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Experiences on the propagation and efficacy of arbuscular mycorrhizal fungi in Latin America

Experiencias sobre la propagación y efectividad de los hongos micorrizógenos arbusculares en Latinoamérica

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Abstract

Arbuscular mycorrhizal fungi (AMF) have great potential to contribute to the solution of multiple problems in agriculture, they are used in agricultural production in the form of bioprotectors, bioregulators, restoratives and other benefits. Mycorrhizal plants present higher values in height, biomass and performance; because the mycelial network is more efficient to avoid nutrient deficiency. AMF propagate in host plants that have the greatest capacity to establish arbuscular mycorrhizal symbiosis; among them it is mainly from the Poacea family and legumes. The host plants are planted in pots containing sterilized substrates of pH 5.2 to 7 and a low phosphorous source, conducted under greenhouse, laboratory or nursery conditions for an average period of six months. The study of mycorrhizae is scanty, the objective of this review compiles experiences on the effect of types of substrates, and hosts that favours the propagation of AMF, which can be used by researchers who are interested in recovering the deteriorated agroecosystem, mitigating pollution and contributing to quality food

Resumen

Los hongos micorrizógenos arbusculares (HMA) tienen gran potencial para contribuir a la solución de múltiples problemas de la agricultura, son utilizados en la producción agrícola en forma de bioprotectores, biorreguladores, restablecedores y otros beneficios. Las plantas micorrizadas presentan mayores valores en altura, biomasa y rendimiento, porque la red micelial es más eficiente para evitar la deficiencia de nutrientes. Los HMA, se propagan en las plantas hospederas que tienen mayor capacidad de establecer la simbiosis micorrizógena arbuscular, entre ellas es principalmente de la familia Poacea y leguminosas. Las plantas hospederas se siembran en macetas que contienen sustratos esterilizados de pH 5.2 a 7 y fuente de fósforo bajo, conducidos en condiciones de invernadero, laboratorio o vivero, por un tiempo promedio de seis meses. El estudio de las micorrizas es poco, más aún sobre la propagación es muy escasa, motivo por la cual, el objetivo de esta revisión es recopilar experiencias sobre tipos de sustratos y hospederos que permiten la propagación de HMA, la cual puede ser útil y empleada por los investigadores que se interesan por recuperar el agroecosistema deteriorado, mitigar la contaminación y contribuir a la alimentación de calidad.

Introduction

Agronomic production face overuse of chemical fertilizers which conducts to environmental pollution[1-4], degradation of soils, in addition to increasing production costs. This leads to search sustainable production systems through use of beneficial microorganisms also known as biofertilizers[2-4], such as arbuscular mycorrhizal fungi (AMF). These fungi improve the soil productivity decreasing the use of synthetic fertilizers alleviating the environmental damage[1,5-9].

AMF belong to the Phylum Glomeromycota, which establish arbuscular mycorrhizae with diverse plants groups[5,10], forming mutualistic associations with most families of plants[5,11-13].

Unlike helpful bacteria that could be isolated and propagated on artificial culture media, the AMF cannot, needing live plants which constitutes the most important limitation for using them. The scarce information about the more favorable substrates and hosts plants for propagation of spores and other propagules in Latin America, was the principal incentive to do this review. The objective is to compilate the experiences about the propagation of AMF with different substrata, and hosts, and the effectiveness of them in Latin America, to provide useful information to researchers interested in the recovery of degraded agroecosystems, alleviation of environmental pollution, and achievement of quality food.

Development

AMF form arbuscular mycorrhiza into the roots of more than 80 % of plants[5-7,11,12], fungi get carbohydrates, plus an appropriate habitat to accomplish their life cycle thanks to the association, contributing to improve nutrimental status and hydration of hosts plants, phytopathogens protection, besides solubilization and easy absorption of soil nutrients, contributing to ecosystems stability[5-7,14].

The deterioration of agroecosystems produces the need to recover them. Biofertilizers such as AMF could be used to achieve that purpose, improving the efficiency of biological processes in soils and plants. However, information about the better substrates for propagation is scarce. This is crucial to achieve the production required for intensive and extensive use of bioinoculants. The organic and biological fertilization sources have important potential use, efficiently providing nutrients and contributing to diminish economic costs during agronomic management, this promotes synthetic fertilizers substitution, and positive impacts on economy, society and environment[15,16]. Provide information about the AMF propagation that favors and helps the development of strategies to improve this kind of bioinoculants propagation is vital. The use of AMF could be one of the most appropriate methods to greenhouse sustainable cultivation of guava[17], and conservation of threat wild species such as Andean magnolias, key plants to recover naturally deteriorated Andean forests[18]. Also important is its use on seashores rehabilitation[19]. Those are examples of the ecologic and economic importance of these fungi[20].

Effectiveness of the AMF. In Cuba, biofertilizers such as AMF have been effective to substitute mineral fertilizers[1], promoting its use in conjunction with organic manures with good results[1]. The reduction or substitution of inorganic fertilization using AMF is a

viable practice that favors profitability and ecologic conservation of production systems[21,22].

In *Musa* AAA cv. Gran Enano (banana) in Colombia, the use of 15 AMF inoculum from banana agroecosystems produced the highest foliar and radical weights[23]. In mixed culture of *Lolium multiflorum* and *Pisum sativum* in Peru, greenhouse mycorrhization favored the highest foliar dry weigh and plant size. The use of EcoMic® and Quito Max bioinoculants, alone and together, increased the production of *Zea mays* versus that of not inoculated plants[24].

The role of arbuscular mycorrhiza to solve many agronomic problems at tropical environments show its great potential as biofertilizers. Nevertheless, it is important to know that the effects produced by arbuscular mycorrhiza are not the same for all the cultured plants or trees, neither for all climatic conditions, the interactions that these fungi establish with other microorganisms are in part responsible for that. AMF could be inoculated as bioprotectors and bioregulators at nursery or rooting of vitroplants, being for several plants an invaluable alternative to solve micropropagation, acclimatization and nutrition problems[14,22]. The positive effect of AMF is evident on dry aerial biomass because the inoculated treatments mostly produce the better values, being significatively different from not mycorrhizated[16]. The production of AMF mycelial network starting from the roots of host plant seems the most efficient and less expensive strategy to get those responses, through hyphal network the fungus explored more soil volume than thinner roots, reaching the smaller spaces, in addition to the AMF efficient physiological mechanisms for nutrients assimilation.

The dual action of AMF that increase the absorption of soluble phosphates, along the role of solubilizing phosphate fungi (P) promotes the solubilization of insoluble phosphate complexes, benefiting the *Lactuca sativa* nutrition, on this species, the plants inoculated with the AMF fungi *Rhizophagus intraradices* (Glomeromycota) and not AMF *Penicillium thomii* (Ascomycota) were significantly higher than not inoculated plants, both fungi on substrata added with phosphoric rocks, in an eight treatments experiment[25].

On traditional seedbeds of tobacco plants, with the purpose to determinate the influence of AMF and mineral fertilizer reduction[26], the results showed that agronomic practices applied during two periods reduced the effectiveness of AMF[27].

Table 1 Effectiveness of the AMF on mycorrhized plants

Mycorrhized plant	Stem height (cm)	Peduncle length (cm)	Dry foliar biomass (g)	Dry root biomass (g)	Reference
Lactuca sativa	not		1.27		25
Musa AAA cv. Gran Enano	not	not	0.78-1.417	1.750-3.287	23
Anthurium andreanum	4.2 - 4.70	18.3 - 23.7	not	not	28
Anthurium andreanum	4.70	23.67	not	not	28
Zea mays	23.2 - 35.6	not	0.73-1.87	0.13-0.29	29
Brachiaria decumbens	not	not	40.8 (g/plant)	not	30
Begonia sp var. Rex	not	16 (leaves)	5.00	0.80	16
Psidium guajava L (Guayaba)	9.3-18.8	not	0.46-2.02	0.4-1.7	17

The AMF consortia from *Coffea arabica* var. Garnica submitted to moderate technological management produced significatively better height and development of plants in nursery and field conditions[31]. AMF inoculated plants of *Amelanchier denticulata* (tlaxistle) and *Eysenhardtia polystachya* (sweet stick) had better responses in diameter, height, aerial biomass ($p<0.001$) and phosphorus content[32].

The addition of 310.50 g m^{-2} (75 %) of mineral fertilizer and 0.50 kg of AMF m^{-2} of soil, positively influenced diameter, stem length, fresh and dry biomass, foliar area, and performance of useful tobacco seedlings/m^2, and increase percentage of mycorrhizal colonization, producing positive economic and environmental effects[26].

Several results show that agricultural practices negatively affect the AMF, reducing their effectiveness. Then, a decreasing of chemical fertilizers and pesticides along with the AMF inoculation could be used to reduce the ecological deterioration of soil, while reducing production costs[27].

When evaluating the effect of different doses of the agronomic bioinoculant MicoFert on dry matter (DM) and foliar phosphorus content in *Leucaena leucocephala* in Cuba, it was found that inoculated plants produced higher content of foliar phosphorus[33].

On *Lolium multiflorum* and *P. sativum* at greenhouse, the AMF positively affected the length and dry foliar biomass, the mycorrhized plants obtained from 21.8 to 30.4 cm, and 69.2 to 82.6 cm heigh, and 13.0 to 16.01 g of dry foliar biomass, respectively.

On tomato (hybrid HA 3108 Hazera), the effects on height, foliar dry matter, final performance and bromatological quality of the fruits, at different doses of AMF, Cubense strain applied through the commercial biofertilizer Ecomic®, and worm humus, alone and combined with the AMF, as mineral nutrition substitutes were evaluated[34]. Only one treatment of inoculation was significant, although all treatments inoculated were numerically better than control. According to above results the application of AMF inoculant was more efficient than worm humus at 25 % of mineral fertilizer.

Substrates for the propagation of AMF. The type of substrate used depends on availability at each place. Next, experiences and results obtained per different searchers are described. Propagation pots were stablished in greenhouse, with 500 g of soil of interest as inoculum source, plus 500 g of the same soil sterilized as substratum[35]. On squash in greenhouse were evaluated the response of compost, vermicompost, tea compost, and vermicompost associated with mycorrhizae. The spores were used to determinate the propagation, using 1 kg of sterilized substratum, plus 200 g of soil sample containing AMF consortia.

On growth of *Anthurium* plants the influence of different pH values in the substrata, and the effects of biofertilization with AMF were evaluated. The mixture of acidic peat+" cachaza" +zeolite, 6.9 pH produced the better response on mycorrhized treatmens[28].

Six substrate combinations produced by the addition of banana soil with AMF natives from it, combined with three inert materials, sand, rice husk and vermiculite, at 70/30 and 50/50 volume proportions of each were prepared. pH 5.2, 4.7 % organic matter, 27 mg·kg^{-1} P, and sandy loam texture were the soil features. The substrata S2 (sand 50-soil 50) and the S6

(vermiculite 50-soil 50) produced the significantly higher responses[23].

Table 2 Substates and proportions that favor higher multiplication of AMF

Substrate	Proportion v/v	Proportion % or weight	pH	Phosphorus source	Reference
"Terrafertil"-volcanic ash	1:1	not	5.7	22.7 mg RP/plant	25
Soil-sand (S2)	50	50	5.2	27 mg kg-1	23
Soil-vermiculite (S6)	50	50	5.2	27 mg kg-1	23
A (agricultural soil-sand-black soil)	3:3:2	not	6.5	0.1% NT	36
C (uncultured soil)	not	100	7.2	0.03% NT	36
1 (acid peat-"cachaza"-soil)	not	40:40:20	7.0	2930 pm	28
2 (acid peat-"cachaza"-zeolite)	not	40:40:20	6.9	3076 pm	28
3 (acid peat-"cachaza"-rice husk)	not	40:40:20	6.8	3028 pm	28
Sand-soil	1:1	not	not	not	17
SC (sugar cane bagasse)-sand-carbonized rice husk	1:1:1	not	6.6	1.1 g k^{-1}	37
Soil + rice husk	2:1	not		Foliar fertilization	29
Red soil: agricultural soil	3:1	not	4.12	10	30
Ferralitic leached soil-"cachaza"-rice straw	not	62:28:10	7	289 mg.kg-1	16

On squash in greenhouse, the response of compost, vermicompost, tea compost and vermicompost associated with mycorrhiza were evaluated. The treatment 4 (75 % river sand+25 % compost+mycorrhizae+tea vermicompost) was set up on vegetative stage, while treatment 6 (75 % river sand+25 % vermicompost+mycorrhizae+tea vermicompost) was set up on reproductive stage[15]. The response in leaf length, leaves quantity, plant vigor, dry aerial biomass, and root biomass of *Begonia* var. Rex, and number of AMF spores were determined according to the substrata produced by the mixture of soil, "cachaza" and rice husk, plus AMF application (EcoMic®)[16]. To propose one substratum appropriate to produce *L. sativa* in pots, eight treatments were established, combining AMF and phosphate solubilizing fungi (S) plus different materials: 1) substratum, 2) substratum+AMF, 3) substratum+S, 4) substratum+AMF+S, 5) substratum: PR (phosphoric rock)+S, and, 8) substratum: PR+AMF+S. The substrate used had 5.7 pH, and 22.7 mg of PR per plant[25].

AMF host plants. The AMF need a host plant to complete their development, until now there are not synthetic culture media to isolate and to multiply these fungi in absence of plant roots[30,38].

Trap crops allow propagation of AMF spores with the morphological features needed to taxonomical identification, also to observe its ontogenetical stages[6]. *Zea mays* (corn) and *Leucaena* sp. (gourd) have been used as host plants in pots with field soil (500 g/pot) in greenhouse conditions, to obtain newly formed spores, by six months of propagation, watered every third day[10].

The propagation of native AMF has been achieved using *L. multiflorum* and *P. sativum* as host plants.

The infective potential of the propagation has been evaluated on corn (*Z. mays* L.) in greenhouse, through the most probable number (MPN) of infective propagules[27]. Pre-germinated *Brachiaria decumbens* (B), *Pueraria phaseoloides* (K), *Sorghum vulgare* (S) and *Tagetes erecta* (T) were used as trap crops, for five months plus 15 extra days to promote sporulation[23].

By their capacity to establish the arbuscular mycorrhizal symbiosis seeds of corn (*Z. mays* L.), English grass (*Lolium perenne* L.), and tomato (*Lycopersicum esculentum* P. Mill.) were selected for propagation. The soil was collected to extract the AMF spores, after two culture cycles of four months each[39].

Table 3 Main AMF host plant species

Species	Family	Number of plants/pot	Greenhouse/field	Country	Reference
Lactuca sativa	Asteraceae	not	greenhouse	Argentina	25
Brachiaria decumbens (B)	Poaceae	not	greenhouse	Colombia	23
Sorgum vulgare (S)	Poaceae	not	greenhouse	Colombia	23
L. multiflorum	Poaceae	1	laboratory	Peru	36
Zea mays	Poaceae	1	laboratory	Peru	36
Anthurium andreanum	Araceae	1	nursery	Cuba	28
Sorghum bicolor	Poaceae	9	field	Brazil	37
Zea mays	Poaceae	1	nursery	Colombia	29
Brachiaria decumbens	Poaceae	10	greenhouse	Honduras	30
Begonia sp var. Rex	Begoniaceae	1	semi-controlled conditions	Cuba	16
Psidium guajava L	Myrtaceae	1	nursery	Mexico	17

Propagation of AMF species and consortia (spore quantity and colonization %). The AMF inocula are generally propagated using sterilized soil and greenhouse conditions[5,40], and quantifying the spore number previous extraction through wet sieving[36,40].

The AMF greenhouse propagation was performed in pots with 500 g, and 200 g of interest soil as inoculum source, plus 1300 g of the same soil, sterilized three days consecutively, for 1 to 1.5 h dairy, at 120 °C and 1.0 kg/cm^2 of pressure[35]. Propagation pots were established with rhizospheric soil (500 g/pot) in greenhouse conditions, during six months, watered with distilled water every third day, the plants were not watered for 15 days to promote the sporulation at the end of the period[10,35].

On *Lolium multiflorum* ("rye grass") + *P. sativum* ("pea") in greenhouse conditions there were 16 to 43 spores of AMF consortia/g of soil. Papaya cultured on very technical field conditions produced 10.9 spores as MPN of infective propagules[27], and 8.04 to 12.62 % of colonization.

Contents of 30 and 60 ppm of P reduce the number of spores from 49.9 to 40, and 31 spores, respectively[30]. However, the biomass increases with those doses of P. The P level must be kept low because high concentrations inhibit the development of the vesicular arbuscular mycorrhiza (MVA)[30].

Table 4 Propagation of AMF species or consortia (spore quantity and colonization %)

AMF species or genus	AMF consortia	Spore number/100 g	MPN of propagules/cm^3	Colonization %	Direct inoculation/trans-plant	Reference
Rhizophagus intraradices-Penicillium thomii	*R. intraradices – P. thomii*	not	not	26	transplant	25
Glomus, Acaulospora and *Entrophospora*	*Glomus, Acaulospora* and *Entrophospora*	966 - 1618	not	49.0 ± 44.8 %	transplant	23
Glomus sp.	not	1050-1633	not	25-40		36
Glomus hoi like	not	not	not	not	5 g per plant	28
Experiment I *Rhizophagus clarus*		162+-82.5	283 a 350	not		37
Experiment II *Claroideoglomus etunicatum*	not	240 +- 169.7	233 a 283	not		37
Rhizophagus clarus	not	11.6±10.0	28	not	transplant	37
Claroideoglomus etunicatum	not	16.3±7.7	8	not	transplant	37
Dentiscutata heterogama	not	0.3±0.5	0.3	not	transplant	37
Preinoculum of cacao culture 5 g/pot	*Glomus, Acaulospora, Entrophospora, Gigaspora, Archaeospora* and *Scutellos-pora*	12 - 364/10g	not	12.16 - 30.5 micelio	direct inoculation	29
MYCORAL inoculant	*Glomus, Acaulospora* and *Entrophospora*	49.9 (25 mL/100g suelo)	not	not	direct inoculation	30
EcoMic® inoculant	EcoMic® inoculant	50%	not	not	transplant	16
Native AMF + INIFAP	Native AMF + INIFAP	3-40/g	not	70		17

One experiment in greenhouse conditions showed the negative effect of agronomic management, after six weeks, when the root system was collected and the colonization potential was evaluated, root samples collected in grassland had higher percentage of mycorrhizal colonization, while a drastic decline of infective propagules, proportional to management intensity was found at cultured plots. These results show that the agronomic practices decline the infectivity of AMF[27].

The colonization percentage has been quantifying removing the root pigments, through acid and basic solutions, the dye trypan blue could be substituted by Parker QuinK water washable ink, also the glycerin and lactic acid[41]. The roots are colored and cut off in 2 cm length pieces, and placed in slides to observe under microscopy, to quantify the AMF colonized and not colonized points.

Between 30 and 60 % of colonization was obtained on three mycorrhization treatments, on papaya cultured under high technification the AMF colonization fluctuated between 8.04 and 12.62 %[25]. AMF average colonization on trap plants was 37.76±21.86 %, the B plants (*Brachiaria decumbens*) and S (*Sorghum vulgare*) were the most favorable to AM symbiosis[23]. The extraction of AMF spores was performed in 100 g of dry soil by sample, through wet sieving and decantation procedures[10,42,43].

The spores were separated from mineral and organic material by mechanical stir and 2500 r.p.m. centrifugation (revolutions per minute) with water, a second centrifugation with 60 % sucrose at 1200 r.p.m., and decantation over 44 µm pore size sieve. Isolation of spores from every extract was placed inside Petri dishes divided in fields (0.5 x 0.5 cm), only turgent and homogeneous colored spores were considered. The abundance of spores in 100 g of soil is established by direct quantification[35,44]. Samples of 20 or 50 g of dry soil could be also considered[26]. Also, 40 and 60 % sucrose solutions could be used to extract spores and separate them from organic and mineral soil material[10,41,44,45], and even 50 % sucrose solution can be used for it[33]. Stereomicroscope is used to separate and to quantify spores[36,38,46,47]. Live and dead spores were quantified, those with cytoplasmatic content and without spore wall damage were alive spores, if these conditions were not meet the spores were dead[47]. The spores were placed in permanent slides with polyvinyl alcohol-lactoglycerol (PVLG) and PVLG + Melzer´s Reagent, 1:1 proportions[36,38,41,46].

Glomus (*G. aggregatum, G. geosporum, G. clarum*), *Acaulospora* (*A. morrowiae, A. mellea, A. gerdemannii*), *Scutellospora* (*S. calospora*) and *Entrophospora* were the AMF species from banana agroecosystems, while *Glomus* sp., *Acaulospora* sp., *Scutellospora* sp., and *Entrophospora* were the AMF from commercial inoculum. From 966 to 1618 spores in 100 g of dry soil and 49.0±44.8 % colonization percentage were obtained[23].

About the abundance, from 216.4±96.6 spores of AMF per 100 g of dry soil have been obtained[35]. Ten genera and 27 morphospecies[44]. The abundance of spores fluctuated from 55 to 198 in 100 g dry soil. *Ambispora reticulata* was a new record for Chiapas and Mexico. *Acaulospora* was the most frequent and diverse genus. Richness of AMF morphospecies, diversity, and evenness made “Chiquihuites” site stood out, low levels of organic matter and PO_4^{-3} in the soil contributed to it[44].

Conclusion

The improvement in the growth and resistance of mycorrhized plants against non-mycorrhized ones show the effectiveness of AMF as bioprotectors, bioregulators, biofertilizers, restorers of polluted soils, among other benefits for plants and soils.

Combinations of inert and organic substrates, slightly acid to neutral (pH 5.2 to 7) and low phosphorus content (20 to 30 ppm) seem the most suitable conditions to propagate AMF.

Plants of the family Poaceae, alone and combined with legumes are appropriate hosts for AMF propagation.

The substrates and the hosts plants influence the propagation of individual AMF species, species mixtures or consortia, therefore it is crucial to consider them for successful propagation.

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Conflicts of interest

This review paper has no conflicts of interest.

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Ethical considerations

This is a review paper, the authors declare it was prepared using the cited literature and giving credits to respective papers and authors.

Authors' contributions

Esquivel Quispe Roberta, planned the paper and wrote the base document including tables, made bibliographic search and catalogued it. *Hernández Cuevas Laura Verónica*, made bibliographic search, contributed with ideas to paper integration and collaborated with the revision and correction of the paper. *Quispe Ochoa Josue Olser*, collaborated with bibliographic search.

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