On-farm production and utilization of arbuscular mycorrhizal fungus inoculum

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Douds, D. D., Jr., Nagahashi, G., Pfeffer, P. E., Kayser, W. M. and Reider, C. 2005. **On-farm production and utilization of arbuscular mycorrhizal fungus inoculum**. Can. J. Plant Sci. **85**: 15–21. Arbuscular mycorrhizal (AM) fungi colonize the roots of the majority of crop plants, forming a symbiosis that potentially enhances nutrient uptake, pest resistance, water relations, and soil aggregation. Inoculation with effective isolates of AM fungi is one way of ensuring the potential benefits of the symbiosis for plant production. Although inocula are available commercially, on-farm production of AM fungus inoculum would save farmers the associated processing and shipping costs. In addition, farmers could produce locally adapted isolates and generate a taxonomically diverse inoculum. On-farm inoculum production methods entail increasing inoculated isolates or indigenous AM fungi in fumigated or unfumigated field soil, respectively, or transplanting pre-colonized host plants into compost-based substrates. Subsequent delivery of the inoculum with seed to the planting hole in the field presents technological barriers that make these methods more viable in labor-intensive small farms. However, a readily available method for utilization of these inocula is mixing them into potting media for growth of vegetable seedlings for transplant to the field. Direct application of these inocula to the field and transplant of seedlings precolonized by these inocula have resulted in enhanced crop growth and yield.

Key words: AM fungi, sustainable agriculture, biofertilizer

Douds, D. D., Jr., Nagahashi, G., Pfeffer, P. E., Kayser, W. M. et Reider, C. 2005. **Production et usage d'inoculums de mycorhizes à arbuscule à la ferme**. Can. J. Plant Sci. **85**: 15–21. Les mycorhizes à arbuscules (MA) colonisent les racines de la majorité des végétaux. Ces champignons vivent en symbiose avec la plante, améliorant l'absorption des éléments nutritifs, la résistance aux ravageurs, l'utilisation de l'eau et l'agrégation des sols. L'inoculation de bons isolats de MA est une façon de veiller à ce que les bienfaits de cette symbiose profitent à la production agricole. Bien qu'on vende des inoculums sur le marché, leur préparation à la ferme permettrait à l'agriculteur d'économiser les frais de conditionnement et d'expédition. L'agriculteur pourrait aussi produire des isolats adaptés aux conditions locales et obtenir un inoculum de composition variée sur le plan taxonomique. La production d'inoculums à la ferme suppose un accroissement du nombre d'isolats ou de MA indigènes inoculés dans le sol des champs fumigés ou pas, respectivement, ou la transplantation de plantes hôtes déjà colonisées dans un substrat enrichi de compost. L'administration d'inoculum avec la semence à la plantation soulève des obstacles techniques qui rendent cette méthode plus rentable dans les petites exploitations faisant appel à une main-d'œuvre abondante. Toutefois, le mélange de l'inoculum au milieu d'empotage dans lequel pousseront les plantules repiquées par la suite en pleine terre est une méthode aisément disponible. L'application directe de l'inoculum au sol et le repiquage de plantules colonisées ont abouti à une meilleure croissance et à un rendement plus important des cultures.

Mots clés: Mycorhizes à arbuscules, agriculture durable, amendement biologique

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Arbuscular mycorrhizal (AM) fungi are soil fungi that colonize roots of the majority of crop plants, forming a mutualistic symbiosis. The fungus takes up fixed carbon as hexose from the apoplast of the root cortex (Shachar-Hill et al. 1995). The extraradical phase of the fungus acts in effect, as an extension of the root system for the uptake of mineral nutrients, especially immobile nutrients such as P, Cu, and Zn, which are transported back to the intraradical structures where they are released by the fungus for uptake by the root cells. In addition to enhanced nutrient uptake, other benefits to the plant that have been ascribed to the symbiosis in experimental situations are enhanced water relations and increased disease resistance (Augé 2000; Linderman 2000). The extraradical hyphae of the AM fungus also stabilize soil aggregates by both enmeshing soil particles (Miller and Jastrow 1992) and excreting a glycoprotein ("glomalin"), which may act as a glue-like substance to adhere soil particles together (Wright and Upadhyaya 1998). These attributes of the symbiosis work together to promote the growth of plants.

"There are two principal ways of ensuring that the benefits in terms of crop production are obtained from mycorrhizal associations: (1) by inoculating with selected efficient mycorrhizal fungi and (2) by promoting the activity of effective indigenous mycorrhizal fungi by proper cultural practices" (Bagayaraj 1992). Cultural practices that increase the activity of indigenous AM fungi are: reduced tillage, crop rotations, cover crops, and phosphorus management (Douds and Johnson 2003). Reduced tillage, especially no-till, leaves the extraradical mycelial network in the soil intact. This promotes rapid colonization of a new crop and enhances early season mycorrhiza-mediated P uptake (McGonigle and Miller 1993).

Abbreviations: **AM**, Arbuscular mycorrhizal

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Crop rotation appears to be essential, from the soil biology viewpoint, to ensure that the AM fungi which proliferate especially well with a given host crop plant do not come to dominate the fungal community. The AM fungi which proliferate with a host plant are not necessarily those best at promoting the growth of that plant but may be effective in promoting the growth of other crops in the rotation (Feldmann et al. 1991; Johnson et al. 1992). Proliferation of such AM fungi has been implicated as a contributor to yield decline in continuous monocultures (Schenck et al. 1989). In addition, the diversity of the AM fungus community is linked to the diversity and productivity of the plant community (van der Heijden et al. 1998; Bever et al. 2001). A more relaxed attitude toward weed management may increase both the diversity and effectiveness of the AM fungus community (Miller and Jackson 1998; Feldmann and Boyle 1999; Jordan et al. 2000). Cover crops promote the activity of indigenous AM fungi by providing living host roots, and therefore a source of carbohydrate, during periods when the soil would otherwise lie bare (Galvez et al. 1995; Boswell et al. 1998). Warm, moist soil conditions after crop senescence and before sowing the next crop cause AM fungus spores to germinate prematurely and soil-borne hyphae to respire. This utilization of storage compounds can limit AM fungus viability and decrease the inoculum potential of soils left bare. Management practices to be avoided, from an AM fungus perspective, are the long fallows used in semi-arid climates (Thompson 1987) and the growth of nonhost crops such as *Brassica* spp. (Blaszkowski 1995).

A different way of thinking about phosphorus management is essential for optimal functioning of the symbiosis (Gransee and Merbach 2000; Kahiluoto et al. 2001). Efficient use of the symbiosis can effectively substitute for P applications up to 222 kg P_2O_5 ha^{-1} (Plenchette and Morel 1996; Kelly et al. 2001). Plants typically limit the colonization of roots by AM fungi in soils high in P (Jasper et al. 1979). This limits the flow of carbon to the fungus and results in lower populations of spores of AM fungi (Douds and Schenck 1990a).

There are instances when inoculation with AM fungi is necessary or desirable. An AM fungus available as inoculum may be more effective on a given crop relative to the effectiveness of the indigenous AM fungus community. Inoculation may be necessary to help overcome the harmful effects of past agricultural management upon the native AM fungus community, e.g. excessive use of fungicides. In extreme instances, inoculation may be necessary to reintroduce AM fungi to severely degraded or reclaimed soils (Cuenca et al. 1998; Requena et al. 2001). One of the main agronomic situations in which inoculation is desirable, however, is to take advantage of the benefits of outplanting a precolonized seedling (Rice et al. 2002; Matsubara et al. 2002).

Even though inocula of AM fungi are commercially available, production of AM fungus inoculum on the farm is an attractive alternative. Purchasing the large amounts of inoculum necessary for large-scale agriculture may be cost prohibitive. Producing the inoculum on-site saves processing and shipping costs included in the price of commercial inocula. These factors are the primary reason why most on-farm methods have been utilized in developing nations. Another benefit of on-farm production of inoculum is that locally adapted isolates, which may be more effective than introduced ones in certain situations (Sreenivasa 1992), can be produced when the farmers' indigenous AM fungus communities are used as starter inocula (see below). Further, a taxonomically diverse inoculum can be produced. This is important in light of recent demonstrations of functional diversity of AM fungi (Wright and Upadhyaya 1996; Smith et al. 2000; Hart and Reader 2002). Commercial inocula may contain only one species.

Methods of On-farm Production of AM Fungus Inoculum

1. Most advances in on-farm production of AM fungi have been made in developing tropical countries. Sieverding (1987, 1991) developed the earliest published method to produce inoculum of an effective strain of the AM fungus *Glomus manihotis* in Columbia. In this method, a 25-m^2 field plot first is tilled and then fumigated. Fumigation kills indigenous AM fungi which may compete with the introduced isolate, kills weed seeds which may contaminate the soil-based inoculum, and kills pathogens present in the soil. One fumigant used here for soil disinfestation was methyl bromide. Use of this fumigant will be banned in 2005 due to its harmful effect upon the ozone layer (US Environmental Protection Agency 2003). Chemical alternatives exist, such as dazomet, chloropicrin, metam sodium, and 1,3-dichloropropene as well as non-chemical means, e.g., solarization. Two to four weeks of solarization can decrease infectivity of AM fungi to near zero in surface soils in Mediterranean climates (Bendavid-Val et al. 1997) while in others it may work indirectly by killing seeds of potential weedy host plants (Schreiner et al. 2001). After the fumigant has dissipated, *G. manihotis* was inoculated into holes drilled in the soil and then seeds of a grass host, typically *Brachiaria decumbens*, are sown. Alternatively, precolonized *B. decumbens* can be transplanted to the plot, minimizing the amount of starter inoculum needed. Flowers are removed as they are produced to avoid *B. decumbens* seeds falling to the soil and becoming a weed problem when the inoculum is used. The soil and roots are harvested to a depth of 20 cm after 4 mo of growth.

Post-harvest analysis of the inoculum demonstrated that fumigation of the soil increased AM fungus spore production g^{-1} soil and increased the relative proportion of spores of the introduced isolate relative to indigenous AM fungi compared to unfumigated, inoculated plots (Table 1). Utilization of this inoculum increased the yield of cassava (*Manihot esculenta*) by 20% over controls. Dodd et al. (1990a,b) used this method, also in Columbia, to produce an inoculum containing three AM fungi. The inoculum contained final concentrations of 250 spores g^{-1} *G. manihotis*, 250 spores g^{-1} *Glomus occultum*, and 10 spores g^{-1} *Entrophospora columbiana*. This inoculum increased the growth of cassava (up to 30%) and *Sorghum* sp. (23–476%) but not *Brachiaria dictyoneura* or *Pueraria phaseoloides* (Dodd et al. 1990a).

Table 1. Production of spores of AM fungi in the on-farm inoculum production method developed in Columbia. *Glomus manihotis* was inoculated into 25-m^2 field plots, with or without fumigation pretreatment of the soil[z]

Treatment	Spores 100 g^{-1}	spores cm^{-3}	% *G. manihotis*
Not fumigated	758	9.1	67
Fumigated[y]	7218	86.6	97

[z]Summary of data from Sieverding (1987).
[y]Means of four fumigation treatments.

2. Adholeya and co-workers in India also have developed methods of on-farm production of AM fungus inoculum. One method entails preparing raised beds of soil 60 cm × 60 cm × 16 cm (Gaur 1997; Douds et al. 2000). After fumigation, AM fungi are inoculated into furrows in the beds. The starter inoculum is pot culture inocula of either introduced isolates or indigenous AM fungi. The primary difference with the method developed in Columbia is that a succession of hosts is grown over the course of 3 yr. For example, *Sorghum sudanese*, *Zea mays*, and *Daucus carota* may be grown in one year, each for 4 mo. The farmer receives an economic return from each host crop, and after the third such cycle the soil in the raised bed is ready to be used as inoculum. The amount of inoculum increases approximately 10-fold from year 1 to year 3, yielding upwards of 2.5×10^6 propagules per bed (Table 2).

Gaur et al. (2000) and Gaur and Adholeya (2002) later modified this method to yield a shorter production cycle without the use of fumigants. Raised beds were prepared as above using a 2:1 (vol/vol) mixture of soil to leaf compost, and are either inoculated or left uninoculated to increase indigenous AM fungi. This time, only one plant growth cycle was used. Forage crops or vegetables were grown as host plants, once again giving the farmer an economic return in addition to inoculum of AM fungi. Inoculum production was 15- to 20-fold greater when starter inoculum was used, but nevertheless, this method produced only 55–69 000 propagules per bed, 40-fold fewer than the 3-yr method, above. This inoculum was effective, however, giving a 51–119% increase in shoot weight of vegetables (Gaur et al. 2000) and 10–70% increase in shoot weight of forage crops (Gaur and Adholeya 2002).

3. We have developed another method for on-farm production of AM fungus inoculum in temperate climates (Douds et al., in preparation). Raised bed enclosures, 0.75 m × 3.25 m × 0.3 m, are constructed with silt fence walls, weed barrier cloth floors, and plastic sheeting dividing walls between 0.75-m square sections. We filled the enclosures to a depth of 20 cm with mixtures of compost and vermiculite. Preliminary experiments examined different dilutions of yard clippings compost with vermiculite. Vermiculite is used as a relatively inert diluent to reduce the nutrient concentration of the compost to levels conducive to AM fungus colonization of roots and proliferation of the fungi. A 1:4 (vol/vol) mixture of compost and vermiculite, respectively, was optimal. Ten bahiagrass (*Paspalum notatum* Flugge) plants, precolonized by AM fungi are transplanted into the

Table 2. Inoculum production in the on-farm method developed in India. Pot culture inocula were introduced into raised beds of soil (60 × 60 × 16 cm) after fumigation[z]

	Propagules	
Inoculum	per bed (× 10^5)	per cm^3
After 1 yr		
Indigenous mix	2.3–3.3	4.0–5.7
Introduced isolates[y]	0.6–2.2	1.0–3.8
After 3 yr		
Indigenous mix	22–25	38–43
Introduced isolates[y]	6–16	10–28

[z]Summary of data from Gaur (1997) and Douds et al. (2000).
[y]Range of seven introduced isolates.

enclosures, one isolate per enclosure section. The enclosures are then tended for one growing season: watered as needed and weeded as seeds in the compost germinate. Experimentation has shown that no supplemental nutrient addition is necessary. The host plant, being a tropical C4 grass, is frost killed naturally so as not to become a weed pest itself. The inoculum over winters in situ and is ready for use the following growing season. We feel potential pest problems with the application of this inoculum should be minimal due to (1) the pathogen suppressive properties of compost (Hiotink and Fahy 1986), (2) no pathogens are introduced with the vermiculite or bahiagrass, and (3) bahiagrass is unlikely to share any pathogens with the unrelated vegetable crop hosts for which the inoculum is intended.

Significant quantities of inoculum of a variety of AM fungi have been produced via this method. An average of 95×10^6 propagules were produced per 0.75 m × 0.75 m enclosure section (Table 3). Comparisons of results of the Most Probable Number bioassays and spore populations indicated that the vast majority of propagules are in a form other than spores, i.e., extraradical hyphae and colonized root pieces. The overwinter survival rate is high. Inoculum tested in December having a propagule density of 260 cm^{-3} had a density of 215 cm^{-3} the following May. Contaminant AM fungi present in soil mixed into the compost as it was turned the previous year indicated another option for this method of on-farm inoculum production. This method could be used to multiply indigenous AM fungi from an uncultivated, natural area of the farm or nearby location to help restore the native AM fungus community.

The efficacy of inoculum produced by this method in 2001 was tested in 2002 in a field trial using potatoes (*Solanum tuberosum* 'Superior'). The experiment was conducted in the Compost Utilization Trial at the Rodale Institute Experimental Farm, Kutztown, PA (Reider et al. 2000). Seed potatoes were inoculated with 15 cm^3 of one of three treatments placed directly below the seed potato. The first was a commercially available AM fungus inoculum (MYKE® Garden, Premier Tech Biotechnologies, Rivière-du-Loup, Quebec) containing 30 propagules of the AM fungus *Glomus intraradices*, as determined by the manufacturer, in a peat-vermiculite mixture. The second was 15 cm^3 of the on-farm inoculum (compost:vermiculite = 1:9 vol/vol) containing 3225 propagules of *Glomus*

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Table 3. Production of inoculum of a variety of AM fungi in a 1:4 (vol/vol) mixture of yard clippings compost and vermiculite. Spores of "other" AM fungi indicates presence of AM fungi not directly inoculated into the media[z]

Precolonized plants	Spores cm^{-3}		Propagules	
	Inoculated	Other	cm^{-3}	Total ($\times 10^6$)[y]
Control	0	17.4	830	93
Glomus mosseae	7.4	0.3	707	80
Glomus etunicatum	5.5	32.4	465	52
Glomus claroideum	4.3	37.2	365	41
Glomus geosporum	2.8	74.1	2150	242
Glomus intraradices	1.3	69.7	950	107
Gigaspora gigantea	4.4	8.2	465	52
Gigaspora rosea	20.9	66.5	–[w]	–[w]

[z]Data of Douds et al. (unpublished).
[y]Propagules per $75 \times 75 \times 20$ cm enclosure section, results of MPN bioassays.
[w]Not determined.

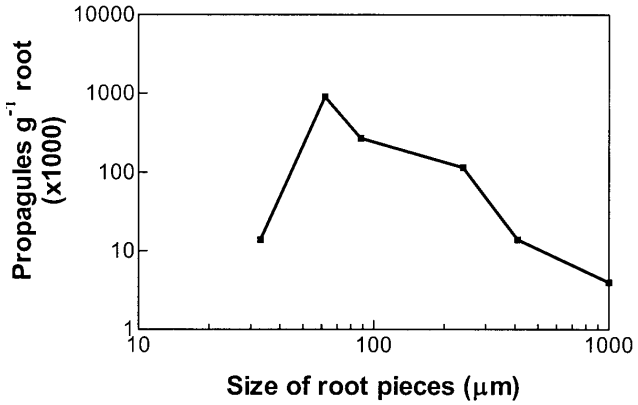

Fig. 1. Propagule densities of sheared root inocula. Sweet potato (*Ipomea batatas*) plants, precolonized by *Glomus* sp. (INVAM 925) were grown aeroponically and sheared for 40 s. Most Probable Number bioassays then were conducted after size fractionation of the roots. Data of Sylvia and Jarstfer (1992).

mosseae, *Glomus etunicatum*, *Glomus claroideum*, and the AM fungi indigenous to the small amount of soil mixed into the compost. The third, control treatment was 15 cm^3 of a fresh 1:9 (vol/vol) mixture of yard clippings compost and vermiculite. There were two soil fertility management regimes, conventional chemical fertilizer and dairy cow manure + leaf compost. Final yields of tubers showed that the mycorrhizal treatments out produced the control by 33–45% and that the on-farm inoculum performed as well as the commercial mix (Douds et al. unpublished data).

Post Inoculum Production Issues

Storage

The general rules for storage of AM fungi in pot culture type media are that cool is better than warm, and dry is better than moist (Daft et al. 1987; Douds and Schenck 1990b). Storage is not a great issue for on-farm production of inocula in the tropics since these inocula are intended for use upon completion of the production cycle. There is no need to maintain viability during shipping, handling, and warehousing as in a commercial inoculum. The compost-based inoculum production system in temperate climates takes advantage of the natural ability of the AM fungi to over winter, and is designed for use immediately thereafter.

Maximizing the Number of Propagules

Fungal spores are only one component of the inoculum of AM fungi in these media. Colonized roots and hyphae are also potential propagules of some AM fungi (Klironomos and Hart 2002). The proper processing of colonized roots as inoculum is essential for recovering all potential propagules. This was best illustrated by Sylvia and Jarstfer (1992) in their work with sheared root inoculum. The aeroponic system with which they worked produced colonized roots containing vesicles and spores in a system free of any sort of potting media (Hung and Sylvia 1988). Shearing the roots in a commercial blender produced root pieces of various sizes. The maximal number of propagules g^{-1} of root was achieved for roots sheared into approximately 60-μm pieces (Fig. 1). This type of processing is not possible with the types of growth substrates and large volumes of inoculum produced in the various on-farm methods. Roots in soil-based media cannot be blended, but this demonstrates that the number of propagules recovered from the on-farm system can be increased by chopping the roots into small pieces (Sylvia and Jarstfer 1992).

Potential for a Growth Response in the Field

There are three important factors that will influence whether any introduced inoculum may be effective at promoting crop growth in the field. The first factor is the mycorrhizal responsiveness of the host plant. Some plant families, such as the Cruciferae, are not colonized by AM fungi and therefore would not respond to inoculation. Others contain species that become colonized but are known to be relatively unresponsive to AM fungi [e.g., various citrus species (Graham et al. 1991)]. One should avoid inoculating a plant unlikely to respond.

The second factor that will influence the efficacy of an inoculum is the available P level of the soil into which the inoculum is introduced. Plants growing in high-P soils limit the colonization of roots by AM fungi. Cutoff limits of available P above which a mycorrhizal growth response may not be seen understandably vary for soil type and host plant and range from 50 μg $NaHCO_3$ extractable P g^{-1} soil for *Linum usitatissimum* (Thingstrup et al. 1998) to 140 μg $NaHCO_3$ extractable P g^{-1} soil for *Allium porrum* (Amijee et al. 1989), both grown in sandy loam soil. This does not consider the potential operation of benefits of mycorrhizae other than enhanced P uptake, such as enhanced disease resistance and water relations or the influence upon soil structure and soil biology. These other factors may have contributed to increased yield of *Capsicum annuum* upon inoculation with AM fungi in soil with available P levels in excess of 350 μg dilute HCl-NH_4F extractable P g^{-1} soil (Douds and Reider 2003).

The third factor that may influence crop response to inoculation with AM fungi is the health or size of the indigenous population of AM fungi. Sieverding (1991) studied this in Columbia and found a decreasing response to inoculation in

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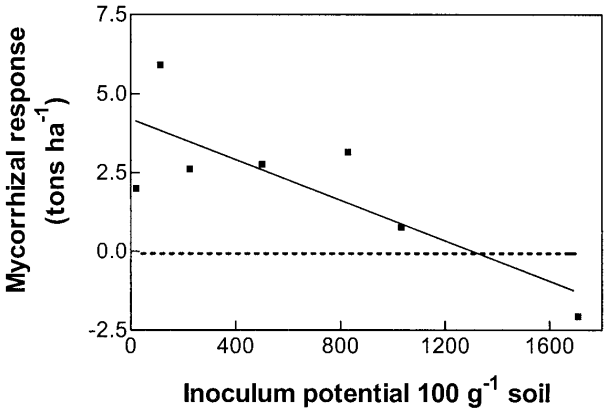

Fig. 2. Response of cassava (*Manihot esculenta*) to inoculation with AM fungi, expressed as change in yield vs. controls, as affected by increasing levels of indigenous AM fungi in the soil. Data of Sieverding (1991).

the presence of an increasing background level of indigenous AM fungi (Fig. 2). Some AM fungi respond to increased propagule density, as would occur with inoculation of the planting hole, by forming mycorrhizas with a greater proportion of extraradical hyphae (the nutrient absorbing organ of the mycorrhiza) relative to intraradical hyphae (Abbott et al. 1992). A greater potential for a growth response would occur via this mechanism in soils of low initial propagule density. The size of the indigenous population of AM fungi is less important when the inoculum is used to produce seedlings precolonized by AM fungi for transplant to the field. Here, the symbiosis is immediately functional and the potential benefits do not have to await colonization by soil born AM fungi.

Delivery of the Inoculum to the Field

Economic, efficient delivery of inoculum of AM fungi to the field has been an obstacle in the utilization of inocula in large scale agriculture, especially for row crops, although significant progress in encapsulating in vitro produced inocula for field application recently has been made (Adholeya 2003). Mycorrhizal fungus inocula have been formulated as powdered seed coatings, encapsulated in alginate, and suspended in carrier for fluid drilling (Bagyaraj 1992; Singh 2003). One innovative method entailed growing the fungi in media containing expanded clay particles (Baltruschat 1987). The AM fungus hyphae penetrated into pores in the particles. Individual colonized particles could then be utilized as inoculum readily applied by standard farm machinery. This method had the additional strength in that the fungus was extremely resilient within these particles and could be stored for long periods of time.

Inocula produced on-farm are not readily processed for mechanical application to field soils. This is another reason these methods have targeted labor intensive farms in developing countries where inocula are applied by hand at low cost and other forms of agriculture in which seedlings are transplanted to the field. The latter situation takes advantage of the most economical way to utilize AM fungus inoculum, i.e., by mixing a concentrated source of AM fungus propagules into a potting mix for growth and precolonization of seedlings for later transplant to the field. Therefore, the temperate climate zone method targets vegetable producers and horticulturists who produce their own seedlings.

CONCLUSION

On-farm production of AM fungus inoculum is a viable option in developing countries characterized by labor-intensive agriculture and for applications in which the inoculum can be mixed into potting media for the production of precolonized seedlings prior to transplant to the field. Significant quantities of a taxonomically diverse inoculum can be produced using materials readily available to farmers. This technique saves the associated costs of processing and shipping, which are included in the price of commercially available inocula. Finally, these factors combined with the demonstrated yield increases indicate the potential for increased economic returns for farmers utilizing AM fungi and the associated environmental benefits accrued from decreased use of fertilizers and pesticides.

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