A Phosphate Transporter from the Root Endophytic Fungus *Piriformospora indica* Plays a Role in the Phosphate Transport to the Host Plant

Vikas Yadav\(^1\), Manoj Kumar\(^1\), Deepak Kumar Deep\(^1\), Hemant Kumar\(^1\), Ruby Sharma\(^1\), Takshashila Tripathi\(^1\), Narendra Tuteja\(^2\), Ajay Kumar Saxena\(^1\) and Atul Kumar Johri\(^1\)*

\(^1\)School of Life Sciences, Jawaharlal Nehru University, New Meharuli Road, New Delhi-110067, India.
\(^2\)International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India.

*Address correspondence to: Atul K. Johri, e-mail: akjohri14@yahoo.com, Fax: 91-11-26742558, Tel: 91-11-26704511. \(^\$\): Contributed equally

ABSTRACT

Because pure cultures and a stable transformation system are not available for arbuscular mycorrhizal fungi, the role of their phosphate transporters for the symbiotic interaction with the plant up till now could not be studied. Here we report the cloning and the functional analysis of a gene encoding a phosphate transporter (*PiPT*) from the root endophytic fungus *Piriformospora indica* which can be grown axenically. The *PiPT* polypeptide belongs to the major facilitator superfamily (MFS). Homology modeling reveals that *PiPT* exhibits twelve transmembrane helices divided into two halves connected by a large hydrophilic loop in the middle. The function of the protein encoded by *PiPT* was confirmed by complementation of a yeast phosphate transporter mutant. The kinetic analysis of *PiPT* (*Km* 25 μM) reveals that it belongs to high affinity phosphate transporter family (Pht1). Expression of *PiPT* was localized to the external hyphae of *P. indica* colonized with maize plant root which suggest that external hyphae are the initial site of phosphate uptake from the soil. To understand the physiological role of *PiPT*, knockdown (KD) transformants of the gene were prepared using electroporation and RNA interference. KD transformants transported a significantly lower amount of phosphate to the host plant than wild type *P. indica*. High amount of phosphate was found in plants colonized with wild type *P. indica* than that of non-colonized and plants colonized with KD-*PiPT* *P. indica*. These observations suggest that *PiPT* is actively involved in the phosphate transportation and in turn *P. indica* helping in improvements of the nutritional status of the host plant.

INTRODUCTION

Phosphorous (P) is one of the most essential mineral nutrients for plant growth and development and constitutes up to 0.5 % of the dry weight of plant cell. It plays diverse regulatory, structural, and energy transfer roles and consequently is required in significant quantities (1,2). In the soil P present mainly in the form of sparingly soluble complexes that are not directly accessible to plants. Thus, it is the nutrient that limits crop production throughout the world (3). In arbuscular mycorrhizal associations, plants acquire phosphate from the extensive network of fine extra radical hyphae of fungus, that extend beyond root depletion zones to mine new regions of the soil (4). Plants possess two distinct modes of phosphate uptake, direct uptake by its own transporters and indirect uptake through mycorrhizal associations. High-affinity phosphate transporters have been identified and characterized in several plant and fungal species, including *Arabidopsis*, *Medicago*...
truncatula, Lycopersicon esculentum, Solenum tuberosum, Saccharomyces cerevisiae, Neurospora crassa (5-12). However, in case of arbuscular mycorrhizal fungal species including Glomus versiforme, G. intraradices and G. mosseae the role of phosphate transporters could not be verified due to the lack of a stable transformation system (4,13,14).

Involvement of P. indica was reported in high salt tolerance, disease resistance and growth-promoting activities leading to enhancement of host plant yield (15-17). However, the role of this root-endophytic fungus in plant nutrition has not yet been demonstrated.

In present study, a high affinity phosphate transporter has been isolated, identified and functionally characterized from root endophyte fungus P. indica. Recently, a transformation system based on the polyethylene glycol method has been established for P. indica (18). Using electroporation for transformation and RNAi approach as powerful tools for gene silencing in fungi (19), we demonstrate that PiPT is essential for phosphate transport to the host plant. We suggest that exploitation of P. indica and its PiPT not only complements crop growing strategies but may also serve as a model system to study molecular mechanism and indirect uptake of phosphate by plants.

**EXPERIMENTAL PROCEDURES**

**Plant, Fungi, Bacteria and Yeast Strains.** Zea mays (var. pro33) plant and fungus P. indica (15) were used throughout the study E. coli XL1-Blue was used for cloning purposes (20). Yeast phosphate transporter mutant MB192 (MATa: pho3-7 pho84::HIS3 ade2 leu2-3,7 12 his3-532 trpl-289 ura3-1,2 can1) was used for complementation studies and uptake kinetics.

Seeds were surface sterilized for 2 min in ethanol followed by 10 min in a NaClO solution (0.75 % Cl) and finally washed six times with sterile water (15). Additionally seeds were also treated with dH₂O at 60 °C for 5 min (17). Seeds were germinated on water-agar plates (0.8 % Bacto Agar; Difco, Detroit, Mich.) at 25°C in the dark (15). Plants were grown under controlled conditions in a green house with 8 h-light (1000 Lux) /16h-dark period at temperature of 28°C±2°C with a relative humidity 60-70% . Surface sterilized pre-germinated maize seedlings were placed in pots filled with a mixture of sterile sand and soil in the ratio of 3:1 (garden soil from Jawaharlal Nehru University campus and acid-washed riverbed sand). After two weeks, P. indica was inoculated, whereas in control plants autoclaved dH₂O was used. Plants were weekly supplied with half-strength modified Hoagland solution containing 50 μM KH₂PO₄ (21) with following composition 5 mM KNO₃, 5 mM Ca (NO₃)₂, 2 mM MgSO₄, 10 μM MgCl₂, 4 μM ZnSO₄, 1 mM CaSO₄, 1 μM NaMoO₄, 50 μM H₃BO₃, 40 mM sucrose and. Plant roots were harvested at 10, 15, 20 and 25 days after inoculation and a random sample of the root system was assessed for colonization. To study colonization, 10 root samples were selected randomly from the maize root. Samples were softened in 10 % KOH solution for 15 min and acidified with 1N HCl for 10 min and finally stained with 0.02 % Trypan blue overnight (17,22). Samples were de-stained with 50 % Lacto-phenol for 1-2 hour prior to observation under light microscope (Leica Microscope, Type 020-518.500, Germany). The distribution of chlamydospores within the root was taken as an index for studying colonization. Percent colonization was calculated for the inoculated plants according to the method described previously (17).

**Isolation of Full Length PiPT cDNA.** To isolate cDNA, that encodes phosphate transporter, we established a cDNA library of P. indica which was grown in low phosphate Aspergillus minimal medium (AMM) for one week (23). Total RNA was isolated by TRIZOL reagent (Gibco) and pooled followed by poly(A)⁺ RNA extraction from 2 mg of
RNA by using Oligo-dT cellulose beads (Amersham). cDNA synthesis was performed using a cDNA synthesis kit (Stratagene). After size fractionation, cDNA >800 bp long were selected to create a library in the λ-ZAP vector and recombinant independent clones (5x10^7) were obtained with lengths ranging from 0.5 to 3 kb.

In order to isolate full length PiPT, homologous probe was made using partial PiPT gene, which was amplified using phosphate transporters degenerate primers (forward 5′-atgggttyggiathggiggigaytaycc and reverse 5′-gtcgtrttiggiccraarttygraaraaa) where y represents a pyrimidine, r represents a purine and i represents inosine (14). In brief, high molecular weight genomic DNA was extracted from about 500 mg of P. indica culture (24). PCR reactions were carried out in a final volume of 50 μl containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl_2; 0.01% (w/v) gelatin; 200 μM of dNTPs; 3 μM of each primer; 3 units of DNA Taq-polymerase and 60-100 ng of genomic DNA (as template). The PCR program used was as follows: 94°C for 3 min (1 cycle), 92°C for 45 sec, 55°C for 45 sec, 72°C for 1 min, 15 sec (30 cycles) and 72°C for 5 min (1 cycle). The PCR product was cloned in p-drive vector (Qiagen) and sequenced by using M13 primers.

After obtaining homologous probe (928 bp), cDNA library was screened to get full length PiPT cDNA by using Hybond-N nylon membranes (Amersham Biosciences) harboring recombinant λ phages, which were hybridized overnight at 60°C in hybridization buffer containing 7 % SDS; 0.5 M phosphate buffer (pH 7.5); 1 mM EDTA (pH 8) and 1 % BSA. Membranes were washed twice at 25°C, once at 60°C in 0.5 X SSC and 0.2 % SDS, before autoradiography. After three rounds of screening, the plaque-purified phages were converted to pBluescript-II SK-derivatives by in vivo excision according to the manufacturer’s instructions and sequenced.

**Northern, Southern Blots and RT-PCR Analyses.** To study the effect of substrate (phosphate) concentration on PiPT transcripts, P. indica culture were grown in MN media (25) containing different concentrations of inorganic phosphorus (KH_2PO_4). Fungus was harvested at 1, 5, 10 and 15 days and total RNA was isolated. Fifteen μg of total RNA was electrophoretically separated on 1.2 % denaturing formaldehyde agarose gels and blotted onto nylon membrane. For Southern blot, genomic DNA (25 μg) was digested with restriction enzymes and separated on 0.8 % agarose gel, denatured and transferred to a nylon membrane. For both the analyses hybridization and washing conditions were kept same as mentioned in the previous text. Homologous probe used before to get full length PiPT gene was also used for Northern blots.

For the expression studies of PiPT, two step RT-PCR was performed with 5 μg of total RNA which was isolated from colonized (with P. indica) and non-colonized maize roots. The first strand of cDNA was synthesized with Superscript® cDNA synthesis kit (Clontech) and this was subsequently used as a template for PCR with gene specific primers (forward 5′-gtcgtcgctg-atctcgacca and reverse 5′-atccggag-atggtaacaatc). The cycling conditions were as follows: denaturation was done at 94°C for 5 min for one cycle however, for 35 cycles denaturation was done for 40 s. Annealing was done at 59°C for 40 s and extension was done at 72°C for 90 s.

Translational elongation factor gene of P. indica (PiTef) was used as a reference gene as described (17).

**Computational Analyses and Homology Modeling.** The functional sites and their pattern were determined using PROSITE data bank (http://www.expasy.ch/prosite/). For identification purposes, blastX algorithm (http://www.ncbi.nlm.nih.gov) was used. For modeling PiPT transmembrane (TM) domains, TM helix segments were predicted with various computer programs e.g., SOSUI, HMMTOP, TMHMM and TMpred (26-29). A consensus of twelve TM helices
emerged and boundary of each predicted helix was identified based on empirical rules derived from known membrane protein structures (30). Two models \textit{PiPT1} and \textit{PiPT2} were constructed based upon homology with known crystal structures of glycerol-3-phosphate transporter (\textit{GpPT}) and lactose permease (\textit{LacY}) of \textit{E. coli} (31,32). Sequence alignments were done with ClustalW and BLOSUM62 with gap penalty of 10 for insertion and 5 for extension (33,34). All predicted TM domains of \textit{PiPT} were used as secondary structural constraints in the input file for modeling program. The final \textit{PiPT} model having 1-522 amino acids was build using MODELLER program based on \textit{GpPT} (35). Statistical validation for bond lengths, bond angles and Ramachandran analysis were obtained with PROCHECK (36). Adjustments were made whenever residues in the \textit{PiPT} model had stereochemical violations.

\textbf{Yeast Complementation.} For this purpose, yeast high-affinity phosphate transporter mutant strain MB192 was used (11). \textit{PiPT} cDNA was cloned into the EcoRI-BamHI site of the yeast expression vector pll2A1NE by using sequence specific primers (forward 5'-cgaattcctcgtcgcgacaccttcattc and reverse 5'-aagatctgtggctacattaatgtatctcattc). MB192 was transformed with recombinant pll2A1NE vector (9) having \textit{PiPT} insert by LiCl-PEG method (37,38). Procedures and medium used for growth and selection of transformants were similar as described elsewhere (11). For repressible acid phosphatase (\textit{rAPase}) assay, cells were grown on YNB medium-high-phosphate (11 mM) containing 3 % glycerol (in place of glucose). \textit{rAPase} was measured after 36 hrs by using intact yeast cells as an enzyme source and p-nitrophenyl phosphate (pNPP) as substrate (39). MB192 cells transformed with empty vector was used as a control.

\textbf{Uptake of Radioactive Orthophosphate.} Transformed MB192 cells (recombinant pll2A1NE vector having \textit{PiPT} insert) were grown to the logarithmic phase (A\textsubscript{650} 0.8-1.2) on modified YNB medium containing 20 µM KH\textsubscript{2}PO\textsubscript{4}. Before uptake assay, transformed MB192 cells were pre-incubated with 10 mM glucose for 5 min. Cells were washed in phosphate-free medium and re-suspended in the same medium (A\textsubscript{650}=1.0). Uptake was performed as described (40). In brief, cells (A\textsubscript{650}=1.0) were incubated in a solution containing 20 µM phosphate together with 100 nCi \textsuperscript{32}phosphorus (Amersham Biosciences). The incorporation reaction was stopped by adding 4 ml ice-cold water, centrifuged (15,800 x g, 10 min) and supernatant was discarded. Pellet was washed twice with 4 ml of ice-cold water, radioactivity incorporated by the cells was determined using a liquid scintillation counter (Beckman Instruments). The uptake assay was performed at room temperature (25 °C). Experiment was repeated three times independently and each time three replicates were taken. MB192 cells transformed with empty vector were used as a control. Amount of phosphate transported by control (background) was deducted from the amount of phosphate transported by complimented MB192 with \textit{PiPT}. GraphPad Prism 4 was used to plot nonlinear regression for phosphate uptake rate.

\textbf{Localization of \textit{PiPT} Expression and Real Time-PCR (rqRT-PCR) Analysis.} To determine the \textit{PiPT} expression in external hyphae and in internal hyphae of the \textit{P. indica} colonized maize plant root, Relative quantitative RT-PCR was performed. For colonization purpose, radical of maize seedlings were first submerged into macerated \textit{P. indica} and were incubated for two more days on water agar plates and finally transferred to MN containing 10 µM phosphate. At day 5 external hyphae projecting out from the surface of the colonized root were collected by forceps. Approximately 2 mg of hyphae was collected per sample. In case of internal hyphae sample collection, first external hyphae were removed using forceps and or brushed off with a
paintbrush as described (4). Small pieces (5-10 mm) of colonized root were collected. Colonization was also confirmed from these collected root pieces as described previously (17). RNA was isolated from these two samples and cDNA was synthesized with oligo(dT) and Superscript II and then subjected to real-time PCR with specific primer pairs and SYBR Green I using a ABI 7500 Real-Time PCR System (Applied Biosystems) according to manufacturer’s instructions. The forward and reverse primer sets for the PiPT and PiTef gene were RT PiPT FOR 5’- ctcgctcagcgaaatcagaagccatg and RT PiPT REV 5’- ccacagtcaagagcttgca and PiTefFOR 5’- tcg-tcgctgtaacaagatg, PiTefREV 5’- gagtgctcagcttggttgt respectively. The reaction mixture was heated at 95 °C for 10 min and then subjected to 40 PCR cycles of 95 °C for 15 s, 61 °C for 1 min and 72 °C for 20 s and the resulting fluorescence was monitored. For the constitutive PiTef gene, the mixture was heated at 95 °C for 10 min, and then subjected to 40 PCR cycles of 95 °C for 15 s, 57 °C for 1 min and 72 °C for 20 s. The heat dissociation curves confirmed that a single PCR product was amplified for each gene. The melting temperatures were 81.3 °C, 85.3 °C for the PCR products of the PiPT and PiTef gene, respectively. The level of target mRNA, relative to the mean of reference housekeeping genes, was calculated by the comparative Ct method as described by the manufacturer.

RNAi Cassette Formation for Knockdown (KD) of PiPT. A 350 bp unique fragment of PiPT gene was selected using BLAST tool and further analyzed for RNA 20 structures. This unique fragment was amplified using specific primers pSD-1GFor 5’-ccggaattccgccgcaaatcagaagcctgatg and pSD-1GRev5’-ccggaattcagcgaaatcagaagccatg and cloned in to pGEM-T cloning vector. The 350 bp insert was digested out from pGEM-T with Eco RI and was sub cloned into pSD-1G vector at unique Eco RI site. (For vector information please see supplementary information, Figure 1). Positive clones were confirmed with PCR, Eco RI digestion and sequencing. This construct was named as pSPIPTD-1G.

Transformation of pSPIPTD-1G into P. indica. For this purpose minimum inhibitory concentration (MIC) of Geneticin (G418) was first determined by growing P. indica at 30 °C at 200 rpm for 7 days. This culture of P. indica was macerated and inoculated in fresh media for 24 hrs. The culture was centrifuged at 1000 g for 5 min, and the mycelia pellet was re-suspended in 1 mL AMM. 100 µl of this culture was mixed with 10 mL top agar containing 100, 300, 500 and 700 µg/ml of G418 in a separate 15 ml tubes. This mixture was poured on AMM agar plates and was incubated at 30 °C for 2 weeks. We have found that P. indica was unable to grow at 700 µg/ml and above of G418. Here in every P. indica electroporation mediated transformation experiment, 1000 µg/ml of G418 was used. pSPIPTD-1G was introduced into the P. indica by using electroporation. For the first time we have developed a transformation protocol using electroporation as follows, seven days old macerated mycelia were grown in AMM for 24 h at 30 °C. To this β-glucuronidase (1 mg/ml) (Sigma: Helix pomatia) was added (41). After 2 h of growth, mycelia were centrifuged at 1000 g for 5 min, washed and suspended in 1 mM HEPES buffer (pH 7.0) and 50 mM mannitol. From this 100 µl was taken in a tube and mixed with 2 to 7 µg of pSPIPTD-1G and subjected to electroporation using the Bio-Rad Gene Pulser apparatus at the field strength to 12.5 KV/cm, at 25 µF capacitance and 5 msec pulse length. After electroporation, 1 ml AMM was added and incubation resumed at 30 °C for 24 h. Transformants were selected and named as “KD-PiPT transformants”.

PiPT Transcripts Abundance and siRNA Analysis in KD-PiPT P. indica. To determine the abundance of PiPT transcripts, rqRT-PCR was done. For this purpose, total
RNA was extracted from wild type and two colonies of KD-PiPT *P. indica* using Trizol reagent. For RNA preparation and PCR analysis, experimental conditions were kept same as mentioned previously in localization of PiPT experiment. After checking the PiPT transcripts abundance analysis, siRNA analysis was performed to show whether KD construct leads to siRNA accumulation or not. For this purpose, small RNAs were extracted and probed with some modifications (42). In brief, total RNA was extracted from KD-PiPT *P. indica* and wild type *P. indica* by using Trizol Reagent. The pellet was dissolved in DEPC water, heated to 65 °C for 5 min, and then placed on ice. Polyethylene glycol (molecular weight 8000; Sigma) was added to a final concentration of 5 % and NaCl to a final concentration of 0.5 M. After 30-min incubation on ice, the RNA was centrifuged at 10,000 g for 10 min. Three volumes of ethanol were added to the supernatant and the RNA was precipitated at -20°C for at least 2 h. The low molecular weight RNAs were pelleted by centrifugation for 10 min at 10,000 g. The pellet was dissolved in DEPC water and heated to 65 °C for 5 min and a one-third volume of 4 X loading solution was added (2 X TBE [1 X TBE is 0.09 M Tris-borate, pH 8.0, and 0.002 M EDTA], 40 % sucrose, and 0.1% bromophenol blue) before loading on 15 % urea PAGE in 1X TBE. The RNA samples were electrophoresed at 2.5 V/cm and then blotted to HybondN+ membrane (Amersham) and UV cross-linked. The membrane was prehybridized in 50 % formamide, 7 % SDS, 50 mM NaHPO₄/NaH₂PO₄, pH 7.0, 0.3 M NaCl, 5 X Denhardt’s solution (1 X Denhardt’s solution is 0.02 % Ficoll, 0.02 % Polyvinyl pyrrolidone (PVP) and 0.02 % BSA), and 100 mg/mL sheared, denatured salmon sperm DNA at 37 °C for at least 3 h. Probe was prepared by end labeling of the PiPT primer (5’-gctcgctgatctgacca) using γ-P³²ATP and Polynucleotide Kinase as per the instructions manual (Molecular Labeling and Detection, Fermentas) and was added to pre-hybridization solution. The hybridization was performed at 37 °C overnight and the membrane was subsequently washed at 37 °C in 2 X SSC [1 X SSC is 0.15 M NaCl and 0.015 M sodium citrate (C₆H₂Na₃O₇.2H₂O)] and 0.2 % SDS for 15 min twice. Final washing was given only with 2 X SSC at room temperature for 10 min and autoradiography was done.

**Role of PiPT in Phosphate Transport from *P. indica* To Host Plant.** To prove the role of PiPT in phosphate transport bi-compartment assay was performed using in-vitro culture system (43) as shown in Figure 9. In bi-compartment experiment, to make a physical barrier between both compartments, a 6 cm Petri dish (compartment 2) was placed inside a 15 cm Petri dish (compartment 1). Murasige-Skoog and AMM was supplied to compartment 1 while compartment 2 was supplied with MN media. Surface sterilized maize seeds were placed in the compartment 1. The leafy shoots protruded through a groove cut in the lid of each dish and were fixed in one position by wrapping a sterile non-absorbent cotton wool around the portion of the subtending rhizome as it passed through the groove. Three sets were prepared for the experiment (a) maize plants colonized with wild type *P. indica* (WT) (b) maize plants colonized with KD-PiPT *P. indica* (KD) and (c) maize plants grown alone without *P. indica* (C). In all the cases 10 µM phosphate concentration was used in compartment 1 as well as in compartment 2. For sets “a” and “b” to establish colonization between maize roots (compartment 1) and *P. indica* (compartment 2) a connective bridge was made by placing a 4-5 cm long agar strip so that *P. indica* can cross into the compartment 1 (Figure 9). In case of set “c” a connecting bridge was also made in order to check any transfer of radioactive phosphate from compartment 2 to 1 due to diffusion and this set was used as a control. As the colonization develops extra-radical hyphae proliferate in the medium surrounding the roots in compartment 1 where they ramify...
and later sporulate. After colonization was established, the MN media in compartment 2 of all three sets was replaced with fresh MN media containing 10 µM phosphate and 1µM of $^{32}$P (specific activity 200 mCi/mmol). Radioactivity was determined in all the three sets by autoradiography and the amount of $^{32}$P incorporated was measured by liquid scintillation analyzer (Peckard). Experiment was conducted three times independently.

**Effect of PiPT on Phosphate Nutrition.** To know whether phosphate has a effect on plant growth, we have determined the total phosphate content in (1) maize plant colonized with wild type *P. indica* (2) only maize plant and (3) maize plant colonized with KD-PiPT *P. indica*. In this case, for colonization, radical of maize seedlings (4 days old) were first submerged into macerated wild type as well KD-PiPT *P. indica* and were incubated for two more days on water agar plates and finally transferred to MN containing 10 µM phosphate and grown for 10 more days. Control plants were grown under similar conditions using autoclaved macerated fungal mycelium. Inorganic phosphate was determined using acid extraction of fresh plant material by a method described by Irving and Bouma (44). Shoots were collected and biomass was measured in terms of fresh weight from all the three sets of same age. To these three set of samples, 40 µL of 5 M H$_2$SO$_4$ was added per 20 mg of each sample. The samples were ground in a hand-held device and to this 3 mL of distilled water was added. The resulting solution was filtered using Whatman No. 4 filter paper and a subsample, ranging from 20 µL to 1.5 mL, (depending on the phosphate concentration) was analyzed for phosphate. The subsample was made up to 1.5 mL with water and to this 0.5 mL of Malachite green reagent was added (45), the total contents were then mixed vigorously. After at least 30 min, the $A_{50}$ of the solution was measured. Standards in the range of 125nM to 50 µM of phosphate as KH$_2$PO$_4$ were used. In order to understand the role of *P. indica* in phosphate nutrition improvements, growth promoting performance of *P. indica* was analyzed at low (10 µM) and at sufficient or high phosphate concentration (1mM). We have selected high phosphate concentration as the same has been used in the Hoagland solution (21). For this purpose, four sets were prepared (1) maize plants grown in low phosphate and treated with autoclaved macerated fungal mycelium (served as a control for low phosphate condition) (2) maize plants colonized with wild type *P. indica* and grown at low phosphate condition (3) maize plants grown at high phosphate condition and treated with macerated fungal mycelium (served as a control for high phosphate condition) (4) maize plants colonized with wild type *P. indica* and grown at high phosphate condition. As mentioned above, all four experimental sets were grown in acid washed sand fertilized with modified 0.5 X Hoagland solution (21) containing respective phosphate concentrations. After 4 weeks, plants were harvested and biomass was measured in terms of fresh weight.

**RESULTS**

**Isolation and Organization of PiPT.** Full-length PiPT (GenBank Accession no. DQ899728) cDNA clone, encoding the *P. indica* phosphate transporter, was isolated from low phosphate supplied *P. indica* cDNA library. PiPT shares 35 % sequence identity with GvPT and PHO84 phosphate transporters, from *G. verisforme* and *S.cerevisiae* respectively. Low sequence identity (30 %) was observed with plant phosphate transporters (Table 1). The PiPT is 1815 bp in length and the open reading frame (ORF) is flanked by 90 bp untranslated sequence, at the 5”end and 156 bp of untranslated sequence including the poly(A$^+$), at the 3’ end. An unrooted phylogenetic tree demonstrates the close relationship between the PiPT protein and members of the plant Pht1 family and high-affinity phosphate transporters of fungi (Figure 1). Southern blot analysis of *P. indica* genomic DNA
hybridized with full-length PiPT cDNA probes, suggesting the presence of a single gene (Figure 2).

**Homology Modelling.** PiPT encodes a polypeptide of 522-amino acids having the relative molecular mass (57.7 kDa). Based on the hydropathy plots, the encoded polypeptide is predicted to be an integral membrane protein containing twelve hydrophobic membrane-spanning domains (TM) having a large hydrophilic loop between TM6 and TM7, resulting in (6+6) configuration. There are five potential protein kinase C-mediated phosphorylation sites at amino acid position 158, 235, 269, 273 and 294 having \{ST\}-(35) consensus motif. There are three potential casein kinase II mediated phosphorylation sites at amino acid position 124, 231 and 277 having \{ST\}-(2)-{DE} consensus motif. We have also observed cAMP- and cGMP-dependent protein kinase phosphorylation site present at amino acid position 165 \{consensus motif (35)-{2}-\{ST\}\}. A seventeen amino acid long sequence (from 376-392aa) shows signature-tag of MFS transporter super family having \{LIVMSTAG\}-\{LIVMFSAG\}-\{SH\}-RDE}-\{LIVMSA\}-\{DE\}-\{TD\}-\{LIVMFY-WA\}-\{R\}-(35)-{4,6}-\{GSTA\} consensus sequence (Figure 3).

Blast search with transporter classification database (TCDB, UCSD) indicate that PiPT belongs to MFS family. PiPT2 model has substantially more gaps in the helical regions; therefore, further analysis was done only with PiPT1 model. Structure based sequence alignment for the TMDS of GlPT and PiPT1 was generated. We observed low homology in the 1-6 TMDs between GlPT and PiPT1. The alignment scheme was generated and 1-6 TMDs sequence segments were then mapped to the reference alignment. We were able to build 522 residues of PiPT1 model based on the GlPT coordinates. Ramachandran analysis suggested that PiPT1 has no residues in the disallowed region and has an excellent score (0.4) for the overall G factor (bond lengths and bond angles). Cartoon representation of three-dimensional structure of the PiPT1 homology model indicates the pattern of characteristic of the MFS fold. The predicted PiPT shape is trapezoidal with dimensions are ~46x27 Å from the bottom and ~33x26 Å from the top and its height is ~42 Å (Figure 4).

**PiPT Expression is Dependent on the Phosphate Availability and Colonization.** Increased expression of PiPT was detected at all the time intervals when 50 μM or less phosphate was supplied (Figure 5a). This increased expression of PiPT under low phosphate supplied conditions reveals the high affinity nature of PiPT. RT-PCR data showed that PiPT is expressed during the interaction with the maize plants colonized with *P. indica* at all the time points i.e., 5 d onwards while no expression was observed in roots of non-colonized plants (Figure 5b). Furthermore, the increased expression of PiPT was observed with gradual increase of colonization with time (Figure 5c).

**PiPT Encodes a High Affinity Phosphate Transporter.** To determine whether PiPT encoded a functional phosphate transporter, the cDNA was ligated into a yeast expression vector p112A1NE and transformed into a yeast strain MB192 carrying the *pho84* mutation. PCR based confirmation was done for the presence of either PiPT cDNA or *PHO84* cDNA in the yeast mutants used for complementation and uptake kinetics. PCR was carried out with plasmids as template (isolated from respective yeast cells) and primers which were specific to either *PHO84* or PiPT. A 1.7 kb DNA fragment was obtained for PiPT while 0.8 kb DNA fragment was obtained for *PHO84*. No amplification was observed when native p112A1NE used as a template. These positive yeast cells were further used for rAPase assay and uptake kinetics of PiPT. Yeast *pho84* mutant lacks the high affinity phosphate transporter and consequently have severely impaired phosphate uptake. Because
they can not accumulate phosphate, *pho84* mutant cells produce a repressible acid phosphatase (rAPase; EC 3.1.3.2) even during growth on high phosphate media containing levels of phosphate sufficient to repress rAPase production in wild type cells. MB192 transformants expressing either *PiPT* or *PHO84* displayed wild type rAPase activity indicating that expression of *PiPT* or *PHO84* complements the *pho84* phenotype. MB192 transformants carrying the expression vector (without transporter) displayed the mutant *pho84* phenotype with high rAPase activity (Figure 6a).

Phosphate uptake by MB192 expressing *PiPT* was further confirmed by measuring uptake of $^{32}$P from solution. MB192 expressing *PiPT* accumulated $^{32}$P at a rate significantly (P<0.001) higher than the background rate shown by control MB192 cells (Figure 6b). The phosphate uptake by MB192 cells expressing *PiPT* follows Michaelis-Menten kinetics with an apparent $K_m$ of $25.27 \pm 3.66$ μM values ($V_{max}$ 105.8 $\pm$ 3.45 pmol/min/A$_{650}$) (Figure 6c).

**Localization of *PiPT* Expression.** To determine the specific localization of *PiPT* expression, transcript abundance was measured in internal hyphae and external hyphae that ramify out of the colonized root into the media using rq-RT-PCR. We have found that *PiPT* transcripts were 18 fold higher in the external hyphae as compared to internal hyphae and this difference was found significant (P<0.01) (Figure 7).

**Characterization of KD-*PiPT* *P. indica.*** We have obtained transformation efficiencies between 5-20 transformants/μg DNA (supplementary information, Figure 2). KD for *PiPT* was analyzed for transcript abundance and siRNA. Real time RT-PCR analysis reveals a lower abundance (60 %) of *PiPT* in KD-*PiPT* *P. indica* as compared to wild type *P. indica* and this difference was found significant (P<0.05) (Figure 8a). Accumulation of siRNA was observed in the Northern blot in case of KD-*PiPT* *P. indica* and no siRNA was detected in case of wild type *P. indica* (Figure 8b).

**Analysis of Fungus-to-Plant Transfer of Phosphate.** In the first set (a) autoradiograph revealed extensive labeling of maize plants by uptake of radiolabelled $^{32}$P from wild type *P. indica* (Figure 10 Aa). The $^{32}$P was transferred to maize plants through the fungal mycelium and across the hyphal bridge between both compartments. Very little radioactive counts were observed in the agar media of second compartment confirming that amount of $^{32}$P present in the maize plants was exclusively transferred by *P. indica* and not because of leaching by the fungus in second compartment. In the set “b” a very less radioactivity was detected in maize plant colonized with KD-*PiPT* transformants of *P. indica*, confirming the direct role of *PiPT* in phosphate transport to maize plants (Figure 10Ab). In case of set “c” no radioactivity was observed (Figure 10Ac) and hence, the movement of P$^{32}$ from one chamber to another is not due to diffusion but through fungus only. We observed that 349 pmole phosphate has been transported by wild type *P. indica* to the host plant as compared to 36 pmole in case of KD-*PiPT* *P. indica* and this difference was found significant (P<0.05) (Figure 10B). We have also observed that the colonization of both wild type and KD transformants of *P. indica* into maize plant was found similar in both the cases i.e., 70 % at 20 days after inoculation (Figure 10Aa-c).

**Role of *PiPT* in Phosphate Nutritional Improvements of Host Plant.** We found that inoculation of *P. indica* significantly increased the average above ground biomass of maize plants by 2.4 and 2.8 fold than that of the non-colonized plants as well as from the KD-*PiPT* *P. indica* colonized plants respectively (Figure 11 a). Further we found 2.5 and 3.75 fold higher phosphate content in plants colonized with wild type *P. indica* as compared to non-colonized plants and KD-*PiPT* *P. indica* colonized plants respectively and this difference was found
statistically significant (P<0.05) (Figure 11 b). In a separate analysis, to know the performance of *P. indica* in growth promotion activity at low and high phosphate concentration (as mentioned in the materials and method), we have found that the growth (in terms of fresh weight) of maize plants colonized with *P. indica* was 1.2 fold higher when grown at high phosphate condition (1 mM), while it was 2 fold higher in case of maize plant colonized with *P. indica* grown at low phosphate condition (10 µM) in comparison to their respective controls (Figure 11 c).

**DISCUSSION**

In present study, a high affinity phosphate transporter has been isolated, identified and functionally characterized in *P. indica*. The deduced amino acid sequences of the *PiPT* share significant similarities (including homology and topology) with those of known high-affinity phosphate transporters reported so far from higher plants and fungi. Based on our phylogenetic dendrogram data we have observed that *PiPT* falls into the group of high affinity phosphate transporters of fungi far distinct to plant phosphate transporters. Hydrophobicity analysis and sequence alignment suggests that *PiPT* contains 12 membrane-spanning domains with a large hydrophilic loop separating them into two groups of six. This is a characteristic of phosphate transporter and many other transporter proteins belongs to the MFS family and thus support our data (46-50). It was suggested that MFS proteins are typically 400 to 600 amino acids long having similar transmembrane topology and signature sequences in two cytoplasmic loops. Additionally, position and spacing of these membrane-spanning regions of *PiPT* are very similar to those reported in other phosphate transporters from higher plants and fungi (51). Based on secondary structure analyses using PROSITE database of protein domains, families and functional sites (52,53) we have observed that both the N and C termini and hydrophilic domains of the polypeptides are present towards the cytoplasmic side of the plasma membrane. The central channel of the *PiPT* is formed by TM 1, TM 2, TM7 and TM 11. Unlike other fungal and plant phosphate transporters, cAMP- and cGMP-dependent protein kinase phosphorylation sites have been observed in *PiPT* polypeptide. Consequently, we speculate the involvement of cAMP- and cGMP-dependent downstream signaling pathway for the phosphate transporter regulation. Though these data are not supported by the experimental evidences and therefore it needs warrant investigation.

So far, there is no report available on crystallization and well-defined structure of inorganic phosphate transporters those can be used for modeling of *PiPT* and therefore we used *GlPT* as a reference template for the modeling of *PiPT* (31). *PiPT* model contains 522 amino acid residues, with an unmodeled loop at N and C termini and polypeptides of 82 amino acids between TM 6 and TM 7. Since *PiPT* is only a modeled structure, hence, a realistic evaluation of its quality should be investigated experimentally. The expression of *PiPT* does not depend on the culture time and a steady state high transcript level of *PiPT* was observed upon µM phosphate supply (50 µM and less). Based on structural similarities and a physiological response to a phosphate deficiency, *PiPT* is clearly part of the high-affinity transporter system. Similar findings have been observed by Chung et al. (54) in algae, *Tetraselmis chui* for a high-affinity phosphate transporter gene (*TcPHO*) and in turn supports our data.

Further, the expression studies showed that the expression of *PiPT* is regulated by the amount of phosphate present in the outside only and it seems that there is no role of intracellular phosphate pool in the regulation of *PiPT*. *PiPT* expression level increases as colonization proceeds as shown in Fig. 5 b,c. We have also observed similar colonization of *P. indica* in maize plant (70 % at 20 days
after inoculation) at low as well as at high phosphate concentration; therefore we suggest that the colonization is independent of phosphate availability. The functionality and kinetics of PiPT was investigated by its ability to complement the yeast high affinity phosphate transporter pho84 mutant. These data suggests that PiPT is a functional stretch which has potential to complement yeast pho84 mutant. Further, kinetic data of PiPT follows Michaelis-Menten kinetics with an apparent Km of 25.27 ± 3.66 μM. These findings along with high level expression of PiPT in μM phosphate supply suggest that PiPT belongs to high-affinity phosphate transporters. Similar results were also reported previously with GvPT and PHO84 for those the Km has been estimated i.e., 18 μM and 8.2 μM respectively (4,11). To localize the PiPT expression in roots of maize plant colonized with P. indica, we have collected external hyphae projecting out from the colonized root as well as internal hyphae (within the root). Quantitative-RT-PCR analysis shown that PiPT transcripts were 18-fold higher in the external hyphae as compared to internal hyphae, indicating that external hyphae is the main site of PiPT expression. Similar findings were also observed by Harrison and Buuren (4) in the external hyphae projecting out from the surface of the mycorrhizal roots of Allium porrum colonized with G.versiformae and in turn support our data.

We have successfully used electroporation mediated transformation system in P. indica and obtained good transformation efficiency, similar findings have been reported by Zuccaro et al. (18) and in turn support our data. However in their study they have used protoplast preparation for transformation. To determine the physiological function of PiPT in vivo, we prepared KD transformants of PiPT and found that the transcript level of PiPT was specifically and effectively reduced. We have also observed the accumulation of siRNA in case of KD-PiPT transformants. The formation of siRNA indicates that in P.indica RNAi machinery exist. We have observed the low incorporation of radioactive phosphate into plant colonized with the KD-PiPT P. indica (bicompartiment experiment) which suggest that RNAi results in the silencing of the PiPT and hence KD-PiPT P. indica has transported reduced amount of phosphate to the host plant as compared to the wild type P. indica.

The mechanism underlying phosphate transfer from the fungus to the plant remains unknown and it is speculated that the process occurs at the plant-fungus interface. This potentially requires two transporters: the first to enable efflux of phosphate from the fungus and the second to mediate uptake of phosphate by the plant (50,51). Our data on involvement of PiPT in indirect phosphate transport to host plant provide the information regarding the molecular mechanism underlying phosphate transport by P. indica to the host plant. However, in a previous study (55) it has been shown that P. indica does not induce potato phosphate transporter; StPT3, and the authors have concluded that P. indica is not involved in the phosphate transfer to host plant. Contrary to this report, we have found that P. indica is involved in the phosphate transfer to the host maize plant. Further we do not ruled out the host specific nature of P. indica as we used maize plants as the only host in the present study. A complete array of different host plants would provide the clear picture whether nature of P. indica and PiPT is host specific or not.

It has been reported that to get colonized in barley P. indica causes root cell death (56) . Here we would like to emphasize that the main part of the root further develops and is not necrotized when colonized by the fungus. Thereby we speculate that once phosphate releases from the fungus into such dead cell, it might be taken up by the non-affected adjacent cells for further distribution into the different parts of the plant as in our functional experiment (Figure 10) we have observed the presence of the radioactive phosphate not only in roots but also in the
RNA silencing has been demonstrated in the filamentous and phytopathogenic fungi (57-68). In order to demonstrate the applicability of RNA silencing as a tool to know genes function in P. indica, the PiPT gene was targeted to prove its role in the transportation of phosphate to the host plant. Our results showed that newly developed electroporation mediated transformation in combination with RNAi mediated gene silencing are useful techniques to study gene function analysis in P. indica which was a limiting factor so far to manipulate this fungus genetically.

In a previous reports it was found that P. indica is not involved in the phosphate improvements of the host plant and therefore authors have concluded that phosphate has no role in the increased biomass of the Nicotiana attenuata and barley (69,70). Contrary to these reports, in the present study it was found that phosphate has an impact on the biomass of the maize plant colonized with P. indica. In our study, total phosphate content as well as biomass was found higher in the plants colonized with wild type P. indica as compared to non-colonized and KD-PiPT P. indica colonized plants, suggesting that phosphate is playing a role in the increased plant yield or biomass and this increase in the biomass is due to the PiPT. Similar findings were also observed by Shahollari et al. (71) in case of Arabidopsis colonized with P. indica. They have reported that P. indica increases the phosphate uptake 2-3 times higher in Arabidopsis seedlings and it was concluded that P. indica stimulates Arabidopsis growth in fashion similar to that described for mycorrizal symbioses. In the present study it is important to note that the growth promoting activity (in terms of biomass of maize plant) of P. indica was more (2 fold) at low phosphate condition as compared to high phosphate condition (1.2 fold), therefore our data suggest that P. indica has an ability to increase the biomass of the maize plant particularly under low phosphate condition. As P. indica can be grown axenically therefore the present study on PiPT can provide a new horizon to understand the whole phosphate transport network either with or without host plant. As P. indica is also reported to help plants in growth promotion, salt tolerance, disease resistance and higher yield (16,17). Thus, we suggest that P. indica could be a good candidate for use in sustainable agriculture to improve plant productivity in the lands deficient in P.

References

FOOTNOTES

We thank Dr. Georg Leggewie (Germany) for PHO84 and p112A1NE plasmid, Dr. Yasuji Oshima, Osaka University and Dr. Hitoshi Nakayashiki, Kobe University, Japan for yeast mutants MB192 and RNAi vectors respectively. The plasmid pSD -1G was obtained from the Fungal Genetics Stock Center, University of Missouri, Kansas, USA. Authors are also thankful to Ajay Vashistha and Amita Joshi, International Center for Genetic Engineering and Biotechnology, New Delhi for their help in cDNA library construction and screening. We also thank Dr. Maria J. Harrison, Cornell University, USA for providing GiPT and GvPT during the initial phase of the present work. A grant from Council of Scientific and Industrial Research (CSIR) and Department of Science and Technology (DST), Govt. of India to AKJ and NT is also acknowledged. VY, MK, HK and DKD are thankful to University Grant Commission (UGC), Indian Council of Medical Research (ICMR), CSIR and Jawaharlal Nehru University (JNU) for providing fellowship. We also greatly acknowledge help from Prof. Rajendra Prasad, Rector, JNU for providing the capacity-buildup fund during the course of investigation.

FIGURE LEGENDS

Figure 1: Unrooted phylogenetic relationship of PiPT with other high affinity phosphate transporters from plants and fungi. Protein names are followed by GenBank (GB) accession numbers: GmPT (DQ074452) from *Glomus mosseae*; GiPT (AF359112) from *Glomus intraradices*; GvPT (U38650) from *Glomus versiforme*; PHO84 (D90346) from *Saccharomyces cerevisiae*; LePT1 (AF022873), LePT2 (AF022874), LePT4 (AY885651) from *Lycopersicon esculentum*; AtPT1 (U62330), AtPT2 (U62331) from *Arabidopsis thaliana*; SiPT1 (X98890), StPT2 (X98891), StPT4 (AY793559), StPT5 (AY885654) from *Solanum tuberosum*; MtPT1 (AF000354), MtPT2 (AF000355), MtPT4 (AY116210) from *Medicago truncatula*; SrPT1 (AJ286743), SrPT2 (AJ286744) from *Sesbania rostrata*; NtPT1 (AF156696), NtPT2 (AB042950), NtPT3 (AB042951), NtPT4 (AB042956) from *Nicotiana tabacum*. The evolutionary history was inferred using the Neighbor-Joining method (72). The tree is drawn to
scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (73) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were done by using MEGA4 (74).

Figure 2: Southern blot analysis of *P. indica* genomic DNA digested with BamHI (Lane1), HindIII (Lane2) or EcoRI (Lane3). The blots were hybridized with labeled *PiPT* cDNA. The *PiPT* gene does not contain EcoRI and Hind III sites.

Figure 3: Alignment of the deduced amino acid sequence of *PiPT* with *G. versiforme GvPT*, yeast *PHO84*, *Pholiota nameko*, *PnPT* and *Magnaporthe grisea*, *MgPT* by using MULTIALIN (45). Degree of sequence conservation at each position amino acids are shown in red, low consensus amino acids are shown in blue color. Membrane-spanning domains (TM) of *PiPT* as predicted by TopPred (75) are shown as helix over the corresponding amino acid sequences indicated by numerals (TM1-TM12). Prediction of functional motifs in *PiPT* polypeptide was performed with PROSITE data base (www.expasy.ch/prosite/) (52) Signature tag of MFS transporter is indicated by *. (Consensus symbols: ! is anyone of IV, $ is anyone of LM, % is anyone of FY, # is anyone of NDQEBZ). Green boxed sequences are potential phosphorylation site for cAMP- and cGMP-dependent protein kinase.

Figure 4: Ribbon representation of *PiPT* model based on homology modeling using *GlPT* as template. The predicted *PiPT* shape is trapezoidal with dimensions are ~46x27 Å from the bottom and ~33x26 Å from the top and its height is ~42 Å.

Figure 5: (a) Effect of different concentrations of phosphate on the expression of *PiPT* transcripts. Northern blot of total RNA isolated from *P. indica* grown in MN media containing the indicated different phosphate concentrations for 1, 5, 10 and 15 days. A picture of the gel showing uniform loading of RNA (b) RT-PCR was used to assess abundance of *PiPT* transcript at 5, 10, 15 and 20 days showed as 680 bp amplified *PiPT* DNA fragment from colonized maize plant roots (+PI). As a negative control, 5 and 20 days plants without *P. indica* (-PI) were taken. DNA size markers were shown in extreme left as M. *PiTef* was used as a reference gene (c) Trypan blue staining of maize plant roots showing the presence of intracellular chlamydospores of *P. indica* in the cortical cells showing the colonization at 5d or onwards (black arrow). High degree of colonization was observed with time and no colonization was observed in control roots.

Figure 6: Complementation of *PiPT* gene using yeast phosphate uptake-mutant (MB192) and kinetics of phosphate uptake. (a) Acid phosphatase activity was checked in yeast, *pho84* mutant containing only vector (p112A1NE), MB192 mutant cells with vector + *PHO84* insert, MB192 mutant cells with vector + *PiPT* insert grown on high phosphate medium for 36 hrs. (b) Phosphate uptake into yeast MB192 mutant cells transformed with vector (no phosphate transporter gene) (black), *PHO84* (line) and with *PiPT* cDNA (grey). Means and standard errors of means of three replicate determinations consisting of three measurements each are shown in a and b. Time period for the phosphate accumulation was 3 min (c) Nonlinear regression of phosphate uptake of MB192 transformed with *PiPT* versus external phosphate concentration at pH 4.5. *PiPT* and *PHO84* (positive control) indicates that the data are statistically significant (P< 0.001) as compared to control i.e., MB192 mutant cells only with vector (no insert). Significance has been calculated using t-test (Sigma Stat version 2.0 USA).
Figure 7: Localization of PiPT expression in external (EH) and internal hyphae (IH) from maize plant root colonized with *P. indica*. For the determination of relative expression of PiPT level, cDNA was synthesized from RNA isolated from EH and IH and subjected to Real Time PCR using specific primers and SYBR green I. The comparative Ct method was applied to analyze the data. For experimental samples, targeted (PiPT) quantity was determined and divided by the target quantity of the calibrator (Tef). Thus, the calibrator becomes the 1 X sample and all other quantities are expressed as an n-fold difference relative to the Tef. The values obtained for PiPT expression for EH and IH were 1 and 0.055 fold respectively relative to Tef. The means plus SD of three independent determinations are presented. Asterisks * indicate significant differences from EH at P<0.01.

Figure 8: Characterization of KD-PiPT *P. indica*. (a) The transcript levels of the PiPT gene in KD and wild type *P. indica*. Wild type control (WTC); transformed colony 2 (TC 2) and TC 5, with an RNAi construct. The *P. indica* colonies were first grown in AMM, at day 4 AMM was replaced with fungal minimal media with a 10 µM supplement of phosphate and RNA was extracted from *P. indica* colonies at 3 days after transfer of media. Expression level of PiPT was determined by Real Time PCR as described in legend of Figure 7. The values obtained for PiPT expression for WTC, TC2 and TC5 were 1, 0.4 and 0.39 fold respectively relative to Tef. The means plus SD of three independent determinations are presented. Asterisks * indicate significant differences from WTC at P<0.01. (b) Northern blot analysis of siRNAs of the PiPT in *P. indica* transformants. Blot was exposed overnight to show detectable siRNA accumulation in the KD-PiPT *P. indica* (TC2 and TC5), C, wild type *P. indica*. DNA oligonucleotides (16 and 22nt) were used as molecular size markers for siRNA analysis. Equal loading of total RNA was estimated by ethidium bromide staining of rRNAs, predominant RNAs.

Figure 9: Bi-compartment Petri dish culture system to study the transport of radiolabeled (32P) orthophosphoric acid to maize plants via *P. indica*. (A) Lateral view. (B and C) Top view showing both compartments separated by small glass Petri plate (used as a compartment 2). Black arrow indicates the *P. indica* growth in compartment 1 and white arrow indicates the growth of *P. indica* in compartment 2 (D) Colonization of the maize roots by *P. indica*.

Figure 10: (A) Transport of phosphorus to maize plants by *P. indica* carried out in bi-compartment Petri dish culture system. Radioactivity incorporated in plant was demonstrated by autoradiography. Radioactivity counts intensities are shown in false color code (vertical bar, low to high). (i) Whole maize plant before autoradiography. (ii) False-color autoradiograph of the maize plant obtained after 3 h of exposure of the maize plant. (iii) Microscopic view of a sample of plant root before autoradiography. a. maize plants were colonized with wild type *P. indica* (WT) b. maize plants were colonized with KD *P. indica* (KD) c. maize plants were grown alone without *P. indica* (C). (B) Amount of 32P transferred to the maize plant components by *P. indica*. Radioactivity was measured three times independently (number of transformants used n=2). The mean plus SD of three independent measurements are shown. Bar labeled with the (*) represents significance as compared with the wild type *P. indica* (P < 0.05).

Figure 11: Effect of PiPT on phosphate nutrition and plant growth (a) Biomass (b) Total phosphate content. Error bars denote the SD of the mean from plants of three replicate plates. * represent the significant difference from the maize plant colonized with wild type *P. indica* (taken as a control), (c) Growth promoting performance of *P. indica* at low (10 µM) and at high phosphate concentration (1mM). The mean plus SD of three independent measurements are
shown. Bar labeled with the (*) represents significant (P < 0.05) as compared with their respective control.

Tables

Table 1: Summary of amino acids identity (%) between P. indica PiPT and other fungal and plant phosphate transporters

<table>
<thead>
<tr>
<th>Name of the Organism</th>
<th>Phosphate transporter (number of amino acids)</th>
<th>GenBank Accession Number</th>
<th>Identity with PiPT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piriformospora indica*</td>
<td>PiPT (522)</td>
<td>DQ899728</td>
<td>100</td>
</tr>
<tr>
<td>Glomus versiforme*</td>
<td>GvPT (521)</td>
<td>U38650</td>
<td>35</td>
</tr>
<tr>
<td>Glomus intraradices*</td>
<td>GiPT (521)</td>
<td>AF359112</td>
<td>35</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae*</td>
<td>PHO84 (596)</td>
<td>D90346</td>
<td>35</td>
</tr>
<tr>
<td>Lycopersicon esculentum**</td>
<td>LePT1 (538)</td>
<td>Y14214</td>
<td>30</td>
</tr>
<tr>
<td>Arabidopsis thaliana**</td>
<td>AtPT2 (534)</td>
<td>U62331</td>
<td>30</td>
</tr>
<tr>
<td>Solenium tuberosum**</td>
<td>StPT3 (535)</td>
<td>AJ318822</td>
<td>30</td>
</tr>
</tbody>
</table>

Note: * Fungi, ** Plant
Figure 1
Figure 3
Figure: 4

Periplasm

N-term

Cytoplasm

C-term

~33x26 Å

~46x27 Å

42 Å
Figure 5
Figure 6
Figure 7

Relative Expression of PiPT gene

- EH
- HI

*
Figure 8

(a) Relative Quantity of PiPT Gene

(b) Predominant RNA
Figure 10
Figure 11

(a) Plant colonized with wild type *P. indica* vs. Only Plant vs. Plant colonized with KD-PiPT-*P. indica*

(b) Total Phosphate content (µM/shoot) for Plant colonized with wild type *P. indica* vs. Only Plant vs. Plant colonized with KD-PiPT-*P. indica*
Figure 11:

- Control plant at low phosphate
- P. indica Colonized plant at low phosphate
- Control plant at High phosphate
- P. indica Colonized plant at High phosphate

Biomass (g)
A phosphate transporter from the root endophytic fungus Piriformospora indica plays a role in the phosphate transport to the host plant
Vikas Yadav, Manoj Kumar, Deepak Kumar Deep, Hemant Kumar, Ruby Sharma, Takshashila Tripathi, Narendra Tuteja, Ajay Kumar Saxena and Atul Kumar Johri

J. Biol. Chem. published online May 17, 2010

Access the most updated version of this article at doi: 10.1074/jbc.M110.111021

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2010/05/17/M110.111021.DC1