NBU Journal of
Plant Sciences

Vol 3, March 2009
ISSN No 0974 6927

Arbuscular mycorrhizal fungal association in rhizosphere of *Hevea brasiliensis*

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Abstract

Occurrence of VAM spores in *Hevea brasiliensis* growing soils and percent colonization in roots of three varieties (RRII 105, RRIM 600 and GT 1) were studied. Spore population varied from 30 to 45/g soil and the root infection of all the three varieties ranged from 70 to 100 % in both mature and young plants. Percent of root infection was found to be more in the drought resistant varieties RRII 105 followed by RRIM 600 and the less drought resistant variety GT-1. Nine different types of glomalean spores were recovered from the soil samples, including five from the genus *Glomus*, three from Acaulospora, one from *Gigaspora* and few undefined species of *Sclerocystis* . The scanning electron microscopic observations of the most commonly occurring spores of *Glomus fasciculatum* revealed smooth wall character with number of pits

Keywords: AM Fungi, *Hevea brasiliensis* .

A mycorrhiza is a mutualistic symbiosis where a fungus and a colonized plant root co-exist for long periods during physiological, ecological and reproductive stages of both partners (Harley 1989). The most widespread type of mycorrhiza is that characterized by obligate formation of fungal arbuscules in the root cortex. Classification of the Mycorrhizal fungi is based mainly on the morphological characters of the spores (Morton and Benny 1990). The most consistent of those features appears to be related to the developmental functions, such as type, number and positions of the spore walls, events in spore development and germination processes (Morton 1988).These characters remain structurally constant even under diverse environmental conditions (Morton 1985, 1990). Hence the present investigation was carried out to understand the presence of the dominant VAM population in the rhizosphere of three important commercially grown varieties of *Hevea brasiliensis*.

Material and Methods

Isolation of VAM. Soil samples and roots of different varieties of *Hevea brasiliensis* were collected from two different plantation sites (sector 1 & 2); Sector 1 with mature trees of six years old and sector 2 with less mature trees of four years old .Top soil (up to 15 cm) and feeder roots from the mature trees from both the sites of three varieties (RRII 105, RRIM 600 and GT 1) were taken, packed in polythene bags and stored in cold (-4°C) till further processing.

Collection of VAM spores. VAM spores were collected from the soil samples by wet sieving and decanting method (Gerdemann and Necolson 1963) 5 Kg of soil sample was homogenized by mixing with 10 L of water and allowed to stand for 2-3 h, after the soil particles had settled down the top water was then passed through the sieves of pore size marked as BS 200, BS 150, BS 100, BS 80 and BS 60 piled one above other in a order of decreasing pore size from top to bottom.(wet sieving apparatus). The spores were collected from each tier with the help of a fine brush and suspended in sterile water for further observation.

Histopathological analysis. The root specimen were taken from field and then processed following the method of Phillips and Hayman (1970) for histopathological observations. The root were cut into pieces, after washing treated with KOH(10%), kept in water bath for 1h, then 1% HCL was added to neutralize the alkalinity. The root pieces were then washed with water (after 30 min) and staining was done by simmering the roots in cotton blue: lactophenol(1:4) for 3-4min with mild heating. Degree of contrast between fungal tissues and back ground plant cells was obtained according to the duration of storage of tissues. 1% HCl was added to acidify the tissues, as most histological stains are acidic. The anatomical study of the roots of *Hevea brasiliensis* for the percent VAM infection was also conducted. The percentage VAM infection was calculated as follows:

VAM infection(%) = root segments infected / all root segments X 100

Scanning electron microscopy of VAM spores. Scanning electron microscopy of the predominat VAM spores was performed. Samples were prefixed in 2.5 % glutaraldehyde in 0.1 M Phosphate buffer, pH 6.8 in vaccum (Shetty *et al* 2003). The samples were coated with 20 nm gold-palladium alloy in a sputter coater and examined in a JEOL JSM 5200 Scanning Electron Microscope (Tokyo Japan)

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Result and Discussion

Survey of the soils from the rubber growing region showed 85 to 95 % mycorrhizal infection in the feeder roots and the spore count ranged from 30 to 47 spores/ g dry soil in all three variaties (Table 1). Histopathological staining of roots colonized with VAM showed vesicles and arbuscules in the developing stage (Fig. 1 F,G,H & I). Most of the vesicles formed in the cortical cells showed more or less paris type of development . It has been reported in earlier studies that VAM development and spore production are influenced by the species involved, host plant under consideration, as well as edaphic and environmental factors and also VAM species vary considerably in their efficiency to infect and influence plant growth (Carling and Brown, 1980). In the present investigation we found that the VAM population varied in different varieties of *Hevea brasiliensis*. Maximum spore population recorded in the soils obtained from the rhizosphere of drought resistant variety RRII 105 (47 spores/g dry soil) and highest percentage of mycorrhizal infection among these varieties was 95%. Less tolerant variety GT-1 also showed relatively more number of spore/g dry soil.

Some of the frequently occurring spores observed were :

Table 1. AM infection and spore distribution in rubber growing soils

Variety	Plantation site*	AM- Root infection(%)	Spore count/100g soil
RRII 105	Sector A	85	47
	Sector B	82	42
RRIM 600	Sector A	85	40
	Sector B	84	39
GT 1	Sector A	80	33
	Sector B	82	35

RRII 105, RRIM 600 & GT 1- Clonal varieties of Rubber.
*Two different plantation sites, sector A & B were chosen. All the values are mean of ten replicates

Table 2: Distribution of VAM fungal species in the rhizosphere of *H.brasiliensis*

AM fungi	Rubber variety		
	RRII 150	RRIM 600	GT 1
Glomus fasciculetum	+	+	+
Glomus microcarpum	+	+	+
Glomus aggregatum	+	-	+
Glomus sp 4	+	-	-
Glomus sp 5	+	+	-
Acaulospora sp 1	+	+	+
Acaulospora sp 2	-	+	+
Acaulospora sp 3	+	+	-
Gigaspora margarita	+	+	+

(+) Present; (-) Absent. All the values are mean of ten replicates Species indicated were present or absent irrespective of the age of the plants in both the sampling sites

Glomus fasciculatum. Spores oval to ellipsoid, pale yellow to pale brown, smooth borne singly on straight and cylindrical hyphae with slight thickening at the base. More than two wall layers, outer wall thinner and brownish yellow at maturity, spore size ranges from 60-130 µm (Fig.1, J).

Glomus microcarpum. Spores globose to oval, smooth, yellowish to orange brown and borne singly on cylindrical hyphae and hyphal constriction observed at the point of attachment, spore size 30-55 µm in diameter.

Glomus aggregatum. Spores globose golden brown , develops singly or aggrate in groups, spores with single subtending hyphae at maturity, 130-150 µm diameter, spore surface smooth with a wall thickness of an average of 5-8 µm.(Fig 1.D).

***Glomus* sp 3.** Spores globose to oval, golden brown, smooth, 180- 220 µm diameter, spore wall brown with an average thickness of 18-20 µm, attached to two or more cylindrical and brownish subtending hyphae with the hyphal attachment curved along the spore surface.

***Acaulospora* sp. 1** Spores globose and hyaline, smooth, measuring an average of 40- 50µm in diameter, composite wall layer thickness about 2-2.5µm, spore wall hyaline and attached to a hyaline vesicle.

***Acaulospora* sp. 2,** spores globose and are brownish in colour, diameter ranging from 300-400 µm, surface ornamentation slightly rough and reticulated as seen under the light microscope, wall layers brownish and wall thickness measures an average of 20-25 µm, attached to the vesicles.

Gigaspora margarita. Spores globose to oval and yellowish brown with an average diameter of 300- 400 µm, hyphae with bulbous attachment with a base width of 20- 30 µm showing filiform attachment, spores with single wall layer.(Fig 1,C)

Sclerocystis sp. Numerous spores arranged radially to form a sporocarp 200 to 300 µm size, honey brown in colour, each spore has a length of 30- 40 µm and a width of 10 – 20 µm.(Fig 1,E).

VAM association in *H. brasiliensis* and their role in plant growth are well established (Ikram et al 1996). AM fungi in mature ecosystem are not regularly correlated with the abundance of VAM root infection (Moose and Brown, 1968). There was no significant correlation between the spore count from the soil and VAM colonization in the roots in the present investigation. It has been reported that the classification of the members of the order Glomales is based primarily on the light microscopy of the wall structure of the dormant spores (Morton and Benny 1990). In our SEM study, spores of *Glomus fasciculatum* revealed smooth wall character with number of pits (Fig1, K). In a similar SEM study, warty spore surface covering the protuberanes of the wall layer of *Glomus spinosum* was evident (Hu 2002). Spores of *Glomus spinosum* resemble those of *G. aggregatum* (Koske 1985). Under SEM mature chlamydospores of *Glomus deserticola* showed smooth wall without any ornamentation, the

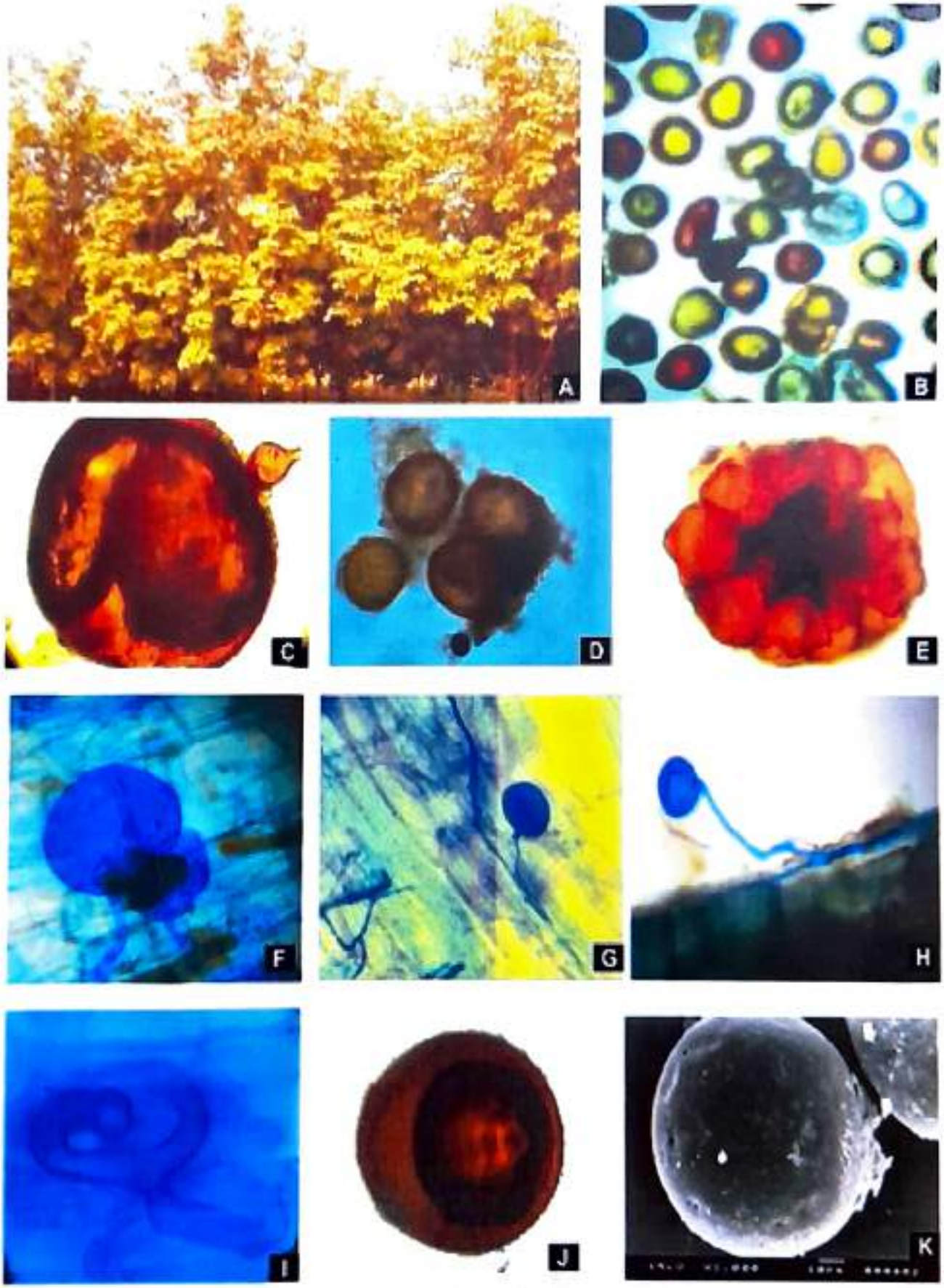

Fig1: (A) Plantation site of H. *brasiliensis*. (b) Light microscopical picture of different types of VAM spores. (C-E) Light microscopic view (40X) of different types of spores obtained C: *Gigaspore margarita*, D: *Glomus aggregatum* and E: *Sclerocystis* sp. (F-I) Light microscopic view (100X) of the development of arbuscules and vesicles within the cortical cells. (J) Light microscopic view of spores of *G. fasciculatum* (X40). (K) SEM of spores of *G. fasciculatum* (X1000) showing smooth surface and pits.

attachment was simple and pits or roughness in the spore wall was not observed.(Nagarajan and Mohan 2004).

The results suggests the nature of VAM diversity found in the rhizosphere of *H. brasiliensis*. The occurrence and distribution of AM fungi are affected by the physicochemical factors of the soil (Selvaraj and Bhaskaran, 1996) and also on the mutual interaction of the excretion of the host roots and VAM (Vierheilig *et al* 1998). In the present investigation VAM distribution was found to be varying with the varieties selected. However, population of *Glomus sp.* was found to be predominant in all the three varieties.

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