

Screening of the Arbuscular Mycorrhizae in the rhizosphere soils of three Rattan species of Charmady Kanpadi Reserve forest of Karnakata, India

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Abstract: Mycorrhizae represent mutualistic association between plant root and specialized soil fungi are a gift to the plants as they promote their growth. The present study was undertaken to screen the presence of Arbuscular Mycorrhizae (AM) in there rattan species namely *Calamus thwaitesii* Becc. & Hook.f. , *Calamus nagabettai* Fernandez & Dey and *Calamus prasinus* Lakshmana & Renuka of Charmady Kanpady Reserve Forest of Karnataka, India. A total of 38 species of AM fungi belonging to three genera namely *Acaulospora*, *Glomus* and *Scutellospora* were isolated from the rhizosphere soils of rattans.

Keywords: Arbuscular Mycorrhizae, rattans, rhizosphere, root colonization, vesicular colonization.

I. INTRODUCTION

Rattans is a collective term commonly used for the spiny climbing palms, classified under the subfamily Calomoideae of the family Arecaceae. They are an important non-wood forest resource of the tropics particularly of the Asian and African countries. The woody stem of this climbing palm provides traditional raw material for furniture and novelty items. The rattans of the world include 13 genera and 568 species (Uhl & Dransfield, 1987). Of the 13 genera of climbing palms, *Calamus* is the largest genus with about 370 species (Renuka, 1991). In India, 51 species of rattans belonging to four genera, viz. *Calamus*, *Daemonorops*, *Plectocomia* and *Korthalsia* have been reported (Lakshman, 1993 & Renuka, 1991). Among the Indian rattans 25 species are considered to be endemic. Evergreen forests of the Western Ghats form the largest natural home of rattans in south India, includes the only one genus of rattan i.e., *Calamus*. There are 19 species of *Calamus* have been reported from Western Ghats. Of these 17 species are endemic to these regions. Only two species viz., *Calamus thwaitesii* Becc. & Hook.f. and *C. rotang* have their distribution to Sri Lanka (Renuka, 1991). Of all the states falling in the jurisdiction of Western Ghats, Karnataka has the maximum number of species of *Calamus* (13 species), of which five species are endemic to this region viz. *C. karnatakensis*, *C. lacciferus*, *C. lakshmana*, *C. prasinus*, and *C. stoloniferus* (Lakshmana, 1993). In Karnataka the rattans are distributed in the Kodagu, Dakshina Kannada, Chikmagalur, Hassan and Uttara Kannada. The highest number species of *Calamus* are found in Kodagu District (Lakshmana, 1993).

Mycorrhizae represent mutualistic association between plant root and specialized soil fungi are a gift to the plants as they promote their growth. Mycorrhizal colonization increases phosphorous (P) uptake of many plants and has been shown to increase nitrogen uptake in some species as well (Smith and Read 1997). Thapar and Khan (1985) studied the distribution of VA mycorrhizal fungi in forest soils in India. Kharbuli and Mishra (1982) did the survey of mycorrhizal association in some trees of North Eastern India. There are reports on the occurrence of vesicular arbuscular mycorrhizal fungi in the

soils of Western Ghats forests (Muthukumar and udaiyan, 2000 & 2001); (Muthukumar *et al.*, 1994). Raman and Nagarajan (1999) studied the mycorrhizal association of orchids in tropical forests of southern India. Verma and Jamaluddin (1995) did the mycorrhizal study of Teak plants in the central India.

Scanty literature is available on the mycorrhizal study of Rattans. Gong *et al* (1994 & 1995) studied the VA mycorrhizae in the rhizosphere soils of four rattan species (*Daemonorops margaritae*, *Calamus simplicifolius*, *C. tetradactylus*, and *C. tetradactyloides*). Zakaria (1991) did the preliminary studies on growth dependency of in vitro micropropagated *Calamus manan* on VA mycorrhiza (VAM) prior to transplanting to the field.

The rattans are mainly used for making furniture, novelty items etc. Hence the demand for eco-friendly rattans is increasing day by day. Research worldwide is concentrated on improving the quality of rattans through techniques such as tissue culture, hybridization, clonal selection etc. Mycorrhiza is found to play a vital role in the growth of many plant species and can also be used as one of the methods for crop improvement of rattans. Study of diversity of indigenous mycorrhizae in the rhizospheric soil and their colonization in the roots of rattans in the preliminary task, hence this study is been undertaken.

II. MATERIALS AND METHODS

For the mycorrhizal study three species of rattans (*Calamus*) were selected. They are

1. *Calamus thwaitesii* Becc. & Hook.f. : High climbing and robust cane. Stems clustering, 20 m or more in length, with sheath up to 18 cm girth, without sheath 10 cm. Distributed throughout the Western Ghats in moist deciduous, semi-evergreen forests of Goa, Karnataka, Kerala and Tamil Nadu.
2. *Calamus nagabettai* Fernandez & Dey : Climbing cane with clustering stems, reaching up to 25 m or more; clumps with 10-15 rhizomes; stems with leaf sheath up to 12-16 cm girth, without sheath up to 10-12 cm girth. Distributed in evergreen forests of Kodagu and Dakshina Kannada districts of Karnataka.
3. *Calamus prasinus* Lakshmana & Renuka: High climbing cane, solitary stem with sheaths up to 9 cm girth, without sheath up to 3 cm. Distributed in evergreen forests of Mangalore and Sampaje divisions of Karnataka.

Among these *Calamus nagabettai* and *C. prasinus* are endemic to Western Ghats and *C. thwaitesii* is non-endemic (Lakshmana, 1993). All the three species are economically important.

The study was conducted in the Charmadi Kanpadi Reserve forest in the Western Ghats of Karnataka is confined between 13° 0' 20" to 13° 0' 30" N latitude and 75° 15' 35" to 75° 15' 50" E longitude, situated at an elevation of 1380 m, about 85 km from Mangalore in Dakshina Kannada District of Karnataka.

The soil samples were collected after removing one to two cm topsoil from a depth of 10-15 cm close to the plant. Four samples were collected from the rhizosphere of each plant and like-wise the samples collected from five plants (20 samples) mixed to form a composite sample. The samples were air dried, labeled and stored at 4°C. Non-rhizosphere soil samples were collected and processed as above.

Actively growing roots were collected from different plants of each species along with the soil samples. Individual root samples of 1 to 2 grams (fresh weight) were fixed in small plastic vials with Formalin-Acetic-Alcohol (FAA).

The spores of AMF were isolated following wet sieving and decanting method (Gerdemann and Nicolson, 1963) and were identified following key of Schenck and Perez (1990). Root samples were processed following the method suggested by Phillips & Hayman (1970) and colonization assessment was done according to Giovannetti and Mosse, 1980.

III. RESULTS AND DISCUSSION

All the three rattan species were found to be mycorrhizal, but the number of AM species varied from species to species. In the present study maximum number of AM species were isolated from the rhizosphere of *Calamus nagabettai* with 29 species followed by *Calamus prasinus* with 24 species and least number of species were isolated from *Calamus thwaitesii* with 17 species (Fig. 1). In this study the total number of AM fungi recorded in the rhizosphere soil samples (*Calamus thwaitesii*, 17 species; *C. nagabettai*, 29 species and *C. prasinus*, 24 species) was more than the total number species recorded in the non-rhizosphere soil samples (17 species).

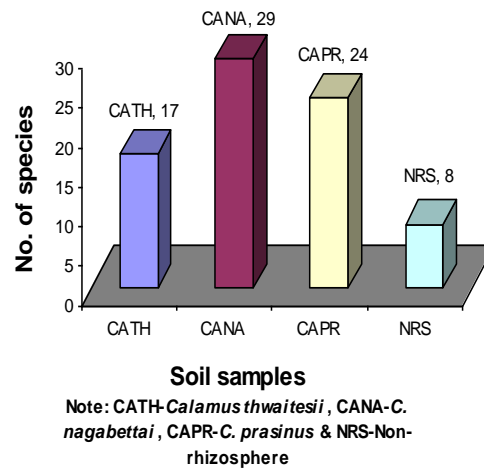


Fig.1 . The record of AM species recorded from rhizosphere & non-rhizosphere region

A total of 38 species of AM fungi belonging to three genera namely *Acaulospora*, *Glomus* and *Scutellospora* were isolated (Table 1). *Glomus* was found to be dominant and is represented by 27 species (71% of total species recorded) followed by *Acaulospora* and *Scutellospora* with 6 and 5 species respectively (Fig. 2).

Table 1: Consolidated list of AM species isolated from the rhizosphere and non-rhizosphere soil samples of Charmady region

Sl.No.	Name of the AM species
1.	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss
2.	<i>A. denticulata</i> Sieverding and Toro
3.	<i>A. mellea</i> Spain and Schenck
4.	<i>A. scrobiculata</i> Trappe
5.	<i>A. spinosa</i> Walker and Trappe
6.	<i>A. tuberculata</i> Janos and Trappe
7.	<i>Glomus aggregatum</i> Schenck and Smith
8.	<i>G. albidum</i> Walker and Rhodes
9.	<i>G. ambisporum</i> Smith and Schenck
10.	<i>G. australe</i> (Berkeley) Berch
11.	<i>G. botryoides</i> Rothwell and Victor
12.	<i>G. citricolum</i> Tang and Zang
13.	<i>G. claroideum</i> Schenck and Smith
14.	<i>G. epigeum</i> Daniels & Trappe
15.	<i>G. etunicatum</i> Becker and Gerdemann
16.	<i>G. fasciculatum</i> (Thaxter sensu Gerdemann) Gerdemann and Trappe
17.	<i>G. flavisporum</i> (Lange and Lund) Trappe and Gerdemann
18.	<i>G. fragilistratum</i> Skou and Jakobsen
19.	<i>G. geosporum</i> (Nicolson and Gerdemann) Walker
20.	<i>G. glomerulatum</i> Sieverding
21.	<i>G. heterosporum</i> Smith and Schenck
22.	<i>G. hoi</i> Berch and Trappe
23.	<i>G. intraradices</i> Schenck and Smith
24.	<i>G. macrocarpum</i> Tulasne and Tulasne
25.	<i>G. magnicaule</i> Hall
26.	<i>G. microaggregatum</i> Koske, Gemma and Olexia
27.	<i>G. monosporum</i> Gerdemann and Trappe
28.	<i>G. mosseae</i> (Nicolson and Gerdemann) Gerdemann and Trappe
29.	<i>G. multicaule</i> Gerdemann and Bakshi

30.	<i>G. pulvinatum</i> (Hennings) Trappe and Gerdemann
31.	<i>G. pustulatum</i> Koske, Friese, Walker and Dalpé
32.	<i>G. radiatum</i> (Thaxter) Trappe and Gerdemann
33.	<i>G. reticulatum</i> Bhattacharjee and Mukerji
34.	<i>Scutellospor coralloidea</i> (Trappe, Gerdemann and Ho) Walker and Sanders
35.	<i>S. heterogama</i> (Nicolson and Gerdemann) Walker and Sanders
36.	<i>S. nigra</i> (Redhead) Walker and Sanders
37.	<i>S. pellucida</i> (Nicolson and Schenck) Walker and Sanders
38.	<i>Scutellospora</i> sp.1

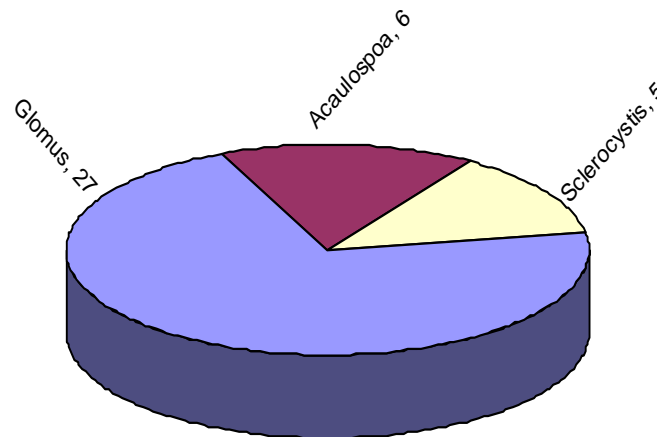


Fig. 2. Generic level of species distribution

Nagabhushanam *et al.*, (1999) isolated AM fungi associated with some common legume trees in the order

Glomus → Acaulospora → Gigaspora → Scutellospora → Entrophospora → Sclerocystis. Rodrigues (2006) isolated AM fungi from the iron ore mine waste lands in Goa in the order: Glomus → Acaulospora → Gigaspora → Scutellospora

In the present study the isolated AM fungal species were in the following order:

Glomus → Acaulospora → Scutellospora (27 → 6 → 5)

In the present study the genus *Glomus* was found to be dominant in all the three rattan species. Similar results were obtained by Sastry and Johri, 1999; Rodrigues and Jaiswal, 2001; Rani and Manoharachary, 1994; Prasad, 1999; Nagabhushanam *et al.*, 1999 and Uniyal and Uniyal, 2000.

The spore density (SD) ranged between 8 and 51 spores/10g of soil. The maximum SD (51 spores/10g of soil) was recorded from the rhizosphere of *Calamus nagabettai* and minimum (8 spores/10g of soil) from the non-rhizosphere soil samples. This spore number is very less when compared to other workers. Muthukumar and Udaiyan (2001) reported the spore density ranging from 12 to 72 spores/10g soil and Johnson and Wedin (1997) have isolated 1376 and 920 spores/10g soil from a seasonally dry forest and grassland respectively from tropical America. Muthukumar *et al.*, (1994) recorded the spore number ranging from 150-1650 spores/10g soil in certain tropical wild legumes.

The degree of infection varied among rattan species. The hyphal colonization (HC) ranged between 86.79% and 92.38%. The maximum HC (92.38%) was recorded from the rhizosphere of *Calamus prasinus* and minimum (86.79%) from the rhizosphere of *Calamus nagabettai*. The arbuscular colonization (AC) ranged between 23.21% and 43.54%. The maximum AC (43.54%) was recorded from the rhizosphere of *Calamus thwaitesii* and minimum (23.21%) from the rhizosphere of *Calamus nagabettai*. The vesicular colonization (VC) ranged between 52.92% and 53.96%. The maximum VC (53.96%) was recorded from the rhizosphere of *Calamus thwaitesii* and minimum (23.21%) from the rhizosphere of *Calamus prasinus*. Similar results were obtained by Nagabhushanam *et al.*, (1999). They have investigated the association of VAM fungi with 40 legume trees growing in the Godavari belt. All the trees showed the VAM infection, but with varying incidence. Thapar *et al.* (1992) also have recorded varying degree of infection among different plants with in a family.

Table 2: Mycorrhizal parameters of rhizosphere and non-rhizosphere soil samples

Sl.No.	Parameters	CATH	CANA	CAPR	NRS
1	Spores/10g of soil	29.325	51.75	28.88	8.208
2	Hyphal colonization %	92.168	86.79	92.38	-
3	Arbuscular colonization %	43.54	23.21	30.33	-
4	Vesicular colonization %	53.958	52.92	55.62	-

Note: CATH: *Calamus thwaitesii*, CANA: *Calamus nagabettai*, CAPR: *Calamus prasinus*, NRS: Non-rhizosphere soil

IV. CONCLUSION

All the rattan species namely *Calamus thwaitesii*, *Calamus nagabettai* and *Calamus prasinus* showed AM colonization with variation in the spore density and spore diversity in the rhizosphere soils. The hyphal, vesicular and arbuscular colonization in the roots also varied among the three species of rattans. Though the spore density was minimum compared to other species; maximum spore density was recorded in *Calamus nagabettai*. Significant arbuscular colonization was recorded in the roots of *Calamus thwaitesii*. Glomus was found to be dominant in the rhizosphere soils of all the three species of rattans.

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REFERENCES

- [1] Gerdemann, J.W. and Nicolson, T.H., 1963. Spores of Mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46: 235-244
- [2] Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84: 487-500
- [3] Gong, M.Q, F.Z. Wang and Y. Chen. 1995. Successful inoculation on rattan seedlings with VA mycorrhizal fungus, *Forest Research*, 8(3):247-251.
- [4] Gong, M.Q., Y. Chen and F.Z. Wang. 1994. Study on VA mycorrhizae of rattan species, *Forest Research*, 7(4):359-363.
- [5] Johnson, N.C. and Wedin, D.A. 1997. Soil carbon, nutrients and mycorrhizae during conversion of dry tropical forest to grassland. *Ecol. Appl.* 7: 171-182
- [6] Kharbuli. P.P. and Misra, R.R. 1982. Survey of mycorrhizal association in some trees of North-Eastern India. *Act. Bot. Ind.* 10: 192-195
- [7] Lakshmana, A.C. (1993), *Rattans of South India*, Evergreen Publishers, Bangalore. P: 180
- [8] Madhava, D. Mehrotra (1991), *Mycorrhizae of Indian Forest trees*. Indian Council of Forestry Research & Education, New Delhi. P: 294
- [9] Muthukumar, T. and Udaiyan, K. 2001. Arbuscular mycorrhizae of plants growing in the Western Ghats region, Southern India. *Mycorrhiza* 9:297-313
- [10] Muthukumar, T. and Udaiyan, K. 2001. Vesicular arbuscular mycorrhizal association in medicinal plants of Mauthamalai Hills, Western Ghats, Southern India. *J. Mycol. Pl. Pathol.* Vol. 13(2): 180-184.
- [11] Muthukuar, T., Udaiyan, K. and Manian, S. 1994. Vesicular arbuscular mycorrhizae in certain tropical wild legumes. *Ann. For.* 2(1): 33-43
- [12] Nagabhusanam, P., Reddy, S.M. and Reddy, S.R. 1999. VAM fungi associated with some common legume trees of Godavari belt. *Indian J. Forestry.* Vol. 22(2): 129-131.

- [13] Nagarajan, N, and Raman, N. 1999. Mycorrhizal associations of Orchids in a tropical forest of Southern India. T. Trop. For. Sci. 11(3): 548-553
- [14] Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc.,55: 158-161
- [15] Prasad, K. 1999. Vesicular-arbuscular mycorrhizal fungi (VAMF) associated with *Prosopis juliflora* (L.) J. Trop. Ecol. 15: 233-236
- [16] Rani, S.J. and Manoharachary, C. 1994. VA mycorrhizal association of Safflower in relation to Physico-chemical characters. J. Soil Biol. Ecol. 14(1): 41-45
- [17] Renuka, C., 1991. How to establish a cane plantation. KFRI Info. Bull. No.10.
- [18] Renuka, C., 1992. Rattans of Western Ghats - a taxonomic manual. KFRI, Peechi. 60 p.
- [19] Thapar HS, Khan SN. 1985. Distribution of VA mycorrhizal fungi in forest soils of India. Indian J For, 8: 5-7.
- [20] Rodrigues, B.F. and Jaiswal, V. 2001. Arbuscular mycorrhizal (AM) fungi from coastal sand dune vegetation of Goa. Indian J. For. 24(1): 18-20
- [21] Sastry, M.S.R. and Johri, B.N. 1999. Arbuscular mycorrhizal diversity of stressed soils of Bailadila iron ore sites in Bastar region of Madhya Pradesh. Curr. Sci. 77(8): 1095-1100
- [22] Schenck, N.C. and Perez, Y.,1990 . Manual for the identification of VAM fungi [N.C.Schenck, N.C. and Perez(Eds.)],INVAM, university of florida , Gainesville , Florida , U.S.A.INVAM, university of florida ,pp.241.
- [23] Schubler, A., Schwarzott, D. and Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105(12): 1413-1421.
- [24] Smith, S.E. and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd edition. Academic press, San Diego, CA, pp. 605.
- [25] Thapar, H.S., Vijayan, A.R. and Uniyal, K. 1992. VA mycorrhizal association and root colonization in some important tree species. *Indian Forester*. 118(3): 207-212.
- [26] Uhl, N.W. and Dransfield, J., 1987, Geenera Palmarum. The L.H. Bailey Hortorium and the International Palm Society, Kansas.
- [27] Uniyal, K. and Uniyal, D.P. 2000. Population dynamics of arbuscular mycorrhizal fungi in *Dalbergia sissoo* Roxb. *Indian Forester* (July): 782-787
- [28] Verma, R.K. and Jamaluddin 1995. Association and activity of Arbuscular mycorrhizae of Teak (*Tectona grandis*) in Central India. *Indian Forester* (June): 533-539
- [29] Zakaria, M. 1991. Preliminary studies on growth dependency of in vitro micropropagated *Calamus manan* on VA mycorrhiza (VAM) prior to transplanting to the field, RIC Bulletin, 10(1):6-7.