REVIEW

Purple acid phosphatases: roles in phosphate utilization and new emerging functions

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Abstract

Plants strive for phosphorus (P), which is an essential mineral for their life. Since P availability is limiting in most of the world's soils, plants have evolved with a complex network of genes and their regulatory mechanisms to cope with soil P deficiency. Among them, purple acid phosphatases (PAPs) are predominantly associated with P remobilization within the plant and acquisition from the soil by hydrolyzing organic P compounds. P in such compounds remains otherwise unavailable to plants for assimilation. PAPs are ubiquitous in plants, and similar enzymes exist in bacteria, fungi, mammals, and unicellular eukaryotes, but having some differences in their catalytic center. In the recent past, PAPs' roles have been extended to multiple plant processes like flowering, seed development, senescence, carbon metabolism, response to biotic and abiotic stresses, signaling, and root development. While new functions have been assigned to PAPs, the underlying mechanisms remained understood poorly. Here, we review the known functions of PAPs, the regulatory mechanisms, and their relevance in crop improvement for P-use-efficiency. We then discuss the mechanisms behind their functions and propose areas worthy of future research. Finally, we argue that PAPs could be a potential target for improving P utilization in crops. In turn, this is essential for sustainable agriculture.

Introduction

Phosphorus (P) is a crucial macronutrient and essential for life. Being the key constituent of nucleic acids, ATP, phospholipids, etc., P plays a critical role in cell structure and functions, including energy metabolism, metabolic pathways, and signal transduction (Raghothama 2005). Plants can absorb and assimilate only soluble inorganic phosphate (Pi; $H_2PO_4^-$) which remains limited in the natural environment due to its reactive nature as it forms insoluble complexes with cationic minerals (Fe or Al in acidic soils; Ca or Mg in alkaline soils) (Oelkers and Valsami-Jones 2008; Arai and Sparks 2007). The P-rich biomolecules in decaying biological matter add organic phosphate (Po) to the soils. Po remains unavailable for root absorption unless hydrolyzed by

enzymes to release Pi, which is acquired by Pi transporters (PTs) present in the plasma membrane of root epidermal cells (Dissanayaka et al. 2018). The availability of Pi in the soils remains at a suboptimal level (1–10 μM) which is many magnitudes lower than the Pi concentration in a plant cell (5–20 mM) (Shen et al. 2011). Hence, Pi uptake against the steep concentration gradients occurs at the expense of plant ATPs (Srivastava et al. 2018). Plants initiate an array of adaptive responses to combat Pi deficiency, broadly known as phosphate starvation response (PSR). These include (i) morphological alterations (root architecture, root to shoot ratio, and symbiotic associations with mycorrhizae), (ii) molecular adaptations (activation of Pi starvation-induced genes and associated regulators), (iii) physiochemical adaptations (enhanced internal Pi recycling, anthocyanin accumulation, and alterations in root exudates) (Fig. 1). The coordinated PSR results in increased Pi acquisition via roots and optimization in plant internal Pi utilization. The hydrolases (such as phospholipases, ribonucleases, acid phosphatases, etc.) and organic acids (citrate, malate, oxalate, etc.) in root exudates help liberate Pi from P complexes in the rhizosphere for roots acquisition (Raghothama 1999).

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The Pi uptake and transport are tightly regulated via high-affinity transporters present in the root epidermal cells and distributed to organelles/tissues by a specific Pi transporter networks (Poirier and Jung 2015; Gu et al. 2016). These coordinated actions ensure the optimum Pi uptake and utilization in plants.

Acid phosphatases (APases) are a family of hydrolases ubiquitously present in living organisms and hydrolyze orthophosphate monoesters (Po) to release Pi at acidic pH (Fig. 2) (Olczak et al. 2003). The increase in APases expression and activity is a characteristic response of Pi deprived plants (Tran et al. 2010a; Zhang et al. 2014a; Secco et al. 2013; Mehra et al. 2016). Purple acid phosphatases (PAPs), a distinct group of APases, are widely studied for their roles in plant acclimation to Pi deficiency. PAPs are broadly involved in liberating Pi from phospho-monoesters in soil and senescing tissues, thus help in Pi uptake and distribution in the plant (Olczak et al. 2003;

Tran et al. 2010a; López-Arredondo et al. 2014; Scheible and Rojas-Triana 2015; Gu et al. 2016). Besides, a few PAPs are also known to exhibit peroxidase activity in addition to the phosphatase activity (Klabunde et al. 1995; Del Pozo et al. 1999; Bozzo et al. 2002). Recent reports highlight their unanticipated roles in multiple stress responses and plant development (flowering, seed germination, etc.). This review aims to explore the recent advances made on the understanding PAPs' role in Pi utilization and new emerging roles. We also discuss PAPs' role in Pi signaling pathways, their regulation and utilization in crop improvement for Pi-use efficiency.

Fig. 1 Schematic depiction of the different phosphate starvation responses and functions of APases. Under Pi deprived conditions, plants adaptations include morphological, molecular, and physiochemical changes. The morphological (such as root system architecture) and physiochemical (organic acid and APases secretion) alterations result in enhanced availability of Pi in soil. The molecular activities drive the acquisition and translocation of Pi in the above-ground parts of plants
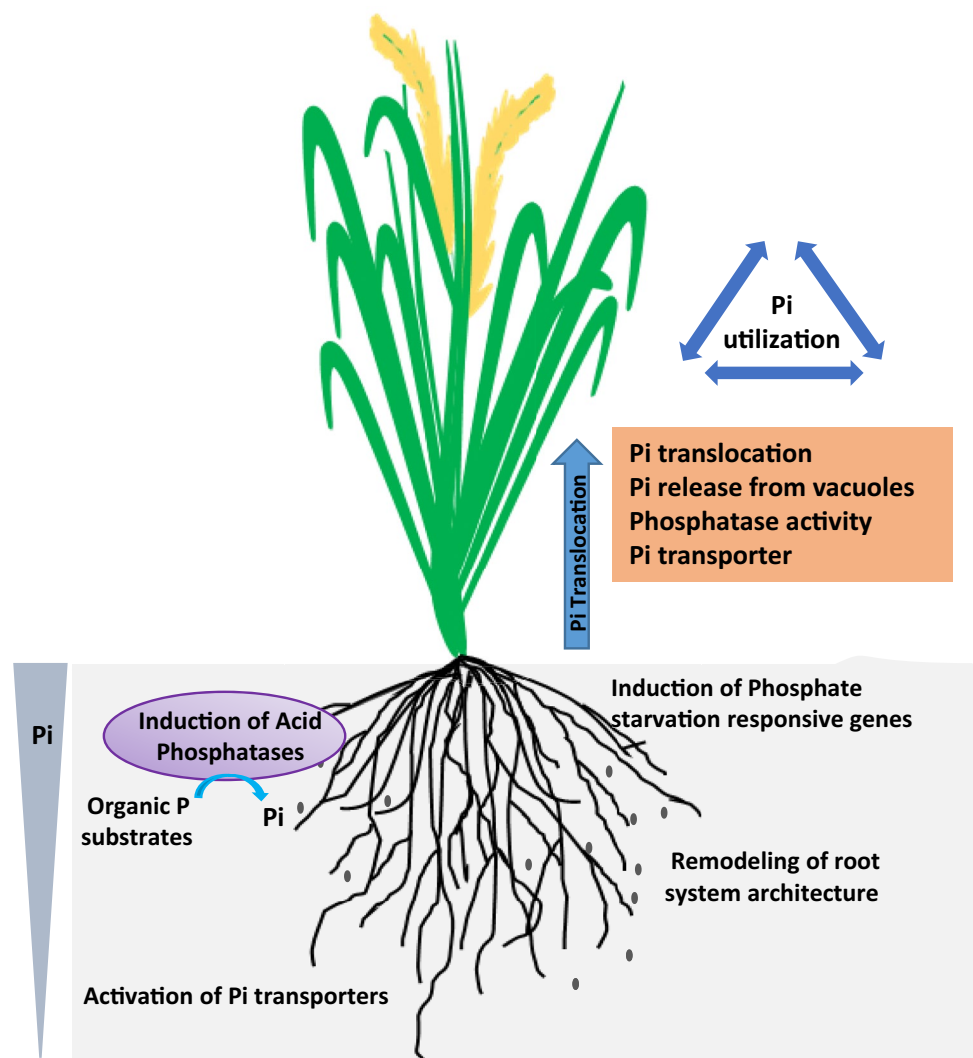
Fig. 2 Schematic representation of active site, domain architecture and reaction mechanism of PAPs. **A** The active site of red kidney bean purple acid phosphatase (rkbPAP), a representative binuclear metallohydrolase with Zn and Fe ions (adapted from Schenk et al., 2008). **B** Schematic illustrations showing domain architecture/motifs present in PAPs. (i) The amino acid sequence of PAPs contain metallophos domain across the species and five conserved motifs with seven invariant amino acids. (ii) Few PAPs have FN3 domain (fibronectin domain) at N-terminal along with metallophos domain and might have a role in substrate selectivity. (iii) PAPs with phytase activity have REKA (Arg, Glu, Lys, Met, Ala) motif at C-terminal. **C** PAPs hydrolyze phosphate monoesters in the presence of a water molecule to release alcohol and inorganic phosphate
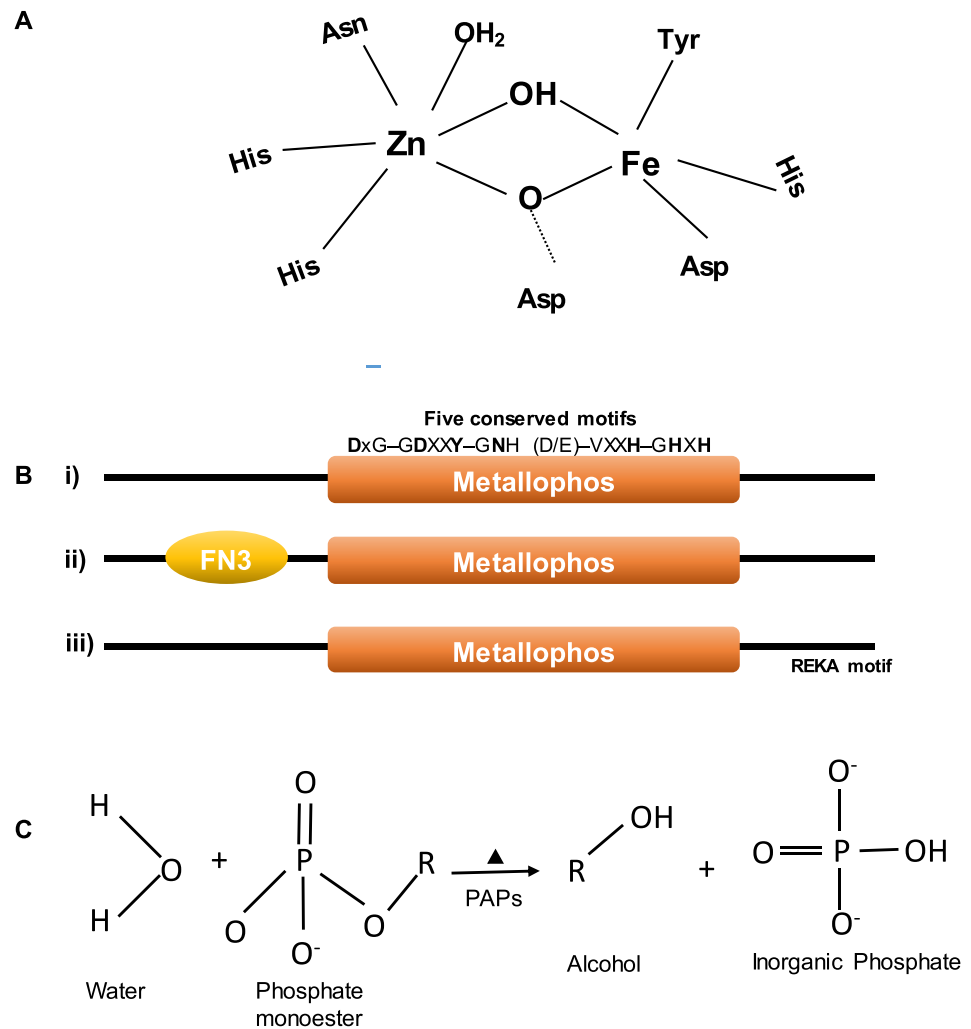
Purple acid phosphatases (PAPs): ubiquitous family of APases

PAPs belong to the metallophosphatase superfamily proteins carrying a metallophos domain and bimetallic reaction center at their active site (Fig. 2) (Schenk et al. 2013). These enzymes are broad substrate-specific, function at pH 4–6, and temperature range of 25–60 °C (Tian and Liao 2015). PAPs give pink or purple color in an aqueous solution due to the electron transfer reaction from tyrosine to Fe(III) at 560 nm (Olczak et al. 2003). The electron transfer at catalytic center results in the inactivation of the enzyme (purple color), and the redox reaction (Fe^{3+}–Fe^{2+}) changes it to an active form (pink color) (Olczak et al. 2003). PAPs are also known as TRAPs (Tartrate-Resistant Acid Phosphatases) due to their resistance to tartrate-mediated inhibition of catalytic activity. The term "TRAPs" is mostly used for mammalian PAPs which are functional in different mammalian tissues such as the bovine spleen, hairy leukaemia cells,

porcine uterine fluid, bone, lungs, and placenta (Sundararajan and Sarma 1954; Chen et al. 1975; Efstratiadis and Moss 1985; Ketcham et al. 1985, 1989). Their functions include bone resorption, antigen presentation, generation of ROS, and iron transport (Hayman et al. 1996; Halleen et al. 2003). Other than mammals, PAPs have been identified in plants, bacteria, and fungi. Different *Aspergillus* species have sequences closely related to plant PAPs and one such PAP (85 kDa) from *Aspergillus ficuum* has been characterized (Ullah and Cummins 1988; Schenk et al. 2000a). A few bacteria also have genes encoding PAPs, i.e., cyanobacterium *Synechocystis* sp., *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Burkholderia cenocepacia*, and *Burkholderia pyrrocinia* (Schenk et al. 2000a; Yeung et al. 2009; Zhu et al. 2019). Bioinformatic analysis also revealed 57 PAP-like sequences in 43 bacterial genomes and 4 in cyanobacteria (Yeung et al. 2009; Zhu et al. 2019). Surprisingly, a phospholipase D from *Streptomyces chromofuscus* (scPLD) also showed structural and mechanistic similarities

to iron-dependent PAPs (Zambonelli and Roberts 2003). Although, PAPs are reported in a limited number of microorganisms; however, structural similarities suggest their catalytic roles similar to plants' PAPs (Schenk et al. 2000a).

PAPs have been functionally characterized from several plants, including Arabidopsis, rice, tomato, kidney bean, tobacco, sweet potato, soybean, duckweed, potato, chickpea, and yellow lupin (Table 1). Recently PAPs have also been explored in model unicellular organisms, i.e., *Phaeodactylum tricornutum* (diatom) and *Chlamydomonas reinhardtii* (green algae), performing a similar function and providing new insights into algal P metabolism (Rivera-Solís et al. 2014; Wang et al. 2021). Among plants, PAPs exist as multigene family in Arabidopsis (29 *AtPAPs*), rice (26 *OsPAPs*); maize (33 *ZmPAPs*); soybean (35 *GmPAPs*), chickpea (25 *CaPAPs*); *Jatropha curcas* (25 *JCrPAPs*), *Camellia sinensis* (19 *CsPAPs*), and tomato (25 *SlPAPs*) (Li et al. 2002, 2012a; Zhang et al. 2011; Bhadouria et al. 2017; González-Muñoz et al. 2015; Venkidasamy et al. 2019; Yin et al. 2019; Srivastava et al. 2020). PAPs were also recently reported in *Brassicaceae*, *Solanaceae,* and *Cucurbitacea*e (Xie and Shang 2018). A subfamily of PAPs also exists, namely phosphodiesterase (PPD) in legumes, hydrolyze phosphodiesters, monoesters, and anhydrides (Olczak et al. 2009; Wang et al. 2015). However, there are still many organisms yet to be explored for the presence of PAPs. Nevertheless, the existence of PAPs multigene families in many plants suggests their conserved and diverse roles.

Structural and functional conservation among PAPs

Different phylogenetic studies divided the members of PAPs multigene family into groups, and the members of the same group largely have similar properties such as molecular weight, biochemical activity, subcellular localization, etc. (Li et al. 2002, 2012a; Tian and Liao 2015; Bhadouria et al. 2017; Srivastava et al. 2020). The functional conservation among the clade members thus aids in assigning a putative function to the new PAP identified in a clade. However, PAPs' evolutionary history is not very clear; only a recent phylogenetic study proposed that functional diversification of plant PAPs occurred before the existence of early angiosperm (Xie and Shang 2018).

Further, the amino acid sequence analysis of mammals and plants PAPs revealed the presence of five conserved domains/motifs (**D**x**G**–G**D**XX**Y**–**GNH** (D/E)–VXX**H**–**GHXH**) with seven invariant amino acid residues (bold letters) (Fig. 2) (Koonin 1994; Zhuo et al. 1994; Klabunde et al. 1995). However, variations in the number, position, and amino acid residues at the conserved motifs have been reported for different PAPs (Li et al. 2002, 2012a;

Zhang et al. 2011; Bhadouria et al. 2017). These invariant residues contribute to the enzyme activity as a mutation in them either eliminates or reduces the catalytic activity (Koonin 1994; Zhuo et al. 1994; Funhoff et al. 2001; Mitić et al. 2009). The metallic center consists of invariable Fe^{3+} and a varying second metal ion M^{2+} (M = Fe/Zn/Mn) (Fig. 2) (Klabunde et al. 1995; Schenk et al. 1999; 2008; Boudalis et al. 2007). The in-vitro experiments show catalytic activity reconstitution with varied metal ions, but PAPs are more specific towards specific metal ions when studied in-vivo (Beck et al. 1986; Mitić et al. 2006, 2009). The ferrous ion produces hydroxyl radicals on reacting with hydrogen peroxide, thus imparts alternative function to TRAPs, i.e., generating reactive oxygen species (ROS). Similar to TRAPs, some plant PAPs also catalyze a peroxidation reaction in addition to dephosphorylation (Del Pozo et al. 1999; Bozzo et al. 2002, 2004). Both activities are independent of each other as phosphatase activity inhibitors such as molybdate, do not interfere with peroxidase activity. These enzymatic activities also have different pH optima with phosphatase activity being optimal at acidic pH (~ pH 5), whereas peroxidation occurs at alkaline pH (~ pH 8.5) (Bozzo et al. 2002, 2004). Only a few plants' PAPs show peroxidase activity which is intriguing, and its relevance should be explored further.

PAPs are broadly divided based on their protein size as small (~ 35 kDa) and large (~ 80 kDa) molecular weight PAPs. The small PAPs were initially identified in mammals and large ones were identified in plants. Later, both types of PAPs were reported in mammals and plants (Schenk et al. 2000a, b; Flanagan et al. 2006). PAPs are reported to exist as monomer, dimer, tetramers, and oligomers (Table 1). Although, the presence of metallophos domain is a characteristic feature of PAPs; lately, other domains are also reported in some PAPs (Fig. 2). For instance, a structure similar to sterol desaturases at C-terminal is present in a PAP from *Lupinus albus* (Miller et al. 2001). However, the relevance of such a domain remains unexplored. A transmembrane domain at C-terminal is also present in a few plant PAPs, e.g., GmPAP35 in soybean, AtPAP2 and AtPAP9 in Arabidopsis, OsPAP9a in rice (Li et al. 2002, 2012a; Sun et al. 2012a; Zhang et al. 2011). The presence of only one such member in each plant except for Arabidopsis (Li et al. 2002, 2012a; Sun et al. 2012a; Zhang et al. 2011) is intriguing. It also remains to see if all these PAPs localize to the membrane. The physiological impact of this finding is yet to be studied in detail.

Kidney bean PAP (KbPAP) has been widely explored for protein structure (Sträter et al. 1995). Later, structure of human, pig, rat, sweet potato, and yellow lupin PAPs has also been solved (Sträter et al. 1995; Klabunde et al. 1996; Lindqvist et al. 1999; Guddat et al. 1999; Uppenberg et al. 1999; Schenk et al. 2008; Antonyuk et al. 2014). Broadly, PAPs' protein structure consists of two domains (i.e.,

Table 1 Molecular properties, subcellular localization, and proposed functions of PAPs in different plants

Gene	Localization	Expression	S/I	Function	References
Arabidopsis thaliana					
AtPAP2	Chloroplast, mitochondria, plasma membrane	Root, stem, pollen	S	Carbon metabolism; Aids in import of selected proteins into chloroplast	Sun et al. (2012a, b, 2013, 2018), Zhang et al. (2016), Zhu et al. (2005)
AtPAP5		Only in P deficient seedlings		Plant defense response	Ravichandran et al. (2013, 2015)
AtPAP9-1	Plasma membrane and cell wall	Stipule and vascular tissues in response to fungal infection		Signaling, defense response	Zamani et al. (2014)
AtPAP10		Constitutive expression	S	Pi acquisition	Wang et al. (2011, 2012, 2014c), Zhang et al. (2014b), Zhu et al. (2005)
AtPAP12		Constitutive expression	S	Pi scavenging, recycling from endogenous phosphomonesters	Tran et al. (2010b), Wang et al. (2014c), Zhu et al. (2005)
AtPAP15	Putative vacuole/tonoplast	Constitutive expression	S	Mobilize phosphate reserve during seed and pollen germination	Zhang et al. (2008), Kuang et al. (2009), Wang et al. (2009), Zhu et al. (2005)
AtPAP17	Cell wall or plasma membrane	Constitutive expression	I	Pi mobilization and metabolism of reactive oxygen species under stress or senescent condition	Del Pozzo et al. (1999), Farhadi et al. (2020), Zhu et al. (2005)
AtPAP18	Apoplast and vacuoles	Constitutive expression	S	Pi acquisition and remobilization	Zamani et al. (2012), Younessi-Hamzekhanlu et al. (2018), Zhu et al. (2005)
AtPAP23		Flowers			Zhu et al. (2005)
AtPAP25	Cell Wall	Flowers		Signaling role as a phosphoprotein phosphatase under Pi deficiency	Del Vecchio et al. (2014), Zhu et al. (2005)
AtPAP26	Vacuolar, secretory	Constitutive expression	S/I	Pi metabolism, Alkaline peroxidase activity	Hurley et al. (2010), Tran et al. (2010b), Veljanovski et al. (2006), Robinson et al. (2012), Farhadi et al. (2020), Zhu et al. (2005)
Oryza sativa					
OsPAP2	nd	Roots		–	Hur et al. (2010)
OsPAP10a	nd	Roots	S	Pi acquisition, ATP hydrolysis	Tian et al. (2012)
OsPAP21b	Endomembrane (probably Golgi bodies)	Roots	S	Utilization of external organic P	Mehra et al. (2017)
OsPAP23 (OsPHY1)	nd	Germinating seeds		Degradation of phytins during seed germination	Li et al. (2012b)
OsPAP26	Apoplast	Constitutive expression	S	Pi remobilization from senescing to non-senescing leaves; and organic P utilization	Gao et al. (2017)
Glycine Max					
GmPAP4	Plasma membrane or cytoplasm	Flowers, Pods	S	Pi acquisition, in particular phytate utilization	Kong et al. (2014)
GmPAP7a	nd	Roots		Extracellular ATP utilization	Zhu et al. (2020)
GmPAP7b	nd	Roots			
GmPAP14	nd	Roots	S	Utilizing external organic P (phytate)	Kong et al. (2018)
GmPAP1 like	Cell wall localized	Roots		Utilizing external organic P (dNTP)	Wu et al. (2018)

Table 1 (continued)

Gene	Localization	Expression	S/I	Function	References
GmPAP21	nd	Roots, old leaves and nodules	I	Pi recycling and metabolism in nodules	Li et al. (2017)
GmPAP3	Mitochondria	–	I	Stress tolerance by enhancing ROS activity	Liao et al. (2003), Li et al. (2008), Deng et al. (2014)
GmPAP33	Plasma membrane of arbuscule containing cells	Roots	I	Arbuscule degeneration during AM symbiosis via involvement in phospholipid hydrolysis	Li et al. (2019)
GmPAP29 (GmPhy)	nd	Germinated seeds	I	Seed germination	Hegeman and Grabau (2001)
Phaseolus vulgaris					
PvPAP3	Plasma membrane and apoplast	Roots	I	Utilization of extracellular ATP	Liang et al. (2010)
KbPAP (PvPAP2)	Cytoplasm	Cotyledons		Pi acquisition	Cashikar et al. (1997), Liang et al. (2012)
Lycopersicon esculentum					
LeSAP1	–		S	Proposed to be involved in the hydrolysis of extracellular organic P compounds	Bozzo et al. (2002, 2006)
LeSAP2	–		S	Proposed to be involved in the hydrolysis of extracellular organic P compounds	Bozzo et al. (2002, 2006)
IAP	–		I	Hydrolyzes intracellular phosphate esters, shows peroxidase activity, therefore, involved in ROS metabolism	Bozzo and Plaxton et al. (2008)
SlPAP1		Germinated seeds	I	Expressed during seed germination	Suen et al. (2015)
Nicotiana tabaccum					
NtPAP	Secretory	–	S	Proposed to mobilize organic-P in the rhizosphere	Lung et al. (2008)
NtPAP12	Cell wall	–	S	Regulation of these carbohydrate synthases by a phosphorylation or dephosphorylation process, as well as the role of wall-bound phosphatases in the regulation of cell wall biosynthesis	Kaida et al. (2008, 2009, 2010)
Medicago truncatula					
MtPAP1	Apoplast	Roots	S	Utilizing organic phosphorus	Xiao et al. (2006), Ma et al. (2012)
MtPHY	–	Roots	–	Utilizing organic phosphorus	Xiao et al. (2005), Ma et al. (2009)
Spirodela oligorrhiza					
GPI linked PAP	Cell wall	Outermost cortical cells of roots	–	Acquiring inorganic phosphate	Nakazato et al. (1998), Nishikoori et al. (2001)

Table 1 (continued)

Gene	Localization	Expression	S/I	Function	References
Astragalus sinicus					
AsPPD1	Plasma membrane and cytoplasm	Roots and nodules (on rhizobia inoculation)		Maintaining symbiotic ADP level, energy charge and nodule organogenesis and nitrogen fixation	Wang et al. (2015)
Lupinus luteus					
LlPPD1		–		Hydrolyzes pyrophosphate or phospho-diester bond in diphosphonucleotides and phosphodiesters	Olczak et al. (2002, 2009)

S/I secretory or intracellular

N-terminal and C-terminal) joined by the disulfide bridge. PAPs' catalytic site is present at C-terminal domain, whereas the N-terminal domain (~ 120 residues) does not have any known function (Sträter et al. 1995). Mammalian PAPs have only one domain similar to the C-terminal domain (Sträter et al. 1995; Guddat et al. 1999). The C-terminal domain consists of two β sheets alternating with α helix, conserved in both animals and plants. The metal ions are in contact with carboxyl ends of the β strands (Sträter et al. 1995; Guddat et al. 1999). The N-terminal subdomain consists of β sheets and does not participate in catalysis. Later, the structural study of PPD1 (PAP from yellow lupin) revealed that its N-terminal has a unique domain called fibronectin III (FN3) like domain (two β-sheets making up a seven-stranded β barrel) spanning the whole N-terminal (1–150 residues) (Fig. 2). However, an FN3 domain (150–250 residues) is also present in other PAPs including red kidney PAP and sweet potato PAP (Sträter et al. 1995; Tsyguelnaia and Doolittle 1998). This domain is proposed to participate in substrate selectivity (Antonyuk et al. 2014). The FN3 domains in animals are known to mediate protein–protein interactions or cell adhesion (Craig et al. 2004), and might also have similar functions in plants. PAPs' catalytic mechanism will not be discussed in detail here as an excellent review is available (Schenk et al. 2013). Briefly, the non-esterified oxygen atoms in the phosphate group attack one of the metal ion (M^{2+}: Fe/Mn/Zn) and the other oxygen atom attacks Fe^{3+} ion. Thus, a transition state is formed, changing the position of the phosphate group. The nucleophilic attack by the hydroxyl group present between two ions releases the leaving group (ROH). The water molecules attack this transition state that results in the release of the phosphate group (Schenk et al. 2008, 2013). Recently, the crystal structure of rkbPAP in complex with vanadate derivative of ATP has been identified, which mimics the natural enzyme–substrate complex. This structure facilitates viewing the trajectory of substrate binding and targets release from the catalytic site, confirming the earlier predicted mechanism (Feder et al. 2020).

PAPs localize to various subcellular organelles

PAPs are commonly classified as intracellular and secreted based on their functioning in cytoplasm or in extracellular spaces, respectively. The intracellular PAPs are localized in different cell compartments like cytoplasm, plasma membrane, apoplast, tonoplast, mitochondria, chloroplast, golgi bodies, and endoplasmic reticulum (Table 1). The secreted PAPs are either associated with root surface or secreted out in the soil, and aid in Pi solubilization in the rhizosphere. Some PAPs even have dual localization, which in some

cases, changes based on prevailing condition or stress. For instance, AtPAP26 exist as both vacuolar and secreted protein (Veljanovski et al. 2006; Hurley et al. 2010). AtPAP2 localizes to the outer envelope of chloroplast, mitochondria, and plasma membrane (Sun et al. 2012b, 2018). AtPAP9 is localized to the plasma membrane and cell wall (Zamani et al. 2012). Both AtPAP2 and AtPAP9 have a transmembrane domain at C-terminal (Sun et al. 2012b; Zamani et al. 2012). AtPAP18 is also a dual-targeted protein, as its transient expression in onion epidermal cells resulted in localization to apoplast after 24 h and vacuoles after 72 h (Zamani et al. 2012). Vacuoles are the cellular reservoir of P and they behave as a source or sink in a condition-dependent manner. Therefore, a wide localization profile of PAPs may help plants in maintaining organelles' P balance. However, it remains to be explored what controls the localization of PAPs in different cellular chambers.

As far as the synthesis of PAPs is considered, induction of PAPs occurs first in roots compared to shoot on Pi deprivation (Bozzo et al. 2004, 2006; Secco et al. 2013). Reports suggest that the synthesis of intracellular APases occurs prior to secreted APases under Pi deprivation condition, as shown in tomato suspension cells (Bozzo et al. 2004, 2006). It appears that plants primarily rely on cytosolic P and activate secreted PAPs later, to acquire P from the rhizosphere.

## Complex molecular regulation of PAPs expression and activity

Most PAPs are reported to be induced transcriptionally in response to Pi deficiency (Zhang et al. 2011; Li et al. 2012a; González-Muñoz et al. 2015; Mehra et al. 2016). The MYB domain transcription factor, PHR1 and its homologue (PHL1, PHL2, and PHL3) are involved in transcriptional regulation of various PSR genes, including PAPs such as *AtPAP10*, *OsPAP21b* (Sun et al. 2016; Mehra et al. 2017). The PHR1 or its homologues positively regulate the expression of APases including PAPs, as *PHR1* overexpression enhances APase activity and mutants displayed a reduced APase activity (Rubio et al. 2001; Zhang et al. 2011; Yao et al. 2014a; Sun et al. 2016; Mehra et al. 2017). Most of the PAPs have PHR1 binding site, i.e., P1BS elements in their promoter, but not all, yet most are responsive to Pi deficiency (Bhadouria et al. 2017). Moreover, only a few studies have shown the physical binding of PHRs at the *PAP's* promoter (Mehra et al. 2017). Other cis-regulatory elements such as NIT2, PHO, helix-loop-helix, TC elements, TATA box-like, etc., are present in the promoter of many Pi responsive gene suggesting an additional regulation (Mukatira et al. 2001, Hammond et al. 2004). WRKY75 and ZAT6 are other transcription factors that are induced under Pi deprivation and positively regulate the expression of PSI genes such

as phosphatases in Arabidopsis (Devaiah et al. 2007a, b). Besides, altered expression of PAPs is also reported in over-expression/mutant lines of other transcription factors such as OsMYB2P-1, StMYB44, and AP2/ERF, implying that other members of this regulatory pathway need to be identified for having clearer information on the regulation of PAPs (Dai et al. 2012; Chen et al. 2018; Zhou et al. 2017). Intriguingly, the expression of *OsPAP10* was enhanced in the mutants of SPX domain-containing transporters, *OsSPX-MFS1* (Wang et al. 2012). Further, the nuclear translocation of PHR1 is facilitated by SPX protein in Pi dependent manner (Wang et al. 2014a; Puga et al. 2014). Under Pi sufficient condition, SPXs remain bound to PHR1; therefore, inhibit its binding to promoter of the target PSR genes. In Pi deficient condition, the affinity of SPX towards PHRs decreases leading to the liberation of PHRs for the induction of PSR genes (Wang et al. 2014a; Puga et al. 2014). The SPX dependent regulation varies in different crops as PvSPX1, GmSPX3, and AtSPX1 positively regulate the expression of *PAP*s (*PvPAP3*, *GmPAP21*, *AtPAP17*, *AtPAP2*) in *Phaseolus vulgaris*, soybean, and Arabidopsis, whereas rice SPX1-5 negatively regulates PAPs expression (Duan et al. 2008; Liu et al. 2010a; Shi et al. 2014; Yao et al. 2014a, b; Wang et al. 2014a; Qi et al. 2017). In another study, the overexpression of *BnA2SPX1* in Arabidopsis resulted in the downregulation of *AtPAP10* expression (Du et al. 2017). These results imply that a complex regulatory mechanism underlying SPX members exists in different plants (Fig. 3) (Lv et al. 2014; Liu et al. 2010a; Shi et al. 2014).

Plant hormones are known to influence the expression of many Pi deficiency responsive genes, including PAPs. Auxins usually participate positively in Pi deficiency signaling. However, rice mutants of auxin-responsive transcription factor, *osarf12*, had an elevated expression of PAPs like, *OsPAP9b*, *OsPAP10a*, *OsPAP10c*, and *OsPAP27a,* suggesting a negative regulation in WT (Wang et al. 2014b). On the other hand, cytokinins regulate PAPs (*AtPAP17* or *OsPAP10*) expression, negatively exhibited by the exogenous cytokinins application (Fang et al. 2009). Ethylene is a component of local cellular signaling (in response to Pi levels in rhizosphere) and influences the *AtPAP10* regulation in Pi dependent manner. The *AtPAP10* expression, protein accumulation, and activity increase under P sufficient condition in the presence of ACC (ethylene precursor) (Fig. 3) (Zhang et al. 2014a). These studies suggest the involvement of local Pi signaling in PAPs regulation.

Moreover, systemic signaling components (in response to cellular Pi levels) also influence PAPs behavior. Sucrose, a component of systemic signaling is required for the transcription of *AtPAP10* (Zhang et al. 2014a). The presence of sucrose also affects the miRNA399 expression, a positive regulator of PSR (Liu et al. 2010b). The miR399 functions downstream to PHR1 (Bari et al. 2006). *PHO2* (*UBC24*) is

Fig. 3 Molecular regulation of PAPs. Under Pi deficient conditions, SPX proteins do not bind to PHR/PHL transcription factor, thus activating PAPs expression (monocots), whereas in dicots SPX1 positively regulates PHR1. PHR controls the miR399-PHO2 pathway, which is also affected by sucrose. OsSPX-MFS1 and OsMYB2P-1 affect the expression of PAPs negatively and positively, respectively. The post-translational modifications such as glycosylation affect the activity and targeting of PAPs. Serine proteases are involved in feedback inhibition of PAPs' activity by Pi. Auxin signaling also affects Pi signaling through OsARF12. Cytokinin level and PAPs activity are inversely related. Ethylene, as local sensing molecule provides stability to PAPs at protein level. Arrows indicate activation, perpendicular lines show inhibition and dotted lines show indirect effect
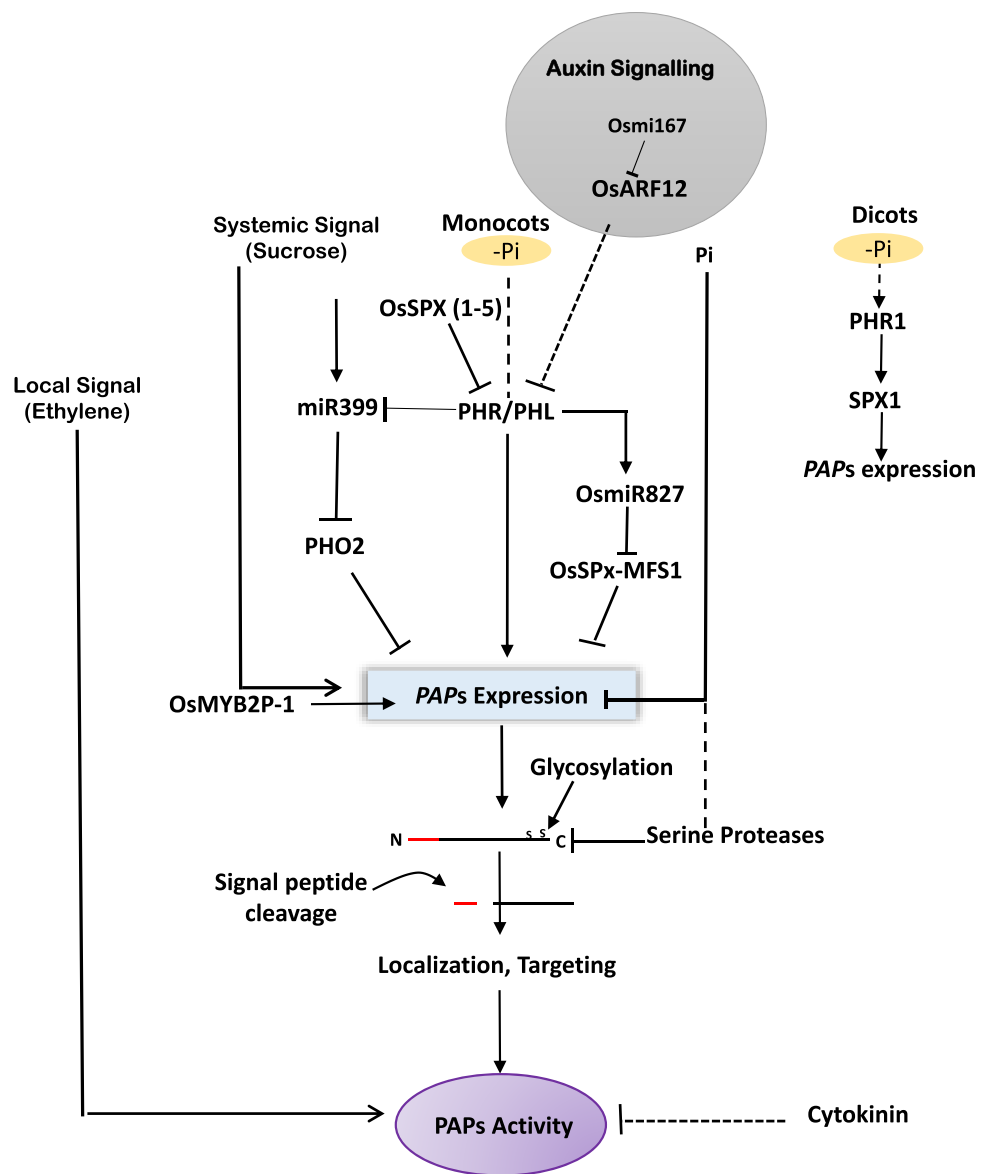
a member of the E2 ubiquitin conjugase family which assists the degradation of Pi transporters in sufficient conditions. PHO2 expression is suppressed by miR399s under Pi deficiency, thus enhancing Pi acquisition (Wu et al. 2013). The *miRNA399* overexpression or mutation in *PHO2* in Arabidopsis or rice results in an increased root-associated APase activity than WT (Zhang et al. 2011; Tao et al. 2016). This suggests that miRNA399-PHO2 pathway also affects root-associated APase activity, most likely indirectly.

Furthermore, PAPs are also regulated at the post-transcriptional level under Pi deficiency. It is reported that a high Pi concentration inhibits the PAPs activity, possibly through a feedback mechanism (Zhang et al. 2014b; Mehra et al. 2017). The P resupply to the Pi deficient tomato suspension cells also resulted in the degradation of APases by enzymes such as serine proteases (Bozzo et al. 2004,

2006). Transcripts profiling further revealed a reduction in the expression of different PAPs such as *OsPAP3b*, *OsPAP21b*, *OsPAP23*, on Pi resupply (Secco et al. 2013). On the other hand, few PAPs such as *OsPAP10d, OsPAP7, OsPAP20b*, and *OsPAP21a* show constitutive expression under P sufficient or starvation, but their transcripts decrease on P resupply (Secco et al. 2013). Other PAPs like *AtPAP10, StPAP1, OsPAP26* or *AtPAP26* do not show transcript induction under Pi deprivation; instead respond post-transcriptionally (Zimmermann et al. 2004; Veljanovski et al. 2006; Tran et al. 2010b; Wang et al. 2011; Gao et al. 2017). The constitutive mRNA level of these PAPs may help the cellular machinery cope quickly under stress conditions (Veljanovski et al. 2006; Wang et al. 2011). Since PAPs form a multigene family in plants,

sub-functionalization or neo-functionalization may be defining their varied behavior in response to Pi deficiency.

Post-translational modification (PTM) has been reported to influence PAPs' activity. Glycosylation affects protein structure, folding, enzyme activity, trafficking, interaction, and localization (Spiro 2002). Glycosylation also plays an important role in PAPs functioning (Fig. 3). PAPs exist as glycosylated protein, which may assist in their targeting into the different cellular organelles and enzymatic activities. The majority of PAPs carry xylosylated or fucosylated amino acids, while others have mannose as sugar moiety (Olczak et al. 2003). Plant PAPs consist of almost 10% sugar and have three to five glycosylation sites (Plaxton and Shane 2015). Two PAPs present in *P. vulgaris* and *L. luteus* have been characterized, and glycosylation at C-terminal was found crucial for the secretion of the enzyme (Olczak and Olczak 2007). Notably, two glycoforms of AtPAP26, i.e., S1 and S2 were identified differing in glycosylation pattern and both are secreted (Veljanovski et al. 2006). It was further revealed that their differential glycosylation pattern affects substrate specificities, as AtPAP26-S2 can hydrolyze phenyl-P substrates, but AtPAP26-S1 cannot (Tran et al. 2010b). AtPAP26-S2 is a high mannose glycoform compared to AtPAP26-S1. AtPAP26-S2 was found to interact with AtGAL1 (Galanthus nivalis agglutinin-related and apple domain lectin-1) due to high mannose glycans, enhancing its catalytic activity and physiochemical properties (Ghahremani et al. 2019). This explains the role of glycosylation in the regulation of enzymatic activity and substrate specificities. Understanding the glycosylation pattern of PAPs and other PTMs can help reveal novel functions of PAPs and their subcellular localization. Most of the information on PAPs regulation has been generated in Arabidopsis or rice. Whether there is a major difference in PAPs' regulation in other plants is a subject of future research.

The diverse functions of PAPs

PAPs' functions are mainly linked to Pi homeostasis. However, PAPs transcripts are also reported to be induced by other abiotic (N or K deficiency, salt stress) and biotic stresses (pathogen attack) (Bhadouria et al. 2017; Deng et al. 2014; Ravichandran et al. 2013). Additionally, PAPs are linked with root growth, symbiotic association, carbon metabolism, phospholipid hydrolysis, defense response, and cellular signaling (Kaida et al. 2009; Sun et al. 2012b; Ravichandran et al. 2015; Del Vecchio et al. 2014; Mehra et al. 2017; Xue et al. 2018; Li et al. 2019). PAPs' functional horizon is expanding; it is now evident that there is more to reveal about them besides their roles in Pi deficiency adaptation.

PAPs' roles in mitigating P deficiency stress

PAPs' broad substrate specificity helps plants survive Pi deficiency by hydrolyzing various organic P compounds for Pi supply. Hence, overexpression of PAP encoding genes is a common approach for enhancing plant Pi deficiency tolerance (Table 1). Usually, the PAPs overexpression improves performance in terms of biomass and total P accumulation, when plants grown on media supplied with an external Po (ATP, dNTPs, DNA) or in soil with organic manure (Table 1). For instance, *OsPAP10a*, *OsPAP10c*, and *OsPAP21b* overexpression helped plants hydrolyze the externally supplied ATP and other Po (Tian et al. 2012; Lu et al. 2016; Mehra et al. 2017; Deng et al. 2020). Kidney bean (*PvPAP1*, *PvPAP3*) and *Stylosanthes* spp. (*SgPAP7*, *10*, *26*) PAPs could also utilize dNTPs as Pi source; reflected by an increase in total P content and biomass on their overexpression in hairy roots (Liang et al. 2012; Lu et al. 2016). In Arabidopsis, AtPAP12 and AtPAP26 are prominent PAPs involved in Pi utilization, as *atpap12/atpap26* mutant shows an inability to utilize the herring sperm DNA or glycerol-3-phosphate, which otherwise were hydrolyzed by WT Arabidopsis (Robinson et al. 2012). Expectedly, overexpression of *AtPAP12* or *26* resulted in better growth on Pi deficient or ADP/ Fru-6P supplied media; however, there was no difference in total P content compared to WT (Wang et al. 2014c). Such PAPs might be involved in enhancing internal Pi recycling from P-rich biomolecules. Thus, both secreted and intracellular PAPs aid the plant performance in Pi limiting conditions.

Besides conventional APase activity, few PAPs such as LlPPD1 (*Lupinus luteus*) and AsPPD1 (*Astragalus sinicus*), can also hydrolyze phosphodiesters (Olczak et al. 2000, 2009; Wang et al. 2015). Moreover, a few PAPs display phytase activity, i.e., hydrolyzing phytates. Phytate is a complex of Ca or Mg with myo-inositol and constitutes 70–80% of organic P present in the soil. PAPs with phytase activity differ from other phytases in their catalytic mechanism and also have REKA motif at C-terminal (AtPAP15, NtPAP, GmPHY, and OsPHY), as identified, recently (Fig. 2) (Yao et al. 2012; Feder et al. 2020). The REKA motif at C-terminal is responsible for phytase activity, as identified by the study of NtPAP–phytate complex using homology modeling (Feder et al. 2020). These PAPs are categorized as phytases because of their higher affinity towards phytates (Hegeman and Grabau 2001; Jeong et al. 2017; Kuang et al. 2009; Dionisio et al. 2011; Liu et al. 2018). A handful of PAPs having phytase activity are secreted, whereas others are mostly active during seed germination (Hegeman and Grabau 2001; Kuang et al. 2009; Bhadouria et al. 2017). Secreted PAPs with phytase activity are *NtPAP*, *AtPAP15*, *MtPAP1*, *MtPHY1*, *GmPAP4*, and *GmPAP14* (Table 1). *CaPAP7, SlPAP1*, *OsPAP23*, and *GmPHY* (*GmpPAP29*) are

intracellular PAPs exhibiting phytase activity (Hegeman and Grabau 2001; Li et al. 2012b; Suen et al. 2015; Bhadouria et al. 2017). The exogenous application of CaPAP7 purified protein leads to an increase in biomass and Pi content of Arabidopsis seedlings grown on media containing phytate as the sole P source (Bhadouria et al. 2017). The overexpression of PAPs with phytase activity (*MtPAP1*, *MtPHY1, OsPAP23, GmPAP14*, etc.) led to better growth and higher P content when phytate was provided as the sole P source (Xiao et al. 2005, 2006; Ma et al. 2009; Li et al. 2012b; Kong et al. 2014, 2018). Thus, PAPs with phytase activity increase the substrate profile and strengthen the PAPs utility in improving crop P-use-efficiency.

The developing seeds store P in the form of phytate, which becomes the P source during germination when roots are not fully established. Seed germination involves phytates hydrolysis by phytases and simultaneous induction of APase activity (Gibson and Ullah 1988). The PAPs with phytase activity might be critical for seed germination, as many of them were isolated from germinating seeds (Hegeman and Grabau 2001; Li et al. 2012b). Such PAPs might be involved in seed size regulation, as suggested by CaPAP7 (chickpea PAP with phytase activity) higher expression in genotypes with lower seed weight or phytate content. Since seed phytate levels are directly proportional to seed weight, *CaPAP7* might influence seed phytate content, thus seed weight (Bhadouria et al. 2017). PAPs with phytase activity help in seed germination; however, it would be interesting to investigate whether they also assist in P mobilization towards developing seeds, thus influencing crop yield.

PAPs with dual functionality

Some of the PAPs (KbPAP, AtPAP17, LeSAP1, and LeSAP2) consist of two catalytic sites displaying phosphatase and peroxidase activity (Klabunde et al. 1995; Del Pozo et al. 1999; Bozzo et al. 2002). The phosphatase activity is involved in Pi mobilization during its deprivation and tissue senescence (Del Pozo et al. 1999). The peroxidase activity is involved in ROS generation or scavenging, as shown in the case of mammalian PAPs (Del Pozo et al. 1999; Schenk et al. 2013). An increased ROS production is often reported under Pi deficiency that induces the antioxidant pathway genes (Pandey et al. 2013). Klabunde and co-workers had also reported that KbPAP acts as an antioxidant, thus reducing the ROS in seed (Klabunde et al. 1995). Both the activities appear to be independent of each other as APase inhibitors do not affect the peroxidase activity (Bozzo et al. 2002).

PAPs' peroxidase activity appears to be very appealing as it may help provide tolerance against both Pi deficiency and oxidative stress. Tomato PAPs, LeSAP1, and LeSAP2 are predicted to be involved in ROS production

and associated with oxidative burst accompanying pathogen attack. Additionally, various APases are also induced on pathogen infection (Bozzo et al. 2002). There are also few reports which assert the role of PAPs in improving resistance against biotic stress. *AtPAP5*, a low Pi inducible PAP is required for basal resistance against *Pseudomonas syringae* pv. *Tomato* DC3000 (*Pst* DC3000) (Ravichandran et al. 2013). The higher susceptibility of *atpap5* mutants to *Pst* DC3000 complements the earlier results. Another PAP, *AtPAP9* transcripts get induced to fungal infection in stipule and vascular tissues and, is surprisingly not responsive to Pi deficiency (Zamani et al. 2014). Lastly, a study identified the PAP localized in the QTL associated with FHB (*Fusarium* head blight) resistance in wheat (Liu et al. 2019). Plants have extensive antioxidant defense machinery; the evolution of a few PAPs with peroxidase activity might help plant tolerate oxidative stress that co-exists with Pi deficiency. Although the precise function and mechanism of peroxidase activity in PAPs remain obscure and need further investigation.

P remobilization from senescing tissues and flag leaf

It has been hypothesized that the plant remobilizes Pi from older tissues in response to senescence or cellular P levels dependent manner, i.e., Pi deficiency triggers the Pi remobilization from older tissues. Plants such as *Banksia serrata* and *Hakea prostrata* grown in severely Pi depleted soils, and soybean may recycle up to 85% and 50% of total P from senescing leaves, respectively (Crafts-Brandner 1992; Lambers et al. 2015). Shane and co-workers have isolated two senescence induced intracellular PAP isoforms (*HpPAP1* and *HpPAP2*) functioning in senescing leaves of *H. prostrate* (Shane et al. 2014). Similarly, OsPAP26 activity increases in rice during senescence, and the Arabidopsis *atpap26* mutant showed delayed senescence (Robinson et al. 2012; Gao et al. 2017). Furthermore, Gregerson and Holm (2007) had reported the upregulation of one PAP in the senescing flag leaf of wheat. Rice grains receive most P by the mobilization from plant tissues via flag leaf (Wang et al. 2016). Also, PAPs mediated P mobilization towards developing seeds has been uncovered recently (Gregersen and Holm 2007; Jeong et al. 2017). Lastly, many Pi starvation-induced genes were also reported to be induced in the flag leaf at 15 DAA (days after anthesis), including four PAPs (*OsPAP3*, *OsPAP9b*, *OsPAP10a*, and *OsPAP26* thus, emphasizing their role in seed P loading (Jeong et al. 2017). Although there seems to be functional conservation among the PAPs; however, it remains to be distinguished the PAPs' roles between senescence and Pi deficiency driven Pi remobilization while the former represents the developmental necessity; the latter is a survival adaptation.

PAPs involvement in response to other abiotic stresses

Emerging evidence shows that PAPs can also help plants tolerate salt and osmotic stress. The overexpression of *AtPAP15* provides tolerance to salt and osmotic stress in addition to reduced phytate content in the seeds (Zhang et al. 2008). *AtPAP17* is also responsive to salt, ABA and low Pi (Del Pozo et al. 1999). Similarly, *GmPAP3* overexpression reduces the damaging effects of salt stress by decreasing oxidative damage (Liao et al. 2003; Deng et al. 2014). Similarly, in *Pennisetum glaucum*, *PgPAP18* like (purple acid phosphatase 18 like) confers tolerance to multiple environmental stress like heat, salt stress, PEG, and $CuSO_4$ in *E. coli* (Reddy et al. 2017). Such tolerance to oxidative stress may be due to the peroxidase activity of PAPs, as in the case of GmPAP3 or AtPAP17, but it needs experimental validation.

PAPs' role in root growth modulation

The change in root system architecture (RSA) is one of the primary responses plants exhibit in response to Pi deficiency. In a few cases, PAPs are associated with a change in root growth patterns in transgenics. For instance, *AtPAP10* overexpression resulted in increased root growth, such as primary and lateral root (LR) length and density under Pi deficiency (Wang et al. 2011). The overexpression of *AtPAP12* or *AtPAP26* also had similar results. Expectedly, the *atpap10* or *atpap12/atpap26* mutants resulted in attenuated root growth (Wang et al. 2011; Tran et al. 2010b). An increase in root growth is also reported on the overexpression of *AtPAP15* and *AtPAP18* in soybean or tobacco, respectively (Wang et al. 2009; Zamani et al. 2012). In rice also, *OsPAP21b* overexpression resulted in longer primary and lateral roots, whereas root biomass decreased in knockdown lines (Mehra et al. 2017). The *GmPAP4* overexpression in Arabidopsis affected lateral root length and number positively (Kong et al. 2014). The overexpression of *JcERF035* (an AP2/ERF transcription factor in *Jatropha curcas* L.) in Arabidopsis resulted in the suppression of *AtPAP17* expression and lateral root formation (Chen et al. 2018). This transcription factor could be a missing link between PAPs expression and increased lateral root formation, but it needs further validation. A few genes like Arabidopsis *LPR1*, *PDR2*, and *SHR* are known to affect both low Pi sensing and root architecture modulation (Pandey et al. 2013). It is also proposed that PAP may alter root development via cell wall modulation (Mehra et al. 2017; Deng et al. 2020). However, there are no reports explaining PAPs' direct roles in root architecture alteration and the mechanism behind it. It could be possible that their overexpression leads to the induction of Pi signaling, which in turn modulates root architecture and growth.

PAPs role in microbial associations

Symbiotic associations with bacteria or fungi are an inseparable part of plant's life, and PAPs are also found to affect such associations. In legumes, root nodules are the site for nitrogen fixation in the soil, and PAPs such as *GmPAP21*, *AsPPD1* are expressed in root nodules under Pi deficiency (Wang et al. 2015; Li et al. 2017). The overexpression of such PAPs negatively affected nodule formation. Whereas the silencing of *AsPPD1* resulted in early senescence in nodules and reduced nitrogenase activity (Wang et al. 2015). Recently, an RNA-seq analysis also indicated the role of 16 PAPs in maintaining Pi homeostasis in soybean nodules (Xue et al. 2018). PAPs (e.g., AsPPD1, GmPAP21) are involved in maintaining ATP/ADP ratio and energy charge, affecting the nodule formation and nitrogenase activity (Wang et al. 2015; Li et al. 2017). Pi demand for root nodules is high; thus, PAPs activity may help supply much-needed Pi and maintain nitrogenase activity.

Besides, PAPs are also involved in regulating another important symbiotic association, i.e., mycorrhiza. GmPAP33 has been reported to play an important role in arbuscular mycorrhizal symbiosis. *GmPAP33* gets induced on arbuscular mycorrhizal inoculation independent of P status (Li et al. 2019). The overexpression of *GmPAP33* resulted in improved yield, high P content and large arbuscules, whereas knockdown resulted in an increase in phospholipid concentration and smaller arbuscules. This study further revealed that the role of *GmPAP33* in arbuscule degeneration occurs through phospholipid hydrolysis (Li et al. 2019). However, PAPs' precise mechanism controlling the size, shape, or number of nodules/arbuscule remained unexplored.

Carbon metabolism

Recently, carbon metabolism was linked to further improvement of yield in high yielding green revolution rice varieties (Li et al. 2018). Some of the PAPs, like AtPAP2, are reported to be involved in carbon metabolism (Sun et al. 2012a). In Arabidopsis, *AtPAP2* does not respond to Pi starvation; instead, it is involved in carbon metabolism as *AtPAP2* overexpression in Arabidopsis resulted in the enhanced biomass, seed yield and higher sugar content. Further, overexpression lines showed induction of sucrose phosphate synthase (Sun et al. 2012a, 2018). Similar results were also obtained on overexpressing *AtPAP2* in *Camelina sativa*, i.e., improved growth rate and seed yield, thus enhancing oil yield/unit area (Zhang et al. 2012). Similarly, overexpression of *NtPAP12*, a

cell wall-bound phosphatase localized in apoplast, activates callose and cellulose synthases by dephosphorylation/photophosphorylation, thereby influencing cell wall synthesis (Kaida et al. 2009). Recently, overexpression of *PtPAP1* in *P. tricornutum* cells (diatoms) showed an increase in carbon content and led to the reallocation of this carbon flux to lipogenesis as depicted by higher lipid content. Furthermore, the *PtPAP1* overexpression showed higher photosynthetic efficiency and expression of genes involved in photosynthesis (Wang et al. 2021). These results assert the involvement of PAPs in carbon metabolism, and open up the new avenue for enhanced biomass production using PAPs.

PAPs' role in reproductive development

Interestingly, many PAPs showed high expression in reproductive tissues (Wang et al. 2011; Zhang et al. 2011; Mehra et al. 2015; Bhadouria et al. 2017). Zhu et al. (2005) reported that 28 *AtPAP*s showed expression in floral organs with varied intensity. Of these, seven members (*AtPAP 6*, *11*, *14*, *19*, *23*, *24*, and *25*) were expressed mainly in flowers. Even in chickpea, most of the *CaPAP*s are expressed in flower buds (Bhadouria et al. 2017). The detailed study of *AtPAP23* expression showed its presence in the floral apical meristem, immature flower, petals, and anthers in Arabidopsis (Zhu et al. 2005). Unexpectedly, *AtPAP23* transgenics (overexpression and knockdown lines) did not show any change in flower development. On the other hand, the overexpression of *OsPAP10a* and *OsPAP21b* resulted in early flowering (Tian et al. 2012; Mehra et al. 2017). These transgenics' early flowering phenotype can be correlated with improved P levels, as Pi deficiency is known to delay the flowering. However, a direct mechanism linking the activity of PAPs and flowering is yet to be unravelled.

PAPs expression modulates cellular signaling

The PAPs overexpressing plants had only a marginally increased APase activity over the untransformed plants but displayed a marked increase in organic P utilization. PAPs (such as AtPAP25, OsPAP21b) can dephosphorylate a wide range of substrates, including phosphoserine, phosphotyrosine, and phosphothreonine. These metabolites play an important role in multiple cellular signaling processes (Del Vecchio et al. 2014; Mehra et al. 2017). Thus, besides working as phosphatase, PAPs may also influence cellular signaling. Tobacco PAP, NtPAP12 localizes to the apoplast and is involved in dephosphorylation of a-xylosidase and b-glucosidase. Its overexpression leads to decreased glycosidases activity and a higher level of xyloglucan and cello- oligosaccharides (Kaida et al. 2010). AtPAP25 and NtPAP12, both are cell wall localized protein and can be studied for their role in signaling or altering cell wall phosphoproteome under Pi deprivation. The signaling role of PAPs has not been explored and can be an interesting area for future research. The PAPs like OsPAP21b also forms higher molecular weight complexes while interacting with other unknown proteins (Tran et al. 2010b; Mehra et al. 2017). It is possible that such a big protein complex might be linked with some of the PAPs' signaling roles.

Future perspectives

PAPs are central to plants response to Pi deficiency and adaptation. The emerging roles of PAPs in plant development processes like symbiotic/non-symbiotic association, flowering, seed germination, carbon metabolism, maintaining redox balance, nodule formation, oxidative stress, and defense mechanism suggest that they play greater roles in addition to Pi deficiency. The availability of whole-genome sequence of an ever-increasing number of plants has aided the identification of PAPs multigene family in diverse plants. Functional redundancy is expected among the members of a multigene family. However, emerging evidence reveals some level of sub- or neo-functionalization among PAPs and future research will strengthen PAPs' wider role besides Pi mobilization. The recent advancements in cellular transcriptomics would greatly help assign more specific roles to PAPs. Moreover, the proteome profiling, especially phosphoproteome of P deficient or plants subjected to other stresses in spatiotemporal manner will further aid in unraveling the mechanistic of PAPs functions.

Overexpression of PAPs has been a common strategy to understand their functions. The utilization of CRISPR/cas9 to generate PAPs knockout will provide more precise information on their roles in plants. Most studies used a constitutive promoter for overexpression, which sometimes results in undesired effects in P sufficient conditions. As demonstrated recently, using a native promoter can be a better way to study their roles (Deng et al. 2020). Only a handful of studies have tested PAPs overexpression transgenics in soil using organic-P as a sole P source (Mehra et al. 2017; Deng et al. 2020). The transgenics overexpressing PAPs should be assessed for agronomic traits using organic fertilizers in fields to evaluate their real potential for improving crop P-use-efficiency. This will help figure out the best candidate to be used for developing P efficient crops. To develop Pi efficient crops, the breeding programme often utilizes QTL approach. Although there are few reports where PAPs have been identified falling in QTLs associated with low Pi tolerance, such as HvPAPa is associated with *mqphy* QTL (associated with phytase activity), few ZmPAPs also co-localize with low Pi responsive cQTLs in a meta-analysis (Dai et al. 2011; Zhang

et al. 2014b; González-Muñoz et al. 2015). However, the information on PAPs association with P responsive QTL is at a nascent stage, and no PAP has been identified as a candidate gene so far. However, initial reports are encouraging and given their key role in P homeostasis, PAPs may be identified as an important candidate in future for molecular breeding to develop Pi efficient crops.

There remain many unanswered questions on PAPs biochemistry and new roles. What is the role of PAPs' N-terminal domain? Is it responsible for protein–protein interactions leading to oligomerization as reported in TRAPs? How post-translation modifications influence their catalytic behavior and how are they regulated? Answering these questions should be a target of future research. It should also be investigated how PAPs having roles other than P utilization are regulated at molecular levels? Lastly, PAPs are degraded on sufficient P supply (Mehra et al. 2017) losing the advantages to plants growing in P supplied soils. Pinning down amino acids responsible for their interaction with proteasome machinery and mutating them using gene-editing may help further improve P-use-efficiency. Considering the advantages provided by the PAPs to the crops (e.g., improved P-use-efficiency, hydrolyzing organic P, better yield), they could be potential targets for improving low P tolerance using molecular breeding and transgenesis.

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