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Root proteome of rice studied by iTRAQ provides integrated insight into aluminum stress tolerance mechanisms in plants

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ABSTRACT

One of the major limitations to crop growth on acid soils is the prevalence of soluble aluminum ions (Al^{3+}). Rice (*Oryza sativa* L.) has been reported to be highly Al tolerant; however, large-scale proteomic data of rice in response to Al^{3+} are still very scanty. Here, we used an iTRAQ-based quantitative proteomics approach for comparative analysis of the expression profiles of proteins in rice roots in response to Al^{3+} at an early phase. A total of 700 distinct proteins (homologous proteins grouped together) with >95% confidence were identified. Among them, 106 proteins were differentially expressed upon Al^{3+} toxicity in sensitive and tolerant cultivars. Bioinformatics analysis indicated that glycolysis/gluconeogenesis was the most significantly up-regulated biochemical process in response to excess Al^{3+}. The mRNA levels of eight proteins mapped in the glycolysis/gluconeogenesis were further analyzed by qPCR and the expression levels of all the eight genes were higher in tolerant cultivar than in sensitive cultivar, suggesting that these compounds may promote Al tolerance by modulating the production of available energy. Although the exact roles of these putative tolerance proteins remain to be examined, our data lead to a better understanding of the Al tolerance mechanisms in rice plants through the proteomics approach.

Biological significance
Aluminum (mainly Al^{3+}) is one of the major limitations to the agricultural productivity on acid soils and causes heavy yield loss every year. Rice has been reported to be highly Al tolerant; however, the mechanisms of rice Al tolerance are still not fully understood. Here, a combined proteomics, bioinformatics and qPCR analysis revealed that Al^{3+} invasion caused complex proteomic changes in rice roots involving energy, stress and defense, protein turnover, metabolism, signal transduction, transport and intracellular traffic, cell structure, cell growth/division, and transcription. Promotion of the glycolytic/gluconeogenetic pathway in roots appeared crucially important for Al tolerance. These results lead to a

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better understanding of the Al tolerance mechanisms in rice and help to improve plant performance on acid soils, eventually to increase the crop production.

1. Introduction

Aluminum (mainly Al^{3+}) limits the agricultural productivity on more than one third of the world's total arable and potential arable lands, as the rhizotoxic species Al^{3+} is released into soil solutions when the soil pH is below 5.0, accumulating to levels that inhibit root elongation and growth by damaging the root cells structurally and functionally [1–4]. It has been well demonstrated that the root apex is the most sensitive part of the root to Al^{3+} because it is the site of cell division and cell elongation [5,6]. Since Al^{3+} is so reactive, it can interact with multiple structures in the apoplast and symplasm of root cells [3,7]. For instance, in the cell wall, Al^{3+} primarily binds to the pectin matrix and thereby alters the physical properties of the cell wall [7–9]. In the symplasm, sites of Al^{3+} interaction include membrane constituents, ion channels, metabolic enzymes, components of signaling pathways, members of the cytoskeleton, and even the DNA [3,7,10]. Furthermore, Al^{3+} usually results in an "acid soil syndrome" that includes poor plant water and nutrient uptake, which makes plants sensitive to various stresses, especially drought stress [11]. Therefore, Al toxicity has been recognized as a major factor limiting crop production worldwide on acid soils, particularly in the developing countries.

In the long-term coevolution, some plant species or cultivars have developed two primary strategies to cope with Al toxicity, which are so-called "internal detoxification mechanisms" and "external detoxification mechanisms" [12,13]. Internal detoxification is mainly achieved by sequestration of Al^{3+} into the vacuoles in the form of Al–organic acid complexes or redistribution to the shoots through the symplastic pathway [3], which is seen in some Al-accumulating plants such as hydrangea (*Hydrangea macrophylla*) and buckwheat (*Fagopyrum esculentum*). Over the past years, several mechanisms for the external detoxification have been proposed, but the most-studied one is the secretion of organic acid anions from the roots in response to Al stress in both monocots and dicots [3,4,14]. The organic acid anions secreted from roots include citrate, oxalate, and malate, depending on plant species. All of them are able to chelate toxic Al^{3+}, thereby detoxify Al^{3+} in the rhizosphere [2,3,15]. Recently, genes responsible for Al-induced secretion of malate and citrate have been identified, such as *ALMT1* in wheat [16], *HvMATE* in barley [17] and *SbMATE* in sorghum [18]. Transforming these genes into Al-sensitive cultivars has been proved to increase Al resistance significantly [19].

Rice (*Oryza sativa* L.) is an important crop worldwide with a high basal level of tolerance to Al compared with other small-grained cereals [20–22]. As a model species, rice is an attractive system for mutational and genetic analyses at both gene and protein levels. It has been well demonstrated that different from those crops which employ secretion of organic acid anions as a main mechanism of Al tolerance, organic acid anion secretion is not the major mechanism for high Al tolerance in rice because the amount of secretion is very small [4,21–23]. Recently, through genome-wide association analysis (GWA) and quantitative trait loci (QTL) mapping, 48 QTLs associated with Al^{3+} tolerance have been identified in rice [24]. Furthermore, several Al-resistant genes have also been cloned by mutant approaches in rice, for example, *ART1* (*Al^{3+} resistance transcription factor 1*) [4,25], *STAR1/STAR2* (*sensitive to Al rhizotoxicity 1 & 2*) [26], *Nrat1* (*Nramp aluminum transporter 1*) [27], *FRDL4* (*ferric reductase defective3-like 4*) [28], *ALS1* (*aluminum sensitive 1*) [29] and *MGT1* (*magnesium transporter 1*) [30]. All of these genes are specifically induced by Al and knockout of any of them results in the decreasing in Al tolerance, indicating their huge contributions to the high Al tolerance observed in rice. More recently, through comparative genome-wide transcriptional analysis of wild type and *star1* mutant, a novel platform has been provided for further work on Al tolerance in rice [31]. However, the mechanisms underlying high Al tolerance in rice are still not well understood at the molecular level. So, a more thorough analysis at proteome or metabolome levels is required to clarify the mechanisms of Al tolerance in rice.

Although transcriptomics provides a useful tool for unraveling gene expression networks, proteomics links these networks to protein products and provides further insight into posttranscriptional modifications that regulate cellular functions, thereby complementing genomics analysis [32]. In the past two decades, two-dimensional electrophoresis (2-DE) has been widely used for protein separation and analysis, while iTRAQ (isobaric tags for relative and absolute quantification) is a robust mass spectrometry technology that allows quantitative comparison of protein abundance by measuring peak intensities of reporter ions released from iTRAQ-tagged peptides by fragmentation during MS/MS [33]. Given iTRAQ's ability to perform relative and absolute quantification in up to four or even eight phenotypes, accuracy at quantification, and high-resolution precursor ion selection and extended protein and peptide fractionations [34], iTRAQ-based proteomics has been widely applied in systems ranging from microorganism stress responses [35] to evaluating plants in response to deficient or excess nutrient elements [36,37] in the past decade. However, this technique has not been used to investigate the plant responses to Al^{3+} toxicity.

Here we employed an iTRAQ-based quantitative proteomics approach to elucidate the effects of Al^{3+} toxicity on the protein levels in rice cultivars differing in Al tolerance in detail. With these measurements, we aimed to identify protein abundance changes in response to Al with the potential mechanistic relevance to the early phase of Al^{3+} toxicity. We found that glycolysis/gluconeogenesis was the most significantly up-regulated biochemical process in response to excess Al^{3+}. The mRNA levels of eight proteins mapped in this pathway were further analyzed by qPCR and the expression levels of all the eight genes were higher in tolerant cultivar than in sensitive cultivar, suggesting that these compounds may promote Al tolerance by modulating the production of available energy and the tolerant cultivar might have a higher potential to produce more energy to counteract the toxic effect of Al^{3+}.