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REVIEW

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Transcriptional responses and regulations to deficient phosphorus in plants

Abstract Significant progress has been made over the past several years in the understanding of phosphorus (Pi)-starvation responses in plants and their regulation. The transcriptional changes that occur in response to Pi starvation are beginning to be revealed, although much is left to understand about their significance. In this paper, the recent progresses on the gene expression changes under deficient-Pi, cis-regulatory elements involved in response to deficient-Pi, the transcriptional control of Pi-starvation responses in eukaryotes, transcription factors involved in response to Pi-starvation, the role of MicroRNA on regulation of phosphate homeostasis, and phosphate sensing and signal transduction in plants have been summarized. The purpose of this review is to provide some basis for further elucidation of the transcriptional responses and regulations, and the networks of Pi sensing and signal transduction under deficient-Pi in plants in the future.

Keywords deficient phosphorus, transcriptional response, transcriptional factor, Pi sensing and signal transduction

1 Introduction

Phosphorus is an essential macronutrient for plants and other living organisms. Though this mineral is quite abundant in the soil, it is frequently a limiting factor for crop growth, development, and productivity, due to the immobility of more than 80% of soil orthophosphate that is not readily available to plant roots (Holford, 1997). It is estimated that crop yield is limited by P availability in 30%–40% of arable lands (Runge-Metzger, 1995; Von Uexküll and Mutert, 1995).

In a long time of evolution, plants have evolved an ability to adapt to the starved-Pi condition at the developmental and biochemical levels (Raghothama, 1999; Abel et al., 2002; Rausch and Bucher, 2002; Vance et al., 2003). Generally, developmental responses for plants to low Pi status are related to the changes in root architecture, such as increases in the root surface/soil volume ratio, the root-to-shoot growth ratio, the number of lateral roots and the number and length of root hairs, which can improve the ability of plants to access soil phosphate (Bates and Lynch, 1996; Ma et al., 2001; Williamson et al., 2001; López-Bucio et al., 2002). In some plant species, other mechanisms have evolved, such as forming clusters of lateral roots and establishing symbiotic associations with mycorrhizal fungi (Harrison, 1999; Watt and Evans, 1999; Burleigh et al., 2002; Vance et al., 2003).

At the molecular level, lots of genes involved in Pi-starvation responses have been cloned. It is also found that the Pi responsiveness of plants can be largely regulated by transcriptional control. Though the regulatory system of plants to Pi is complicated, much information is presently available with regards to the control of Pi-starvation responses in plants.

2 Gene expression changes in plants under deficient-Pi

Most of the genes that respond to Pi-starvation have been cloned or isolated in the past several years, including the genes of RNases, phosphatase, high affinity Pi transporters, and some members of Mt4/TPSI1 family. These genes, directly or indirectly involved in the Pi acquisition of recycling, are generally shown to be induced (Raghothama, 1999; Abel et al., 2002), indicating that many genes in the Pi-starvation rescue system are controlled transcriptionally.

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Generally, the transcripts of Pi-responsive genes could be detected at low levels before Pi-starvation, and increase significantly with the onset of the stress.

Recently, with the advances in post-genomic study approaches, micro-array technology has been used to simultaneously catalogue the effects of P deficiency on the expression of thousands of genes (Wang et al., 2002; Hammond et al., 2003; Wasaki et al., 2003; Wu et al., 2003). It is thought that the expression of these genes is coordinated by both general stress-related and low-P-specific signaling cascades (Hammond et al., 2003; Vance et al., 2003). The genes identified with response to P deficiency can be grouped into 'early' genes that respond rapidly and often non-specifically to P deficiency, and 'late' genes that alter the morphology, physiology or metabolism of plants upon prolonged P deficiency. These 'late' genes generally improve the acquisition of P or promote the efficient use of P in plants (Vance et al., 2003).

3 Cis-regulatory elements involved in response to deficient-Pi

The cis-regulatory elements located in the promoters of genes play an important role in up-regulating their expressions under Pi-starvation condition, by interacting with the DNA binding domain in the transcription factors. To date, several common cis-regulatory elements have been identified in the promoters of deficient-Pi induced genes. Analysis of the promoters of the Pho regulon genes, the Pi-starvation responsiveness genes in yeast, reveal two consensus cis-regulatory elements, GCACGTGGG and GCACGTTTT, for Pho2 and Pho4 binding and control of gene expression in response to P deficiency (Oshima, 1997).

In plant species, based on the analysis of the Arabidopsis mutants defective, an imperfect-palindromic sequence (GNATATNC) located at the promoter regions of several P-responsive genes (Rubio et al., 2001; Hammond et al., 2003), could be bounded by transcription factor AtPHR1. Other potential cis-regulatory elements have also been identified in the promoter sequences of P-responsive genes (Mukatira et al., 2001; Rubio et al., 2001; Tang et al., 2001; Hammond et al., 2003). Hammond et al. (2003) reported the occurrence of putative Pho-like (CGCGTGGG) and TATA box-like (TATAAATA) cis-regulatory elements in the promoter regions of Arabidopsis genes under the function of the positive regulator of the yeast Pho regulon, Pho4, which contains a basic helix-loop-helix (bHLH) DNA binding domain. Alternatively, the core sequences of the Arabidopsis Pho-like elements (CACGTG) are also similar to ABRE-like and G-box elements; proteins from the bZIP class of transcription factors might bind to them (Schindler et al., 1992). Based on the analysis of the promoter regions from genes described as differentially regulated under P deficiency, Wu et al. (2003) identified two more novel putative cis-regulatory elements that occurred more frequently in the promoters of genes up-regulated in leaves 48 h after withdrawing P (TCTCTCT) and down-regulated in leaves 24 h after withdrawing P (AAAATATC). Except those isolated in Arabidopsis, a cis-regulatory element, similar to the yeast *Pho4* binding site (CACGT(G/T)), was also identified in two other plant P-responsive genes, *TPSI1* from tomato and *Mt4*, *MtPT1*, and *MtPT2* from *Medicago truncatula* (Liu et al., 1997; Burleigh and Harrison, 1999; Xiao et al., 2006).

All the putative cis-regulatory elements identified in groups of genes that respond rapidly to P deficiency could act as binding sites for proteins to control the initial responses of plants to P deficiency. Because of the interactions between the cis-regulatory elements and the transcription factors, further analysis is now required to identify the novel elements involved in transcriptional regulation of Pi-responsive genes.

4 Transcriptional control of Pi-starvation responses in eukaryotes

There has been a clear understanding of transcriptional control under deficient-Pi in eukaryotes in yeast. In this organism, there are two transcription factors Pho2 and Pho4, belonging to the homeodomain and basic-helix-loop-helix families, respectively, to play dominant roles in transcriptional control (Lenburg and O'Shea, 1996). Aside from them, there are three other components, including a cyclin-dependent kinase CDK (Pho85), a cyclin (Pho80) and a CDK inhibitor (Pho81), which is possibly the Pi sensor in the Pi-starvation signal transduction pathway (Lenburg and O'Shea, 1996). When Pi concentration in the cytoplasm is high enough, Pho80 and Pho85 are assembled the as Pho80/Pho85 complex. This complex phosphorylates Pho4, resulting in the latter to be exported from the nucleus, through the co-operation process between the complex and the nuclear export protein Mns5 and a small Ran-GTPase (Kaffman et al., 1998a). Phosphorylation of Pho4 under high Pi condition prevents the interaction of this transcription factor with Pho2, a step necessary for transcriptional activation. As a result, transcription of Pi responsive genes is prevented. Under deficient-Pi condition, the CDK inhibitor, Pho81, inhibits the Pho80/Pho85 complex formation through an unknown phosphatase. Pho4 becomes dephosphorylated and enters the nucleus via the import protein Pse1 (Kaffman et al., 1998b), where it co-operates with Pho2 to activate transcription of Pi-starvation responsive genes.

In plant species, the closely related members of Pho regulatory genes have not fully revealed, owing to the fact that plants have evolved to a different regulatory system to control Pi-starvation responses and preclude extrapolation from the yeast system, or the similar regulatory genes to be

identified in the future. But, the possibility that transcriptional repressors in plants are involved in the control of Pi-starvation responsive genes has been raised by assaying the mobility shift, with promoter fragments from two Pi-starvation responsive genes and the DNA binding proteins detected in nuclear extracts from plants grown on Pi-rich medium, which Pi-starved plants are in lack of (Mukatira et al., 2001). The finding of high Pi-status specific DNA binding proteins that specifically interact with promoter sequences of Pi-starvation responsive genes, suggests the involvement of transcriptional repressors in the regulation of Pi-starvation responses. Up to now, molecular genetic evidence has highlighted the role of several transcription factors involved in the regulation of Pi starvation in plants.

5 Transcription factors involved in response to deficient-Pi

5.1 Role of *PHR1* in response to Pi-starvation

A MYB transcription factor, PHR1, has been isolated in Arabidopsis and shown to be involved in a regulation system responsive to Pi starvation (Rubio et al., 2001). In the Pi starvation condition, the *PHR1* mutant did not show the typical Pi starvation phenotypes (accumulation of anthocyanins and reduction of root-to-shoot ratio) and changes in the expression of Pi starvation marker genes. Because the expression of *PHR1* is not sensitively affected by the status of Pi supply levels and the PHR1 is located in the nucleus independent of Pi status, it is proposed that either PHR1 activity is regulated posttranscriptionally or a second Pi starvation regulatory protein is needed to mount a proper Pi starvation response (Franco-Zorrilla et al., 2004).

The PHR1 protein is 409 amino acids in size and contains two domains, a MYB-related domain, characteristic of DNA-binding proteins, and a predicted coiled-coil domain, potentially involved in protein-protein interactions. In line with the presence of these two domains, DNA-binding assays show that PHR1 binds to DNA as a dimer in a sequence-specific manner. Thus, the MYB domain is likely to be involved in sequence-specific recognition, and the coiled-coil domain may be the dimerization domain. In agreement with this interpretation, a deletion derivative of PHR1 lacking part of the coiled-coil domain shows impaired high-affinity sequence-specific DNA binding. PHR1 is highly related to the *Chlamydomonas reinhardii* gene, PHOSPHORUS STARVATION RESPONSE1 (PSR1; Wykoff et al., 1999). PHR1 and PSR1 share, in addition to a MYB DNA binding domain, a second domain predicted to form a coiled coil (CC), a fold usually involved in protein–protein interactions. It is found that PHR1 binds as a dimer to an imperfect palindromic DNA sequence presenting in the promoter of many 'late' Pi-starvation genes (Rubio et al., 2001), the mutation of the two PHR1-binding sequences (P1BS sequences) presents in the promoter of the Pi-starvation response gene, and AtIPS1, impairs its Pi-starvation responsiveness, which establishes the importance of PHR1 (and related transcription factors) in the control of Pi-starvation responsive genes (Rubio et al., 2001). Therefore, plants have derived a system regulating Pi-starvation responses from a transcriptional regulatory system that is already present in their unicellular ancestors.

Recently, two *OsPHR* genes from rice have been isolated and designated as *OsPHR1* and *OsPHR2* based on amino acid sequence homology to *AtPHR1*. Similar to PHR1 in Arabidopsis, both *OsPHR1* and *OsPHR2* are involved in Pi-starvation signaling pathway by regulating the expression of Pi starvation-induced genes, while only *OsPHR2* overexpression results in the excessive accumulation of Pi in shoots under Pi-sufficient conditions. Under Pi-sufficient conditions, overexpression of *OsPHR2* mimics Pi-starvation stress in rice with enhanced root elongation and proliferated root hair growth, suggesting the involvement of *OsPHR2* in Pi dependent root architecture alteration by both systematic and local pathways. In *OsPHR2*-overexpression plants, some Pi transporters are up-regulated under Pi-sufficient conditions which correlates with the strongly increased content of Pi. The identification and cloning of PHR1 and the homologous of PHR1 in other plant species provide a potentially useful tool for engineering plants that require less phosphate fertilizer (Zhou et al., 2008).

5.2 Function of HD-ZIP proteins in response to deficient-Pi

Previous expression analysis found that the vegetative storage protein *B* gene (*VspB*) was regulated by wound, water deficit, light, metabolites such as carbon and nitrogen, and by the plant regulators jasmonic acid, and auxin during plant development (Mason and Mullet, 1990; Staswick, 1990; Mason et al., 1992; DeWald et al., 1994; Creelman and Mullet, 1997). After that, it is found that *VspB* expression was also induced by deficient P, showing the similar expression pattern as some Pi-starvation responsive genes (Tang et al., 2001). DNA and protein interaction analysis suggested that the transcription factor HD-ZIP proteins involved in the transcription control of *VspB*, inferred by a 50 bp fragment of *VspB* promoter (–536 to –484), could bind the HD-ZIP binding domain in low-phosphate concentrations, based on gel-shift and DNase-I footprinting assays. Further analysis found that two HD-ZIPs (GmHDL56 and GmHDL57) encoded by two soybean genes functioned as the transcription factors interacting with the Box II (TAATTAAT) located at the 50 bp fragment of *VspB* promoter (Sessa et al., 1993; Meijer et al., 1997; Sessa et al., 1998).

Studies of this class of transcription factors in Arabidopsis reveal the existence of four different groups of HD-ZIP proteins that can be distinguished in part based on their

binding site specificity (Sessa et al., 1994). Box II is similar to the sequences that bind to members of the first class of these proteins (HD-ZIP I, binding to CAAT (A/T) ATTG). One member of this class of genes is activated by abscisic acid and water deficit (Soderman et al., 1996) and ectopic expression of Athb-1 alters leaf cell fate (Aoyama et al., 1995). It is interesting that a member of the second class of HD-ZIP proteins, ATHB-2, functions as a negative regulator of gene expression and is involved in mediating specific auxin responses (Steindler et al., 1999). Therefore, it seems that further systematic examination of *VspB* promoter activity in plants overexpressing each HD-ZIP protein (GmHDL56 and GmHDL57) will be required to identify the specificity in the regulation of response to deficient-Pi in the future.

5.3 Role of zinc finger transcription factor ZAT6 in response to Pi-starvation

A total of 176 proteins that contain one or more zinc finger domains have been reported in *Arabidopsis*, making ZFPs one of the largest families of putative transcriptional regulators (Englbrecht et al., 2004). Based on the previous reports, the ZFPs were involved in floral organogenesis, leaf initiation, lateral shoot initiation, and gametogenesis (Englbrecht et al., 2004). Presently, in *Arabidopsis*, a cysteine-2/histidine-2 zinc finger transcription factor, *ZAT6* (zinc finger of Arabidopsis 6), is found to be responsive to Pi stress (Devaiah et al., 2007a). ZAT6 has been classified as part of the 20 member C1-2 subclass of C2H2 ZFPs (Englbrecht et al., 2004). C1 refers to ZFPs that do not have tandem arrays of zinc fingers, where the two invariant zinc coordinating His residues are separated by three amino acids.

The overexpression of *ZAT6* can affect root development and retard seedling growth as a result of decreased Pi acquisition, resulting in altering root architecture of older plants, with consequent changes in Pi acquisition. In addition, the expression of several Pi starvation-responsive genes is decreased in *ZAT6* overexpressing plants, thereby indicating that *ZAT6* is a repressor of some Pi starvation-responsive genes involved in the primary root growth and Pi homeostasis regulation (Devaiah et al., 2007a). These results indicate that *ZAT6* plays an important role in the regulation of root development, Pi acquisition and homeostasis.

All plant ZFPs with the DLN box, also called the EAR motif, function as transcriptional repressors (Ohta et al., 2001). It is particularly interesting to note the presence of a DLN box, defined by the consensus sequence L/FDLNL/F (X) P, on the deduced amino acid sequence of ZAT6, suggests that ZAT6 can function as a transcriptional repressor, and regulate partly the expression of its downstream Pi responsive genes. Therefore, the characterization of *ZAT6* sheds new light on the current understanding regarding the regulation of root development during Pi starvation and the subsequent changes in Pi homeostasis. Besides this, the regulation of root architecture by *ZAT6*, coupled with its responsiveness to other major nutrient stresses, suggests an important role for *ZAT6* in the regulation of multiple nutrient stresses.

5.4 Possible role of WRKY75 under deficient-Pi

To be nuclear-localized and induced differentially in plants during Pi deficiency, *WRKY75* is one of several transcription factors induced during Pi deprivation in plant species (Devaiah et al., 2007b). Suppression of *WRKY75* expression through RNAi silencing may result in early accumulation of anthocyanin, indicating that the RNAi plants are more susceptible to Pi stress. Further analyses reveal that the expression of several genes involved in Pi starvation responses, including phosphatases, *Mt4/TPS1*-like genes, and high-affinity Pi transporters, can be decreased when *WRKY75* is suppressed. Consequently, Pi uptake of the mutant plant may also decrease during Pi starvation. In addition, when *WRKY75* expression is suppressed, lateral root length and number, as well as root hair number, may be significantly increased (Devaiah et al., 2007b). However, changes in the root architecture are obvious under both Pi-sufficient and Pi-deficient conditions. This indicates that the regulatory effect of *WRKY75* on root architecture could be independent of the Pi status of the plant.

The localization pattern of *WRKY75* is similar to that of PHR1, a Myb transcription factor from *Arabidopsis* that has been shown to be involved in regulating several Pi starvation responses (Rubio et al., 2001). However, the expression of *WRKY75* is strongly induced upon Pi starvation, and is rapidly suppressed by Pi resupply, as well as its possible involvement in regulating root growth independent of the Pi status of plants, suggesting that sumoylation may not be involved in the regulation of WRKY75. This is not the same as the *PHR1*, as the expression is not induced upon Pi starvation and is localized to the nucleus independent of the Pi status of the plant, resulting in its regulation to be posttranslational.

WRKY transcription factors are known to regulate specific genes by binding to the conserved TTGAC/T W boxes on their promoter regions (Ulker and Somssich, 2004). The differential level of suppression in the *WRKY75* RNAi plants is also correlated to a combination of the number and type of W boxes predicted in the promoter regions of Pi-responsive genes by silico analysis. Therefore, although the details of the actual regulatory mechanism are yet to be dissected, it is clear that WRKY75 can be working as a positive regulator of several genes involved in the global Pi starvation response. *WRKY75* may be a potentially useful tool in understanding multiple stress responses as it plays an important role in regulating root

architecture, which is critical for many other nutrient stresses besides P.

5.5 A role of OsPTF1 under phosphate starvation

In rice, a basic helix-loop-helix (bHLH) domain transcription factor, OsPTF1, is Pi starvation induced in roots while constitutively expressed in shoots (Yi et al., 2005). Like the yeast Pho4 and other homologous bHLH transcription factors, His-5, Glu-9, Arg-12, and Arg-13 are present in the basic region of the OsPTF1 protein. These residues constitute the recognition motif for the G-box (CACGTG), which is one type of E-box (CANNTG; Atchley et al., 1999; Massari and Murre, 2000). The electrophoretic mobility shift assays showed that OsPTF1 is able to bind to the E-box and the G-box, but not to the mutated G-box DNA (CAATTG). Interaction analysis between OsPTF1 and G-box DNA found that the G-box-OsPTF1 interaction is specific. Overexpression of OsPTF1 may result in about 30% of increase in tillering ability, root and shoot biomass, and phosphorus content of transgenic rice plants higher than those of the wild-type plants in Pi-deficient conditions.

Microarray data show that 158 genes are found to be regulated by overexpression of *OsPTF1*. Among those, 14 genes are up-regulated in both leaves and roots. Forty-three genes are up-regulated in either leaves or roots. 27–30 genes are down-regulated in leaves and roots. Ninety-eight genes are classified based on a database search from the 158 genes. The function-classified genes include nutrient transporters and metabolism, carbon metabolism, ATP-binding protein, oxidoreductase, protease, disease resistance protein, RNase, H^+-transporting ATPase, vacuolar H^+-pyrophosphatase, senescence-associated protein, receptor-like kinase, and several cytochrome P450 genes. Many function-unknown or putative genes can be strongly up-and down-regulated by overexpression of *OsPTF1* (Yi et al., 2005). Taken together, the transcription factor OsPTF1 can be involved in deficient Pi response and has a potential function on improving plant phosphorus acquisition capabilities.

6 Role of MicroRNA on regulation of phosphate homeostasis

MicroRNAs (miRNAs) represent a class of noncoding small RNAs that generally function as posttranscriptional negative regulators through base pairing to nearly complementary sequences in the target mRNAs (Carrington and Ambros, 2003; Bartel, 2004). Most plant miRNAs regulate their targets by directing mRNA cleavage in the coding regions (Bartel, 2004; Dugas and Bartel, 2004). Several plant miRNAs were demonstrated to be critical in regulating leaf or flower development by targeting the relevant transcription factors (Reinhart et al., 2002). Other than transcription factors, miRNAs may target a wide range of transcripts (Adai et al., 2005; Axtell and Bartel, 2005). Their expression has been shown to be regulated by development in a tissue-specific manner or in response to a range of environmental stresses (Reinhart et al., 2002; Sunkar et al., 2005). The diverse expression patterns and large varieties of potential target genes of miRNAs suggest that miRNAs can regulate various developmental and physiological processes and might play a direct role in cell-to-cell signaling in plants (Kidner and Martienssen, 2005).

It is found that a specific microRNA can be involved in regulating inorganic phosphate (Pi) homeostasis in plants. The suppression of an ubiquitin-conjugating E2 enzyme by a specific microRNA, miR399, plays a vital role for plants to adapt themselves to environmental changes in Pi availability. Upon Pi starvation, the miR399 is upregulated while its target gene, an ubiquitin-conjugating E2 enzyme, is downregulated in Arabidopsis. Accumulation of the E2 transcript is suppressed in transgenic Arabidopsis overexpressing miR399. Transgenic plants can accumulate five to six times as much as the normal Pi level in shoots, displaying Pi toxicity symptoms that are phenocopied by a loss-of-function E2 mutant. Pi toxicity may be caused by increased Pi uptake and by translocation of Pi from roots to shoots and retention of Pi in the shoots. Moreover, unlike wild-type plants, in which Pi in old leaves are readily retranslocated to other developing young tissues, the remobilization of Pi in miR399-overexpressing plants is easily impaired (Chiou et al., 2005). These results provide evidence that miRNA can control Pi homeostasis by regulating the expression of a component of the proteolysis machinery in plants.

Recently, the mechanism that microRNA399 (miR399) controls inorganic phosphate (Pi) homeostasis has been explored. It is found that the control of Pi homeostasis by miR399 can be regulated by an ubiquitin-conjugating E2 enzyme gene (*UBC24*), based on follow results: in the Pi overaccumulator mutant, *Pho2*, which was caused by a single nucleotide mutation resulting in early termination within the *UBC24* gene, the level of full-length *UBC24* mRNA was reduced and no UBC24 protein was detected (Aung et al., 2006). Accumulation of *UBC24* mRNA was suppressed by the targeting of miR399, whose expression is up-regulated by Pi starvation (Fujii et al., 2005; Chiou et al., 2005). Significantly, overexpression of miR399 or loss of function of the UBC24 gene led to accumulation of high Pi content to a toxic level in leaves, which resulted from the increased uptake of Pi from roots, the increased translocation of Pi from roots to shoots, and the retention of Pi in the leaves (Chiou et al., 2005). Moreover, impairment of Pi remobilization from old to young leaves accelerated toxicity in old leaves (Chiou et al., 2005). These observations suggest that interaction between miR399 and UBC24 can regulate Pi homeostasis at the systemic level. It is interesting to note that the *Pho2* mutant (Pi overaccumulator), miR399-overexpressing, and UBC24

loss-of-function plants all display similar phenotypes, except that the defect in Pi remobilization within leaves has not been described in *Pho2*.

7 Phosphate sensing and signal transduction

Similar to other abiotic stress, several studies have demonstrated that there were complicates and intricate regulatory systems responding to the Pi stress in plants, including those for signal sensing and signal transduction. As a multicellular organism, the plants have evolved two types of the Pi phosphate system, if classified based on the distance of the signal transduction pathway in plants.

7.1 Pi signaling and signal transduction at whole plant level

The Pi signal sensing and signal transduction were first elucidated at the level of whole plant by split root experiments, in which the roots of Pi-starved plants were divided and one part was exposed to a high Pi medium, while the other was left in a low Pi medium. It was found that the part of the roots growing in Pi-sufficient condition was systemically repressed when the other part of the roots were exposed to low Pi medium (Liu et al., 1997; Burleigh and Harrison, 1998; Baldwin et al., 2001). This result implicates that there were long-distance signal transductions of Pi at the whole plant level.

In the split root experiment, it is speculated that the long-distance systemic signal seems not to be Pi itself (Burleigh and Harrison, 1998). In the *Pho1*, a mutant with impaired Pi loading in the xylem and a low overall Pi content (Poirier et al., 1991) showed no Pi-starvation genes expression in the non-starved cells in the root, such as those of the epidermis and cortex (Martín et al., 2000). This indicates that the plant regulator cytokinins is the repressor for long-distance-controlled Pi responses. Taken together, it is suggested that the cytokinin signaling pathway is a candidate component of this systemic repression signal transduction system (Martín et al., 2000).

It is found that the long-distance systemic repression signaling and signal transduction are involved in the regulation of the 'late' Pi-starvation-induced genes, in which PHR1 and other non-identified transcription factors are perhaps the intermediate components. More signal transduction components in long-distance need to be identified and further characterized.

7.2 Pi signaling and signal transduction at cellular level

The other type of phosphate sensing and signal transduction identified in plants was the local, in which the signaling and its transduction were carried out at the cellular level. Köck et al. (1998) has analyzed the Pi local sensing system by using the Pi-starvation rescue approach in tomato. In Pi-sufficient condition, it is observed that the tomato cells grown were incubated with compounds that sequester intracellular Pi by being phosphorylated after uptake. In the meantime, the Pi-starvation responsive RNase genes were rapidly induced, suggesting that Pi sensing itself and signal transduction is intracellular (Köck et al., 1998).

In the roots, the responses of genes whose expression was generally controlled by external Pi concentration are elicited to be rapidly responding to the onset of Pi starvation (Wang et al., 2002; Wu et al., 2003). Contrary to the roots, the shoot Pi-status could not be decreased sufficiently only 1 h after Pi starvation. Meanwhile, it appears to occur in an intermediate situation in root tips, which are controlled both by external and whole plant Pi status. The growth through a high-Pi patch could reduce primary root growth after the root tip has left the patch, indicating that the root tip is responsive to local Pi status (Linkohr et al., 2002). The *Pho2* mutant, which hyper-accumulates Pi in the shoot while having a Pi content similar to the wild type in its roots (Delhaize and Randall, 1995), displays a higher primary root growth than the wild type, indicating that the shoot Pi status has an effect on root growth (Williamson et al., 2001). Similar to long-distance signaling and signal transduction of Pi in plants, the local Pi signaling and signal transduction pathway are also largely unknown and should be explored further in the future.

8 Perspectives

Phosphorous (P) is one of the mineral nutrients essential for plant growth, development, and reproduction. Not only is it a major component of fundamental macromolecules, such as nucleic acids and phospholipids, but it also plays an important role in energy transfer and the regulation of enzyme reactions and metabolic pathways (Raghothama, 1999). The manipulation of the genes enabling growth in low-P environments has provided a new approach on improving the P-use-efficiency of plants and reduced the P-fertilizer requirement of crops (Vance et al., 2003).

Given the complexity of the Pi-starvation response, the exploitation of the full potential of this response will depend on the manipulation of its regulatory system. Current knowledge of the control of Pi-starvation responses is, however, insufficient to make this possibility feasible. Therefore, it is necessary to have a better understanding of the regulatory system of the Pi-starvation response, by which to shed light on the significance of the different transcriptional programmes. It is also needed to further elucidate the pathways and networks of Pi sensing and signal transduction under Pi deficiency in plants. Meanwhile, modern biotechnology will provide a novel approach for improvement of crops with high Pi utilization efficiency based on a better understanding of the molecular mechanisms on Pi starvation responses in plants in the future.

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