Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review

Sonal Srivastava1,2, Jasvinder Kaur1,3, Vandana Anand1,2, Vidisha Bist1,2, Pallavi Singh1, Satyam Rastogi1, Sumit Yadav1, Suchi Srivastava1,2,*

DOI: 10.18811/ijpen.v8i01.03

ABSTRACT
Phosphorus (P) is an essential macronutrient, crucial for intensification of agricultural production. Although P is a vital nutrient for all life forms but in natural ecosystem it is least available owing to its high reactive nature. Plants have developed diverse adaptive strategies to improve Pi acquisition and utilization under inadequate conditions. They undergo numerous modifications at morphological, physiological, and biochemical level to maintain P homeostasis under deplete conditions of P. In last decades, notable development has been made in utilizing microbes for regulating P supply in soil solution. In this review, we focused on the understanding of P uptake from soil, mobilization within plants, phosphate starvation responses and efficacy of microorganisms in facilitating external P to plants. Moreover, other metabolism in microorganism viz., phosphate accumulation is an important trait of microbes to maintain labile P in soil solution. Application of phosphate accumulating microbes is well known in enhanced biological phosphorus removal (EBPR) system and heavy metal remediation. This review also discussed the phosphate accumulation mechanism in microbes and their importance in regulating P supply in soil. Current knowledge and further exploration of mechanism involved by P accumulating microbes may offer better insight of the regulatory events controlling P homeostasis. It will be beneficial for developing more efficient sustainable technologies to augment P use efficiency in soil for better plant growth promotion and nutrient starvation alleviation.

Keywords: Phosphorus; Phosphate starvation response; Phosphate transporters; Phosphate solubilization; Phosphate accumulation.

INTRODUCTION
Phosphorus (P) is the second most important macronutrient for plant growth and development (Sharma et al., 2017). It is a vital component of cell membrane and necessary for photosynthesis and energy creation (Vance et al., 2003). However, P is least available in soil due to its highly reactive nature. According to earlier reports, 40% of land across the world is P deficient, which leads to reduce crop yield in 30-40% of arable land (Vance et al., 2003).

In plants, inorganic P (Pi) is involved in an array of metabolic processes, while its limited availability immensely affects plant physiology, metabolism and crop performance. Plant involves multiple adaptive mechanisms to regulate internal Pi use and maximize external Pi acquisition under starved conditions. Accumulation of sugar, anthocyanins, and other secondary metabolites in plants under Pi stressed condition facilitate the recycling of substantial amount of Pi from phosphorylated precursors (Leong et al., 2018; Pant et al., 2015). Plants also induce secretion of phosphohydrolases, organic acids, phosphatases, and phenolic compounds to mobilize P from different organic and inorganic source in soil (Srivastava and Srivastava, 2020). Pi deficiency predominantly affects root system architecture (RSA), which involves attenuated primary root growth and enhanced lateral branching to promote Pi sensing and acquisition (Xu et al., 2020). Redesigning of RSA occurs through involvement of hormonal signaling network such as auxin and ethylene. Regulated uptake and subsequent transport of Pi throughout the plant is mediated by different transport systems which enable Pi to move across different plant membranes.

A significant amount of P fertilizer is applied to agricultural land (Scheible and Rojas-Triana, 2018), whereas P use efficiency in the agricultural field is low (15–20%) which indicates lower availability of P to the plants. Root associated microbiota play a crucial role in improving nutrient status of soil. Rhizospheric microbes are key component of P cycle and play a significant role in mediating P availability to the plants (Richardson and Simpson, 2011). They involve different mechanisms viz., increasing root growth, alteration in sorption equilibria, mineralization and solubilization of unavailable P by inducing their metabolic processes (Bargaz et al., 2018; Ryan et al., 2001; Khan et al., 2014). Soil microbes are known to accumulate phosphate from soil in the form of polyphosphate, a linear polymer of orthophosphates linked through high energy phosphoanhydride bond which serve as a source of inorganic P under starvation condition (Rao et al., 2009). Enzymes responsible for synthesis of polyP is
polyphosphate kinase (ppk) which catalyzes the conversion of terminal P of ATP/GTP to polyp and exopolyphosphatase (ppx) hydrolyses the terminal P of polyP chain to release Pi (Dhir, 2019). This review recapitulates P availability in soil, its transportation and starvation responses in plants. Additionally, we also summarize role of soil microflora in improving P availability to the plants and discuss new microbial trait which can be beneficent to plants in phosphate starved conditions.

**Impact of Phosphorus Limitation in Plants**

Phosphate deficiency is a common nutritional stress to the plants. It has been reported to alter the photosynthetic apparatus of plant involving reduced carbon dioxide assimilation, photoinhibition of photosystem II and downregulation of photosynthesis-related genes (Hernández and Munné-Bosch, 2015). Low P limits the concentration of orthophosphates in stroma of chloroplast and inhibit ATP synthase activity which causes restricted ATP production leading to reduced CO₂ fixation (Carstensen et al., 2018). In addition to reduced CO₂ assimilation, Pi deficiency also reduces stomatal conductance in plants (Zhang et al., 2014). Reduced assimilation of CO₂ is associated with different biochemical limitations viz. RuBP regeneration or carboxylation efficiency and mesophyll limitation to CO₂ diffusion (Singh et al., 2013; Zhang et al., 2014). Photoassimilates partitioning in plants is also affected due to altered metabolism under Pi starved condition such as higher accumulation of starch in chloroplast and reduced sucrose, fructose and glucose in cytosol (Nelson et al., 2007; Hernandez and Munné-Bosch, 2015). To counteract photo-oxidative stress, plants have evolved a wide range of mechanisms such as formation of reactive oxygen species (ROS) and their subsequent quenching (Pintó-Marijuan and Munné-Bosch, 2014). Plants have developed complex antioxidant defense mechanisms against these radicals, which involve different enzymes such as ascorbate peroxidase, catalase, and superoxide dismutase to prevent lipid peroxidation and maintain the level of ROS in photosynthetic membrane (Kruk et al., 2014; Miret and Munné-Bosch, 2015).

**Phosphate Starvation Responses in Plants**

Plants evolve a complex range of morphological, physiological and biochemical adaptations under phosphate deprived conditions collectively known as phosphate starvation response (PSRs) (Fig. 1). These responses enhance the acquisition, distribution and mobilization of P in plants under starved conditions. Major modulations in plants grown under low Pi conditions are discussed below:

**Modification in root system architecture**

Under low Pi condition plants adapt different strategies to increase the P acquisition which leads to the morphological and architectural changes in roots as well as in root hair formation (Abel et al., 2002). Roots are the major site for entry of P in the plants and the remodeling in root architecture is important for efficient nutrient uptake. Modification in root architecture mainly involves the reduced primary and increased secondary root growth, enhanced root hair length, density and reduced root gravitropism which altogether result in increase in surface area of root for better Pi acquisition (Desnos, 2008; Rouached et al., 2011). In support of above hypothesis under P deficiency conditions dense proteoid root formation was observed in white lupin (Lupinus albus L.) which showed increased exudation of organic acids viz. citric and malic acid (Udhe-stone et al., 2003). Phosphate starvation mediated change in root morphology and development results in increased root to shoot ratio, number of lateral roots and decreased root diameter (Bustos et al., 2010).

**Alteration in Root Exudation Pattern**

At physiological and biochemical level, plants stimulate the production and release of organic anions in soil to solubilize unavailable inorganic P. To recycle Pi from organic pool, plants release enzymes viz. phosphodiesterases, phosphatases (acid phosphatase, alkaline phosphatase, purple acid phosphatases), ribonucleases and nucleases (Plaxton and Tran, 2011; Scheible and Rojas-Triana, 2018). Phosphate starvation induced release of nucleases, phosphodiesterase and phosphatases degrades extracellular nucleic acids contained in decaying organic matter, thereby releasing Pi which is subsequently taken by the plants through high affinity phosphate transporters (Plaxton, 2004).

**Modulation in Plant Metabolic Activity**

Plant cells themselves contain bulk of organically bound P in the form of nucleic acid, phospholipids and phosphorylated metabolites (Plaxton & Tran, 2011; Lambers et al., 2011). This form of P is tightly controlled by the plants and are remodelised during limitation condition (Scheible and Rojas-Triana, 2018). Other responses in plants involve accumulation of sugars, anthocyanin, starch, triacylglycerols and amino acids (Pant et al., 2015; Morcuende et al., 2007; Hammond & White, 2008). Under P starved condition, plants replace phospholipids from sulfolipids and galactolipids to remobilize the P from internal sources. Metabolic profiling shows the reduction of phosphorylated compounds and enhanced carbohydrates, amino acids, nitrogenous compounds and organic acids under P deprived conditions. Santosh et al. (2018) showed increased accumulation of carbohydrates (glucose and fructose) and amino acids in root and leaves of tea plants grown under P deprived condition. Upregulated expression of anthocyanidin reductase, leucoanthocyanidin dioxygenase and glycosyltransferases in phosphate deprived conditions has also been shown. The increased accumulation of disaccharides and trisaccharides such as sucrose, maltose, raffinose, lactose, and 6-kestose in the shoots of P-deficient plants emphasizes hindrance in the glycolysis pathway, according to metabolite profiling of two different P-sensitive and resistant varieties of Zea mays studied under P starvation conditions (Ganie et al., 2015). Plants begin to adapt to low P availability by modifying gene expression as well as metabolic pathways (Thibaud et al., 2010). Li et al., 2007 found the downregulation of ATP-dependent RNA helicase and upregulation of adenosylsuccinate synthetase, key enzyme involved in AMP biosynthesis. They also reported the expression of genes involved in cell cycle, transcriptional regulation, phytohormone biosynthesis and signal transduction which play significant role in plant adaptation during P starvation condition by P homeostasis.
Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review

Phytohormone Signaling

Phytohormone signaling under low Pi conditions triggers an array of processes in plants. Auxin is regulatory phytohormones classically entailed in plants’ growth and development. Furthermore, multiple studies have shown that auxin plays a role in plant response to abiotic stressors by altering the plant’s architecture and anatomy (Chien et al., 2018). These stress induced morphogenic responses include reorganization of growth pattern by altering cell differentiation, cell elongation inhibition and localized cell division activation (Potters et al., 2007). In response to low Pi, modulation in auxin results in alteration in root system architecture. Wang et al. (2014) reported that primary root growth in auxin responsive factor (arf) gene mutants (osarf12 and osarf12/25) was more responsive as compared to wild type under low Pi conditions. In addition to root remodeling, auxin responsive factor 7 and 19 are responsible for induced expression of key regulatory component to Pi starvation i.e. phosphate starvation response1 (PHR1). Expression of PHR1 in A. thaliana is positively regulated by auxin signaling in plants during Pi deprived condition (Huang et al., 2018).

Strigolactones (SLs) are a signaling molecule between root to shoot during low Pi conditions. Level of SLs magnify under Pi starved condition along with its transport from root

Fig. 1: Physiological and morphological alteration in plants grown under phosphate starved condition.

Fig. 2: Schematic representation of phosphorus dynamics in soil.
to aboveground plant tissues to control shoot architectural response under stress (Kohlen et al., 2011; Foo et al., 2013). Increased exudations of strigolactones promote beneficial plant-microbe interaction under Pi deficient condition; results improved Pi acquisition by plants (Umehara et al., 2010; Foo et al., 2013). Revalska et al. (2018) demonstrated that auxin-SLs interaction upregulate auxin transport which results in enhanced number of lateral roots leading to Pi stress alleviation in Medicago truncatula. Study also reported upregulation of auxin influx transmembrane carrier MutLAX3 and two SLs associated genes MtMAX2 and MtMAX3 in plants grown under Pi deprived condition.

Repressed synthesis of gibberellic acid (GA) in response to low Pi participates in phosphate stress responses in A. thaliana (Devaiah et al., 2009; Jiang et al., 2007). Application of GA or mutation in DELLA proteins (key component of GA signaling) lowers the phosphate stress responses in both root and shoot under Pi deficient condition. However, enhanced level of DELLA proteins triggers the anthocyanin accumulation in leaves in response to phosphate deprivation (Jiang et al., 2007). Increased DELLA proteins under stress are also linked to reduced ROS production and active defence network in plants (Achard et al., 2009). Study also showed that delayed ROS induced cell death owing to an elevated quantity of DELLA proteins in ga1-3 mutant (deficient in GA synthesis). Cytokinin (CK) is known for its diverse actions in plant growth, development, nutrient homeostasis and stress response (Pavlu et al., 2018). In plants, Pi deprivation results in downregulation of IPT3 gene involved in CK biosynthesis leading to its repressed synthesis along with downregulation of CK signaling component (Hirose et al., 2008). Repression of CK upregulate the expression of Pi transporters and mediate the remobilization of Pi within plants (Pavlu et al., 2018). On the contrary, expression of ethylene biosynthesis genes gets upregulated under low Pi conditions (Zhang et al., 2014). However, Kim et al. (2008) showed reduced ethylene level in tomato under Pi starved condition. Ethylene in plants is involved in modulation of root architecture along with remobilization of Pi from source to sink by inducing the secretion of phosphatases, however, anthocyanin accumulation is negatively correlated with ethylene production (Chapin and Jones, 2009). Like cytokinins, ethylene modulates ROS induced cell death together with ABA, salicylic and jasmonic acid (Hernández and Munné-Bosch, 2015) (Fig.2; Table 2).

**Phosphorus Acquisition and Transportation in Plants**

Efficient uptake and translocation of Pi is essential for normal functioning in plants. They acquire high and low affinity transportation mechanism for Pi uptake depending on the concentration of available Pi (orthophosphate (H₂PO₄⁻ and HPO₄²⁻)) in soil (Misson et al., 2004; Smith et al., 2003). The transportation of Pi via high affinity transporters is enhanced during P starvation conditions while low affinity transportation express constitutively in plants (Wu et al., 2011; Raghothama and Karthikeyan, 2005). Kinetic characterization of Pi translocation from soil to plant suggests that high affinity transport operates when P is available in low micromolar range whereas low affinity at higher concentration i.e. millimolar range (Chiou et al., 2001; Table 1: Phosphate transporters in Arabidopsis thaliana

<table>
<thead>
<tr>
<th>Localization</th>
<th>Phosphate transporters</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHT1</td>
<td>Plasma membrane</td>
<td>ATPH1;1 &amp; ATPH1;4 High-affinity phosphate transporters, mediate Pi acquisition in both low- and high- P environments</td>
<td>Li et al., 2019; Shin et al., 2004; Misson et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH1;2 &amp; ATPH1;3 Highly expressed under low-Pi conditions; contribute to Pi acquisition</td>
<td>Shin et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH1;5 Mobilize Pi from older leaves to young organs and roots; Pi homeostasis</td>
<td>Nagarajan et al., 2011; Smith et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH1;6 Induce during Pi transportation; Pi uptake and translocation</td>
<td>Lapis-Gaza et al., 2014;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH1;8 &amp; ATPH1;9 translocation of orthophosphate root to shoot</td>
<td>Lapis-Gaza et al., 2014;</td>
</tr>
<tr>
<td>PHT2</td>
<td>Plastids</td>
<td>ATPH1;2 High affinity transporter, Pi uptake and translocation</td>
<td>Rausch et al., 2004; Roch et al., 2019</td>
</tr>
<tr>
<td>PHT3</td>
<td>Mitochondria</td>
<td>ATPH3;1, ATPH3;2 &amp; ATPH3;3 Pi uptake into mitochondria</td>
<td>Hamel et al., 2004; Roch et al., 2019</td>
</tr>
<tr>
<td>PHT4</td>
<td>Plastids and Golgi apparatus</td>
<td>ATPH4;1 Phosphate compartmentation in chloroplast and ATP synthase mediates Na⁺-dependent Pi transport in root plastids of A. thaliana</td>
<td>Guo et al., 2008; Karlsson et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH4;2</td>
<td>Miyaji et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH4;4 Ascorbate transportation in A. thaliana</td>
<td>Irigoyen et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH4;6 Transport Pi out of golgi lumen under low Pi condition for Pi recycling</td>
<td>Cubero et al., 2009</td>
</tr>
<tr>
<td>PHT5</td>
<td>Tonoplast/ vacuolar membrane</td>
<td>ATPH5;1 Pi import to vacuole, prevents accumulation of Pi in cytoplasm</td>
<td>Liu et al., 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATPH5;3 Pi partitioning from cytosol to vacuole</td>
<td>Luan et al., 2019</td>
</tr>
</tbody>
</table>
Huang et al., 2011). Pi is symplastically translocated from the roots to the xylem, where it is distributed further via another type of transporter-like protein (Wu et al., 2011). In P-deficient and P-sufficient plants, P content in xylem ranges from 1 mm to 7 mm respectively (Mimura et al., 1996). The translocation of Pi in plants from roots to young leaves grown under P-enriched conditions occurs via xylem. Pi supply in P deficient plant is restricted from root to shoot and P is supplemented to new leaves and shoots from old leaves. The whole process involves the breakdown of organic P and depletion of Pi stored in old leaves.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Stress</th>
<th>Plant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter ludwigi; Bacillus megaterium</td>
<td>Drought</td>
<td>Alfalfa (Medicago sativa)</td>
<td>Kang et al., 2021</td>
</tr>
<tr>
<td>Bacillus licheniformis; Enterobacter asburiae</td>
<td>Saline</td>
<td>Quinoa (Chenopodium quinoa)</td>
<td>Mahdi et al., 2021</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa; Bacillus subtilis</td>
<td>Insoluble P source (TCP)</td>
<td>Rice (Oryza sativa)</td>
<td>Gupta et al., 2021</td>
</tr>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>Salinity and drought</td>
<td>Pepper (Capsicum annuum)</td>
<td>Kazerooni et al., 2021</td>
</tr>
<tr>
<td>Enterobacter ludwigi</td>
<td>Cadmium</td>
<td>Rice (Oryza sativa)</td>
<td>Adhikari et al., 2021</td>
</tr>
<tr>
<td>Bacillus aryabhattai; Arthrobacter woluwensis</td>
<td>Salinity</td>
<td>Soybean (Glycine max L.)</td>
<td>Khan et al., 2021</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>Phosphate starved salinity stress</td>
<td>Arabidopsis thaliana</td>
<td>Srivastava and Srivastava, 2020</td>
</tr>
<tr>
<td>Pseudomonas pseudoalcaligenes; Bacillus subtilis</td>
<td>Salinity</td>
<td>Soybean (Glycine max L.)</td>
<td>Yasmeen et al., 2020</td>
</tr>
<tr>
<td>Streptomyces laurentii; Penicillium sp.</td>
<td>Drought</td>
<td>Great millet (Sorghum bicolor L.)</td>
<td>Kour et al., 2020a</td>
</tr>
<tr>
<td>Pseudomonas libanensis</td>
<td>Drought</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>Kour et al., 2020b</td>
</tr>
<tr>
<td>Pseudomonas sp.; Bacillus simplex</td>
<td>Insoluble P source</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>Rezakhani et al., 2019</td>
</tr>
<tr>
<td>Enterobacter aerogenes; Pantoea sp.; Enteriobacter sp</td>
<td>Calcareous soils</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>Mohamed et al., 2019</td>
</tr>
<tr>
<td>Pseudomonas pseudoalcaligenes; Pseudomonas putida</td>
<td>Salinity</td>
<td>Coriander (Coriandrum sativum)</td>
<td>Al-Garni et al., 2019</td>
</tr>
<tr>
<td>Bacillus cereus; Bacillus subtilis; Bacillus circulans</td>
<td>Salinity</td>
<td>Arabidopsis thaliana</td>
<td>Bokhari et al., 2019</td>
</tr>
<tr>
<td>Aneurinibacillus aneurinilyticus Paenibacillus sp.</td>
<td>Salinity</td>
<td>French bean (Phaseolus vulgaris)</td>
<td>Gupta and Pandey, 2019</td>
</tr>
<tr>
<td>Pseudomonas fluorescens; Azosprillum brasiliense</td>
<td>Salt</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>Kadmiri et al., 2018</td>
</tr>
<tr>
<td>Bacillus megaterium; Staphylococcus haemolyticus; Bacillus licheniformis</td>
<td>Metal resistant</td>
<td>Mung beans (Vigna radiata)</td>
<td>Biswas et al., 2018</td>
</tr>
<tr>
<td>Funneliformis mosseae; Pseudomonas fluorescens, Bacillus subtilis; Paenibacillus sp.</td>
<td>Water deficit</td>
<td>Maize (Zea mays)</td>
<td>Ghorchiani et al., 2018</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>Alkaline conditions</td>
<td>Cotton</td>
<td>Ahmad et al., 2018</td>
</tr>
<tr>
<td>Acinetobacter sp.; Bacillus sp.</td>
<td>Herbicide (glyphosate)</td>
<td>Chickpea (Cicer arietum)</td>
<td>Shahid and Khan, 2018</td>
</tr>
<tr>
<td>Bacillus polymyxa</td>
<td>Salt</td>
<td>Berry</td>
<td>Joe et al., 2016</td>
</tr>
</tbody>
</table>

Table 2: Phosphate solubilizing bacteria mediated stress alleviation in plants
Synchronized coordination of proteins related to Pi transport activities is required for efficient uptake and mobilization of Pi in plants. The uptake of phosphorus from soil mostly relies on the PHT1 transporters (Nussaume et al., 2011). Besides, its expression in other plant organs and tissue types also affirm its participation in internal distribution of Pi (Hamburger et al., 2002; Mudge et al., 2002). Phosphate transporters of Glycine max (GmPT1 and GmPT2) belonging to PHT1 family reported to be expressed in root and shoot under both phosphate enriched and deficient condition, confirming their role in Pi translocation within plants (Wu et al., 2011). Under Pi sufficient conditions, PHT1:5 is only expressed in shoot required for Pi remobilization. However, during limiting conditions it is expressed in both root and shoot tissue, thereby, playing pivotal role in Pi distribution (Nagarajan et al., 2011). Similarly an enhanced expression of PHT1:2 and PHT1:6 was found under Pi deficient condition in both root and shoot tissues in rice (Ai et al., 2009). Besides PHT1, proteins having SPX domain linked to their N-terminal are also known to be implicated in Pi sensing and transportation in plants (Secco et al., 2012). Arabidopsis PHO1 (encodes a protein of the SPX-EXS subfamily) localized in the root pericycles determines the Pi loading in xylem and any impairment in PHO1 prevents translocation of Pi from root to shoot (Hamburger et al., 2002; Arpat et al., 2012). Similar to AtPHO1, three genes OsPHO1;1, OsPHO1;2, and OsPHO1;3 are phylogenetically associated with SPX-EXS subfamily in rice (Secco et al., 2010). Among these three genes OsPHO1;3 is mostly induced under Pi starvation condition and OsPHO1;2 have been demonstrated as functional ortholog of AtPHO1 (Secco et al., 2010). Different proteins involved in ubiquitination process mediate transportation of Pi in both sufficient and starved condition in plants. Different phosphate transporters in A. thaliana are enlisted in Table 1.

Soil microorganisms regulate the expression of genes involved in nutrient uptake and plant growth promotion. Different microbes are reported to play critical role in modulation of phosphate transporters in plants under stress conditions. Srivastava et al. (2018) reported higher expression of phosphate transporter OsPT11 in rice plant subjected to arsenic stress in presence of Chlorella vulgaris. Bacterial strain P. putida modulated the expression of PT1 and PHO2 gene in A. thaliana to regulate Pi supply under phosphate starved-salinity stress condition (Srivastava and Srivastava, 2020). Another study reported the higher transcript of P transporters (Pht1 and PT2-1) in AMF and PGPR inoculated plants (Saia et al., 2015).

**Microbe mediated Uptake in plants**

P dynamics in soil are influenced by a variety of biological (immobilization-mineralization) and physicochemical (sorption-desorption) processes (Tian et al., 2021). Although soil is a significant reservoir of P, much of it gets sorbed onto soil particles or erodes and leaches, making it unavailable to plants (Rittman et al., 2011). Generally, soil contain between 100-3000 kg/hectare of P but the concentration of bioavailable form of phosphorus in soil is very low (1.0 mg/kg) (Tairo and Ndakidemi, 2013; Bünemann and Condron, 2007).

Availability of P is substantially influenced by soil pH i.e. 6.0 to 7.5 pH favors the maximum availability of P in soil (Zhang et al., 2010; Sharma et al., 2013). P solubility diminishes in acidic soil due to P fixation in the form of aluminum/iron phosphates. Large surface areas of Fe/Al oxides offer, large number of adsorption sites which further enhance with increasing ionic strength (Shen et al., 2011). Further reactions also result in occlusion of P in nanoparticles that occurs in Fe/Al oxides, resulting in P unavailability to plants (Arai and Sparks, 2007). Moreover, p unavailability also occurs due to precipitation reactions (Devau et al., 2010; Shen et al., 2011). Phosphate precipitates with calcium, resulting in dicalcium phosphate which is available to the plants. Furthermore, dicalcium phosphate is further transformed; more stable forms of P such as octocalcium phosphate and hydroxyapatite (HA) are formed, which are less accessible to plants at alkaline pH. Hydroxyapatite typically accounts for 50% of total Pi in calcareous soil and their solubility increase with acidification of soil (Wang and Nancollas, 2008).

Soil is also enriched with organic P which usually accounts for 30 to 65% of total soil P, other than inorganic pool. In soil, organic pool exists in two form viz. stable (inositol phosphates and phosphonates) and active forms (organic polyphosphates and mono/diester of orthophosphates) (Turner et al., 2002; Condon et al., 2005). Organic P undergoes mineralization by the action of phosphatase enzymes released by soil microorganisms or plant roots. The overall bioavailability of P in the soil is substantially influenced by organic P biotransformation (Turner et al., 2007). All these reactions occurring in the soil is highly influenced by few factors such as soil pH, moisture, temperature, surface physicochemical properties (Shen et al., 2011).

Microorganisms are integral component of soil phosphorus cycle as they broadly affect the availability of phosphorus to the plant by their transformation. Soil microorganisms has tendency to solubilize and mineralize the phosphorus from their inorganic and organic pool respectively, through release of organic acids and phosphatases (Sharma et al., 2013). These processes dominantly occur in rhizospheric region of plants and also help in improving the plant nutrition. Mechanism of microbe mediated Pi availability in soil is summarized below:

**Phosphate Solubilization by Soil Microorganism**

Phosphate fertilizers, including rock phosphate ores such as fluorapatite and francolite, are bonded in the form of calcium phosphate in alkaline conditions and are readily unavailable for plants (Mohammadi, 2012). Numerous microorganisms in soil involve P-solubilization activity and constitute for 1-50% and 0.1-0.5% for bacteria and fungus of the total soil population, respectively (Sharma et al., 2013). P solubility in soil increases by release of organic acids and lowering of soil pH and dissociation of calcium bound phosphates (Villegas and Fortin, 2002; Fankem et al., 2006). P-solubilizing potential of microbes is significantly influenced by the availability of nutrient (carbon and nitrogen source) and temperature (Ahuja et al., 2007). These insoluble form of phosphate after solubilization form soluble complexes with metal ions, competing with P for adsorption sites on soil and by chelating cations bound to P (Wakelin et al., 2004; Adeleke et al., 2017).

**Phosphate Mineralization by Soil Microorganism**

P mineralization is also known as organic P solubilization from the organic pool of soil which plays an imperative function in phosphorus availability in farming system. Organic P
Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review

constitutes upto 4–90% of total soil P (Khan et al., 2009) and is released in the soil by the act of phosphatases (Srivastava and Srivastava, 2020). Phosphatases are released in the soil from both plant roots and microbes, but the major portion of it is derived from microbial population. Phosphatase enzymes are characterized into two types, i.e., acidic and alkaline depending on their pH optima (Turner, 2010). Typically alkaline phosphatases dominate in alkaline and neutral soil, whereas acidic phosphatases act in acidic soil (Sharma et al., 2013). The phoasoester and phosphoanhydride bonds of organic matter are dephosphorylated by the action of phosphatase enzyme, which releases inorganic P from the organic pool (Nannipieri et al., 2011). Phosphatases of microbial origin has greater affinity of P mineralization as compared to release from plants (Tarafdar et al., 2001) and upto 60% of the organic P is reported to be hydrolyzed by phosphatases (Bunemann, 2008). Besides, there are other enzymes which are involved in P mineralization viz. phytases, phosphonoates and C–P lyases.

Phytate is a key component of organic P in soil and is a major form of P stored in plant seeds and pollen. Role of microorganisms in regulating the mineralization of phytate in soil has been demonstrated by the assessment of rhizospheric soil after plant growth, which showed loss of phytase-labile P as compared to control conditions (Richardson et al., 2009, Richardson and Simpson, 2011). Phosphonatases and C–P lyases cleave the C–P bond of organophosphonates (Rodriguez et al., 2006).

Microbial Immobilization of Phosphorus

Role of microbial biomass in phosphate cycling of soil is an important mechanism to maintain labile form of P within soil. P immobilized within microbial biomass is generally equivalent or greater than that of plants. Microbes have potential to accumulate approx. 2 to 10% of total soil P within their biomass which may exceed to 50%, depending on the soil type (Achat et al., 2010). Microorganisms and plants both compete for available orthophosphate from soil solution and microbes immobilize significant amount of P which temporarily becomes unavailable to the plant. Microbial form of P are prevented from soil reactions and over a long period of time immobilized P becomes available to the plants, thereby, P immobilization is important for maintaining labile P pool in soil (Olander and Vitousek, 2004, Richardson and Simpson, 2011). The release of microbial P occurs during microbial biomass turnover and it depends on several environmental factors viz. soil moisture, fertilizer application and season (Bunemann et al., 2009; Zhang et al., 2018). Achat et al. (2010) in his study show faster cycling of ~80% of microbial P pool in less than 10 days in a forest soil dominant in organic P. In a natural ecosystem, this turnover can range from 10 days to one year(Bunemann et al., 2009).

Polyphosphate: An Accumulated P within Microbial Biomass

Microorganisms accumulate phosphorus in the form of inorganic polyphosphate (polyP) which are most ancient and conserved molecule composed of linear molecules of orthophosphate linked with high energy phosphoanhydride bond (Brown and Kornberg, 2004; Gray et al., 2014). It was first discovered by Arthur Meyer in microbes and named volutin after stained to pink color by blue dyes in 1904, further identified as polyP by J. M. Wiame in 1947. Polyphosphates is found in all tree domains of life (archaea, bacteria and eukarya) (Varela et al., 2010). They are present in plants, animals, fungus, bacteria but the highest amount of polyP is found in microbial cells. These storage granules of energy and phosphatatedegrees to release energy for the synthesis of amino acids, sugars, lipids and nitrogenous bases. P released from polyP chain is reported to play a vital role in gene regulation (Achbergerová and Nahálka, 2011). Polyphosphate granules are also involved in improving the cell’s resilience to a variety of stressors, including heat shock, heavy metal exposure, peroxide, and starvation (Dahl et al, 2015) as microorganisms are highly dependent on environmental conditions for their survival (Kulaev and Kulakovskaya, 2000).

In bacteria, polyP accumulates in granules and in eukaryotes in acidocalcisomes (Docampo et al., 2005, Seufferheld et al., 2008). PolyP are engaged in a variety of functions in microorganisms, including phosphate and energy storage, enzyme activity modulation, formation of membrane channel, sequestration and storage of cation, and gene activity control (Vagabov et al., 2008). Under P starvation conditions the accumulated P serves as a source of P and promote biosynthesis of nucleic acid and phospholipids (Mühlroth et al., 2017). Poly P also plays a key role in motility, competence, biofilm formation and virulence. The disruption in ppk gene causes susceptibility to different stress conditions such as heat, starvation, and oxidative stress (Nikel et al., 2013; Alacantara et al., 2014).

Polyphosphate Metabolism in Microorganism

PolyP metabolism in microorganisms is a two-step process, i.e., synthesis and its degradation. Detailed mechanism is discussed below:

Synthesis of Polyphosphate Chain

Polyphosphates are abundantly found in yeast and bacteria, comprises of long-chain (upto 1000 residues) of polyphosphates, whereas, in a mammalian cell, short length of polyphosphate chain is found (Azevedo and Saiardi, 2014). The polyphosphate metabolism in prokaryotes and eukaryotes differs. In bacteria, the synthesis of polyP is regulated by polyphosphate kinase (ppk), which catalyzes the polymerization of the terminal phosphate of ATP/ GTP in polyphosphates to extend the polymer. Besides, ppk gene is also found in Dictyosteliida amoebae termed as DdPPK1 assuming that being a bacteria feeder the gene has been transferred in it through horizontal gene transfer (Docampo et al., 2010; Zhang et al., 2007). Another class of polyphosphate synthesizing enzyme in amoeba has been observed DdPPK2 termed as actin like protein complex. The deletion of PPK1 in D. discoideum doesn’t affect the synthesis of polyP immensely due to presence DdPPK2 (Gomez-Garcia and Kornberg, 2004).

Hydrolysis of Polyphosphate Chain

Degradation of polyP chain in bacteria is catalysed by exopolyphosphatase (pxp), which hydrolyses the terminal Pi from polyphosphate chain under phosphate starvation condition (Varela et al., 2010; Lindner et al., 2009). Therefore,
ppx are involved in maintaining Pi homeostasis in cell (Bru et al., 2017). Both ppx and ppx genes are important for bacterial persistence under varied environmental conditions (Chuang et al., 2015). Inhibition of ppx gene by the alarmon e (p)pppGpp is being reported to enhance the level of polyP in E. coli (Ayrapetyan et al., 2015). Chuang et al. (2015) reported that knockdown of ppx in Mycobacterium tuberculosis resulted in slowed growth, enhanced tolerance to heat and high pH, increased intracellular survival during macrophage infection. Study also showed reduced susceptibility to isoniazid owing to increased cell wall thickness in ppx mutant of M. tuberculosis. In another study, deletion mutant of ppx gene showed defects in motility, nutrient stress survival, biofilm formation, invasion and intracellular survival in Campylobacter jejuni (Malde et al., 2014). However, deletion mutants were tolerant to human complement-mediated killing. Similarly, Shi et al. (2004) reported defect in motility, biofilm formation and sporulation in ppx mutant of Bacillus cereus. In addition, ppx gene in P. aeruginosa is reported to be associated with production of virulence factors such as pyocyanin, pyoverdin, biofilm, rhamnolipids and quorum sensing acyl homoserine lactones involved in both chronic and acute infection (Gallarato et al., 2014).

Application of Phosphate Accumulating Microbes

Polyphosphate accumulating microbes are well known for removal of large amount of P from wastewater through the process of EBPR (Wang et al., 2019). The phosphate accumulating organisms (PAOs) of the bacterial community are enriched in the EBPR treatment system to accumulate significant quantities of polyP in their cells, enhancing biological phosphorus removal from wastewater (Tao et al., 2020). Till now Candidatus Accumulibacter has been considered the most important known PAO in EBPR system (Nielsen et al., 2019), however, Tetrasphaera are present in higher abundance. Based on sequencing methods, genera Dechloromonas and Tessaracoccus are in ample number in the system, while, their role in P removal is low (Albertsen et al., 2015; Stokholm-Bjerregaard et al., 2017). Although the pure culture of Accumulibacter are still not available, but they are frequently enriched in EBPR systems and typically represent 5-20% of bacterial community (He et al., 2008; Nielsen et al., 2010). Tarayre et al. (2017) reported the presence of bacteria belonging to phyla α-Proteobacteria, β-Proteobacteria, and Sphingobacteria in EBPR. Several other bacterial; strains belonging to genera Acinetobacter, Corynebacterium, and Pseudomonas have been reported with the presence of polyphosphate granules of size nearly 100 nm as evident through electron microscopy (Tarayre et al., 2017). Staphylococcus aureus can store up to 93 mM phosphates in their storage granules; it has been recorded to remove up to 81% of phosphate from the polyphosphate accumulating medium. However, the increased glucose source removes 78% of the phosphate, with phosphate absorption of 13.24 mg PO4/g (Sumathi and Vasudevan, 2018). Several studies have reported PAOs mediated removal of heavy metals from tainted systems. Boswell et al. (2001) noted removal of lanthanum from bound heavy metal Lanthanum in a bioreactor by polyphosphate synthesizing and Pi releasing Acinetobacter johnsonii. In addition, Anand and Aayogi (2019) proposed a TBO-based method, which can be helpful in determining the inherent capacities of P accumulation in PAOs and to assess their suitability in controlling hyperphosphatemia occurring due to chronic kidney dysfunction.

P immobilization within microbial biomass is critical mechanism for maintaining P supply in soil solution and regulating its availability to the plants (Richardson and Simpson, 2011; Esberg et al., 2010). Previous study has shown efficient transfer of polyP accumulated within arbuscular mycorrhizal fungi to the plants under phosphate deficient condition (Peppe et al., 2020; Etesami and Jeong, 2021). P. putida characterized for proline, IAA production, phosphatase activity and polyP accumulation alleviated phosphate starved-salinity stress in A. thaliana by modification in root architecture, acid phosphatase activity, hormonal signaling and enhanced P uptake (Srivastava and Srivastava, 2020). Recently, Srivastava et al. (2022) has reported abundance of Pseudomonas genera as high polyP accumulator in soil. Study also reported the salinity stress alleviation potential of polyP accumulating bacteria in A. thaliana. Despite important aspect, studies exploring the efficacy of polyP accumulating microbes in plant growth promotion and stress alleviation is scarce.

Conclusion and Future Perspective

Phosphorus unavailability in soil is major issue resulting in reduced P use efficiency and increased cost of fertilizer. Phosphate solubilizing and mineralizing microbes are an asset to agricultural production in terms of both phosphate availability and stress alleviation in plants. Numerous studies are performed in terms of utilizing P-solubilizing microbes for improved plant growth, however, only limited are accessible commercially. The development of P solubilizing microbes based bioformulations with higher shelf life and efficacy will be boon to agriculture sector to sustain crop productivity and food security.

In soil, phosphate accumulating microbes play a crucial role in P cycling, however, their role in regulating P supply and plant growth promotion is underexplored. In future, it will be enthralling to investigate the correlation of polyP accumulation with other plant growth promotory traits viz. solubilization and mineralization. It will be interesting to explore the detailed mechanism involved during the interaction of polyP accumulating microbes with plants under P deficient conditions. These studies may open new avenues for development of sustainable technologies for maximizing use of unavailable P and reducing chemical input in the soil.

Acknowledgement

So. S is thankful to Department of Science and Technology, India for research fellowship. VB and VA thanks CSIR for awarding senior research fellowship. VB, VA and SS also thank AcSIR for academic support.

References

Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review

International Journal of Plant and Environment, Volume 8 Issue 1 (2022)


Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review


Phosphate Starvation Responses in Plants and Microbe Mediated Phosphorus Recycling in Soil: A Review


