The Planta RBS, Vol.-5, Issue-2, October 2024: 1623 – 1629 // ISSN: 3048:8494
KUMAR *et. al.*, (Series Chapter No. – 180 // ISBN: 978-81-965701-9-4)
https://www.pgrindias.in

MOLECULAR MECHANISMS UNDERLYING PHOSPHATE SENSING, SIGNALING AND ADAPTATIONS IN PLANTS

D. Manoj Kumar[1], T. Srinivas[2], Y. Suneetha[3] and Dungu Vasudeva Reddy[4]

[1]Agricultural Research Station, Ragolu, ANGRAU
[2,3]Regional Agricultural Research Station, Maruteru, ANGRAU
[4]Agricultural Cultural College, Bapatla, ANGRAU
Corresponding E-mail: manojgene7@gmail.com

Abstract

Phosphorus (P) is fundamental nutrient to crop development because they form the basic component of many organic molecules, nucleic acids, proteins, further P plays a pivotal role in local and systemic signaling of disease incidence and triggers the gene regulation to incite systemic disease resistance from plant immune system. Phosphorus (P) is taken up by the plant in phosphate (Pi) form and is utilized to promote tillering, root development, early flowering and ripening. It is also the second most limiting mineral nutrient in almost all soils, and more than 30% of the world's arable land has low P. Phosphorus availability is particularly limiting on highly weathered acid soils of the tropics and subtropics due to its fixation by Al and Fe oxides on the surface of clay minerals. P deficiency under field condition results in stunted plants, reduced tillering ability and flowering. Researchers had identified many underlying mechanisms in plants to utilize and fixed phosphorus in the soil and alternate mechanisms to rely inside the plants.

Key words: Plants, Nutrients, Phosphorus, Sensing, Signaling and Adaptations

Citation: Kumar, D. Manoj, T. Srinivas, Y. Suneetha and Dungu Vasudeva Reddy (2024), Molecular Mechanisms Underlying Phosphate Sensing, Signaling and Adaptations in Plants, *The PLANTA Research Book Series*, 5(2), 1623-1629. www.pgrindias.in

Introduction

Phosphorus (P) is one of the least available of all essential nutrients in the soil and its concentration is generally below that of many other macronutrients. In most soils, the concentration (>2 µM) of available P_i in soil solution is several orders of magnitude lower than that in plant tissues (5–20 mM) (Raghothama, 1999). Global consumption of P_i fertilizer is currently approximately 50 million tons, with a projected annual increase of 20 million tons by 2030 (Cakmak, 2002). For available P in Indian soils, 51 percent of the districts are in the low category, 40 percent are medium and 9 percent in the high P category (Dey *et al.*, 2017).Currently, P deficiency occurs in about 50 per cent of the agricultural soils pertaining to Asia, Africa and South American countries (Lynch, 2011). Choudhury *et al.* (2007) reported that rice yield increased linearly with an increase in soil P content. Fertilizer use has therefore, become extremely important for ensuring food security under present conditions (Vinod and Heuer, 2012). Fertilizer prices are however, increasing due to high energy costs and limited natural resources. Further, the Covid-19 pandemic had plunged nations into a financial crisis with little cross-border trade and drastically curtailed the current

global supply. Therefore, the balanced and sustainable use of P fertilizer is of paramount importance (Shukla *et al.*, 2022). Additionally, limited fossil fuels and fertilizer stocks are available; thus, it's necessary to study the P utilizing mechanisms in plants. In order to adapt to the heterogeneous nutrient availability in soils, plants have developed complex mechanisms to integrate local and systemic sensing and signaling systems for maintenance of cellular nutrient homeostasis, at the whole plant level. Roots perceive fluctuations in extracellular nutrient levels and send signals to the shoot *via.*, the xylem, as a warning of impending limitation in the supply of the particular nutrient. Shoots sense these roots derived nutrient signals and send signals both to the shoot apices and roots, *via.*, the phloem, to regulate P_i uptake, mobilization and redistribution (Zhang *et al.*, 2014).

Local Pi-Sensing Pathways in Yeast and Plants

In yeast, Pho84 serves as both a plasma-membrane (PM)-localized high-affinity Pi transporter and as a Pi-sensing "transceptor." Under high Pi conditions, Pho84 senses high external Pi levels and activates Protein Kinase A (PKA) that then phosphorylates it, leading to ubiquitination and subsequent recruitment of the endocytic pathway for delivery to the vacuole for degradation. Under low external Pi levels, an internal sensor(s) is thought to upregulate expression of PSI genes, leading to enhanced expression of Pho84 and targeting of Pho84 to the PM. In plants exposed to low Pi levels, inputs from an internal sensor(s) andPHT1 family of PM-localized Pi transporters activate PSI genes, leading to higher levels of PHT1 in the PM.

Local Pi Sensing and Signaling Controls Primary Root Development

1. Local Pi sensing and signaling controls primary root development. The stem cell niche (SCN) in the primary root (PR) requires plasmodesmata (PD)-mediated trafficking of transcription factors (TFs), such as SHORT ROOT (SHR), for normal developmental progression.
2. Upon experiencing Pi-limiting conditions in the soil, two important pathways become activated in the PR SCN. The STOP1 pathway activates ALMT1 expression and the subsequent insertion of ALMT1, a malate channel, into the plasma membrane then facilitates malate release into the apoplasm.
3. In parallel, the endoplasmic reticulum (ER)-located PDR2 is inactivated, likely allowing for the intracellular trafficking of LPR1 from the ER to the plasma membrane, where it serves as a cell-wall associated ferroxidase that oxidizes Fe2+ to Fe3+. This enzymatic activity of LPR1 results in Fe3 +accumulation in the apoplasm, where it forms a complex with the released malate to then activate ROS production which triggers callose deposition in SCN cell wall.
4. This process blocks PD trafficking of SHR and other TFs, which leads to PR apical meristem exhaustion. These STOP1-ALMT1 and PDR2-LPR1 signaling pathways also play a role in cell wall stiffening in the root transition zone, leading to inhibition of cell expansion and subsequently root elongation.

Signaling and Transport Systems Involved in Cellular-Level Pi Homeostasis

1. Under Pi-sufficient conditions, PHT1 proteins can be phosphorylated, which then prevents the normal interaction with PHF1, resulting in their retention in the endoplasmic reticulum (ER).

2. Plasma membrane (PM)-localized PHT1 proteins are also recycled by ubiquitination-mediated degradation through receptor-mediated targeting to the vacuole. Fewer PHT1 proteins within the plasma membrane lead to low Pi influx. Tonoplast-located PHT5 transporters function in Pi influx into the vacuole; here, PHT5;1 is the major contributor. Rice OsSPX-MFS1 similarly acts as a vacuolar Pi influx transporter.
3. Pi transport across the tonoplast is energized by the H+ electrochemical potential gradient generated by the H+-ATPase (grey oval) and H+-pyrophosphatase (PPase, blue oval). Under low Pi conditions, phosphate starvation-response (PSR) genes are activated, leading to regulatory changes, including an increase in PHF1-mediated delivery of non-phosphorylated PHT1 proteins to the plasma membrane, down regulation of PHT1 recycling, and an increase in H+-ATPase and H+-PPase activities.
4. In parallel, OsSPX-MFS3 and the other unidentified vacuolar Pi efflux transporter(s) (VPiTs) mediate Pi efflux from vacuole to the cytoplasm. These regulatory responses facilitate cytoplasmic Pi homeostasis through Pi delivery from the apoplasm and vacuole.
5. Under Pi-insufficient conditions SPX-domain-mediated inositol polyphosphate (InsP) sensing that functions in Pi homeostasis. Under sufficient-Pi conditions, 5-InsP7 (one of several InsP isomers) binds to the SPX-domain within the PHO1 protein, thereby allowing Pi export into the xylem vessel lumen for Pi delivery through the xylem transpiration stream from the root to the shoot.
6. The 5-InsP7-mediated SPX4-PHR2 interaction prevents PHR2 entry into the nucleus, thus blocking binding to cis-regulatory elements P1BS that control expression of PSR genes. Under Pi-stress conditions, the absence of 5-InsP7 results in PHO1 inactivation, which impairs Pi transport into the xylem.
7. In addition, 5-InsP7-diassociated SPX4 allows PHR2 release from their complex in the cytoplasm, followed by its entry into the nucleus to initiate the PSR signaling cascade. The SPX4 is likely degraded during long-term Pi-stress. 5-InsP7: 5-diphosphoinositol pentakisphosphate; P1BS: PHR1 binding site.

Role of Plant Vascular Systemin Maintaining Whole-Plant Level P Homeostasis

(a) Pi Sensing and Signaling in the Plant.

Low Pi availability is sensed at the primary root (PR) tip, and triggers an alteration in RSA by modulating root meristem development, including the PR arrest and lateral root (LR) initiation. Pi-stress signals [e.g. Pi, cytokinin (CK), and/or strigolactone (SL)], derived from the root system, are transported to the shoots, via the xylem, to elicit the molecular PSRs in the various target tissues. Source shoot tissues perceive the xylem-based Pi-stress signals and generate phloem-mediated systemic output signals (e.g. Pi, RNAs, proteins, and/or metabolites) for regulating various responses in the shoot and root sink tissues, including developing leaves, and shoot and root apices. For example, under Pi-stress conditions, shoot-derived systemic signals function in adjusting the RSA to maximize Pi uptake in the topsoil.

(b) Pi Signaling Regulatory Network at Cellular Level

Various RNA species (21–24 nt sRNAs, miRNAs and mRNAs) are generated in source tissues which serve as the sites for sensing of root-derived Pi-stress signals. These RNAs form stable ribonucleoprotein (RNP) complexes, in source phloem, which then deliver the cargo RNAs into sink organs to regulate Pi signaling pathways. The PHO2 mRNA

degradation in roots is mediated by the shoot-derived miR399. Pi-stress related mobile 21-nt and 24-nt sRNAs, involved in post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS), respectively, target specific RNAs and gene/transposable element (TE) loci to regulate their expression in sink tissues. Currently, the mechanism by which phloem-mobile mRNAs might mediate in regulating Pi signaling pathways, within their targeted sink tissue(s), remains largely unknown.

Hormone-Mediated Pi Signaling Pathways Controlling RSA and PSI Gene Expression

Auxin, ethylene, CKs, SLs, GA, and ABA have all been implicated in the regulation of RSA and PSR genes.

1. Auxin, ethylene, and SLs levels are induced by low Pi conditions and act as positive regulators of the Pi-starvation signaling pathways.
2. GA and CKs levels are decreased by low Pi availability and operate as negative regulators of the Pi-starvation signaling pathways.
3. ABA is induced under low Pi conditions, but acts as a repressor to suppress PSI gene expression.
4. PDR2 and LPR1/2 function to promote and inhibit primary root growth under low Pi conditions, respectively.

Transcriptional and Posttranscriptional Regulation of PSRs

1. Under low Pi conditions, changes in RSA, PSI gene expression, anthocyanin accumulation, sugar/starch accumulation, Pi uptake, and subsequent xylem loading are transcriptionally and post transcriptionally regulated by a combination of transcription factors, SIZ1, components of chromatin remodeling complexes and long non-coding RNAs.
2. Based on available transcriptomics databases, many transcription factors appear to be differentially expressed under Pideficient conditions, implicating them in transcriptional regulation of PSR genes. Among these Pi deficiency responsive transcription factors, six families are representative, namely those of the NAC, MYB, ERF/AP2 (Ethylene Response Factor/ APETALA2), zinc finger, WRKY, and CCAAT-binding families.

Morphological Approaches to Improve P acquisition Efficiency

1. Root hairs: Under low P, uptake is closely related to root surface area, root hair length and root hair density.
2. Root diameter: Root diameter denotes the volume of soil penetrated by root.
3. Root whorl number: Basal root whorl or node number exhibits enhanced soil P exploration.
4. Shallow root growth angle: The tops soil strata have higher available P than the subsoil strata owing to deposition by plant and fertilizer residues over time.
5. Root biomass: Besides above traits, increases in root biomass under P stress are common phenomena resulting in greater root-to-shoot ratio.

Changes in Root Physiology to Improve P acquisition Efficiency

1. Root-induced proton extrusion: Plant roots release protons under P deficiency leading to acidification of the rhizosphere.
2. Organic acid exudation: A major fraction of exudates released by plant roots during P starvation comprises the organic acids (OA) such as citrate and malate. These mobilize both Porg and Pi by displacing phosphate from soil matrix through ligand exchange.
3. Enzyme exudation: Enzymes such as Phosphatases, Phosphodiesterases, Apases, Rnases, Nucleases and Phytases are released by plant roots into rhizosphere where they hydrolyse soil Porg pools.

Root–microbiome Interactions

1. Coordinated upregulation of both SL-biosynthesis genes and PLEIOTROPIC DRUG RESISTANCE1 (PDR1), an ABC transporter located in the root epidermal plasma membrane, allow for SL secretion into the rhizosphere, a condition favoring colonization by AMF .
2. During the process of AMF colonization, fungal hyphae penetrate through the epidermis to cortical cells where they form an arbuscule, comprised of hyphae ensheathed by a specialized form of the cortical plasma membrane, termed peri-arbuscular membrane.
3. The fungal Pi transporter (FPiT) releases Pi across the fungal plasma membrane into the peri-arbuscular space.
4. Transfer of Pi into the cortical cell cytoplasm, across the peri-arbuscular mem-brane, takes place through AM-inducible plant Pi transporters (AMPiTs) belonging to the PHT1 transporter family.

Plants however, possess several adaptive strategies to enhance P acquisition ability which include changes in root architecture such as increased root-to-shoot ratio, lateral root number, root hair length and density. Specialized root structures called ‘proteoid’ or ‘cluster’ roots which exudate carboxylates. Increased secretion of organic acids and induction of high-affinity P_i transporter in roots. Association between plant roots and arbuscular mycorrhizal fungi (AMF) is another means of improved P acquisition. (Elanchezhian*et al.*, 2015). Plants also increase the efficiency of P_i use during P_i starvation by Phosphate recycling, mobilization, scavenging, alternative reactions of cytosolic glycolysis and tonoplast proton pumping, anthocyanin accumulation, replacing their membrane phospholipids with amphipathic sulfolipids and galactolipids. Reductions in intracellular levels of ATP, ADP and related nucleoside Ps occur along with increase in utilization of PP_i. (Plaxton and Tran, 2011.)

Shujie and Yunfa, (2011) reported positive relationship between RPAE (relative phosphorus absorption efficiency) and P concentration in shoot and root material at all P levels, irrespective of soybean genotype. An exponential relationship was found between PUE (phosphorus utilization efficiency) and P concentrations in shoots and roots. In order to select soybean genotype with high P efficiency one should pay attention to PUE combined with high RPAE. Nanamori *et al*., 2004, in their studies on *Brachiaria* and Ricefound out that the distribution ratio to sugars was 68% in rice and 50% in the *Brachiaria* hybrid in the 0 μM P treatment. P_i concentration, expressed on the basis of dry weight also decreased

markedly with P deficiency in both test crops, with P_i concentration being higher in leaves than in roots. The ^{14}C distribution ratio to the amino acid and organic acid pools was greater in the *Brachiaria* hybrid than in rice, and slightly increased with P deficiency in the *Brachiaria* hybrid.

Ebrowska *et al.* (2017) in their study on mechanisms of oat (*Avena sativa* L.) acclimation to phosphate deficiency reported that P_i deficiency caused inhibition of shoot growth, but generally it did not affect root elongation; root diameter was decreased, root/shoot ratios increased. Photosynthesis rate and productivity parameters decreased under low P_i nutrition, however, sugar content generally increased. Study showed that oat, in contrast to other plants, can use phytates as the sole source of P. P_i starvation significantly increased the activity of extracellular and intracellular acid phosphatases (APases) in comparison to the control plants. Three major A Pase isoforms were detected in oat tissues and the isoform pattern was similar in all studied conditions. Hernandez *et al.,* (2007) functional genomics studies used to investigate global gene expression and metabolic responses of bean plants (*Phaseolus vulgaris*) grown under P-deficient and P-sufficient conditions revealed a total of 126 genes, representing different functional categories and showed significant differential expression in response to P: 62% of these were induced in P-deficient roots. A set of 372 bean transcription factor (TF) genes, coding for proteins with Inter-Pro domains characteristic or diagnostic for TF, were identified.

Hufnagel *et al.,* 2014 investigated the role of homologs of OsPSTOL1in sorghum (*Sorghum bicolor*) performance under low P. SbPSTOL1 alleles reducing root diameter were associated with enhanced P uptake under low P in hydroponics, whereasSb03g006765 and Sb03g0031680 alleles increasing root surface area also increased grain yield in a low-P soil. SbPSTOL1 genes colocalizedwith quantitative trait loci for traits underlying root morphology and dry weight accumulation under low P *via*., linkage mapping.Chin *et al.,* 2011 in their studies on Developing Rice with High Yield under Phosphorus Deficiency: Pup1 sequence to application. To facilitate targeted introgression of Pup1 into intolerant varieties, the gene models predicted in the Pup1 region in the donor variety Kasalath were used to develop gene-based molecular markers that are evenly distributed over the fine-mapped 278-kb QTL region. Following a marker-assisted backcrossing approach, Pup1 was introgressed into two irrigated rice varieties and three Indonesian upland varieties.Utilization of resistant varieties with agricultural management practices has been reported to be a more effective way through resistance breeding (Duppala*et al.*, 2023). In the past, moderate resistance genes (*MR*) and resistance quantitative trait loci (QTLs) have been used in rice improvement by conventional breeding. However, conventional breeding is painstaking and time-consuming and may not be applicable for certain types of quantitative resistance. Marker assisted selection (MAS) can be a “shortcut” in resistance breeding programs because it reduces the number of generations. This technology has already proven to be a useful tool for rice breeding (Manojkumar*et al.*, 2023).

References:

Cakmak,I. 2002. Plant nutrition research: priorities to meet human needs for food insustainableways. *Plant Soil.* 247: 3–24.

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.

Choudhury, A.T.M.A., Kennedy, I.R., Ahmed, M.F and Kecskes, M.L. 2007. Phosphorus fertilization for rice and control of environmental pollution problems. *Pakistan journal of biological sciences.* 10 (13): 2098-2105.

Dey, P., Santhi, R., Maragatham, S and Sellamuthu, K.M. 2017. Status of Phosphorus and Potassium in the Indian Soils *vis-a-vis* World Soils. *Indian Journal of Fertilisers*. 13 (4): 44-59.

Duppala, M.K., Srinivas, T., Rao, L.S., Suneetha, Y., Sundaram, R.M., Kumari, V.P., Bhuvaneswari, V and Ganesh, B. 2023.Screening of recombinant inbred lines for resistance to bacterial leaf blight pathotypes in rice (*Oryza sativa* L.). *Plant Science Today*. 10(3): 343–353.

Elanchezhian, R., Krishnapriya, V., Pandey, R., Subbarao, A and Abrol, Y.P. 2015. Physiological and molecular approaches for improving phosphorus uptake efficiency of crops.*Current Science*. 108(7): 127-1279.

Hernandez, G., Ramirez, M., Lopez, O.V., Tesfaye, M., Graham, M.A., Czechowski, T., Schlereth, A., Wandrey, M., Erban, A., Cheung, F., Wu, H.C., Lara, M., Town, C.D., Kopka, J., Udvardi, M.K and Vance, C.P. 2007. Phosphorus Stress in Common Bean: Root transcript and metabolic responses. *Plant Physiology*. 144:752–767.

Hufnagel, B., DeSousa, S.M., Assis, L., Guimaraes, C.T., Leiser, W., Azevedo, G.C., Negri, B., Larson, B.G., Shaff, J.E., Pastina, M.M., Barros, B.A., Weltzien, E., Rattunde, H.F.W., Viana, J.H., Clark, R.T., Falcao, A., Gazaffi, R., Garcia, A.A.F., Schaffert, R.E., Kochian, L.V and Magalhaes, J.V. 2014. Duplicate and Conquer: Multiple homologs of Phosphorus-Starvation Tolerance, Enhance phosphorus acquisition and sorghum performance on low phosphorus Soils. *Plant Physiology*. 166: 659–677.

Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant physiology.*156: 1041-1049.

Manojkumar, D, Srinivas, T., Suneetha, Y and Suresh, G. 2023. Generation Mean Analysis for quantitative traits in the population of AKDRMS 21-54 x YH3 cross of rice (*Oryza sativa* L.). *Journal of Environmental Biology*. 44(1): 826-832.

Nanamori, M., Shinano, T., Wasaki, J., Yamamura, T., Rao, I.M. and Osaki, M. 2004. Low Phosphorus Tolerance Mechanisms: Phosphorus recycling and photosynthate partitioning in the tropical forage grass, *Brachiaria* Hybrid cultivar Mulato compared with Rice. *Plant Cell Physiology.* 45(4): 460–469.

Plaxton, W.C and Tran, H.T. 2011. Metabolic Adaptations of Phosphate-Starved Plants. *Plant Physiology.* 56: 1006–1015.

Raghothama, K.G. 1999. Phosphate acquisition. Annual Reviewsof Plant Biology. 50: 665–693.

Shujie, M and Yunfa, Q. 2011. Effects of phosphorus concentration on adaptive mechanisms of high- and low-P efficiency soybean genotypes when grown in solution. *Plant Soil Environment*. 57 (2): 61–66.

Shukla, A.K., Behera, S.K., Chaudhari, S.K and Singh, G. 2022. Fertilizer use in Indian agriculture and its impact on human health and environment. *Indian Journal of Fertilisers.* 18 (3): 218-37.

Vinod and, A.T.M.A., Kennedy, I.R., Ahmed, M.F and Kecskes, M.L. 2007. Phosphorus fertilization for rice and control of environmental pollution problems. *Pakistan journal of biological sciences.* 10 (13): 2098-2105.

Zebrowska, E., Milewska, Mand Ciereszko, I. 2017. Mechanisms of oat (Avena sativa L.) acclimation to phosphate deficiency. *PeerJournal.* 5: 1-29.

Zhang, Z., Liao, H and Lucas, W.J. 2014. Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. *Journal of Integrative Plant Biology*. 56(3):192–220.