Influence of extramatrical hyphae on mycorrhizal dependency of wheat genotypes


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FACTORS AFFECTING ARBUSCULAR 
MYCORRHIZAL DEPENDENCY OF 
WHEAT GENOTYPES WITH DIFFERENT 
PHOSPHORUS EFFICIENCIES

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ABSTRACT

Three wheat (Triticum aestivum L.) genotypes with high, inter-
mediate and low phosphorus (P) efficiency were grown in a pot 
experiment with low P supply and adequate P supply either 
inoculated with the arbuscular mycorrhizal fungus Glomus 
versiforme or uninoculated. The mycorrhizal dependency of the 
genotype with relatively high P efficiency was lower than that 
of the genotypes with lower P efficiencies. Linear correlation 
analysis revealed that mycorrhizal dependency was primarily 
controlled by P uptake efficiency. More carbohydrate was trans-
located to the roots of genotypes with low P efficiency than of 
those with high P efficiency. In a second pot experiment the 
same three genotypes were grown in low P soils. Higher hyphal 
length density arising from carbohydrate translocation led to 
more P uptake, and this may account for higher mycorrhizal 
dependency.
INTRODUCTION

It is well established that host plants can benefit from colonization by arbuscular mycorrhizal fungi, and inoculation with these fungi can significantly increase host plant growth (1). In fact, the degree to which host plants of different species benefit is highly variable. To describe and quantify the degree of host plant growth stimulation, the term ‘mycorrhizal dependency’ was defined by Gerdemann (2) and the formula for calculating this was subsequently presented by Menge et al. (3). The mycorrhizal dependencies of many plants were investigated, and the values were found to range from 0 to 99% (4). The factors affecting mycorrhizal dependency were also evaluated in order to understand the mechanism responsible for this highly variable attribute. Root architecture and root hair characteristics of host plants were considered to be the most important fundamental factors affecting mycorrhizal dependency (5,6). Plants with magnolioid roots are the most dependent on arbuscular mycorrhizae, while plants with graminoid roots are the least dependent (7). The assimilation rate of CO₂, transpiration rate and growth rate of host plants are also reported to be related to mycorrhizal dependency (8).

In most cases the benefit to the host plant from the arbuscular mycorrhizal association is derived from an increase in P uptake via the mycorrhizal fungi, especially in conditions of low available soil P. Plant P efficiency might therefore be expected to be closely related to mycorrhizal dependency. Phosphorus utilization efficiency and P uptake efficiency are the two components of plant P efficiency. Graham and Syvertsen (8) found that P utilization efficiency had a negative effect on the mycorrhizal dependency of citrus plants while other workers have reported a positive influence of P utilization efficiency on mycorrhizal dependency (9). This inconsistency may be the result of reduced influence of arbuscular mycorrhizae on P utilization efficiency of the host plant. It is well known that the main effect of inoculation with arbuscular mycorrhizal fungi is to improve the capacity of the host plant to take up P from the soil. It would therefore be logical to suppose that plant P uptake efficiency might be closely related to mycorrhizal dependency.

In many experiments designed to study mycorrhizal dependency, numerous different plant species have been selected as test plants. However, different genotypes of the same species with different P efficiencies are more likely to reveal the relationship between P efficiency and mycorrhizal dependency. We therefore selected three wheat genotypes for our experiments. This paper reports two experiments designed to test the hypothesis that P uptake efficiency is the fundamental determinant of mycorrhizal dependency, and further to elucidate the effects of arbuscular mycorrhizal fungi on host plant P uptake efficiency.
MATERIALS AND METHODS

Plant Growth

Three wheat (*Triticum aestivum* L.) cultivars, namely 81(85), Fengxiao 8, and NC37 with high, moderate, and low P efficiency, respectively (based on relative yield), were selected. Inoculum of the arbuscular mycorrhizal fungus *Glomus versiforme* was produced in standard pot culture using red clover (*Trifolium pratense* L.) as host plant. Wheat seeds were surface sterilized with 10% (v/v) H₂O₂ followed by washing with tap water. After germination in the dark on moist filter paper at 28°C to the stage of embryo emergence, seeds were grown in pots as described below. A calcareous Luvisol soil collected from the demonstration farm of China Agricultural University, Beijing District, with a low Olsen-P of 3.4 mg kg⁻¹ and pH (in water) of 8.1 was used as the growth medium. Basal fertilizers were applied at rates of 200 mg nitrogen (N), 20 mg phosphorus (P), and 200 mg potassium (K) kg⁻¹ soil. Conventional plastic pots were used in Experiment 1. Each pot contained 1.5 kg autoclaved (121°C, 1.1 kg cm⁻²) 1:1 v/v soil:sand mixture. Additional P was added to some pots to bring the soil status up to an adequate level of 100 mg P kg⁻¹ (with other factors kept constant) so that the P efficiency of each genotype could be calculated. The aim of this experiment was to determine the factors responsible for differences in mycorrhizal dependency among the genotypes. In the second experiment three-compartment rhizoboxes were used, with a central root compartment containing 150 g soil separated by a 30 μm pore size nylon mesh from the two outer hyphal compartments, each containing 240 g soil. The plants were grown in low P conditions only, and the experiment was designed to test the hypothesis that there were differences in hyphal density and P uptake among the genotypes that resulted in different mycorrhizal dependencies.

Mycorrhizal infection was established by mixing 20 g or 15 g fresh inoculum with the soil under the seeds. Non-mycorrhizal controls were set up using autoclaved inoculum together with 10 ml of a water filtrate of the inoculum to introduce a similar general microbial population to that in the inoculated pots. At the 2–3 leaf stage of growth, ten seedlings were left in each container in Experiment 1 and seven in Experiment 2. The plants were grown in a glasshouse in a randomized block design with four replicate pots per treatment.

Harvest and Analysis

The plants were harvested after eight weeks of growth. Shoots and roots were separated and the oven dry weights (70°C for 48 h) were recorded. Samples of ground plant material were dry ashed at 560°C, dissolved in HCl and analyzed.
for a range of nutrient elements by inductively coupled plasma-atomic emission spectroscopy. Roots were carefully washed and cut into bundles about 2 cm long for staining with Trypan blue using the method of Phillips and Hayman (10). The proportion of root length infected was determined by the grid-line intersection method (11). In Experiment 1 the shoots were cut and inserted into bottles containing 10 ml EDTA-Na₂ to sample the phloem sap (12). The amount of sugar translocated from shoot to root in the sap was determined by the method of Van Handel (13). In Experiment 2 the hyphae in the hyphal compartments were recovered by a flotation and decanting technique on to a screen. Hyphal length was determined under the microscope by the grid-line intersection method and hyphal density was calculated according to Frey and Schüepp (14).

Statistical Analysis

Data were subjected to analysis of variance using the SAS™ software (15). Pairs of treatment means were compared by calculation of least significant difference (LSD) at the 5% level. Mycorrhizal dependency was compared with P efficiency indices using linear correlation analysis.

RESULTS

Plant Growth Response

No root colonization by mycorrhizal fungi was observed in the non-mycorrhizal controls. The proportion of root length infected in inoculated plants in Experiment 1 ranged from 50 to 54% and there was no significant difference in infection rate among the three wheat genotypes (Table 1). However, the three

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Infection Rate, %</th>
<th>Biomass, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Mycorrhizal</td>
<td>Mycorrhizal</td>
</tr>
<tr>
<td>81(85)</td>
<td>51.4 a b</td>
<td>8.10 a</td>
</tr>
<tr>
<td>Fengxiao 8</td>
<td>49.8 a</td>
<td>5.63 b</td>
</tr>
<tr>
<td>NC37</td>
<td>53.7 a</td>
<td>6.15 ab</td>
</tr>
</tbody>
</table>

*P < 0.05; NS, not significant.

*aSignificance (by ANOVA) of difference between mycorrhizal and non-mycorrhizal plants.

*bWithin columns, means with the same letter are not significantly different by LSDₐ = 0.05.
ARBUSCULAR MYCORRHIZAL DEPENDENCY

Genotypes showed different growth responses. Genotype 81(85) had a higher yield than Fengxiao 8 or NC37 when uninoculated, but the biomass of all three genotypes was the same when they were mycorrhizal. This was because genotypes Fengxiao 8 and NC37 responded to mycorrhizal inoculation with a significant increase in yield but cultivar 81(85) showed no response. The mycorrhizal dependency of Fengxiao 8 was consequently higher than that of 81(85), and NC37 was intermediate.

Plant P Nutrition and P Efficiency

At low P supply in Experiment 1, mycorrhizal inoculation improved the P status of genotypes 81(85) and Fengxiao 8 (Table 2). Plant P concentration ranged from 0.7 to 1.1 mg kg\(^{-1}\) and did not differ among the genotypes either when they were mycorrhizal or when non-mycorrhizal. There was therefore no difference in P utilization efficiency among the genotypes. However, mycorrhizal inoculation decreased the P utilization rate of 81(85) and Fengxiao 8. In contrast, P uptake rate differed among the genotypes, with 81(85) showing the highest P uptake rate, Fengxiao 8 the lowest, and NC37 having an intermediate uptake rate (Table 2). Although mycorrhizal inoculation increased the P uptake rate of all three genotypes, the differences among the genotypes still occurred after mycorrhizal colonization.

Phosphorus efficiency was calculated as the ratio of biomass at low P supply to biomass at adequate P supply. This expression can be used to represent the ability of the plants to take up and utilize P at low P supply, and the results

<table>
<thead>
<tr>
<th>Genotype</th>
<th>P Concentration, mg g(^{-1})</th>
<th>P Utilization Rate, g mg(^{-1})</th>
<th>P Uptake Rate, mg P g(^{-1}) Root (Dry Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Mycorrhizal</td>
<td>Mycorrhizal Sig.(^a)</td>
<td>Non-Mycorrhizal</td>
</tr>
<tr>
<td>81(85)</td>
<td>0.77 a(^b)</td>
<td>1.03 a *</td>
<td>1.26 a</td>
</tr>
<tr>
<td>Fengxiao 8</td>
<td>0.70 a</td>
<td>0.98 a *</td>
<td>1.56 a</td>
</tr>
<tr>
<td>NC37</td>
<td>0.91 a</td>
<td>1.06 a NS</td>
<td>1.10 a</td>
</tr>
</tbody>
</table>

\(^a\)Significance (by ANOVA) of difference between mycorrhizal and non-mycorrhizal plants.
\(^b\)Within columns, means with the same letter are not significantly different by LSD\(_{\alpha}=0.05\).
from Experiment 1 are shown in Table 3. In the non-mycorrhizal condition, the P efficiency of genotype 81(85) was higher than that of Fengxiao 8, with NC37 giving an intermediate value. In the mycorrhizal plants there was no difference in P efficiency of the three genotypes because inoculation significantly enhanced the P efficiency of genotypes Fengxiao 8 and NC 37, but not of cultivar 81(85).

### Relationship Between Mycorrhizal Dependency and P Efficiency Indices

Although wheat showed a relatively low mycorrhizal dependency when mycorrhizal at low P supply, there were still differences in dependency between the genotypes because of differences in their P nutrition characteristics. Thus, linear correlation analysis was used to compare mycorrhizal dependency with each of the three P efficiency indices in Experiment 1 and the correlation coefficients are shown in Table 4. The results show that P efficiency was

<table>
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<tr>
<th>Table 3. P Efficiency of Three Wheat Genotypes</th>
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<tr>
<td></td>
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<tr>
<td><strong>P Efficiency, Ratio of Biomass at Low P Level to Biomass at Adequate P Level</strong></td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>81(85)</td>
</tr>
<tr>
<td>Fengxiao 8</td>
</tr>
<tr>
<td>NC37</td>
</tr>
</tbody>
</table>

*P < 0.05; NS, not significant.

*aSignificance (by ANOVA) of difference between mycorrhizal and non-mycorrhizal plants.

*bWithin columns, means with the same letter are not significantly different by LSDₐ = 0.05.

<table>
<thead>
<tr>
<th>Table 4. Linear Correlation Coefficients (r, 10 Degrees of Freedom) Between Mycorrhizal Dependency, P Uptake Efficiency, and P Utilization Efficiency of Three Wheat Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>r, 10 Degrees of Freedom</td>
</tr>
<tr>
<td>P Efficiency</td>
</tr>
<tr>
<td>Mycorrhizal dependency</td>
</tr>
</tbody>
</table>

***P < 0.01; NS, not significant.
negatively correlated with mycorrhizal dependency, and P uptake rate (one of the components of P efficiency) was also negatively related to mycorrhizal dependency. However, P utilization rate (the other component of P efficiency) showed no significant relationship with mycorrhizal dependency.

**Carbohydrate Allocation to Roots**

When the amount of sugar translocated to the roots in the phloem was determined in Experiment 1 (Table 5), it was evident that genotypes Fengxiao 8 and NC37 (with low P efficiencies) transported more carbohydrate from shoots to roots than did genotype 81(85) (a high P efficiency genotype). This is important because the translocated carbohydrate is potentially available for the construction of a larger root system for greater nutrition acquisition. As expected, the trend in root-to-shoot ratio (R/S) was very similar to the pattern of sugar translocation rate, with R/S values of 0.27, 0.55, and 0.83 for genotypes 81(85), Fengxiao 8, and NC37 respectively.

**Hyphal Length Density and Hyphal P Influx Rate**

In the hyphal compartments in Experiment 2 the genotypes with low P efficiency had higher hyphal length density than did high P efficient genotypes (Table 6). There was no difference in hyphal P influx rate among the three genotypes. During the growth of the wheat plants, P uptake by the hyphae was significantly lower in genotype 81(85) than in the other two genotypes, with a contribution of hyphae to total plant P uptake of 29.5, 38.8, and 37.6% for 81(85), Fengxiao 8, and NC37, respectively.

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**Table 5. Sugar Translocation Rate and Root-to-Shoot Ratio of Three Non-Mycorrhizal Wheat Genotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sugar Translocation Rate (mg cm⁻² h⁻¹)</th>
<th>Root-to-Shoot Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>81(85)</td>
<td>0.47 b*</td>
<td>0.27 c</td>
</tr>
<tr>
<td>Fengxiao 8</td>
<td>1.44 a</td>
<td>0.55 b</td>
</tr>
<tr>
<td>NC37</td>
<td>1.06 a</td>
<td>0.83 a</td>
</tr>
</tbody>
</table>

*Within columns, means with the same letter are not significantly different by LSDₜ = 0.05.
### Table 6. Hyphal Length Density, Hyphal P Influx Rate, and Hyphal P Uptake of Three Mycorrhizal Wheat Genotypes in Experiment 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hyphal Length Density mg g(^{-1})</th>
<th>Hyphal P Influx Rate (\times 10^{13}) mol m(^{-1}) s(^{-1})</th>
<th>Hyphal P Uptake mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>81(85)</td>
<td>0.19 b(^a)</td>
<td>1.40 a</td>
<td>1.06 b</td>
</tr>
<tr>
<td>Fengxiao 8</td>
<td>0.51 a</td>
<td>0.86 a</td>
<td>1.78 a</td>
</tr>
<tr>
<td>NC37</td>
<td>0.62 a</td>
<td>0.72 a</td>
<td>1.79 a</td>
</tr>
</tbody>
</table>

\(^a\)Within columns, means with the same letter are not significantly different by LSD._0.05_

### DISCUSSION

Although the three wheat genotypes have been classified as high (81(85)), moderate (Fengxiao 8), and low (NC37) P efficient varieties according to their relative yields, their P efficiencies at the seedling stage (8 weeks) were high (81(85)), low (Fengxiao 8), and moderate (NC37) according to the relative biomass. Although the P efficiency of genotype 81(85) was the highest, differences in P efficiency among the three genotypes were not significant when the plants were mycorrhizal. Because mycorrhizal dependency was closely related to plant P status, all those factors contributing to plant P status may affect mycorrhizal dependency. It has been reported that for many plants root dry weight, root hair length, and root hair density are negatively related to mycorrhizal dependency (16). In the present work, root P uptake rate was negatively related to mycorrhizal dependency (Table 4). It is well established that plants with high yield, long root hairs, or dense root hairs are usually characterized by higher root P uptake rate (17). This may imply that root P uptake rate is the most important fundamental factor responsible for mycorrhizal dependency. Our results agree with those of other workers by suggesting that differences in mycorrhizal and non-mycorrhizal plant growth reflect differences in the uptake of P rather than its utilization within the plant (18). This is the case especially at the seedling stage because plant uptake rate contributes much more than plant utilization rate does at early stages of growth.

In the present experiments, the mycorrhizal dependency of genotype 81(85) was lower due to its higher root P uptake rate when non-mycorrhizal. Although the three genotypes took up very similar amounts of P, their root dry weights were different, and this resulted from differences in carbohydrate allocation. In genotype 81(85), a slow sugar translocation rate in the phloem led to lower root dry weight and thus higher root P uptake rate. Such a phenomenon could result from long evolutionary history. On one hand, genotype 81(85) does not necessarily spend much carbohydrate constructing a large root system for mineral
nutrition acquisition because of its high root P uptake rate (19). On the other hand, more carbohydrate allocated to the shoots contributes to a larger leaf area and more assimilated photosynthate.

After inoculation with the mycorrhizal fungus, there was poor hyphal growth in genotype 81(85) (Table 6), with a hyphal length density of about one third of that in the other two genotypes. This may be attributed to the smaller allocation of carbohydrate to the roots of genotype 81(85) compared to the other two genotypes. This result is in close agreement with the report of Same et al. (20) that the development of arbuscular mycorrhizal colonization was inhibited by carbohydrate supply when the leaves were subjected to a shading treatment. The subsequent difference in hyphal length of our three genotypes resulted in different P uptake by the mycorrhizal hyphae.

CONCLUSIONS

The results indicate that the mycorrhizal dependency of the genotype with the highest P efficiency was lower than that of the genotypes with lower P efficiencies. Linear correlation analysis suggested that mycorrhizal dependency was primarily controlled by P uptake efficiency. More carbohydrate was translocated to the roots of genotypes with low P efficiency than of those with high P efficiency. Higher hyphal length density arising from carbohydrate translocation led to more P uptake, and this may account for higher mycorrhizal dependency.

ACKNOWLEDGMENTS

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