Phosphorus acquisition from non-labile sources in peanut and pigeonpea with mycorrhizal interaction

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Abstract

Both peanuts and pigeonpeas are believed to utilize non-labile phosphate (P) due to the dissolving abilities of their root exudates, in which the mycorrhizal interaction is rarely considered. We examined a hypothesis that such effective P acquisition would not appear without the mycorrhizal function. First, mycorrhizal responses of peanuts and pigeonpeas were compared with soybeans or a non-mycorrhizal species of yellow lupin in a P-limited soil amended with a mixture of three non-labile P sources (Fe-P, Al-P, and Ca-phytate). Inoculation with Gigaspora margarita greatly increased P uptake in pigeonpeas (10-fold) and peanuts (6-fold), more than in soybeans (3-fold), indicating greater mycorrhizal dependency in both these plants. Second, the mycorrhizal responses of peanuts and pigeonpeas were compared in a soil amended with three P sources individually. P uptake responses were significant, and similar for all the P sources in pigeonpeas. In peanuts, the response was significant for both Al-P and Fe-P, but not for Ca-phytate because of greater P uptake in the non-inoculated plants, implying a direct utilization of Ca-phytate by peanuts. Third, interactive effects of the pigeonpeas’ rhizosphere and extraradical hyphae were investigated in a compartment container, where root exudate movement as well as hyphal penetration into the compartment soil, amended with Al-P, was controlled. A significant increase in P uptake was found only when both the exudates and hyphae were permitted access into the compartment soil, suggesting the importance of the interactive effect. We concluded that the mycorrhiza can accelerate P acquisition by the plants from non-labile sources in soil through interaction between the roots and hyphae. It is a novel finding that peanuts can utilize Ca-phytate without mycorrhiza, but the mechanism is still unclear.

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1. Introduction

A substantial amount of phosphate (P) fertilizer is often needed to obtain sufficient crop yield. However, most of it, often 90% or more, is not taken up by the crop but is retained in non-labile P sources (Stevenson and Cole, 1999). Consequently, an excessive amount of P fertilizer is often used, and there are concerns about depletion of commercially available rock phosphate as a source of the fertilizer. Recently, increasing attention has been paid to the effect of excessive use of P fertilizer for economic and environmental reasons. In fact, heavy application of P sometimes causes eutrophication of lakes and rivers (Sharpley, 1995). Hence, it is important to utilize retained soil P efficiently for crop production to conserve P resources as well as to reduce environmental risks.

Some plant species are known to utilize non-labile P effectively in either inorganic or organic forms. The white lupin develops proteoid roots under P-deficient...
conditions. These roots excrete large quantities of citrate (Gardner et al., 1983; Dinkelaker et al., 1989), which mobilize calcium-bound phosphate (Ca-P). The pigeonpea is also reported to utilize iron-bound phosphate (Fe-P) by excreting piscidic acid, which chelates Fe (Ae et al., 1990). Otani and Ae (1996) and Otani et al. (1996) also reported high utilization of aluminum-bound phosphate (Al-P) by this plant. For its part, the peanut has a low response to P fertilizer, suggesting that it utilizes non-labile P accumulated in soils. Peanuts exude several organic acids from their roots (Ohwaki and Hirata, 1992), but these exudates are not fully related to solubilization of non-labile P (Otani and Ae, 1996). The 15–80% of total P in agro- cultural soils is present as organic P (Stevenson and Cole, 1999), the largest fraction being phytate (Dalal, 1977). Several studies have shown the significance of acid phosphatase exuded from roots for mineralizing organic P to allow its acquisition by plants (Tadano and Sakai, 1991; Gilbert et al., 1999).

The abilities of those root exudates to dissolve non-labile P have been examined in solution culture systems. However, little is known about the efficacy of these exudates in soil (Uren, 2001; Jones et al., 2003), where competition for the released P ions, either abiotically or biotically, is likely to be important. Even though the concentration of P ions in the rhizosphere could be increased by those agents, the effect might be negated when rhizosphere organisms and P-fixing cations immediately immobilized the released P ions. If such competition for the released P ions was serious in soil, the extraradical mycorrhizal hyphae would be essential for P acquisition from non-labile sources. For this purpose, we used a compartment system to control movement of root exudates as well as hyphal penetration into the soil amended with Al-P. Based on these three experiments, we aimed to clarify the contribution of mycorrhiza to P acquisition from non-labile sources.

2. Materials and methods

2.1. Experiment 1

A low-nutrient soil (an artificially aggregated subsoil, Humic Andosol, pH 6.0 in water-extraction) was used with only 0.04 mg available P (Truog-P) per kg dry soil. Plastic pots (15.8 cm diameter, 19.5 cm high) were filled with dry soil (2.3 kg per pot), and a mixture of three different non-labile phosphates was added. The amount per pot of the mixture consisted of 0.43 g Ca-phytate, 0.63 g FePO₄·4H₂O (Fe-P), and 0.34 g AlPO₄ (Al-P) (Katayama Chemical, Japan). Each P source contained 87 mg P, giving 114 mg P per kg dry soil. Nitrogen and potassium were also applied as (NH₄)₂SO₄ (130 mg N per kg dry soil) and KCl (180 mg K per kg dry soil). Simultaneously, half of the pots were inoculated with 20 g of a mycorrhizal fungal inoculum (Cerakinkong, Central Glass Co. Ltd., Japan) per pot, containing spores of Gigaspora margarita Becker & Hall. The other half received the same amount of inoculum sterilized in an autoclave. Filtered leachates of the inoculum (No.

6 filter paper, Toyo Roshi Co. Ltd., Japan) were then applied to each pot.

Seeds of peanuts (*Arachis hypogaea* (L.) cv. Tarapoto), pigeonpeas (*Cajanus cajan* (L.) Millsp., Snow Brand Seed. Co. Ltd., Japan), soybeans (*Glycine max* (L.) Merrill. cv. Okuharawase), and yellow lupins (*Lupinus luteus* (L.), Snow Brand Seed. Co. Ltd., Japan) were used in this experiment. The seeds were surface-sterilized with 1% sodium hypochlorite for 5 min and then rinsed with tap water. Three seeds were sown in each pot and thinned to one plant per pot a week later. Plants were grown in a growth chamber (30–25 °C, 16–8 h day–night regime, 60% relative humidity) under natural illumination. Nutrients other than N, P, and K were applied with 100 ml of Hoagland solution (Hoagland and Arnon, 1938). At 42 days after sowing, the plants were harvested.

2.2. Experiment 2

Although similar procedures to experiment 1 were adopted, the addition of P was different. The pots (15.8 cm diameter, 19.5 cm high) were filled with dry soil (2.3 kg per pot) containing one of the three P sources as follows: 1.29 g Ca-phytate or 1.89 g FePO$_4$·4H$_2$O or 1.02 g AlPO$_4$ (Katayama Chemical, Japan) (114 mg P per kg dry soil, respectively). Half of the pots received 20 g of the fungal inoculum as inoculation treatment, while the other half received the same amount of inoculum sterilized in an autoclave. Leachate of inoculum was again applied to the pots. Peanut (*Arachis hypogaea* (L.) cv. Tarapoto) and pigeonpea (*Cajanus cajan* (L.) Millsp.) seeds were used in this experiment. The seeds were surface-sterilized as above. Three seeds were sown in each pot and thinned to one plant per pot a week later. Plants were grown in a growth chamber (30–25 °C, 16–8 h day–night regime, 60% relative humidity) under natural illumination. At 49 days after sowing, the plants were harvested.

2.3. Experiment 3

Root boxes as shown in Fig. 1 were used. Each box was divided into two compartments with nylon mesh (50 μm openings) to restrict roots to one compartment (root compartment), but the mycorrhizal hyphae could enter the other compartment (hyphal compartment) across the mesh. In addition to the nylon mesh, half of the boxes had a stainless wire mesh (6 mm openings, 1.4 mm wide) to provide an air-gap between the compartments in order to restrict movement of root exudates into the hyphal compartment (Gap+), while the other half did not (Gap−). Each root box was filled with soil sterilized with an autoclave (121 °C, 60 min). In each root compartment, KNO$_3$ (2.5 g per container) and mycorrhizal inoculum (10 g per container) were mixed into the soil. For the soil in each hyphal compartment, AlPO$_4$ (0.7 g per container) was added. Filtered leachate of the inoculum was supplied to both compartments. Surface-sterilized seeds of pigeonpeas were sown into the root compartment of each root box and thinned to one plant. Plants were grown in a growth chamber (30–25 °C, 16–8 h day–night regime, 60% relative humidity) under natural illumination. For half of the boxes in both air-gap treatments, a hyphal-cut treatment was conducted by installing a thin plastic plate between the compartments at a 2–3 days interval (Cut+), while that was not done for the other half (Cut−). Tap water was supplied to both compartments in each box immediately after the cutting to retain field capacity. In this way, four combinations were established: Gap+Cut+, Gap+Cut−, Gap−Cut+, Gap−Cut−. All the plants were harvested at 77 days after sowing.

2.4. Determination of dry matter and P content

Harvested shoots were separated into stems and leaves which were dried at 80 °C for 48 h and weighed. The root systems were carefully washed free of soil with tap water. Each root system was divided into two parts of similar volume. One was maintained in FAA (formalin:acetic acid:70% ethanol = 5:5:90 in volume) to measure the root length and AM colonization. The other part was dried at 80 °C for 48 h and weighed. The dried leaves, stems, and roots were then ground. The P contents were determined colorimetrically by the phosphovanado-molybdate method (Hanson, 1950) once the plant materials had been digested by nitric acid and perchloric acid.

2.5. Measurement of root length and AM colonization

The length of the root samples stored in FAA was measured with a root length scanner (Commonwealth
Aircraft Co. Ltd., Australia). The roots were then cut into approximately 1 cm segments, which were collected and cleared in 10% KOH (Phillips and Hayman, 1970). They were then bleached by 10% H2O2 to ensure clearing and stained with 0.05% trypan blue or chlorazole black E (Brundrett et al., 1983) in lactoglycerol. The proportion of the total root length colonized was estimated using the grid intersect method (Giovannetti and Mosse, 1980).

2.6. Statistical analysis

Four replications were prepared per treatment for all experiments. The data were analyzed by two-way analysis of variance (ANOVA), in which the variation sources consisted of the four plant species and the mycorrhizal inoculation in experiment 1, the three P sources and the inoculation within each peanut and pigeonpea in experiment 2, and the air-gap and hyphal-cut treatments in experiment 3.

3. Results

3.1. Experiment 1

Inoculation enhanced the shoot dry weights by a factor of 5.4 for peanuts and by a factor of 9.0 for pigeonpeas, while in the soybean and lupin, there was no significant increase (Table 1). Inoculation also enhanced root dry weights of peanuts and pigeonpeas. Similar trends were observed for the total root length (Table 1). Inoculation resulted in extensive colonization of root lengths, ranging from 32 to 45%. Low colonization was found in the non-inoculated plants, due to the unsterilized soil that was used. In lupins, no mycorrhizal colonization was found even in inoculated plants. P uptake from the soil was calculated by subtracting the initial seed P content from the total P content of harvested plants. Inoculation significantly increased the P uptake, by approximately 6.4-, 12.5-, and 4.0-fold for peanuts, pigeonpeas and soybeans, respectively.
Table 1
Effects of inoculation with mycorrhizal fungus on shoot and root dry weight, total and mycorrhizal root length in 42-day-old legume plants grown in soil amended with a mixture of Fe-P, Al-P and Ca-phytate

<table>
<thead>
<tr>
<th>Plant</th>
<th>Shoot Dry weight (g per plant)</th>
<th>Length (m per plant)</th>
<th>P uptake (mg P per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Total root</td>
</tr>
<tr>
<td>Peanut</td>
<td>13.5 ± 1.0</td>
<td>3.6 ± 0.6</td>
<td>307 ± 97</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>2.5 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>126 ± 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeonpea</td>
<td>4.5 ± 1.5</td>
<td>1.7 ± 0.7</td>
<td>230 ± 84</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>41 ± 24</td>
</tr>
<tr>
<td>Soybean</td>
<td>4.4 ± 3.9</td>
<td>2.1 ± 2.0</td>
<td>197 ± 187</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>1.5 ± 1.0</td>
<td>0.6 ± 0.4</td>
<td>83 ± 56</td>
</tr>
<tr>
<td>Lupin</td>
<td>0.7 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>1.3 ± 0.5</td>
<td>0.5 ± 0.2</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>Sources of variation</td>
<td>Probability (using ANOVA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inoculation</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction</td>
<td>&lt;0.001</td>
<td>0.039</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of four replicates.

respectively (Table 1). Lupins showed no P content change due to inoculation.

3.2. Experiment 2

The growth of non-inoculated plants, particularly pigeonpeas, tended to be better in experiment 2 than in experiment 1, probably due to the longer growth period in experiment 2 at the exponential growth stage. In peanuts, inoculation increased the shoot dry weight with all the P sources and root dry weight with Al-P, but there was no significant difference between added P sources in either inoculated or non-inoculated plants (Table 2). The total root length significantly increased due to inoculation. Mycorrhizal colonization of inoculated roots ranged from 35 to 46%.

There was again some colonization in non-inoculated peanut roots. In pigeonpeas, the shoot and root dry weights increased greatly due to mycorrhizal inoculation, but there was no difference between added P sources in either inoculated or non-inoculated plants (Table 2). The total root length and mycorrhizal root length also significantly increased due to inoculation, and colonization ranged from 27 to 42% in inoculated plants and from 10 to 16% in non-inoculated plants.

Mycorrhizal response in P uptake for each of the sources differed between peanuts and pigeonpeas. P uptake was similarly high in inoculated peanuts among the three P sources, approximately 25 mg P per plant, whereas some variation was observed in non-inoculated ones according to the P source (Fig. 2). Non-inoculated peanuts showed high P uptake in soil amended with Ca-phytate, resulting in an insignificant difference between inoculated and non-inoculated plants. For Al-P or Fe-P, however, P uptake by non-inoculated peanuts was small and the inoculation increased P uptake significantly. In contrast, such a source-dependency of the mycorrhizal response in P uptake was not observed in pigeonpeas. P uptake by inoculated pigeonpeas was approximately 20 mg P per plant, while that by non-inoculated ones was around 5 mg per plant in any soils amended with the three P sources.

3.3. Experiment 3

The shoot dry weight was greater in Gap−Cut−, followed in descending order by Gap+Cut−,
Table 2
Effects of inoculation with mycorrhizal fungus on shoot and root dry weight, total and mycorrhizal root length in 49-day-old legume plants grown in soil amended with different sources of non-labile P

<table>
<thead>
<tr>
<th>P sources</th>
<th>Peanut</th>
<th>Pigeonpea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Ca-phytate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>11.8 ± 2.6</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>6.4 ± 2.1</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Fe-P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>12.7 ± 3.7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>4.9 ± 1.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Al-P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>13.8 ± 3.4</td>
<td>2.4 ± 1.0</td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>3.7 ± 0.7</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

Sources of variation Probability (using ANOVA)

<table>
<thead>
<tr>
<th>P sources</th>
<th>Gap</th>
<th>Cut</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-phytate</td>
<td>0.952</td>
<td>0.090</td>
<td>0.064</td>
</tr>
<tr>
<td>Fe-P</td>
<td>0.001</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Al-P</td>
<td>0.021</td>
<td>0.383</td>
<td>0.765</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of four replicates.

Fig. 2. P uptake by peanut and pigeonpea grown in soil amended with different sources of non-labile P (means ± S.D.).
1, without mycorrhizal inoculation, both peanuts and pigeonpeas showed poor P uptake similar to soybeans or lupins (Table 1), showing that effective P acquisition by peanuts and pigeonpeas as reported in solution culture systems does not occur in soil. Furthermore, mycorrhizal inoculation enhanced P acquisition 6-fold in peanuts and 10-fold in pigeonpeas, far more strongly than the 3-fold increase in soybeans. Thus, it was apparent that both peanuts and pigeonpeas are highly responsive to the inoculated fungus in regard to utilization of non-labile P.

In this study, unsterilized soil was used, so some mycorrhizal formation was also observed in non-inoculated plants (Tables 1 and 2). The indigenous mycorrhizal fungi in the soil differed from Gigaspora (Yano et al., 1998). Considering the low P uptake by non-inoculated plants with low levels of AM colonization (Tables 1 and 2), P transport through the indigenous fungi was obviously very limited, and inoculated Gigaspora margarita seemed to play a significant role.

There are some reports to suggest that AM fungal hyphae can solubilize Ca-P (Li et al., 1991; Yao et al., 2001), and Al-P (Yao et al., 2001), or mineralize organic P (Tarafdar and Marschner, 1994; Kusle and Kahir, 2000). If the hyphae alone strongly mobilized non-labile P, then P uptake responses due to inoculation would be similar between peanuts and pigeonpeas for any P source. However, we detected interspecific difference in the mycorrhizal response in experiment 2, particular for Ca-phytate (Fig. 2). Based on the interspecific differences, it is assumed that some root factor, including mycorrhizosphere effects (Toro et al., 1998; Vázquez et al., 2000; Barea et al., 2002), was significant for plant P acquisition from non-labile sources.

In contrast to AM fungal hyphae, there is considerable evidence that plant roots can dissolve non-labile P sources with root exudates such as proton (Grinsted et al., 1982; Zhu et al., 2002), organic acids (Gardner et al., 1983; Lipton et al., 1987; Hoffland et al., 1989; Ae et al., 1990; Ohwaki and Honda, 1992; Johnson et al., 1994; Otani et al., 1996; Gaume et al., 2001; Shen et al., 2002), and acid phosphatase (Tadano and Sakai, 1991; Wasaki et al., 1997; Gilbert et al., 1999; Richardson et al., 2000). However, the efficacy of those P-solubilizing agents has been rarely examined in soil. López-Bucio et al. (2000) used a P-poor, sterilized soil, and demonstrated that a transgenic citrate-overproducing tobacco can grow and reproduce while the wild type fails to reproduce. This study reveals the efficacy of exuded citrate in soil but in the absence of either competition for the released P ions or degradation of the exuded citrate by soil microbes, the plants could fully acquire P without such constraints. In that report, the effect of mycorrhizal inoculation was also examined, but the enhancing response was smaller compared to our results. Probably due to a lack of constraints under sterilized conditions, mycorrhizal interaction to overcome the constraints might not be required.

The importance of overcoming such constraints seems to be supported by our data in experiment 3. Pigeonpeas did not seem to acquire P from the hyphal compartment with the rhizosphere effects alone without the extraradical AM hyphae (Gap→Cut+), considering the similar P uptake to that without the effects (Gap+Cut+); nevertheless, greater P uptake and a significant interaction between the rhizosphere and the extraradical hyphae was observed when both effects were present (Gap→Cut→). This indicates that P uptake in pigeonpeas became greater only when both intact extraradical hyphae and the root exudates were allowed to penetrate the hyphal compartment amended with Al-P. While we could not confirm that all the increase in P uptake was derived from Al-P without using P-labelling techniques, we have assumed that (1) the hyphal capture of P solubilized by the exudates is significant, otherwise (2) the presence of the extraradical hyphae stimulates soil microorganisms with phosphate-solubilizing capacity (Toro et al., 1997, 1998; Barea et al., 2002).

The results of experiment 2 revealed an interesting phenomenon in Ca-phytate utilization by peanuts. While mycorrhizal responses in pigeonpeas to all three P sources were significant to a similar extent, significant responses in peanuts were found for Al-P and Fe-P but not for Ca-phytate (Fig. 2, Table 2). Previously, Ae et al. (1996) found the unique phenomenon that root cell walls of peanuts have a strong ability to solubilize Al-P or Fe-P (Ca-phytate was not examined), but this was not found in pigeonpeas. This implies that peanut has a reaction site solubilizing Al-P or Fe-P at the rhizoplane (cell wall on the root.
surface); suggesting the need for a lower contribu-
tion of the extraradical hyphae of AM fungi. Our re-
results, however, do not support this since AM fungal
inoculation increased acquisition of P from Al-P and
Fe-P (Fig. 2). In contrast, the P-solubilizing site of
pea and peanut. This work was supported by a Grant-in-Aid for Science
making the rhizosphere (several mm-width from the rhizoplane), where AM hyphae
can contribute to soil exploration and exploitation;
thus significant enhancement in P uptake would ap-
pear similarly for all the three P sources. However, the
root cell wall of peanuts must have an efficient mech-
anism for acquiring P from Ca-phytate since there
was no significant increase in P uptake by inocu-
lated peanuts over their non-inoculated counterparts
(Fig. 2). As far as we know, it is a novel finding that
the peanut has the ability to utilize Ca-phytate quite
effectively.

In conclusion, we found that mycorrhizal inocula-
tion with Gigaspora margarita increases the utiliza-
tion of non-labile P sources, but the extent depends
on the host plants as well as the P sources. Peanuts and
pea and peanuts, which are reported to be plants that
utilize sparingly soluble P sources effectively, demon-
strated high mycorrhizal responses in P uptake and
growth. For a plausible mechanism of the accelerated
P acquisition, we have assumed that an interaction
effect between the roots and hyphae is involved in
the accelerated P acquisition; the roots and/or soil
microorganisms associating with the extraradical hy-
phae dissolved non-labile P and then the released P
can be captured by the hyphae more efficiently. Fur-
thermore, we found that non-inoculated peanuts were
superior in acquiring P from Ca-phytate with a lower
mycorrhizal response, suggesting that an unknown
mechanism on-root is involved.

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