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Isolation and characterization of purple acid phosphatase gene during seedling development in mungbean

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Abstract

Purple acid phosphatases (PAPs), which are normally found in plant tissues, can hydrolyze a broad spectrum of phosphate esters. In this study, a mungbean [*Vigna radiata* (L.) Wilczek cv. KPS1] acid phosphatase gene (*VrPAP1*) was isolated from seedling cotyledons. The full-length of *VrPAP1* cDNA contained an open reading frame of 1 644 bp encoding 547 amino acid residues with a predicted molecular mass of 62.07 kDa. Sequence analysis showed that VrPAP1 is purple acid phosphatase. RNA blot analyses indicated that the *VrPAP1* accumulated during the first hour in cotyledons of germinating seeds and reached a maximum expression after 24 h and then decreased. The *VrPAP1* mRNA was observed in cotyledons, hypocotyls and leaves but not in radicles or dry seeds. DNA blot analysis indicated that *VrPAP1* is a single copy gene in the mungbean genome.

Additional key words: gene expression, RACE, RT-PCR, *Vigna radiata.*

Introduction

Phosphorus source from seed is essential for plant germination. In mature cereals and legumes, the major storage form of P is phytate (Kumar *et al*. 2010) and its content is 0.5 to 5.0 % of dry mass (Laboure *et al*. 1993, Greiner *et al*. 2000). Phytate and phosphate esters can be hydrolyzed by the purple acid phosphatase (PAP) to provide P for plant metabolism. PAP, belonging to metallophospho-esterases family, catalyzes the hydrolysis of phosphate monoesters within an acidic pH range. PAPs have a pattern of five consensus motifs D*X(G/H*), GD*XX(X/Y), GN*H(E/D), VXXH*, and GH*XH* including seven conserved amino acid residues (asterisks). These seven amino acid are involved in the coordination of the dimetal nuclear center which is Fe(III)-Zn(II) in plant PAPs (Sträter *et al*. 1995). The physiological roles of plant PAPs have been reported in several species.

Members of plant PAP can hydrolyze phytate to inorganic phosphate and phosphoric esters of myo-inositol as P source for seed germination. These are called PAP phytases and have been identified in a wide range of plant seeds. The maximum phytase activity was reached after 4 d of germination in faba bean (Greiner *et al*. 2001) whereas in broad bean after 8 d (Kyriakidis *et al*. 1998). Azeke *et al*. (2011) reported that in rice, maize, millet, sorghum, and wheat phytase activity reached maximum after 7, 6, 5, 7, and 8 d of germination, respectively. The temporal pattern of *phytase* mRNA expression during seed germination had been reported in soybean and maize. The highest steady state

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Abbreviations: PAP - purple acid phosphatase; RT-PCR - reverse transcriptase-polymerase chain reaction; RACE - rapid amplification of cDNA end; VrPAP1 - mungbean purple acid phosphatase.
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RNA level was detected after 8 d of germination in soybean (Hegeman and Grabau 2001) whereas after 2 d in maize (Maugenest *et al*. 1997).

Additionally, some PAP members are involved in plant adaptation to abiotic stresses. For example, in soybean, *GmPAP3* is involved in adaptation to NaCl and oxidative stress (Liao *et al.* 2003). In common bean, *PvPAP3* functions in the adaptation to P deficiency by enhancing utilization of extracellular ATP as P source (Liang *et al.* 2010).

Up to now, there have been studies on differential expression on *PAP* in several plant species but a few of them have been carried out in mungbean (Olczak *et al*. 2003). Therefore, in this study, we have characterized a *PAP* gene from cotyledons of germinating mungbean seeds and studied its differential expression during seed germination.

Materials and methods

The germination of mung bean [*Vigna radiata* (L.) Wilczek cv. KPS1] seeds followed the method of Mandal and Biswas (1970). The dry seeds were washed with water for 5 min and then completely covered with water in a large Petri dish and kept at 35 °C in an incubator. After the seeds were fully soaked (about 12 h), the water level in the Petri dish was adjusted such that the soaked seeds were less than half immersed in water. The mungbean cotyledons were harvested at 12-h intervals until 120 h after germination. Leaves, hypocotyls, and radicles were obtained 72 h after germination. All samples were immediately frozen in liquid N and kept at -80 °C until use.

Total RNA was extracted according to Salzman *et al.* (1999). RNA used for RT-PCR amplification was isolated from cotyledons of seedling after 48 h of germination. For RNA blot analysis, cotyledons were sampled after 12, 24, 36, 48, 60, 72, 96, and 120 h of germination.

The reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify the mungbean *PAP* cDNA using the degenerate primers specific to this gene. Primers were based on highly conserved regions of several plant *PAP* genes reported in GenBank.

RNA (5 μg) was used to produce cDNA using the *Super Script*III one step RT-PCR (*Invitrogen*, Carlsbad, CA, USA). Degenerated primers Pf5F 5'-TGG(A/G)T (A/C/T)TCTTGGGT(C/T)ACAGG-3' and Pf6R 5'-GCT (G/A)TACCA(A/T)GGTGGATGC-3' were used to amplify an internal fragment from the mungbean *purple acid phosphatase* (*VrPAP1*) gene. The RT-PCR reaction was carried out according to the *Invitrogen* protocol. The mixture was operated at 50 °C for 30 min, pre-denatured at 94 °C for 2 min, then was used 40 cycles at 95 °C for 1 min, 60 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR product was cloned into the *pGEM-T* easy vector (*Promega*, Madison, WI, USA) and sequenced using *BigDye*™ terminator (*Macrogen*, Seoul, South Korea).

To obtain a full-length *VrPAP1* cDNA sequence, the 5' part of the gene was amplified using a rapid amplification of cDNA end (RACE) kit (*GeneRacer* kit, *Invitrogen*). The 5' end degenerated primer (5'-GGC CTTCAAA(A/G)GGATA(G/C)A(A/G) CTG-3') was used in combination with the *GeneRacer*™ 5'-Primer (*Invitrogen*). The 3' part of this gene was obtained by one step RT-PCR. Gene-specific 3' end primer (5'- CCTCGA TGGGATGGGTGGGGAAGGTT-3') and adaptor M13-Oligo (dT_{18}) primer (5'-CATGGTCATAGCTGTTTC CTG-oligodT_{18}-3') were used to amplify 3'RACE from the *VrPAP1*. The reaction was carried out according to the *Qiagen* (Hilden, Germany) *OneStep* RT-PCR kit protocol. All PCR products were cloned into the *pGEM-T* easy vector (*Promega*) and sequenced.

N-terminal region of *VrPAP1* was predicted having a signal peptide by using *SignalP 4.0* (Nielsen *et al.* 1997, Petersen *et al.* 2011) and *TargetP 1.1* (Emanuelsson *et al.* 2000). Sequence identity analyses were performed using the *BLASTP* (Altschul *et al*. 1997, 2005) search obtained from National Center for Biotechnology Information (NCBI). The phylogenetic relationships between VrPAP1 and other homologues from different plant species were carried out using the protein alignment tool from *MUSCLE* (http://www.ebi.ac.uk/Tools/msa/muscle/) and further analyzed using the neighbour-joining method (Saitou and Nei 1987) in *MEGA 5* software (Tamura *et al*. 2011). The phylogenetic tree of plant PAPs was obtained by using *PhyloWidget* (Jordan and Piel 2008).

For RNA gel blot analysis about 20 μg of total RNA obtained from cotyledons of seedling and other mungbean tissues (leaves, hypocotyls and radicles), was separated by formaldehyde agarose gel electrophoresis (1 % agarose) prior to transfer to nylon membranes (*Schleicher & Schuell*, Dassel, Germany). This membrane was hybridized at 50 °C for 16 h with a 862-bp internal fragment probe of the VrPAP1 cDNA labelled with DIG-11-UTP (*Roche*, Basel, Switzerland) in a hybridization buffer composed of: 7 % sodium dodecylsulphate (SDS), 50 % deionized formamide, 5× standard saline citrate (SSC), 2 % blocking reagent (*Roche*), 50 mM sodium phosphate, pH 7.0, and 0.1 % (m/v) N-lauroyl-sarcosine. After washing in a washing solution (1× SSC plus 0.1 % SDS) at room temperature, three further washes were performed with the same washing buffer at 65 °C. The target RNA was detected by chemiluminescence using CDP-star (*Roche*) and *BioMax Light* film (*Kodak*).

For DNA gel blot analysis, genomic DNA was isolated from cotyledons of seedling based on a protocol described by Dellaporta et al. (1990). Approximately 15 g of genomic DNA was digested with restriction enzymes (BamHI, EcoRI, and HindIII), then separated in a 1 % agarose gel and transferred to a nylon membrane

(*Schleicher & Schuell*). This membrane was hybridized using the same probe used for RNA gel blot analysis at 60 °C for 16 h in a hybridization buffer containing 5× SSC, 0.1 % N-lauroylsarcosine, 0.02 % SDS and 2 % blocking agent (*Roche*). The remaining procedures for detection were the same as for the RNA blot hybridization above mentioned.

Results and discussion

A full-length *VrPAP1* gene was isolated from cotyledons of germinating mungbean seeds (48 h after germination) by using 5' and 3' RACE protocol. The nucleotide sequences of *VrPAP1* gene was deposited in the GenBank database under accession number EU871632.

The full-length cDNA of *VrPAP1* gene revealed to be 1 864 bp nucleotides. This sequence has an open reading frame of 1 644 bp starting with an initiation codon ATG at position 26 and ending with termination codon TAA at position 1 667 (Fig. 1). The 5' and 3' untranslated sequences were 25 and 198 bp long, respectively. The putative protein encoded by *VrPAP1* has 547 amino acid residues (GenBank acc. No. ACF75910) with a predicted molecular mass of 62.07 kDa and a deduced isoelectric point of 5.02. The result from *SignalP 4.0* and *TargetP 1.1* showed that the N-terminal region of VrPAP1 protein has characteristics of a signal peptide with a probable cleavage site between Ala_{26} and Val_{27}.

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GCCATTCCTTCATTTTGTGCCAGAAATGAAAATCTGCACCACCTTATGCATGCTGGCCATGGTTTTGGTGATGATGAGCACCGACTTCATC
                        M  K  I  C  T  T  L  C  M  L  A  M  V  L  V  M  M  S  T  D  F  I
ACCGTCATGGCCGTGACTGAGAGTCACATTCCCACCACTTTGGATGGCCCATTTGAGCCTGTGACCCGCCGGTTCGACCCAACACTGCGA
 T  V  M  A  V  T  E  S  H  I  P  T  T  L  D  G  P  F  E  P  V  T  R  R  F  D  P  T  L  R
CGGGGCAGTGATGACCTGCCCATGACTCATCCAAGGCTAAGAAAGAATGTCACTTTGAATTTCCCTGAACAGATTGCTCTTGCAATTTCT
 R  G  S  D  D  L  P  M  T  H  P  R  L  R  K  N  V  T  L  N  F  P  E  Q  I  A  L  A  I  S
TCACCAACTTCTATGTGGGTTTCTTGGGTTACTGGGGATGCACAGATTGGCCTCAACGTGACGCCAGTAGATCCTGCATCTATTGGAAGT
 S  P  T  S  M  W  V  S  W  V  T  G  D  A  Q  I  G  L  N  V  T  P  V  D  P  A  S  I  G  S
GAAGTGTGGTATGGAAAAGAGAGTGGCAAGTACACAAGTGTTGGGAAAGGTGATTCTGTTGTTTACAGTCAGTTGTATCCCTTTGAAGGC
 E  V  W  Y  G  K  E  S  G  K  Y  T  S  V  G  K  G  D  S  V  V  Y  S  Q  L  Y  P  F  E  G
CTATGGAATTACACCTCTGGTATCATTCATCATGTGAAACTTGAAGGTCTTGAACCTGGAACAAGATATTATTACAAATGTGGGGATAGT
 L  W  N  Y  T  S  G  I  I  H  H  V  K  L  E  G  L  E  P  G  T  R  Y  Y  Y  K  C  G  D  S
TCTATTCCAGCCATGAGCCAAGAGCGTTTTTTTGAGACTTTTCCTAAACCTAGTCCAAATAATTATCCAGCTAGAATAGCAGTTGTTGGA
 S  I  P  A  M  S  Q  E  R  F  F  E  T  F  P  K  P  S  P  N  N  Y  P  A  R  I  A  V  V  G
GATCTGGGCCTCACAAGAAATTCTACATCAACTATTGATCATCTAATTCATAATGATCCCTCAATGATTCTAATGGTTGGAGATTTGACA
 D  L  G  L  T  R  N  S  T  S  T  I  D  H  L  I  H  N  D  P  S  M  I  L  M  V  G  D  L  T
TATGCAAATCAGTATCTTACAACTGGTGGAAAGGGAGTTTCATGTTATTCATGTGCATTTCCAGATGCTCCTATTAGAGAAACGTATCCT
 Y  A  N  Q  Y  L  T  T  G  G  K  G  V  S  C  Y  S  C  A  F  P  D  A  P  I  R  E  T  Y  P
CGATGGGATGGGTGGGGAAGGTTTATGCAAAACCTAATTTCTAAAGTTCCAATAATGGTGGTAGAAGGAAATCATGAAACAGAAGAACAG
 R  W  D  G  W  G  R  F  M  Q  N  L  I  S  K  V  P  I  M  V  V  E  G  N  H  E  T  E  E  Q
GCCGATAACAAAACATTTGTGGCCTACAGTTCTAGGTTTGCATTCCCCTCTGAAGAAAGTGGATCTTTATCCACATTATACTACTCTTTC
 A  D  N  K  T  F  V  A  Y  S  S  R  F  A  F  P  S  E  E  S  G  S  L  S  T  L  Y  Y  S  F
AATGCTGGGGGCATTCATTTTATTATGCTCGGAGCTTATATTGATTATTATAAAAACGGTGAACAATACAAATGGCTGGAGAGGGATCTG
 N  A  G  G  I  H  F  I  M  L  G  A  Y  I  D  Y  Y  K  N  G  E  Q  Y  K  W  L  E  R  D  L
GCAAGTGTTGATAGATCAATAACTCCCTGGCTTATAGCTACTTGGCATCCACCATGGTATAGTTCTTATGAAGTTCATTATAAAGAAGCA
 A  S  V  D  R  S  I  T  P  W  L  I  A  T  W  H  P  P  W  Y  S  S  Y  E  V  H  Y  K  E  A
GAGTGCATGAGGGTGGAGATGGAAAACCTGTTATACTCGTATGGTGTGGATATAGTATTTAATGGACATGTTCATGCTTATGAGAGGTCC
 E  C  M  R  V  E  M  E  N  L  L  Y  S  Y  G  V  D  I  V  F  N  G  H  V  H  A  Y  E  R  S
AATCGGGTTTACAATTACAGTTTAGATCCCTGTGGTCCTGTGCATATTGCAGTAGGGGATGGGGGTAACAGAGAGAAGATGGCAATCAAA
 N  R  V  Y  N  Y  S  L  D  P  C  G  P  V  H  I  A  V  G  D  G  G  N  R  E  K  M  A  I  K
TTTGCAGACGAGCCTGGTCATTGTCCTGATCCATTAAGTACTTCTGATCATTTTATGGGTGGCTTTTGTGCAACAAATTTTACATTTGAC
 F  A  D  E  P  G  H  C  P  D  P  L  S  T  S  D  H  F  M  G  G  F  C  A  T  N  F  T  F  D
CAAGAGAGTGAGTTTTGTTGGGATCACCAGCCAGATTACAGTGCTTTCAGAGAAACTAGCTTTGGCTATGGAATTCTAGAGGTGAAAAAT
 Q  E  S  E  F  C  W  D  H  Q  P  D  Y  S  A  F  R  E  T  S  F  G  Y  G  I  L  E  V  K  N
GAAACGTGGGCTTTGTGGAGTTGGTATCGTAATCAGGATTCGTACAAGGAAGTTGGGGATCAAATTTACATAGTGAGACAACCTGATATA
 E  T  W  A  L  W  S  W  Y  R  N  Q  D  S  Y  K  E  V  G  D  Q  I  Y  I  V  R  Q  P  D  I
TGTGATGTCCCTCGGAAGGTGTGCAGAGATTTTACTGCTTCGATTTAAGAGGCACAAGGGAGTTCATTTGGTGTAATTGATGAATATGTC
 C  D  V  P  R  K  V  C  R  D  F  T  A  S  I  *
ACAACAGCTGAATATTAACTTCTGTGGAAACCACAGTGCGCTAGATGTCACTAAGCATTAACATATGCTACCTAGCACTGGTACCCAATA
TGGTAAACACTCTTGTTCGGTAACTATCAATATTCAGAATGTTTAACAAAAAAAAAAAAAAAAAAA
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Fig. 1. Nucleotide and deduced amino acid sequences of mungbean cv. KPS1 cDNA fragment encoding full open reading frame of *VrPAP1*. The start and stop condons are *underlined*.

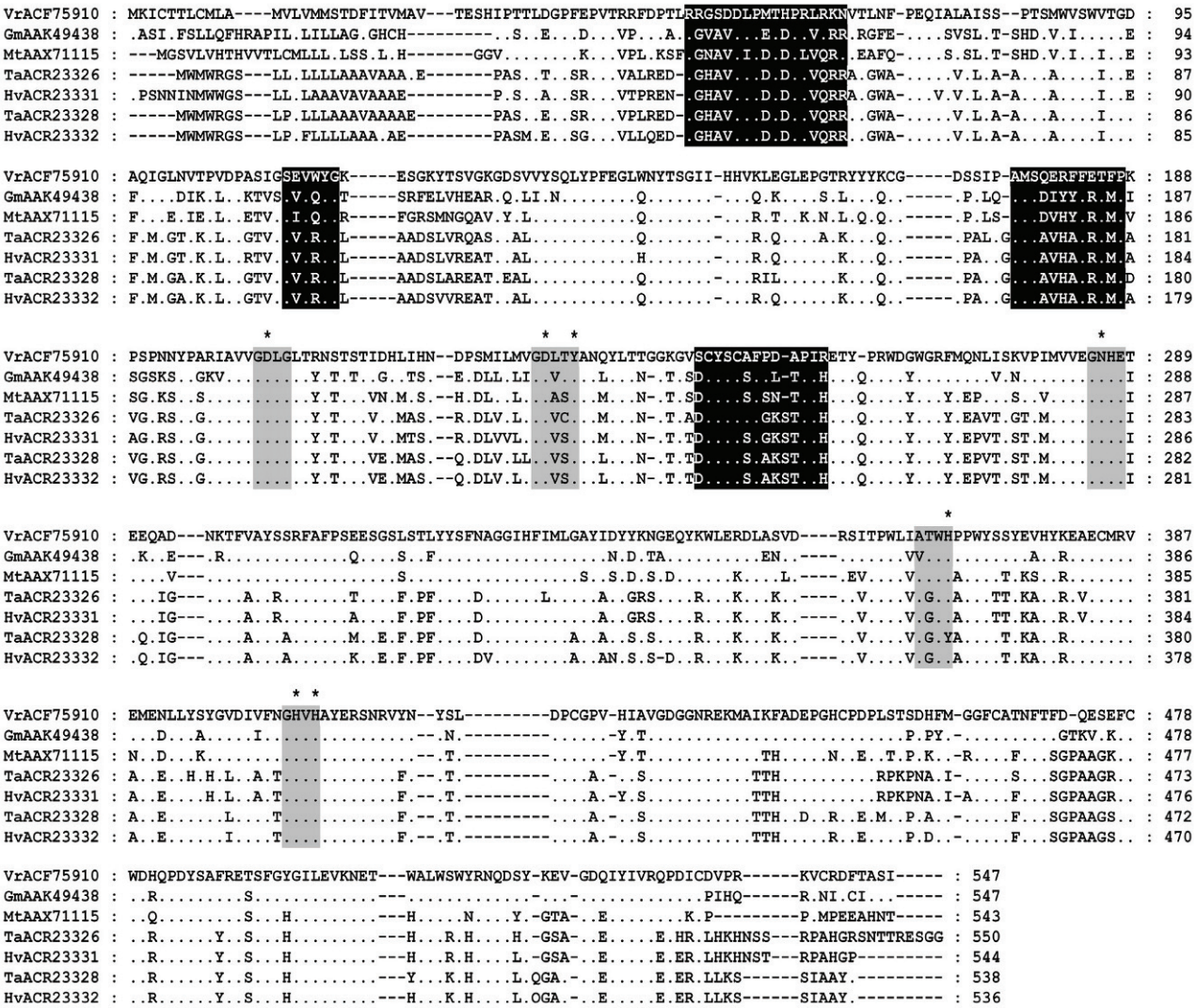

Fig. 2. Alignment of the deduced amino acid sequences of VrPAP1 with other PAPs from several plant species. Amino acid identical to VrPAP1 residues are indicated by *dots*. *Gray shading* - PAPs conserved regions; *asterisk* - the metal-binding residues; *black shading* - PAPhy motifs from PAPs with known phytase activity. The first two letters of each protein label represent the abbreviated species name followed by the GenBank accession number: Vr - *Vigna radiata,* Gm - *Glycine max,* Mt - *Medicago truncatula,* Ta - *Triticum aestivum*, Hv - *Hordeum vulgare*.

Comparison between VrPAP1 and other plant PAP sequences was done at amino acid level by using *BLASTP* search obtained from *NCBI*. VrPAP1 predicted protein showed 72, 68 and 65 % identity with soybean phytase (*GenBank* acc. No. AAK49438), *Medicago truncatula* PAP (acc. No. AAX71115) and *Nicotiana tabacum* PAP (acc. No. ABP96799), respectively. The sequence identity between VrPAP1 and monocot PAP phytase group (Dionisio *et. al*. 2011) exhibited identity ranged between 63 to 65 %. In addition, VrPAP1 also showed identity with other plant PAPs such as *Medicago truncatula* PAP (acc. No. XP_003618565), *Arabidopsis thaliana* PAP23 (acc. No. NP_193106), and *Ricinus communis* PAP (acc. No. XP_002521081) with 70, 69, and 67 % of identity, respectively.

Alignment of amino acid from VrPAP1 and other plant PAP were done by using *MUSCLE* tool, *MEGA 5*, and *GeneDoc* (Nicholas *et al.* 1997) programs (Fig. 2). The result showed that the VrPAP1 polypeptide contains purple acid phosphatase conserved motifs which were similarly reported in several plant PAPs such as soybean, wheat, barley, maize, and rice (Hegeman and Grabau 2001, Dionisio *et. al*. 2011). The conserved motifs contain five sequence blocks comprising two motifs (D*X[G/H*] - (X_n) - GD*XX[Y/X] - (X_n) - GN*H[E/D], and VXXH* - (X_n) - GH*XH*). They contain a characteristic set of seven amino acid residues (asterisks) involved in metal-ligating required for enzyme function (Fig. 2; Koonin 1994, Zhou *et al*. 1994, Lohse *et al*. 1995). In addition, VrPAP1 amino acid sequence showed partial homology with four conserved motifs, *1*) R-G-(H/V/Q/N)-A-(V/I)-D-(L/I)-P-(D/E)-T-D-P-(R/L)-V-Q-

R-(R/N/T); *2*) S-(V/I) V-(R/Q)-(Y/F)-G; *3*) A-M-S-X-X-(H/Y)-(A/Y/H)-F(R/K) -T-M-P; and *4*) D-C-Y-S-C-(S/A)-F-X-X-X-T-P-I-H which were previously reported in wheat, barley, maize, and rice PAP phytase (Dionisio *et. al*. 2011) (Fig. 2).

A phylogenetic tree was generated from the amino acid sequence alignment of the VrPAP1 and other PAP homologues from different plant species (Fig. 3). The PAP proteins (28 in total) were divided into three major groups: I - PAPs with known phytase activity, II - short plant PAPs and III - other PAPs and PAP-like proteins. Based on this analysis, the VrPAP1 protein, closely related to *G. max* phytase (acc. No. AAK49438; Hegeman and Grabau 2001), was placed into the group I. Sequence and phylogenetic tree analysis indicate that VrPAP1 is a PAP which showed identical and shared conserve regions with plant PAP phytases rather than other plant PAPs. However, the function of VrPAP1 remains to be identified .

The RNA was isolated from cotyledons after 12, 24, 36, 48, 60, 72, 96 and 120 h of germination and from mature seed in order to analyze the temporal accumulation of *VrPAP1* transcript. The results show that *VrPAP1* transcripts appeared after 12 h of germination, reaching the highest amount after 24 h and then decreased progressively from 48 to 72 h and could not be detected onward. In soybean, *GmPhy* was detected at all the time during embryogenesis (4, 6, 8, 10, and 12 d after germination), with its highest amounts detected after 8 d of germination (Hegeman and Grabau 2001). In germinating maize, maximum *PhyS11* mRNA was detected the 2nd day and it decreased in young seedling (Maugenest *et al*. 1997). In kidney bean, *KeACP* gene was expressed at the early hours after the imbibitions in embryonic axes (Yoneyama *et al.* 2004). The qRT-PCR analysis of barley and wheat *PAPhy* isogene b showed high expression in the early germinating grain (Dionisio *et. al*. 2011). As homologues to the b-type PAPs, the *VrPAP1* temporal expression has also shown that *VrPAP1* gene was expressed very early in cotyledons of germinating seeds.

Spatial occurrence of expression was determined after 72 h of germination. The *VrPAP1* transcripts could be found in cotyledons and hypocotyls. Only a very slight hybridization was observed with mRNA from leaves whereas *VrPAP1* transcript was undetected in dry seeds or radicles (Fig. 4*B*).

The number of the *VrPAP1* genes in the mungbean

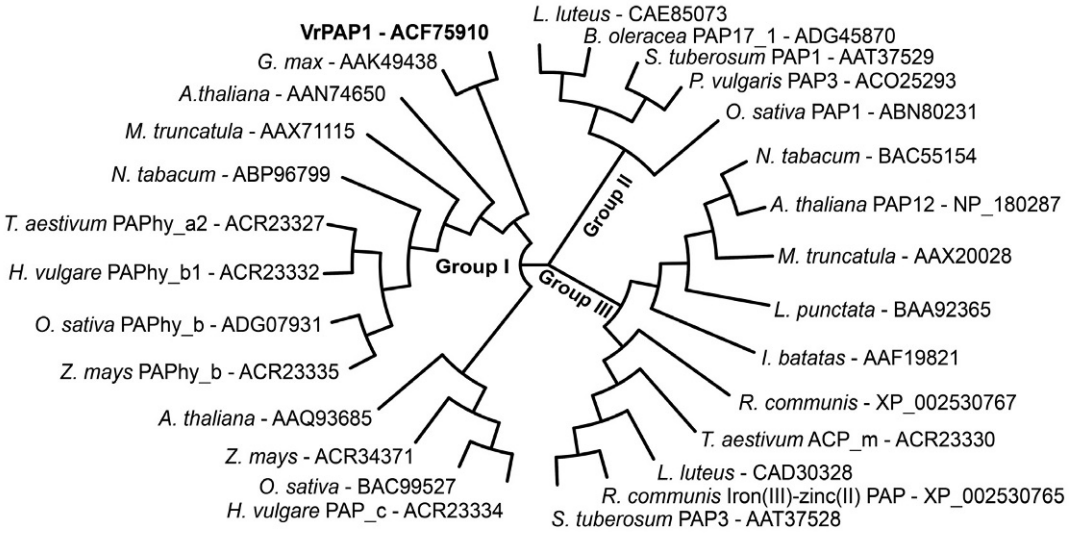

Fig. 3. Neighbour-joining phylogenetic tree of VrPAP1 with other homologuous PAP proteins of plants constructed using *MEGA 5* and *PhyloWidget*.

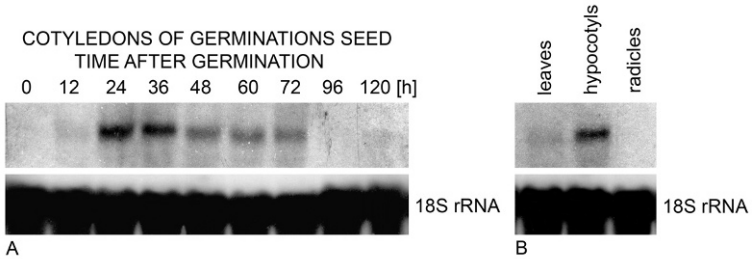

Fig. 4. RNA gel blot analyses of *VrPAP1* expression during seed germination. *A* - Pattern of the *VrPAP1* transcripts in cotyledons of germinating mungbean seeds from dry seed (0 h of germination) to 120 h of germination. *B* - Pattern of the *VrPAP1* transcripts in plant parts of munngbean seedling (72 h of germination).

genome was estimated by DNA gel blot analysis. Genomic DNA isolated from cotyledons of seedling (after 4 h of germination) was digested with *Bam*HI, *Eco*RI, and *Hind*III and probed with the internal fragment from the *VrPAP1* gene. The presence of only one strongly hybridized band in each digest (Fig. 5) indicated that a single gene encoding the *VrPAP1* is present in mungbean genome. In *Medicago truncatula* genome, two copies of PAP (Xiao *et al*. 2006a) and PAP phytase (Xiao *et al*. 2006b) genes exist whereas at least 2 - 3 copies of *NtPAP4*, *NtPAP12*, *NtPAP19*, and *NtPAP21* genes (Kaida *et al.* 2003) are present in the *Nicotiana tabacum* genome. The results obtained in this study show that VrPAP1 belongs to the purple acid phosphatase family. It possesses well conserved PAP motifs and even though the PAPhy motifs (Dionisio *et al*. 2011) are not matching the known PAP phytases. Unless an *in vitro* proof of VrPAP1 phytase activity, these cannot be discarded from being also PAPhy motifs but different from the majority of the PAPhy motifs of the PAP phytase known to date. The phylogenetic analysis definitevely groups VrPAP1 into the dicot part of the group I PAPs. The mRNA expression of *VrPAP1* was only detected in the early stages of germination in the cotyledons and hypocotyls. The tight gene expression and its organ specific localization during seedling development let us suppose that the *VrPAP1* could be involved in phosphorus mobilization.

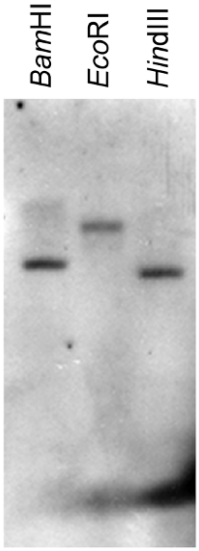

Fig. 5. DNA gel blot analyses. Mungbean genomic DNA was digested with the restriction enzymes *Bam*HI, *Eco*RI, and *Hin*dIII. Gel blot was probed with the internal fragments from the *VrPAP1* gene.

In maize, two *phytase* genes (*PHYT I* and *PHYT II*) are expressed in germinating seeds, whereas only *PHYT I* is expressed in adult roots and leaves (Maugenest *et al*. 1997, 1999). The *KeACP* gene of kidney bean showed high transcription in embryonic axes whereas low transcription in stems, leaves and roots (Yoneyama *et al.* 2004). In barley and wheat, the *PAPhy_a* was detected in developing grains while *PAPhy_b* were mainly found in germinated grains though not in the primary leaf and root (Dionisio *et. al*. 2011). Our results support the fact that the expression of *VrPAP1* gene is tissue specific and cotyledons are the main storage compartment for phytic acid. In soybeans, phytic acid is deposited in protein storage vacuoles (PSVs) (Prattley and Stanley 1982) present in the endosperm cells in contrast with barley and wheat where phytic acid is present only in aleurone cells in the PSVs. Dionisio *et al*. (2011) found that PAPhy of mature wheat, barley, maize, and rice grain was also localized in the PSVs. Therefore, the early and seed-specific expression of *VrPAP1* indicates that it could serve in the process of phosphorus mobilization and supply to other plant tissues during seedling growth. To validate our hypotheses, further research is needed to investigate the subcellular localization and the biochemical function of mungbean PAP phytase.

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