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REPLACE, REUSE, RECYCLE: IMPROVING THE SUSTAINABLE USE OF PHOSPHORUS BY PLANTS

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Novelty statement.

The phosphate problem is one of matching supply and demand.
Understanding molecular mechanisms of phosphate response can help in addressing recapture as well as efficiency of use to increase sustainability.
Abstract

The ‘phosphorus (P) problem’ has recently received strong interest with two distinct strands of importance. The first is too much P is entering into waste water creating a significant economic and ecological problem. Secondly, whilst agricultural demand for phosphate fertiliser is increasing to maintain crop yields, rock phosphate reserves are rapidly declining. Unravelling the mechanisms by which plants sense, respond to and acquire phosphate can address both problems, allowing the development of crop plants that are more efficient at acquiring and using limited amounts of phosphate whilst at the same time improving the potential of plants and other photosynthetic organisms for nutrient recapture and recycling from waste water. In this review we attempt to synthesise these important but often disparate parts of the debate in a holistic fashion, since solutions to such a complex problem require integrated and multidisciplinary approaches that address both P supply and demand. Rapid progress has recently been made in our understanding of local and systemic signalling mechanisms for phosphate and expression and regulation of membrane proteins that take phosphate up from the environment and transport it within the plant. We discuss the current status of understanding of such mechanisms involved in sensing and responding to phosphate stress. We also discuss approaches to improve the P use efficiency of crop plants and future direction for sustainable use of P including use of photosynthetic organisms for recapture of P from waste waters.

6 key words in alphabetical order: fertilisers, phosphate, nutrient recycling, membrane transporters, phosphate signalling, transcription factors

Abbreviations:

AMF Arbuscular Mycorrhizal Fungi, MAB marker assisted breeding, miRNA micro RNA, NATS natural antisense transcripts, OA organic acids, PAE, the amount of P taken up as a function of biomass. PUE the amount of productivity or yield per unit P. Pi, inorganic phosphate. SPX, protein domain named for founding members Syg1, Pho81, XPR1. TF transcription factors
Introduction

Phosphate is a non-substitutable plant nutrient, essential for global agriculture. There are two key reasons why the sustainable use of phosphate is of importance; the supply is running out and paradoxically much of what is produced is wasted and results in environmental damage. Rock phosphate is crucial for the production of inorganic phosphate fertilisers but reserves are finite and the supply is expiring rapidly (Cooper and Carliell-Marquet, 2013). In 2010 global extraction was c. 176Mt and demand is increasing, with global peak phosphorus use expected to be reached by 2030 (Cordell et al., 2009). The best estimates for longevity of reserves are around 200 years and the worst are at 50 years (Rosemarin et al., 2011). Moreover, the process of mining rock phosphate and manufacturing fertiliser is expensive and energy intensive (Elser and Bennett, 2011).

In 2013 the UK imported and consumed c. 140,000 tonnes of phosphate, with 86,000 tonnes used for crop fertiliser and animal feeds (Cooper and Carliell-Marquet, 2013). It is estimated that 2-3 tonnes of phosphate per million people per day enter the UK’s watercourses as treated sewage where it is lost to the environment (Kato et al., 2007) and can negatively impact on ecosystems. This equates to 70,000 tonnes or half the country’s annual requirement. Prices for diammonium phosphate fertiliser in 2014 were $500 tonne$^{-1}$ (Argus, 2014) leading to the potential loss of $35M (£22M) every year.

The majority of phosphate inputs to the environment are from land application as fertilisers (Smil, 2000), animal-generated wastes (Goopy and Murray, 2003) and waste water from human conurbations (organic waste and detergents). These inputs supply waste water treatment plants with concentrations of dissolved phosphate that is difficult and expensive to remove (Britton et al., 2005) yet provide a potential supply of this resource. Phosphorus (P) is an essential element in many cellular macromolecules such as nucleic acids, phospholipids, and metabolites such as nucleoside triphosphates and phosphorylated intermediates in many biochemical pathways, therefore capacity to replace phosphorus (as phosphate) is limited. Consequently, the key to sustainability must be to reuse and recycle phosphorus efficiently both within the environment (Elser and Bennett, 2011) and within the plant (Veneklaas et al., 2012). Although several excellent reviews are available on efficient utilization of P nutrition for sustainable crop production (Chiou and Lin, 2011; Lopez-Arredondo et al., 2014; Nussaume et al., 2011; Raghothama, 1999; Richardson et al., 2011; Rouached et al., 2010; Zhang et al., 2014) in this article we
present a more holistic view that considers the potential to apply recently developed molecular understanding of plant phosphate responses to reducing crop phosphate requirements and environmental phosphate remediation.

**Chemical and biological technologies for capturing phosphate**

Methods employed in capturing phosphate from waste outputs depend on available space, cost and load applied. In many cases, the addition of metal salts such as $\text{Al}_2(\text{SO}_4)_3$, $\text{CaCl}_2$ or $\text{FeCl}_3$ is used to precipitate out the phosphorus (Bashan and Bashan, 2004). Struvite ($\text{NH}_4\text{MgPO}_4\cdot 6\text{H}_2\text{O}$) formation is an alternative method used for nutrient recovery from anaerobic digestates (Britton *et al.*, 2005). These technologies have been used for many years with variable success in achieving low phosphate discharges ($\text{c.}<1\text{mg P L}^{-1}$), but carry the burden of cost variations due to fluctuating prices of iron, magnesium and aluminium (Farchy, 2013; Vidal, 2008). A further issue to tackle when adding these salts is the discharge consent on the salts themselves—high concentrations of iron are not permitted as it can cause as much harm to the environment as high concentrations of phosphate. In an attempt to tackle the issues surrounding the chemical removal of phosphates, in recent years much research has been carried out employing biological alternatives.

Phosphate can be removed from waste streams via several different biological methods. These include microbiological, algal, plants (terrestrial and aquatic) and combinations of these. Some are energy-requiring processes and some are not. As well as nutrient removal capacities, biological methods often provide extra benefits such as production of bioenergy crops and animal fodder. Here we focus on the potential for plant based remediation.

Microalgae such as *Chlorella sp.* or *Scenedesmus sp.* can be utilised to remove phosphate from wastes (Larsdotter, 2006). Systems include waste water ponds used for nutrient capture (Chopin *et al.*, 2012) or photobioreactors which are generally more focused on maximal biomass generation (Michels *et al.*, 2014). The latter tubular systems are energy intensive (artificial lights and temperature control in laboratory settings), while the former makes use of solar energy. While algal and mixed bacterial-algal assemblages have been shown to capture high concentrations of phosphates (Muñoz and Guieysse, 2006), a drawback is the difficulty of harvesting which can prove uneconomical (Michels *et al.*, 2014).
Terrestrial and aquatic (rooted and free-floating) higher plants (and combinations of all) can be implemented for the capture of several compounds including phosphates (Vermaat and Khalid Hanif, 1998). Waste water stabilisation ponds on farmland, constructed and engineered wetlands as well as constructed tanks for phytoremediation are all employed globally. Water hyacinth, knotgrass and cattail can all be grown to capture nutrients in natural or managed wetlands (Fedler and Duan, 2011). Floating macrophytes such as duckweed (E.g. *Lemna sp.* or *Spirodelapsp.*) have also shown promise in the uptake of phosphates from waste water, in large scale batch or variable flow rate tank systems (Abuaku *et al.*, 2006; Alaerts *et al.*, 1996; Farrel 2012). The large quantities of phyto-biomass produced by phyto-remediative systems (Verma and Suthar, 2014) generally all have beneficial by-products as energy sources such as for biogas, biodiesel (Fujita *et al.*, 1999), or feed for fish or cattle (Goopy and Murray, 2003). The other obvious advantage of using plants in outdoor settings to recapture phosphate is that they are solar powered. However studies are often descriptive in nature and difficult to compare in terms of efficacy as very different systems, organisms and conditions have been used, and often control over important variables is lacking, especially in low cost open systems. Where more controlled studies are performed results are frequently extrapolated from small scale to tonnes/ha with the associated potential for multiplication of errors. Nevertheless, the drawbacks of chemical removal practices and the energetic inputs required by some biological phosphate removal processes highlight the benefits of low energy phytoremediation. The beneficial by-products from plant nutrient capture systems must also not be overlooked. A clearer understanding of the molecular mechanisms of phosphate uptake in plants would provide great benefits, not least in their manipulation for greater and more reliable phosphate capture from high P waste waters as well as the converse goal of maintaining crop plant productivity with reduced P inputs.

**Plant responses to low phosphate.**

Plants operate molecular signalling networks to detect and respond to Pi starvation. Many recent studies have helped to underpin the molecular signalling networks involved in P homeostasis (reviewed in Chiou and Lin, 2011). Plants sense and respond to the Pi status both locally and systematically, with separate molecular mechanisms being involved in local and long distance Pi signalling to maintain homeostasis under Pi starvation (Lin *et al.*, 2014; Lopez-Arredondo *et al.*,...
2014; Thibaud et al., 2010). Typical levels of inorganic phosphate (Pi) in soils are
low µM, whereas levels in the cytosol of plants under optimal conditions are mM,
requiring the ability to acquire and buffer cytosolic Pi at concentrations 3 orders of
magnitude above that in the environment. Plants respond to low P stress in a
number of ways (Figure 1). These include: release of Pi from vacuolar stores for
example; remodelling of membranes to reduce reliance on phospholipids (reviewed
in Nakamura, 2013) and redistribution of Pi from old(ER) source tissues to young,
actively growing sink tissues. Remodelling the root system increases the surface
area for Pi uptake. Moreover, the secretion of organic acids (OAs) increases Pi
solubility, especially in acidic soils and the secretion of phosphatases releases Pi
from soil organic matter. The majority of plant species form mutualistic associations
with soil microorganisms, especially with Arbuscular Mycorrhizal Fungi (AMF)
expanding the volume of soil that can be explored and allowing interchange of
nutrients in both directions. Membrane proteins are central to many of these
adaptations and examples to be explored in this review are members of the PHT1
family that are important in both acquisition of Pi from the soil and its recycling
within the plant, members of the PHO1 family some of which are involved in export
of Pi from roots to shoots, and membrane proteins involved in secretion of organic
acids. The elaborate machinery, that regulates these (and other phosphate
response genes) at multiple levels from transcription through to protein location and
stability, is also discussed in this article.

Transcriptional regulation of P responses

PHR1 and its regulatory network

PHOSPHATE STARVATION RESPONSE 1 (PHR1) belongs to the MYB
family of DNA-binding proteins and is a major transcription factor (TF) involved in Pi
signalling (Figure 2). It binds to the phosphate starvation related regulatory element
(P1BS) motif (GNATATNC) in the promoter region of Pi stress responsive genes
(Rubio et al., 2001). PHR1 is localized to the nucleus and a SUMO E3 ligase (SIZ1)
is known to control Pi homeostasis at the posttranslational level through
sumoylation of PHR1 (Miura et al., 2005). PHR1 is involved in the activation of
multiple P starvation-inducible genes including phosphate transporter1 (PHT1),
PHO1, At4 and micro-RNA399 (miRNA-399) (Chen et al., 2011a; Rubio et al., 2001;
Shin et al., 2006). The miRNA-399 has been implicated in Pi starvation related
signalling in many plants (Lin et al., 2008; Pant et al., 2008; Liu and Vance, 2010;
Liu et al., 2010; Xu et al., 2013) by regulating the levels of PHO2 mRNA which
produces ubiquitin-conjugating enzyme E2 24 ((UBC24) (Pant et al., 2008)). Some
of these molecules move within the vasculature and therefore function as systemic
signals integrating activities in different tissues (Lin et al., 2014).

PHR1 both regulates and is in turn regulated by SPX domain proteins
(Secco et al., 2012) (Figure 2). These proteins are strongly involved in Pi starvation
responses. The transcript abundance of AtSPX1-AtSPX3 is significantly enhanced
while the expression level of AtSPX4 is reduced to half of that before Pi deprivation
(Duan et al., 2008). The regulation of the AtSPX genes was shown to be controlled
by PHR1 with AtSPX1 being proposed to be a transcriptional regulator, given its
nuclear localization and capacity of up-regulating the expression of downstream PSI
(Phosphate Starvation Inducible) genes when over-expressed (Duan et al., 2008).
However, recent studies have shown that instead of directly regulating the PSI
genes expression, AtSPX1/ AtSPX2 are involved in the formation of a protein
complex with AtPHR1 in a Pi dependent manner (Puga et al., 2014). Upon Pi
starvation, the interaction between AtSPX1/ AtSPX2 and AtPHR1 is replaced by the
binding of AtPHR1 to the P1BS (PHR1 Binding Site) from PSI genes, thus activating
the expression of these genes (Puga et al., 2014). A similar Pi dependent
interaction between OsSPX1/OsSPX2 and OsPHR2 was also detected in rice
(Wang et al., 2014). OsPHR2 is also regulated post transcriptionally by OsSPX4,
which binds to and prevents its translocation into the nucleus under high Pi
conditions. However under low Pi conditions OsSPX4 is degraded by the
proteasome allowing OsPHR2 to traffic to the nucleus and activate gene expression
(Lv et al., 2014). Given the fact that transcription of PHR1/PHR2 is not greatly
influenced by Pi levels, these observations indicate a Pi sensing and signaling
function of SPX proteins, although further research is needed to clarify how Pi level
affects the interaction between SPX proteins and PHR1/PHR2. The functional
similarities of SPX proteins between monocotyledons and dicotyledons also suggest
the highly conserved SPX domain could be of great significance in a prevalent Pi
sensing and signaling pathway.

Transgenic manipulation of PHR1

Several studies have looked at the impact of over expressing PHR1 of
Arabidopsis (Nilsson et al., 2007), ZmPHR1 of maize (Wang et al., 2013b), OsPHR2
of rice (Zhou et al., 2008) BnPHR1 of oil seed rape (Ren et al., 2012) and TaPHR1-
A1 of wheat (Wang et al., 2013a). These studies all observed up regulation at the
transcriptional level of several low phosphate response genes such as phosphate transporters and non-coding RNA miRNA399, and corresponding down regulation of PHO2, and showed increased levels of Pi in tissues. In several of these studies the PHR1 over expressing plants showed improved growth under low Pi conditions (Wang et al. 2013a, b, Ren et al., 2012 Zhou et al., 2008). In some studies reduced plant growth and performance and Pi toxicity symptoms were observed under high phosphate growth conditions (Nilsson et al., 2007, Zhou et al., 2008, Ren et al., 2012) but not in others (Wang et al., 2013a,b). This is perhaps not surprising as over expressing some of the Pi-starvation responsive genes that are downstream of PHR1 such as OsmiR399 (Hu et al., 2011) and OsSPX1 (Wang et al., 2009a) caused Pi toxicity in transgenic plants. In all cases constitutive strong promoters (35S or maize Ubiquitin) were used for over expression of PHR1 and the level of over expression determined by measuring transcript abundance. Since active PHR1 is controlled primarily at the post transcriptional level this may not be a reliable method of estimating the true level of transcriptionally active PHR1. In the studies where growth inhibition at high Pi was not reported, tissue levels of Pi showed only relatively modest increases. The beneficial effects of PHR1 over expression included increased root growth/branching (Wang et al., 2013a) and proliferation of root hairs (Zhou et al., 2008).

Other transcription factors

Other TFs involved in P signalling are WKRY75, ZAT6, BHLH32, PTF1, MYB2P-1 and MYB62 (reviewed in (Lopez-Arredondo et al., 2014) (Figure 2). Both WKRY75 and ZAT6 are up-regulated during Pi starvation and are found to be involved in regulating the modification of root architecture (Devaiah et al., 2007a; Devaiah et al., 2007b). In contrast, the BHLH32 TF is down regulated during Pi starvation and has been found to be associated with the modifications of root architecture and carbon metabolism in response to Pi stress (Chen et al., 2007b).

Over expression of OsMYB2P-1 conferred Pi-starvation tolerance in rice (Dai et al., 2012). Transgenic plants had shorter roots than wild type controls on P sufficient medium and longer roots and more tillers on Pi deficient medium. The OsMYB2P-1 over expressing plants had retarded growth and lower biomass on high Pi, but better growth than wild type on low Pi (Dai et al., 2012). As with PHR1 over expressing plants, the OsMYB2P-1 over expressing transgenics had enhanced expression of Pi responsive genes including IPS and miRNA399 in both Pi sufficient and deficient conditions. PHO2 was repressed and OsPT2 was upregulated under
Pi deficient conditions and the transgenics had increased Pi levels compared to wild type (Dai et al., 2012).

Transgenic plants over expressing *Oryza sativa Phosphate Starvation–Induced Transcription Factor 1* (OsPTF1) showed improved growth and yield characteristics in hydroponics, pots and field. At low Pi root and shoot biomass and Pi content was higher, as was the number of tillers, reproductive development and yield (Yi et al., 2005). Over expression of maize ZmPTF1 also showed improved tolerance to Pi starvation and resulted in increased partitioning of carbohydrate to the roots leading to larger root biomass on low Pi (Li et al., 2011). Interestingly, over expression of PTF1 up regulated a different set of genes to those under PHR1 control and included genes involved in gluconeogenesis (phosphoenolpyruvate carboxykinase PEPCK) and sucrose synthesis (sucrose synthase 2) as well as phosphate scavenging RNAse and vacuolar pyrophosphatase (Yi et al., 2005, Li et al., 2011). These results emphasise the interaction between phosphate levels and carbohydrate metabolism and point to the importance of carbohydrate supply to maintain growth under low Pi stress. Sugars are hence another important group of metabolites involved in Pi starvation related signalling which influence the expression of many Pi stress related genes in a number of species (Liu et al., 2005; Karthikeyan et al., 2007; Hammond and White, 2008; Hernandez et al., 2009).

**Other regulatory genes**

It is well established that an important response to Pi stress is through changes in root architecture. Plants produce more lateral roots and root hairs in response to Pi stress which expands the adsorptive area in the soil (reviewed in Rouached et al., 2010). The phenotypic changes of root architecture are genotype dependent and have been shown to be important for overcoming Pi stress in bean, soybean, maize and barley (reviewed in Zhang et al., 2014). Key regulatory genes involved in Pi starvation associated signalling linked to root system architecture changes are *LOWPHOSPHATE ROOT* (LPR1, LPR2 and LPR3) and the *PHOSPHATE DEFICIENCY RESPONSE 2* (PDR2) genes (figure 2). Both LPR and PDR2 are involved in root architecture modification in response to Pi starvation. LPRs encode multi copper oxidases expressed in the meristematic regions of the root tip, including root cap, and have been demonstrated to reduce the primary root growth capacity under Pi starvation (Svistoonoff et al., 2007). PDR2 encodes a P5-type ATPase that functions in the endoplasmic reticulum and is involved in close
monitoring of Pi status around the roots (Ticconi et al., 2004). PDR2 is essential for
the expression of SCARECROW (SCR) which is a key regulator for root morphology
during Pi starvation (Ticconi et al., 2009). In the root tip, both PDR2 and LPR1
function to sense the external Pi status and regulate the root architecture through an
endoplasmic reticulum-resident pathway (Rouached et al., 2010). Interactions with
auxin and sugar signalling are also of critical importance in modulation of root
architecture in response to phosphate deprivation (reviewed in Rouached et al.,
2010).

**PHT1 phosphate transporters**

P enters into the plant as Pi via plasma membrane transporters of the
PHOSPHATE TRANSPORTER1 (PHT1) family and the process is affected by soil
pH which influences the predominant form of Pi (HPO$_4^{2-}$ or H$_2$PO$_4^-$) available
(Schachtman et al., 1998). Following the first identification and characterization of
PHT1 family members in Arabidopsis (Muchhal et al., 1996), subsequent PHT1
members have been characterized in many plants including potato, white lupin,
tomato, Madagascar periwinkle, barrel medic, barley, tobacco, rice, maize and
wheat (Table 1) (Nussaume et al., 2011).

The PHT1 proteins belong to the Major Facilitator Super family (MFS), which
is the largest superfamily of active transporters and these are generally symporters
or antiporters driven by proton or sodium gradients. The PHT1 proteins are
predicted to contain 12 trans-membrane alpha helices divided into two domains (N
and C) of 6 transmembrane helices each (Karandashov and Bucher, 2005). The
PHT1s are encoded by a family of genes found in each plant species, for example
the Arabidopsis genome contains 9 genes (Mudge et al., 2002), rice has 13 genes
(Paszkowski et al., 2002), soybean has 14 genes (Fan et al., 2013), barley (Rae et
al., 2003) and foxtail millet (Ceasar et al., 2014) contain 12 genes each. The first
crystal structure of a eukaryotic fungal (Piriformospora indica) high-affinity
phosphate transporter was recently solved at 2.9 Å in an inward-facing occluded
state (Pedersen et al., 2013). Pi is located between the two domains buried in the
middle of the membrane at a location similar to the substrate binding sites in other
major facilitators. The same study also proposed a model for the mechanism of Pi
import into the cell (Pedersen et al., 2013).
PHT1 proteins transport Pi into the epidermal cortical cells of the root via a proton-Pi co-transport mechanism (Ullrich and Novacky, 1990). Different members of the PHT1 gene family show different patterns of expression with respect to tissue and phosphate status (reviewed in Nussaume et al., 2011). The PHT1s have been predominantly found to be expressed in roots, especially in epidermal cells and the outer cortex of the root hair (Misson et al., 2004; Mudge et al., 2002; Schunmann et al., 2004; Xiao et al., 2006). For example 8 out of 9 PHT1s in Arabidopsis have been found to be expressed in roots (Karthikeyan et al., 2002; Mudge et al., 2002). Further, localization studies on these transporters in different plant species confirmed that PHT1 is most specifically targeted to the plasma membrane (Bayle et al., 2011; Fan et al., 2013; Gonzalez et al., 2005; Jia et al., 2011; Preuss et al., 2011). In addition members of the PHT1 family have been found to be expressed in aerial parts including shoot, leaves and flowers suggesting their involvement in both acquisition and remobilization of Pi in the plant. For example in Arabidopsis, AtPHT1;5 is involved in removing Pi from senescing leaves (Nagarajan et al., 2011) and AtPHT1;6 has been found to be expressed in pollen (Karthikeyan et al., 2002; Mudge et al., 2002).

The PHT1s show a range of affinities for Pi and are divided into high and low affinity transporters. The affinities of PHT1s have been characterized by expressing in heterologous systems including the S. cerevisiae pho84 mutant which lacks the equivalent endogenous phosphate transporter (Bun-Ya et al., 1991) and Xenopus oocytes. The high-affinity PHT1s are usually expressed at low Pi concentrations and have a $K_m$ ranging from 3 to 10 $\mu$M, whereas the low-affinity ones functional at high Pi availability have a $K_m$ ranging from 50 to 300 $\mu$M (Lopez-Arredondo et al., 2014; Raghothama and Karthikeyan, 2005). These expression patterns and kinetic properties of PHT1s suggest that they play multiple roles for Pi acquisition and remobilization with respect to external Pi status and tissue specificity. Most of the PHT1s are found to be expressed in response to Pi starvation. Examples of PHT1 transporters expressed under Pi starvation and their affinities where known are listed in Table1.

*Post translational regulation of PHT1 levels*

Besides regulation at the transcriptional level in response to phosphate levels, PHT1 transporters undergo regulated trafficking and degradation. These mechanisms have been studied in detail in Arabidopsis thaliana and to a lesser extent in rice.
PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR 1 (PHF1) was identified through a genetic screen as an ER localised factor required for PHT1;1 targeting to the plasma membrane (Gonzalez et al., 2005). It was subsequently shown to enhance plasma membrane localisation of PHT1;2 and PHT1;4 as well (Bayle et al., 2011). PHF1 shares some sequence similarity to S. cerevisiae Sec12p and its overexpression, like that of Sec12p, inhibited export of COPII dependent cargo from the ER (Gonzalez et al., 2005), however PHF1 did not co-localise with other COPII components suggesting a distinct role (Bayle et al., 2011). PHT1-GFP fusions are detectable in sorting endosomes regardless of the external Pi concentration, but Pi starvation stabilised the GFP signal at the plasma membrane. In the presence of high Pi and Concanavalin A, which inhibits the vacuolar ATPase, GFP fluorescence was detected in vacuole-like structures, consistent with a model where PHT1 is endocytosed and targeted to the vacuole for degradation under high P conditions (Bayle et al., 2011) (Figure 3A).

NITROGEN LIMITATION ADAPTATION (NLA) is an E3 ligase which also contains an SPX domain (Table 3) that interacts with PHT1 at the plasma membrane. It targets PHT1;1 and PHT1;4 leading to their ubiquitination and subsequent endocytosis and degradation in the vacuole (Lin et al., 2013) (Figure 3). nla mutants over accumulate Pi and show toxicity symptoms (Kant et al., 2011). Thus NLA is an important component of a regulatory system that prevents Pi over accumulation under conditions of surplus. The ubiquitination pathway requires sequential action of E1, E2 and E3 enzymes with UBC8 being the E2 that interacts with NLA (Peng et al., 2007). A further enzyme, PHO2, an ER and Golgi localised peripheral membrane protein which may be a chimeric E2-E3 enzyme (Liu et al., 2012) is also important in phosphate deficiency responses and regulates PHT1 transporters (and also other targets such as PHO1 (Liu et al., 2012) and PHF1 (Huang et al., 2013) via ubiquitination (Figure 3B). However double mutants in nla and pho2 showed aggravated phenotypes. They were smaller, accumulated higher levels of Pi in shoots and had much higher steady state levels of PHT1;1/2/3, suggesting they function independently in regulation of phosphate transporter levels (Lin et al., 2013). When Pi is limiting AtNLA is down regulated by miRNA827 (Hsieh et al., 2009) relieving this inhibition, whilst PHO2 is a target of miRNA399 (Aung et al., 2006). One interesting observation is that PHO2 is predominantly in the vasculature based on studies with promoter reporter fusions; however PHT1s are predominantly expressed in the epidermal, cortex and root hair cells. This discrepancy in potential localisation has led to the proposal that PHO2 mRNA or
PHO2 protein may undergo cell to cell trafficking (Huang et al., 2013), adding yet another layer of complexity to regulation of phosphate transporters.

Lin et al showed that NLA regulation of PHT1 levels is also conserved in rice (Lin et al., 2013), and in S. cerevisiae Pho84p is internalised and degraded subsequent to phosphorylation and ubiquitination (Lundh et al., 2009). Interestingly PHT1;1 and PHT1;4 also show increased phosphorylation under Pi replete conditions. A phosphorylation mimicking mutation Ser514 to Asp promoted intracellular localisation, probably through inhibiting ER exit. Phosphorylation of Ser 320 also increased under Pi replete conditions, but whether this affected endocytosis was not tested (Bayle et al., 2011). In Rice OsPHF1 also regulates trafficking of phosphate transporters (Chen et al., 2011b) whereas in S. cerevisiae pho86 mutants retain Pho84p in the ER (Kota and Ljungdahl, 2005). Thus an ancient conserved mechanism for regulation of phosphate transporter activity appears to operate across kingdoms although the molecular components differ.

Manipulation of PHT1 expression levels

Several studies have investigated the effects of up regulating expression of phosphate transporters on the ability of plants to grow on low levels of Pi. OsPHT1;1 (OsPT1) is widely expressed in rice plants and not markedly induced by Pi deficiency (Seo et al., 2008; Sun et al., 2012). In these studies transgenic plants that express OsPHT1;1 under the control of the CaMV 35S promoter (Seo et al., 2008) or the ubiquitin promoter (Sun et al., 2012) were characterised. In both cases plants with increased level of OsPHT1;1 transcript were selected, and these plants accumulated higher levels of Pi in shoots under Pi sufficient conditions. However, under Pi limiting conditions no difference in Pi content was seen in 21 day old plants (Sun et al., 2012). In older plants grown in fertilised soil, Pi levels were almost double the levels in the xylem of transgenic compared to control plants (Sun et al., 2012) and field grown plants grown on unfertilised soil had much higher Pi content as well as 20% more panicles at harvest, although the plants were 30% shorter (Seo et al., 2008). The OsPHT1;1 overexpresser lines took up more phosphate and also produced more root hairs than control plants, even under Pi replete conditions (Sun et al., 2012). A similar enhancement of root hair production even under high Pi was seen when arabidopsis PHT1;5 was expressed under the control of the Actin 2 promoter (Nagarajan et al., 2011). AtPHT1;5 is expressed in root and leaf and moderately upregulated under Pi deficiency, and characterisation of mutants in this gene point to an important role in the allocation of Pi to shoots under P limitation.
conditions and in transfer of Pi from shoots to roots under Pi sufficient conditions (Nagarajan et al., 2011). At PHT1;5 over expressers showed reduced Pi uptake but increased biomass and leaf area, dry weight and stalk thickness under both long and short days. However, over expression lines senesced earlier (Nagarajan et al., 2011). OsPHT1;8 (OsPT8) is also a widely expressed phosphate transporter that is not strongly induced by low Pi (Jia et al., 2011). Over expression of OsPHT1;8 under the control of the maize ubiquitin promoter resulted in increased Pi uptake, high level accumulation of Pi in roots and shoots and toxicity symptoms under conditions of high Pi supply. The transgenic plants displayed stunted growth under both high and low Pi conditions (Jia et al., 2011). Overexpression of AtPHT1;9 under the 35S promoter resulted in increased Pi uptake, high level accumulation of Pi in roots and shoots and toxicity symptoms under conditions of high Pi supply. The transgenic plants displayed stunted growth under both high and low Pi conditions (Jia et al., 2011). Overexpression of AtPHT1;9 under the 35S promoter resulted in enhanced tolerance to Pi deficiency in seedlings with plants in soil growing similarly to controls (Remy et al., 2012). However in contrast to the effects of overexpressing AtPHT1;5 (Nagarajan et al., 2011) and OsPHT1;1 (Sun et al., 2012) AtPHT1;9 overexpression resulted in no difference in root hair density on high Pi and less proliferation of lateral roots under Pi deficiency (Remy et al., 2012) while over expression of BnPHT1;4 in Arabidopsis resulted in longer primary roots and reduced lateral root density in low Pi compared to control plants (Ren et al., 2014).

AMF interactions with PHT1 genes

AMF play an important role in mobilization of Pi from new sites in soil to Pi depletion zones that form around the root surface by extending their hyphae far beyond the Pi depletion zone (Becquer et al., 2014). The AMF in turn receive carbon photosynthetically manufactured by the host plant (Smith and Read, 2008). A comprehensive discussion of the role of AMF in increased P uptake is beyond the scope of this review, however it should be noted that several PHT1 genes are mycorrhiza-specific and inducible only upon inoculation of AMF. PHT1s that are known to be induced by AMF are given in Table 2. There is a complex and still not well understood interplay between plant and fungus. Barel medic MtPHT1;4 is specifically localised to the plant-derived periarbuscular membrane and the specific delivery to this membrane is proposed to arise through a transient reorientation of polarised secretion to this membrane during arbuscle development (Pumplin et al., 2012). MtPHT1;4 is essential for the acquisition of Pi delivered by the AM fungus and also critical for AM symbiosis. Loss of MtPHT1;4 function leads to premature death of the arbuscules; the fungus is unable to proliferate within the root and symbiosis is terminated (Javot et al., 2007). Similarly in rice both OsPHT1;11 and
OsPHT1;13 are important for AM symbiosis although only OsPHT1;11 is required for Pi transfer to the plants (Yang et al., 2012).

PHO1 and its homologues

Since the concentration of bioavailable Pi in the soil solution is frequently 1000 fold lower than that in the plant intracellular compartments (Bieleski, 1973), an efficient Pi homeostasis system requires not only the acquisition of Pi but also the reallocation of this element. The Arabidopsis pho1 mutant displays a series of Pi deficiency symptoms including a prominent decrease in leaf Pi content (Poirier et al., 1991). Intriguingly, it was also found in the same study that mutating pho1 does not impact the root Pi uptake or shoot Pi movement, thus suggesting PHO1 is specifically playing a crucial role of exporting Pi from root cortical cells to the xylem before the element is delivered to the shoot (Poirier et al., 1991). This proposed Pi exporting function of PHO1 was later confirmed by transgenic overexpression of PHO1 in Arabidopsis shoot tissues, resulting in enhanced shoot Pi content and intense release of Pi into the extracellular medium (Arpat et al., 2012; Stefanovic et al., 2011). Transient expression of AtPHO1 in tobacco leaves, revealed that the protein was predominantly localised to the Golgi/trans-Golgi network, but a certain proportion of total PHO1 was re-localised to the plasma membrane upon high Pi infiltration (Arpat et al., 2012). PHO1 may be more than a Pi exporter. Arabidopsis lines with reduced levels of PHO1 (2-10 fold decrease compared to wild type), showed reduction of shoot Pi levels comparable to pho1 mutants. However, unlike the pho1 mutant, growth rates similar to those of wild type were maintained and gene expression profiles indicative of Pi stress were not observed, showing that it is possible to uncouple Pi levels in the shoot from changes in gene expression (Rouached et al., 2011a). The authors propose that PHO1 may also be involved in transporting a root to shoot signal (other than Pi) that leads to induction of the suite of Pi deficiency responses in shoot and it is this transcriptional response rather than low Pi per se which leads to growth inhibition in the pho1 mutant (Rouached et al., 2011a).

Arabidopsis genomic sequence analysis identified 10 homologues of PHO1. These genes encode proteins (PHO1;H1-PHO1;H10) each of which has a well-conserved hydrophilic SPX domain at the N-termini and a hydrophobic EXS domain with six to eight potentially membrane-spanning segments at their C-termini (Hamburger et al., 2002). Among these 10 PHO1 homologues, PHO1;H1 and
PHO1;H10 are shown to exhibit the same Pi stress inducible expression as PHO1 (Yuan and Liu, 2008), while only PHO1;H1 has a similar Pi exporting function and restores the Pi xylem-loading in pho1 mutant plants (Stefanovic et al., 2007). However, expression of PHO1;H1 and PHO1 has been shown to be dependent on either the regulation of transcription factor PHR1, or controlled by PHO2 mediated endomembrane degradation, respectively (Figures 2 and 3) (Liu et al., 2012; Stefanovic et al., 2007). Such observations suggest that when facing Pi stress, plants utilise complex signaling pathways at multiple levels of regulation with potentially complex cross-talking among these pathways to maintain the intracellular Pi level. Three similar PHO1 family members, OsPHO1;1-OsPHO1;3, have also been found in rice. So far, OsPHO1;2 has been the only member found to resemble AtPHO1 in Pi transfer from roots to shoots, although all three rice PHO1 members are phylogenetically close to AtPHO1 and AtPHO1;H1 (Secco et al., 2012a) and are potentially regulated by their cis-Natural Antisense Transcripts (NATs) under Pi deprivation (Secco et al., 2010). The closest mammalian homolog of PHO1, xenotropic and polytropic retrovirus receptor XPR1, has also recently been demonstrated to exhibit Pi export activity when expressed in metazoan cells (Giovannini et al., 2013) and ectopically expressed in tobacco epidermal cells (Wege and Poirier, 2014).

Despite all Arabidopsis PHO1 family members containing some common primary structural features and RT-PCR analysis indicating a broad range of gene expression throughout the plant corpus (Wang et al., 2004), to date, only AtPHO1 and AtPHO1;H1 have been shown to play critical roles in Pi signaling and transport. AtPHO1;H4, otherwise known as SHB1 (Short Hypocotyl Under Blue1) has been demonstrated to control hypocotyl elongation under blue light through the formation of a protein complex (Zhou and Ni, 2010), while homologue AtPHO1;H10 is intensely induced upon various abiotic and biotic stresses apart from Pi starvation (Ribot et al., 2008). The relatively high level of similarity among PHO1 family members and the conservation of their N-terminal SPX domain throughout homologues from different species indicate an important role of SPX domain–possessing proteins and this domain itself in Pi homeostasis maintenance (Table 3).

**Secretion of organic acids to enhance P availability**

Acid soils suffer from Pi deficiency as it is sequestered by positively charged components of the soil (Figure 4), such as the toxic Al$^{3+}$ ions that become mobilised
at soil pH below 4.5. Importantly, approximately 50% of the world's potentially arable soils are acidic (von Uexküll and Mutert, 1995) and 60% of these are in developing nations, so this is a widespread problem compromising a large portion of potentially arable land (Kochian, 1995). Thus, plants have developed a range of mechanisms to deal with growth on acidic soils, chief among these is organic acid (OA) exudation. By this mechanism plants release organic anions, such as malate and citrate, into the soil and these anions overcome the dual problems of soil that is both deficient in phosphate and replete with Al\(^{3+}\) ions by protecting the plant from Al\(^{3+}\) ion toxicity and helping to mobilise phosphate as shown in Figure 5. Phosphate can be mobilised by organic anions either by anion exchange, freeing bound Pi, or by chelation of the metal ions that immobilise Pi in the soil (Sas et al., 2001). OA exudation is well established as a major trait in plants with resistance to Al-toxicity and improved PUE such as wheat (Ryan et al., 2001). The importance of OA exudation can be seen by the fact that up to 20% of a plant's carbon usage can be invested in OA exudation in the roots (Lynch et al., 2005) and this loss of carbon may account for some of the loss in yield of P-starved plants.

There are two main families of membrane proteins involved in OA exudation, the channels of the Aluminium-activated Malate Transporter (ALMT) family, and transporters of the Multidrug and Toxic compound Extrusion (MATE) family, which export malate and citrate, respectively (Ryan et al., 2011). These proteins are alpha-helical membrane proteins that form pores through the plasma membrane of root epidermal cells in order to release OAs into the soil. The MATE family is large, with many members still uncharacterised, however a sorghum homolog (SbMATE) has been shown to confer Al\(^{3+}\) tolerance by facilitating the release of citrate into the rhizosphere in response to Al\(^{3+}\) (Magalhaes et al., 2007). In addition, the barley gene HvAACT1 has been identified as a plasma-membrane-localised MATE transporter expressed at the root tips of barley root epidermal cells responsible for citrate efflux in the presence of Al\(^{3+}\) (Furukawa et al., 2007).

The first gene of the ALMT family to be characterised was ALMT1 in wheat and it has been shown that TaALMT1 releases malate in an Al-activated manner (Zhang et al., 2008). The protein senses free Al\(^{3+}\), which is a signifier of acidic soils, and releases malate through its central pore, down a concentration gradient into the soil. It acts as a channel, passively releasing the malate, rather than a transporter. There is a pressing need for a greater understanding of the structure and
mechanism of these channels; although some research has attempted to explore
topology via either immunocytochemical or bioinformatics approaches no settled
model has yet been agreed upon (Dreyer et al., 2012; Motoda et al., 2007). Recent
work has shown that the first 48 residues and a C-terminal helix of TaALMT1 are
vital for its function in oocytes (Sasaki et al., 2014). As yet several areas remain
unresolved including: the mechanisms by which these proteins are activated, how
they function at a molecular level, and their atomic-level structure. Interestingly,
although TaALMT1 has been shown to be constitutively expressed before being
directly activated by Al³⁺, activity of the Arabidopsis homologue AtALMT1 is
controlled at the transcriptional level by transcription factors STOP1 (Sawaki et al.,
2009) and WRKY1 (Ding et al., 2013) in response to the presence of Al³⁺.

Manipulation of organic acid exudation through transgenic modification

Transformation of barley (Hordeum vulgare L.), (which does not have a
functional equivalent) with TaALMT1 from wheat resulted in plants that were able to
take up more phosphate from the soil and which thrived when grown in acid, highly-
P-fixing ferrosol (Delhaize et al., 2009). This boost in yield was seen both in short-
term 26-day pot trials and a longer term experiment to physical maturity after 156
days. The improvement is due to a combination of effects. Firstly, the transgenic
plants were able to thrive in acid soil, enabling more root growth and so increasing
the area of its rhizosheath. Even in limed conditions the wild type barley had a
severely restricted rhizosheath, while ALMT1-transformed plants grown in both limed
and non-limed conditions produced a larger rhizosheath. Secondly, there was an
increase in phosphate uptake per unit root length indicating that the PAE was
increased by the release of malate into the soil by mobilisation of Pi. These
experiments show that the creation of a transgenic line with just a single gene
addition (that of TaALMT1) was able to more than double the grain yield of barley
plants grown in acid soil, producing yields close to growth in ideal non-acidic
conditions (with no loss of productivity on limed soil). This large effect is very
promising for the potential production of transgenic crops with improved PAE and
PUE on acid soils.

In connection with the effects on the rhizosheath it is notable that even on limed soil
and soil with added P, the deeper regions of the soil remain depleted of P. Wild type
barley roots were near-non-existent below 50 cm, but growth below this depth could
be enabled by TaALMT1. This restricted root growth impairs yield due to decreased
uptake of nutrients such as P, but also by restricting access to deep water sources. These transgenic approaches also impact on water usage and drought susceptibility, facilitating integration with other transgenic crop approaches. Although work assessing transgenic barley has been promising, a question remains over the viability of a transgenic strategy to increase yields as no work has been undertaken at field-scale.

Exploitation of knowledge for crop improvement

The results of manipulation of levels of specific membrane transporters, channels and transcription factors suggest that such an approach could be beneficial to both PAE and PUE. However, it is still unclear exactly how plants sense Pi levels internally and the contribution of levels of phosphate in specific cell types and subcellular compartments to perception and response. As excess accumulation of phosphate results in toxicity, simply driving plants to take up more is not necessarily the solution and runs the risk of further depletion from the soil. It is also difficult to compare results of different studies when different growth conditions and developmental stages of plants are used. More sophisticated approaches using targeted gene expression in specific tissues, analysis of protein levels (which may not reflect transcript levels because of the extensive post transcriptional regulation) and whole lifecycle comparisons of control and transgenic plants under conditions more closely replicating those in the field are required. Perturbation of phosphate transporter expression clearly alters these balances in as yet unpredictable ways and provokes changes in transcription of other genes as reported (Jia et al., 2011, Nagarajan et al., 2011, Sun et al., 2012). The uncoupling of transcriptional responses to phosphate starvation from phosphate levels that was seen in Arabidopsis lines with reduced PHO1 expression (Rouached et al., 2011a) may present a useful tool for further investigation as does the recent discovery of a small molecule ‘phosphatin’ that can attenuate Pi starvation responses and partially uncouple growth inhibition from Pi levels (Arnaud et al., 2014). Furthermore, as it is becoming apparent that there is significant cross talk between phosphate and other nutrient pathways such as nitrogen (Kant et al., 2011), sulfur (Moseley et al., 2009; Rouached et al., 2011b), iron (Bournier et al., 2013; Thibaud et al., 2010) and zinc (Khan et al., 2014) a more holistic approach that considers multiple nutrients may be necessary. However, there may also be specific instances where over expression of a single gene or combination of relatively few genes could make a
significant contribution such as the expression of TaALMT in barley (Delhaize et al., 2009).

As an alternative to targeting individual genes, plant breeders have developed crops with improved tolerance to acid soils, which are also improved in P uptake efficiency (David and Brett, 2003). Screening for QTLs for low Pi tolerant varieties is also a useful method of identification of new components in the P homeostasis pathway and potential means of marker assisted breeding. Several studies have been conducted for phenotyping the root traits and marker development in order to produce the low Pi tolerant varieties (reviewed in (Richardson et al., 2011). In rice, a major QTL, phosphate uptake1 (Pup1) was identified from aus type Pi starvation-tolerant Indian rice variety Kasalath (Chin et al., 2010), and this has been recommended for MAB. This gene was named for phosphate starvation tolerance locus (PSTOL1) and was missing in the non-tolerant rice genome, Nipponbare; expression of PSTOL1 is also found to be up regulated under Pi starvation (Gamuyao et al., 2012). In barley, increased level of expression of a low affinity PHT1 transporter HvPHT1;6 and HvPHT1;3 was correlated with genotypes with higher PUE (Huang et al., 2011).

Identification of root trait variations among the genotypes has been another important area of study to identify and develop Pi stress tolerant varieties (Lynch, 2007). Variation in root growth angles has been identified as an important trait for Pi-deficiency tolerance in maize (Zhu et al., 2005b), bean (Bonser et al., 1996; Liao et al., 2001) and wheat (Manske et al., 2000). Root hair variation has also been considered as an important trait for improving the Pi stress tolerance. Several studies have been conducted to assess the genotype variation for root hair density and root hair length (reviewed in Richardson et al., 2011) and QTLs associated with root hairs have also been identified in maize (Zhu et al., 2005a) and common bean (Yan et al., 2004). More studies are needed to utilize MAB to release new varieties with increased PAE and PUE.

Concluding statements

The development of integrated and sustainable approaches to agriculture is essential to meet humankind’s future needs. Increased understanding and exploitation of genes, transcription factors and proteins involved in uptake, utilization and signalling of Pi will be useful for efficient utilization of P in future. Transgenic
approaches to modulate the expression levels of some of these genes holds promise but needs to be decoupled from detrimental knock on effects on other aspects of plant physiology. Marker assisted breeding and improvement is a complementary approach for the production of Pi efficient crops. As well as improved farming methods and improved crop varieties with superior PAE and PUE it will be crucial to develop more efficient and environmentally benign methods to recover nutrients including P from waste and here too plants have a role to play. Thus, phosphorus sustainability is a major challenge requiring the efforts of government and industries, engineers, soil scientists, plant scientists, agronomists, plant breeders and farmers.

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### Tables

**Table 1. PHT1 genes up regulated by low Pi in different plants and their affinities**

Some of the PHT1s reported to be induced by Pi starvation are listed along with the name of the host plant, site of expression and affinities of the known transporters with reference. The expression patterns of these transporters have been analysed by RT-PCR, qRT-PCR and promoter GUS or GFP fusion studies.

<table>
<thead>
<tr>
<th>Name of the PHT1 gene</th>
<th>Plant</th>
<th>Affinity</th>
<th>Site of induction by low Pi</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtPHT1;1</td>
<td>Arabidopsis</td>
<td>High affinity</td>
<td>-</td>
<td>Mitsukawa <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>AtPHT1;7, AtPHT1;8, AtPHT1;9</td>
<td>Arabidopsis</td>
<td>-</td>
<td>Root</td>
<td>Mudge <em>et al.</em>, 2002</td>
</tr>
<tr>
<td>CfPHT1;1, CfPHT1;2</td>
<td>Cayenne pepper</td>
<td>-</td>
<td>Only in AMF inoculated roots</td>
<td>Chen <em>et al.</em>, 2007a</td>
</tr>
<tr>
<td>CfPHT1;3, CfPHT1;4, CfPHT1;5</td>
<td>Soybean</td>
<td>High affinity</td>
<td>Root</td>
<td>Fan <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>GmPHT1;1 to GmPHT1;12</td>
<td>Soybean</td>
<td>-</td>
<td>Root</td>
<td>Fan <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>HvPHT1;1</td>
<td>Barley</td>
<td>High affinity</td>
<td>Root</td>
<td>Rae <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>HvPHT1;6</td>
<td>Barley</td>
<td>Low affinity</td>
<td>Moderately induced in root and shoot</td>
<td>Rae <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>HvPHT1;9</td>
<td>Barley</td>
<td>-</td>
<td>Roots</td>
<td>Huang <em>et al.</em>, 2011</td>
</tr>
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<td>MtPHT1;1</td>
<td>Barrel medic</td>
<td>Low affinity</td>
<td>-</td>
<td>Liu <em>et al.</em>, 1998b</td>
</tr>
<tr>
<td>OsPHT1;2, OsPHT1;6</td>
<td>Rice</td>
<td>Low affinity</td>
<td>-</td>
<td>Ai <em>et al.</em>, 2009</td>
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<tr>
<td>OsPHT1;8</td>
<td>Rice</td>
<td>High affinity</td>
<td>Root</td>
<td>Jia <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>OsPHT1;8</td>
<td>Rice</td>
<td>High affinity</td>
<td>Shoot</td>
<td>Secco <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>PtaPHT1;1, PtaPHT1;2, PtaPHT1;3, PtaPHT1;7</td>
<td>Hardy orange</td>
<td>-</td>
<td>Roots</td>
<td>Shu <em>et al.</em>, 2012</td>
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<tr>
<td>PsPHT1;2</td>
<td>Kidney bean</td>
<td>-</td>
<td>Roots</td>
<td>Tian <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>SiPHT1;2</td>
<td>Foxtail millet</td>
<td>-</td>
<td>Leaf</td>
<td>Ceasar <em>et al.</em>, 2014</td>
</tr>
<tr>
<td>Name of the PHT1 gene</td>
<td>Plant</td>
<td>Affinity</td>
<td>Site of induction by low Pi</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>-----------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>SiPHT1;4</td>
<td>Foxtail millet</td>
<td>-</td>
<td>Root</td>
<td>Ceasar et al., 2014</td>
</tr>
<tr>
<td>SiPHT1;1, SiPHT1;2</td>
<td>Tomato</td>
<td>-</td>
<td>Roots</td>
<td>Liu et al., 1998a</td>
</tr>
<tr>
<td>SmPHT1;1, SmPHT1;2, SmPHT1;3, SmPHT1;4, SmPHT1;5</td>
<td>Eggplant</td>
<td>-</td>
<td>Leaf and roots</td>
<td>Chen et al., 2007a</td>
</tr>
<tr>
<td>StPHT1;2</td>
<td>Potato</td>
<td>Low affinity</td>
<td>Roots</td>
<td>Leggewie et al., 1997</td>
</tr>
<tr>
<td>ZmPHT1;1, ZmPHT1;2, ZmPHT1;3, ZmPHT1;6</td>
<td>Maize</td>
<td>-</td>
<td>Root and leaf: ZmPHT1;1 ZmPHT1;2; All parts: ZmPHT1;3; Old leaves: ZmPHT1;6</td>
<td>Nagy et al., 2006</td>
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</tbody>
</table>
Table 2. PHT1 genes induced by AMF in various plants

The PHT1s reported to be induced by inoculation with AMF have been listed with the name of the plant and the name of the AMF species used with references. The expression patterns of these transporters have been analysed by RT-PCR, qRT-PCR after inoculating the roots with specific AMF.

<table>
<thead>
<tr>
<th>Name of the PHT1 gene</th>
<th>Plant species</th>
<th>AMF species used</th>
<th>Reference</th>
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<tbody>
<tr>
<td>AsPHT1;1</td>
<td>Chinese Milkvetch</td>
<td>Gigaspora margarita and Glomus intraradices</td>
<td>Xie et al., 2013</td>
</tr>
<tr>
<td>AsPHT1;3</td>
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<tr>
<td>AsPHT1;4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BdPHT1;3</td>
<td>Purplefalsebrome</td>
<td>Glomus candidum</td>
<td>Hong et al., 2012</td>
</tr>
<tr>
<td>BdPHT1;7</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>BdPHT1;12</td>
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<td>CjPHT1;3</td>
<td>Red pepper</td>
<td>Glomus intraradices</td>
<td>Chen et al., 2007a</td>
</tr>
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<td>CjPHT1;4</td>
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<tr>
<td>HvPHT1;8</td>
<td>Barley</td>
<td>Glomus intraradices, Glomus sp, WFVAM23 and Scutellospora calospora</td>
<td>Glassop et al., 2005; Sisaphaithong et al., 2012</td>
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<td>HvPHT1;11</td>
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<td>LjPHT1;3</td>
<td>Miyakogusa</td>
<td>Glomus mosseae, Glomus intraradices</td>
<td>Maeda et al., 2006</td>
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<td>LjPHT1;4</td>
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<td>MtPHT1;1</td>
<td>Barrel Clover</td>
<td>Glomus versiforme</td>
<td>Harrison et al., 2002; Javot et al., 2007</td>
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<td>PhPHT1;3</td>
<td>Petunia</td>
<td>Glomus intraradices</td>
<td>Wegmuller et al., 2008</td>
</tr>
<tr>
<td>PhPHT1;4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhPHT1;5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PtaPHT1;4</td>
<td>Hardy orange</td>
<td>Glomus etunicatum, Glomus diaphanum and Glomus versiforme</td>
<td>Shu et al., 2012</td>
</tr>
<tr>
<td>PtaPHT1;9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PtaPHT1;10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PtaPHT1;12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiPHT1;8</td>
<td>Foxtail millet</td>
<td>Glomus mosseae</td>
<td>Ceasar et al., 2014</td>
</tr>
<tr>
<td>SiPHT1;9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiPHT1;3</td>
<td>Tomato</td>
<td>Glomus margarita, Glomus caledonium and Glomus intraradices</td>
<td>Nagy et al., 2005</td>
</tr>
<tr>
<td>SiPHT1;4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiPHT1;5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name of the PHT1 gene</td>
<td>Plant species</td>
<td>AMF species used</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>-----------</td>
</tr>
<tr>
<td><em>SmePHT1;3</em>&lt;br&gt;<em>SmePHT1;4</em>&lt;br&gt;<em>SmePHT1;5</em></td>
<td>Eggplant</td>
<td><em>Glomus intraradices</em></td>
<td>Chen et al., 2007a</td>
</tr>
<tr>
<td><em>StPHT1;3</em>&lt;br&gt;<em>StPHT1;4</em>&lt;br&gt;<em>StPHT1;5</em></td>
<td>Potato</td>
<td><em>Glomus intraradices</em></td>
<td>Rausch et al., 2001; Nagy et al., 2005</td>
</tr>
<tr>
<td><em>TaPHT1;8</em>&lt;br&gt;<em>TaPHT1;10</em>&lt;br&gt;<em>TaPHT1;11</em>&lt;br&gt;<em>TaPHT1;12</em></td>
<td>Wheat</td>
<td><em>Glomus sp.WFVAM23, Scutellospora calospora and Glomus intraradices</em></td>
<td>Glassop et al., 2005; Sisaphaithong et al., 2012</td>
</tr>
<tr>
<td><em>ZmPHT1;6</em></td>
<td>Maize</td>
<td><em>Glomus intraradices</em></td>
<td>Nagy et al., 2006</td>
</tr>
</tbody>
</table>
Table 3. SPX domain-containing proteins in *Arabidopsis* and rice for which location or functional information is known

Profile of SPX domain-containing proteins in *Arabidopsis* and rice (Modified from Secco *et al.*, 2012b)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function/Regulation profile</th>
<th>Subcellular localization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtPHO1</td>
<td>Pi transfer from root to shoot; Pi loading into the xylem vessel</td>
<td>Largely at Golgi/trans-Golgi network and uncharacterized vesicles; A minor fraction at plasma membrane</td>
<td>Stefanovic <em>et al.</em>, 2011; Rouached <em>et al.</em>, 2011a; Liu <em>et al.</em>, 2012</td>
</tr>
<tr>
<td></td>
<td>Possible transcriptional signal transporting from root to shoot.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controlled by PHO2 mediated endomembrane degradation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtPHO1;H1</td>
<td>Pi transfer from root to shoot</td>
<td></td>
<td>Stefanovic <em>et al.</em>, 2007</td>
</tr>
<tr>
<td></td>
<td>Regulated by PHR1 and influenced by phosphite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtPHO1;H4</td>
<td>Control hypocotyl elongation under blue light</td>
<td>Nucleus</td>
<td>Zhou &amp; Ni, 2010</td>
</tr>
<tr>
<td>(AtSHB1)</td>
<td>Form a large protein complex through SPX and EXS domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regulate endosperm development relevant genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtPHO1;H10</td>
<td>Involved in abiotic/biotic stresses response (including wounding, dehydration, cold, salt and pathogen attack)</td>
<td></td>
<td>Ribot <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>OsPHO1:2</td>
<td>Pi transfer from root to shoot</td>
<td></td>
<td>Secco <em>et al.</em>, 2010</td>
</tr>
<tr>
<td></td>
<td>Gene expression regulated by its cis-natural antisense transcripts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtSPX1</td>
<td>Positive regulator of plant adaptation to Pi starvation</td>
<td>Nucleus</td>
<td>Duan <em>et al.</em>, 2008; Puga <em>et al.</em>, 2014</td>
</tr>
<tr>
<td></td>
<td>Interacts with PHR1 in a Pi dependent manner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtSPX2</td>
<td>Interacts with PHR1 in a Pi dependent manner</td>
<td>Nucleus</td>
<td>Duan <em>et al.</em>, 2008; Puga <em>et al.</em>, 2014</td>
</tr>
<tr>
<td><strong>AtSPX3</strong></td>
<td><strong>Negative regulator of some PSI genes</strong></td>
<td><strong>Cytoplasm speckles</strong></td>
<td><strong>Duan et al., 2008</strong></td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>AtSPX4</strong></td>
<td>-</td>
<td>Plasma membrane</td>
<td><strong>Duan et al., 2008</strong></td>
</tr>
<tr>
<td><strong>OsSPX1</strong></td>
<td>Positive regulator of plant adaptation to Pi starvation</td>
<td>Nucleus</td>
<td><strong>Wang et al., 2009a</strong>; <strong>Wang et al., 2014</strong></td>
</tr>
<tr>
<td></td>
<td>Interacting with PHR2 in a Pi dependent manner</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OsSPX2</strong></td>
<td>Interacting with PHR2 in a Pi dependent manner</td>
<td>Nucleus</td>
<td><strong>Wang et al., 2009b</strong>; <strong>Wang et al., 2014</strong></td>
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</tr>
<tr>
<td><strong>OsSPX3</strong></td>
<td>Negative regulator of some PSI genes</td>
<td>Cytoplasm speckles</td>
<td><strong>Wang et al., 2009b</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>OsSPX4</strong></td>
<td>Interacting with PHR2 mainly in cytoplasm and preventing its translocation into the nucleus</td>
<td>Nucleus/Cytoplasm</td>
<td><strong>Wang et al., 2009b</strong>; <strong>Lv et al., 2014</strong></td>
</tr>
<tr>
<td></td>
<td>Controlled by Pi dependent 26S Proteasome Pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AtSPX-MFS3</strong></td>
<td>-</td>
<td>Tonoplast</td>
<td><strong>Secco et al., 2012b</strong></td>
</tr>
<tr>
<td><strong>OsSPX-MFS1</strong></td>
<td>Pi transport and relocation in leaves</td>
<td>-</td>
<td><strong>Lin et al., 2010</strong></td>
</tr>
<tr>
<td></td>
<td>Gene expression controlled by miR827</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OsSPX-MFS2</strong></td>
<td>Gene expression controlled by miR827</td>
<td>-</td>
<td><strong>Lin et al., 2010</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AtNLA</strong> (AtBAH1)</td>
<td>Involved in the nitrogen starvation response</td>
<td>Endomembrane system</td>
<td><strong>Peng et al., 2007</strong>; <strong>Kant et al., 2011</strong></td>
</tr>
<tr>
<td></td>
<td>Regulating Pi homeostasis by ubiquination of PHT1 family members Gene expression regulated by a miR827 in a Pi dependent manner</td>
<td></td>
<td><strong>Lin et al., 2013</strong></td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Schematic representation of plant responses to low Pi stress.  
Plants respond in multiple ways to low Pi. Some of these responses are local whilst others are systemic. Some respond to external Pi concentration whilst others respond to internal Pi levels. OA, organic acids; AMF, Arbuscular Mycorrhizal Fungi; RSA, Root System Architecture.

Figure 2. Regulation and control of key genes in the model plant *Arabidopsis* during phosphate (Pi) starvation responses. Blue arrowheads and red blunt-ends show positive and negative regulation, respectively. In the presence of lowered environmental Pi concentrations, Root system remodeling is introduced, followed by the up-regulation of high affinity Pi transport systems (PHT1s) to increase Pi uptake from the soil, while specialized transporters (AtPHO1, AtPHO1;H1) are induced for the movement of Pi within the plant. A rigorous regulation system consisting of key transcriptional factor PHR1, post transcriptional regulation by non-coding RNAs and post translational regulation by protein trafficking and degradation is also involved for the functional integration of such transporters in response to Pi starvation.

Figure 3. Regulation of phosphate transporters by post translational mechanisms.  
A. In epidermal cortex and root hair cells PHT1 transporters under transcriptional control of PHR1 are translated in the cytosol and targeted to the endoplasmic reticulum (ER) where they pass through the endomembrane system before localisation at the plasma membrane. Export from the ER is enhanced by PHF1. PHT1 is present in sorting endosomes but localisation to the plasma membrane is enhanced under low Pi conditions. Under high Pi conditions the level of PHT1 at the plasma membrane is down regulated by multiple mechanisms. NLA dependent ubiquitination at the plasma membrane results in vacuolar targeting and degradation. Export from the ER is reduced by PHT1 phosphorylation and PHO2 targeting of PHF1.  
B. In root cortical cells PHO2 also targets PHO1 for ubiquitination and degradation in high Pi conditions. NLA nitrogen limitation adaption; PHT1, phosphate transporter1;
PHO1, phosphate1; PHO2, phosphate2; PHF1 phosphate transporter traffic facilitator1; PHR1 phosphate starvation response1; WRKY6 a transcription factor.

Figure 4. Factors affecting Pi availability in soil
Bioavailability of Pi in the soil is affected by physicochemical and biological factors such as soil pH, soil type and concentrations of cations such as various metals that can complex phosphate as well as microbial activity. Plants counteract these limitations through different strategies that may include exudation of phosphatases to liberate phosphate from organic molecules, organic anions to chelate metal cations and increase phosphate solubility and increasing the volume of soil that can be explored through modifications to the architecture of the root system and interaction with arbuscular mychorrizal fungi.

Figure 5. Organic acid (OA) exudation is an important mechanism to improve Pi availability on acid soil.

A) Acid soil sensitive plants are compromised on acid soils by toxic Al$^{3+}$ restricting root growth and low availability of Pi in the soil lowering yields. B) When acid soil tolerant varieties are grown (whether transgenic or not) transcription factors, such as STOP1 in Arabidopsis, upregulate genes involved in protection from Al$^{3+}$ toxicity. Mechanisms differ between different plant species but responses include release of OAs such as malate, citrate or oxalate by ALMT or MATE genes, depending upon the plant species, which leads to lower free Al$^{3+}$ and higher free Pi in the soil and thus higher yields. The upregulation of OA secretion can be by transcriptional or post transcriptional mechanisms. C) A structure to show malate chelating aluminium, sequestering it to reduce its toxicity.

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Low Pi stress

- AMF
- RSA changes
- OA exudation
- Induction of high affinity Pi transporters
- Export of Pi from the vacuole
- Membrane remodelling
- Redistribution of Pi from source to sink
Soil Chemistry (pH, Cations & Microbial activity)

OA exudates
Phosphatases
Root System Architecture
Mycorrhizae

pi availability