Review Article

Progress in Biological Sciences

Vol. 4, Number 1, Winter / Spring 2014/1-32

Phosphate: the silent challenge

Received: December 4, 2013; Accepted: March 20, 2014

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ABSTRACT

Phosphorus (P) is one of the most vital elements for all living organisms which acts as a constituent of essential biomolecules such as nucleic acids, phospholipids, and phosphosugars, and as a major contributor to almost all metabolic reactions including photosynthesis, respiration, and energy delivery. It is one of the most needed nutrients for plant growth and development. Despite high levels of P in the soil, plants absorb it only in the soluble inorganic form of free phosphate ion (Pi) which is scarce in soil. Therefore, there has been a large demand for Pi fertilizers to secure crop yields, yet its deposition in soil and gradual run-off into water reservoirs lead to chains of events that cause irreversible damages to ecosystems. Researches, including genome-wide data analyses, have revealed interesting molecular aspects of plant adaptive strategies to deal with low Pi concentrations in soil. These include the higher expression of acid phosphatases and Pi transporters as well as the secretion of organic acids in the rhizosphere that maintain cellular Pi homeostasis in order to keep metabolic reactions running. Describing the cycle of Pi exchange between physical and biological worlds, the extent to which current agricultural practices are disturbing the cycle, the necessity of introducing less-destructive methods of providing Pi, and alternative measures and solutions for sustainable agriculture will be discussed in this review.

Keywords: biofertilizer, fertilizer, phosphatase, phosphate, phosphate rock, phosphate-solubilizing microorganism.

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Introduction

The use of chemical fertilizers has been an integrated part of the Green Revolution in intensive agricultural practices to meet the increasing demands for food. In reality, disruptions in the natural cycles of elements have caused environmental challenges some leading to irreversible damages to ecosystems, and the mining resources for these elements on the earth are being depleted. Phosphorus (P) is one of 17 essential elements required for plant growth. In many ecosystems and arable lands, inadequate bioavailability of P is often the second most limiting factor for biomass production (1). In fact, since the optimal growth of all plants is tightly dependent on P, an adequate supply of the element is crucial for food production. In practice, mined P rock converted to phosphoric acid is primarily used for agricultural fertilizers (~80%). The remainder is used in animal feed (~5%), beverages, meat treatment, toothpastes, or stabilizers (~15%) as well as in industrial applications such as detergents, electrical sensors, and semi-conductors, flame retardants, and as dispersing agents in paints and metal surface treatments (2, 3). Providing P remains one of the main challenges in the world of agriculture. The importance of P for plant nutrition, challenges related to its use, and solutions for this global issue are reviewed here from several points of view. This review is an update to previously published reviews (4) with some further details.

Importance of Pi in plants

P, in the form of phosphate ion (Pi), is the most vital element for all living organisms in the structures of essential biomolecules such as nucleic acids, sugar phosphates, phospholipids, and phosphoproteins and in energy-rich compounds like adenosine triphosphate (ATP), adenosine diphosphate, and NADPH (5, 6). It plays crucial roles in almost all metabolic reactions including photosynthesis and respiration, energy generation, nucleic acid synthesis, membrane synthesis, enzyme activation/inactivation, and redox reactions (6). Additionally, Pi is an important signaling molecule capable of modulating multiple cellular functions in signal transduction pathways and gene expression in cells (7).

Total P level in plants ranges from 0.5 to 5 mg P g^{-1} dry weight (DW) (8). It exists either as free inorganic Pi or in esterified forms. The main pools for esterified Pi are nucleic acids, phospholipids, phosphorylated water-soluble low molecular mass metabolites, and phosphorylated proteins. The nucleic acid pool (mostly RNA) is typically the largest (40–60%) organic Pi pool in plant cells, generally ranging between 0.3 to 2 mg P g^{-1} DW (9).

The metabolically active Pi pool is of the order of 0.1–0.8 mg P g^{-1} DW, depending on the species, tissue, and P supply (9). In live tissue, Pi homeostasis must be kept within a fairly narrow range of 5 to 20 mM in the cytoplasm to ensure appropriate functioning of metabolic reactions. If there is excess cellular Pi, it is stored in vacuoles and used to buffer the cytoplasmic Pi homeostasis (9).

Plants absorb P only in its soluble inorganic forms of Pi, $H_2PO_4^-$, or HPO_4^{2-}, which occur in the soil at concentrations between 0.1 and 1 μM (10-12). Therefore, plants as sessile organisms have evolved adaptive traits to acquire Pi from soil and to ensure maintenance of homoeostatic levels of the element. The uptake of soluble soil Pi needs to occur against a relatively high concentration gradient as the soluble Pi

concentration in the rhizosphere can be up to 10,000 times lower than that of root cells (5, 13). Pi/H^+ symporters are energized by ATP-dependent proton efflux that actively assimilate Pi against a steep concentration gradient. PHT1, PHT2, and PHT3 transporters play important roles in intracellular Pi translocation, or transport between plant organs (14). The high-affinity Pi transporters of Arabidopsis belong to the PHT1 family of transporters, which has nine members. Each transporter has 12 membrane-spanning domains. While all nine members are responsive to Pi starvation, each appears to exhibit a certain degree of tissue-specific expression, i.e. some are expressed in epidermal and root hair cells or expressed in root stellar cells (15, 16).

Plant adaptation to Pi deficiency

It is known that plants have adopted a wide range of morphological and physiological mechanisms (17-19) to cope with P deficiency in soil. Modifications of root growth and architecture are the most-documented plant responses to Pi starvation. Plants adjust their root system architecture (RSA) to maximize surface area in order to enhance foraging of topsoil where Pi tends to build up (1). Substantial differences exist in the extent of topsoil foraging within and among species (20). Architectural traits associated with enhanced topsoil foraging include the shallower growth angles of axial roots, enhanced adventitious rooting, a greater number of axial roots, and greater dispersion of lateral roots (20).

RSA in dicots is characterized by the inhibition of primary root growth accompanied by the enhancement of lateral root and root hair formation to increase topsoil foraging (Figure 1) (21). Additionally, under conditions of low P availability, root hairs become longer and denser. Root hair elongation is regulated by Pi availability in a dose-dependent manner. P deprivation can also cause an increase of up to five-fold in root-hair density. These responses occur via an increase in the number of epidermal cells that differentiate into trichoblasts (22). Interestingly, root hairs disappeared entirely in tomato and Arabidopsis under severe Pi starvation, (23; Lohrasebi *et al*., unpublished data).

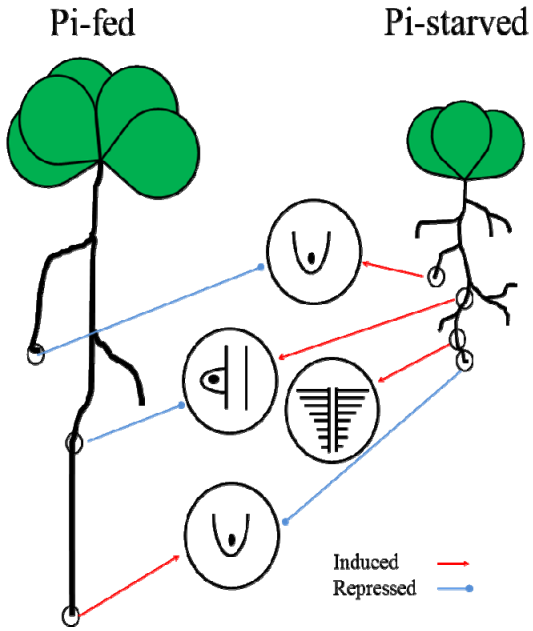

Figure 1. Alterations in root architecture and root hair density in response to available Pi. See text for details.

Symbiotic associations with fungi (arbuscular mycorrhizae or ectomycorrhizae) are widespread and efficient responses to increased Pi uptake (24, 25). In plants adapted to grow in extremely Pi-poor soils, such as Proteaceae and white lupin, characteristic cluster roots (proteoid roots) are formed (26) as an alternative strategy to arbuscular mycorrhiza formation (27). These clusters of rootlets are induced by rhizosphere bacteria and are specialized for the synthesis and excretion of organic acids (e.g., citrate)

that help mobilize Pi from Ca, Fe, and Al salts (6, 28). Exudation of organic acids from roots is not limited to proteoid roots, and is in fact a more general strategy to solubilize Pi by chelating Ca, Fe, and Al cations (17, 29). Moreover, the secretion of phosphatases or phytases by roots increases mobilization of Pi bound to organic components (e.g., phytic acid) which are considered to be the greatest Pi pool in soils (13, 30).

Transcriptomics approaches and mutation analyses have revealed some relevant genes that are repressed or induced during Pi starvation. For example, the Pi root development (PRD) gene is rapidly repressed in roots under low Pi conditions (31-34). In this context, PRD repression mediates primary root growth arrest (35). Another example is altered Pi starvation response1(APSR1) expression, a protein involved in root meristem maintenance. Expression of the *APSR1* gene decreases in response to Pi starvation. The loss of function mutant *apsr1-1* showed a reduction in primary root length and root apical meristem size, short differentiated epidermal cells, and long root hairs (36).

Many of the responses of root tissues to Pi deficiency are co-ordinated by local and systemic signals involving gibberellins, auxin, cytokinins, ethylene, and strigalactones (SL) as well as the translocation of regulatory miRNAs and excess sucrose transport from shoot to root in the phloem (37). For instance, SLs are plant hormones that regulate root hair elongation and lateral root formation in response to low Pi conditions. This regulation is mediated by more auxiliary growth-2 (MAX2) factors and correlated with transcriptional induction of the auxin receptor transport inhibitor response-1 (TIR1) (38).

Increased lateral root formations in Pi-deprived Arabidopsis seedlings was related, at least in part, to the response to auxin in root cells and to the expression of TIR1 auxin receptor (39). In response to low Pi, both ethylene biosynthesis and responsiveness are also enhanced. Ethylene regulates root hair growth and density by shortening trichoblast cells to increase the number of H cells - an adaptation that may involve auxin (40). Gibberellic acid (GA) response is mediated by transcription factors containing DELLA peptide motifs that act as negative regulators. Under Pi-starvation conditions, genes that activate GAs are repressed, and thus cause a reduction in bioactive GA levels (41). Interestingly, abscisic acid hormone played no role in the regulation of many Pi-responsive genes (42).

As mentioned, Pi plays a crucial role in almost all metabolic processes in plants. A remarkable alteration in plant metabolism is the bypass of Pi- or adenylate-dependent reactions. As a consequence of harsh Pi stress, reductions of up to 80% in intracellular levels of ATP, ADP, and related nucleotide occur (43, 44). Protein phosphorylation and glycosylation were found responsible for controlling the activity and/or subcellular targeting of the enzymes involved in bypassed metabolic reactions in response to Pi deprivation (13, 45, 46). The accumulation of starch in plant cells in response to Pi starvation is well known (47). The inhibition of a major regulatory enzyme of starch biosynthesis, ADPGlc pyrophosphorylase, has also been shown to occur by Pi provision (47, 48).

A well-known feature of plant responses to long-term Pi starvation is the development of dark-green or purple shoots due to the accumulation of anthocyanin caused by the Pi-induced expression of a set of enzymes that catalyze the synthesis of cyanidin, pelargonidin, flavonoids, and anthocyanin (6, 15, 42).

Another known response to Pi starvation is the replacement of phospholipids with sulfolipid and/or galatolipids. SQD1 and SQD2 are Pi starvation inducible enzymes required for sulfolipid biosynthesis in Arabidopsis (15, 16). This is supported by reduced growth rate of a *sqd2* T-DNA insertion mutant under Pi starvation conditions (49). Similarly, galactolipid accumulation was reduced in the roots of Pi-starved *pldz1* single and *pldz1/pldz2* double mutants (50). Also, a non-specific phospholipase C5 was found to be responsible for phospholipid degradation in leaves during Pi starvation (51).

The role of acid phosphatases

Plants increase the efficiency of Pi use during Pi starvation via the up-regulation of a wide array of hydrolases that scavenge and recycle Pi from intra- and extracellular in/organic Pi compounds (6, 15, 52, 53). The induction of secreted ribonucleases (RNases), nucleases, phosphodiesterases, and acid phosphatases (APases) is important for systematic metabolism Pi scavenging by roots from a broad range of exogenous organic P substrates, including RNA, DNA, ATP, 3-phosphoglycerate, and various hexose Ps such as Glc-6-P (54-56).

Plants genomes carry many APase-encoding genes grouped into distinct homology classes. For example, the Arabidopsis genome encodes 58 putative acid phosphatases including 29 purple acid phosphatases, 12 phospholipid phosphatases, 2 SurE acid phosphatases, 12 HAD acid phosphatases, and 3 histidine acid phosphatases (Fiezi and Malboobi, unpublished data). The genetic and functional redundancy of plant acid phosphatases make it difficult to investigate their roles and contributions to cellular Pi provision. There is a wide tissue- and/or cellular compartment-specificity for isozymes with variations in their physical and kinetic properties. Purple acid phosphatases (PAPs) comprise the largest class of plant APases. Arabidopsis, rice, and soybean genomes encompass 29, 26, and 35 PAP family members, respectively (57-59). They exhibit a characteristic purple or pink color in solution resulting from a charge transfer transition at 560 nm from the metal-coordinating Tyr to the metal ligand Fe (III).

Upon Pi deprivation, intracellular (vacuolar) and secreted APases that hydrolyze Pi from a broad and overlapping range of Pi monoesters with an acidic pH optimum are up-regulated.

Transcript profiling of the AtPAP family revealed that while most are expressed in all tissues (58, 60), some AtPAP transcripts are highly expressed in response to stress in specific cells or in certain compartments (61). For example, some have been localized to mitochondria and chloroplasts (AtPAP2; 62, 63), vacuole (AtPAP18 and AtPAP26; 64, 65), cell wall and plasma membrane (AtPAP9, AtPAP10, NtPAP12, AtPAP25 and PvPAP3; 66-69, 56), and secretome (AtPAP10, AtPAP12, AtPAP26 and AtPAP18; 67, 53, Zamani *et al.*, unpublished data).

It is noteworthy that almost all characterized PAPs contain transit peptides and are glycosylated, implying that they are mainly targeted to the golgi where glycosylation occurs. Although some PAPs, like AtPAP2 (63, 70), AtPAP5 (71), AtPAP9 (63, 66), AtPAP10 (67), AtPAP15 (30, 72), AtPAP18 (65), AtPAP23 (60), and AtPAP25 (69), have been functionally characterized using T-DNA insertional mutation and overexpressed lines, their cumulative physiological roles have not been resolved yet. All that is presently known is that there is a tight relationship in their expression levels such that knock-out of one gene results in

higher expression of some others (Lohrasebi and Malboobi, unpublished data).

Organic acid biosynthesis and secretion

Carboxylate exudation from roots participates in Pi provision to varying degrees, particularly in plants that do not form cluster roots. The profile of organic acids secreted is species- and cultivar-specific, with citrate and malate being the most abundant (26).

Several mechanisms have been described for their roles in Pi acquisition. Citrate might increase the availability of P in the soil by binding Ca, Fe, and Al and thus reducing the formation of insoluble complexes with Pi (73, 74), which explains the positive relation between citrate secretion and Pi concentration. Furthermore, citrate may bind to Fe, forming a citrate-Fe-P polymer that is soluble and can diffuse to the roots. The root reduces the Fe, thus breaking the polymer and releasing P directly at the root surface (75).

Genomic analysis of Pi adaptation mechanisms

In the last decade, several genome-wide studies have been conducted to figure out how plants handle low availability of Pi. These studies have shown that changes in gene expression profiles occur as early as 72 hours after plants are subjected to Pi starvation (31, 32, 76). Furthermore, expression patterns in leaves and roots in responses to Pi starvation were found to be distinct for both Arabidopsis and rice seedlings (31, 77).

Wu *et al*. (31) reported the expressions of 1800 of 6172 examined genes, including over 100 that encoded transcription factors and cell signaling proteins, were altered over two-fold in response to Pi starvation. Using a whole genome Arabidopsis Affymetrix chip representing 22,810 genes, Misson *et al*. (32) found that the expression of 612 genes is induced under Pi-limiting conditions. The expressed genes include those that encode Pi transporters, RNases, and APases, and genes that play roles in sulfate and iron transport and homeostasis, Pi salvaging from organic compounds, phospholipids degradation and galacto- and sulfolipid synthesis, anthocyanin synthesis, phytohormone responses, signal transduction, transcriptional regulation, protein degradation, and cell wall metabolism. The repressed genes (245) are involved in lipid synthesis, reactive oxygen controlling, and protein synthesis. Two years later, Morcuende *et al*. (33) reported alterations in the expression levels of over 1000 Arabidopsis genes involved in Pi uptake, the mobilization of organic Pi, the conversion of phosphorylated glycolytic intermediates to carbohydrates and organic acids, the replacement of Pi-containing phospholipids with galactolipids, and the repression of genes responsible for nucleotide/nucleic acid synthesis. The responses were reversed within 3 h after Pi re-supply. Müller *et al*. (78) investigated the effects of the interaction between Pi and sucrose signals on the gene expression pattern in Arabidopsis. They found several genes that had been previously identified to be responsive to sugar or Pi. In the time course microarray experiments conducted by Lin *et al*. (79), Pi-responsive genes were clustered based on co-expression patterns by pairwise comparison of genes against a customized data base. Three major clusters enriched with genes functioning in transcriptional regulation, root hair formation, and developmental adaptations were distinguished in this analysis.

Genome-wide profiling by micro- and tiling arrays revealed a remarkable difference in the molecular responses of roots and of

shoots both during and after Pi starvation and a minimal overlap between root and shoot transcriptomes suggesting two independent Pi-starvation regulons in both a time- and organ-dependent manner (80). Novel expression patterns were detected for over 1000 candidate genes, and the genes were classified as either initial, persistent, or latent responders. Comparative analysis to AtGenExpress identified a number of genes co-regulated across multiple stimuli. Analysis of co-regulation enabled the determination of specific versus generic members of closely related gene families with respect to Pi-starvation.

Recently, the response of rice to low Pi was examined using oligonucleotide microarrays, and the results showed that about 1494 (35%) of the examined genes were affected by Pi deficiency (81). To explore the role of Pi in rice, 25 pathways were selected based on the number of affected genes. The largest category of genes was related to sucrose degradation to ethanol and lactate pathways. Cytosolic glycolysis contained the most down-regulated genes while having the fewest up-regulated genes. This is consistent with decreased glucose, pyruvate and chlorophyll and chlorophyllide *a* biosynthesis and increased sucrose and starch levels. The dynamics of the rice transcriptome were analyzed under conditions of Pi starvation by Affymetrix GeneChip rice genome arrays as well (82). Pi starvation altered the expression of 2,317 genes, representing 7.2% of the expressed genes. These changes were mostly transient and affected various cellular metabolic pathways including stress responses, primary and secondary metabolism, molecular transport, regulatory processes, and developmental processes. Only 130 (5.6% of 2,317) transcripts were expressed similarly both in roots and shoots under Pi starvation.

Oono *et al.* (83) analyzed the inducible transcripts associated with Pi starvation and over-supply of Pi to characterize the transcriptome in rice seedlings using the mRNA-Seq method. They detected 10,388 transcripts with no match to any Rice Annotation Project transcript. The transcripts that showed specific responses to Pi stress include some with open reading frames (83). Comparative analysis of the RNA-Seq profiles of four rice cultivars revealed similarities as well as distinct differences in expression of these responsive transcripts (83). Many annotated, unannotated, and unaligned responsive transcripts were identified which are responsible for scavenging, mobilizing, acquiring, and utilizing Pi under stress conditions. Differences in the expression of these transcripts provided an overall view on how genotypes with different levels of tolerance to Pi stress respond to its availability.

O'Rourke *et al*. (84) utilized RNA-Seq technology to assess global gene expression in white lupin cluster roots, normal roots, and leaves in response to Pi supply and identified a total of 2,128 differentially expressed sequences with a change of at least two-fold in response to Pi deficiency. In Pi-deficient leaves, 987 transcripts were down-regulated while 355 were up-regulated. Also, these researchers found 396 and 535 genes were up-regulated in Pi-sufficient and Pi-deficient roots, respectively.

Comparative analysis between rice and Arabidopsis identified 37 orthologous groups that responded to Pi starvation, demonstrating the existence of a conserved Pi responsiveness mechanism among dicot and monocot plants (82). Further analysis of transcription profiles

of microRNAs revealed differential expressions of miR399 and miR169 under Pi starvation suggesting their potential roles in plant nutrient homeostasis (82).

Recently, a comparison of wheat- and rice-responsive transcripts of orthologous genes under Pi-starvation conditions was performed (85). This pervasive analysis revealed commonly up-regulated transcripts, most of which appeared to be involved in a general response to Pi starvation, namely, a PHR1-IPS1-miR399-UBC24/PHO2 signaling cascade and its downstream functions such as Pi remobilisation, Pi uptake, and changes in Pi metabolism. These results will be useful in deciphering gene networks involved in low Pi stress and in identifying genes that could be exploited in breeding for Pi-efficient and high-yielding cultivars under Pi starvation conditions (85).

Similarly a set of 200 genes were identified that show differential expression patterns between fertilized and unfertilized potato plants and identified novel components to previously known Arabidopsis and rice gene expression profiles, e.g., patatin gene (86). Twelve sequences were consistently expressed differentially in three species, Arabidopsis, potato, and white lupin, making them ideal candidates to monitor the Pi status of plants.

A metabolomics analysis has demonstrated that Pi deprivation is accompanied by the accumulation of carbohydrates, organic acids, and amino acids in Arabidopsis (33). In an interesting study, Lan *et al.* (87) investigated the correlations between Pi deficiency-induced changes in transcriptome and proteome profiles in Arabidopsis roots. They arranged for an exhaustive tandem mass spectrometry-based shotgun proteomics and whole-genome RNA sequencing to generate a nearly complete catalog of expressed mRNAs and proteins. A set of 13,298 proteins and 24,591 transcripts were identified in which subsets of 356 proteins and 3106 mRNAs were differentially expressed during Pi deficiency. The most dramatic changes were noticed in genes involved in Pi acquisition and in processes that either release Pi or bypass Pi/ATP-dependent metabolic steps. The concordance between the abundance of mRNAs and their encoded proteins was generally high for highly up-regulated genes. However, the analysis also revealed numerous inconsistent changes in mRNA/protein pairs, indicating the involvement of both transcriptional and post-transcriptional regulations. This study endorses the need for integrated measurements and interpretations of transcript and protein abundance for generating biosystems simulating plant response to environmental stimuli.

Pi sensing and gene expression

The genome-wide transcriptional approach has been used to infer possible scenarios for signaling and adaptation of plants to Pi availability. Signaling and subsequent metabolic adjustments to Pi limitation are largely triggered by internal Pi status and involve microRNAs, non-coding RNAs, and PHO_2 downstream of PHR as revealed in recent studies. PHR1 is one of 15 members of the MYB-CC gene family in Arabidopsis. It is localized in the nucleus and binds to a palindromic 8-bp sequence (GNATATNC) called P1BS (PHR1-binding sequence) in the promoters of many Pi responsive genes (88-90). Despite that, the *phr1* mutant showed no major phenotypic defects except for a slight difference in the root-to-shoot ratio and in root hair induction (88). Considering PHR1 interaction with another MYB factor, PHL1,

Bustos *et al.* (90) showed about two-thirds of the genes repressed in Pi-deprived wild type seedlings were markedly de-repressed in *phr1phl1* double mutants.

A number of miRNAs have been shown to be specifically induced by Pi limitation, including miRNA399, miRNA778, miRNA827, and miRNA2111 in Arabidopsis (91-93), though only the role of miRNA399 in the regulation of Pi response has been elucidated (16, 94).

Furthermore, the Pi-signaling network involves IPS (Induced by Pi Starvation) genes that carry short open reading frames (95, 96). They may function as 'target mimicry' since they carry a conserved 23-bp region complementary to certain gene sequences (97). This must help fine-tuning of PHO2–miRNA399 pathway which is well conserved in numerous plants (98).

P cycles

P circulates through the environment in several subcycles (Figure 2).

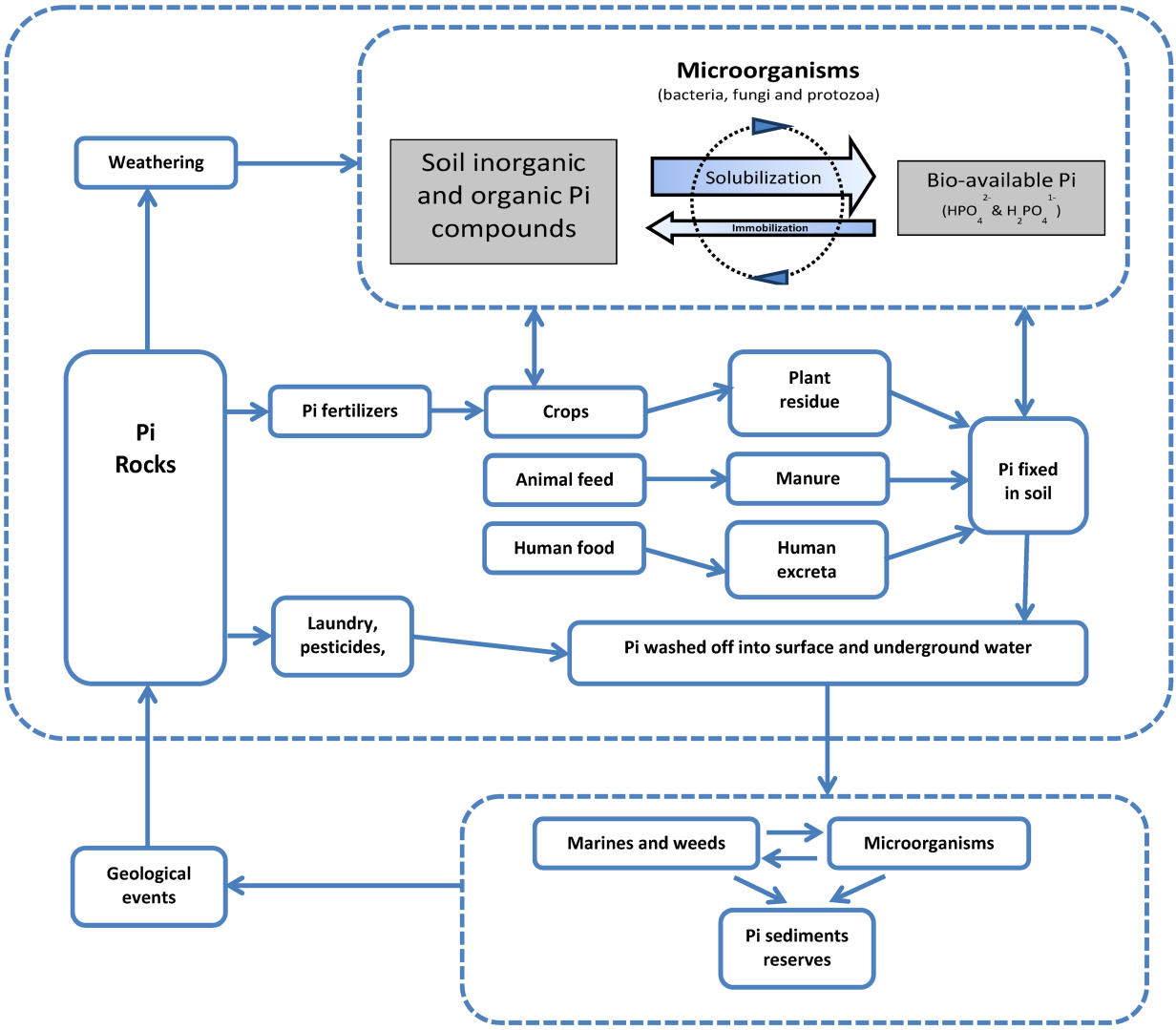

Figure 2. Pi flow through food production and consumption.

Pi is taken up by plants from soil, utilized by animals that consume plants, and returned to the soil as an organic residue. Most of the Pi used by living organisms becomes incorporated into organic compounds. When plant materials are returned to the soil, the organic Pi will slowly be released as inorganic Pi or incorporated into more stable organic matters (99). Depending on temperature, moisture, pH, and soil microorganism populations, Pi is released from these compounds and becomes prone to leaching, erosion, or run-off to surface or underground waters at an estimated rate of 18.7 to 31.4 MMT P/yr. It eventually gets discharged into lakes, seas, and oceans in the range of 12 to 21 MMT P/yr (100). Then, another sub-cycle between marine organisms and Pi sediments occurs.

Our perception is that Pi moves quickly through plants and animals; however, the processes that move Pi through the soil or ocean are very slow, making the Pi cycle overall one of the slowest biogeochemical cycles (101). Human activities, including the utilization of Pi in fertilizers, pesticides, detergents, and the food industry are the main sources of disturbance in the natural cycles (102). As a result, erosion, pollution, and fertilizer runoff, have become two to three times higher than what it was in pre-human times.

Agricultural aspects of Pi

Soil scientists recognize three kinds of Pi: (1) deposited Pi as chemical compounds in the soil; (2) available Pi or free Pi ion in the soil; and (3) absorbable Pi or free Pi ion in the vicinity of roots. As fertilizer granules are added to the soil, Pi is diffused into the soil and might expand as far as 3 to 5 cm, depending on the soil moisture (103). Depending on the type of soil and the composition of its cations, the released Pi can re-precipitate within weeks or months (103, 104). On the other side, there is a correlation between the removal of Pi and the amounts of harvested products. Roughly, the harvest of every ton of cereal grain is accompanied by the removal of 10 Kg P_2O_5 per hectare (105). Similar estimates are true for all other crops. To compensate for this, the lost Pi must be matched by corresponding inputs of fertilizer and manure.

Fate of Pi in soil

Total P amount in soils, in various inorganic and organic P forms, range from 35 to 5300 mg kg^{-1} with an average of 800 mg kg^{-1} (106). The ratio of inorganic to organic compounds is governed by dynamism in climatic, edaphic, agronomic, and biological factors. In most soils, 50 to 75% of inorganic P compounds are bonded with Al, Fe, or Ca in the form of complexes, depending on soil pH (107).

In most soils, organic Pi constitute 30% to 65% of total P, but the concentrations may be as low as 5% or as high as 95% (108). Organic Pi pools in soil generally occur, in order of abundance, as inositol phosphate > polymer organic phosphate > nucleic acid P > phospholipid P (109). Inositol hexaphosphate, or phytate, is mostly synthesized by plants and accounts for about 40% of the organic P found in soil.

A notable characteristic of P is its slow diffusion rate (10^{-12} to 10^{-15} m^2 s^{-1}); this, concomitant with high plant uptake rates, causes a depletion zone around the roots (110). Moreover, the concentration of available soil Pi seldom exceeds 10 µM (10, 108) that is much lower than it is in plant tissues, approximately 5 to 20 mM Pi (17). Because of the low concentration and poor mobility of plant-available P in soils, chemical P fertilizers are needed to improve crop growth and yield.

Pi production and consumption trends

Mined Pi rock is principally used for agricultural fertilizers (80%), the remainder being used for animal feed additions (5%) and industrial applications (15%) including detergents and metal treatments (111). According to FAO (112), the current consumption rate of about 20 MT P/year is growing mainly in developing countries (Figure 3), including China and India who are the largest consumers with 34% and 19% usage of total Pi fertilizers in the world, respectively (113). Between 2002 and 2009, the global use of Pi fertilizers increased by 12%, affecting market price increases and environmental pollution (see below). In contrast, Europe decreased its consumption rate to about 20% from 2002 to 2009 due to raising environmental concerns (Figure 3).

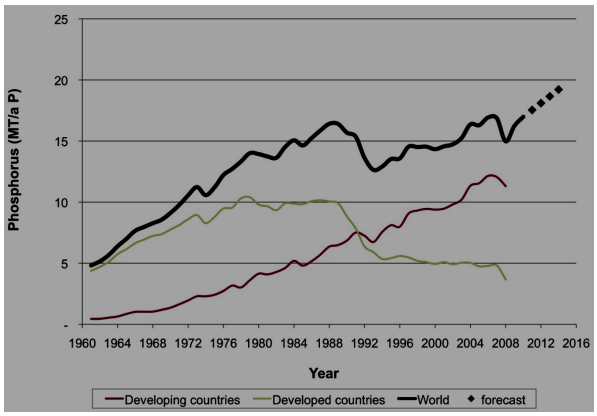

Figure 3. Global trend of P consumption in developed and developing counties (Quoted from 114).

It is estimated that high quality P rock reserves mines will be depleted in the next 40 to 60 years, while demands are progressively increasing for Pi due to the adoption of diets higher in meat content and the increasing use of bio-energy in the coming years (113). For example, in 2009, 32% of all corn grown in the US was used for ethanol production, representing 10% of Pi fertilizer used in the US that year (111).

Environmental impacts of Pi production and consumption

Both production and consumption of Pi are damaging to the environment. Removing huge amounts of Pi rock (PR) from mining sites, using huge amounts of water for extensive washing, and, importantly, the co-produced materials all cause great changes in ecosystems. For each ton of P_2O_5 extracted, five tons of phosphogypsum and Pi slags are produced, from which only 10% is used for the purposes of road construction, cement, and housing; the rest remains stacked (115).Moreover, PRs contain mainly uranium (20-300 ppm) and thorium (1 to 5 ppm) and their decay products. This means that 0.35 kg U_2O_8 per Mt P_2O_5, or 2100 tons, of unused uranium is accumulated annually (115).

The increase in demand for Pi fertilizer has resulted in the development of fast and high yield production methods that do not necessarily consider all aspects and consequences of their application. Such practices not only alter chemical and physical properties of soils, but also remarkably affect macro and micro flora (115).

The application of Pi fertilizers to soil does not necessarily guarantee plant uptake due to their polycrystalline structures and their tendency to precipitate. Only 15-30% of the applied Pi fertilizer is taken up by harvested crops in the year of its application (9). Thirty-three percent of the P added to soil is lost by erosion or water wash-off, and the rest accumulates in the soil. Losses due to livestock production and improper management of manure cause about 45% of P to enter the livestock system. According to Cordell *et al*. (116), more than 7 million tons of P is released into the environment annually through animal manure and human excreta, causing major water-quality problems. Taken

together, about 90% of the Pi that enters the food system is lost into the environment (111). Furthermore, the sodium triphosphate of discharged detergents accounts for a portion of the Pi inputs to surface and underground waters (115).

Eutrophication, or over-enrichment of aquatic ecosystems with nutrients, mostly Pi and nitrate, leads to algal blooms and anoxic events and may initiate irreversible environmental damages. The oxygen shortage after the decomposition of aquatic plants impairs the needed support for aquatic life leading to overall loss of biodiversity. Blooms of cyanobacteria are a particular problem in fresh water and are associated with sudden fish kills, changes in taste and odor, and the formation of carcinogens (e.g. trihalomethane) during the chlorination of potable water. Water-soluble neurotoxins arising from cyanobacteria can also kill livestock and harm humans (117). Although some lakes may recover after nutrient inputs are reduced, recycling Pi from sediments causes lakes to remain eutrophic for years. The estimated cost of damage mediated by eutrophication in the U.S. alone is approximately $2.2 billion annually (118).

Health impacts of Pi

The long-term application of Pi fertilizers on cropproduction fields has raised concerns about the potential health risks of heavy metals such as U, Cd, As, Cr, Cu, Pb, and Zn (119). A major contaminant is fertilizer-derived uranium into ground, surface, and marine coastal waters. In Germany, the use of Pi fertilizer from 1951 to 2011 resulted in the accumulation of approximately 14,000 tons of uranium in agricultural lands corresponding to an average cumulative loading of 1 kg of uranium per hectare. Uranium may damage biological systems through its chemical toxicity as well as its radioactivity. The main health effects of uranium are renal, developmental, reproductive, and bone growth impairments as well as DNA and brain damage (120).

Cadmium (Cd) buildup in topsoil is tightly correlated with the application of Pi fertilizer in intensive agriculture. With respect to human health impacts, food is the only major route of Cd accumulation to high concentrations, particularly in the kidney. Cd has also been shown to be an endocrine-disrupting chemical with estrogenic properties and a potential prostate carcinogen. High Cd pollution has been recognized as the cause of a painful bone disease known as 'itai-itai' (121).

Alternative solutions

Several approaches have been sought to deal with limiting Pi resources. These include direct use of Pi rock, recycling Pi, genetic variations through breeding, mutagenesis, transgenesis, and the use of microorganisms for Pi provision as described below.

1. *Recycling Pi*

So far, only a few techniques have been developed for recycling Pi from sewage systems and agricultural and industrial treatment units in developed countries such as Sweden, Japan, and Germany (115, 122). Crystallization of Pi in wastewater as struvite (ammonium magnesium Pi), and the use of separated urine and sanitized faecal matters in municipalities are among the costly methods that can be used to recover or reuse wasted Pi (116).

2. *The use of plant species with high Pi efficiency*

Pi-efficient plants are defined as plants that can produce higher yields per unit of applied

or absorbed nutrient compared to other plants grown under similar agroecological conditions (123). Screening for plant species or genotypes with increased Pi absorption capabilities involves criteria such as:

i) Greater root architectures leading to extended soil exploration. An example is a soybean variety named 'BX10' which has superior root traits that support better adaptation to low-P soils (124).

ii) The exudation of proton and organic acids that increases the solubility of Pi by decreasing pH and/or chelating elements. An example is a wheat variety, Xiaoyan 54, that secretes more carboxylates (e.g. malate and citrate) into the rhizosphere than Pi-inefficient genotypes (125).

iii) The secretions of elevated levels of APase enzymes that break down Pi compounds robustly (126). There are many examples of over-expressed secreted phosphatases (Table 1).

A range of quantitative trait loci (QTL) has been identified for tolerance mechanisms to low Pi in various food crops (127). A major QTL pup1, on rice chromosome 12 was found to be associated with tolerance in low Pi soils. This locus includes one known and 30 putative genes (128).

3. *Mutagenesis*

Mutations altering root architecture have been of interest in the selection of plants capable of acquiring more Pi from soil. For example, barley genotypes with long root hairs have higher yields than genotypes with no root hairs on soils with low P availability. Also, genotypes of bean, maize, and brassicas with larger root systems have better growth rates in Pi-limiting conditions (129).

Considering that about 75 percent of total seed P exists as phytic acid, mutations that block the synthesis or accumulation of phytic acid during seed development, often referred to as low phytic acid (*lpa*) mutations, has been of interest. Such mutations have been introduced into maize, barley, rice, wheat, and soybean (130). Genetic factors that decrease or increase total seed P are valuable for enhancing food and feed quality, for the environmental management of Pi, and for optimizing the utilization of Pi in agriculture (131).

4. *Transgenesis*

Many attempts to develop Pi-efficient plants have been made through the transfer of genes with bacterial, fungal or plant origins as a strategy toward sustainable agriculture (132). As classified in Table 1, the expression of transcription factors may upregulate the expression of a cluster of Pi responsive genes. Overexpression of plant Pi transporters in homogenous or heterogeneous genetic background altered Pi uptake levels or translocation rates, and these changes were accompanied by improved biomass. Many studies have reported transgenic plants that overexpress phytase in various food crops. Phytases are secreted to the rhizosphere, where the hydrolysis of phytate and release of Pi is very beneficial (133). Overexpression of phytase in potato, clover, soybean, and tobacco resulted in increased Pi acquisition and content. Similarly, the manipulation of biochemical pathways to increase the biosynthesis and exudations of organic acids improved Pi acquisition from soils as well. Engineered tobacco plants produced higher levels of organic acids, specifically citrate (134). The promising result was that these transgenic plants yielded more leaf and fruit biomass than controls when grown under Pi-limiting conditions (134, 135).

5. Direct application of PR

The direct application of ground PR has been practiced since the early 19th century as a relatively inexpensive source of Pi. However, this method is limited to specific soils with acidic pH (below 5.5) and low exchangeable Ca^{2+} or to some plants with higher capabilities in mobilizing Pi such as legumes and some crops of the *Cruciferae* family which excrete organic acids (136).

Table 1. An inventory of reported transgenic plants intended for the enhancement of Pi metabolism.

Gene name	Gene origin	Transformed plant	Main effect	Reference
Transcription factor				
AtPHR1	Arabidopsis	Torenia	Increased Pi content	(137)
ZmPHR1	Maize	Arabidopsis	Increased Pi content and biomass	(138)
ZmPTF1	Maize	Maize	Increased biomass	(139)
OsPTF1	Rice	Rice	Increased tillering ability, Pi content and biomass	(140)
OsPHR2	Rice	Rice	Increased Pi content in shoots	(98)
Ta-PHR1	Wheat	Wheat	Improved Pi use efficiency and yield performance	(141)
AtMYB2	Arabidopsis	Arabidopsis	Increased miR399f expression and tissue Pi contents	(142)
Transporter				
OsPht1;1	Rice	Rice	Modulates Pi uptake and translocation	(143)
Pht1;5	Arabidopsis	Arabidopsis	Pi translocation from root to shoot	(144)
GmPT5	Soybean	Soybean	Increased biomass	(145)
TaPht1;4	Wheat	Wheat	Increased Pi content and biomass	(146)
LePT1	Tomato	Tobacco	Increased Pi content and biomass	(147)
GmPT1	Soybean	Tobacco	Increased Pi content and biomass	(148)
NtPT1	Tobacco	Rice	Increased Pi uptake	(149)

Gene name	Gene origin	Transformed plant	Main effect	Reference
Phosphatase & phytase				
Phytase	Synthetic	Potato	Increased Pi content and biomass	(150)
Phytase (ex:phyA)	*Aspergillus niger*	Tobacco	Increased Pi content	(151)
Phytase (phyA)	*Aspergillus niger*	Arabidopsis	Increased Pi content and growth	(152)
appA	*E. coli*	Potato	Increased Pi content and biomass	(153)
phyA & appA	*Aspergillus niger* & *E. coli*	*Brassica napus*	Increased Pi content and biomass	(154)
phyA2	*Aspergillus niger*	Maize	Increased Phytase activity in seeds	(155)
168phyA	*Bacillus subtilis*	Tobacco & Arabidopsis	Increased Pi content and biomass	(156)
(AVP1) Type I H+-pyrophosphatase	Arabidopsis	Rice & tomato	More robust roots, higher shoot mass, higher yields	(157)
(TsVP) H+-pyrophosphatase	*Thellungiella halophila*	Maize	Increased Pi uptake and grain yield	(158)
PvPS2:1	*Phaseolus vulgaris*	Arabidopsis & Bean	Increased Pi acquisition	(159)
Phytase (phyA)	*Aspergillus ficuum*	Cotton	Increased Pi content and biomass	(160)
OsPHY1	Rice	Tobacco	Increased Pi content and biomass	(161)
OsPAP2	Rice	Arabidopsis	Increased phosphatase activity	(162)
OsPAP10a	Rice	-	Improved ATP hydrolysis and utilization	(163)
AtPAP2	Arabidopsis	*Camelina sativa*	Increased seed yield and seed size	(70)
AtPAP2	Arabidopsis	Arabidopsis	Increased seed yield	(63)
AtPAP10	Arabidopsis	Arabidopsis	Increased biomass	(67)
AtPAP15	Arabidopsis	Soybean	Increased Pi content and biomass	(30)
AtPAP18	Arabidopsis	Tobacco	Increased Pi content and biomass	(65)
AtPAP26	Arabidopsis	Arabidopsis & tobacco	Increased Pi uptake, Pi content and biomass	(Sabet *et al.*, unpublished)

Gene name	Gene origin	Transformed plant	Main effect	Reference
LASAP2	White lupin	Tobacco	Improved phosphorus uptake and growth	(164)
LASAP3	White lupin	Tobacco	Increased Pi content and biomass	(165)
MtPHY1 & MtPAP1	*Medicago truncatula*	*Trifolium repens*	Increased ability to utilize organic phosphorus	(166)
MtPHY1 & MtPAP1	*Medicago truncatula*	Alfalfa	Increased Pi content and biomass	(167)
MtPAP1	*Medicago truncatula*	Arabidopsis	Increased Pi content and biomass	(168)
Organic acid				
Citrate Synthase	*Daucus carota*	Arabidopsis	Increased Pi content and biomass	(169)
Citrate synthase	*Pseudornonas aeruginosa*	Tobacco	Increased biomass	(135)
Citrate synthase	*Pseudomonas aeruginosa*	*Brassica napus*	Improved Pi uptake Increased Pi content in shoots and seeds if $FePO_4$ was used as the sole Pi source	(170)
Malate dehydrogenase	*Penicillium oxalicum*	Tobacco	Increased Pi content and biomass	(171)
Others				
TaALMT1	Wheat	Barley	Increased Pi uptake and grain production (acid soils)	(172)
ath-miR399d	Arabidopsis	Tomato	Increased leaf Pi concentration	(173)

6. *The use of organic materials*

Because of problems associated with chemical fertilizers, the utilization of organic fertilizers as an alternative source of plant nutrients has been reconsidered. Organic matters may be used in the form of manure (un/processed), compost, humic acid, or biochar.

Major sources of manure are animal waste, human excreta, town refuse, sewage and sludge, slaughterhouse waste, byproducts of agro industries, crop waste, and green manure crop materials.

The quality of farmyard manure depends on the type of animal, the age of the animal, the kind of feed given to the animals, the kind of litter used, the age of the manure, and the method of storage. Manure is rich in nitrogen and potassium but poor in Pi; yet, the availability of soil Pi is enhanced by organic materials due to chelating polyvalent cations,

organic acids, and other decay products that help the survival and growth of beneficial microorganisms.

Similarly, compost or decomposed organic material is a combination of digested and undigested food; its constituents depend on type of material, air, moisture, temperature, and soil pH. Despite reducing the need for Pi, variability in quality, cost, and the unbalanced levels of elements in these organic matters have hindered their usage compared to chemical fertilizers. In recent decades, a number of studies have tested the availability of Pi in organic manure and the effect of its wash off into watersheds from soils where manure is used (174).

Humic acids containing large numbers of negatively charged carboxyl and hydroxyl groups are formed by the microbial degradation of dead plant matter, such as lignin, over a long period of time (175). The precise properties and structure of a given sample depends on the water or soil source and the specific conditions of extraction. Nevertheless, the average properties of humic substances from different sources are remarkably similar (176). Humic acids have recently been recommended as organic material that may facilitate the adsorption of Pi fertilizers.

Biochar, particularly that of animal bone origin, is another sustainable solution for the provision of Pi. Biochar, primarily aimed at carbon sequestration, is the product of pyrolysis, a thermochemical decomposition of organic materials performed at 400-700°C in the absence of oxygen for a few minutes. A process developed by researchers in University of Florida removes three-quarters of Pi from the biochar of sugar beet residues (177). The solubilization of animal bone char with high Pi content by micro-organisms that produce organic acids has been proposed as a multi-functional biofertilizer (178).

7. *The use of Pi solubilizing microorganism*

Soil microorganisms, including bacteria and fungi, are keys to Pi dynamics in soil and the subsequent availability of Pi to plants through a number of mechanisms. These include i) hydrolysis of in/organic Pi compounds; ii) increase in the surface area of roots by either an extension of existing root systems (e.g., mycorrhizal associations) or by enhancement of root branching and root hair development by inducing the production of phytohormones; and iii) stimulation of metabolic processes that are effective in altering the distribution of microorganism populations (Figure 2).

Among them, Pi solubilizing bacteria (PSB) play direct roles in hydrolyzing Pi from organic and inorganic compounds in soil. PSB strains from *Pseudomonas*, *Bacillus*, *Rhizobium* or *Pantoea* genera are the most powerful ones (179-181). The positive effect of *Pseudomonas putida* and *Pantoea agglomerans* inoculation on plant growth has been reported in many field trials (181-183).

Different kinds of organic acids, namely citric acid, gluconic acid, lactic acid, succinic acid, propionic acid, and three unknown organic acids have been distinguished in the cultures of isolate microorganisms (184, 185). Organic anions and associated protons secreted by microorganisms solubilize Pi in the surrounding soil by the simple hydrolysis of inorganic compounds (e.g., Fe- and Al-P in acid soils, Ca-P in alkaline soils), by chelating associated metal ions, or by ion exchange reactions (29,186).

A more promising approach to the use of PSB is the mineralization of soil organic Pi compounds by the production of phosphatases (179). Microorganisms may produce both acid and alkaline phosphatase

(187), while plants secrete APases only (188). The largest portion of extracellular soil phosphatases is derived from microbial populations (189). The degradability of organic Pi compounds depends mainly on the physicochemical and biochemical properties of the molecules. Nucleic acids, phospholipids, and sugar phosphates are easily broken down, but phytic acid, polyphosphates, and phosphonates are decomposed more slowly (190, 191). It is noteworthy that microorganisms, especially bacteria, have a relatively high P requirement (1.5–2.5% P by dry weight compared to 0.05–0.5% for plants). Therefore, in closed ecosystems with insignificant Pi inputs, soil organisms could be highly competitive with higher plants for Pi (Figure 2) unless they are sufficiently robust (109).

Rhizobacteria may indirectly influence the Pi acquisition by plants through the production of phytohormones such as auxin and gibberellic acid as well as vitamins that stimulate root growth (192). Therefore, some researchers prefer naming such bacteria plant growth promoting rhizobacteria (PGPR; 193).

Another valuable plant–microbe interaction, namely mycorrhizal symbiosis, is based on the mutualistic exchange of carbon from the plants in return for Pi and other nutrients from the fungi. Influx of Pi in roots colonized by mycorrhizal fungi can be 3–5 times higher than in non-mycorrhizal roots (194). The symbiotic relationship between Mycorrhiza and plants is one of the most abundant symbiotic activities in the plant kingdom and exists in most ecosystems (195). Unfortunately, the neglectful interference of human activities such as the over-application of fungicides and frequent applications of chemical Pi fertilizers has seriously threatened this advantageous symbiosis.

Concluding remarks

As the world is concerned about the limited resources of Pi and, at the same time, environmental pollution due to the excessive use of fertilizers and heavy metals impurities, collaborative efforts are needed to reduce the use of these fertilizers while meeting the progressive needs for food demands. Understanding the biology of the Pi metabolism cycle and its cycles between the biological and physical worlds would greatly help the efficient utilization of Pi. Alternative methods of extracting PR for Pi provision by hydrolyzing soil Pi compounds is also indispensable to reduce the need for Pi fertilizers and to lessen environmentally harmful input. Now it is time to develop more comprehensive formulations as integrated plant nutrition management systems which involve the use of certain ratios of chemical fertilizer, organic matters, and biofertilizers. However, there are a lot of uncertainties that require global data sharing and collaborative research programs. The Phosphate Knowledge Center web site (http://www.GreenPi.info) has been established to pave the way for both. It would also assist the management of huge amounts of compiled data as well as the introduction of a biomodel to describe the complexity of Pi roles in biological systems.

REFERENCES

1. Abel, S. (2011) Phosphate sensing in root development. *Current opinion in plant biology*, **14**, 303-309.
2. Smit, A.L., Bindraban, P.S., Schröder, J., Conijn, J. and Van Der Meer, H. (2009) Phosphorus in agriculture: global resources, trends and developments. *Report to the Steering Committee Technology Assessment of the Ministry of Agriculture, The Neetherlands, Wageningen*, in press.
3. Childers, D.L., Corman, J., Edwards, M. and Elser, J.J. (2011) Sustainability challenges of phosphorus and food: solutions from closing the human phosphorus cycle. *BioScience*, **61**, 117-124.
4. Malboobi, M.A., Samaeian, A., Sabet, M.S. and Lohrasebi, T. (2012) *Plant Phosphate Nutrition and Environmental Challenges*. In: Plant Science Book. Edited by Nabin Kumar Dhal, InTech Press. Croatia. pp. 1-33.
5. Czarnecki, O., Yang, J., Weston, D.J., Tuskan, G.A. and Chen, J.G. (2013) A dual role of strigolactones in phosphate acquisition and utilization in plants. *International journal of molecular sciences*, **14**, 7681-7701.
6. Vance, C.P., Uhde-Stone, C. and Allan, D.L. (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, **157**, 423-447.
7. Spina, A., Sorvillo, L., Chiosi, E., Esposito, A., Di Maiolo, F., Sapio, L., Caraglia, M. and Naviglio, S. (2013) Synergistic cytotoxic effects of inorganic phosphate and chemotherapeutic drugs on human osteosarcoma cells. *Oncology reports*, **29**, 1689-1696.
8. Vance, C.P. (2010) Quantitative trait loci, epigenetics, sugars, and microRNAs: quaternaries in phosphate acquisition and use. *Plant physiology*, **154**, 582-588.
9. Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A., Scheible, W.R., Shane, M.W. and White, P.J. (2012) Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist*, **195**, 306-320.
10. Bieleski, R. (1973) Phosphate pools, phosphate transport, and phosphate availability. *Annual review of plant physiology*, **24**, 225-252.
11. Hinsinger, P. (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and soil*, **237**, 173-195.
12. Conyers, M. and Moody, P. (2009) A conceptual framework for improving the P efficiency of organic farming without inputs of soluble P fertiliser. *Crop and Pasture Science*, **60**, 100-104.
13. Plaxton, W.C. and Tran, H.T. (2011) Metabolic adaptations of phosphate-starved plants. *Plant Physiology*, **156**, 1006-1015.
14. Poirier, Y. and Bucher, M. (2002) Phosphate transport and homeostasis in Arabidopsis. *The Arabidopsis book/American Society of Plant Biologists*, **1**.
15. Lin, W.-Y., Lin, S.-I. and Chiou, T.-J. (2009) Molecular regulators of phosphate homeostasis in plants. *Journal of experimental botany*, **60**, 1427-1438.
16. Fang, Z., Shao, C., Meng, Y., Wu, P. and Chen, M. (2009) Phosphate signaling in *Arabidopsis* and *Oryza sativa*. *Plant Science*, **176**, 170-180.

17. Raghothama, K. (1999) Phosphate acquisition. *Annual review of plant biology*, **50**, 665-693.

18. Shahbaz, A.M., Oki, Y., Adachi, T., Murata, Y. and Khan, M.H.R. (2006) Phosphorus starvation induced root-mediated pH changes in solublization and acquisition of sparingly soluble P sources and organic acids exudation by Brassica cultivars. *Soil Science and Plant Nutrition*, **52**, 623-633.

19. Lambers, H. and Shane, M. (2007) Role of root clusters in phosphorus acquisition and increasing biological diversity in agriculture. *Frontis*, **21**, 235-248.

20. Lynch, J.P. (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiology*, **156**, 1041-1049.

21. Péret, B., Clément, M., Nussaume, L. and Desnos, T. (2011) Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends in plant science*, **16**, 442-450.

22. López-Bucio, J., Cruz-Ramírez, A. and Herrera-Estrella, L. (2003) The role of nutrient availability in regulating root architecture. *Current opinion in plant biology*, **6**, 280-287.

23. Basirat, M., Malboobi, M.A., Mousavi, A., Asgharzadeh, A. and Samavat, S. (2011) Effects of phosphorous supply on growth, phosphate distribution and expression of transporter genes in tomato plants. *Australian Journal of Crop Science*, **5,** 537-543.

24. Péret, B., Svistoonoff, S. and Laplaze, L. (2009) When plants socialize: symbioses and root development. *Annu. Plant Rev*, **37**, 209-238.

25. Smith, S.E. and Smith, F.A. (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual review of plant biology*, **62**, 227-250.

26. Alexova, R. and Millar, A.H. (2013) Proteomics of phosphate use and deprivation in plants. *Proteomics*, **13**, 609-623.

27. Niu, Y.F., Chai, R.S., Jin, G.L., Wang, H., Tang, C.X. and Zhang, Y.S. (2013) Responses of root architecture development to low phosphorus availability: a review. *Annals of botany*, **112**, 391-408.

28. Watt, M. and Evans, J.R. (1999) Proteoid roots. Physiology and development. *Plant Physiology*, **121**, 317-323.

29. Jones, D. and Brassington, D. (1998) Sorption of organic acids in acid soils and its implications in the rhizosphere. *European Journal of Soil Science*, **49**, 447-455.

30. Wang, X., Wang, Y., Tian, J., Lim, B.L., Yan, X. and Liao, H. (2009) Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. *Plant Physiology*, **151**, 233-240.

31. Wu, P., Ma, L., Hou, X., Wang, M., Wu, Y., Liu, F. and Deng, X.W. (2003) Phosphate starvation triggers distinct alterations of genome expression in Arabidopsis roots and leaves. *Plant Physiology*, **132**, 1260-1271.

32. Misson, J., Raghothama, K.G., Jain, A., Jouhet, J., Block, M.A., Bligny, R., Ortet, P., Creff, A., Somerville, S. and Rolland, N. (2005) A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11934-11939.

33. Morcuende, R., Bari, R., Gibon, Y., Zheng, W., Pant, B.D., BLÄSING, O., Usadel, B., Czechowski, T., Udvardi, M.K. and Stitt, M. (2007) Genome-wide reprogramming of metabolism

and regulatory networks of Arabidopsis in response to phosphorus. *Plant, Cell & Environment*, **30**, 85-112.

34. Thibaud, M.C., Arrighi, J.F., Bayle, V., Chiarenza, S., Creff, A., Bustos, R., Paz-Ares, J., Poirier, Y. and Nussaume, L. (2010) Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. *The Plant Journal*, **64**, 775-789.
35. Sánchez-Calderón, L., López-Bucio, J., Chacón-López, A., Gutiérrez-Ortega, A., Hernández-Abreu, E. and Herrera-Estrella, L. (2006) Characterization of low phosphorus insensitive mutants reveals a crosstalk between low phosphorus-induced determinate root development and the activation of genes involved in the adaptation of Arabidopsis to phosphorus deficiency. *Plant physiology*, **140**, 879-889.
36. González-Mendoza, V., Zurita-Silva, A., Sánchez-Calderón, L., Sánchez-Sandoval, M.E., Oropeza-Aburto, A., Gutiérrez-Alanís, D., Alatorre-Cobos, F. and Herrera-Estrella, L. (2013) APSR1, a novel gene required for meristem maintenance, is negatively regulated by low phosphate availability. *Plant Science*, **205**, 2-12.
37. White, P.J. and Veneklaas, E.J. (2012) Nature and nurture: the importance of seed phosphorus content. *Plant and soil*, **357**, 1-8.
38. Mayzlish-Gati, E., De-Cuyper, C., Goormachtig, S., Beeckman, T., Vuylsteke, M., Brewer, P.B., Beveridge, C.A., Yermiyahu, U., Kaplan, Y. and Enzer, Y. (2012) Strigolactones are involved in root response to low phosphate conditions in Arabidopsis. *Plant physiology*, **160**, 1329-1341.
39. Pérez-Torres, C.-A., López-Bucio, J., Cruz-Ramírez, A., Ibarra-Laclette, E., Dharmasiri, S., Estelle, M. and Herrera-Estrella, L. (2008) Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. *The Plant Cell Online*, **20**, 3258-3272.
40. Nagarajan, V.K. and Smith, A.P. (2012) Ethylene's role in phosphate starvation signaling: more than just a root growth regulator. *Plant and Cell Physiology*, **53**, 277-286.
41. Jiang, C., Gao, X., Liao, L., Harberd, N.P. and Fu, X. (2007) Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in Arabidopsis. *Plant physiology*, **145**, 1460-1470.
42. Trull, M., Guiltinan, M., Lynch, J. and Deikman, J. (1997) The responses of wild-type and ABA mutant *Arabidopsis thaliana* plants to phosphorus starvation. *Plant, Cell & Environment*, **20**, 85-92.
43. Duff, S.M., Moorhead, G.B., Lefebvre, D.D. and Plaxton, W.C. (1989) Phosphate starvation inducible'bypasses' of adenylate and phosphate dependent glycolytic enzymes in *Brassica nigra* suspension cells. *Plant Physiology*, **90**, 1275-1278.
44. Plaxton, W.C. and Podestá, F.E. (2006) The functional organization and control of plant respiration. *Critical Reviews in Plant Sciences*, **25**, 159-198.
45. Gregory, A., Hurley, B., Tran, H., Valentine, A., She, Y., Knowles, V. and Plaxton, W. (2009) *In vivo* regulatory phosphorylation of the phosphoenolpyruvate carboxylase AtPPC1 in phosphate-starved *Arabidopsis thaliana*. *Biochem. J*, **420**, 57-65.
46. Tran, H.T., Qian, W., Hurley, B.A., SHE, Y.M., Wang, D. and Plaxton, W.C. (2010) Biochemical

and molecular characterization of AtPAP12 and AtPAP26: the predominant purple acid phosphatase isozymes secreted by phosphate-starved *Arabidopsis thaliana*. *Plant, cell & environment*, **33**, 1789-1803.

47. Hammond, J.P. and White, P.J. (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *Journal of experimental botany*, **59**, 93-109.
48. Geigenberger, P. (2011) Regulation of starch biosynthesis in response to a fluctuating environment. *Plant Physiology*, **155**, 1566-1577.
49. Yu, B., Xu, C. and Benning, C. (2002) Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. *Proceedings of the National Academy of Sciences*, **99**, 5732-5737.
50. Li, M., Qin, C., Welti, R. and Wang, X. (2006) Double knockouts of phospholipases Dζ1 and Dζ2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. *Plant physiology*, **140**, 761-770.
51. Gaude, N., Nakamura, Y., Scheible, W.R., Ohta, H. and Dörmann, P. (2008) Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of Arabidopsis. *The Plant Journal*, **56**, 28-39.
52. Nilsson, L., Müller, R. and Nielsen, T.H. (2010) Dissecting the plant transcriptome and the regulatory responses to phosphate deprivation. *Physiologia plantarum*, **139**, 129-143.
53. Tran, H.T., Hurley, B.A. and Plaxton, W.C. (2010) Feeding hungry plants: the role of purple acid phosphatases in phosphate nutrition. *Plant Science*, **179**, 14-27.
54. Ticconi, C.A. and Abel, S. (2004) Short on phosphate: plant surveillance and countermeasures. *Trends in plant science*, **9**, 548-555.
55. Richardson, A.E., Hocking, P.J., Simpson, R.J. and George, T.S. (2009) Plant mechanisms to optimize access to soil Phosphorus. *Crop Past. Sci*., **60**, 124-143.
56. Liang, C., Tian, J., Lam, H.-M., Lim, B.L., Yan, X. and Liao, H. (2010) Biochemical and molecular characterization of PvPAP3, a novel purple acid phosphatase isolated from common bean enhancing extracellular ATP utilization. *Plant physiology*, **152**, 854-865.
57. Zhang, Q., Wang, C., Tian, J., Li, K. and Shou, H. (2011) Identification of rice purple acid phosphatases related to posphate starvation signalling. *Plant Biology*, **13**, 7-15.
58. Li, D., Zhu, H., Liu, K., Liu, X., Leggewie, G., Udvardi, M. and Wang, D. (2002) Purple acid phosphatases of *Arabidopsis thaliana* comparative analysis and differential regulation by phosphate deprivation. *Journal of Biological Chemistry*, **277**, 27772-27781.
59. Li, C., Gui, S., Yang, T., Walk, T., Wang, X. and Liao, H. (2012) Identification of soybean purple acid phosphatase genes and their expression responses to phosphorus availability and symbiosis. *Annals of botany*, **109**, 275-285.
60. Zhu, H., Qian, W., Lu, X., Li, D., Liu, X., Liu, K. and Wang, D. (2005) Expression patterns of purple acid phosphatase genes in Arabidopsis organs and functional analysis of AtPAP23 predominantly transcribed in flower. *Plant molecular biology*, **59**, 581-594.
61. Del Pozo, J.C., Allona, I., Rubio, V., Leyva, A., De La Peña, A., Aragoncillo, C. and Paz-Ares, J.

(1999) A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilising/oxidative stress conditions. *The Plant Journal*, **19**, 579-589.

62. Sun, F., Carrie, C., Law, S., Murcha, M.W., Zhang, R., Law, Y.S., Suen, P.K., Whelan, J. and Lim, B.L. (2012) AtPAP2 is a tail-anchored protein in the outer membrane of chloroplasts and mitochondria. *Plant signaling & behavior*, **7**, 927-932.
63. Sun, F., Suen, P.K., Zhang, Y., Liang, C., Carrie, C., Whelan, J., Ward, J.L., Hawkins, N.D., Jiang, L. and Lim, B.L. (2012) A dual-targeted purple acid phosphatase in *Arabidopsis thaliana* moderates carbon metabolism and its overexpression leads to faster plant growth and higher seed yield. *new phytologist*, **194**, 206-219.
64. Hurley, B.A., Tran, H.T., Marty, N.J., Park, J., Snedden, W.A., Mullen, R.T. and Plaxton, W.C. (2010) The dual-targeted purple acid phosphatase isozyme AtPAP26 is essential for efficient acclimation of Arabidopsis to nutritional phosphate deprivation. *Plant physiology*, **153**, 1112-1122.
65. Zamani, K., Sabet, M.S., Lohrasebi, T., Mousavi, A. and Malboobi, M.A. (2012) Improved phosphate metabolism and biomass production by overexpression of AtPAP18 in tobacco. *Biologia*, **67**, 713-720.
66. Zamani, K., Lohrasebi, T., Sabet, M.S., Malboobi, M.A. and Mousavi, A. (2014) Expression pattern and subcellular localization of Arabidopsis purple acid phosphatase AtPAP9. *Gene Expression Patterns*, **14**, 9-18.
67. Wang, L., Li, Z., Qian, W., Guo, W., Gao, X., Huang, L., Wang, H., Zhu, H., Wu, J.-W. and Wang, D. (2011) The Arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. *Plant Physiology*, **157**, 1283-1299.
68. Kaida, R., Satoh, Y., Bulone, V., Yamada, Y., Kaku, T., Hayashi, T. and Kaneko, T.S. (2009) Activation of β-glucan synthases by wall-bound purple acid phosphatase in tobacco cells. *Plant physiology*, **150**, 1822-1830.
69. Del Vecchio, H. (2012) Biochemical and Molecular characterization of AtPAP25, a novel cell wall-localized purple acid phosphatase isozyme upregulated by phosphate-starved *Arabidopsis thaliana*. in press.
70. Zhang, Y., Yu, L., Yung, K.F., Leung, D.Y., Sun, F. and Lim, B.L. (2012) Over-expression of AtPAP2 in *Camelina sativa* leads to faster plant growth and higher seed yield. *Biotechnology for biofuels*, **5**, 1-10.
71. Ravichandran, S., Stone, S.L., Benkel, B. and Prithiviraj, B. (2013) Purple Acid Phosphatase5 is required for maintaining basal resistance against Pseudomonas syringae in Arabidopsis. *BMC plant. Biol*., **13**, 107.
72. Zhang, W., Gruszewski, H.A., Chevone, B.I. and Nessler, C.L. (2008) An Arabidopsis purple acid phosphatase with phytase activity increases foliar ascorbate. *Plant physiology*, **146**, 431-440.
73. Dinkelaker, B., Römheld, V. and Marschner, H. (1989) Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant, Cell & Environment*, **12**, 285-292.

74. Gerke, J., Beißner, L. and Römer, W. (2000) The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. I. The basic concept and determination of soil parameters. *Journal of Plant Nutrition and Soil Science*, **163**, 207-212.

75. Gardner, W., Barber, D. and Parbery, D. (1983) The acquisition of phosphorus by*Lupinus albus* L. *Plant and soil*, **70**, 107-124.

76. Hammond, J.P., Bennett, M.J., Bowen, H.C., Broadley, M.R., Eastwood, D.C., May, S.T., Rahn, C., Swarup, R., Woolaway, K.E. and White, P.J. (2003) Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. *Plant Physiology*, **132**, 578-596.

77. Wang, X., Yi, K., Tao, Y., Wang, F., Wu, Z., Jiang, D., Chen, X., Zhu, L. and Wu, P. (2006) Cytokinin represses phosphate-starvation response through increasing of intracellular phosphate level. *Plant, cell & environment*, **29**, 1924-1935.

78. Müller, R., Morant, M., Jarmer, H., Nilsson, L. and Nielsen, T.H. (2007) Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiology*, **143**, 156-171.

79. Lin, S.I., Chiang, S.F., Lin, W.Y., Chen, J.W., Tseng, C.Y., Wu, P.C. and Chiou, T.J. (2008) Regulatory network of microRNA399 and PHO2 by systemic signaling. *Plant physiology*, **147**, 732-746.

80. Woo, J., MacPherson, C.R., Liu, J., Wang, H., Kiba, T., Hannah, M.A., Wang, X.J., Bajic, V.B. and Chua, N.H. (2012) The response and recovery of the *Arabidopsis thaliana* transcriptome to phosphate starvation. *BMC plant biology*, **12**, 62.

81. Park, M.R., Baek, S.-H., De los Reyes, B.G., Yun, S.J. and Hasenstein, K.H. (2012) Transcriptome profiling characterizes phosphate deficiency effects on carbohydrate metabolism in rice leaves. *Journal of plant physiology*, **169**, 193-205.

82. Cai, H., Xie, W., Zhu, T. and Lian, X. (2012) Transcriptome response to phosphorus starvation in rice. *Acta Physiologiae Plantarum*, **34**, 327-341.

83. Oono, Y., Kawahara, Y., Kanamori, H., Mizuno, H., Yamagata, H., Yamamoto, M., Hosokawa, S., Ikawa, H., Akahane, I. and Zhu, Z. (2011) mRNA-Seq reveals a comprehensive transcriptome profile of rice under phosphate stress. *Rice*, **4**, 50-65.

84. O'Rourke, J.A., Yang, S.S., Miller, S.S., Bucciarelli, B., Liu, J., Rydeen, A., Bozsoki, Z., Uhde-Stone, C., Tu, Z.J. and Allan, D. (2013) An RNA-seq transcriptome analysis of orthophosphate-deficient white lupin reveals novel insights into phosphorus acclimation in plants. *Plant physiology*, **161**, 705-724.

85. Oono, Y., Kawahara, Y., Yazawa, T., Kanamori, H., Kuramata, M., Yamagata, H., Hosokawa, S., Minami, H., Ishikawa, S. and Wu, J. (2013) Diversity in the complexity of phosphate starvation transcriptomes among rice cultivars based on RNA-Seq profiles. *Plant molecular biology*, **83**, 523-537.

86. Hammond, J.P. and White, P.J. (2011) Sugar signaling in root responses to low phosphorus availability. *Plant Physiology*, **156**, 1033-1040.

87. Lan, P., Li, W. and Schmidt, W. (2012) Complementary proteome and transcriptome profiling in

phosphate-deficient Arabidopsis roots reveals multiple levels of gene regulation. *Molecular & Cellular Proteomics*, **11**, 1156-1166.

88. Rubio, V., Linhares, F., Solano, R., Martín, A.C., Iglesias, J., Leyva, A. and Paz-Ares, J. (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & development*, **15**, 2122-2133.
89. Nilsson, L., Müller, R. and Nielsen, T.H. (2007) Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in *Arabidopsis thaliana*. *Plant, cell & environment*, **30**, 1499-1512.
90. Bustos, R., Castrillo, G., Linhares, F., Puga, M.I., Rubio, V., Pérez-Pérez, J., Solano, R., Leyva, A. and Paz-Ares, J. (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. *PLoS genetics*, **6**, e1001102.
91. Fujii, H., Chiou, T.J., Lin, S.I., Aung, K. and Zhu, J.K. (2005) A miRNA Involved in Phosphate-Starvation Response in Arabidopsis. *Current Biology*, **15**, 2038-2043.
92. Hsieh, L.C., Lin, S.I., Shih, A.C.C., Chen, J.W., Lin, W.Y., Tseng, C.Y., Li, W.H. and Chiou, T.J. (2009) Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. *Plant physiology*, **151**, 2120-2132.
93. Pant, B.D., Musialak-Lange, M., Nuc, P., May, P., Buhtz, A., Kehr, J., Walther, D. and Scheible, W.R. (2009) Identification of nutrient-responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. *Plant Physiology*, **150**, 1541-1555.
94. Doerner, P. (2008) Phosphate starvation signaling: a threesome controls systemic Pi homeostasis. *Current opinion in plant biology*, **11**, 536-540.
95. Liu, C., Muchhal, U.S. and Raghothama, K. (1997) Differential expression of TPS11, a phosphate starvation-induced gene in tomato. *Plant molecular biology*, **33**, 867-874.
96. Burleigh, S.H. and Harrison, M.J. (1997) A novel gene whose expression in *Medicago truncatula* roots is suppressed in response to colonization by vesicular-arbuscular mycorrhizal (VAM) fungi and to phosphate nutrition. *Plant molecular biology*, **34**, 199-208.
97. Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., Leyva, A., Weigel, D., García, J.A. and Paz-Ares, J. (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. *Nature genetics*, **39**, 1033-1037.
98. Zhou, J., Jiao, F., Wu, Z., Li, Y., Wang, X., He, X., Zhong, W. and Wu, P. (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiology*, **146**, 1673-1686.
99. Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W. and Zhang, F. (2011) Phosphorus dynamics: from soil to plant. *Plant physiology*, **156**, 997-1005.
100. Compton, J.S., Mallinson, D.J., Glenn, C.R., Filippelli, G., Follmi, K., Shields, G. and Zanin, Y. (2000) Variations in the global phosphorus cycle. In Marine authigenesis: From global to microbial, edited by C. R. Glenn, p. 21–33. Tulsa, Oklahoma: Society for Sedimentary Geology (SEPM).
101. Liu, Y., Villalba, G., Ayres, R.U. and Schroder, H. (2008) Global phosphorus flows and

environmental impacts from a consumption perspective. *Journal of Industrial Ecology*, **12**, 229-247.

102. Turner, B.L., Frossard, E. and Baldwin, D.S. (2005) *Organic phosphorus in the environment*. CABI. Wallingford, UK; p 432.

103. Havlin, J.L., Beaton, J.D., Tisdale, S.L., Nelson, W.L. (2005) Soil fertility and fertilizers: an introduction to nutritional management. New Jersey: Pearson.

104. Johnston, A.E., Ehlert, P.A.I., Kueche, M., Amar, B., Jaggard, K.W. and Morel, C. (2001) The effect of phosphate fertilizer management strategies on soil phosphorus status and crop yields in some european countries. evaluation report (1991-1996). Rabat (Morocco), Actes/Imphos, 2001, pp. 59-102.

105. Johnston, A.E. and Steén, I. (2000) *Understanding phosphorus and its use in a agriculture*. European Fertilizer Manufacturers Association.

106. Bowen, H.J.M. (1979) *Environmental chemistry of the elements*. Academic Press, London.

107. Sharpley, A. (1995). *Fate and transport of nutrients: Phosphorus*. USDA. Jordan Hill, Oxford OX2 8DP, UK.

108. Sharpley, A. (1995) Fate and transport of nutrients: Phosphorus. Working Paper No. 8

109. Condron, L.M. and Tiessen, H. (2005) Interactions of organic phosphorus in terrestrial ecosystems. *Organic phosphorus in the environment*, in press., 295-307.

110. Paul E.A. (2007) Soil Microbiology and Biochemistry. Third edithion. Linacre House, Jordan Hill, Oxford OX2 8DP, UK.

111. Holford, I. (1997) Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research*, **35**, 227-240.

112. Tirado R. and Allsopp M. (2012) Phosphorus in agriculture, Problems and solutions. Greenpeace International Technical Report.

113. FAO (2011) Current World Fertilizer Trends and Outlook to 2015. FAO; Rome.

114. FAO STAT (2012). http://faostat.fao.org/site/575/default. Last access date 02/05/2012

115. FAO (2008) Current World Fertilizer Trends and Outlook to 2011/12. FAO; Rome.

116. IMPHOS (2009) Addressing Environmental Issues Associated with Phosphate. IMPHOS phosphate newsletter; 26. Available: www.imphos.org.

117. Cordell, D., Drangert, J.O. and White, S. (2009) The story of phosphorus: Global food security and food for thought. *Global environmental change*, **19**, 292-305.

118. McDowell, R.W. and Hamilton, D.P. (2013) Nutrients and eutrophication: introduction. *Marine and Freshwater Research*, **64**, iii-vi.

119. Chislock, M.F., Doster, E., Zitomer, R.A. and Wilson, A.E. (2013) Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nature Education knowledge* **4**,10.

120. Cheraghi, M., Lorestani, B., Merrikhpour, H. and Rouniasi, N. (2013) Heavy metal risk assessment for potatoes grown in overused phosphate-fertilized soils. *Environmental monitoring and assessment*, **185**, 1825-1831.

121. Schnug, E., Bernd, G. and Lottermoser (2013) Fertilizer-derived Uranium and its threat to human health. *Environ. Sci. Technol.,* **47**, 2433-2434.

122. Pan, J., Plant, J.A., Voulvoulis, N., Oates, C.J. and Ihlenfeld, C. (2010) Cadmium levels in Europe: implications for human health. *Environmental geochemistry and health*, **32**, 1-12.

123. Schipper, W., Klapwijk, A., Potjer, B., Rulkens, W., Temmink, B., Kiestra, F. and Lijmbach, A. (2001) Phosphate recycling in the phosphorus industry. *Environmental technology*, **22**, 1337-1345.

124. Fageria, N., Baligar, V. and Li, Y. (2008) The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of plant nutrition*, **31**, 1121-1157.

125. Yan, X., Wu, P., Ling, H., Xu, G., Xu, F. and Zhang, Q. (2006) Plant nutriomics in China: an overview. *Annals of botany*, **98**, 473-482.

126. Li, J., Liu, X., Zhou, W., Sun, J., Tong, Y., Liu, W., Li, Z., Wang, P. and Yao, S. (1995) Technique of wheat breeding for efficiently utilizing soil nutrient elements. *Science in China (Scienctia Sinica) Series B*, **38**, 1313-1320.

127. Miyasaka, S.C. and Habte, M. (2001) Plant mechanisms and mycorrhizal symbioses to increase phosphorus uptake efficiency. *Communications in Soil Science and Plant Analysis*, **32**, 1101-1147.

128. Doerge, R.W. (2002) Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics*, **3**, 43-52.

129. Chin, J.H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetiyono, J., Moeljopawiro, S., Wissuwa, M. and Heuer, S. (2011) Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application. *Plant physiology*, **156**, 1202-1216.

130. Gregory, P.J. and George, T.S. (2011) Feeding nine billion: the challenge to sustainable crop production. *Journal of experimental botany*, **62**, 5233-5239.

131. 131. Jain, S.M. and Suprasanna, P. (2011) Induced mutations for enhancing nutrition and food production. *Geneconserve*, **40**, 201-215.

132. Bilyeu, K.D., Zeng, P., Coello, P., Zhang, Z.J., Krishnan, H.B., Bailey, A., Beuselinck, P.R. and Polacco, J.C. (2008) Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant physiology*, **146**, 468-477.

133. Gaxiola, R.A., Edwards, M. and Elser, J.J. (2011) A transgenic approach to enhance phosphorus use efficiency in crops as part of a comprehensive strategy for sustainable agriculture. *Chemosphere*, **84**, 840-845.

134. Anderson, G. (1980) Assessing organic phosphorus in soils. *The role of phosphorus in agriculture*, in press, 411-431.

135. Anderson, G. (1980) Assessing Organic Phosphorus in Soil. In: Khasawneh FE., Sample EC., Kamprath EJ. (Eds.), The Role of Phosphorus in Agriculture. American Society of Agronomy, Madison, WI, USA; 1980. p411–431.

136. Ramaekers, L., Remans, R., Rao, I.M., Blair, M.W. and Vanderleyden, J. (2010) Strategies for improving phosphorus acquisition efficiency of crop plants. *Field Crops Research*, **117**, 169-176.

137. López-Bucio, J., de la Vega, O.M., Guevara-García, A. and Herrera-Estrella, L. (2000) Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. *Nature biotechnology*, **18**, 450-453.

138. Rajan, S. and Watkinson, J. (1992) Unacidulated and partially acidulated phosphate rock: Agronomic effectiveness and the rates of dissolution of phosphate rock. *Fertilizer research*, **33**, 267-277.

139. Matsui, K., Togami, J., Mason, J.G., Chandler, S.F. and Tanaka, Y. (2013) Enhancement of phosphate absorption by garden plants by genetic engineering: a new tool for phytoremediation. *Biomed Res Int.,* **2013**, 182032.

140. Wang, X., Bai, J., Liu, H., Sun, Y., Shi, X. and Ren, Z. (2013) Overexpression of a Maize transcription factor ZmPHR1 improves shoot inorganic phosphate content and growth of Arabidopsis under low-phosphate conditions. *Plant Mol. Biol. Rep.,* **31**, 665-677.

141. Li, Z., Gao, Q., Liu, Y., He, C., Zhang, X. and Zhang, J. (2011) Overexpression of transcription factor ZmPTF1 improves low phosphate tolerance of maize by regulating carbon metabolism and root growth. *Planta*, **233**, 1129-1143.

142. Yi, K., Wu, Z., Zhou, J., Du, L., Guo, L., Wu, Y. and Wu, P. (2005) OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. *Plant physiology*, **138**, 2087-2096.

143. Wang, J., Sun, J., Miao, J., Guo, J., Shi, Z., He, M., Chen, Y., Zhao, X., Li, B. and Han, F. (2013) A phosphate starvation response regulator Ta-PHR1 is involved in phosphate signalling and increases grain yield in wheat. *Annals of botany*, **111**, 1139-1153.

144. Baek, D., Kim, M.C., Chun, H.J., Kang, S., Park, H.C., Shin, G., Park, J., Shen, M., Hong, H. and Kim, W.-Y. (2013) Regulation of miR399f transcription by AtMYB2 affects phosphate starvation responses in Arabidopsis. *Plant physiology*, **161**, 362-373.

145. Sun, S., Gu, M., Cao, Y., Huang, X., Zhang, X., Ai, P., Zhao, J., Fan, X. and Xu, G. (2012) A constitutive expressed phosphate transporter, OsPht1; 1, modulates phosphate uptake and translocation in phosphate-replete rice. *Plant physiology*, **159**, 1571-1581.

146. Nagarajan, V.K., Jain, A., Poling, M.D., Lewis, A.J., Raghothama, K.G. and Smith, A.P. (2011) Arabidopsis Pht1; 5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. *Plant Physiology*, **156**, 1149-1163.

147. Qin, L., Zhao, J., Tian, J., Chen, L., Sun, Z., Guo, Y., Lu, X., Gu, M., Xu, G. and Liao, H. (2012) The high-affinity phosphate transporter GmPT5 regulates phosphate transport to nodules and nodulation in soybean. *Plant physiology*, **159**, 1634-1643.

148. Liu, X., Zhao, X., Zhang, L., Lu, W., Li, X. and Xiao K. (2013) TaPht1;4, a high-affinity phosphate transporter gene in wheat (*Triticum aestivum*), plays an important role in plant phosphate acquisition under phosphorus deprivation. *Funct Plant Biol.,* **40**, 329-341.

149. Zhang, J., Zhang, X., Duan. Y. and Han, Y. (2013) Construction of a phosphate transporter gene expression vector and its usage for tobacco transformation. *Russ. J. Plant. Physiol.,* **60**, 290-294.

150. Song, H., Yin, Z., Chao, M., Ning, L., Zhang, D. and Yu, D. (2014) Functional properties and

expression quantitative trait loci for phosphate transporter *GmPT1* in soybean. *Plant. Cell. Environ.*, **37**, 462-472.

151. Park, M.R., Baek, S.-H., Benildo, G. and Yun, S.J. (2007) Overexpression of a high-affinity phosphate transporter gene from tobacco (NtPT1) enhances phosphate uptake and accumulation in transgenic rice plants. *Plant and soil*, **292**, 259-269.

152. Zimmermann, P., Zardi, G., Lehmann, M., Zeder, C., Amrhein, N., Frossard, E. and Bucher, M. (2003) Engineering the root–soil interface via targeted expression of a synthetic phytase gene in trichoblasts. *Plant Biotechnology Journal*, **1**, 353-360.

153. George, T.S., Simpson, R.J., Hadobas, P.A. and Richardson, A.E. (2005) Expression of a fungal phytase gene in *Nicotiana tabacum* improves phosphorus nutrition of plants grown in amended soils. *Plant Biotechnology Journal*, **3**, 129-140.

154. Richardson, A.E., Hadobas, P.A. and Hayes, J.E. (2001) Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate. *The Plant Journal*, **25**, 641-649.

155. Hong, Y.F., Liu, C.Y., Cheng, K.J., Hour, A.L., Chan, M.T., Tseng, T.H., Chen, K.Y., Shaw, J.F. and Yu, S.M. (2008) The sweet potato sporamin promoter confers high-level phytase expression and improves organic phosphorus acquisition and tuber yield of transgenic potato. *Plant molecular biology*, **67**, 347-361.

156. Wang, Y., Ye, X., Ding, G. and Xu, F. (2013) Overexpression of phyA and appA genes improves soil organic phosphorus utilisation and seed phytase activity in Brassica napus. *PloS one*, **8**, e60801.

157. Chen, R., Xue, G., Chen, P., Yao, B., Yang, W., Ma, Q., Fan, Y., Zhao, Z., Tarczynski, M.C. and Shi, J. (2008) Transgenic maize plants expressing a fungal phytase gene. *Transgenic research*, **17**, 633-643.

158. Lung, S.C., Chan, W.L., Yip, W., Wang, L., Yeung, E.C. and Lim, B.L. (2005) Secretion of beta-propeller phytase from tobacco and Arabidopsis roots enhances phosphorus utilization. *Plant science*, **169**, 341-349.

159. Yang, H., Knapp, J., Koirala, P., Rajagopal, D., Peer, W.A., Silbart, L.K., Murphy, A. and Gaxiola, R.A. (2007) Enhanced phosphorus nutrition in monocots and dicots over-expressing a phosphorus-responsive type I H^+-pyrophosphatase. *Plant biotechnology journal*, **5**, 735-745.

160. Pei, L., Wang, J., Li, K., Li, Y., Li, B., Gao, F. and Yang, A. (2012) Overexpression of Thellungiella halophila H^+-pyrophosphatase gene improves low phosphate tolerance in maize. *PloS one*, **7**, e43501.

161. Liang, C.Y., Chen, Z.J., Yao, Z.F., Tian, J. and Liao, H. (2012) Characterization of Two Putative Protein Phosphatase Genes and Their Involvement in Phosphorus Efficiency in Phaseolus vulgarisF. *Journal of integrative plant biology*, **54**, 400-411.

162. Liu, J., Zhao, C., Ma, J., Zhang, G., Li, M., Yan, G., Wang, X. and Ma, Z. (2011) Agrobacterium-mediated transformation of cotton (*Gossypium hirsutum* L.) with a fungal phytase gene improves phosphorus acquisition. *Euphytica*, **181**, 31-40.

163. Li, R.-J., Lu, W.-J., Guo, C.-J., Li, X.-J., Gu, J.-T. and Xiao, K. (2012) Molecular

Characterization and Functional Analysis of OsPHY1, a Purple Acid Phosphatase (PAP)–Type Phytase Gene in Rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, **11**, 1217-1226.

164. Hur, Y.J., Jin, B.R., Nam, J., Chung, Y.S., Lee, J.H., Choi, H.K., Yun, D.J., Yi, G., Kim, Y.H. and Kim, D.H. (2010) Molecular characterization of OsPAP2: transgenic expression of a purple acid phosphatase up-regulated in phosphate-deprived rice suspension cells. *Biotechnology letters*, **32**, 163-170.

165. Tian, J., Wang, C., Zhang, Q., He, X., Whelan, J. and Shou, H. (2012) Overexpression of OsPAP10a, a root-associated acid phosphatase, increased extracellular organic phosphorus utilization in rice. *Journal of integrative plant biology*, **54**, 631-639.

166. Wasaki, J., Maruyama, H., Tanaka, M., Yamamura, T., Dateki, H., Shinano, T., Ito, S. and Osaki, M. (2009) Overexpression of the LASAP2 gene for secretory acid phosphatase in white lupin improves the phosphorus uptake and growth of tobacco plants. *Soil science and plant nutrition*, **55**, 107-113.

167. Maruyama, H., Yamamura, T., Kaneko, Y., Matsui, H., Watanabe, T., Shinano, T., Osaki, M. and Wasaki, J. (2012) Effect of exogenous phosphatase and phytase activities on organic phosphate mobilization in soils with different phosphate adsorption capacities. *Soil Science and Plant Nutrition*, **58**, 41-51.

168. Ma, X.-F., Wright, E., Ge, Y., Bell, J., Xi, Y., Bouton, J.H. and Wang, Z.-Y. (2009) Improving phosphorus acquisition of white clover (*Trifolium repens* L.) by transgenic expression of plant-derived phytase and acid phosphatase genes. *Plant Science*, **176**, 479-488.

169. Ma, X.-F., Tudor, S., Butler, T., Ge, Y., Xi, Y., Bouton, J., Harrison, M. and Wang, Z.-Y. (2012) Transgenic expression of phytase and acid phosphatase genes in alfalfa (*Medicago sativa*) leads to improved phosphate uptake in natural soils. *Molecular Breeding*, **30**, 377-391.

170. Xiao, K., Katagi, H., Harrison, M. and Wang, Z.Y. (2006) Improved phosphorus acquisition and biomass production in Arabidopsis by transgenic expression of a purple acid phosphatase gene from *M. truncatula*. *Plant Science*, **170**, 191-202.

171. Koyama, H., Kawamura, A., Kihara, T., Hara, T., Takita, E. and Shibata, D. (2000) Overexpression of mitochondrial citrate synthase in Arabidopsis thaliana improved growth on a phosphorus-limited soil. *Plant and Cell Physiology*, **41**, 1030-1037.

172. Wang, Y., Xu, H., Kou, J., Shi, L., Zhang, C. and Xu, F. (2013) Dual effects of transgenic Brassica napus overexpressing CS gene on tolerances to aluminum toxicity and phosphorus deficiency. *Plant and soil*, **362**, 231-246.

173. Lü, J., Gao, X., Dong, Z., Yi, J. and An, L. (2012) Improved phosphorus acquisition by tobacco through transgenic expression of mitochondrial malate dehydrogenase from Penicillium oxalicum. *Plant cell reports*, **31**, 49-56.

174. Delhaize, E., Taylor, P., Hocking, P.J., Simpson, R.J., Ryan, P.R. and Richardson, A.E. (2009) Transgenic barley (*Hordeum vulgare* L.) expressing the wheat aluminium resistance gene (TaALMT1) shows enhanced phosphorus nutrition and grain production when grown on an acid soil. *Plant biotechnology journal*, **7**, 391-400.

175. Gao, N., Su, Y., Min, J., Shen, W. and Shi, W. (2010) Transgenic tomato overexpressing ath-miR399d has enhanced phosphorus accumulation through increased acid phosphatase and proton secretion as well as phosphate transporters. *Plant and soil*, **334**, 123-136.

176. Sims, J., Simard, R. and Joern, B. (1998) Phosphorus loss in agricultural drainage: Historical perspective and current research. *Journal of Environmental Quality*, **27**, 277-293.

177. Stevenson, F.J. (1994) *Humus chemistry: genesis, composition, reactions*. John Wiley & Sons.

178. Sîrbu, C., Cioroianu, T. and Rotaru, P. (2010) About the humic acids and thermal behaviour of some humic acids. *Physics AUC*, **20**, 120-126.

179. Environmental Protection (2013) "Biochar"more effective, cheaper at removing phosphate from water". http://eponline.com/articles/2011/05/18/biochar-more-effective-cheaper-at-removing-phosphate-from-water.aspx.

180. Vassilev, N., Martos, E., Mendes, G., Martos, V. and Vassileva, M. (2013) Biochar of animal origin: a sustainable solution to the global problem of high-grade rock phosphate scarcity? *Journal of the Science of Food and Agriculture*, **93**, 1799-1804.

181. Rodríguez, H. and Fraga, R. (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology advances*, **17**, 319-339.

182. Malboobi, M.A., Owlia, P., Behbahani, M., Sarokhani, E., Moradi, S., Yakhchali, B., Deljou, A. and Heravi, K.M. (2009) Solubilization of organic and inorganic phosphates by three highly efficient soil bacterial isolates. *World Journal of Microbiology and Biotechnology*, **25**, 1471-1477.

183. 183. Malboobi, M.A., Behbahani, M., Madani, H., Owlia, P., Deljou, A., Yakhchali, B., Moradi, M. and Hassanabadi, H. (2009) Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. *World Journal of Microbiology and Biotechnology*, **25**, 1479-1484.

184. Khan, A.A., Jilani, G., Akhtar, M.S., Naqvi, S.S. and Rasheed, M. (2009) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *J Agric Biol Sci*, **1**, 48-58.

185. Mishra, D., Rajvir, S., Mishra, U. and Kumar, S.S. (2013) Role of bio-fertilizer in organic agriculture: a review. *Research Journal of Recent Sciences ISSN*, **2277**, 2502.

186. Mishra, D.J., Rajvir, S., Mishra, U.K. and Kumar, S.S, (2013) Role of bio-fertilizer in organic agriculture: a review. *Res. J. Recent Sci.,* **2(ISC-2012)**, 39-41.

187. Chen, Y., Rekha, P., Arun, A., Shen, F., Lai, W.-A. and Young, C. (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied soil ecology*, **34**, 33-41.

188. Brahmaprakash, G. and Sahu, P.K. (2012) Biofertilizers for sustainability. *Journal of the Indian Institute of Science*, **92**, 37-62.

189. Richardson, A.E., Barea, J.M., McNeill, A.M. and Prigent-Combaret, C. (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and soil*, **321**, 305-339.

190. Greiner, R. and Konietzny, U. (2006) Phytase for Food Application. *Food Technology & Biotechnology*, **44**, 125-140.

191. Mullaney, E.J. and Ullah, A.H. (2007) Phytases: attributes, catalytic mechanisms and applications. In: Turner, B. L., Richardson, A. E., andMullaney E. J. (ed.), *Inositol phosphates: Linking agriculture and the environment*. CAB INternational Press. London, UK. pp. 97-110.

192. Dodor, D.E. and Tabatabai, M.A. (2003) Effect of cropping systems on phosphatases in soils. *Journal of Plant Nutrition and Soil Science*, **166**, 7-13.

193. McGrath, J.W., Wisdom, G.B., McMullan, G., Larkin, M.J. and Quinn, J.P. (1995) The Purification and Properties of Phosphonoacetate Hydrolase, a Novel Carbon-Phosphorus Bond-Cleavage Enzyme from Pseudomonas Fluorescens 23F. *European Journal of Biochemistry*, **234**, 225-230.

194. Ohtake, H., Wu, H., Imazu, K., Anbe, Y., Kato, J. and Kuroda, A. (1996) Bacterial phosphonate degradation, phosphite oxidation and polyphosphate accumulation. *Resources, conservation and recycling*, **18**, 125-134.

195. Arpana, N., Kumar, S. and Prasad, T. (2002) Effect of seed inoculation, fertility and irrigation on uptake of major nutrients and soil fertility status after harvest of late sown lentil. *Journal of Applied Biology*, **12**, 23-26.

196. Hodge, A., Berta, G., Doussan, C., Merchan, F. and Crespi, M. (2009) Plant root growth, architecture and function. *Plant and Soil*, **321**, 153-187.

197. Pinton, R., Varanini, Z. and Nannipieri, P. (2007) *The rhizosphere: biochemistry and organic substances at the soil-plant interface*. CRC press.

198. Mehrvarz ,S. and Chaichi, M,R. (2008) Effect of Pi solubilizing microorganisms and phosphorus chemical fertilizer on forage and grain quality of Barely (*Hordeum vulgare* L.). *Amer-Eurasian J Agri & Environ Sciences.,* 3, 855-860.