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Molecular mechanisms in response to phosphate starvation in rice

Madhusmita Panigrahy, D. Nageswara Rao, N. Sarla *

Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, 500030, India

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ABSTRACT

Phosphorus is one of the most important elements that significantly affect plant growth and metabolism. Among the macro-nutrients, phosphorus is the least available to the plants as major phosphorus content of the fertiliser is sorbed by soil particles. An increased knowledge of the regulatory mechanisms controlling plant's phosphorus status is vital for improving phosphorus uptake and P-use efficiency and for reducing excessive input of fertilisers, while maintaining an acceptable yield. Phosphorus use efficiency has been studied using forward and reverse genetic analyses of mutants, quantitative genomic approaches and whole plant physiology but all these studies need to be integrated for a clearer understanding. We provide a critical overview on the molecular mechanisms and the components involved in the plant during phosphorus starvation. Then we summarize the information available on the genes and QTLs involved in phosphorus signalling and also the methods to estimate total phosphate in plant tissue. Also, an effort is made to build a comprehensive picture of phosphorus uptake, homeostasis, assimilation, remobilization, its deposition in the grain and its interaction with other micro- and macro-nutrients as well as phytohormones.

Contents

1. Introduction

Phosphorus is an essential element required to sustain plant life, as it is the key substrate in energy metabolism in the form of adenosine triphosphate (ATP). It is one of the major constituents of nucleic acids in the form of sugar-phosphate backbone and membranes in the form of phospholipids bilayer. It is needed in cell division, in the formation of bio-membranes, in the transformation of starch and sugar, in seed germination, in flowering and fruit formation, and in almost every phase of plant's vital processes. Several reviews deal with phosphorus (P) in plants and soil (Yuan and Liu, 2008; Ticconi and Abel, 2004; Hammond et al., 2004; Abel et al., 2002; Marschner, 1995). P is the most important inorganic nutrient after nitrogen (N), for plant growth, and often limits primary productivity in natural and cropping systems unless supplied as fertiliser (Vance et al., 2003). P is the least available, highly immobile and

* Corresponding author. Biotechnology Laboratory, Directorate of Rice Research, Rajendranagar, Hyderabad-500030, India. Tel.: +91 40 2401 5036; fax: +91 40 2401 5308.
E-mail addresses: madhusmita_2000@yahoo.com (M. Panigrahy), dokula@rediffmail.com (D.N. Rao), nsarla@drricar.org, sarla_neelamraju@yahoo.com (N. Sarla).

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non-renewable resource. About 80–90% of the P applied as fertiliser is sorbed by soil particles and makes it unavailable for plants that lack specific adaptation to access sorbed P (Gerke et al., 1994). Humid tropical and subtropical climate, warm, moist conditions result in weathered acid soils (mostly ultisols and oxisols), in which free iron and aluminium oxides bind native and applied P into forms unavailable to plants (Yan et al., 2006). In the calcareous soils (mostly aridisols), the amounts of calcium and magnesium compounds are usually high which bind inorganic phosphates into forms highly unavailable to plants. The high P-fixing capacity of the soils results in very low P uptake by the plants. Moreover, global P resources are rapidly being depleted. Estimates indicate that presently mined rock phosphate (inorganic P) reserves will easily be halved by 2060 (Steen, 1997). Demand for low-input sustainable crop cultivation is increasing to meet the need for environment-friendly agriculture. Consequently, developing crop germplasms better adapted to low P conditions with better uptake and efficient nutrient P-use efficiency has now become one of the major objectives of crop breeding programmes. Also, part of the applied P in intensive cropping systems enters the waterways through runoff and erosion, contributing to pollution and eutrophication of surrounding lakes and marine environments (Filippelli, 2008). Therefore, improving P acquisition and use by crops is critical for economical and environment-friendly crop agriculture. Rice is the model crop in monocots; its genome has been sequenced and has accelerated studies on P. In this review, recent developments and future prospects for obtaining a better understanding of the regulation of phosphorus use efficiency and phosphate metabolism are discussed with special emphasis on rice. The processes involved in phosphorus uptake, transport, homeostasis, assimilation, remobilization within the plant and its deposition in the grain are discussed. A table containing all the genes and QTLs involved in phosphorus signalling is presented. Finally, our knowledge on how the different genes may act during phosphate starvation is summarized. The interaction of phosphorus with other micro- and macro-nutrients as well as phytohormones is explained. Reference to work on other cereals, dicots and lower plants is made when similar information is not available for rice.

2. Mechanism of Pi uptake, transport and P homeostasis in plants

Phosphorus is absorbed by plants mainly in the form of phosphate ion with molecular symbol $(H_2PO_4)^{2-}$ or $(HPO_4)^{2-}$. Several forms of Pi exist in the soil such as $(H_2PO_4)^{-}$, $(H_2PO_4)^{2-}$ and $(PO_4)^{3-}$, but the dihydrogen form of orthophosphates ($H_2PO_4^-$) is most readily transported into the plant. Different methods of determining total Pi content in plant tissue, seeds and soil is described in Supplementary file S2. In plants, uptake of Pi under non-limiting conditions appears to be regulated by internal levels of P. Mass flow generally delivers only 1–5% of P demand and much of the required phosphate reaches the root by diffusion. But, the diffusion coefficient of Pi is very low ($0.3–3.3\times10^{-13}$ m^2 s^{-1}) as compared with those of other nutrients (Clarkson, 1981). Generally there is a very low concentration of available Pi in the soil solution and the concentration of Pi in the cytosol is in milli-molar range (Marschner, 1995). Roots have to acquire Pi against a steep concentration gradient (100-fold or higher) because of the high demand for P in the plant. An energy mediated co-transport process, driven by protons generated by plasma membrane H^+ ATPase has been proposed for Pi uptake in plants (Sakano et al., 1992; Ullrich-Eberius et al., 1981 and Ullrich-Eberius et al., 1984). Pi absorption is accompanied by H^+ influx with a stoichiometry of 2 to 4 H^+ per $H_2PO_4^-$ transported. The Pi acquired by roots is rapidly loaded on to xylem and then transported to different parts of the plant according to the metabolic need.

Two categories of transporters are expressed for phosphate transport across the plant. Low-affinity transport system is expressed constitutively, whereas, high-affinity transport system is regulated by the availability of Pi in the plant. The Pi transporters are actually proton/phosphate ($H^+/H_2PO_4^-$) symporters. The low-affinity transporters are active in vascular loading and unloading, internal distribution and remobilisation of acquired P (Smith et al., 2001) whereas, the high-affinity Pi transporters play an important role in acquisition of P. There appear to be two major check points of regulation for ion transport across roots: the initial uptake across the plasma membrane into the symplast of the epidermal and cortical cells and the subsequent release into xylem. Under Pi-limiting condition, increase in capacity of roots for Pi uptake occurs accompanied by increase in capacity of the transfer of absorbed phosphate to shoots by increasing phosphate release into xylem (Drew and Saker, 1984). Plants exhibit a strong tendency to maintain constant cytoplasmic concentration of ions such as N, P, and K, irrespective of the large fluctuations in the external concentrations. This is referred to as homeostasis.

Higher plants generally store Pi as polyphosphates in vacuoles (Kuqa et al., 2008). Phosphate homeostasis in cytoplasm is maintained by Pi transport across the tonoplast. Tonoplast H-ATPase or pyrophosphates provide required energy to maintain electrochemical gradient for Pi transport. The transport mechanism for the tonoplast Pi transporters is unclear but vacuoles probably play the dual role of sink and source for Pi in plant cells. In P-non-limiting conditions, vacuoles play the role of sink by storing the phosphate and in P-limiting condition, they serve as source to fulfil the demand of P. Changes in the cytosolic or vacuolar Pi can trigger a signal transduction pathway that activates Pi-starvation rescue system. Roots are generally considered as a source of Pi for other plant parts and become a sink in Pi starvation (Raghothama, 1999). Plants have developed adaptive processes to facilitate external Pi acquisition, limit consumption of Pi and adjust recycling internally to maintain cellular Pi homeostasis in case of inadequate Pi availability (Lin et al., 2009).

3. Physiological changes in P deficiency to enhance Pi acquisition

When P becomes limiting, the P already contained in the plant especially in the less active older leaves (as P is a mobile element within plant tissue) is redirected or translocated to the younger, more active cells and then at maturity it is redirected to the seed at its active stage of formation. In response to low level of available Pi, plants have developed highly specialised morphological, physiological and biochemical adaptations to acquire and utilise Pi from the environment. These adaptations include many features like enhanced uptake ability through activation of high-affinity transporters, adaptive root development leading to altered root morphology and root architecture, induction of phosphate scavenging and recycling enzymes, induction of alternative pathways of cytosolic glycolysis, induction of tonoplast H^+-pumping pyrophosphatase, alternative pathways of respiratory electron transport, and other metabolic pathways associated with signal transduction and transcription regulation (Misson, 2005; Wu et al., 2003; Hu et al., 2001).

In response to low Pi availability, plants develop more proliferated root system that is efficient in uptake of soil Pi (Shenoy and Kalagudi, 2005). This includes increased influx rates, high root to shoot ratio, increased root mass, decrease in root diameter and increase in absorptive surface area relative to root volume (Yuan and Liu, 2008). Low P supply in *Hordeum vulgare* causes enhanced root length and increased root turnover allows greater amount of uptake of the immobile resource P (Steingrobe, 2001).

Another feature in low Pi availability is mycorrhizal symbiosis that enhances the Pi uptake and growth, especially when the root system is relatively coarse with fewer root hairs e.g. in *Citrus* (Graham and Eissenstat, 1994). Mycorrhiza formation involves a complex interaction between fungi and plant roots and P appears to play a key role in this association (Ming et al., 2006). Formation of specialised root clusters like proteoid roots is one of the several characteristics under low Pi conditions in some mycorrhizal and non-mycorrhizal species such as white *Lupin* (Keerthisinghe et al., 1998) as it has been shown

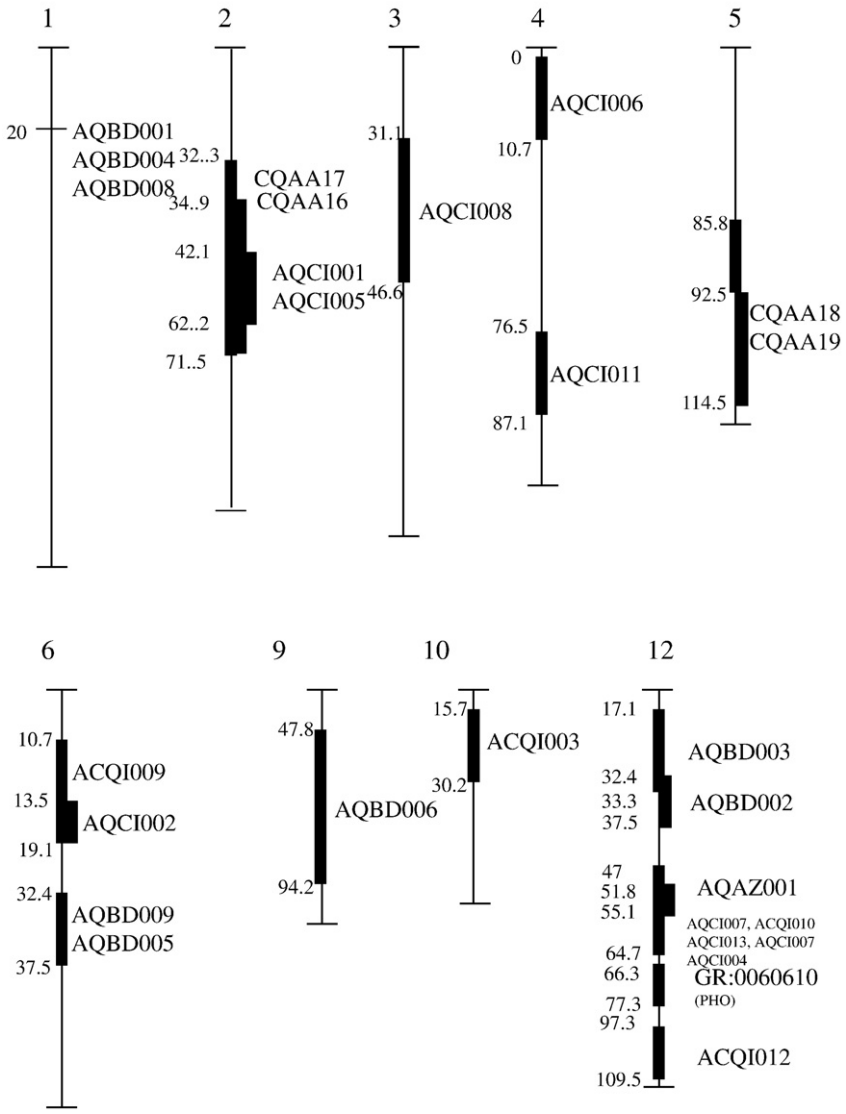

Fig. 1. Chromosome wise representation of the QTLs reported for P-deficiency tolerance in rice. Numbers on the top represents the number of the chromosome. Numbers on the left side are the position on the physical map in Mb and indicate the start and end position of the QTL on physical map. Names on the right are the accession IDs of the QTLs. Figure is not to scale.

that proteoid roots are efficient in absorbing Pi at a faster rate than non-proteoid roots (Vorster and Jooste, 1986). The organic acids secreted from proteoid roots, help to release Pi from calcium (Ca), iron (Fe) and aluminium (Al) phosphates by chelation of the metal.

Malic acid, citric acid and carboxylate are the predominant acids secreted by roots under Pi deficiency. These acids are secreted into the rhizosphere and solubilize Pi from rock phosphate (Hoffland et al., 1989). Some species like pigeon pea and alfalfa make biochemical changes to extract Pi associated with iron. These changes include phenolic compounds like pisidic acid (p-hydroxyl tartaric acid) in pigeonpea and alfafuran (2-3, 5′ dihydroxy phenyl)-5, 6 dihydroxy benzofuron in the alfalfa (Masaoka et al., 1993). Another biochemical adaptation during P deficiency is the conversion of sucrose to hexose phosphate via a pyrophosphate (PPi) dependent pathway requiring UDP-glucose pyrophosphorylase, specifically in *Arabidopsis*. The activity of phosphoenol pyruvate carboxylase (PEPCase) appears to be central in regulating many metabolic adaptations to P deficiency (Hammond et al., 2004).

Phosphite (PO_3^-) is a structural analog of phosphate, which can be absorbed but cannot be metabolised as nutrient. Phosphite can repress P-starvation responses in *Brassica* and *Arabidopsis* (Ticconi and Abel, 2004). In *Arabidopsis*, phosphite can repress the Pi-starvation induced root hair elongation, anthocyanin accumulation, and secretion of RNases. Phosphite can be transported through plant phosphate transporters but cannot be recognized as a substrate by the metabolic enzymes. How phosphite represses the Pi-starvation induced responses is not clear.

4. Genetic studies on P uptake and P-use efficiency

Majumder et al. (1989) and Chaubey et al. (1994) analysed general combining ability (gca) and specific combining ability (sca) effects in diallel crosses of P-efficient and P-inefficient rice cultivars and concluded that P-deficiency tolerance is a quantitatively inherited trait with outstanding parents being carriers of mostly additive genes. Advances in molecular marker technology have led to the construction of detailed molecular linkage map in rice (McCouch et al., 1988). Dense linkage maps have made it possible to identify and locate genes controlling quantitatively expressed traits and their dissection into Mendelian factors referred to as quantitative trait loci (QTL) with links to a known map position (Paterson et al., 1988). P-deficiency tolerance in rice is determined by regulation of P uptake (external efficiency)

and P-use efficiency (internal efficiency) and can be monitored indirectly by two parameters i.e. dry weight and tiller number (Wissuwa et al., 1998). But it was found that tolerance to P deficiency was largely caused by genotypic differences in P uptake and that P-use efficiency had negligible effect. Dry weight has a high correlation with P uptake. A major QTL for P uptake in P deficiency was identified on the long arm of chromosome 12 with 13.2 cM marker interval in rice using back cross inbred lines derived from *japonica*×*indica* cross. The QTL fine mapped from this region with 3 cM distance from marker C443 was named as phosphorus uptake1 (pup1) (Wissuwa et al., 2002; Wissuwa and Ae, 2001). Details of the markers linked to pup1 and the map position are given in Supplementary file S1. Four more minor QTLs were detected for P uptake on chromosomes 2, 6, 9, and 10 (Wissuwa et al., 1998; Ni et al., 1998). The QTLs for dry weight of whole plant on chromosomes 6 and 12 coincided with the QTL for P uptake. The QTL for tiller number on chromosome 12 also coincided with the QTL for P uptake on chromosome 12 (Wissuwa et al., 1998). The collocation of QTLs for tiller number and P uptake also indicates the tight correlation between biomass, tiller number and P uptake.

Another major QTL designated as PHO for P-deficiency tolerance, was mapped on short arm of chromosome 12 flanked by markers RG9 and RG241 (Ni et al., 1998). The QTLs involved in P uptake and P-deficiency tolerance are shown chromosome wise in Fig. 1. Chromosomes 2, 6 and 12 have a higher number of QTLs involved in P-deficiency tolerance, chromosome 12 bearing the highest. Chromosome 12 bears the only known QTL for P uptake, the pup1 (accession ID AQAZ001). So it appears that chromosome 12 is the most important chromosome for P-deficiency tolerance. The detailed information of all the QTLs presented in Fig. 1 is given in Supplementary file S1. Table S1 also contains sequence information of genes and QTLs related to phosphate regulation in rice. The sequence information was obtained from NCBI (www.ncbi.nih.gov), TIGR database (http://rice.plantbiology.msu.edu), RAP database (http://rapdb.dna.affrc.go.jp) and GRAMENE database (www.gramene.org) (Feb 2009).

5. Genetic regulation of Pi-starvation induced changes

Pi starvation in plants leads to co-ordinated gene expression, which includes induction of many enzymes and genes but the functions of only a few are known. Some genes function to acquire and utilise Pi efficiently, others are involved in regulating the expression of Pi-starvation induced genes.

Regulatory mechanisms to respond to Pi starvation have been studied in detail in *Escherichia coli* and yeast, much before the detailed processes were known in higher plants like *Arabidopsis*. In *E. coli*, at least 30 genes are involved in response to Pi during starvation. PhoB, a regulator, and PhoR, a sensor, act in concert as a two-component system that responds to the cell's need for Pi (Wanner, 1997). In yeast, the PHO-regulon responds to changes in Pi concentration. It includes both acidic and alkaline phosphates (*PHO5*, *PHO8*, *PHO10*, *PHO11*), high-affinity Pi transporters (*PHO84*) and negative regulators (*PHO80*, *PHO85*) (Johnston and Carlson, 1992; Oshima, 1982; Oshima et al., 1996; Toh-e, 1989). In yeast, a basic helix–loop–helix (bHLH) transcription factor *PHO4* generally interacts with the second transcription factor *PHO2* to induce the expression of the Pi-starvation induced acid phosphates and high-affinity Pi transporters (*PHO5* and *PHO84*) (Sengstag and Hinnen, 1988). In Pi-sufficient condition, one negative regulator *PHO80* in association with *PHO85* protein represses the *PHO* gene transcription probably by hyper-phosphorylation of *PHO4*. *PHO81* is a positive regulator, which inhibits *PHO80*/*PHO85* function in the absence of Pi. In the presence of Pi the *PHO81* is inactivated and its synthesis is reduced. Under these conditions *PHO80*/*PHO85* levels prevent Pho4 and Pho2 from activating *PHO* transcription.

Some similarities exist between yeast and higher plants in their response to Pi starvation. The tomato *TPSI1* and *Medicago truncatula* *Mt4* promoter sequences contain cis-elements similar to those found on PHO-regulon genes in yeast (Burleigh and Harrison, 1998). *TPSI1* induced during early changes in Pi starvation is temporarily regulated by changes in Pi concentration (Liu et al., 1997). Expression of *Mt4* is suppressed either by supply of P or by formation of mycorrhizal association. Moreover based on the similarity of one region that is characteristic to *TPSI1*/*Mt4* family, two genes were cloned from a Pi-starvation induced cDNA library of rice roots using subtractive hybridisation (Hou et al., 2005), which are as follows. The first one, *OsIPS1*, had sequence similarity with *Arabidopsis IPS1*. The second gene *OsIPS2* was reported later as *OsPI1* (*Oryza sativa* phosphate limitation inducible gene). In Pi starvation, higher accumulation of *OsIPS1* was found in roots compared to shoots. Using transgenic plants, it was shown that *OsIPS1* and *2* are independently responsive to Pi signalling and are mainly expressed in lateral roots and in the vascular bundle of the primary root. Both *OsIPS1*/*2* are responsive to both systemic and local responses to Pi starvation. During long-term Pi starvation, *OsPI1* (previously reported as *OsIPS2*), showed most significant increase in transcription in both roots and leaves (Wasaki et al., 2006) and was repressed by P re-supply. There was a large difference between the response in roots or in leaves in rice exposed to Pi-deficient condition. The response in roots seems to be more dramatic than in leaves. The major responses in leaves were of genes involved in internal P utilisation, whereas, in roots not only internal P utilisation but also many genes involved in effective P uptake were up regulated.

It is suggested that the regulatory gene *Phosphate starvation response 1* (*PHR1*) plays an essential role in co-ordinated regulation of many late Pi-starvation genes. The *PHR1* encodes a MYB transcription factor with homology to *PSR1* from *Chlamydomonas* (Rubio et al., 2001). Two types of transcriptional regulations in response to Pi starvation have been reported i.e. transiently induced genes during early stages of Pi stress and highly induced genes during prolonged Pi stress. The promoter of early response genes are enriched with sequence motifs, the *PHO* like CBKGTGG (B = G or T or A; K = C or T or A) and the TATA box like (TATAAATA) elements. The late starvation induced genes contain 10 base pair sequence CGCATATTCC, which is considered as the *PHR1* binding site. *Arabidopsis PHR1* binds to the *P1BS* (PHR1 binding sequence) which is present in the promoters of the genes controlled by *PHR1* i.e. *AtACP5* (acid phosphatase), *AtIPS1_3*, *At4*, and *RNS1* (Rubio et al., 2001; Bariola et al., 1994). *Arabidopsis phr1* mutants show less accumulation of anthocyanin, less root–shoot ratio compared to wild type. These results indicate that *PHR1* encodes a positive regulator of Pi-starvation responses. But, *AtPHR1* does not control the increased root hair number and length indicating that PHR1 controls only a subset of responses. Rice genome database search showed two genes *OsPHR1* and *OsPHR2* homologous to *Arabidopsis PHR1* (Rubio et al., 2001). Zhou et al. (2008) investigated the function of *OsPHR1* and *OsPHR2* in Pi signalling in rice using transgenic plants. The authors showed that both *OsPHR1* and *OsPHR2* are involved in Pi-signalling pathway by regulating the expression of Pi-starvation induced genes. The *OsPHR2* is involved in Pi dependent root architecture alteration in both systematic and local pathways. Both *OsPHR1* and *OsPHR2* are expressed constitutively in all tissues with higher expression levels in roots and leaves. *OsPHR2* also encodes a MYB transcription factor and both *OsPHR1* and *OsPHR2* are nuclear proteins.

A second gene *SIZ1* (SUMO/Smt3 ligase) is involved in regulation of phosphate starvation response, The SIZ1 was identified as the suppressor of *sos3* (salt over sensitive) in *Arabidopsis*. But later it was found to be involved in regulation of Pi-starvation responses (Miura et al., 2005). *SIZ1* encodes a *s*mall *u*biquitin-like *mo*difier (SUMO) E3 ligase. Sumoylation has many regulatory roles, one of which is to protect proteins from ubiquitin-mediated degradation. PHR1 has two predicted sumoylation sites and PHR1 has been shown to be sumoylated by SIZ1 in vitro. The *siz1* mutation reduces the induction

of *AtIPS1* and *AtIPS2* under Pi-starvation conditions. As the induction of *AtIPS1* and *AtIPS2* in Pi starvation is controlled by PHR1, sumoylation of PHR1 may play a positive role for the Pi-starvation induced expression of *AtIPS1* and *AtIPS2* (Yuan and Liu, 2008).

A third gene, *P*i-starvation induced *t*ranscription *f*actor 1 (*OsPTF1*) belonging to basic helix–loop–helix (bHLH) group of genes was cloned from a cDNA library constructed by the suppression subtractive hybridisation (SSH) method from a P-efficient indica landrace, Kasalath, (Yi et al., 2005). This gene corresponds to a QTL for P efficiency mapped in rice. The *OsPTF1* contains reorganization motif for G-box. Over-expression of *OsPTF1* enhances the tolerance to Pi deficiency. Tiller number, shoot biomass, panicle weight, and P content are about 20 to 30% higher in Pi-deficient conditions in hydroponic experiments, soil pot or field experiments in the *OsPTF1* over-expressing plants as compared to the wild type plants. To investigate further the downstream genes regulated by *OsPTF1*, a microarray analysis was performed using rice whole-genome oligo-chips. The microarray data showed that expression of 158 genes was up regulated more than two-fold in roots, shoots or both in the transgenic plants. The marked induction of the *PHO* genes, such as *RNS1* and H^+-transporting ATPase, in the transgenic rice plants under Pi-supplied conditions strongly suggests that over-expression of *OsPTF1* triggered a rescue system in response to Pi starvation and played an important role in the increased tolerance to Pi deficiency (Yi et al., 2005).

In addition to transcription factors, other major genes induced under Pi starvation have been identified e.g. *RNS1* and *RNS2*, two senescence associated RNase genes (Bariola et al., 1994; Dodds et al., 1996). The RNases were assumed to release Pi from RNA molecules in the extra-cellular matrix including those derived from other organisms in the rhizosphere or present within cells.

Another Pi-starvation inducible gene, *PSR3*, encodes a polypeptide that is homologous to β-glucosidase and plays a role in deglycosylation and regulation of acid phosphates during Pi stress (Malboobi and Lefebvre, 1997). Genes like RNases, phosphatases and *TPSI1*/*Mt4* family contain binding sequences to *PHR1*, the major regulatory gene (Franco-Zorrilla et al., 2004; Hammond et al., 2004).

6. Pi translocation and Pi transporters and regulation of Pi homeostasis

The P transport process begins with loading soil acquired P into root xylem, and in the form of inorganic phosphate. The high-affinity Pi transporters are expressed in epidermal cells, root hair and outer layer of cortex (cells in close contact with soil). Plant Pi transporters are members of subfamily 9 of the major facilitator super family of proteins. Pi transporters are membrane associated proteins consisting of 12 membrane spanning regions. Nine high-affinity Pi transporters from *Arabidopsis thaliana* have been isolated. These are PHT1 to PHT9, also known as AtPTs and AtPHTs (Muchhal et al., 1996; Okumura et al., 1998; Smith et al., 1997). Two Pi transporters *Le*PT1 and *Le*PT2 have been isolated from tomato. Using genome-wide approach in rice, Paszkowski et al. (2002) identified a set of 13 Pi transporter genes named as *OsPT1–OsPT13*. They found the highest degree of sequence identity in *OsPT4* and *OsPT5* with corresponding *Arabidopsis* Pi transporter genes. They demonstrated that only OsPT11 out of the 13 Pi transporters was specifically induced during arbuscular mycorrhizal symbiosis and this induction was highly specific to root system. Over-expression of *OsPT11* complements the defect in Pi uptake in *pho84* mutant strain of yeast. Ming et al. (2006) identified the possible high-affinity Pi transporter encoding gene *OsPT6-1* from leaves of *O. sativa* using PCR with specific primers. Introduction of *OsPT6-1* could complement the Pi uptake in a mutant yeast strain deficient in high-affinity phosphate transporters. It was demonstrated using in situ hybridisation and RT-PCR that *OsPT6-1* is expressed in both shoots and leaves, but the peak expression was observed in mesophyll cells under low phosphate condition. Ai et al. (2009) demonstrated the expression, localization and function of the two phosphate transporters OsPT2 and OsPT6. The authors described the OsPT2 as low-affinity phosphate transporter functioning in the transport of stored Pi in the plant, and OsPT6 as a high-affinity phosphate transporter playing a broad role in Pi uptake and translocation across the plant (Ai et al., 2009).

The *pho1* mutant of *Arabidopsis* which is deficient in loading Pi into xylem has provided a deeper understanding of loading of Pi acquired by the roots on to xylem. *PHO1* was identified as a member of the EXS transporter family (Hamburger et al., 2002). The EXS domain has been identified in yeast (*Saccharomyces cerevisiae*) as proteins involved in either phosphate transport or sensing (Wang et al., 2004). There are 10 homologues of *PHO1* gene in *Arabidopsis*. Though they have partial functional redundancy, only three of them (*PHO1*, *PHO1;H1*, *PHO1;H10*) are induced in Pi starvation. These three genes are induced through different pathways, which are PHR1 dependent or independent (Ribot et al., 2007; Stefanovic et al., 2007).

Unlike *pho1* mutant, *Arabidopsis* mutant *pho2* has 2- to 4-fold increased levels of Pi in leaves and unaltered levels in the roots. Bari et al. (2006) suggested that *PHO2* plays an important role in regulation of Pi allocation between roots and shoots. *PHO2* encodes an unusual E2 conjugase. Recently it was shown that Pi modulates the level of microRNA, which targets an E2 conjugase (Chiou et al., 2006). The miRNAs are a group of small RNAs processed from the stem-loop region of single-stranded endogenous transcripts and are involved in posttranscriptional gene regulation (Bonnet et al., 2006; Jones-Rhoades et al., 2006; Mallory and Vaucheret, 2006; Sunkar et al., 2007). Up regulation of miR399 is a specific response to deficiency of Pi but not other nutrients and *PHO2* expression is modulated by Pi dependent miR399 (Bari et al., 2006). There is a lot of interest in the role of microRNA in P-starvation response (Pant et al., 2008; Buhtz et al., 2008). It was reported that miR399 is a long-distance signal for the regulation of Pi homeostasis, because of its detection in the phloem sap. It is suggested that mi399 moves from shoot to root, where it is perceived by PHO2, leading to expression of transporters and other genes that are involved in the movement of Pi from root to shoot. This result was further supported by the experiments of Lin et al. (2008). The miR399 a to f are approximately 21 nucleotide motifs, whose over-expression led to reduction of *PHO2* transcripts. Induction of miR399 transcript is highly specific to Pi deprivation. *PHO2* transcript contains five binding sites for miR399 in the 5′UTR. It is suggested that during Pi deprivation, induction of miR399 inhibits *PHO2* and hence transfer of Pi to shoot is stimulated. Lin et al. (2008) further demonstrated the miR399-guided cleavage of *PHO2* mRNA. Two miRNA-directed cleavages may generate siRNAs (Axtell et al., 2006). Therefore, cleavage of *PHO2* transcript by miR399 causes the production of *PHO2* siRNAs. These small RNAs are double stranded; about 20 nucleotides long, present in both roots and shoots, and most likely function as siRNAs to reinforce the repression of *PHO2*. The authors suggest that PHR1 is upstream to PHO2 and miR399 in Pi signalling. Recently it was shown that *AtPHR1* was sumoylated by SUMO E3 ligase SIZ1 (Miura et al., 2005). In *Arabidopsis*, mutations in the *PHO3*, *PSR1*, *PDR2* and *PHR1* genes impair Pi-starvation signalling, whereas *PHO1*, *PHO2*, and *PUP1* mutations attenuate Pi uptake and distribution within tissues but this kind of regulation is yet to be demonstrated in rice.

Another gene called rice SPX (SYG/PHO81/XPR1) (*OsSPX1*) was specifically induced by Pi starvation in roots. Suppression of *OsSPX1* caused over-accumulation of Pi similar to *pho2* mutant and OsPHR2 over-expressers. The expression of OsPT2 and OsPT8 was induced significantly in the knockout plants of *Os*SPX1. In contrast, over-expression of OsSPX1 caused suppressed induction of all the 10 PSI genes tested (IPS1, Induced by Phosphate Starvation 1, IPS2, *Os*PAP10 Purple Acid Phosphatase 10, *Os*SQD2 Sulfoquinovosyldiacylglycerol 2, miR399 microRNA399, *Os*PT2, *Os*PT3, *Os*PT6, and *Os*PT8) (Wang et al.,

2009). This suggests that *Os*SPX1 acts in a negative feedback loop to optimise plant growth under phosphate starvation condition. More mutants in different steps of phosphate regulation during starvation are likely to help dissect the complex interactions taking place at cellular and whole plant level.

7. Phosphorus deposition in grain

Young plants have much (75–80%) of their phosphate in inorganic form and are water soluble, whereas, in mature grain it is largely organic phosphate which is accumulated. Most of the phosphate in grain is stored in the form of phytin or inositol hexa phosphate. The enzyme phytase releases the P from the grain during germination. Therefore, seed phytate is the major source of P that supports seedling growth on P-deficient soil (Marshner, 1995). One common QTL for total P concentration and phytate is located on chromosome 5 flanked by markers RM 305 and RM 178 (James et al., 2007). Another QTL for phytate content in grain is located on chromosome 12 flanked by markers RM 247 and RM 179. The low phytic acid mutation 1 (*lpa1*) causes reduction in phytic acid content in seed from 71 to 39% and increase in inorganic phosphorus from 5 to 32% with little effect in the total seed P. One of the *lpa* mutation was mapped on chromosome 2 flanked by microsatellite markers RM 3542 and RM 482 (Liu et al., 2007), which was earlier shown to be in the same region (Andaya and Tai, 2005). Another *lpa* mutation was mapped on chromosome 3, tightly linked to RM3199 with a genetic distance of 1.198 cM. This latter mutation was very likely to be the LOC_Os03g52760, a homolog of the maize myo-inositol kinase (EC 2.7.1.64) gene. Thus 4 QTLs/genes for phytic acid in seed are known in rice on chromosomes 2, 3, 5 and 12.

8. Interaction of P with other micro- and macro-nutrients

Phosphate uptake and homeostasis is affected by the presence of several other elements and physical soil factors (Jain et al., 2007; Zhang et al., 2006). The interaction with some major elements is considered here.

8.1. Interactions with nitrogen

The balance between N and P is important (Ziadi et al., 2008), as it has been found that the contribution of synergistic interaction between N and P in cereals can be 13–89% of the yield depending on yield potential and soil fertility (Moiser et al., 2004). In addition to enhanced crop yield, nutrient recoveries are higher in plots treated with both N and P than with N or P alone (Moiser et al., 2004). Increase in N and P application without potassium (K) application limits the crop yield beyond certain level in rice (Aulakh and Malhi, 2005). Another study demonstrated that the synergistic interaction between N and P could be more synergistic with application of K in adequate levels (Aulakh and Malhi, 2005).

8.2. Interactions with iron

Iron has always been a concern for P studies because iron in the form of ferritin interacts with P in soil or root surface or within the plant or in growth medium. In nutrient medium, Fe interacts with P to form precipitates and makes both the nutrients unavailable to the plant. Ward et al. (2008) have shown the interaction of phosphorus and iron in primary root response in seedlings of *A. thaliana*. They presented evidence that primary root growth inhibition in *A. thaliana* (Col-0) during P deficiency is due to iron toxicity in root tip. When Fe is removed in P-deficient medium, primary root continues to grow without any other alterations. The response was attributed to excess Fe caused by an increase in Fe bioavailability in P-deficient medium. This growth response showed no correlation with other P-deficiency responses like dry weight, tissue P concentration or regulation of P-responsive genes. The authors explained that primary root response in *A. thaliana* during P deficiency is completely separate from systemically controlled changes in gene expression. They suggested that reduced primary root growth is due to Fe-toxicity, while P governs changes in the gene expression involved in P nutrition. Thus effects of Fe-toxicity need to be considered in studies on P deficiency. On the contrary in rice, there is elongation of primary roots during P deficiency which is unlike in *A. thaliana* (Shimizu et al., 2004; Wissuwa et al., 2005). Nevertheless, iron toxicity does cause significant reduction in yield in some cases in low land rice cultivation on acidic water-logged soils (Marschner, 1995). This indicates that high Fe concentration could be associated with fixing of P in the soil so that it is unavailable for uptake by the plant.

8.3. Interactions with silica

Silicon (Si) is the second most abundant element in soil present in the form of silicic acid (H_4SiO_4) in soil water or solution. Silicon is readily absorbed by plant in the form of monosilicic acid. Silicon benefits the plants as it contributes to the structure of cell walls, roots and leaves. Si acts as a mechanical barrier against both, water loss and pathogens (Marschner, 1995). In Marandu palisadegrass (*Brachiaria brizantha*), the influences on productivity and physiological attributes following changes of phosphorus availability in the soil through the applications of phosphate and silicate were investigated. It was found that, the use of calcium silicate ($CaSiO_3$) or magnesium silicate ($MgSiO_3$) in acid soils enhances phosphorus availability to the plants by decreasing the fixation of the orthophosphate ion (Pereira de Melo et al., 2007). Positive silicate effects are usually associated with increase in soil pH, there are more erect leaves, with higher availability of phosphorus, and tolerance by plants to excess aluminium and iron in the soil.

8.4. Interactions with arsenic

Irrigation of arsenic contaminated water for rice in Bangladesh is the likely cause of elevated arsenic concentration in rice grain. This high arsenic concentration in rice and drinking water is a risk for human health in Bangladesh. Several surveys have been undertaken to address the issue of arsenic in rice grains, (Shah et al., 2004; USAID, 2004; Meharg and Rahman, 2003). It was demonstrated that arsenic uptake in different rice varieties are influenced by iron plaque formation and regulated by plant phosphorus status (Liu et al., 2004). Arsenic status in the plant is affected by phosphorus status in the rhizosphere of rice, as it is known that interactions exist between arsenic and phosphate uptake (Meharg, 2004). It is also suggested that arsenate is transported in the plant by the transporters for phosphate. In an 18 day hydroponic experiment it was shown in a fern *Pteris vittata* that, increasing the concentration of phosphate decreased the arsenate uptake with greater effect on root arsenate concentration than on shoot arsenate concentration (Wang et al., 2002). Therefore, increased concentration of arsenic can limit the phosphate transport across the plant. In another study, arsenic resistance in the mutants of *Chlamydomonas reinhardii* resulted in higher intracellular level of P (Kobayashi et al., 2005).

Arsenic uptake by rice can be reduced by using more aerobic cultivation practices, as under flooded condition, reduction of iron oxides releases arsenic from soils/sediments, which is then more available for uptake by the rice plant (Ross et al., 2006). It was demonstrated by Xie and Huang (1998) that addition of MnO_2 or gypsum (Lombi et al., 2004) effectively reduced arsenic concentration in soil solution. The problem of arsenic uptake needs to be addressed in the context of P uptake as the two processes use the same transporters in the plant.

In addition to the interactions with the micro- and macro-nutrients in the soil, phosphorus interacts with the phytohormones inside the plant (Zhang et al., 2003). For example, cytokinin is shown to play a role

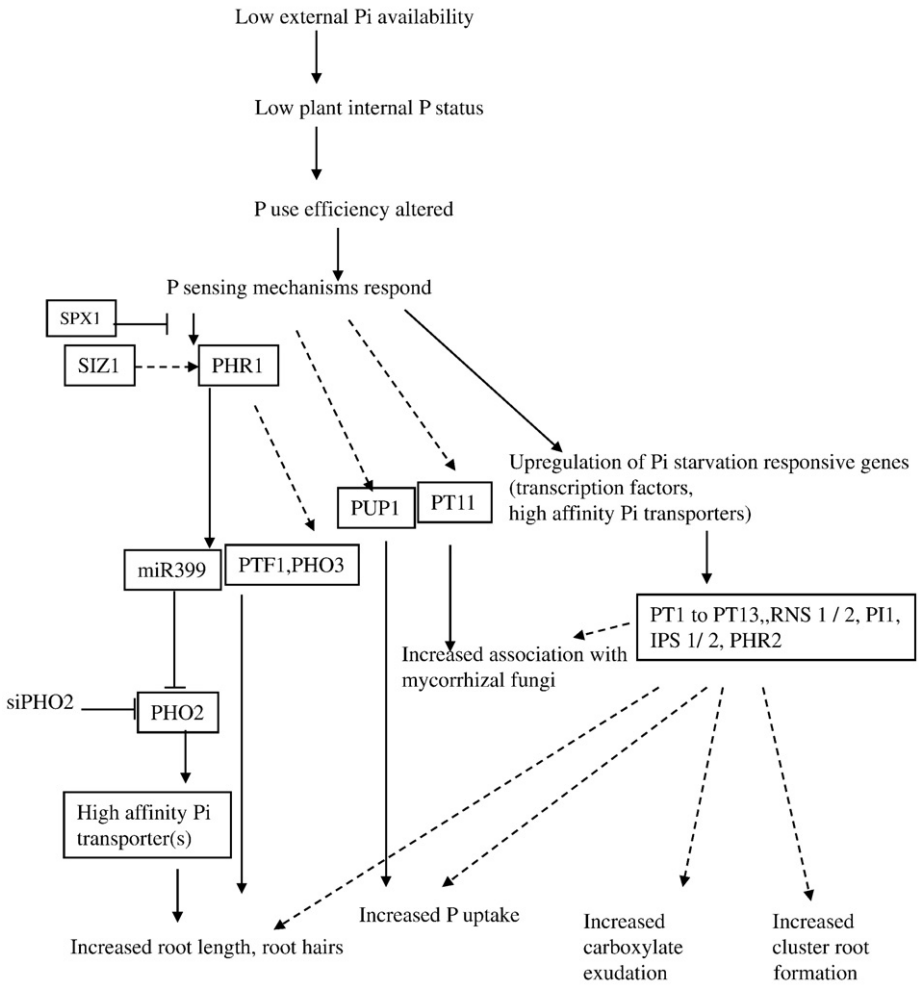

Fig. 2. Flow chart showing molecular events controlling phosphate uptake and transport during P starvation leading to different responses in rice. The known regulation is represented by solid arrows and unknown, predicted regulation is represented by dashed arrows.

in monitoring the status of phosphorus in *Arabidopsis* and in biomass partitioning between roots and shoots in low Pi supply (Franco-Zorrilla et al., 2004; Kupier et al., 1989). The repression of phosphate starvation signalling responses by exogenous application of cytokinin was partially explained by a significant increase of intracellular Pi content. Microarray data also revealed that a small number of genes have different cytokinin response patterns under different Pi levels, suggesting a subtle interaction between cytokinin and Pi-starvation signalling pathway (Wang et al., 2006).

9. Perspective

The process of phosphate regulation in rice or other monocots is not fully known. This discussion is based on the information available from rice and a projection is made for signalling events based on the information known from other plants. Some steps of phosphate signalling pathway can be constructed and these molecular events are depicted in Fig. 2. In P-non-limiting condition, the roots of the plant generally acquire phosphate by simple diffusion or mass flow. Low-affinity Pi transporters are constitutively expressed and take part in the transport of Pi to different parts of the plant in Pi-non-limiting condition. Phosphate is transported in the xylem. In Pi-limiting condition, highly specific signalling pathways come into play to acquire more Pi from the soil environment. These signalling events ultimately lead to many morphological adaptive changes in root, shoot structure and growth, which are known as Pi-deficiency response. Pi deficiency in cells is sensed by the internal P-use efficiency. Deprivation of P in the root cells possibly changes their biochemical environment. Therefore, roots are the first organs to sense the limited exogenous Pi supply as shown in *Arabidopsis* (Svistoonoff et al., 2007). The root then responds by sending two kinds of signals — local root born signal (LRS) and systemic root born signal (SRS) in *Arabidopsis* (Atkins and Smith, 2007; Schachtman and Shin, 2007). LRS is directed to the roots, which are in direct contact with the rhizosphere medium. SRS is directed to the shoots through xylem and causes the induction of mature miR399. The mature miR399 moves from shoot to root through phloem sap. In the shoot, it generates a third signal, systemic shoot signal (SSS), which along with the LRS stimulates the expression of miR399 now in roots. Accumulation of miR399 in roots inhibits the expression of PHO2 expression ultimately leading to enhanced P uptake and translocation. Generation of siPHO2 by the cleavage of PHO2 transcript through miR399 may cause further suppression of PHO2 at a later stage. The miR399 is specifically induced after Pi deprivation. But, the Pi deprivation induced miR399 is substantially reduced in *phr1* mutant. This suggests that miR399/PHO2 acts downstream of PHR1 in *Arabidopsis* but, this is yet to be shown in rice. Induction of PHR1 occurs probably after generation of the SRS in shoots and before the induction of miR399. PHR1 could thus serve as the key monitor of the Pi-status of the plant. During prolonged Pi stress, changes occur in the biochemical environment. Then, the downstream elements like RNS1 and RNS2, IPS1 and IPS2 probably bind to the 10 base pair binding element of *PHR1* gene and start a cascade of response. In rice, PHO2 and PHO3 control Pi-deficiency induced root elongation. In rice, a highly specific phosphate transporter PI11 is specifically induced during mycorrhizal symbiosis of the roots, however the immediate upstream or downstream events are not yet known. Finally, two major QTLs i.e. *pup1*

and *pho* are stimulated during Pi deficiency for Pi uptake. Several P-efficient wheat genotypes in germplasm collected from different areas of China secreted more organic acids (e.g. malic acid and citric acid) into the rhizosphere than the P-inefficient genotypes (Yan et al., 2006). The results demonstrate the feasibility of developing wheat or rice varieties with improved P-use efficiency by engineering more efficient organic acid secretion under P deprivation.

Molecular analysis of P-efficient wheat cultivar, Xiaoyan 54 showed that a phosphate transporter is highly transcribed in this variety under both normal and phosphate-deprived conditions (Davies et al., 2002). Genetic studies indicated that many root traits are closely associated with several major QTL that can be used to facilitate selection and breeding for higher P efficiency in crops (Yan et al., 2004). This has resulted in plant molecular breeding programmes aimed at genetic improvements of root traits.

Gramene database search showed that the QTL for phytate content of the rice grain, which is located on chromosome 12, flanked by the markers RM 247 and RM 179 was found to be located close to the region of the only known QTL for P uptake (*pup1*). The marker RM 179 is at 14,450,698–14,450,887 bp whereas; pup1 is located at 13,101,084–15,120,848 bp on the physical map (Gramene database). Thus these two QTLs for phytate content and P uptake appear to be contiguous with a partial overlap. The expression of these two QTLs deserves further study to get a better understanding of the relationship between P uptake and phytate content.

Moreover, the identification of the effect of OsPTF1 on P nutrition may speed up molecular breeding programmes for P efficiency in rice. As OsPTF1 was derived from rice itself, combining traditional breeding with molecular techniques could help develop new rice varieties containing the efficient allele of the gene. This study provides evidence that genetic modification of a key regulator involved in the Pi-signalling pathway may greatly facilitate P uptake and utilisation in plants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biotechadv.2009.02.006.

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