Environmental and Experimental Botany 114 (2015) 57–64

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Molecular mechanisms of phosphate and zinc signalling crosstalk in plants: Phosphate and zinc loading into root xylem in Arabidopsis

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ARTICLE INFO

Article history:
Available online 4 June 2014

Keywords:
Phosphate
Zinc
Membrane transporters
Xylem loading
Signalling crosstalk

ABSTRACT

Inorganic phosphate (Pi) and zinc (Zn) are an essential macro- and micronutrients for plant survival. Control of Pi and Zn content in tissues is of major importance for normal plant growth and development. Zn deficiency typically leads to Pi over-accumulation in shoots (and *vice versa*), signifying the presence of complex interactions that link the homeostatic regulation of these two nutrients. Despite their primary importance, the molecular bases of these interactions remains poorly understood. Recent research has placed the co-regulation of these two elements at a limiting step in Pi and Zn distribution within plants, *e.g.* the loading of Pi and Zn into root xylem. In *Arabidopsis thaliana*, this process mainly involves members of the Phosphate 1 (*PHO1* and *PHO1;H1*) family (for Pi) and the heavy metal ATPases protein (*HMA2* and *HMA4*) family (for Zn). This review examines recent progress in determining the molecular mechanisms that regulate the loading of Pi and Zn into root xylem, by individually describing these specific genes. The first molecular evidence for their signalling crosstalk at this particular step of their transport in plants is also presented, with an emerging role for *PHO1;H3*. This recent progress is important for biotechnological and agronomic strategies aimed at enhancing Pi and Zn transfer to the aerial part of plants.

1. Introduction

Pi and Zn are essential nutrients for all the living organisms. The macronutrient Pi is an essential structural component of RNA and DNA, as well as phospholipids. Pi is involved in many key biological processes in the cell, including numerous enzymatic reactions (Westheimer, 1987; Poirier and Bucher, 2002; Rouached et al., 2010). Several signal transduction cascades also rely on Pi *via* the modulation of enzyme activity by protein phosphorylation, which can be adversely altered in situations where Pi is present in too low a concentration (Poirier and Bucher, 2002). The micronutrient Zn is required for proper cell functioning (Berg and Shi, 1996; Salgueiro et al., 2000; Sinclair and Kramer, 2012), as it is a highly effective cofactor for hundreds of enzymes, the structural Zn-finger domains that mediate DNA-binding of transcription factors, and protein–protein interactions (Coleman, 1998; Shahzad et al., 2014). Due to the central roles of Pi and Zn in numerous aspects of plant metabolism, it is not surprising that plants are profoundly affected by Pi or Zn starvation, and that their deficiencies provoke a coordinated series of morphological, physiological and biochemical adaptations (Mikulska et al., 1998; Poirier and Bucher, 2002; Misson et al., 2005; Rouached et al., 2010; Jain et al., 2013). It is therefore of great importance for cells to tightly control Pi and Zn homeostasis, which likely interact *via* a complex process (Cakmak and Marschner, 1986; Khan et al., 2014).

In plants, Pi and Zn are taken up at the root–soil interface, predominantly as free ions (Guerinot, 2000; Shahzad et al., 2014; Nussaume et al., 2011; Milner et al., 2013). In recent years, significant progress has been made in our knowledge of the regulation of Pi and Zn acquisition in plants, and this phenomenon has been documented in many research publications and elegantly summarised in multiple reviews (Sinclair and Kramer, 2012; Nussaume et al., 2011). Arabidopsis genome contains nine PHT1 family members and most of them are controlled by the endogenous Pi status of the plant (Poirier and Bucher, 2002; Nussaume et al., 2011). Some PHT1 genes are preferentially expressed in roots, and function as a high-affinity Pi uptake transporter (Muchhal et al., 1996; Misson et al., 2005; Remy et al., 2012; Bayle et al., 2011; Nussaume et al., 2011). Shin et al. (2004) provided genetic evidences proven that *PHT1;1* and *PHT1;4* play crucial role in Pi

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http://dx.doi.org/10.1016/j.envexpbot.2014.05.013
0098-8472/

transport in roots during growth under both low- and high-Pi environments because the double mutant *pht1;1 pht1;4* shows a 75% reduction in Pi uptake capacity relative to the wild type (Shin et al., 2004). Research results are summarised on this subject in many reviews (Poirier and Bucher, 2002; Nussaume et al., 2011). For Zn, many Zn uptake transporters have been identified and belong to the Zrt/IRT-like protein (ZIP) family of Zn transporters. In *Arabidopsis thaliana*, the ZIP family contains 15 members (Maser et al., 2001), including the *AtIRT1* which localises preferentially to the plasma membrane of root epidermal cells (Vert et al., 2002). It has been reported that *irt1* mutant accumulates less Zn as compared to wild type, revealing its implication in Zn uptake (Henriques et al., 2002). For ample information on the regulation of Zn uptake in Arabidopsis readers are referred to Sinclair and Kramer (2012). After their acquisition at the root periphery, each element can be fixed into the root *via* transport into vacuoles. Alternatively, their symplastic journey, thought to be mediated by the plasmodesmata, ends with their loading into root xylem. For Pi, *PHO1* and its closest homologue *PHO1;H1* have been identified as key genes in the long-distance transfer of Pi from the root to the shoot (Poirier et al., 1991; Hamburger et al., 2002; Stefanovic et al., 2007; Stefanovic et al., 2011). For Zn, two members of the Arabidopsis P_{1B}-ATPase subfamily: *HMA2* and its most closely related sequence in the *HMA* cluster, *HMA4* play crucial role in Zn loading into xylem (Hussain et al., 2004; Verret et al., 2004; Hanikenne et al., 2008; Siemianowski et al., 2011; Wong et al., 2009). The most recent reports on their biological functions and the molecular mechanisms of their regulation in *A. thaliana* will be reviewed below.

Research efforts over the past 10 years have contributed the first studies on Pi and Zn deficiency signalling pathways (Chiou and Lin, 2011; Assuncao et al., 2013) However, it is clear that these results are just part of a very complex process.

The Pi long-distance signalling network includes the MYB transcription factor PHR1, the ubiquitin E2 conjugase PHO2, and the miRNA A399 (Pant et al., 2008). In response to Pi deficiency, miRNA399 is transcriptionally regulated by PHR1, and then translocated from shoot to root by the phloem, where it targets the *PHO2* transcript (Bari et al., 2006; Lin et al., 2008; Pant et al., 2008). The repression of *PHO2* expression causes an increase in the expression of root Pi-uptake transporters (PHT1;8 and PHT1;9), and therefore an increase in Pi acquisition by the roots as well as its translocation to the shoot (Bari et al., 2006; Lin et al., 2008). The Zn deficiency appears to be first sensed in shoots; the signal is then transmitted to the roots, where these cation transporters function (Assuncao et al., 2010, 2013). This suggests the presence of long-distance Zn deficiency signalling molecules (which are yet to be identified). A recent working model of Zn deficiency signalling (Assuncao et al., 2013) proposes that the Arabidopsis transcription factors bZIP19 and bZIP23 play important roles in the response to Zn deficiency by regulating downstream genes, including ZIP members (*i.e.* the Zrt/Irt-like proteins, candidates that mediate root Zn uptake and transport) (Guerinot, 2000; Assuncao et al., 2010).

Interactions between Pi and Zn in plants have been reported in numerous plant species (Reed, 1946; Verma and Minhas, 1987; Webb and Loneragan, 1988; Tagwira et al., 1993; Loneragan et al., 1982; Gianquinto et al., 2000; Huang et al., 2000; Zhu et al., 2001; Shi et al., 2008). Such interaction is integrative as a plant loses its capacity to regulate Pi transport under Zn deficiency, despite the presence of an adequate Pi supply. Pi–Zn intercation is specific. Such specificity has been demonstrated by the fact that in barley only Zn deficiency could induce Pi uptake and not nitrogen, sulfur, nor manganese deficiency (Huang et al., 2000). Similarly, cotton or tomato plants do not show an over-accumulation of Pi under iron or copper deficiency (Cakmak and Marschner, 1986; Liu et al., 1998). This interaction is of agronomic importance and can account for the shortcomings of current models that are typically focused on improving the assimilation of the individual elements. Earlier aforementioned studies have provided physiological evidence for the importance of Zn deficiency in Pi translocation to the shoots. More recently, Khan et al. (2014) have provided direct molecular evidence for the crosstalk between Pi and Zn nutrition in *A. thaliana*, by identifying genes involved in this crosstalk. Results from mining the transcriptomics data support the existence of genetic programmes that regulate Pi–Zn nutrition interaction in plants, as well as providing new research channels to elucidate this phenomenon (Misson et al., 2005; van de Mortel et al., 2006).

In this review, a molecular evidence for the Pi–Zn homeostasis and interaction in *A. thaliana* with an emerging role for PHO1;H3 will be discussed. Given importance of genes involved in the regulation of Pi loading into roots xylem under Zn deficiency, a large part of this review is dedicated for detailing the current understanding on the molecular mechanisms that regulate this process. In preceding context roles of the Phosphate 1 (*PHO1* and *PHO1;H1*) family (for Pi) and the heavy metal ATPases protein (*HMA2* and *HMA4*) family (for Zn) will be reviewed. In addition, to further probe the regulation of these genes, results from data mining based on meta-analysis tools using the available sets of Arabidopsis microarray data will be presented.

2. Converging signalling pathways that regulate Pi and Zn loading into the root xylem: an emerging role for PHO1;H3

The existence of complex interactions that link the homeostatic regulations of Pi and Zn has long been recognised (Reed, 1946; Verma and Minhas, 1987; Webb and Loneragan, 1988; Tagwira et al., 1993; Loneragan et al., 1982; Cakmak and Marschner, 1986; Gianquinto et al., 2000; Huang et al., 2000; Zhu et al., 2001; Shi et al., 2008). In particular, Zn deficiency is associated with over-accumulation of Pi in the shoots of both dicotyledons and monocotyledons (Huang et al., 2000; Misson et al., 2005; Khan et al., 2014), although the genes underlying mechanisms of this process remain to be identified. Very recently, Khan et al. (2014) identified genes that are necessary for the increase in Pi over-accumulation in response to Zn deficiency in Arabidopsis. These genes include *PHR1*, *PHO1* and its homologue *PHO1;H3*. *PHR1* was already known as a major regulator of Pi deficiency signalling through its involvement in the so-called *PHR1-miRNA399-PHO2* regulatory pathway (Bari et al., 2006). However, this regulatory pathway is not involved in the over-accumulation of Pi in the shoot in response to Zn deficiency (Khan et al., 2014), and therefore a Zn-responsive signalling pathway involving *PHR1* remains to be elucidated. *PHO1* is most likely one of the final targets of the Zn-deficiency signalling pathway. Since its expression level does not change in response to Zn deficiency, it is likely that its activity is regulated through a protein–protein interaction, considering that a similar mechanism involving PHO1 and PHO2 has already been reported (Liu et al., 2012). Finally, *PHO1;H3* is involved in the control of Pi accumulation in response to Zn deficiency, and thus appears to be involved in the regulation of Pi transport (Khan et al., 2014), although no biological function has been identified so far. Nevertheless, it was recently reported that it is specifically and strongly induced by Zn deficiency, and that its expression pattern is similar to *PHO1*: both are expressed in cells of the root vascular cylinder and are localised to the Golgi when expressed transiently in tobacco cells (Khan et al., 2014). When grown in Zn-free medium, *pho1;h3* mutant plants displayed higher Pi contents in the shoots than wild-type plants. However, this was not observed in a *pho1 pho1;h3* double mutant, suggesting that PHO1;H3 restricts root-to-shoot Pi transfer that requires PHO1 function for Pi homeostasis in response to Zn deficiency (Khan et al., 2014). This makes *PHO1;H3* an interesting entry point to study Pi–Zn crosstalk in the root xylem. Future research to