The molecular basis of zinc homeostasis in cereals

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Abstract

Plants require zinc (Zn) as an essential cofactor for diverse molecular, cellular and physiological functions. Zn is crucial for crop yield, but is one of the most limiting micronutrients in soils. Grasses like rice, wheat, maize, and barley are crucial sources of food and nutrients for humans. Zn deficiency in these species therefore not only reduces annual yield but also directly results in Zn malnutrition of more than two billion people in the world. There has been good progress in understanding Zn homeostasis and Zn deficiency mechanisms in plants. However, our current knowledge in monocots, including grasses, remains insufficient. In this review, we provide a summary of our knowledge on molecular Zn homeostasis mechanisms in monocots, with a focus on important grass crops. We additionally highlight divergences in Zn homeostasis of monocots and the dicot model *Arabidopsis thaliana*, as well as important gaps in our knowledge that need to be addressed in future research on Zn homeostasis in cereal monocots.

Keywords

Zinc, Uptake, Chelation, Transport, Monocot, Dicot, Fe uptake strategy, Rice, Wheat, Barley

Introduction

Gramineae (Grasses), like all life forms, demand optimal zinc (Zn) supply to maintain growth and reproduction (Broadley *et al.*, 2007). Zn is a key cofactor for enzymes involved in various cellular pathways such as gene expression, DNA replication, cell development, and protein synthesis (Gupta *et al.*, 2016). These enzymes contribute to several physiological processes including hormone regulation, chlorophyll synthesis, lipid metabolism, carbohydrate metabolism, pollen formation, and seed development (Vallee and Falchuk, 1993; Hafeez, Khanif and Saleem, 2013; J. Zhang *et al.*, 2019). Zn requirement for plants was first discovered more than a century ago in maize (*Zea mays*, Zm) and then in barley (*Hordeum vulgare*, Hv) (Mazé, 1915; Sommer and Lipman, 1926). Zn deficiency has since been recognized as one of the most common micronutrient deficiencies in crops (Cakmak, 2004; Alloway, 2008). Zn malnutrition has similarly the largest impact among all micronutrients on human health (Black *et al.*, 2008). There is mild to high risk of Zn deficiency on all continents, accounting for half of the world population (Gregory *et al.*, 2017). The annual death of ~800,000 children worldwide is, for instance, attributed to acute Zn deficiency (Akhtar, 2013). Populations that rely primarily on cereal-based diets suffer the most from this so-called hidden hunger (Rawat *et al.*, 2013). Zn uptake from the soil is influenced by a number of characteristics including soil concentration of water-soluble Zn, soil pH, type, moisture, and mineral blend (Broadley *et al.*, 2007; Alloway, 2009; Shahzad, Rouached and Rakha, 2014). Unfortunately, a considerable portion of Zn, *i.e.* two-thirds in wheat (*Triticum aestivum*, Ta), one-third in rice (*Oryza sativa*, Os), and three-quarters in barley, is polished off the seeds before being consumed, while Zn retained in the endosperm is partially bound to phytate and thus poorly bioavailable to humans (Lombi *et al.*, 2011; Swamy *et al.*, 2016; Balk *et al.*, 2019). Moreover, forage grasses constitute a significant share of livestock and poultry feed, and are equally critical for meat-rich diets.

Grasses are the second largest and economically most important family of monocotyledons, where cereal grasses, in particular rice, wheat, maize, and barley, represent the majority of the biomass produced by agriculture (Eckardt, 2000; http://www.fao.org/publications/sofa/2016/en/). However, compared to the dicotyledonous model Arabidopsis (*Arabidopsis thaliana*, At), the molecular basis of Zn homeostasis, unlike iron (Fe) (Kobayashi, 2019), remains poorly known in monocots. Here, we review the current knowledge on the Zn pathway from roots to the seeds in cereals (Fig. 1-3, Box 1), focusing on potential differences within monocots as well as with dicots (Box 2, Box 3). Whenever possible, we place characterized molecular actors along this Zn pathway (Fig. 1-3). We shed light on existing gaps in our current knowledge on Zn homeostasis in staple grain crops, and provide a perspective for basic research and future development of Zn biofortification strategies.

Zn chelation in monocots

Metal chelators such as nicotianamine (NA), free histidine, phytochelatins, glutathione, phosphate and organic acids (malate, citrate, and oxalate), increase metal solubility inside and outside the cells, buffer intracellular metal ion concentrations, and control unwanted interactions with cellular components, thus contributing to metal movement and alleviating metal toxicity (Salt *et al.*, 1999; Sinclair and Krämer, 2012; Olsen and Palmgren, 2014; Graham and Stangoulis, 2018). Among those, NA is very important for Zn homeostasis and detoxification, while citrate and histidine also play their part (Clemens, 2019). For many of the others, information is scarce.

Unlike dicots, grasses produce an additional type of metal chelators: phytosiderophores (PSs, Fig. 1A) (Caldelas and Weiss, 2017). Synthesized from NA (Shojima *et al.*, 1990), mugineic acids (MAs) are a group of PSs that fulfill two roles: i) upon secretion from roots into the rhizosphere, mobilize, chelate and stabilize Fe(III) and Zn[1] before uptake, and ii) as internal metal chelators, enable metal movement across membranes via specific transporters (Fig. 1B). NA can similarly serve both roles (Clemens, 2019).

The dual role of NA, both as metal chelator and PS precursor, leads to different physiological functions for NA Synthase (NAS) in Arabidopsis and grasses (Fig. 1C,D). This is reflected by differences between NAS subcellular localization in Arabidopsis and rice/maize/barley. AtNAS1 to AtNAS4 and ZmNAS1 are distributed uniformly in the cytoplasm when expressed in tobacco cells, while OsNAS2, ZmNAS2, ZmNAS3, and HvNAS1, together with two intermediate MA biosynthesis enzymes, NA aminotransferase-A (HvNAAT-A) and deoxymugineic acid synthase 1 (HvDMAS1), localize at rough endoplasmic reticulum (rER)-derived vesicles (Mizuno *et al.*, 2003; Nozoye *et al.*,

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[1] Zn is meant for Zn(II). The oxidation state of Fe will be specified in the text, as it can occur in two valences.

2014). Similar vesicles appear in rice and barley root cells just before deoxymugineic acid (DMA) secretion into the rhizosphere (Nishizawa and Mori, 1987; Negishi *et al.*, 2002). It is suggested that in grasses, NA and MAs are synthesized and stored in these vesicles, before being translocated to the required locations (Fig. 1B). This may prevent Fe and Zn deficiency responses triggered by metal chelation in the cytoplasm (Nozoye *et al.*, 2014).

PSs, especially DMA, play a key role in monocots as part of a chelation-based Fe-uptake system (Strategy II). In contrast, dicots use a reduction-based Fe strategy (Strategy I), including Fe(III) to Fe(II) reduction and Fe(II) uptake (Fig. 1C) (Marschner, Romheld and Kissel, 1986; Kobayashi *et al.*, 2012). The use of two strategies, ending up with the uptake of two distinct Fe forms, determines distinct interactions between Zn and Fe homeostasis (Hanikenne *et al.*, 2021). Rice takes advantage of both strategies (Fig. 3, Box 3) (Ishimaru *et al.*, 2006).

In addition to Fe uptake, PSs have a critical role in Zn uptake from the rhizosphere in monocots (Kawakami and Bhullar, 2018). Zn deficiency induces root PS secretion in barley (Suzuki *et al.*, 2006), and Zn-PS complexes are taken up by maize roots (Fig. 1A) (Von Wirén, Marschner and Römheld, 1996). Consistently, Zn and Fe deficiencies result in the upregulation of maize MA pathway genes (*e.g. ZmNAS1* and *ZmNAS3*) (Mallikarjuna *et al.*, 2020). Differences in Zn uptake efficiency among rice and wheat genotypes are attributed to their PS secretion rate into the rhizosphere (Cakmak *et al.*, 1996; Arnold *et al.*, 2010; Neeraja *et al.*, 2018). However, decreased secretion of DMA into the rice rhizosphere under Zn deficiency is in contrast with the elevated MA secretion observed in wheat and barley in these conditions (Ishimaru *et al.*, 2008). Consistently, increasing DMA alone (by overexpression of *OsNAS1*) in rice does not increase Zn accumulation in the embryo and endosperm, while coupled increase in DMA and NA (by overexpressing *HvNAATb* and *OsNAS1* together) results in Zn enrichment in both tissues (Díaz-Benito *et al.*, 2018). Fe concentration in contrast increases in seeds in both cases. Fe(III)-DMA and Zn-NA may thus be the dominant forms transported and stored in rice grains (Díaz-Benito *et al.*, 2018).

Consistent with their role in Zn uptake, NA and DMA contribute to Zn excess tolerance in rice, particularly in intracellular and intercellular Zn mobility in roots toward pericycle cells, and therefore in root-to-shoot metal translocation. Under Zn excess, rice genes involved in NA and DMA synthesis (*OsNAS1*, *OsNAS2*, *OsNAAT1* and *OsDMAS1)*, are upregulated in roots, but not in shoot (Ishimaru *et al.*, 2008). NA and DMA as well as their transporters (Table 1) consequently play an important role in controlling the balance between Zn sequestration in root vacuoles and Zn radial transport towards the xylem, and therefore with other players (see Zn transport Section), regulate shoot Zn accumulation (Ishimaru *et al.*, 2008; Haydon *et al.*, 2012; Sinclair and Krämer, 2012).

NA is also crucial for Zn long-distance transport in phloem and xylem (Fig. 1A, C) (Olsen and Palmgren, 2014). Overexpression of *OsNAS1, 2* and *3* as well as *HvNAS1* increases Zn content of polished and whole grains in transgenic lines of the respective species (Lee *et al.*, 2009, 2011; Masuda *et al.*, 2009; Wirth *et al.*, 2009; Johnson *et al.*, 2011; Banakar *et al.*, 2017; Singh *et al.*, 2017). In many monocotyledonous and dicotyledonous species, Zn can be transported as free Zn ions and mostly as bound to histidine and NA in the xylem sap (White and Broadley, 2009; Flis *et al.*, 2016; Caldelas and Weiss, 2017), while citrate can also be a dominant Zn ligand in the xylem in other species (Cornu *et al.*, 2015; Flis *et al.*, 2016). *OsNAS3*, expressed in cells adjacent to the xylem (Inoue *et al.*, 2003), is upregulated by Zn deficiency and downregulated by Zn excess (Ishimaru *et al.*, 2008; Suzuki *et al.*, 2008). *OsNAS3* transcript levels, as well as NA and DMA contents, are higher in developing panicles of high-Zn-grain rice strains than low-Zn strains (Neeraja *et al.*, 2018).

For translocation to developing grains, NA is the dominant Zn chelator in rice phloem (Nishiyama *et al.*, 2012). *NAS* and NA-metal transporter genes are highly expressed in barley seed transfer cells (Tauris *et al.*, 2009): Zn remobilization from the phloem to transfer cells may occur as NA-Zn, whereas NA- and PS-Zn complexes would be present in the transfer cell cytosol. TaNAAT2 and TaNAS3 similarly have an active role in chelating metals during senescence in wheat, when nutrients are (re-)allocated to reproductive organs (Fig. 1A, C) (Pearce *et al.*, 2014).

In addition to organic metal chelators, a class of proteins, metallothioneins (MT) plays a role in metal chelation (Sinclair and Krämer, 2012). HvMT3 and HvMT4 can bind Zn, and HvMT4 may contribute to Zn storage in barley grains (Hegelund *et al.*, 2012). HvMT2b2 is strongly induced in the embryo and the aleurone of barley grains upon foliar Zn application, and may be a chelator under excess Zn. HvMT2a was also proposed as a key Zn chelator in the endosperm (Tauris *et al.*, 2009). In rice, *OsMT1a* is induced by Zn and its overexpression increases Zn concentration in both vegetative tissues and grains (Z. Yang *et al.*, 2009), whereas OsMTI-1b was shown to bind Zn (Malekzadeh, Shahpiri and Siapoush, 2020).

Zn Transport in monocots

Zn cellular import/export and intracellular trafficking in plants require a passage through numerous biological membranes (Fig. 1-3, Box 1), ensured by many proteins active transport of Zn and other divalent cations. The function of several of these proteins in Zn homeostasis is tightly linked to Fe homeostasis and contributes to interactions between these two nutrients (Hanikenne *et al.*, 2021).

ZIP family

Zn-Regulated Transporter (ZRT), Iron-Regulated Transporter (IRT)-Like Protein (ZIP) transporters are generally mediating cellular uptake of Zn and other divalent metals like manganese (Mn), Fe or even cadmium (Cd), with a transport direction from outside the cell or from an organelle to the cytoplasm (Guerinot, 2000). Several rice ZIPs are linked to Zn movement throughout the plant towards grains. For instance, *OsZIP3* and *OsZIP1* are among rice marker genes for Zn status, and show a good association between gene expression level and grain Zn concentration (Swamy *et al.*, 2016). *OsZIP1* is expressed in the root exodermis and endodermis (Fig. 1B) and is upregulated in roots and shoot under Zn deficiency (Ramesh *et al.*, 2003). Higher *OsZIP1* expression is associated with higher tolerance to Zn deficiency among cultivars (Lu *et al.*, 2021). OsZIP1, and ZmZIP5, display a dual localization in the ER and plasma membrane (PM) (Fig. 1B) (S. Li *et al.*, 2013; Li *et al.*, 2019; Liu *et al.*, 2019; Mallikarjuna *et al.*, 2020). Interestingly, a role of the ER in facilitating root-to-shoot Zn movement has been proposed in Arabidopsis (Fig. 1D) (Sinclair *et al.*, 2018). OsZIP1 and ZmZIP5 may therefore not only detoxify excess Zn out of cytosol, but also contribute to Zn root-to-shoot translocation.

OsZIP8 is highly induced in roots and shoot by Zn deficiency and complements a Zn-deficient yeast strain (X. Yang *et al.*, 2009; Lee, Kim, *et al.*, 2010). The OsZIP8 protein is localized at the PM in root cells. *OsZIP8* overexpression in rice results in root Zn accumulation, while shoot and seeds are deficient (Lee, Kim, *et al.*, 2010). Similar results were reported for *OsZIP5* (Lee, Jeong, *et al.*, 2010). OsZIP5 and OsZIP9 are PM localized and involved in Zn and Cd influx into root cells, with the corresponding genes being induced by Zn deficiency (Tan *et al.*, 2020). *oszip5*, *oszip9*, and *oszip5oszip9* mutants accumulate lower Zn concentrations and display growth retardation under Zn deficiency. The *oszip9* and *oszip5oszip9* mutants have the most severe phenotypes (Tan *et al.*, 2020). *OsZIP9* is expressed in root epidermal and exodermal cells. Its root expression level is highly associated with Zn content of milled grains (Swamy *et al.*, 2016; Yang *et al.*, 2020). In contrast to *OsZIP5*, *OsZIP9* is controlled by shoot Zn status (Tan *et al.*, 2020), suggesting a long-distance systemic regulation as reported for *AtMTP2* and *AtHMA2* in Arabidopsis (Sinclair *et al.*, 2018). *OsZIP8, OsZIP5* and *OsZIP9* are therefore involved in Zn uptake into root cells (Fig. 1B).

OsZIP3 is expressed in the xylem transfer cells in enlarged vascular bundles and transports Zn (Fig. 2) (Sasaki *et al.*, 2015). The *OsZIP3* gene has higher expression in flag leaves than roots, and is slightly induced by Zn deficiency in shoot (Ramesh *et al.*, 2003).

The *OsZIP4* gene functions in root-to-shoot Zn translocation (Bashir, Takahashi, Nakanishi, *et al.*, 2013). It is regulated by Zn availability (Suzuki *et al.*, 2012). OsZIP4 is localized at the PM (Ishimaru *et al.*, 2005, 2008) and is likely involved in transporting Zn from the enlarged to the diffuse vascular bundle in the basal node and to panicles (Box 1, Fig. 2) (Mu *et al.*, 2021). Consistently, the expression level of *OsZIP4* in flag leaves correlates with rice grain Zn content (Swamy *et al.*, 2016).

OsZIP7 rescues the Zn uptake-defective *zrt1zrt2* yeast mutant under Zn deficiency, but not under Fe deficiency (Ricachenevsky *et al.*, 2018; Tan *et al.*, 2019). OsZIP7 localizes at the PM in rice protoplasts (Tan *et al.*, 2019). The *OsZIP7* gene is highly expressed in rice roots and less in flag leaves (Chandel *et al.*, 2010), while it is upregulated by Zn deficiency in root stele and parenchyma cells of enlarged vascular bundle in the basal node (Fig. 1B and Fig. 2) (Tan *et al.*, 2019). An *oszip7* mutant displays Zn accumulation in roots and basal nodes and reduced Zn delivery to upper plant parts (Tan *et al.*, 2019; Gindri *et al.*, 2020). Over-expression of *OsZIP7* in Arabidopsis significantly increases leaf, but decreases root, Zn concentration (Ricachenevsky *et al.*, 2018). Moreover, *OsZIP7*-overexpressing Arabidopsis lines are hypersensitive to high Zn and display significantly increased Zn seed concentrations (Ricachenevsky *et al.*, 2018). Accordingly, OsZIP7 plays an important role in Zn xylem loading in roots, inter-vascular transport in the basal node, and consequently Zn translocation towards leaves and rice grains (Tan *et al.*, 2019). The barley ortholog of OsZIP7, HvZIP7, is similarly localized at the PM and was implicated in root-to-shoot Zn translocation (Fig. 1) (Tiong *et al.*, 2014). *TaZIP7*, another orthologous gene in wheat, is highly expressed during senescence in leaves (Pearce *et al.*, 2014), suggesting a role in Zn translocation towards the phloem and reproductive organs. Finally, and similar to *OsZIP7*, *ZmZIP7* increases Zn concentration in seeds when expressed in Arabidopsis (Li *et al.*, 2016). Unlike rice/wheat, *ZmZIP7* is more expressed in flag leaves than in baby kernels, and is among low-expressed ZIPs in maize (Mondal *et al.*, 2014). Their Arabidopsis homologs, *AtIRT3* and *AtZIP4* (Tiong *et al.*, 2015; Evens *et al.*, 2017), are respectively suggested to function in Zn xylem unloading (Lin *et al.*, 2009), and Zn uptake into root endodermis and pericycle cells to mediate Zn xylem loading (Lin *et al.*, 2016). This subgroup of plant ZIPs, including AtIRT3, AtZIP4 as well as the rice, barley, wheat, and maize ZIP7 proteins (Evens *et al.*, 2017), thus appear to share a role in mediating root-to-shoot Zn translocation.

Other ZIPs were shown to be Zn-responsive in monocots, but their contribution to Zn uptake and distribution remains unclear. For instance, *OsZIP6* is expressed in roots and shoot, and is highly upregulated in shoot under Zn, Fe, and Mn deficiency (Kavitha, Kuruvilla and Mathew, 2015). Its orthologs *HvZIP6* and *TaZIP6* are only induced in roots by Zn deficiency (Tiong *et al.*, 2015; Evens *et al.*, 2017). In this respect, the monocotyledonous *ZIP6* genes differ from their Arabidopsis ortholog, *AtZIP6*, which is unresponsive to the Zn status (Talke, Hanikenne and Krämer, 2006; Spielmann *et al.*, 2020). The *HvZIP2*, *HvZIP3, HvZIP5*, *HvZIP8*, *HvZIP10*, and *HvZIP13* genes are upregulated by Zn starvation, and HvZIP3, HvZIP5, and HvZIP8 transport Zn in yeast (Pedas, Schjoerring and Husted, 2009; Tiong *et al.*, 2015; Nazri *et al.*, 2017). Reports on wheat *ZIP* genes are rare and with a few exceptions (see *TaZIP6* and *TaZIP7* above), their role remains to be studied. However, Zn transport activity in yeast was shown for TaZIP3, TaZIP6, TaZIP7, TaZIP9, and TaZIP13 proteins (Evens *et al.*, 2017).

Finally, in rice, *OsIRT1* and *OsIRT2* are upregulated under Fe deficiency and the corresponding proteins are known as Fe transporters, contributing to the dual Fe uptake strategy in rice (Ishimaru *et al.*, 2006; Che *et al.*, 2019). Their transcript levels exhibit good association with grain Zn content (Swamy *et al.*, 2016). Rice plants overexpressing *OsIRT1* accumulate higher Fe and Zn in roots, shoot and seeds, and are sensitive to excess Zn (Lee and An, 2009), which suggest that in addition to Fe, OsIRT1 can also transport Zn (Fig. 1B). ZmIRT1 complements yeast strains defective in Zn or Fe uptake under low Zn or Fe supply, respectively, and does so more efficiently than any other ZmZIP transporter (ZmZIP1 to 8) and OsIRT1 (S. Li *et al.*, 2013). Overexpression of *ZmIRT1* increases Zn and Fe content in Arabidopsis roots and shoot (Li *et al.*, 2015). Several barley IRTs also rescue a yeast strain defective in Zn transport (Pedas *et al.*, 2008; Pedas, Schjoerring and Husted, 2009). Although it is involved in Mn uptake in barley (Pedas *et al.*, 2008), expression of *HvIRT1* is induced by excess Zn (Kaznina *et al.*, 2019). AtIRT1 to 3 are the best-characterized ZIPs in Arabidopsis. They are mainly known for Fe homeostasis but they have broad substrate range and were reported to transport Zn as well (Korshunova *et al.*, 1999). AtIRT1 is key for Strategy I Fe(II) uptake in Arabidopsis (Kobayashi *et al.*, 2012), and it takes up Zn, Cd, Mn, Co and nickel (Ni) from soil (Fig. 1D) (Rogers, Eide and Guerinot, 2000; Vert *et al.*, 2002; Fukada *et al.*, 2011). Its transcript level is upregulated by Fe deficiency and excess Zn (Connolly, Fett and Guerinot, 2002; Becher *et al.*, 2004). In dicots, IRT1-like proteins play an important role in the interactions between Zn and Fe homeostasis (Hanikenne *et al.*, 2021). In contrast to its Arabidopsis homolog, the *OsIRT1* transcript level is not induced by Zn excess (Ishimaru *et al.*, 2008), indicating a distinct interaction between Zn and Fe in the two species.

HMA family

A subset of Heavy Metal ATPases (HMAs) of the P_{1B}-type ATPases has a Zn transport function (Williams and Mills, 2005; Hanikenne and Baurain, 2014). These pumps are unique in plants among eukaryotes for Zn cellular export (Olsen *et al.*, 2016). Hence, *OsHMA9* is expressed in root vascular bundles and anthers, and is induced by excess Zn, Cu and Cd (Lee *et al.*, 2007). *oshma9* mutants are hypersensitive to excess Zn and Cu. Localized at the PM, OsHMA9 is probably involved in Zn and Cu efflux from root cells (Lee *et al.*, 2007). A similar function was recently proposed for OsZIP1 under elevated metal levels (Liu *et al.*, 2019).

OsHMA3 was identified as a major determinant of Cd accumulation variation in leaves and grains among rice cultivars (Ueno *et al.*, 2010; Miyadate *et al.*, 2011). Its homolog, *AtHMA3*, has a similar function in Arabidopsis (Morel *et al.*, 2009; Chao *et al.*, 2012). Mostly expressed in roots, HMA3 proteins mediate Cd and Zn sequestration into vacuoles (Fig. 1B) (Morel *et al.*, 2009; Ueno *et al.*, 2010; Cai *et al.*, 2019). In addition to Cd, *OsHMA3* may be involved in Zn homeostasis: it transports Zn when expressed in Arabidopsis (Miyadate *et al.*, 2011), and *OsHMA3* overexpression in rice leads to an enhanced Zn concentration, as well as induction of the *OsZIP4*, *OsZIP5*, *OsZIP8*, *OsZIP9* and *OsZIP10* genes, in roots (Sasaki, Yamaji and Ma, 2014). As a higher Zn sequestration in root vacuoles by OsHMA3 results in cytosolic Zn depletion, this *ZIP* upregulation corresponds to a Zn-deficiency response, which enables unchanged Zn concentration in aerial parts of the overexpressor lines (Sasaki, Yamaji and Ma, 2014). Conversely, *OsHMA3* loss of function results in enhanced sensitivity to excess Zn, and reduced Zn accumulation in root and shoot vacuoles. Triggered by the higher Zn availability in root cell cytosol, *OsZIP4*, *OsZIP5*, *OsZIP8* and *OsZIP10* in roots, as well as *OsZIP5* and *OsZIP8* in shoot, are downregulated in the *oshma3* mutant, which may be responsible for the lower Zn concentration in shoot (Cai *et al.*, 2019). Altogether, *OsHMA3* is involved in Zn detoxification in rice roots, and in Zn sequestration into root vacuole as a reserve for Zn-deficient conditions (Cai *et al.*, 2019).

The AtHMA2 and AtHMA4 pumps play a key role in Zn root-to-shoot translocation (Fig. 1D) (Hussain *et al.*, 2004). It is interesting to note that monocots, in most cases, have only one such pump whereas this function is duplicated in Brassicaceae with AtHMA2/4-like proteins (Baxter *et al.*, 2003; Hanikenne and Baurain, 2014). As its Arabidopsis homologs which are involved in active Zn export from the symplast to the xylem by pumping it out of adjacent cells in the roots (Hussain *et al.*, 2004), OsHMA2 is expressed in the root vasculature and is responsible for root-to-shoot Zn translocation (Fig. 1B) (Satoh-Nagasawa *et al.*, 2012). Rice *hma2* mutants accordingly display decreased root-to-shoot Zn transfer (Satoh-Nagasawa *et al.*, 2012). *OsHMA2* is also involved in xylem-to-phloem transport of Zn at nodes: it is expressed in the phloem of enlarged and diffuse vascular bundles (Fig. 2) (Takahashi *et al.*, 2012; Yamaji *et al.*, 2013). A wheat homolog of OsHMA2, TaHMA2, is also localized at the PM. The *TaHMA2* gene is highly expressed in nodes, and its overexpression increases Zn root-to-shoot translocation in wheat, rice and tobacco (Tan *et al.*, 2013). *TaHMA2* and its paralog, *TaHMA2-like,* are significantly upregulated during senescence (Pearce *et al.*, 2014), suggesting a role in source-to-sink remobilization of Zn and/or other metals, which coincides with senescence (Fig. 3E). Moreover, expression of *HvHMA2* complements the shoot Zn-deficiency phenotype of the *hma2hma4* Arabidopsis double-mutant (Mills *et al.*, 2012).

In addition to a defect in Zn root-to-shoot translocation, the Arabidopsis *hma2hma4* double mutant fails to direct Zn towards the grain endosperm and embryo, and Zn accumulates in maternal seed coat, where AtHMA4 was proposed to play a central role in mediating post-phloem translocation of Zn to the seed endosperm (Olsen *et al.*, 2016). A similar role may be fulfilled by *OsHMA2* (Østerberg and Palmgren, 2018) and *HvHMA2*, which are expressed in the transfer cells of developing seeds (Fig. 3 D,H) (Tauris *et al.*, 2009; Olsen and Palmgren, 2014). In contrast, *TaHMA2*-overexpressing wheat lines show decreased Zn concentration in the endosperm combined to increase Zn in the embryo and the aleurone layer (Tan *et al.*, 2013). This discrepancy might result from ectopic overexpression of *TaHMA2* with a ubiquitin promoter in the transgenic lines. Alternatively, TaHMA2 may play a different role than its homologs in Arabidopsis and rice. It is also possible that Zn transport in rice grains is different from wheat and barley due to anatomical differences (Box 2).

HvHMA1 was reported to mediate export of Zn and other metals, especially copper (Cu), out of chloroplasts in barley (Fig. 1) (Mikkelsen *et al.*, 2012). Its homolog in Arabidopsis, *AtHMA1*, has been described both as a Zn exporter out of chloroplast (Kim *et al.*, 2009) and a Cu importer into the chloroplast (Seigneurin-Berny *et al.*, 2006). *HvHMA1* expression is induced in leaves by Zn deficiency and suppressed by excess Zn. HvHMA1, besides leaf plastids, localizes to intracellular compartments of grain aleurone cells, and was suggested to be involved in Zn and Cu homeostasis during grain filling (Mikkelsen *et al.*, 2012).

MTP family

Cation Diffusion Facilitators (CDF), called Metal Tolerance/Transport Protein (MTP) in plants, are involved in metal transport out the cytoplasm. A MTP sub-family mediates Zn vacuolar sequestration (Montanini *et al.*, 2007). *Oryza sativa Zn Transporter 1* (*OZT1*), also called *OsMTP1*, is induced by Zn excess in roots and shoot. OsMTP1 localizes to the tonoplast *in planta* and confers Zn resistance when expressed in yeast (Lan *et al.*, 2013; Menguer *et al.*, 2013). *OsMTP1* RNAi lines are sensitive to Zn (Yuan *et al.*, 2012). Similar to its Arabidopsis homologs, AtMTP1 and AtMTP3, OsMTP1 has a role in vacuolar Zn sequestration (Fig. 1B). The AtMTP1, AtMTP2 and AtMTP3 proteins are respectively involved in Zn vacuolar sequestration (Desbrosses-Fonrouge *et al.*, 2005), in Zn transport into the ER to facilitate cell-to-cell Zn movement in roots, consequently facilitating root-to-shoot translocation (Sinclair *et al.*, 2018) (Fig. 1D) and in vacuolar storage enabling Zn removal from the root-to-shoot pathway under excess Zn (Arrivault, Senger and Krämer, 2006). The AtMTP2 function is possibly conserved in monocots via OsZIP1 and ZmZIP5 for instance (see above). HvMTP1, which transports Zn in yeast, localizes to the tonoplast and is expressed in roots, shoot and grains, especially in the aleurone layer (Tauris *et al.*, 2009; Podar *et al.*, 2012). In developing seeds, HvMTP1 may sequester Zn in vacuoles and thus control the amount of Zn transported across post-phloem apoplastic barriers (Fig. 3G) (Olsen and Palmgren, 2014). A similar role was proposed for HvMTPc4 in barley grains (Fig. 3G). Overexpression of *HvMTP1* under an endosperm-specific promoter in barley increases Zn, Fe, Cu and Mn concentrations in grains under control growth conditions (Menguer *et al.*, 2018). Moreover, *HvMTP5* transcripts are significantly less abundant in barley lines naturally accumulating high Zn in seeds compared to low-Zn lines. It might thus sequester Zn in maternal tissues and prevent Zn loading to filial tissues (Detterbeck *et al.*, 2019).

In rice, Zn starvation induces the expression of multiple *MTP* genes: *OsMTP6*, *OsMTP7*, *OsMTP8.1* and *OsMTP12* in roots, and *OsMTP9* and *OsMTP12* in shoot (Ram *et al.*, 2019). *OsMTP1*, *OsMTP5-like* and *OsMTP7-like* are in addition upregulated in rice cultivars with higher tolerance to Zn deficiency (Lu *et al.*, 2021). Although their corresponding genes are regulated by Zn, OsMTP9 and OsMTP8.1 are involved in Mn homeostasis. OsMTP9 localizes at the PM of root cells and has a critical role in Mn translocation out of the exodermis and endodermis to the root stele and thus to shoot (Ueno *et al.*, 2015). *OsMTP8.1* is reported to be a Mn-specific transporter involved in Mn tolerance in rice shoot (Chen *et al.*, 2013). A similar Mn-specific role was previously reported for *AtMTP11* in Arabidopsis (Delhaize *et al.*, 2007; Peiter *et al.*, 2007). These rice MTP proteins in fact belong to distinct CDF sub-families, defined by phylogenetic analyses, with different substrate specificities: *OsMTP9* and *OsMTP8.1* belong to a Mn sub-family, *OsMTP6* and *OsMTP7* to a Zn/Fe sub-family, and *OsMTP12* to a Zn sub-family (Ram *et al.*, 2019). Taken together, these Zn-induced *OsMTP* genes may act directly or indirectly (*i.e.* by contributing to the homeostasis of other metals) in Zn homeostasis in rice.

ZIF family

The Zn-Induced Facilitator 1 (ZIF1)-Like (ZIFL) protein family, belonging to Major Facilitator Superfamily (MFS) of transporters, is involved in metal homeostasis, especially Zn in Arabidopsis (Sinclair and Krämer, 2012). AtZIF1 localizes to the tonoplast and transports NA into the vacuole. *AtZIF1* expression is increased by high Zn or Fe deficiency. An *atzif1* mutant is hypersensitive to Zn, while *AtZIF1* overexpression enhances vacuolar Zn and NA accumulation and leads to Zn- and Fe-deficiency symptoms (Haydon and Cobbett, 2007; Haydon *et al.*, 2012). Based on sequence similarities to Arabidopsis ZIFL proteins, 10 to 15 members of this family are identified in rice, maize and wheat (Ricachenevsky *et al.*, 2011; Sharma *et al.*, 2019). The higher number of monocot *ZIFL* genes compared to dicots may result from early segmental and tandem genomic duplications in the monocot lineage (Tang *et al.*, 2010; Ricachenevsky *et al.*, 2011). Six rice ZIFLs, *OsZIFL2*, *OsZIFL4* (or Transporter Of Mugineic acid 1, *OsTOM1*), *OsZIFL5* (or *OsTOM2*), *OsZIFL7* (or *OsTOM3*), *OsZIFL10* and *OsZIFL12*, are significantly upregulated in roots upon Zn excess. Fe deficiency also induces *OsTOM1* to *-3* and *OsZIFL12* expression in roots, but not in shoot (Ricachenevsky *et al.*, 2011; Che *et al.*, 2019). OsTOM1 is involved in root secretion of DMA and Fe(III) stabilization in the rhizosphere (Fig. 3A) (Nozoye *et al.*, 2011). Compared to WT plants, *ostom2* RNAi rice lines accumulate slightly higher Zn concentration in leaves and roots (Nozoye *et al.*, 2015). Eight out of 15 wheat *ZIFL* genes are similarly induced by excess Zn, five of which are also induced by Fe-deficient conditions (Sharma *et al.*, 2019). The PM Efflux Transporter of NA 1 (OsENA1), another MFS/TOM protein, transports NA out of cells into the apoplasm (Nozoye *et al.*, 2019), where it may be important for Fe mobilization. As Zn supplementation complements the shorter and hairy root phenotypes of *OsENA1*-overexpressing rice plants, OsENA1, on top of Fe, may also contribute to Zn homeostasis (Nozoye *et al.*, 2019).

OsZIFL12, also called *OsVMT* (Vacuolar Mugineic acid Transporter), is induced by Fe deficiency in roots. It is highly expressed in roots and in parenchyma cell bridges of node I (Che *et al.*, 2019), which are part of the nutrient pathway between the enlarged and diffuse vascular bundle xylem (Yamaji *et al.*, 2015). The corresponding protein localizes at the tonoplast and is proposed to sequester DMA into vacuoles, enabling the formation of Fe(III)-DMA complexes in this compartment (Fig. 2) (Che *et al.*, 2019). The Zn concentration is higher in node I of brown rice and polished grains in *vmt* mutant lines compared to WT plants. A higher DMA concentration is also observed in polished grains of *vmt* mutants, and, as DMA can chelate Zn, this was proposed to be responsible for the higher Zn concentration in nodes and grains (Che *et al.*, 2019).

Finally, using laser capture microdissection, different parts of barley grains, grown in soil under control condition and upon foliar Zn application, were dissected and transcription of potential Zn homeostasis-related genes was analyzed (Tauris *et al.*, 2009). *HvZIF1* was the only Zn homeostasis-related transporter gene upregulated by foliar Zn application in the embryo (Tauris *et al.*, 2009), and may contribute to Zn sequestration in barley grains (Fig. 3F).

YSL family

Yellow Stripe-Like (YSL) transporters are members of the OligoPeptide Transporter (OPT) superfamily, and are known to transport PS/NA-metal complexes into the cytosol by proton-coupled symport (Sinclair and Krämer, 2012). ZmYS1 was the first identified YSL and, as part of the Strategy II, has a central role in PS-Fe(III) uptake into the maize root epidermis (Fig. 1B) (Curie *et al.*, 2001). In addition to this crucial role in Fe nutrition, it was later shown to transport DMA complexes with several metals including Zn (Schaaf *et al.*, 2004). Furthermore, *YS1* mutation in maize significantly reduces Zn uptake and the root-to-shoot translocation rate of PS-Zn complexes (Von Wirén, Marschner and Römheld, 1996).

HvYSL2, and to a lower extent HvYS1, are transporting DMA-Zn/Fe/Mn complexes (Araki, Murata and Murata, 2011). The corresponding genes, especially *HvYSL2*, are highly induced by Zn and/or Fe deficiency in roots of barley seedlings: *HvYSL2* is more induced by Zn than Fe deficiency, and vice-versa for *HvYS1*, and combined Fe and Zn deficiencies highly induce both. These genes are not regulated significantly by Zn and/or Fe starvation in shoot. HvYSL2 appears to be involved in PS-metal transport in the endodermis and in xylem metal loading (Fig. 1B). HvYS1 rather plays a role in Fe uptake from the rhizosphere (Araki, Murata and Murata, 2011). YSL transporters were suggested as potential candidates for a role in Zn accumulation in barley grains (Detterbeck *et al.*, 2019).

Rice *YSL*s are also linked to Zn homeostasis. Upon Zn excess, *OsYSL2* and *OsYSL15* are upregulated in roots (Ishimaru *et al.*, 2008). *OsYSL15* is upregulated in shoot under Fe deficiency, as well (Anuradha *et al.*, 2012; Che *et al.*, 2019). Moreover, *OsYSL15*, *OsYSL13-like* and *OsYSL6* are more highly expressed in rice cultivars with higher tolerance to Zn deficiency (Lu *et al*., 2020). If the Arabidopsis AtYSL1 to 3 are NA-metal (including Zn) transporters (Sinclair and Krämer, 2012), it is still not clear whether monocotyledonous YSL transporters can transport NA-bound Zn.

VIT family

Vacuolar Iron Transporter (VIT) proteins can transport Fe, Mn and Zn into the vacuole for storage and detoxification (Brear, Day and Smith, 2013). *AtVIT1* is important for Fe accumulation and partitioning in Arabidopsis seeds (Kim *et al.*, 2006). Two rice proteins, OsVIT1 and OsVIT2, localize to the tonoplast and although they have the ability to transport Zn in yeast, both appear to be mainly involved in Fe vacuolar sequestration in flag leaves (Fig. 3A) (Zhang *et al.*, 2012). Note however that (i) the *OsVIT1* and *OsVIT2* expression is regulated by Zn and Fe supply (Zhang *et al.*, 2012); (ii) *OsVIT2* is among highly expressed genes in rice cultivars tolerant to Zn deficiency (Lu *et al*., 2020); and (iii) the corresponding mutants display decreased and increased Zn accumulation in flag leaves and grain embryo, respectively (Zhang *et al.*, 2012; Bashir, Takahashi, Akhtar, *et al.*, 2013). Wheat homologs of *OsVITs*, *TaVIT1*, and *TaVIT2,* are downregulated during senescence (Pearce *et al.*, 2014). Reduced vacuolar storage can facilitate nutrient remobilization from senescing tissues to reproductive organs. *TaVIT2* overexpression enhances Zn levels in barley grain endosperm but has no effect in wheat (Connorton *et al.*, 2017). It is proposed to be mainly involved in Fe and Mn transport.

Finally, another rice *VIT* gene, *OsVIT5*, is highly expressed in panicles of cultivars with high Zn in grains and may contribute to grain Zn accumulation (Fig. 3C) (Neeraja *et al.*, 2018).

NRAMP family

The Natural Resistance-Associated Macrophage Protein (NRAMP) family members act in proton/transition metal cation co-transport or antiport (Bozzi and Gaudet, 2021). *OsNRAMP5*, like *OsNAS3* and *OsVIT5*, is highly expressed in developing panicles of cultivars containing high Zn in grains (Neeraja *et al.*, 2018). This gene however contributes mainly to Mn homeostasis and Cd accumulation (Sasaki *et al.*, 2012).

OsNRAMP1 is highly induced in both roots and shoot under Zn excess and Fe deficiency. The Zn excess response is likely not a Zn-induced Fe-deficiency secondary effect (Ishimaru *et al.*, 2008). However, OsNRAMP1 also significantly contributes to Mn and Cd uptake in rice (Chang *et al.*, 2020). In maize, *ZmNRAMP1* was recently shown to be induced by both Zn and Fe deficiencies in roots and shoot (Mallikarjuna *et al.*, 2020).

Regulation of Zn homeostasis

Several transcription factors (TFs) belonging to basic Leucine Zipper (bZIP) and basic Helix-Loop-Helix (bHLH) widely found in Eukaryotes, as well as the plant-specific NAM, ATAF and CUC (NAC) and Abscisic acid Insensitive 3/Viviparous 1 (ABI3/VP1), families contribute to the regulation of Zn homeostasis in monocots.

The Arabidopsis bZIP19 and bZIP23 represent the main regulatory mechanism of the Zn homeostasis network in plants known to date (Assunção, Schat and Aarts, 2010). Through Zinc-Deficiency Response Element (ZDRE) *cis* motifs, AtbZIP19 and AtbZIP23 control the upregulation of the Arabidopsis *IRT3*, *ZIP1*, *ZIP3*, *ZIP4*, *ZIP5*, *ZIP9*, *ZIP10*, *ZIP12*, *NAS2* and *NAS4* genes in response to Zn deficiency (Assunção *et al.*, 2010; Castro *et al.*, 2017). The two TFs have partially overlapping functions in controlling Zn homeostasis (Inaba *et al.*, 2015; Lilay *et al.*, 2019) and act directly as Zn sensors, via Zn binding by their Cys/His-rich motif that inactivates the TFs under sufficient Zn concentrations (Lilay *et al.*, 2021).

Several F-group bZIPs, as AtbZIP19 and AtbZIP23, are encoded in monocotyledonous plant genomes. Their expression in the Arabidopsis *bzip19bzip23* double mutant background has been extensively used to examine their function: the expression of *HvbZIP56* and *HvbZIP62* (Nazri *et al.*, 2017), *TabZIPF1* and *TabZIPF4* (Evens *et al.*, 2017), and *OsbZIP48* and *OsbZIP50* (Lilay *et al.*, 2020) genes rescue the severe growth defect of the mutant upon Zn deficiency. *HvbZIP56*-overexpressing Arabidopsis lines also showed that AtbZIP19/23 target genes are efficiently regulated by the ectopic expression of monocot *bZIP* genes (Nazri *et al.*, 2017). These evidences are indicative of a conserved Zn deficiency regulatory network in plants (Castro *et al.*, 2017).

A wheat NAC TF, Grain Protein Content 1 (GPC1) or NAM-B1, named after its Arabidopsis homolog No Apical Meristem (NAM), was suggested to be a central regulator of multiple processes during leaf senescence, including nutrient (Zn, Fe) and protein source-to-sink remobilization during grain filling (Uauy *et al.*, 2006). The OsNAC5 TF, highly expressed in flag leaves during grain filling, was suggested to have a similar role in rice (Sperotto *et al.*, 2009). RNAi or mutant lines for *GPC1* display delayed senescence and a 36% reduction of Zn concentration in wheat grains in lab and field conditions (Pearce *et al.*, 2014). In *gpc1* plants, downregulation of the *TaHMA2-like* (ortholog of *OsHMA2*), *TaZIP3* (ortholog of *OsZIP3*), *TaZIP7* (ortholog of *OsZIP7*), *TaZIP10-like1* (ortholog of *OsZIP10*), *TaZIP13-like2* (ortholog of *OsZIP8*) and *TaNAAT2* (ortholog of *OsNAAT1*) genes is observed, while they are all highly expressed during senescence (Pearce *et al.*, 2014). These genes are possible downstream targets of TaGPC1.

Several bHLH TFs including FIT, bHLH38, 39, 100, 101, and PYE are known to mediate Fe deficiency responses through regulating Fe acquisition-related genes such as *AtFIT*, *AtNAS4*, *AtIRT1*, or the ferric chelate reductase *AtFRO2*/*AtFRO3* in Arabidopsis (Brumbarova, Bauer and Ivanov, 2015; Gao *et al.*, 2019; Kobayashi *et al.*, 2019). Similarly, OsIRO2, a bHLH TF, is a regulator of Fe deficiency-inducible genes in rice, including NA and DMA synthesis genes such as *OsNAS1*, *OsNAS2*, *OsNAAT1* and *OsDMAS1*, and a DMA transporter gene *OsYSL15* (Ogo *et al.*, 2007). Zn excess induces *OsIRO2*, its downstream genes, and consequently NA and DMA production in rice roots. In contrast, OsIRT1 is not a target of OsIRO2 and is not induced by Zn excess (Ishimaru *et al.*, 2008).

The Iron Deficiency-responsive Element-binding Factor 1 (IDEF1) TF, belonging to the ABI3/VP1 family, is a positive regulator of OsIRO2 (Kobayashi *et al.*, 2007). OsIDEF1 is a Fe deficiency-responsive TF that binds to Fe and other divalent metals, including Zn, and is important for Fe deficiency-responses and Fe tolerance in rice (Kobayashi *et al.*, 2007, 2012). Some of these *ZIFL* genes are induced by both Fe deficiency and Zn excess (Ricachenevsky *et al.*, 2011), and thus IDEF1 may be involved in regulation of *ZIFL* family genes and Zn homeostasis in rice.

Besides trans-regulatory factors, epigenetic and post-transcriptional regulation, as well as hormones, contribute to the control of metal homeostasis in monocots. For instance, *OsMTP11* is highly induced in roots and shoot by Zn and Mn excess as well as Cd and Ni. This induction by metals may be regulated by changes in DNA methylation status of the *OsMTP11* promoter (Zhang and Liu, 2017). Modifications of histone methylation at the *OsZIP1* locus are suggested to control Cd stress response in rice (Liu *et al.*, 2019). In *Sorghum bicolor*, 19 microRNAs (miRNAs) respond to Zn deficiency in roots or shoot. Interestingly, bZIP-binding ZDRE motifs were predicted in the promoter region of some of these miRNAs (Y. Li *et al.*, 2013), suggesting a potentially important role for miRNAs in regulating Zn deficiency responses in Sorghum. Similarly, involvement of several miRNAs has been reported in regulation of Fe (Buhtz *et al.*, 2010; Waters, McInturf and Stein, 2012), Mn (Valdés-López *et al.*, 2010) and Cu (Sunkar, Kapoor and Zhu, 2006; Abdel-Ghany and Pilon, 2008; Pilon, 2017) homeostasis in Arabidopsis (Paul, Datta and Datta, 2015).

Recently, hormone biosynthetic and metabolic processes were shown to be enriched in the transcriptomic responses of Zn-deficient rice (Lu *et al*., 2020) and maize (Mallikarjuna *et al.*, 2020) plants, as well as Zn-resupplied Zn-deficient *Brachypodium distachyon* plants (Amini *et al.*, 2021). In these reports, jasmonic acid, auxin or gibberellic acid signaling pathways are suggested to be involved in the regulation of Zn homeostasis. Previously, auxin signaling was linked to Zn uptake and transport under Zn deficiency in rice (Begum *et al.*, 2016).

Interdependence of Zn homeostasis and other nutrients

Plant nutrition, due to a sophisticated interaction network, requires a fine-tuned balance for the acquisition and distribution of micro- and macronutrients. In rice, for instance, Fe deficiency results in highly increased Zn content in roots and especially in shoot (Saenchai *et al.*, 2016). Zn excess increases Fe concentration in roots, but results in slightly lower Fe concentration in shoot, where the expression of the Fe-responsive *OsFER1* is inhibited, indicating Fe deficiency in rice shoot (Ishimaru *et al.*, 2008). However, the Fe excess-responsive marker gene, *OsFER1*, is not induced in rice roots, suggesting that Fe accumulates in the apoplast rather than in the cell cytoplasm (Ishimaru *et al.*, 2008). On the other hand, the Fe-deficiency-induced *OsIRO2* gene is upregulated by Zn excess (Ishimaru *et al.*, 2008), indicating that the OsIRO2 TF is a potential mediator of Fe-Zn crosstalk in rice (Ricachenevsky *et al.*, 2011). The presence of predicted IRO2-binding sites upstream of some *TaZIFL* genes was also suggested as a potential link between Fe and Zn responses (Ricachenevsky *et al.*, 2011; Sharma *et al.*, 2019). However, IRO2-binding sites were not found in the promoter region of rice *ZIFL* genes, and IDEF1 was alternatively proposed as regulator of these genes (Ricachenevsky *et al.*, 2011).

Fe-Zn interactions are well documented in dicots (Hanikenne *et al.*, 2021), including a complex regulation of AtIRT1 at the transcript and protein level (Connolly, Fett and Guerinot, 2002; Fukao *et al.*, 2011; Shanmugam, Tsednee and Yeh, 2012; Dubeaux *et al.*, 2018). The transcript levels of Zn detoxification genes are on the other hand, upregulated in a FIT-dependent manner under Fe deficiency when *AtIRT1* is strongly upregulated (Colangelo and Guerinot, 2004; Arrivault, Senger and Krämer, 2006; Sinclair and Krämer, 2012). Conversely, Fe supplementation attenuates Zn toxicity caused by Zn excess (Fukao *et al.*, 2011; Shanmugam *et al.*, 2011; Zargar *et al.*, 2015; Lešková *et al.*, 2017). Ferric Reductase Defective 3 (AtFRD3), which transports citrate into the xylem where it is required for Fe translocation to shoot (Durrett, Gassmann and Rogers, 2007), is also involved in Fe-Zn interaction (Pineau *et al.*, 2012; Charlier *et al.*, 2015; Scheepers *et al.*, 2020). In contrast, *OsIRT1* is not regulated by Zn excess (Ishimaru *et al.*, 2008) and the functional homolog of FRD3, OsFRDL1 (FRD-Like 1), seems to be less crucial in Fe/Zn homeostasis (Inoue *et al.*, 2004; Yokosho *et al.*, 2009; Yoneyama, Ishikawa and Fujimaki, 2015; Yokosho, Yamaji and Ma, 2016), suggesting a distinct impact of Zn on Fe homeostasis in Arabidopsis and rice.

Indeed, in monocots that use a chelation-based Fe uptake strategy, the competition between Zn and Fe(III) uptake from the soil, due to their different oxidation state, is expected to be less prevalent, or at least differ from the Zn/Fe(II) competition in reduction-based systems (Fukao *et al.*, 2011). However, as evidenced by the response of Fe homeostasis genes to Zn excess in several species described above, it is clear that Zn excess nevertheless perturbs Fe in monocots. This is particularly true on calcareous soils, where grasses are more sensitive to Zn toxicity than dicots, as Fe deficiency-induced PSs produced by roots strongly solubilize Zn (Chaney, 1993). In an investigation for Zn/Fe QTLs in wheat, only four QTL-rich clusters (QRC) were specific for Zn or Fe, and 10 out of 14 QRC were common for Zn and Fe, indicating a high interdependence between the homeostasis of these metal ions in wheat (Tong *et al.*, 2020).

Similar to Fe, Mn concentration is increased and decreased respectively in rice roots and shoot upon Zn excess (Ishimaru *et al.*, 2008). Conversely, the Mn concentration is reduced in roots of Zn-deficient wheat plants (Evens *et al.*, 2017). Therefore, it is necessary to consider the interplay between Zn and other metals, in particular Fe, Mn and Cu when investigating metal homeostasis networks and regulatory pathways (Antosiewicz, Barabasz and Siemianowski, 2014; Hanikenne *et al.*, 2021).

Zn and phosphate (P) interaction is another well-studied nutrient cross-homeostasis in Arabidopsis (Bouain *et al.*, 2014; Khan *et al.*, 2014; Kisko *et al.*, 2018; Hanikenne *et al.*, 2021). For instance, the AtPHR1 TF is involved in coordination of both P and Zn homeostasis. Zn deficiency leads on the other hand to upregulation of the *AtPHT1;1* gene, encoding a phosphate transporter (Jain *et al.*, 2013), and the consequent over-accumulation of P in Arabidopsis shoot (Khan *et al.*, 2014). The Arabidopsis bZIP19/23 TFs were hypothesized, besides Zn, to be involved in Mn, Cu and P homeostasis (Lilay *et al.*, 2019). Zn deficiency was previously demonstrated to induce P transporters in barley (Huang *et al.*, 2000). Zn-deficient Sorghum plants accumulate a higher concentration of P and this is gradually reversed by Zn resupply (Y. Li *et al.*, 2013). In rice, P deficiency leads to a reduced Zn concentration in shoot (Saenchai *et al.*, 2016). In contrast, P excess substantially decreases Zn concentration in wheat shoot and grains (Ova *et al.*, 2015). This P-Zn interaction in wheat is only observed in soil and was proposed to be mycorrhiza-dependent (Ova *et al.*, 2015). On the other hand, synergistic interactions between Fe and P homeostasis were also reported (Saenchai *et al.*, 2016), and therefore a complex crosstalk network between Zn, Fe and P homeostasis and signaling likely takes place and needs further examination. The *OsPHO1;1* gene, encoding a protein with a putative role in P transport through the endomembrane system, seems to be involved in such tripartite signaling in rice (Saenchai *et al.*, 2016).

Finally, there is a strong interaction between Zn/Fe and nitrogen (N), especially for Zn and Fe storage in cereal grains (Guttieri, Stein and Waters, 2013; Persson *et al.*, 2016). Zn concentration of wheat and maize grains increases considerably when N is supplied adequately (Cakmak *et al.*, 2010; Xue *et al.*, 2019; Xia *et al.*, 2020). This interaction might be, at least partly, due to the role of N in the synthesis of transporter proteins and NA, but there is no report on a clear role of N in Zn homeostasis (Persson *et al.*, 2016).

Conclusions

Although comprehensive data are lacking to compare gene expression profiles during Zn deficiency responses in multiple species, it appears that key players and gene families, *i.e.* chelator synthesis proteins, transporters and TFs, are conserved between Arabidopsis and grasses. Based on transcriptomic profiling of Fe-deficient rice and Arabidopsis plants, co-expression gene networks were built and compared (Grillet and Schmidt, 2019): the Fe uptake network is strikingly conserved in the two species and rice and Arabidopsis share more commonalities than differences. It will be relevant to conduct similar analyses with monocot species that specifically use the chelation-based strategy for Fe uptake and to extend it to other nutrients such as Zn or Cu. Yet, there are two notable differences between monocots and dicots concerning their Zn homeostasis, as noted above: the presence of PSs and an exodermis cell layer with its Casparian strip in grasses (Fig. 1 B,D).

While the gene families and their function remain similar, the main sources of differences in Zn homeostasis within monocots, and between monocots and dicots, stem from different growth environments, anatomical differences, and the use of NA and/or PS molecules.

Outlook

Two major obstacles hindering investigations of the Zn homeostasis molecular network in grasses are: (i) the lack of a proper model plant that represents wheat and barley, and (ii) most of our knowledge in rice, barley and wheat directly or indirectly originates from Arabidopsis. In particular, intracellular Zn trafficking transporters, to the mitochondria, vesicles or the Golgi complex, sensing, signaling and regulatory pathways behind Zn uptake, long-distance transport and redistribution toward seeds remain poorly known in both monocots and dicots. Application of comprehensive omics studies combined with targeted genetic approaches would help uncover distinct features of monocotyledonous Zn homeostasis, like the discovery of a novel NAC family TF in wheat (Uauy *et al.*, 2006). Furthermore, using laser capture microdissection technology (Tauris *et al.*, 2009; Che *et al.*, 2019) or state-of-the-art single-cell tools (Denyer *et al.*, 2019; Rodriguez-Villalon and Brady, 2019; Ryu *et al.*, 2019; T. Q. Zhang *et al.*, 2019) in studying the ionome and genetics of distinct cell and tissue types, will help to discover transporters that contribute to Zn transport across the biological barriers towards grains. As a consequence of these distinctions between rice and other grasses, it seems indispensable to perform independent studies in wheat and barley rather than translating the knowledge from rice (Box 2 and Box 3). Alternatively, Brachypodium may be used as a promising model system for wheat and barley plants (Vogel *et al.*, 2010; Brkljacic *et al.*, 2011), and was proposed as a model for Fe and Cu homeostasis studies in grasses (Yordem *et al.*, 2011; Jung *et al.*, 2014). This can be extended to Zn homeostasis (Amini et al., 2021). Improving Zn content of polished grains to tackle the hidden hunger in the world necessitates an even better understanding of molecular Zn players in cereal grasses.

Data availability statement

Data sharing not applicable – no new data generated.

Author Contribution

SA, MH and BA conceptualized the manuscript. SA made the figures with input from MH and BA. SA wrote the initial draft of the manuscript. MH, BA and SA edited the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

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Box 1. The Zn pathway from roots to seeds in monocots.

Once taken up into the root, Zn uses two different but interconnected pathways to move from root epidermal cells to the xylem (Fig. 1 B,D). In the symplastic pathway, Zn is transported via the cytoplasmic continuum of cells, linked by plasmodesmata, and passes through cortical and endodermal cells. This pathway is initiated at the root epidermis by plasma membrane transporters, and further influenced by plasma and vacuolar membrane transporters, as well as chelators, which altogether determine the balance between radial Zn transport to the xylem and Zn storage (Fig. 1). In the apoplastic pathway, Zn is pumped extracellularly into the stellar apoplast in regions where the Casparian strip is not fully formed (White, 2001; Sinclair and Krämer, 2012). As a layer of lignin that surrounds the cells of the root endodermis (Fig. 1), the Casparian strip forms an impermeable diffusion barrier dividing in two the root apoplast. Zn movement towards the stele thus requires passage to the symplast. The transport into and out of the apoplast is considered a major bottleneck in micronutrient homeostasis in plants (Olsen and Palmgren, 2014).

Once it reaches the central cylinder, Zn flux into above-ground tissues occurs via active loading of the root xylem, where Zn moves as free cation due to the acidic pH (Olsen and Palmgren, 2014). In wheat and barley, root-to-shoot translocation is a bottleneck, especially for grain Zn filling (Palmgren *et al.*, 2008). However, this is not the case for rice as excess Zn is stored at relatively similar levels in stems and roots (Stomph, Jiang and Struik, 2009). After being transported into the shoot xylem, Zn is taken up into living xylem parenchyma and mesophyll cells, where it is necessary for photosynthesis, and is subsequently exported into the leaf apoplast for phloem loading. In grasses, xylem-phloem exchange of Zn solutes occurs at nodes (Fig. 2). Due to low solubility at the alkaline pH, Zn has to be chelated in the phloem.

Finally, Zn is provided to developing seeds via long-distance transport in the phloem (Fig. 3), which has two distinct sources in grasses: i) phloem loading after remobilization from vegetative tissues, especially flag leaves, and/or ii) direct and continuous xylem flow from roots followed by xylem-to-phloem transfer in the stem and nodes (Waters *et al.*, 2009). Depending on the species, this transport occurs either symplastically or from the apoplast through cellular uptake (Turgeon and Wolf, 2009; Sinclair and Krämer, 2012; Olsen and Palmgren, 2014).

Box 2. Plant anatomy and Zn homeostasis

Zn homeostasis has distinct features in different species, stemming from anatomical differences. First, while both monocots and dicots have an apoplastic barrier in the endodermis, monocots have another layer of Casparian strip at the exodermis; the outermost layer of the cortex (Fig. 1) (Olsen and Palmgren, 2014). Second, in grasses, nodes act as hubs to facilitate the distribution of Zn to young vegetative organs and reproductive tissues (Fig. 2) (Yamaguchi *et al.*, 2012), which demands intervascular Zn transfers at nodes, including from the xylem of enlarged vascular bundles (that goes toward flag leaves) to the xylem of diffuse vascular bundles (that goes toward productive tissues) (Kawakami and Bhullar, 2018). Third, distinct grain anatomy is responsible for major differences in Zn homeostasis among monocot species: at the base of rice grains, the xylem of vascular bundles is continuous and Zn can be loaded directly to the nucellar projection and the aleurone layer (Fig. 3B) (Stomph, Jiang and Struik, 2009). In contrast, xylem-to-phloem Zn transport is required at the base of seeds in wheat and barley. In wheat, several apoplastic barriers need to be crossed for Zn to reach the grain: i) between the stem xylem and the grain base phloem (Olsen and Palmgren, 2014), ii) between the vascular parenchyma and nucellar projections, and iii) between maternal transfer cells and the filial endosperm (Wang, Offler and Patrick, 1994). Active transport of free and chelated Zn ions, between symplast and apoplast, is therefore required to efflux Zn from these bottlenecks until it reaches the grain aleurone layer, endosperm, and embryo (Fig. 3F) (Palmgren et al., 2008). In wheat grain, Zn is more concentrated in the embryo, while the aleurone layer contains a higher Zn concentration than the endosperm, and Fe is the highest in aleurone layer (Ajiboye *et al.*, 2015; Balk *et al.*, 2019). The rice seed, like the wheat grain, has an apoplastic barrier between maternal tissues and filial endosperm and embryo tissues, and Zn transporters have a critical role in these symplastic pathways (Kawakami and Bhullar, 2018). The rice endosperm also has the lowest Zn concentration in grain, and it seems that differences in nutrient transport pathways at the grain base of rice and wheat are not leading to distinct distribution of Zn in the seeds (Stomph, Jiang and Struik, 2009).

Box 3. Rice and Zn homeostasis in monocots

As it feeds a large part of the human population, rice is extensively used as a model plant for grasses (Sasaki and Burr, 2000). The number of rice (52), barley (27), wheat (16), and maize (8) genes cited in this manuscript to be linked with Zn homeostasis/tolerance (Table 1), reflects that a substantial amount of studies on the molecular basis of Zn homeostasis in cereals has been dedicated to rice. However, rice is not a perfect model for wheat and barley, especially for uncovering metal homeostasis mechanisms. It was indeed suggested that bottlenecks for Zn transport from the rhizosphere to grain are different in rice and other cereals (Stomph, Jiang and Struik, 2009).

First, rice is primarily cultivated in paddy fields, in conditions that prevent Fe oxidation and precipitation (Grillet and Schmidt, 2019), and in contrast to other cereals, it uses both Fe uptake strategies (Fig. 3A) (Ishimaru *et al.*, 2006). It can therefore be speculated that a number of metal homeostasis genes play dissimilar roles in rice and other grasses. Differential DMA-secretion patterns in rice compared to wheat and barley in response to Zn deficiency (Suzuki *et al.*, 2008), may also reflect the presence of a dual Fe uptake strategy in rice.

Second, rice has different Zn allocation strategies to grains. Unlike wheat, rice leaves are not a Zn source for grains and rice plants dominantly use Zn uptake from soil and xylem transport post-flowering to provide Zn to grains. In general, differential mechanisms of Zn translocation to rice grains under sufficiency and deficiency are reported (Wu *et al.*, 2010; Sperotto, 2013). For instance, when Zn is not supplied sufficiently, stored Zn in roots, stem, and sheath, but not leaves, is reported to remobilize to rice grains (Jiang *et al.*, 2007; Sperotto *et al.*, 2009; Stomph, Jiang and Struik, 2009). In contrast, in field conditions, pre-anthesis remobilization of deposited Zn from vegetative tissues via phloem transport has been reported as the main Zn source for grains in wheat, while maize grains accumulate Zn post-silking from the soil (Xue *et al.*, 2019). The contribution of Zn sources, soil, or remobilization from other tissues, in grain Zn accumulation, can also depend on factors like water availability, timing of senescence, length of grain-filling period, and nitrogen status (Cakmak and Kutman, 2018).

Third, Zn root-to-shoot transport is not (or is less of) a bottleneck in rice compared to other cereals. The continuous Zn uptake from soil in rice might also be explained by a highly effective root-to-shoot Zn transport system, compared to wheat and barley where Zn excess is stored in root vacuoles (Stomph, Jiang and Struik, 2009). OsHMA2 may be the key for this feature in rice. The control of HMAs on Zn xylem loading is indeed crucial for Zn root-shoot partitioning (Hanikenne *et al.*, 2008; Claus, Bohmann and Chavarría-Krauser, 2013). However, differences in Zn radial transport in rice roots remain to be investigated.

Finally, rice has a different grain anatomy than wheat, barley, and maize, which modify the Zn pathway to the grain (Fig. 3 B,F, see Box 2 for details) (Hands and Drea, 2012). The genes responsible for this transport in wheat and barley are not identified yet.

Table 1. List and function of genes linked with Zn homeostasis/tolerance in monocots.

	Rice		**Barley**		**Wheat**		**Maize**	
Chelator synthesis	*OsNAS1*	NA/MA synthesis, Zn enrichment in grain	*HvNAS1*	NA/MA synthesis, Zn enrichment in grain	*TaNAS3*	NA/MA synthesis	*ZmNAS1*	NA/MA synthesis
	OsNAS2	NA/MA synthesis, Zn enrichment in grain	*HvNAAT-A*	NA/MA synthesis	*TaNAAT2*	NA/MA synthesis	*ZmNAS2*	NA/MA synthesis
	OsNAS3	NA/MA synthesis, Zn enrichment in grain	*HvNAAT-b*	NA/MA synthesis			*ZMNAS3*	NA/MA synthesis
	OsNAAT1	NA/MA synthesis	*HvDMAS1*	NA/MA synthesis				
	OsDMAS1	NA/MA synthesis	*HvMT2a*	Zn chelator in grain endosperm				
	OsMT1a	Zn enrichment	*HvMT2b2*	Zn chelator in grain under excess Zn				
	OsMTI-1b	Zn/Cd chelator	*HvMT3*	-				
			HvMT4	Zn storage in grain				
ZIP	*OsZIP1*	Root Zn uptake, Zn transport into endodermis/out of exodermis, Zn detoxification into ER	*HvZIP2*	-	*TaZIP3*	-	*ZmZIP5*	Root Zn uptake, Zn root-to-shoot translocation
	OsZIP3	Zn translocation in nodes	*HvZIP3*	-	*TaZIP6*	-	*ZmZIP7*	-
	OsZIP4	Zn translocation in nodes	*HvZIP5*	-	*TaZIP7*	Zn translocation to phloem and leaves	*ZmIRT1*	Fe/Zn uptake
	OsZIP5	Root Zn/Cd uptake	*HvZIP6*	-	*TaZIP9*	-		
	OsZIP6	-	*HvZIP7*	Zn transport into phloem and root-to-shoot translocation	*TaZIP10-like1*	-		
	OsZIP7	Zn/Cd xylem loading in root, Zn/Cd translocation in nodes	*HvZIP8*	-	*TaZIP13*	-		
	OsZIP8	Root Zn uptake	*HvZIP10*	-	*TaZIP13-like2*	-		
	OsZIP9	Root Zn/Cd/Co uptake	*HvZIP13*	-				
	OsZIP10	-	*HvIRT1*	-				
	OsIRT1	Fe/Zn uptake						

Table 1. (*Continued*)

	Rice		**Barley**		**Wheat**		**Maize**
HMA	*OsHMA2*	root-to-shoot Zn translocation, xylem-to-phloem transport of Zn at nodes, Zn translocation to the seed endosperm	*HvHMA1*	Zn/Cu export out of chloroplast	*TaHMA2*	Zn root-to-shoot translocation at nodes	
	OsHMA3	Zn/Cd sequestration into root vacuoles, Zn detoxification in root	*HvHMA2*	root-to-shoot Zn translocation, Zn translocation to the seed endosperm	*TaHMA2-like*	-	
	OsHMA9	Zn/Cu efflux from root cells					
MTP	*OsMTP1* (*OsOZT1*)	vacuolar Zn sequestration	*HvMTP1*	vacuolar Zn sequestration in seeds			
	OsMTP5-like	Involved in Zn tolerance	*HvMTPc4*	vacuolar Zn sequestration in seeds			
	OsMTP6	-	*HvMTP5*	-			
	OsMTP7	-					
	OsMTP7-like	Involved in Zn tolerance					
	OsMTP8.1	-					
	OsMTP9	-					
	OsMTP11	-					
	OsMTP12	-					
MFS (ZIF/ZIFL)	*OsZIFL2*	-	*HvZIF1*	Zn sequestration in grains			
	OsZIFL4 (*OsTOM1*)	DMA secretion from root					
	OsZIFL5 (*OsTOM2*)	-					
	OsZIFL7 (*OsTOM3*)	-					
	OsZIFL10	-					
	OsZIFL12 (*OsVMT*)	DMA sequestration into vacuole					
	OsENA1	transports NA out of cells into the apoplasm					

Table 1. (*Continued*)

	Rice		**Barley**		**Wheat**		**Maize**	
YSL	*OsYSL2*	-	*HvYS1*	transport DMA-Zn/Fe/Mn complexes			*ZmYS1*	transport PS-Zn complex
	OsYSL6	-	*HvYSL2*	transport DMA-Zn/Fe/Mn complexes in the endodermis, xylem DMA-metal loading				
	OsYSL13-like	-						
	OsYSL15	DMA transport						
VIT	*OsVIT1*	Zn/Fe sequestration in vacuoles of flag leaves			*TaVIT1*	-		
	OsVIT2	Zn/Fe sequestration in vacuoles of flag leaves			*TaVIT2*	-		
	OsVIT5	grain Zn accumulation						
NRAMP	*OsNRAMP1*	-					*ZmNRAMP1*	-
	OsNRAMP5	-						
Transcription Factors	*OsbZIP48*	Regulation of Zn homeostasis	*HvbZIP56*	Regulation of Zn homeostasis	*TabZIPF1*	Regulation of Zn homeostasis		
	OsbZIP50	Regulation of Zn homeostasis	*HvbZIP62*	Regulation of Zn homeostasis	*TabZIPF4*	Regulation of Zn homeostasis		
	OsNAC5	Regulation of Zn/Fe remobilization to developing grains			*TaNAM-B1* (*TaGPC1*)	Regulation of Zn/Fe remobilization to developing grains		
	OsIDEF1	regulation of *ZIFL* genes						
	OsIRO2	-						
Other genes	*OsFRDL1*	Zn/Fe homeostasis, citrate transport into the xylem						
	OsPHO1;1	Zn/Fe/P cross-homeostasis						

The gene inventory is limited to genes discussed in the document. The genes are categorized by gene family and species.
Genes may have other functions that are not Zn-related and not noted in the table.
Dash (-): no reported/proposed function.
Blank cells: no available information.
Genes on each row are not orthologs.
bZIP = basic Leucine Zipper, **DMA** = Deoxymugineic Acid, **DMAS** = Deoxymugineic Acid Synthase, **ENA** = Efflux transporter of NA, **ER** = endoplasmic reticulum, **FRDL** = Ferric Reductase Defective-Like, **GPC** = Grain Protein Content, **HMA** = Heavy Metal ATPases, **Hv** = *Hordeum vulgare*, **IDEF** = Iron Deficiency-responsive Element-binding Factor, **IRO** = Iron-related transcription factor, **IRT** = Iron-Regulated Transporter,

MA = Mugineic Acid, **MFS** = Major Facilitator Superfamily, **MT** = Metallothioneins, **MTP** = Metal Transport Protein, **NA** = Nicotianamine, **NAAT** = NA Aminotransferase, **NAC** = NAM, ATAF and CUC, **NAM** = No Apical Meristem, **NAS** = Nicotianamine Synthase, **NRAMP** = Natural Resistance-Associated Macrophage Protein, **Os** = *Oryza sativa*, **OZT** = *Oryza sativa* Zn Transporter, **PHO** = PHOSPHATE, **PS** = Phytosiderophore, **Ta** = *Triticum aestivum*, **TOM** = Transporter Of Mugineic acid, **VIT** = Vacuolar Iron Transporter, **VMT** = Vacuolar Mugineic acid Transporter, **YS** = Yellow Stripe, **YSL** = Yellow Stripe-Like, **ZIF** = Zn-Induced Facilitator, **ZIFL** = Zn-Induced Facilitator-Like, **ZIP** = Zn-Regulated Transporter (ZRT), Iron-Regulated Transporter (IRT)-Like Protein, **Zm** = *Zea mays*.

Figure legends

Figure 1. Schematic of root and molecules involved in Zn homeostasis in monocots and dicots. Whole-plant and root layers of monocots (A and B) and the dicot *Arabidopsis thaliana* (C and D) are shown with the most important Zn transporters and chelators that a play role in Zn uptake from rhizosphere into roots and Zn movement to the root stele. Differences in Fe-uptake strategies between monocots and dicots are illustrated as well as the additional cell layer in monocots, the exodermis. Black boxes in the exo- and endodermis represent apoplastic barriers. PS = Phytosiderophore, NA = Nicotianamine, ER = endoplasmic reticulum, rER = rough ER, NAS = Nicotianamine Synthase, DMA = Deoxymugineic Acid, ZIP = Zn-Regulated Transporter (ZRT), Iron-Regulated Transporter (IRT)-Like Protein, YS = Yellow Stripe, YSL = Yellow Stripe-Like, FRO = Ferric Reductase Oxidase, HMA = Heavy Metal ATPases, MTP = Metal Transport Protein, Os = *Oryza sativa*, Hv = *Hordeum vulgare*, Zm = *Zea mays*, At = *Arabidopsis thaliana*.

Figure 2. Schematic of a rice node and molecules involved in the distribution of Zn to the leaf and the panicle. The Zn pathway from the root vasculature to the node where Zn is to be distributed between leaf and panicle is illustrated. Black boxes in Bundle sheath represent the apoplastic barrier. DMA = Deoxymugineic Acid, ZIP = Zn-Regulated Transporter (ZRT), Iron-Regulated Transporter (IRT)-Like Protein, HMA = Heavy Metal ATPases, VMT = Vacuolar Mugineic acid Transporter (also called OsZIFL12), Os = *Oryza sativa*.

Figure 3. Schematic of seed and molecules involved in Zn homeostasis in monocot grains. Whole-plant and seed anatomy of rice (A-D) and other grasses like wheat and barley (E-H) are shown with the most important players Zn homeostasis in the seed. Differences in the anatomy of rice and barley seeds are illustrated at the base of grains. Different growth environments between the two species and their impact of Fe/Zn uptake are also shown. For each rice and other grasses the middle/top figure shows:whole seeds (B, F), transverse section (C, G) as indicated at the top, and a zoom on the indicated boxes on the the transverse section (D, H). PS = Phytosiderophore, NA = Nicotianamine, YSL = Yellow Stripe-Like, ZIF = Zn-Induced Facilitator, TOM = Transporter Of Mugineic acid (also called ZIFL), HMA = Heavy Metal ATPases, MTP = Metal Transport Protein, Os = *Oryza sativa*, Hv = *Hordeum vulgare*, Ta = *Triticum aestivum*.