



RESEARCH ARTICLE



Biochemical characterization of common bean PvPAP26, a ubiquitous purple acid phosphatase that is highly expressed during seedling development

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Abstract

Phosphatases are important enzymes involved in phosphate acquisition. Seedlings require high amounts of phosphate during early development, as it is a key component of nucleic acids and other essential molecules. However, knowledge about phosphate recycling during seedling development in plants is still limited. In *Phaseolus vulgaris* (common bean), total phosphatase activity increases during post-germinative development. The major phosphatase, detected by in-gel assays, was purified from embryonic axes. The purified protein was analyzed by MALDI-TOF/TOF mass spectrometry, which enabled identification of the corresponding gene through database searches. This gene was classified as a member of the Purple Acid Phosphatase (PAP) family. Inhibitor studies performed with the purified protein further confirmed its classification as a PAP. The PAP gene family in common bean consists of 26 members. Among the PAP proteins identified in *Arabidopsis thaliana*, the purified protein showed highest sequence homology with AtPAP26 and was therefore named as PvPAP26. The gene *PvPAP26* was expressed in all the tissues analyzed, both in seedlings and mature plants, with the highest expression observed in cotyledons during the phase of intense nutrient mobilization. Gene expression in seedlings was not affected by treatments with methyl jasmonate, phosphate supplementation, or salt stress. In leaves, gene expression was not altered by salt stress, wounding, or dark-induced senescence. These results indicate that *PvPAP26* is ubiquitously expressed across

all examined tissues, with markedly higher expression in cotyledons shortly after germination. A potential role for this protein in nutrient mobilization during early seedling development is discussed.

Keywords Phosphorus · Purple acid phosphatase · Seedlings development · PvPAP26

Introduction

Phosphorus is one of the most important essential nutrients for plant growth and development (Vance 2001). It is a structural component of key biomolecules such as nucleic acids, plays a critical role in the synthesis of phospholipids for biological membranes, and is an integral part of ATP, NADP, and numerous other compounds. Inorganic phosphate (Pi) serves both as a substrate and a product in many metabolic reactions. During glycolysis, several enzymes require phosphate or adenylates as co-substrates. In addition, triose phosphate and glucose phosphate translocators mediate the transport of triose phosphate or glucose phosphate across membranes in exchange for phosphate (Flores-Tornero et al. 2017). Phosphorus also acts as a regulator of the activity of various enzymes.

The bioavailability of phosphate in soil is typically low, ranging between 1 and 10 μM , which is far below the intracellular concentrations found in plant cells, generally in the millimolar range (Shen et al. 2011). As a result, phosphate fertilization is often necessary in agricultural systems, since the natural phosphate content of most soils is insufficient to meet the optimal requirements for plant growth (Vance et al. 2003). However, only a small fraction of the phosphorus applied through fertilizers is taken up by plants (Ojeda-Rivera et al. 2022), while the majority can leach into water bodies, leading to significant environmental concerns

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(Zak et al. 2018). Additionally, rock phosphate—the primary source for fertilizer production—is a non-renewable resource and is being rapidly depleted (George et al. 2016). Therefore, improving our understanding of the mechanisms that enhance phosphorus use efficiency in crops is essential (Chen et al. 2023). It has been proposed that plants employ two main strategies to increase phosphorus efficiency: improving phosphate acquisition efficiency (PAE) and/or enhancing phosphate utilization efficiency (PUE) (Adem et al. 2020; Han et al. 2022; Zou et al. 2022; Chen et al. 2023). The PAE strategy involves the release of root exudates, modifications in root morphology, and the establishment of symbiotic relationships with microorganisms. In contrast, the PUE strategy focuses on the recycling and remobilization of the internal phosphorus pool between organs, tissues, or subcellular compartments.

Plant acid phosphatases catalyze the hydrolysis of phosphate esters, releasing inorganic phosphate under acidic conditions, and play a crucial role in phosphate recycling (Li et al. 2002; Bozzo et al. 2006). These enzymes may function intracellularly to recycle and remobilize phosphate, or extracellularly after being secreted, where they scavenge phosphate from the external environment.

The availability of whole-genome sequences has enabled the identification of numerous genes encoding proteins with phosphatase activity. In the *Arabidopsis thaliana* genome, more than 50 putative acid phosphatases have been predicted (Li et al. 2002). Among them, the most prominent group is the Purple Acid Phosphatases (PAPs), which comprise 29 members in *Arabidopsis* (Li et al. 2002). PAPs are members of the metallophosphatase superfamily and are defined by the presence of a metallophos domain and a bimetallic reaction center at their active site (Schenk et al. 2013). PAPs contain five conserved domains required for metal binding (**DXG/GDXXY**/GNH(D/E)/VXXH/**GHXH**), with the bolded amino acids involved directly in the binding (Schenk et al. 2005). These seven amino acids, along with a conserved histidine in the third domain, constitute the active center of the enzyme. The PAP family in *Arabidopsis* has been classified into three groups based on the amino acid sequence similarity (Tran et al. 2010). Groups I and II consist of oligomeric, high-molecular weight PAPs, whereas group III includes monomeric, low molecular weight AtPAPs (Tran et al. 2010). Multigene PAP families have also been described in *Arabidopsis* (Li et al. 2002), rice (Zhang et al. 2011), soybean (Li et al. 2012), and maize (González-Muñoz et al. 2015). Several members of these superfamilies have been functionally characterized for their roles in phosphorus mobilization and utilization, such as AtPAP26 (Veljanovski et al. 2006) and AtPAP10 (Wang et al. 2011) in *Arabidopsis*, and OsPAP10a (Tian et al. 2012) and OsPAP10c (Lu et al. 2016) in rice. While most PAPs

are associated with responses to phosphate deficiency, some are also induced under salt stress (Liao et al. 2003; Reddy et al. 2017), osmotic stress, and oxidative stress (Li et al. 2008). Salinity and osmotic stress in plants can trigger the production of reactive oxygen species (ROS), which in turn induce PAP expression, suggesting a potential role for these enzymes in abiotic stress responses.

Germination and post-germinative growth are two developmental phases in which phosphorus plays a crucial role. Newly emerging seedlings require high levels of phosphorus, which are primarily supplied by reserves accumulated in the cotyledons. During seed development on the mother plant, storage compounds such as starch, proteins, and lipids are deposited and later used to support early seedling growth (Bewley 1997). Developing embryonic axes have particularly high phosphorus demands due to the need for nucleic acid synthesis. In this context, the availability of nucleotides during the early stages of seedling development is essential for successful germination (Stasolla et al. 2003). The main storage form of phosphorus in seeds is phytic acid, which can represent up to 75% of total P (Raboy 2020). Therefore, the utilization of accumulated phytate must be the most important source of phosphate for the developing axes. Another potential source of phosphate and/or nucleotides could be the nucleic acids present in dried seeds. For instance, the RNA carried out from seed development in mother plants present in seeds as residual messages (Nonogaki et al. 2010). We have observed the induction of enzymatic activities that catalyze nucleic acids degradation, as well as the expression of genes encoding these enzymes during early post-germinative development in common beans (Lambert et al. 2014, 2016; Diaz-Baena et al. 2021). The metabolism of the released nucleotides must involve a phosphatase that removes the phosphate from nucleotides, releasing nucleoside.

In this work, we analyzed phosphatase activity during the post-germinative development of common bean, with particular focus on the cotyledons as a source of nutrients for the developing embryonic axis. To perform this analysis, the major phosphatase activity in cotyledons was identified and purified, and the gene encoding this enzyme was cloned. In addition, we examined its potential involvement in various physiological processes characterized by high nutrient mobilization.

Materials and methods

Plant material

Phaseolus vulgaris plants of the Great Northern variety were used. The seeds were sterilized by immersion in

ethanol for 30 s, followed by treatment with 0.2% (w/v) NaClO for 10 min. The seeds were then rinsed at least 6 times with distilled water. Sterilized seeds were placed in 120 mm diameter Petri dishes containing 3 paper discs moistened with 10 ml sterile distilled water and seeds were covered with a fourth layer of paper disc moistened with 2 ml water. Unsealed Petri dishes were placed in a growth chamber under a 14 h light / 10 h dark photoperiod, with daytime temperature set at 26 °C, nighttime at 20 °C, and constant relative humidity of 70%. Photosynthetic photon flux density was 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The amount of water inside the plates was maintained by adding distilled water daily.

For experiments involving common bean plants older than 6 days post-imbibition, seedlings were transferred at day 3 to pots (18 cm diameter, 15 cm high) containing a perlite:vermiculite substrate (1:3, v/v), with 3 seedlings per pot. Plants were maintained in a growth chamber under the conditions described above until sample collection. The plants were watered 3 times per week with culture medium for plants (Harper and Gibson 1984) diluted 1/4 and supplemented with 10 mM KNO_3 as a nitrogen source.

The analysis of expression in common bean radicles subjected to different treatments was performed in radicles isolated from seedlings at 6 days post imbibition and grown in petri dishes. Seedlings were grown under standard conditions until day 5 after imbibition, at which time they were subject to different treatments for 24 h: sterile water supplemented with methyl jasmonate 250 μM (MeJA), NaCl 200 mM (NaCl), phosphate 5 mM (P) and subjected to needle puncture every 5 mm. As control seedlings were maintained with distilled water (–).

The analysis of expression in common bean leaves subjected to different treatments was performed in the first trifoliate leaves at 28-day post imbibition in plants growth with 10 mM as nitrogen source except for fixation treatment that were obtained from plants in nitrogen fixing conditions. Dark-induced senescence was obtained by wrapping the leaves in aluminium foil 6 days before sample collection. Salt effect was analysed after supplementing the culture medium with 50 mM NaCl from the time of sowing. Wounded leaves were obtained after damaging the leaves with forceps 24 h before collecting the samples.

Unless otherwise indicated, plant material was collected, frozen by immersion in liquid nitrogen and stored at – 80 °C until use.

Preparation of crude extracts

Frozen plant material was ground to a fine powder using a mortar and liquid nitrogen, and stored at – 80 °C until use. For extraction, an aliquot of the powdered material was

homogenized in extraction buffer (TES-NaOH 50 mM pH 7, Deoxycholate 0.15% (w/v)). The suspension was centrifuged at 24,000g for 10 min at 4 °C, and the supernatant was used as the crude extract.

Protein purification

All purification steps were performed between 0 and 4 °C using working buffer (50 mM Tes-NaOH, pH 7). Crude extracts were obtained as described above from 37 g of embryonic axes collected from seedlings at 6 days after imbibition. Fresh plant material was homogenized in a mortar using working buffer supplemented with 0.15% deoxycholate (w/v) as extraction buffer, in a ratio of 5 ml per gram of fresh tissue. The homogenate was centrifuged at 22,000g for 10 min at 4 °C, and the resulting supernatant was used as the crude extract for further purification.

Ion exchange chromatography

The crude extract was subjected to ion exchange chromatography using DEAE-Sephacel column (7.5 × 2 cm, Sigma-Aldrich). Chromatography was performed at a flow of 20 ml/h and using the working buffer. The column was pre-equilibrated by successive washed with 4 volumes of deionized water, 5 volumes of working buffer supplemented with 1 M NaCl, and 10 volumes of working buffer. After protein loading, the column was washed with 4 volumes of working buffer containing 0.1 M NaCl. Elution was carried out using a first continuous gradient of 15 volumes from 0.1 to 0.3 M NaCl (260 ml) in working buffer, followed by a second gradient of 7 volumes from 0.3 to 1 M NaCl (120 ml) in the same buffer. Fractions of 7 ml were collected, and protein concentration and phosphatase activity were measured in the presence and absence of molybdate. Fractions showing molybdate-sensitive phosphatase activity were pooled and used in the next purification step.

Affinity chromatography

The enzyme preparation obtained after ionic exchange chromatography was subjected to affinity chromatography using a ConA-Sepharose column (1.5 × 3.5 cm, GE Healthcare). The column was first activated with 0.02 M Tris buffer (pH 7.4) containing 0.5 M NaCl and 1 mM MgCl_2 . The column was then equilibrated with 5 volumes of working buffer. Chromatography was performed at a flow rate of 1 ml/min. Bound proteins were eluted with a gradient from 0 to 0.5 M methyl α -D-mannopyranoside in working buffer, at the same flow rate. Fractions of 2 ml were collected.

Phenyl sepharose chromatography

The fractions with phosphatase activity from the previous chromatography were pooled, and ammonium sulfate was added until reaching a final concentration of 1 M. This mixture was loaded onto a Phenyl Sepharose column (1.5×3.5 cm, GE Healthcare) that had previously been equilibrated with working buffer containing 1 M ammonium sulfate. Elution was performed at a flow rate of 1 ml/min in a gradient of 30 column volumes from 1 to 0 M ammonium sulfate in working buffer. Fractions of 2 ml were collected in which phosphatase activity was measured.

Identification of the protein by peptide fingerprinting (MALDI-TOF)

The 95 kDa protein was digested with trypsin, and the resulting peptides were purified using a C18 resin micro-column (Millipore) and eluted directly with a matrix solution (3 mg/ml alpha-cyano-4-hydroxycinnamic acid in 70% acetonitrile/0.1% TFA) onto a MALDI plate in a volume of 1 µl. After co-crystallization on the plate, the samples were analyzed by MALDI-TOF/TOF mass spectrometry to obtain the peptide fingerprint (MS) in a mass spectrometer (4800 Plus MALDI TOF/TOF Analyzer (AB Sciex)) equipped with delayed extraction, reflector, and positive mode, in a mass/charge (m/z) range of 800 to 4000 Da, with an accelerating voltage of 20 kV. Internal calibration of the spectra was performed, achieving a precision in the m/z measurement of ± 20 ppm. Fragmentation spectra (MS/MS) were obtained for the five most intense m/z . Protein identification was performed by combining the MS spectra and their corresponding MS/MS spectra using MASCOT as the search engine. The analysis was carried out by the Central Research Support Service (SCAI).

Determination of phosphatase activity

In vitro

Phosphatase activity was determined by measuring the release of inorganic phosphate in the reaction mixture, as described by Cabello-Díaz et al. (2012). Unless otherwise indicated, the standard assay conditions were 50 mM MES buffer (pH 5.5), 5 mM pNPP as substrate, and a suitable amount of crude extract or purified protein. The reaction began with the addition of an appropriate amount of enzyme preparation and was incubated at 37 °C. At 0 and 30 min after start of reaction, 0.2 ml aliquots were transferred to 1.5 ml vials containing 0.6 ml of 1 N sulfuric acid and phosphate concentration was determined. To each vial, 0.1 ml of 2.5% (w/v) ammonium molybdate dissolved in 3

N H₂SO₄ and 0.1 ml of reducing solution were added. The reducing solution consisted of 2% (w/v) ascorbic acid and 2% hydrazine sulfate dissolved in 0.1 N H₂SO₄. The tubes were shaken and after 50 min of incubation at room temperature, the absorbance was determined at 820 nm. The molar extinction coefficient was 19 mM⁻¹ cm⁻¹. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 µmol of phosphate per minute.

In gel

The crude extract was mixed with loading buffer (without reducing agent) at a 1:3 ratio and loaded onto an acrylamide gel without reducing agent and was not heated. After SDS-PAGE, the gels were washed two times for 10 min each with 50 mM MES pH 5.5 and incubated at 37 °C without shaking in a reaction mixture described above for the in vitro activity. The reaction mixtures were then removed, and a phosphate precipitation solution (1% (w/v) ammonium molybdate, 100 mM triethylamine, and 1 M HCl) was added to precipitate the phosphate released during the enzymatic reaction. When a visible band appeared, the solutions were removed, and the reactions were stopped by adding distilled water.

Biochemical characterization of the purified enzyme

Determination of the optimum temperature

Activity was determined under standard conditions, but the assay was performed at different temperatures (30, 40, 50, 60, 65, and 70 °C) for a reaction time of 30 min. Non-enzymatic phosphate release was determined at each temperature.

Determination of the optimum pH

Activity was determined in a buffer mixture composed of succinate-MES-TES, 50 mM of each, adjusting the pH values between 4 and 9. Activity was determined using standard conditions. Non-enzymatic phosphate release was determined at each temperature.

Effect of inhibitors and cations

Enzyme activity was assayed in the standard conditions in the presence of several potential inhibitors and cations at a final concentration of 1 mM. Non-enzymatic phosphate release was determined at each temperature.

Substrate specificity

Activity was assessed using various phosphorylated compounds as substrates at a final concentration of 5 mM in the reaction mixture.

Determination of apparent K_m

Phosphatase activity was tested in reaction mixtures containing various phosphorylated compounds as substrates at concentrations between 0 and 5 mM. The assay was performed under standard conditions but with a reaction time of only 10 min.

Protein determination

Protein concentration was determined by Bradford method (Bradford 1976) using the Bio-Rad reactive and using bovine serum albumin as standard.

RNA isolation and cDNA synthesis

Total RNA was extracted from 50 mg of pulverized material using NZYol reagent (NZYTECH, Lisbon, Portugal), following the manufacturer's instructions with the exception of an additional LiCl precipitation step at the end of the procedure to improve RNA quality. RNA concentration was measured using a NanoVue Plus spectrophotometer (GE Healthcare, Little Chalfont, United Kingdom). The purity of the RNA was determined by measuring absorbance at 230, 260, and 280 nm in the NanoVue Plus spectrophotometer. The integrity of the RNA was determined by electrophoresis on agarose gels and visualization of 28 and 18S rRNA. cDNA synthesis was carried out as described in Díaz-Baena et al. (2021).

Quantitative PCR

Gene expression was analyzed by quantitative RT-PCR as previously described (Díaz-Baena et al. 2021). Ubiquitin

and actin 2 were used as reference genes. All primers used are listed in Table 1. Primer specificity for each pair was confirmed to be greater than 90%.

Statistic

All results represent the averages of three independent biological experiments, each with two technical replicates. The statistical analyses used are described in the corresponding figure legends.

Data bases

The Phytozome database v.14.0 (<http://phytozome.jgi.doe.gov/pz/portal.html>) was used to identify PAP genes in *Phaseolus vulgaris* and *Arabidopsis thaliana*. Chromosomal location was determined based on the genomic data available in the same database (Phytozome v14.0). Amino acid sequences were aligned using the MUSCLE algorithm (Edgar 2004) implemented in MEGA-X version 10.0.5 (Kumar et al. 2018). Phylogenetic relationships were reconstructed through the Neighbor-Joining approach (Saitou and Nei 1987), applying the Poisson correction model and conducting 1000 bootstrap replicates to assess branch support. Subcellular localization, protein type, and presence or absence of a signal peptide were predicted using DeepLoc-2.1 (Ødum et al. 2024) and SignalP 6.0 (Teufel et al. 2022). Localizations with a probability under the threshold established by the program were named as undetermined (n.d.). Molecular weight predictions were performed using SnapGene Viewer v5.2.4.

Results

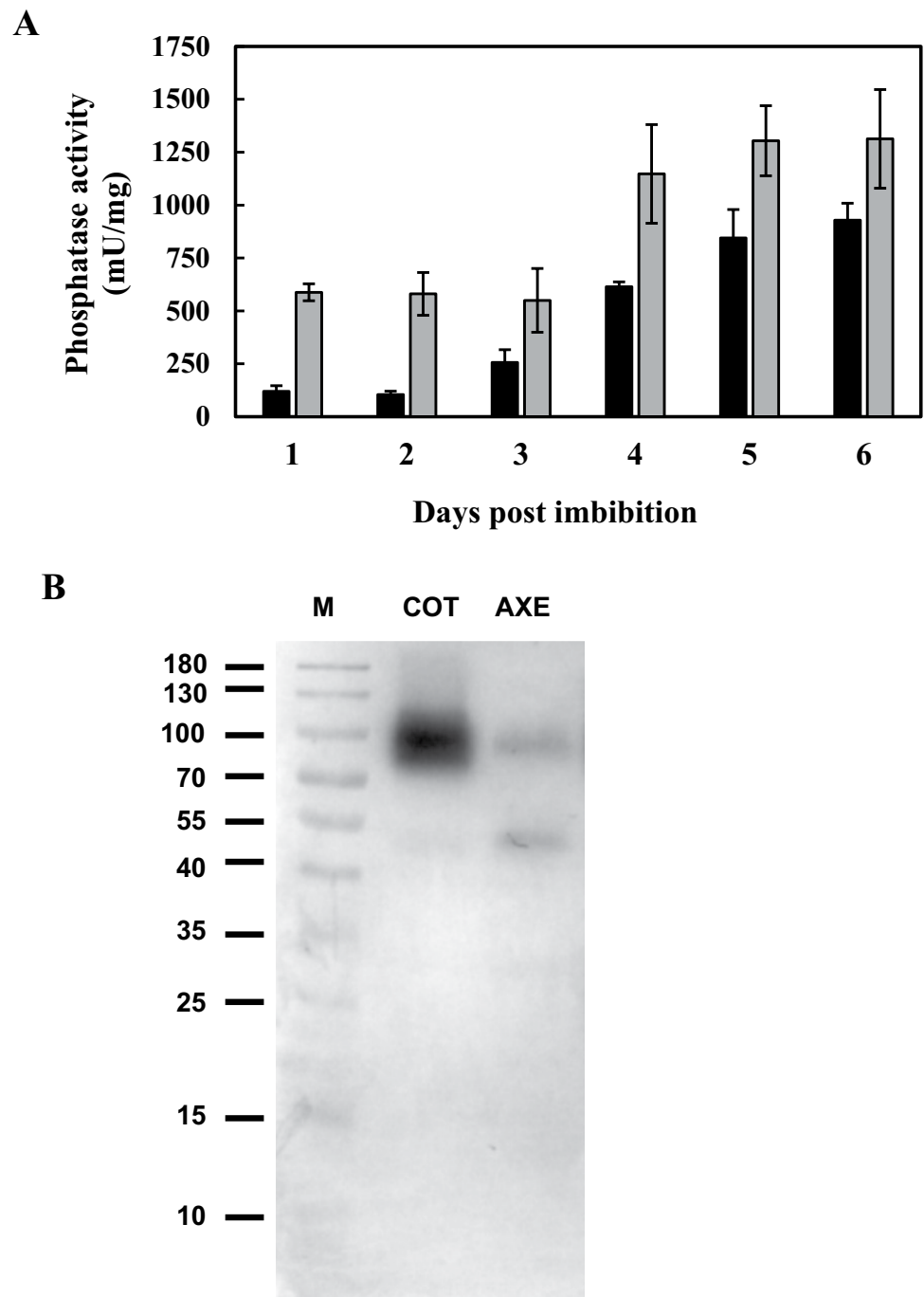
Phosphatase activities during seedling development in common bean

Total phosphatase activity was measured with pNPP as substrate in crude extracts from cotyledons and embryonic axes at different stages of germination and seedling development in common bean. Radicle emergence occurred between the second and third day after the start of imbibition, marking the end of germination and the beginning of post-germinative development. Total phosphatase activity remained constant during germination in both tissues but increased during post-germinative growth (Fig. 1A). Specific activity was consistently higher in cotyledons than in embryonic axes (Fig. 1A). In gel activity assays under non-denaturing SDS-PAGE conditions (absence of heat and reducing agents but in the presence of SDS) revealed the existence of a phosphatase activity band of approximately 95 kDa in cotyledons

Table 1 Primers used in qRT PCR

	Forward 5'>3'	Reverse 5'>3'
<i>PvPAP26</i>	AAGTTGSCCGGATGCTTCC	ACAGTC TCCGCT CCACTC TG
<i>ACTIN</i>	GCAATTCAGGCTGTCTTGCT TTGT	TAAATC ACGGCC AGCAAG ATCC
<i>UBIQUITIN</i>	TACATGCGATCTTGACTGGC	GGGGCT TTTCTGG GTAGTCT

Fig. 1 Phosphatase activity in embryonic axes and cotyledons of common bean during germination and postgerminative development. **A** Total phosphatase activity was measured in crude extracts of embryonic axes (black bars) and cotyledons (grey bars) at the indicated days post-imbibition using pNPP as substrate. Data are presented as mean $\pm$ SD of three biological replicates, with two technical replicates. **B** In gel phosphatase activity in crude extracts from cotyledons (COT) and embryonic axes (AXE) 6 days after start of imbibition. The molecular weight of the markers is indicated at the left



(Fig. 1B). In embryonic axes an additional second activity band of approximately 50 kDa was also detected (Fig. 1B), which corresponds to the phosphatase previously purified and characterized by Cabello-Díaz et al. (2012).

Purification and identification of the 95 kDa phosphatase in common bean seedlings

The phosphatase with an apparent molecular mass of approximately 95 kDa was purified to electrophoretic homogeneity

from developing embryonic axes collected 6 days post imbibition (Table 2). This tissue was selected for purifying the protein due to its extracts stability and lower lytic activity compared to cotyledons. Under non-reducing and non-denaturing SDS-PAGE conditions, the purified protein migrated as a single band of approximately 95 kDa (Fig. 2). Upon treatment with reducing agents and heat, the protein resolved into two bands of 57 and 52 kDa, indicating that the enzyme is a dimer (Fig. 2). The final purified enzyme exhibited a specific activity of 192 U/mg protein (Table 2),

Table 2 Purification procedure for 95 kDa phosphatase. The main steps are indicated in Material and Methods

	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification factor
Crude extract	208	670.70	0.3	100	1
DEAE	174	49.15	3.5	83.8	11.4
Concanavalin-A	25	0.13	190.0	12.2	613.1
Phenyl Sepharose	16	0.08	192.2	7.8	620.1

corresponding to a turnover number of $20,000 \text{ min}^{-1}$ for the 95 kDa complex. The complete purification protocol resulted in a final yield of 7.8% and a purification factor of 620, suggesting that the phosphatase represents approximately 0.16% of the total protein content in embryonic axes of common bean.

The purified phosphatase exhibited optimal activity in vitro at 50°C (Fig. 3A), with an activation energy value of 48.3 kJ mol^{-1} and a Q_{10} value of 1.78 between 40 and 50°C . The enzyme showed moderate thermal stability, retaining nearly full activity after incubation at 50°C for 20 min. However, incubation at temperatures above 60°C resulted in a substantial loss of phosphatase activity. The purified protein displayed maximal activity at pH 6, with very low activity at pH 4 and pH 8 (Fig. 3B). Phosphatase activity was completely inhibited by molybdate, fluoride, vanadate, zinc and copper (Fig. 3C). Substrate specificity assays revealed that the enzyme was active on a broad range of phosphorylated compounds, including several nucleotides (Fig. 3D). Highest activity was observed with the artificial substrate pNPP, as well as with phosphoenolpyruvate (PEP), pyrophosphate (PPi), and phosphotyrosine (TyrP). Kinetic parameters were determined for these substrates and compared with values obtained for two nucleotides, AMP and IMP. The enzyme followed Michaelis–Menten kinetics with all substrates tested. K_m values of $180 \mu\text{M}$ and $190 \mu\text{M}$ were obtained for PEP and TyrP, respectively, indicating the highest substrate affinity for these compounds. K_m values of 420, 510 and $490 \mu\text{M}$ were obtained for PPi, AMP and IMP, respectively. Furthermore, the catalytic efficiency constant for nucleotides (4 and $6 \mu\text{M}^{-1} \text{ min}^{-1}$ for IMP and AMP) was lower than for the other phosphorylated compounds assayed (46 , 88 and $99 \mu\text{M}^{-1} \text{ min}^{-1}$ for PPi, TyrP and PEP) indicating that nucleotides are poor efficient substrates for purified enzyme.

The purified 95 kDa protein was subjected to MALDI TOF/TOF analysis. After trypsin fragmentation, a series of peptides was obtained (Fig. 4A) that constitute its peptide fingerprint. In addition, the 5 peptides with the highest signal were fragmented to obtain their fragmentation spectra (Fig. 4A, peptides in bold). The sequences of 18 peptides enabled identification of the gene Phvul.003G170500 in the

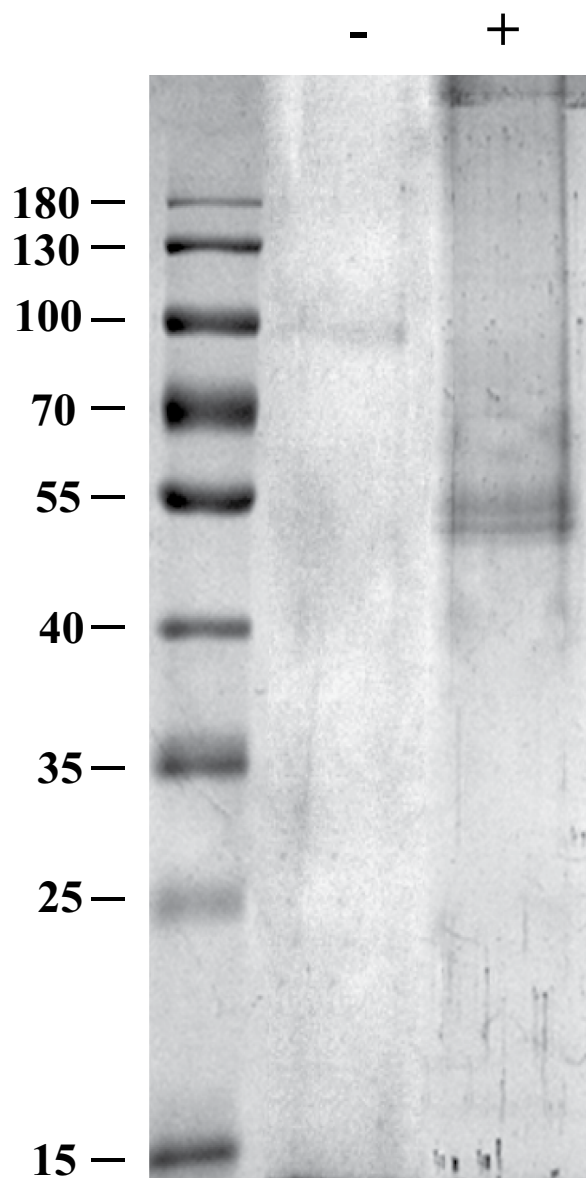


Fig. 2 Electrophoresis in polyacrylamide gel of purified phosphatase of *Phaseolus vulgaris*. -) Purified protein (40 ng) was mixed with SDS containing loading buffer in the absence of reducing agents and heat and subjected to electrophoresis on a 10% acrylamide gel. +) Purified protein (40 ng) was mixed with loading buffer with SDS and DTT, the mixture was built for 5 min and analysed by SDS-PAGE. After electrophoresis the gels were silver-stained. The whole gel from starts of separating to the dye front was photographed. Molecular weight markers are shown on the left side of the figure (PageRuler prestained protein ladder, Thermo Scientific)

genome database (Phytozome) with a probability of 100% (total ion C.I. 100%). The identified peptides are underlined in Fig. 4B, and cover approximately 41% of the deduced protein sequence (Fig. 4). The corresponding gene identified is categorized as a putative purple acid phosphatase (PAP).

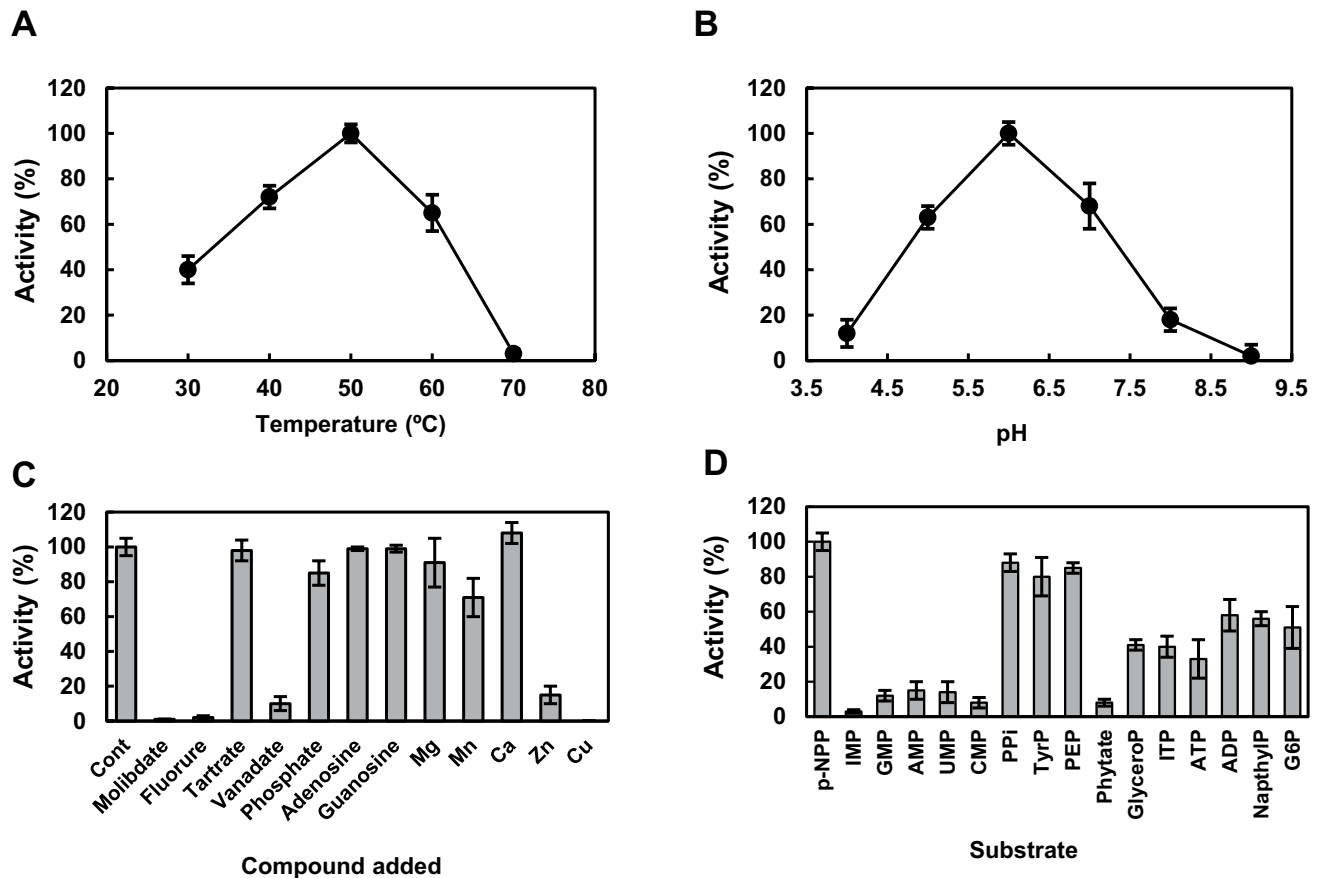


Fig. 3 Characterization of phosphatase activity from the purified protein. **A** Activity was measured using pNPP as substrate at the indicated temperatures. **B** Activity was measured using pNPP as a substrate in a buffer mixture containing succinate-MES-TES (50 mM of each) adjusted to the indicated pH values. **C** Effect of various compounds on purified protein phosphatase activity. Enzyme activity was determined in the presence of the indicated compounds at a final concentration of

1 mM. The value of the activity in the absence of compounds was taken as 100% (Cont). **D** Substrate specificity of purified phosphatase activity. Enzyme activity was measured using the indicated compounds at a final concentration of 5 mM as substrate. Results are expressed relative to the activity obtained with pNPP (set as 100%). Data are presented as mean $\pm$ SD from four independent determinations

Analysis of the PAP gene family in common bean

A search for putative PAP genes in the *Phaseolus vulgaris* genome using *Arabidopsis thaliana* PAP sequences as queries identified 26 candidate genes (Supplementary Table 1). In common bean, these genes are distributed across all 11 chromosomes, and in several cases, their genomic arrangement suggests that they may have arisen from gene duplication events, as some are located in tandem on the same chromosome (Supplementary Fig. 1). The cDNAs encode peptides with molecular masses for the predicted mature proteins between 25.5 KDa for Phvul.003G170632 and 74.9 KDa for Phvul.003G042300 (Supplementary Table 1). Most of the predicted proteins 21 out of 26 are predicted to contain a signal peptide (Supplementary Table 1). Subcellular localization and classification as soluble or membrane-associated proteins were inferred using DeepLoc v1.0 (Armenteros et al. 2017) (Supplementary Table 1). For 12 proteins the

probability obtained was below the threshold indicated in the program and, therefore, is indicated as undetermined. Most of the proteins are predicted to be in the lysosome/vacuole or in the extracellular space. A phylogenetic tree constructed using the PAP proteins from *Arabidopsis* and common bean is shown in Fig. 5. The highest identity from the protein deduced for Phvul.003G170500 corresponds to AtPAP26. Among them, the identity at the amino acid level corresponds to 79% and, therefore, based on this result, the protein was identified as PvPAP26. The deduced signal peptide is 28 amino acids long coding for a mature protein of 52.55 kDa that with a probability of 0.62 is located in the lysosome-vacuole.

A

Sequence	Calc. Mass	Obsrv. Mass	Start Seq.	End Seq.	C.I. %
WDTWGR	820.374	820.377	211	216	96
TKYYR	893.452	893.457	132	137	
SVFESWFIK	1142.588	1142.586	337	345	
EFWFETPPK	1180.567	1180.578	146	154	
THAIYHWNR	1197.591	1197.596	430	438	100
FLDPQPEYSAFR	1469.706	1469.718	404	415	100
ASAHIVLSSYSPFVK	1718.948	1718.947	275	290	
THAIYHWNRNDDGK	1726.804	1726.811	430	443	
SGYIHHCVIEGLEK	1804.869	1804.872	117	131	
RASAHIVLSSYSPFVK	1875.049	1875.056	274	290	
YTPQYMWLQEELKR	1884.931	1884.952	291	304	
YKVDVIFAGHVHAYER	1903.981	1903.986	346	361	
IGSGDSSREFWFETPPK	1939.919	1939.934	138	154	
SAPVYITVGDGGNQEGLASK	1962.977	1962.982	384	403	100
GYNAPQQVHITQGDYDGK	1990.925	1990.934	56	73	100
SGYIHHCVIEGLEKTK	2034.011	2034.021	117	133	
EFWFETPPKVDPDASYK	2055.970	2055.979	146	162	
SEWPAVDIPLDHEAFVVPK	2121.065	2121.076	37	55	

B

1-	MERRVQTMLL	KFVLASFVLL	VSIRDGSAGI	TSSFIRSEWP	AVDIPLDHEA
51-	FAVPKGYNAP	QQVHITQGDY	DGKAVIISWV	TPDEPGPNHV	QYGTSESKFQ
101-	TSLEGTVTNY	TFYEEKSGYI	HHCVIEGLEK	TKYYRIGS	GDSSREFWFE
151-	TPPKVDPDAS	YKFGIIGDLG	QTFNSLSTLE	HYIQSGAETV	LFVGDLCYAD
201-	RYEYNDVGLR	WDTWGRFVER	STAYHPWIWA	AGNHEIDYMP	YMGEVVPFKN
251-	FLYRYTTPYL	ASNSSNPLWY	AVRRASAHII	VLSSYSPFVK	YTPQYMWLQE
301-	ELKRVDREKT	PWLIVLMHVP	LYNSNGAHYM	EGESMRVFE	SWFIKYKVDV
351-	IFAGHVHAYE	RSYRFSNIDY	NITNGNRYPL	PDKSAPVYIT	VGDGGNQEGL
401-	ASKFLDPQPE	YSAFREASYG	HSTLEIKNRT	HAIYHWNRRND	DGKKVPTDSF
451-	VLHNQYW				

Fig. 4 Identification of the purified protein. **A** Peptide sequences obtained by MS and MS/MS. Fragmentation spectra and resulting data were analyzed using the MASCOT software. Peptides further fragmented are in bold. The calculated and observed mass and the peptide position in the deduced protein are indicated. For fragmen-

tated peptides the C.I. in percentage is shown. **B** Identification of the corresponding protein containing the peptides identified by MS/MS analysis after database search. The identified peptides are underlined. The amino acids involved in metal binding highlighted in grey

Expression analysis of *PvPAP26* during germination and early seedling development

The expression of *PvPAP26* was analysed in French bean seedlings during germination and early post-germinative

development (Fig. 6A). In embryonic axes, transcript levels were very low at 1 day post imbibition (DPI), but increased significantly by day 2, reaching a maximum at that stage (Fig. 6A, black bars). In cotyledons, *PvPAP26* expression was also low at 1 DPI, increased at 2 DPI, and remained

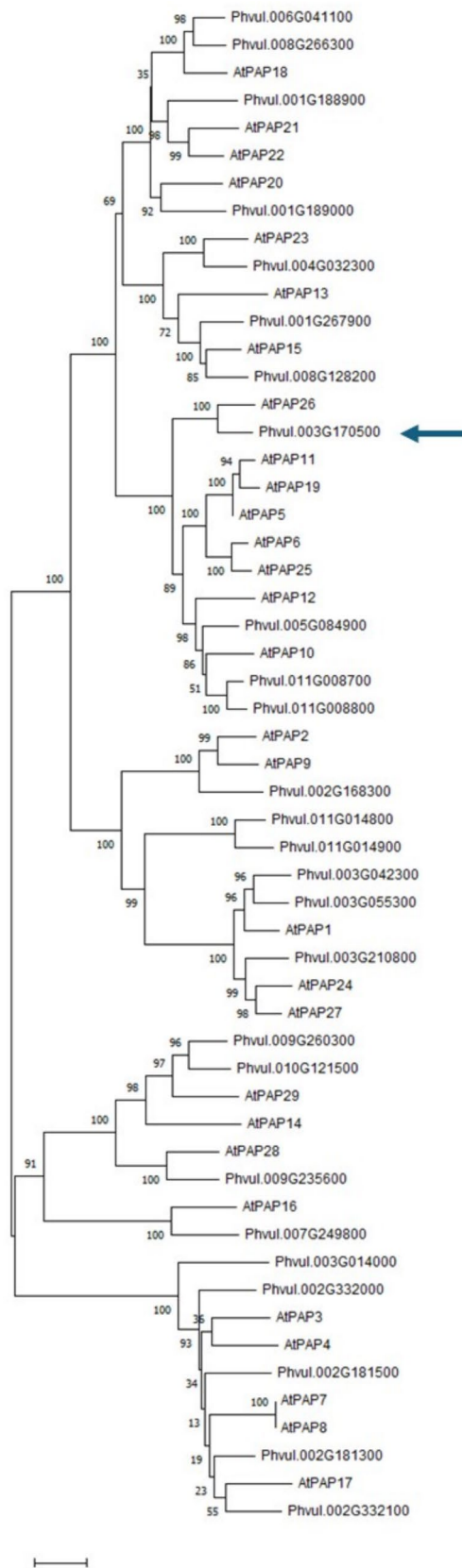


Fig. 5 Phylogenetic tree of Purple Acid Phosphatases (PAPs) identified in the *Phaseolus vulgaris* genome and PAPs from *Arabidopsis thaliana*. The position of the purified protein (PvPAP26) is indicated by an arrow. Sequence alignment and tree construction were performed as described in Materials and Methods

relatively constant throughout subsequent development (Fig. 6A, grey bars).

The expression of *PvPAP26* was analysed in cotyledons and radicles of common bean seedlings under conditions associated with changes in nutrient mobilization. In cotyledons, expression remained high during the phase of active globulins mobilization (6 to 9 DPI) and declined markedly by 11 DPI, when globulins reserves were nearly depleted (Lambert et al. 2016) (11 DPI, Fig. 6B). In radicles, *PvPAP26* expression was not significantly affected by salt stress, methyl jasmonate treatment, or mechanical wounding (Fig. 6C).

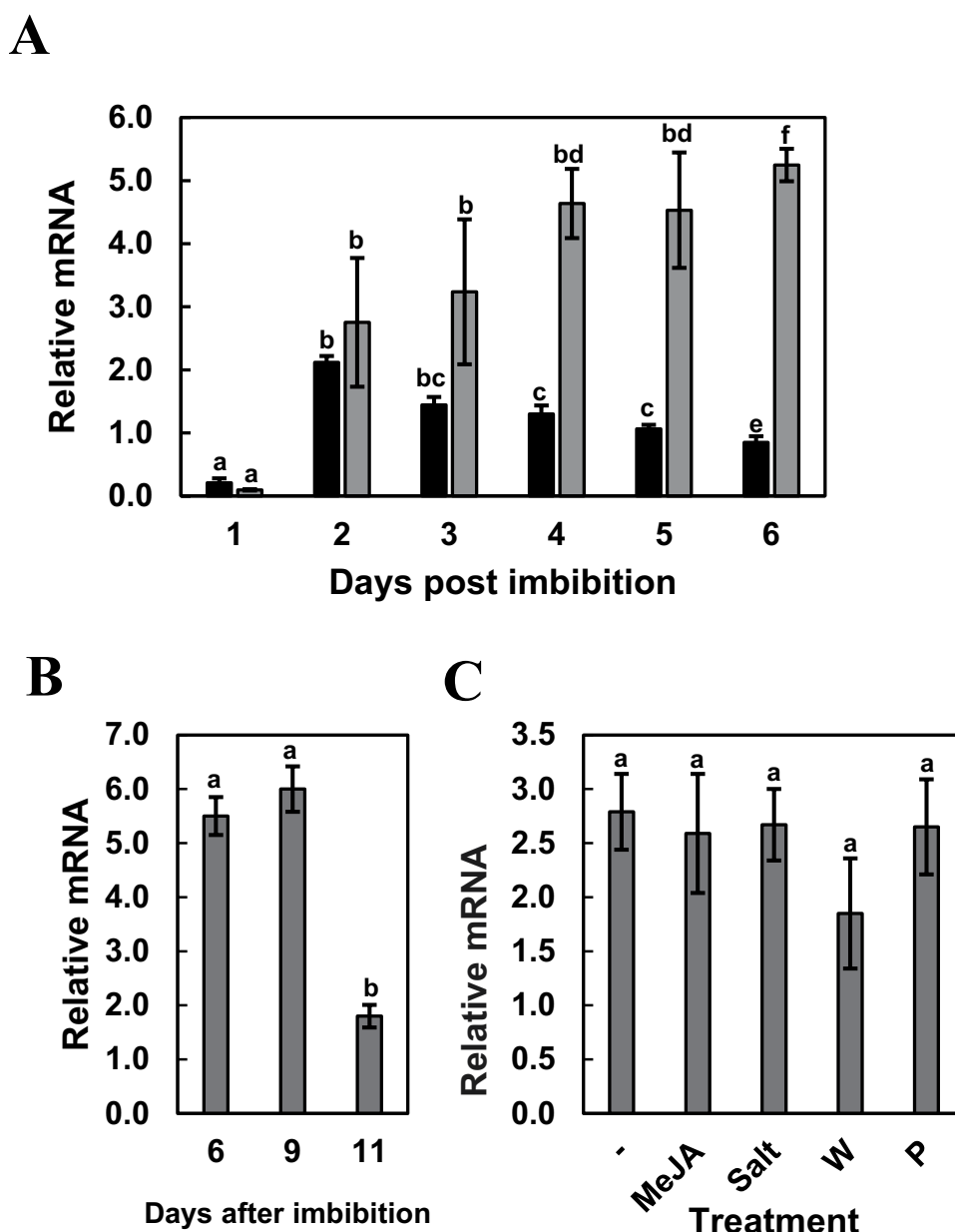
Expression analysis of *PvPAP26* in adult plants

The expression of *PvPAP26* was also analyzed in various tissues of adult plants. In all cases, *PvPAP26* was expressed with highest values in pods and leaves, but these values remained lower than those observed in cotyledons at 6 days after imbibition (Fig. 7A). To evaluate whether physiological conditions associated with altered nutrient mobilization affect gene expression, *PvPAP26* transcript levels were measured in leaves subjected to dark-induced senescence, salt stress, biological nitrogen fixation, or mechanical wounding. None of these treatments significantly altered *PvPAP26* expression in leaves (Fig. 7B). Since fruit development involves intense nutrient redistribution, *PvPAP26* expression was also analyzed in pods and seeds during developing and seed filling stages (Fig. 7C). No big changes in transcript levels were observed in pods during these developmental phases (Fig. 7C). The expression in seeds during seed filling phase was lightly lower than the expression in pods (Fig. 7C).

Discussion

Phosphorus is an essential macronutrient and a structural component of key molecules involved in plant metabolism, including nucleic acids, phospholipids, and energy carriers such as ATP. During the early stages of seedling development, the demand for phosphorus is particularly high, and this requirement must be met by the reserves stored in cotyledons during seed maturation. The main storage form of phosphorus in seeds is phytate (Rousseau et al. 2020; Jha et al. 2022). Despite the importance of phosphorus at this stage, little is known about the mechanisms of phosphate

Fig. 6 *PvPAP26* expression in common bean seedlings. **A** Expression analysis in developing axes (black bars) and cotyledons (grey bars) by qRT PCR at the indicated days after the start of imbibition. **B** Expression of *PvPAP26* in cotyledons at the different days after the start of imbibition. **C** Effect of treatment with MeJA, salt, wounding and phosphate on *PvPAP26* expression in radicles of bean seedlings. Treatments were 250 μ M MeJA for 24 h, 200 mM salt for 24 h, wounding for 24 h, 5 mM phosphate. Data are presented as mean $\pm$ SD of three biological replicates, with two technical replicates. Significant differences, according to Tukey's test, are indicated with different letters ($p < 0.05$)



mobilization in germinating common bean seedlings. In this study, total phosphatase activity was determined in both embryonic axes and cotyledons of French bean seedlings during germination and early post-germinative development. The in gel assay analysis allowed us to identify two main bands. The lower band with an apparent molecular mass of 55 kDa was previously purified and characterized as a phosphatase resistant to molybdate and with high affinity for nucleoside monophosphate as substrates (Cabello-Díaz et al. 2012). In this study, we have carried out the purification and characterization of the activity band with an apparent molecular mass of around 95 kDa. The purified phosphatase from embryonic axes likely corresponds to the major molybdate-sensitive phosphatase activity

previously detected in this tissue (Cabello-Díaz et al. 2012), in addition to the molybdate-resistant enzyme purified and characterized (Cabello-Díaz et al. 2012). The enzyme was strongly inhibited by molybdate and vanadate, two common inhibitors of acid phosphatases (Duff et al. 1994), but was resistant to tartrate, a characteristic feature of purple acid phosphatases (PAPs) (Anand and Srivastava 2012).

The ability of the purified enzyme to bind concanavalin A suggests that it is glycosylated, which is a common feature among PAPs. It has been proposed that between 5 and 10% of the total molecular mass of PAPs is attributable to carbohydrate content (Schenk et al. 2013). The 95 kDa purified protein consists of two subunits of approximately 57 and 52 kDa indicating a dimeric structure. This organization is

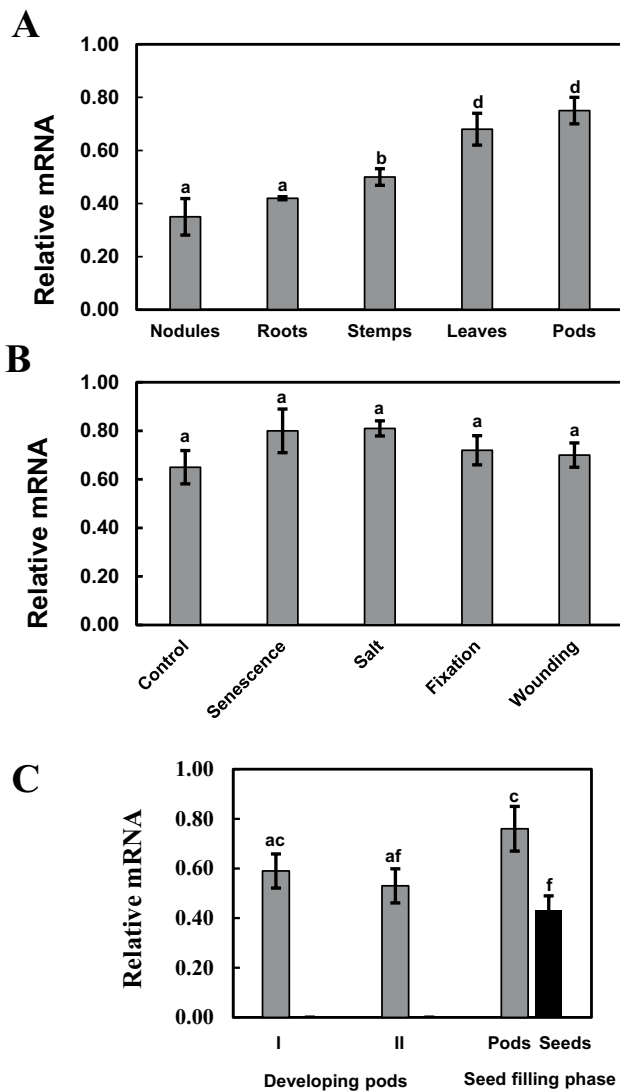


Fig. 7 Relative expression of the *PvPAP26* in different organs of adult common bean plants. **A** Relative expression in the indicated vegetative organs of adult plants. **B** Expression in leaves subjected to different treatments, compared to 28-day-old control leaves from plants grown with 10 mM nitrate as the nitrogen source. **C** Expression in reproductive organs. Developing pods correspond to whole fruits at 6 (I) and 14 (II) days after anthesis. Fruits at the seed filling stage were separated into pods and seeds. Data are presented as mean $\pm$ SD of three biological replicates, with two technical replicates. Significant differences, according to Tukey's test, are indicated with different letters ($p < 0.05$)

consistent with observations in tomato, where PAPs have been reported as heterodimers composed of 63 and 57 kDa subunits (Bozzo et al. 2004), and in Arabidopsis, where differentially glycosylated isoforms of AtPAPs have been described (Tran et al. 2010). Proteomic analysis performed with pure protein suggests that, in common bean, the two subunits could be homodimers, and it is plausible to consider that both subunits could represent differently glycosylated forms of the same protein. The common bean protein thus belongs to the high molecular weight (HMW) group of

PAPs, most of which are homodimers stabilized by disulfide bonds (Tran et al. 2010; Schenk et al. 2013).

The purified enzyme displayed a sharp pH activity profile, with an optimum centered around pH 6.0. In vitro substrate assays revealed that the enzyme exhibits catalytic activity toward a wide range of phosphorylated compounds, with highest activity observed for pNPP, PEP, TyrP, and PPI. Moderate activity was also detected with the nucleoside diphosphate ADP, whereas activity with the nucleoside monophosphates tested was very low. The broad substrate range precludes clear identification of a physiological substrate based on specificity alone. The apparent K_m values for PEP were comparable to those reported for PAPs from tomato (Bozzo et al. 2004) and Arabidopsis (Tran et al. 2010). The low activity with nucleoside monophosphates as substrates is a clear difference to the other main phosphatase purified from common bean embryonic axes, which showed highest activity with these compounds as substrates (Cabello-Díaz et al. 2012).

Proteomic analysis of the purified protein enabled identification of the corresponding gene, which contains the conserved domains characteristic of purple acid phosphatases (PAPs), confirming its classification as a PAP. This conclusion is consistent with the inhibitor profile observed in biochemical assays. In this study, we identified 26 putative PAP genes in common bean by similarity to Arabidopsis PAPs using genome database searches. PAPs form a multigene family in plants, with 29 members in Arabidopsis (Li et al. 2002), 26 in rice (Zhang et al. 2011), 35 in soybean (Li et al. 2012), 33 in maize (González-Muñoz et al. 2015), 25 in chickpea (Bhadouria et al. 2017), 25 in *Jatropha curcas* (Venkidasamy et al. 2019), 19 in *Camellia sinensis* (Yin et al. 2019), and 25 in tomato (*Solanum lycopersicum*) (Srivastava et al. 2020). The widespread occurrence of PAP multigene families across species suggests a high level of functional diversification. While PAPs have traditionally been associated with phosphate starvation responses, emerging evidence indicates their involvement in a wide range of physiological processes, including symbiotic and non-symbiotic interactions, flowering, seed germination, carbon metabolism, redox balance, nodule development, oxidative stress response, and plant defense (Bhadouria and Giri 2022). In our analysis, 7 genes of the PAP family in common bean appear to have originated from gene duplication events, supporting the expansion and potential functional specialization of this gene family. Phylogenetic comparison with Arabidopsis PAPs revealed that the purified bean protein is most closely related to AtPAP26, supporting its designation as *PvPAP26*.

The expression of *PvPAP26* was detected in all tissues of common bean, suggesting a ubiquitous expression pattern. Notably, *PvPAP26* expression was highest in cotyledons

during the period of intense globulins (Lambert et al. 2016) and rRNA degradation (Diaz-Baena et al. 2023), indicating a potential functional role at this developmental stage. Although PAPs have been primarily studied in the context of phosphate starvation, emerging evidence suggests broader roles during plant development. Our data show that *PvPAP26* is highly expressed in cotyledons and embryonic axes of developing seedlings—an understudied context for PAP function.

While phytate is widely considered the major phosphate reserve in seeds (Rousseau et al. 2020; Jha et al. 2022), nucleic acids, particularly rRNA, may also serve as significant phosphorus reserves during early seedling development. In cotyledons, rRNA degradation parallels that of storage proteins (Diaz-Baena et al. 2021), and the nucleotides released could represent a readily accessible phosphate pool. Nucleic acids account for approximately 50% of the total phosphate content in plant cells, with rRNA comprising around 80% of this pool (Stigter and Plaxton 2015). Therefore, rRNA recycling could make a substantial contribution to phosphorus use efficiency during germination and post-germinative growth. This hypothesis is speculative and requires further investigation. Furthermore, nucleic acids such as RNA and DNA oligonucleotides derived from decaying organic matter are abundant in many soils. It has been proposed that root-secreted nucleases, ribonucleases, and phosphatases can contribute to the mobilization of inorganic phosphate (Pi) from these extracellular nucleic acid sources (Dissanayaka et al. 2021). In support of this, Arabidopsis was shown to grow equally well when supplied with purified DNA or inorganic phosphate as the sole external phosphorus source (Robinson et al. 2012). Given its strong expression and activity during early seedling development, it is plausible that *PvPAP26* may contribute to the degradation of phosphorylated compounds in the immediate environment of the seedling. This idea is supported by previous reports indicating that *AtPAP26* can be secreted into the extracellular space (Veljanovski et al. 2006), potentially facilitating phosphate acquisition during seedling establishment. More recently, *AtPAP26* has been implicated in phosphate recycling from RNA during autophagy, particularly under dark-induced conditions, where it localizes to the vacuole (Firdoos et al. 2025). The putative vacuolar localization for *PvPAP26* would support this role in phosphate recycling from compounds derived to the vacuole as could be the case with nucleic acid derivatives.

In summary, we purified and identified the main phosphatase activity in embryonic axes of common bean as a purple acid phosphatase (PAP), and by proteomic analysis we identified the gene that codes this protein, which showed highest similarity to Arabidopsis *AtPAP26*. The gene is ubiquitously expressed, with maximal expression in

cotyledons during the phase of active nutrient mobilization, but expression level was not affected by senescence, salt stress, or wounding. The high expression *PvPAP26* in cotyledons is an aspect that will require attention in the future and more targeted functional studies in legumes.

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References

- Adem GD, Ueda Y, Hayes PE, Wissuwa M (2020) Genetic and physiological traits for internal phosphorus utilization efficiency in rice. *PLoS ONE* 15:e0241842. <https://doi.org/10.1371/journal.pone.0241842>
- Anand A, Srivastava PK (2012) A molecular description of acid phosphatase. *Appl Biochem Biotechnol* 167:2174–2197. <https://doi.org/10.1007/s12010-012-9694-8>
- Armenteros JJA, Sønderby CK, Sønderby SK, Nielsen H, Winther O (2017) DeepLoc: prediction of protein subcellular localization using deep learning. *Bioinformatics* 33:3387–3395. <https://doi.org/10.1093/bioinformatics/btx431>
- Bewley JD (1997) Seed germination and dormancy. *Plant Cell* 9:1055–1066. <https://doi.org/10.1105/tpc.9.7.1055>
- Bhadouria J, Giri J (2022) Purple acid phosphatases: roles in phosphate utilization and new emerging functions. *Plant Cell Rep* 41:33–51. <https://doi.org/10.1007/s00299-021-02773-7>
- Bhadouria J, Singh AP, Mehra P, Verma L, Srivastawa R, Parida SK, Giri J (2017) Identification of purple acid phosphatases in chickpea and potential roles of *CaPAP7* in seed phytate accumulation. *Sci Rep* 7:1–12. <https://doi.org/10.1038/s41598-017-11490-9>
- Bozzo GG, Raghothama KG, Plaxton WC (2004) Structural and kinetic properties of a novel purple acid phosphatase from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. *Biochem J* 377:419–428. <https://doi.org/10.1042/bj20030947>
- Bozzo GG, Dunn EL, Plaxton WC (2006) Differential synthesis of phosphate-starvation inducible purple acid phosphatase isozymes in tomato (*Lycopersicon esculentum*) suspension cells and seedlings. *Plant Cell Environ* 29:303–313. <https://doi.org/10.1111/j.1365-3040.2005.01422.x>

- Bradford MM (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Anal Biochem* 72:248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cabello-Díaz JM, Quiles FA, Lambert R, Pineda M, Piedras P (2012) Identification of a novel phosphatase with high affinity for nucleotides monophosphate from common bean (*Phaseolus vulgaris*). *Plant Physiol Biochem* 53:54–60. <https://doi.org/10.1016/j.plaphy.2012.01.012>
- Chen Z, Wang L, Cardoso JA, Zhu S, Liu G, Rao IM, Lin Y (2023) Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. *Front Plant Sci* 14:1094157. <https://doi.org/10.3389/fpls.2023.1094157>
- Díaz-Baena M, Delgado-García E, Pineda M, Galvez-Valdivieso G, Piedras P (2021) S-like ribonuclease T2 genes are induced during mobilisation of nutrients in cotyledons from common bean. *Agronomy* 11:490. <https://doi.org/10.3390/agronomy11030490>
- Díaz-Baena M, Galvez-Valdivieso G, Delgado-García E, Piedras P (2023) High ribonuclease activity in the testa of common bean seedlings during germination: implication and characterization of the ribonuclease T2 PvRNS3. *Agronomy* 13:2750. <https://doi.org/10.3390/agronomy13112750>
- Dissanayaka DMSB, Ghahremani M, Siebers M, Wasaki J, Plaxton WC (2021) Recent insights into the metabolic adaptations of phosphorus-deprived plants. *J Exp Bot* 72:199–223. <https://doi.org/10.1093/jxb/eraa482>
- Duff SMG, Sarath G, Plaxton WC (1994) The role of acid phosphatases in plant phosphorus metabolism. *Physiol Plant* 90:791–800. <https://doi.org/10.1111/j.1399-3054.1994.tb02539.x>
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Firdoos N, Krumwiede L, Medina-Escobar N, Treichel L, Fischer L, Herde M, Witte C-P (2025) The vacuolar phosphatases purple acid phosphatase 26 and haloacid dehalogenase IIA2.1 hydrolyze 5′-, 3′-, and 2′-nucleotides derived from RNA degradation. *Plant Physiol* 197:kiaf025. <https://doi.org/10.1093/plphys/kiaf025>
- Flores-Tornero M, Anoman AD, Rosa-Téllez S, Toujani W, Weber APM, Eisenhut M, Kurz S, Alseekh S, Fernie AR, Muñoz-Bertomeu J, Ros R (2017) Overexpression of the triose phosphate translocator (TPT) complements the abnormal metabolism and development of plastidial glycolytic glyceraldehyde-3-phosphate dehydrogenase mutants. *Plant J* 89:1146–1158. <https://doi.org/10.1111/tj.13452>
- George TS, Hinsinger P, Turner BL (2016) Phosphorus in soils and plants-facing phosphorus scarcity. *Plant Soil* 401:1–6. <https://doi.org/10.1007/s11104-016-2846-9>
- González-Muñoz E, Avendaño-Vázquez AO, Montes RA, de Folter S, Andrés-Hernández L, Abreu-Goodger C, Sawers RJ (2015) The maize (*Zea mays* ssp. *mays* var. B73) genome encodes 33 members of the purple acid phosphatase family. *Front Plant Sci* 6:341. <https://doi.org/10.3389/fpls.2015.00341>
- Han Y, White PJ, Cheng L (2022) Mechanisms for improving phosphorus utilization efficiency in plants. *Ann Bot* 129:247–258. <https://doi.org/10.1093/aob/mcab145>
- Harper JE, Gibson AH (1984) Differential nodulation tolerance to nitrate among legume species. *Crop Sci* 24:797–801. <https://doi.org/10.2135/cropsci1984.0011183X002400040040x>
- Jha R, Yadav HK, Raiya R, Singh RK, Jha UC, Sathee L, Singh P, Thudi M, Singh A, Chaturvedi SK, Tripathi S (2022) Integrated breeding approaches to enhance the nutritional quality of food legumes. *Front Plant Sci* 13:984700. <https://doi.org/10.3389/fpls.2022.984700>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lambert R, Quiles FA, Cabello-Díaz JM, Piedras P (2014) Purification and identification of a nuclease activity in embryo axes from French bean. *Plant Sci* 224:137–143. <https://doi.org/10.1016/j.plantsci.2014.04.017>
- Lambert R, Cabello-Díaz JM, Quiles FA, Piedras P (2016) Identification of nucleases related to nutrient mobilization in senescing cotyledons from French bean. *Acta Physiol Plant* 38:266. <https://doi.org/10.1007/s11738-016-2287-3>
- Li D, Zhu H, Liu K, Liu X, Leggewie G, Udvardi M, Wang D (2002) Purple acid phosphatases of *Arabidopsis thaliana*. Comparative analysis and differential regulation by phosphate deprivation. *J Biol Chem* 277:27772–27781. <https://doi.org/10.1074/jbc.M204183200>
- Li WYF, Shao G, Lam HM (2008) Ectopic expression of GmPAP3 alleviates oxidative damage caused by salinity and osmotic stresses. *New Phytol* 178:80–91. <https://doi.org/10.1111/j.1469-8137.2007.02356.x>
- Li C, Gui S, Yang T, Walk T, Wang X, Liao H (2012) Identification of soybean purple acid phosphatase genes and their expression responses to phosphorus availability and symbiosis. *Ann Bot* 109:275–285. <https://doi.org/10.1093/aob/mcr246>
- Liao H, Wong FL, Phang TH, Cheung MY, Li WY, Shao G, Yan X, Lam HM (2003) GmPAP3, a novel purple acid phosphatase-like gene in soybean induced by NaCl stress but not phosphorus deficiency. *Gene* 318:103–111. [https://doi.org/10.1016/S0378-1119\(03\)00764-9](https://doi.org/10.1016/S0378-1119(03)00764-9)
- Lu L, Qiu W, Gao W, Tyerman SD, Shou H, Wang C (2016) OsPAP10c, a novel secreted acid phosphatase in rice, plays an important role in the utilization of external organic phosphorus. *Plant Cell Environ* 39:2247–2259. <https://doi.org/10.1111/pce.12794>
- Nonogaki H, Bassel GW, Bewley JD (2010) Germination - still a mystery. *Plant Sci* 179:574–581. <https://doi.org/10.1016/j.plantsci.2010.02.010>
- Ødum MT, Teufel F, Thumhuri V, Almagro-Armenteros JJ, Johansen AR, Winther O, Nielsen H (2024) DeepLoc 2.1: multi-label membrane protein type prediction using protein language models. *Nucleic Acids Res* 52:W215–W220. <https://doi.org/10.1093/nar/gkae237>
- Ojeda-Rivera JO, Alejo-Jacuinde G, Nájera-González HR, López-Arredondo D (2022) Prospects of genetics and breeding for low-phosphate tolerance: an integrated approach from soil to cell. *Theor Appl Genet* 135:4125–4150. <https://doi.org/10.1007/s00122-022-04095-y>
- Raboy V (2020) Low phytic acid crops: observations based on four decades of research. *Plants* 9:140. <https://doi.org/10.3390/plants9020140>
- Reddy CS, Kim KM, James D, Varakumar P, Reddy MK (2017) PgPAP18, a heat-inducible novel purple acid phosphatase 18-like gene (PgPAP18-like) from *Pennisetum glaucum*, may play a crucial role in environmental stress adaptation. *Acta Physiol Plant* 39:54. <https://doi.org/10.1007/s11738-017-2348-2>
- Robinson WD, Park J, Tran HT, Del Vecchio HA, Ying S, Zins JL, Patel K, McKnight TD, Plaxton WC (2012) The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by *Arabidopsis thaliana*. *J Exp Bot* 63:6531–6542. <https://doi.org/10.1093/jxb/ers309>
- Rousseau S, Pallares A, Vancoillie F, Hendrickx M, Grauwet T (2020) Pectin and phytic acid reduce mineral bioaccessibility in cooked common bean cotyledons regardless of cell wall integrity. *Food Res Int* 137:109685. <https://doi.org/10.1016/j.foodres.2020.109685>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>

- Schenk G, Gahan LR, Carrington LE, Mitic N, Valizadeh M, Hamilton SE, de Jersey J, Guddat LW (2005) Phosphate forms an unusual tripodal complex with the Fe-Mn center of sweet potato purple acid phosphatase. *Proc Natl Acad Sci U S A* 102:273–278. <https://doi.org/10.1073/pnas.0407239102>
- Schenk G, Mitic N, Hanson GR, Comba P (2013) Purple acid phosphatase: a journey into the function and mechanism of a colorful enzyme. *Coord Chem Rev* 257:473–482. <https://doi.org/10.1016/j.ccr.2012.03.020>
- Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F (2011) Phosphorus dynamics: from soil to plant. *Plant Physiol* 156:997–1005. <https://doi.org/10.1104/pp.111.175232>
- Srivastava R, Akash PAP, Chauhan PK, Kumar R (2020) Identification, structure analysis, and transcript profiling of purple acid phosphatases under Pi deficiency in tomato (*Solanum lycopersicum* L.) and its wild relatives. *Int J Biol Macromol* 165:2253–2266. <https://doi.org/10.1016/j.ijbiomac.2020.10.080>
- Stasolla C, Katahira R, Thorpe TA, Ashihara H (2003) Purine and pyrimidine nucleotide metabolism in higher plants. *J Plant Physiol* 160:271–1295. <https://doi.org/10.1078/0176-1617-01169>
- Stigter KA, Plaxton WC (2015) Molecular mechanisms of phosphorus metabolism and transport during leaf senescence. *Plants* 4:773–798. <https://doi.org/10.3390/plants4040773>
- Teufel F, Almagro-Armenteros JJ, Johansen AR, Gislason MH, Pihl SI, Tsigiris KD, Winther O, Brunak S, von Heijne G, Nielsen H (2022) SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nat Biotechnol* 40:1023–1025. <https://doi.org/10.1038/s41587-021-01156-3>
- Tian J, Wang C, Zhang Q, He X, Whelan J, Shou H (2012) Overexpression of OsPAP10a, a root-associated acid phosphatase, increased extracellular organic phosphorus utilization in rice. *J Integr Plant Biol* 54:631–639. <https://doi.org/10.1111/j.1744-7909.2012.01143.x>
- Tran HT, Hurley BA, Plaxton WC (2010) Feeding hungry plants: the role of purple acid phosphatases in phosphate nutrition. *Plant Sci* 179:14–27. <https://doi.org/10.1016/j.plantsci.2010.04.005>
- Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. *Plant nutrition in a world of declining renewable resources*. *Plant Physiol* 127:390–397. <https://doi.org/10.1104/pp.010331>
- Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol* 157:423–447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Veljanovski V, Vanderbeld B, Knowles VL, Snedden WA, Plaxton WC (2006) Biochemical and molecular characterization of AtPAP26, a vacuolar purple acid phosphatase up-regulated in phosphate-deprived Arabidopsis suspension cells and seedlings. *Plant Physiol* 142:1282–1293. <https://doi.org/10.1104/pp.106.087171>
- Venkidasamy B, Selvaraj D, Ramalingam S (2019) Genome-wide analysis of purple acid phosphatase (PAP) family proteins in *Jatropha curcas* L. *Int J Biol Macromol* 123:648–656. <https://doi.org/10.1016/j.ijbiomac.2018.11.027>
- Wang LS, Li Z, Qian WQ, Guo WL, Gao X, Huang LL, Wang H, Zhu HF, Wu JW, Wang DW, Liu D (2011) The Arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. *Plant Physiol* 157:1283–1299. <https://doi.org/10.1104/pp.111.183723>
- Yin C, Wang F, Fan H, Fang Y, Li W (2019) Identification of tea plant purple acid phosphatase genes and their expression responses to excess iron. *Int J Mol Sci* 20:1954. <https://doi.org/10.3390/ijms20081954>
- Zak D, Kronvang B, Carstensen MV, Hoffmann CC, Kjeldgaard A, Larsen SE, Audet J, Egemose S, Jorgensen CA, Feuerbach P, Gertz F, Jensen HS (2018) Nitrogen and phosphorus removal from agricultural runoff in integrated buffer zones. *Environ Sci Technol* 52:6508–6517. <https://doi.org/10.1021/acs.est.8b01036>
- Zhang Q, Wang C, Tian J, Li K, Shou H (2011) Identification of rice purple acid phosphatases related to phosphate starvation signaling. *Plant Biol* 13:7–15. <https://doi.org/10.1111/j.1438-8677.2010.00346.x>
- Zou T, Zhang X, Davidson EA (2022) Global trends of cropland phosphorus use and sustainability challenges. *Nature* 611:81–87. <https://doi.org/10.1038/s41586-022-05220-z>

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