

Mycorrhizal and rhizosphere fungi of some endemic trees of Westernghats

K. VIJAYAKUMAR AND T.K. ABRAHAM

Division of Microbiology, Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, Kerala

A survey of mycorrhizal and rhizosphere fungi of some endemic trees of Western Ghats was made. Significant variations were observed in the species composition of the rhizospheric fungi and AM fungi associated with different tree species selected, seasonal variation of the spore population in the rhizosphere zone was observed. Lowest spore population in the rhizosphere zone was observed during June and July and the spore population was highest during December to February. *Glomus* was the most commonly occurring AM fungus of the plants selected in this study.

Key words: Mycorrhizae, rhizosphere microorganism, western ghat trees.

INTRODUCTION

The AM fungi with their cosmopolitan nature are known to form extensive mycorrhizae in natural condition (Hayman, 1983). Importance of mycorrhizae in natural ecosystem is well recognized and studies were conducted with mycorrhizal fungi to improve the early tree growth. The indigenous population of AM fungi may be suitable for inoculation purpose on account of their broad host compatibility, wide distribution, and efficiency in improving nutrient absorption and growth. The knowledge about the distribution of AM fungi in forest soil is important for understanding their ecology and may be relevant in the introduction of more efficient species in growth enhancement studies. The prevailing soil characteristics and the abundance of non-mycorrhizal fungi may also influence the root infection and mycorrhizal development. A survey was therefore conducted to study the AM and rhizosphere fungal population in the natural forests of Western Ghats.

MATERIALS AND METHODS

The plants selected for this study are *Gluta travancorica* Bedd., *Bentinckia condapanna* Berry ex Roxb. and *Myristica malabarica* Lam., growing in the Agasthyamala hills of Western Ghats. Agasthyamala hills situated at the southern most ends of Western ghats exhibits remarkable diversity in vegetation due to variability in

altitude, topography and rainfall. The selected plants, *G. travancorica* and *M. malabarica* are seen in lower altitudes of 500-800 mts, while *B. condapanna* grow in higher altitude of 1000-1300. *B. condapanna* is a beautiful palm confined to the steep slopes. *G. travancorica* and *M. malabarica* are hard wood trees and the wood of *G. travancorica* is extensively used in carving industry.

Rhizosphere soil samples of the trees were collected for isolating mycorrhizal fungal propagules and non-mycorrhizal fungi. Random sampling method was employed for the collection of soil. Four soil samples were collected for a plant growing at different altitudes. For quantitative studies, at least 4 subsamples were replicated per site around a plant. The subsamples were mixed thoroughly and 50 gm of the composite soil was kept for spore isolation. The mycorrhizal spores were recovered by wet-sieving and decanting method (Gerdemann and Nicolson, 1963). Rhizosphere fungi were isolated from one gram of the composite soil by standard methods. The root bits were washed in water and surface sterilized in 10% sodium hypochlorite solution. These root bits were aseptically transferred into 10 ml. sterile distilled water and agitated on a wrist shaker for 3 min. each root piece was cut into 0.5 cm and four root bits were placed equidistantly on Czapeck - Dox agar medium (Harley and Waid, 1955). Four replicates were prepared for each plant. The plates were incubated at 28 ± 2 °C for 5

days and total number of colonies appeared were counted and identified. The remaining root of each plant were bulked, washed in water and mycorrhizal colonization was measured following the method of Philips and Hayman (1970). Grid-line intersect method (Giovannetti and Mosse, 1980) was employed for the estimation of root colonization. Root samples and soil samples were collected at monthly intervals from January to December 1997. Soil P was estimated by using Bray extractant method (Jackson, 1976) and pH was estimated using an Elico pH meter. The total count of AM spores and rhizosphere fungi and number of individual species of each sample were determined. Using this data the frequency of occurrence of both AM and rhizosphere fungi were calculated as follows.

Frequency (%)

No. of sampling in which a particular fungus was recorded

Total no. of sampling made

RESULTS AND DISCUSSION

Rhizosphere is the most active part of soil surrounding the plant roots harbouring different types of microorganisms. Higher number of fungal organisms were present in the rhizosphere of the trees. This may be due to availability of energy rich substrates and extra nutrients available in that region. Garbaye (1992) had reported that the number of microorganisms per gram of soil is larger by two or three orders of magnitude in the rhizosphere than in the surrounding soil. Differences in the number of microorganisms present in the rhizosphere of selected trees may be due to the differences in growth and metabolic activities of these plants. Variation in pH value of the soil samples was negligible (5.1-5.9) and could not be correlated with the distribution of fungal species.

Variations were observed in the species composition of the fungal organisms. Some of the fungal organisms present in the rhizosphere were found to be specific to some of the plants. This may be due to the stimulation of growth of specific groups of organisms in the rhizosphere. Martin (1989) had also observed this type of specificity. *Penicillium* and *Aspergillus* were the dominant fungi in the rhizosphere of all the trees studied (Table 1). Altitudinal variation had not shown any effect on the abundance or on the distribution of rhizosphere fungi. This is evident

from the results obtained from the samples collected from altitudes varying from 600m-125m. But seasonal variation affect the proliferation of the fungi in the rhizosphere. It was also observed that the fungal organisms were higher in number during winter season compared to the rainy season. Interestingly, *Fusarium oxysporum*, the root pathogen, was isolated from the rhizosphere of all the selected trees except in few collections. Infection of the pathogen was also observed on plant roots.

Table 1. Occurrence of fungi in the rhizosphere of the selected plants

Name of plant and altitude	Number of fungi (g soil ⁻¹ × 10 ⁴)	Rhizosphere fungi	Frequency (%)
<i>Gluta travancorica</i>	600 m 700 m 800 m	<i>Alternaria</i> sp.	16
		<i>Cladosporium</i> sp.	25
		<i>Curvularia lunata</i>	33
		<i>Fusarium oxysporum</i>	75
		<i>Penicillium janthinellum</i>	50
		<i>Penicillium</i> sp.	83
<i>Myristica malabarica</i>	600 m 700 m 800 m	<i>Trichoderma viride</i>	33
		<i>Aspergillus flavus</i>	91
		<i>Curvularia lunata</i>	41
		<i>Fusarium oxysporum</i>	83
		<i>Gliocladium</i> sp.	25
		<i>Memoniella echinulata</i>	33
<i>Bentinckia condapanna</i>	1000 m 1100 m 1200 m	<i>Penicillium comemberti</i>	83
		<i>Tricoderma viride</i>	33
		<i>Aspergillus fumigatus</i>	75
		<i>Eurotium</i> sp.	41
		<i>Fusarium oxysporum</i>	75
		<i>Gliocladiopsis sagariensis</i>	66
		<i>Penicillium janthinellum</i>	83
		<i>Penicillium frequentans</i>	66
		<i>Verticillium tenerum</i>	58

The number of AM fungi showed high variation among the selected plants. Lowest spore density was observed in *G. travancorica* (Fig 1). Maximum

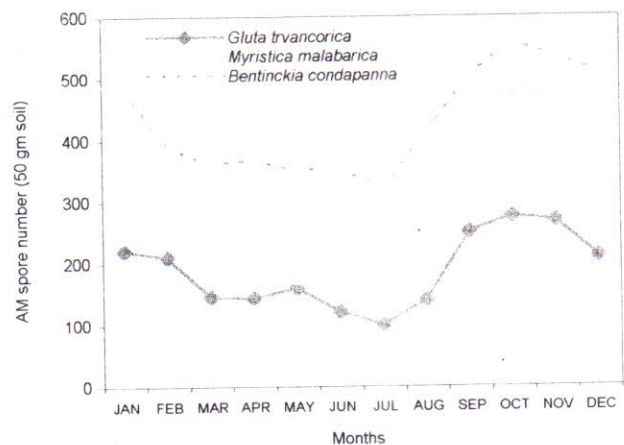


Fig 1. AM spore germination on the rhizosphere of the selected plants

spore density was observed during the months from August to November in all the trees. The spore population declined from the month of December and the lowest spore population was observed in the months of June and July. Mycorrhizal colonization was on the higher side from December to February and the lowest colonization percentage was recorded between the months of May and October (Fig 2). This may be

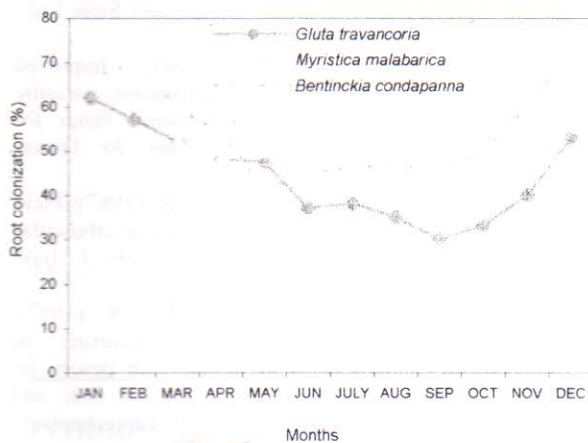


Fig 2. AM root colonization of the selected plants

due to the high moisture content in the soil during the rainy season. Highest root colonization was observed in *B. condapanna* growing in higher altitudes. It is reported that in native plant communities the AM spore density and colonization vary both seasonally and among the plant species (Brundrett and Kendrick, 1988).

The observed variation in spore number and colonization among the trees may be related to their topographic position. Higher AM development associated with *B. condapanna* growing at high altitude (1000-1300) shows the influence of topographic variation on mycorrhizal development. Both *G. travancorica* and *M. malabarica* are commonly found in riverine habitat (500-800M). Lower level of AM development and spore density were observed in these plants. Earlier workers also showed that AM development was lower in moist low land topographic positions (Demars and Boerner, 1995).

It was observed that the level of soil P was greater in rainy season (32 kg/ha.) and high level of soil P suppressed the root infection. During the months from August to November, there was a reduction in acidity and soil P and this might have favored the sporulation. Porter et al., (1989) stated that soil pH could have influence on AM fungal

spores. Hence AM spore formation is mainly determined by the nutritional status of the soil as well as that of the plants.

Although different AM species were observed in the rhizosphere, *Glomus* was encountered very frequently compared to others (Table 2). *Glomus* was reported to be the most commonly occurring AMF genus in many studies (Srivastava et al., 1997).

Table 2. Occurrence of AM fungi in the rhizosphere of the selected plants

Name of plant and Altitude	Number of AM spores (50 g. soil)	AM fungi	Frequency (%)
<i>Gluta travancorica</i>	600 m	<i>Acaulospora</i> sp.	25
		<i>Glomus clarum</i>	58
		<i>G. fasciculatum</i>	75
		<i>G. intraradices</i>	25
700 m	188	<i>G. mosseae</i>	83
		<i>Glomus</i> sp.	50
800 m	246	<i>G. aggregatum</i>	58
		<i>G. clarum</i>	50
<i>Myristica malabarica</i>	600 m	<i>G. constrictum</i>	42
		<i>G. fasciculatum</i>	83
		<i>G. intraradices</i>	67
		<i>Glomus</i> sp.	50
700 m	272	<i>Glomus</i> sp.	50
		<i>Sclerocystis</i> sp.	33
800 m	388	<i>G. aggregatum</i>	50
		<i>G. fasciculatum</i>	67
<i>Bentinckia condapanna</i>	1000 m	<i>G. intraradices</i>	75
		<i>G. monosporum</i>	33
		<i>G. mosseae</i>	50
		<i>Glomus</i> sp.	58
1100 m	480	<i>Glomus</i> sp.	58
		<i>Sclerocystis</i> sp.	50
1200 m	585	<i>Glomus</i> sp.	58
		<i>Sclerocystis</i> sp.	50

Species composition of AM fungi varied markedly between the rhizosphere of these trees. *Glomus intraradices* was the dominant species in the rhizosphere of *B. condapanna*, while this fungus occupied only the second position in case of *M. malabarica*. In case of *G. travancorica*, the presence of *Glomus intraradices* was less than 30% and *G. mosseae* emerged as the dominant species. Lack of host specificity may be one of the reasons for the wide occurrence of these fungi in different host plants.

Many free-living microorganisms in the rhizosphere are antagonists of mycorrhizal fungi during their growth on the surface of root and can severely reduce mycorrhizal formation and thereby indirectly reduce plant growth (Keast and Tonkin, 1983). In the present study, even though substantial population of *Fusarium oxysporum* was isolated from the root system, disease symptoms were not apparent on these trees. Mycorrhizal colonization in these roots were high,

but the spore load in the rhizosphere were not reduced. A detailed study is necessary to find out the nature of interaction between the rhizosphere and AM fungi.

ACKNOWLEDGEMENT

The authors are grateful to Director, TBGRI, for encouragement and for providing necessary facilities.

REFERENCES

- Brundrett, M and Kedrick, B. (1988). The Mycorrhizal status, root anatomy and phenology of plants in a sugar maple forest. *Can. J. Bot.* **66** : 1153 - 1173.
- De Mars, B.G. and Boerner, R.E.J. (1955). Mycorrhizal dynamics of three wood land herbs on contrasting phenology along topographic gradients. *Amer. J. Bot.* **82** : 1426 - 1431.
- Garbaye, J. (1991). Biological interactions in the mycorrhizosphere. *Experientia* **47** : 370 - 375.
- Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of endomycorrhizal Endogone species extracted from soil by wet sieving and decanting *Trans. Br. Mycol. Soc.* **46** : 235 - 244.
- Giovannetti, M. and Moss, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84** : 489 - 500.
- Harley, J.L. and Waid, J.S. (1955). A method of studying active mycelia on living roots and other surfaces of the soil. *Trans. Br. Mycol. Soc.* **38** : 104 - 118.
- Hayman, D.S. (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Can. J. Bot.* **61** : 944 - 963.
- Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice-Hall India, New Delhi.
- Keast, D and Tonkin, C. (1983). Antifungal activity of Western Australian soil actinomycetes against *Phytophthora* and *Pythium* species and a mycorrhizal fungus *Laccaria laccata*. *Aust. J. Biol. Sci.* **36** : 191 - 203.
- Martin, W. (1989). *Soil Biology*. Blackie and Sons Ltd., London.
- Philips, J. M. and Hayman, D.S. (1970). Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55** : 158 - 160.
- Porter, W.M., Robson, A.D. and Abbott, L.K. (1987). Field survey of the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *J. Appl. Ecol.* **24** : 659 - 662.
- Srivastava, H.P., Chandel, S and Kaushal, S. (1997). Arbuscular mycorrhizal fungi occurring in vegetated sand dunes of Indian Thar desert. In: Reddy, S.M. Srivastava, H.P., Purohit, D.K. and Ram Reddy (eds). *Microbial Biotechnology*. Scientific Publishers, India. pp. 87 - 96.

(Accepted for publication December 20, 2001)