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REVIEW

Signal transduction during aluminum-induced secretion of organic acids in plants

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Abstract

An excess of aluminum (Al) is a major factor limiting crop production in acidic soils. Secretion of organic acids (OAs) from the root apex of diverse plant species or genotypes *via* activation of anion channels has been recognized as the most important mechanism of Al exclusion. Citric, oxalic, and malic acids are the most effective OAs in detoxifying Al. In this review, we summarize biochemical properties of OAs secreted by plants. We also highlight the molecular mechanisms of Al signal perception, Al transport, signal regulators associated with OAs secretion, as well as interactions between Al and hormone signaling pathways. Based on a comprehensive understanding of the relationship between signal modulators and regulation of expression of relevant genes, a signal transduction model for Al-induced OAs secretion is proposed.

Additional key words: abscisic acid, Al detoxification, Al tolerance mechanism, salicylic acid, signal transduction.

Introduction

Aluminum (Al) is one of the most abundant elements in the earth crust. When soil pH is less than 5.0, soil Al is released from the solid phase to the soil solution or adsorbed on the cation exchange sites of soil particles, and the activity of soil Al increases, which limits crop production (Kochian 1995). Active Al^{3+} is harmful to plant root growth and inhibits absorption of water and nutrients (Delhaize *et al.* 2004). The root apex is a major part of Al perception and induction of stress response. The primary and most obvious symptom of Al toxicity is the suppression of root elongation (Horst *et al.* 1999, Kochian *et al.* 2004). As a result, plant roots become stunted and brittle, and the root apices become swollen and damaged (Clarkson 1965).

Plants have evolved different Al tolerance mechanisms. In addition to the internal Al tolerance mechanism based on Al-inducible changes in organic acids (OAs) syntheses and Al compartmentation (Pineros *et al.* 2002), the majority of plants resist Al toxicity through external exclusion mechanisms. As the relationship between OAs secretion and Al tolerance was proved, OAs secretion has been recognized as the most important mechanism of the tolerance so far (Kochian *et al.* 2004). In this review, biochemical properties of OAs are introduced. We focus on the mechanisms of Al signal perception, Al transport, and signal modulators associated with OAs secretion. Interactions between Al and hormone signaling pathways are also discussed. Based on a comprehensive understanding of the relationship between signal modulators and expression regulation of relevant genes, a model for signal transduction pathways is proposed.

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Abbreviations: ABA - abscisic acid; ALS - aluminum sensitive; DAG - diacyl glycerol; DTZ - distal transition zone; EZ - elongation zone; IP3 - inositol-1,4,5-triphosphate; MAPK - mitogen-activated protein kinase; MATE - multi-drug and toxic compound extrusion; miRNAs - microRNAs; Nramp - natural resistance-associated macrophage protein; Nrat - Nramp aluminum transporter; OA - organic acid; PA - polyamine; PI - phenylisothiocyanate; PIP2 - phospholipid phosphatidylinositol-4,5-bisphophate; PLC - phospholipase C; PM - plasma membrane; ROS – reactive oxygen species; SA - salicylic acid; SNP - sodium nitroprusside; STAR - sensitive to Al rhizotoxicity.

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Properties of OAs secreted by plants

Aluminium stress induces the secretion of OAs such as malic, citric, and oxalic acids. According to the chelate ability of OAs on Al^{3+}, OAs are arranged as follows: citric acid > oxalic acid > malic acid (Ma 2000). The patterns of Al-induced OAs secretion differ significantly in different plant species. Citric acid is secreted from maize (Kollmeier *et al.* 2001), soybean (Yang *et al.* 2001), barley (Zhao *et al.* 2003), *etc*., malic acid is secreted from wheat (Delhaize *et al.* 1993) and *Arabidopsis* (Hoekenga *et al.* 2003), and citric acid and malic acid are synchronously secreted from sunflower (Saber *et al.* 1999), rye (Li *et al.* 2000), triticale (Hayes and Ma 2003), *etc*. Oxalic acid is the main OA secreted from buckwheat (Zheng *et al.* 1998), *Cassia tora* (Ma and Miyasaka 1998), and spinach (Yang *et al.* 2005).

According to a response relationship between Al stress and the time of OAs secretion, the secretion is divided into two patterns (Ma 2000). In pattern I, plants respond to Al stress quickly, and OAs are released to the medium in tens of minutes through the activation of anion channels in the plasma membrane (PM). Wheat, buckwheat, tobacco, and spinach belong to pattern I plants. In pattern II, the plant response to Al stress has a significant lag period. Distinct OAs secretion normally takes a few hours after Al treatment. This pattern may relate to induction of Al-tolerant genes. Pattern II plants include maize, *Cassia*, rye, triticale, and soybean.

The Al^{3+} can activate anion channels in the cells of wheat root apices (Ryan *et al.* 1997). Especially in the cells of Al-tolerant wheat cultivars, Al^{3+}-stimulated malate efflux *via* anion channels has been verified by the suppression effect of several antagonists (Ryan *et al.* 1995). The types of OAs secreted by different plants under Al stress are not the same; they depend on characteristics of channel proteins responsible for secretion of OA anions in the PM of plant roots. With a patch-clamp technique, it was found that an Al-activated anion channel may be permeable to OAs mediating Al-induced OAs secretion (Pineros and Kochian 2001). An Al^{3+}-induced malate release is different in Al-tolerant and Al-sensitive genotypes, which depends on the sustainability of Al^{3+}-activated malate permeable cation channels (Zhang *et al.* 2001). An Al-induced citrate accumulation may be the integrated result of an increase in citrate synthase activity and a decrease in aconitase activity in root tips of *Cassia* (Yang *et al*. 2004). Why can an anion channel in an Al-tolerant genotype be activated and secret OAs when in an Al-sensitive genotype OAs secretion is little or none? At present, this problem cannot be answered. Presumably, it may result from the difference of membrane anion channel proteins, permeability of channels to organic anions, or channel protein responses to Al^{3+} stimulation. However, the signal transduction of Al-initiated OAs secretion is little known.

Perception of Al signal

Phospholipids are vital components of cell membranes. The breakdown of phosphatidylinositol-4,5-bisphophate (PIP2) into inositol-1,4,5-triphosphate (IP_3) and diacyl glycerol (DAG) by the action of phospholipase C (PLC) plays an important role in signal transduction pathways. Aluminium may interfere with the phosphoinositide signaling pathway. $AlCl_3$ and Al-citrate specifically inhibit PLC activity in a dose-dependent manner. Aluminium exposure may specifically target PLC (Poot-Poot and Hernandez-Sotomayor 2011). The intracellular target site of Al^{3+} may be integrally involved in root growth (Jones and Kochian 1995). The most abundant protein on the PM, H^+-ATPase, is involved in multiple stress responses, including Al stress, by activating a series of secondary transporters (Sussman 1994). The increase of abscisic acid (ABA) content could enhance the activity of H^+-pumping (Kasai *et al.* 1993) caused by Al. The effects of Al stress on citrate secretion are mediated *via* modulation of the activity of PM H^+-ATPase (Shen *et al.* 2005). Aluminium activates threonine-oriented phosphorylation of PM H^+-ATPase in dose- and time-dependent manners. Under Al treatment, Mg addition helps maintain the activity of PM H^+-ATPase and enhance Al-dependent citrate efflux, which protects plants against Al stress (Yang *et al.* 2007). Aluminium induces the expression of cell wall-associated receptor kinase 1 (WAK1) in *Arabidopsis* roots. Expression of WAK1 is associated with signal transduction between the root and shoot. Overexpression of *WAK1* enhances plant Al tolerance (Sivaguru *et al.* 2003a). There is also evidence that a glutamate-like ligand efflux from an anion channel can bind to a glutamate receptor, thereby initiate signaling in response to Al (Sivaguru *et al.* 2003b). However, the glutamate receptor in plants has not been confirmed.

Gene expression induced by Al^{3+}

Over the past 20 years, some genes contributing to Al-induced OAs efflux have been identified (Table 1). An Al-activated malate transporter (ALMT) and multi-drug and toxic compound extrusion (MATE) proteins facilitate organic anion outflow from roots. The *ALMT* family genes have been characterized in wheat (Sasaki *et al.* 2004), *Arabidopsis* (Hoekenga *et al.* 2006), rye (Collins *et al.* 2008), and *Brassica napus* (Ligaba *et al.* 2006). An *AtALMT1*, a homologous gene of wheat *TaALMT1*, has been cloned in the model plant *Arabidopsis*. The similarity of amino acid sequence between them is only 40 %. The *AtALMT1* is located in an Al-tolerant quantitative trait locus on chromosome 1. Its expression and protein activity are regulated by Al, which is different in expression pattern from *TaALMT1* (Hoekenga *et al.* 2006). As shown in Fig. 1, AtALMT1 possesses five transmembrane domains with the N-terminal end which is relatively conserved containing 19 completely conservative amino acids (DKWTEGNRGFYTRGPW GHP) (Delhaize *et al.* 2007). The hydrophilic C-terminal end is orientated extracellularly. It is predicted that S196 and T308 may be putative phosphorylation sites. Aluminium tolerance in *Arabidopsis* depends on *AtALMT1*-mediated malate anion exudation from roots. In addition, as a vacuole-localized malate channel protein, AtALMT9 may be involved in an internal Al-resistant mechanism (Kovermann *et al.* 2007). The MATE proteins function as transporters using the electrochemical gradient of Na/proton exchange to export a wide variety of substrates including secondary metabolites and xenobiotics. The *MATE* family genes have been implicated in the Al^{3+} tolerance of wheat (Ryan *et al.* 2009), maize (Maron *et al.* 2010), rye (Yokosho *et al.* 2010), *Arabidopsis* (Liu *et al.* 2009), and sorghum (Magalhaes *et al.* 2007).

Aluminumresistance transcription factor 1 (*ALS1*) encodes the membrane-spanning domain of an ABC transporter and is usually located in the tonoplast, but *AtALS1* is constitutively expressed in vascular tissues, hydathodes, and root apices. The OsALS1 is a tonoplast-localized ABC transporter which is required for internal detoxification of Al in rice (Huang *et al*. 2012). The Al^{3+} up-regulates the expression of *OsALS1* (Larsen *et al.* 2007). As a half-type ABC-transporter, ALS3 is involved in the redistribution of Al^{3+} within a plant to be far away from sensitive tissues (Larsen *et al.* 2005). In rice, citrate

Table 1. Genes associated with organic acids secretion and Al-tolerance.

Gene	Plant species	Protein function	Reference
ALMT	*Triticum aestivum, Arabidopsis thaliana, Secale cereale, Brassica napus*	malate transport-efflux	Sasaki *et al*. 2004, Hoekenga *et al*. 2006, Ligaba *et al*. 2006, Collins *et al*. 2008
MATE	*Sorghum bicolor, Zea mays, Arabidopsis thaliana, Secale cereale, Triticum aestivum*	citrate transport-efflux	Magalhaes *et al*. 2007, Liu *et al*. 2009, Ryan *et al*. 2009, Maron *et al*. 2010, Yokosho *et al*. 2010
ALS1	*Arabidopsis thaliana*	partial ABC protein	Larsen *et al*. 2007
ALS3	*Arabidopsis taliana*	partial ABC protein	Larsen *et al*. 2005
FRDL4	*Oryza sativa*		Yokosho *et al*. 2011
Nrat1	*Oryza sativa*		Xia *et al*. 2010
STAR1	*Arabidopsis thaliana, Oryza sativa*	UDP-glucose transport	Huang *et al*. 2009, 2010
STAR2	*Oryza sativa*	UDP-glucose transport	Huang *et al*. 2009

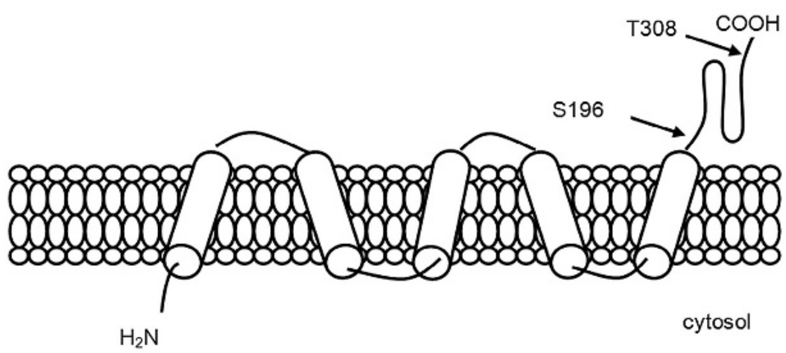

Fig. 1. A diagram depicting the secondary structure of AtALMT1. The AtALMT1 (493 amino acids) is predicted to possess 5 transmembrane regions with the N-terminal ends orientated in the cytosol. The C-terminal ends are orientated extracellularly. The phosphorylation sites of genes were predicted according to http://kinasephos.mbc.nctu.edu.tw/. Modified from Ryan *et al*. (2011).

efflux from an Al^{3+}-activated MATE transporter, OsFRDL4, contributes to Al tolerance (Yokosho *et al.* 2011). Aluminum resistance transcription factor 1 (ART1) regulates *OsFRDL4*, expression of which is greatly enhanced by a short exposure to Al. Among different genotypes of rice, the content of *OsFRDL4* attributes to some of the variation in tolerance. Nramp aluminum transporter 1 (Nrat1) is one member of a natural resistance-associated macrophage protein (Nramp) family (Xia *et al.* 2010). The expression of *Nrat1* is up-regulated by Al^{3+}. Knockout mutations of *Nrat1* decrease Al^{3+} uptake, increase Al^{3+} binding to cell walls, and result in an enhanced sensitivity to Al^{3+}.

As an Al^{3+} tolerance gene in rice, sensitive to Al rhizotoxicity 1 (*OsSTAR1*), encodes a nucleotide-binding domain of a bacterial-type ABC transporter, whereas *OsSTAR2* encodes a transmembrane domain of an ABC transporter. The interaction between STAR1 and STAR2 forms an STAR1-STAR2 complex (Huang *et al.* 2009). The STAR1-STAR2 complex facilitates the export of UDP-glucose, the delivery of which to the apoplast modifies the cell wall and prevents Al^{3+} accumulation and reduces damage. Unlike *OsSTAR1*, *Arabidopsis AtSTAR1* encodes an ATP-binding domain of an ABC transporter. Its loss-of-function mutation increases Al^{3+} sensitivity in *Arabidopsis* (Huang *et al.* 2010). Homologues of *STAR1* and *STAR2* confer Al^{3+} tolerance to plants. The identification of these genes provides an opportunity for enhancing Al^{3+} tolerance of crop species through transgenic methods.

Regulators of OAs secretion

Coupling with intracellular responses, mitogen-activated protein kinase (MAPK) cascades are implicated in a vast array of plant functions. Pretreatment or treatment with K-252a, a protein kinase (PK) inhibitor, severely inhibits Al-induced citrate efflux accompanying by an increased Al accumulation and root growth inhibition. The results show that K-252a-sensitive PKs play a pivotal step in modulating the activity of anion channels (Shen *et al.* 2004). As an essential residue of protein phophorylation, serine 384 **(**S384) regulates *TaALMT1* activity, which precedes the enhancement of Al^{3+} transport activity (Ligaba *et al.* 2009).

Transcription factors that are associated with OAs secretion are Cys_2His_2-type zinc-finger protein families including sensitive to proton rhizotoxicity 1 **(**STOP1) and ART1. The STOP1 regulates *Arabidopsis* responses to Al^{3+} toxicity (Iuchi *et al.* 2007). When exposed to Al, STOP1 is initially attributed to the up-regulation of *AtALMT1* expression. Subsequently, STOP1 also controls *Arabidopsis thaliana* multi-drug and toxic compound extrusion 1 (*AtMATE1*) and aluminum sensitive 3 (*ALS3*) (Liu *et al.* 2009, Sawaki *et al.* 2009). The ART1 is a C_2H_2 zinc-finger transcription factor constitutively expressed in rice roots. By interacting with a *cis*-element - GGN(T/g/a/C)V(C/A/g)S(C/G), ART1 regulates the expression of multiple Al^{3+} tolerance genes (at least 31) in rice (Yamaji *et al.* 2009, Tsutsui *et al.* 2011).

Plant responses to Al require a precise regulation of gene expression at transcriptional and post transcriptional levels. MicroRNAs (miRNAs) are 20 - 23 nucleotides long non-coding RNAs which promote the cleavage of target mRNAs. Some Al-responsive miRNAs identified, such as miR319, miR390, miR393, miR319a.2, and miR398, play important regulatory roles in an Al stress signaling network (He *et al.* 2014). Plant miRNAs may modify the expression of their target gene to regulate defense against oxidative stress and signal transduction of biological responses (Gielen *et al.* 2012). However, whether miRNAs regulate OAs secretion has not been reported.

Interactions between Al and hormonal signaling pathways

In general, there are five major classes of plant hormones: ABA, auxins, cytokinins, ethylene, and gibberellins. Phytohormones play important roles in responses to various biotic and abiotic stresses. Aluminium treatment increases an endogenous ABA content in soybean roots in dose- and time-dependent manners. By increasing the activity of citrate synthase, the application of exogenous ABA decreases the accumulation of Al^{3+}. Therefore, ABA may be involved in the early response to Al (Shen *et al.* 2004). Aluminium pretreatment blocks auxin-induced reorientation of microtubules in the outer cortex indicating that Al toxicity is very closely correlated with reorganization and stabilization of the cytoskeleton in maize roots (Blancaflor *et al.* 1998). There is a signal pathway in the root apex mediating Al signal transmission between the distal transition zone (DTZ) and the elongation zone (EZ) through basipetal auxin transport. The genotypic differences in Al resistance are expressed in the DTZ (Kollmeier *et al.* 2000). Aluminium may inhibit the synthesis and translocation of cytokinins to the meristem region of shoots and subsequently affect shoot growth (Pan *et al.* 1989). Enhanced ethylene formation possibly does not play a role in the Al-induced inhibition of root elongation or in the induction of a resistance mechanism (Gunse *et al.* 2000).

In addition, other plant growth regulators including salicylic acid (SA), polyamines (PAs), nitric oxide (NO), *etc*., have been involved in mediation of various biotic and abiotic stress-induced physiological responses in plants. Aluminium significantly enhances an endogenous SA content in root tips, and an exogenous SA increases citrate efflux in a concentration-dependent manner. The SA promotes Al-induced citrate efflux which is not associated with citrate accumulation (Yang *et al.* 2003). The PAs are essential for plant growth and development, and they affect mitosis and meiosis. They exist in a conjugated or free form, but free PAs have no significant response to an Al signal as has been shown in spruce suspension cells (Minocha *et al.* 2004). Inhibition of nitric oxide synthase activity results in the reduction of an endogenous NO content, which could underline the Al-induced inhibition of root elongation in *Hibiscus moscheutos* (Tian *et al.* 2007). The Al toxicity results from endogenous NO content being lower than required for root elongation in plants, and NO donor, sodium nitroprusside (SNP), alleviates the inhibitory effect of Al on root elongation (He *et al.* 2012). In *Cassia*, NO reduces Al-induced lipid peroxidation, production of reactive oxygen species ROS), and activation of lipoxygenase and antioxidant enzymes (Wang *et al.* 2005). The alleviation of Al-induced inhibition of root elongation is associated with an increased endogenous NO content in root tip cells. Nitric oxide and jasmonate regulate peroxidase activity and lignin synthesis of the cell wall in roots exposed to Al (Xue *et al.* 2008). Nevertheless, whether NO is involved in Al-induced OAs secretion in plants is still unclear.

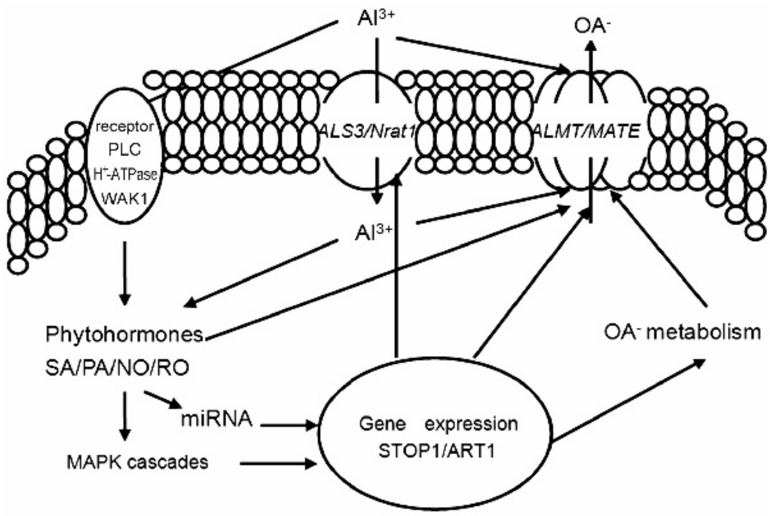

Fig. 2. The signalling network of Al-induced OAs secretion in plants. The modified picture from Liu *et al*. (2014).

Conclusions and perspectives

According to the above-mentioned correlation among different regulators, the signal transduction pathway may be activated during Al-induced secretion of OAs in plants (Fig. 2). On the one hand, an Al stress signal may be perceived by the PLC signal pathway or H^+-ATPase activity in the cell membrane, subsequently disrupt the homeostasis of phytohormones and ROS. The activation of MAPK cascades alters the expression of transcription factors (*STOP1* and *ART1*), which lead to protein phosphorylation of downstream genes such as *ALMT*, *MATE*, *ALS3*, *Nrat1*, *citrate synthase* (*CS)*, *etc*. The expression changes of miRNAs also modify Al-responsive genes at the post-transcriptional level. The expressions of genes *ALMT* and *MATE* are associated with OAs secretion and metabolism. Aluminium may directly activate OAs secretion by means of PM anion channels. On the other hand, Al^{3+} may be transported into the cytosol under the action of *ALS3* or *Nrat1*. Extracellular or cytoplasmic Al^{3+} can also directly regulate the expression of genes associated with OAs secretion. Finally, the integration of two signal pathways activates anion channels in the cell membrane, promotes OA anions exudation from plant roots, and resists Al toxicity.

Although Al-induced OAs secretion from the roots has been widely recognized as an important mechanism for Al tolerance in plants, the processes leading to OAs secretion are still not completely known. For instance, is OAs secretion an active secretion or a passive diffusion? Does active Al directly interact with an anion channel protein or does it indirectly trigger OAs secretion? The crystal structure of the anion channel protein also remains to be resolved. With the application of patch clamp and molecular biology techniques, the study of Al tolerance mechanism will be deepening.

Hereafter, four aspects of research should be carried out. First, as a receptor-like kinase, the function and responding mechanism of WAK1 in Al-induced OAs secretion still need further exploration. To better understand the regulatory process causing OAs secretion, we will have to explore thoroughly signaling molecules involved in Al-induced OAs secretion and their interactions with anion channels. Second, the isolation and identification of genes associated with oxalic acid secretion will help clarify the molecular mechanisms of specific OAs synthesis and release. Third, the relationship between some signal regulators (miRNAs, NO, PA) and OAs secretion remains to be further verified. Fourth, a citrate carrier inhibitor, phenylisothiocyanate, can inhibit Al-stimulated citrate efflux (Yang *et al.* 2006), so the isolation and identification of a mitochondria membrane carrier or transport protein will become a hot spot of research.

On the basis of a clear mechanism, the isolation, identification, transfer, and expression of Al-resistant genes will facilitate the breeding of plant genotypes or cultivars suitable for acidic Al toxic soils through biotechnology methods.

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