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MYCORRHIZAL INOCULUM PRODUCTION TECHNIQUE FOR LAND REHABILITATION

Teknik Produksi Inokulum Mikoriza Untuk Rehabilitasi Lahan

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ABSTRAK

Spora cendawan mikoriza arbuskula (CMA) telah dikoleksi dan diisolasi dari berbagai jenis tanah masam berfikasi P-tinggi di Indonesia. Lima isolate CMA lokal telah berhasil diperoleh dari *spora tunggal dengan teknik "test tube culture" dan dikembangkan-biakan dengan sistem "open pot culture". Kelima isolate tersebut adalah Acaulospora delicata (EJ*-01). Acaulospora tuberculata (INDO-2), Glomus manihotis (INDO-1), Glomus mosseae (PAL-03) dan Entrophospora colombiana (LAM-36). Kelima biakan isolate CMA tersebut, dipelihara dengan tanaman inang Kudzu pada media zeolit yang diperkaya nutrisi. Dengan mempelajari berbagai tipe media, ukuran partikel, pH dan komposisi nutrisi, isolate CMA terpilih telah berhasil dapat diperbanyak dalam skala industri dengan menggunakan inang sorghum. Dengan tehnik pra-inokulasi di persemaiaan maka penggunaan mikoriza dalam skala besar di bidang kehutanan dapat direalisasikan.

INTRODUCTION

The novel functions of Arbuscular Mycorrhizal Fungi (AMF) as biological agent for improving growth and health of plants and help early seedling establishment on degraded reforestation sites are recognized. Most tree species in tropical regions are colonized by mycorrhizal fungi, however, the exploitation of these potential benefits of AMF has yet to be widely adopted, and they are not yet used as part of standard silviculture management in Indonesia. This may be related to the limited availability of inoculum of AMF and the lack of data demonstrating a benefit from large scale application of AMF inoculum in the field.

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, and although several attempts at axenic culturing of these fungi have been made, sustained axenic culture has not yet been achieved (Piche et al., 1994). Thus they cannot be multiplied on artificial media without a living host (Sieverding, 1991). For reproduction, these fungi depend on a supply of photosynthate from the host plant or on Ri T-DNA-transformed root components when the roots are grown meristimatically on artificial growth media under axenic conditions (Becard and Piche, 1990; Williams,1992).

Nevertheless, several techniques for producing and formulating inoculum of AMF in dual culture with plant hosts are now available i.e. aeroponic cultures (Hung and Sylvia, l988), nutrient film techniques (Elmes et al., 1983), Ri T-DNA transformed roots (Williams, 1992),

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alginate beads (Strullu and Plenchette, 1991), and hydrogels (Hung et al., 1991). The culturing of AMF on plants grown in disinfected soil or in inert soil-less media, however, remains the most frequently used technique (Feldmann and Idczak,1992; Sylvia and Jarstfer,1994).

Soil-less cultures can be used to produce high quality inoculum of AMF, with a propagule number much greater than that achieved in the soil-based methods used in the past. Using soil-less media also avoids the need for sterilization of the media necessary to remove detrimental organisms, and also allows better control over the physical and chemical characteristics of the growth media. Soil-less media are more uniform in composition, and better aeration is often possible compared with soil (Sylvia and Jarstfer, 1994).

Limited success in the propagation of tropical AMF has frequently be reported and this is often due to an inappropriate selection of host and medium, or the use of unsuitable environmental conditions (Dodd and Thompson, 1994). The current procedures for the establishment of pure pot cultures of AMF have mainly been developed for temperate species collected from soils of neutral pH with moderate to high fertility. The adoption of similar procedures for the culture of AMF collected from tropical conditions (especially from acidic poor soils) is not always satisfactory.

This paper explained the practical procedure for bulking up of mycorrhizal inoculum for tropical conditions, that may be used for reforestation activities, thus application of mycorrhizal inoculum as an alternative low input technology for rehabilitated degraded forest can be recommended.

MATERIALS AND METHOD

Tube Culture Establishment

Spore preparation

Spores of tropical AMF were extracted by wet sieving and decanting from kudzu (Pueraria javanica) pot cultures. Under a dissecting microscope, apparently healthy spores were selected, and transferred into a small Petri dish (3 cm diam). While transferring the spores, transference of debris such as fine colonised roots, small fragments of hyphae, and organic matter was avoided, as this can introduce other AMF as contaminants. Spores were then surface-sterilized with 0.05% (w/v) sodium hypochlorite for 3 min and washed in several changes of distilled water.

Seed germination and inoculation

Scarified seeds of kudzu and bahia grass (Paspalum notatum), were surface-sterilized with 30% (v/v) chlorox for 15 min, and washed several times prior to germination on steam-sterilised terrageen. The seedlings with radicles 2-3 cm long were gently removed from the substrate (see section 2.5), and immersed in sterile water to remove the adherent particles from the root surface. The seedlings were then transferred to Petri dishes (9 cm diam) layered with sterile dry tissue paper (Whatman no 1).

A single surface-sterilized spore was then placed near the root tip of kudzu, or on the roots of bahia grass using fine forceps. This procedure was carried out under the dissecting microscope (x40 magnification) to ensure the spores were clearly placed near the root tips.

Culturing in test tubes

Large test tubes (2 cm diam x 15 cm length) were partially filled with 13 g of sieved (1.4 mm mesh sieve) terragreen. This media was moistened with 12 ml half-strength Hoaglands (-P) solution which had been adjusted to a pH similar to that of the original soil from which the AMF originated. The tubes were then steam-sterilized at 121 ^{o}C for 15 min. After the tube had cooled, inoculated seedlings were transplanted into each test tube by placing the seedling between the glass and substrate. The tubes were then plugged with cotton wool and the rooting zone covered in aluminium foil. The tubes were placed in the growth room (temp 20^{o}C, PAR 300 µmol m^{-2} s^{-1}, with continuous light) for two weeks before being transferred to the greenhouse for a further 4-6 weeks under ambient tropical conditions. The tubes were re-moistened every two weeks by gently removing the cotton wool plug and pipetting 5 ml of sterile water onto the growth medium.

Checking the rate of colonization by AMF

Two to three weeks after inoculation, root colonization was checked by opening the foil and exposing the root zone. The unopened tubes were directly examined under a dissecting microscope, and root colonization was recognized by observing the presence of extramatrical mycelium near the root or on the glass surface. After 6-8 weeks in the glass tube, the colonized roots of seedlings were checked by removing them from the tube and examining them under a dissecting microscope. Those confirmed to have extramatrical mycelium of AMF were transplanted into open pot cultures in the greenhouse as described below.

Pot culture Establishment

Substrate preparation

In earlier work, untreated terragreen had been used successfully for culturing tropical AMF. The particle sizes of this substrate, however, are heterogenous. In order to improve the homogeneity, the substrate was sieved through a 1.4 mm mesh. Particles retained in the sieve were then used as a substrate for culturing tropical AMF. To remove any remaining fine particles (which also contain a high level of plant-available nutrients), the substrate was washed in running water until the wash water was clean. It was discovered that the nutrient content of terragreen after washing was not sufficient to support the growth of the host plant as well as supporting mycorrhizal development. To overcome this deficiency, the terragreen was soaked overnight in half-strength Hoagland's solution minus-P (INVAM, 1991) with the pH of the solution adjusted as described previously.

After the excess solution was drained, the substrate was dried and pasteurized in the oven at 80-90 ^{0}C for 24 h. After cooling the treated terragreen was used directly as a suitable medium for establishment of AMF in pot culture.

Harvesting of tube cultures and potting cultures establishment

After 6-8 weeks growth in the test tube, colonized seedlings were harvested in one of two ways. Firstly, by direct transplanting, after shaking the tube and gently removing the whole seedling using forceps. Each seedling was then transplanted into a plastic pot (capacity 150 ml) containing moistened treated terragreen. The second technique involved the harvesting of the colonized roots of the seedling. The contents of the tube were allowed to dry, and were then shaken out, and the entire root of the seedling was placed in a petri dish and carefully washed to remove the adherent particles. It was then transferred into another Petri dish containing distilled water and examined under a dissecting microscope. Colonized roots with mycelium and young spores were selected and cut into 1-2 fragments. These fragments were used as inoculum for starting new pot cultures of AMF. Two pieces of root fragments were inoculated onto roots of one-week old bahia grass or kudzu seedlings, then transplanted into plastic pots contained treated terragreen (capacity 150 ml).

Maintaining pot cultures and harvesting inoculum of AMF as starter

The pots containing inoculated plants were placed in the greenhouse. Nutrients were added every week using 25 mls half-strength Hoagland's solution (-P) per pot, in addition to daily watering. One month after inoculation, root colonization and culture purity were checked by taking a small root sample and placing it into a Petri dish and checking for the presence of extramatrical hyphae under a dissecting microscope. Plants which had good colonization and were free of contamination were carefully taken out. The ends of the roots were trimmed before each plant was re-potted into bigger plastic pots (capacity 0.6-3 L) containing treated terragreen. They were maintained for a further 2-3 months in the greenhouse. After this period the plant shoots were pruned, and the substrate allowed to dry in situ for one week. The substrate containing roots and spores was then ready for use as STARTER inoculum. This inoculum was used as starter for bulking-up tropical AMF inoculum.

Bulking-up of Inoculum of Tropical AMF

The starter inoculum of the four indigenous AMF i.e. G. manihotis (INDO-1), G. mosseae (PAL-03), A. tuberculata (INDO-2) and A. delicata (EJ-01) and also three exotic AMF i.e. Gigaspora rosea Becker & Hall (INVAM-FL105), Glomus etunicatum Becker & Gerd (INVAM-FL216), Glomus clarum Nicolson & Schenck (originally from Assateague island, USA collected by C.Walker) were bulked-up using the procedure described above. A slight modification involved the use of treated zeolite as the medium instead of terragreen and the use of the fertilizer VITAFEED-102 (18-0-36) at the rate of 333 mg L^{-1} as the nutrient solution instead of half-strength Hoagland's solution (-P). Plastic cups (capacity 250 ml) were filled with treated zeolite and moistened with VITAFEED-102 solution. Using sterile forceps, a hole approximately 2 cm in diameter and 3 cm deep was made on the surface of zeolite in each pot. One teaspoonful (about 10 g) of the crude inoculum of indigenous or exotic AMF were then placed in the hole. Each pot was then planted with pre-germinated bahia grass or kudzu. The plants were then arranged in the greenhouse at the Faculty of Forestry IPB, Bogor.Maintenance, including watering, addition of nutrient solution, checking for root colonization, transplanting to bigger plastic pots (capacity 1 L), drying and harvesting (after 4 months), were carried out as previously described.

Effect of Different Host and Medium Combinations on Sporulation of Tropical AMF

To improve inoculum production, different combination of host and local medium was tested following procedure above.

Inoculum preparation

The colonized roots were harvested and separated from the starter inoculum and cut into small (0.5-1 cm length) fragments for use as inoculum in this experiment.

Substrate preparation

Three different local substrates, zeolite, inolite and river sand were sieved, washed and fertilized with VITAFEED-102, then pasteurised. The prepared substrates were then placed in 250 ml plastic cups.

Seed germination and pre-transplant inoculation

Plastic boxes (24 cm long, 15 cm wide and 6.5 cm deep) were filled with pasteurized zeolite to a depth of 3 cm. To permit drainage, 6 holes (diam 4 mm) were made in the base of the box. The chopped root inoculum was evenly spread over the surface of the zeolite and then a further 2 cm depth of zeolite was placed over it. This was used as the seed bed on which seeds of kudzu, bahia grass or sorghum were germinated.

Potting and maintenance

Two-week-old seedlings of bahia grass, kudzu and sorghum were gently removed from the seed bed using sterile forceps, and transferred to Petri dishes containing distilled water. The roots of each seedling were checked under a dissecting microscope for the presence of extramatrical hyphae. Colonized seedlings were transplanted into 250 ml plastic cups containing either treated zeolite, inolite or river sand previously moistened with VITAFEED-102 solution. Pots were placed in the laboratory at room temperature for 3 days before being laced in the greenhouse at the Faculty of Forestry IPB,Bogor. Every week, the plants were fertilized with 25 ml of VITAFEED-102 solution, in addition to daily watering.

Data collection and analysis

Nine treatment combinations e.g. three substrates (zeolite, inolite and river sand) and three hosts (kudzu, bahia grass and sorghum) were used for each fungus. Each experiment was laid out in a randomized block design with five replicates. Two weeks after transplanting, and at 4 week intervals thereafter, spore development was monitored by taking and checking root samples. The samples were transferred into Petri dishes containing water and observed either directly under the dissecting microscope or by mounting the samples on slides in PVLG and observing them under the x40 objective of a compound microscope. After 14 weeks the plants were dried in situ for 2 weeks and then harvested. Roots of the host plant were gently separated from the substrate which was then mixed thoroughly. Twenty five grams sample of crude inoculum was then taken to count the number of extraradical spores. The spores were extracted by wet-sieving and decanting and transferred to a Petri dish on which a gridline scale had been previously marked on the base. They were then counted under a dissecting microscope. Only free spores were counted which may lead to an underestimate for spores of G. clarum (CLAR-17) and G. manihotis (INDO-1) which can also be formed inside the roots. Counts of spore numbers were subjected to ANOVA and the treatment means were further separated by Duncan's multiple range test (DMRT) for the significant differences at $P < 0.05$.

RESULTS

Test tube Culture Technique

By using the test tube culture techniques (Setiadi, 1996), three species of indigenous AMF i.e. G. manihotis (INDO-2), A. tuberculata (INDO-2), G. mosseae (PAL-03) and one species of exotic AMF G.clarum (CLAR-17) were successfully obtained in pure pot-culture. The success rate (number of tubes colonized) using this technique varied according to the isolates and the host (see Table-1). Using bahia grass as a host, 80% of the plants in tubes inoculated with G. manihotis were colonized, whilst the corresponding figure for A. tuberculata was 55%, for G. mosseae it was 45%, and for G. clarum it was 70%. However, if kudzu was used as the host the success rate was reduced i.e. for G. manihotis, 25% success, for A. tuberculata 15% and for G. mosseae 10%. Single-spore cultures of A. delicata failed to establish using this technique both on bahia grass and on kudzu. To check the suitability of the test tube technique as a culture system of AMF, multispore (20 spores per plant) cultures were also tested. The success rate using multispore inoculum was increased relative to single spore inoculum for each fungus tested using either host (see Table-1). A 100% success rate was achieved for both G. manihotis and A. tuberculata cultured on either kudzu or bahia grass. A success rate of 100% was also achieved for G. clarum cultured on bahia grass. For G. mosseae cultured on bahia grass and kudzu the success rates were 60% and 20% respectively.

Although the culturing attempts for A. delicata using single spores failed, when multispores were used the success rate was increased to 40% on bahia grass and 20% in kudzu. In general, in all the attempts at culturing of single or multispore inoculum, the number of host plants of bahia grass colonized was higher than for kudzu, for each fungus tested.

Table-1. Number of successful cultures for single and multispore attempts using AMF in test tube cultures using kudzu or bahia grass as a host

Culture type	Host	Number of Plant Colonized/Number of Plant Inoculated				
		CLAR-17	INDO-1	INDO-2	EJ-01	PAL
Single spores	bahia grass	15/19	16/20	11/20	0/20	9/20
	kudzu	ND	5/20	3/20	0/20	2/20
Multi-spores	bahia grass	10/10	10/10	10/10	4/10	6/10
	kudzu	ND	10/10	10/10	2/10	2/10

ND = no data

Starter Inoculum Development

Starter inoculum was initiated by transferring a colonized seedling from the test tube into an open pot (150 ml). The success rates after transplanting, either from direct transplanting or by using colonized roots of the seedlings are presented in Table-3. Using colonized roots, a success rate of 100% was achieved for isolates INDO-1, INDO-2, EJ-01 and PAL-03 either using bahia grass or kudzu. A success rate of 100% was also achieved for isolate CLAR-17 on bahia grass. However, a lower success rate was obtained when direct transplanting was used. For example a success rate of 90% was obtained for isolate INDO-1 using bahia grass as a host and 40% using kudzu as a host. For isolate INDO-2, the corresponding values were 60% for bahia grass and 40% for kudzu. The cultures established on these hosts (as shown in Table-3) were maintained as starter cultures to be bulked-up. In general, the success rate when transferring colonized plants (from the test tube) using colonized roots of either host was higher than for direct transplanting. But, when direct transplanting was carried out, higher success rates were obtained for bahia grass than for kudzu. It was also noted that in acid media (pH 4.3-6.4), bahia grass grew relatively better than kudzu, but in alkaline media (pH 7.2-8.2) kudzu grew more strongly than bahia grass (Table- 2)

Table- 2. Comparison of bahia grass and kudzu for culturing tropical AMF isolates

Cultural Practices	Tropical AMF Host	
	Kudzu	Bahia grass
Growth on acid media (pH 4.3-6.4)	++	+++
Growth on alkaline media (pH 7.2-8.2	+++	+
Relative drought tolerance	+++	++
Relative high temperature tolerance	++	+++
Germination treatment necessary	yes	no
Time for seed to germinate	3-6 days	8-14 days
Seed viability after storage	high-moderate	low-very low
Need Rhizobium symbiont	yes	no
Maintenance inputs	medium	low
Sensitivity to spider mites in greenhouse	high	low

Note: '+' is intended to be indicative of comparative relative values

Bulking-up of Tropical AMF Inoculum

The four indigenous AMF isolates (INDO-1, INDO-2, PAL-03 and EJ-01) and also G. clarum (CLAR-17) were bulked-up using either kudzu or bahia grass as the host. Soilles medium of zeolite was used as a potting medium in open pot culture in either 250 ml or 1000 ml pots, following the procedure described previously. The number of spores produced in these two different pot sizes are presented in Table- 3. In general, the density of spores produced in the small pots were almost 2-3 times higher than the density of spores produced in the bigger pots for each species of AMF using either bahia grass or kudzu. The density of spores in small pots, however, varied according to the AMF tested and the host, but the density of spores of each AMF isolate produced in bigger pots using kudzu as a host, were always higher than those obtained on bahia grass but these differences were not significant.

Table- 3. Number of successful cultures establishment following transfer of tropical AMF by direct transplanting or by using colonized roots

Host	Transfer Technique	Number of Plants Colonized/Number of Plants Inoculated				
		INDO-1	INDO-2	EJ-01	PAL-03	CLAR
Bahiagrass	Direct	9/10	6/10	ND	ND	ND
	Root	10/10	10/10	10/10	10/10	10/10
Kudzu	Direct	4/10	4/10	ND	ND	ND
	Root	10/10	10/10	10/10	10/10	ND

ND = no data

Table-4. Number of spores of AMF per 25g inoculum, after bulking-up in 250 ml and 1000 ml pots containing zeolite using kudzu or bahia grass as hosts

AMF Species	Host	Pot Size	
		250 ml	1000 ml
G.manihotis (INDO-1)	bahia grass	580 a	263 a
	kudzu	671 a	272 a
A.tuberculata (INDO-2)	bahia grass	782 a	226 a
	kudzu	834 a	273 a
G.mosseae (PAL-03)	bahia grass	279 a	84 a
	kudzu	158 a	125 a
A.delicata (EJ-01)	bahia grass	1068 a	555 a
	kudzu	992 a	657 a
G.clarum (Clar-17)	bahia grass	239 a	75 a
	kudzu	304 a	87 a

Means with the same letter within columns of each isolate of AMF are not significantly different at $P < 0.05$

Effect of Different Host and Medium Combinations on Sporulation of Indonesian AMF

The nine combinations of three different local substrates and three AMF host plants were tested for the promotion of sporulation of the AMF (Table-5). Statistical analysis showed that the choice of host and the medium, either as single factors or in combination, significantly affected the sporulation of each species of AMF. Colonization of roots by AMF and subsequent sporulation were shown to have successfully occurred in all 295 of the pot cultures containing the various host-substrate combinations. However, spore production of each AMF varied according to the host and substrate combination (Table-5).

Table-5 The effect of substrates and host on the sporulation of Indonesian AMF

Inoculation treatment	Substrate	Number of spores per gram of inoculum		
		Kudzu	Sorghum	Bahia grass
A. delicata (EJ- 01)	inolite	216 a	70 def	166 b
	zeolite	107 c	100 cd	105 c
	sand	61 ef	91 cde	55 f
G. manihotis (INDO-1)	inolite	42 b	35 b	38 b
	zeolite	18 b	41 b	22 b
	sand	33 b	81 a	24 c
A. tuberculata (INDO-2)	inolite	49 bc	33 cd	25 cd
	zeolite	61 ab	41 bc	45 bc
	sand	83 a	32 cd	12 d
G. mosseae (PAL – 03)	inolite	62 a	18 b	18 b
	zeolite	4 b	10 b	4 b
	sand	21 b	30 b	15 b

Means followed by same letters within the columns and rows for each fungus are not significantly different at $P < 0.05$

Comparing the nine host-substrate treatments on spore production of G. manihotis, the highest spore densities (81 spores g^{-1} of substrate) were produced in the combination of sand-sorghum, whilst the lowest spore densities (24 spores g^{-1} of substrate) were produced in sand-bahia grass. No significant differences were observed between the rest of the treatments. The highest spore production of A. tuberculata was achieved either in sand-kudzu (83 spores g^{-1} of substrate) or in zeolite-kudzu (61 spores g^{-1} of substrate) combinations. Kudzu-inolite was significantly the best combination for producing spores of both A. delicata and G. mosseae (i.e. 216 spores g^{-1} of substrate and 62 spores g^{-1} of substrate respectively). Combinations of sand and bahia grass were the two materials which had been previously used as a substrate medium and plant host respectively for establishing open pot cultures of AMF at the IUC-IPB in Indonesia. Table-5, shows that modification of this host-substrate combination can significantly increase the production of spores.

DISCUSSION

The initial attempts to obtain pot cultures of Indonesian AMF from spores recovered directly from the field had limited. This may be due to several reasons: e.g. the spores may have had low germination capacity (Tommerup, 1992); the spores may have been parasitised by indigenous microflora (INVAM, 1990); the spores may not have been adapted to the environment used for isolation; the spore may have been constitutively dormant (INVAM, l991); or the selection of host and soil medium or the growth room and greenhouse conditions may have been inappropriate (INVAM, l991; Dodd and Thomson, l994). To improve the success rate of pot-culturing of tropical AMF, the trapping of the indigenous propagules present in the soil to induce their sporulation prior to pot culture establishment is recommended (Dodd and Thomson, l994). To provide optimum conditions for the trap cultures, a suitable medium (substrate, nutrition and pH) and appropriate mycotrophic host plants is also recommended (INVAM, l991). Pot cultures of AMF can then be initiated using spores recovered from the trap cultures. Following consideration of these statements, it was decided to select different substrate mixes, to adjust the pH and to adapt culture procedures to tropical conditions.

Initial testing of twelve substrates, it was found that terragreen was the most suitable medium for both the growth of the host and for colonization by the tropical AMF. The main advantages in using this clay-based substrate for pot-culturing of AMF is the physical structure it possesses which promotes good aeration and root development, coupled with the adsorptive capacity of the clay. This latter property means that this substrate slowly releases added nutrients, particulary phosphate, thus reducing the need for frequent application of fertilizer.

The limited success in obtaining subcultures of tropical AMF using other soil-less media may be due in particular to the inappropriate pH of the medium and pH of water used for watering for both the growth of the host and colonization by AMF. Previous studies have shown the importance of medium pH for germination and establishment of AMF (INVAM, 1992). Some fungi particulary those isolated from tropical soil, prefer low pH conditions (Sieverding, 1991; Dodd, pers. comm.), whilst others may require alkaline conditions. For example, spore germination of Acaulospora laevis was optimum at pH 4.5 and this germination capacity was reduced by 10% in neutral or alkaline conditions (Hepper 1984). In contrast, some Glomus spp. have a neutral to alkaline pH for optimum germination (Siquera et al.,1984). Mixed soil-less substrates used in the experiment, except terragreen, had a pH greater than 7.4. This may mean that they were too alkaline for both growth of the host and of the indigenous AMF.

In these results, using kudzu as a host for culturing the tropical species of AMF which were recovered from acid soil (G. manihotis, E. colombiana and A. tuberculata) and a slightly acid soil (A. delicata), the fungi failed to colonize the host under alkaline conditions. In contrast, when a medium with lower pH was used (i.e. the terragreen substrate), successful colonization by these species occurred. Of the two host plants tested, kudzu grew better across the pH range of 4.3-8.2, whilst bahia grass grew well only in acidic conditions (Table-2). This may be a reason for the failure of subcultures of tropical AMF to establish when using bahia grass grown in alkaline conditions. Due to this sensitivity of the host to pH, special consideration is required when choosing a suitable host for specific isolation of AMF. The success rate in culturing indigenous AMF can be increased if the pH of the growth substrate is adjusted close to that from where the spores were extracted. A practical consequence is that information about the origin of the isolate (edapho-climatic, host preference, pH and substrates) is required for successful subculturing species of AMF. Further studies to determine the effect of different soil-less media on tropical AMF colonization using an