MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF PLANT NUTRITION, 25(7), 1389–1405 (2002)

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

Rosa Rubio,[1] Fernando Borie,[1,*] Carlos Schalchli,[1] Carlos Castillo,[1] and Rosario Azcón[2]

[1]Departamento de Ciencias Químicas, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile
[2]Departamento de Biología de Suelos y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, Granada 18008, Spain

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

*Corresponding author. E-mail: fborie@ufro.cl

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

Available P[a]	Total P[b]	Org. P[c]	pH	SOM	K	Na	Ca	Mg	Al	CEC	Al Sat
	($\mu g\,g^{-1}$)		H_2O	%	(cmol (+) kg^{-1})						(%)
4.0	2540	1480	5.42	18	0.70	0.07	9.33	1.23	0.07	11.33	0.61

[a]$NaHCO_3$-extractable; [b]Dick and Tabatabai;[21] [c]Borie and Barea.[22]

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°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°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

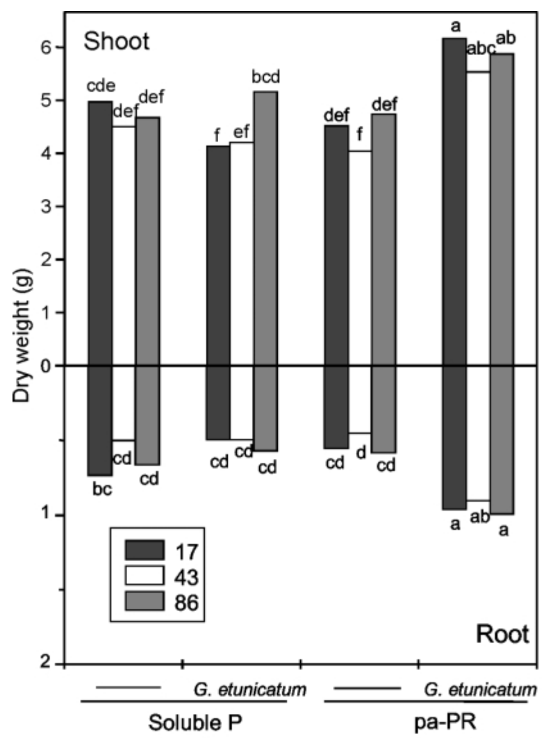

Figure 1. Effect of *G. etunicatum* inoculation on shoot and root biomass (g) of wheat plants growing in an natural soil fertilized with soluble P or pa-PR applied at 17, 43, and 86 kg P ha^{-1} and inoculated or not with *G. etunicatum.* Bars with different letters indicate significantly by different means ($P \leq 0.05$) by Duncan's Multiple Range Test.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

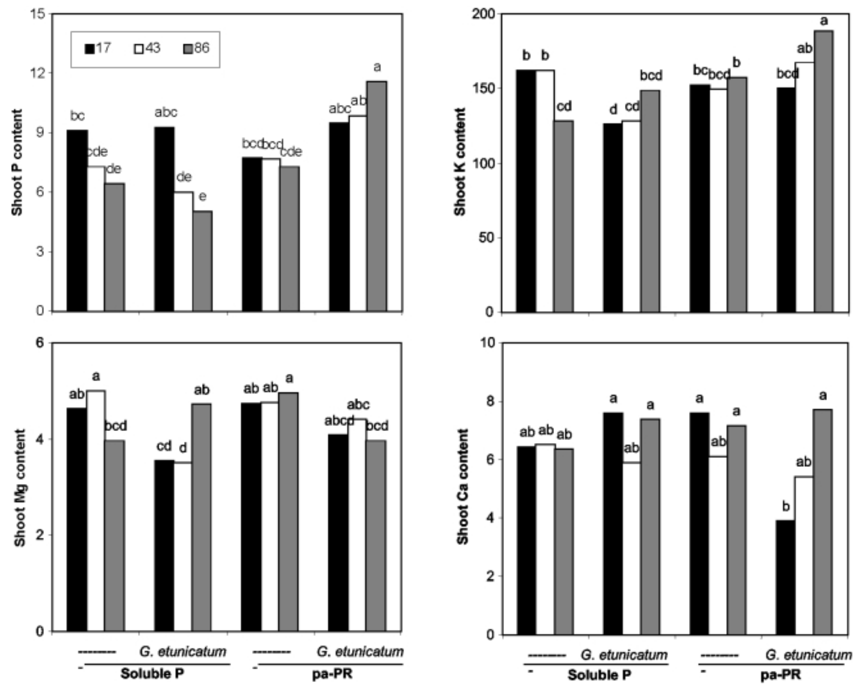

Figure 2. Effect of *G. etunicatum* inoculation on shoot P, K, Mg, and Ca content (mg) in wheat plants growing in an natural soil fertilized with soluble P or pa-PR applied at 17, 43, and 86 kg P ha^{-1} and inoculated or not with *G. etunicatum.* For each graph, bars with different letters indicate 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.[31] Limited information is available for enhanced mineral nutrients acquisition by mycorrhizal plants grown on acidic soils.[32] The inoculated AM fungus, *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^{-1}, the Zn and Ca contents were also enhanced by the inoculated fungus. In general, Ca shoot contents were not increased by *G. etunicatum* inoculation although this nutrient is normally low and deficient in acidic soils.[33] Nutrients showing low availability such as P, Zn, and Cu, have commonly reported to be higher in mycorrhizal plants but in the experimental soil used here, Zn plant acquisition was not increased in AM inoculated plants under any P treatment (Fig. 2). In the present pot experiment, the treatments differed essentially in the type and/or

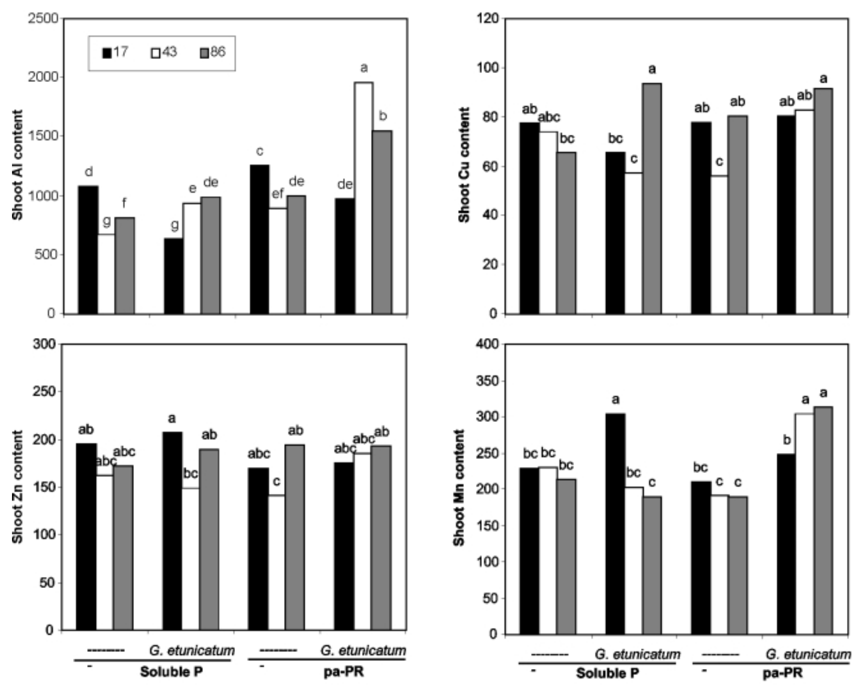

Figure 3. Effect of *G. etunicatum* inoculation on shoot Al, Cu, Zn, and Mn content (μg) in plants growing in an natural soil fertilized with soluble P or PR applied at 17, 43, an 86 kg P ha^{-1} and inoculated or not with *G. etunicatum.* For each graph, bars with different letters indicate significantly by different means ($P \leq 0.05$) by Duncan's Multiple Range Test.

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.[18] 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.[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.[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 *Glomus mosseae* hyphae have shown to be effective in hydrolizing organic P to enhance P in wheat.[37,38] But no generalization can be made since AMF species appear to have different mechanisms for P metabolism and transport.[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.[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.[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 ($\mu g\, g^{-1}$) 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 *G. etunicatum*

	Soluble P		pa-PR	
	—	*G. etunicatum*	—	*G. etunicatum*
		pH		
17	5.62f	5.46g	5.77bcd	6.12a
43	5.71de	5.68ef	5.75cde	5.84b
86	5.63f	5.73cde	5.80bc	5.81bc
		P-Olsen		
17	31.1bc	10.9g	27.7cd	25.1de
43	34.3b	23.1e	30.4bc	17.1f
86	31.9b	43.2a	40.7a	14.1fg

Values with different letters indicate significantly different means ($P \leq 0.05$) by Duncan's Multiple Range Test.

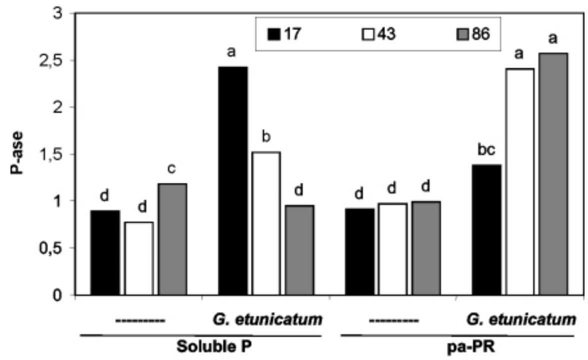

Figure 4. Effect of *G. etunicatum* inoculation on soil phosphatase activity (P-ase, mg g^{-1}) at harvest of wheat plants growing in a natural soil fertilized with soluble P or pa-PR applied at 17, 43, and 86 kg P ha^{-1} and inoculated or not with *G. etunicatum.* Bars with different letters indicate significantly by 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

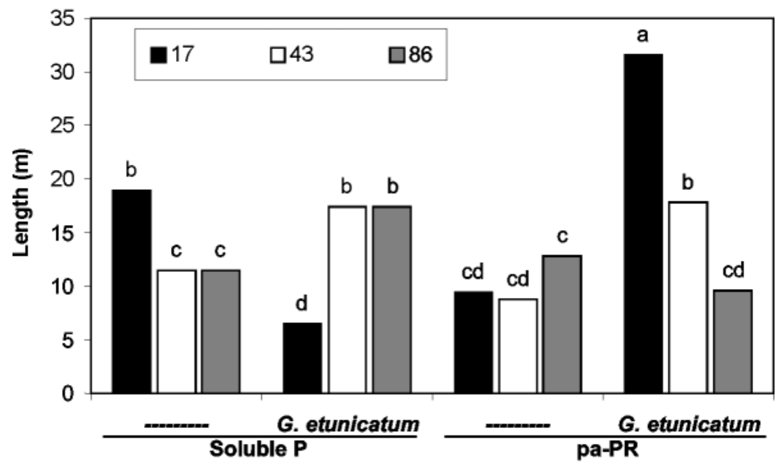

Figure 5. Effect *G. etunicatum* inoculation on AM colonized root length (m) at harvest of wheat plants growing in a natural soil fertilized with soluble P or pa-PR applied 17, 43, and 86 kg P ha^{-1} and inoculated or not with *G. etunicatum.* Bars with different letters indicate significantly by different means ($P \leq 0.05$) by Duncan's Multiple Range Test.

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.[43] This mycorrhizal effect has been attributed to the fact that polyphosphates from fungal mycelium could sequester metals reducing the transfer into roots.[44] Recently, Mehravaran et al.[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.[46,47] Thus, the acquisition of these micronutrients in AM plants is routinely enhanced.[16] Clark and Zeto[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[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,[1,6,7]. In 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.[49] Medeiros et al.[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 $86\,kg\,P\,ha^{-1}$ 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.[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 ($47\,kg\,ha^{-1}$) or soluble P ($86\,kg\,ha^{-1}$) applications. These results do not agree with those reported by Li et al.[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.[51] Results here obtained are coincident with those reported by Graw,[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.

ACKNOWLEDGMENTS

We would like to thank the CONICYT for supporting this research through Research Grant Fondecyt 1990756 and also for providing funds to R. Azcón for travel and lodging in Chile through C.I. 7990086 Grant.

REFERENCES

1. Clark, R.B.; Zobel, R.W.; Zeto, S.K. Effects of Mycorrhizal Fungus Isolates on Mineral Acquisition by *Panicum virgatum* in Acidic Soil. Mycorrhiza **1999**, *9*, 167–176.
2. Ruiz-Lozano, J.M.; Azcón, R. Hyphal Contribution to Water Uptake in Mycorrhizal Plants as Affected by the Fungal Species and Water Status. Physiol. Plant. **1995**, *95*, 472–478.
3. Ruiz-Lozano, J.M.; Azcón, R.; Gómez, M. Alleviation of Salts Stress by Arbuscular-Mycorrhizal *Glomus* Species in *Lactuca sativa* Plants. Physiol. Plant. **1996**, *98*, 767–772.

4. Borie, F.; Rubio, R. Effects of Arbuscular Mycorrhizae and Liming on Growth and Mineral Acquisition of Aluminum-Tolerant and Aluminum-Sensitive Barley Cultivars. J. Plant Nutr. **1999**, *22*, 121–137.
5. Clark, R.B.; Zeto, S.K. Growth and Root Colonization of Mycorrhizal Maize Grown on Acid and Alkaline Soil. Soil Biol. Biochem. **1996**, *28*, 1505–1511.
6. Medeiros, C.A.B.; Clark, R.B.; Ellis, J.R. Effects of Excess Aluminum on Mineral Uptake in Mycorrhizal Sorghum. J. Plant Nutr. **1994**, *17*, 1399–1416.
7. Medeiros, C.A.B.; Clark, R.B.; Ellis, J.R. Effects of Excess Manganese on Mineral Uptake in Mycorrhizal Sorghum. J. Plant Nutr. **1994**, *17*, 2203–2219.
8. Mendoza, J.; Borie, F. The Effects of *Glomus etunicatum* Inoculation on Aluminum, Phosphorus, Calcium and Magnesium Uptake in Two Barley Genotypes with Different Aluminum Tolerance. Commun. Soil Sci. Plant Anal. **1998**, *9*, 681–695.
9. Bethlenfalvay, G.J.; Linderman, R.G. Mycorrhizae in Sustainable Agriculture; American Society of Agronomy: Madison, WI, 1992; Spec. Publ. 54, 124 pp.
10. Bethlenfalvay, G.J. Mycorrhizae and Crop Productivity. In *Mycorrhizae in Sustainable Agriculture*; Bethlenfalvay, G.J., Linderman, R.G., Eds.; American Society of Agronomy: Madison, WI, 1992; Spec. Publ. 54, 1–28.
11. Joner, E.J.; Jakobsen, I. Contribution by Two Arbuscular Mycorrhizal Fungi to P Uptake by Cucumber (*Cucumis sativus* L) from ^{32}P-Labelled Organic Matter During Mineralization in Soil. Plant Soil **1994**, *163*, 203–209.
12. Schreiner, R.P.; Bethlenfalvay, G.J. Mycorrhizal Interactions in Sustainable Agriculture. Crit. Rev. Biotech. **1995**, *15*, 271–285.
13. Jeffries, P.; Barea, J.M. Biogeochemical Cycling and Arbuscular Mycorrhizas in the Sustainability of Plant-Soil Systems. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*; Gianinazzi, S., Schüepp, H., Eds.; Birkhäuser Verlag: Basel, 1994; 101–115.
14. Bagyaraj, D.J. Ecology of Vesicular-arbuscular Mycorrhizae. In *Handbook of Applied Mycology: Soil and Plants*; Arora, D.K., Raj, B., Mukerji, K.G., Knudsen, G.R., Eds.; Marcel Dekker, Inc.: New York, 1990; 3–33.
15. Sieverding, E. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft Technische Zusammenarbeit (GTZ) GmbH: Eschborn, Germany, 1991; 371 pp.
16. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd Ed.; Academic Press: London, 1995; 889 pp.
17. Chien, S.H.; Menon, R.G.; Billingham, K.S. Phosphorus Availability from Phosphate Rock as Enhanced by Water-soluble Phosphorus. Soil Sci. Soc. Am. J. **1996**, *60*, 1173–1177.
18. Toro, M.; Nedialkova, K.; Azcón, C.; Barea, J.M. Establishment of Two Rock Phosphate Solubilizing Bacteria in the Rhizosphere of Mycorrhizal

Onion Plants and their Effect on Plant Growth in a Microcosm. In *Mycorrhizas in Integrated Systems: From Genes to Plant Development*; Azcón-Aguilar C., Barea, J.M., Eds.; ECSC-EC-EAEC, Bruselas, 1996; 665–669.

19. Toro, M.; Azcón, R.; Barea, J.M. The Use of Isotopic Dilution Techniques to Evaluate the Interactive Effects of *Rhizobium* Genotype, Mycorrhizal Fungi, Phosphate Solubilizing Rhizobacteria and Rock Phosphate on Nitrogen and Phosphorus Acquisition by *Medicago sativa.* New Phytol. **1998**, *138*, 265–273.
20. Barea, J.M.; Azcón-Aguilar, C.; Azcón, R. Interactions Between Mycorrhizal Fungi and Rhizosphere Microorganisms Within the Context of Sustainable Soil-Plant Systems. In *Multitrophic Interactions of Terrestrial Systems*; Gange, A.C., Brown, V.K., Eds.; Blackwell Science: Cambridge, 1997; 65–77.
21. Dick, W.A.; Tabatabai, M.A. An Alkaline Oxidation Method for Determination of Total Phosphorus in Soils. J. Soil Sci. Am. Soc. **1977**, *41*, 511–514.
22. Borie, F.; Barea, J.M. Fósforo Orgánico en Suelos Volcánicos Chilenos. Agric. Tech. (Chile) **1983**, *43*, 239–248.
23. Zadoks, J.; Chang, T.; Konzak, C.F. A Decimal Code for the Growth Stages of Cereals. Weed Res. **1974**, *14*, 415–421.
24. Hewitt, E.J. *Sand and Water Culture Methods Used in the Studies of Plant Nutrition*; Commonwealth Agriculture Bureau: London, 1966; Tech. Commun. 22, 430–434.
25. Phillips, J.M.; Hayman, D.S. Improved Procedures for Clearing and Staining Roots and Staining Parasitic and Vesicular-Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. Trans. Brit. Mycol. Soc. **1970**, *55*, 158–161.
26. Tennant, D.A. Test of Modified Line Intersect Method of Estimating Root Length. J. Ecol. **1975**, *63*, 995–1001.
27. Giovanetti, M.; Mosse, B. An Evaluation of Techniques for Measuring Vesicular-Arbuscular Mycorrhizal Infection in Roots. New Phytol. **1980**, *84*, 489–500.
28. Olsen, S.R.; Sommers, L.E. Phosphorus. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, A.L., Ed.; American Society of Agronomy and Soil Science Society of America: Madison, WI, 1982; Agron. No. 9, 403–430.
29. Tabatabai, M.A.; Bremner, J.M. Use of p-Nitrophenyl Phosphate for Assay of Soil Phosphatase Activity. Soil Biol. Biochem. **1969**, *1*, 301–307.
30. Rubio, R.; Moraga, E.; Borie, F. Acid Phosphatase Activity and Vesicular-Arbuscular Infection Associated with Roots of Four Wheat Cultivars. J. Plant Nutr. **1990**, *13*, 585–598.

31. Kroehler, C.J.; Linkins, A.E. The Root Surface Phosphatases of *Eriophorum vaginatum*: Effects of Temperature, pH, Substrate Concentration and Inorganic Phosphorus. Plant Soil **1988**, *105*, 3–10.
32. Clark, R.B.; Zeto, S.K. Mineral Acquisition by Arbuscular Mycorrhizal Plants. J. Plant Nutr. **2000**, *23*, 867–902.
33. Clark, R.B. Arbuscular Mycorrhizal Adaptation, Spore Germination, Root Colonization, and Host Plant Growth and Mineral Acquisition at Low pH. Plant Soil **1997**, *192*, 15–22.
34. Tadano T.; Osawa, K.; Sakai, H.; Osaki, M.; Matsui, H. Secretion of Acid Phosphatase by the Roots of Crop Plants Under Phosphorus-Deficient Conditions and Some Properties of the Enzyme Secreted by Lupin Roots. Plant Soil **1993**, *156*, 95–98.
35. Boddington, C.L.; Dodd, J.C. A Comparison of the Development and Metabolic Activity of Mycorrhizas Formed by Arbuscular Mycorrhizal Fungi from Different Genera on Two Tropical Legumes. Mycorrhiza **1998**, *8*, 149–157.
36. Joner, E.J.; Johansen, A. Phosphatase Activity of External Hyphae of Two Arbuscular Mycorrhizal Fungi. Mycol. Res. **2000**, *104*, 81–86.
37. Tarafdar, J.C.; Marschner, H. Efficiency of VAM Hyphae in Utilisation of Organic Phosphorus by Wheat Plants. Soil Sci. Plant Nutr. **1994**, *40*, 593–600.
38. Tarafdar, J.C.; Marschner, H. Phosphatase Activity in the Rhizosphere and Hyphosphere of VA Mycorrhizal Wheat Supplied with Inorganic and Organic Phosphorus. Soil Biol. Biochem. **1994**, *26*, 387–395.
39. Boddington, C.L.; Dodd, J.C. Evidence that Differences in Phosphate Metabolism in Mycorrhizas Formed by Species of *Glomus* and *Gigaspora* Might be Related to Their Life-Cycle Strategies. New Phytol. **1999**, *142*, 531–538.
40. Dodd, J.C.; Boddington, C.L.; Rodriguez, A.; Gonzalez-Chavez, C.; Mansur, I. Mycelium of Arbuscular Mycorrhizal Fungi (AMF) from Different Genera: Form, Function and Detection. Plant Soil **2000**, *226*, 131–151.
41. Yao, Q.; Li, X.; Feng, G.; Christie, P. Mobilization of Sparingly Soluble Inorganic Phosphates by the External Mycelium of an Arbuscular Mycorrhizal Fungus. Plant Soil **2001**, *230*, 279–285.
42. Saggin, O.J., Jr.; Siqueira, J.O.; Guimaraes, P.T.G.; Oliveira, E. Inoculation of Coffee Trees with Different Mycorrhizal Fungi: Effects on Seedling Raising and on Outplants Growth in Fumigated Soil. R. Bras. Ci. Solo **1995**, *19*, 213–220.
43. Khotari, S.K.; Marschner, H.; Römheld, V. Direct and Indirect Effects of VA Mycorrhiza and Rhizosphere Microorganisms on Mineral Nutrient Acquisition by Maize (*Zea mays* L.) in a Calcareous Soil. New Phytol. **1990**, *116*, 637–645.

44. Turnau, K.; Kottke, L.; Oberwinkler, F. Element Localization in Mycorrhizal Roots of *Pteridium aquilinum* L. Kuhn Collected from Experimental Plots Treated with Cadmium Dust. New Phytol. **1993**, *123*, 313–324.
45. Mehravaran, H.; Mozafar, A.; Frossard, E. Uptake and Partitioning of P-32 and Zn-65 by White Clover as Affected by Eleven Isolates of Mycorrhizal Fungi. J. Plant Nutr. **2000**, *23*, 1385–1395.
46. Barea, J.M. Vesicular-arbuscular Mycorrhizae as Modifiers of Soil Fertility. Adv. Soil Sci. **1991**, *15*, 1–40.
47. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D.; Havlin, J.L. *Soil Fertility and Fertilizers*; MacMillan: New York, 1993; 785 pp.
48. Graham, J.H.; Abbott, L.K. Wheat Responses to Aggressive and Non-Aggressive Arbuscular Mycorrhizal Fungi. Plant Soil **2000**, *220*, 207–218.
49. Thompson, J.P. Correction of Dual Phosphorus and Zinc Deficiencies of Linseed (*Linum usitatissimum* L.) with Cultures of Vesicular-Arbuscular Mycorrhizal Fungi. Soil Biol. Biochem. **1996**, *28*, 941–951.
50. Li, X.-L.; George, E.; Marschner, H. Acquisition of Phosphorus and Copper by VA-mycorrhizal Hyphae and Root-to-shoot Transport in White Clover. Plant Soil **1991**, *136*, 49–57.
51. Bürkett, B.; Robson, A. ^{65}Zn Uptake in Subterranean Clover (*Trifolium subterraneum* L.) by Three Vesicular-arbuscular Micorrhizal Fungi in a Root-Free Sandy Soil. Soil Biol. Biochem. **1994**, *26*, 1117–1124.
52. Graw, D. The Influence of Soil pH on the Efficiency of Vesicular-Arbuscular Mycorrhiza. New Phytol. **1979**, *82*, 687–695.
53. Raju, P.S.; Clark, R.B.; Ellis, J.R.; Maranville, J.W. Effects of VA Mycorrhizae on Growth and Mineral Uptake of Sorghum Grown at Varied Levels on Soil Acidity. Commun. Soil Sci. Plant Anal. **1988**, *19*, 919–931.
54. Medeiros, C.A.B.; Clark, R.B.; Ellis, J.R. Growth and Nutrient Uptake of Sorghum Cultivated with Vesicular-Arbuscular Mycorrhiza Isolates at Varying pH. Mycorrhiza **1994**, *4*, 185–191.
55. Nurlaeny, N.; Marschner, H.; George, E. Effects of Liming and Mycorrhizal Colonization on Soil Phosphate Depletion and Phosphate Uptake by Maize and Soybean Grown in Two Tropical Acid Soils. Plant Soil **1997**, *192*, 63–68.