Hakea sericea Schrad. - A Model to Study Phosphate Uptake in Proteoid Roots

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INTRODUCTION

Phosphorus (P) is one of the most important plant macronutrients, playing a key role in many metabolic processes such as in energy transfer, signal transduction, biosynthesis of macromolecules, photosynthesis or respiration (Raghothama, 1999). Despite of this, P is one of the most unavailable and inaccessible mineral nutrients, frequently being the limiting nutrient for plant growth. The form of P most readily accessed by plants is Pi, the concentration of which rarely exceeds 10 µM in soil (Schachtman et al., 1998). Many of the morphological and biochemical changes that are induced in roots growing in Pi-deficient conditions are geared towards enhancing Pi uptake, including not only the ability of increasing soil Pi availability but also the induction of high-affinity Pi uptake systems. Although some progress has been done on the elucidation of phosphate transport in plants, there are still few studies concerning biochemical and molecular characterization of phosphate uptake in proteoid roots. Here we present data on the mechanisms involved in Pi acquisition from soil by Hakea sericea Schrad. (Proteacea), an Australian invader of natural habitats, which is able to develop proteoid roots as a response to P deficiency (Fig. 1).

Figure 1. *Hakea sericea* Schrad. schrub (A) and Serra d'Arga (Northern Portugal) landscape where the spreading of *Hakea sericea* has become a major problem (B).

RESULTS

Pi Transport

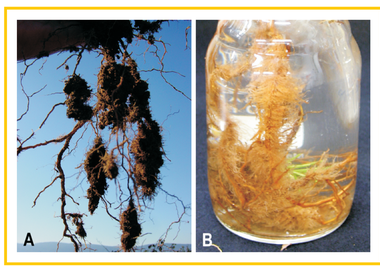
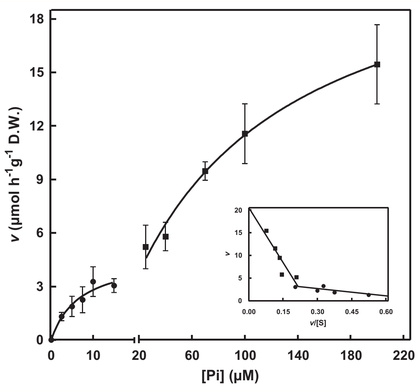


Figure 2. *Hakea sericea* proteoid roots harvested from shrubs growing in Serra d'Arga, Northern Portugal (A) and after being washed in the lab, exhibiting densely spaced rootlets (B).



Two mediated Pi transport systems:

	K_m	V_{max}
High Affinity	6 µM	5 µmol h^{-1} g^{-1} FW
Low Affinity	100 µM	24 µmol h^{-1} g^{-1} FW

Figure 3. Initial uptake rates of Pi by proteoid roots of Hakea sericea. Kinetic studies were performed after washing proteoid roots with mineral medium without Pi and cross-sectioning. Roots were incubated with 2.5-200 µM NaH_2PO_4 and depletion of Pi from the external medium (pH 6.0) was determined by the colorimetric method of Adams (1991). Values are mean ± S.E., N=3. Insert: Eadie-Hofstee plot of the initial Pi uptake rates.

Phosphite behaved as a competitive inhibitor

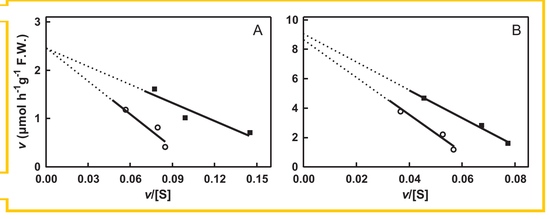
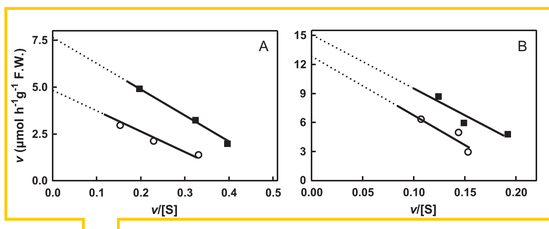


Figure 4. Eadie-Hofstee plots of the initial uptake rates of 5-20 µM Pi (high-affinity range) (A) and 20-100 µM Pi (low-affinity range) (B) in the absence (■) or in the presence of 600 µM phosphite (○).



CCCP inhibited the initial uptake rates of both high- and low-affinity ranges up to 60%, suggesting the involvement of a H^+-dependent transport

Figure 5. Eadie-Hofstee plots of the initial uptake rates of 5-25 µM Pi (high affinity range) (A) and 25-70 µM Pi (low affinity range) (B) in the absence (■) or in the presence of 50 µM CCCP (○).

Mersalyl (100 µM) reduced by 50% the initial uptake rates of 10 µM Pi.

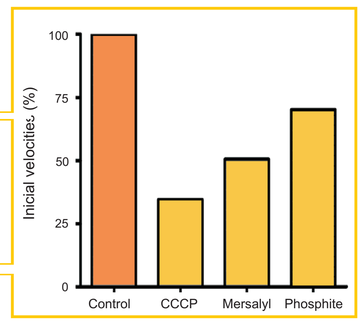


Figure 6. Initial uptake rates of 10 µM Pi in the absence or in the presence of 50 µM CCCP, 150 µM mersalyl and 600 µM phosphite.

Table I. Michaelis Menten constants (K_m) of *H. sericea* high-affinity Pi transport system at different extracellular pH values (pH_{ext}).

pH_{ext}	K_m (µM)				
	Total phosphate	H_3PO_4	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}
4.5	4.95	6.4×10^{-2}	4.88	3.2×10^{-3}	7.7×10^{-12}
5	6.57	2.7×10^{-2}	6.53	1.2×10^{-2}	1.9×10^{-10}
6.0	6.11	5.8×10^{-4}	5.75	0.35	1.7×10^{-7}
6.5	11.25	4.0×10^{-4}	9.42	1.84	2.8×10^{-6}

For both transport systems *K*m variation is lower when Pi concentration is expressed as $[H_2PO_4^-]$ suggesting that $H_2PO_4^-$ is the transported form

Table II. Michaelis Menten constants (K_m) of *H. sericea* low-affinity Pi transport system at different extracellular pH values (pH_{ext}).

pH_{ext}	K_m (µM)				
	Total phosphate	H_3PO_4	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}
4.5	66.79	0.87	65.88	$4{,}1 \times 10^{-2}$	$2.0\ 10^{-10}$
5	78.69	0.33	78.21	0,15	$2.3\ 10^{-9}$
6.0	106.4	1.0×10^{-2}	100.2	6.18	$3.0\ 10^{-6}$
6.5	97.76	$3{,}4 \times 10^{-3}$	81.81	15.95	$2.4\ 10^{-5}$

Search for phosphate transporter genes *(PiT)* in *H. sericea* Schrad.

H. sericea homolog probe

For the identification of *PiT* genes encoding *H. sericea* Pi/H^+ symporters, degenerated primers were designed (Fig. 7) and used in PCR amplification of *H. sericea PiT* fragments (Fig. 8). After cloning, two different *PiT* fragments were discriminated (Fig. 9). Sequence analysis (Fig. 10-12) revealed that at least two different *PiT* genes are present in *H. sericea* genome. *Hakea sericea* genomic library was prepared.

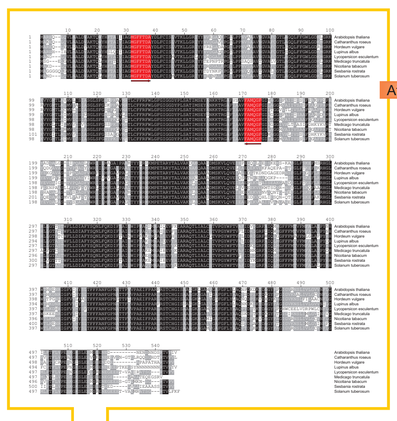


Figure 7. Amino acid sequence alignment of higher plant Pi/H^+ symporters. The residues conserved among the majority of the sequences are shadowed in gray, and those conserved in all sequences are shadowed in black. The sequences used for degenerated primer design are depicted in red.

After PCR

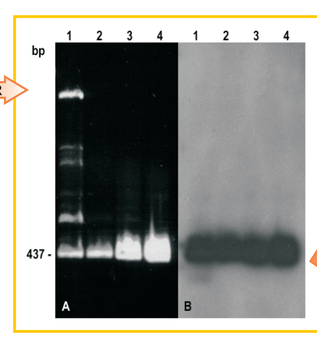
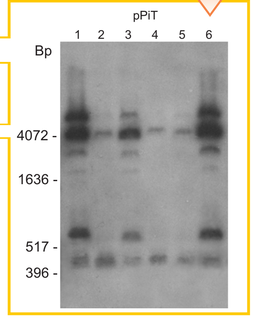


Figure 8. Southern analysis of PCR amplification products of *H. sericea* genomic DNA using degenerated primers for the conserved regions of *PiT* genes from higher plants. Annealing temperatures of 40°C (1); 45°C (2); 50°C (3) and 55°C (4) were used. A - Electrophoretic analysis (1.2% agarose gel); B - Southern blot analysis performed using [α-^{32}P]dCTP labeled *Lupinus albus LaPT1* gene.

Fragments were cloned into pPCR-Script Amp SK(+) (Stratagene)

Figure 9. Restriction pattern of the recombinant plasmids containing *PiT* fragments after digestion with *EcoR*I/*Sac*I. Southern blot analysis performed using [α-^{32}P]dCTP labeled *Lupinus albus LaPT1* gene.



During PCR amplification two fragments with the same molecular weight, but corresponding to different *PiT* genes of *H. sericea*, could have been amplified in the same PCR reaction.

Two distinct restriction patterns were obtained. The first included pPiT1, pPiT3 and pPiT6 while the second one was observed for pPiT2, pPiT4 and pPiT5.

Sequence analysis

Inserted *PiT* fragments in pPiT2 and pPiT6, both containing 437-bp, share 77.4% identity with each other and are homologous with phosphate transporters of higher plants.

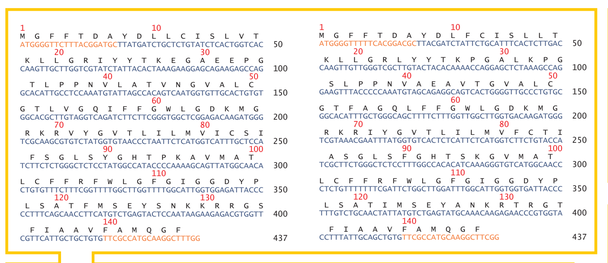


Figure 10. Partial nucleotide and deduced amino acid sequences of identified *H. sericea PiT* fragments [pPiT2 insert (A) and pPiT6 insert (B)]. The deduced amino acid sequence is represented above the nucleotide sequence, in the one letter code. The numbers on the right are related with the nucleotides and the numbers above are related with the amino acids. The sequences corresponding to the primers used in the amplification are represented in orange.

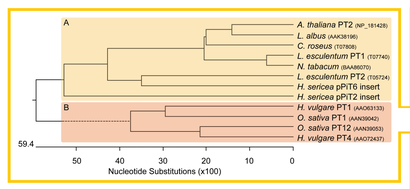


Figure 11. Phylogenetic tree representing the relation between *H. sericea PiT* fragments and other phosphate transporters of higher plants. The amino acid sequences were aligned with the program MegAlign (DNAStar). The length of each pair of branches represents the length between pairs of sequences. The scale bellow the tree measures the distances between the sequences. The accession of each sequence follows the species name.
A - Eudicotyledons; B - Liliopsida (monocotyledons).

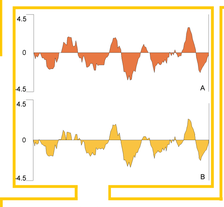


Figure 12. Hydrophobicity plot (Kyte-Doolitle) of *H. sericea PiT* fragments deduced amino acid sequences [pPiT2 insert (A) and pPiT6 insert (B)]. Five transmembrane domains are observed in both peptides.

Screening of *H. sericea* gDNA library

In order to obtain the complete sequences of phosphate transporter genes, *Pit2* and *PiT6* fragments are currently being used as homologous probes in the screening of the gDNA library of *H. sericea*.

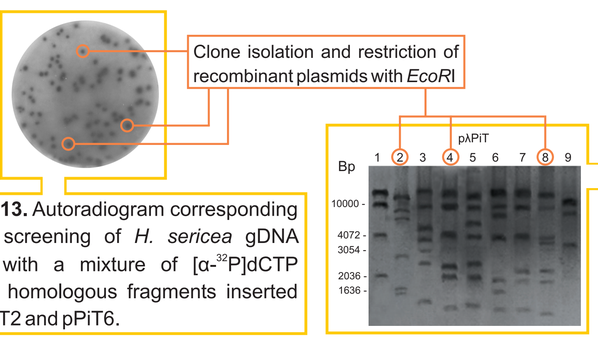


Figure 13. Autoradiogram corresponding to the screening of *H. sericea* gDNA library with a mixture of [α-^{32}P]dCTP labeled homologous fragments inserted into pPiT2 and pPiT6.

Figure 14. Restriction pattern of the recombinant plasmids containing λ*PiT* fragments after digestion with *EcoR*I. At least 8 clones present different restrictions patterns. Southern blot analysis using a mixture of [α-^{32}P]dCTP labeled homologous fragments inserted into pPiT2 and pPiT6 is currently underway.

Concluding Remarks

-*H. sericea* proteoid roots have highly efficient transporters for acquisition of Pi from soil;

-Pi uptake was inhibited by CCCP, suggesting the involvement of H^+- dependent transport;

-The Pi transported form is likely $H_2PO_4^-$;

-The high affinity Pi transport system has a *K*m of about 6 µM, a typical soil Pi concentration;

-Screening of genes encoding *H. sericea* Pi transporters is now underway.

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