Orthophosphate uptake in proteoid roots of naturally occurring Hakea sericea Schrad.

M. F. Sousa1, P. Silva2, A. R. Façanha2, R. M. Tavares1, T. Lino-Neto1 and H. Gerós1

Centro de Biologia | Departamento de Biologia | Universidade do Minho | Campus de Gualtar | Braga | Portugal

1Centro de Biotecnologia e Biodiversidade | Universidade Estadual do Norte Fluminense Darcy Ribeiro | Campos dos Goytacazes | Brasil

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 (Raghavendra, 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 by soil by Hakea sericea Schrad. (Proteaceae), an Australian invader of natural habitats, which is able to develop proteoid roots as a response to Pi deficiency (Fig. 1).

RESULTS

Pi transport

Proteoid roots were harvested from adult H. sericea shrubs growing in Serra d’Arga, Northern Portugal (Fig. 2), washed with mineral medium without Pi, and cross-sectioned. To study Pi transport roots were incubated with 2.5-200 µM NaH2PO4, and the depletion of Pi from the external medium was determined by the colorimetric method of Adams (1991).

Figure 3. Initial uptake rates of Pi, at pH 7.0, by proteoid roots of Hakea sericea. Values are mean ± S.E., N=3. Insert: Eadie-Hofstee plot of the Pi transport rates.

The protonophore CCCP (50 µM) inhibited the initial uptake rates of 5-25 µM Pi (high-affinity range) and 20-100 µM Pi (low-affinity range) up to 60%, suggesting the involvement of a H+-dependent transport (Fig. 3 and 4). To determine which Pi form is preferentially transported, kinetic studies were conducted at pH 4.5, 5.0, 6.0 and 6.5. For both transport systems, this pH range, Kc variation is lower when Pi concentration is expressed as [H2PO4-] (Table I and II), suggesting that H2PO4- is the transported form.

Figure 4. Eadie-Hofstee plots of the initial uptake rates of 5-20 µM Pi (A) and 20-100 µM Pi (B) in the absence (A) or in the presence of 600 µM phosphite (B). The measurement of initial uptake rates of 5-20 µM Pi (high-affinity range) and 20-100 µM Pi (low-affinity range) µM Pi in the presence of 600 µM phosphite (PiPho) showed that this substrate behaved as a competitive inhibitor (Fig. 4 and 5), indicating that it is also a substrate for both Pi transport systems. Monensyl (100 µM) reduced by 50% the initial uptake rates of 10 µM Pi (Fig. 5).

Figure 5. Initial uptake rates of 10 µM Pi in the absence or in the presence of 10 µM CCCP, 150 µM monensyl and 600 µM phosphite.

Phosphate uptake was inhibited by CCCP, suggesting the involvement of a H+-dependent transport. The Pi transported form is likely HPO42-. The Pi transported form is likely HPO42-.

Figure 6. Eadie-Hofstee plots of the initial uptake rates of 5-20 µM Pi (A) and 20-70 µM Pi (B) in the absence (A) or in the presence of 50 µM CCCP (D). The measurement of initial uptake rates of 5-20 µM Pi (high-affinity range) and 20-70 µM Pi (low-affinity range) µM Pi in the presence of 600 µM phosphite (PiPho) showed that this substrate behaved as a competitive inhibitor (Fig. 6 and 7), indicating that it is also a substrate for both Pi transport systems. Monensyl (100 µM) reduced by 50% the initial uptake rates of 10 µM Pi (Fig. 7).

Figure 7. Southern analysis of PCR amplification products of H. sericea genomic DNA using degenerate primers for the conserved regions of PiT genes from higher plants. Annealing temperatures of 45°C (1), 42°C (2), 37°C (3) and 33°C (4) were used. A - Electrophoretic analysis (2 X agarose gel); B - Southern blot analysis performed using [a-32P]dCTP-labeled Lupinus albus LpiT7 gene.

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

For the identification of PiT genes encoding H. sericea PiH+ symporters, a gDNA library was constructed using Lambda DASH III/Bam HI vector kit (Stratagene). In order to obtain homologous PiT probes, PCR amplifications of H. sericea gDNA were performed in the presence of degenerated primers designed for the conserved regions of PiT genes from higher plants (Fig. 7).

As a result of the use of degenerated primers, several fragments with the same molecular weight, but corresponding to different PiT genes of H. sericea, could have been amplified in the same PCR reaction. Electrophoretic and Southern analyses of these fragments were performed after cloning them into pPCR-Script Amp SK (+) (Stratagene) and digesting the recombinant plasmids with EcoRl/SacI (Fig. 8). Two distinct restriction patterns were obtained. The first included PiT1, PiT3 and PiT6 while the second one was observed for PiT2, PiT4 and PiT5.

Figure 8. Restriction pattern of the recombinant plasmids containing PiT fragments after digestion with EcoRl/SacI. Southern blot analyses performed using [a-32P]dCTP labeled Lupinus albus LpiT7 gene.

After screening PiT2 and PiT6, two 437-bp fragments sharing 77.4% identity with each other and homologous to phosphate transporters of higher plants were obtained (Figure 9). The deduced amino acid sequences were aligned with amino acid sequences of phosphate transporters from other higher plants and a phylogenetic tree was created (Fig. 10). The phylogenetic analysis revealed the presence of five transmembrane domains in both PiT2 and PiT6 peptides (Fig. 11).

Figure 9. Partial nucleotide and deduced amino acid sequences (PiT2 - A and PiT6 - B) identified in Hakea sericea. The deduced amino acid sequences 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 green.

In order to obtain the complete sequences of phosphate transporters, PiT2 and PiT6 fragments are currently being used as homologous probes in the screening of the gDNA library of H. sericea (Fig. 11).

Figure 10. Phylogenetic tree representing the relation between PiT2 and PiT6 of H. sericea 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 bar below the tree indicates the distance between the sequences. The accession of each sequence follows the species name. A - Eucalyptus/ B - Lippia/ C - Monocotyledons.

CONCLUDING REMARKS

- H. sericea proteoid roots have highly efficiencrete transporters for acquisition of Pi from soil.
- Pi uptake was inhibited by CCCP, suggesting the involvement of a H+-dependent transport.
- The Pi transported form is likely H2PO4-.
- The high affinity Pi transport system has a Kc of about 6 µM, a typical soil Pi concentration.
- Screening of genes encoding H. sericea Pi transporters is now under way.

REFERENCES


CONCLUSIONS

- Hakea sericea Schrad. (Proteaceae), an Australian invader of natural habitats, which is able to develop proteoid roots as a response to Pi deficiency (Fig. 1).
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