CHAPTER - II
TAXONOMY OF VA-MYCORRHIZAL FUNGI
INTRODUCTION

The fungi involved in mycorrhizal association generally produce spores outside the roots. The morphology of these spores mainly forms the basis of taxonomy of these fungi. The microscopic characters which include finer details complicates the identification of different species. As a result the taxonomical studies on endogonaceous members have been fragmentary, though, these fungi are frequently encountered in nature. Peyronel (1923) was the first to recognise that vesicular-arbuscular mycorrhizal fungi were members of Endogonales. Gerdemann and Nicolson (1963) developed procedure for collecting spores of these fungi from soil and described new species of genus *Endogone*. The family endogonaceae was monographed in (1974) by Gerdemann and Trappe and the genus *Endogone* was segregated into seven genera. Trappe (1982) recognised nine genera of VA mycorrhizal fungi.


Studies on taxonomy of endogonaceae from Indian soils are meagre (Bakshi, 1974; Bhattcharjee et al., 1980 (a,b), 1982; Gerdemann and Bakshi, 1976; Singh and Varma,
1981; Thapper and Khan, 1985). Moreover north-eastern region of India remained untouched. During the present investigation on mycorrhizal association of tree species in a forest ecosystem of Meghalaya, ten different VAM fungi were identified and are described in this chapter.

(A) **Methods of Isolation and Identification of Endogonaceous fungi:**

The spores of endogonaceous fungi were isolated from the soil by the method of Gerdemann and Nicolson (1963). On the filter paper the spores were separated from soil debris and transferred to watch glasses containing water. The identical spores were separated and stored in glass vials in Ringer's solution. The different types of spores were mounted in Polyvinyl lactophenol medium on glass slides for microscopic observations. Ringer's solution and PVL medium were prepared as follows.

(i) **Ringer's solution**

\[
\text{Sodium Chloride} = 7.5 \text{ g.} \\
\text{Potassium Chloride} = 0.075 \text{ g.} \\
\text{Calcium Chloride} = 0.1 \text{ g.} \quad \text{(anhydrous)} \\
\text{Sodium bicarbonate} = 0.1 \text{ g.} \quad \text{(anhydrous)}
\]

All the above constituents were dissolved in a litre of water and pH of solution was adjusted to 7.4 with
IN NaOH/IN Hcl.

(ii) PVL medium: Polyvinyl lactophenol medium was prepared by dissolving 15ml of Polyvinyl alcohol granules in 100ml of distilled water on a water bath at 80°C for 2 days. The resulting solution was stored in brown bottle. 50 parts of this solution were mixed with 22 parts each of lactic acid and phenol. The resulting mixture is PVL medium and was used for permanent mount of endogonaceous spores.

(iii) Histochemical tests: Histochemical tests of spores were performed with Meltzer's reagent which was prepared as follows.

\[
\begin{align*}
\text{Iodine} & = 0.5 \text{ g.} \\
\text{Potassium Iodide} & = 1.5 \text{ g.} \\
\text{Chlora hydrate} & = 20 \text{ g.} \\
\text{Distilled water} & = 20 \text{ ml.}
\end{align*}
\]

The constituents were mixed and the reagent stored in dark bottle. The spores were mounted directly in the reagent.

(B) Establishment of culture of VA mycorrhizal fungi:

Isolated spores of Glomus spp. were inoculated in autoclaved garden soil on maize plants and grown till the host matured and dried. The method suggested by Gilmore (1968) was used. The suspension of isolated spores of VA endophyte Glomus spp. was poured in a hole in autoclaved
garden soil taken in a plastic pot. A germinated seeds of maize were placed over the inoculum in the hole. The host plant was grown for a period of 2 months and allowed to dry. The roots of maize host were chopped and mixed with soil. This soil-root mixture was used as an inoculum.

(C) Description of endogonaceous fungi:

Ten species of endogonaceous fungi were isolated and identified from the rhizospheric soil samples of various tree species. These species are described below.

Genus Acaulospora is represented by 2 spp.

1. Acaulospora laevis Gerdemann and Trappe.

Sporocarps unknown, spores forming singly, sessile, borne laterally on a wide thin walled hyphae 20 μm in diameter that terminate nearby in a globose vesicle of the size of spore, shrunken at spore maturity, spores smooth, 300-400 μm, globose or sub-globose, dull yellow, spore wall continuous except for the occluded opening 5μm thick. Hypha attached below spores branched.

This form was collected from the rhizospheric soil of Machilus kingi. The characters of the present isolate resemble type description of Gerdemann and Trappe (1974) (Plate 2.1).

2. Acaulospora scrobiculata Trappe.

Sporocarp unknown, spores forming singly in soil,
sessile, borne laterally on thin walled hyaline hyphae that terminate in a thin walled vesicle, collapsed vesicle attached to spore, spore shape varies from globose to ellipsoidal, 100-200 μm, olive to light brown, spore surface evenly pitted. This form was isolated from the rhizospheric soil of *Alnus nepalensis* and resemble the type description of Trappe (1977). (Plate 2.1).

*Genus sclerocystis* is represented by three species.

3. *Sclerocystis coremioides* Berk and Broome.

Sporocarp sub-globose, 200-250μm, in gregarious, dark-brown, peridium of interwoven hyphae (5 μm diam.) present. Chlamydospores arranged in a single layer around a central plexus of hyphae, obovoid to ellipsoidal to broadly clavate, 50-75 x 40-53 μm, brown, spore wall 5 μm thick (Plate 2.2).

4. *S. rubiformis* - Gerdemann & Trappe, sp. nov.

Sporocarp brown, subglobose, 400x450μm consisting of a single layer of chlamydospores surrounding a central plexus of hyphae, peridium absent or poorly developed of loose interwoven hyphae. Chlamydospores brown, obovoid to ellipsoidal or sub-globose, 33-45x25-35 μm, spore wall laminate 3 μm thick with perforated projections on the inner surface.

This form was isolated from the rhizosphere of *Machilus kingii* and is also reported by Bhattacherjee *et al.*
(1980) from agricultural field at Bangalore.

5. *S. microcarpus* Iqball & Bushra

Sporocarp brown, 112 μm in diam., globose. Chlamydospores formed radially in a single tightly packed layer around a central plexus of hyphae. Peridium absent. Chlamydospores 58-75x18-20 μm, narrowly clavate tapering towards basal hyphal attachment 5-8 μm in diam., spore wall 8-13μm thick at apex and 3-8 μm thick at the base.

The description of the present isolate resembles the type description given by Iqball and Parveen (1981). This species has not been reported from India earlier and constitute a new record. (Plate 2.2).

Genus *Glomus*: Two species of *Glomus* were identified namely, *G. macrocarpus* (Nicol & Gerd.) and *G. mosseae* (Nicol & Gerd.).

6. *Glomus macrocarpus* var. geosporus (Nicol. & Gerd.)

Chlamydospores formed singly in soil, globose or subglobose, 110-150x110-143μm, smooth, dark brown to black, spore wall 10-15μm thick, perforated, hyphal attachment 12-15μm in diam.

This form was isolated from the rhizosphere of *Manglietia insignis* and has also been described in agricultural fields by Bhattacharjee et al. (1980) (Plate 2.3).

7. *G. mosseae* (Nicol. & Gerd.)

Sporocarp not found. Chlamydospores.
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7. G. mosseae (Nicol. & Gerd.)

Sporocarp not found. Chlamydospores yellow to brown,
68

globose to obovoid 90-110 µm in diam. Spore wall 7 µm thick.

This species was isolated from the rhizosphere of Cinnamomum tamala and has also been reported from agricultural fields by Bhattacharjee et al. (1980) (Plate 2.3)

Genus Gigaspora: Three species of Gigaspora were identified from the collection. These are G. gregaria Nicol. & Schenck, G. calospora and G. gigantea.

8. Gigaspora gregaria Nicol & Schenck

Azygosporos formed singly in soil, 200-300 µm indiameter, globose, dark brown, spore wall 12-25 µm thick, irregular, polygonal projections on the surface present. The suspensor like cell 50 µm in diam. Concolorous with spore and giving rise to a slender hyphae that projects to the spore surface.

This species was isolated from the rhizosphere of Alnus nepalensis and differ from G. coralloidea in having smaller spores with surface projections. This species is being reported from India for the first time (Plate 2.4)

9. G. calospora (Nicol & Gerd.)

Azygosporos formed singly in soil, globose or sub-globose, 150-160 µm in diam., pale yellow, spore wall 2-5 µm thick, continuous, suspensor like cell 50 µm in diam. bulbous and concolorous with spore wall. This form was
isolated from the rhizosphere of *Schima khasiana* and resembles the type description of Gerdemann & Trappe (1974). However, azygospores were more or less globose. This form has been collected from the forest and agricultural land in Uttar Pradesh by Bakshi (1974) (Plate 2.4).

10. *G. gigantea* (Nicol. & Gerd.)

Azygospores formed singly in soil, 260-370x260-350 μm, globose, greenish yellow, spore wall 12-25 μm thick, suspensor like cell bulbous, 42-48 μm in diam. giving rise to a slender hypha which projects to the spore, vesicles not observed in the collection.

This form was isolated from the rhizosphere of *Manglietia insignis* and has also been reported in woodland by Bakshi (1974) (Plate 2.4).
Plate 2.1

1. Spores of *Acaulospora scrobiculata* showing surface pits (X 100); 2. Spore with two distinct walls (X 400); 3. Sessile spore of *A. laevis* and shrunken vesicle (X 100); 4. Spore with hyphal attachment (X 400); (h=hypha; i=inner wall; o=outer wall; p=surface pits; s=spore; v=vesicle; w=spore wall).
Plate 2.2

1. Sporocarp of *Sclerocystis coremioides* (X 100);  
2. V.S. sporocarp of *S. microcarpus* (X 100);  
3. Spores of *S. microcarpus* (X 400);  
4. Sporocarp of *S. rubiformis* (X 100). (b=basal end; c=central plexus; h=hyphal attachment; S=spore; Sh=peridium; w=spore wall).
Plate 2.3

1. Spore of *Glomus macrocarpus* var. geosporus (X 100);
2. Spore of *G. mosseae* (X 100). (h=hyphal attachment; s=spore).
Plate 2.4

1. Spore of *Gigaspora gigantea* (X 100); 2. Spore of *G. calospora* (X 100); 3. Spore of *G. gregaria* (X 100)
(b=bulbous base; h=hyphal attachment; s=spore).