OsZIP4, a Novel Zinc Transporter, Regulates Zinc Localizations in Rice

Yasuhiro Ishimaru[1], Motofumi Suzuki[1], Hiroshi Masuda[1], Michiko Takahashi[1], Hiromi Nakanishi[2], Satoshi Mori[2], Naoko K. Nishizawa[1, 3]

[1] Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, JAPAN (aishimar@mail.ecc.u-tokyo.ac.jp)
[2] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, JAPAN
[3] Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation, JAPAN

INTRODUCTION

Zinc is an essential nutrient that plays important roles in numerous physiological processes in plants, serving as a cofactor for many enzymes and as the key structural motifs in transcriptional regulatory proteins. A deficiency of Zn, therefore, decreases growth, but excess Zn has significant toxicity to biological systems through metal-based cytotoxic reactions. Therefore, the uptake and transport of Zn must be strictly regulated. Intracellular Zn homeostasis is achieved through the coordinated regulation of specific transporters engaged in Zn influx, efflux, and intracellular compartmentalization.

The ZIP family (Zn-regulated transporter/Fe-regulated transporter-like protein) of Zn transporters is found in plants, bacteria, fungi, and humans (Gaither and Eide 2001). In rice, ZIP transporters have been reported for OsZIP1 and OsZIP3, involved in Zn transport.

In this study, we isolated and characterized OsZIP4 and found that *OsZIP4* encoded a Zn transporter localized to the plasma membrane and regulated by the plant's Zn status. Overexpression of OsZIP4, moreover, altered the Zn concentration in shoots and roots, and revealed that OsZIP4 was an important Zn transporter for Zn distribution.

RESULTS AND DISCUSSION

We isolated *OsZIP4* that exhibits sequence similarity to the rice ferrous ion transporter, *OsIRT1* (Ishimaru et al. 2006). Northern blot analysis revealed that *OsZIP4* was highly expressed under conditions of Zn deficiency in roots and shoots (Fig. 1). Also, OsZIP4 complemented a Zn-uptake-deficient yeast (*Saccharomyces cerevisiae*) mutant, Δ*zrt1*Δ*zrt2*, indicating that OsZIP4 is a functional transporter of Zn. The OsZIP4-sGFP fusion protein was transiently expressed in onion epidermal cells localized at the plasma membrane. *In situ* hybridization analysis revealed that *OsZIP4* in Zn-deficient rice was expressed in the meristem of Zn-deficient roots and shoots, and in phloem cells of roots and shoots. These results suggested that OsZIP4 is a Zn transporter that may be responsible for the translocation of Zn within rice plants.

Furthermore, we have produced transgenic rice plants over-expressing *OsZIP4* under the control of the CaMV 35S promoter. Zinc concentration in 35S-OsZIP4 transgenic plants was higher in roots and lower in shoots compared to controls, suggesting that *OsZIP4* expression driven by 35S promoter in *35S-OsZIP4* root may be involved in re-absorption of Zn from xylem or phloem for transport to shoots, in addition to Zn accumulation in the cortex and the epidermis (Fig. 2). Northern blot analysis revealed that transcripts of *OsZIP4* expression driven by CaMV 35S promoter were detected in roots and shoots of *35S-OsZIP4* transgenic plants, but endogenous *OsZIP4* transcripts were little in roots, and much in shoots.

Microarray analysis revealed that the genes expressed in shoots of *35S-OsZIP4* coincided with genes induced in shoots of Zn deficient plants. These results indicated that OsZIP4 changed the Zn distribution inside rice plant, and that OsZIP4 is a critical Zn transporter that should be strictly regulated.

FIGURES

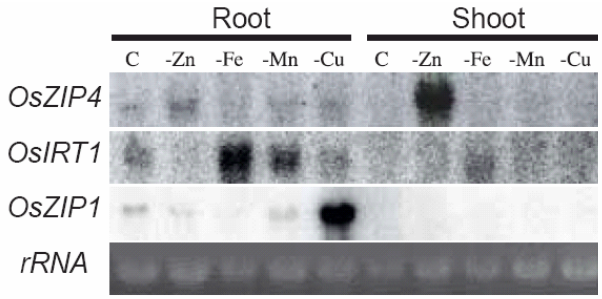

Figure 1. Expression pattern of *OsZIP4*. Northern blot analysis of the *OsZIP4*, *OsIRT1* and *OsZIP1* in the roots and leaves of rice plants grown under trace metal deficient conditions. Total RNA (10 μg) extracted from plants grown in normal nutrient solution (control) (C) or under conditions of low zinc (-Zn), iron (-Fe), manganese (-Mn), or copper (-Cu) supply was blotted on each line. Ethidium bromide-stained *rRNA* is shown as a control for loading.

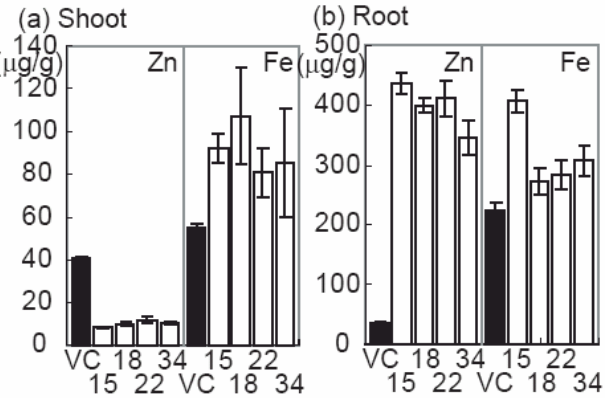

Figure 2. Zn concentrations of 35S-OsZIP4 rice plants.
(a), Zinc and iron concentration of control (VC) and 35S-OsZIP4 (15, 18, 22, 34) in shoot. (mean ± standard deviation, n = 6). (b), Zinc and iron concentration of control (VC) and 35S-OsZIP4 (15, 18, 22, 34) in root. (mean ± standard deviation, n = 6).

CONCLUSIONS

The OsZIP4 is a Zn transporter that is responsible for the translocation of Zn within rice plants and needs to be tightly regulated.

ACKNOWLEDGEMENTS

We thank Dr. Nagamura and the Rice Genome Project (National Institute of Agrobiological Sciences, Tsukuba, Japan) for microarray analysis.

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