Journal of Experimental Botany, Vol. 65, No. 20, pp. 5725–5741, 2014
doi:10.1093/jxb/eru314 Advance Access publication 30 July, 2014

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Phosphate and zinc transport and signalling in plants: toward a better understanding of their homeostasis interaction

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Received 10 May 2014; Revised 18 June 2014; Accepted 20 June 2014

Abstract

Inorganic phosphate (Pi) and zinc (Zn) are two essential nutrients for plant growth. In soils, these two minerals are either present in low amounts or are poorly available to plants. Consequently, worldwide agriculture has become dependent on external sources of Pi and Zn fertilizers to increase crop yields. However, this strategy is neither economically nor ecologically sustainable in the long term, particularly for Pi, which is a non-renewable resource. To date, research has emphasized the analysis of mineral nutrition considering each nutrient individually, and showed that Pi and Zn homeostasis is highly regulated in a complex process. Interestingly, numerous observations point to an unexpected interconnection between the homeostasis of the two nutrients. Nevertheless, despite their fundamental importance, the molecular bases and biological significance of these interactions remain largely unknown. Such interconnections can account for shortcomings of current agronomic models that typically focus on improving the assimilation of individual elements. Here, current knowledge on the regulation of the transport and signalling of Pi and Zn individually is reviewed, and then insights are provided on the recent progress made towards a better understanding of the Zn–Pi homeostasis interaction in plants.

Key words: Cross-talk, homeostasis, phosphate, signalling, transport, zinc.

Introduction

Plants require an adequate supply of inorganic phosphate (Pi) and zinc (Zn) for their survival, while also serving as the major entry point for these two elements into the food chain. For this reason, the effects of phosphorus (P) and Zn deficiencies on crop yield have become a worldwide concern in recent years, in terms of food availability and malnutrition. Specifically, three issues present a major concern. First, Pi sustainability, as the world is facing 'a potential P crisis' because of the rapid depletion of current Pi reserves (Abelson, 1999). The second problem is low Zn content in the food that is a major public health issue (Shahzad *et al.*, 2014), since it causes Zn malnutrition which is associated with retarded growth, skeletal abnormalities, delayed wound healing, increased abortion risk, and diarrhoea (Salgueiro *et al.*, 2000*a*, *b*). Thirdly, at the end of the plant life cycle, Pi is mainly stored in the grain as phytic acid (PA), which is composed of six Pi molecules linked to a myo-inositol backbone. The abundance of PA in crop seeds used as feed and food constitutes a long-standing issue. High PA content in cereal seeds is one of the major P sources that contribute to eutrophication (Bali and Satyanarayana, 2001). PA also has antinutritional properties since it acts as a chelating factor

for several essential cations such as Zn, reducing its bioavailability in the human digestive system, and thus leading to Zn deficiency (Maga *et al*., 1982; Raboy, 2009; Veum *et al.*, 2009). To counter these alarming situations, substantial efforts have been made to improve Pi and Zn nutrition in plants, based on determining how plants respond to Pi and Zn deficiency at the physiological and molecular levels. This knowledge has been documented in many research publications and elegantly summarized in multiple reviews (Poirier and Bucher, 2002; Rouached *et al.*, 2010; Sinclair and Kramer, 2012; Shahzad *et al.*, 2014). Both Pi and Zn are taken up at the root–soil interface, predominantly as free ions. Under Pi or Zn deficiency, root architecture changes and increases its capacity for nutrient import (Nussaume *et al.*, 2011; Jain *et al.*, 2013). Once within the root symplast, each element can be stored in the root through transport into vacuoles. Alternatively, they can undergo symplastic transport towards and into the vascular cylinder, for subsequent transfer to the shoot. Export from cells is required to load Pi or Zn into the dead xylem, and thus for their translocation from the root to the shoot via the xylem (Hamburger *et al.*, 2002; Hanikenne *et al.*, 2008; Rouached *et al.*, 2010; Sinclair and Kramer, 2012). Numerous genes controlling these crucial steps of Pi or Zn transport in plants have been identified, and characterized at the molecular level, including the high affinity phosphate transporter (PHT1) (Nussaume *et al.*, 2011), phosphate exporter (PHO1) (Rouached *et al.*, 2010), transporters belonging to ZIP (ZRT, IRT-like protein) and members of P_{1B}-ATPases (HMA2 and HMA4) (Sinclair and Kramer, 2012). Despite accumulating information on the identity of these genes, developing crop plants with improved P-use efficiency and Zn content by breeding or engineering remains a target, yet not achieved. A complex yet neglected interaction between Pi and Zn nutrition *in planta* is a part of the explanation of the shortcomings of current agronomic models targeted at improving the nutrition of each element individually.

The interconnection between the homeostasis of these two nutrients has been observed in many crop species and can be summarized as follows: Zn-deficient plants overaccumulate Pi in the shoot and, conversely, Pi-deficient plants overaccumulate Zn in the shoot (Reed, 1946; Bingham and Martin, 1956; Loneragan *et al*, 1982; Cakmak and Marchsner, 1986; Norvell and Welch, 1993; Huang *et al.*, 2000; Zhu *et al.*, 2001; Misson *et al.*, 2005; Khan *et al.*, 2014). In >90% of land plants, symbiotic associations are formed with mycorrhizal fungi (Schachtman *et al.*, 1998). Mycorrhizae plays an important role in the acquisition of P for the plant (Smith and Read, 1997), and they appeared also to facilitate Zn transport in the soil–fungi–plant continuum (Chen *et al.*, 2003). Nevertheless, the molecular basis of the Pi–Zn interaction in plants remains poorly understood in both mycorrhizal (Cakmak and Marchsner, 1986; Huang *et al.*, 2000; Zhu *et al.*, 2001; Watts-Williams *et al.*, 2013) and non-mycorrhizal genera (Khan *et al.*, 2014). To gain insight into this phenomenon, in this review we focus mainly on *Arabidopsis* (a non-mycorrhizal genus) for which more physiological and 'omics' data on the regulation of Zn and Pi homeostasis are available. The published transcriptomics data (Misson *et al.*, 2005; Van de Mortel *et al.*, 2006; Bustos *et al.*, 2010; Rouached *et al.*, 2010) and characterization of some *Arabidopsis* mutants (Khan *et al.*, 2014) have highlighted the presence of a genetic programme that co-regulates the homeostasis of the P and Zn elements as an integral part of the adaptive responses to these stresses. These important phenomena have consequences in terms of comprehending the regulation of homeostasis for each of these individual elements. Therefore, future work that aims to elucidate the regulation of Pi or Zn homeostasis should first consider the possibility of cross-talk between the regulatory pathways of the two elements. The aim of this article is to present a condensed summary of the most relevant findings on the regulation of Pi and Zn transport and signalling, and to review the growing body of evidence supporting the Pi and Zn homeostasis interaction in plants.

Phosphate in plants

Pi: an essential macronutrient for plant growth

Pi is an essential nutrient that ensures various basic biological functions. It is a component of RNA and DNA, as well phospholipids. Pi plays an essential role in the energy metabolism of the cell: the Pi–Pi bond present in molecules such as ATP is energy rich, and is essential to numerous enzymatic reactions. Pi also participates in several signal transduction cascades via the modulation of enzyme activity by protein phosphorylation (Poirier and Bucher, 2002). Due to the central role of Pi in numerous aspects of plant metabolism, it is not surprising that their metabolism is profoundly affected by Pi starvation, and that Pi deficiency is associated with a coordinated series of morphological, transcriptomic, and metabolic adaptations (Poirier and Bucher, 2002; Misson *et al.*, 2005; Lan *et al.*, 2012).

The Pi transport system in plants

Pi is relatively inaccessible to plant roots because of its low solubility and high capacity for adsorption to soil particles. Plants must therefore use a complex series of Pi transporters that are involved in Pi acquisition from the soil, and its subsequent distribution to all tissues and subcellular organelles. Pi acquisition at the root periphery in plants is coupled to proton entry ($Pi:H^+$ symporter) and mediated by members of the *PHT1* gene family (Schachtman *et al.*, 1998). The *Arabidopsis* and rice genomes contain nine and 13 PHT1 family members, respectively (Paszkowsky *et al.*, 2002; Poirier and Bucher, 2002). The PHT1 genes are preferentially expressed in roots, and the encoded proteins function as high-affinity Pi uptake transporters (Muchhal *et al.*, 1996; Misson *et al.*, 2005; Bayle *et al.*, 2011; Nussaume *et al.*, 2011; Remy *et al.*, 2012). The expression of *PHT1* genes is, however, not restricted to roots and can be detected in other vegetative and reproductive tissues, implicating their role beyond Pi uptake at the root surface (Mudge *et al.*, 2002; Karthikeyan *et al.*, 2002; Nagarajan *et al.*, 2011).

Regulation at the transcriptional as well as post-transcriptional levels has been documented for the *PHT1* genes At

the transcriptional level, *PHT1* gene expression is influenced by the internal Pi concentration of cells, with Pi deficiency leading to the accumulation of a steady-state level of mRNA for several *PHT1* genes (Muchhal *et al.*, 1996; Muchhal and Raghothama, 1999; Misson *et al.*, 2005). The transcript levels of *PHT1* family members are also influenced by active photosynthesis, exogenous sugars, or even phytohormone application (Martin *et al.*, 2000; Franco-Zorrilla *et al.*, 2005; Karthikeyan *et al.*, 2007; Hammond and White, 2008; Lejay *et al.*, 2008; Rubio *et al.*, 2009; Hammond and White, 2011). A number of genes encoding transcription factors are differentially regulated by phosphate starvation, including *WRKY75* (Devaiah *et al.*, 2007*a*), *MYB62* (Devaiah *et al.*, 2009), *ZAT6* (Devaiah *et al.*, 2007*b*), and *PHR1* (Bustos *et al.*, 2010). Molecular studies have now revealed that PHR1 recognizes an imperfect palindromic motif (GNATATNC) found in the promoter sequence of some *PHT1* genes (Rubio *et al.*, 2001; Franco-Zorrilla *et al.*, 2004). Bustos *et al.* (2010) demonstrated that mutation of this motif in the promoter of two Pi starvation-responsive genes impaired their responsiveness to Pi starvation, thus revealing the importance of the PHR1 binding sequence as an integrating *cis*-regulatory motif (Bustos *et al.*, 2010).

A post-translational regulatory level has also been proposed for *PHT1*. It was recently shown that the C-terminal phosphorylation of PHT1 proteins is required for their access to the plasma membrane (Bayle *et al.*, 2011), with the assistance of phosphate transporter traffic facilitator1 (PHF1) (Gonzalez *et al.*, 2005). The presence of PHT1 at the plasma membrane is a highly regulated process. When Pi is present in the medium, PHT1 is degraded through the protein ubiquitination machinery. Under Pi deficiency, the abundance of ubiquitin E2 conjugase *PHO2* transcripts and the *NITROGEN LIMITATION ADAPTATION* (*NLA*) gene is reduced through the action of two microRNAs (miR399 and miR827, respectively), leading to an increase in PHT1 (Lin *et al.*, 2013).

Once within the root system, Pi is transferred from roots to shoots via its loading into the root xylem vessels by the PHO1 protein. PHO1 is preferentially expressed in the root vascular cylinder (Poirier *et al.*, 1991; Hamburger *et al.*, 2002; Stefanovic *et al.*, 2007; Arpat *et al.*, 2012), and the protein is primarily localized to the Golgi and *trans*-Golgi network (Hamburger *et al.*, 2002; Arpat *et al.*, 2012). PHO1 could be partially relocated to the plasma membrane in the presence of a high Pi concentration (Arpat *et al.*, 2012). Ectopic expression of *Arabidopsis* PHO1 in tobacco leaves, or in *Arabidopsis* leaves or mesophyll protoplasts, has shown that PHO1 mediates Pi export into the apoplasts, thus establishing PHO1 as a Pi exporter (Arpat *et al.*, 2012). The *Arabidopsis PHO1* gene family is composed of 11 members, namely *PHO1*, and 10 homologues named *PHO1;H1* to *PHO1;H10* (Wang *et al.*, 2004). Interestingly, of the 11 genes comprising the *PHO1* family, only *PHO1* and its closest homologue *PHO1;H1* could rescue the phenotype of the *pho1* mutant, in terms of shoot growth and shoot Pi content (Stefanovic *et al.*, 2007). Like *PHT1*, *PHO1* is regulated at the transcriptional and post-transcriptional levels. In *Arabidopsis*, Pi deficiency leads to a strong increase in the activity of the *PHO1;H1* promoter, and a less intense increase in the *PHO1* promoter. The increase in *PHO1;H1* transcript level under Pi deficiency is under the control of the PHR1 transcription factor, and sequences recognized by PHR1 are found in the promoter of that gene (Stefanovic *et al.*, 2007). In contrast, stimulation of *PHO1* transcription by phosphate deficiency occurs independently of PHR1 (Stefanovic *et al.*, 2007). In agreement with this distinct regulation of *PHO1*, the WRKY6 transcription factor was found to bind to the W box of the *PHO1* promoter, where its transcription regulates *PHO1* transcription activity (Chen *et al.*, 2009). At the post-transcriptional level, PHO1 has been shown to be a target for PHO2-mediated degradation (Liu *et al.*, 2012). Study of the expression of the *OsPHO1;2* homologue of *Arabidopsis PHO1* in rice has revealed a unique mode of post-transcriptional regulation (Jabnoune *et al.*, 2013). A *cis*-natural antisense transcript was found associated with the sense *OsPHO1;2* gene, and was expressed in the same tissues as *OsPHO1;2*. Overexpression of the *cis*-natural antisense transcript under Pi deficiency was shown to stimulate the translation of the sense *OsPHO1;2* mRNA, leading to an increase in OsPHO1;2 protein level (Jabnoune *et al.*, 2013).

Following Pi uptake into the root and its loading into the root xylem vessel, Pi must be reabsorbed into the leaf cells. Furthermore, Pi is highly mobile between tissues, with a flux between leaves and roots, leaves and seeds, and old source leaves and young sink leaves. This Pi distribution characteristically involves the movement of Pi in and out of the phloem. Although our current view of the long-distance movement of Pi between organs is incomplete, it is likely to involve members of the *PHO1* and *PHT1* gene families. In addition to its distribution between cells, Pi must also be transported into all subcellular compartments of the cell, including plastids and mitochondria. These processes involve genes belonging to the *PHT2*, *PHT3*, *PHT4*, and *pPT* gene families (Poirier and Bucher, 2002; Nussaume *et al.*, 2011). Briefly, *Arabidopsis* PHT2 is predominantly expressed in green tissue and found associated with chloroplasts (Versaw and Harrison, 2002). Expression of PHT2 in yeast revealed that it is a low-affinity H^+/Pi symporter, with a potential role in Pi transfer from the cytosol to the chloroplast (Daram *et al.*, 1999; Versaw and Harrison, 2002; Rausch *et al.*, 2004; Guo *et al.*, 2013). Members of the PHT3 family probably function as mitochondrial Pi transporters (Kiiskinen *et al.*, 1997; Millar and Heazlewood, 2003). Members of the PHT4 family are localized in either the chloroplasts or Golgi (Roth *et al.*, 2004; Guo *et al.*, 2008). PHT4;6 has been implicated in the transfer of Pi out of the Golgi (Cubero *et al.*, 2009). The pPT family mediates the exchange of Pi with other metabolites across the plastid membrane and is composed of four main groups: (i) TPT, a chloroplast triose-phosphate/Pi translocator precursor (Flugge, 1999); (ii) PPT, a plastidic phosphoenolpyruvate/Pi translocator (Fischer *et al.*, 1997); (iii) AGPT, a third group of plastidic Pi antiporters (Kammerer *et al.*, 1998); and (iv) XPT, a plastidic Pi translocator (Eicks *et al.*, 2002).

Pi sensing and signalling pathways

Some key pieces in the Pi signalling puzzle have now been identified, revealing a complex regulatory network that plants activate in response to Pi deficiency stress. Progress in the field of Pi homeostasis regulation in plants has been summarized in several recent reviews (Doerner, 2008; Rouached *et al.*, 2010; Chiou and Lin, 2011). The most documented mechanism is the Pi long-distance signaing network, which includes PHR1, the ubiquitin E2 conjugase PHO2, and mi399 (Pant *et al.*, 2008). It has been demonstrated that miR399 is transcriptionally regulated by the MYB transcription factor PHR1, in response to Pi deficiency (Bari *et al.*, 2006). Furthermore, miR399 mediates long-distance Pi signalling from the shoot to the root via the phloem, and targets the transcription of *PHO2* (Lin *et al.*, 2008; Pant *et al.*, 2008). The repression of *PHO2* transcription causes an increase in the expression of root Pi uptake transporters, and hence an increase in the acquisition of Pi by the roots and its translocation to the shoot (Bari *et al.*, 2006; Lin *et al.*, 2008). The presence of a conserved 23 bp region complementary to miR399—with a mismatch at its expected cleavage site in the transcript of the non-coding gene *IPS1* (INDUCED BY PHOSPHATE STARVATION1)—appears to contribute to the regulation of the PHO2–miR399 pathway via a mechanism known as 'target mimicry' (Franco-Zorrilla *et al.*, 2007). This finding highlights the involvement of non-coding RNAs in Pi signalling. Many other Pi-responsive miRNAs have been identified (Fujii *et al.*, 2005; Hsieh *et al.*, 2009; Pant *et al.*, 2009). Although some targets have been proposed for these miRNAs, it is necessary to confirm them in order to appreciate fully the role of these miRNAs in Pi signalling. A recent report has provided new insights into the role of miR82, which is induced by Pi deprivation and targets the *NITROGEN LIMITATION ADAPTATION* (*NLA*) gene (Hsieh *et al.*, 2009) during Pi homeostasis regulation (Lin *et al.*, 2013). This demonstrates that Pi deficiency signalling networks are complex and appear to involve cross-talk between two miRNA regulatory pathways (Lin *et al.*, 2013). In *Arabidopsis*, the signalling network for Pi is also coordinated with other minerals, such as sulphate (Rouached *et al.*, 2011*a*, *b*), iron (Ward *et al.*, 2008), and Zn (Jain *et al.*, 2013; Khan *et al.*, 2014).

In recent years, the importance of proteins having an SPX domain in Pi homeostasis and signalling has been reported. In *Arabidopsis*, expression of all four *Arabidopsis* genes, named *AtSPX1–AtSPX4*, is regulated by Pi starvation. Whereas *AtSPX1*, *AtSPX2*, and *AtSPX3* are induced by Pi starvation, expression of *AtSPX4* is suppressed by Pi starvation (Duan *et al.*, 2008). Furthermore, expression of all the members was found to be under the control of PHR1 (Rubio *et al.*, 2001; Miura *et al.*, 2005).

A number of signalling players and networks have begun to surface for the regulation of the Pi deficiency response, revealing coordination between the Pi, phytohormone, and phytoassimilate signalling pathways. The cross-regulation between Pi starvation responses and phytohormones is perhaps best illustrated by the case of Pi and cytokinins. It has been shown that cytokinin supply can repress the expression of Pi uptake transporters and many other Pi starvation-induced genes (Martin *et al.*, 2000; Brenner *et al.*, 2005). Such an interaction involves the cytokinin receptors CYTOKININ RESPONSE 1/WOODEN LEG/ARABIDOPSIS HISTIDINE KINASE 4 (CRE1/WOL/AHK4) (Martin *et al.*, 2000; Karthikeyan *et al.*, 2002; Franco-Zorrilla *et al.*, 2005; Hou *et al.*, 2005; X. Wang *et al.*, 2006). The interaction between the Pi and gibberellin (GA) signal transduction pathways has also been proposed based on results showing that the overexpression of the transcription factor gene *MYB62*, that is induced in response to Pi deficiency, resulted in a reduction in the expression not only of several Pi starvation-induced genes but also of some GA biosynthetic genes, and that the observed GA deficiency phenotype of the MYB62 overexpressor lines could be partially rescued by external GA application (Devaiah *et al.*, 2009). Finally, auxin and ethylene are known to modulate the developmental adaptations of roots under Pi limitation (Rubio *et al.*, 2009). The Pi signalling pathway also interacts with those regulating the carbon status of the plants (Wissuwa *et al.*, 2005). Lejay *et al.* (2003) reported that some high-affinity Pi transporters are regulated diurnally and induced by sucrose supply, and that two other members of the same family are induced by sucrose supply and are proposed to be upstream of hexokinase (HXK) sugar-sensing pathways (Lejay *et al.*, 2008). Taken together, these observations provide strong evidence for the existence of an interconnection between hormones, sucrose, and Pi starvation signalling in plants.

Zinc in plants

Zn: an essential micronutrient for plant growth

Zn is the only metal represented in all six classes of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Coleman, 1998), and it also plays a structural role in regulatory proteins (Berg and Shi, 1996). In plants, Zn deficiency is described as causing reduction of biomass, interveinal necrosis, deformed and chlorotic leaves, and reduction in yield. In most crops, the typical leaf Zn concentration required for adequate growth is approximately between 15 mg kg^{-1} and 20 mg kg^{-1} dry weight (Marschner, 1995). Despite its essentiality, high concentrations of Zn in the growth medium can cause toxicity in plants. At the cellular level, elevated Zn concentration causes oxidative stress, a decrease in accumulation of ATP, disintegration of cell organelles, and development of vacuoles (Sresty and Madhava Rao, 1999; Xu *et al.*, 2013).

Diverse techniques with different thresholds in their spatial resolution or sensitivity have been used to investigate Zn^{2+} cellular distribution in plant cells and tissues. These include electron-spectroscopic or energy-dispersive X-ray analysis imaging (Lanquar *et al.*, 2005), elemental mapping using synchrotron radiation-induced X-ray fluorescence and particle-induced X-ray emission (Kim *et al.*, 2006; Punshon *et al.*, 2009; Schnell Ramos *et al.*, 2013), or inductively coupled plasma atomic emission spectroscopy (ICP-AES) after subcellular

fractionation (Seigneurin-Berny *et al.*, 2006; Lanquar *et al.*, 2010). However, these approaches provide only static information on the total Zn concentration. Interestingly, the recent development of tools to image/monitor the dynamics of the cytosolic Zn, namely the combination of genetically encoded Förster resonance energy transfer (FRET) sensors and Root Chip technology (Lanquar *et al.*, 2014), offers a great opportunity to measure the intracellular free Zn concentrations in plants cells. These tools will certainly help to study Zn^{2+} homeostasis, transport, and signalling in plant cells.

Zn transport system in plants

Soil is the source of Zn for plants; thus an efficient Zn uptake by plants is crucial. However, Zn uptake is not closely related to the Zn concentration in the soil solution (Ernst and Nelissen, 2000). The metal uptake by plants depends upon the solubility of metals in soils which varies considerably depending on the soil composition, soil pH, and soil P (Tagwira *et al.*, 1992, 1993; Sauvé *et al.*, 2000; Loosemore *et al.*, 2004; Houben *et al.*, 2013). The relationship between Zn availability and soil pH and P staus will be detailed below. The precise type(s) of root cells and mechanisms underlying Zn uptake are not completely known. Transport across the plasma membrane is thought to be mediated by a transport system and intracellular high-affinity binding sites. Transporters belonging to the ZIP (ZRT, IRT-like protein) family are thought to be the primary Zn transporters involved in Zn uptake. The ZIP family comprises 15 members in *A. thaliana* (Maser *et al.*, 2001). AtIRT1, a member of the ZIP family from *A. thaliana*, localizes to the plasma membrane especially of root epidermal cells (Vert *et al.*, 2002; Barberon *et al.*, 2011), and *A. thaliana* mutant for IRTI accumulates less Zn as compared with the wild type, indicating its physiological role in Zn uptake (Henriques *et al.*, 2002). Some other transporters of the ZIP family are also thought to be involved in Zn uptake based on results obtained during transcriptomic studies and in heterologous systems. Functional heterologous expression of six *A. thaliana* ZIP genes, ZIP1, ZIP2, ZIP3, ZIP7, ZIP11, and ZIP12, complemented the *Saccharomyces cerevisiae* Zn-uptake deficient mutant, *zrt1 zrt2 (*Milner *et al.*, 2013). At the transcriptional level, *ZIP1*, *ZIP3*, and *ZIP4* are induced in Zn-starved *A. thaliana* roots (Grotz *et al.*, 1998), while *ZIP1*, *ZIP3*, *ZIP4*, *ZIP9*, and *ZIP10* are constitutively overexpressed in the Zn hyperaccumulator species, *A. halleri*, as compared with *A. thaliana* (Talke *et al.*, 2006). It is worth mentioning that a passive Zn^{2+} influx can occur through the depolarization-activated non-selective cation channel (DA-NSCC) type as reported in the Zn-hyperaccumulating *Thlaspi caerulescens* (Piñeros and Kochian 2003). The transport of Zn^{2+} can also be mediated by the voltage-independent NSCCs (VI-NSCCs) which depend on the insensitivity/permeability of these channels to Zn^{2+} ion (Demidchik and Maathuis, 2007).

In addition to these transporters, Zn movement in roots is also thought to be mediated by metal chelators: phytosiderophores (PSs), phytochelatins (PCs), and metallothioneins (MTs) (Palmgren *et al.*, 2008). For the most part, they keep the free metal concentrations very low, and interact with and donate metals to apometalloproteins or transport proteins that mediate the sequestration or efflux of metal ions (Callahan *et al.*, 2006). PSs, hexadenate metal chelators, are compounds of the mugineic acid (MA) family capable of forming complexes with iron [stable Fe(III) chelates] (Tagaki *et al.*, 1984) and probably with Zn (Von Wirén *et al.*, 2000). The graminaceous monocot plants (Strategy II-plants: secrete PSs) and most other plants (Strategy I-plants: do not secrete PSs) can be distinguished based on these two distinct strategies that they use for uptake of sparingly soluble iron from the soil. Strategy I-plants use the reduction strategy which is characterized by plasma membrane-localized ferric reductases coupled with Fe(II) transporters. Strategy II-plants use the chelation strategy through the secretion of PSs and the absorption of the Fe(III)–PS complex via a specific uptake systems located at the root surface (Romheld and Marchner, 1986). Interestingly, the release of PSs is induced under Zn-deficient conditions in cereals (Tolay *et al.*, 2001; Suzuki *et al.*, 2008; Arnold *et al.*, 2010); nevertheless, whether this observation can be explained by an induced physiological deficiency for Fe remains an open question (Pedler *et al.*, 2000). It has been suggested that Strategy II-plants may take up Zn as Zn–PS complexes (von Wiren *et al.*, 1996; Erenoglu *et al.*, 2000; Tolay *et al.*, 2001). von Wiren *et al.* (1996) have demonstrated that Zn enters Strategy II-plants via a non-PS-mediated route.

Nicotianamine (NA) is the precursor of MA-family PSs, and has been proposed to form stable complexes with many heavy metals including Zn and to play a role in their circulation in the xylem and phloem (Stephan and Scholz, 1993). Interestingly, endogenous or exogenous expression of *NA synthase* (*NAS*) genes from various species under the control of either constitutive or tissue-specific promoters resulted in an increase in PS excretion, Zn uptake, and translocation, and Zn accumulation in polished rice grains (Masuda *et al.*, 2009, 2012, 2013*a*, *b*; Johnson *et al.*, 2011; Lee *et al.*, 2011). Taken together, these data clearly illustrate the importance of NA in uptake and translocation of Zn in plants. NA is present in both Strategy I- and Strategy II-plants but does not give rise to MA in Strategy I-plants. It has been reported that Zn deficiency stimulates the synthesis of MA forms, resulting in more PS secretion in barley and rice (Suzuki *et al.*, 2006, 2008). Under this condition (–Zn), barley takes up more Zn–MA complex than Zn^{2+} ions (Suzuki *et al.*, 2006). The Zn–MA chelates may possibly be taken up into roots by YSL transporters. In line with this idea, it has been shown that the maize *ys1* mutant absorbs less Zn(II)–MA than wild-type plants (von Wiren *et al.*, 1996), thus revealing a physiological relevance of ZmYS1 in Zn–MA chelate uptake into roots. PCs are oligomers of glutathione, produced by the enzyme phytochelatin synthase. A role for PCs in Zn sequestration has been described in different mutants of yeast and *A. thaliana* (Tennstedt *et al.*, 2009). MTs are small cysteine-rich proteins involved in metal homeostasis. Very recently, Schiller *et al.* (2014) reported that MT proteins regulate the concentrations of Zn and other metal ions, in particular during germination and grain filling in barley. *Arabidopsis* transgenic

lines overexpressing a barley *MT2a* gene exhibited more sensitivity to Zn excess (Schiller *et al.*, 2014). It has been shown using *in vitro* assays that AtMT2 could chelate Zn (Robinson *et al.*, 1996). Functional heterologous expression of *AtMT2* could partly complement Zn hypersensitivity in mutants of *Synechococcus*. A study in rice also reported the involvement of *OsMT1a* in Zn homeostasis (Yang *et al.*, 2009). *OsMT1a* was found to be predominantly expressed in the roots and was induced by Zn treatment. Overexpression of OsMT1a caused an increase in Zn accumulation in the *OsMT1a*-overexpressing rice lines (Yang *et al.*, 2009). Taken together, these data prove the involvement of the MTs in regulating Zn homeostasis in plants. A novel class of proteins 'plant defensins type I (PDF1s)' were described for their involvement in cellular Zn tolerance (Mirouze *et al.*, 2006). PDF1s are small cysteine-rich peptides (Lay and Anderson, 2005), and are constitutively highly overexpressed in the Zn hyperaccumulators; *A. halleri* and *T. caerulescens* (Van de Mortel *et al.*, 2006; Shahzad *et al.*, 2013). Functional heterologous expression of PDF1s from *A. halleri* in yeast as well as in *A. thaliana* increased their Zn tolerance (Mirouze *et al.*, 2006; Shahzad *et al.*, 2013). The mechanism by which PDF1s play a role in Zn tolerance is still unknown. However, it seems likely that Zn might be chelated by the cysteines present in PDF1s. Bioinformatics analysis indeed revealed that an atom of Zn could be chelated by PDF1 (L. Marquès, personal communication).

Specific molecular mechanisms for Zn loading/unloading into/from the xylem have also been proposed. The plasma membrane transporters, AtHMA2 and AtHMA4, members of P_{1B}-ATPases, have been well documented for their role in Zn loading into the xylem (Hussain *et al.*, 2004; Verret *et al.*, 2004). Moreover, using *A. thaliana hma2 hma4* double mutants or overexpressors of *HMA4* in *A. thaliana* has suggested the role of these gene in Zn interorgan distribution (Hussain *et al.*, 2004; Verret *et al.*, 2004; Hanikenne *et al.*, 2008). In the roots of *A. thaliana* and *A. halleri*, specific mRNA activity of *HMA4* was observed in the root pericycle and xylem parenchyma (Hanikenne *et al.*, 2008). It is noteworthy that the localization of *HMA2* and *HMA4* promoter activity in the vascular tissues of shoots and leaves indicates their possible involvement in xylem unloading as well (Hussain *et al.*, 2004; Hanikenne *et al.*, 2008). In addition, the localization of *HMA4* expression in the xylem parenchyma and the leaf cambium suggests a possible role for *HMA4* in Zn distribution within the leaf (Hanikenne *et al.*, 2008). Once loaded into the dead xylem, free Zn may move upward to shoots along the xylem sap. However, ligands of Zn with citrate or NA have also been proposed to be involved in long-distance transport of Zn in the dead xylem (Suzuki and Ishioka, 2008; Rellán-Álvarez *et al.*, 2010; Pineau *et al.*, 2012).

Proper functioning of cellular machinery requires a certain amount of Zn in the cytosol to serve the needs of cell organelles. Therefore, the Zn in excess of the nutritional needs is sequestered to avoid cytotoxic effects which can be remobilized when required. Vacuoles are assumed to be major sites of Zn sequestration and thereby detoxification (Martinoia *et al.*, 2007). In *A. thaliana*, metal tolerance protein 1 (MTP1) and metal tolerance protein 3 (MTP3), members of the cation diffusion facilitator (CDF) family, heavy metal associated 3 (HMA3), a member of the P(1B-2) subgroup of the P-type ATPase family, and Zn-induced facilitator1 (ZIF1), a member of a vacuolar membrane major facilitator superfamily, have been shown to be implicated in vacuolar Zn sequestration (Drager *et al.*, 2004; Desbrosses-Fonrouge *et al.*, 2005; Arrivault *et al.*, 2006; Kawachi *et al.*, 2009; Morel *et al.*, 2009; Shahzad *et al.*, 2010; Haydon *et al.*, 2012). Under Zn-deficient conditions, the Zn stored in the vacuole has to be remobilized to the cytosol to serve the needs of the cell. We still have very poor knowledge of the transporters that could be involved in this process. It is thought that possible candidates should belong to either ZIP family transporters, yellow stripe1 (YSL1) family, or natural resistance-associated macrophage proteins (NRAMPs). A transporter involved in iron remobilization, AtNRAMP4, might also be involved in Zn remobilization. AtNRAMP4 is a tonoplastic member of the *NRAMP* gene family (Lanquar *et al.*, 2005). Heterologous expression of *AtNRAMP4* complemented the growth phenotype of a *zrt1 zrt2 S. cerevisiae* mutant, suggesting its role in Zn transport across the membranes (Lanquar *et al.*, 2004). Transcript analysis revealed that *AtNRAMP4* was induced in the roots under excess Zn conditions (Van de Mortel *et al.*, 2006). The previous findings have established the role of AtNRAMP4 in the remobilization of iron from the vacuole. Whether NRAMP4 or some other member of the NRAMP family could also play a role in Zn remobilization still remains to be carefully examined. All these findings support the critical role of vacuoles that needs to be considered while developing Zn-biofortified cereals (Shahzad *et al.*, 2014).

The *Arabidopsis ZIF1* gene and the two other ZINC-INDUCED FACILITATOR-LIKE genes (*AtZIFL1* and *AtZIFL2*) form a distinct membrane protein family involved in regulating Zn homeostasis (Haydon and Cobbett, 2007). The expression of the *ZIF1* gene is up-regulated by Zn excess, and its mutation affects Zn distribution (Haydon and Cobbett, 2007; Haydon *et al.*, 2012). *ZIFL* genes are thought to accomplish a role in Zn translocation to the seeds. In *Arabidopsis*, *ZIF1* and *ZIFL1* are listed as candidates for seed Zn concentrations through a study of quantitative trait loci (QTLs) (Waters and Grusak, 2008). In barley, transcriptomic data analyses revealed that a *ZIF1-like* gene is expressed in the aleurone layer of seeds and its expression is induced in the embryo upon foliar Zn application (Tauris *et al.*, 2009). For further information on the *ZIFL* gene family (68 family members) in plants, readers are referred to Ricachenevsky *et al.* (2011). The remobilization of metal reserves such as Zn from leaves and its continuous uptake during seed filling constitutes the major source of metal supply to the seed (Curie *et al.*, 2001; Waters *et al.*, 2008; Inou *et al.*, 2009). Reverse genetics have revealed a role for the YSL members in the remobilization of Zn from senescing leaves, in the formation of pollen. and in the Zn loading of seeds. In *Arabidopsis*, expression of *YSL1* and *YSL3* has been observed in leaves during senescence (Waters *et al.*, 2006). Both genes are also expressed in pollen grains (Le Jean *et al.*, 2005; Waters *et al.*, 2006). Genetic evidence for the involvement of *YSL1* and *YSL3* in

pollen development was revealed through the characterization of the double *ysl1ysl3* loss-of-function mutant (Waters *et al.*, 2006). All together, these data support the involvement of YSL1 and YSL3 in seed formation.

In conclusion, it has become clear that Zn transport and homeostasis in plants involve a large number of membrane proteins and chelators. It is necessary to undertake the task of determining the subcellular localization of the proteins involved in Zn transport, which would pave the way to understanding the physiological significance of their co-expression, and bring us one step closer to answering questions regarding their functional redundancy and/or interplay. Future challenges will be the purification of these large membrane proteins and obtaining their crystal structures. Such progress is necessary to obtain a better understanding of the molecular mechanisms regulating Zn transport in plants.

Zn sensing and signalling pathway

How plants sense and transmit the signal of Zn deficiency remains poorly understood. Nevertheless, using available data on the identified transcriptions factors and targets genes, together with their effects on Zn transport and accumulation in plants, a putative working model on Zn deficiency signalling has been proposed (Assuncao *et al.*, 2013). The two members of the bZIP transcription factors in *Arabidopsis*, bZIP19 and bZIP23, have been identified to play an important role in the response to Zn deficiency through the regulation of downstream genes (Assuncao *et al.*, 2010*a*). These target genes include members of the ZIP family of cation transporters (Guerinot, 2000). As mentioned above, ZIP members are likely candidates mediating root Zn uptake and transport, and hence are important players in the Zn homeostasis network. Because the Zn deficiency seems to be first sensed in shoots and the signal is then transmitted to the roots where these cation transporters are fuctionning (Assuncao *et al.*, 2013), suggests the presence of a long-distance Zn deficiency signalling molecule, yet to be identified. Within the set of bZIP19/23 target genes there are also the NAS genes *NAS2* and *NAS4*, encoding enzymes that catalyse the synthesis of NA (Assuncao *et al.*, 2010*a*, *b*). Both categories of supposed target genes (*ZIP* and *NAS*) of the bZIP19 and bZIP23 transcription factors are induced in response to Zn deficiency and contain a palindromic 10 bp motif, RTGTCGACAY, in their promoter region. An experimental demonstration of the importance of this *cis*-regulatory motif associated with genes that are induced by Zn starvation is awaited. Further research would focus on and should lead to the identification and characterization of additional transcription factors controlling the expression of key Zn-responsive genes identified by microarray approaches (Van de Mortel *et al.*, 2006). This could be achieved through direct genetics approaches. An *A. thaliana* transgenic line expressing a reporter gene (GFP or luciferase) under the control of the Zn-responsive promoter could be mutagenized using ethyl methanesulphonate (EMS), and used to screen for (i) mutant(s) impaired in the (reporter gene) response to Zn deprivation and (ii) mutant(s) showing unusual constitutive expression of the reporter gene under control conditions (in the absence of Zn deprivation). Then, positional cloning or sequencing approaches can be used to identify one of the genes harbouring the mutations responsible for the altered response(s). To characterize these transcription factors functionally and gain more insights into how these regulatory proteins modulate the Zn signalling pathways, the TRANSPLANTA collection of *Arabidopsis* lines can be used (Coego *et al.*, 2014). This collection is a powerful resource; each line expressing one of 949 transcription factors under the control of a β-oestradiol-inducible promoter. Such an approach would allow the problem of severe developmental defects caused by constitutive overexpression of transcription factors to be overcome, and would permit a controlled conditional overexpression. In conclusion, discovering and characterizing transcription factors governing the expression of Zn-responsive genes will provide new insights into Zn homeostasis signalling pathways.

Interaction between zinc and phosphorus homeosatsis.

P interacts with many micronutrients such as manganese (Mn), iron (Fe), copper (Cu), and Zn (Murphy *et al.*, 1981; Broadley *et al.*, 2010; Perez-Novo *et al.*, 2011; Pedas *et al.*, 2011). Here, we will focus on the interplay between P and Zn at the physiological and molecular levels.

The Pi and Zn interaction occurs in the soil, the natural medium for plant growth. Numerous studies performed in various crop species indicate the existence of a negative relationship between Pi and Zn accumulation in plants (Reed, 1946; Bingham and Martin, 1956; Loneragan *et al.*, 1982; Cakmak and Marchsner, 1986; Norvell and Welch, 1993; Huang *et al.*, 2000; Zhu *et al.*, 2001). Lately such a negative relationship was also reported in *A. thaliana* (Misson *et al.*, 2005; Khan *et al.*, 2014). These studies have revealed the effect of Zn starvation on the overaccumulation of Pi nutrition in plants, which can lead to the appearance of Pi toxicity symptoms in high external Pi concentrations (Loneragan *et al.*, 1982). However, to date, the effect of Pi starvation on Zn nutrition has not been studied extensively, and very little information is available, such as a higher accumulation of Zn being observed during long-term Pi deprivation (Misson *et al.*, 2005). As already mentioned, the molecular bases of the Pi–Zn interactions remain poorly understood. In a attempt to better understand this interconnection, we will present below the available data on Pi–Zn interaction in soil, in yeast, and in plants. The evidence for the genetic basis of the Pi–Zn co-regulation that mediates the adaptation of a plant to the available Pi and Zn will be reviewed.

Pi–Zn interaction in soil: influences of soil pH and root exudates

The bioavailability of Pi and Zn in soil is critical for plant growth. Both these elements must be available, continuously, and in balanced proportions to support the metabolic demands of plants. Nevertheless, the bioavailability of Pi

and Zn concentrations in soil is low. For Pi, the low mobility can be attributed to its strong reaction in both solution and solid phases of the soil (Tagwira *et al.*, 1992, 1993; A.S. Wang *et al.*, 2006). Regarding Zn, its reduced bioavailability is often induced owing to its interactions with P, which become stronger in acidic soils (Tagwira *et al.*, 1992, 1993). Variations in soil pH affect Zn availability. At high pH, Zn availability in soil is low; while at low pH, Zn becomes bioavailable but the plant's capacity to absorb Zn becomes a limiting factor. To cope with low availability of Zn in alkaline soils, the plant excretes organic acids such as citrate, which lowers the soil pH, thus ultimately increasing the Zn availability. For instance, if a Zn deficiency occurs due to high pH and P levels in soil during the growing season, a foliar application of Zn is highly recommended (Tagwira *et al.*, 1992, 1993; A.S. Wang *et al.*, 2006). In wheat plants grown in calcareous soil with high pH, where Zn is not readily available, addition of Zn gives a higher Zn concentration in grains despite the decrease in Zn uptake caused by a simultaneous Pi supply (Lu *et al.*, 2011). The presence of P, especially at high concentrations, was found to boost Zn adsorption (Perez-Novo *et al.*, 2011). It is noteworthy that the choice of the Pi fertilizers can either raise or lower soil pH (Butcher *et al.*, 1989; Kashem and Singh, 2002) and consequently increase or decrease (Clark and McBride, 1984; Thakur *et al.*, 2006) the mobility of Zn. Interestingly, low Zn availability in soil can be linked to the type of Pi fertilizer that can decrease (monoammonium phosphate) or increase the pH (diammonium phosphate), presenting a typical reaction of Pi-induced Zn deficiency (Levi-Minzi and Petruzzelli, 1984). Application of superphosphate at different rates appears to have no effects on soil pH, whereas the application of Pi fertilizer contained in mixtures has been related to a decrease in soil pH (Hamon *et al.*, 1998; Kashem and Singh, 2002). From these considerations, it is clear that the type of Pi fertilizer is an important parameter to be taken into account in the fertilization models to increase the bioavaliabity of Pi and Zn and to regulate plant growth capacity. At the rhizosphere, the capacity of plants to modulate the development of the root architecture including root elongation and the frequency of lateral root formation also influences the uptake rate of Pi and Zn. The plant roots play an important role in producing exudate such as organic acids that chelate metal ions as a part of the mechanisms improving ion uptake (Curie *et al.*, 2001). For example, in white lupin, Pi deficiency provokes the formation of proteoid roots known to exude large quantities of citric acid (Gardner *et al.*, 1983; Tang *et al.*, 2013). Citrate exuded into the rhizosphere enhances Pi availability to the plant by solubilizing P in the soil that is bound to metal ions such as Zn (Gardner *et al.*, 1983; Fox *et al.*, 1990; Tang *et al.*, 2013), and this may explain the high productivity of white lupin on soils that have low plant-available P. Nevertheless, it remains unclear whether Zn transport occurs in chelated forms with organic acids such as citrate from the rhizosphere into plant roots, a phenomenon known for the absorption of the iron–MA complexes via YSL1 (Curie *et al.*, 2001). Therefore, additional work will be required to identify the chemical profile of these secreted compounds under Pi or Zn deficiency, and the understanding of their biosynthetic pathways and their transport system to explore their possible roles in plant response to Pi and Zn homeostasis cross-talk.

The molecular mechanisms underlying how plants integrate multiple nutritional stimuli into root developmental programmes remains poorly understood (Gruber *et al.*, 2013; Kellermeier *et al.*, 2014). Pi deficiency has a marked effect on primary root growth, lateral root formation, and root hair production, elongation, and density (Bates and Lynch, 1996). Close inspection revealed that the Pi limitation causes a reduction in cell elongation as well as cell cycle activity in the root meristem, and consequently reduces the growth capacity of primary roots (Poirier and Bucher, 2002). Zn-deficient seedlings showed a reduction in primary root length and an increase in first- and higher order lateral root number (Jain *et al.*, 2013). In contrast to Pi starvation, Zn deficiency does not affect meristematic cells in the primary root (Jain *et al.*, 2013). It will be interesting to assess how plants regulate root growth in response to combined Pi and Zn deficiencies in comparison with Pi or Zn single stress.

Involvement of phytic acid in the P and Zn transport and signaling

As already mentioned in the Introduction, up to 90% of the total seed P is stored in the grain as PA, accounting for at least 1% of seed dry weight (Bohn *et al.*, 2008). PA is mainly found in the protein bodies of the embryo and aleurone layers (Steadman *et al.*, 2001), where other minerals are also preferentially deposited at a high concentration. The negatively charged Pi in PA strongly binds to metal cations such as Zn, to form a mixed salt called 'phytin' or 'phytate' (Bohn *et al.*, 2008). In cereals, numerous low PA plants have been generated thorough mutagenesis, such as wheat (Guttieri *et al.*, 2004), rice (Liu *et al.*, 2007), maize (Raboy *et al.*, 2000; Pilu *et al.*, 2003; Shi *et al.*, 2003), and barley (Rasmussen and Hatzack 1998). Silencing of a multidrug resistance-associated protein (MRP) transporter in sorghum causes a decrease in the PA concentration in seeds up to 86%, and consequently an increase in Zn absorption when analysed in Caco-2 cell lines (Kruger *et al.*, 2013). Future work would provide more insights into the effects of low PA cereals on Zn bioavailability in animal systems. The combined effect of supplying P and Zn to wheat in field conditions has been investigated and revealed that with added P the PA concentration in the grain was increased (Lu *et al.*, 2011). Moreover Zn–PA complexes were found in the vacuolar compartments of chalazal endosperm which is known to play a role in loading of minerals into the embryo at different stage of its development. For instance, in *A. thaliana*, Zn–PA crystals were shown to disappear from the endosperm early, between the globular and heart stages during bent-cotyledon stages (Otegui *et al.*, 2002). Beyond seeds, another study has shown that PA synthesis can occur in cell suspension culture of *Catharanthus roseus* and accumulates in vacuoles (Mitsuhashi *et al.*, 2005). This observation suggests the presence of a mechanism for PA transport from the cytosol, where it is synthesized into other organelles; however, so far, no plant protein has yet

been identified that mediates subcellular sequestration of PA. The same study also showed that plants supplemented simultaneously with high P and Zn could accumulate PA in non-vacuolar compartments, presumably the cytosol, probably because of the insolubility of phytate–Zn chelates. The precise mechanisms involved in PA–Zn subcellular location and its intracellular transport are not clearly understood and require further attention.

In a number of Zn-tolerant species such as *A. halleri* and *A. lyrata*, PA is implicated in binding and storage of Zn in roots (Rauser, 1999; Haydon and Cobbett, 2007). Sarret *et al.* (2002) reported a positive correlation between Zn and P concentrations in roots, and suggested that Zn is coordinated to P as Zn–PA in roots of both species and in leaves of *A. lyrata*. These data further support the presence of co-regulation of Pi–Zn nutrition in vegetative cells of intact plants, which may influence both P and Zn uptake and transport within a plant body. However, while the role of PA in regulation of Pi homeostasis and signalling has been demonstrated in a low PA *Arabidopsis* mutant (INOSITOL-PENTAKISPHOSPHATE 2-KINASE 1) *ipk1* (Stevenson-Paulik *et al.*, 2005), no available evidence on its implication in the regulation of Zn transport and/or signalling has been recorded. Therefore, further work should reconsider the role of PA in co-regulating Pi and Zn transport and signalling cross-talk.

Insights on the Pi–Zn interaction in plants from transcriptomics

Microarray technology has been successfully used to investigate the global transcriptional changes in roots and/or shoots in Pi-starved *Arabidopsis* plants (Hammond *et al.*, 2003; Wu *et al.*, 2003; Misson *et al.*, 2005; Muller *et al.*, 2007; Rouached *et al.*, 2011*b*; Bustos *et al.*, 2010; Woo *et al*, 2012). In contrast, limited information is available on the effects of Zn deficiency on the global gene expression in *Arabidopsis*. Indeed, in the literature, only transcriptomic data in roots of *A. thaliana* Zn-deficient plants have been reported (Wintz *et al.*, 2003; Van de Mortel *et al.*, 2006). Therefore, obtaining transcriptomic data from shoots of Zn-deficient plants and/or plants exposed to Pi and Zn combined stresses is necessary to bring us closer to answering questions regarding the functional categories of genes involved in Zn and Pi cross-talk.

In the frame of this review, we considered transcriptomic data published by Bustos *et al.*, (2010) for Pi and by van de Mortel *et al.* (2006) for Zn deficiency in *A. thaliana*, and the expression levels of both Pi- and Zn-related genes were compared. On the one hand, analysis revealed that many Pi-related genes were induced in Zn-deficient *Arabidopsis* roots (van de Mortel *et al.*, 2006), consisting of those known to be involved in several physiological and biochemical processes tightly connected to Pi homeostasis, including signal transduction and sugar metabolism such as inositol-3-phosphate synthase isozyme 1 (At4g39800) and trehalose-6-phosphate phosphatase (At2g22190), respectively. On the other hand, large number of genes involved in heavy metal homeostasis were found to be up-regulated in *Arabidopsis* Pi-starved plants, including those encoding a member of the MATE (multidrug and toxic compound extrusion) efflux family, FRD3 (AT3G08040) (Pfam profile: PF01554), the metal-associated domain-containing protein (Pfam profile PF00403), ABC-like transporter or ABC transporter such as NRAMP4 (AT3G13100), many Zn-finger proteins (e.g At5g54040, At3g52800, At3g19580, At5g18550, At1g76410, At5g04340, At2g19810, and At5g59820), and C2H2-type Zn finger proteins (ZFPs). These genes are known to be involved in the maintenance of metal homeostasis, and their induction may reflect the molecular response to Zn overload in plants induced by Pi deficiency (Misson *et al.*, 2005). The involvment of some of these genes in the regulation of Pi homeostasis in *Arabidopsis* has been demonstrated, for instance a gene from the subclass of C2H2 ZFP transcription factors (ZAT6) regulates Pi homeostasis through the control of root architecture (Devaiah *et al.*, 2007*b*).

Expression levels of Zn deficiency response genes identified by van de Mortel *et al.* (2006) in the microarray data obtained from the *Arabidopsis* wild type deficient for Pi and the *phr1* mutant were further analysed (Bustos *et al.*, 2010) (Fig. 1). This expression data analysis showed that the Pi and Zn deficiencies often act in the opposite directions on these sets of genes. Many genes induced by Zn deficiency were repressed by Pi deficiency (Fig. 1). Interstingly most of these genes were further repressed in the *phr1* mutant background, which further support the implication of the PHR1 transcription factor in the regulation of Pi-related genes under Zn deficiency. This observation is consistent with recent reports by Khan *et al.* (2014) which revealed the involvement of PHR1 in Pi–Zn homeostasis cross-talk in *Arabidopsis*. Our analysis also revealed the presence of gene clusters that are induced by both Zn and Pi deficiency (Fig. 1), including the putative Zn transporter (At1g05300), transcriptional regulator protein WRKY1-like (At3g01970), superoxide dismutase (At4g25100), and ferritin 1 precursor (At5g01600). Taken together, this expression analysis confirms the presence of a cross-talk between Pi and Zn regulatory networks in plants. Additional research efforts will be needed to determine how the Pi and Zn signals are relayed to the transcription machinery and to provide new insights into Pi–Zn signalling cross-talk.

Pi–Zn interaction at the cellular level; lessons from studies in yeast

The interaction between Pi and Zn occurs in yeast and has been revealed through studying the effect of Zn availability on the expression of the Pi transporter PHO84 and its activity. The *PHO84* gene encodes a cell surface high affinity transporter for Pi, and it is rapidly activated with relatively small changes in intracellular Pi (Petersson *et al.*, 1999; Ogawa *et al.*, 2000; Wykoff and O'Shea, 2001; Springer *et al.*, 2003; Wykoff *et al.*, 2007). Mutation in the transcription factor pho80 causes a constitutive expression of PHO84 (Petersson *et al.*, 1999). Interestingly, it was observed that either *pho80* mutation or Zn deficiency stress causes a common effect on PHO84 expression. The relationships between Zn nutrition and Pi transport in yeast involving PHO84 is supported by

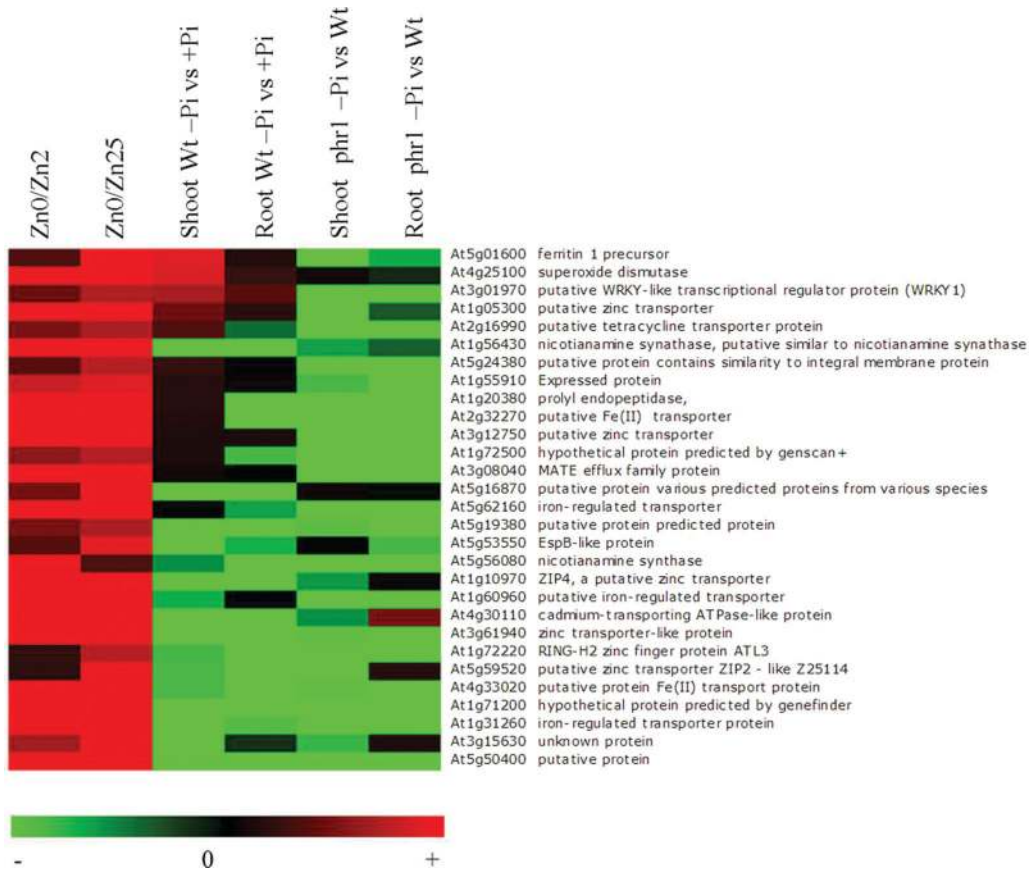

Fig. 1. Effects of varying concentrations of zinc and phosphate on the expression levels of Zn starvation response genes in *Arabidopsis*. Genes induced in roots of Zn- deficient *Arabidopsis* plants identified by Van de Mortel *et al.* (2006) are presented. The expression profile of these genes in response to Pi starvation in shoots and roots of the *Arabidopsis* wild type and the *phr1* mutant are listed (Bustos *et al.*, 2010). The red colour represents an increase in expression relative to the wild type; the green colour represents a decrease in expression relative to the wild type. Zinc concentrations in the medium were 25 μM (Zn25), 2 μM (Zn2) or 0 μM (Zn0).

additional lines of evidence; for instance, yeast cells lacking *PHO84* (*Δpho84*) are resistant to Zn toxicity (Jensen *et al.*, 2003). In this context, PHO84 not only controls cellular Pi but can also influence the uptake or homeostasis of Zn (Jensen *et al.*, 2003). It is clear that to complement heterologous studies in yeast, future work should aim to explore the role of genes involved in the maintenance of Pi homeostasis signalling in the regulation of Zn transport/accumulation *in planta*. Additional regulatory mechanisms to those found in yeast might exist in plants, considering their complex morphology and biochemistry. More recently, Khan *et al.* (2014) provided evidence of the implication of *PHO* genes in the Pi–Zn interaction in *Arabidopsis* (detailed below).

Pi–Zn interaction in plants: effect of Zn deficiency on Pi transport system and its accumulation in shoots

The connection between the effects of Zn availability on Pi uptake and accumulation and vice versa has been widely described in various studies (Reed, 1946; Loneragan *et al.*, 1982; Cakmak and Marchsner, 1986; Norvell and Welch, 1993; Huang *et al.*, 2000; Zhu *et al.*, 2000; Khan *et al.*, 2014). The Pi concentration decreases with the application of Zn (Robson and Pitman, 1983; Verma and Minhas, 1987), while application of Pi causes a decrease in Zn concentration in crop plants (e.g. wheat and maize) (Robson and Pitman, 1983). Experiments performed in well-controlled mineral solutions have shown that the physiological interaction between Pi and Zn must occur to explain such antagonistic effects (Cakmak and Marschner, 1987). Very recently, Bouain *et al.* (2014) showed that the increase in Pi concentration in the growth medium decreases Zn accumulation in lettuce roots. These data suggest that Pi nutrition may directly influence the Zn uptake mechanisms in plants.

The expression of *PHT1* genes encoding high-affinity Pi transporters in roots is tightly controlled at the transcription level by the Pi availability (Muchhal and Raghothama, 1999). Interestingly, in barley, Zn deficiency causes an up-regulation in the expression of the PHT1 Pi transporter and its activity despite an adequate supply of Pi (Huang *et al.*, 2000). In *A. thaliana*, Jain *et al.* (2013) and Khan *et al.* (2014) have reported that Zn deficiency could induce the expression of *PHT1;1* in shoots with concurrent down-regulation in roots. This clearly indicates that the expression of specific *PHT1;1* is controlled by both the Pi and Zn status of the plants, probably through a coordination between Zn and Pi signalling pathways. Thus, under Zn deficiency, the plants lose the capacity to down-regulate the expression of genes encoding high-affinity Pi transporters in roots. Interestingly, this Zn–Pi relationship appears to be specific, since the expression of Pi

transporters is not induced by other macro- or micronutrients tested in barley roots (Huang *et al.*, 2000), in cotton (Cakmak and Marschner, 1986), or in tomato roots (Liu *et al.*, 1998). However, the intricate and complex regulation of Zn–Pi homeostasis is yet to be investigated to underpin the signalling pathways, which are activated by Zn deficiency to regulate Pi homeostasis. Furthermore, as the PHT1s are regulated post-transcriptionally in response to Pi starvation, how the activity of these transporters is regulated at the post-translational level under Zn deficiency remains to be elucidated.

The accumulation of Pi in the shoots of plants grown under Zn deprivation has been frequently observed, and the potential role of the shoot-derived signal as a determinant of this important process has been suggested (Cakmak and Marschner, 1986); however, no genes have been identified so far acting in the phenomenon. Very recently, Khan *et al.* (2014) demonstrated that *A. thaliana* Zn-deficient plants accumulate Pi in the shoots, and identified PHO1 and its homologue PHO1;H3, responsible for Pi export into the xylem, as key players in this process. Interestingly, when grown in Zn-free medium, the *pho1;h3* mutant plants display a higher Pi content in the shoots than wild-type plants (Khan *et al.*, 2014). Thus PHO1;H3 appears to act as a negative regulator of the Pi transfer to the shoot under Zn deficiency, even though this process requires PHO1. It is noteworthy that the expression patterns of PHO1;H3 and PHO1 show co-localization in the endomembrane system of the plant cells. Additionally, the same authors showed that the transcription factor PHR1 is also involved in the Pi–Zn cross-talk, and excluded the role of PHO2 in this Pi–Zn interaction, since the *pho2* mutant exhibited the same phenotype as wild-type plants, overaccumulating Pi in their shoots when grown under Zn deficiency. Based on the evidence mainly from studies in *A. thaliana*, it is becoming possible to propose an integrative model for the regulation of genes involved in intracellular and interorgan Pi and Zn homoeostasis under the deficiency of either one. PHR1 acts positively to regulate the root Pi uptake and its translocation under Pi deficiency, including *PHT1* gene family members and the *PHO1;H1* gene. *PHR1*, *PHO1*, and *PHO1;H3* are the common genes that link Zn and Pi nutrition and homeostasis (Khan *et al.*, 2014). Regulation of *PHO1;H3* expression by Zn deficiency involves as yet unidentified transcriptions factor, and deserve further investigation. Figure 2 represents the illustration of a molecular basis for the Zn–Pi signalling cross-talk.

Conclusion

Continuing research in the field has made it increasingly evident that maintaining Zn and Pi homeostasis in plants is interconnected and requires tightly controlled mechanisms. This phenomenon is likely to provide great advantages for plants to cope with the fluctuations in the availability of Pi and Zn in various environments and stress conditions. Recents studies have provided evidence in favour of a genetic basis for the interconnection of these two elements. Bouain *et al.* (2014) showed that the co-regulation of Pi–Zn homeostasis may vary in varieties of the same plant specue, for example lettuce plant

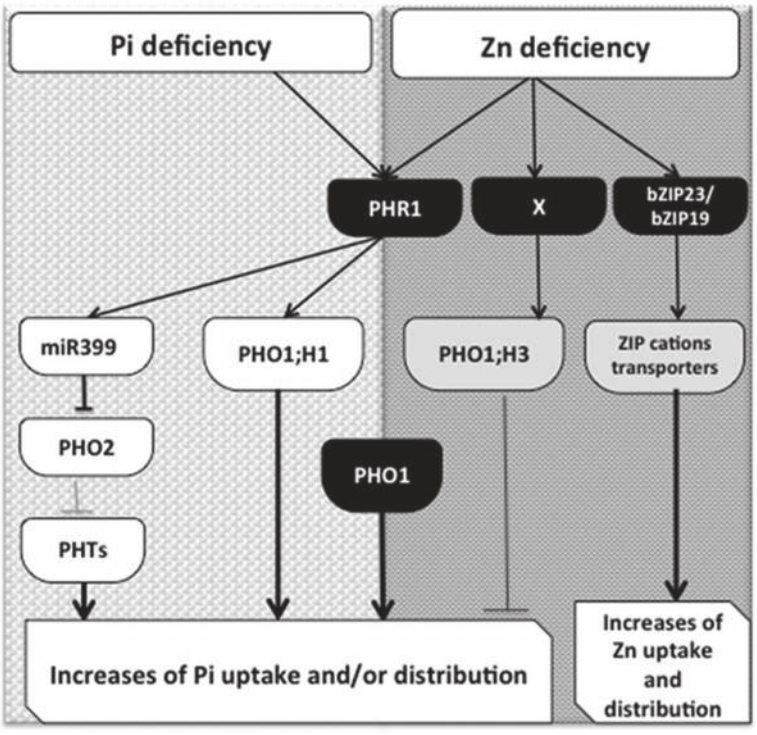

Fig. 2. Schematic representation of the regulatory pathways and their interconnections involved in plant phosphate and Zn deficiency responses. Under Pi deficiency, the PHR1–miR399–PHO2–PHT pathway is activated. Under Zn deficiency, the signalling pathways that involve bZIP23 and bZIP19 and specific cation transporter genes are activated (Assuncao *et al.*, 2013). *PHO1;H3*, which is induced by Zn deficiency, and the PHR1 and PHO1 proteins contribute to the Zn–Pi interaction. Negative and positive regulatory effects are indicated by grey lines and arrowheads, respectively.

varieties, which showed a contrasting behaviour in terms of the co-regulation of Pi and Zn homeostasis. Broadley *et al.*, (2010) reported that Zn deficiency leads to a substantial increase in the shoot P concentrations of many *Brassica oleracea* L. genotypes, thus proving to be a sufficient natural genetic variation to study some of the interactions between Zn and P nutrition. The future challenge will be to develop a comprehensive understanding of the coordination mechanisms of the homeostasis of these two elements by discovering new signalling and regulatory networks and elucidating how Zn and Pi signalling pathways are integrated into functional networks and to help plants grow better. The combination of transcriptomics and metabolomics can be one of the effective tools used to clarify cross-talk between metabolic pathways of Pi and Zn and could help the assigned metabolites to play negative and/or positive regulatory roles in the co-regulation of Pi and Zn nutrition. Finally, the development of a comprehensive understanding of Pi–Zn signalling cross-talk will be of great scientific interest, and crucial for sustainable agriculture worldwide.

Acknowledgements

This work was funded by grants from the Swiss National Foundation (31003A-138339) to YP, and the 'Institut National de la Recherche 457 Agronomique'–France to HR. The authors would like to thank Brandon Loveall for critical reading of the manuscript. The authors apologize to colleagues whose relevant work has not been mentioned due to word limitations. We are grateful to Dr Sikander Pal for helpful comments on the manuscript. We deeply acknowledge each of the referees for their helpful comments and constructive suggestions, which greatly improved the content and presentation of this article.

References

Abelson PH. 1999. A potential phosphate crisis. *Science* **283,** 2015.

Arnold T, Kirk GJ, Wissuwa M, Frei M, Zhao FJ, Mason TF, Weiss DJ. 2010. Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. *Plant, Cell and Environment* **33,** 370–381.

Arpat AB, Magliano P, Wege S, Rouached H, Stefanovic A, Poirier Y. 2012. Functional expression of PHO1 to the Golgi and trans-Golgi network and its role in export of inorganic phosphate. *The Plant Journal* **71,** 479–491.

Arrivault S, Senger T, Kramer U. 2006. The Arabidopsis metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *The Plant Journal* **46,** 861–879.

Assuncao AG, Herrero E, Lin YF, *et al*. 2010*a*. Arabidopsis thaliana transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. *Proceedings of the National Academy of Sciences, USA* **107,** 10296–10301.

Assuncao AG, Persson DP, Husted S, Schjorring JK, Alexander RD, Aarts MG. 2013. Model of how plants sense zinc deficiency. *Metallomics* **5,** 1110–1116.

Assuncao AG, Schat H, Aarts MG. 2010*b*. Regulation of the adaptation to zinc deficiency in plants. *Plant Signaling and Behavior* **5,** 1553–1555.

Bali A, Satyanarayana T. 2001. Microbial phytases in nutrition and combating phosphorus pollution. *Everyman's Science* **4,** 207–209.

Barberon M, Zelazny E, Robert S, Conejero G, Curie C, Friml J, Vert G. 2011. Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) transporter controls iron uptake in plants. *Proceedings of the National Academy of Sciences, USA* **108,** 450–458.

Bari R, Datt Pant B, Stitt M, Scheible WR. 2006. PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology* **141,** 988–999.

Bates TR, Lynch JP. 1996. Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell and Environment* **19,** 529–538.

Bayle V, Arrighi JF, Creff A, Nespoulous C, Vialaret J, Rossignol M, Gonzalez E, Paz-Ares J, Nussaume L. 2011. Arabidopsis thaliana high-affinity phosphate transporters exhibit multiple levels of posttranslational regulation. *The Plant Cell* **23,** 1523–1535.

Berg JM, Shi Y. 1996. The galvanization of biology: a growing appreciation for the roles of zinc. *Science* **271,** 1081–1085.

Bingham FT, Martin JP. 1956. Effect of soil phosphorus on growth and minor element nutrition of citrus. *Soil Science Society of America Proceedings* **20,** 382–385.

Bohn L, Meyer AS, Rasmussen SK. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B* **9,** 165–91.

Bouain N, Kisko M, Rouached A, *et al.* 2014. Phosphate/zinc interaction analysis in two lettuce varieties reveals contrasting effects on biomass, photosynthesis, and dynamics of Pi transport. *BioMedical Research International* Article ID 548254.

Brenner WG, Romanov GA, Kollmer I, Burkle L, Schmulling T. 2005. Immediate-early and delayed cytokinin response genes of Arabidopsis thaliana identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. *The Plant Journal* **44,** 314–333.

Broadley MR, Lochlainn SO, Hammond JP, Bowen HC, Cakmak I, Eker S., Erdem H, King GJ, White PJ. 2010. Shoot zinc (Zn) concentration varies widely within Brassica oleracea L. and is affected by soil Zn and phosphorus (P) levels. *Journal of Horticultural Science and Biotechnology* **85,** 375–380.

Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Perez-Perez J, Solano R, Leyva A, Paz-Ares J. 2010. A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. *PLoS Genetics* **6,** e1001102.

Butcher B, Davidoff MC, Amacher B, Hinz C, Iskandar IK, Selim HM. 1989. Correlation of Freundlich Kd and n retention parameters with soils and elements. *Soil Science* **148,** 370–379.

Cakmak I, Marschner H. 1986. Mechanism of phosphorus induced zinc deficiency in cotton. I. Zinc deficiency-enhanced uptake rate of phosphorus. *Physiologia Plantarum* **68,** 483–490.

Cakmak I, Marschner H. 1987. Mechanism of phosphorus induced Zn deficiency in cotton III. Changes in physiologial availability of Zn in plants. *Physiologia Plantarum* **70,** 13–20.

Callahan DL, Baker AJ, Kolev SD, Wedd AG. 2006. Metal ion ligands in hyperaccumulating plants. *Journal of Biological Inorganic Chemistry* **11,** 2–12.

Chen BD, Li XL, Tao HQ, Christie P, Wong MH. 2003. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. *Chemosphere* **50,** 839–846.

Chen YF, Li LQ, Xu Q, Kong YH, Wang H, Wu WH. 2009. The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. *The Plant Cell* **21,** 3554–3566.

Chiou TJ, Lin SI. 2011. Signaling network in sensing phosphate availability in plants. *Annual Review of Plant Biology* **62,** 185–206.

Clark CJ, McBride MB. 1984. Cation and anion retention by natural and synthetic allophane and imogolite. *Clays and Clay Minerals* **32,** 291–299.

Coego A, Brizuela E, Castillejo P, *et al*. 2014. The TRANSPLANTA collection of Arabidopsis lines: a resource for functional analysis of transcription factors based on their conditional overexpression. *The Plant Journal* **77,** 944–53.

Coleman JE. 1998. Zinc enzymes. *Current Opinion in Chemical Biology* **2,** 222–234.

Cubero B, Nakagawa Y, Jiang XY, Miura KJ, Li F, Raghothama KG, Bressan RA, Hasegawa PM, Pardo JM. 2009. The phosphate transporter PHT4;6 is a determinant of salt tolerance that is localized to the Golgi apparatus of Arabidopsis. *Molecular Plant* **2,** 535–552.

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL. 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature* **409,** 346–349.

Daram P, Brunner S, Rausch C, Steiner C, Amrhein N, Bucher M. 1999. Pht2;1 encodes a low-affinity phosphate transporter from Arabidopsis. *The Plant Cell* **11,** 2153–2166.

Desbrosses-Fonrouge AG, Voigt K, Schroder A, Arrivault S, Thomine S, Kramer U. 2005. Arabidopsis thaliana MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Letters* **579,** 4165–4174.

Demidchik V, Maathuis FJM. 2007. Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. *New Phytologist* **175,** 387–404.

Devaiah BN, Karthikeyan AS, Raghothama KG. 2007. WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. *Plant Physiology* **143,** 1789–1801.

Devaiah BN, Madhuvanthi R, Karthikeyan AS, Raghothama KG. 2009. Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in Arabidopsis. *Molecular Plant* **2,** 43–58.

Devaiah BN, Nagarajan VK, Raghothama KG. 2007. Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6. *Plant Physiology* **145,** 147–159.

Doerner P. 2008. Phosphate starvation signaling: a threesome controls systemic P(i) homeostasis. *Current Opinion in Plant Biology* **11,** 536–540.

Drager DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Kramer U. 2004. Two genes encoding Arabidopsis halleri MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. *The Plant Journal* **39,** 425–439.

Duan K, Yi KK, Dang L, Huang HJ, Wu W, Wu P. 2008. Characterization of a sub-family of Arabidopsis genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus starvation. *The Plant Journal* **54,** 965–975.

Eicks M, Maurino V, Knappe S, Flugge UI, Fischer K. 2002. The plastidic pentose phosphate translocator represents a link between the cytosolic and the plastidic pentose phosphate pathways in plants. *Plant Physiology* **128,** 512–522.

Erenoglu B, Eker S, Cakmak I, Derici R, Romheld V. 2000. Effect of iron and zinc deficiency on release of phytosiderophores in barley cultivars differing in zinc efficiency. *Journal of Plant Nutrition* **23,** 1645–1656.

Ernst WH, Nelissen HJ. 2000. Life-cycle phases of a zinc- and cadmium-resistant ecotype of Silene vulgaris in risk assessment of polymetallic mine soils. *Environmental Pollution* **107,** 329–338.

Fischer K, Kammerer B, Gutensohn M, Arbinger B, Weber A, Hausler RE, Flugge UI. 1997. A new class of plastidic phosphate translocators: a putative link between primary and secondary metabolism by the phosphoenolpyruvate/phosphate antiporter. *The Plant Cell* **9,** 453–462.

Flugge UI. 1999. Phosphate translocators in plastids. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 27–45.

Fox TR, Comerford NB, McFee WW. 1990. Phosphorus and aluminium release from a spodic horizon mediated by organic acids. *Soil Science Society of America Journal* **54,** 1763–1767.

Franco-Zorrilla JM, Gonzalez E, Bustos R, Linhares F, Leyva A, Paz-Ares J. 2004. The transcriptional control of plant responses to phosphate limitation. *Journal of Experimental Botany* **55,** 285–293.

Franco-Zorrilla JM, Martin AC, Leyva A, Paz-Ares J. 2005. Interaction between phosphate-starvation, sugar, and cytokinin signaling in Arabidopsis and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiology* **138,** 847–857.

Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, Rubio-Somoza I, Leyva A, Weigel D, Garcia JA, Paz-Ares J. 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nature Genetics* **39,** 1033–1037.

Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK. 2005. A miRNA involved in phosphate-starvation response in Arabidopsis. *Current Biology* **15,** 2038–2043.

Gardner W, Barber D, Parbery D. 1983. The acquisition of phosphorus by Lupinus albus L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant and Soil* **70,** 107–124.

Gonzalez E, Solano R, Rubio V, Leyva A, Paz-Ares J. 2005. PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in Arabidopsis. *The Plant Cell* **17,** 3500–3512.

Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D. 1998. Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. *Proceedings of the National Academy of Sciences, USA* **95,** 7220–7224.

Gruber BD, Giehl RF, Friedel S, von Wirén N. 2013. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiology* **163,** 161–179.

Guerinot ML. 2000. The ZIP family of metal transporters. *Biochimica et Biophysica Acta* **1465,** 190–198.

Guttieri MJ, Bowen D, Dorsch JA, Raboy V, Souza E. 2004. Identification and characterization of a low phytic acid wheat. *Crop Science* **44,** 418–424.

Guo B, Jin Y, Wussler C, Blancaflor EB, Motes CM, Versaw WK. 2008. Functional analysis of the Arabidopsis PHT4 family of intracellular phosphate transporters. *New Phytologist* **177,** 889–898.

Guo C, Zhao X, Liu X, Zhang L, Gu J, Li X, Lu W, Xiao K. 2013. Function of wheat phosphate transporter gene TaPHT2;1 in Pi translocation and plant growth regulation under replete and limited Pi supply conditions. *Planta* **237,** 1163–1178.

Hamburger D, Rezzonico E, MacDonald-Comber Petetot J, Somerville C, Poirier Y. 2002. Identification and characterization of the Arabidopsis PHO1 gene involved in phosphate loading to the xylem. *The Plant Cell* **14,** 889–902.

Hammond JP, Bennett MJ, Bowen HC, Broadley MR, Eastwood DC, May ST, Rahn C, Swarup R, Woolaway KE, White PJ. 2003. Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. *Plant Physiology* **132,** 578–596.

Hammond JP, White PJ. 2008. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *Journal of Experimental Botany* **59,** 93–109.

Hammond JP, White PJ. 2011. Sugar signaling in root responses to low phosphorus availability. *Plant Physiology* **156,** 1033–1040.

Hamon R, McLaughlin, MJ. Naidu R, Correll R. 1998. Long-term changes in cadmium bioavailability in soil. *Environmental Science and Technoloy* **32,** 3699–3703.

Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Kramer U. 2008. Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. *Nature* **453,** 391–395.

Haydon MJ, Cobbett CS. 2007. Transporters of ligands for essential metal ions in plants. *New Phytologist* **174,** 499–506.

Haydon MJ, Kawachi M, Wirtz M, Hillmer S, Hell R, Kramer U. 2012. Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in Arabidopsis. *The Plant Cell* **24,** 724–737.

Henriques R, Jasik J, Klein M, Martinoia E, Feller U, Schell J, Pais MS, Koncz C. 2002. Knock-out of Arabidopsis metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. *Plant Molecular Biology* **50,** 587–597.

Hou XL, Wu P, Jiao FC, Jia QJ, Chen HM, Yu J, Song XW, Yi KK. 2005. Regulation of the expression of *OsIPS1* and *OsIPS2* in rice via systemic and local Pi signalling and hormones. *Plant, Cell and Environment* **28,** 353–364.

Houben D, Evrard L, Sonnet P. 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere* **92,** 1450–1457.

Hsieh LC, Lin SI, Shih AC, Chen JW, Lin WY, Tseng CY, Li WH, Chiou TJ. 2009. Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. *Plant Physiology* **151,** 2120–2132.

Huang C, Barker SJ, Langridge P, Smith FW, Graham RD. 2000. Zinc deficiency up-regulates expression of high-affinity phosphate transporter genes in both phosphate-sufficient and -deficient barley roots. *Plant Physiology* **124,** 415–422.

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobbett CS. 2004. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. *The Plant Cell* **16,** 1327–1339.

Inou H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK. 2009. Rice OsYSL15 is an iron-regulated iron (III)-deoxymugineic acid transporter

expressed in the roots and is essential for iron uptake in early growth of the seedlings. *Journal of Biologial Chemistry* **284,** 3470–3479.

Jabnoune M, Secco D, Lecampion C, Robaglia C, Shu Q, Poirier Y. 2013. A rice cis-natural antisense RNA acts as a translational enhancer for its cognate mRNA and contributes to phosphate homeostasis and plant fitness. *The Plant Cell* **25,** 4166–4182.

Jain A, Sinilal B, Dhandapani G, Meagher RB, Sahi SV. 2013. Effects of deficiency and excess of zinc on morphophysiological traits and spatiotemporal regulation of zinc-responsive genes reveal incidence of cross talk between micro- and macronutrients. *Environmental Science and Technology* **47,** 5327–5335.

Jensen LT, Ajua-Alemanji M, Culotta VC. 2003. The *Saccharomyces cerevisiae* high affinity phosphate transporter encoded by PHO84 also functions in manganese homeostasis. *Journal of Biologial Chemistry* **278,** 42036–42040.

Johnson AA, Kyriacou B, Callahan DL, Carruthers L, Stangoulis J, Lombi E, Tester M. 2011. Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One* **6,** e24476.

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge U-I. 1998. Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose 6-phosphate/phosphate antiporter. *ThePlant Cell* **10,** 105–117.

Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG. 2007. Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. *Planta* **225,** 907–918.

Karthikeyan AS, Varadarajan DK, Mukatira UT, D'Urzo MP, Damsz B, Raghothama KG. 2002. Regulated expression of Arabidopsis phosphate transporters. *Plant Physiology* **130,** 221–233.

Kashem MA, Singh BR. 2002. Solubility in soil solution and availability to plants of Cd, Ni and Zn as affected by fertilizer additions. *Nutrient Cycling and Agroecosystems* **62,** 287–296.

Kawachi M, Kobae Y, Mori H, Tomioka R, Lee Y, Maeshima M. 2009. A mutant strain Arabidopsis thaliana that lacks vacuolar membrane zinc transporter MTP1 revealed the latent tolerance to excessive zinc. *Plant and Cell Physiology* **50,** 1156–1170.

Khan GA, Bouraine S, Wege S, Li Y, de Carbonnel M, Berthomieu P, Poirier Y, Rouached H. 2014. Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1;H3 in *Arabidopsis*. *Journal of Experimental Botany* **65,** 871–884.

Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A. 2014. Analysis of the root system architecture of Arabidopsis provides a quantitative readout of crosstalk between nutritional signals. *The Plant Cell* **26,** 1480–1496.

Kiiskinen M, Korhonen M, Kangasjarvi J. 1997. Isolation and characterization of cDNA for a plant mitochondrial phosphate translocator (*Mpt1*): ozone stress induces *Mpt1* mRNA accumulation in birch (*Betula pendula* Roth). *Plant Molecular Biology* **35,** 271–279.

Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, Ecker JR, Kaplan J, Guerinot ML. 2006. Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. *Science* **314,** 1295–1298.

Kruger J, Taylor JRN, Du X, De Moura FF, Lönnerdal B, Oelofse A. 2013. Effect of phytate reduction of sorghum, through genetic modification, on iron and zinc availability as assessed by an *in vitro* dialysability bioaccessibility assay, Caco-2 cell uptake assay, and suckling rat pup absorption model. *Food Chemistry* **141,** 1019–1025.

Lan P, Li W, Schmidt W. 2012. Complementary proteome and transcriptome profiling in phosphate-deficient Arabidopsis roots reveals multiple levels of gene regulation. *Molecular and Cellular Proteomics* **11,** 1156–1166.

Lanquar V, Lelièvre F, Barbier-Brygoo H, Thomine S. 2004. Regulation and function of AtNRAMP4 metal transporter protein. *Soil Science and Plant Nutrition* **50,** 477–484.

Lanquar V, Lelievre F, Bolte S, *et al*. 2005. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO Journal* **24,** 4041–4051.

Lanquar V, Ramos MS, Lelievre F, Barbier-Brygoo H, Krieger-Liszkay A, Kramer U, Thomine S. 2010. Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is required for optimal photosynthesis and growth under manganese deficiency. *Plant Physiology* **152,** 1986–1999.

Lanquar V, Grossmann G, Vinkenborg J., Merkx M., Thomine S, Frommer WB. 2014. Dynamic imaging of cytosolic zinc in Arabidopsis roots combining FRET sensors and RootChip technology. *New Phytoogist*, **202,** 198–208.

Lay FT, Anderson MA. 2005. Defensins—components of the innate immune system in plants. *Current Protein and Peptide Science* **6,** 85–101.

Lee S, Persson DP, Hansen TH, *et al*. 2011. Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase. *Plant Biotechnology Journal* **9,** 865–873.

Lejay L, Gansel X, Cerezo M, Tillard P, Mueller C, Krapp A, von Wiren N, Daniel-Vedele F, Gojon A. 2003. Regulation of root ion transporters by photosynthesis: functional importance and relation with hexokinase. *The Plant Cell* **15,** 2218–2232.

Lejay L, Wirth J, Pervent M, Cross JM, Tillard P, Gojon A. 2008. Oxidative pentose phosphate pathway-dependent sugar sensing as a mechanism for regulation of root ion transporters by photosynthesis. *Plant Physiology* **146,** 2036–2053.

Le Jean M, Schikora A, Mari S, Briat JF, Curie C. 2005. A loss-of-function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. *The Plant Journal* **44,** 769–82.

Levi-Minzi R, Petruzzelli G. 1984. The influence of phosphate fertilizers on Cd solubility in soil. *Water, Air, and Soil Pollution* **23,** 423–429.

Lin SI, Chiang SF, Lin WY, Chen JW, Tseng CY, Wu PC, Chiou TJ. 2008. Regulatory network of microRNA399 and PHO2 by systemic signaling. *Plant Physiology* **147,** 732–746.

Lin WY, Huang TK, Chiou TJ. 2013. NITROGEN LIMITATION ADAPTATION, a target of microRNA827, mediates degradation of plasma membrane-localized phosphate transporters to maintain phosphate homeostasis in Arabidopsis. *The Plant Cell* **25,** 4061–4074.

Liu QL, Xu XH, Ren XL, Fu HW, Wu DX, Shu QY. 2007. Generation and characterization of low phytic acid germplasm in rice (Oryza sativa L.). *Theoretical and Applied Genetics* **114,** 803–814.

Liu TY, Huang TK, Tseng CY, Lai YS, Lin SI, Lin WY, Chen JW, Chiou TJ. 2012. PHO2-dependent degradation of PHO1 modulates phosphate homeostasis in Arabidopsis. *The Plant Cell* **24,** 2168–2183.

Loneragan J, Grunes D, Welch R, Aduayi E, Tengah A, Lazar V, Cary E. 1982. Phosphorus accumulation and toxicity in leaves in relation to zinc supply. *Soil Science Society of America Journal* **46,** 345–352.

Loosemore N, Straczek A, Hinsinger P, Jaillard B. 2004. Zinc mobilisation from a contaminated soil by three genotypes of tobacco as affected by soil and rhizosphere pH. *Plant and Soil* **260,** 19–32.

Lu X, Tian X, Cui J, Zhao A, Yang X, Mai W. 2011. Effects of combined phosphorus–zinc fertilization on grain zinc nutritional quality of wheat grown on potentially zinc-deficient calcareous soil. *Soil Science* **176,** 684–690.

Maga J. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *Journal of Agricultural and Food Chemistry* **30,** 1–9.

Marschner H. 1995. *Mineral nutrition of higher plants*. London: Academic Press.

Martin AC, del Pozo JC, Iglesias J, Rubio V, Solano R, de la Peña A, Leyva A, Paz-Ares J. 2000. Influence of cytokinins on the expression of phosphate starvation-responsive genes in *Arabidopsis*. *The Plant Journal* **24,** 559–567.

Martinoia E, Maeshima M, Neuhaus HE. 2007. Vacuolar transporters and their essential role in plant metabolism. *Journal of Experimental Botany* **58,** 83–102.

Maser P, Thomine S, Schroeder JI, *et al*. 2001. Phylogenetic relationships within cation transporter families of Arabidopsis. *Plant Physiology* **126,** 1646–1667.

Masuda H, Aung MS, Nishizawa NK. 2013*a*. Iron biofortification of rice using different transgenic approaches. *Rice* **6,** 40.

Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Nishizawa NK. 2012. Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Scientific Reports* **2,** 1–6.

Masuda H, Kobayashi T, Ishimaru Y, Takahashi M, Aung MS, Nakanishi H, Mori S, Nishizawa NK. 2013*b*. Iron biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Plant Physiology* **4,** 1–12.

Masuda H, Usuda K, Kobayashi T, Ishimaru Y, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Mori S, Nishizawa NK. 2009. Overexpression of the barley nicotianamine synthase gene HvNAS1 increases iron and zinc concentrations in rice grains. *Rice* **2,** 155–166.

Millar AH, Heazlewood JL. 2003. Genomic and proteomic analysis of mitochondrial carrier proteins in Arabidopsis. *Plant Physiology* **131,** 443–453.

Milner MJ, Seamon J, Craft E, Kochian L. 2013. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *Journal of Experimental Botany* **64,** 369–381.

Mirouze M, Sels J, Richard O, *et al*. 2006. A putative novel role for plant defensins: a defensin from the zinc hyper-accumulating plant, Arabidopsis halleri, confers zinc tolerance. *The Plant Journal* **47,** 329–342.

Misson J, Raghothama KG, Jain A, *et al*. 2005. A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences, USA* **102,** 11934–11939.

Mitsuhashi N, Ohnishi M, Sekiguchi Y, Kwon Y-U, Chang Y-T, Chung S-K, Inoue Y, Reid RJ, Yagisawa H, Mimura T. 2005. Phytic acid synthesis and vacuolar accumulation in suspension-cultured cells of Catharanthus roseus induced by high concentration of inorganic phosphate and cation. *Plant Physiology* **138,** 1607–1614.

Miura K, Rus A, Sharkhuu A, *et al*. 2005. The Arabidopsis SUMO E3 ligase SIZ1 controls phosphate deficiency responses. *Proceedings of the National Academy of Sciences, USA* **102,** 7760–7765.

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P. 2009. AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. *Plant Physiology* **149,** 894–904.

Muchhal US, Pardo JM, Raghothama KG. 1996. Phosphate transporters from the higher plant *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **93,** 10519–10523.

Muchhal US, Raghothama KG. 1999. Transcriptional regulation of plant phosphate transporters. *Proceedings of the National Academy of Sciences, USA* **96,** 5868–5872.

Mudge SR, Rae AL, Diatloff E, Smith FW. 2002. Expression analysis suggests novel roles for members of the *Pht1* family of phosphate transporters in Arabidopsis. *The Plant Journal* **31,** 341–353.

Muller R, Morant M, Jarmer H, Nilsson L, Nielsen TH. 2007. Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiology* **143**. 156–171.

Murphy LS, Ellis R, Adriano, DC. 1981. Phosphorus–micronutrient interaction effects on crop production. *Journal of Plant Nutrition* **3,** 593–613.

Nagarajan VK, Jain A, Poling MD, Lewis AJ, Raghothama KG, Smith AP. 2011. Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. *Plant Physiology* **156,** 1149–1163.

Norvell WA, Welch RM. 1993. Growth and nutrient uptake by barley (Hordeum vulgare L. cv Herta): studies using an N-(2-hydroxyethyl) ethylenedinitrilotriacetic acid-buffered nutrient solution technique (I. Zinc ion requirements). *Plant Physiology* **101,** 619–625.

Nussaume L, Kanno S, Javot H, Marin E, Pochon N, Ayadi A, Nakanishi TM, Thibaud MC. 2011. Phosphate import in plants: focus on the PHT1 transporters. *Frontiers in Plant Science* **2,** 83.

Ogawa N, DeRisi J, Brown PO. 2000. New components of a system for phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae* revealed by genomic expression analysis. *Molecular Biology of the Cell* **11,** 4309–4321.

Otegui MS, Capp R, Staehelin LA. 2002. Developing seeds of Arabidopsis store different minerals in two types of vacuoles and in the endoplasmic reticulum. *The Plant Cell* **14,** 1311–1327.

Palmgren MG, Clemens S, Williams LE, Kramer U, Borg S, Schjorring JK, Sanders D. 2008. Zinc biofortification of cereals: problems and solutions. *Trends in Plant Science* **13,** 464–473.

Pant BD, Buhtz A, Kehr J, Scheible WR. 2008. MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *The Plant Journal* **53,** 731–738.

Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, Kehr J, Walther D, Scheible WR. 2009. Identification of nutrient-responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. *Plant Physiology* **150,** 1541–1555.

Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **99,** 13324–13329.

Pedas P, Husted S, Skytte K, Schjoerring JK. 2011. Elevated phosphorus impedes manganese acquisition by barley plants. *Frontiers in Plant Science* **2,** 37

Pedler JF, Parker DR, Crowley DE. 2000. Zinc deficiency-induced phytosiderophore release by the Triticaceae is not consistently expressed in solution culture. *Planta* **211,** 120–126.

Perez-Novo C, Bermudez-Couso A, Lopez-Periago E, Fernandez-Calvino D, Arias-Estevez M. 2011. Zinc adsorption in acid soils: influence of phosphate. *Geoderma* **162,** 358–364.

Petersson J, Pattison J, Kruckeberg AL, Berden JA, Persson BL. 1999. Intracellular localization of an active green fluorescent protein-tagged Pho84 phosphate permease in *Saccharomyces cerevisiae*. *FEBS Letters* **462,** 37–42.

Pilu R, Panzeri D, Gavazzi G, Rasmussen SK, Consonni G, Nielsen E. 2003. Phenotypic, genetic and molecular characterization of a maize low phytic-acid mutant (lpa241). *Theoretical and Applied Genetics* **107,** 980–987.

Pineau C, Loubet S, Lefoulon C, Chalies C, Fizames C, Lacombe B, Ferrand M, Loudet O, Berthomieu P, Richard O. 2012. Natural variation at the FRD3 MATE transporter locus reveals cross-talk between Fe homeostasis and Zn tolerance in Arabidopsis thaliana. *PLoS Genetics* **8,** e1003120.

Piñeros M, Kochian L. 2003. Differences in whole-cell and single-channel ion currents across the plasma membrane of mesophyll cells from two closely related Thlaspi species. *Plant Physiology* **131,** 583–594.

Poirier Y, Bucher M. 2002. Phosphate transport and homeostasis in Arabidopsis. *Arabidopsis Book* **1,** e0024.

Poirier Y, Thoma S, Somerville C, Schiefelbein J. 1991. Mutant of Arabidopsis deficient in xylem loading of phosphate. *Plant Physiology* **97,** 1087–1093.

Punshon T, Guerinot ML, Lanzirotti A. 2009. Using synchrotron X-ray fluorescence microprobes in the study of metal homeostasis in plants. *Annals of Botany* **103,** 665–672.

Raboy V. 2009 Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Science* **177,** 281–296.

Raboy V, Gerbasi P, Young KA, Stoneberg S, Pickett SG, Bauman AT, Murthy PPN, Sheridan WF, Ertl DS. 2000. Origin and seed phenotype of maize low phyticacid 1-1 and low phytic acid 2-1. *Plant Physiology* **124,** 355–368.

Rasmussen SK, Hatzack F. 1998. Identification of two low-phytate barley (Hordeum vulgare L.) grain mutants by TLC and genetic analysis. *Hereditas* **129,** 107–12.

Rausch C, Zimmermann P, Amrhein N, Bucher M. 2004. Expression analysis suggests novel roles for the plastidic phosphate transporter Pht2;1 in auto- and heterotrophic tissues in potato and *Arabidopsis*. *The Plant Journal* **39,** 13–28.

Rauser WE. 1999. Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin and metallothioneins. *Cell Biochemistry and Biophysics* **31,** 19–48.

Reed H. 1946. Effects of zinc deficiency on phosphate metabolism of the tomato plant. *American Journal of Botany* **33,** 778–784.

Rellan-Alvarez R, Giner-Martinez-Sierra J, Orduna J, Orera I, Rodriguez-Castrillon JA, Garcia-Alonso JI, Abadia J, Alvarez-Fernandez A. 2010. Identification of a tri-iron(III), tri-citrate complex in the xylem sap of iron-deficient tomato resupplied with iron: new insights into plant iron long-distance transport. *Plant and Cell Physiology* **51,** 91–102.

Remy E, Cabrito TR, Batista RA, Teixeira MC, Sa-Correia I, Duque P. 2012. The Pht1;9 and Pht1;8 transporters mediate inorganic phosphate acquisition by the Arabidopsis thaliana root during phosphorus starvation. *New Phytologist* **195,** 356–371.

Ricachenevsky FK, Sperotto RA, Menguer PK, Sperb ER, Lopes KL, Fett JP. 2011. ZINC-INDUCED FACILITATOR-LIKE family in plants: lineage-specific expansion in monocotyledons and conserved genomic

and expression features among rice (*Oryza sativa*) paralogs. *BMC Plant Biology* **11,** 20.

Robinson NJ, Wilson JR, Turner JS. 1996. Expression of the type 2 metallothionein-like gene MT2 from Arabidopsis thaliana in Zn(2+)-metallothionein-deficient Synechococcus PCC 7942: putative role for MT2 in Zn2+ metabolism. *Plant Molecular Biology* **30,** 1169–1179.

Robson A, Pitman M. 1983. Interactions between nutrients in higher plants. In: Lauchli A, Bieleski RL, eds. *Encyclopaedia of plant physiology New series*. Berlin: Springer-Verlag **15A,** 287–312.

Roth C, Menzel G, Petetot J, Rochat-Hacker S, Poirier Y. 2004. Characterization of a protein of the plastid inner envelope having homology to animal inorganic phosphate, chloride and organic-anion transporters. *Planta* **218,** 406–416.

Römheld V, Marschner H. 1986. Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. *Plant Physiology* **80,** 175–80.

Rouached H, Arpat AB, Poirier Y. 2010. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. *Molecular Plant* **3,** 288–299.

Rouached H, Secco D, Arpat B, Poirier Y. 2011*a*. The transcription factor PHR1 plays a key role in the regulation of sulfate shoot-to-root flux upon phosphate starvation in Arabidopsis. *BMC Plant Biology* **11,** 19.

Rouached H, Stefanovic A, Secco D, Bulak Arpat A, Gout E, Bligny R, Poirier Y. 2011*b*. Uncoupling phosphate deficiency from its major effects on growth and transcriptome via PHO1 expression in Arabidopsis. *The Plant Journal* **65,** 557–570.

Rubio V, Bustos R, Irigoyen ML, Cardona-Lopez X, Rojas-Triana M, Paz-Ares J. 2009. Plant hormones and nutrient signaling. *Plant Molecular Biology* **69,** 361–373.

Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes and Development* **15,** 2122–2133.

Salgueiro MJ, Zubillaga M, Lysionek A, Cremaschi G, Goldman CG, Caro R, De Paoli T, Hager A, Weill R, Boccio J. 2000*a*. Zinc status and immune system relationship: a review. *Biological Trace Element Research* **76,** 193–205.

Salgueiro M, Zubillaga M, Lysionek A, Sarabia MI, Caro R, De Paoli T, Hager A, Weill R, Boccio J. 2000*b*. Zinc as an essential micronutrient: a review. *Nutrition Research* **20,** 737–755.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MA, Manceau A. 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **130,** 1815–1826.

Sauvé S, Hendershot W, Allen HE. 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. *Environmental Science and Technology* **34,** 1125–1131.

Schachtman DP, Reid RJ, Ayling SM. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology* **116,** 447–453.

Schiller M, Hegelund JN, Pedas P, Kichey T, Laursen KH, Husted S, Schjoerring JK. 2014. Barley metallothioneins differ in ontogenetic pattern and response to metals. *Plant, Cell and Environment* **37,** 353–367.

Schnell Ramos M, Khodja H, Mary V, Thomine S. 2013. Using µPIXE for quantitative mapping of metal concentration in Arabidopsis thaliana seeds. *Frontiers in Plant Science* **4,** 168.

Seigneurin-Berny D, Gravot A, Auroy P, *et al.* 2006. HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. *Journal of Biologial Chemistry* **281,** 2882–289.

Shahzad Z, Gosti F, Frerot H, Lacombe E, Roosens N, Saumitou-Laprade P, Berthomieu P. 2010. The five AhMTP1 zinc transporters undergo different evolutionary fates towards adaptive evolution to zinc tolerance in Arabidopsis halleri. *PLoS Genetics* **6,** e1000911.

Shahzad Z, Ranwez V, Fizames C, *et al*. 2013. Plant Defensin type 1 (PDF1): protein promiscuity and expression variation within the Arabidopsis genus shed light on zinc tolerance acquisition in Arabidopsis halleri. *New Phytologist* **200,** 820–833.

Shahzad Z, Rouached H, Rakha A. 2014. Combating mineral malnutrition through iron and zinc biofortification of cereals. *Comprehensive Reviews in Food Science and Food Safety* **13,** 329–346.

Shi J, Wang H, Wu Y, Hazebroek J, Meeley RB, Ertl DS. 2003. The maize low-phytic acid mutant lpa2 is caused by mutation in an inositol phosphate kinase gene. *Plant Physiology* **131,** 507–515.

Sinclair SA, Kramer U. 2012. The zinc homeostasis network of land plants. *Biochimica et Biophysica Acta* **1823,** 1553–1567.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*, 2nd edn. Academic Press, London.

Springer M, Wykoff DD, Miller N, O'Shea EK. 2003. Partially phosphorylated Pho4 activates transcription of a subset of phosphate-responsive genes. *PLoS Biology* **1,** E28.

Sresty TVS, Madhava Rao KV. 1999. Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea. *Environmental and Experimental Botany* **41,** 3–13.

Steadman KJ, Burgoon MS, Lewis BA, Edwardson SE, Obendorf RL. 2001. Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *Journal of the Science of Food and Agriculture* **81,** 1094–1100.

Stefanovic A, Ribot C, Rouached H, Wang Y, Chong J, Belbahri L, Delessert S, Poirier Y. 2007. Members of the PHO1 gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. *The Plant Journal* **50,** 982–994.

Stephan UW, Scholz G. 1993. Nicotianamine: mediator of transport of iron and heavy metals in the phloem? *Physiologia Plantarum* **88,** 522–529.

Stevenson-Paulik J, Bastidas RJ, Chiou S-T, Frye RA, York JD. 2005. Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. *Proceedings of the National Academy of Sciences, USA* **102,** 12612–12617.

Suzuki M, Takahashi M, Tsukamoto T, *et al*. 2006. Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *The Plant Journal* **48,** 85–97.

Suzuki M, Tsukamoto T, Inoue H, Watanabe S, Matsuhashi S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2008. Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. *Plant Molecular Biology* **66,** 609–17.

Suzuki S, Ishioka T. 2008. Normal mode analysis of zinc caprate (Zn)1/2O2C(CH2)8CH3. *Spectrochimica. Part A, Molecular and Biomolecular Spectroscopy* **70,** 195–197.

Tagwira F, Piha M, Mugwira L. 1992. Effect of pH, and phosphorus and organic matter contents on zinc availability and distribution in two Zimbabwean soils. *Communications in Soil Science and Plant Analysis* **23,** 1485–1500.

Tagwira F, Piha M, Mugwira L. 1993. Zinc studies in Zimbabwean soils: effect of lime and phosphorus on growth, yield, and zinc status of maize. *Communications in Soil Science and Plant Analysis* **24,** 717–736.

Takagi SI, Nomoto K, Takemoto T. 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *Journal of Plant Nutrition* **7,** 1–5.

Talke IN, Hanikenne M, Kramer U. 2006. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator Arabidopsis halleri. *Plant Physiology* **142,** 148–167.

Tang H, Li X, Zu C, Zhang F, Shen J. 2013. Spatial distribution and expression of intracellular and extracellular acid phosphatases of cluster roots at different developmental stages in white lupin. *Journal of Plant Physiology* **15,** 1243–50.

Tauris B, Borg S, Gregersen PL, Holm PB. 2009. A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. *Journal of Expermimental Botany* **60,** 1333–1347.

Tennstedt P, Peisker D, Bottcher C, Trampczynska A, Clemens S. 2009. Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiology* **149,** 938–948.

Thakur SK, Tomar NK, Pandeya SB. 2006. Influence of phosphate on cadmium sorption by calcium carbonate. *Geoderma* **130,** 240 –249.

Tolay I, Erenoglu B, Romheld V, Braun HJ, Cakmak I. 2001. Phytosiderophore release in Aegilops tauschii and Triticum species under zinc and iron deficiencies. *Journal of Experimental Botany* **52,** 1093–1099.

van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Ver Loren van Themaat E, Koornneef M, Aarts MG. 2006. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots

of Arabidopsis thaliana and the related metal hyperaccumulator Thlaspi caerulescens. *Plant Physiology* **142,** 1127–1147.

Verma T, Minhas R. 1987. Zinc and phosphorus interaction in a wheat–maize cropping system. *Fertilizer Research* **13,** 77–86.

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P. 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Letters* **576,** 306–312.

Versaw WK, Harrison MJ. 2002. A chloroplast phosphate transporter, PHT2;1, influences allocation of phosphate within the plant and phosphate-starvation responses. *The Plant Cell* **14,** 1751–1766.

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat JF, Curie C. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell* **14,** 1223–1233.

Veum TL, Ledoux DR, Shannon MC, Raboy V. 2009. Effect of graded levels of iron, zinc, and copper supplementation in diets with low-phytate or normal barley on growth performance, bone characteristics, hematocrit volume, and zinc and copper balance of young swine1. *Journal of Animal Science* **87,** 2625–2634.

von Wiren N, Khodr H, Hider RC. 2000. Hydroxylated phytosiderophore species possess an enhanced chelate stability and affinity for iron(III). *Plant Physiology* **124,** 1149–1158.

von Wiren N, Marchner H, Romheld V. 1996. Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. *Plant Physiology* **111,** 1119–1125.

Wang AS, Angle JS, Chaney RL, Delorme TA, Reeves RD. 2006. Soil pH effects on uptake of Cd and Zn by Thlaspi caerulescens. *Plant and Soil* **281,** 325–337.

Wang X, Yi K, Tao Y, Wang F, Wu Z, Jiang D, Chen X, Zhu L, Wu P. 2006. Cytokinin represses phosphate-starvation response through increasing of intracellular phosphate level. *Plant, Cell and Environment* **29,** 1924–1935.

Wang Y, Ribot C, Rezzonico E, Poirier Y. 2004. Structure and expression profile of the Arabidopsis PHO1 gene family indicates a broad role in inorganic phosphate homeostasis. *Plant Physiology* **135,** 400–411.

Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG. 2008. The effect of iron on the primary root elongation of Arabidopsis during phosphate deficiency. *Plant Physiology* **147,** 1181–1191.

Waters BM, Chu H-H, DiDonato RJ, Roberts LA, Eisley RB, Lahner B, Salt DE, Walker EL. 2006. Mutations in Arabidopsis yellow stripe-like1 and yellow stripe-like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiology* **141,** 1446–1458.

Waters BM, Grusak MA. 2008. Quantitative trait locus mapping for seed mineral concentrations in two Arabidopsis thaliana recombinant inbred populations. *New Phytologist* **179,** 1033–1047.

Waters BM, Uauy C, Dubcovsky J, Grusak MA. 2008. Effect of wheat NAM genes on remobilization of Fe and Zn and translocation of minerals to grain during grain fill. In: *XIV International Symposium on Iron Nutrition and Interactions in Plants*, p. 51.

Watts-Williams S, Patti AF, Cavagnaro TR. 2013. Arbuscular mycorrhizas are beneficial under both deficient and toxic soil zinc conditions. *Plant and Soil* **371,** 299–312.

Wintz H, Fox T, Wu YY, Feng V, Chen W, Chang HS, Zhu T, Vulpe C. 2003. Expression profiles of Arabidopsis thaliana in mineral deficiencies reveal novel transporters involved in metal homeostasis. *Journal of Biologial Chemistry* **278,** 47644–47653.

Wissuwa M, Gamat G, Ismail AM. 2005. Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany* **56,** 1943–1950.

Woo J, MacPherson CR, Liu J, Wang H, Kiba T, Hannah MA, Wang XJ, Bajic VB, Chua NH. 2012. The response and recovery of the Arabidopsis thaliana transcriptome to phosphate starvation. *BMC Plant Biology* **12,** 62.

Wu P, Ma L, Hou X, Wang M, Wu Y, Liu F, Deng XW. 2003. Phosphate starvation triggers distinct alterations of genome expression in Arabidopsis roots and leaves. *Plant Physiology* **132,** 1260–1271.

Wykoff DD, O'Shea EK. 2001. Phosphate transport and sensing in *Saccharomyces cerevisiae*. *Genetics* **159,** 1491–1499.

Wykoff DD, Rizvi AH, Raser JM, Margolin B, O'Shea EK. 2007. Positive feedback regulates switching of phosphate transporters in *S. cerevisiae*. *Molecular Cell* **27,** 1005–1013.

Xu Q1, Chu W, Qiu H, Fu Y, Cai S, Sha S. 2013. Responses of Hydrilla verticillata (L.f.) Royle to zinc: in situ localization, subcellular distribution and physiological and ultrastructural modifications. *Plant Physiology and Biochemistry* **69,** 43–48.

Yang Z, Wu Y, Li Y, Ling HQ, Chu C. 2009. OsMT1a, a type 1 metallothionein, plays the pivotal role in zinc homeostasis and drought tolerance in rice. *Plant Molecular Biology* **70,** 219–229.

Zhu YG, Smith SE, Smith FA. 2001. Plant growth and cation composition of two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. *Journal of Experimental Botany* **52,** 1277–1282.