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Phosphate transport by proteoid roots of *Hakea sericea*

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Abstract

Up to now the higher capacity of proteoid roots to absorb inorganic phosphate from P_i-poor soils has been related mainly to their increased root surface area and higher exudation of organic acids and phosphatases, while much less attention has been directed to their mechanisms of P_i uptake. Here we report a characterization of the P_i uptake kinetics of the proteoid root-forming species *Hakea sericea* Schrad. This Proteaceae is an Australian native, which is disseminating very fast through forests of the European south. Dense mats of proteoid roots were observed in the upper soil layers of the invaded area in Portugal where availability of P and N was shown to be very low. Plants grown hydroponically under low-P_i also developed proteoid roots, and the proteoid clusters presented a major role in P_i absorption in comparison to the non-proteoid portions of the root system as revealed by their higher $^{32}P_i$ labeling. The $^{32}P_i$ uptake by proteoid roots was dependent on H^+ gradient and yielded a biphasic kinetics, suggesting the involvement of H^+/P_i co-transport systems with K_m values of 0.225 and 40.8 μM P_i. The analogs phosphite (Phi) and arsenate, but not vanadate, inhibited competitively the P_i absorption. Such biphasic P_i uptake pattern with the highest affinity at submicromolar range is likely to be of critical importance for the capacity of this plant species to invade and proliferate throughout vast areas of nutrient-deprived soils.

Keywords: *Hakea sericea*; Invasive species; Phosphate uptake; Phosphorus-poor soils; Proteaceae; Proteoid roots

1. Introduction

Phosphorus (P) is an important plant macronutrient, making up about 0.2% of plant dry weight. P deficiency limits plant growth more frequently than any other nutrients except nitrogen [1–3]. Although total soil P content typically varies from 500 to 2000 mg kg^{-1}, total bioavailable P may be only a few mg kg^{-1} [2]. The form of P most readily accessed by plants is P_i, the concentration of which rarely exceeds 10 μM in soil solutions [4]. The P_i level in soil solution is regulated mainly by its interaction with organic or inorganic surfaces in the soil. Aluminium and iron ions in acid soils, and calcium ions in alkaline soils, interact strongly with P_i and render it unavailable to plants. Consequently, plants have developed numerous morphological, physiological, biochemical and molecular adaptations to acquire P_i [2,3].

Proteoid roots (or cluster roots) are considered, along with mycorrhizas and nitrogen-fixing nodules, to be one of the major adaptations to enhance nutrient acquisition [5]. Along a proteoid root, discrete clusters of closely spaced rootlets develop. The rootlets emerge in continuous rows from the cortex and are covered with root hairs, increasing the absorption surface area [6]. Species with proteoid roots can grow in soils with poorly available nutrients, and their induction has been reported mainly in response to low availability of P and Fe [7]. Until now the ability of proteoid roots in improving P_i mobilization from soil has been related mainly to the increase of root surface area and exudation of carboxylic acids, acid phosphatases, phenolics, mucilages, and water [6,8,9].

Hakea sericea Schrad. is a Proteaceae native in Southeastern Australia and its ability to produce proteoid roots, together with the production of large number of seeds protected by woody follicles, efficient dispersal of seeds and rapid germination, have enabled the species to become well-adapted to cope with

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Phi, phosphite; TPP^+, tetraphenylphosphonium.

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fires and P_i-poor soils. These advantages have contributed to make *H. sericea* an aggressive invader of natural vegetation in the Mediterranean basin, similarly to what has been reported in New Zealand and South Africa [10]. It has been postulated that Proteaceae species could present enhanced P_i absorption capacity. This feature, in association with a deficiency for down-regulating their P_i transport, may result in P_i toxicity symptoms [11,12]. Nevertheless, up to now, detailed data on kinetics and energetics of P_i uptake by proteoid roots of *Hakea* spp. are still lacking, the only approach published so far showing transport depending linearly on the external P_i concentration [13]. The present work reports a kinetic characterization of the P_i uptake system of the *H. sericea* proteoid roots and its relationship with the adaptive aspects of this invasive species in nutrient-poor soils.

2. Material and methods

2.1. Sampling area

Proteoid roots and follicles from wild *H. sericea* plants, as well as soil samples, were collected in Serra de Arga mountains (430–450 m elevation), Northern Portugal, centred at 8°45′06″W, and 41°51′20″N.

2.2. Soil analysis

Samples were collected in 10–40 cm of depth in the soil, air-dried and passed through a 2 mm sieve prior to analysis. Extractable P content was determined in the modified Egner–Riehm extract [14]; P_i was measured colorimetrically by the ascorbic acid method [15]. Total P was extracted with *aqua regia* (HCl:HNO_3, 3:1) and measured colorimetrically. Mineral N content was determined in an aqueous extract of the soil (1:5) after reduction with $FeSO_4$ and Ag^+ followed by alkalinization. NH_3 was separated by distillation and titrated. Total N was determined by the Kjeldhal method [16].

2.3. Hydroponic culture of H. sericea

To obtain hydroponically grown plants, follicles were opened at 120 °C for 1 h and seed dormancy was broken in the dark, at 4 °C for 1 week. Seeds were embedded for 24 h, and germination occurred in quartz sand. One-month seedlings were transferred to vessels containing 8 l mineral medium with 200 μM $Ca(NO_3)_2$, 100 μM $MgSO_4$, 330 μM KNO_3, 50 μM NH_4NO_3, 18 μM H_3BO_3, 8 μM $MnSO_4$, 0.16 μM $CuSO_4$, 0.32 μM ZnSO4, 0.4 μM Na_2MoO_4, 20 μM FeEDTA, and 1, 100 or 1000 μM NaH_2PO_4 (depending on the experiment), at pH 5.8. This nutrient solution was aerated and changed biweekly. The hydroponic system was kept in a green-house with a photoperiod of 16 h and a quantum irradiance of 200 μmol m^{-2} s^{-1}, at 25 °C. After 6 weeks, cotyledons were removed. To evaluate the effect of P_i availability on the induction of proteoid roots, two sets of seedlings were grown in different nutrient solutions containing 1 μM or 100 μM of NaH_2PO_4. To study the correlation between plant P status and P_i uptake, plants grown in the 1 μM P_i nutrient solution were transferred to a 1 mM P_i nutrient solution for 2 days prior to uptake experiments.

2.4. Measurement of $^{32}P_i$ uptake by proteoid roots

Radioactive phosphate [$^{32}P_i$] uptake experiments were carried out according to a modified protocol of Sentenac and Grignon [17]. The assays were performed in fresh proteoid root segments (excised proteoid roots) collected from hydroponically grown *H. sericea* plants. After sampling, proteoid roots were carefully washed with deionised water and immediately used in $^{32}P_i$ uptake experiments. In order to correlate the results obtained using roots from hydroponically grown plants with the P_i uptake phenomena occurring in plants growing in their natural environment, proteoid roots excised from wild-grown plants were also used in $^{32}P_i$ uptake experiments. However, technical issues related with detachment of soil particles from the roots and extensive washing, together with the heterogeneity exhibited by the plant material, impaired detailed analysis of ^{32}Pi uptake.

In order to estimate initial $^{32}P_i$ uptake rates, 50–70 mg FW of root segments were incubated in 20 ml of transport solution (10 mM $CaCl_2$, 20 mM MES and 0.1–100 μM H_3PO_4 labeled with $^{32}P_i$), adjusted to pH 4.5, 5.5, 6.0, or 6.5 to impose transmembrane proton gradients, at 25 °C. $^{32}P_i$ uptake was also measured in root segments pre-incubated with 100 μM of the protonophore CCCP and 100 μM TPP^+ for 10 min to evaluate the dependence on proton electrochemical gradient. After shaking for 2 min, a period that ensures linearity of uptake, the reaction was stopped by washing the root segments with ice-cold 2 mM $CaCl_2$ under vacuum. Root segments were transferred to 50 ml Falcon tubes containing 5 ml of 2% (w/v) Triton X-100 and incubated for 12 h. Aliquots of extracts (0.5–2 ml) were transferred to vials containing scintillation fluid (OptiPhase HiSafe II; LKB Scintillation Products). The radioactivity was measured in a Packard Tri-Carb 2200 CA liquid scintillation counter (Packard Instruments Co. Inc., Rockville, MD). Non-specific binding of labeled P_i to the filters and/or root cells was determined after root segments had been incubated with 2% (w/v) Triton X-100 for 12 h prior to uptake experiments and these results were used to correct P_i uptake data.

2.5. Effect of P_i analogs and mercurial reagents on $^{32}P_i$ uptake

Inhibition of P_i transport by Phi, arsenate, or vanadate was assayed by adding simultaneously 2 μM $^{32}P_i$ and 1 mM of these compounds. The transport reaction was stopped as described previously after 2 min of incubation. Competition between P_i and the analogs Phi and arsenate was tested by running competitive uptake kinetics. The transport reaction was started after the addition of the unlabeled substrates (250 μM). $^{32}P_i$ transport was also measured in the presence of 100 μM $HgCl_2$ or 250 μM mersalyl (mersalic acid), after proteoid roots had been pre-incubated during 10 min with the mercurial reagents.