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Zn Uptake and Translocation in Rice Plants

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Abstract Zinc (Zn) is an essential micronutrient with numerous cellular functions in plants, and its deficiency represents one of the most serious problems in human nutrition worldwide. Zn deficiency causes a decrease in plant growth and yield. On the other hand, Zn could be toxic if excess amounts are accumulated. Therefore, the uptake and transport of Zn must be strictly regulated. In this review, the dominant fluxes of Zn in soil–root–shoot translocation in rice plants (*Oryza sativa*) are described, including Zn uptake from soils in the form of Zn^{2+} and Zn-DMA at the root surface, and Zn translocation to shoots. Knowledge of these fluxes could be helpful to formulate genetic and physiologic strategies to address the widespread problem of Zn-limited crop growth.

Keywords Zn · Rice · Metal transport

Zinc in biology

The variety of roles that zinc (Zn) plays in cellular processes is a good example of the diverse biological utility of metal ions. Zn is involved in protein, nucleic acid, carbohydrate, and lipid metabolism. In addition, Zn is critical to the control of gene transcription and the coordination of other biological processes regulated by proteins containing DNA-binding Zn-finger motifs (Rhodes and Klug 1993), RING fingers, and LIM domains (Vallee and Falchuk 1993). Several molecules associated with DNA and RNA synthesis are also Zn metalloenzymes, such as RNA polymerases (Wu et al. 1992), reverse transcriptases, and transcription factors (Wu and Wu 1989). Zn is a non-redox-active ion and is therefore targeted to transcription factors and other enzymes involved in DNA metabolism, as the use of redox-active metal ions for these tasks could lead to radical reactions and nucleic acid damage. However, these processes must be tightly regulated to ensure that the correct amount of Zn is present at all times. Although it is an essential nutrient, Zn could be toxic if it accumulates in excess. The precise cause of Zn toxicity is unknown, but the metal may bind to inappropriate intracellular ligands, or compete with other metal ions for enzyme active sites or transporter proteins. In order to play such diverse roles in cells, and because it cannot passively diffuse across cell membranes, Zn must be transported into the intracellular compartments of the cell where it is required for these Zn-dependent processes. A group of proteins called Zn transporters is dedicated to the transport of Zn across biological membranes.

Studies of Zn uptake in biology are critical because Zn is essential for all organisms, including humans (Hambidge 2000). As Zn plays multiple roles in plant biochemical and physiological processes, even slight deficiency causes a decrease in growth, yield, and Zn content of edible plant parts. Zn deficiency is a serious agricultural problem as around one half of the cereal-growing soils in the world contain low Zn in the soil (Graham and Welch 1996; Cakmak et al. 1999). In soil, Zn is present in various forms. Among the total soil Zn content, Zn primary minerals constitute around 15%, Zn organic matter complexes around 45%, outer-sphere complexes around 20%, Zn-sorbed phosphate around 10%, and Zn-sorbed iron oxyhydroxides around 10% (Sarret et al. 2004). The solubility and availability of Zn is

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determined by various factors like high CaCO3, high pH, high clay soils, low organic matter, low soil moisture, and high iron and aluminum oxides (Cakmak 2008).In low Zn soils, the Zn uptake may be enhanced by the exudation of low-molecular-weight compounds like malate and mugineic acid family phytosiderophores (Arnold et al. 2010; Cakmak et al. 1994; Peng et al. 2009; Suzuki et al. 2006; Walter et al. 1994; Widodo et al. 2010; Zhang et al. 1989).

Food Zn content is very important for human health as the artificial supplementation of foods with essential minerals is often difficult to achieve, particularly in developing countries. Therefore, it has been suggested that increased levels of Zn in staple foods, e.g., rice may play a role in reducing Zn deficiency (Ruel and Bouis 1998; Graham et al. 1999; Welch and Graham 1999). Therefore, it is essential to understand the molecular mechanism through which plants mobilize, take up, translocate, and store Zn.

ZIP transporters

Higher plants take up Zn from the rhizosphere via transporters, and molecular aspects of this phenomenon are now being clarified. In the *Arabidopsis thaliana* genome, a large number of cation transporters potentially involved in metal ion homeostasis have been identified (Maser et al. 2001). Several members of the Zn-regulated transporters in the iron (Fe)-regulated transporter-like protein (ZIP) gene family (Guerinot 2000) have been characterized and shown to be involved in metal uptake and transport in plants (Eide et al. 1996; Korshunova et al. 1999; Vert et al. 2001, 2002; Connolly et al. 2002). The ZIP proteins are predicted to have eight transmembrane domains, with their amino- and carboxyl-terminal ends situated on the outer surface of the plasma membrane (Guerinot 2000). These proteins vary considerably in overall length due to a variable region between the transmembrane domains (TM) TM-3 and TM-4, which is predicted to be on the cytoplasmic side, providing a potential metal-binding domain rich in histidine residues. The most-conserved region of these proteins lies in a variable region that has been predicted to form an amphipathic helix, containing a fully conserved histidine that may form part of an intramembranous metal-binding site involved in transport (Guerinot 2000). The transport function is disabled when the conserved histidines or certain adjacent residues are replaced by mutation (Rogers et al. 2000).

ZIP1, ZIP3, and ZIP4 from *Arabidopsis* restore Zn uptake to the yeast (*Saccharomyces cerevisiae*) Zn-uptake mutant, Δ*zrt1*Δ*zrt2*, and have been proposed to play a role in Zn transport (Grotz et al. 1998; Guerinot 2000). *ZIP1* and *ZIP3* are expressed in roots in response to Zn deficiency, suggesting that they transport Zn from the soil to the plant, while *ZIP4* is expressed in both roots and shoots, suggesting that it transports Zn intracellularly or between plant tissues (Grotz et al. 1998; Guerinot 2000). ZIP2 and ZIP4 rescue yeast mutants deficient in copper (Cu) transport, and *ZIP4* is up-regulated in Cu-deficient roots (Wintz et al. 2003). Yeast Zrt1 and Zrt2 are high- and low-affinity Zn transporters, respectively (Eide 1998; Guerinot 2000). The proposed role of ZIP transporters in Zn nutrition has been further supported by the characterization of homologs from several plant species. For example, GmZIP1 has been identified in soybean (*Glycine max*; Moreau et al. 2002), and functional complementation of Δ*zrt1*Δ*zrt2* yeast cells showed that GmZIP1 is highly selective for Zn, but not for Fe or manganese (Mn). GmZIP1 is expressed specifically in nodules, but not in roots, stems, or leaves, and the protein is localized to the peribacteroid membrane, suggesting a role in symbiosis. In barley, Zn transporters are induced by low pH (Pedas and Husted 2009).

In rice plants (*Oryza sativa*), several *ZIP* transporter genes have been reported, e.g., *OsIRT1*, *OsIRT2*, *OsZIP1*, *OsZIP3*, *OsZIP4*, and *OsZIP5* (Fig. 1; Ishimaru et al. 2006; Lee et al. 2010; Ramesh et al. 2003; Yang et al. 2009). *OsZIP1*, *OsZIP3*, *OsZIP4*, and *OsZIP5* were found to be rice Zn transporters induced by Zn deficiency (Ramesh et al. 2003; Lee et al. 2010; Ishimaru et al. 2006). *OsZIP1*, *OsZIP3*, and *OsZIP4* are expressed in the vascular bundles in shoots and in the vascular bundles and epidermal cells in roots (Ramesh et al. 2003; Ishimaru et al. 2006). In situ hybridization analysis revealed that *OsZIP4* in Zn-deficient rice was expressed in the meristem of Zn-deficient roots and shoots, and also in vascular bundles of the roots and shoots. These results suggest that OsZIP4 is a Zn transporter that may be responsible for Zn translocation to the plant parts that require Zn.

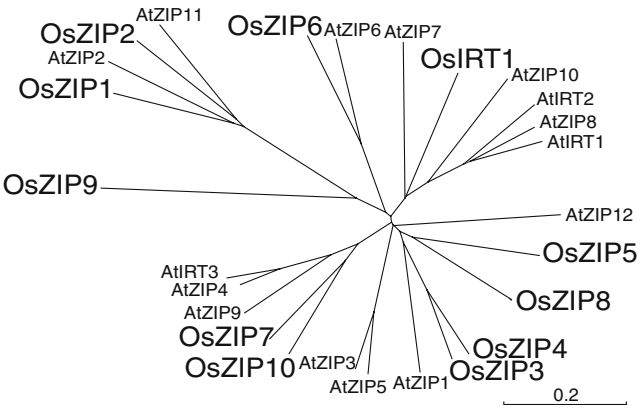

Fig. 1 Unrooted phylogenic tree that highlights the relationship among the ZIP transporter proteins in rice plants and *A. thaliana*. Unrooted phylogenic tree for the OsIRT1, OsZIPs, AtIRTs, and AtZIPs. Calculations were performed using the CLUSTAL W neighbor-joining method and the tree was visualized with TreeView.

Furthermore, we have produced transgenic rice plants overexpressing *OsZIP4* under the control of the CaMV 35S promoter (Ishimaru et al. 2007). Compared to control plants, Zn concentration in *35S-OsZIP4* transgenic plants was higher in roots and lower in shoots, suggesting that *OsZIP4* expression driven by the 35S promoter in the *35S-OsZIP4* root may be involved in xylem unloading and reducing the Zn transport to shoots. Northern blot analysis revealed that transcripts of *OsZIP4* expression driven by the CaMV 35S promoter were detected in roots and shoots of *35S-OsZIP4* transgenic plants, but endogenous *OsZIP4* transcripts were rare in roots, and abundant in shoots. Microarray analysis revealed that the genes expressed in shoots of *35S-OsZIP4* transgenic plants coincided with those induced in shoots of Zn-deficient plants. Similar results were reported for *OsZIP5* overexpression plants, which accumulated more Zn in roots, whereas the shoot Zn accumulation decreased. The *OsZIP5OX* plants were sensitive to Zn excess, while the *Oszip5* knock out plants were tolerant to Zn excess. *OsZIP1* and *OsZIP3* are primarily associated with Zn uptake in roots and Zn homeostasis in shoots (Ramesh et al. 2003). *OsZIP4* is involved in the translocation of Zn, particularly into vascular bundles and meristem (Ishimaru et al. 2005).

Contribution of MAs to Zn uptake and translocation

The mugineic acid family phytosiderophores (MAs), play a major role in Fe acquisition from their roots to solubilize sparingly soluble Fe in the rhizosphere. Welch (1995) proposed that MAs may also contribute to the acquisition of Zn and other metal nutrients by graminaceous plants. It was reported that Zn deficiency increases the secretion of MAs from wheat (*Triticum* spp.) and barley (*Hordeum vulgare*) roots into the rhizosphere (Cakmak et al. 1994; Walter et al. 1994; Zhang et al. 1989, Suzuki et al. 2006). In rice line RIL46, Zinc-deficiency tolerance to some extent is due to the increased efflux of MAs (Widodo et al. 2010). The biosynthesis of MAs and their corresponding genes has been characterized. MAs are synthesized from methionine (Mori and Nishizawa 1987; Fig. 2). *S*-adenosyl-*L*-methionine (SAM) synthetase converts methionine into SAM. Subsequently, three molecules of SAM are combined to form one molecule of nicotianamine (NA) by NA synthase (NAS; EC 2.5.1.43). NA is then converted to 3″-keto acid by NA aminotransferase (NAAT; EC 2.6.1.80), and 2′-deoxymugineic acid (DMA) is synthesized by DMA synthase (DMAS; Bashir et al. 2006; Bashir and Nishizawa 2006). The synthesized MAs are secreted to the rhizosphere by TOM1 (Nozoye et al. 2011). In some graminaceous species, including barley, DMA is further hydroxylated by two dioxygenases, IDS2 (EC 1.14.11.25) and IDS3 (EC 1.14.11.24; Nakanishi et al. 2000; Kobayashi et al. 2001). The genes encoding the enzymes involved in DMA synthesis have been well characterized in Fe-deficient rice and barley. The expression of *HvNAS1*, *HvNAAT-A*, *HvNAAT-B*, *HvDMAS1*, *HvIDS2*, and *HvIDS3* is elevated in Fe-deficient barley roots (Nakanishi et al. 1993; Okumura et al. 1994; Higuchi et al. 1999; Takahashi et al. 1999; Bashir et al. 2006). In rice, the expression of *OsNAS1*, *OsNAS2*, *OsNAAT1*, *OsDMAS1* and *TOM1* increases in both roots and shoots by Fe deficiency (Inoue et al. 2003; Inoue et al. 2008; Bashir et al. 2006; Nozoye et al. 2011). The expression of *OsNAS3* increases in Fe-deficient roots, but decreases in Fe-deficient shoots (Inoue et al. 2003). Promoter-β-glucuronidase (GUS) analysis suggests that *OsNAS1* and *OsNAS2* and TOM1 are involved in DMA secretion as these genes are expressed in all root cells (Bashir et al. 2006; Inoue et al. 2003; Nozoye et al. 2011). However, *OsNAS3* may not be involved in DMA secretion because its expression is restricted to the pericycle and companion cells of the roots (Inoue et al. 2003).

Recently, we showed that the expression of *NASHOR2*, a *NAS* gene in barley (Herbik et al. 1999), and *HvNAAT-B* was elevated in Zn-deficient barley shoots, but that the expression of *IDS2* and *IDS3* was not detected in Zn-deficient shoots (Suzuki et al. 2006). However, the expression of *HvNAS1*, *HvNAAT-A*, *HvNAAT-B*, *IDS2*, and *IDS3* was elevated in both Zn- and Fe-deficient roots, while the expression of these genes was not observed in Fe-deficient shoots (Suzuki et al. 2006). Therefore, we suggest that Zn deficiency induces DMA synthesis in barley shoots, while both Zn and Fe deficiency induce MAs synthesis and secretion in barley roots.

YSL transporter

A *yellow stripe 1* (*YS1*) gene important for the uptake of Fe^{3+}-MAs has been identified in maize (Curie et al. 2001). *ZmYS1* functionally complements yeast strains that are defective in Fe uptake on media containing Fe^{3+}-MAs, but

Fig. 2 DMA biosynthesis in rice plants. The enzymes are in *boxes*. *SAM S*-adenosyl-methionine, *NA* nicotianamine, *DMA* deoxymugineic acid.

not on media containing Fe^{3+} citrate, suggesting that *ZmYS1* is a MAs-dependent Fe transporter (Curie et al. 2001). *ZmYS1* has a broad specificity for metals and ligands, and can transport MAs-bound metals, including Zn, Cu, and nickel (Schaaf et al. 2004, Murata et al. 2006). On the other hand, Roberts et al. (2004) reported that *ZmYS1* transported Fe^{3+}- and Cu-MAs, but not Zn-MAs.

Our search for *YS1* homologs in the *O. sativa* L. ssp. *japonica* (cv. Nipponbare) rice genomic database identified 18 putative *OsYSL*s that exhibit 36–76% sequence similarity to *YS1* (Koike et al. 2004). The phloem-specific expression of *OsYSL2* suggests that it is involved in the phloem transport of Fe. OsYSL2 transports Fe^{2+}-NA and Mn^{2+}-NA, but did not transport Fe^{3+}-MAs. These results suggest that *OsYSL2* is a rice metal-NA transporter that is responsible for phloem transport of Fe and Mn. OsYSL15 and OsYSL18 transports Fe (III)-DMA (Aoyama et al. 2009; Inoue et al. 2009).

Until now, a Zn-NA transporter or Zn-MAs transporter involved in Zn translocation has not been identified in rice plants. However, the *ZmYS1*-transport Zn-MAs, NA, and DMA were synthesized in Zn-deficient shoots, and rice plants have 18 putative *OsYSL*s. These data suggest the existence of Zn-NA or Zn-MAs transporters for Zn translocation in rice shoots.

Zn translocation of Zn^{2+} and Zn-DMA

Recently, we showed that Zn-deficient barley roots absorb more Zn-DMA than Zn^{2+} (Suzuki et al. 2006). In contrast, less Zn-DMA than Zn^{2+} was absorbed from the roots of Zn-deficient rice. These findings are correlated with a change in the amount of MAs secreted under Zn deficiency. In barley, MAs secretion is increased by Zn deficiency, and this contributes to the absorption of Zn from the soil. However, in rice, the level of DMA secretion was slightly reduced (Suzuki et al. 2008b); therefore, rice may prefer to absorb Zn^{2+} rather than Zn-DMA.

Moreover, we also showed that the amount of endogenous DMA in rice shoots increase due to Zn deficiency, corresponding to the increased expression of *OsNAS3*, *OsNAAT1*, and *OsDMAS1* in Zn-deficient shoots (Suzuki et al. 2008a, b). In accordance with the dramatic increase in *OsNAAT1* expression in Zn-deficient shoots, the amount of endogenous NA in shoots was remarkably reduced by Zn deficiency. In contrast to the increase in MAs secretion caused by Zn deficiency in barley, the amount of DMA secreted from rice roots slightly decrease. Furthermore, our PETIS experiment clearly show that more ^{62}Zn is translocated to the newest leaf when ^{62}Zn-DMA is supplied compared to the conditions when $^{62}Zn^{2+}$ was supplied, while the opposite tendency was observed at the bottom of the leaf sheath (Suzuki et al. 2008a, b), suggesting that Zn-DMA is the preferred form for long-distance transport of Zn in Zn-deficient rice.

An appreciation for the importance of Zn in molecular biology, structural biology, and nutritional sciences has grown rapidly over the past 15 years (Berg and Shi 1996). However, many questions about the mechanisms that Zn uses in plants still remain, such as the Zn efflux system, Zn homeostasis inside the cell, and transcriptional and post-transcriptional regulation. Further studies will enable us to clarify the mechanism of Zn transport and to manipulate the distribution of Zn to produce crops that can tolerate stress due to Zn deficiency, as well as enhance the Zn content of food crop seeds.

Seed Zn localization

Seed germination is triggered by an array of complex process governed by numerous factors mainly, hormone signaling pathways, light, and water. Germination also involves the movement of metal ions like Zn, so that it may be utilized efficiently. Rice seeds contain not only Zn but also NA and DMA, and the amount of DMA is significantly higher than that of NA (Usuda et al. 2009; Masuda et al. 2009). NA, DMA, and Zn is mobilized during germination in rice (Takahashi et al. 2009). In rice, Zn localizes to embryo, endosperm and to the aleurone layer of the seeds. The Zn content is particularly high in embryo (Takahashi et al. 2009). During germination, Zn in the endosperm decreases while in the embryo, high levels of Zn accumulates in the radicle and leaf primordium (Fig. 3). With time, Zn increases in the scutellum and the vascular bundle of the scutellum. In the scutellum, Zn accumulates to the endosperm similarly to Fe (Takahashi et al. 2009; Bashir et al. 2010). Zn is distributed in the leaf primordium and the root tip, 36 h after germination. Zn is observed to the area assumed to be the junction between the embryo and the dorsal vascular bundle. In a previous report we discussed (Takahashi et al. 2009) that proteins abundant in seeds decrease 1 to 2 days after sowing while biological functions such as respiration become active 3 days after sowing. Microarray analysis of germinating rice seeds suggest that ZIP family members decrease during germination (Nozoye et al. 2007). As Zn accumulation in meristematic tissues is limited in the embryo (Fig. 3). A decrease in *OsZIP* family transcripts might be necessary for this type of partial localization of Zn. Similarly to other members of the rice ZIP family genes, OsZIP4 expression in whole seeds decreased in the 2–3 days after sowing.

Rice genotypes differ greatly in Zn use efficiency and grain Zn contents and this aspect has been investigated by various researchers (Graham et al. 1999; Nagarathna et al. 2010; Neue, et al. 1998; Refuerzo et al. 2009; Wissuwa et

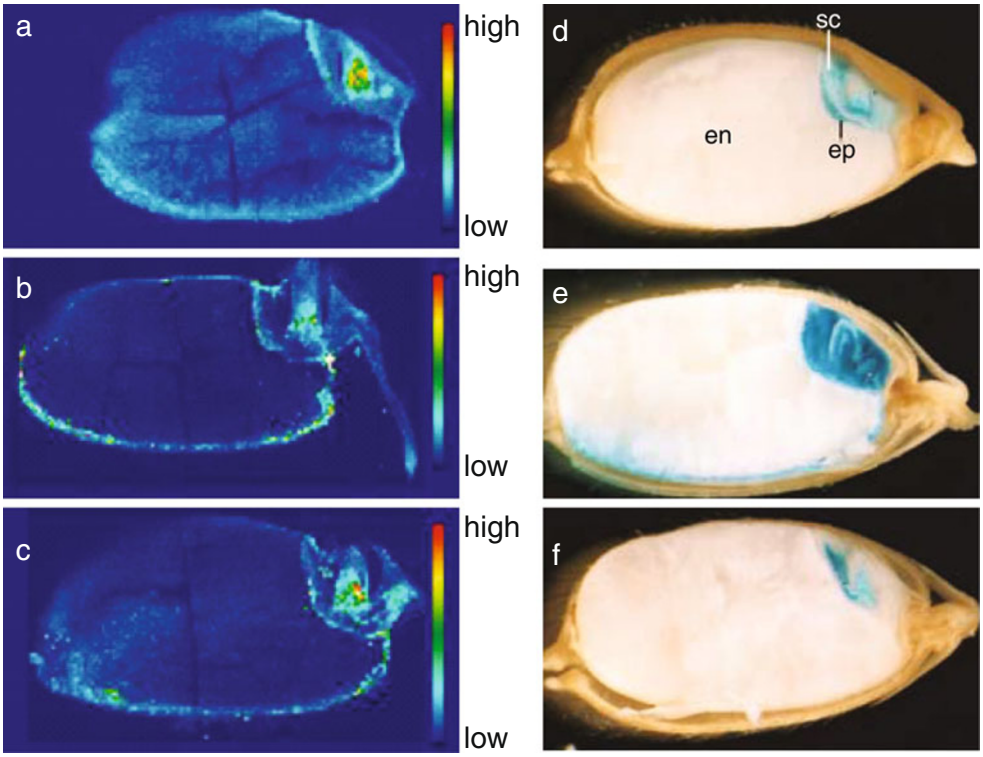

Fig. 3 Zn localization and histochemical localization of GUS activity derived from OsZIP4 promoter. **a**–**c** The normalized X-ray fluorescence intensities scaled from *blue* (minimum) to *red* (maximum). **a** 12 h; **b** 24 h; **c** 36 h after sowing. **d**–**f** ZIP4 promoter-derived GUS expression. **d** 0 days and seeds 1–2 days (**b**–**c**) after sowing. *sc* scutellum, *en* endosperm, *ep* epithelium.

al. 2006; Wissuwa et al. 2008; Yang et al. 1998). Rice grain Zn concentrations ranged from 15.9 to 58.4 mg kg^{-1} (Graham et al. 1999), suggesting ample variation for this trait that might be exploited through conventional breeding. Another study revealed that native soil Zn status is the dominant factor to determine grain Zn concentrations followed by genotype and fertilizer. Depending on soil Zn status, grain Zn concentrations could range from 8 to 47 mg kg^{-1} in a single genotype (Wissuwa et al., 2006; Wissuwa et al., 2008).

The localization and genetic control mechanisms of Zn in Zn-deficient rice plants is summarized in Fig. 4. Using this knowledge, different approaches have been adopted to increase the Zn in rice seed. Transgenic lines harboring *HvNAS1* driven by the *35S* or *actin1* promoter accumulated more Fe and Zn in polished T2 seeds, showing a positive correlation between Fe and NA/DMA concentrations in seeds (Masuda et al. 2009). These results suggest that NA overproduction enhances the translocation of Fe and Zn to rice grains. Higuchi et al. (2001a, b) produced rice lines expressing *HvNAS1*, resulting in an increased NA content in the roots and leaves under Fe-sufficient conditions. However, the seed Fe and Zn concentrations did not increase in plants grown under Fe-deficient conditions in calcareous paddy fields or under Fe-sufficient conditions in andosol paddy fields (Masuda et al. 2008; Suzuki et al. 2008a). Recently, we demonstrated that the expression of *OsYSL2*, if driven by a suitable promoter, resulted in a significant increase in grain Fe (Ishimaru et al. 2010). The expression of *OsYSL2* when controlled by the sucrose transporter promoter increased the Fe concentration in polished rice up to 4.4-fold compared to WT. These results suggest that controlling the temporal and spatial expression could be effective to increase the Zn in rice seeds.

Various groups have already demonstrated the potential to increase the Zn concentration of rice grains. Traditional breeding, maker-assisted breeding, and plant transformation techniques and a combination of these techniques can be further exploited to mitigate the Zn deficiency in plants and humans.

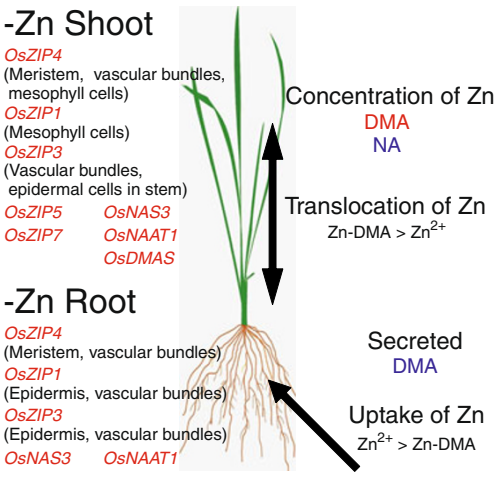

Fig. 4 Summary of the localization and genetic control mechanisms of Zn in Zn-deficient rice plants. *Red letters*: genes or phytosiderophores induced by Zn deficiency. *Blue letters*: phytosiderophores suppressed by Zn deficiency.

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