OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN TREE SPECIES FROM WESTERN GHATS OF GOA, INDIA

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KHADE, S. W. & RODRIGUES, B. F. 2003. Occurrence of arbuscular mycorrhizal fungi in tree species from Western Ghats of Goa, India. We surveyed the prevalence of arbuscular mycorrhizal (AM) fungi in Mollem forest area of Western Ghats of Goa. A total of 25 tree species belonging to 18 families was screened. AM colonisation in the tree species varied with family and host genera. The highest mean root colonisation (100%) was recorded in *Macaranga peltata, Xylia xylocarpa, Zanthoxylum rhetsa* and *Randia ruglosa.* Maximum mean spore density of 745 spores 100 g^{-1} rhizosphere soil was recorded in *Leea indica.* A total of 18 AM fungi belonging to five genera, namely, *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* was found to be associated with the tree species studied.

Key words: AM fungi - *Acaulospora* - *Gigaspora* - *Glomus* - root colonisation - *Sclerocystis* - *Scutellospora* - spore density - tree species

KHADE, S. W. & RODRIGUES, B. F. 2003. Kejadian kulat mikoriza arbuskular dalam spesies pokok dari Ghats Barat di Goa, India. Kami mengkaji kelaziman kulat mikoriza arbuskular (AM) di hutan Mollem di Ghats Barat, Goa. Sejumlah 25 spesies pokok daripada 18 famili disaring. Pengkolonian AM dalam spesies pokok berubah-ubah mengikut famili dan genera perumah. Purata pengkolonian akar tertinggi (100%) dicatatkan dalam *Macaranga peltata, Xylia xylocarpa, Zanthoxylum rhetsa* dan *Randia ruglosa.* Purata kepadatan spora sebanyak 745 spora 100 g^{-1} tanih rizosfera dicatatkan dalam *Leea indica.* Sejumlah 18 kulat AM daripada lima genera, iaitu *Acaulospora, Gigaspora, Glomus, Sclerocystis* dan *Scutellospora* didapati hidup bersama tiga spesies yang dikaji.

Introduction

Arbuscular mycorrhizal (AM) fungi can be found in most ecosystems throughout the world ranging from the Artic to the tropical rain forests (Janos 1980a, Beldose *et al.* 1990). AM fungi play a vital role in natural ecosystems like tropical forests by influencing the composition and succession of plants (Janos 1980b, Giovanetti & Gianinazzi-Pearson 1994). Besides this, increase uptake of nutrients, especially phosphorus (Bolan *et al.* 1987, Jayachandran *et al.* 1989), nutrient cycling (Newman & Eason 1989) and exudates in the mycorrhizosphere (Linderman 1988) are major attributes of AM fungal association.

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AM association with forest tree species has been studied in India, covering various areas including subtropical evergreen forest and arid zone (Sharma *et al.* 1984, Thapar & Khan 1985, Tarafdar & Rao 1990, Thapar & Vijayan 1990, Raman *et al.* 1992, Santhaguru *et al.*1995, Vijaya *et al.*1995). The Western Ghats of southern India has also been the focus of AM studies by many workers. Kandasamy *et al.* (1988) carried out an intensive survey of the prevalence of AM fungi in forest tree species occurring at different altitudes in the Western Ghats of Nilgiri District. Ecological studies on AM fungal association with plant species from Kalakad forest reserve located in the Western Ghats, Tamil Nadu has been documented (Mohankumar & Mahadevan 1988, 1989). The authors investigated the influence of edaphic factors and seasonal variation on the distribution of AM fungi in six well-defined ecosystems, namely, evergreen, semi-evergreen, mixed deciduous, teak forests, scrub-jungle and grassland, at high and low altitudes. Muthukumar and Udaiyan (2000) have documented AM fungal association in four vegetation types, namely, forest, grassland, scrub and cultivated lands. AM fungal association in medicinal plants of Maruthamalai Hills in Western Ghats has also been studied (Muthukumar *et al.* 2001).

The state of Goa (14° to 16° N latitude and 73° to 75° E longitude) lies in the central portion of Western Ghats that extends from the Tapti river (Gujarat) in the north down to the peninsular tip of south India. It is one of the biodiversity hotspots of the world. No work on AM association in tree species from this portion of Western Ghats has been reported so far. In this paper, we report the occurrence and distribution of AM fungi in tree species from Mollem forest area located in the Western Ghats of Goa.

Materials and methods

Mollem forest, the largest sanctuary in Goa region, was selected for studying the occurrence of AM fungi in tree species. It is located at latitude 15° 29' N and longitude 74° 13' E (Figure 1). This sanctuary is spread over an area of 240 km^2 and encompasses rich forests varying from moist deciduous to semi-evergreen with the highest altitude of about 891 m above mean sea level. The climate of the tract is tropical with three main seasons, namely, monsoon (June till October), winter (November till January) and summer (February till May). The annual rainfall recorded is 5569 mm with maximum humidity of 96%. The mean maximum and minimum temperatures recorded are 37.2 and 15.9 °C respectively. The soil is moderately drained, gravelly with silty clay loam texture with pH of 5.4 to 6.2 and low in nutrients especially available phosphorus (16 kg ha^{-1}) and total nitrogen (0.24%). The forest tree species are more than 200 years old with an average bole diameter of 4.2 m. *Terminalias* are the dominant canopy species with *Careya arborea, Lagerstroemia lanceolata, Dillenia pentagyna, Microcos paniculata, Strychnos nux-vomica* and *Calycopteris floribunda* being the frequently occurring species in the forest extension areas.

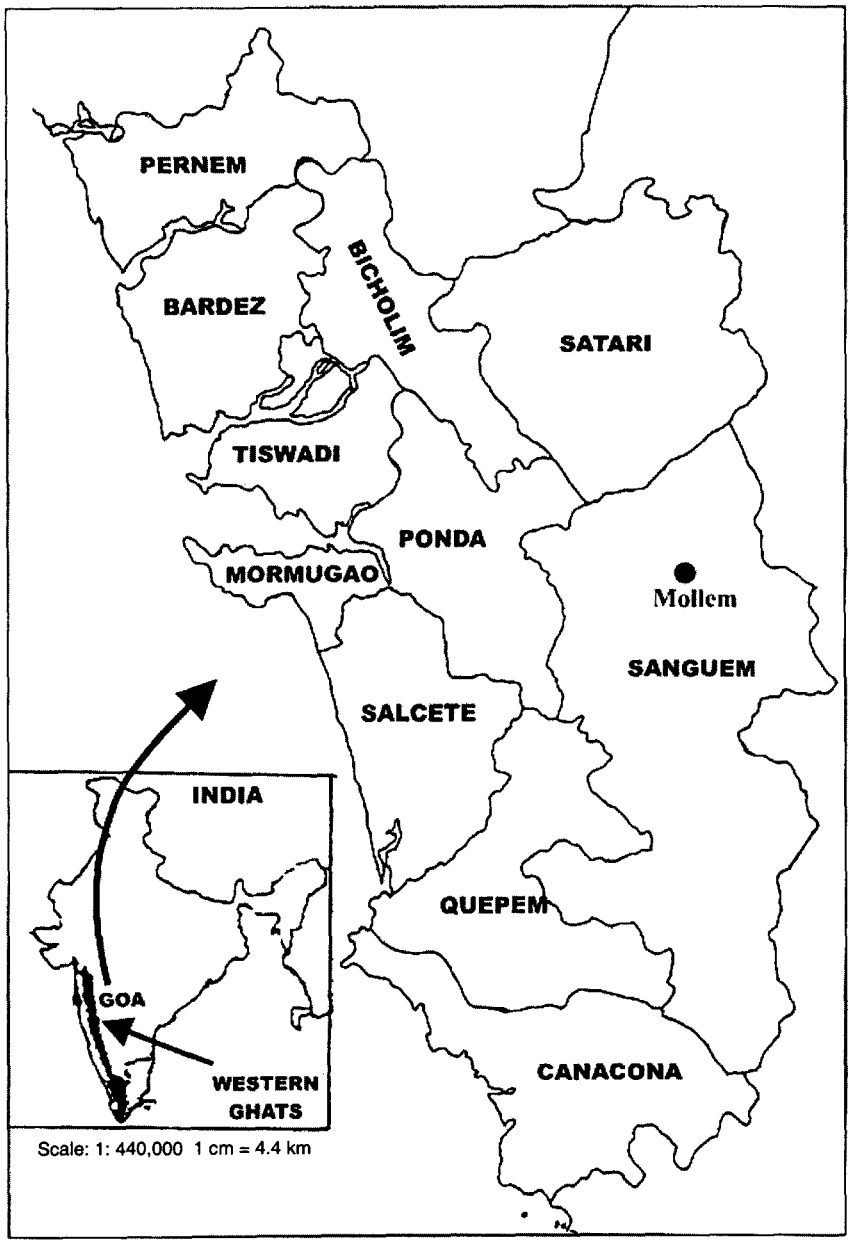


Figure 1 The study site of Mollem forest in Western Ghats of Goa

Root and rhizosphere soil samples of 25 tree species belonging to 18 families were randomly collected from forest extension area of Mollem between October and November 2000. For each tree species, three plants were sampled. During sampling, care was taken to trace back the feeder roots of the selected tree species. Samples were packed in polyethylene bags and transported to the laboratory. Root samples were freshly processed whereas the soil samples were stored at 4 °C until analysed.

The root samples were washed with water, cleared with 10% KOH, acidified in 1 N HCl and then stained in lactoglycerol trypan blue (0.05%) according to Phillips and Hayman (1970). Quantification of AM fungal colonisation was carried out by using the slide method (Giovannetti & Mosse 1980). Presence of arbuscules, vesicles and hyphae connecting to the fungal structures were taken into consideration while estimating the degree of root colonisation of AM fungi. For isolation of AM fungal spores/sporocarps, wet sieving and decanting method proposed by Gerdemann and Nicolson (1963) was followed and quantification of spore density was carried out as described by Gaur and Adholeya (1994). Intact and unparasitised spores were used for the quantification of spore density and taxonomy of AM fungi. AM fungi were identified according to their spore morphology and wall characters (Morton & Benny 1990, Schenck & Perez 1990, Walker & Trappe 1993, Wu 1993).

Identification of tree species was carried out based on Rao (1985) and Mathew (1991). Standard deviation was calculated for mean root colonisation and mean spore density. Pearson's correlation test was performed to assess the relationship between levels of AM fungal root colonisation and number of spores in the rhizosphere soil. Data on root colonisation was arcsin transformed whereas spore numbers were log transformed prior to correlation analysis.

Results

The results of the analysis of roots and rhizosphere soil samples indicate the widespread occurrence of AM fungal association in different tree species occurring in Mollem forest (Table 1, Figure 2). All the tree species selected for the study were found to exhibit AM fungal colonisation. Hyphae and vesicles were predominant AM fungal structures whereas the arbuscules were observed in root samples of seven tree species. In the present study, mean root colonisation and mean spore density varied with families and host genera. An average root colonisation of 50.7% was recorded; the mean root colonisation levels ranged from 17 to 100%. Maximum AM fungal root colonisation was recorded in *Macaranga peltata, Xylia xylocarpa, Zanthoxylum rhetsa* and *Randia ruglosa.* A colonisation of 17% was recorded in *Dillenia pentagyna, S. nux-vomica* and *Lagerstroemia lanceolata.*

An average of 220 spores 100 g^{-1} soil was recorded in the rhizosphere soil of the tree species studied. AM fungal spores ranged between 18 spores 100 g^{-1} soil for *S. nux-vomica* and 745 spores 100 g^{-1} soil for *Leea indica* and were not correlated with mean root colonisation ($r = 0.255$; $p > 0.05$).

Species composition of AM fungi in rhizosphere soil of different tree species revealed the presence of 18 species belonging to five genera, namely, *Acaulospora* (5), *Glomus* (8), *Gigaspora* (1), *Sclerocystis* (3) and *Scutellospora* (1) with the species number given in parenthesis (Table 2, Figure 2).

Table 1 Arbuscular mycorrhizal status of tree species in Mollem forest, Western Ghats of Goa

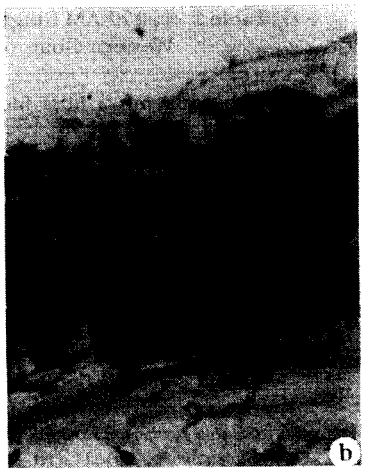
Tree species	Family	Root colonisation of AM fungi			*Mean root colonisation[a](%)	*Mean spore density 100 g^{-1}soil[b]
		Hypha	Arbuscule	Vesicle		
Alstonia scholaris (L.)R.Br.	Apocynaceae	+	–	+	40 ± 3.50 (39.22)	460 ± 40.55 (2.66)
Ervatamia heyneana (Wall.)Cooke.	Apocynaceae	+	–	+	42 ± 3.10 (40.39)	273 ± 26.90 (2.43)
Holarrhena antidysenterica Wallich ex A. DC.	Apocynaceae	+	–	+	67 ± 5.85 (54.93)	350 ± 37.15 (2.54)
Buchanania cochinchinensis (Lour.) Almeida.	Anacardiaceae	+	–	+	18 ± 0.08 (25.10)	72 ± 8.02 (1.85)
Lamnea coromandelica (Houtt.) Merrill.	Anacardiaceae	+	–	+	25 ± 1.50 (29.99)	78 ± 6.92 (1.89)
Calycopteris floribunda (Roxb.) Lamk.	Combretaceae	+	–	+	52 ± 4.80 (46.14)	500 ± 51.25 (2.69)
Terminalia paniculata Roth.	Combretaceae	+	+	+	50 ± 5.50 (44.99)	550 ± 56.03 (2.74)
Terminalia crenulata Roth.	Combretaceae	+	–	+	84 ± 8.31 (66.41)	340 ± 2.58 (2.53)
Dillenia pentagyna Roxb.	Dilleniaceae	+	–	+	17 ± 1.20 (24.34)	27 ± 1.72 (1.43)
Macaranga peltata (Roxb.) Muell. Arg.	Euphorbiaceae	+	+	+	100 ± 11.73 (89.98)	70 ± 5.85 (1.84)
Phyllanthus emblica L.	Euphorbiaceae	+	–	+	25 ± 3.10 (29.99)	575 ± 58.20 (2.75)
Leea indica (Burm. f.) Merrill.	Leeaceae	+	–	+	52 ± 4.50 (46.14)	745 ± 70.15 (2.87)
Careya arborea Roxb.	Lecithidaceae	+	–	+	50 ± 5.28 (44.99)	42 ± 3.67 (1.62)
Acacia pennata (L.)Willd.	Mimosaceae	+	+	+	66 ± 5.55 (66.41)	20 ± 1.52 (1.30)
Strychnos nux-vomica L.	Loganiaceae	+	–	+	17 ± 1.35 (24.34)	18 ± 1.88 (1.25)
Lagerstroemia lanceolata Wall. ex Wt. & Arn.	Lythraceae	+	–	+	17 ± 0.09 (24.34)	52 ± 3.95 (1.71)
Xylia xylocarpa Taub.	Mimosaceae	+	+	+	100 ± 11.32 (89.98)	240 ± 23.11 (2.38)
Ziziphus rugosa Lamk.	Rhamnaceae	+	+	+	60 ± 5.85 (50.76)	42 ± 3.85 (1.62)
Randia ruglosa (Thw.)Hook. f.	Rubiaceae	+	–	+	100 ± 9.33 (89.98)	630 ± 62.25 (2.79)
Zanthoxylum rhetsa (Roxb.)DC.	Rutaceae	+	+	+	100 ± 12 (89.98)	27 ± 1.72 (1.43)
Helicteres isora L.	Sterculiaceae	+	–	+	17 ± 1.80 (24.34)	25 ± 1.79 (1.39)
Microcos paniculata L.	Tiliaceae	+	–	+	33 ± 2.8 (35.05)	70 ± 8.10 (1.84)
Gmelina arborea Roxb.	Verbenaceae	+	+	+	82 ± 7.93 (64.88)	200 ± 19.02 (2.30)
Ficus sp.	Urticaceae	+	–	+	33 ± 2.48 (35.05)	30 ± 4.15 (1.47)
Hopea wightiana Wall ex Wt. & Arn.	Dipterocarpaceae	+	–	+	20 ± 1.82 (26.56)	69 ± 5.80 (1.83)

*Mean of three independent observations

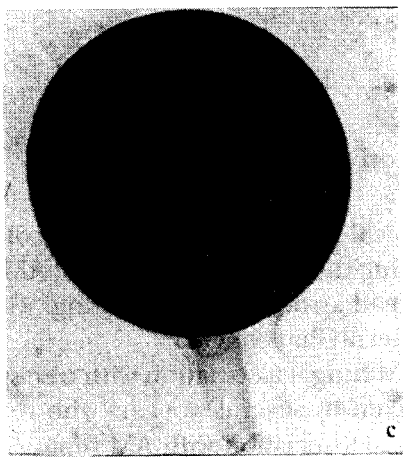
[a]Values in parentheses are arcsin transformed for root colonisation

[b]Values in parentheses are log transformed for spore density

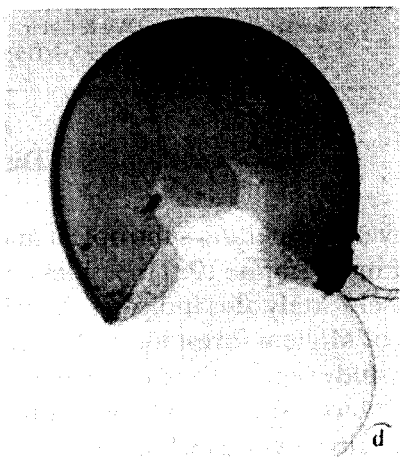
(a) Arbuscular colonisation (400×)



(b) Vesicular colonisation (400×)

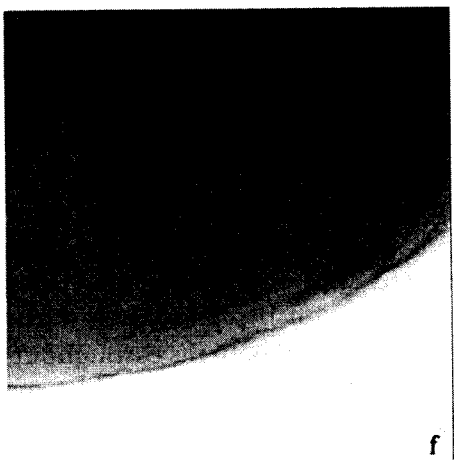


(c) Spore of *Glomus monosporum* (400×)



(d) Spore of *Gigaspora margarita* (400×)

(e) Spores of *Sclerocystis taiwanensis* (400×)



(f) A portion of spore of *Acaulospora foveata* (400×)

Figure 2 AM fungi in tree species of Mollem forest

Predominance of vesicles in the roots of the forest tree species studied indicates that conditions were favourable for their formation and that mycorrhizal strategies of plants might be correlated with environmental conditions.

Presence of arbuscules was recorded in the roots of only seven out of the 25 tree species studied. A possible reason for this small number of arbuscules could be that most of the roots were in an inactive stage at the time of sampling. Arbuscules, being ephemeral structures, may be absent if the roots are inactive (Brundrett 1991). In addition, co-existing plants in natural communities may avoid competition for the nutrients by having roots that are active at different times of the year (Veresoglou & Fitter 1984, Fitter 1986).

Arbuscules are the main sites for host-fungus nutrient exchange and their presence is normally used to designate AM association (Smith & Gianinazzi-Pearson 1988). Nevertheless, the presence of AM fungal hyphae and vesicles have been used as evidence of AM association (Brundrett 1991). However, they are unreliable indicators since they also occur in the senescent roots of non-host species and rhizome scale leaves (Hirrel *et al.* 1978, Staz & Sakai 1984). Thus, in ecosystems surveys, it may be best to define AM colonisation levels as a proportion of the plant's root system that, when susceptible to colonisation, supports an active colonisation with arbuscules (Brundrett & Kendrick 1988). This requires prior understanding of host-root phenology or, alternatively, collection of root samples throughout the year (Brundrett 1991).

The average root colonisation (50.7%) recorded in the present study is in agreement with the findings of Mohankumar and Mahadevan (1988). The range of root colonisation of AM fungi (17 to 100%) in our study is also in accordance with the earlier reports on AM association of plants from Western Ghats (12 to 90%) by Muthukumar *et al.* (2001).

Large variation in spore numbers (18 to 745 spores 100 g^{-1} rhizosphere soil) recorded in the rhizosphere soil of different tree species in the present study can be attributed to several reasons. Firstly, the occurrence of several AM fungi in the soils or within roots suggests that interspecific competition between them is possible (Brundrett & Kendrick 1990). Secondly, subsequent variation in the timing of spore production occurs among AM fungi associated with host plants, suggesting that competition between fungi and environmental factors probably also influence spore production in natural communities (Gemma & Koske 1988). Also, peak period of spore production is generally thought to coincide with the period of fungal resource remobilisation from senescing roots (Sutton & Barron 1972) and is greatest in natural communities when root activity is interrupted by a long dry season (Janos 1980b).

Spore density range recorded in the present study is much higher than the figures reported by Mohankumar and Mahadevan (1988) in mixed deciduous, evergreen and semi-evergreen forest ecosystems of Kalakad forest reserve in Western Ghats (79 to 130 spores 100 g^{-1} rhizosphere soil) and by Raman *et al.* (1992) in Mamandur forest of Tamil Nadu (30 to 301 spores 100g^{-1} rhizosphere soil) in southern India. The occurrence of higher spore density in this study compared with other localities in the Western Ghats could be due to varying ecoedaphic factors.

In our study no definite correlation could be established between AM fungal root colonisation and spore numbers. Our results are in agreement with observations made by Rani *et al.* (1995) but contrast with the findings of Muthukumar *et al.* (2001), who have reported positive correlation between the two in medicinal plants from Maruthamalai hills in Western Ghats. The poor correlation between spore density and root colonisation could be because sporulation of AM fungi is dependent on a wide range of environmental factors (Muthukumar *et al.* 2001) and their germination potential varies at different times of the year (Tommerup 1983, Gemma & Koske 1988). Also, soils in an ecosystem often contain low numbers of living spores of AM fungi (Brundrett & Kendrick 1988, Janos 1980b) and these spores may not function as propagules if they are quiescent or have an innate period of dormancy. Thus, initiation of AM colonisation in occurring ecosystem suggests that pre-existing network of hyphae is often the main AM inoculum (Brundrett 1991).

Our study recorded the presence of 18 AM fungal species from rhizosphere soil of 25 tree species from Mollem forest. Johnson *et al.* (1991) reported similar findings in their study of plant and soil controls of mycorrhizal fungal communities. They reported the presence 12 to 22 different species of AM fungi per study site. However, Muthukumar *et al.* (2001) reported only 35 AM fungal species from the rhizosphere soil of about 329 plant species from Western Ghats. Recovery of relatively higher number of species in the present study is in agreement with Francis and Read (1994) who have reported that high species diversity, characteristic of phosphorus-deficient grassland ecosystem dominated by plant species with arbuscular mycorrhizae, may be attributed to a low level of host specificity.

AM fungi belonging to the genus *Glomus* were the most representative types in our study. The predominance of this genus in tropical soils has been reported by other workers (Thapar & Khan 1985, Raghupathy & Mahadevan 1993).

Much attention is focused on the conservation of forest macro flora and fauna compared with the vast world of microbes. Nevertheless, there is an urgent need for detailed inventory of these microbes including AM fungi. In addition, measures for their conservation at ecosystem levels are pertinent (Bisht *et al.* 1995). Western Ghats of Goa has rich biodiversity. Thus, more extensive sampling over a longer period is required to determine the species diversity of AM fungi (Walker *et al.* 1982). AM fungi are the key links in regulating the patterns of energy and nutrient flux in terrestrial ecosystem which often transcend the pattern of community distribution and species abundance in the biosphere (Fahey 1992). The present work is just one step in this direction and contributes data necessary for further studies on AM fungi from this region of which very little has been explored.

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