PLANT GROWTH RESPONSES IN NATURAL ACIDIC SOIL AS AFFECTED BY ARBUSCULAR MYCORRHIZAL INOCULATION AND PHOSPHORUS SOURCES

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ABSTRACT

The effect of arbuscular-mycorrhizal (AM) fungus Glomus etunicatum inoculation in interaction with two sources of phosphorus (P) [soluble P and partially acidulated phosphate rock (pa-PR)] at three rates (17, 43, and 86 kg P ha⁻¹) was studied in an acidic natural soil using wheat (Triticum aestivum L.) as host plant. Shoot and root dry biomass, AM colonized root length, macro-micronutrients content and soil phosphatase (P-ase) activity were determined after six months of plant growth. The inoculated G. etunicatum fungus, a fungal strain adapted to the prevailing soil conditions, enhanced plant growth (shoot and

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root biomass) and mineral acquisition of some elements when plants were fertilized with pa-PR but not with soluble P. The nutrient acquisition by AM inoculated plants varied with the source and amount of applied P. When pa-PR was supplied, the inoculated AM fungus enhanced P, potassium (K), aluminum (Al), and manganese (Mn) plant acquisition in comparison with indigenous endophytes alone. Shoot zinc (Zn) and copper (Cu) uptakes were also enhanced by *G. etunicatum* inoculation only at the intermediate assayed pa-PR level (43 kg ha\(^{-1}\)). AM root colonization in the efficient pa-PR treatments, did not relate well to the plant growth and nutrient acquisition in most cases. Nutrients (Ca and Mg) that increased in AM inoculated plants were not those commonly deficient in acidic soils. Nevertheless, some nutrients, which often become limiting under low pH conditions such as P and K were increased by *G. etunicatum* inoculation plus pa-PR. Changes in rhizospheric soil pH under pa-PR application may be involved in these mycorrhizal effects on nutrient acquisition. The increases in plant biomass as a result of mycorrhizal inoculation do not seem to account for all the changes observed in mineral acquisition. The highest soil P-ase activity was observed at the lowest pa-PR dose showing a negative relationship with P-availability. The inoculation of *G. etunicatum* was effective in this natural acidic soil in overcoming factors that restrict plant growth and nutrition when pa-PR was applied.

**INTRODUCTION**

The main AM benefits on plant growth stimulation are attributed to increasing the root uptake of essential plant nutrients and reducing the acquisition of those elements in toxic levels which are unfavorable to plant growth.

In most soils, available phosphorus is rather low, especially in acidic soils due to its very high P-adsorption capacity. Mycorrhizae constitute efficient root extension organs involved in uptake and translocation of phosphate and other nutrients with low diffusion rates. Nevertheless, mycorrhizal fungal isolates show differences in effectiveness for overcoming mineral deficiencies in plants grown under limited nutritional conditions in acidic soils.\(^{[1]}\) Although an increased growth in AM plants is mainly attributed to enhanced phosphorus uptake, the AM symbiosis is believed to be involved not only in the amelioration of plant mineral nutrition, but also in withstanding stress tolerances, such as drought\(^{[2,3]}\) and high Al\(^{[4–6]}\) or Mn levels.\(^{[7,8]}\) It can be generalized that modern high-input agricultural practices generally are detrimental to AM fungi in soil, while low-input
sustainable agriculture methods enhance the performance of the symbiosis.[9] Mycorrhizal associations occur in almost all crops and play a direct role in nutrient cycling patterns and rates in agrosystems environments[10,11] being considered as key factors for successful low-input farming. Thus, the formation and functioning of the AM symbiosis is expected to play an important role in sustainable agriculture.[12,13] To increase productivity in cropping systems, either nutrients have to be artificially supplied or conditions for mycorrhizal formation have to be favored, but high levels of synthetic mineral fertilizers can inhibit AM infection. Thus, AM symbiosis is influenced by various management practices such as the rates and type of fertilizers. In arable land, fertilizer supplies are often added to replenish mineral nutrients, but combining compatible AM fungus (i) with mineral fertilizers is required for successful results. Soils with high and intensive input agriculture have a greatly decreased capacity to initiate AM colonization and excessive use of fertilizers can also negatively affect mycorrhizal formation[14] and plant development; consequently, crops become more and more dependent on artificial fertilizers for high production. Low amounts of nutrient applications do not appear to affect AM symbiosis adversely and do not damage mycorrhizal fungi, exhibiting no inhibitory effect, or they can even stimulate root infection in host plants resulting in more abundant mycorrhizal population.[15]

The use of phosphate rock (PR), be it alone or partially acidulated (pa-PR), has been proposed for restoring the phosphate reserves in agricultural systems where P is scarce.[16,17] Generally speaking, this sparingly soluble form of P has a low effectiveness on non-acidic soils. Interactive actions involving PR and AM fungi have been proposed to improve P plant nutrition.[18,19] In fact, current development in sustainability involves soil microbial activities[20] and the use of less expensive sources of nutrients as phosphate rock.

This study presents results from plant growth and mineral nutrition of wheat growing in a pot culture using a natural acidic soil and the effect of P levels and sources application when inoculated with a local adapted AM isolate of *Glomus etunicatum*.

**MATERIALS AND METHODS**

**Test Plant and Soil**

Wheat (*Triticum aestivum* L. cv Otto) was used as the test plant. Surface sterilized (2% Cloramine T, 2–3 min) seeds were thoroughly washed with distilled water and after germination in a culture chamber four seedlings were transplanted into 1 L plastic pots containing an agricultural acidic soil collected from Vilcún series (a Typic Dystrandepts). The characteristics of the test soil, an Andisol, are described in Table 1.
Table 1. Selected Chemical Properties of Soil Used in This Study

<table>
<thead>
<tr>
<th>Available P&lt;sup&gt;a&lt;/sup&gt; (µg g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Total P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Org. P&lt;sup&gt;c&lt;/sup&gt;</th>
<th>pH H&lt;sub&gt;2&lt;/sub&gt;O</th>
<th>SOM %</th>
<th>K (cmol (+) kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>Al</th>
<th>Al Sat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>2540</td>
<td>1480</td>
<td>5.42</td>
<td>18</td>
<td>0.70</td>
<td>0.07</td>
<td>9.33</td>
<td>1.23</td>
<td>0.07</td>
<td>11.33</td>
</tr>
</tbody>
</table>

<sup>a</sup>NaHCO<sub>3</sub>-extractable; <sup>b</sup>Dick and Tabatabai; <sup>c</sup>Borie and Barea.
The experimental soil was fertilized with soluble P (TSP) or partially acidulated phosphate rock (pa-PR) at the equivalent rates of 17, 43, and 86 kg P ha\(^{-1}\). Commercial pa-PR originating from Israel contained 50% water soluble-P.

The AM strain used was \(G.\ etunicatum\) CH 110 obtained from INVAM collection (Morgantown, WV) an ecotype isolated in 1992 from Chilean Andisols and which had shown its effectiveness as inoculum in a previous assay on barley.\(^{[4]}\) Forty grams of solid fungal inoculum of \(G.\ etunicatum\) containing spores, mycelium and mycorrhizal root fragments were thoroughly mixed with the soil in the pot. For reinforcing the AM inoculation a 10 g inoculum layer was placed under the seedlings. In the non-mycorrhizal treatment the same amount and distribution of autoclaved inoculum was applied to the soil.

Twenty days after transplanting, the seedlings were thinned to two plants per pot. The plants grew in a greenhouse under controlled environment conditions with a 16/8 h day/night regime, 450 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) photon flux density, 75% relative humidity and day/night temperature of 25/15\(^\circ\)C. Plants were N fertilized with a KNO\(_3\) (at equivalent of 200 kg N ha\(^{-1}\)) solution; one third being applied one week after transplanting and the rest at the beginning of tillering (stage 21).\(^{[23]}\) Pots were watered to field capacity on a mass basis three times per week and every two weeks over the duration of the experiment 10 mL aliquots of nutrient solution less P\(^{[24]}\) were added.

The experiment was carried out for six months between 19 June and 13 December 1999 and plants were harvested after anthesis, at the milky stage of growth (stage 71),\(^{[23]}\) severing shoots from roots. The roots were thoroughly washed for removing adhering soil under a stream of cold water. Samples of washed roots were cut into segments about 1–2 cm long, cleared with 10% potassium hydroxide (KOH) in boiling water for 15 minutes, and stained with trypan blue for measurements of mycorrhizal colonization.\(^{[25]}\) Under microscopic examination root length colonization was calculated by the gridline-intersect technique\(^{[26]}\) and AM colonization estimated by the method described by Giovanetti and Mosse.\(^{[27]}\) Dry weights of shoots and roots were determined after drying at 65\(^\circ\)C for 48 hours. Root weights were corrected for including fresh weight samples taken for determination of mycorrhizal colonization.

After acid digestion treatment, P in plant tissue was determined colorimetrically using the vanado-molybdate method and K, calcium (Ca), magnesium (Mg), Al, Zn, Mn, and Cu were quantified by atomic absorption spectroscopy.

Soil pH and available-P were determined in a soil:water suspension (1 : 2.5) and extracted with a solution of 0.5 M NaHCO\(_3\) at pH 8.5,\(^{[28]}\) respectively. Acid phosphatase (P-ase) in the soil was determined using p-nitrophenylphosphate (PNPP) according to procedure described by Tabatabai and Bremner\(^{[29]}\) with modifications reported by Rubio et al.\(^{[30]}\) for volcanic soils.
The data were statistically analyzed by an analysis of variance after arcsin transformation. When a significant \((P \leq 0.05)\) treatment effect was found, the mean values were compared using the Duncan’s Multiple Range Test \((P \leq 0.05)\).

**RESULTS AND DISCUSSION**

Results from this study show the interactive effect of *G. etunicatum* inoculation and pa-PR application on plant growth (Fig. 1) and nutrients acquisition (Fig. 2 and 3). The *G. etunicatum* inoculation was not effective at any of the three levels of applied soluble P (Figs. 1–3). The amount of available P for

![Figure 1](image)

*Figure 1.* Effect of *G. etunicatum* inoculation on shoot and root biomass (g) of wheat plants growing in an natural soil fertilized with soluble P or pa-PR applied at 17, 43, and 86 kg P ha\(^{-1}\) and inoculated or not with *G. etunicatum*. Bars with different letters indicate significantly by different means \((P \leq 0.05)\) by Duncan’s Multiple Range Test.
plant uptake was very low, nearly 5–100 mM routinely encountered in arable soils.\textsuperscript{[31]} Limited information is available for enhanced mineral nutrients acquisition by mycorrhizal plants grown on acidic soils.\textsuperscript{[32]} The inoculated AM fungus, \textit{G. etunicatum}, enhanced P, K, Mn, and Al plant acquisition in comparison with indigenous endophytes alone when pa-PR (at the highest level) was the applied P source. At the intermediate pa-PR level of 43 kg P ha\textsuperscript{-1} and inoculated or not with \textit{G. etunicatum}. For each graph, bars with different letters indicate significantly by different means ($P \leq 0.05$) by Duncan’s Multiple Range Test.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Effect of \textit{G. etunicatum} inoculation on shoot P, K, Mg, and Ca content (mg) in wheat plants growing in an natural soil fertilized with soluble P or pa-PR applied at 17, 43, and 86 kg P ha\textsuperscript{-1} and inoculated or not with \textit{G. etunicatum}. For each graph, bars with different letters indicate significantly by different means ($P \leq 0.05$) by Duncan’s Multiple Range Test.}
\end{figure}

In the present pot experiment, the treatments differed essentially in the type and/or
amount of applied P fertilizer since non-mycorrhizal control was absent and indigenous AM endophytes were present in all the treatments. Thus, we can only compare the ability of G. etunicatum inoculation to change macro and micronutrients acquisition to naturally occurring mycorrhizae associated wheat plants under pa-PR or soluble P application.

Wheat P acquisition by roots of mycorrhizal plants (by autochthonous and inoculated endophytes) decreased proportional to P increased in the medium. This negative effect of P fertilizer was not detected in pa-PR supplied plants in which AM inoculation was effective in increasing shoot P content even at the highest applied level (Fig. 2).

Increased availability of less soluble sources of P has been attributed to AMF particularly in interaction with P-solubilizing microorganisms. Root-induced changes of rhizosphere pH, caused by processes such as differential
uptake of anions and cations, root respiration or organic acid exudation, might strongly affect P availability and hence, its uptake.\textsuperscript{[16]} In this study, the application of pa-PR increased soil pH (Table 2) and this change can also affect oxidation-reduction potential. It is important to remark that such increase on soil pH with pa-PR application was not observed when sterile soil was used (data not shown).

Under low or P deficiency conditions, the phosphatase activity in the rhizosphere could potentially solubilize soil organic P.\textsuperscript{[34–36]} AMF mycelium and AMF-roots can contribute to increase such activity in soil as the results show (Fig. 4). P-ase activity of \textit{Glomus mosseae} hyphae have shown to be effective in hydrolizing organic P to enhance P in wheat.\textsuperscript{[37,38]} But no generalization can be made since AMF species appear to have different mechanisms for P metabolism and transport.\textsuperscript{[35,39]} In fact, P uptake by specific form and function of AM mycelia according to fungal species or genus should differently enhance P acquisition by the plant root.\textsuperscript{[40]}

Procedures providing a greater surface area for scavenging mineral nutrients that are deficient or with a low mobility in the acid soil here used may be involved in the mycorrhizal contribution to nutrient uptake by wheat growing in pa-PR added medium. Thus, Yao et al.\textsuperscript{[41]} have argued that the different sources of P may have different effects on hyphal development and consequently, the P uptake could be related to the amount or length of hyphae present, irrespective of the solubility of the form of P supplied. In the present

Table 2. Soil pH and P-Olsen (\(\mu g \cdot g^{-1}\)) at Harvest of Wheat Plants Growing in a Natural Soil Fertilized with Soluble P or pa-PR Applied at 17, 43, and 86 kg P ha\(^{-1}\) and Inoculated or Not with \textit{G. etunicatum}  

<table>
<thead>
<tr>
<th>Soluble P</th>
<th>pa-PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>— G. etunicatum</td>
<td>— G. etunicatum</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5.62f</td>
</tr>
<tr>
<td>43</td>
<td>5.71de</td>
</tr>
<tr>
<td>86</td>
<td>5.63f</td>
</tr>
<tr>
<td>P-Olsen</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>31.1bc</td>
</tr>
<tr>
<td>43</td>
<td>34.3b</td>
</tr>
<tr>
<td>86</td>
<td>31.9b</td>
</tr>
</tbody>
</table>

Values with different letters indicate significantly different means \((P \leq 0.05)\) by Duncan’s Multiple Range Test.
experiments, the external mycelium was increased in *G. etunicatum* inoculated pots at any level of added pa-RP (results not shown here).

Additionally, root weights of inoculated plants and fertilized with pa-PR were higher than those fertilized with soluble P and could potentially absorb more nutrients. Nevertheless, in most of the treatments, some nutrient (Cu, Zn, Ca, and Mg) absorptions were not significantly affected whatever the source of applied P was. In soil supplied with 86 kg P ha$^{-1}$, inoculated and non-inoculated plants having similar shoot and root weight also differed in nutrient uptake with Al, Mg and Cu being increased and Mn being decreased in *G. etunicatum* colonized plants. Such differences cannot be attributed to differences in mycorrhizal root length on AM colonization between both treatments. In this study, *G. etunicatum* effectiveness in pa-PR added medium did not closely rely on the improvement of the amount of mycorrhizal root length (Fig. 5). Medeiros[6] and Clark and Zeto[5] also reported that plant biomass yield did not correlate well with AM colonized root length. Increases in root biomass as result of *G. etunicatum* inoculation seem neither to account for all the changes in mineral acquisition observed in pa-PR inoculated treatments since the content of Ca (17 kg P ha$^{-1}$) and Mg (86 kg ha$^{-1}$) were decreased. Acidic soils are generally low in cationic bases, which are often limiting plant growth in such soils, and enhancement of K, Ca, and Mg uptake is expected. However, AMF isolates have exhibited variability in, Ca and Mg uptake in plants grown in acidic soils.[42]

Plant growth in acidic soils often exhibits P, Ca and Mg deficiencies, while Al or Mn phytotoxicities occur. Under such conditions, the mycorrhizal
colonization function effectively alleviates the mineral stresses and such AM activities are not always explained by the effect on plant growth. It is generally assumed that at high micronutrients levels in the soil, the acquisition of these minerals are reduced in mycorrhizal plants.\cite{43} This mycorrhizal effect has been attributed to the fact that polyphosphates from fungal mycelium could sequester metals reducing the transfer into roots.\cite{44} Recently, Mehravaran et al.\cite{45} reported that some fungal isolates may affect the relative partitioning of P and Zn between plant roots and tops.

Mobility of Cu, Zn, and Mn in soil is low and the uptake of these micronutrients by roots is diffusion limited.\cite{46,47} Thus, the acquisition of these micronutrients in AM plants is routinely enhanced.\cite{16} Clark and Zeto\cite{5} reported that many of the differences in plant enhancement or mineral acquisition were closely associated with the specific fungal isolate colonizing roots. However, Graham and Abbott\cite{48} reported no differences over the control on shoot P concentration of wheat when plants were inoculated with ten separate AM fungal isolates. But the present results are an indication about the different ability of mycorrhizal roots for taking nutrients via extraradical hyphae, which varied according to associated endophytes (natural or inoculated).

In this study, the effective inoculation of G. etunicatum in pa-PR treated soil resulted more active for Al and Mn plant uptake than for Zn or Cu acquisition in contrast to the effect reported for effective AMF in acidic medium.\cite{1,6,7}
general, the inoculated fungus did not affect Zn or Cu acquisition in relation to native fungus in spite of the stimulating effect on root growth and AM colonization found in these treatments. This fact may be indicating of Zn and Cu sufficiency in the soil used.\cite{49} Medeiros et al.\cite{6,7} reported that an isolate of *G. etunicatum* was effective in alleviating Al and Mn toxicities but the soil here used had a low Al content (Table 1), which can explain the *G. etunicatum* inoculation response found on this element. Information about AM isolates differences to mineral acquisition is limited and more extensive studies are required. Variation in the activity of AM isolates can justify these differences particularly using a natural soil having a mixed AM population.

Depending on the P source applied, the inoculated fungus increased (with pa-PR) or decreased (with soluble P) Mn uptake under a fertilization of 86 kg P ha\textsuperscript{-1} having similar mycorrhizal root length. It is known that Mn availability will depend on soil pH value and the observed pH changes of both treatments (Table 2) could explain changes in red-ox potential. Manganese, in the reduced form, is more available to plants.\cite{16} Root exudation, which is affected by root growth and plant P content (values that increased in pa-PR inoculated plants), is an important factor for Mn uptake by plants. Regarding Cu content, the overall effect of P sources was not relevant on this element and the effect of AM inoculation was only observed under particular levels of pa-PR (47 kg ha\textsuperscript{-1}) or soluble P (86 kg ha\textsuperscript{-1}) applications. These results do not agree with those reported by Li et al.\cite{50} who found that mycorrhizal mycelium increased Cu uptake when increasing P levels in the medium. The regulatory effect of P on the effectiveness of AM symbiosis is known, but the knowledge on the effects of other soil interacting components is still limited.

Regarding results, nutrients acquisition by mycorrhizal wheat does not only depend on the availability of nutrients in soil solution, but also on the effectiveness of the root uptake according to the mycorrhizal fungi involved.\cite{51} Results here obtained are coincident with those reported by Graw\cite{52} who found that plants growing with different sources of P varied in P uptake under AM conditions in an acidic soil.

In most published studies, the effect of AM fungi on plant nutrients uptake has been evaluated comparing mycorrhizal and non-mycorrhizal plants but here the main concern is to stimulate natural mycorrhizal effect by inoculation of an adapted endophyte in the compatibility with P sources supply. Thus, available results reported on nutrients acquisition by AM colonization in acidic soils cannot be compared with results presented here. In fact, in this study the greatest effect of *G. etunicatum* in pa-PR added soil was increasing P, K, Al, and Mn acquisition. Different isolates of AM fungi often function differently for micronutrients acquisition under different experimental conditions, such as rhizosphere pH and P sources and/or levels. The increased K as affected by pa-PR as well as AM inoculation were more relevant at the highest pa-PR level.
In other work, Raju et al. [53] determined higher P, K, and Cu and lower Mn and Al uptake in mycorrhizal sorghum plants grown in soil at pH 4.5. Medeiros et al. [54] found that certain AM fungal isolates (e.g., G. etunicatum) enhanced the acquisition of P, K, Ca, and Mg as well as Zn, Cu, and Mn at pH 4.0. In contrast to these two studies, Nurlaeny et al. [55] reported that the content of Cu, Zn, and Mn in maize and soybean were similar in mycorrhizal and non-mycorrhizal plants growing in acid soil. Regarding available information, [32] it is difficult to draw general conclusions, since changes in soil nutrient content, pH, plant species or even cultivars within plant species and AM fungi are involved in the behavior and effectiveness of AM colonization overcoming those factors restricting plant growth and nutrition in acidic soils.

In conclusion, plant growth on acidic soils commonly undergoes P limitation and AM inoculation in pa-PR added medium could reduce external P plant requirements. However, an accurate knowledge of the beneficial effect of the inoculation of any mycorrhizal strain would require the identification of all natural endophytes present in the soil but such matter was not the main objective of this work. From a practical point of view, it is interesting to know that, in this particular Andisol, the P, K, and micronutrients increasing due to inoculation of G. etunicatum CH-110 an adapted endophyte present in most Chilean volcanic soils required a pa-PR supply as fertilizer for reaching successful mycorrhizal performance. Therefore, more studies are required into factors affecting mycorrhizal activities in natural systems especially those which concern to the synergistic effects of phosphate related free-living microorganisms.

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REFERENCES

18. Toro, M.; Nedialkova, K.; Azcón, C.; Barea, J.M. Establishment of Two Rock Phosphate Solubilizing Bacteria in the Rhizosphere of Mycorrhizal


ARBUSCULAR MYCORRHIZAL INOCULATION AND P SOURCES


