REVIEW

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

H. HE¹,²*, L. HE², and M. GU²

Cash Crops Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R. China¹
College of Agronomy, Guangxi University, Nanning 530004, P.R. China²

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 Al³⁺ 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.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (Grant Nos. 30960181 and 31260296) and the 2011 Guangxi Innovation Program for Graduates (GXU11T31076).

* Corresponding author; fax: (+86) 771 3235212 801, e-mail: wingtiger2008@hotmail.com

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-bisphosphate; PLC - phospholipase C; PM - plasma membrane; ROS – reactive oxygen species; SA - salicylic acid; SNP - sodium nitroprusside; STAR - sensitive to Al rhizotoxicity.
Properties of OAs secreted by plants

Aluminium 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.

Perception of Al signal

Phospholipids are vital components of cell membranes. The breakdown of phosphatidylinositol-4,5-bisphosphate (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 (DKWTEGNGFYYTRGPW 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 Al³⁺ 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).

Aluminum resistance 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 Al³⁺ up-regulates the expression of OsALS1 (Larsen et al. 2007). As a half-type ABC-transporter, ALS3 is involved in the redistribution of Al³⁺ 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.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Plant species</th>
<th>Protein function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS1</td>
<td>Arabidopsis thaliana</td>
<td>partial ABC protein</td>
<td>Larsen et al. 2007</td>
</tr>
<tr>
<td>ALS3</td>
<td>Arabidopsis thaliana</td>
<td>partial ABC protein</td>
<td>Larsen et al. 2005</td>
</tr>
<tr>
<td>FRLD4</td>
<td>Oryza sativa</td>
<td>partial ABC protein</td>
<td>Larsen et al. 2007</td>
</tr>
<tr>
<td>Nrat1</td>
<td>Oryza sativa</td>
<td>UDP-glucose transport</td>
<td>Xia et al. 2010</td>
</tr>
<tr>
<td>STAR1</td>
<td>Arabidopsis thaliana, Oryza sativa</td>
<td>UDP-glucose transport</td>
<td>Huang et al. 2009, 2010</td>
</tr>
<tr>
<td>STAR2</td>
<td>Oryza sativa</td>
<td>UDP-glucose transport</td>
<td>Huang et al. 2009</td>
</tr>
</tbody>
</table>

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).
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 phosphorylation, serine 384 (S384) regulates TaALMT1 activity, which precedes the enhancement of Al\(^{3+}\) transport activity (Ligab et al. 2009).

Transcription factors that are associated with OAs secretion are Cys\(_2\)His\(_2\)-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 efflux from an Al\(^{3+}\)-activated MATE transporter, OsFRDL4, contributes to Al tolerance (Yokosh 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.

Interactions between Al and hormonal signaling pathways

In general, there are five major classes of plant hormones: ABA, auxins, cytokinins, ethylene, and gibberellins. Phytoreturns 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, Al 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), putrescine, spermidine and spermine (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 lipoxigenase 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.

![Diagram](image)

**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, Al³⁺ may be transported into the cytosol under the action of ALS3 or Nrat1. Extracellular or cytoplasmic Al³⁺ 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.

References


Sivaguru, M., Pike, S., Gassmann, W., Bashin, T.I.: Aluminum rapidly depolymerizes cortical microtubules and depolarizes the plasma membrane: evidence that these responses are mediated by a glutamate receptor. - Plant Cell Physiol. 44: 667-675, 2003b.