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REVIEW

Molecular mechanisms regulating Pi-signaling and Pi homeostasis under OsPHR2, a central Pi-signaling regulator, in rice

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Abstract **Phosphorus (P) is one of the most important major mineral elements for plant growth and metabolism. Plants have evolved adaptive regulatory mechanisms to maintain phosphate (Pi) homeostasis by improving phosphorus uptake, translocation, remobilization and efficiency of use. Here we review recent advances in our understanding of the OsPHR2-mediated phosphate-signaling pathway in rice. OsPHR2 positively regulates the low-affinity Pi transporter *OsPT2* through physical interaction and reciprocal regulation of OsPHO2 in roots. OsPT2 is responsible for most of the OsPHR2-mediated accumulation of excess Pi in shoots. OsSPX1 acts as a repressor in the OsPHR2-mediated phosphate-signaling pathway. Some mutants screened from ethyl methanesulfonate (EMS)–mutagenized M2 population of *OsPHR2* overexpression transgenic line removed the growth inhibition, indicating that some unknown factors are crucial for Pi utilization or plant growth under the regulation of OsPHR2.**

Keywords *Oryza sativa* L., OsPHR2, OsPT2, OsSPX1, Pi homeostasis

Introduction

The molecular mechanisms involved in regulating Pi-signaling in plants remain to be elucidated, although several transcription factors have identified that each influence only a part of the profile of changes induced by Pi starvation in *Arabidopsis*. These transcription factors include PHR1 (PHOSPHATE STARVATION RESPONSE 1) (Rubio et al., 2001), WRKY75 (Devaiah et al., 2007a), ZAT6 (Devaiah et al., 2007b), MYB62 (Devaiah et al., 2009), BHLH32 (Chen et al., 2007), and WRKY96 (Chen et al., 2009). In rice (*Oryza sativa* L.), a bHLH transcription factor, OsPTF1 (Pi starvation-induced transcription factor 1) has been reported to be involved in Pi-signaling and Pi uptake (Yi et al., 2005). AtPHR1 plays a key role in the phosphorus (P) signaling system in *Arabidopsis* (*Arabidopsis thaliana*). AtPHR1 is a transcription factor with a MYB domain and a predicted coiled-coil (CC) domain, and is defined as a member of the MYB-CC family. *AtPHR1* and *CrPSR1* (PHOSPHORUS STARVATION RESPONSE 1) belong to a large gene family (including another 14 proteins in *Arabidopsis*) with a conserved MYB DNA binding domain (BD) and a predicted CC domain in each family member (Rubio et al., 2001). Based on the protein sequence similarity of AtPHR1, two homologous single copy genes were isolated in rice (*Oryza sativa* L.) and designated *OsPHR1* and *OsPHR2* (Zhou et al., 2008). Although both are involved in Pi-signal transduction, only the ectopic overexpression of *OsPHR2* results in excessive shoot Pi accumulation and plant growth inhibition, especially under Pi abundant conditions (Zhou et al., 2008). This raises the intriguing question of whether the increase in Pi uptake and translocation in plants would upset the nutrient homeostasis in plant cells required for normal metabolism in plant growth. We prefer the hypothesis that the excessive Pi in shoots results in Pi-toxicity. In support of this, ectopic overexpression of a low affinity Pi transporter, OsPT2, creates excessive shoot Pi accumulation and inhibition of plant growth (Ai et al., 2009; Liu et al. 2010). However, at shoot Pi concentration levels similar to wild type plants, the OsPHR2-overexpressed plants still showed inhibition of plant growth. This raises the possibility that OsPHR2 regulates some unknown factors crucial for plant growth or phosphate physiologic utilization (Wu and Xu, 2010). In this review, we focus on the molecular mechanisms involved in regulating Pi-signaling and Pi homeostasis under OsPHR2, as well as on

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the recent progress in discovering new signaling players involved in the Pi-starvation responses in rice.

OsPHR2 regulates low affinity Pi-transporter through both physiologic and reciprocal regulation of OsPHO2

Downstream of AtPHR1, *miRNA399,* as a target of PHR1, is specifically induced by Pi starvation. *miRNA399* reciprocally regulates the gene *PHO2* at the post-transcriptional level (Fujii et al., 2005; Bari et al., 2006; Chiou et al., 2006). PHO2 functions as a ubiquitin-conjugating E2 enzyme (UBC24; Aung et al., 2006; Bari et al., 2006), and the loss of function of PHO2/UBC24 leads to excessive accumulation of Pi in the shoot tissue (Fujii et al., 2005; Chiou et al., 2006). In addition, repression of OsPT2, the low affinity of Pi transporter (Ai et al., 2009), in a background of *OsPHR2* overexpression, remarkably reduces the excessive shoot Pi accumulation, but this is not observed in a *pho2* mutant (Liu et al. 2010). These results provide evidence that OsPT2 makes a major contribution to excessive Pi accumulation in shoots driven by OsPHR2 and makes us speculate that OsPHR2 may physically regulate OsPT2, in addition to the reciprocal regulation of PHO2 at the transcriptional level (Liu et al., 2010).

The cis-element of AtPHR1 (designated as P1BS) is conserved in promoters of many Pi-signaling responsive genes (Rubio et al., 2001). In the promoter of *OsPT2*, one P1SB element is found between −346 and −338 bp upstream of the ATG of *OsPT2*. The expression and response of *OsPT2* to Pi-starvation signaling was greatly reduced in a T-DNA insertion mutant at −569 bp of *OsPT2* promoter. This result suggests that the T-DNA insertion interferes with binding of OsPHR2 to the cis-element or that the cis-element alone is not sufficient for *OsPT2* to respond to Pi-starvation signaling (Liu et al., 2010). In fact, the P1BS element exists in many promoters of genes both responsive and non-responsive to Pi-starvation stress. It will be interesting to clarify what elements coordinate Pi-signal transduction in plants.

OsSPX1 is a suppressor on function of OsPHR2

The hydrophilic and poorly conserved SPX domain (SYG1/Pho81/XPR1) is found at the N-termini of various proteins, particularly signal transduction proteins (Barabote et al., 2006). Most identified plant SPX gene products are involved in responses to environmental cues or internal regulation of nutrition homeostasis. Barley IDS4 (iron-deficiency specific clone 4) contains part of the SPX domain and is preferentially expressed in Fe-deficient roots (Nakanishi et al., 1993). *Arabidopsis* PHO1, harboring both SPX and EXS domains, plays a role in loading root Pi into the xylem vessels, and loss of PHO1 function in *pho1* mutants results in Pi deficiency in above-ground tissues (Poirier et al., 1991; Hamburger et al., 2002). A PHO1 homolog in *Arabidopsis* may have a similar role in Pi loading and signaling (Wang et al., 2004). The product of the tomato IDS4-like gene interacts with the leucine zipper domain of a hypoxia-induced transcription factor involved in the low-oxygen response (Sell and Hehl, 2005). Homologous to yeast SYG1, the *Arabidopsis* SHORT HYPOCOTYL UNDER BLUE 1 (SHB1) protein acts in cryptochrome signaling and seed development (Kang and Ni, 2006; Zhou and Ni, 2009; Zhou et al., 2009; Zhou and Ni, 2010).

Four genes with unique SPX domains in *Arabidopsis* were identified to be involved in Pi-signaling pathways controlled by PHR1 and SIZ1 (Duan et al., 2008). The rice (*Oryza sativa* L.) genome contains at least six genes exclusively with the SPX (SYG1/PHO81/XPR1) domain at the N-terminal, designated as OsSPX1–6. The diverse expression patterns of the *OsSPX* genes in different tissues and their responses to Pi-starvation have been reported (Wang et al., 2009b). Among them, five genes, *OsSPX1*, *2*, *3*, *5* and *6* are responsive to Pi-starvation in shoots and/or in roots. Subcellular localization analysis indicates that OsSPX1 and OsSPX2 is exclusively located in nucleus, OsSPX3 is in cytoplasm and/or in nucleus, and OsSPX4 is a membrane-localized protein. OsSPX1 regulates *OsSPX2, 3* and *5* at the transcriptional level and is positively involved in the responses of the genes to Pi-starvation. Overexpression of *OsSPX3* regulates *OsSPX5* in shoots under Pi sufficiency, and OsSPX3 negatively regulates PSI (Pi-starvation induced) genes (Wang et al., 2009b). The effect of OsSPX1 on Pi-signaling and its negative regulation on shoot Pi excessive accumulation has also been reported (Wang et al., 2009a). Because of the reciprocal effect of OsPHR2 and OsSPX1 on shoot Pi accumulation, it could be reasoned that OsSPX1 may be a genetic repressor of function of OsPHR2 on Pi uptake and translocation.

Evidence from the physiological analysis of transgenic plants with double overexpression of *OsPHR2* and *OsSPX1* supports the above hypothesis (Liu et al., 2010) and reveals that OsSPX1 has a counteracting effect on the upregulation of *OsPT2* in roots driven by *OsPHR2* overexpression and the accumulation of excess shoot Pi under abundant Pi. In contrast, OsSPX1 did not, however, show the counteracting effect on the negative regulation of OsPHO2 on *OsPT2*. The results suggest that OsSPX1 may be a repressor involved in the physical regulation of OsPHR2 on OsPT2, but not in the reciprocal regulation of OsPHO2 on OsPT2, although a feedback Pi-signaling network is defined by OsPHR2, OsSPX1 and OsPHO2 in roots under abundant Pi (Fig. 1).

OsPHR2 may control some unknown factors crucial for physiologic utilization of cell Pi

The growth inhibition of transgenic plants with shoot Pi

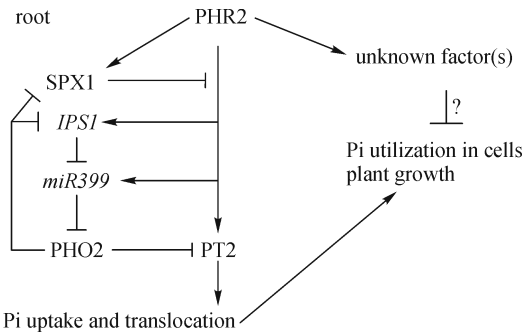

Figure 1 A proposed model for the regulation of low-affinity Pi transporter OsPT2 and the feedback Pi-signaling network defined by OsPHR2, OsSPX1 and OsPHO2 in roots under abundant Pi (Liu et al., 2010), and the unknown factor(s) negatively regulating physiologic utilization of cell Pi in shoots under control of OsPHR2.

excessive accumulation caused by ectopic overexpression of *OsPHR2* raises an intriguing question: whether the excessive Pi in shoots exerts a physiologic toxicity? Our favored hypothesis is that the growth inhibition mediated by overexpression of *OsPHR2* is caused by toxic physiologic effects due to excessive Pi accumulation in shoots (Pi toxicity). In fact, toxic symptoms become diminished with decreased Pi levels in growth medium (Zhou et al., 2008). However, under low Pi supply conditions, the shoot Pi concentration in plants with *OsPHR2*-overexpression is similar to that in wild type plants under higher Pi supply conditions, but the growth of the transgenic plant is still retarded (Wu and Xu, 2010). Therefore, it could be reasoned that the inhibition of plant growth caused by overexpression of *OsPHR2* may be due to the repression of physiologic utilization of cell Pi or to some inhibitor(s) under regulation of OsPHR2. This hypothesis is supported by the fact that some mutants isolated from an EMS-generated seed stock under background of overexpression of *OsPHR2* showed diminished growth inhibition (Fig. 2). Although experimental evidence is still required to confirm this hypothesis, a complex network of Pi uptake, translocation and utilization under the control of OsPHR2 has emerged (Fig. 2).

Prospects

It is increasingly clear that the response of plants to Pi-signaling and the maintenance of Pi homeostasis in cells are defined by a complex feedback loop. Currently, the regulatory systems and their molecular basis are still poorly understood, in spite of their importance for biotechnological and agronomic strategies aimed at improving crops with more efficient utilization of Pi nutrition as well as other nutrients. It is obvious that many pieces of this puzzle representing the Pi regulatory network are missing, and future exciting research will aim to resolve the mystery of how plants sense Pi, transmit signals both locally and at long distance, and maintain the nutrient homeostasis to efficiently utilize Pi coordinated with other nutrients. Mutants isolated from EMS-generated seed stock show rescue of growth inhibition caused by overexpression of *OsPHR2* (Fig. 2), giving us new insight into the understanding of molecular network regulating Pi uptake, translocation and utilization in plants. Loss-of-function of some repressor(s) or gain-of-function of some gene(s) may be involved in the rescue of plant growth inhibition due to Pi excessive accumulation driven by

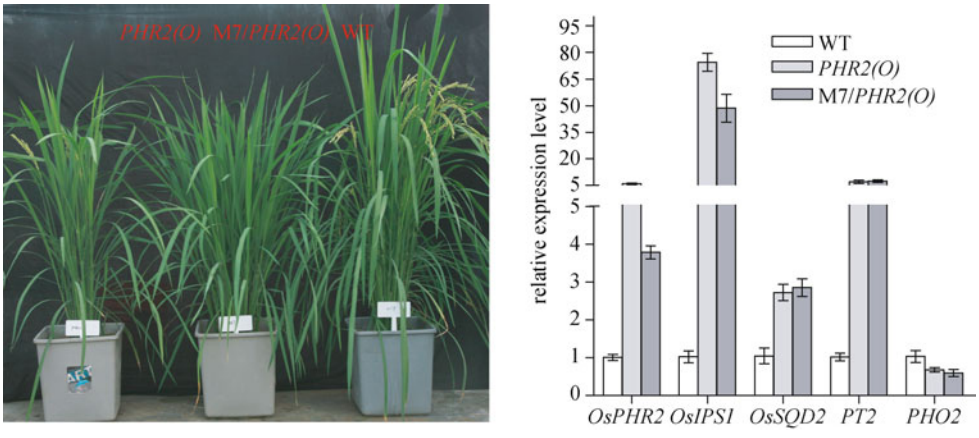

Figure 2 Growth performance of a mutant designated as M7 in a pot experiment with Pi supplied condition (1 g of Ca_2PO_4/kg dried soil) and isolated from an EMS-generated seeds stock under the background of *OsPHR2* overexpression (left), and quantitative RT-PCR analysis of the expression levels of *PHR2*, *IPS1*, *SQD2*, *PT2* and *PHO2* in the M7 mutant (right). In the pot experiment from left to right: transgenic plants with *OsPHR2* (*PHR2(O)*) overexpression, M7 mutant under the background of of *OsPHR2(O)* overexpression, and the wild type (WT) plant (Nipponbare).

OsPHR2, which is worthy of further study. Finally, a curious question is how SPX1 negatively regulates the function of OsPHR2 on Pi transporters.

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