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Factors affecting arbuscular mycorrhizal dependency of wheat genotypes with different phosphorus efficiencies

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Mobilization of sparingly soluble inorganic phosphates by the external mycelium of an arbuscular mycorrhizal fungus

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Abstract

Red clover (*Trifolium pratense* L.) and *Glomus versiforme* (Karsten) Berch growing in rhizoboxes were employed in two glasshouse experiments to study the mobilization of sparingly soluble phosphates by arbuscular mycorrhizal fungal (AMF) mycelium. In one experiment, four inorganic sources of phosphate, $\text{CaH}_2(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ ($\text{Ca}_2\text{-P}$), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ($\text{Ca}_8\text{-P}$), $\text{Ca}_{10}(\text{PO}_4)_6 \cdot \text{F}_2$ ($\text{Ca}_{10}\text{-P}$) and $\text{AlPO}_4 \cdot n\text{H}_2\text{O}$ (Al-P), were chemically synthesized, labelled with ^{32}P in an atomic pile and applied to the hyphal compartments of the rhizoboxes. Shoot yield, ^{32}P and total P uptake were measured in clover growing in the root compartments. A similar experiment was conducted simultaneously using the same phosphate sources unlabelled and clover mycorrhizal infection and soil pH were determined. Although AMF inoculation increased the P uptake and biomass of clover shoots, the contribution of AMF to shoot P uptake and biomass varied with phosphate source, and was greatest with $\text{Ca}_2\text{-P}$ and least with $\text{Ca}_{10}\text{-P}$. ^{32}P measurements indicated that external hyphae could mobilize $\text{Ca}_2\text{-P}$, $\text{Ca}_8\text{-P}$ and Al-P , but not $\text{Ca}_{10}\text{-P}$. This indicates that AMF not only mobilize the same types of phosphates that plants mobilize under stress conditions of low P, but give increased contact with phosphates in the soil compared with non-mycorrhizal root systems.

Introduction

Although chemical fertilizers have greatly increased crop production in the last few decades, there have also been undesirable consequences of fertilizer use, especially the accumulation in intensively managed soils and transfer to surface waters of excessive amounts of nutrients such as nitrogen (N) and phosphorus (P). The development of sustainable agricultural systems will require new techniques that help to minimize fertilizer application rates while maintaining adequate crop yields. The application of biological resources to explore soil nutrients is currently an active field of research that may hold promise for the future (Baligar et al., 1990; Jeffries and Barea, 1994). Breeding new plant varieties that are tolerant to low nutrient conditions is one useful technique (Li et al., 1995).

Utilizing soil micro-organisms to increase nutrient uptake by plants may represent another potential approach, for example symbiotic nitrogen fixing bacteria contribute to the N nutrition of legumes and arbuscular mycorrhizal fungi can increase plant P uptake under conditions of low P availability (Li et al., 1991a).

It is well established that arbuscular mycorrhiza can greatly enhance the uptake of relatively immobile soil nutrients, especially P, by plant root systems (Bolan, 1991). Consequently, colonization of the roots by AMF can increase plant growth, with a more pronounced effect in low fertility soils or soils with patchy nutrient distribution. Several mechanisms may underlie the mycorrhizal effect on P uptake, including (i) exploitation of a larger soil volume and thus greater P uptake by the extensive external hyphae (Li et al., 1991b), (ii) different kinetics of uptake by mycorrhizal roots compared with non-mycorrhizal roots (Cress et al., 1979), (iii) solubilization of organic P by phos-

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phatase enzymes derived from hyphae (Ezawa et al., 1995), and (iv) mobilization of sparingly soluble inorganic P by AMF (Bolan, 1991). Although there have been numerous reports presenting evidence to support some of these mechanisms, experiments have provided little evidence for the fourth mechanism. Murdoch et al. (1967) demonstrated that mycorrhizal maize plants responded to application of tricalcium phosphate while non-mycorrhizal plants showed no response. Wibawa et al. (1995) observed similar results using cocoa and palm plants with application of rock phosphate. An experiment by Bolan et al. (1984) employing ^{32}P labelling techniques demonstrated that mycorrhizal subterranean clover could mobilize iron phosphate that non-mycorrhizal plants could not take up, but the mycorrhizal and non-mycorrhizal plants showed no difference in specific activity. Results showing no differences in labelling do not eliminate the possibility that mycorrhizal plants take up phosphate that is unavailable to non-mycorrhizal plants.

In previous work in our laboratory, red clover was grown in three-compartment rhizoboxes with *Glomus mosseae*, and external hyphae were separated from the root system with 30- μm -pore size nylon mesh (Yao et al., 2000). Fertilizer $\text{KH}_2^{32}\text{PO}_4$ was applied to the hyphal compartments at the rate of 5 mg kg $^{-1}$ together with one of five inorganic sources of phosphate and there was a control with no P addition. P in the plants could be divided into three origins: From the soil, from the fertilizer and from the inorganic P sources. The proportion of total plant P derived from the fertilizer was calculated as the specific activity of ^{32}P in the plant divided by that in the original fertilizer, and the proportions from soil and the various inorganic P sources were also calculated as described by Yao et al. (2000). This procedure was described as 'indirect' labelling because only the spike of KH_2PO_4 was labelled, not the various other inorganic P sources. The results indicated that the external hyphae mobilized and took up P from $\text{Ca}_2\text{-P}$, $\text{Ca}_8\text{-P}$, Al-P and Fe-P , but not from $\text{Ca}_{10}\text{-P}$. The contribution of the various P sources to clover P uptake was calculated to be in the order of $\text{Ca}_2\text{-P} > \text{Ca}_8\text{-P} = \text{Al-P} > \text{Fe-P}$.

The present investigation was designed to provide more reliable evidence to test the hypothesis that AMF can mobilize sparingly soluble inorganic phosphates by using direct ^{32}P labelling of the inorganic P sources in an atomic pile before application to the hyphal compartments. The same inorganic P sources were investigated as before, except for $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ (Fe-P), which was not included because interference from

the radioactive isotope of Fe could not be eliminated. Both of the glasshouse experiments involved the synthesis of pure inorganic phosphates and the physical separation of plant roots and associated AMF external hyphae, and one experiment employed ^{32}P labelling techniques.

Materials and methods

Preparation of ^{32}P -labelled phosphates

The inorganic phosphates $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ($\text{Ca}_2\text{-P}$), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ($\text{Ca}_8\text{-P}$), $\text{Ca}_{10}(\text{PO}_4)_6 \cdot \text{F}_2$ ($\text{Ca}_{10}\text{-P}$) and $\text{AlPO}_4 \cdot n\text{H}_2\text{O}$ (Al-P) were obtained from the Chinese Academy of Sciences Institute of Soil Science, Nanjing 210008, China. Portions were placed in an atomic pile for direct radiation with a radiation flux of $1 \times 10^{13} \text{ N cm}^{-2} \text{ s}^{-1} \text{ kW}^{-1}$ for 30 h (Guo et al., 1993) at the Chinese Institute of Atomic Energy in Beijing. This provided ^{32}P -labelled samples of the four inorganic phosphates with a specific radioactivity of $2.08 \times 10^4 \text{ Bq mg}^{-1} \text{ P}$.

Plant growth

Red clover (*Trifolium pratense* L.) was grown in three-compartment rhizoboxes in symbiosis with the AMF *Glomus versiforme*. Fungal inoculum was obtained from Professor Honggang Wang, Institute of Soils and Fertilizers, Chinese Academy of Agricultural Sciences, 30 Baishiqiao Road, Haidian District, Beijing 100081. A mixture of spores, infected root fragments and soil substrate was used as inoculum. Each PlexiglasTM rhizobox comprised a 2-cm-wide root compartment for plant growth, a 3-cm-wide central separation compartment to ensure that root growth did not extend to the hyphal compartment, and a 2-cm-wide hyphal compartment. The length and height of each compartment were 8 cm (Figure 1). The three compartments were separated from each other by a nylon net of 30 μm pore size to prevent root penetration but allow hyphal penetration.

Soil in the root and hyphal compartments was a neutral sandy soil with Olsen-P of 12.3 mg kg $^{-1}$ and pH (in H_2O) of 6.7 and that in the separation compartments was a neutral clay soil with Olsen-P of 3.6 mg kg $^{-1}$ and pH (H_2O) of 6.5. The soils were autoclaved at 121 °C for 2 h and amended with 200 mg N kg $^{-1}$ as NH_4NO_3 and 200 mg K kg $^{-1}$ as K_2SO_4 . Forty mg P kg $^{-1}$ as KH_2PO_4 were applied to each root compartment and one of the four inorganic

Figure 1. Diagram showing the design of the rhizoboxes.

sources of P was applied to the hyphal compartment at the rate of 400 mg P kg^{-1} . Controls with no P applied to the hyphal compartment were included. In one experiment, the inorganic phosphates applied to the hyphal compartments were ^{32}P -labelled and there were five replicates of each treatment. In the other experiment, unlabelled phosphates were applied to the hyphal compartments and there were four replicates of each treatment. Fifty clover seeds were sown evenly in the root compartment of each rhizobox. Mycorrhizal and non-mycorrhizal rhizoboxes of all treatments were established. The root compartments of the mycorrhizal pots were inoculated with 15 g of inoculum placed about 0.5 cm under the clover seeds. The rhizoboxes were arranged in five or four blocks with one replicate of each treatment fully randomized within each block. The water content of the rhizoboxes was adjusted regularly by weight. Water was added to the central separation compartment to minimize movement of P from the hyphal compartment to the root compartment by mass flow or diffusion. The plants grew in an unheated glasshouse at China Agricultural University in Beijing without supplementary illumination for 8 weeks from 26 April to 21 June 1999.

Harvest and analysis

Clover shoots were removed at soil level and oven dried (70°C for 48 h) for yield determination. Radioactivity of ^{32}P in the shoots from the labelling

experiment was measured using a low background Model BH1216 scintillation counter. None of the various Ca nuclides generated in the atomic pile (with the sole exception of ^{45}Ca) would be expected to affect the measurement of ^{32}P . An aluminium sheet was used as a filter covering the sample during measurement to eliminate interference by ^{45}Ca (Guo et al., 1993). Shoot dry weights were recorded. Samples of ground oven-dried shoots were dry ashed at 560°C , dissolved in HCl and analysed for nutrients by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The contribution of mycorrhiza to shoot biomass and P uptake was calculated by subtracting the non-mycorrhizal from the mycorrhizal value and expressing the result as a percentage of the mycorrhizal value. Root samples from the unlabelled experiment were stained by the method of Phillips and Hayman (1970) and infection rate was examined using the standard grid-line intersection method of Giovannetti and Mosse (1980). Fifty 2-cm-long root segments were examined microscopically and 200–300 intersections were recorded from each pot. After harvest, soil in the hyphal compartments of the unlabelled experiment was cut into four 0.5-cm-thick portions, the centres of which were distances of 0.25, 0.75, 1.25 and 1.75 mm from the nylon net adjacent to each separation compartment. The pH (in H_2O) of each soil portion was measured using a Model PHS-3TC pH meter. Data are presented as mean values after testing

Table 1. Mean proportion of clover root length colonized by mycorrhizal fungi, shoot biomass, and apparent contribution of mycorrhiza to shoot biomass

Treatment	Infection rate (% of root length infected)	Shoot biomass (g DM pot ⁻¹)		Mycorrhizal contribution to Shoot biomass (%)
		Mycorrhizal	Non-mycorrhizal	
Control	58.5	0.55	0.68	19.1
Ca ₂ -P	64.4	0.54	1.27	57.5
Ca ₈ -P	53.3	0.48	1.19	59.7
Ca ₁₀ -P	52.6	0.54	0.77	29.9
Al-P	54.2	0.59	1.15	48.7
ANOVA				
Source of variation:				
Mycorrhiza	ND ^a		<i>p</i> <0.001	ND
P type	NS ^b		<i>p</i> <0.001	ND
Interaction	ND		<i>p</i> <0.001	ND

^aND, not determined.

^bNS, not significant.

by analysis of variance (ANOVA) using the GENSTAT package (GENSTAT Committee, 1993).

Results

Shoot yields

Plants were well colonized in the inoculated treatments and no colonization was observed in any of the non-mycorrhizal treatments. Although the proportion of root length infected ranged from 53 to 64%, there was no significant difference among the five phosphate treatments (Table 1). Colonization by AMF increased plant shoot biomass, with the highest rise in the Ca₂-P treatment from 0.54 g to 1.27 g pot⁻¹, and the lowest rise in the control from 0.55 to 0.68 g pot⁻¹. The contribution of AMF to shoot biomass differed among the phosphate sources. The greatest contribution was in soil amended with C₈-P and least in unamended soil (Table 1).

Contribution of AMF to plant P nutrition

Promotion of plant growth by AMF resulted mainly from an increase in P uptake (Table 2). Shoot P concentrations in non-mycorrhizal plants were similar for different P sources (range 0.70–0.72 mg kg⁻¹) while those in mycorrhizal plants varied from 0.75 to 0.85 mg kg⁻¹ (Table 2). AMF inoculation enhanced shoot P concentrations, and as a consequence shoot P uptake also increased. The contribution of AMF to

shoot P uptake was greatest in soil amended with C₂-P and C₈-P and least in unamended soil (Table 2).

³²P uptake by external hyphae

Shoot ³²P activities were calculated on the assumption that shoot ³²P activity in the control treatment was 0 (Table 3). Non-mycorrhizal shoots had values of only up to 31 cpm pot⁻¹, while mycorrhizal plant shoots had much higher activities with the sole exception of the Ca₁₀-P treatment. When shoot ³²P uptake was expressed as a percentage of shoot total P uptake (Table 4), non-mycorrhizal plants had absorbed almost no ³²P from the hyphal compartment, accounting for only 0.24–1.15% of total P uptake in the shoots. In contrast, the values for mycorrhizal plants ranged from 13 to 18% with the sole exception of the Ca₁₀-P treatment in which there was no increase in mycorrhizal plants (Tables 3 and 4). Both mycorrhizal and non-mycorrhizal plants in the Ca₁₀-P treatment absorbed very little ³²P, indicating that the external hyphae could not mobilize P from Ca₁₀-P, but could increase the mobilization of P from Ca₂-P, Ca₈-P and Al-P compared with non-mycorrhizal plants.

Effect of external hyphae on soil pH

Soil pH in the hyphal compartments was consistently lower in the inoculated treatments than in non-mycorrhizal rhizoboxes (data not shown). Soil pH did not change significantly with distance from the separation compartment.

Table 2. Influence of mycorrhizal colonization on mean clover shoot P concentration and shoot P uptake and apparent contribution of mycorrhiza to shoot P uptake

P type	Shoot P concentration (mg g ⁻¹)		Shoot P uptake (mg pot ⁻¹)		Mycorrhizal contribution to shoot P uptake (%)
	Non- mycorrhizal	Mycorrhizal	Non- mycorrhizal	Mycorrhizal	
Control	0.71	0.75	0.39	0.51	23.5
Ca ₂ -P	0.71	0.85	0.39	1.08	63.9
Ca ₈ -P	0.72	0.82	0.35	0.97	63.9
Ca ₁₀ -P	0.70	0.77	0.38	0.60	36.7
Al-P	0.71	0.82	0.42	0.94	55.3
ANOVA					
Source of variation:					
AMF	<i>p</i> <0.001		<i>p</i> <0.001		ND
P type	<i>p</i> <0.05		<i>p</i> <0.001		ND
Interaction	NS		<i>p</i> <0.001		ND

Table 3. Mean clover shoot ³²P activity and ³²P uptake

P type	Shoot ³² P activity (cpm pot ⁻¹)		Shoot ³² P uptake (mg pot ⁻¹)	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Ca ₂ -P	18	1159	0.0036	0.1967
Ca ₈ -P	15	675	0.0030	0.1336
Ca ₁₀ -P	31	23	0.0052	0.0048
Al-P	30	747	0.0050	0.1273
ANOVA				
Source of variation:				
Mycorrhiza	<i>p</i> <0.001		<i>p</i> <0.001	
P type	<i>p</i> <0.001		<i>p</i> <0.001	
Interaction	<i>p</i> <0.001		<i>p</i> <0.001	

Table 4. Mean clover shoot ³²P uptake expressed as a proportion of total P uptake

P type	Shoot ³² P uptake as a percentage of shoot total P uptake (%)	
	Non-mycorrhizal	Mycorrhizal
Ca ₂ -P	0.24	18.45
Ca ₈ -P	0.85	13.22
Ca ₁₀ -P	0.70	0.81
Al-P	1.15	14.01
ANOVA		
Source of variation:		
AMF	<i>p</i> <0.001	
P type	<i>p</i> <0.001	
Interaction	<i>p</i> <0.001	

Discussion

It is well documented that AMF can increase P uptake by host plants (Smith and Read, 1997). In the present experiments, clover shoot biomass increased because of an increase in P uptake following inoculation with *G. versiforme*. We found that the apparent contribution of AMF to plant P uptake was higher than that of AMF to shoot biomass. This may be the result of increased plant P concentration because the P utilization rate of plants usually decreases with increased P concentration (Baon et al., 1993).

In the present experiments, non-mycorrhizal clover plants absorbed P only from the soil and from P fertilizer in the root compartments through their root systems while mycorrhizal clover absorbed additional P from the hyphal compartments through the external hyphae of *G. versiforme*. In the control treatment, both shoot P concentration and shoot P uptake of mycorrhizal plants were higher than those of non-mycorrhizal plants, with a difference in uptake of 0.13 mg P, which was extracted from soil in the three rhizobox compartments by the external hyphae. Shoot P uptake rates of mycorrhizal plants supplied with $\text{Ca}_2\text{-P}$, $\text{Ca}_8\text{-P}$ and Al-P (but not $\text{Ca}_{10}\text{-P}$) were much higher than those of non-mycorrhizal plants. If the differences in P uptake between the four phosphate sources and the control treatment is regarded as equivalent to hyphal P uptake from added phosphate, then the P uptake values may have been overestimated. The reason is that enhanced P nutrition in the mycorrhizal plants would have increased the growth of their root systems and hence the external mycelium, and more P would have been absorbed from soil and P fertilizer in the root compartments. The data obtained from the ^{32}P activity measurements were much lower than those obtained by subtraction. The results based on ^{32}P activity indicate that the external mycelium mobilized the different sources of inorganic phosphates in the order: $\text{Ca}_2\text{-P} > \text{Ca}_8\text{-P} = \text{Al-P}$, but did not mobilize $\text{Ca}_{10}\text{-P}$. This sequence is in good agreement with our previously reported results derived from 'indirect' ^{32}P labelling (Yao et al., 2000). Taken together, the data from these experiments suggest that AMF may have access to only those sources of phosphate which plant root systems may also mobilize. However, the external hyphae may offer the advantage of increased contact with phosphates due to the additional soil volume explored by the hyphae. Consequently, AMF may contribute greatly to plant P nutrition even when supplied

with only sparingly soluble inorganic phosphates as sources of P.

It could be argued that the different sources of P may have had different effects on hyphal development in the hyphal compartments and thus the uptake of P could be related to the amount or length of hyphae present, irrespective of the solubility of the form of P supplied. Unfortunately, root and hyphal lengths were not determined. Jakobsen et al. (1992) showed that different AMF species could supply different amounts of P to the host plant and these differences were related to different amounts of external mycelium produced by the AMF species. However, it is likely that in our study the solubilities of the different P sources were more important than hyphal development because the differences in shoot ^{32}P uptake and in the contribution of AMF to shoot ^{32}P uptake were very large (Tables 3 and 4). We could not measure the activity of ^{32}P in the roots or hyphae because of the large ^{32}P activity in the soil of the hyphal compartments from inorganic P residues and in the root compartments from root exudates. If we could have detected ^{32}P activity in roots and hyphae, this would have given us unequivocal evidence of fungal mobilization of P from the inorganic sources.

There is a wealth of information in the literature regarding the mechanisms underlying the mobilization of sparingly soluble phosphates by mycorrhizal hyphae. The most important mechanism involves the excretion of H^+ following the utilization of NH_4^+ by hyphae (Smith et al., 1985). This leads to a decrease in soil pH. Li et al. (1991c) reported that the pH in the hyphosphere was lowered by up to 0.6 units when mycorrhizal clover plants were supplied with $(\text{NH}_4)_2\text{SO}_4$. In the present experiments, the soil pH in the hyphal compartments was lowered by 0.2–0.5 units when the plants were supplied with NH_4NO_3 . Among the four inorganic phosphates examined, the soil pH in the $\text{Ca}_2\text{-P}$ treatment decreased the least while that in $\text{Ca}_{10}\text{-P}$ treatment was lowered the most. This may have been due to the lack of mobilization of the $\text{Ca}_{10}\text{-P}$ by AMF despite its higher Ca^{2+} content (which might buffer soil pH if it were released into the soil solution) compared with the $\text{Ca}_2\text{-P}$. Another mechanism that could be involved in the mobilization of phosphates is the exudation of hydroxyacids that chelate metal ions (Parfitt, 1979), but this would need to be verified by additional experiments. It is unlikely that the pH effects that we observed were caused solely by the mycorrhizal inoculum because the soil used for inoculum production had a pH (in

water) of 8.1 and the soil pH in the rhizoboxes was 6.5–6.7. Furthermore, the non-mycorrhizal treatments received sterilized inoculum. In conclusion, our results indicate that AMF mobilized the same types of phosphate as non-mycorrhizal plants under conditions of low P availability, but gave increased contact with phosphates in the soil and may also have solubilized more P by producing more acidic conditions compared with non-mycorrhizal root systems.

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