MYCORRHIZAE FROM SELECTED TREE SPECIES IN MT. PANGASUGAN, LEYTE, PHILIPPINES AND THEIR EFFECT ON TREE GROWTH

RUBEN M. GAPASIN, JESUSITO L. LIM and CARLITO V. RANCHES
Department of Plant Protection
Visayas State College of Agriculture (ViSCA)
Baybay, 6521-A Leyte

ABSTRACT

A study was conducted to determine and identify indigenous mycorrhizae associated with selected tree species growing in Mt. Pangasugan, evaluate grasses as trap crop for their culture and mass production, test their efficacy for tree growth improvement and evaluate the most efficient delivery system for the mycorrhizae. There were 14 tree species sampled and based on root sample assay seven (7) were found positive for mycorrhizal association. These were Kaliandra (Calliandra calothrsus), Antoso-an (Cassia javanica L.), Paguringon (Craoxylum celebicum Blume). Fire tree (Delonix regia (Bojer Hook) Raf.), Yamane (Gmelina arborea Roxb.), Ipil-ipil (Leucaena leucocephala (Lamarck) de Wit) and Narra (Pterocarpuz indicus Willd). However, all the soil samples contained vesicular-arbuscular mycorrhizal (VAM) spores with Paguringon having the highest spore count of 359 while Dao had the lowes count of 65 spores. Four major genera of VAM fungi were identified associated with the tree species namely: Glomus, Gigaspora, Acaulospora and Sclerocystis. All the three grasses evaluated as trap crops supported the build up of mycorrhizal fungi with Napier grass producing the heaviest roots (21.57 g) and containing the highest mycorrhizal spores (476). In terms of inoculum produced in the soil there was an increase of 61.6%, 48.1% and 39.8% for Paragrass, Napier grass and Guinea grass, respectively after 3 months. Mycorrhizal roots and soil containing mycorrhizae as inoculum increased both the height (cm) and stem diameter of tree seedlings tested in the screenhouse and field. The general observation was that the soil with mycorrhizae had greater effect on seedling growth.

Keywords: *Mycorrhizae, Vesicular-arbuscular-mycorrhizae, Glomus, gigaspora, Acaulospora, Sclerocystis, Seedling growth, Mycorrhizal spores, Tree species Trap crop*

INTRODUCTION

Mycorhiza is a symbiotic relationship between a fungus and the roots of plants. This association is widespread in the plant kingdom. The fungus derives all its food requirements from the plants. In return, the fungus provides benefit to the plants such as: increased absorption of nutrients and water, increased drought resistance of plants, control of root pathogen infection, production of growth promoting substances, promotion of the activity of other beneficial organisms and improvement of soil structure and soil aggregation (Kothari et al., 1990 and Dela Cruz, 1993).

Mycorhizal fungi are classified into ectomychorrhiza and endomycorrhiza. Endomycorrhiza is found in many trees such as narra, ipil-ipil, kakawate, yemane, falcata, mangium, rain tree and others. The ectomycorrhizal fungi form a loose network of hyphae on the root surface. Endomycorrhiza invade the cortical cells where they develop spherical to ovate structures (vesicles) and multibranched hyphae (arbuscules), thus called vesicular-arbuscular mycorrhiza (VAM). The VAM fungi belong to the family Endoganaceae a lower form of fungi resembling molds.

There has been considerable interest in vesicular-arbuscular (VA) mycorrhizal fungi in the past two decades because of the overwhelming published evidence indicating their growth promoting aspects. In a study conducted by Bagyaraj et al. (1989) they screened seven VA mycorrhizal fungi for symbiotic response with Hawaiian giant cultivars of Leucaena leucocephala. They found three promising ones from a preliminary trial conducted with fungi obtained from different parts of the world and the other four were local isolates obtained from the rhizosphere of Leucaena. A local isolate of Glomus mosseae was found to be the best mycorrhizal fungi for inoculation of Leucaena in order to obtain healthy, vigorously growing seedlings.

According to Kothari et al. (1990) root infection with vesicular-arbuscular (VA) mycorrhizal fungi can increase efficiency of nutrient absorption and enhance growth and development of mycorrhizal plant particularly in soil with low phosphorus. They noted that VA mycorrhizal association may alter plant-water relations since more rapid recovery from water stress and higher soil moisture extraction at low water potential have been observed in mycorrhizal plants. On the other hand, Dela Cruz (1993) considered mycorrhiza as a biofertilizer because it contains beneficial fungi that can increase the absorption of nutrients that are already in the soil. He said that the fungus that can increase the absorption of nutrients that are already in the soil. He said that the fungus has the ability to increase efficiency and recover of bound nutrients from the soil. The addition of chemical fertilizers had helped improve the survival and growth rates of tree seedlings in reforestation sites. However, the cost of chemical fertilizers is very expensive. It is also detrimental to the soil microfauna and other soil-inhabiting beneficial microorganisms when continuously used. Other alternative technologies to replace these chemical fertilizers is important.

Mycorrhiza is one of the alternative technologies known to substitute for chemical fertilizers. Once the fungus invades plant roots, it can proliferate within the roots as the latter grows. There is no need to reinoculate the plants especially in the field. It is convenient to inoculate reforestation seedlings during the nursery phase. In addition, mycorrhiza is safe to use since it is part of the natural reforestation. This study was conducted with the following objectives: (1) to determine and identify indigenous mycorrhizae associated with selected tree species growing in Mt. Pangasugan, (2) assess trap crop for the culture and mass production of potential mycorrhizae, (3) test their efficacy for tree growth improvement and (4) evaluate the most efficient delivery system for these mycorrhizae.

MATERIALS AND METHODS

Different tree species at the closed canopy area in Mt. Pangasugan were tagged. Soil and root samples were collected from them. The root samples were then processed following the standard procedures by Kormanik and McGraw (1983). Likewise, the soil samples were processed for mycorrhiza following the sucrose-flotation method of Allen et al. (1979).

Soil collected from different tree species was thoroughly mixed to 1 part sand and was placed in 20 cm pot. The potted sand-soil mixture was planted with Guinea grass (*Panicum maximum* Jacq.); Napier grass (*Pennisetum purpureum* Schumach.) and Paragrass (*Brachiaria mutica* (Forrsk.) Stapf.) as hosts for mycorrhizal culture and mass production. The initial mycorrhizal spore count in the soil prior to planting the grasses was determined. Build up of mycorrhizal fungi in these three grass species was assessed three months after planting. Percent increase of mycorrhizal fungi population in the soil was computed.

Screenhouse Experiment

Tree seedlings namely: Dao (*Dracontomelon dao* (Branco) Merril et Rolfe, Paguringon *(Cratoxylum celebicum)* Blume, Hindang, Bagalunga (*Melia dubia* Caranilles), and Kalumpit (*Terminalia microcarpa* Decaisne) acquired from the GTZ nursery area were evaluated for mycorrhizal infectivity using mycorrhizal roots and mycorrhizal soil as inocula. Data on plant height and stem diameter were taken before VAM inoculation and at monthly interval until five months after VAM inoculation. Roots and soil samples were collected and processed following the procedures stated earlier. Infection of VAM in roots was determined by randomly picking 30- 1 cm root segments, placed on a slide and observed under the microscope and the percent infection was computed. VAM spores extracted from soil samples were counted under the microscope with the aid of a multiple hand tally counter. The following treatments were used: T1-uninoculated; T2-inoculated with 100 g soil with mycorrhizae and T3-inoculated with 25 g mycorrhizal roots. Each treatment was replicated three times.

Field Experiment

Evaluation of the effective mycorrhizal fungi for tree growth and development under field condition was conducted. The experiment was set up at Punta, Baybay, Leyte using Narra and Mahogany seedlings. Soil samples were randomly collected in the area. These were processed in the laboratory to assess the population of native VAM fungi present in the area. One month after transplanting, the seedlings were inoculated with VAM fungi. Data on plant height and stem diameter were taken before and after inoculation until 12 months. The following treatments were used; T1-uninoculated control; T2-inoculated with 25 g mycorrhizal roots; T3-inoculated with 25 g mycorrhizal roots + 50 g MYCOVAM 1 and T4-inocculated with 50 g MYCOVAM 1. Each treatment was replicated five times.

RESULTS AND DISCUSSION

Determination and Identification of Mycorrhizae

There were fourteen (14) tree species sampled namely: Ayangili (*Acacia confusa* Merr.), Kariskis (*Albiziza lebbekoides* (D.C.) Berth.), Kaliandra (*Calliandra calothrsus*), Antoso-an (*Cassia javanica* L.), Kapok (*Ceiba pentandra* (L.) Gaertu), Paguringon (*Cratoxylum celebicum* Blume), Fire tree (*Delonix regia* (Bojer Hook) Raf.), Dao (*Dracontomelon dao* (Branco) Merrill et Rolfe), Ani-I (*Erythrina fusca* (Lour.,)), Dapdap (*Erythrina orientalis* L. (Murr.), Yemane (*Gmelina arborea* Roxb., Ipil-ipil (*Leucaena leucocephala* (Lamarck) de Wit), Narra (*Pterocarpus indicus* Willd) and Anabiong (*Trema orientalis* (L.) Blume). Of these tree species, seven (7) were identified to have mycorrhizal association through root sample assay. These included: Kaliandra, Antoso-an, Paguringon, Fire tree, Yemane, Ipil-ipil and Narra. However, all the soil samples (150 g) collected from these tree species contained various VAM spores with the soil sample from Paguringon having the highest spore count of 359 spores while the soil sample from Dao had the least count of 65 spores (Table 1). Mean spores count in soil samples collected from these tree species was 180 spores.

Four major genera of VAM fungi were identified associated with trees in Mt. Pangasungan namely: *Glomus, Gigaspora, Acaulospora and Sclerocystis.* Description of each genera is presented in Table 2.

Mass Production/Culture of Mycorrhizae

All the three grasses tested as trap crops supported the build up of mycorrhizal fungi as revealed by VAM spore count in both the soil and root samples. Napier grass produced the heaviest roots (21.57 g) and contained the highest mycorrhizal spores in the roots (476 spores). This was significantly different to Guinea and Para grasses (Table 3). In terms of inoculum production in the soil, spore count after 3 months was 467, 374 and 299 spores or an increase of 61.62%, 48.12% and 39.80% for Paragrass, Napier grass and Guinea grass, respectively.

Table 1. Number of mycorrhizal spores from the soil and roots collected from selected tree species at the closed canopy area in Mt. Pangasugan, Leyte, Philippines.

Scientific Name	Common Name	Mycorrhizal Spores Soil[1]	Roots[1]
Acacia confusa Merr.	Ayangili	325	–
Albizia lebbekoides (D.C.) Berth	Kariskis	187	–
Calliandra calothyrsus.	Kaliandra	331	+
Cassia javanica (L.)	Antoso-on	213	+
Ceiba pentandra (L.) Gaertu.	Kapok	80	–
Cratoxylum celebicum Blume	Paguringon	359	+
Delonix regia (Bojer Hook) Raf.	Fire tree	141	+
Draxontomelon dao (Branco) Merrill et Rolfe	Dao	65	–
Eryhrina fusca Lour.	Ani-i	151	–
E. orientalis (Murr) L.	Dapdap	84	–
Gmelina arborea Roxb.	Yemane	97	+
Leucaena leucocephala (Lamarck) de Wit	Ipil-ipil	178	+
Pterocarpus indicus Willd	Narra	167	+
Trema orientalis (L.) Blume	Anabiong	144	–

[1]Based from 150 g soil sample
[2]+and-indicates presence or absence of mycorrhiza

Table 2. Description of genera of vesicular arbuscular mycorrhiza (VAM) fungi associated with selected tree species in Mt. Pangasugan, Leyte, Philippines

GENERA	DESCRIPTION
Acaulospora	Azygospore produced singly in soil, large generally globose or subglobose, with oily content, borne laterally on the stalk of a large, terminal, thin-walled vesicles. Vesicles about the same size as the spore. Spore walls continuous except for a small occluded spore. Germ tubes produced directly through walls near spore base. Forming endomycorrhizae with lobed vesicles and arbuscules.
Gigaspora	Azygospore produced singly in soil, large, generally globose or subglobose, with oily contents, borne terminally on a bulbous suspensor-like cell to the spore. Spore wall continuous except for a small occluded spore. Germ tubes produced directly through wall near spore base, Thin-walled vesicles borne in soil on coiled hyphae, forming singly or in clusters. Forming endomycorrhizae with arbuscles.
Glomus	Chlamydospores borne terminally on single (rarely two) undifferentiated, nongametangial hyphae in sporocarps or individually in soil. Spore contents at maturity separated from attached hyphae by a septum or occluded by spore wall thickening.
Sclerocystis	Chlamydospores arranged side by side in a single layer, elongate, radiating out from a central plexus of hyphae.

Table 3. Fresh root weight (g) and mycorrhizal spores in three grass species three months after planting to soil collected in Mt. Pangasugan closed canopy area.[1]

Grass Host	Fresh Root Weight	Mycorrhizal Spores	
		Soil[2]	Roots[3]
Guinea Grass (*Panicum maximum* Jacq.)	14.6 b	299.0 c	394.0 b
Napier Grass (Pennisetum purpureum Schumach.)	21.6 a	347.0 b	476.0 a
Para Grass (Brachiaria mutica (Forssk.) Stapf.	11.6 b	469.0 a	341.0 b

[1]Average of 10 replications. Means within a column followed by the same letter are not significantly different at 5% level (DMRT)
[2]Based from 150 g soil sample.
[3]Based from ten 1 cm root sample.

Screenhouse Experiment

The different tree seedlings showed varied growth response to VAM inoculation. Inoculation of VAM regardless of type of delivery system increased both plant height and stem diameter for all tree species tested except for the plant height of Bagalunga seedling. It was observed that Dao had the greatest growth response in terms of plant height increase (12.34 to 31.09) compared to the other tree seedlings. However, in terms of stem diameter increase, Hindang had the highest stem diameter growth (13.05 to 31.05) (Table 4). The use of soil with mycorrhizae (T2) as inoculum had a greater effect on seedling growth compared to the use of mycorrhizal roots (T3). Although results are not statistically different from that of the uninoculated treatment (T1) a variable trend was observed as to the effect of VAM inoculation on both growth parameters studied. This may be due to the fact that the inoculum used contained different VAm fungi species since it was field collected. According to Daniels and Menge (1981) mycorrhizal species differ significantly in their ability to stimulate growth to host plant because of differing rate of infection, greater infectivity related to spore size, more external hyphae or more rapid translocation of essential nutrient elements.

As presented in Table 4, the highest percent root infection was observed in Hindang followed by Paguringon and Dao seedlings inoculated with soil with mycorrhizae (T2). The treatments inoculated with mycorrhizal roots (T3) gave the second highest root infection to the same tree species. It was noted that roof infection was higher in Hindang, Paguringon and Dao seedlings and minimal root infection was observed that there was an infection even on the uninoculated treatment (T1) for Paguringon, Hindang, Dao and Bagalunga except for Kalumpit.

Table 4. Plant height (cm) and stem diameter (mm) growth of five tree species, perent root infection and spore count in soil as affected by VAM fungi inoculation five months after under screenhouse condition.[1]

Treatment[3]	Growth[2] Plant Height	Stem Diameter (cm)	% Infection[4] (mm)	Spore in Soil[5]
PAGURINGON				
Uninoculated	32.4	2.0	5.6 c	116.0 b
100 g soil with mycorrhiza	36.3 (10.8)	2.7 (25.1)	60.0 a	1,186.7 a
25 g mycorrhizal roots	33.7 (3.8)	2.8 (29.3)	40.0 b	1,075.3 a
HINDANG				
Uninoculated	9.2	3.3	6.7 b	355.0 b
100 g soil with mycorrhiza	12.2 (24.8)	4.8 (31.1)	75.6 a	1,431.7a
25 g mycorrhizal roots	9.8 (6.4)	3.8 (13.1)	67.8 a	1,361.3 a
DAO				
Uninoculated	16.3	3.8	5.6 b	49.0 b
100 g soil with mycorrhiza	23.7 (31.1)	5.3 (28.1)	38.9 a	195.0 a
25 g mycorrhizal roots	18.6 (12.3)	4.5 (14.9)	27.8 a	167.0 a
KALUMPIT				
Uninoculated	12.8	2.7	0.0 b	18.7 b
100 g soil with mycorrhiza	17.2 (25.5)	2.8 (5.7)	7.0 ab	32.0 ab
25 g mycorrhizal roots	16.7 (23.3)	3.0 (11.0)	12.2 a	39.7 a
BAGALUNGA				
Uninoculated	13.7	1.3	2.2 b	16.0 b
100 g soil with mycorrhiza	14.2 (3.7)	1.7 (20.4)	8.9 ab	34.7 a
25 g mycorrhizal roots	13.2 (-3.6)	1.7 (20.4)	12.1 a	37.0 a

[1]Average of three replications. Means within a column followed by the same letter are no significantly different at 5% level (DMRT)

[2]Figures in parenthesis denote the percent increased based from the uninoculated control.

[3]Inoculum level of 370 spores per 150 g soil.

4Based from 30 1 cm root segment.

5Based from 300 g soil sample.

The reason for this may be because the seedlings acquired were already potted in small plastic bags and the soil used for potting was not sterilized and may have contained native VAM fungi spores.

In terms of VAM spore recovery from the soil, Hindang and Paguringon gave the highest spore recovery even in uninoculated treatment compared to the other tree seedlings. There was inoculation but very low spore recovery from Kalumpit and Bagalunga (lower than that of the inoculum level) was noted. In other words, the said tree seedlings may be poor host of the VAM fungi used. Abbott et al. (1988) mentioned that in many soils, spore number is not a good predictor of VAM formation. Also, the total number of infective propagules in a soil has little predictive value for subsequent mycorrhizal formation, particularly where there are different species of VAM fungi and where there are also different types of propagules of individual VAM fungi and where there are also different types of propagules of individual VAM fungi species.

Field Experiment

Table 5 shows the result of the field experiment conducted at Punta, Baybay, Leyte, Philippines. Inoculation of VAM fungi increased both plant height and stem diameter of Narra and Mahogany. Plant height and stem diameter increase (%) due

Table 5. Plant height (cm) and stem diameter (mm) growth of Narra and Mahogany as affected by VAM fungi inoculation 12 months after under field condition.[1]

Treatment	Growth[2] Plant Height (cm)	Stem Diameter (mm)
NARRA		
Uninoculated	88.4 b	8.0 b
25 g mycorrhizal roots	95.2 b (7.2)	10.4 b (23.1)
25 g myeorrhizal roots + 50 g Mycovam 1	193.4 a (54.3)	21.4 a (62.6)
50 g Mycomvam 1	142.9 ab (38.1)	18.8 b (32.2)
MAHOGANY		
Uninoculated	70.2	12.2
25 g mycorrhizal roots	91.9 (23.6)	14.2 (14.1)
25 g mycorrhizal roots + 50 g Mycovam 1	117.8 (40.4)	17.0 (28.2)
50 g Mycovam 1	103.9 (332.4)	4.7 (17.0)

[1]Average of five replications. Means within a column followed by the same letter are not significantly different at 1% level (DMRT)

[2]Figures in parenthesis denote the percent increased based from the uninoculated control.

to VAM fungi application ranged from 7.18-54.29 and stem diameter increase ranged from 23.07-62.64 for Narra. For Mahogany, the increase ranged from 23.55-40.39 and 14.08-28.23 for plant height and stem diameter, respectively.

LITERATURE CITED

Allen, M.F., T.S. Moore, Jr., M. Christensen and N. Stanton. 179. Growth of vesicular-arbuscular mycorrhizal and non-mycorrhizal *Bouteloua gracilis* in a defined medium. *Mycologia* 71:660-669.

Bagyaraj, D.J., M.S. Byra Reddy and P.A. Nalini. 1989. Selection of effectiveness inoculant VA mycorrhizal fungi for Leucaena. *Forest Ecology and Management* 27:81-85.

Daniels, B.A. and A.J. Menge, 1981. Evaluation of the commercial potential of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. *New Phytol.* 81:345-354.

Dela Cruz, R.E. 1993. Enhancing reforestation through mycorrhiza. Paper presented during the NRCP Cluster III Pre-annual meeting. UPLB-CF. College, Laguna. 21 pp.

Kormanik, P.P. and A.C. McGraw. 1983. Quantification of vesicular-arbuscular mycorrhizae in plant roots. Methods and Principles of Mycorrhizal Research. The Amer. Phytopathol. Soc. St. Paul, Minnesota. 37-45 pp.

Kothari, S.K., H. Marschner and E. George. 1990. Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. *New Phytol.* 116:303-311.