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Review

Organic acid anions: An effective defensive weapon for plants against aluminum toxicity and phosphorus deficiency in acidic soils

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ABSTRACT

Aluminum (Al) toxicity and phosphorous (P) deficiency are two major limiting factors for plant growth on acidic soils. Thus, the physiological mechanisms for Al tolerance and P acquisition have been intensively studied. A commonly observed trait is that plants have developed the ability to utilize organic acid anions (OAs; mainly malate, citrate and oxalate) to combat Al toxicity and P deficiency. OAs secreted by roots into the rhizosphere can externally chelate Al^{3+} and mobilize phosphate (Pi), while OAs synthesized in the cell can internally sequester Al^{3+} into the vacuole and release free Pi for metabolism. Molecular mechanisms involved in OA synthesis and transport have been described in detail. Ensuing genetic improvement for Al tolerance and P efficiency through increased OA exudation and/or synthesis in crops has been achieved by transgenic and marker-assisted breeding. This review mainly elucidates the crucial roles of OAs in plant Al tolerance and P efficiency through summarizing associated physiological mechanisms, molecular traits and genetic manipulation of crops.

1. Introduction

High soil acidity, which is found in about 40% of the world's arable land, is one of the most important factors limiting crop productivity (Ma, 2000; Kochian et al., 2004). In acidic soils, where the soil pH is commonly below 5.0, a large number of soluble ionic aluminum (mainly Al^{3+}) is released, which rapidly inhibits root elongation and subsequently affects the uptake of water and nutrients (Kochian, 1995; Ryan et al., 2001; Ma, 2007). Concurrently, phosphorus (P) fixation with Al and iron (Fe) oxides becomes stronger when soil pH drops (Barber, 1995; Kochian et al., 2004), leading to the severe limitation of available phosphate (Pi) in acidic soils. Consequently, plants surviving on acidic soils are threatened simultaneously with both Al toxicity and P deficiency.

Plants have evolved diverse strategies to cope with Al toxicity and P deficiency (Marschner, 2012; Liang et al., 2013). Multiple mechanisms for Al tolerance in plants have been elucidated, including various forms of external and internal detoxification. Many plant species keep Al^{3+} in the rhizosphere by exuding Al-chelating substances, including organic acid anions (OAs), phenolic compounds and mucilage (Ma et al., 2001; Kochian et al., 2004; Ma, 2007). Within plant tissues, Al^{3+} also can be detoxified by complexation and sequestration upon entry into the cytoplasm (Kochian, 1995). Both forms of Al tolerance are illustrated in buckwheat (*Fagopyrum tataricum*), which is able to secret oxalate from roots to chelate Al^{3+} into non-toxic compounds in the rhizosphere, while also maintaining the ability to accumulate as much as 15 mg g^{-1} (dry weight) of Al in leaves by sequestering a large amount of Al^{3+} into vacuoles (Ma et al., 1997, 2001). On acidic soils, mechanisms for increasing P acquisition efficiency in plants under P deficiency conditions include modification of root morphology and architecture, exudation of P-solubilizing compounds (like OAs and phosphatases), and association with arbuscular mycorrhizal fungi (AMF) (Wang et al., 2009; Tian et al., 2012; Liang et al., 2014).

OAs, such as tricarboxylic acid (TCA) cycle intermediates (e.g., malate and citrate), and carbon metabolism products (e.g., oxalate), have been reported as key components involved in Al detoxification and P acquisition (López-Bucio et al., 2000a,b), due to not only external chelation of Al^{3+} and mobilization of Pi, but also internal detoxification of Al^{3+} and release of free Pi in the cytosol (Ma, 2000; Ryan et al., 2001). In recent decades, considerable numbers of genes involved in OA synthesis and transport have been characterized

Abbreviations: Al, aluminum; ALMT, aluminum-activated malate transporter; MATE, multidrug and toxic compound extrusion; OAs, organic acid anions; P, phosphorus; Pi, phosphate.

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through molecular and genetic approaches, which accelerates breeding and genetic improvement efforts to produce crops adapted to acidic soils (Inostroza-Blancheteau et al., 2010). An important prerequisite to fully realize potential gains from such adaptation is to sufficiently understand the physiological, genetic and molecular mechanisms of Al tolerance and P efficiency in crop species. This review emphasizes the significance of OAs for plant adaptation to acidic soils while approaching the topic from several angles.

2. OAs are optimal options for plants adapted to acidic soils

Root exudation is a general strategy employed by plants to counteract environmental stresses. Within this broad response category, particular root exudates are usually produced in response to specific stress factors. For example, mugineic acid secretion is specifically induced by Fe deficiency in monocot roots (Singh et al., 2000); OA secretion in the form of dicarboxylates and tricarboxylates, is specifically induced by P deficiency and Al toxicity (Delhaize et al., 1993; Ligaba et al., 2004). Although OAs constitute 5%–10% of the total organic carbon in the soil solution, the main form typically found in soils is monocarboxylates (Jones, 1998). The mono-OAs (e.g., acetate^{-}) chelate far fewer cations than tri-OAs (e.g., citrate^{3-}) and di-OAs (e.g., malate^{2-}, and oxalate^{2-}) (Ryan et al., 2001). Therefore, OAs secreted from roots, such as citrate^{3-}, malate^{2-} and oxalate^{2-}, can more effectively mediate plant responses in acidic soil than the mono-OAs typically found in soil. An additional consideration is that OAs in the soil are readily degraded by microorganisms (Lundström et al., 1995), which leads to less available OAs in the rhizosphere. To overcome this degradation, both Al^{3+} toxicity and P deficiency induce considerable OA secretion into the rhizosphere, with high concentrations of OAs densely accumulating for Al^{3+} chelation and P solubilization (Fig. 1).

All plants are capable of synthesizing the malate and citrate involved in the TCA cycle. They exist mainly in the form of citrate^{3-} and malate^{2-} in the cytosol, where the pH is near neutral (Ryan et al., 2001). However, excessive accumulation of citrate and malate could cause cytosol acidification (Neumann et al., 2000). Meanwhile, over-accumulation of oxalate in the cytosol results in physiological Ca and Fe deficiency due to its strong metal chelating ability (Libert and Franceschi, 1987). Thus, using redundant OAs to chelate Al^{3+} in the cytosol and vacuole might be more economical and labor-saving for plants than responding to side effects likely resulting from over-accumulation of one OA. On the other hand, such detrimental side effects may be rare since large differences in the concentrations of OAs (cytosol: millimolar *vs* rhizosphere: micromolar) and electrical potential (cytosol: negative *vs* rhizosphere: positive) between the cytosol and rhizosphere greatly promote the passive movement of excessive OAs out of cells (Ryan et al., 2001; Marschner, 2012), which is often mediated by anion channels, such as the Al^{3+}-activated protein ALMT1 (aluminum-activated malate transporter) in wheat (*Triticum aestivum*) (Sasaki et al., 2004).

OAs also affect the activity of microorganisms in the rhizosphere, which indirectly influences the bioavailability of nutrients in soils (Fig. 2). It has been revealed that plant growth, P uptake and crop yield are remarkably improved when soil is inoculated with beneficial microorganisms (Kucey et al., 1989; Illmer et al., 1995; Whitelaw, 2000). Since microbes can directly use OAs as carbon sources, these positive effects could derive from reciprocal exchanges between host plants supplying carbohydrates and microbes providing mineral nutrients. In the case of AMF, elongation of AMF extraradical hyphae can extend the P depletion zone beyond the rhizosphere, and the absorbed P by fungi is transferred across the mycorrhizal interface to the host, which subsequently improves P efficiency and growth of host plants. In return, host plants offer enough carbohydrates to support fungus growth (Wright et al., 1998; Kiers et al., 2011; Liang et al., 2014). On the other hand, exudates created by microbes themselves such as OAs, proton and acid phosphatases, also can decrease metal phytotoxicity and increase available P in the rhizosphere, which indirectly helps plants adapt to acidic soils (Osorio, 2011; Cabral et al., 2015).

3. External release of OAs to improve Al tolerance and P availability

Al phytotoxicity is caused by the high concentration of monomeric Al^{3+} in soil solution. Plant roots specifically release OAs to form Al-OA complexes in the rhizosphere in response to Al toxicity, thus rendering Al non-toxic and making the roots 5–20 times more resistant to Al stress (Fig. 1; Delhaize et al., 1993). The Al^{3+}-activated OA exudation is a major Al tolerance mechanism in some plant species, such as wheat and barley (*Hordeum vulgare*) (Sasaki et al., 2004; Furukawa et al., 2007). In addition, root OAs can form stronger complexes with Al^{3+}, Fe^{3+} and Ca^{2+} than Pi, rather than competing with Pi for soil binding sites (Neumann and Martinoia, 2002; Shane and Lambers, 2005), which increases Pi availability for plant acquisition by stimulating its release from bound forms in soils (Fig. 1). In some plant species, the more OAs secreted from roots, the higher Al tolerance and P efficiency have been achieved (Delhaize et al., 1993; Jones, 1998; Hinsinger, 2001), suggesting that the release of OAs from roots is an effective strategy employed by plants for adapting to acidic soils.

The exudation of OAs varies widely among both plant species and environmental conditions (Jones and Brassington, 1998; Jones, 1998; Dakora and Phillips, 2002). For instance, oxalate exudation from buckwheat was rapidly induced by Al^{3+} toxicity, but not by 11-day Pi starvation (Ma et al., 1997; Zheng et al., 1998); citrate exudation was significantly induced by Pi starvation in maize (*Zea mays*) (Gaume et al., 2001), and was not apparently affected by toxic concentrations of Al^{3+} in wheat (Ryan et al., 2009); within rice (*Oryza sativa*) and stylo (*Stylosanthes guianensis*), both Al toxicity and P deficiency can independently induce citrate secretion (Kirk et al., 1999; Li et al., 2009; Yokosho et al., 2011). Close inspection also reveals variations among plant species in the particular OAs involved in Al detoxification and P solubilization. Toxic Al^{3+} can induce secretion of both malate and citrate from wheat and *Arabidopsis* roots (Hoekenga et al., 2006; Ryan et al., 2009; Liu et al., 2012), but only citrate from rice and sorghum (*Sorghum bicolor*) roots (Magalhaes et al., 2007; Yokosho et al., 2011). P deficiency triggers the exudation of malate and citrate from soybean (*Glycine max*), but only citrate from rice (Kirk et al., 1999; Liao et al., 2006). Generally, these variations can be explained by the presence of specific types of membrane-localized transporters that are responsible for OA secretion as described below.

Genes responsible for OA secretion have been identified in many plant species, with two gene families predominating, namely ALMT and MATE/AACT (multidrug and toxic compound extrusion/aluminum-activated citrate transporter) (Ryan et al., 2011; Delhaize et al., 2012). Proteins encoded by ALMT and MATE are all localized to the plasma membranes of root cells, and transport malate and citrate, respectively (Fig. 2). Phytotoxic concentrations of Al^{3+} (1–100 μmol/L) can either rapidly activate these transporters for facilitating OA release (Sasaki et al., 2004; Furukawa et al., 2007; Yang et al., 2011a), or first activate the expression of these genes in roots within hours, leading to time-lag release of OAs for Al detoxification (Magalhaes et al., 2007; Yang et al., 2011b; Chen et al., 2013). For low-P stress in acidic soils, whether plant P efficiency is associated with the expression of *ALMT* and *MATE* has not been clearly evaluated. To date, only two genes, *GmALMT1* and

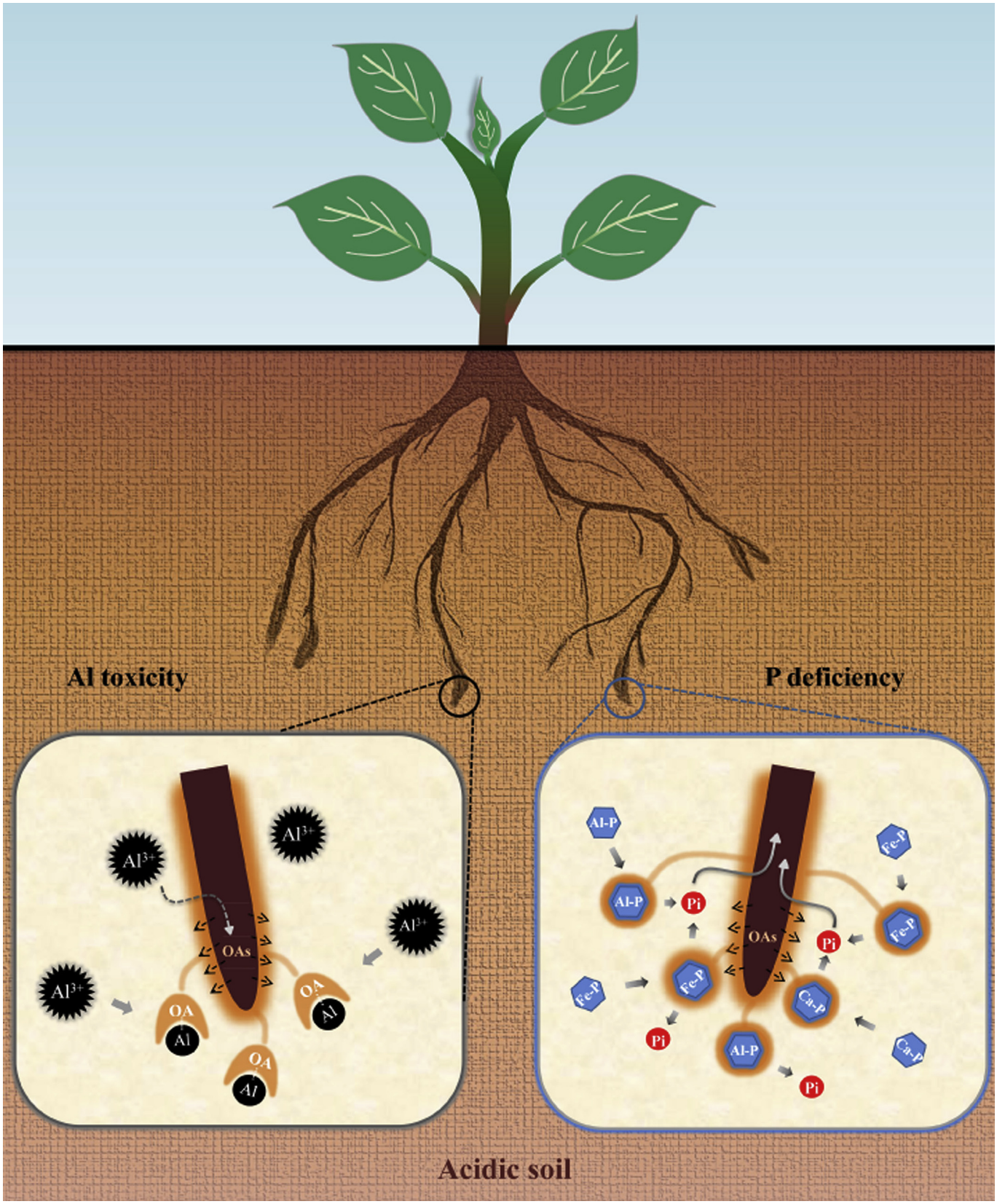

Fig. 1. The roles of organic acid anions (OAs) in Al detoxification and P solubilization on acidic soils. The OAs secreted from roots function as a defensive weapon for plants against Al toxicity and P deficiency symptoms when grown on acidic soils. OAs form sufficiently strong complexes with Al^{3+} to protect roots from Al toxicity in the rhizosphere (left), and solubilize rhizosphere Pi *via* complexation with Al, Fe and Ca oxides and hydroxides on mineral surfaces (right).

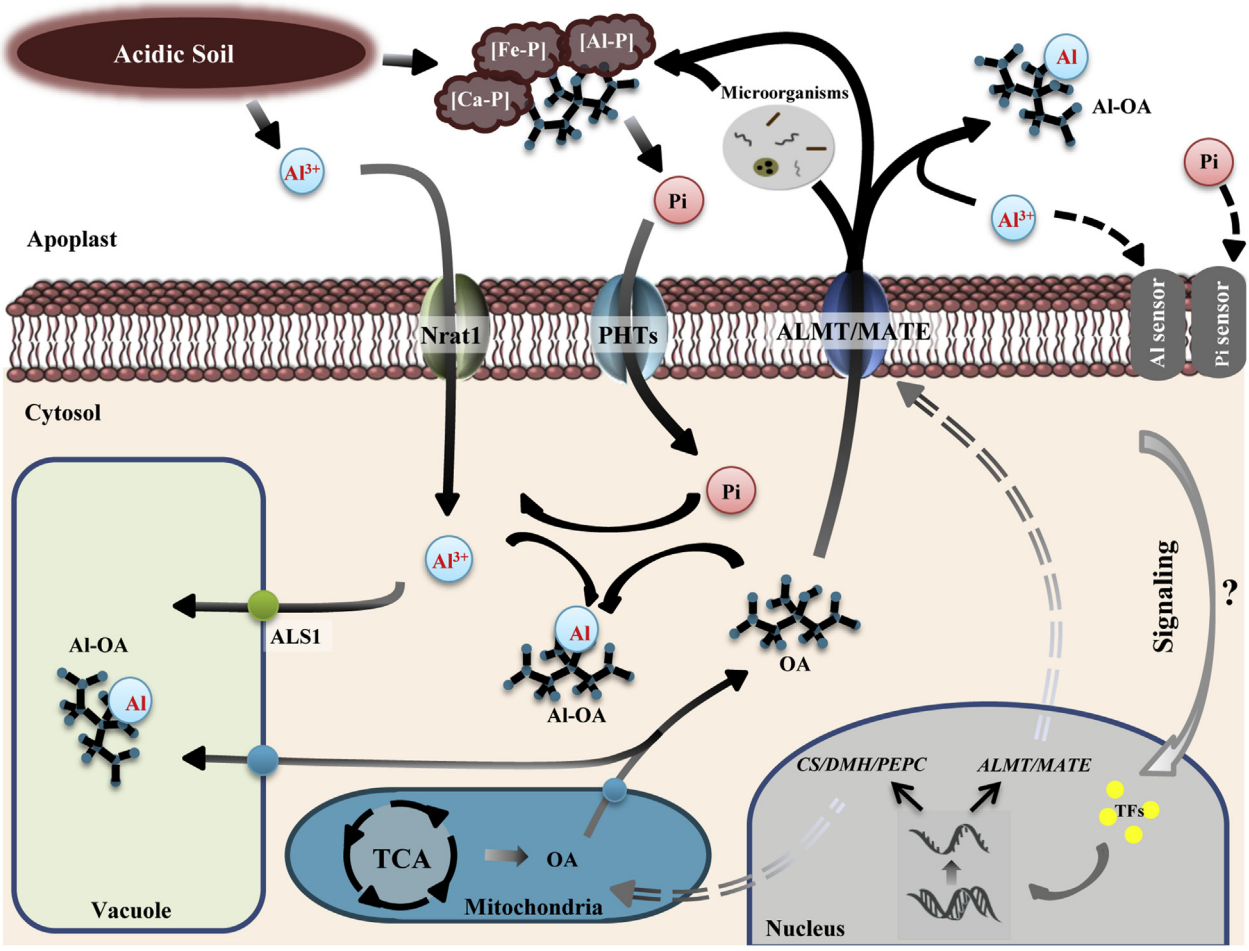

Fig. 2. Schematic model for high Al^{3+}/low P-activated synthesis and transport of organic acid anions (OAs) in plant root cells. Phytotoxic Al^{3+} is abundant and Pi is extremely limited in acidic soils. High Al^{3+} and low P signaling activate the expression of genes involved in OA synthesis (e.g., *CS*, *DMH* and *PEPC*) and transport (e.g., *ALMT* and *MATE*). External Al first enters into cytosol through Nrat1 and then is sequestrated into vacuoles through ALS1 to form Al-OA. The remaining Al^{3+} in cytosol could be chelated by OAs into non-toxic compounds, which protects roots from Al^{3+} injury and releases more free Pi for metabolism. On the other hand, OA transporters, ALMT and MATE, facilitate the exudation of OAs into rhizosphere, thus enhancing external Al detoxification and P acquisition. Nrat1, plasma membrane-localized Al transporter; ALS1, tonoplast-localized Al transporter; ALMT, aluminum-activated malate transporter; MATE, multidrug and toxic compound extrusion; CS, citrate synthase; MDH, malate dehydrogenase; PEPC, phosphoenol pyruvate carboxylase; PHT, phosphate transporter; TCA, tricarboxylic acid; TF, transcription factor.

TaALMT1, were speculated to be involved in plant P efficiency. In the soybean P-efficient genotype HN89, *GmALMT1* was shown to be upregulated along with the increased release of malate from roots in response to P deficiency (Liang et al., 2013). Over-expression of *TaALMT1* from wheat in barley promoted P acquisition of transgenic plants grown on acidic soils (Delhaize et al., 2009). Notably, both GmALMT1 and TaALMT1 have been identified as malate transporters involved in Al tolerance (Liang et al., 2013; Sasaki et al., 2004). The logical implication of the preceding observations is that P efficiency and Al tolerance might have co-evolved in plants through environmental selection of *ALMT* variants. Indeed, P-efficient genotypes (e.g., HN89 and BX10) in soybean are commonly more resistant to Al than the P-inefficient genotypes (e.g., BD2 and HN112) (Dong et al., 2004; Liao et al., 2006). Similarly, an Al-resistant cowpea (*Vigna unguiculata*) genotype was observed to be better adapted to P-deficient soils than an Al-sensitive one due to enhanced OA exudation stimulated by combined high Al^{3+} and low P stresses (Jemo et al., 2007).

The root region responsible for OA exudation differs between high Al^{3+} and low P conditions. Since the main target of Al toxicity in plants is the root apex, Al^{3+}-induced OA secretion occurs primarily in root apices (Ryan et al., 1993; Liao et al., 2006; Wang et al., 2007). This is consistent with the tissue-specific expression of Al-responsive *ALMT* and *MATE* genes in the outer layer of root apices (Furukawa et al., 2007; Chen et al., 2013). A sheath of OAs is formed to protect each root apex from toxic Al^{3+}, thus largely alleviating Al^{3+}-inhibition of root growth. Limiting the release of OAs to the apical zone might reduce the metabolic cost for Al detoxification (Ma et al., 2001). Contrasting with Al^{3+}-stress responses, low P-induced OA secretion occurs mainly in lateral roots. For example, white lupin (*Lupinus albus*) develops specialized bottle brush-like lateral roots known as cluster roots, which release large amounts of OAs under P-deficient conditions (Gardner et al., 1982; Shen et al., 2003; Wang et al., 2007). Since P is rarely mobile in soils (Lynch, 2011), plant P acquisition efficiency is mainly determined by the root exploration of soils. In order to acquire more P from soils, plants need to increase the amount of OAs secreted from roots, as well as the volume of soil influenced by these OAs. Collectively, it is reasonable to speculate that plants grown on acidic soils might have evolved to share the same OA transport systems for responses to Al^{3+} and low P stresses. It is thought that the expression patterns of OA transporter genes can distinguish these responses; however, the tissue specificity of gene expression in response to P deficiency has not yet been clearly

characterized.

4. OAs detoxify Al internally and enhance Pi release in the cytosol

The roles for OAs are not only limited to external detoxification of Al and improvement of P availability in the rhizosphere. These compounds are also involved in internal sequestration of Al^{3+} and release of free Pi within cells. Al^{3+} can be rapidly taken up into root cells (Lazof et al., 1994). In rice, this process is mediated by a member of the Nramp transporter family, Nrat1 (Fig. 2; Xia et al., 2010; Famoso et al., 2011). Most of the cytosolic Al^{3+} is rapidly sequestrated into vacuoles through a tonoplast-localized half-size ABC transporter, ALS1, to form Al-OA (Fig. 2; Huang et al., 2012). For instance, Al^{3+} is sequestered in vacuoles as both Al-oxalate and Al-citrate for internal detoxification in buckwheat, an Al-accumulating plant species with high Al tolerance (Shen et al., 2002, 2004). However, the remaining Al^{3+} in cytosols might be still phototoxic due to its strong affinity for oxygen donor compounds, such as inorganic Pi, ATP, RNA, DNA, proteins, and phospholipids (Martin, 1988; Chen et al., 2012). Cytosolic OAs can chelate Al^{3+} into non-toxic compounds, and therefore protect roots from Al^{3+} injury and in turn release more free Pi from Al-P precipitation for metabolic use (Fig. 2).

Additionally, since a large amount of Pi is stored in vacuoles, P deficiency drives vacuolar Pi transport into cytosols to meet metabolic demands (Lee et al., 1990; Schachtman et al., 1998). Since vacuolar OAs act as ligands to replace Pi in bonds with excessive metal ions such as Ca^{2+} and Fe^{3+} (Macklon et al., 1996), thereby facilitating free Pi release in vacuole and subsequent transport into cytosols. This might be one reason why Al toxicity and P deficiency can independently stimulate the increase of OA concentrations in plant species well adapted to acidic soils (Silva et al., 2001; Kihara et al., 2003; Rangel et al., 2010).

OA synthesis induced by excessive Al^{3+} or low P can be achieved by increasing the activities of enzymes involved in OA metabolism, such as citrate synthase (CS), malate dehydrogenase (MDH), and phosphoenol pyruvate carboxylase (PEPC) (Tesfaye et al., 2001; Ryan et al., 2011; Lü et al., 2012). Alterations in the expression of corresponding genes can affect OA synthesis and exudation, with effects observable in Al tolerance and P efficiency. For instance, over-expression of *MDH* in alfalfa (*Medicago sativa*) increases the activity of MDH and the synthesis of OAs, leading to enhanced P uptake and resistance to Al toxicity as indicated by increases in root elongation and biomass (Tesfaye et al., 2001). Moreover, toxic levels of Al^{3+} and low P availability not only affect associated genes at the transcriptional level, but also at the translational level. In stylo under Al stress, the gene expression and protein accumulation of *SgME1* (*malic enzyme*) were both increased in an Al-tolerant and P-efficient genotype, which was promptly accompanied by elevated malate synthesis and exudation, and thereby conferred adaptation of stylo to acidic soils (Sun et al., 2014). Overall, it seems that both the synthesis and transport of OAs are key regulatory steps for Al tolerance and P efficiency. Nevertheless, activities of related enzymes do not always correlate with the OA exudation under toxic Al^{3+} or low P stress (Keerthisinghe et al., 1998; Watt and Evans, 1999), suggesting that Al and P responsive regulation networks vary among plant species and/or genotypes.

5. Genetic manipulation of OA synthesis and transport for more sustainable agriculture

Liming and P fertilization are conventional strategies for farmers to ameliorate Al toxicity and P deficiency symptoms in plants grown on acidic soils. However, continuous input of lime and P fertilizer are not only costly, but also environmentally risky (Vance et al., 2003; Kochian et al., 2004). Therefore, improving Al tolerance and P efficiency in crops is necessary for sustainable agriculture. The existence of genetic variation among plant species and genotypes suggests that manipulation of plant tolerance to Al toxicity and P deficiency is attainable. The traditional selection of superior genotypes under field conditions has some disadvantages, such as unreliable phenotypic selection caused by genotype-environment interactions, especially when evaluating abiotic stresses like Al toxicity and P deficiency (Inostroza-Blancheteau et al., 2010). The initial identification of genes and loci related to Al detoxification and P efficiency as described above makes genetic manipulation more realistic for Al tolerance and P efficiency in crops.

Since genes encoding OA transporters (ALMT and MATE) have the common characteristic of monogenic inheritance, many attempts have been made to over-express these genes in order to enhance OA secretion in crops. A great potential to improve Al tolerance exists in barley, because of its relatively high sensitivity to Al. Over the last decade, *TaALMT1* from wheat, and *HvALMT1* and *HvAACT1* from barley have been successfully over-expressed in barley, respectively (Delhaize et al., 2009; Gruber et al., 2010; Zhou et al., 2013). Increased malate/citrate exudation and higher Al tolerance were noted for each of these transgenic barley lines. Among them, transgenic plants carrying *TaALMT1* also exhibited an increased ability to acquire P from acidic soils (Delhaize et al., 2009). Nevertheless, increasing OA exudation alone is not the only effective way to enhance tolerance to high Al^{3+} and low P stresses. Many efforts have also been made to increase OA synthesis in plants. For example, Al tolerance was significantly improved by over-expressing *CS* from *Pseudomonas aeruginosa* in tobacco (*Nicotiana tabacum*) and nodule-enhanced forms of *MDH* in alfalfa, which increased both the OA contents in the cytosol and OA exudation from the roots (de la Fuente et al., 1997; Tesfaye et al., 2001). More recently, over-expression of *VuFDH* (*formate dehydrogenase*) from rice bean (*Vigna umbellata*) in tobacco results in increased tolerance to Al^{3+} and H^+ stresses, possibly attributed to reduced accumulation of formate (Lou et al., 2016). Over-expression of *DcCS* from carrot (*Daucus carota*) in *Arabidopsis* resulted in increased P efficiency and better growth under low P conditions, accompanied with enhanced P contents *in vivo* and citrate exudation *in vitro* (Koyama et al., 2000). Notably, over-expressing *CS* from *P. aeruginosa* in canola (*Brassica napus*) not only leads to increased citrate synthesis and exudation but also changes malate metabolism, which confers improved tolerances to Al toxicity and P deficiency (Wang et al., 2013). On the other hand, many failures in related attempts to enhance plant productivity in acidic soils have been observed (Delhaize et al., 2001; Ryan et al., 2011), possibly due to disruptions of carbon metabolism. It is, therefore, reasonable to conclude that regulatory networks involved in plant responses to high Al^{3+} and low P have yet to be fully elucidated, and that these networks can only be thoroughly understood and manipulated in the context of the entire plant metabolic and regulatory system.

Although transgenic approaches are gaining in popularity among researchers, marker-assisted breeding is still a more common strategy in the agronomic practice. Introduction of identified Al-tolerant or P-efficient loci (single or multiple) into sensitive cultivars through marker-assisted selection remains promising. In sorghum, a series of Al-tolerant NILs (near isogenic lines) of BR012 (an Al-sensitive line) have been successfully obtained by introgression of the Alt_{SB} locus (regulating *SbMATE* expression) from Al-tolerant lines (Melo et al., 2013). However, there is still no progress in the improvement of P efficiency by manipulation of genetic loci participating in OA synthesis and transport (Zhang et al., 2014).

6. Conclusions and prospects

A well-developed root system that can adapt to acidic soils should be able to both tolerate high Al^{3+} and efficiently acquire low-available Pi. OAs potentially perform a diverse range of functions in the rhizosphere, especially serving as a defensive weapon for plants against both Al toxicity and P deficiency on acidic soils. These compounds can chelate Al^{3+} into non-toxic complexes for both intracellular and extracellular detoxification. They are also able to increase P acquisition efficiency by mobilizing P in the rhizosphere and increasing P utilization efficiency by releasing free Pi in the cytosol. Consequently, external and internal mechanisms have evolved in plants to coordinately accelerate the synthesis and exudation of OAs (Fig. 2).

There are several common characteristics on root growth altered by P deficiency and Al toxicity, including inhibition of primary roots and promotion of lateral roots (Sun et al., 2016). It has been revealed that phytohormones, especially auxin and ethylene, play important roles in controlling root responses to both P deficiency and Al toxicity, suggesting a co-evolving signaling pathway regulating both stresses for plants on acidic soils (Sun et al., 2016). However, little information is available about the interactions between P deficiency and Al toxicity in terms of exudation and metabolism of OAs. Pi limitation remarkably reduces Al^{3+}-induced citrate exudation in white lupin at the seedling stage, but enhances malate exudation in the presence of Al^{3+} in an Al-resistant genotype of cowpea (Wang et al., 2007; Jemo et al., 2007). Moreover, OA concentrations in roots responding to P and Al interactions also varies among plant species (Chen et al., 2009; Dong et al., 2004). These observations suggest that the Al-P interactions in plants might be regulated by sophisticated mechanisms that cannot be simply explained by Al-P chemical precipitation. Notably, while the same OAs are involved in both responses, different patterns of OA synthesis and exudation in response to Al toxicity and P deficiency suggest that these responses might be regulated by different signaling pathways (Fig. 2). It is known that Al^{3+}-responsive *ALMT* and *MATE* genes are regulated by the C_2H_2-type zinc finger transcription factor ART1/STOP1 (Al Resistance Transcription Factor 1/Sensitive to Proton Rhizotoxicity 1) in plants (Delhaize et al., 2012). However, it is still unknown how Al^{3+} signaling regulates ART1/STOP1 and in turn OA exudation. P deficiency also triggers a number of transcription factors, such as WRKY and MYB family members, to control root development and Pi uptake (Rouached et al., 2010; Liang et al., 2014), but whether low-P induced OA exudation is regulated by these factors still requires further investigation.

Exploitation of new technologies and methods can contribute to understanding and manipulation of high Al^{3+} and low P stress-related pathways in molecular engineering and breeding efforts. DNA replacement by the CRISPR/Cas9 system is a promising genome-editing tool that can introduce Al-tolerant and P-efficient genes as crop genetic improvements (Zhao et al., 2016). Besides OA-related genes, many genes involved in Al tolerance and P efficiency have not yet been identified. Developing new methods to facilitate the discovery and identification of candidate genes for better cropping is still expected.

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