Transcriptional Profiling of Aluminum Toxicity and Tolerance Responses in Maize Roots

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ABSTRACT

In most crop species there is considerable genotypic variation for Al tolerance in acid soils. In particular, Al tolerance in maize is a complex phenomenon, involving multiple genes and physiological mechanisms. To elucidate the molecular basis of this phenomenon, we performed a detailed analysis of root gene expression under Al stress using microarrays with Al-tolerant and Al-sensitive genotypes. A number of candidate genes encoding membrane transporters of the MATE family were identified among the ESTs that exhibited significantly higher expression in the tolerant genotype in response to Al.

INTRODUCTION

Plants have evolved different mechanisms to overcome Al toxicity, which is the primary factor limiting crop yield on acid soils. The most prevalent of these mechanisms of Al tolerance is the exclusion of Al from the root tip based on the release of organic acids, which chelate Al^{3+} forming stable, nontoxic complexes. Release of malate, citrate and/or oxalate from roots upon exposure to Al has been correlated with differential Al tolerance in a large number of monocot and dicot species (Kochian et al., 2004). In maize, although Al tolerance is strongly associated with high rates of root citrate release (Piñeros et al., 2002), it appears that in contrast to other species, Al tolerance in maize is a rather complex phenomenon involving multiple genes and physiological mechanisms. A recent study on QTL mapping identified five distinct genomic regions with importance to Al tolerance in maize (Ninamango-Cárdenas et al., 2003).

A number of genes have been shown to be differentially regulated by Al stress in different plant species (Kochian et al., 2004). However, these genes are mostly related to a general stress response resulting from the toxic effects of Al, and are unlikely to play a significant role in Al tolerance. The use of more sensitive, high-throughput expression profiling techniques applied in comparative studies will be crucial to reveal the role of differential gene regulation in Al tolerance. With that in mind, we performed a detailed comparative investigation of the changes in gene expression taking place in roots of an Al-tolerant and an Al-sensitive genotype of maize under short-term Al stress using microarrays.

MATERIALS AND METHODS

Seeds of the tropical maize inbred line C100-6 (Al-tolerant) were provided by CBMEG (University of Campinas, Campinas, Brazil), and seeds of Al237 (Al-tolerant) and L53 (Al-sensitive) by EMBRAPA Maize and Sorghum (Sete Lagoas, Brazil). The maize microarrays were provided by the Maize Oligonucleotide Array Project (www.maizearray.org). For a detailed description of the genotypes and experiment see Maron et al. (2008).

RESULTS AND DISCUSSION

Al stress altered the expression of a significantly larger number of genes in roots of the Al-sensitive genotype L53 (Figure 1), probably as a result of more severe Al toxicity. Nevertheless, a number of genes were identified which were up-regulated by Al exclusively in roots of the Al-tolerant genotype, C100-6. Only small changes in the expression of genes involved in organic acid synthesis and metabolism were observed (results not shown), indicating that the expression of these genes is not likely to be implicated in regulating the Al-induced citrate release response in maize roots. In contrast, a number of genes showing homology to MATE transporters were differentially regulated by Al, exhibiting various patterns of expression (results not shown). A member of this family of transporters has been recently identified as the Al tolerance gene in sorghum (Magalhaes et al., 2007). A closer examination of the ESTs from which the oligonucleotides printed in the array originated revealed that 3 of these ESTs in fact represented the same MATE gene in the maize genome (www.maizesequence.org). In

addition, the chromosomal location of this gene coincided with one of the major QTL for maize Al tolerance identified by Ninamango-Cárdenas et al. (2003).

The full length cDNA and genomic ORFs of this candidate MATE gene were cloned from C100-6 and L53, as well as from the Al-tolerant parent of the mapping population used for QTL analysis by Ninamango-Cárdenas et al. (2003). Mapping of a 160-bp deletion in the 3′ UTR confirmed the chromosomal position of the gene near a major Al tolerance QTL (results not shown). Sequence comparisons showed that the MATE isolated from maize (*ZmMATE1*), although a member of the same family of membrane transporters, is not a close homolog of *SbMATE*, the Al tolerance gene recently identified in sorghum.

ZmMATE1 encodes a 563-amino acid protein, with a predicted secondary structure consisting of 12 transmembrane domains (Figure 2). The expression pattern of *ZmMATE1* was analyzed using real-time PCR (Figure 3). *ZmMATE1* expression in root tips was higher in the Al-tolerant genotypes (C100-6 and Al237) in the absence of Al (0h). In addition, expression was strongly upregulated by Al stress in all 3 genotypes, but more so in the tolerant ones. The spatial pattern of *ZmMATE1* expression was also analyzed (Figure 3b), and showed that *ZmMATE1* was highly expressed in the root tips (i.e., the site of Al toxicity). *ZmMATE1* expression was also present at the upper part of the root, while its lowest expression levels were observed in the shoot. The cellular localization of the *ZmMATE1* protein was investigated using transient expression assays of a translational fusion with GFP in *Arabidopsis* protoplasts. In cells transformed with *ZmMATE1* :: *GFP* fluorescence was associated with the cell periphery (Figure 4a, b), indicating that the protein is localized to the plasma membrane. In contrast, in control cells transformed with empty vector GFP fluorescence was observed in the cytoplasm (Figure 4c, d). These results are consistent with secondary structure predictions showing that *ZmMATE1* is a transmembrane protein.

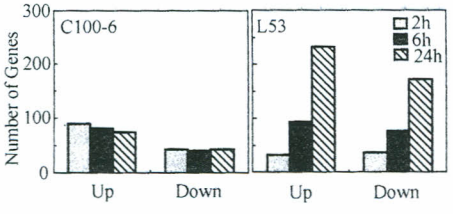

Figure 1 Number of genes differentially regulated in roots of C100-6 (Al-tolerant) and L53 (Al-sensitive) under Al stress

CONCLUSIONS

With the application of a sensitive, high-throughput technology such as microarrays in a comparative way, the present study was able to identify a candidate Al tolerance gene in maize belonging to the same family of membrane transporters responsible for Al tolerance in sorghum. Further investigations are currently underway to examine the potential role of *ZmMATE1* in Al-activated citrate release response in maize roots.

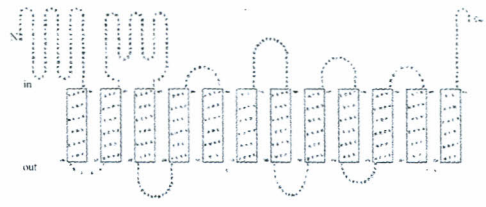

Figure 2 Predicted structure of *ZmMATE1*

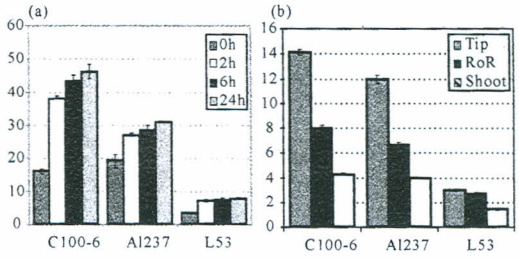

Figure 3 Gene expression analysis by real-time PCR. (a) time course of *ZmMATE1* expression in root tips exposed to 39 μM Al^{3+}; (b) *ZmMATE1* expression in different parts of the plant: root tip, rest of root (RoR) and shoot

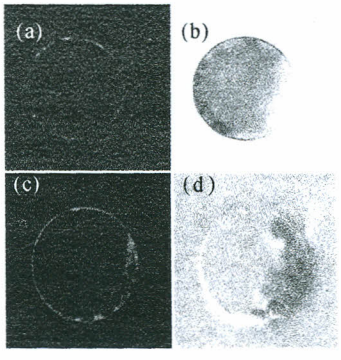

Figure 4 Cellular localization of the *ZmMATE1* protein in Arabidopsis protoplasts. GFP fluorescence (a) and bright-field image (b) of protoplasts transformed with *ZmMATE1* :: GFP. (c) and (d) show GFP fluorescence and bright-field image of protoplasts transformed with a cytoplasmic GFP control

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