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SHORT COMMUNICATION

Rice reduces Mn uptake in response to Mn stress

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ABSTRACT

Rice (*Oryza sativa* L) is one of the most Mn-tolerant crops that can grow in submerged paddy fields, where the Mn concentration in soil solution is very high due to reduction. Although a large part of Mn is transferred from the roots to the shoot in rice, the roots are constantly exposed to high Mn concentrations in submerged paddies. Thus, mechanisms for preventing Mn overaccumulation in the cytoplasm of root cells are necessary. Recently, we showed that two cation diffusion facilitators, MTP8.1 and MTP8.2, play a crucial role in Mn tolerance in rice roots by sequestering Mn in vacuoles. Moreover, we observed that disruption of *MTP8.1* and *MTP8.2* resulted in reduced Mn accumulation under excess Mn. In the present study, we examined the effects of disruption of *MTP8.1* and *MTP8.2* on Mn uptake and determined that this phenotype is caused by a rapid and significant reduction of Mn uptake in response to excess Mn. Previously, we showed that Mn export from root cells through MTP9 was promoted by high Mn. Together, these findings suggest that optimal Mn concentration in rice roots is maintained by reduced uptake, vacuolar sequestration, and extrusion by cation diffusion facilitators.

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Mn detoxification is quite an important task for rice growing in submerged paddy fields with low redox potential. It has been demonstrated that rice plants transfer a large part of Mn from the roots to the shoot by the activity of metal tolerance protein 9 (MTP9),[1] unload it from xylem at the nodes, and distribute it to old tissues via natural resistance-associated macrophage protein 3 (NRAMP3) in the presence of high Mn concentrations.[2] We recently reported that Mn in old leaves is detoxified by two vacuolar transporters, MTP8.1 and MTP8.2.[3,4] Both *MTP8.1* and *MTP8.2* are constitutively expressed at higher levels in old than in young leaves, but the level of *MTP8.1* is several times higher than that of *MTP8.2*.[4] Knockout of *MTP8.1* resulted in significant growth reduction in addition to chlorosis and necrosis in leaves under high Mn conditions,[3] whereas no obvious Mn toxicity symptoms were presented in *mtp8.2* leaves.[4] However, when *MTP8.2* was knocked down in *mtp8.1*, the mutants (*mtp8.2i*/*mtp8.1*) exhibited higher Mn sensitivity than did *mtp8.1*,[4] indicating that *MTP8.2* is also important for Mn tolerance in shoots. In roots, *MTP8.1* and *MTP8.2* were expressed at similar levels regardless of Mn condition.[4] *MTP8.1* and/or *MTP8.2* disruption led to impaired root elongation under excess Mn,[4] indicating that vacuolar sequestration by the transporters plays a crucial role in Mn detoxification in roots. Interestingly, the *mtp8.2i*/*mtp8.1* mutants accumulated significantly lower amounts of Mn than did the wild type under excess Mn.[4] However, these transporters are not involved in Mn uptake by roots. We hypothesized that rice plants may respond to excess Mn by reducing Mn uptake, thereby enhancing Mn tolerance. In the present study, we present the effect of disruption of *MTP8.1*/*MTP8.2* on Mn uptake and a possible mechanism of Mn tolerance in rice roots.

To examine our hypothesis, we performed a short-term (3-h) uptake experiment that examined whether the *MTP8.1*/*8.2* disruption reduced Mn uptake, according to Ueno et al.[5] with some modifications. Seeds of wild-type Nipponbare and *mtp8.2i*/*mtp8.1* plants were germinated in flowing tap water for 4–7 days, and then pre-cultured in 1/2 Kimura B solution with 0.1 μM Mn for 26 days. To investigate Mn uptake, the roots of the seedlings were pre-incubated in 0.5 mM $CaCl_2$ solution for 30 min at either 30°C or 4°C. They were then exposed to 0.5 mM $CaCl_2$ solution containing varying concentrations of Mn (0.5, 2, 10, 50, 100, and 200 μM) for 3 h at 30°C or 4°C. Mn concentrations in roots and shoots obtained at 4°C were subtracted from those at 30°C to determine net root uptake and translocation to the shoot. The results showed that at elevated Mn levels (> 10 μM), *mtp8.2i*/*mtp8.1* exhibited Mn uptake rates that were up to five times lower than in the wild type (Fig. 1A). In contrast, there was no significant difference in Mn translocation to the shoots between the mutant and wild-type plants (Fig. 1B). The inhibition of Mn uptake may be a response from NRAMP5 to

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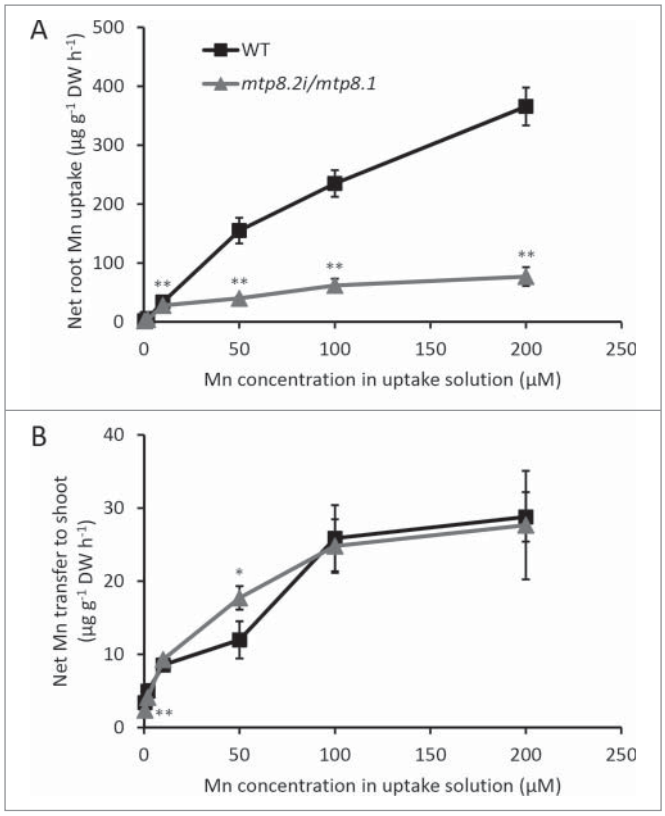

Figure 1. Kinetics of short-term Mn uptake by roots of wild-type (square symbols) and *mtp8.2i/mtp8.1* (triangle symbols) plants. Seedlings were exposed to 0.5 mM $CaCl_2$ solution containing varying concentrations of Mn (0.5, 2, 10, 50, 100, and 200 μM) for 3 h at 30°C or 4°C temperatures. Net root Mn uptake (A) and Mn transfer to shoot (B) were calculated by subtracting values at 4°C from those at 30°C. Data represent the mean ± SD ($n = 3$). $^{**}P < 0.01$, $^{*}P < 0.05$, Tukey's test.

compensate for the lack of vacuolar sequestration under *MTP8.1/8.2* disruption. NRAMP5 is the major transporter for Mn uptake that localizes in the distal side of the plasma membrane in exodermal and endodermal cells.[6] The dramatic increase in Mn in the cytosol of *mtp8.2i/mtp8.1* root cells can stimulate the response to Mn toxicity by inhibiting further uptake of Mn through NRAMP5. Because *NRAMP5* transcription does not respond to the Mn level,[6,7] even in *mtp8.2i/mtp8.1* (data not shown), this inhibition may be related to post-transcriptional degradation of NRAMP5. Correlation between Mn tolerance and restriction of Mn uptake has been reported in some plant species. Robson and Loneragan demonstrated that the higher Mn tolerance of subterranean clover than that in toothed medick was attributed to a lower uptake rate.[8] In addition, Culvenor noted that Mn tolerance in bulbous canary grass was associated with low Mn uptake.[9]

In addition to vacuolar sequestration and reduced uptake, Mn concentration in root cells may be controlled by extrusion. The accumulation of Mn exporter MTP9, which is localized in the proximal side of the exodermis/endodermis and mediates Mn uptake in co-ordination with NRAMP5, is promoted in response to Mn.[1] However, a similar amount of Mn was transferred to the shoots in *mtp8.2i/mtp8.1* and wild type (Fig. 1B), suggesting that this regulation may arise after the onset of NRAMP5 inhibition. The expression of *Cucumis sativus MTP9* is upregulated under excess Mn.[10] Further, overexpression of *CsMTP9* in *Arabidopsis thaliana* enhanced Mn tolerance in roots. Although accumulation of oxidized Mn in the apoplast is known to be a cause of Mn toxicity,[11,12] these studies suggest that extrusion of Mn from cells by MTP9 also mediates Mn tolerance in roots.

The overall evidence suggests that high Mn tolerance in rice roots is associated with stringent control of cytosolic Mn concentration via reduced uptake, constitutive vacuolar sequestration by MTP8.1 and MTP8.2, and enhanced extrusion from cells (Fig. 2). The fast restriction of Mn uptake when exposed to excess Mn may be attributed to post-transcriptional regulation of NRAMP5. Molecular mechanisms for Mn sensing by the roots require further investigation.

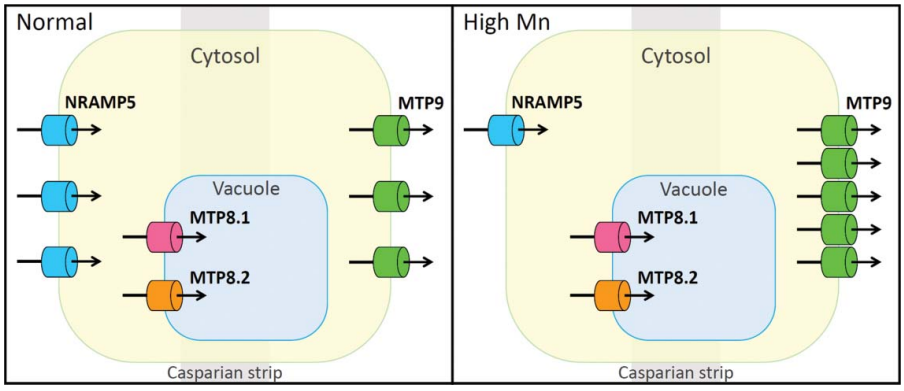

Figure 2. Putative mechanism of Mn tolerance in rice root. Right and left panels show normal and high Mn conditions, respectively. NRAMP5 is localized at the distal side of the exodermis and endodermis, while MTP9 is localized at the proximal side of both layers. MTP8.1 and MTP8.2 are localized to the tonoplast. Optimal Mn concentration in the root may be maintained via reduced uptake caused by the degradation of NRAMP5 and enhanced extrusion from cells caused by an increase in MTP9 accumulation, as well as constitutive vacuolar sequestration by MTP8.1 and MTP8.2.

Abbreviations

MTP metal tolerance protein
NRAMP natural resistance-associated macrophage protein

Disclosures

The authors have no conflict of interest to declare.

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