

# Mycobization as a biotechnological tool: a challenge

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## ABSTRACT

Mycobization involves the inoculation of agronomically important crops with different functional groups of fungi. Taking into consideration that phosphorus (P) is an essential nutrient for plant growth and that most soils show a deficit in assimilable P, in this chapter we will evaluate the interactions between fungi able to solubilize P from insoluble sources such as phosphoric rock, and fungi able to mobilize known phosphates such as arbuscular mycorrhiza-forming fungi. Mycobization represents a low-cost technological tool that does not have a negative impact for systems of intensive use such as organic orchards.

## INTRODUCTION

At the beginning of the twentieth century, agricultural products provided food to a world population of about 1200 million people, whereas nowadays they do for a population of about 6000 million. Due to the increasing population growth rate -which is about 60 million people/year-, by 2020 the population will be around 8000 million. Some projections indicate that by then the world production of the main crops will have to be increased by about 700 million tons. The speed of these changes leads to the need to pay attention to the consequences brought about by this demand, such as the massive use of agrochemicals, which, in turn do not allow the maintenance of soil productivity.

The stability and productivity of an agroecosystem or of a natural system depend, to a large extent, on the quality of the soil, not only to obtain profitable crops, which guarantee the sustainability of the agroecosystem, but also to minimize the environmental impact [1].

In the past, much attention was paid to investigate the physical and chemical components of the soil [1], whereas little attention was paid to its biological properties. However, in the last years, more studies have emphasized the importance of keeping a diverse and active microbial community [2, 3], in order both to guarantee the quality of the soil and to obtain greater amounts of economical and healthy foods [4].

Microorganisms populations are involved in a net of interactions that affect soil-plant development, and some of the beneficial activities of the microorganisms can be exploited as low-cost biotechnological tools to solve sustainability problems [2]. The development reached by means of these biotechnological techniques has opened new possibilities to produce a wide range of different products. Currently, there are applications related with human health, feeding and agriculture, and industrial and mining processes, which take care and preserve the environment.

Among beneficial soil organisms, we can mention arbuscular mycorrhiza fungi that belong to the phylum Glomeromycota [5], which form a universal symbiosis established with more than 80% of plant species, including most agricultural and horticultural crops, and herbaceous and arbustive species in natural ecosystems [6].

Arbuscular mycorrhizal fungi are key components in all ecosystems, not only because of the biomass they provide to the soil, but also because they are closely related to processes that take place in the rhizosphere, establishing synergic relationships with other microorganisms that benefit the plant. They act actively in the uptake and translocation of nutrients, especially of phosphorus, benefiting both plant growth and production. In addition, they provide greater tolerance to pathogens, thus contributing to the plant's health. They decrease the incidence of diseases due to changes in the immunological system and to a better nutritional state [7]. As a result, mycorrhized plants frequently exhibit an increase in the growth, production and survival as compared to non-mycorrhizal ones.

Considering all the beneficial aspects mentioned, arbuscular mycorrhizal fungi are potentially useful as inoculants to achieve a sustainable production, and substantially decrease the need for agrochemicals and guarantee the ecological equilibrium.

At a commercial scale, important benefits can be derived from their use, including a better plant growth and greater production, an improvement in the uniformity of the crop, a reduction in phosphorous fertilization, a decrease in root damage, and a reduction in harvest time and environmental stress.

## POTENTIAL HOST PLANTS

As previously mentioned, arbuscular mycorrhizal fungi have an extremely wide range of host plants, although not all plants present the same degree of mycorrhizal dependence. Janos [8] defined mycorrhizal dependence as “the inability of plants without mycorrhizas to grow or survive without some increase in soil fertility”.

Mycotrophic plants differ in their colonization percentages. Some crops, such as sweet potato, soybean, maize, sorghum, barley, sugarcane, tobacco, cotton, and cacao, frequently exhibit high colonization rates under natural conditions. Other crops such as wheat, beans, coffee and tomato can have more moderate colonization rates. In addition, some small intraspecific differences can be observed in the colonization percentages between different ecotypes, cultivars or clones of the same crop. Additionally, the environmental conditions also affect the formation of arbuscular mycorrhizas and/or the root extent. For instance, crops with high supplies of fertilizers rich in nitrogen, phosphorus and potassium, inhibit mycorrhizal development.

Although most agricultural crops are potential host plants for arbuscular mycorrhizal fungi, their use as biofertilizers is limited by several reasons: one of them is that most agricultural soils present a varied and abundant mycorrhizal biota, generally adapted to the crop's condition, which can compete until eliminating the biological inoculant added. Due to the large extensions of cultivated areas, in order to guarantee a successful colonization, it is necessary to apply a great amount of inoculant to prevent its dilution; besides, as previously mentioned, it should be considered that if the system has important supplies of phosphorus, this will either decrease or inhibit the colonization.

Instead, their use is mainly restricted to horticultural crops and to diverse plants that either propagate by stolons or micropropagate -aromatic and ornamental plants- and share one characteristic: they go through a pot period where the inoculation strategies are simpler, environmental variables can be controlled, and a starter substrate completely free of microorganisms can be selected in order to guarantee a successful colonization.

## THE USE OF PHOSPHORUS IN THE SOIL

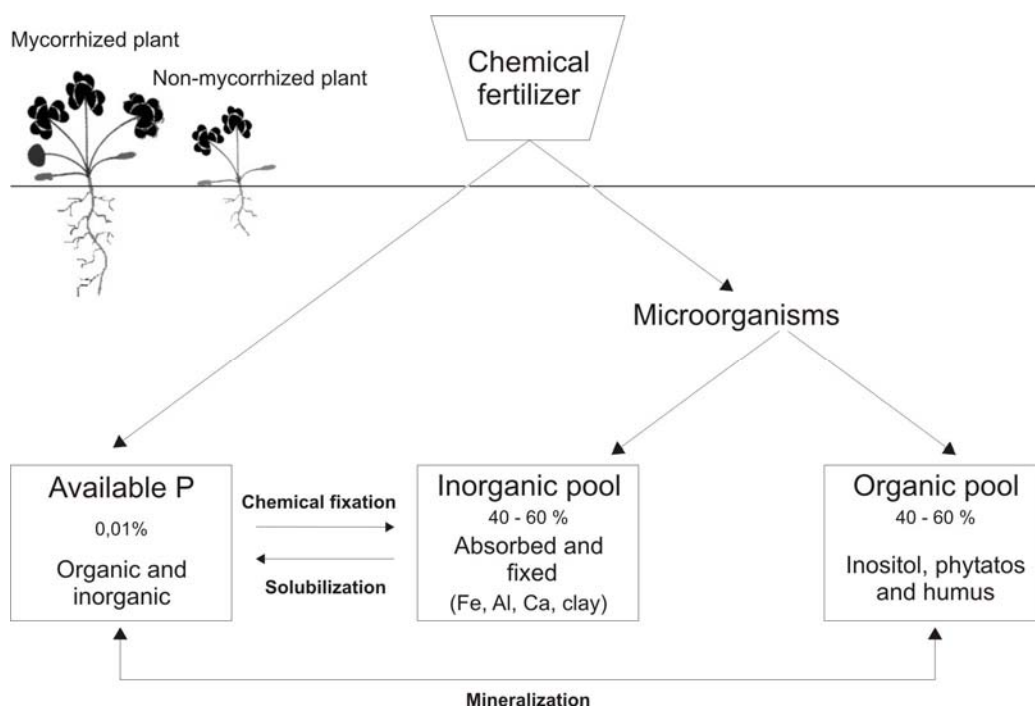
Phosphorus is the most important plant growth-limiting nutrient in soil besides nitrogen. It is estimated that 5.7 billion hectares in the world do not contain the necessary amounts of available phosphorus to sustain the productivity of their crops [9].

Phosphates in the soil are present under three forms: 1) soluble inorganic phosphorus in the soil solution, 2) insoluble inorganic phosphorus in crystalline nets, and 3) phosphorated organic compounds such as phytate [10]. They are relatively immobile and diffuse slowly to the plant roots [11] and capture phosphorus at a greater speed than that at which the nutrient diffuses. In soils with a low availability of phosphorus, this generates areas of depletion around the roots [12], which is a critical problem for the uptake and cycling of nutrients in soil-plant systems [5]. Here is where the mycorrhizal activity is centered, since the external hyphae can explore a higher volume of the soil, overcoming the depletion areas (Fig. 1).

It is important to point out that both mycorrhized and non-mycorrhized roots absorb phosphorus from the same pool available in the soil [13, 14, 15]. Mycorrhizal fungi are not able to solubilize the non-available forms of phosphorus, but can increase its absorption by providing them a greater exploration of the soil through the external mycelium. On the other hand, mycorrhizal hyphae have a higher affinity for phosphate, as expressed in the Michaelis-Menten equation by a lower  $K_m$  value, and absorb phosphorus at lower solution concentrations

than roots do [16].

As regards phosphate cycling, two general types of microbiological processes have been described: 1) those that promote the solubilization of non-soluble phosphorus sources in the soil, and 2) those that increase the uptake of soluble phosphates around the plant. The former is carried out by solubilizer microorganisms, among which there is a great number of phosphate-solubilizing bacteria [17], actinomycetes [18] and fungi [19]. These organisms are capable of solubilizing the phosphate content in minerals by means of secretion of carboxylic acids, enzymes and bacterial mucilage [19, 20, 21]. The second mechanism is carried out by means of mobilizing microorganisms, which are basically arbuscular mycorrhiza-forming fungi [7].



**Fig. 1** Phosphorus cycle in the soil. The values are estimative percentages in the mineral soil.

## INTERACTIONS IN THE MYCORRHIZOSPHERE

In the soil, the populations of microorganisms concentrate around the roots, stimulated by root excretions and exudates. In this area defined as the rhizosphere [22, 23, 24, 25], there is an increase in the activity of microorganisms such as bacteria, saprotrophic fungi, pathogens, and symbionts, which are involved in a wide range of important functions involved in plant growth. The development of the rhizosphere is a dynamic process that incorporates physical, chemical and biological modifications that occur in the root-soil interphase. An important consequence of the increase in microbial activity of the rhizosphere derives from the ability of microorganisms to alter the availability of nutrients for the plant.

The interactions that occur at the soil-root interphase are essential to understand the dynamics of the processes that are characteristic of the rhizosphere [26, 27]. A great number of microorganisms of the rhizosphere develop activities that alter the availability of nutrients and act synergistically with mycorrhizal fungi to increase plant growth. These interactions are critical because they regulate the formation and development of mycorrhizas, which, in turn, can affect the microbial population of the rhizosphere. This deserves special attention for the development of biotechnological techniques that make use of the microorganisms of the rhizosphere.

Below we will detail some of the most relevant interactions between arbuscular mycorrhizal fungi and microorganisms of the rhizosphere.

## Saprotrophic fungi

Saprotrophic fungi from the soil that inhabit in the rhizosphere interact with arbuscular mycorrhizal fungi in different ways, and can have either positive, negative or neutral effects in the establishment and later development of the symbiosis. Understanding this synergism is extremely important because mycobization -co-inoculation of selected strains of saprotrophic and mycorrhizal fungi- in agriculturally important host plants might favor faster root colonization and thus benefit plant nutrition. On the other hand, the selection of saprotrophic fungi with inhibitory effects on the mycorrhizal symbiosis may allow a greater control on the quality and purity of a mycorrhizal inoculum.

Numerous investigations have evaluated the interaction between mycorrhizal and saprotrophic fungi. However, there are only few studies about the synergistic effect between yeast and arbuscular mycorrhizas in spite they are two important components in the microbial communities of the soil. Singh et al. [28] studied the effects of inoculation with the commercial yeast *Saccharomyces cerevisiae* Hensen in leguminous plants (*Leucaena leucocephala*, *Glycine max*, *Cajanus cajan*, *Phaseolus mungo*, *P. aureus* and *Vigna unguiculata*) growing on a substrate with native arbuscular mycorrhizal fungi. They evidenced a significant increase in plant biomass, as well as an increase in the parameters of the mycorrhizal colonization, in the number of spores and the formation of arbuscules and vesicles in the presence of *S. cerevisiae*. Fracchia et al. [29], on the other hand, found that the yeast *Rhodotorula mucilaginosa* (Jörg) Harrison stimulated hyphal growth of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Gigaspora rosea* Nicol. & Schenk and that the exudates produced by *R. mucilaginosa* increase the colonization of soybean by *G. mosseae* and of red clover by *G. rosea*.

Fracchia et al. [30] also investigated different combinations between the saprotrophic *Fusarium oxysporum* Schlecht. Fr. and several species of arbuscular mycorrhizal fungi such as *G. mosseae*, *G. fasciculatum* Gerd. & Trappe, *G. intraradices* Schenk & Smith, *G. clarum* Nicol. & Schenk and *G. deserticola* Trappe, Bloss & Menge on agricultural (maize, sorghum and wheat) and horticultural (lettuce, tomato, lentil and pea) crops. They found that *F. oxysporum* influences the mycorrhizal colonization and the growth response in host plants, but that this capacity varies depending on the plant and mycorrhizal fungus studied. These results suggest a direct interaction between saprotrophic and mycorrhizal fungi.

## Phosphorus solubilizers

In the soil, there are also different microorganisms and other fungi capable of solubilizing phosphates, as well as of increasing the absorption of phosphorus in the plant [31].

Velázquez et al. [32] have proposed the term “Mycobization” to denominate the inoculation of plants with different functional groups belonging to the Kingdom Fungi, as is the case of the co-inoculation with solubilizing saprotrophs and mobilizing symbiotic biotrophs.

The combined inoculation of arbuscular mycorrhizal fungi and phosphorus-solubilizing microorganisms has allowed a better absorption of native phosphorus and of the phosphorus in the rock phosphate [33, 34]). Rock phosphate can be a fertilizer of effective agronomic use and low cost mainly in highly meteorized acid soils, since it provides phosphorus and calcium, two elements that are generally lacking in these soils [35, 36, 37]).

Numerous studies involve the use of rock phosphate as a source of phosphorus. For instance, Graham & Timmer [38, 39]) have evaluated the interaction of the arbuscular mycorrhizal fungus *G. intraradices* in different citrus trees (citrange, lemon rouge and orange sour), with different levels of superphosphate and rock phosphate. They demonstrated a more significant growth of the plants fertilized with rock phosphate, being this form of P more permanent and available for a longer time than the P supplied in the superphosphate.

On the other hand, Omar [40] evaluated the capacity of solubilizing P from rock phosphate of the geofungi *Aspergillus niger* van Tieghem and *Penicillium citrinum* Thom, in wheat plants inoculated with the mycorrhizal fungus *Glomus constrictum* Trappe. Positive effects were recorded in plant growth and content of phosphorus in those plants co-inoculated with *G. constrictum* and *A. niger* or *P. citrinum* and in those in which rock phosphate was used as the

phosphorus source.

In addition, increases have been recorded in the growth of wheat plants growing in a sandy substrate with a deficit in nutrients when inoculated with two phosphoric rock-solubilizing microorganisms -*Bacillus circulans* Jordan and *Cladosporium herbarum* Link: Fries- with a mycorrhizal fungus belonging to the *Glomus* genus [41].

The combination of rock phosphate, arbuscular mycorrhizal fungi and phosphorus-solubilizing fungi presents an interesting alternative to the use of fertilizers worldwide [32, 33, 42].

Due to the importance of microbial solubilization and phosphorus mobilization processes, Cabello et al [33] evaluated the effects of the co-inoculation of *G. mosseae* -a symbiont biotroph- and *P. thomii* Maire -a solubilizing saprotrophic fungus- in the growth of *Mentha piperita* L. plants and the percentage of root mycorrhizal colonization along two harvest times. This experiment involved a series of five treatments on perlite:vermiculite (inert substrate) with different fungal combinations, rock phosphate and non-inoculated controls. It was found that *P. thomii* neither affected nor stimulated the capacity of *G. mosseae* for colonizing roots (Table 1). The maximum growth in both non-inoculated plants and plants inoculated with *G. mosseae* and rock phosphate was observed after the first harvest (Table 2). These results are in agreement with those found by Graham & Timmer [38], where the maximum values of growth were recorded at the moment in which phosphorus was added to the medium. The fact that biomass increased in mycorrhized plants after the first harvest represents important economic and agro-economic benefits, since *M. piperita* is a perennial plant that can be harvested up to four times along its life cycle.

On the other hand, the phosphorus present in the plant tissues was greater after the first harvest in all the treatments. It was also observed that phosphorus was solubilized in both the mycorrhized and the non-mycorrhized root systems without the solubilizing fungus. This confirms that the abiotic factors can solubilize part of the P present in the system [43]. The roots can also contribute to the mobility and bioavailability of the phosphorus in the rhizosphere by means of the induction of areas of depletion/accumulation of phosphorus, acidification/alkalinization and exudation of organic acids/anions [9].

|              | R.P + Gm | R.P + Gm + Pt |
|--------------|----------|---------------|
| Colonization | 94 a     | 95 a          |
| Hyphae       | 27 a     | 23 a          |
| Arbuscules   | 31 a     | 64 a          |
| Vesicles     | 14.6 a   | 4 b           |

**Table 1.** Percentage of root colonization, hyphae, arbuscules and vesicles. Gm, *Glomus mosseae*; Pt, *Penicillium thomii*; RP, rock phosphate. Values are means  $\pm$  SD for three pots. The same letter above the bar indicates that values did not differ significantly between treatments according to ANOVA and LSD test ( $P \leq 0.05$ ). (Adapted from Cabello et al. [33]).

|                              | p:v   |       | p:v+RP |        | p:v+ RP +Gm |         | p:v+ RP +Pt |        | p:v+RP+Gm+Pt |        |
|------------------------------|-------|-------|--------|--------|-------------|---------|-------------|--------|--------------|--------|
|                              | I°    | II°   | I°     | II°    | I°          | II°     | I°          | II°    | I°           | II°    |
| Dry weight (g/plant)         | 2.14a | 1.90a | 5.84c  | 2.45a  | 4.42ab      | 6.54b   | 3.66b       | 4.72ab | 4.59bc       | 4.74ab |
| P substrate (mg/kgsubstrate) | 3.9a  | 3.75a | 12.2a  | 16.36b | 11.4a       | 12.74ab | 37.03c      | 18.26c | 64.83b       | 40.19b |

**Table 2.** Dry weight in plant and P content in substrate (I°: first harvest and II°: second harvest). p:v (perlite:vermiculita); RP (rock phosphate); Gm (*Glomus mosseae*) and Pt (*Penicillium thomii*). Values are means  $\pm$  SD for three pots. The same letter above the bar indicates that values did not differ significantly (Adapted from Cabello et al. [33])

Velázquez et al. [32] combined the use of rock phosphate as a source of phosphorus, *G. mosseae* as a mobilizing microorganism, and *Aspergillus niger* as a solubilizer, in *Lycopersicon esculentum*

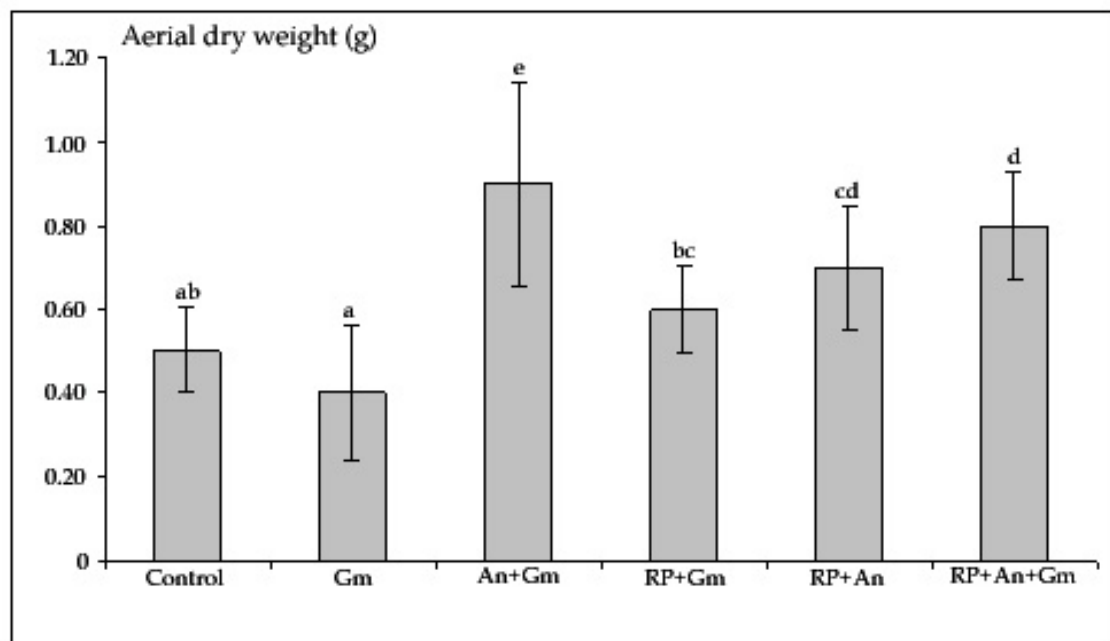
Mill plants. The experiments involved different treatments developed on a perlite:vermiculite:sand substrate (inert substrate) with different fungal combinations, rock phosphate and non-inoculated controls. They found that the number of *G. mosseae* spores was significantly different between treatments: the treatment inoculated with *G. mosseae* and without rock phosphate presented a higher number of spores, whereas the treatment inoculated with *G. mosseae* and rock phosphate evidenced the lowest value in the number of spores (Table 3). This result correlates with the nutritional deficit of the host plant, also reflected in the growth parameters evaluated and the low content of phosphorus in the aerial biomass. In conditions of nutritional stress, the flow of carbohydrates from the plant to the fungus decreases, and, as a consequence, sporulation is stimulated. Mycorrhizal colonization was positive in all the treatments inoculated with *G. mosseae*, although the colonization rates decreased when the content of P in the soil increased.

This negative correlation between the availability of phosphorus and the mycorrhizal colonization has been well documented [7]. A greater aerial biomass in the co-inoculated treatments, either with or without the application of rock phosphate has also been recorded (Fig. 2). The greater values of phosphorus concentrations in the substrate were obtained in the treatment where rock phosphate and the solubilizer *A. niger* was added, thus confirming the solubilizing capacity of this microorganism.

This work confirmed the synergistic effects between mycorrhiza-forming fungi and solubilizing microorganisms. It is also important to point out that the solubilizing capacity of the microorganisms tested *in vitro* does not always reflect the potential solubilizing capacity of these microorganisms in field conditions [19, 44].

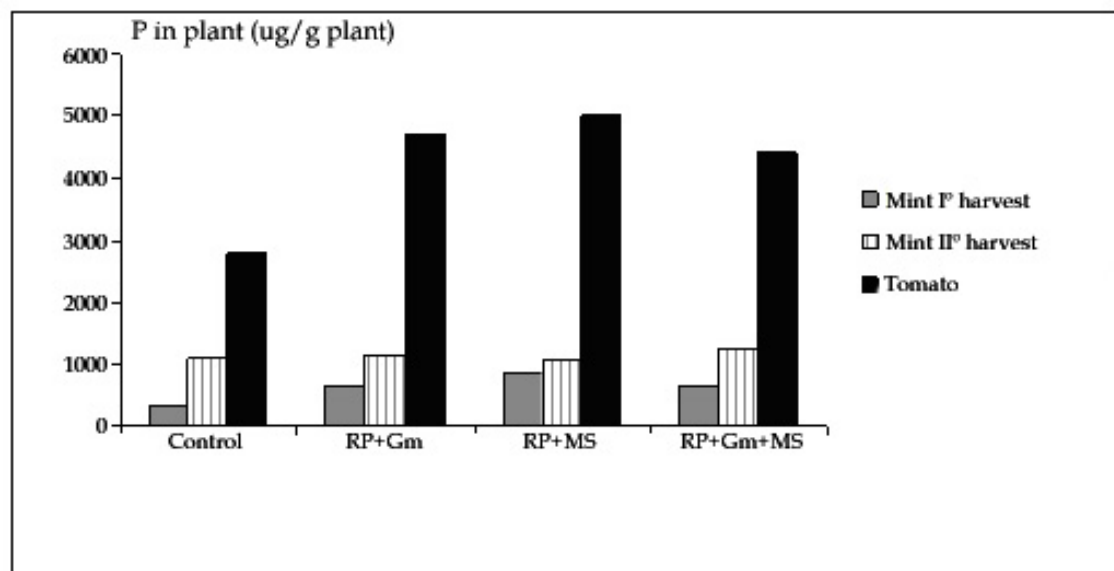
| <i>Treatment</i> | <i>N° of spores/100 g dry substrate</i> | <i>Percentage root colonized</i> |
|------------------|-----------------------------------------|----------------------------------|
| Control          | -                                       | -                                |
| Gm               | 1496 a                                  | 40% ab                           |
| An + Gm          | 738 b                                   | 50% a                            |
| RP + Gm          | 421 b                                   | 41% ab                           |
| RP + An          | -                                       | -                                |
| RP + An + Gm     | 598 b                                   | 32% b                            |

**Table 3.** Number of spores in 100 g dry substrate and percentage of root colonization. Gm, *Glomus mosseae*; An, *Aspergillus niger*; RP, rock phosphate. Treatment number between brackets. Values are means  $\pm$  SD for five pots. The same letter above the bar indicates that values did not differ significantly between treatments according to ANOVA and LSD test ( $P \leq 0.05$ ). (Adapted from Velázquez et al. [32].



**Figure 2.** Aerial dry weight in tomato plants. Gm, *Glomus mosseae*; An, *Aspergillus niger*; RP, rock phosphate. Treatment number between brackets. Values are means  $\pm$  SD for five pots. The same letter above the bar indicates that values did not differ significantly between treatments according to ANOVA and LSD test ( $P \leq 0.05$ ). (Adapted from Velázquez et al. [32]).

In both *M. piperita* - *G. mosseae* - *P. thomii* and *L. esculentum* - *G. mosseae* - *A. niger* systems, the synergism between P-mobilizing and P-solubilizing microorganisms allowed the increment of P content in both plants (Fig. 3). It can be observed that co-inoculation of *G. mosseae* - *A. niger* was more effective in increasing P contents in the plant than the co-inoculation with *G. mosseae* - *P. thomii*, for which it is important to point out that each soil-plant-fungus system requires special attention.



**Figure 3.** P content in *M. piperita* and *L. esculentum* plants. RP (rock phosphate), Gm (*G. mosseae*), MS (solubilizing microorganism). (Adapted from Cabello et al. [33], Velázquez et al. [32]).

## FINAL CONSIDERATION

Considering that P is an essential nutrient for plant growth and taking into consideration that most soils are deficient in assimilable phosphorus, this review highlights the most important

achievements related with the interactions between rhizospheric, P-solubilizing and P-mobilizing microorganisms and their potential as biofertilizers. Mycobization is a low-cost technological tool that does not have a negative impact on systems of intensive use such as organic orchards. However, further studies are necessary to evaluate the effects of mycobization in large-scale extensive crops in soils with a nutritional deficit under different agroclimatic conditions. In a first stage, we should evaluate the potentiality of the native microbiota in each environment in order to manage it by means of appropriate agronomical practices, without introducing foreign microorganisms, and thus be able to obtain a sustainable production and guarantee either a low or no environmental impact.

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## REFERENCES

- [1] Parr JF, Papendick RI, Hornick SB, Meyer RE. *American Journal Alternative Agriculture* 1992, 7:2-3.
- [2] Kennedy AC, Smith KL. *Plant Soil* 1995, 170:75-86.
- [3] Barea JM. In: *Plant growth-promoting rhizobacteria, present status and future prospects*, Ogoshi A, Kobayashi L, Homma Y, Kodama F, Kondon N, Akino S (eds.), OCDE, Paris, 1997, 150-158.
- [4] Dodd J. *Outlook on Agriculture* 2000, 29:63-70.
- [5] Schüßler A, Schwarzott D, Walker C. *Mycological Research* 2001, 105:1413-1421.
- [6] Barea JM, Azcon R, Azcon-Aguilar C. In: *Microorganisms in Soils: Roles in Genesis and Functions*, Buscot F, Varma A (eds.), Springer-Verlag, Berlin Heidelberg, Germany, 2005, 196-212.
- [7] Smith SE, Read DJ. *Mycorrhizal Symbiosis*. Academia Press London, UK, 1997.
- [8] Janos DP. *Mycorrhiza* 2007, 17:75-91.
- [9] Hinsinger P. *Plant and Soil* 2001, 237:173-195.
- [10] Tinker PBH. In: *Endomycorrhizas*, Sanders FE, Mosse B, Tinker PB (eds.), Academic Press, London, 1975.
- [11] Nye PH, Tinker PB. *Solute movement in the Soil-Water System*. Blackwell Scientific, Oxford, 1977.
- [12] Jeffries P, Barea JM. In: *Fungal associations*, Hock B (ed.), Springer, Berlin Heidelberg New York, 2001, 95-113.
- [13] Mosse B. *New Phytologist* 1973, 72:127.
- [14] Nelson CE, Bolgiano NC, Furutani SC, Safir GR, Zandstra BH. *Journal of the American Society for Horticultural Science* 1981, 106:786.
- [15] Joner EJ, Jakobsen I. *Plant and Soil* 1995, 172:221-227.
- [16] Lange Ness RL, Vlek PLG. *Soil Science Society of American Journal* 2000, 64:949-955.
- [17] Taha SM, Mahmoud SAZ, Halim El-Damaty A, Abd El-Hafez AM. *Plant and Soil* 1969, 31:149-160.
- [18] Rao AV, Venkateswarlu, Kaul P. *Current Science* 1982, 51:1117-1118.
- [19] Whitelaw MA. *Advances in Agronomy* 2000, 69:100-153.
- [20] Deubel A, Merbach W. In: *Microorganisms in Soils*, Buscot F, Varma A (eds.), Springer-Verlag, Berlin Heidelberg, Germany, 2005, 177-191.
- [21] Kucey RMN. *Applied and Environmental Microbiology* 1987, 53:2699-2703.
- [22] Bowen GD. In: *Contemporary Microbial Ecology*, Ellwood DC, Latham MJ, Hedger JN, Lynch JN, Slater JM (eds.), Academic Press, London, 1980, 283-304.
- [23] Foster RC, Bowen GD. In: *Phytopathogenic Prokaryotes*, Mount R, Lacey C (eds.), Academic Press, New York, 1982, 159-185.
- [24] Lynch JM. *Soil Biotechnology. Microbiological Factors in Crop Productivity*. Blackwell Scientific Publications, Oxford, 1983.
- [25] Curl EA, Truelove B. *The Rhizosphere*. Springer-Verlag, Berlin, 1986.
- [26] Newman EI. *Biological Reviews* 1978, 53:511-554.
- [27] Suslow TV. In: *Phytopathogenic Prokaryotes*, Mount R, Lacey C (eds.), Academic Press, New York, 1982, 187-223.
- [28] Singh CS, Kapoor A, Wange SS. *Plant and Soil* 1991, 131:129-133.
- [29] Fracchia S, Godeas A, Scervino M, Sampedro I, Ocampo JA, Garcia-Romero. *Soil Biology and*

*Biochemistry* 2003, 35:701-707.

- [30] Fracchia S, Garcia-Romera I, Godeas A, Ocampo JA. *Plant and Soil* 2000, 223:175-184.
- [31] Kucey RMN, Janzea HH, Leggett ME. *Advences in Agronomy* 1989, 42:199-228.
- [32] Velázquez MS, Eliades LA, Irrazabal GB, Saparrat CM, Cabello MN. *Journal of Agricultural Technology* 2005, 1(2):315-326.
- [33] Cabello M, Irrazabal G, Bucsinszky AM, Saparrat M, Schalamuck S. *Journal of Basic Microbiology* 2005, 45:182-189.
- [34] Goenadi DH, Siswanto, Sugiarto Y. *Soil Science Society of American Journal* 2000, 64:927-932.
- [35] He ZL, Baligar BC, Martens DC, Ritchey KD. *Soil Science Society of American Journal* 1996, 60:589-1595.
- [36] He ZL, Baligar BC, Martens DC, Ritchey KD, Wemper WD. *Soil Science Society of American Journal* 1996, 60:1596-1601.
- [37] Wright RJ, Baligar VC, Belesky DP. *Soil Science* 1992, 153:25-36.
- [38] Graham JH, Timmer LW. *Journal of the American Society for Horticultural Science* 1984, 109:118-121.
- [39] Graham JH, Timmer LW. *Journal of the American Society for Horticultural Science* 1985, 110:489-492.
- [40] Omar SA. *World Journal of Microbiology and Biotechnology* 1998, 14:211-218.
- [41] Singh S, Kapoor KK. *Biology and Fertility of Soils* 1999, 28:139-144.
- [42] Antunes V, Cardoso EJBN. *Plant and Soil* 1991, 131:11-19.
- [43] He ZL, Baligar VC, Martens DC, Ritchey KD, Elrashidi M. *Plant and Soil* 1999, 170:131-140.
- [44] Villegas J, Fortín JA. *Canadian Journal of Botany* 2002,80:571-576.