Control of phosphate homeostasis through gene regulation in crops
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Phosphorus (P) is an essential yet frequently deficient element in plants. Maintenance of phosphate (Pi) homeostasis is crucial for crop production. In comparison with the model plant Arabidopsis, crops face wider ranges and larger fluctuations in P supply from the soil environment, and thus develop more complicated strategies to improve Pi acquisition and utilization efficiency. Undergirding these strategies, there are numerous genes involved in alternative metabolism pathways that are regulated by complex Pi signaling networks. In this review, we intend to summarize the recent advances in crops on control of Pi homeostasis through gene regulation from Pi acquisition and mobilization within plants, as well as activation of rhizosphere P and P uptake through symbiotic associations.

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Introduction
As an essential nutrient for plant growth and development, phosphorus (P) is also the second most frequently required fertilizer component after nitrogen (FAOSTAT, http://faostat.fao.org, 2014). In order to feed the expanding population, crops require ever larger amounts of P to realize required yields. Most of the P acquired by plants is taken up as inorganic phosphate (Pi). Although, P fertilizers might meet crop P demands temporally, over the course of a growing season, P availability is still often limiting due to the high rate fixation and the slow diffusion of P in most soils. With this in mind, maintaining Pi homeostasis under fluctuating and often limiting Pi conditions becomes an essential consideration for improving crop productivity.

Over the last few decades, the gene regulation network underlying maintenance of Pi homeostasis has been well documented in the model plant, Arabidopsis [1,2]. The Pi signaling network, including candidate signaling molecules such as Pi, sugars, hormones, and microRNAs (miRNAs) has also been summarized in the informative reviews above. However, due to agricultural practices (e.g. fertilization and cultivation), crops face more fluctuations of the P supply than Arabidopsis does. These fluctuations might trigger even more complicated physiological, biochemical and molecular responses. The responses that have been outlined for improved P acquisition include alteration of root morphology and architecture, increase of root exudation and induction of high-affinity Pi transporters. Once acquired, P utilization can be enhanced through modification of metabolic pathways and reallocation of Pi among different organs and tissues, as well as sub-cellular compartments. Moreover, symbiotic associations with rhizosphere microorganisms, such as mycorrhizal fungi and rhizobia, also play important roles in the regulation of crop Pi homeostasis (Figure 1).

Considering the large knowledge gap existing between crops and Arabidopsis, in this review, we mainly highlight the recent advances in understanding gene regulation networks controlling Pi homeostasis in crops. In the meantime, progress in understanding these processes in Arabidopsis will be noted.

Gene regulation networks involved in P acquisition
Plants have developed several strategies to enhance P acquisition from P-limited soils (Figure 1). The most obvious change is the remodeling of root morphology and architecture, which provides an ideal structure for scavenging P. Complementing an optimized root architecture, increased root exudation can facilitate Pi release from unavailable soil P forms around roots. Then, the subsequent induction of high-affinity Pi transporters works to direct efficient uptake of rhizosphere Pi.

Remodeling of root architecture for better P acquisition
Remodeling of root system architecture (RSA) to cope with P deficiency is separately or coordinately controlled by complex regulation networks, which have been well summarized [1,2]. Functional characterization of crop species homologues of key Arabidopsis transcription factors/regulators has revealed molecular mechanisms of
Mechanisms for maintaining Pi homeostasis under Pi starvation in crop plants. Enhanced P acquisition is achieved by remodeling of root system architecture, forming symbiotic associations with rhizosphere microorganisms, increasing root exudates and inducing high-affinity Pi transporters. Increased Pi utilization efficiency can be facilitated by increasing the efficiency of Pi recycling and remodeling.

RSA Pi starvation-responses in crops. These transcription factors/regulators include OsPHR2, OsPTF1, OsARF12/16, OsMYB2P-1 and OsMYB4P in rice (*Oryza sativa*), *GhWRKY1* in cotton (*Gossypium barbadense*), *TaPHR1* in wheat (*Triticum aestivum*) and *ZmPTF* in maize (*Zea mays*) [1,3–8] (Figure 2). Overexpression of most of the above genes has resulted in augmented root growth under Pi starvation. Moreover, downstream genes are also reported to control RSA Pi starvation-responses in several crop species. Among them, *OsLTI1* (*OsPHO2*) in rice acts as a negative regulator of RSA under Pi starvation, while *GmEXPB2* in soybean (*Glycine max*), *LaGPX-PDE1/2* in white lupin (*Lupinus albus*) and the recently identified *PSTOL1* in rice all act as positive regulators [9–11,12] (Figure 2). Remodeling of RSA mediated by the above transcription factors/regulators in turn improves plant Pi status as supported by increased shoot Pi concentrations [3,6,11], which suggests vital roles for these genes in controlling root development for the purpose of exploring more to soils, thus increasing Pi acquisition efficiency and maintaining Pi homeostasis in crops.

Control of rhizosphere P activation

A large fraction of soil P is fixed into organic (e.g. phytate) or inorganic (e.g. Ca-P, Fe-P, Al-P) forms, which cannot be directly utilized by plants. It is generally believed that root exudates, such as acid phosphatases (APases), RNases, protons (H+) and organic acids, are intricately involved in P activation from insoluble P pools in the rhizosphere [13] (Figure 1).

Secreted purple acid phosphatases (PAPs) have been documented to function in Pi release from other organic P forms. For example, AtPAP10, AtPAP12 or AtPAP26 release Pi from ADP, glycerol-3-P or DNA in Arabidopsis [14], while *PvPAP* and *PvPAP3* release Pi from dNTPs in common bean (*Phaseolus vulgaris*) [15]. Recently, *PcPAP1* and *PcPAP3* have been suggested to be downstream genes of *PvSPX1* in common bean [16]. However, in Pi starved rice, *OsPAP10a* expression has been reported as enhanced by *OsMYB2P-1, OsPHR2* and *OsSPX-MSF1*, and suppressed by *OsSPX1, OsSPX5* and *OsARF12* [4,17,18,19,20]. This suggests the existence of a complicated gene network that coordinately regulates the activation of organic rhizosphere P with multiple other processes in response to low P stress in crops.

Increased exudation of H+ and organic acids has been found to activate the fixed inorganic P from soils [21]. A recent study showed that a tomato (*Lycopersicon esculentum*) 14-3-3 member, TPF1, functioned as a root plasma membrane H+-ATPase that increases the release of H+ under low P conditions [22]. However, further work is still required to fully uncover information at molecular level on the regulation of Pi starvation responsive H+ and organic acid exudation.
Regulation of root system architecture modification in Arabidopsis and crops. Black lines represent the regulation network in Arabidopsis, while blue lines represent the regulation network in crops. Arrows indicate positive effects, whereas lines ending with a short bar indicate negative effects. Solid lines indicate no effects observed. Dashed lines indicate undefined relations. At, Arabidopsis thaliana; Os, Oryza sativa; Gm, Glycine max; Zm, Zea mays; Ta, Triticum aestivum; Gb, Gossypium barbadense; La, Lupinus albus.

**Transcriptional and posttranscriptional regulation of Pi transporters**

Direct Pi uptake from rhizosphere occurs mainly through Pi:H+ cotransporters (PHT). After the first PHT genes (AtPT1 and AtPT2) were cloned from Arabidopsis, numerous PHT homologues have been characterized as regulators of Pi uptake in various crop species. Notable examples include OsPT1/6/8/9/10 in rice [20,23] and TaPt1.4 in wheat [24] (Figure 3).

The transcriptional network regulating Pi uptake through PHTs in Arabidopsis has been recently summarized with AtPHR1 playing a central role [1]. Similarly, OsPHR2 in rice, TaPHR1 in wheat and PvPHR1 in common bean have also been found to upregulate the expression of PHTs [7,20,25]. Moreover, in other crop species (e.g. barley and wheat), the PHR binding sequence (P1BS/ P1BS-like) exists in the promoter regions of some PHTs [26], which suggests roles for PHR in direct regulation of PHTs in these species as well.

The full extent of PHT regulation and connections with other pathways in crops has yet to be elucidated. In rice, OsPHR2 enhances the accumulation of OsmiR399 and subsequently suppresses the mRNA of OsPHO2. Mutation of OsPHO2 caused upregulation of several PHT genes (e.g. OsPT1/8), and thus resulted in an increase of Pi uptake under low P conditions [11]. Several PHO2 genes have also been identified as targets of miR399 in other crops, such as soybean [27]. Whether PHO2 affects PHTs in these crops, either directly or indirectly, remains unclear. Furthermore, in Arabidopsis, other transcription factors, including ZAT6, MYB62, WRKY75 and WRKY45, have also been reported as modulators of PHT expression [1,28] (Figure 3). In crops, however, their homologues have not been identified or functionally characterized.

In addition to transcriptional regulation, complex posttranscriptional regulation networks of PHT proteins have been gradually uncovered. In Arabidopsis, PHT proteins are regulated by the PT Traffic Facilitator (AtPHF1) during intracellular trafficking to the plasma membrane under low P conditions [1]. Similar activity is found in rice, where mutation of OsPHF1 results in the retention of OsPT2 and OsPT8 in the endoplasmic reticulum [29]. Thus, when overexpressing OsPHF1, Pi-uptake capacity of rice is enhanced [29].
Regulation of Pi uptake and translocation in Arabidopsis and crops. Black lines represent the regulation network in Arabidopsis, while red lines represent the regulation network in crops. Arrows indicate positive effects, whereas lines ending with a short bar indicate negative effects. Solid lines indicate no effects observed. Dashed lines indicate undefined relations. At, Arabidopsis thaliana; Os, Oryza sativa; Pv, Phaseolus vulgaris; Ta, Triticum aestivum.

**Pi signaling and gene regulation involved in Pi mobilization**

To maintain Pi homeostasis in plants, Pi mobilization must operate efficiently. This includes recycling of P from old/inactive tissues/cell compartments to young/active tissues/cell compartments, as well as, translocation of Pi within the whole plant (Figure 1).

**Systemic control of root-shoot Pi translocation**

Unlike Pi uptake from the rhizosphere, Pi translocation in different plant compartments is conducted through both PHT and SPX domain proteins. The sophisticated regulation network driving this Pi translocation in response to Pi starvation in Arabidopsis has been discussed by Chiong and Lin [1]. Here, Pi translocation in crop species is addressed.

Besides functioning in Pi uptake from soils, three rice PHTs (OsPT2/6/8) have also been found to play a role in Pi translocation. Knockdown OsPT2 and OsPT6 results in decreased long-distance Pi transport from roots to shoots, while overexpression of OsPT8 increases Pi accumulation in both roots and shoots [20]. Interestingly, OsPT8 also mediates Pi translocation from vegetative tissues to reproductive organs. These data shed light on the multiple functions of PHTs in crops. Additionally, SPX domain proteins might be also involved in Pi translocation, especially in the xylem loading of Pi. Evidence for this comes from the observation that mutation of OsPHO1:2 results in the strong reduction of Pi transport from roots to shoots. In addition, another SPX domain protein OsSPX-MFS1 has been identified as a Pi transporter functioning in leaf Pi reallocation [20]. In Arabidopsis, the chloroplast Pi transporter AtPHT2:1, and Golgi Pi transporter AtPHT4:6 have been suggested to affect the Pi subcellular compartments [30,31]. However, molecular mechanisms underlying Pi redistribution among subcellular compartments remain largely unknown in crops.

Regulation of Pi translocation has been widely reported in rice and Arabidopsis (Figure 3). In rice, OsSPX3/5 might be the functional repressors of OsPHR2 and...
Phosphoenolpyruvate carboxylase (PEPC)-malate dehydrogenase-malic enzyme glycolytic bypass, which reduced the requirement of Pi, is a well documented alteration of glycolysis under Pi starvation. Upregulation of PEPC and PEPC kinase (PPCK) by P deficiency has been documented in Arabidopsis, rice, white lupin, orange (Citrus sinensis L.) and melon (Cucumis melo L.) [13,32,33]. The expression of PPCKs is repressed by the basic helix loop helix transcription factor BHLH32 in Arabidopsis, but upregulated by another BHLH protein, PTF1, in both rice and maize [3,13]. Thus, it can be concluded that either multiple BHLH proteins regulate PPCKs in opposite directions or Arabidopsis and rice have evolved different regulation pathways in Pi conservation.

Another strategy for P recycling under low P stress is the conversion of membrane phospholipids to sulfolipids and galactolipids, which could save Pi for vital metabolisms. This conversion process consists of two steps: first, the phospholipids are hydrolyzed to yield diacylglycerol (DAG) and release free Pi; second, galactolipids and sulfolipids are synthesized using DAG as a substrate [34]. Two sets of rice genes involved in the synthesis of either sulfolipids (SOD1 and SOD2) or galactolipids (DGD1 and DGD2) are found to be upregulated by Pi starvation [35]. In white lupin, phospholipids are totally decomposed under Pi starvation via highly induced expression of two glycerophosphodiesterase (GPDE) genes, GPX-PDE1 and GPX-PDE2, thus recycling Pi for plant growth [9].

Enhanced P recycling from a broad range of organic-P in inactive/senescent tissues/cell compartments is proposed to be a universal strategy of plant adaptation to Pi starvation. In this response, intercellular acid phosphatase (IAPase) and ribonuclease are thought to play crucial roles. Increased IAPase activity is observed in some P deficient plants, along with induced expression of various IAPase coding genes, such as *Cm-PAP1* [32]. However, among the functionally identified IAPase genes, only *AtPAP26* is proven to be involved in P recycling from senescing leaves [36]. The ribonuclease genes RN32 from Arabidopsis have also been found associated with Pi starvation and senescence [37]. These results indicate that both acid phosphatases and ribonucleases are important for P remobilization from senescing leaves, particularly under low P stress.

### Genes involved in P recycling and conservation

Plants also increase P use efficiency during Pi starvation via promotion of intracellular P recycling by upregulation of a variety of genes. The resulting responses include alteration of metabolic pathways, replacement of membrane phospholipids and recycling of P from a broad range of organic P forms [13].

Regulation of Pi homeostasis in symbiosis

Two important symbiotic associations affecting plant Pi homeostasis are known as mycorrhizae, and nodules on legumes. Forming symbiotic relationships with arbuscular mycorrhizal fungi (AMF) is an important strategy by crops in adaptation to P deficiency [38]. Elongation of AMF extraradical hyphae can extend the P depletion zone beyond the rhizosphere, and subsequently improve the Pi-status and growth of host plants (Figure 1). Maintenance of plant Pi homeostasis in mycorrizal symbiosis is a complex process involving the multidirectional regulation of various genes. For example, the expression of pre-miR399 in Medicago leaves is upregulated by AM symbiosis [39]. Enhanced accumulation of miR399 in shoots might increase its transport to roots, and thus increase mature miR399 abundance in roots. Elevated miR399 in roots is postulated to keep a low level of *MtPho2*, and thereby prevents suppression of symbiotic Pi uptake [39].

The symbiotic Pi uptake mediated by AM-associated Pi transporters is essential for maintaining Pi homeostasis in the AM symbiosis system [40], such as OsPT11 and OsPT13 in rice [41**], LePT3 and LePT4 in tomato [42], AsPT4 in *Astragalus sinicus* [43] and ZmPHT1;6 in maize [44]. Interestingly, the P1BS cis-element is present in the promoter of many AM-related Pi transporters (*e.g.* *StPT3*, *StPT4*, *LePT4*, *MtPT4*, *OsPT11* and *OsPT13*) [45]. This indicates that these AM-related genes might be regulated by PHR transcript factors. However, details of this signaling network remain to be revealed.

Legumes can interact with rhizobia to form nodules for fixing atmospheric nitrogen. Functional characterization of a Pi transporter, GmPT5, indicates that Pi homeostasis in nodules is largely dependent on Pi transport from roots rather than direct Pi uptake by nodules during Pi starvation [46**]. Although a group of Pi starvation responsive genes and proteins have been identified through modern transcriptomic and proteomic analysis in nodules [47,48], molecular information on rhizobial effects on Pi homeostasis remains fragmentary. Further functional characterization of the genes/proteins identified in these experiments might be helpful for constructing a more complete model of the gene regulation network controlling Pi homeostasis in nodules.

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Prospects
In order to maintain Pi homeostasis under low P stress, plants have adapted various strategies involving multidimensional gene regulation networks. However, Pi homeostasis in plants cannot be taken in isolation from other nutrient elements. Rather, evidence for coordination of homeostasis among different nutrients is emerging. In Arabidopsis, Pi starvation downregulates the expression of RING-type ubiquitin E3 ligase NITROGEN LIMITATION ADAPTION (NALA), which is involved in N recycling during N limitation stress. When Pi is sufficient, NLA and UBC24 (PHO2) might work in tandem to mediate polyubiquitination of AtPHT1;4 for degradation [49,50]. In addition, P application facilitates N fixation in soybean nodules [46**]. These results together suggest that even more complex gene regulation networks work to maintain Pi homeostasis in plants.

On the other hand, in agricultural production, heavy application of P fertilizers has been carried out to avoid losses of crop yield due to P deficiency, but it might lead to P toxicity in crops. Very little attention has been paid on how crops cope with overwhelming P application. Recently, it has been documented that OsSPX4 could maintain Pi homeostasis under P sufficient conditions mainly through inhibition of the targeting of OsPHR2 to the nucleus [51]. However, more studies are still needed to understand the regulatory network about maintenance of Pi homeostasis under excess P conditions. Therefore, in order to achieve sustainable agriculture, further studies need to address not only how crops adapt to Pi starvation, but also how this fits in with complex interacting networks regulating homeostasis of multiple nutrients, as well as, how crops can maintain Pi homeostasis under P intensive conditions.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
- of special interest
- of outstanding interest


The authors sequenced a major quantitative trait locus for phosphorus-deficiency tolerance, Pup1, and thus identified a protein kinase, PSTOL1 in rice. Further analyses showed that PSTOL1 acts as an enhancer of early root growth, thereby enabling plants to acquire more phosphorus and other nutrients.


This study showed that the auxin response factor OsARF12 regulated rice Pi homeostasis by remodeling the transcription levels of some
Pi-responsive genes, as well as OsPHR2, and its downstream components. The results indicated a regulation function of OsARF12 in the crosstalk between auxin and Pi starvation signaling.


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46. Qin L, Zhao J, Tian J, Chen L, Sun Z, Guo Y, Lu X, Gu M, Xu G, Liao H: The high-affinity phosphate transporter GmpPT5 regulates phosphate transport to nodules and nodulation in soybean. Plant Physiol 2012, 159:1634-1643. The authors identify a nodule high-affinity phosphate (Pi) transporter gene, GmpPT5, whose expression is upregulated by P deficiency. Furthermore, they demonstrate that GmpPT5 mainly functions in transferring Pi from roots to plant cells in nodules. This study for first time provides direct evidence that legume Pi transporter involves in maintaining Pi homeostasis in nodules by controlling Pi entry from roots to nodules.


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phosphorus deficiency through proteomic analysis. 


This study showed that NLA and UBC24 (PHO2) might work in tandem to mediate polyubiquitination of AtPHT1;4 for degradation. The results provide additional molecular insight for the Pi signaling/homeostasis regulation.