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Direct evidence on participation of root hairs in phosphorus (^{32}P) uptake from soil

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Abstract

Root hairs substantially extend root surface for ion uptake. Although many reports suggest a relationship between root hairs and phosphorus (P) uptake of plants, the role of root hairs in phosphorus uptake from soils is still debated. We measured uptake of phosphorus from soil directly via root hairs. Root hairs only were allowed to penetrate through a tightly stretched nylon screen (53 μm) glued to the bottom of a PVC tube. The penetrating root hairs grew for 2 and 4 days in soil labelled with radioisotope phosphorus (P) tracer ^{32}P (185 kBq g^{-1} dry soil) filled in another PVC tube. Transparent plastic rings of thickness ranging from 0.25 mm to 2.0 mm were inserted between the two PVC tubes. This provided slit width for microscopic observations *in situ*, which confirmed that only root hairs were growing into the ^{32}P labelled soil. In some cases no rings were inserted (slit width = 0) where both root hairs and root surface were in contact with the labelled soil (total ^{32}P uptake). The uptake of ^{32}P from soil via the root hairs only was quantified by measuring activity of ^{32}P in the plant shoot (^{32}P uptake only via root hairs).

The results showed that when 70 percent of the root hairs grew into the labelled soil, they contributed to 63 percent of the total P uptake. With decreasing number of root hairs growing into the ^{32}P labelled soil, the quantity of ^{32}P in the plant shoot decreased. In this study, P uptake via root hairs was measured in a soil-based system, where root hairs were the only pathway of ^{32}P from soil to the plant shoot. Therefore, this study provides a strong evidence on the substantial participation of root hairs in uptake of phosphorus from soil.

Introduction

It is well known that root epidermis cells absorb ions and that they may differentiate into root hairs. Root surface area for ion uptake may increase many-fold due to the presence of root hairs. The existence of variation in root hairs of legumes (Caradus, 1979) and cereal varieties (Gahoonia et al., 1997) suggests a great potential for genetic improvement of plant uptake of diffusion limited soil nutrients (Lewis and Quirk, 1965) like phosphorus (P) and most trace elements. Many electrophysiological studies using vibrating microelectrodes (Jones et al., 1995; Kochian et al., 1992; Schiefelbein et al., 1992;) have clearly demonstrated the influx of calcium and potassium ions into isolated root and root hair cells.

The role of root hairs in phosphorus uptake from soil still remains a matter of debate. Theoretical calculations (Nye, 1966), autoradiographs (Lewis and Quirk, 1967), correlation of root hair length and number with the quantity of P in the plant (Föhse et al., 1991) all suggested a relationship between root hairs and P uptake from soil. Roots and associated rhizosphere micro-organisms may secrete mucilage (Greaves and Darbyshire, 1972) and organic compounds (Rovira et al., 1978) improving diffusion of phosphorus in soil close to the root surface. Therefore, it remains unclear whether the increased P availability in the previous reports was due to the improved P diffusion in soil or due to the enhancement of P uptake solely via root hairs. In this paper, we aimed to provide

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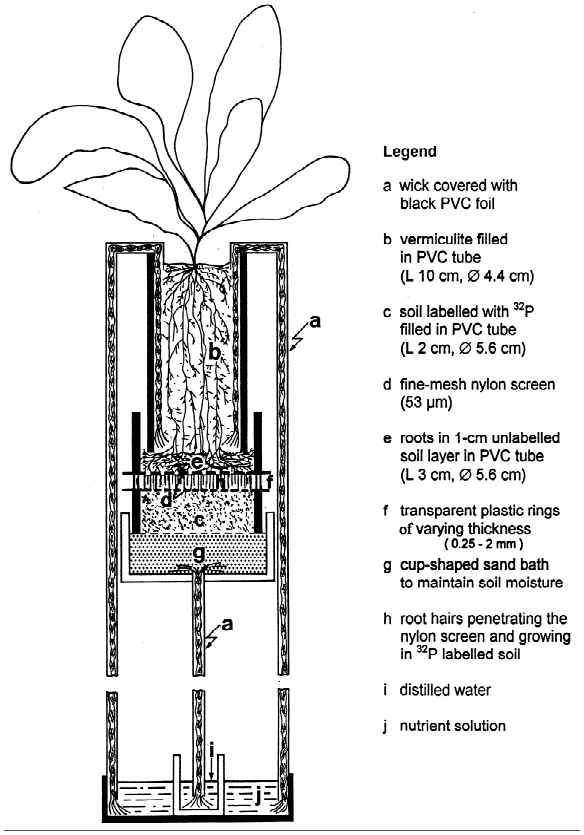

Figure 1. Schematic illustration of the plant growing system used to separate phosphorus uptake via root hairs from that of remaining root surface (modified after Gahoonia and Nielsen, 1991).

direct evidence on the role of root hairs in P uptake by measuring the uptake of ^{32}P from labelled soil via root hairs separately from that of remainder of the root surface.

Material and methods

Soil

The properties of the soil used are as followed: Inorganic phosphorus extracted with 0.5 *M* $NaHCO_3$ ($NaHCO_3$-P_i) = 1.6 mmoles P kg^{-1} soil; P in soil solution = 10 μM; sand 72%, silt 14%, clay 13%; organic matter 2.4%, pH 6.7 in 0.01 *M* $CaCl_2$, CEC = 13 ceq kg^{-1} soil at pH 7. Phosphorus (P) here refers to inorganic P.

Separation of ^{32}P uptake via root hairs from remaining root surface

Rye plants (*Secale cereale* L. cv. Petkus II) were pre-grown for 9 days in vermiculite-filled PVC tubes (length 10 cm, diameter 4.4 cm) closed at the bottom by nylon cloth impervious to roots (Gahoonia and Nielsen, 1991). After removing the nylon cloth, the tubes with plants were transferred in a system allowing penetration of root hairs only through a tightly stretched nylon screen of mesh size of 53 μm, glued to the bottom of a 3-cm long PVC tube of diameter 5.6 cm. The 3-cm PVC tube contained 1-cm layer of unlabelled soil. Another similarly made 2-cm PVC tube was filled with ^{32}P labelled soil (185 kBq g^{-1} dry soil). To maintain defined soil moisture (θ = 0.19, volume/volume), the 2-cm soil columns were placed over a small cup-shaped sand bath fitted with a wick dipping into a reservoir of distilled water at 22 cm water tension. The two PVC tubes were separated by inserting transparent plastic rings of various thickness to provide slit widths ranging from 0.25 to 2 millimetres between them (Figure 1). In this only root hairs were capable of growing into the ^{32}P labelled soil. This was confirmed by *in situ* microscopic observations (Plate 1). Such pictures also allowed an estimate of length classes (see below) of root hairs growing into the ^{32}P labelled soil using image analysis (Leica, Quantimet 500_+). The root hairs were also checked for the presence of water film or mucilage on them. In some cases, no rings were inserted between the two PVC tubes, so that both root hairs and root surface could participate in ^{32}P uptake. Unplanted controls provided checks for an alternative path (other than root hairs) of ^{32}P movement to the root surface in 1-cm soil layer above the rings. The root hairs were allowed to uptake ^{32}P for 2 and 4 days, then the shoots were cut for determining their radioactivity with liquid scintillation counting.

Based on the indications from a previous study (Gahoonia et al., 1994) for controlling soil moisture in the microcosms used here, the additional experiments revealed that with θ = 0.19, the ^{32}P did not contaminate the 1-cm soil layer above the screen with most rings. When the slit width was 0.25 mm but only then, we detected some ^{32}P (21 Bq g^{-1} dry soil) in the 1-cm soil layer above the screen when unplanted vermiculite-filled PVC tubes were placed over it. This was most likely due to screen touching the ^{32}P labelled soil column, as indicated by brown soil patches on it in some cases. At this soil moisture level with the slit widths greater than 0.25 mm, we did not detect radioactivity in

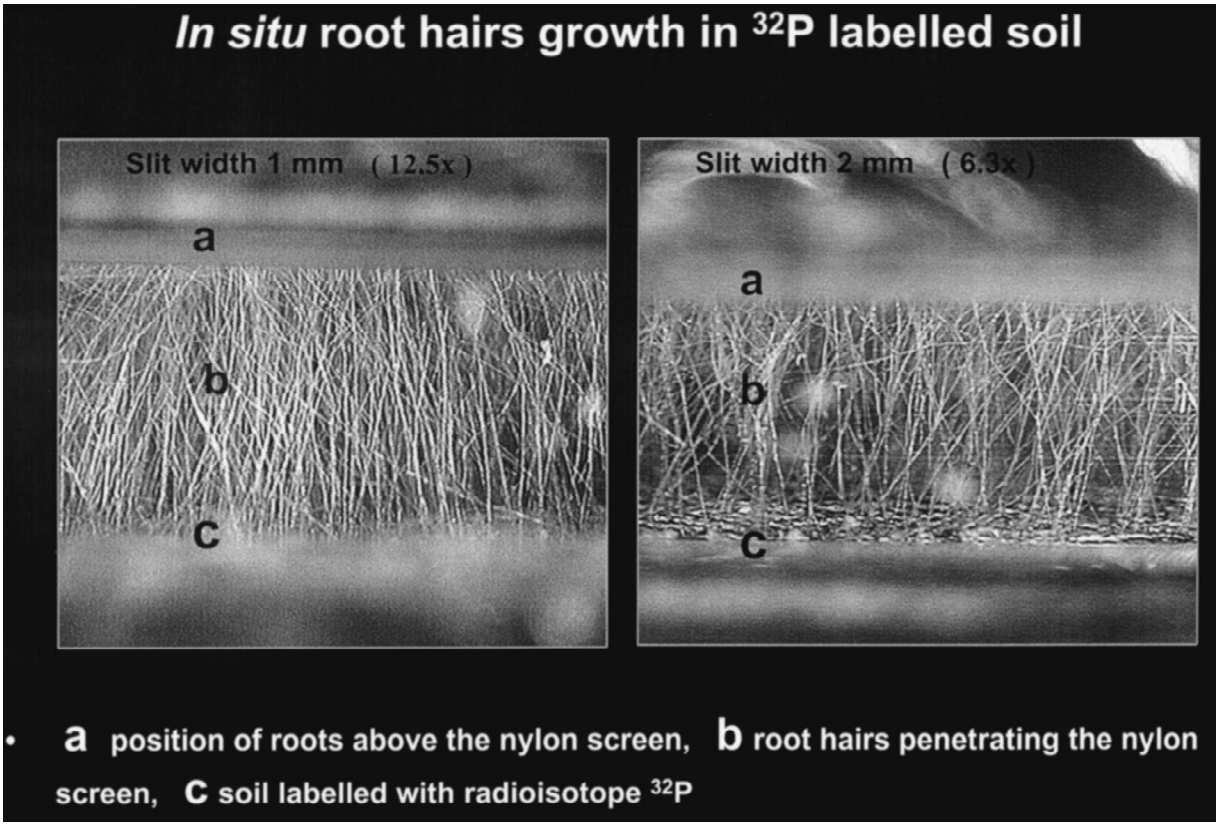

Plate 1. Root hairs growing in a slit of moist air between two soil layers. The lower soil layer was labelled with ^{32}P. (The white spots are the images of dust particles on the inner surface rings used to provide the slit, which could not be cleaned without disturbing the system).

the 1-cm soil layer above the screen. However, with θ = 0.25, contamination of the 1-cm soil layer above the screen with ^{32}P extended up to a slit width 0.75 mm, though the theoretical background of this observation is unclear. So we strictly maintained soil moisture (θ = 0.19). It also reduced the possibility of water film on the root hairs. At each harvest we checked the screen and root hairs for brown colour. Brown colour only on root hairs indicated that root hairs grew into the soil without the screen touching the labelled soil. Roots of control tubes with plants (after 4 days) were stained with Trypan blue and examined under microscope. No mycorrhizal hyphae were observed. The experiments were conducted under controlled conditions (light intensity 280 μE s^{-1} m^{-2}, light/dark period 16/8 h, temperature 18/15 °C, relative humidity 75%). More details of the plant growing technique are given in Gahoonia and Nielsen (1991, 1992).

Soil ^{32}P labelling

All materials were checked for radioactivity before use. The 2-cm PVC tubes were filled with soil (passed through 2 mm sieve) by shaking to obtained uniform soil bulk density (1.3 g cm^{-3}). Radioisotope ^{32}P (as orthophosphate in aqueous solution, carrier free, Amersham) was then directly pipetted on the 2-cm soil columns to achieve 185 kBq ^{32}P g^{-1} dry soil. This procedure gives a rather uniform distribution of ^{32}P in soil layer less than 3 cm (Jupp et al., 1987). The columns were allowed to attain equilibrium for 24 h, then placed over small cup-shaped sand baths to achieve the defined soil moisture as described above.

Determination of ^{32}P activity

After the root hairs had grown in ^{32}P labelled soil for 2 and 4 days, shoots were cut, dried at 80 °C, weighed and placed in crucibles for dry ashing in the muffle at

550 °C for 2 h. The ash was dissolved in 2.5 mL of 6 *M* HCl. Activity of ^{32}P was measured with liquid scintillation counting (Wallac, WinSpectral^{TM} 1414). Only shoots were analyzed, because of the problems associated with cleaning of roots containing ^{32}P and because some ^{32}P would have been lost from roots during washing. This put a constraint on determination of total ^{32}P influx into the plants. After corrections for isotopic decay and counting efficiency, the counts were calculated to mole P.

Determination of percentage of root hairs growing into the ^{32}P labelled soil

In situ pictures of root hairs penetrating the nylon screen (Plate 1) allowed an estimate of lengths of the root hairs penetrating the screen and visible close to the periphery of rings of thickness 1.5 and 2.0 mm. The lengths of 100 randomly selected root hairs were measured from pictures with slit widths 1.5 and 2.0 mm (where most of the root hairs were hanging in moist air), using Quantimet 500$_+$ Image Processing and Analysis System (Leica) (procedure 1). Root hairs determination after dismantling the microcosms seemed not to provide reliable data, because some root hairs growing into the soil with lower slit widths broke during dismantling. With slit widths of 1.5 and 2.0 mm, the root hairs very soon became curly, probably due to change in air moisture. This made it difficult to determine their actual lengths.

To obtain additional information on root hairs (potentially growing in the soil) under identical conditions, we made more measurements of root hairs length on root segments growing in moist air as follows: the plants were grown in a similar way to that in procedure 1. The 2-cm PVC tubes were *not* filled with soil. Six holes (ca. 1 mm) were made in the nylon screen so that ca. six roots penetrated and grew in moist air. After 4 days the penetrating root segments were cut and placed in water in Petri dishes. Again the lengths of 100 randomly selected root hairs on the root segments was measured (procedure 2). Each procedure gave very similar results. The data of root hairs from each procedure was grouped into length classes (length of the root hairs grouped into classes of 0.5, 1.0, 1.5 and, 2.0 mm). The number of root hairs in each length class of the two procedures were averaged and the standard deviation calculated. All the root hairs of lengths equal to or greater than 0.5, 1.0, 1.5 and 2.0 mm were classed as growing in ^{32}P labelled soil with the respective slit widths. At slit = 0 mm, all (100%) root hairs and root

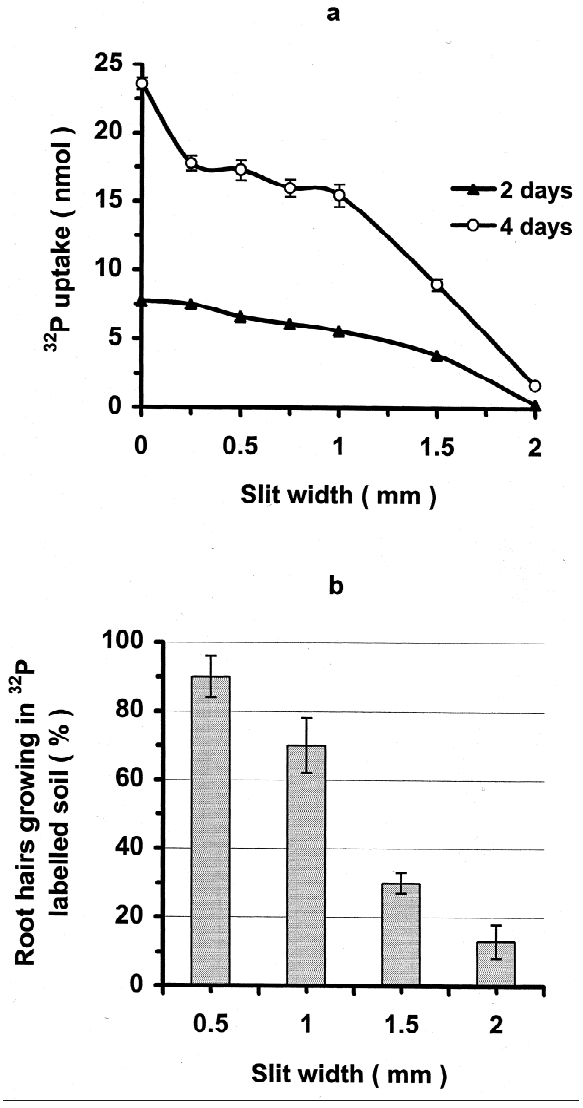

Figure 2. Phosphorus (^{32}P) uptake (a) and the percentage of root hairs growing in ^{32}P labelled soil (b) in 4 days.

surface would grow into and participate in ^{32}P uptake from the labelled soil. As with both the procedures 100 root hairs were scored, the number of root hairs in each class was expressed as a percentage.

The entire surface of the nylon screen (Fig. 1) with each slit width was fully covered with a mat of roots bearing root hairs. Therefore, the root hair length classes seemed to provide a good estimate of root hairs growing into the ^{32}P labelled soil. Statistical analyses were performed using Statistical Analysis System (SAS Institute, 1989) and Microsoft Excel as appropriate.

Results and discussion

The presence of ^{32}P in the shoot in just 2 days showed that the plants absorbed P via root hairs (Figure 2a) and that it was translocated to the shoot. Microscopic examination revealed that in 2 days root hairs had grown in the labelled soil. ^{32}P uptake decreased with increasing slit width, because of the concomitant decrease in number of root hairs participating in P uptake (Figure 2b). With a slit width of one millimetre, 70% of the root hairs grew in ^{32}P labelled soil (Figure 2b) and they contributed to 63% of the total ^{32}P uptake in shoots (the data points at 0 mm in Figure 2a show uptake of P via both root hairs and root surface) in the plants in 4 days. Though the functional life time of root hairs is not well documented, there are indications (Fussender, 1987) that they may be functional for 2–3 days as evidenced by examination of root hair cell integrity under the electron microscope. The plant microcosms used in our study imply that during the experimental period (4 days) new functional root hairs were continuously penetrating the nylon screen and growing into the ^{32}P labelled soil.

Unplanted controls did not show contamination of the upper unlabelled 1-cm soil layer with ^{32}P when ring thickness was greater than 0.25 millimetre. With ring thickness of 0.25 millimetre, we detected some ^{32}P (21 Bq g^{-1} dry soil) in the unlabelled soil, most likely because the screen had touched the ^{32}P labelled soil as indicated by its brown colour. We did not observe water film or mucilage on the root hairs (Plate 1), thus the capillary movement of ^{32}P along the outer surface of root hairs to the remaining root epidermis cells seems unlikely. In our study, it was difficult to envisage an alternative route of tracer ^{32}P in the plant shoot, except root hairs. Therefore, the presence of radioisotope P in the shoots shows a substantial participation of root hairs in uptake of phosphorus from soil.

This report provides direct evidence of participation of root hairs in uptake of phosphorus from soil. The technique applied is simple, providing good reproducibility between replicates (Figure 2a). It may help to determine the role of root hairs in uptake of other plant nutrients from soils by using appropriate radioisotopes. Root hairs are also the site of infection in *Rhizobium*-legume symbiosis (Downie, 1997). The convenient way of observing root hairs *in situ* described above may be helpful in studying the infection process.

Low availability of soil P is widespread in developing countries, where it limits crop production. Even in well developed agriculture systems, it is desirable to reduce fertilisers inputs for economical and environmental reasons. The abundance of root hairs increases, when P supply is lowered (Bates and Lynch, 1996). A wide variation seems to exist in root hairs of cereal varieties (Gahoonia et al., 1997) and it appears to be controlled by simple genetics (Hochmuth et al., 1985; Schiefelbein and Somerville, 1990). The broad sense heritability of root hairs in white clover ranged 0.33–0.44 (Caradus, 1979). The advantage of increasing root surface area for ion uptake with abundant root hairs may be that less photosynthetic carbon may have to be invested in more efficient root systems (Clarkson, 1995). In addition to this, the geometrical arrangement of root hairs on roots implies that they increase P uptake from that soil (rhizosphere soil), where it can be released from soil by other root-induced processes (Ae et al., 1990). Improvement of nutritional characteristics of plants by selection and breeding is an emerging research area. Direct evidence of substantial contribution of root hairs in P uptake from soil will encourage interdisciplinary research to improve nutrient uptake efficiency of plants, adapting them to low-input agriculture.

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