academicJournals

Vol. 7(26), pp. 3331-3340, 25 June, 2013
DOI: 10.5897/AJMR2012.2363
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Interactions between native arbuscular mycorrhizal fungi and phosphate solubilizing fungi and their effect to improve plant development and fruit production by *Capsicum annuum* L.

Claudia Castillo[1,2], Alfredo Morales[2,3], Rosa Rubio[2,3], José Miguel Barea[4]* and Fernando Borie[2,3]

[1]Escuela de Agronomía, Facultad de Recursos Naturales, Universidad Católica-Temuco, Chile.
[2]Center of Amelioration and Sustainability of Volcanic Soils, BIOREN_UFRO, Universidad de La Frontera, Temuco, Chile.
[3]Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile.
[4]Estación Experimental del Zaidín, Granada, España.

Accepted 22 May, 2013

A study was performed to determine the effect of arbuscular mycorrhizal (AM) fungal inoculation, using a locally isolated *Claroideoglomus claroideum* (Gc) ecotype, on the seedling development of chilean pepper plants, and to select an appropriate growth substrate. The first experiment consisted of two stages: (i) a seedling production stage of pepper plants were germinated and grown, inoculated or not with *C. claroideum*; and (ii) a seedling transplanting stage, in which seedlings were transplanted to a wider capacity containers, using the same substrates as in the seedling production stage. These were inoculated or not with *C. claroideum* and/or *Penicillium albidum* (Pa). A soil mixture was selected as the target substrate for further studies. A second experiment was then carried out and three new phosphate-solubilizing *Penicillium* that is, *Penicillium frequentans*, *Penicillium jensenii* and *Penicillium restrictum*, were incorporated as inoculation variables, in addition to the already tested *P. albidum,* inoculated either alone or in co-inoculation with *C. claroideum.* After 28 weeks, plants were harvested and fruit number, weight, and length were recorded. A synergistic interaction between *C. claroideum* and *P. albidum* to improve fruit weight and phosphorous (P) concentration was evidenced, suggesting a sustainable alternative for chilean pepper production.

Key words: *Penicillium,* saprophytic fungi, *Claroideoglomus claroideum*, phosphorus, substrate.

INTRODUCTION

An increasing demand for low-input agriculture has promoted the interest on prospective and application studies of the subset of soil microorganisms known to improve plant growth and health (Willis et al., 2013). Actually, certain beneficial microbial activities can be exploited, as a low-input biotechnology, to benefit sustainable developments in agriculture (Jeffries and Barea, 2012). Particularly relevant to these concerns are the microbiologically mediated processes involved in nutrient cycling, as those responsible for increasing the phosphate availability in soil (Barea et al., 2008; Marschner, 2012). Two general types of processes have been described in this context: those promoting the solubilisation of unavailable P-sources in soils and those improving plant uptake of the P ions already available in the soil solution. The solubilisation/mineralization of

*Corresponding Author E-mail: josemiguel.barea@eez.csic.es, Tel: 34958181600.

unavailable P compounds is carried out by diverse saprophytic bacteria (including actinomycetes) and fungi (Borie et al., 1983; Franco-Correa et al., 2010; Khan et al., 2010), acting by diverse mechanisms (George and Richardson, 2008; Marschner, 2012). The other microbiologically-mediated mechanism involved in P cycling in the plant-soil system is based on improving plant uptake of already solubilised P ion, a characteristic function of arbuscular mycorrhizal (AM) fungi. The AM fungi colonize plant roots and develop an external mycelium which is a bridge connecting the plant with soil microhabitats. This structure explores phosphate ions from soil solution beyond the Pi-depletion zone surrounding the roots, to transfer them into the plant (Barea et al., 2008). It is well documented that AM fungi assist the plant in its uptake of mineral nutrients (other than P, like N) and water (Brundrett, 2002). In addition, the AM symbiosis also improves plant health through increased protection against environmental stresses including biotic, as derived from pathogen attack (López-Ráez et al., 2012) or abiotic, as caused by drought (Ruíz-Lozano et al., 2008), salinity (Oztekin et al., 2013), heavy metals (Azcón et al., 2009) or organic pollutants (Leyval et al., 2002). Additionally, AM associations improve soil structure through aggregate formation, necessary for appropriate soil quality (Curaqueo et al., 2011). Therefore, AM fungi are important in ecological agriculture because of the benefits they provide to the majority of cultivars and the conservation of the environment by acting as biofertilizers, bioprotectors and biocontrol agents (Jeffries and Barea, 2012).

With regard to those microorganisms able to release available P from sparingly soluble P-sources most published information refers to bacteria, but there are several reports concerning fungi (Morales et al., 2007; Barroso and Nahas, 2007), with *Penicillium* and *Aspergillus* spp. as the more often described genera (Silva Filho et al., 2002; Morales et al., 2007). Inoculation with phosphate-solubilizing fungi enhanced plant growth and increased P uptake by the plants, both under greenhouse and field conditions (Asea et al., 1988; Whitelaw, 2000; Wakelin et al., 2004; Morales et al., 2007). Furthermore, synergic interactions between free-living phosphate-solubilising fungi and mycorrhizas to benefit plant growth and P acquisition have been described (Osorio and Habte, 2001; Barea et al., 2008). These studies support the use of such mycorrhizosphere associations as biological substitutes for phosphate-based mineral fertilizers. However, this is a research topic demanding further attention.

Due to difficulties in the massive production of mycorrhizal inocula the incorporation of AM technology to crop production systems is more feasible for those requiring a nursery stage (Gianinazzi and Vosatka, 2004). A characteristic example on this context is the case with horticultural systems where AM inoculation has been challenged for diverse crops (Azcón-Aguilar and Barea, 1997), including chili pepper, *Capsicum annuum* L. (Castillo et al., 2009a, b), a traditional crop having a great interest in Chile, which will be the target of inoculation experiments that are described here.

Chili pepper production is quite relevant in Chile where around 5,000 ha are cultivated and about nine thousand tons of pepper fruit are marketed each year. Particularly, the Chilean Araucanía Region offers favourable growth conditions, especially for local ecotypes that small-scale indigenous Mapuche farmers have been growing for decades. The fruits are used mainly in the production of "merkén", an ancestral condiment of great cultural and economic value. The variety "Cacho de Cabra" accounts for approximately 1,500 tons of the local cultivars (Castillo et al., 2009a). Given the economic and cultural importance of this local cultivar, a series of experiments are being carried out to investigate the impact of AM inoculation on its growth and development (Castillo et al., 2009a, b). However, the effect of dual inoculation with AM and saprophytic phosphate-solubilizing fungi on pepper crops has never been investigated. Accordingly, an experiment was designed with two main aims: (1) To assess the effect of such a dual microbial inoculation, using locally isolated fungi, on the development and fruit production by this local cultivar of chilean pepper plants; and (2) to gain information on the interaction between these symbiotic and saprophytic fungi, involved in P cycling and capture, as a general topic of interest in mycorrhizosphere research. The results of this experiment are reported and discussed here in the context of basic mycorrhizosphere research, and as a strategy for its application from both the economic and cultural point of view.

MATERIALS AND METHODS

Two sequential and related experiments were carried out (Experiments 1 and 2). Both of them have in common: (i) the test plant, an autochtonous high-value local pepper variety of *C. annuum* L. (*cv.* "Cacho de Cabra"); (ii) the AM fungal inoculum, based on the native fungus *Glomus claroideum* (Gc) actually *Claroideoglomus claroideum* (Oehl et al., 2011) and (iii) the native phosphate solubilizing saprophytic fungus *Penicillium albidum*, but additional phosphate-solubilizing fungal strains were also tested in Experiment 2.

Isolation and inocula preparation

The AM fungus *C. claroideum* was isolated from a soil from La Araucania Region, Chile where Mapuche farmers cultivate peppers following an ancestral system based on small scale organic cultivation (Castillo et al., 2009a, b). For inoculum production three substrata that is, volcanic scoria, a soil mixture and perlite (as described in Experiment 1) were assayed. Maize and tagetes were used as host plants. After six month of plant growth, the inoculum containing spores, hyphae and AM root fragments was harvested. In the case with the soil mixture, this inoculum cantained 57 spores per g of dry soil on average. A 10 % v/v of this inoculum was used in further experiments.

The phosphate solubilizing saprophytic fungus *P. albidum,* and *Penicillium frequentans*, *Penicillium jensenii* and *Penicillium restrictum* also tested in Experiment 2, were isolated from volcanic ash-derived soils from La Araucanía region, Chile (Morales et al., 2011). *Penicillium albidum* was isolated from an Ultisol serie Vilcún, where wheat was cropped under no-tillage. *Penicillium frequentans, P. jensenii* and *P. restrictum,* were isolated from an Andisol serie Freire where maize was cropped under organic farming. Rhizosphere soil samples were suspended in sterile distilled water to prepare serial dilutions. Appropriate dilution aliquot was spread onto agar-rose Bengal medium added with calcium phosphate or calcium phytate as either inorganic or organic P sources. The cultures were incubated for 5 days at 28°C. The development of a clear zone around the colony on the culture plates was taken as an index of phosphate solubilization. After that, those halo forming fungal colonies on the calcium phytate added medium were analysed for phosphatase secretion, using phenolftalein phosphate and ammonium vapours. The fungi were selected according to their capacity for phosphate solubilization and phosphatase production. For inocula formulation, the selected fungi were grown on Sabouraud dextrose broth at 20°C for 7 days in dark using an orbital shaker system. The fungal biomass was filtered, washed and dispersed using a blender. Fungal inoculum suspen-sions were then prepared to be inoculated at doses equivalent to: 1.3 x 10^6 C.F.U for *P. albidum*; 1.4 x 10^6 C.F.U. for *P. restrictum;* 1.4 x 10^6 C.F.U. for *P. frequentans*; and 1.5 x 10^6 C.F.U. *P. jensenii*, on a per plant basis.

Experiment 1. Substrate selection and production of quality seedling

This experiment involves the use of three substrates, that are, volcanic scoria (Sc); a soil mixture (S), and perlite (P). The characteristics of these substrata were as follows: the volcanic scoria, (< 5 mm), were taken from the Llaima volcano situated at La Araucanía Region (71º34`W; 38º41`S). There were young red-colored pyroclastic fragments having a high drainage capacity and a low density. The soil used for the soil mixture substrate was an Inceptisol from region (72º27`W; 38º34`S) mixed with sand and vermiculite in a proportion (by volume) of 7:2.5:0.5 (pH: 5.3; bulk density: 0.87; OMS: 7.6 %; Olsen-P: 1.6 mg kg^{-1}). The substrate was tindalized for 1 h during three consecutive days (Castillo et al., 2009b).

The experiment consisted of two stages: (i) a seedlings production stage where pepper plants were germinated and grown, inoculated or not with *C. claroideum*, in 100 mL containers; and (ii) a seedling transplanting stage, in which seedlings were transplanted to 2 L capacity containers, using the same substrates as in the seedling production stage. The transplanted seedlings were grown, either non-mycorrhizal (C) or mycorrhizal (Gc), and were also co-inoculated with the native phosphate solubilizing saprophytic fungus *P. albidum* (Pa). Plants grew under greenhouse conditions (16:8 h light/darkness; 25/15ºC day/night). Irrigation was carried out manually, maintaining a constant humidity by daily weighing. Time-course measurements were performed.

Experiment 2. Inoculation effect on plant development and fruit production in soil mixture

According to the results obtained in Experiment 1, the soil mixture was selected as the target substrate for this and other further studies. Three new phosphate-solubilizing *Penicillium* fungi, that is, *P. frequentans*, *P. jensenii* and *P. restrictum*, were incorporated as inoculation variables, in addition to the already tested *P. albidum.* These were inoculated either alone or in co-inoculation with *C. claroideum.* Plants were grown under the same conditions as in Experiment 1. After 17 weeks of plant growth, the number of leaves was counted and after 28 weeks, plants were harvested. Shoot height and root and shoot weight were measured. Fruits were separately harvested. Fruit number, weight, length were recorded and the P content determined.

Root sub-samples were cleared and stained with trypan blue (Phillips and Hayman, 1970) and assessed for percentage of mycorrhizal root length (Giovanetti and Mosse, 1980), using a stereoscopic system (Nikon SMZ-2B). The establishment of inoculated *Penicillium* spp. that remained colonizing the rhizosphere/ mycorrhizosphere of pepper plants was determined at harvest. Appropriate dilution aliquot was spread onto agar-rose Bengal medium and grown at 28°C for 5 days.

Statistical analysis

The data obtained were submitted to the Shapiro-Wilk normality test and then an ANOVA of one factor was carried out using the Duncan *a posteriori* multiple range means separation test ($P < 0.05$).

RESULTS

Substrate selection and production of quality seedling

Figure 1 summarizes data from the measurements of some morphological traits of chili pepper plantlets at the end of the seedling production stage. Inoculation with the native AM fungus *C. claroideum* improved significantly both height and weight of pepper seedlings growing in the three tested substrates (volcanic scoria, a soil mixture, and perlite). The soil mixture appears as the more favorable substrate for plant growth. These effects were further corroborated by transplanting the produced seedlings to 1 L containers. The time-course responses (plant height) of chili pepper plantlets growing either non-mycorrhizal or mycorrhizal, and co-inoculated or not with the native phosphate solubilizing saprophytic fungus *P. albidum* are recorded in Figure 2. These data showed that inoculation with *Penicillium* was not effective to improve plant growth. However, the AM fungal inoculation benefited plant development in the soil mixture and in the perlite substrata. According to the data summarized in Figures 1 and 2, the soil mixture was selected as the target substrate for further studies.

Inoculation effect on plant development and fruit production in soil mixture

As indicated before, in Experiment 2 three new phosphate-solubilizing *Penicillium* fungi, *P. frequentans*, *P. jensenii* and *P. restrictum*, were incorporated as inoculation variables, in addition to the already tested *P. albidum.* Data recording the microbial inoculation effects on some plant growth response variables are summarized in Figure 3 (leaf number) and 4 (weight). While AM application was effective to improve the tested growth response variables in most cases, the free-living fungi were not effective. None of the inoculated *Penicillium* strains benefited the growth improvement produced by AM inoculation. Moreover, *P. frequentans*, *P. jensenii* and *P. restrictum* diminished the growth improvement effect of *C. claroideum*.

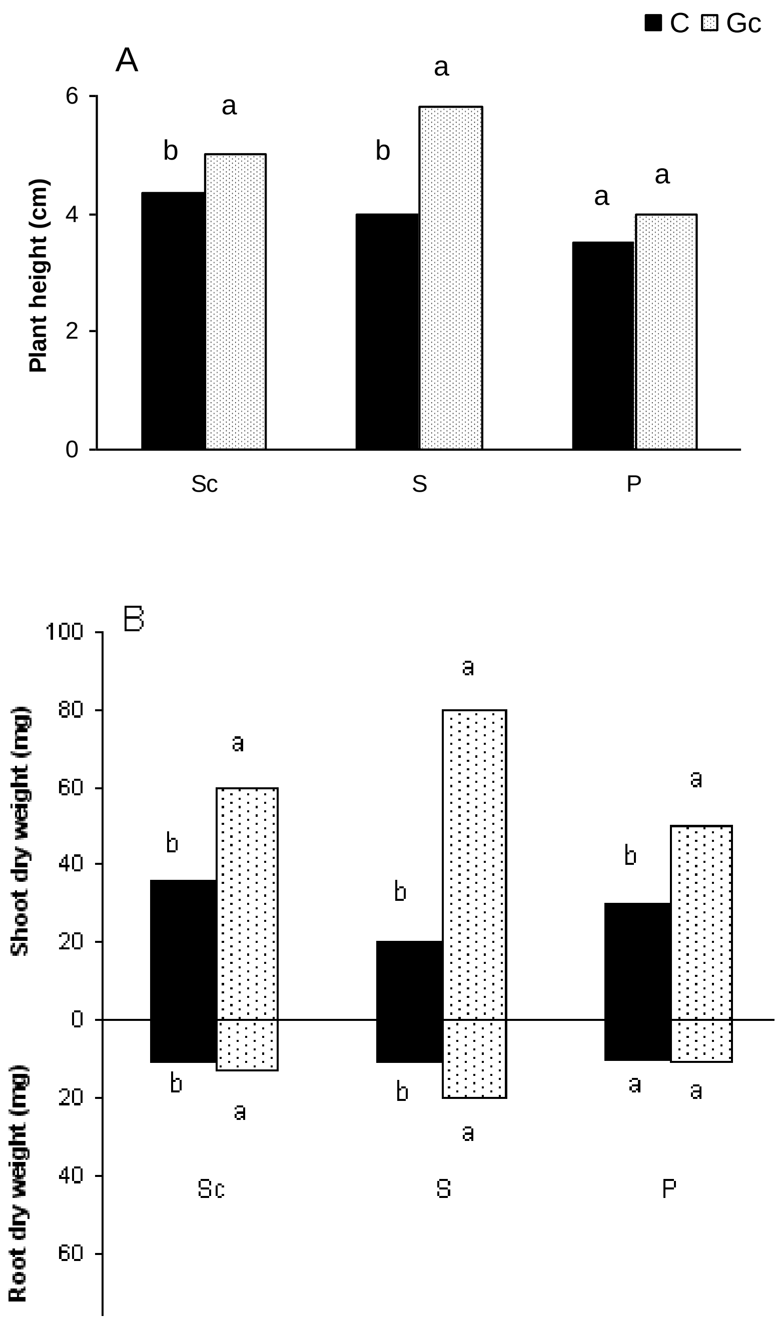

Figure 1. Height (A) and weight (B) of chili pepper plantlets, at the end of seedling production stage, as affected by the mycorrhizal treatment [inoculated with *Claroideoglomus claroideum* (Gc) or non-inoculated (C)] and by the growth substrate [volcanic scoria (Sc), a soil mixture (S) or perlite (P)]. For each response variable, columns not sharing a letter in common differ significantly according to the Duncan test ($P < 0.05$).

Figure 5 records the effects of the inoculation treatments on the production and characteristic of fruits by chili pepper plants. As in the case of the effects on plant growth, the inoculation of AM fungi was beneficial in most instances, but none of the *Penicillium* strains inoculated in non-AM plants produced any positive effect on fruit number. However, there was a synergistic interaction between *C. claroideum* and *P. albidum* to improve fruit weight. This beneficial co-operative effect can be related with an increase in fruit P concentration (Figure 6). It is noteworthy the enhancement of AM colonization level in chili pepper plants by the co-inoculation *P. albidum* and

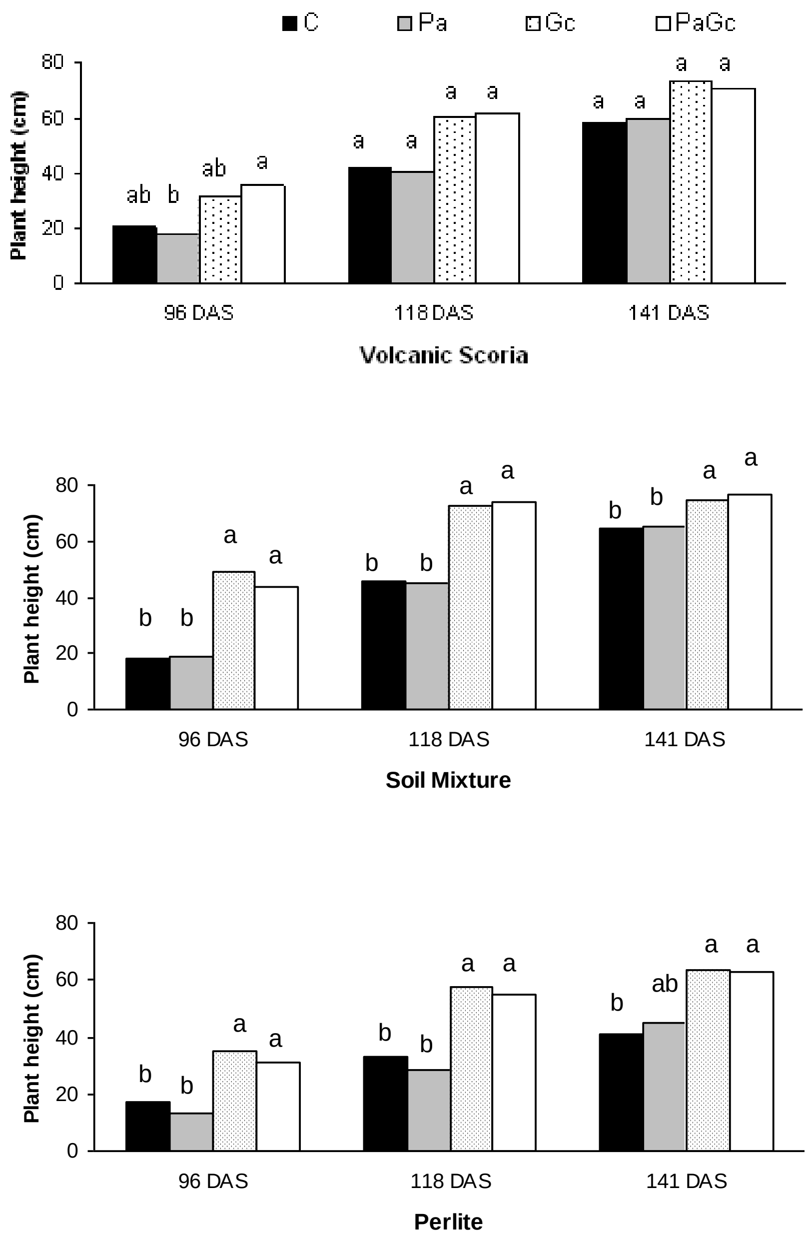

Figure 2. Time-course response (plant height) of chili pepper plants growing on the three tested substrates inoculated with either *Penicillium albidum* (Pa), *Claroideoglomus claroideum* (Gc), *Penicillium albidum* + *Claroideoglomus claroideum* (PaGc) or non-inoculated (C). DAS = days after sowing. For each substrate and sampling time, columns not sharing a letter in common differ significantly according to the Duncan test ($P < 0.05$).

P. frequentans (Figure 7). Inoculated *Penicillium* established in pepper rhizosphere/mycorrhizosphere at levels ranging from 3.10×10^5 to 9.90×10^4, but there were not statistically differences among treatments.

DISCUSSION

The aims of the experiments described in this article were to explore the feasibility of an appropriate management of beneficial soil microbes to reduce the use of chemicals and energy in agricultural production systems concerning a strategic crop for the region: a local cultivar of chilli pepper. The experimental approaches are based on sustainability issues, which try to reach a more economical and sustainable production while minimizing environmental degradation (Jeffries and Barea, 2012). Such biological interventions are becoming more attractive as the use of chemicals for fumigation and disease control is

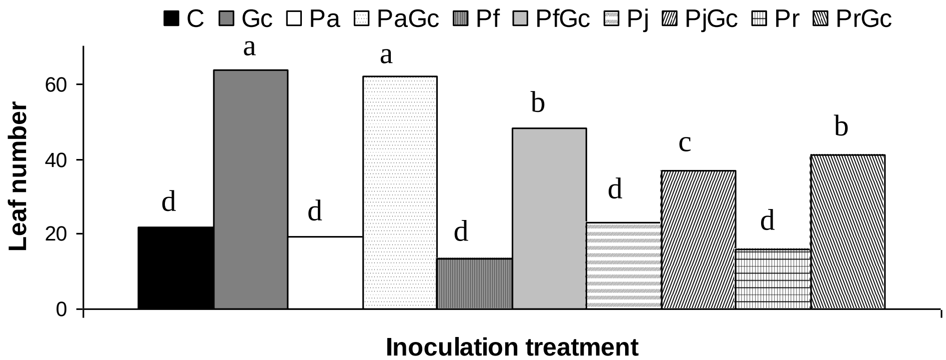

Figure 3. Leaf number of chili pepper plants growing for 17 weeks on the soil mixture inoculated with either *C. claroideum* (Gc), *P. albidum* (Pa), *P. frequentans* (Pf), *P. jensenii* (Pj), *P. restrictum* (Pr), with the dual corresponding co-inoculation treatments PaGc, PfGc, PjGc and PrGc, or non-inoculated (C). Columns not sharing a letter in common differ significantly according to the Duncan test (P < 0.05).

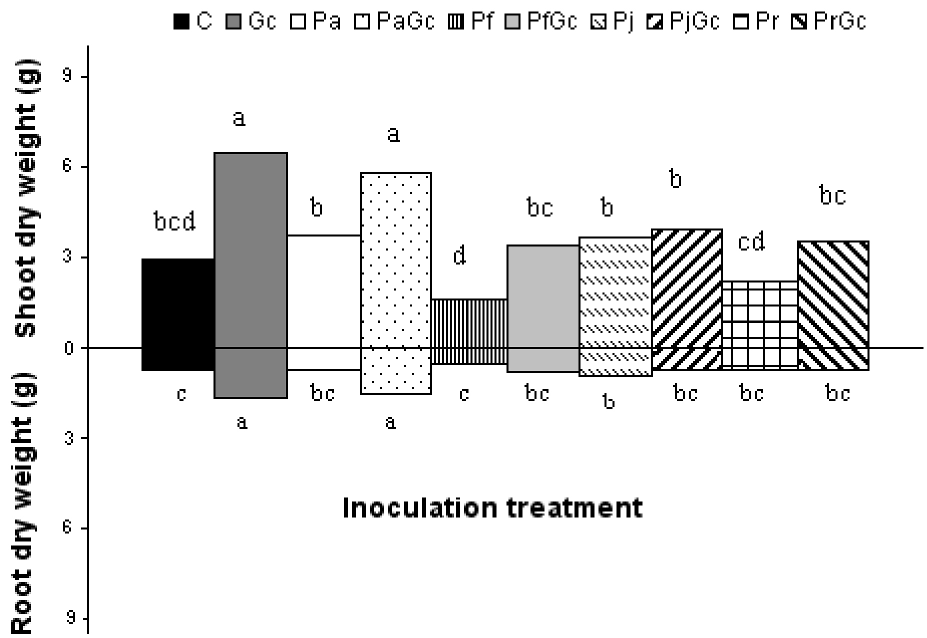

Figure 4. Dry weight at harvest of chili pepper plants grown for 28 weeks on the soil mixture substrate inoculated with either *C. claroideum* (Gc), *P. albidum* (Pa), *P. frequentans* (Pf), *P. jensenii* (Pj), *P. restrictum* (Pr), the dual corresponding co-inoculation treatments PaGc, PfGc, PjGc and PrGc or non-inoculated (C). Columns not sharing a letter in common differ significantly according to the Duncan test (P < 0.05).

progressively discouraged and fertilizers have become more and more expensive (Atkinson, 2009). Sustainable systems seek to maximize the beneficial effects of the natural soil microbiota, in general, however, there is a particular emphasis on AM fungi, phosphate-solubilising microorganisms and N_2- fixing rhizobial bacteria (Gianinazzi et al., 2010; Azcón and Barea, 2010).

Given the economic and cultural importance of the local cultivar of chili pepper "Cacho de Cabra", analysing the effect of dual inoculation of AM and saprophytic phosphate-solubilizing fungi on this strategic crop appeared as a tantalising idea. Accordingly, appropriate experiments were designed and carried out to test this hypothesis, as reported in this article. Among the information generated, we have considered that some of the research topics investigated deserve particular discussion: (i) production of AM fungal inoculum; (ii) production of quality seedlings of chilean pepper, as affected by microbial inoculation, and selection of a target substrate, compatible for both test plant and microorganisms; and

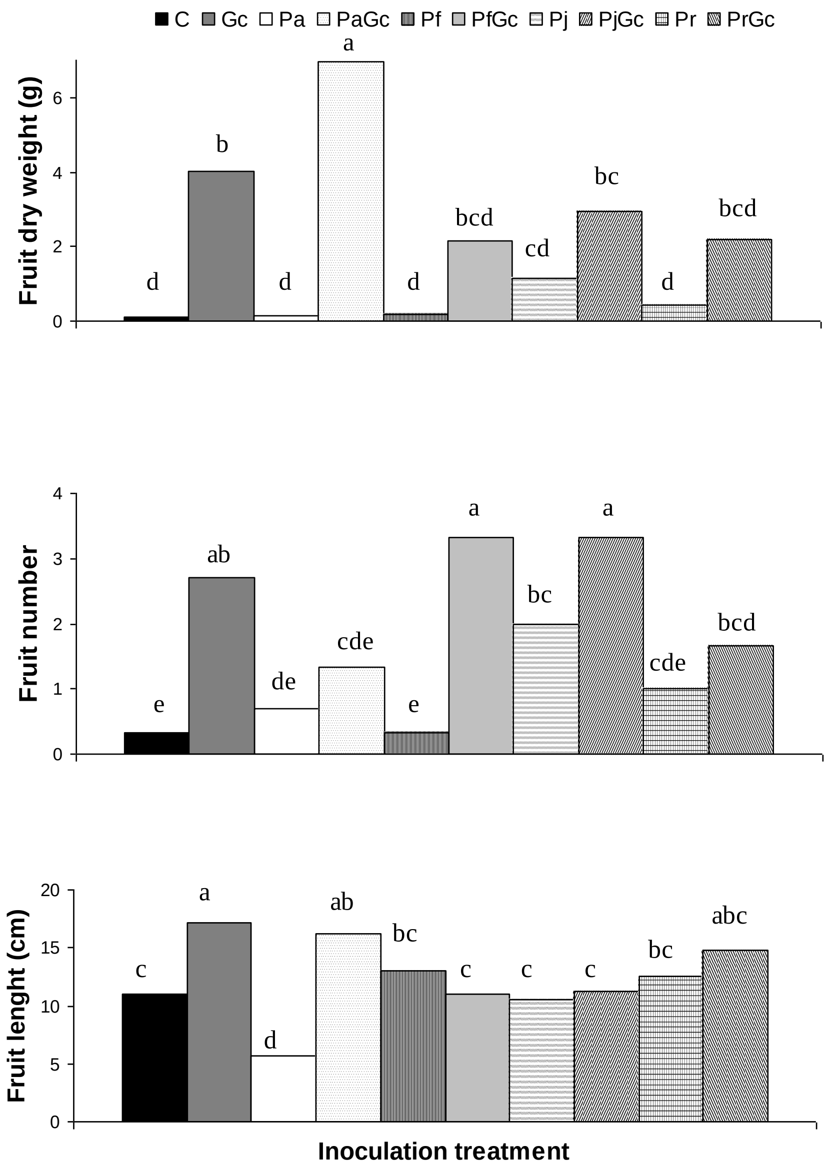

Figure 5. Fruit production by chili pepper plants grown for 28 weeks on the soil mixture substrate inoculated with either *C. claroideum* (Gc), *P. albidum* (Pa), *P. frequentans* (Pf), *P. jensenii* (Pj), *P. restrictum* (Pr), the corresponding dual co-inoculation treatments PaGc, PfGc, PjGc and PrGc or non-inoculated (C). Columns not sharing a letter in common differ significantly according to the Duncan test ($P < 0.05$).

(iii) the interactive effects of these microbial inoculants on plant development and fruit production by chilean pepper.

AM fungal inocula production

The unculturability of the AM fungi (obligate symbionts) continues to be a major barrier for inoculums production (Smith and Read, 2008), however, current agro-biotechnological approaches include the use of AM inoculants, usually relying on plant-based inocula (Gianninazi and Vosátka, 2004; Baar, 2008; IJdo et al., 2011). Recent developments in AM-inoculum production systems range from nursery plots (Koltai et al., 2008; Cuenca et al., 2008) to *in vitro* monoxenic root organ cultures (Bago and Cano, 2005; IJdo et al., 2011).

Selection of appropriate AM fungi and substrata is fundamental to produce high quality and effective inocula (Estaún et al., 2002). The use of locally isolated AM fungi is recommended because they are already adapted to the target soil-plant system and to prevailing conditions at the field site (Pelligrino et al., 2011). However, their effectiveness for the target host has to be unequivocally demonstrated under realistic conditions (Azcón-Aguilar and Barea, 1997).

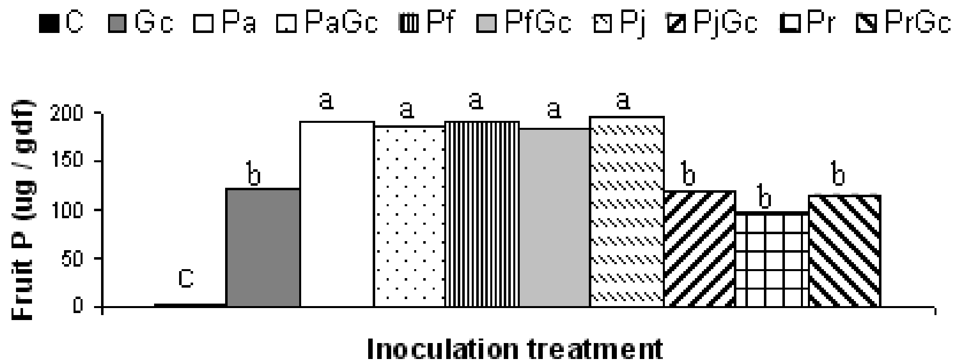

Figure 6. Concentration of P in the fruit of chili pepper plants grown for 28 weeks on the soil mixture substrate inoculated with either *C. claroideum* (Gc), *P. albidum* (Pa), *P. frequentans* (Pf), *P. jensenii* (Pj), *P. restrictum* (Pr), the corres-ponding dual co-inoculation treatments PaGc, PfGc, PjGc and PrGc or non-inoculated (C). Columns not sharing a letter in common differ significantly according to the Duncan test (P < 0.05).

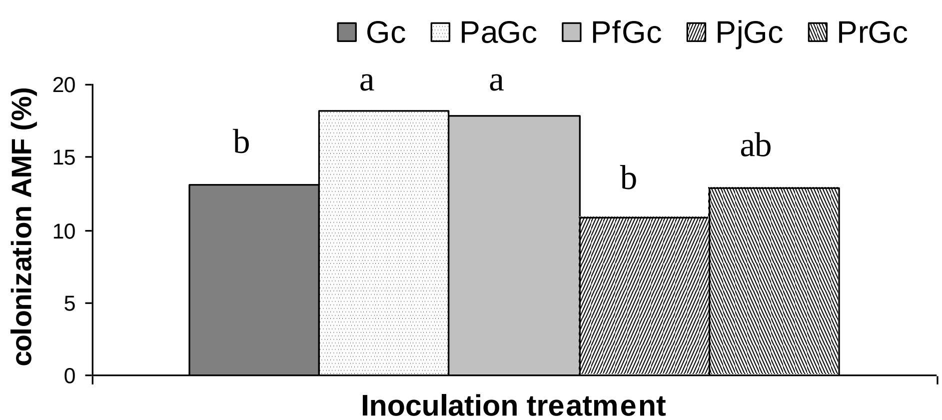

Figure 7. AMF root colonization in chili pepper plants grown for 28 weeks on the soil mixture substrate (S) inoculated with either *C. claroideum* (Gc), and the corresponding dual co-inoculation treatments *P. albidum* (Pa) Gc, *P. frequentans* (Pf) Gc, *P. jensenii* (Pj) Gc, *P. restrictum* (Pr) Gc. Columns not sharing a letter in common differ significantly according to the Duncan test (P < 0.05).

On the basis of these premises, the native AM fungus was used once its effectiveness for the target crop was tested (Castillo et al. 2009a, b). Three locally available substrates were evaluated all of them resulted appro-priate for inoculum production but that based on a soil mixture gave the higher yield of AM propagules, that is, spores, hyphae, root fragments among others. In spite, the use of soil-less substrates is usually recommended (Azcón-Aguilar and Barea, 1997; IJdo et al., 2011), at a relatively small-scale, such as nursery production or in many relatively small, low input systems, the use of soil mixtures for AM inoculation is feasible and advantageous (Sieverding and Barea, 1991; Azcón-Aguilar and Barea, 1997; IJdo et al., 2011). This is the case with the present study where transplanting is a part of the normal pro-duction system, as in many horticultural and plantation crops.

Production of quality seedlings, as affected by microbial inoculation, and selection of a substrate compatible for both test plant and microorganisms

Mycorrhizal inoculation appears critical for pepper seedling growth in the tested substrates. It is a self-evident and widely-described AM effect (Jeffries and Barea, 2012) that does not need further discussion. Particularly, the effect of AM inoculation in chili pepper has already been described (Castillo et al., 2009a, b). As in the case with AM inoculum production, the substrate based on the soil mixture seems the most permissive for both AM inoculum and seedling production. This was postulated also for other similar horticultural production systems (Sieverding and Barea, 1991; Azcón-Aguilar and Barea, 1997; IJdo et al., 2011). Accordingly, the soil mixt-ure was the selected substrate for further experimentation.

Inoculation with the phosphate-solubilizing fungus *P. albidum* was not effective to improve plant growth whether or not mycorrhizal. Probably, this lack of effectiveness could be due to short growth period since in longer experiments some inoculated saprophytic fungi were effectives. All in all, AM inoculation usually select a large and efficient population of phosphorus solubilizing microorganisms including fungi (Toro et al., 1997; Matias et al., 2009), but this was not tested in the present experiments.

The interactive effects of these microbial inoculants on plant development and fruit production by chilean pepper

The most relevant information is that AM application was effective to improve the tested growth response variables (leaf number, plant weight and fruit production) in chilli pepper in most cases. In the absence of AM inoculation, the free-living fungi were not effective to improve plant growth, as in other situation. None of the inoculated *Penicillium* strains benefited the growth improvement produced by AM inoculation.

However, the inoculation on P acquisition and the production of fruits (weight) by chili pepper plants was effective. These positive effects were described for other phosphate-solubilizing fungi, crops and cultural conditions (Singh and Reddy, 2011; Yadav et al., 2011). In addition, there was a synergistic interaction between AM fungi and *P. albidum* to improve fruit weight and P concentration in plant, as found for other phosphate-solubilizing fungi, crops and cultural conditions (Cabello et al., 2005; Huanshi et al., 2011). It is noteworthy that the enhancement of AM colonization level in chili pepper plants by the co-inoculation *P. albidum* and *P. frequentans*, as found in other situations (Huanshi et al., 2011). Persistence of inoculated phosphate-solubilizing saprophytic microorganisms in chili pepper rhizosphere/mycorrhizosphere (Toro et al., 1997) has a relevant agro-biotechnological interest.

Conclusion

The dual inoculation of the autochtonous high-local pepper variety of *C. annuum* L. with an AM fungi *C. claroideum* and a native phosphate solubilizing saprophytic fungus *P. albidum* performed a synergistic interacttion to improve fruit weight and quality. Of the three tested substrates: volcanic scoria, soil:sand mixture and perlite, the soil mixture was the most favorable substrate for chili pepper plant growth. These findings are especially interesting for Mapuchean local farmers which have been cultivating for many decades local ecotypes of chili pepper cv. Cacho de Cabra to make “merken”, an exotic condiment that it is consumed locally and exported. As this vegetable requires a nursery stage dual microbial inoculation would allow obtaining nutritional benefits to achieve a better transplanting adaptation especially under organic agriculture.

ACKNOWLEDGEMENTS

This research was supported by Grants DIUFRO DI08-0054 and Fondecyt 1070283, provided by Dirección de Investigación, Universidad de La Frontera and the Comisión Nacional Científica y Tecnológica, Chile, respectively.

REFERENCES

Asea PEA, Kucey RMN, Stewart JWB (1988). Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol. Biochem. 20: 459-464.

Atkinson D (2009). Soil microbial resources and agricultural policies. In Azcón-Aguilar et al. (eds) Mycorrhizas functional processes and ecological impact, Springer-Verlag, Berlin, Heidelberg, pp 33-45.

Azcón R and Barea JM (2010). Mycorrhizosphere interactions for legume improvement, In Khan et al. (eds) Microbes for Legume Improvement. Springer, Vienna, pp. 237-271.

Azcón R, Medina A, Roldán A, Biró B, Vivas A (2009). Significance of treated agrowaste residue and autochthonous inoculates (Arbuscular mycorrhizal fungi and *Bacillus cereus*) on bacterial community structure and phyto extraction to remediate heavy metals contaminated soils. Chemosphere. 75:327-334.

Azcón-Aguilar C and Barea JM (1997). Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci. Hort. 68: 1-24.

Baar J (2008). From production to application of arbuscular mycorrhizal fungi in agricultural systems: requirements and needs. In Varma (ed) Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. 3rd Ed. Springer-Verlag, Berlin, Heidelberg, pp. 361-373.

Bago B and Cano C (2005). Breaking myths on arbuscular mycorrhizas in vitro biology. In Declerck et al. (eds) In Vitro Culture of Mycorrhizas, vol 4. Soil Biology. Springer-Verlag, Berlin, Heidelberg, pp. 111-138.

Barea JM, Ferrol N, Azcón-Aguilar C, Azcón R (2008). The Ecophysiology of Plant-Phosphorus Interactions. Mycorrhizal Symbioses. Plant Ecophysiol. 7: 143-163.

Barroso CB and Nahas E (2007). Solubilization of hardly soluble iron and aluminum phosphates by the fungus *Aspergillus niger* in the soil. In Vélasquez et al. (eds) First international meeting on microbial phosphate solubilization. Developments in plant and soil sciences. vol.102. Springer, Dordrecht, pp. 193-198.

Borie F, Quinteros J, Aguilera M (1983). Bioquímica de suelos derivados de cenizas volcánicas. IV. Solubilización de fosfatos por hongos del suelo. Agric. Téc. 43: 371–376.

Brundrett MC (2002). Coevolution of roots and mycorrhizas of land plants. New Phytol. 154: 275-304.

Cabello M, Irrazabal G, Bucsinszky AM, Saparrat M, Schalamuk S (2005). Effect of an arbuscular mycorrhizal fungus, *Glomus mosseae*, and a rock-phosphate-solubilizing fungus, *Penicillium thomii*, on *Mentha piperita* growth in a soilless medium. J. Basic Microbiol. 45:182-189.

Castillo CG, Sotomayor L, Ortiz C, Leonelli G, Borie F, Rubio R (2009a). Effect of arbuscular mycorrhizal fungi on an ecological crop of chili peppers (*Capsicum annuum* L.). Chilean J. Agric. Res. 69:79-87.

Castillo CG, Ortiz C, Borie F, Rubio R (2009b). Respuesta de Ají (*Capsicum annuum* L.) cv. “Cacho de Cabra” a la inoculación con Hongos Micorrícicos Arbusculares. Información Tecnológica 20: 3-14.

Cuenca G, Cáceres A, González MG (2008). AM inoculation in tropical agriculture: field results. In Varma (ed) Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. 3rd Ed. Springer-Verlag, Berlin, Heidelberg, pp. 403-417.

Curaqueo G, Barea JM, Acevedo E, Rubio R, Cornejo P, Borie F (2011). Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agroecosystem in central Chile. Soil Till. Res. 113:11-18.

Estaún V, Camprubí A, Joner EJ (2002). Selecting arbuscular mycorrhizal fungi for field application. In Gianinazzi et al. (eds) Mycorrhiza technology in agriculture: from genes to bioproducts. Basel, Switzerland: Birkhäuser Verlag, pp. 249-259.

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea JM (2010). Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl. Soil Ecol. 45: 209-217.

George TS and Richardson AE (2008). Potential and limitations to improving crops for enhanced phosphorus utilization. In White et al. (eds) Ecophysiology of plant–phosphorus interactions. Springer, Dordrecht, pp 247-270.

Gianinazzi S and Vosatka M (2004). Mycorrhizal inocula for agricultural systems: achievements and perspectives. Can. J. Bot. 82: 1264-1271.

Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010). Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20: 519-530.

Giovanetti M and Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Huanshi Z, Xianghua W, Gang L, Pei Q (2011). Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol. Fertil. Soils 47: 543-554.

IJdo M, Cranenbrouck S, Declerk S (2011). Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21:1-16.

Jeffries P and Barea JM (2012). Arbuscular mycorrhiza - a key component of sustainable plant-soil ecosystems. In The Mycota, vol IX. Fungal Associations, 2nd edition. (Hock, B. Ed.) Springer-Verlag, Berlin, Heidelberg (in press).

Khan K, Zaidi A, Ahemada M, Ovesa M, Wani PA (2010)**.** Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch. Agron. Soil Sci. 56: 73-98.

Koltai H, Meier D, Shlomo E, Resnik N, Ziv O, Wininger S, Ben-Dor B, Kapulnik Y (2008). Exploiting arbuscular mycorrhizal technology in different cropping systems under greenhouse conditions in semi-arid regions. In Kubota et al.(eds). Book Series. Acta horticulturae. Proceedings of the International workshop on greenhouse environmental control and crop production in semi-arid region. ISHS published, Tucson, AZ, USA, pp. 223-228.

Leyval C, Joner EJ, del Val C, Haselwandter K (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In Gianinazzi et al. (eds) Mycorrhizal Technology in Agriculture. Birkhäuser Verlag, Basel, Switzerland, pp. 175-186.

López-Ráez JA, Bouwmeester H, Pozo MJ (2012). Communication in the rhizosphere, a target for pest management. In Lichtfouse (ed) Agroecology and Strategies for Climate Change. Springer, volume 8, pp 109-133.

Marschner H (2012). Mineral nutrition of higher plants (3rd edn.). Academic Press, London.

Matias SR, Pagano MC, Muzzi FC, Oliveira CA, Carneiro AA, Horta SN, Scotti MR (2009). Effect of rhizobia, mycorrhizal fungi and phosphate-solubilizing microorganisms in the rhizosphere of native plants used to recover an iron ore area in Brazil. European J. Soil Biol. 45: 259–266.

Morales A, Alvear M, Valenzuela E, Rubio R, Borie F (2007). Effect of inoculation with *Penicillium albidum*, a phosphate-solubilizing fungus, on the growth of *Trifolium pratense* cropped in a volcanic soil. J. Basic Microbiol. 47: 275–280.

Morales A, Alvear M, Valenzuela E, Castillo CG, Borie F (2011). Screening, evaluation and selection of phosphate-solubilising fungi as potential biofertiliser. J. Soil Sci. Plant Nutr. 11: 89-103.

Oehl F, da Silva GA, Sánchez-Castro I, Goto BT, Maia LC (2011). Revision of *Glomeromycetes* with entrophosporoid and glomoid spore formation with three new genera. Mycotaxon. 117: 297-316.

Osorio NW and Habte M (2001). Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in an oxisol. Arid Land Res. Manage. 15: 263–274.

Oztekin GB, Tuzel Y, Tuzel IH (2013). Does mycorrhiza improve salinity tolerance in grafted plants? Sci. Hortic. 149: 55-60.

Pelligrino E, Bedini S, Avio L, Bonari E, Giovannetti M (2011). Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. Soil Biol. Biochem. 43:367-376.

Phillips JM and Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transac. British Mycol. Soc. 55: 158-161.

Ruíz-Lozano JM, Porcel C, Aroca R (2008). Evaluation of the possible participation of drought-induced genes in the enhanced tolerance of arbuscular mycorrhizal plants to water deficit. In Varma (ed). Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. Springer-Verlag, Berlin, Heidelberg, pp. 185-207.

Sieverding E and Barea JM (1991). Perspectivas de la inoculación de sistemas de producción vegetal con hongos formadores de micorrizas VA. En Olivares et al. (eds) Fijación y Movilización Biológica de Nutrientes. Colección Nuevas Tendencias. Vol. II CSIC, pp. 221-245.

Silva Filho GN, Narloch C, Scharf R (2002). Solubilização de fosfatos naturais por microorganismos isolados de cultivos de *Pinus* e *Eucalyptus* de Santa Catarina. Pesqui. Agropecu. Bras. 37: 847-854.

Singh H and Reddy MS (2011). Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. European J. Soil Biol. 47: 30-34.

Smith SE and Read DJ (2008). Mycorrhizal Symbiosis, 3rd ed. Academic Press, Elsevier, New York, 787 pp.

Toro M, Azcón R, Barea JM (1997). Improvement of arbuscular mycorrhizal development by inoculation with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. Appl. Environ. Microbiol. 63: 4408-4412.

Wakelin SA, Warren RS, Harvey PR, Ryder M (2004). Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol. Fertil. Soils 40: 36–43.

Whitelaw MA (2000). Growth promotion of plant inoculated with phosphate-solubilizing fungi. In Sparks (ed). Advances in Agronomy, Academic Press 69: 99–151.

Willis A, Rodrigues BF, Harris PJC (2013). The ecology of arbuscular mycorrhizal fungi. Crit. Rev. Plant Sci. 32: 1-20.

Yadav J, Verma JP, Tiwari KN (2011). Plant growth promoting activities of fungi and their effect on chickpea plant growth**.** Asian J. Biol. Sci. 4: 291-299.