Review

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The complex role of mitochondrial metabolism in plant aluminum resistance

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The majority of soils in tropical and subtropical regions are acidic, rendering the soil a major limitation to plant growth and food production in many developing countries. High concentrations of soluble aluminum cations, particularly Al^{3+}, are largely responsible for reducing root elongation and disrupting nutrient and water uptake. Two mechanisms, namely, the exclusion mechanism and tolerance mechanism, have been proposed to govern Al^{3+} resistance in plants. Both mechanisms are related to mitochondrial activity as well as to mitochondrial metabolism and organic acid transport. Here, we review the considerable progress that has been made towards developing an understanding of the physiological role of mitochondria in the aluminum response and discuss the potential for using this knowledge in next-generation engineering.

Aluminum tolerance in land plants

Acid soils impose multiple stresses on plants, including proton toxicity, nutrient deficiencies (especially calcium, magnesium, and phosphorus) and metal ion toxicities, especially aluminum (Al) and manganese. Together with water limitation, phytotoxicity caused by the Al trivalent cation (Al^{3+}) represents a serious constraint in crop production, particularly on acid soils [1–4], which comprise a large proportion of arable land worldwide [5,6].

In acid soils, the harmless mineral forms of Al dissolve, readily releasing Al into the soil solution. When the pH is 4.5 or below, the Al^{3+} species predominates, but as the pH increases, other mononuclear Al species are formed, such as $Al(OH)^{2+}$ and $Al(OH)^{3+}$. Al^{3+} is highly toxic to many plants, but considerable uncertainty remains regarding the relative toxicity of the hydroxyaluminum species [7–9]. Although crop production on acid soils can be sustained by application of lime for the amelioration of Al toxicity, the associated run-off pollution is undesirable [10]. Thus, developing cultivars with improved tolerance to acid soil stress is an appealing approach to addressing this problem, with recent years witnessing extensive research in this area with regard to (i) the evaluation of germplasm bank for Al tolerance; (ii) the biochemistry and physiology of Al toxicity and tolerance; and (iii) the genetic basis of Al tolerance.

Currently, there are several existing hypotheses to explain the intraspecific and interspecific differences in resistance to Al stress [4–6]. Generally, resistance mechanisms are classified into one of two categories, both related to mitochondrial metabolism (Figure 1). By integrating molecular and metabolic changes that occur under Al stress with physiological studies, we aim to show that respiratory metabolism is strongly linked to Al tolerance and resistance in a variety of contexts and in a range of plant tissues. For this purpose, we will discuss the contribution of mitochondrial metabolism with particular emphasis on the tricarboxylic acid (TCA) cycle and its role in Al resistance and tolerance in plants as well as the molecular hierarchy governing the Al^{3+} response in higher plants. Furthermore, we will briefly discuss the currently accepted mechanisms of Al^{3+} tolerance, describing the genes characterized as being associated with these mechanisms. Additionally, we will provide circumstantial evidence suggesting that targeted genetic manipulation of key enzymes of the TCA cycle in higher plants and related organic acid transporter proteins may constitute a suitable approach to improving Al^{3+} tolerance.

How is mitochondrial metabolism involved in Al^{3+} resistance?

Plant mitochondria play an essential role in the biosynthesis of cellular ATP through oxidative phosphorylation in heterotrophic tissues. Additionally, the TCA cycle in the mitochondria is also extremely important in oxidizing acetyl-CoA into CO_2 to produce NADH, $FADH_2$, and

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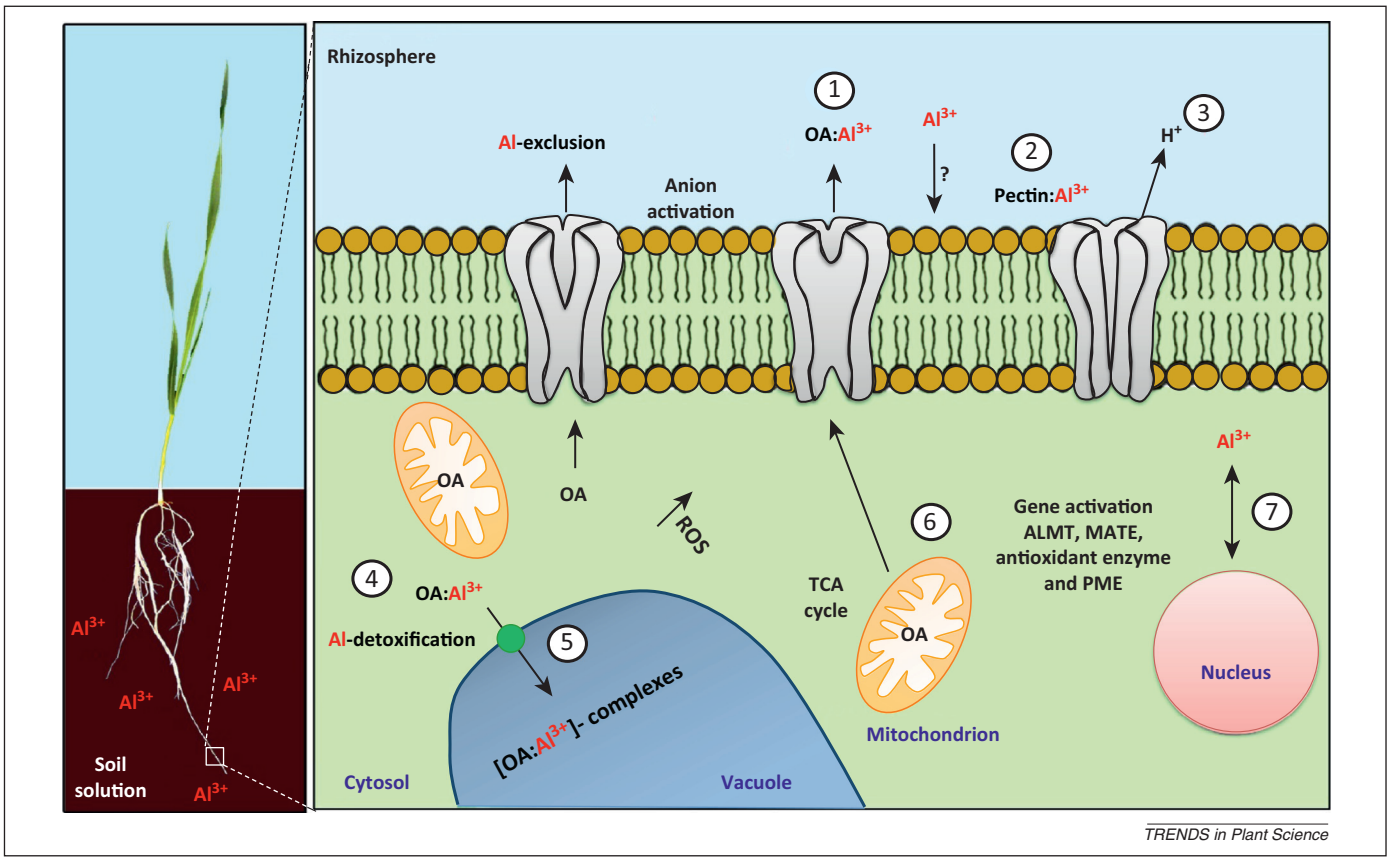

Figure 1. Models of aluminum resistance in plant cells. Aluminum (Al) activates an anion channel on the plasma membrane and stimulates the secretion of organic acid (OA) anions from root cells. The presence of OA in the rhizosphere possibly prevents Al from entering the cell (exclusion mechanism): (1) release of OA anion and chelation of Al^{3+} [OA:Al^{3+}]; (2) binding of Al^{3+} to pectin in the cell wall; (3) decreasing pH of the rhizosphere. By contrast, if Al has entered the cell, an internal tolerance mechanism is generated in the cytoplasm (inclusion mechanism): (4) Al^{3+} is chelated in the cytosol by the OA anion [OA:Al^{3+}]; (5) Al is sequestrated and compartmentalized in organelles such as the vacuole; (6) changes in organic acid metabolism in the tricarboxylic acid (TCA) cycle caused by excess Al; (7) activation of metabolic pathways involved in Al resistance, including the enhanced expression of genes encoding transporter membrane proteins, the biosynthesis of antioxidant enzymes, and the regulation of pectin content in the cell wall, as well as the expression of pectin methylesterase (PME), which is the enzyme responsible for the demethylation of pectin. Abbreviations: Al, aluminum; ALMT, aluminum-activated malate transporter; MATE, multidrug and toxic compound extrusion family; OA, organic acid; PME, pectin methylesterase; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

ATP and carbon skeletons to be used in several other metabolic processes [11,12]. Not surprisingly, plant mitochondria appear to be involved in many other aspects of plant growth and performance, such as signaling, cellular differentiation, and cell death, as well as the control of the cell cycle, cell growth, circadian rhythms, and in response to environmental stresses [13,14].

Al causes damage not only to the roots, which are permanently exposed to Al, but also to the aerial parts of plants [15–17]. Two major physiological mechanisms of Al tolerance are currently known: those which operate to exclude Al from the root apex and those which allow the plant to tolerate Al accumulation in the root or shoot symplasm (see detailed discussion below in the section on Mechanisms of Al tolerance and resistance; Figure 1). The tolerance mechanism associated with Al exclusion includes the exudation of organic acid (i.e., citrate, malate, or oxalate) activated by the presence of Al in the root apex, and it is postulated that this exudate chelates Al [10], thus preventing its uptake across the plasma membrane. The organic acids citrate, malate, and oxalate were previously identified as the main metabolites released from the root apex in response to Al^{3+} [3,18]. Increments in organic acid exudation following genetic manipulation of organic acid metabolism have been extensively tested as possible means to enhance Al tolerance. For example, enhanced citrate synthesis could be achieved by increasing the activities of enzymes involved in citrate and malate synthesis, such as citrate synthase [19–21], malate dehydrogenase [22,23], malic enzyme [24], and pyruvate phosphate dikinase [25]. It should be mentioned that although malate and citrate are the most commonly exuded acids, oxalate efflux has also been detected in several species [2–4]. Importantly, in most cases, the organic anions are not released continuously from the roots but require Al^{3+} to trigger the response [2–4]. Additionally, internal Al^{3+} might also be chelated by these organic acids intracellularly [24,26,27].

Interestingly, noncyclic modes of the TCA cycle have been observed in most organisms, thus leading to the accumulation of specific metabolites, which appears to be key in adapting to hostile environments [28–31]. Notably, the operation of an incomplete TCA cycle has been observed in microbial systems in response to Al toxicity [30,31]. This modular structure of the TCA cycle in *Pseudomonas fluorescens* facilitates the production of ATP, NADPH, and oxalate, thus allowing for the survival of *P. fluorescens* challenged by Al toxicity. Additionally, it