided. Also, the highest Al-induced ROS production was colocated to this same DTZ region. The timing of the Al-associated ROS production in the DTZ preceding *SbMATE* expression led Sivaguru et al. (121) to speculate that ROS production could be involved in Al signaling, with the high ROS levels acting to trigger downstream events that then lead to increased SbMATE protein abundance and protection of this specific root region.

Regulation of Aluminum Resistance Gene Expression by Aluminum

The identification and subsequent characterization of an assortment of plant Al resistance genes have improved our understanding of how the expression of these genes is regulated in response to Al stress. The key features of Al resistance gene expression understood to date include the following: (*a*) Resistance gene expression is higher in resistant genotypes; (*b*) gene expression is localized to the root tip, which is the site of Al toxicity; (*c*) gene expression is often increased by Al stress (e.g., Al-induced increase in *ALMT* and *MATE* gene expression has been documented for *AtALMT*, *BnALMT1*, *ScALMT*, *SbMATE*, *VuMATE1*, *ZmMATE1*, *OsFRDL4*, and *ScFRDL2*) (13, 34, 47, 57, 60, 71, 74, 139, 141, 142); and (*d*) constitutively higher gene expression occurs in wheat and barley for the Al resistance genes *TaALMT1*, *TaMATE1B*, and *HvAACT1* (29, 109, 114, 125). These patterns of gene expression correlate well with the physiological characteristics of root malate and citrate efflux. For the plant species where the *ALMT* and *MATE* genes are constitutively expressed, malate and citrate efflux in the presence of Al occurs with no discernible lag between root Al exposure and OA release. For those species where the gene expression is induced by Al, there is a lag in the time to maximal root OA release that is in the range of 2–6 h in species where gene expression is induced rapidly, such as *Arabidopsis* and maize (60, 74), and in the range of 4–6 days in sorghum (71).

Research into the molecular and biochemical basis for Al induction of gene expression has identified several *cis*-elements and *trans*-acting factors involved in Al-induction pathways (for a recent review on *cis* and *trans* regulation of Al resistance gene expression, see 19). With regard to transcription factors involved in Al induction of Al resistance gene expression, AtSTOP1 and OsART1 are two related members of the C_2H_2-type zinc-finger transcription factor family depicted in **Figure 2** that positively regulate Al-induced expression of Al resistance genes in *Arabidopsis* and rice, respectively (39, 135). AtSTOP1 was identified via positional cloning of a low-pH-sensitive *Arabidopsis* mutant (39) that is also hypersensitive to Al toxicity but not to other toxic metals. AtSTOP1 is involved in the Al-induced expression of several *Arabidopsis* Al resistance genes, including *AtALMT1*, *AtMATE1*, and *AtALS3* (60, 116). AtSTOP1 is constitutively expressed in *Arabidopsis*, indicating that its involvement in the Al induction of gene expression must involve posttranslational processes. Almost nothing is known about how this might occur. It is possible that

AtSTOP1 binds Al, which then triggers the initiation of transcriptional activation. Alternatively, because it has been shown that phosphorylation may play a role in Al induction of *AtALMT1* expression, Al exposure may lead to AtSTOP1 phosphorylation, which activates AtSTOP1 to participate in the transcription of *AtALMT1* and other *Arabidopsis* Al resistance genes (39). Very recently, the same research group identified AtSTOP2, a homolog of AtSTOP1 that may partner with AtSTOP1 in regulating the expression of some of the Al and low-pH resistance genes in *Arabidopsis* (48).

A possible AtSTOP1 partner, AtWRKY46, is a member of the WRKY domain–containing family of transcription factors and was recently shown to be a negative regulator of *AtALMT1* gene expression (22). In that publication, the authors noted that, based on the information in public gene expression databases, *AtWRKY46* expression colocalizes with *AtALMT1* expression in the root, and its expression response to a number of abiotic stresses is the converse of *AtALMT1* expression in response to the same stresses. In response to Al, decreased root *AtWRKY46* expression correlates with Al-increased *AtALMT1* expression. Knocking out *AtWRKY46* led to increases in *AtALMT1* expression, root malate exudation, and *Arabidopsis* Al resistance. The *AtALMT1* promoter sequence harbors several putative WRKY box domains, some of which were shown to be bound by the WRKY46 protein. These findings led the authors to hypothesize that WRKY46 is a negative regulator of *AtALMT1* expression, which may help finely control the release of malate as a valuable carbon commodity for the plant (22).

OsART1 is quite similar in sequence to AtSTOP1 and serves a similar function in rice (135). Like AtSTOP1, it is constitutively expressed in rice roots, but unlike AtSTOP1, it is involved only in Al resistance. Comparative gene expression profiling between wild-type plants and an *OsART1* knockout line identified 31 genes that are upregulated by OsART1 in an Al-dependent manner (135). These included *OsSTAR1* and *OsSTAR2*, which the authors had previously shown were rice Al resistance genes, as discussed above. The genes whose Al-induced expression is regulated by OsART1 have been very effectively mined to identify a number of novel rice Al resistance genes/proteins, including OsNrat1, OsMGT1, OsCDT3, and OsFRDL4 (12, 132, 134). The OsART1 protein and the promoter for the Al-responsive Al resistance gene *OsSTAR1* were used to carry out gel mobility shift-based mapping of the *OsSTAR1* promoter to identify an OsART1 binding motif, GGN(T/g/a/C)V(C/A/g)S(C/G), as a canonical OsART1-binding motif in the promoters of 29 of the 31 genes regulated by ART1 (126).

Other research on promoter *cis*-elements in Al resistance genes has focused on the constitutively high expression of *TaALMT1* in root tips of tolerant wheat lines, where the promoters of Al-resistant *TaALMT1* alleles contain duplicated and triplicated tandem repeats, which could function as enhancers of gene expression (110). This was verified via expression of *TaALMT1* promoter:GUS reporter constructs in transgenic rice callus. Another possible example of sequence repeats enhancing resistance gene expression involves the sorghum Al resistance gene *SbMATE*, where a tourist-like miniature inverted repeat transposable element (MITE) was found in the promoter (71). Sequence analysis of the *SbMATE* promoter in a panel of sorghum lines varying widely in Al resistance showed that the size of the MITE was quite variable and correlated with gene expression and Al resistance, such that the lines with higher *SbMATE* expression harbored larger MITE insertions in the promoter. Sequence analysis of the small, medium-sized, and large MITE insertions showed that the MITEs had a repeated sequence structure and that the increase in MITE size was caused by an increased number of these repeats.

The MATE citrate transporter involved in barley Al resistance, HvAACT1, is an interesting example of alteration of gene expression and transporter function due to the insertion of a transposable element in the promoter. Fujii et al. (27) found that a major function of HvAACT1 is to release citrate into the xylem, where it complexes iron for translocation to the shoot. A second

HvAACT1 allele was identified with a 1-kb transposable element in the *HvAACT1* promoter, which increased gene expression and moved it to the cells at the surface of the root tip, where the citrate efflux confers Al resistance. Tovkach et al. (125) found a similar situation in the promoter of the wheat citrate transporter gene *TaMATE1B*, in which insertion of a transposon-like element extended *TaMATE1B* expression into the root apex.

Copy-number variation plays a role in elevated Al resistance gene expression for *ScALMT* in rye (13) and *ZmALMT1* in maize (73). Maron et al. (73) demonstrated that Al-resistant maize genotypes contain three functional copies of *ZmMATE1* that are identical and part of a tandem triplication. This copy-number expansion is a rare event in maize Al tolerance, and it is interesting that the three maize lines that carry the three-copy allele share the same geographical origin in acid-soil regions of the South American tropics.

Quantitative inheritance: inheritance characterized by a continuous distribution of the phenotypes in the offspring; it is usually controlled by multiple genes that each have minor effects

Qualitative inheritance: inheritance in which the phenotypes of the offspring are simply inherited and fall into a few discrete and easily distinguishable classes; it is usually conferred by single genes with major effects

Aluminum Activation of Aluminum Resistance Protein Function

Physiological studies of root ALMT and MATE malate/citrate transporters involved in Al resistance have clearly shown that Al is needed for proper transporter protein function. Al interacts directly and/or indirectly with the transporters to activate or enhance malate and citrate efflux from the root. This is consistent with the plant exerting significant control over the release of important organic molecules that play multiple roles in the plant, so that they are released into the rhizosphere only in the presence of Al toxicity. Electrophysiological analysis of the TaALMT1 and AtALMT1 transporters in *Xenopus* oocytes has verified the Al enhancement of malate transport, demonstrating that their transport activity in oocytes is enhanced by the presence of extracellular Al (94). This is analogous to ligand-gated channels, with Al acting as an agonistic ligand when interacting with the ALMT protein. The binding of Al to the transporter may result in a conformational change that favors its open state, consequently increasing its transport activity and facilitating anion flux. A detailed analysis of TaALMT1 structure and function (malate permeation and Al activation) combined ALMT phylogenetic studies with electrophysiological analysis of TaALMT1 variants generated from site-directed mutagenesis of all 43 of the negatively charged amino acids as well as serial truncations of the long C-terminal tail (56). The N-terminal region of the protein, which contains the six predicted TMDs and is predicted to form the conductive pathway, was sufficient to mediate malate anion transport even in the absence of the C-terminal domain. However, it is clear that peptide regions in both the N- and C-terminal domains are involved in Al binding and responsiveness, and gaining a clearer understanding of the role of the TaALMT1 in Al responsiveness will require determining its three-dimensional structure.

Evidence suggesting the roles of other proteins interacting with MATE citrate transporters to facilitate Al activation comes from electrophysiological and physiological assays of SbMATE, AtMATE, and VuMATE expression in oocytes. In each case, the MATE transporter mediated constitutive citrate efflux in the absence of Al in oocytes (71, 96, 139). This suggests that in roots, the Al-activated root citrate efflux may be due to the interaction of a second plant protein with the MATE citrate transporter in planta.

THE GENETIC BASIS FOR CROP ALUMINUM RESISTANCE

The genetic basis for Al resistance in crop plants is usually categorized based on either quantitative or qualitative inheritance. However, careful examination of the literature indicates that at the species level, this simplistic view is inadequate. The Al resistance genes identified in wheat (114), sorghum (71), and barley (29) clearly underlie Al resistance loci with major effects that display monogenic inheritance in the few mapping populations that were studied. However, normal

frequency distributions in sorghum crosses where the Al resistance locus Alt_{SB} plays a role in Al resistance and the presence of apparent transgressive segregation suggest that a more complex inheritance pattern takes place in sorghum (9). Further support for this assertion is based on the incomplete transfer of Al resistance from parents to near-isogenic lines that has been observed in both sorghum (77) and wheat (40, 123). Additionally, in wheat, Ryan et al. (109) have reported a second Al resistance mechanism (in addition to TaALMT1-mediated malate release) that involves citrate exudation controlled by a MATE homolog, indicating that there is more diversity in wheat Al resistance than initially thought. This suggests that even in the case of major genes in crop species, allelic variation at auxiliary loci may give rise to polygenic inheritance of Al resistance in certain crosses.

Although in-depth intraspecific investigations emphasized the importance of *TaALMT1* and *SbMATE* in wheat and sorghum Al resistance, respectively (8, 10, 110), other important Al resistance genes may remain unidentified in those species. Perhaps the clearest case of monogenic inheritance of Al resistance is found in barley, where Al resistance in genotypes of distinct genetic origins has long been known to be due largely to an allelic series at the *Alp* locus, resulting in little potential for improvement beyond this locus (81). However, although a GWAS in barley confirmed that *Alp* plays an important role in the cultivated gene pool, novel regions on barley chromosomes 2H and 7H possibly play a role in Al resistance in wild Tibetan barleys (6). Therefore, Al resistance in crop plants should be considered intrinsically quantitative in nature and should be tackled as such from genetic and physiological perspectives. This view warrants specific strategies to explain in detail the molecular basis of quantitative variation for crop Al resistance, which have been lacking.

The majority of studies on the genetics of crop Al resistance have focused on cereal crops. Because of space limitations, we concentrate here on sorghum and wheat as crops traditionally considered to exhibit qualitative genetic inheritance and on rice and maize as species with quantitative inheritance for Al resistance.

Sorghum

High levels of Al resistance are rare in the sorghum germplasm, occurring at a frequency of approximately 5% (8). As such, breeding programs targeting sorghum adaptation to Al-toxic acid soils must rely on the deliberate identification and introduction of Al resistance donors. A major, semidominant Al resistance locus, Alt_{SB}, was mapped to the end region of sorghum chromosome 3 (70), and this work set the stage for the map-based cloning of the gene underlying this locus, *SbMATE* (71).

An investigation of the genetic control of Al resistance in different sorghum accessions indicated that there is potential for improving sorghum Al resistance by both exploring allelic heterogeneity at Alt_{SB} and identifying other Al resistance loci (9). Most importantly, the detection of transgressive segregation suggests that different Al resistance genes may act additively to result in high levels of sorghum Al resistance. A strong correlation has been found between *SbMATE* expression and Al resistance in sorghum (71, 77), and additional loci acting via transcriptional regulation of *SbMATE* seem likely to play a role in sorghum Al resistance. When Melo et al. (77) used marker-assisted backcrossing to transfer only the Alt_{SB} locus from tolerant donors into an Al-sensitive recurrent parent, they observed incomplete transfer of the Al resistance phenotype as well as reduced *SbMATE* expression in the derived near-isogenic lines harboring the Al-tolerant Alt_{SB} alleles. This reinforces the existence of additional Al resistance loci in the sorghum genome that act by modulating *SbMATE* expression. Although a strong reduction in Al resistance and *SbMATE* expression was observed for certain Al resistance donors, other genotypes appear to

control *SbMATE* expression largely in *cis* and are thus important donors of Al resistance in marker-assisted backcross strategies, particularly as the identity of these accessory loci remains unknown.

Linkage disequilibrium: the tendency for two genetic loci to be inherited together more frequently than would be predicted by random chance

With the cloning of major Al resistance genes in crop species, molecular breeding strategies based on causative polymorphisms or polymorphisms in tight linkage disequilibrium with causative ones are now possible. This may allow for allele-mining approaches to identify sources of sorghum Al resistance directly in locally adapted cultivars, circumventing the need to introduce Al resistance from exotic sources. A diversity analysis demonstrated that sorghum Al resistance is a rare trait and is found specifically in guinea and caudatum sorghum subpopulations (8). This study found that when exploring Alt_{SB} in hybrid production, it is not sufficient to select the allele associated with the highest Al resistance phenotype; it is also vital to consider the diversity in mode of gene action related to Alt_{SB}. Subsequent association mapping by the same research group (10) revealed a very high level of linkage disequilibrium decay across the Alt_{SB} locus, reaching basal levels at approximately 1,000 base pairs, which allowed the researchers to detect intralocus recombination events. A haplotype analysis suggested a recent origin of Al resistance mutations within Alt_{SB}, possibly in primordial guinea domesticates in West Africa. The single-nucleotide polymorphism (SNP) in the second *SbMATE* intron that has the strongest association signal with Al resistance recovered 9 of the 14 highly Al-tolerant accessions in the association panel, indicating that it could be a useful marker for allele-mining approaches for sorghum Al resistance.

Figure 3 depicts a breeding scheme aimed at improving Al resistance in sorghum based on the findings described above. First, the population structure is assessed based on racial classifications and marker-based techniques. High-throughput genotyping is then performed using the low-frequency alleles at the SNP loci associated with Al resistance to identify Al-tolerant breeding lines. This approach allows for rapid screening of a large number of lines to identify the few Al-tolerant accessions (10), resulting in a substantial enrichment of accessions showing high or intermediate Al resistance. The final step is the confirmatory Al resistance phenotyping of selected accessions, which is necessary owing to background effects that may reduce *SbMATE* expression (77). If a breeding program does not contain Al-resistant sorghum accessions, introduction of known Al resistance donors followed by marker-assisted selection is advised. Hence, allele-specific and flanking markers (8–10, 71) are used along with background markers for marker-assisted backcrossing to improve Al resistance.

Wheat

Early reports on Al resistance in wheat suggested a monogenic or digenic inheritance, with dominant gene action (7, 45). However, decreased Al resistance in ditelosomic stocks compared with the euploid parent suggested a more polygenic genetic architecture (1, 2, 91), which might have been missed in biparental populations owing to the segregation of a major Al resistance gene. Indeed, in 1996 Luo & Dvorak (61) and Riede & Anderson (103) genetically mapped the major Al resistance locus, which explained 85% of the variation for Al resistance, to the long arm of wheat chromosome 4D. Finer subsequent mapping of this locus, which has been of historical importance in wheat breeding for Al resistance (30), located it to the region where *TaALMT1* resides (100, 104). As mentioned above, although *TaALMT1* clearly exerts a profound effect on wheat Al resistance, evidence is accumulating for the existence of other wheat Al resistance genes. Although it plays a comparatively minor role in wheat Al resistance, a wheat MATE homolog mediating constitutive citrate exudation has been identified in highly Al-resistant wheat lines (109). This homolog is located on chromosome 4BL, which is consistent with the presence of one or more Al resistance genes on chromosome 4BL that was previously suggested by ditelosomic analysis in Chinese Spring wheat (2). Additionally, a GWAS in wheat—albeit with a limited number of markers—identified regions previously shown to be involved in wheat Al resistance, including the

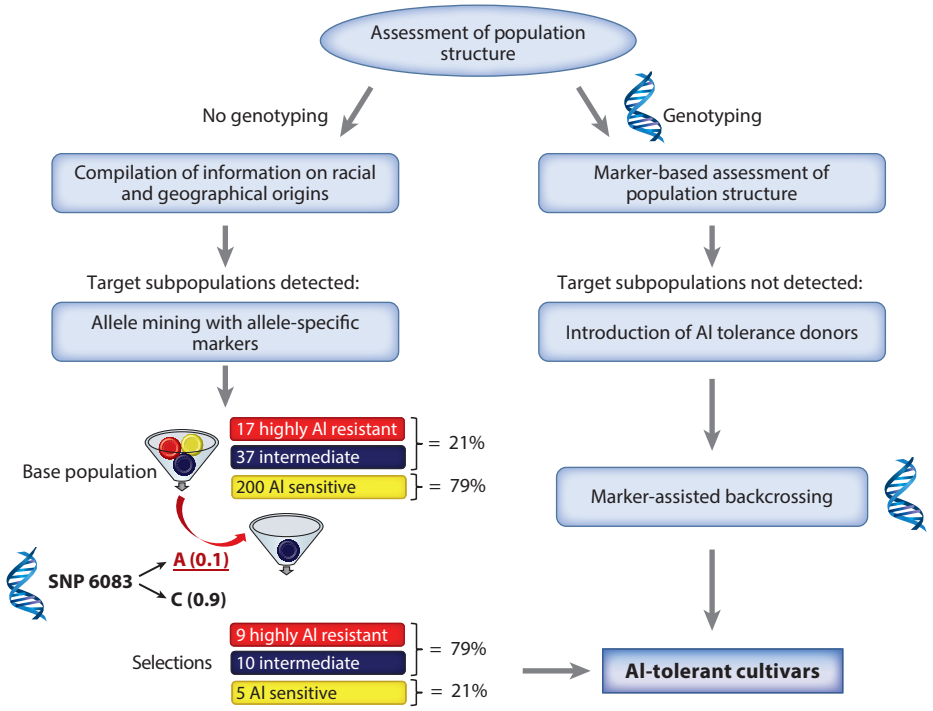


Figure 3

Proposed breeding strategy for improving Al resistance in sorghum based on the Alt_{SB} locus (*SbMATE*). The illustration at the bottom left depicts the proportion of Al-resistant, intermediate, and Al-sensitive accessions in the association panel studied by Caniato et al. (10) (where 21% of the panel consisted of Al-resistant and intermediate accessions and 79% consisted of Al-sensitive accessions) and then in a subset enriched for Al-resistant and intermediate accessions (79%) through selection using the Al-resistant (A) allele of the 6083 SNP [where A and C refer to the two nucleotides (A, adenine; C, cytosine) that can occur at this position in the *SbMATE* DNA sequence] (table 2 in Reference 10). Abbreviations: Al, aluminum; SNP, single-nucleotide polymorphism.

region harboring *TaALMT1* on chromosome 4DL, a quantitative trait locus (QTL) previously reported by Dai et al. (15) on chromosome 3B, and a novel region on chromosome 5B (100). These studies suggest that the time has come to explore how much potential there is to improve wheat Al resistance beyond the use of *TaALMT1*.

Rice

Rice is likely the most Al-resistant cereal (25, 26) and also the cereal where Al resistance is the most genetically complex (68, 87, 130). Although Al resistance QTLs located on conserved positions with the major wheat resistance locus [rice chromosome 3 (87, 130)] and Alt_{SB} in sorghum [rice chromosome 1 (71)] were detected in rice, it appears that the major determinants of the high rice Al resistance are not the root Al exclusion mechanism based on OA release employed by wheat and sorghum (25, 66, 68). However, Yokosho et al. (142) found that a *MATE* homolog located on rice chromosome 1, *OsFRDL4*, encodes a citrate transporter that plays a role in rice Al resistance. Furthermore, the rice homolog of *SbMATE* noted by Magalhaes et al. (71), Os01g69010, is now known to be *OsFRDL4*. The question then becomes how to reconcile the presence of functional *MATE* homologs involved in rice Al resistance with the previously reported lack of correlation

between Al-activated OA exudation and Al resistance (25, 68). It appears that other mechanisms of Al resistance may in certain rice genetic backgrounds overshadow the impact of root citrate exudation on Al resistance. To clarify this issue, specific genetic stocks (such as near-isogenic lines for the different QTLs) can be used to assess the actual contribution of each QTL and related physiological mechanisms in rice Al resistance.

A joint GWAS–linkage analysis of rice Al resistance using a 383-accession association panel and two biparental mapping populations provided links between rice Al resistance and functional variation for genes known to be related to Al resistance (26). In that study, subpopulation structure explained 57% of the Al resistance variation, which is similar to the known relationship between Al resistance and population structure in sorghum (8). Hence, most of the 48 regions associated with Al resistance were subpopulation specific. Evidence for associations with rice Al resistance from GWAS or biparental QTL mapping was found for genes previously implicated in Al resistance, namely those encoding ART1 (135), the ABC transporter STAR2 (37), and the Nramp Al transporter Nrat1 (132). The scenario regarding rice Al resistance strongly suggests that its high levels of Al resistance may be due to the pyramiding of complementary genes and mechanisms.

Maize

The genetic control of Al resistance in maize is generally considered to be polygenic (14, 32, 72, 88, 119). Physiological evidence supports these findings, as it is clear that maize employs an Al exclusion mechanism based on Al-activated root citrate release (92, 98); however, this mechanism is not sufficient to explain all of the natural variation observed for Al resistance, leading to the hypothesis that there are other Al resistance mechanisms in maize (99). The first report of the relatively complex genetic control for maize Al resistance identified five QTLs on chromosomes 2, 6, and 8 that explained 60% of the phenotypic variation (88). A later study with a different maize population also detected five QTLs whose positions were generally congruent with those from the original study (14). The QTL located on chromosome 6 was apparently conserved in these two studies and thus is likely to harbor genes underlying maize Al resistance. *ZmMATE1*, a maize homolog of the sorghum Al resistance gene *SbMATE*, was subsequently identified as the gene underlying this QTL based on its localization to the major Al resistance QTL identified on chromosome 6, its function as an Al-activated root citrate transporter, and its high Al-induced gene expression in the root tips of Al-resistant maize genotypes (74). Collectively, these results suggest that *ZmMATE1* underlies the major-effect QTL detected on chromosome 6 by Ninamango-Cárdenas et al. (88) and Sibov et al. (119).

A recent linkage mapping study provided additional evidence for multiple Al resistance mechanisms in maize (32). Accordingly, a maize homolog of rice *Nrat1*, *ZmNrat1*, was mapped 40 Mb from an Al resistance QTL and was shown to be expressed in maize root apices, and its expression was upregulated by Al. When Melo et al. (77) introgressed the QTL on chromosome 6 determined by *ZmMATE1* into an Al-sensitive background, they observed a significant increase in Al resistance, and unlike the sorghum *SbMATE* near-isogenic lines discussed above, there was no reduction in *ZmMATE1* gene expression. This reinforces the idea that transcriptional regulation of *ZmMATE1* is controlled largely in *cis*, as previously inferred based on *ZmMATE1* expression QTL (eQTL) mapping and discovery of *ZmMATE1* copy-number variation (73). Thus, it appears that the polygenic control of Al resistance in maize may reflect functional diversity in physiological mechanisms of Al resistance in the species, rather than epistatic pathways affecting *ZmMATE1* expression and Al resistance. *ZmMATE1* expression studies in highly Al-tolerant Kenyan maize lines (76) and some physiological studies of maize Al resistance (e.g., 124) suggest the presence of novel resistance mechanisms, which could provide new avenues for improving this trait.

SUMMARY

In this review, we have presented a cross section of the large body of work on Al resistance genes and mechanisms that has been published in the decade since the last *Annual Review of Plant Biology* article on plant acid soil resistance (49). Some of the major points we have attempted to make regarding our understanding of plant Al resistance involve the multiple Al resistance mechanisms that have been discovered. The Al exclusion mechanism involving Al-activated root OA transporters has been known since the early 1990s (18, 21), and the gene encoding the malate transporter underlying this mechanism in wheat was identified in 2004 (114). But since that time, several other ALMT-type malate transporters and MATE citrate transporters have been identified. The molecular basis of both constitutive and Al-induced *ALMT* and *MATE* expression, involving the identification of both *cis*-elements and *trans*-acting factors, is now beginning to be understood. Additionally, research in rice (the most Al-resistant cereal) along with research on a handful of Al accumulator plants has identified novel Al tolerance mechanisms that involve both modifications to the carbohydrate components of cell walls that alter Al binding in this organelle and interesting and unexpected Al-specific transporters. These Al transporters appear to be involved in Al tolerance mechanisms that reduce the Al toxicity load to the root cell wall, and the absorbed Al is ultimately stored in either root or leaf cell vacuoles, where it is detoxified and sequestered from Al-sensitive components of the cell.

Newly emerging areas of research involve different aspects of Al resistance, such as the identification and functional characterization of Al signaling and regulatory pathways that mediate the transcriptional, posttranscriptional, and posttranslational regulation of Al resistance genes and proteins. Furthermore, the field has advanced to the point where researchers are beginning to use variation in both the structural components and signaling pathways and networks related to differential Al resistance to improve crops via molecular breeding and biotechnology for agriculture on acid soils, which limit agriculture in many developing countries in the tropics and subtropics.

SUMMARY POINTS

1. Multiple Al resistance genes underlying novel mechanisms have been identified over the past ten years.
2. The best-characterized mechanism of Al resistance, root tip Al exclusion through Al-activated organic acid exudation, involves genes encoding ALMT malate transporters and MATE citrate transporters.
3. The molecular basis of both constitutive and Al-induced Al resistance gene expression is beginning to be elucidated, with the identification of both *cis*-elements and *trans*-acting factors involved in the expression of a number of Al resistance genes.
4. Novel Al tolerance mechanisms have been identified involving modifications to the carbohydrate composition of the root cell wall, leading to reduced wall Al accumulation.
5. Other Al tolerance mechanisms involve novel Al uptake transporters, including Nrat1 in rice (which moves Al from the cell wall into root cells, where it is sequestered in the vacuole) and aquaporins (which mediate plasma membrane and tonoplast Al accumulation in an Al accumulator).

6. Major Al tolerance loci that have been pivotal in breeding strategies targeting crop adaptation to Al-toxic soils are determined primarily by the plasma membrane transporters conferring Al-activated organic acid release (ALMTs and MATEs).
7. There is breeding potential in exploring the genetic determinants of transcriptional regulation of *ALMT1* and *MATE* genes in addition to those underlying other Al tolerance mechanisms.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Aniol A. 1990. Genetics of tolerance to aluminum in wheat (*Triticum aestivum* L. Thell). *Plant Soil* 123:223–27
2. Aniol A, Gustafson JP. 1984. Chromosome location of genes controlling aluminum tolerance in wheat, rye, and triticale. *Can. J. Genet. Cytol.* 26:701–5
3. Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373–99
4. Barcelo J, Poschenrieder C. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environ. Exp. Bot.* 48:75–92
5. Berg JM, Tymoczko JL, Stryer L. 2002. The citric acid cycle. In *Biochemistry*, pp. 465–87. New York: Freeman. 5th ed.
6. Cai S, Wu D, Jabeen Z, Huang Y, Huang Y, Zhang G. 2013. Genome-wide association analysis of aluminum tolerance in cultivated and Tibetan wild barley. *PLOS ONE* 8:e69776
7. Camargo CEO. 1981. Melhoramento do trigo. I. Hereditariedade da tolerância à toxicidade do alumínio. *Bragantia* 40:33–45
8. Caniato FF, Guimaraes CT, Hamblin M, Billot C, Rami J-F, et al. 2011. The relationship between population structure and aluminum tolerance in cultivated sorghum. *PLOS ONE* 6:e20830
9. Caniato FF, Guimaraes CT, Schaffert RE, Alves VMC, Kochian LV, et al. 2007. Genetic diversity for aluminum tolerance in sorghum. *Theor. Appl. Genet.* 114:863–76
10. Caniato FF, Hamblin MT, Guimaraes CT, Zhang Z, Schaffert RE, et al. 2014. Association mapping provides insights into the origin and the fine structure of the sorghum aluminum tolerance locus, Alt_{SB}. *PLOS ONE* 9:e87438
11. Chang YC, Yamamoto Y, Matsumoto H. 1999. Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant Cell Environ.* 22:1009–17
12. Chen ZC, Yokosho K, Kashino M, Zhao FJ, Yamaji N, Ma JF. 2013. Adaptation to acidic soil is achieved by increased numbers of *cis*-acting elements regulating *ALMT1* expression in *Holcus lanatus*. *Plant J.* 76:10–23
13. **Collins NC, Shirley NJ, Saeed M, Pallotta M, Gustafson JP. 2008. An *ALMT1* gene cluster controlling aluminium tolerance at the *Alt4* locus of rye (*Secale cereale* L.). *Genetics* 179:669–82**

13. Showed that copy-number variation is involved with Al resistance provided by *TaALMT1* in rye.

14. Conceição LDHCS, Tessele C, Barbosa Neto JF. 2009. Diallel analysis and mapping of aluminum tolerance in corn inbred lines. *Maydica* 54:51–64
15. Dai J, Bai G, Zhang D, Hong D. 2012. Validation of quantitative trait loci for aluminum tolerance in Chinese wheat landrace FSW. *Euphytica* 192:171–79
16. De Angeli A, Baetz U, Francisco R, Zhang J, Chaves MM, Regalado A. 2013. The vacuolar channel VvALMT9 mediates malate and tartrate accumulation in berries of *Vitis vinifera*. *Planta* 238:283–91
17. De Angeli A, Zhang J, Meyer S, Martinoia E. 2013. AtALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in *Arabidopsis*. *Nat. Commun.* 4:1804
18. Delhaize E, Craig S, Beaton CD, Bennet RJ, Jagadish VC, Randall PJ. 1993. Aluminum tolerance in wheat (*Triticum asetivum* L.): I. Uptake and distribution of aluminum in root apices. *Plant Physiol.* 103:685–93
19. Delhaize E, Ma JF, Ryan PR. 2012. Transcriptional regulation of aluminium tolerance genes. *Trends Plant Sci.* 17:341–48
20. Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H. 2004. Engineering high-level aluminum tolerance in barley with the *ALMT1* gene. *PNAS* 101:15249–54
21. **Delhaize E, Ryan PR, Randall PJ. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.): II. Aluminum-stimulated excretion of malic acid from root apices. *Plant Physiol.* 103:695–702**
22. Ding ZJ, Yan JY, Xu XY, Li GX, Zheng SJ. 2013. WRKY46 functions as a transcriptional repressor of *ALMT1*, regulating aluminum-induced malate secretion in Arabidopsis. *Plant J.* 76:825–35
23. Dreyer I, Gomez-Porras JL, Riano-Pachon DM, Hedrich R, Geiger D. 2012. Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs). *Front. Plant Sci.* 3:263
24. Eticha D, Stass A, Horst WJ. 2005. Cell-wall pectin and its degree of methylation in the maize root-apex: significance for genotypic differences in aluminium resistance. *Plant Cell Environ.* 28:1410–20
25. Famoso AN, Clark RT, Shaff JE, Craft E, McCouch SR, Kochian LV. 2010. Development of a novel aluminum tolerance phenotyping platform used for comparisons of cereal aluminum tolerance and investigations into rice aluminum tolerance mechanisms. *Plant Physiol.* 153:1678–91
26. Famoso AN, Zhao K, Clark RT, Tung CW, Wright MH, et al. 2011. Genetic architecture of aluminum tolerance in rice (*Oryza sativa*) determined through genome-wide association analysis and QTL mapping. *PLOS Genet.* 7:e1002221
27. **Fujii M, Yokosho K, Yamaji N, Saisho D, Yamane M, et al. 2012. Acquisition of aluminium tolerance by modification of a single gene in barley. *Nat. Commun.* 3:713**
28. Furuichi T, Sasaki T, Tsuchiya Y, Ryan PR, Delhaize E, Yamamoto Y. 2010. An extracellular hydrophilic carboxy-terminal domain regulates the activity of TaALMT1, the aluminum-activated malate transport protein of wheat. *Plant J.* 64:47–55
29. **Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, et al. 2007. An aluminum-activated citrate transporter in barley. *Plant Cell Physiol.* 48:1081–91**
30. Garvin DF, Carver BF. 2003. Role of the genotype in tolerance to acidity and aluminum toxicity. In *Handbook of Soil Acidity*, ed. Z Rengel, pp. 387–406. New York: Dekker
31. Gruber BD, Ryan PR, Richardson AE, Tyerman SD, Ramesh S, et al. 2010. HvALMT1 from barley is involved in the transport of organic anions. *J. Exp. Bot.* 61:1455–67
32. Guimaraes CT, Simoes CC, Pastina MM, Maron LG, Magalhaes JV, et al. 2014. Genetic dissection of Al tolerance QTLs in the maize genome by high density SNP scan. *BMC Genomics* 15:153–67
33. Henikoff S, Greene EA, Pietrokovski S, Bork P, Attwood TK, Hood L. 1997. Gene families: the taxonomy of protein paralogs and chimeras. *Science* 278:609–14
34. Hoekenga OA, Maron LG, Piñeros MA, Cançado GM, Shaff J, et al. 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. *PNAS* 103:9738–43
35. Huang CF, Yamaji N, Chen Z, Ma JF. 2012. A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. *Plant J.* 69:857–67
36. Huang CF, Yamaji N, Ma JF. 2010. Knockout of a bacterial-type ATP-binding cassette transporter gene, *AtSTAR1*, results in increased aluminum sensitivity in Arabidopsis. *Plant Physiol.* 153:1669–77
37. **Huang CF, Yamaji N, Mitani N, Yano M, Nagamura Y, Ma JF. 2009. A bacterial-type ABC transporter is involved in aluminum tolerance in rice. *Plant Cell* 21:655–67**

21. The seminal paper that demonstrated the major root tip Al exclusion mechanism based on Al-activated OA exudation.

27. Identified a *cis* sequence within the *HvAACT1* promoter that enhances gene expression and drives expression to the root tips.

29. Along with Ref. 71, provided the first identification of the second class of root OA transporters involved in Al exclusion (MATEs).

37. Identified the first rice Al resistance genes (*STAR1* and *STAR2*) involved in transporting cell wall–modifying substrates from the root cytoplasm.

38. Ito D, Shinkai Y, Kato Y, Kondo T, Yoshida K. 2009. Chemical studies on different color development in blue- and red-colored sepal cells of *Hydrangea macrophylla*. *Biosci. Biotechnol. Biochem.* 73:1054–59
39. **Iuchi S, Koyama H, Iuchi A, Kobayashi Y, Kitabayashi S, et al. 2007. Zinc finger protein STOP1 is critical for proton tolerance in *Arabidopsis* and coregulates a key gene in aluminum tolerance. *PNAS* 104:9900–5**
40. Johnson JP, Carver BF, Baligar VC. 1997. Expression of aluminum tolerance transferred from Atlas 66 to hard winter wheat. *Crop Sci.* 37:103–8
41. Jones DL, Blancaflor EB, Kochian LV, Gilroy S. 2006. Spatial coordination of aluminium uptake, production of reactive oxygen species, callose production and wall rigidification in maize roots. *Plant Cell Environ.* 29:1309–18
42. Jones DL, Gilroy S, Larsen PB, Howell SH, Kochian LV. 1998. Effect of aluminum on cytoplasmic Ca^{2+} homeostasis in root hairs of *Arabidopsis thaliana* (L.). *Planta* 206:378–87
43. Jones DL, Kochian LV, Gilroy S. 1998. Aluminum induces a decrease in cytosolic [Ca^{2+}] in BY-2 tobacco cell cultures. *Plant Physiol.* 116:81–89
44. Kang J, Park J, Choi H, Burla B, Kretzschmar T, et al. 2011. Plant ABC transporters. *Arabidopsis Book* 9:e0153
45. Kerridge PC, Kronstad WE. 1968. Evidence of genetic resistance to aluminum toxicity in wheat (*Triticum aestivum* Vill., Host). *Agron. J.* 60:710–11
46. Kidd PS, Llugany M, Poschenrieder C, Gunse B, Barcelo J. 2001. The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *J. Exp. Bot.* 52:1339–52
47. Kobayashi Y, Hoekenga OA, Itoh H, Nakashima M, Saito S, et al. 2007. Characterization of *AtALMT1* expression in aluminum-inducible malate release and its role for rhizotoxic stress tolerance in Arabidopsis. *Plant Physiol.* 145:843–52
48. Kobayashi Y, Ohyama Y, Kobayashi Y, Ito H, Iuchi S, et al. 2014. STOP2 activates transcription of several genes for Al- and low pH-tolerance that are regulated by STOP1 in *Arabidopsis*. *Mol. Plant* 7:311–22
49. Kochian LV, Hoekenga OA, Piñeros MA. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.* 55:459–93
50. Kollmeier M, Dietrich P, Bauer CS, Horst WJ, Hedrich R. 2001. Aluminum activates a citrate-permeable anion channel in the aluminum-sensitive zone of the maize root apex. A comparison between an aluminum-sensitive and an aluminum-resistant cultivar. *Plant Physiol.* 126:397–410
51. Kovermann P, Meyer S, Hortensteiner S, Picco C, Scholz-Starke J, et al. 2007. The Arabidopsis vacuolar malate channel is a member of the ALMT family. *Plant J.* 52:1169–80
52. Larsen PB, Cancel J, Rounds M, Ochoa V. 2007. *Arabidopsis ALS1* encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. *Planta* 225:1447–58
53. Larsen PB, Geisler MJ, Jones CA, Williams KM, Cancel JD. 2005. ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in Arabidopsis. *Plant J.* 41:353–63
54. Li JY, Liu J, Dong D, Jia X, McCouch SR, Kochian LV. 2014. Natural variation underlies alterations in Nramp aluminum transporter (*NRAT1*) expression and function that play a key role in rice aluminum tolerance. *PNAS* 111:6503–8
55. Liang C, Piñeros MA, Tian J, Yao Z, Sun L, et al. 2013. Low pH, aluminum, and phosphorus coordinately regulate malate exudation through *GmALMT1* to improve soybean adaptation to acid soils. *Plant Physiol.* 161:1347–61
56. Ligaba A, Dreyer I, Margaryan A, Schneider DJ, Kochian LV, Piñeros M. 2013. Functional, structural and phylogenetic analysis of domains underlying the Al sensitivity of the aluminum-activated malate/anion transporter, TaALMT1. *Plant J.* 76:766–80
57. Ligaba A, Katsuhara M, Ryan PR, Shibasaka M, Matsumoto H. 2006. The *BnALMT1* and *BnALMT2* genes from rape encode aluminum-activated malate transporters that enhance the aluminum resistance of plant cells. *Plant Physiol.* 142:1294–303
58. Ligaba A, Kochian LV, Piñeros M. 2009. Phosphorylation at S384 regulates the activity of the TaALMT1 malate transporter that underlies aluminum resistance in wheat. *Plant J.* 60:411–23

39. Identified the first transcription factor (STOP1) that regulates the Al-induced expression of a suite of Al resistance genes.

59. Ligaba A, Maron L, Shaff J, Kochian LV, Piñeros M. 2012. Maize ZmALMT2 is a root anion transporter that mediates constitutive root malate efflux. *Plant Cell Environ.* 35:1185–200
60. Liu J, Magalhaes JV, Shaff J, Kochian LV. 2009. Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. *Plant J.* 57:389–99
61. Luo MC, Dvorak J. 1996. Molecular mapping of an aluminum tolerance locus on chromosome 4D of Chinese Spring wheat. *Euphytica* 91:31–35
62. Ma JF. 2000. Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol.* 41:383–90
63. Ma JF. 2007. Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. *Int. Rev. Cytol.* 264:225–52
64. Ma JF, Hiradate S. 2000. Form of aluminium for uptake and translocation in buckwheat (*Fagopyrum esculentum* Moench). *Planta* 211:355–60
65. Ma JF, Hiradate S, Nomoto K, Iwashita T, Matsumoto H. 1997. Internal detoxification mechanism of Al in hydrangea: identification of Al form in the leaves. *Plant Physiol.* 113:1033–39
66. Ma JF, Nagao S, Huang CF, Nishimura M. 2005. Isolation and characterization of a rice mutant hypersensitive to Al. *Plant Cell Physiol.* 46:1054–61
67. Ma JF, Ryan PR, Delhaize E. 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci.* 6:273–78
68. Ma JF, Shen R, Zhao Z, Wissuwa M, Takeuchi Y, et al. 2002. Response of rice to Al stress and identification of quantitative trait loci for Al tolerance. *Plant Cell Physiol.* 43:652–59
69. Ma JF, Zheng SJ, Matsumoto H, Hiradate S. 1997. Detoxifying aluminium with buckwheat. *Nature* 390:569–70
70. Magalhaes JV, Garvin DF, Wang Y, Sorrells ME, Klein PE, et al. 2004. Comparative mapping of a major aluminum tolerance gene in sorghum and other species in the poaceae. *Genetics* 167:1905–14
71. Magalhaes JV, Liu J, Guimaraes CT, Lana UGP, Alves VMC, et al. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat. Genet.* 39:1156–61
72. Magnavaca R, Gardner CO, Clark RB. 1987. Inheritance of aluminum tolerance in maize. In *Genetic Aspects of Mineral Nutrition*, ed. HW Gabelman, BC Loughman, pp. 201–12. Dordrecht, Neth.: Nijhoff
73. Maron LG, Guimaraes CT, Kirst M, Albert PS, Birchler JA, et al. 2013. Aluminum tolerance in maize is associated with higher *MATE1* gene copy number. *PNAS* 110:5241–46
74. Maron LG, Piñeros MA, Guimaraes CT, Magalhaes JV, Pleiman JK, et al. 2010. Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *Plant J.* 61:728–40
75. Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, et al. 2002. Multifunctionality of plant ABC transporters—more than just detoxifiers. *Planta* 214:345–55
76. Matonyei TK, Cheprot RK, Liu J, Piñeros MA, Shaff JE, et al. 2014. Physiological and molecular analysis of aluminum tolerance in selected Kenyan maize lines. *Plant Soil* 377:357–67
77. Melo JO, Lana UGP, Piñeros MA, Alves VMC, Guimaraes CT, et al. 2013. Incomplete transfer of accessory loci influencing SbMATE expression underlies genetic background effects for aluminum tolerance in sorghum. *Plant J.* 73:276–88
78. Meyer S, De Angeli A, Fernie AR, Martinoia E. 2010. Intra- and extra-cellular excretion of carboxylates. *Trends Plant Sci.* 15:40–47
79. Meyer S, Mumm P, Imes D, Endler A, Weder B, et al. 2010. AtALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. *Plant J.* 63:1054–62
80. Meyer S, Scholz-Starke J, De Angeli A, Kovermann P, Burla B, et al. 2011. Malate transport by the vacuolar AtALMT6 channel in guard cells is subject to multiple regulation. *Plant J.* 67:247–57
81. Minella E, Sorrells ME. 1992. Aluminum tolerance in barley: genetic relationships among genotypes of diverse origin. *Crop Sci.* 32:593–98
82. Miyasaka SC, Buta JG, Howell RK, Foy CD. 1991. Mechanism of aluminum tolerance in snapbeans: root exudation of citric acid. *Plant Physiol.* 96:737–43

60. Demonstrated that a transcriptional link between *AtMATE* and *AtALMT1* provided by *STOP1* uncovers common regulatory networks that influence distinct transporter families.

71. Along with Ref. 29, provided the first identification of the second class of root OA transporters involved in Al exclusion (MATEs).

73. Showed that copy-number variation is involved in the transcriptional control of *ZmMATE1* in maize.

83. Motoda H, Sasaki T, Yamamoto Y. 2007. Transmembrane topology of wheat ALMT1 transporter. *Plant Cell Physiol.* 48:S88
84. Mumm P, Imes D, Martinoia E, Al-Rasheid KAS, Geiger D, et al. 2013. C-terminus-mediated voltage gating of *Arabidopsis* guard cell anion channel QUAC1. *Mol. Plant* 6:1550–63
85. Negishi T, Oshima K, Hattori M, Kanai M, Mano S, et al. 2012. Tonoplast- and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. *PLOS ONE* 7:e43189
86. Negishi T, Oshima K, Hattori M, Yoshida K. 2013. Plasma membrane-localized Al-transporter from blue hydrangea sepals is a member of the anion permease family. *Genes Cells* 18:341–52
87. Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT. 2003. Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 106:583–93
88. Ninamango-Cárdenas F, Guimarães CT, Martins PR, Parentoni SN, Carneiro NP, et al. 2003. Mapping QTLs for aluminum tolerance in maize. *Euphytica* 130:223–32
89. Osawa H, Endo I, Hara Y, Matsushima Y, Tange T. 2011. Transient proliferation of proanthocyanidin-accumulating cells on the epidermal apex contributes to highly aluminum-resistant root elongation in camphor tree. *Plant Physiol.* 155:433–46
90. Osawa H, Matsumoto H. 2001. Possible involvement of protein phosphorylation in aluminium-responsive malate efflux from wheat root apex. *Plant Physiol.* 126:411–20
91. Papernik LA, Bethea AS, Singleton TE, Magalhaes JV, Garvin DF, Kochian LV. 2001. Physiological basis of reduced Al tolerance in ditelosomic lines of Chinese Spring wheat. *Planta* 212:829–34
92. Pellet DM, Grunes DL, Kochian LV. 1995. Organic-acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). *Planta* 196:788–95
93. Pereira JF, Zhou G, Delhaize E, Richardson T, Zhou M, Ryan PR. 2010. Engineering greater aluminium resistance in wheat by over-expressing *TaALMT1*. *Ann. Bot.* 106:205–14
94. Piñeros MA, Cançado GM, Kochian LV. 2008. Novel properties of the wheat aluminum tolerance organic acid transporter (TaALMT1) revealed by electrophysiological characterization in *Xenopus* oocytes: functional and structural implications. *Plant Physiol.* 147:2131–46
95. Piñeros MA, Cançado GM, Maron LG, Lyi SM, Menossi M, Kochian LV. 2008. Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of *ZmALMT1*—an anion-selective transporter. *Plant J.* 53:352–67
96. Piñeros MA, Fich E, Offenborn JN, Mähs A, Kudla J, Kochian LV. 2013. *A CBL5/CIPK2 calcium sensor/protein kinase complex modulates the transport activity of MATE-type transporters involved in the key plant aluminum resistance mechanism mediated by root organic acid release*. Presented at Int. Workshop Plant Membr. Biol., 16th, Okayama, Jpn., Mar. 26–31

97. Piñeros MA, Kochian LV. 2001. A patch-clamp study on the physiology of aluminum toxicity and aluminum tolerance in maize. Identification and characterization of Al^{3+}-induced anion channels. *Plant Physiol.* 125:292–305

98. Piñeros MA, Magalhaes JV, Alves VMC, Kochian LV. 2002. The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. *Plant Physiol.* 129:1194–206
99. Piñeros MA, Shaff JE, Manslank HS, Alves VMC, Kochian LV. 2005. Aluminum resistance in maize cannot be solely explained by root organic acid exudation. A comparative physiological study. *Plant Physiol.* 137:231–41
100. Raman H, Stodart B, Ryan PR, Delhaize E, Emebiri L, et al. 2010. Genome-wide association analyses of common wheat (*Triticum aestivum* L.) germplasm identifies multiple loci for aluminium resistance. *Genome* 53:957–66
101. Rengel Z. 1992. Disturbance of Ca^{2+} homeostasis as a primary trigger in the Al toxicity syndrome. *Plant Cell Environ.* 15:931–38
102. Rengel Z, Zhang W-H. 2003. Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. *New Phytol.* 159:295–314
103. Riede CR, Anderson JA. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Sci.* 36:905–9

97. Demonstrated that an Al-activated and electrogenic anion efflux transporter underlies Al-activated citrate efflux and Al resistance in maize.

104. Rodriguez Milla MA, Gustafson JP. 2001. Genetic and physical characterization of chromosome 4DL in wheat. Genome. *Genome* 44:883–92
105. Ryan PR, Delhaize E. 2012. Plant adaptations to aluminium toxicity. In *Plant Stress Physiology*, ed. S Shabala, pp. 171–93. Wallingford, UK: CABI
106. Ryan PR, Delhaize E, Jones D. 2001. Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:527–60
107. Ryan PR, Delhaize E, Randall PJ. 1995. Malate efflux from root apices and tolerance aluminum are highly correlated in wheat. *Aust. J. Plant Physiol.* 22:531–36
108. Ryan PR, DiTomaso JM, Kochian LV. 1993. Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. *J. Exp. Bot.* 44:437–46
109. Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. *Plant Physiol.* 149:340–51
110. Ryan PR, Raman H, Gupta S, Sasaki T, Yamamoto Y, Delhaize E. 2010. The multiple origins of aluminium resistance in hexaploid wheat include *Aegilops tauschii* and more recent *cis* mutations to *TaALMT1*. *Plant J.* 64:446–55
111. Ryan PR, Skerrett M, Findlay GP, Delhaize E, Tyerman SD. 1997. Aluminum activates an anion channel in the apical cells of wheat roots. *PNAS* 94:6547–52
112. Ryan PR, Tyerman SD, Sasaki T, Yamamoto Y, Zhang WH, Delhaize E. 2011. Identification of aluminium-resistance genes in plants provides an opportunity for enhancing the acid-soil tolerance of crop species. *J. Exp. Bot.* 62:9–20
113. Sasaki T, Mori IC, Furuichi T, Munemasa S, Toyooka K, et al. 2010. Closing plant stomata requires a homolog of an aluminum-activated malate transporter. *Plant Cell Physiol.* 51:354–65
114. Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, et al. 2004. A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* 37:645–53
115. Sasidharan R, Voesenek LACJ, Pierik R. 2011. Cell wall modifying proteins mediate plant acclimatization to biotic and abiotic stresses. *Crit. Rev. Plant Sci.* 30:548–62
116. Sawaki Y, Iuchi S, Kobayashi Y, Kobayashi Y, Ikka T, et al. 2009. STOP1 regulates multiple genes that protect Arabidopsis from proton and aluminum toxicities. *Plant Physiol.* 150:281–94
117. Schmohl N, Pilling J, Fisahn J, Horst WJ. 2000. Pectin methylesterase modulates aluminium sensitivity in *Zea mays* and *Solanum tuberosum*. *Physiol. Plant.* 109:419–27
118. Shen RF, Ma JF, Kyo M, Iwashita T. 2002. Compartmentation of aluminium in leaves of an Al-accumulator, *Fagopyrum esculentum* Moench. *Planta* 215:394–98
119. Sibov ST, Gaspar MJ, Ottoboni LMM, Arruda P, Souza AP. 1999. Two genes controlling aluminum tolerance in maize: genetic and molecular mapping analyses. *Genome* 42:475–82
120. Sivaguru M, Horst W. 1998. The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. *Plant Physiol.* 116:155–63
121. Sivaguru M, Liu J, Kochian LV. 2013. Targeted expression of SbMATE in the root distal transition zone is responsible for sorghum aluminum resistance. *Plant J.* 76:297–307
122. Tahara K, Hashida K, Otsuka Y, Ohara S, Kojima K, Shinohara K. 2014. Identification of a hydrolyzable tannin, oenothein B, as an aluminum-detoxifying ligand in a highly aluminum-resistant tree, *Eucalyptus camaldulensis*. *Plant Physiol.* 164:683–93
123. Tang Y, Garvin DF, Kochian LV, Sorrells ME, Carver BF. 2002. Physiological genetics of aluminum tolerance in the wheat cultivar Atlas 66. *Crop Sci.* 42:1541–46
124. Tolrà R, Barcelo J, Poschenrieder C. 2009. Constitutive and aluminium-induced patterns of phenolic compounds in two maize varieties differing in aluminium tolerance. *J. Inorg. Biochem.* 103:1486–90
125. Tovkach A, Ryan PR, Richardson AE, Lewis DC, Rathjen TM, et al. 2013. Transposon-mediated alteration of *TaMATE1B* expression in wheat confers constitutive citrate efflux from root apices. *Plant Physiol.* 161:880–92
126. Tsutsui T, Yamaji N, Feng Ma J. 2011. Identification of a *cis*-acting element of ART1, a C_2H_2-type zinc-finger transcription factor for aluminum tolerance in rice. *Plant Physiol.* 156:925–31
127. von Uexküll HR, Mutert E. 1995. Global extent, development and economic-impact of acid soils. *Plant Soil* 171:1–15

111. Demonstrated that Al-activated anion channels underlie Al-activated malate efflux and Al resistance in wheat.

114. Identified the first Al resistance gene, *TaALMT1*, which encodes an Al-activated malate efflux transporter in wheat root apices.

128. Wong MTF, Asseng S, Robertson MJ, Oliver Y. 2008. Mapping subsoil acidity and shallow soil across a field with information from yield maps, geophysical sensing and the grower. *Precis. Agric.* 9:3–5
129. Wood S, Sebastian K, Scherr SJ. 2000. *Pilot analysis of global ecosystems: agroecosystems*. Tech. Rep., Int. Food Policy Res. Inst. and World Resour. Inst., Washington, DC
130. Wu P, Liao CY, Hu B, Yi KK, Jin WZ, et al. 2000. QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. *Theor. Appl. Genet.* 100:1295–303
131. Xia J, Yamaji N, Che J, Shen RF, Ma JF. 2014. Differential expression of *Nrat1* is responsible for Al-tolerance QTL on chromosome 2 in rice. *J. Exp. Bot.* 65:4297–304
132. Xia J, Yamaji N, Kasai T, Ma JF. 2010. Plasma membrane-localized transporter for aluminum in rice. *PNAS* 107:18381–85
133. Xia J, Yamaji N, Ma JF. 2011. Further characterization of an aluminum influx transporter in rice. *Plant Signal. Behav.* 6:160–63
134. Xia J, Yamaji N, Ma JF. 2013. A plasma membrane-localized small peptide is involved in rice aluminum tolerance. *Plant J.* 76:345–55
135. Yamaji N, Huang CF, Nagao S, Yano M, Sato Y, et al. 2009. A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in rice. *Plant Cell* 21:3339–49
136. Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H. 2003. Oxidative stress triggered by aluminum in plant roots. *Plant Soil* 255:239–43
137. Yang JL, Li YY, Zhang YJ, Zhang SS, Wu YR, et al. 2008. Cell wall polysaccharides are specifically involved in the exclusion of aluminum from the rice root apex. *Plant Physiol.* 146:602–11
138. Yang JL, Zhu XF, Peng YX, Zheng C, Li GX, et al. 2011. Cell wall hemicellulose contributes significantly to aluminum adsorption and root growth in Arabidopsis. *Plant Physiol.* 155:1885–92
139. Yang XY, Yang JL, Piñeros MA, Kochian LV, Li GX, Zheng SJ. 2011. A *de novo* synthesis citrate transporter, *Vigna umbellata* multidrug and toxic compound extrusion, implicates in Al-activated citrate efflux in rice bean (*Vigna umbellata*) root apex. *Plant Cell Environ.* 34:2138–48
140. Yang XY, Zeng ZH, Yan JY, Fan W, Bian HW, et al. 2013. Association of specific pectin methylesterases with Al-induced root elongation inhibition in rice. *Physiol. Plant.* 148:502–11
141. Yokosho K, Yamaji N, Ma JF. 2010. Isolation and characterisation of two MATE genes in rye. *Funct. Plant Biol.* 37:296–303
142. Yokosho K, Yamaji N, Ma JF. 2011. An Al-inducible MATE gene is involved in external detoxification of Al in rice. *Plant J.* 68:1061–69
143. Zhang WH, Ryan PR, Sasaki T, Yamamoto Y, Sullivan W, Tyerman SD. 2008. Characterization of the TaALMT1 protein as an Al^{3+}-activated anion channel in transformed tobacco (*Nicotiana tabacum* L.) cells. *Plant Cell Physiol.* 49:1316–30
144. Zhang WH, Ryan PR, Tyerman SD. 2001. Malate-permeable channels and cation channels activated by aluminium in the apical cells of wheat roots. *Plant Physiol.* 125:1459–72
145. Zheng SJ, Ma JF, Matsumoto H. 1998. Continuous secretion of organic acids is related to aluminum resistance during relatively long-term exposure to aluminum stress. *Physiol. Plant.* 103:209–14
146. Zheng SJ, Ma JF, Matsumoto H. 1998. High aluminum resistance in buckwheat: I. Al-induced specific secretion of oxalic acid from root tips. *Plant Physiol.* 117:745–51
147. Zhu XF, Shi YZ, Lei GJ, Fry SC, Zhang BC, et al. 2012. *XTH31*, encoding an in vitro XEH/XET-active enzyme, regulates aluminum sensitivity by modulating in vivo XET action, cell wall xyloglucan content, and aluminum binding capacity in *Arabidopsis*. *Plant Cell* 24:4731–47
148. Zhu XF, Sun Y, Zhang BC, Mansoori N, Wan JX, et al. 2014. *TRICHOME BIREFRINGENCE-LIKE27* affects aluminum sensitivity by modulating the *O*-acetylation of xyloglucan and aluminum-binding capacity in Arabidopsis. *Plant Physiol.* 166:181–89
149. Zhu XF, Wan JX, Sun Y, Shi YZ, Braam J, et al. 2014. Xyloglucan Endotransglucosylase-Hydrolase17 interacts with Xyloglucan Endotransglucosylase-Hydrolase31 to confer xyloglucan endotransglucosylase action and affect aluminum sensitivity in Arabidopsis. *Plant Physiol.* 165:1566–74

132. Identified the first Al uptake transporter that underlies a novel Al resistance mechanism.

135. Showed that ART1, the STOP1 homolog in rice, is a transcription factor that regulates the Al-induced expression of genes involved in Al resistance.

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Contents