Rice Arsenal Against Aluminum Toxicity

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Abstract One of the major constraints on crop production is the ability of plants to grow in acidic soils, where aluminum (Al) is soluble in its toxic form (Al^{3+}). However, some plants can address this Al toxicity by utilizing different strategies such as exclusion (an external mechanism) and detoxification (an internal mechanism). Rice, an important food source, is one of the most Al-tolerant crops, but the mechanism of this tolerance is not well understood. In this review, we provide an overview of Al-tolerance mechanisms in rice and show that this species can employ several strategies that together provide tolerance to Al toxicity.

1 Introduction

Under acidic conditions, aluminum (Al), which is a major worldwide environmental concern, is solubilized in its trivalent ionic form (Al^{3+}) and interacts with plants. This phenomenon causes major problems in agriculture because Al is the most abundant metal in the soil and because up to 50 % of the world's arable land has acidic soil (Von Uexkull and Mutert 1995).

A plant's root apex plays a major role in Al recognition and response and also serves as the primary site for Al toxicity (Kollmeier et al. 2000). The first symptom of Al toxicity is the inhibition of root growth, which is caused by the inhibition of cell wall expansion and elongation and, after long exposures, cell wall division (Matsumoto 2000; Panda et al. 2009). The effects of these injuries include poor uptake of water and nutrients, the disruption of cytosolic calcium (Ca^{2+}) and proton activity (H^+), oxidative stress, and other problems; these consequences are discussed in detail in Kochian et al. (2005).

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Nevertheless, through the course of evolution, plants have developed mechanisms that allow them to cope with Al in the soil. Two main types of mechanisms account for Al tolerance: external detoxification systems and internal detoxification systems. External detoxification, which is the better-documented mechanism, occurs via the efflux of organic acid anions (malate, citrate, and oxalate) from the roots; these anions form chelating complexes that prevent the entrance of Al into the cells. Internal detoxification mechanisms occur by the chelation of Al with organic acid anions and sequestration in vacuoles. For a detailed review of these Al-tolerance mechanisms, readers can consult (Kochian et al. 2002; Hoekenga and Magalhaes 2011).

Rice (*Oryza sativa*—Poaceae) is the most Al-tolerant crop under field conditions (Foy 1988) and the most Al-tolerant cereal grass under controlled conditions (Famoso et al. 2010). However, an explanation of the exact mechanism of this resistance is still lacking. Both Al internalization and Al exclusion seem to occur in rice (Xia et al. 2010; Yokosho et al. 2011). Indeed, rice is up to six times more tolerant of Al than other members of the Poaceae family, such as maize, wheat, and sorghum (Famoso et al. 2010), suggesting that Al tolerance among these grasses was acquired due to selection during the domestication process.

One can hypothesize that Al-tolerance traits could have been selected in these grasses according to their origin of domestication. For example, maize was domesticated in Mexico from *Balsas teosinte* approximately 8000 years ago (Doebley 2004; Doebley et al. 2006), whereas wheat was domesticated in the Fertile Crescent from *Triticum dicoccoides* approximately 10,000 years ago (Ozkan et al. 2002), and the domestication of cultivated rice occurred in southern China (Huang et al. 2012). A soil pH map[1]indicates that the regions where cultivated rice was domesticated have acidic soil and that the regions where maize and wheat were domesticated have neutral to basic soils (Fig. 1a).

Within *O. sativa*, two main subspecies from several genetically differentiated variety groups exist: japonica and indica (Garris et al. 2005). Based on genetic evidence, all rice varietal groups descended from the wild species *Oryza rufipogon* (Huang et al. 2012). *O. sativa* japonica rice was first domesticated from a specific population of *O. rufipogon* around the center of the Pearl River Basin in southern China, and *O. sativa* indica rice was subsequently developed from crosses between japonica rice and local wild rice as the initial cultivars spread into Southeast and southern Asia (Huang et al. 2012). *O. rufipogon* is, in general, very tolerant to Al (Nguyen et al. 2003; Cao et al. 2011), whereas *O. sativa* japonica and *O. sativa* indica are less tolerant. In all, Indica subspecies are less tolerant of Al than japonica subspecies (Ma et al. 2002; Famoso et al. 2011). Molecular evidence shows that the japonica group has less genetic diversity than the indica group (Garris et al. 2005).

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[1] The soil pH map (from 2000 to 2010) was retrieved from www.globalsoilmap.com and does not represent the pH of the soil 10,000 years ago, during the period in which rice was domesticated. However, until the 1800s, most acidic soil remained untouched and under forest cover. There was only some encroachment into regions with acidic soils in densely populated regions of the world, such as East Asia (Von Uexkull and Mutert 1995). For the map source, please see Hengl (2009).

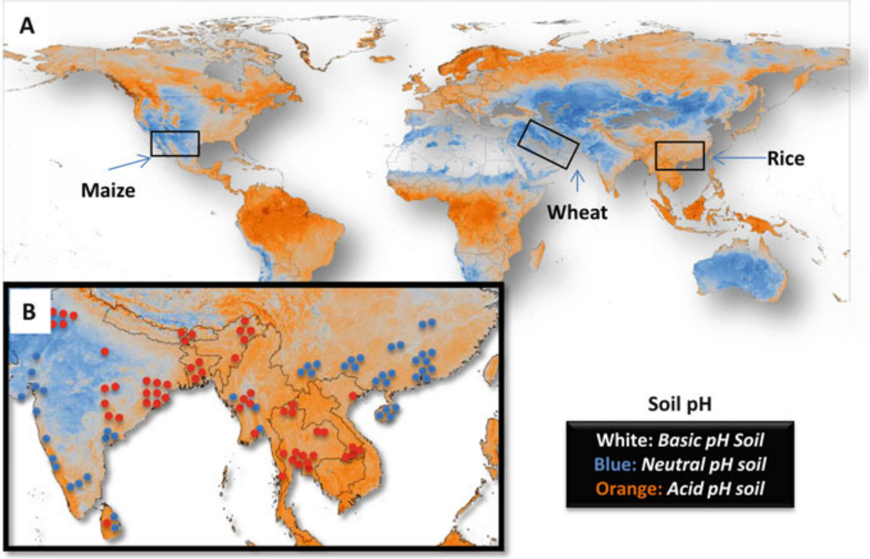

Fig. 1 Global map of soil pH. (**a**) Map representing the mean soil pH, predicted using a correlation with world maps (Hengl 2009). The origins and location of maize, wheat, and rice cultivation are indicated by *black boxes*. Maize and wheat were first cultivated in soil with a neutral to basic pH, while rice was cultivated in acidic soils. (**b**) The *O. rufipogon* accessions that originated *O. Sativa* (Japonica) are shown as *blue spots*, and the *O. rufipogon* accessions that originated *O. Sativa* (*Indica*) are shown as *red spots*. Magnification of the map showing the soil pH in south Asia. The *spot dots* are based on data retrieved from Huang et al. (2012)

A good explanation for this phenomenon was proposed by Kovach et al. (2007). A climate change resulted in the return of glacial-like conditions across Northern Asia from 11,500 to 13,000 years ago (Higham 2002; Lu et al. 2002). The colder weather would have eliminated a large portion of the japonica-like wild ancestors in the Yangtze River Valley. Humans were forced to rely on a narrowing gene pool, forcing a more rapid movement toward the domestication of rice in this region. In contrast, the warmer tropics of South and Southeast Asia would have maintained larger, more diverse populations of indica-like *O. rufipogon,* which could have been foraged by humans for a longer time, resulting in a more gradual domestication process. Crosses between *O. rufipogon* and japonica from this region may have contributed to the less Al-tolerant indica group (Fig. 1b).

Another clue for this comes from Zhao et al. (2013). Nitrogen (N), which plays a crucial role in plant growth, is present in the soils as ammonium and nitrate (two major types of inorganic N sources), with ammonium being more available in acidic soils and nitrate being more available in neutral to basic soils. Indica rice cultivars are generally Al-sensitive and nitrate-preferring, while Japonica cultivars are Al-tolerant and relatively ammonium-preferring. This Al tolerance was negatively correlated with their nitrate preference, suggesting that Al tolerance in rice is antagonistic with nitrate preference and synergistic with ammonium preference under acidic conditions (Zhao et al. 2013). However, acidic soils are distributed in regions where both japonica and indica can grow, suggesting that other factors besides N and Al could have driven the differentiation of japonica and indica and should therefore be evaluated (Zhao et al. 2013).

Despite the lower degree of Al tolerance of the Indica group compared to the japonica group, subspecies of the indica group are still more Al-tolerant than other grasses. How indica subspecies became less tolerant to Al is still an open question.

2 Mechanisms of Al Tolerance in Rice

2.1 Organic Acid Release

The ability of rice to tolerate toxic levels of Al began to be understood in the end of the 1980s (Foy 1988). At the beginning of the 2000s, rice researchers focused on searching for QTLs for Al tolerance (Ma et al. 2002; Nguyen et al. 2002, 2003). The first study regarding Al tolerance in rice demonstrated that the japonica variety was more Al-tolerant than the Indica variety and that japonica accumulated less Al in the root apex, indicating that Al exclusion rather than internal detoxification played major role in rice (Ma et al. 2002). However, recent evaluations of the Al concentration in the root apex showed no difference between japonica and indica varietal groups, demonstrating no relationship between Al exclusion and Al tolerance in rice (Famoso et al. 2010). Organic citrate is secreted in response to Al in both japonica and indica, and the cultivars do not differ significantly in the amount of secreted citrate; there is also no evidence of citrate secretion in response to increasing Al concentrations. Indeed, the amount of citrate that is secreted by rice was not significant, being one tenth of the citrate secreted by rye (Ma et al. 2002), which has a similar Al tolerance to rice (Famoso et al. 2010). This outcome implies that mechanisms to reduce or to mask the toxic forms of Al in the apoplast and symplast other than citrate release may determine the degree of Al tolerance in rice. Meanwhile, it was shown that the expression level of an Al-induced citrate transporter is positively correlated with the amount of citrate secretion in rice cultivars that differ in their Al tolerance (Yokosho et al. 2011).

This contradiction between rice citrate levels and Al tolerance may be explained by the following hypothesis: one portion of the citrate may function in Al exclusion, forming Al-citrate complexes in the rhizosphere, and another portion may function in Al sequestration into vacuoles. Even though the primary mechanism is Al exclusion, rice can tolerate certain intracellular amounts of Al. For example, an Al-tolerant rice cultivar (Pusa Basmati) accumulated more Al in the roots than an Al-sensitive cultivar (Vikas) (Meriga et al. 2004). Indeed, rice plants accumulate Al (Xia et al. 2010) and sequester it into vacuoles by a specific transporter (Huang et al. 2011). The Al hyperaccumulator *Hydrangea macrophylla* detoxifies Al by forming complexes with citrate in a 1:1 molar ratio in the leaves (Ma et al. 1997). In

Fagopyrum esculentum, another Al hyperaccumulator, Al is bound to oxalate ions in the roots and leaves and to citrate ions in the xylem (Ma et al. 1997; Zheng et al. 1998). The most studied Al hyperaccumulators usually use citrate–Al complexes in the xylem, raising the hypothesis that this is a major Al transport route (Grevenstuk and Romano 2013).

2.2 The Antioxidant Defense System, Cell Wall and Plasma Membrane Composition

Under adverse environmental conditions, plants produce increased reactive oxygen species (ROS), leading to the oxidation of biological macromolecules and, as consequence, to lipid peroxidation, membrane damage, and enzyme inactivation. To alleviate oxidative injury, nonenzymatic systems (such as reduced glutathione (GSH), ascorbic acid (AsA), carotenoids, and phenolics) and enzymatic systems [such as superoxide dismutase (SOD), ascorbate peroxidase (APx), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and glutathione POD (GPX)] are activated (Ma et al. 2012). Al stress generates ROS in rice plants and consequently increases the levels of ROS scavenging proteins (Sharma and Dubey 2007; Pandey et al. 2013). Under low concentrations of Al (up to 40 μM), a rice Al-tolerant cultivar will show higher levels of ROS-scavenging enzymes compared to an Al-sensitive cultivar; this will be accompanied by the lignification of the roots in an Al-sensitive cultivar, most likely due to the failure to scavenge ROS products such as hydrogen peroxide (H_2O_2) (Ma et al. 2012).

Moreover, rice plants in which cytosolic APx1/2 is silenced show a higher tolerance of moderate Al concentrations (up to 150 μM) compared to wild type plants (Rosa et al. 2010). However, at high Al concentrations (up to 750 μM), APx1/2-silenced plants become Al sensitive because the low levels of cytosolic APx are not sufficient to compensate for more stressful conditions, resulting in a more Al-sensitive phenotype (Arenhart et al. 2013).

Lipid peroxidation, a symptom of Al toxicity, varies between Al-tolerant and Al-sensitive cultivars. Two scenarios have been proposed: (1) the lipid composition of plants may be variable, making some less susceptible to peroxidation, or (2) plants with highly effective Al exclusion mechanisms suffer less lipid peroxidation because less Al^{3+} reaches the plasma membrane (Hoekenga and Magalhaes 2011). However, decreases in the activities of SOD and APX due to prolonged exposure to Al lead to DNA damage; this suggests that lipid peroxidation is a consequence rather than the cause of Al injury to plant roots (Meriga et al. 2004). The exogenous application of magnesium (Mg), calcium (Ca), salicylic acid (Sa) (Pandey et al. 2013), or nitric oxide (NO) reduced the toxicity of Al in rice seedlings by regulating the expression and activity of antioxidant enzyme activities and reducing ROS levels, but with the exception of NO, the relationship between rice Al tolerance and the endogenous concentrations of these molecules has not been described (Yang et al. 2013).

Nitric oxide, an important signaling molecule, alleviates Al-induced oxidative stress in *Phaseolus vulgaris* and *Cassia tora* (Wang and Yang 2005; Wang et al. 2010). However, endogenous NO responses to Al seem to be related to higher degrees of Al tolerance. For example, in Arabidopsis root apex transition zone, local release of large amounts of NO is blocked by Al treatment (Illés et al. 2006). Nevertheless, in rice, NO increases significantly after Al treatment (Yang et al. 2013). Moreover, rice seedlings that were pretreated with sodium nitroprusside (SNP, a NO donor) were more tolerant of Al treatment (Zhang et al. 2011). Despite the fact that genetic analyses have not implicated ROS scavenging genes or their regulators in natural variation in Al tolerance (Hoekenga and Magalhaes 2011), the ROS scavenging system appears to contribute to internal and external Al tolerance in rice.

Rice plants that were pretreated with SNP became more tolerant of Al and had lower pectin and hemicellulose levels, lower Al accumulation in the root tips and cell walls, a higher degree of methylation of pectin, and a lower cell wall Al-binding capacity than roots that were exposed to Al but not pretreated with SNP (Zhang et al. 2011). Consistent with this, the levels of cell wall polysaccharides (pectin, hemicellulose 1 and 2) in the root apex were reported to be significantly higher in an Al-sensitive cultivar than in an Al-tolerant cultivar in the absence of Al, and Al exposure increased the root apex hemicellulose content more significantly in an Al-sensitive cultivar (Yang et al. 2008). Furthermore, root cell wall pectin methyl esterase activity was constitutively higher in an Al-sensitive cultivar than in an Al-tolerant one, and this was accompanied by a higher proportion of demethylated pectins. The Al adsorption and desorption kinetics of the root tip cell wall also indicated that more Al was adsorbed, and the Al was more tightly bound in Al-sensitive plants. These data were consistent with Al content, pectin methylesterase activity, and pectin demethylesterification, suggesting that cell wall polysaccharides are important in Al exclusion, specifically from the rice root apex (Yang et al. 2008). Moreover, a rice cultivar that contains mutation affecting the root outer cell layers (epidermis, exodermis, and sclerenchyma) accumulated more Al than wild type rice (Huang et al. 2009b). In this mutant, the exodermal cells were changed into sclerenchyma-like cells, experiencing a decrease in cell size and a thickening of cell walls.

In addition to the cell wall, the composition of the plasma membrane seems to play a role in Al tolerance in rice. In rice and other Al-tolerant species, the membrane surface is less negatively charged than in Al-sensitive ones (Wagatsuma et al. 2005). This plasma membrane negative charge is one mechanism that may underlie variations in Al tolerance within species (Khan et al. 2009). An Al-sensitive rice cultivar showed increased plasma membrane permeability and greater Al uptake than an Al-tolerant cultivar. The lipid composition of the plasma membrane differed between these cultivars, with the Al-tolerant cultivar presenting a lower ratio of phospholipids to major neutral lipid Δ^5-sterol than the sensitive cultivar, suggesting that the plasma membrane of the Al-tolerant cultivar is less negatively charged and has reduced permeability compared to that of the Al-sensitive cultivar (Khan et al. 2009).

In another study, four rice cultivars that differ in their Al tolerance were compared, and a decrease in the lipid and fatty acid content was observed in the sensitive cultivars. In the roots of the susceptible cultivars, the levels of phospholipids such as phosphatidylcholine decreased, whereas the amount of lipid remained unchanged in the tolerant cultivars. The study suggests that the stability of lipid composition and the capacity to maintain lipid biosynthetic activities may help rice under Al stress (Huynh et al. 2012).

2.3 The Role of the Root Border Cells

Root border cells (RBCs) are special living cells that are attached to the root apex and play key roles in plant development. RBCs are released from the root tip and secrete a mucilage to protect plants from environmental factors. Recently, Driouich et al. (2013) published detailed review of RBCs and their involvement with plant responses to stress. Mucilage also protects *P. vulgaris* from Al toxicity because the physical removal of RBCs from the root tips resulted in a higher Al accumulation in the root tips and a more severe inhibition of root elongation (Miyasaka and Hawes 2001). In rice, the physical removal of the RBCs from root tips resulted in a more severe inhibition of root elongation and a higher Al accumulation in the root tips in an Al-sensitive cultivar than in an Al-tolerant cultivar (Cai et al. 2011). Furthermore, the Al content of the root tips was lower in roots surrounded by RBCs than that in roots deprived of RBCs, and cell viability and Al-induced mucilage exudation were always higher in the RBCs from the Al-tolerant cultivar than from the Al-sensitive cultivar (Cai et al. 2011).

The dissociation of root cap cells from root tissue is essential for RBC separation. For this, pectate lyases act to depolymerize pectic polysaccharides by cleaving internal linkages. However, these enzymes have little activity on methylated pectin, which predominates in plant cell walls (O'Neill et al. 1990). Pectin methylesterase de-esterifies pectin by removing methoxyl groups to produce methanol and PGA, a substrate that is susceptible to degradation by pectate lyase. In fact, the release of RBCs is dependent on pectin methylesterase activity (Stephenson and Hawes 1994; Wen et al. 1999).

RBCs attached to roots seem to help rice avoid Al toxicity, and one possibility is that Al-tolerant rice cultivars have more attached RBCs than the sensitive cultivars because the Al-sensitive cultivars have a higher degree of pectin methylesterase activity under Al stress (Yang et al. 2008), which may cause premature RBC release. RBCs are most likely one of the first Al barriers in the roots and should be considered as one component of high Al tolerance in rice.

2.4 The Al-Tolerance Genes in Rice

Searches for rice Al-tolerance genes have been the focus of many studies over the last few years, and many candidate genes have been found using various distinct approaches: differential display reverse transcription-PCR (DDRT-PCR) (Zhang et al. 2007), cDNA amplified fragment length polymorphism (cDNA-AFLP) (Mao et al. 2004), proteomic analysis (Yang et al. 2007, 2013), semi-quantitative and real-time polymerase chain reaction (Zhang et al. 2010), microarrays (Yamaji et al. 2009; Tsutsui et al. 2012), genome-wide associations and QTLs (Famoso et al. 2011), and RNA-Seq (Arenhart et al. 2014). Despite the volume of data that has been generated, the comparison of all these data to identify Al-tolerance genes is not an easy task, because different cultivars, time, and Al concentrations were used. Moreover, many of the Al-responsive genes represent reactions to Al-toxicity and are not actually involved in the Al-tolerance mechanisms. Nevertheless, some important rice Al-tolerance genes have been characterized over the past years. For a review of the genes that are involved in Al-tolerance mechanisms in important crop species, please see Delhaize et al. (2012).

At least two transcription factors seem to play a role in Al tolerance in rice: ART1 (Aluminum Resistance Transcription Factor 1) and ASR5 (Aba, Stress and Ripening). ART1 is constitutively expressed in the roots and is not induced by Al. However, an art1-knockout mutant is highly Al-sensitive (Yamaji et al. 2009), indicating a central role for ART1 in the Al-tolerance mechanisms in rice. In the same way, ASR5 is also expressed in the roots, and transcript levels are increased in response to Al (Arenhart et al. 2013). In addition, ASR5-silenced plants are Al-sensitive. Furthermore, both ART1 and ASR5 regulate genes that are important in the response to Al (Yamaji et al. 2009; Arenhart et al. 2014).

Seven ART1-regulated genes have been characterized so far; Nrat1 (Nramp aluminum transporter 1) is a specific Al transporter that is involved in the uptake of Al to cells for sequestration to vacuoles. The silencing of Nrat1 resulted in decreased Al uptake, increased Al binding to the cell wall, and enhanced Al sensitivity (Xia et al. 2010). ALS1 (Aluminum Sensitive 1), a half-size ABC transporter, is also involved in the sequestration of Al to the vacuoles (Huang et al. 2011). MGT1 (Magnesium Transporter 1), a Mg transporter, confers Al tolerance in rice by increasing the concentration of Mg in the cell and decreasing Al-binding sites (Chen et al. 2012). CDT3 (cadmium tolerance 3) anchors to the plasma membrane and functions as a chelator, binding Al and preventing its entrance into the cells (Xia et al. 2013). Silencing CDT3 results in decreased Al accumulation in the root plasma membrane and cell wall but increased Al concentration in the cell sap. FRDL4 (Ferric Reductase Defective3-like 4), a citrate transporter, secretes citrate from roots, chelating Al in the rhizosphere (Yokosho et al. 2011). Finally, STAR1 and STAR2 (sensitive to aluminum rhizotoxicity 1 and 2), an ATP-binding domain and a transmembrane domain, respectively, transport UDP-glucose, which is implicated in cell wall modifications that mask Al-binding sites in the cell wall (Huang et al. 2009a). ASR5 regulates at least 36 genes in response to Al in rice but only STAR1 is well characterized so far (Arenhart et al. 2014).

3 Concluding Remarks

Under acidic conditions in the soil, Al^{3+} (the rhizotoxic form) is formed and incorporated by plants, subsequently inhibiting root elongation and expansion and resulting in several other injuries to the plants. Due to the reactivity of Al, the nucleus, cell wall, plasma membrane, and cytoskeleton can be targets of Al injury (Kochian et al. 2004). A considerable number of different Al-tolerance mechanisms have been proposed, and it is likely that multiple Al-tolerance mechanisms are employed by different plant species (Kochian et al. 2004).

Al transport systems in the plasma membrane/tonoplast of rice appear to be key contributors to the Al tolerance of rice compared to other crops because even the most rice Al-sensitive aus lines, which possess a functionally deficient NRAT1 transporter (Li et al. 2014), are still more Al-tolerant than other cereal species, including maize, sorghum, and wheat (Famoso et al. 2010). Rice, one of the most Al-tolerant crops, seems to employ several mechanisms in response to Al that, when combined, result in a greater tolerance of Al (Fig. 2). A summary of these mechanisms is listed below; note that these mechanisms do not necessarily occur in this order:

3.1 External Detoxification Mechanisms

- *Mucilage secretion*: Rice root border cells attached to the root apices secrete a thicker mucilage that binds to Al and prevents Al entrance into the root cells (Cai et al. 2011).
- *Regulation of plasma membrane lipid composition*: A lower ratio of phospholipids to major neutral lipid Δ^5-sterol leads to a decreased negative charge and a reduced permeability of the plasma membrane, preventing Al entrance into the symplast (Khan et al. 2009). Furthermore, the stability of the plasma membrane lipid composition (e.g., phosphatidylcholine) may act in plasma membrane-mediated prevention of Al binding (Huynh et al. 2012).
- *Regulation of cell wall composition*: a lower content of cell wall polysaccharides such as pectin and Hemicellulose 1 and 2 as well as a higher degree of methylesterification result in fewer carboxylic groups that serves as Al-binding sites, also preventing Al entrance into the root cells (Yang et al. 2008).
- *Regulation of nitric oxide*: After Al exposure, NO levels increase, and NO acts as an antioxidant molecule, helping in the ROS defense system (Yang et al. 2013) and blocking the increase in pectin content (Zhang et al. 2011).

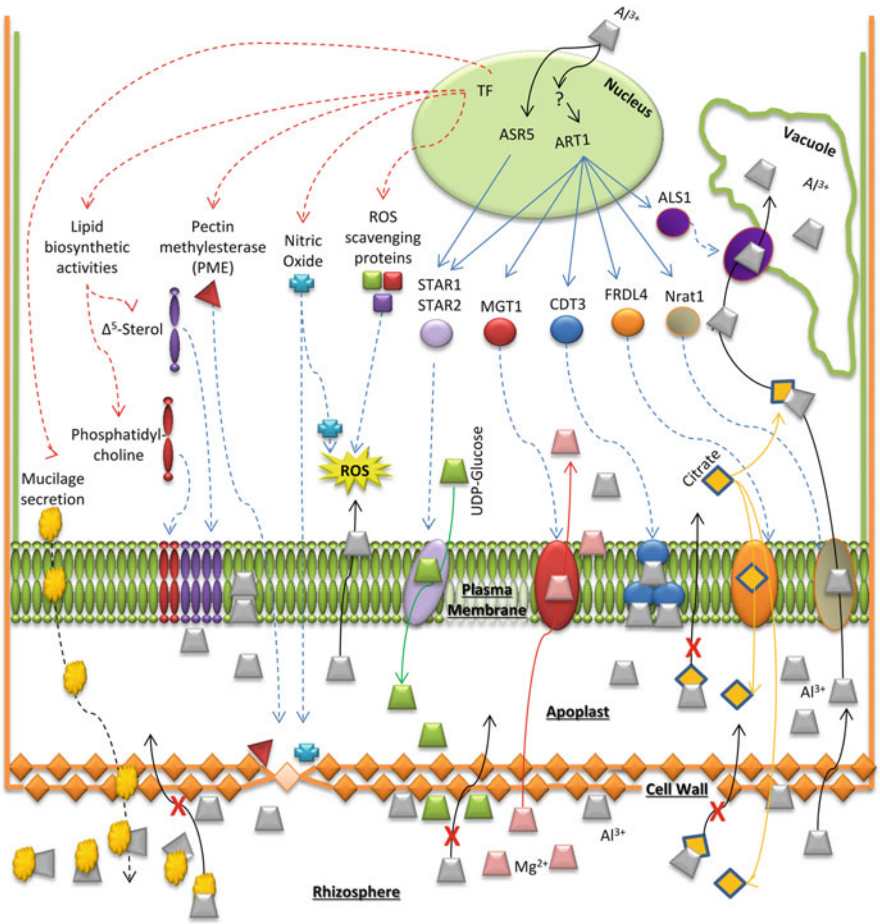

Fig. 2 Summary of rice mechanisms to cope with Al stress. In response to Al, rice utilizes several mechanisms to prevent the entrance of Al to cells. These mechanisms include the production of a mucilage in the attached root border cells to bind Al; the modification of the plasma membrane and cell wall components; increases in the levels of nitric oxide which acts as an antioxidant molecule in ROS defense system and as signaling molecule to block increases in pectin content; the release of citrate into the apoplast and rhizosphere; the release of UDP-Glucose in cell wall to mask Al-binding sites; the regulation of magnesium content in cell to decrease Al-binding sites, and the chelation of Al in plasma membrane by the CDT3 protein. Because some Al may enter the symplast and cause an increase in ROS content, rice can alleviate the toxicity of Al by regulating the expression of ROS scavenging genes. Finally, a portion of the Al in the symplast is transported and accumulated in vacuoles. *Black arrows*: aluminum efflux; *orange arrow*: citrate efflux; *red arrow*: magnesium efflux; *green arrow*: UDP-Glucose efflux; *red dashed arrows*: genes that are possibly regulated by ASR5, ART1, or another transcription factor (TF); *blue arrows*: ART1- and ASR5-regulated genes; *blue dashed arrows*: protein localization in the cell; *black dashed arrow*: mucilage induced by Al in the root border cells

- *Release of citrate:* The release of citrate into the apoplast and rhizosphere leads to Al-citrate complex formation, reducing Al entrance into the root cells (Yokosho et al. 2011).
- *Use of UDP-Glucose in the cell wall:* UDP-Glucose binds to the cell wall, masking potential Al-binding sites (Huang et al. 2009a).
- *Regulation of the magnesium content in the cell sap:* Increasing the Mg content also decreases Al-binding sites on a cell (Chen et al. 2012).
- *Al chelation in the plasma membrane*: A specialized plasma membrane protein is able to chelate Al, preventing its entrance into the cell (Xia et al. 2013).

3.2 Internal Detoxification

- *Induction of ROS scavenging systems*: As consequence of Al toxicity, ROS injury is alleviated by increasing ROS scavenging proteins (Ma et al. 2012).
- *Sequestration of Al into vacuoles*: Specific proteins transport Al to less toxic compartments such as vacuoles (Xia et al. 2010, 2013).

Despite some Al sequestration into vacuoles, rice is essentially an Al-excluder because most of the mechanisms employed by rice attempt to prevent the entrance of Al into the cell. In conclusion, there are many mechanisms that act synergistically to protect rice from Al exposure. Furthermore, with new technologies for broad analysis of data, more Al-regulated genes should be discovered that might help to elucidate this puzzling and complex mechanism.

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