

MtZIP1: A Divalent Metal Transporter from the Model Legume *Medicago truncatula*

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INTRODUCTION

Micronutrients such as Zn, Cu, Fe, and Mn are essential for the efficient functioning of a variety of proteins involved in various plant biological processes. These micronutrients are exclusively obtained from the surrounding rhizosphere by the roots. The micronutrients are transferred to the xylem and transported to the vegetative tissue. However, the plant must regulate the uptake of these metals, since too low or high concentrations of metals in the plant will be detrimental. To achieve this balance, plants utilize and regulate the expression of several types of micronutrient transporters. These transporters are classified under several protein families, including NRAMPs, ZNTs, YSLs and ZIPs.

The ZIP family of proteins includes divalent metal transporters that have been identified in plants, animals, and microbes. All members of this family have sequence similarity to ZRT (zinc-regulated transporter) and IRT (iron-regulated transporter) proteins in *Saccharomyces cerevisiae*. The ZIP transporters have been identified in several plant species, including the model legume *Medicago truncatula*. Seven ZIP genes have been identified in *M. truncatula* (Lopez-Milan et al. 2004) with three of these protein products (MtZIP1, MtZIP5, and MtZIP6) showing an ability to transport Zn (based on yeast complementation studies). We are characterizing the ZIP proteins in *M. truncatula* to achieve a better understanding of Zn uptake from the rhizosphere and partitioning within the plant. In addition, we have identified an MtZIP1 mutant that we are using to characterize whole-plant Zn dynamics.

METHODS

Kinetic analysis of zinc transporters

Constructs containing cDNA sequences for *MtZIP1*, *MtZIP5*, or *MtZIP6* were transformed into the yeast strain ZHY3, which lacks low and high-affinity Zn transporters (ZRT1 and ZRT2). Zinc uptake was assayed using solutions containing 5mM MES (pH 6.0), 2% glucose, and 0.01 μCi ⁶⁵Zn at Zn concentrations of 0.25-10 μM . Cadmium uptake assays were performed in a similar manner with 0.01 μCi ¹⁰⁹Cd and Cd concentrations of 0.5-50 μM .

Isolation of mutations in *MtZIP1*

An MtZIP1 mutant was isolated from an EMS generated population of *M. truncatula*. The reverse genetic approach of TILLING was used to screen for single nucleotide polymorphisms within the coding region of *MtZIP1*. The SNPs were detected using an ABI 3730XL sequencer.

Whole plant partitioning studies

Zinc partitioning studies were performed on *M. truncatula* wild-type (ecotype A17) and homozygous *MtZIP1* mutant plants. Plants were grown in standard hydroponics solution for 3 weeks before being transferred to solution containing minimal nutrients and 1 $\mu\text{Ci/L}$ of ⁶⁵Zn. Plants were harvested every 24 hours for 4 days. Roots and shoots were separated, weighed and analysed for ⁶⁵Zn on a Wizard 1480 gamma counter.

RESULTS AND DISCUSSION

Zinc transport studies with *M. truncatula* family members confirmed that only three members (MtZIP1, MtZIP5 and MtZIP6) transport Zn. MtZIP5 and MtZIP6 are high-affinity transporters with K_m 's around 0.4 μM and V_{max} values around 1 pmol/min/ 10^6 cells. The MtZIP1, on the other hand, is a low-affinity transporter with a K_m of 1.5 μM and V_{max} of 8 pmol/min/ 10^6 cells. The higher K_m and V_{max} values for MtZIP1 suggest that it has a role in the transport of Zn within the plant, rather than being involved in uptake from the rhizosphere. MtZIP5 and MtZIP6 may be both involved in root uptake and internal transport of Zn. The protein localization is unknown at present.

A mutation in *MtZIP1* was identified during the screening of a TILLinG population. The mutant allele had a deletion at base pair 27 of the first exon, which resulted in the insertion of a stop codon and early termination of the translation of MtZIP1. This knockout mutant has been used to characterize the role of MtZIP1 in whole plant Zn homeostasis.

The growth characteristics of the MtZIP1 mutant were similar to wild-type A17 for the first several weeks. However, the plants began to show decreased growth, and the internodes were shorter than those of A17. Mutant plants had to be supplemented with Zn by foliar spray to partially relieve the effects of the mutation. The mutation affected plant productivity and reproductive success. Although the mutant flowered, most flowers aborted and did not set seed. Seed production was enhanced with foliar spraying of Zn.

To further characterize MtZIP1, its role in the partitioning of Zn between roots and shoots was examined. The MtZIP1 mutant partitioned less of its absorbed Zn to shoots relative to wild-type plants (Fig. 1). These results suggest that MtZIP1 functions somewhere between the site of Zn absorption at the root epidermis and the point of Zn entry into the xylem pathway (prior to subsequent root to shoot translocation).

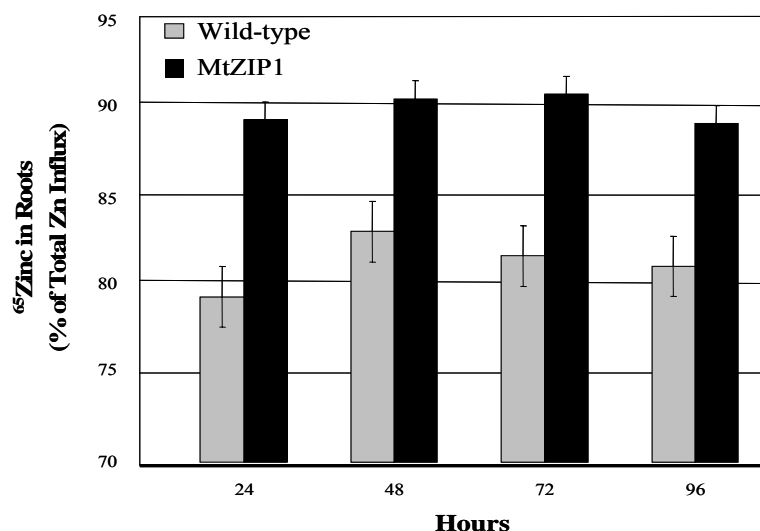


Fig. 1. ⁶⁵Zn in roots as a percent of total zinc uptake in the MtZIP1 mutant and wild-type plants.

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REFERENCES

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