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Opinion

The Key to Mn Homeostasis in Plants: Regulation of Mn Transporters

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Plants only require small amounts of manganese (Mn) for healthy growth, but Mn concentrations in soil solution vary from sub-micromolar to hundreds of micromolar across the growth period. Therefore, plants must deal with large Mn concentration fluctuations, but the molecular mechanisms underlying how plants cope with low and high Mn concentrations are poorly understood. In this Opinion we discuss the role of Mn transporters in the uptake, distribution, and detoxification of Mn in response to changes in Mn concentrations through their regulation at the transcriptional and protein levels, mainly focusing on rice, an Mn-tolerant and –accumulating species. We also propose mechanisms involved in the hyperaccumulation of Mn and future prospects for studying this specific trait.

Plants Accumulate More Mn Than They Require

Manganese (Mn) was demonstrated to be essential for plants in 1922 by McHargue [1] and for animals in 1931 by Kemmerer *et al*. [2]. In plants, Mn is involved in processes including photosynthesis, respiration, protein synthesis, and hormone activation [3]. For example, Mn plays an important role in the oxygen-evolving photosynthetic machinery, catalyzing the water-splitting reaction in photosystem II (PSII) [4]. Mn is also involved in the activation of more than 30 enzymes. Furthermore, a few enzymes such as Mn superoxide dismutase (MnSOD) contain Mn [5]. Although plants only require about 20–40 mg Mn kg^{-1} of dry weight for its various functions [6,7], most plants contain 30–500 mg Mn kg^{-1} dry weight, which is higher than the plant requirement [8]. This could be attributed to variable Mn concentrations in soil solutions and poor regulation of Mn uptake, as discussed below.

When the Mn concentration in the young expanded leaf blade is lower than 10–20 mg kg^{-1}, plants develop Mn-deficiency symptoms regardless of plant species or cultivars. The most visible Mn-deficiency symptom is interveinal chlorosis on young leaves [9]. By contrast, when Mn is present in excess, it is toxic to plants. Toxicity symptoms are characterized by brown spots on the mature leaves as a result of increased peroxidase activity mediated by phenolics and Mn in the **apoplast** (see Glossary) [10,11]. However, in contrast to the critical Mn concentration for Mn deficiency, the critical toxicity concentration of Mn varies widely among plant species. For example, maize (*Zea mays*) growth was inhibited at 200 mg Mn kg^{-1}, while sunflower growth was only inhibited at 5300 mg Mn kg^{-1} [12], and some Mn-hyperaccumulators even accumulate much higher Mn levels, as described below. This Opinion paper discusses the role of Mn **transporters** in the uptake, distribution, and detoxification of Mn, focusing mainly on rice (*Oryza sativa*) where a **node**-localized transporter (OsNramp3; **natural resistance associated macrophage protein**) plays an important role in dealing

Trends

The concentration of Mn, an essential element for plants, varies in soil solution from submicromolar to hundreds of micromolar across the plant growth period as a function of soil conditions.

Recent studies have revealed that some plant species, such as rice, are able to deal with these wide fluctuations of Mn in the environment by differential regulation of transporter expression.

OsNramp3 localized at the nodes responds to different Mn concentrations. It functions as a switch in response to changes in Mn levels.

Other transporters are also implicated in dealing with changes in Mn levels in different plant species, especially Mn-hyperaccumulators, but their exact roles remain to be examined.

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with large changes in environmental Mn concentrations through the regulation of protein expression levels.

Wide Fluctuations of Mn Concentrations in Soil Solution

Mn is the 11th most common element and the second most prevalent transition metal after iron in the Earth's crust. The total Mn in soils varies between 20 and 3000 mg kg^{-1} with an average of ~600 mg kg^{-1}. In soil solutions, divalent Mn^{2+} is the most available form for plants, but its concentration varies between 0.1 and 800 μM depending on soil conditions [13,14]. The Mn^{2+} concentration in soil solution is centrally affected by soil pH and redox conditions [15]. The concentration of Mn^{2+} decreases 100-fold for each unit increase in pH [16]. Therefore, Mn deficiency often occurs in alkaline soils, which comprise 30% of arable land, while Mn toxicity is one of the factors limiting crop production in acid soils that comprise 30–40% of arable land [17]. Furthermore, excess water in soil triggers a progressive decrease in soil redox potential, thus increasing the concentration of Mn^{2+} in soil solution [18]. For this reason Mn toxicity also occurs in poorly drained soils [19]. Because soil conditions are very changeable temporally and spatially, plants must cope with these wide fluctuations of Mn concentrations in soil solution during the growth period. This is especially important for rice because this species is cultivated under both upland and flooded conditions over the whole growth period. Recent studies have revealed that rice has developed strategies to deal with varying Mn concentrations in the environment by regulating Mn transporter expression and activity.

Acquisition of Mn from Soil in Response to Low Mn Availability

Plants have developed several strategies to acquire Mn, which is essential for their growth, from soil with low Mn availability. The best-studied mechanisms involve release of H^+, reductants, and Mn-binding ligands [9,13]. However, the genes involved in these processes have not been identified. Transporters for aluminium (Al)-induced secretion of organic acid anions have been identified [20], but it is unknown whether similar transporters are involved in Mn deficiency-induced secretion of organic acid anions. Recently, leaf Mn accumulation was proposed to be a screening parameter for phosphate (P)-acquisition efficiency [21]. This is based on the finding that some plant species secrete organic acid anions from the roots in response to P deficiency, which also mobilize Mn in addition to P.

Mn Uptake in Rice

Under flooded conditions, the concentrations of both Fe^{2+} and Mn^{2+} dramatically increase because of oxygen depletion, but rice plants preferentially accumulate more Mn than Fe. This is because oxidation of Mn^{2+} requires a higher redox potential than for Fe^{2+} [22]. Oxygen released from the root surface is sufficient to oxidize Fe^{2+}, but not Mn^{2+} [23,24].

Mn uptake by rice increases with increasing external Mn concentrations in the 0.5 μM to 500 μM range without affecting plant growth [25,26]. This high uptake exceeds the requirement of rice for Mn, indicating poor regulation of Mn uptake in this species. Two different types of transporters have so far been identified, OsNramp5 and OsMTP9, that are involved in Mn uptake in rice [27,28]. Both OsNramp5 and OsMTP9 are located at the exodermis and endodermis of mature root zones, but show different polarities; OsNramp5 is at the distal side whereas OsMTP9 at the proximal side of the same cell [27,28]. Therefore, OsNramp5 functions as an influx transporter and is responsible for uptake of Mn from the external milieu (soil solution) to the exodermal cells, as well as from apoplastic solution in aerenchyma to endodermal cells, while OsMTP9 releases Mn from the exodermal and endodermal cells as an efflux transporter towards the stele (Figure 1). This uptake system is very similar to that of silicon (Si, in the form of silicic acids) mediated by OsLsi1 and OsLsi2 [29]. A recent mathematical modeling study showed that **Casparian bands**, localized at the exodermis and endodermis,

Glossary

Apoplast: a space outside the plasma membrane, which is formed by the continuum of cell walls of adjacent cells as well as the extracellular spaces, forming a tissue level compartment comparable to the symplast. The apoplast is important for plant interactions with its environment.

Casparian band: a band of cell-wall material deposited in the radial and transverse walls of the root exodermis and/or endodermis. It is made of lignin and constitutes a physical barrier that prevents water and solutes from freely entering into the cortex (aerenchyma)/stele as well as from leaking back out to the rhizosphere/cortex.

Cation diffusion facilitator (CDF): a family of integral membrane proteins that are widespread in bacteria, fungi, plants, and animals. They transport divalent metals ion such as Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, and Cu^{2+}. CDF proteins function as efflux transporters, and therefore increases tolerance to heavy metals. However, some members are implicated in ion acquisition. In plants the metal tolerance protein (MTP) belongs to the CDF family and is involved in tolerance, translocation, and homeostasis of cations (e.g., Mn^{2+}, Zn^{2+}, Cd^{2+}).

Cation exchanger (CAX): a family of transmembrane proteins that move ions such as Ca^{2+} across a plasma membrane against their concentration gradient as an efflux transporter.

Diffusevascular bundles (DVBs): vascular bundles surrounding the EVBs in nodes. They start at the node and are connected to the upper two nodes or panicle.

Enlarged vascular bundles (EVBs): vascular bundles having a extensive xylem area in the node. They come from the two lower nodes and are connected to the leaf attached to the node.

Natural resistance-associated macrophage protein (Nramp): a divalent cation influx transporter family associated with the uptake of transition metals ions such as Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}.

Node: a junction region of leaves and branches to the stem. In graminaceous plants, each node has a leaf that is connected via the leaf sheath, a tiller or a tiller bud, and

are very important for efficient Si uptake [30], it stands to reason that the bands may also be important for Mn uptake in rice.

Mn is also required for cell division and cell elongation in root tips. Mn seems not to be taken up by the root tips directly but is transported to root tips from the upper parts of the plant via the phloem. Mn cooperatively taken up by OsNramp5/OsMTP9 in the mature root regions is translocated up through the xylem. At the basal nodes, Mn is transferred by OsNramp3 from xylem to phloem, followed by distribution to the root tips and young leaves [31]. This is supported by the fact that knocking out OsNramp3 resulted in brown-colored root tips, a typical sign of Mn deficiency [31].

The transcription of the genes encoding OsNramp5 and OsMTP9 is not affected by external Mn concentrations [27,28]. This constitutive expression indicates poor regulation of Mn uptake at the transcriptional level and differs from that of other nutrient transporters. The expression of most nutrient transporter genes is induced by nutrient deficiency, but suppressed by nutrient excess to maintain their homeostasis [32]. For example, the expression of *IRT1*, a Fe^{2+} transporter gene, was upregulated under Fe-limited conditions [33]. At the protein level, OsMTP9 and OsNramp5 were largely unaffected by high Mn levels ([28], J.F.M. *et al.*, unpublished). This result also differs to the turnover of other transporters such as BOR1 protein, a boron transporter that is rapidly degraded in response to high B [34].

Most Mn taken up is translocated to the shoots, but a fraction will be sequestrated into vacuoles of the root cells. This sequestration is mediated by OsMTP8.1 in rice, which is also expressed in the roots in addition to the shoots (Figure 1), but the expression of OsMTP8.1 is also not affected by environmental Mn changes [26].

Mn Uptake in Other Plant Species

In *Arabidopsis* (*A. thaliana*), Mn uptake is mediated by AtNramp1, a homolog of OsNramp5 but belonging to a different subgroup [35]. In contrast to rice, the expression of *AtNramp1* in *Arabidopsis* is moderately upregulated by Mn deficiency [35]. Two *Arabidopsis* **ZIP** (**Zn-regulated transporter/Fe-regulated transporter-like protein**) family members, AtZIP1 and AtZIP2, are implicated in the transport of Mn in root stellar cells [36]. Both AtZIP1 and AtZIP2 are expressed in the root stele, but AtZIP1 is localized to the tonoplast whereas AtZIP2 is localized to the plasma membrane. AtZIP1 is proposed to remobilize Mn from vacuoles to the cytoplasm, whereas AtZIP2 may mediate Mn uptake into root stellar cells. However, the expression of both AtZIP1 and AtZIP2 is largely unaffected by Mn deficiency [36]. Owing to lack of spatial tissue and cellular localization information of Mn transporters, our understanding of the uptake system of Mn in *Arabidopsis* is not as complete as in rice (Figure 1). In barley (*Hordeum vulgare*) a ZIP family member, HvIRT1, is implicated in Mn uptake [37]. The expression of *HvIRT1* is also moderately enhanced by Mn deficiency [37]. Recently, HvNramp5, a homolog of rice OsNramp5, was reported to be involved in Mn uptake in barley [38]. HvNramp5 is localized to the plasma membrane of the epidermal cells of the root tips. In contrast to *HvIRT1*, its expression is only slightly upregulated by Fe deficiency, but not by Mn deficiency. These differences in response to environmental Mn changes between different species may be attributed to distinct root structures of different species (e.g., monocot versus dicot), Mn uptake ability, and growth conditions (dry soil growth versus flooded growth). Overall, the response of Mn transporter genes to Mn deficiency is mild compared to that in response to Fe deficiency.

Compared to barley and *Arabidopsis*, which are usually grown under upland soil conditions, rice is predominantly grown under flooded conditions. Therefore, fluctuations of Mn levels in soil solution during the growth period are much larger for rice than for barley and *Arabidopsis*.

crown roots or primordia. It plays an important role in the distribution of mineral elements.

Symplast: cytoplasm shared with neighboring cells that are connected via plasmodesmata through which water and low molecular weight solutes can freely diffuse.

Transporter: membrane proteins involved in transport of many molecules including mineral elements. They help molecules to cross biological membranes. In plants, there are many different types of transporters localized at the plasma membrane, vacuolar membrane, and membranes of many organelles.

Zn-regulated transporter/Fe-regulated transporter-like protein (ZIP): influx transporter proteins capable of transporting a variety of cations including Cd^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}.

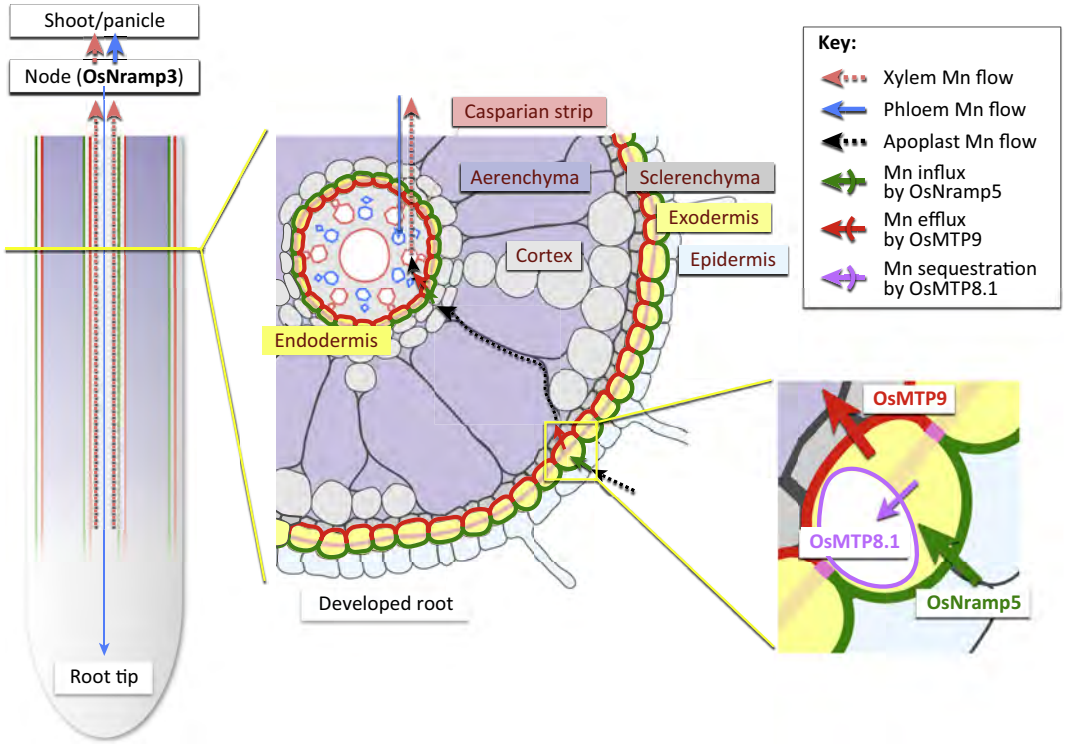

Figure 1. Uptake System of Mn in Rice in Response to Fluctuation in Mn Concentrations. In rice, Mn uptake is mediated by at least two different transporters, OsNramp5 and OsMTP9, that are localized at the distal and proximal sides, respectively, of the same exodermis and endodermis in mature root zones. Part of Mn taken up is sequestered by OsMTP8.1 localized at the tonoplast. These transporters are largely unresponsive to fluctuations in Mn concentrations in the environments at both transcriptional and protein level. Mn in the root tips comes from the upper plant through phloem transport, and is distributed by OsNramp3 at the basal nodes. In plant species other than rice, transporters for Mn uptake have also been reported, but their spatial tissue and cellular localizations remain unknown, and thus the uptake system for Mn is not yet fully understood.

In fact, barley is much less tolerant to high Mn levels and accumulates much less Mn in the shoots than does rice [39]. Rice can tolerate up to 5000 mg Mn kg^{-1} in the shoots without showing any symptoms of toxicity, whereas Mn concentrations of >150 mg Mn kg^{-1} lead to Mn toxicity symptoms in barley [40]. However, rice is able to deal with varying Mn concentrations in the environment through a Mn transporter localized at the node for distribution, as described below.

Altered Distribution of Mn in Response to Environmental Changes in Mn Levels

In rice, after uptake by the roots, most Mn is translocated from the roots to the shoots and then delivered to various tissues. Recent studies show that the node, a junction of vasculatures connecting the leaf, stem, and panicle, plays an important role in distribution of mineral elements in gramineous plants such as rice and barley [41]. Nodes have complex but well-organized vascular bundles that are connected to each other and that are responsible for selective distribution of mineral elements. In rice, a member of the Nramp family, OsNramp3, was found to be involved in Mn distribution [31]. OsNramp3 is a plasma membrane-localized transporter specific for Mn [31]. It is highly expressed in the nodes, and is localized in xylem transfer cells and other parenchyma cells of **enlarged vascular bundles** (**EVBs**), as well as in the phloem region of **diffuse vascular bundles** (**DVBs**) in the basal nodes and upper nodes (Figure 2). Interestingly, OsNramp3 mRNA levels are not affected by Mn concentrations, but the

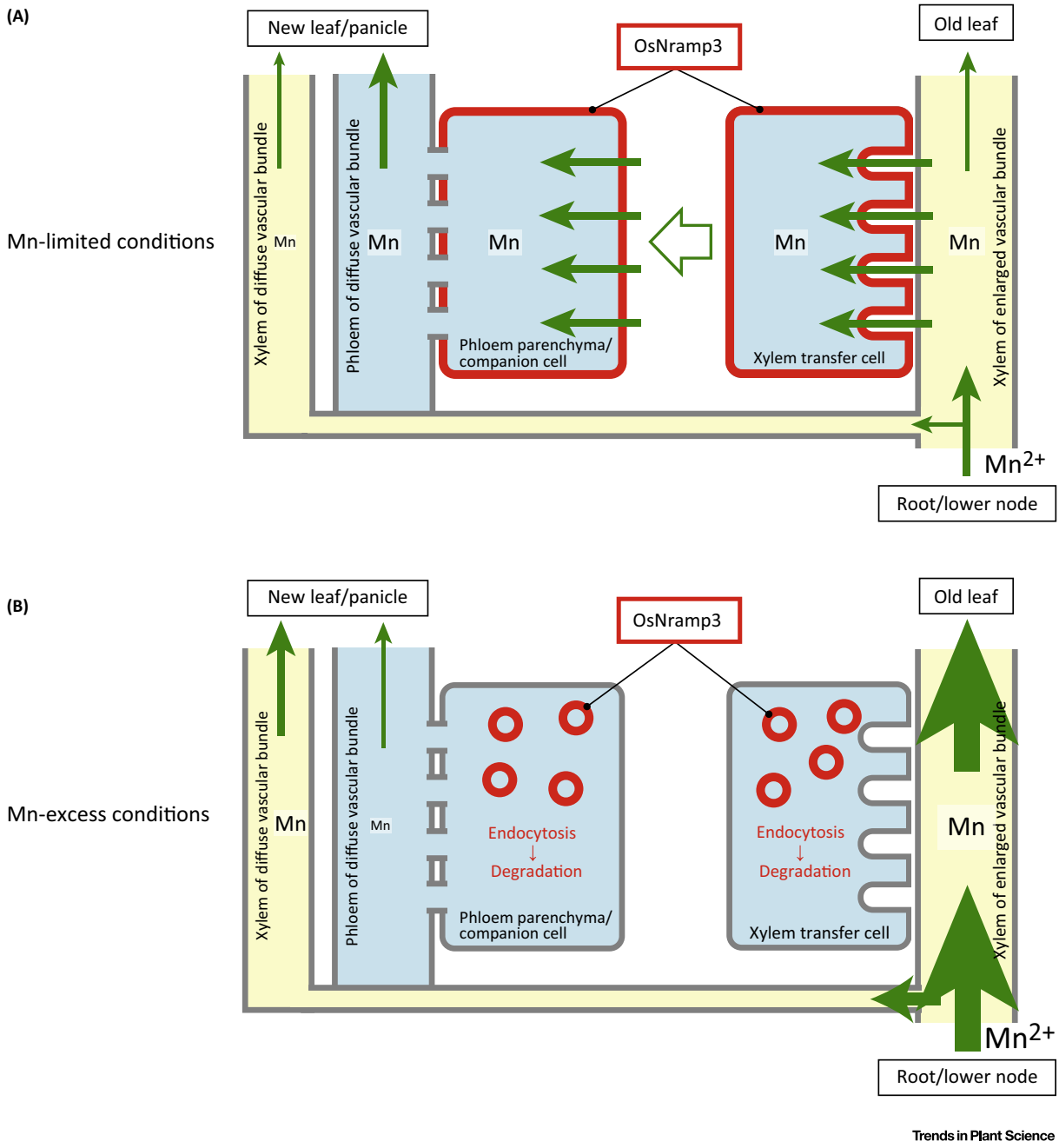

Figure 2. Node-Mediated Regulation of Mn Distribution in Response to Changing Mn Concentrations in Rice. OsNramp3 localized in the nodes functions as a switch for distributing Mn which turns the protein on or off in response to fluctuating Mn concentrations. (A) Under Mn-limited conditions OsNramp3 mediates preferential distribution of Mn to developing tissues, including young leaves, crown root tips, and panicles. (B) By contrast, under Mn-excess conditions, OsNramp3 is rapidly internalized and degraded to avoid Mn toxicity caused by overaccumulation in active developing tissues, and Mn is then delivered to old leaves in a transpiration-dependent manner.

protein is rapidly degraded within a few hours in response to high Mn levels (Figure 2). Phenotypic analysis using knockout lines showed that, at low Mn concentrations, OsNramp3 preferentially transports Mn to young leaves and panicles; however, at high Mn concentrations Mn is delivered to mature tissues. Therefore, OsNramp3 in rice nodes functions as a switch for Mn distribution, which turns on or off the protein in response to fluctuating Mn concentrations.

These findings indicate that rice deals with variable Mn concentrations by regulating Mn distribution through post-translational regulation of OsNramp3-mediated transport rather than via Mn uptake. This is the first example illustrating the response of plants to environmental Mn changes through regulation of the transporter involved in Mn distribution in the nodes. It will be interesting to examine whether a similar regulatory mechanism is present in other plant species.

A different type of transporter in rice, OsYSL2, is also implicated in long-distance transport and distribution of Mn [42]. OsYSL2 is a member of the yellow stripe-like family and transports Mn^{2+}–nicotianamine as well as Fe^{2+}–nicotianamine complexes [43]. This transporter is mainly expressed in leaves, flowers, and developing seeds, but not in roots. Expression of *OsYSL2* is induced by Fe deficiency, but its response to Mn deficiency or excess has not been examined. Overexpression of *OsYSL2* resulted in increased Mn levels in the grain [42]. Because OsYSL2 is localized at the phloem companion cells, it is probably involved in phloem loading of Mn–nicotianamine, although its exact role in rice needs to be further investigated.

Detoxification of Mn in Above-Ground Parts

Plants must detoxify Mn levels which exceed the requirement for healthy growth. Chelation and compartmentalization of Mn in the vacuoles, endoplasmic reticulum (ER), or Golgi play crucial roles in Mn tolerance [44]. Two transporters (OsYSL6 and OsMTP8.1) are involved in Mn detoxification in rice leaves (Figure 3). OsYSL6 transports Mn–nicotianamine complexes [25]

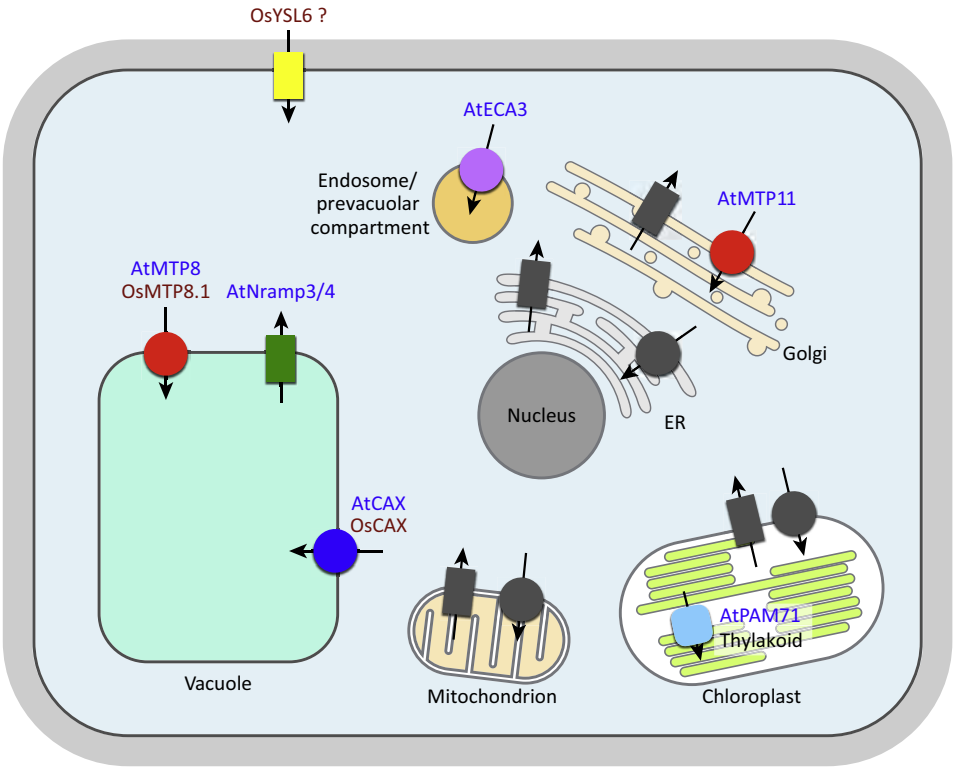

Figure 3. Mn Transporters for Detoxification and Trafficking. OsYSL6 has been implicated in transport of Mn–nicotianamine complexes from apoplast to symplast in rice leaves. Mn in the cytosol is subsequently sequestered by OsMTP8.1/AtMTP8 in rice/*Arabidopsis*, by CAX at the tonoplast, and by AtECA3 to the endosome/prevacuolar compartments. AtNramp3/4 is responsible for export of Mn from the vacuoles, AtMTP11 is involved in the secretory pathway, and AtPAM71 is required for efficient Mn uptake into the thylakoids. Mn is also present in various organelles, but the influx (square) and efflux (circle) transporters have not been identified. Abbreviation: ER, endoplasmic reticulum.

while OsMTP8.1 transports Mn^{2+} [26]. The subcellular localization of OsYSL6 is not known, but it has been suggested to be localized to the plasma membrane based on phenotypic analysis of knockout lines [25]. By contrast, OsMTP8.1, a member of the **cation diffusion facilitator** (**CDF**) family, is localized to the tonoplast [26]. Knockout of either *OsYSL6* or *OsMTP8.1* resulted in increased sensitivity to high Mn concentrations [25,26]. Furthermore, the OsYSL6 mutant shows increased Mn concentrations in leaf apoplasts, but decreased Mn concentrations in **symplasts**. Therefore, in rice OsYSL6 seems to transport Mn–nicotianamine from apoplast to symplast, whereas OsMTP8.1 is responsible for sequestration of Mn into the vacuoles (Figure 3).

The expression of *OsYSL6* did not respond to low (0.05 μM) or high Mn (1000 μM), showing constitutive expression. However, expression levels are higher in older leaves with high Mn concentrations than in young leaves with low Mn concentrations [25]. By contrast, the expression level of *OsMTP8.1* mRNA and its encoding protein level are slightly enhanced by high Mn concentrations [26]. These findings suggest that the transporters involved in Mn detoxification show only a limited response to changing Mn concentrations.

In addition to OsMTP8.1, other CDF members such as ShMTP1 in *Stylosanthes hamata* and AtMTP11 in *Arabidopsis* also confer Mn tolerance. ShMTP1 is localized to the tonoplast like OsMTP8.1 [45], while AtMTP11 is targeted to prevacuolar compartments or Golgi-like compartments [46,47] (Figure 3). They are also involved in sequestration of Mn into vacuoles or the Golgi for detoxification. Recently, AtMTP8 was identified as a vacuolar Mn transporter that could counter Mn toxicity in *Arabidopsis* [48]. Furthermore, there is a strong link between the regulation of this transporter and Fe deficiency. CsMTP8 in cucumber is also involved in Mn tolerance [49]. Although CsMTP8 is also localized to the tonoplast, unlike OsMTP8.1 which is mainly expressed in the shoots, CsMTP8 is highly expressed in the roots. Furthermore, its expression is upregulated by Mn excess [49]. It seems that most MTP members have similar functions in sequestering Mn into organelles, but have different roles depending on the plant species.

Some members of **cation exchanger** (**CAX**) protein family have also been implicated in vacuolar sequestration of Mn. Rice OsCAX1 and OsCAX3 proteins conferred Mn tolerance to yeast cells [50]. Expression of AtCAX2 from *Arabidopsis* in tobacco (*Nicotiana tabacum*) enables more Mn to accumulate in vacuoles and increases Mn tolerance compared to the wild type [51]. However, their exact role *in planta* remains unclear. CAX transporters have low affinity for Mn [52], and therefore CAX proteins may only play a role at high Mn concentrations [53]. By contrast, AtNramp3 and AtNramp4 localized at the tonoplast are necessary for Mn export from the vacuole into the mesophyll cells of adult plant leaves [54]. Their protein level is not affected by Mn supply. On the other hand, it is well known that some plant species, termed Mn hyperaccumulators, are able to accumulate more than 10 000 mg kg^{-1} Mn of dry weight in their above-ground tissues without evident toxicity. For example, several *Gossia* species accumulate 10 000–35 000 mg Mn kg^{-1} in the leaves [55], but little is known about the molecular mechanisms underlying Mn hyperaccumulation [56]. Both proteins and organic acids (e.g., tartaric acid) are suggested to play important roles in detoxification in *Eucalyptus grandis* × *E. urophylla* [57], but their exact roles need to be further confirmed.

Concluding Remarks and Future Perspectives

Over the past 10 years great progress has been made, especially in rice, in understanding plant responses to changes in environmental Mn concentrations. However, our understanding of many aspects of Mn transport and homeostasis remains incomplete (see Outstanding Questions). Various transporter families including Nramp, YSL, ZIP, CAX, CCX (calcium cation

Outstanding Questions

Under submerged condition, both Mn and Fe availability are greatly increased because of reducing anaerobic conditions, but why does rice preferentially accumulate more Mn than Fe?

Why do most plants accumulate higher levels of Mn than they need for their growth?

Mn concentrations in soil solution vary by a factor of 100 over the growth period, but how does rice deal with this large fluctuation?

Some plant species accumulate more than 10 000 mg Mn g^{-1} in the shoots without showing any symptoms of toxicity, what mechanisms underlie internal detoxification of Mn?

exchangers), CDF/MTP, VIT (vacuolar iron transporter), and P-type ATPases have been implicated in Mn uptake and transport within plants [53,58], but the exact roles of these transporters *in planta* are unknown, although some of them have been characterized in heterologous systems (e.g., yeast, *Arabidopsis*). One must be cautious in the interpretation of heterologous results because the ability of these proteins to transport a particular cation does not necessarily mean that this mode of cation transport is physiologically relevant for the plants [53]. At the organ and tissue level, Mn transporters have not so far been identified for xylem loading and unloading, phloem loading and unloading. Efflux transporters for intervascular transfer in the nodes and transporters for loading to the seeds also remain to be identified. At the cellular level, although Mn is found in many organelles including the chloroplast, mitochondria, Golgi apparatus, and vacuoles [53], most transporters for transporting Mn in or out these organelles remain to be identified, except for some transporters such as AtECA3 at endosome/prevacuolar compartments [59], AtMTP11 in the Golgi apparatus [47], and PAM71 in the thylakoids [60]. The response of these elusive transporters to environmental Mn changes also need to be investigated in the future.

At least 22 Mn hyperaccumulator species have been identified so far [61]. However, the mechanisms underlying Mn hyperaccumulation and their responses to changing Mn levels in the environment are poorly understood. Transporters for uptake, translocation, distribution, and internal detoxification are necessary for Mn hyperaccumulation, but none of these has been identified. The distribution of Mn in the hyperaccumulators at both the cellular and organ levels remains poorly understood. Mn in some Mn hyperaccumulators is deposited in non-photosynthetic tissues such as trichomes and epidermal tissues for detoxification [61], but the underlying molecular mechanisms are unknown. Because no genome sequences are available for most of these Mn hyperaccumulators, and there are also no mutant resources, it is difficult to study the molecular mechanisms of Mn hyperaccumulation in these species. However, recent rapid advances in sequencing technologies such as RNA-seq may help to elucidate the mechanisms underlying hyperaccumulation. For example, in *Noccaea caerulescens,* a cadmium (Cd) hyperaccumulator, it was found that the detoxification of Cd is achieved by increasing the copy numbers of a tonoplast-localized Cd^{2+} transporter gene, *NcHMA3* [62]. It would be interesting to investigate in future whether similar mechanisms are present in Mn-hyperaccumulator species. Because both Mn deficiency and toxicity are limiting factors for crop production in agriculture, for the future a better understanding of Mn homeostasis in plants will help to improve Mn use efficiency and/or Mn tolerance of crops on problem soils such as alkaline or reducing soils.

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References

1. McHargue, J.S. (1922) The role of manganese in plants. *J. Am. Chem. Soc* 44, 1592–1598
2. Kemmerer, A. (1931) Studies on the relation of manganese to the nutrition of the mouse. *J. Biol. Chem.* 92, 623–630
3. Burnell, J.C. *et al.* (1988) The biochemistry of manganese in plants. In *Manganese in Soil and Plants* (Graham, R.D., ed.), pp. 125–137, Kluwer Academic Publishers
4. Schmidt, S.B. *et al.* (2016) Manganese deficiency in plants: the impact on photosystem II. *Trends Plant Sci.* 21, 622–632
5. Broadley, M. *et al.* (2012) Function of nutrients: micronutrients. In *Marschner's Mineral Nutrition of Higher Plants* (Marschner, P., ed.), pp. 191–248, Academic Press
6. Mengel, K. and Kirkby, E.A. (1987) Manganese in crop nutrition. In *Principles of Plant Nutrition*, (4th edn), pp. 521–523, International Potash Institute
7. Epstein, E. and Bloom, A.J. (2005) Inorganic components of plants. In *Mineral Nutrition of Plants: Principles and Perspectives*, (2nd edn), pp. 41–66, Sinauer Associates
8. Millaleo, R. *et al.* (2010) Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *J. Soil. Sci. Plant. Nut.* 10, 476–494
9. Eaton, T.E. (2015) Manganese. In *Handbook of Plant Nutrition* (Barker, A.V. and Pilbeam, D.J., eds), pp. 427–485, CRC Press

10. Wissemeier, A.H. and Horst, W.J. (1987) Callose deposition in leaves of cowpea (*Vigna unguiculata* [L] *Walp*) as a sensitive response to high Mn supply. *Plant Soil* 102, 283–286
11. Fuhrs, H. *et al.* (2009) Characterization of leaf apoplastic peroxidases and metabolites in *Vigna unguiculata* in response to toxic manganese supply and silicon. *J. Exp. Bot.* 60, 1663–1678
12. Edwards, D.G. and Asher, C.J. (1981) Tolerance of crop and pasture species to manganese toxicity. In *Proceedings of the Ninth International Plant Nutrition Coloquium* (Scaife, A., ed.), pp. 145–150, Warwick University
13. Rengel, Z. (2015) Availability of Mn, Zn and Fe in the rhizosphere. *J. Soil Sci. Plant Nutri.* 15, 397–409
14. Clarkson, D.T. *et al.* (1988) The uptake and translocation of manganese by plant roots. In *Manganese in Soil and Plants* (Graham, R.D., ed.), pp. 101–111, Kluwer Academic Publishers
15. Marschner, H. (1995) Manganese. In *Mineral Nutrition of Higher Plants*, (2nd edn), pp. 324–333, Academic Press
16. Barber, S.A. (1995) *Soil Nutrient Bioavailability. A Mechanistic Approach,* John Wiley & Sons
17. Kochian, L.V. *et al.* (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol* 55, 459–493
18. Huang, X. *et al.* (2015) Linking waterlogging tolerance with Mn^{2+} toxicity: a case study for barley. *Plant Biol.* 17, 26–33
19. Lynch, J.P. and St Clair, S.B. (2004) Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. *Field Crop Res.* 90, 101–115
20. Ma, J.F. *et al.* (2014) Molecular mechanisms of Al tolerance in gramineous plants. *Plant Soil* 381, 1–12
21. Lambers, H. *et al.* (2015) Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends Plant Sci.* 20, 83–90
22. Mansfeldt, T. (2004) Redox potential of bulk soil and soil solution concentration of nitrate, manganese, iron, and sulfate in two gleysols. *J. Plant Nutri. Soil Sci.* 167, 7–16
23. Zhang, X.K. *et al.* (1998) Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.). Zinc uptake by Fe-deficient rice. *Plant Soil* 202, 33–39
24. Kirk, G.J.D. (2003) Rice root properties for internal aeration and efficient nutrient acquisition in submerged soil. *New Phytol.* 159, 185–194
25. Sasaki, A. *et al.* (2011) OsYSL6 is involved in the detoxification of excess manganese in rice. *Plant Physiol.* 157, 1832–1840
26. Chen, Z.H. *et al.* (2013) Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. *J. Exp. Bot.* 64, 4375–4387
27. Sasaki, A. *et al.* (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell* 24, 2155–2167
28. Ueno, D. *et al.* (2015) A polarly localized transporter for efficient manganese uptake in rice. *Nat. Plants* 1, 15170
29. Ma, J.F. and Yamaji, N. (2015) A cooperative system of silicon transport in plants. *Trends Plant Sci.* 20, 435–442
30. Sakurai, G. *et al.* (2015) *In silico* simulation modeling reveals the importance of the casparian strip for efficient silicon uptake in rice roots. *Plant Cell Physiol.* 56, 631–639
31. Yamaji, N. *et al.* (2013) A node-based switch for preferential distribution of manganese in rice. *Nat. Commun.* 4, 2442
32. Sasaki, A. *et al.* (2014) Transporters involved in mineral nutrient uptake in rice. *J. Exp. Bot.* 67, 3645–3653
33. Barberon, M. *et al.* (2011) Monoubiquitin-dependent endocytosis of the IRON-REGULATED TRANSPORTER 1 (IRT1) transporter controls iron uptake in plants. *Proc. Natl. Acad. Sci. U. S. A.* 108, E450–E458
34. Takano, J. *et al.* (2005) Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *Proc. Natl. Acad. Sci. U. S. A.* 102, 12276–12281
35. Cailliatte, R. *et al.* (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. *Plant Cell* 22, 904–917
36. Milner, M.J. *et al.* (2013) Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *J. Exp. Bot.* 64, 369–381
37. Pedas, P. *et al.* (2008) Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. *Plant Physiol.* 148, 455–466
38. Wu, D. *et al.* (2016) The HvNramp5 transporter mediates uptake of cadmium and manganese, but not iron. *Plant Physiol.* 172, 1899–1910
39. Fuhrs, H. *et al.* (2010) Physiological and proteomic characterization of manganese sensitivity and tolerance in rice (*Oryza sativa*) in comparison with barley (*Hordeum vulgare*). *Ann. Bot.* 105, 1129–1140
40. Vlamis, J. and Williams, D.E. (1964) Iron and manganese relations in rice and barley. *Plant Soil* 20, 221–231
41. Yamaji, N. and Ma, J.F. (2014) The node, a hub for mineral nutrient distribution in graminaceous plants. *Trends Plant Sci.* 19, 556–563
42. Ishimaru, Y. *et al.* (2010) Rice metal–nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *Plant J.* 62, 379–390
43. Koike, S. *et al.* (2004) OsYSL2 is a rice metal–nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J.* 39, 415–424
44. Williams, L.E. and Pittman, J.K. (2010) Dissecting pathways involved in manganese homeostasis and stress in higher plants. In *Cell Biology of Metals and Nutrients* (Hell, R. and Mendal, R.R., eds), pp. 95–117, Springer-Verlag
45. Delhaize, E. *et al.* (2003) Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *Plant Cell* 15, 1131–1142
46. Delhaize, E. *et al.* (2007) A role for the AtMTP11 gene of *Arabidopsis* in manganese transport and tolerance. *Plant J.* 51, 198–210
47. Peiter, E. *et al.* (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *Proc. Natl. Acad. Sci. USA* 104, 8532–8537
48. Eroglu, S. *et al.* (2016) The vacuolar manganese transporter MTP8 determines tolerance to iron deficiency-induced chlorosis in *Arabidopsis*. *Plant Physiol.* 170, 1030–1045
49. Migocka, M. *et al.* (2014) Cucumber metal transport protein MTP8 confers increased tolerance to manganese when expressed in yeast and *Arabidopsis thaliana*. *J. Exp. Bot.* 65, 5367–5384
50. Kamiya, T. *et al.* (2005) Expression profile of the genes for rice cation/H^+ exchanger family and functional analysis in yeast. *Plant Cell Physiol.* 46, 1735–1740
51. Hirschi, K.D. *et al.* (2000) Expression of *Arabidopsis* CAX2 in tobacco. Altered metal accumulation and increased manganese tolerance. *Plant Physiol.* 124, 125–133
52. Shigaki, T. *et al.* (2003) Manganese specificity determinants in the *Arabidopsis* metal/H^+ antiporter CAX2. *J. Biol. Chem.* 278, 6610–6617
53. Pittman, J.K. (2005) Managing the manganese: molecular mechanisms of manganese transport and homeostasis. *New Phytol.* 167, 733–742
54. Lanquar, V. *et al.* (2010) Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is required for optimal photosynthesis and growth under manganese deficiency. *Plant Physiol.* 152, 1986–1999
55. Fernando, D.R. *et al.* (2009) Foliar Mn accumulation in eastern Australian herbarium specimens: prospecting for 'new' Mn hyperaccumulators and potential applications in taxonomy. *Ann. Bot.* 104, 357–357
56. Fernando, D.R. and Lynch, J.P. (2015) Manganese phytotoxicity: new light on an old problem. *Ann. Bot.* 116, 313–319
57. Xie, Q.Q. *et al.* (2015) A newly identified passive hyperaccumulator *Eucalyptus grandis* × *E. urophylla* under manganese stress. *PLoS One* 10, e0136606
58. Socha, A.L. and Guerinot, M.L. (2014) Mn-euvering manganese: the role of transporter gene family members in manganese uptake and mobilization in plants. *Front. Plant Sci.* 5, 106

59. Li, X. *et al.* (2016) A distinct endosomal Ca^{2+}/Mn^{2+} pump affects root growth through the secretory process. *Plant Physiol.* 147, 1675–1689

60. Schneider, A. *et al.* (2016) The evolutionarily conserved protein PHOTOSYNTHESIS AFFECTED MUTANT71 is required for efficient manganese uptake at the thylakoid membrane in *Arabidopsis*. *Plant Cell* 28, 892–910

61. Fernando, D.R. *et al.* (2013) Microbeam methodologies as powerful tools in manganese hyperaccumulation research: present status and future directions. *Front. Plant Sci.* 4, 319

62. Ueno, D. *et al.* (2011) Elevated expression of *TcHMA3* plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *Plant J.* 66, 852–862