Introduction

Cadmium (Cd), one of the most toxic heavy metals for both plants and humans, accumulates in the human body through the food chain and causes serious health problems. In recent years, the accumulation of Cd in rice grains has become an important agricultural problem in Japan because the Cd content of rice grains sometimes exceeds the limit proposed by the Codex Alimentarius Commission. In addition, Cd intake from rice accounts for about one-half of the intake from food in Japan according to the National Institute of Health Sciences. Therefore, new technologies for reducing the Cd content in rice grains are urgently required.

Although the mechanism underlying the uptake and translocation of Cd in plants is not completely understood, some iron (Fe) transporters, such as OsIRT1 and OsIRT2, are reported to uptake Cd as well as Fe (Nakanishi et al. 2006). AtNramp3 and AtNramp4 from *Arabidopsis*, which belong to the Nramp metal transporter family, function as Fe and Cd transporters (Curie et al. 2000; Thomine et al. 2000). Rice has seven *Nramp* genes (Belouchi et al. 1997; Narayanan et al. 2007), and OsNramp1 has been reported to function as an Fe transporter (Curie et al. 2000). In this study, we investigated the possibility that OsNramp1 also transports Cd.

Materials and Methods

Full-length *OsNramp1* was amplified by RT-PCR using total RNA prepared from hydroponically grown rice shoots (*Oryza sativa* L. cv. Nipponbare).

The subcellular localization of OsNramp1 was determined by monitoring the expression of an OsNramp1::GFP fusion protein in onion epidermal cells transformed by DNA particle bombardment. *GFP*, contained in the vector pH7FWG2, was fused to the 3'-terminus of *OsNramp1* using the Gateway system (Invitrogen).

To test the growth of OsNramp1-expressing yeast, full-length *OsNramp1* cDNA was inserted into the expression vector pYH23. The construct was then introduced into yeast strain *ycf1* using the lithium acetate method. *Ycf1* lacks the YCF1 transporter, which functions in the compartmentation of Cd into vacuoles. Yeast cells transformed with empty pYH23 were used as a control. The transformed yeast cells were grown in synthetic defined (SD) medium and spotted onto SD agar containing $CdCl_2$.

To measure the metal content of the OsNramp1-overexpressing rice, plants were

grown in Cd-contaminated soil (0.1 M HCl-extractable Cd = 1.8 ppm) for 6 months in a greenhouse. Harvested leaf blades were dried at 70˚C for 1 week. Sample digestion and measurement of the metal content were performed as described previously (Ishimaru et al. 2007), except that the digestion time and temperature were changed to 2 h at 230˚C.

Results

Subcellular localization of OsNramp1

We investigated the subcellular localization of OsNramp1 using an OsNramp1::GFP fusion protein. Green fluorescence was observed in the peripheral region of the cells, suggesting that OsNramp1 localizes to the plasma membrane.

Growth of OsNramp1-expressing yeast in the presence of Cd

Ycf1 yeast cells expressing OsNramp1 showed increased sensitivity to Cd, suggesting that OsNramp1 transports Cd and alters the accumulation of Cd in the cytoplasm.

Metal content of OsNramp1-overexpressing rice

When grown in Cd-contaminated soil, the OsNramp1-overexpressing rice exhibited an increased leaf blade Cd content compared to the non-transformed rice. The Fe content also increased in the OsNramp1-overexpressing rice, whereas the Zn, Mn, and Cu contents were nearly identical between the OsNramp1-overexpressing and non-transformed plants. These results suggest that OsNramp1 increases the uptake and transport of Fe and Cd in plants.

Conclusions

The results of this study indicate that OsNramp1 increases the cellular uptake of Cd. Furthermore, OsNramp1-overexpressing rice accumulated a larger amount of Cd in their leaf blades. Therefore, the regulation of OsNramp1 expression may be an efficient way to reduce the Cd content in rice grains.

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