

## Arbuscular mycorrhizal fungi associated with some pteridophytes from western ghat region of Goa

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**Abstract:** Commonly occurring pteridophytes from two sites namely Mollem and Chorlem located in western ghats region of Goa were selected for the present study. All the pteridophytic species examined during the study, exhibited the presence of arbuscular mycorrhizal association. The highest arbuscular mycorrhizal root colonization (75%) was recorded in *Pityrogramma calomelanos* whereas the highest spore density (155 spores 100 g<sup>-1</sup> rhizosphere soil) was recorded in *Adiantum lunulatum*. A fairly good diversity of AM fungi was observed in the rhizosphere of pteridophytes of this region. A total of 17 AM fungal species belonging to five genera namely *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocystis* and *Scutellospora* were recorded.

**Resumen:** Para el presente estudio se seleccionaron pteridofitas cuya presencia es común en dos sitios, Mollem y Chorlem, localizados en la región de Goa en los Gates Occidentales. Todas las especies de pteridofitas examinadas durante el estudio mostraron la presencia de asociaciones micorrízicas arbusculares. En *Pityrogramma calomelanos* se registró la mayor colonización por micorrizas arbusculares (75%), mientras que la mayor densidad de esporas (155 esporas 100 g<sup>-1</sup> de suelo de la rizosfera) fue registrada en *Adiantum lunulatum*. Se observó una diversidad bastante alta de hongos MA en la rizosfera de las pteridofitas de esta región. Se registraron en total 17 especies de hongos MA pertenecientes a cinco géneros: *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocystis* y *Scutellospora*.

**Resumo:** No estudo presente foram selecionadas Pteridófitas ocorrendo vulgarmente no Gates ocidentais da região de Goa em duas estações localizadas em Mollem e Chorlem. Todas as espécies de Pteridófitas examinadas durante o estudo, exibiram a presença de associações micorrízicas arbusculares associadas. A colonização radicular mais elevada (75%) foi registrada em *Pityrogramma calomelanos* enquanto que a maior densidade de esporos (155 esporos por 100g de solo da rizosfera) foi encontrada na *Adiantum lunulatum*. Uma razoavelmente boa diversidade de fungos micorrízicos arbusculares (AM) foi observada na rizosfera das Pteridófitas desta região. Foram registados um total de 17 fungos AM pertencendo a cinco géneros *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocystis* e *Scutellospora*.

**Key words:** *Acaulospora*, AM fungi, *Glomus*, *Gigaspora*, pteridophytes, root colonization, *Sclerocystis*, spore density, *Scutellospora*.

### Introduction

Pteridophytes constitute a significant and important group in the plant kingdom as early land plants. They show various adaptations as they

have evolved to fill different habitats thereby providing an ecological niche for many microorganisms like AM fungi that abound the soil. The earliest reports on mycorrhizal colonization in pteridophytes were mostly based on the samples removed

from herbarium specimens (Boullard 1957). Later, Newman & Reddell (1987) examined 180 field specimens of fern species for mycorrhizal colonization. Mycorrhizal surveys were also reported from New Zealand ferns (Cooper 1976), and Ontario ferns and its allies (Berch & Kendrick 1982). More recently Gemma & Koske (1990) and Gemma *et al.* (1992) reported mycorrhizal status of Hawaiian pteridophytes.

In India, Mishra *et al.* (1980) and Ragupathy & Mahadevan (1993) have documented studies on arbuscular mycorrhizal colonization of pteridophytes. Work on arbuscular mycorrhizal status of pteridophytes from western ghats of Southern India has also published. Raja *et al.* (1995) reported AM fungal association in 43 pteridophytes from the Nilgiris and Kodaikanal hills of western ghats. Recently, Muthukumar & Udaiyan (2000) reported the arbuscular mycorrhizae of pteridophytes growing in different habitats and substrata. They surveyed 71 pteridophytic species belonging to 30 families from six different localities in western ghats of Southern India.

The state of Goa lies in the heart of western ghats, which is one of the hotspots of biodiversity. The major portion of the slopes of western ghat belt falls in this region (Rao 1985), encompassing luxuriant forest with good diversity of pterido-

phytic flora (Tewari 1995). The present paper is an attempt to enumerate the status of arbuscular mycorrhizal fungi associated with the pteridophytes of western ghat region of Goa.

## Materials and methods

### *Sample collection*

Five root samples and rhizosphere/substratum samples for each of the ten commonly occurring pteridophytic species were randomly collected during September 2000, from two sites in western ghat region of Goa namely, Mollem and Chorlem (Table 1). The plants were completely uprooted for collection of samples. Samples were placed in the polyethylene bags, labelled and then transported to the laboratory. Root samples were freshly processed, whereas rhizosphere soil samples were stored in deep freezer at 4°C until they were analyzed.

### *Estimation of root colonization*

The root samples were stained by using modified procedure for staining roots (Koske & Gemma 1989). Root samples of each pteridophyte species were washed gently under tap water and cleared in 2.5% KOH, acidified in 5 N HCl and stained in lactoglycerol with 0.05% Trypan blue. The stained roots were examined under compound microscope (x40 – x100). Hundred root segments for each sample were randomly selected for microscopic observation and the degree of colonization was estimated using slide method (Giovannetti & Mosse 1980).

### *Isolation and quantification of spores and sporocarps*

For isolation of spores/sporocarps, wet sieving and decanting procedure (Gerdemann & Nicolson 1963) was followed. For this, 100 g of rhizosphere soil/substratum was dispersed in 1000 ml of water and the coarse particles were allowed to settle for 15-20 seconds. The soil suspension was then decanted through stacked sieves kept in descending order of pore size (500 µm – 45 µm). The above step was repeated twice so that the majority of spores were recovered from the soil. The debris on the sieves was then collected in the beaker and filtered through Whatman no. 1 filter paper. The filter paper was kept in Petridish and care was taken to see that it remains moist. The contents

**Table 1.** List of pteridophytes collected for the study.

Name of pteridophyte	Family	Habitat
Site – Mollem		
<i>Sellaginella</i> sp.	Sellaginellaceae	Soil
<i>Lygodium flexuosum</i> L.	Lygodiaceae	Soil
<i>Lindsaea heterophylla</i> Beddome	Landseaceae	Soil
<i>Pteris vittata</i> L.	PtRIDACEAE	Rock
<i>Adiantum lunulatum</i> Burm.	Adiantaceae	Soil
Site – Chorlem		
<i>Athyrium hohenackeranum</i> (Kunze) Moore	Athyriaceae	Soil
<i>Blechnum orientale</i> L.	Blechnaceae	Soil
<i>Gleichenia dichotoma</i> Willd.	Gleicheniaceae	Soil
<i>Pityrogramma calomelanos</i> Link.	Hemionitidaceae	Soil
<i>Christella dentata</i> (Forssk.) Brownsey & Jermy.	Thelepteridaceae	Soil

were then examined for spores and sporocarps under stereomicroscope, and the quantification of spores and sporocarps was carried out using procedure described by Gaur & Adholeya (1994).

### Identification of AM fungi

Diagnostic slides with spores/sporocarps were prepared using polyvinyl alcohol lactoglycerol (PVLG) as mountant. Both broken and unbroken spores were observed. Spores were examined using compound microscope (x40 – x100). The genera and the species of AM fungi were identified using bibliographies provided by Morton & Benny (1990), Schenck & Perez (1990), Walker & Vestberg (1998) and Wu (1993). Names and epithet of AM fungi are enlisted according to Walker & Trappe (1993).

### Identification of pteridophytes

Identification of pteridophytes was carried out using 'The Manual of Pteridophyte Flora of Western Ghat, South India' (Manickam & Irudayaraj 1992).

### Statistical analysis

Standard deviation was calculated for mean spore density and mean root colonization. Pearson's one tailed correlation test was performed for both AM fungal parameters (mean spore density and mean root colonization). Analysis of variance (ANOVA) was also performed to study the influence of each host type of the colonization of AM

fungi. Prior to ANOVA test, the root colonization values were subjected to square root transformations in order to fit in the statistical package (mstata) format.

## Results

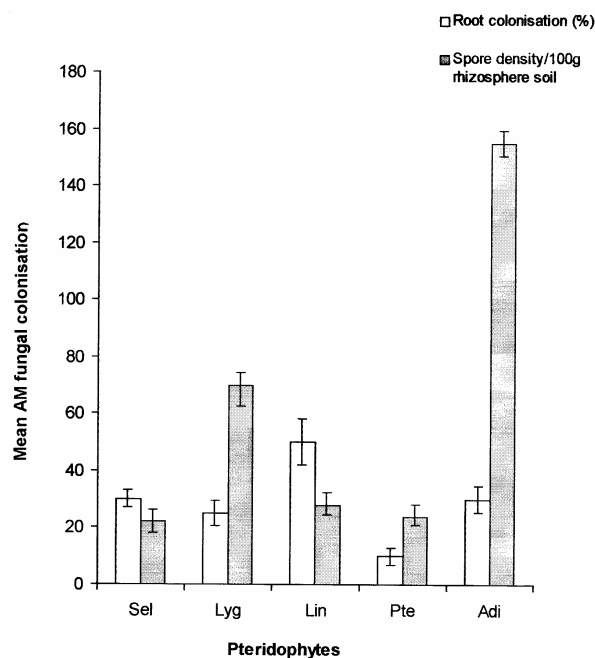
In the present study, the survey of pteridophytes for AM fungi showed variability in colonization and spore density. All the pteridophytes selected for study exhibited the presence of AM fungal association. Hyphal and vesicular stages of colonization were seen in all the pteridophytes. However, arbuscular colonization was seen only in *Lindsaea heterophylla* (Table 2).

In Mollem area, mean root colonization and mean spore density of AM fungi in pteridophytes showed no correlation ( $r = 0.008$ ,  $P > 0.05$ ,  $n = 4$ ). Here, the highest root colonization and spore density was recorded in *Lindsaea heterophylla* (50%) and *Adiantum lunulatum* (155 spores 100 g<sup>-1</sup> rhizosphere soil), respectively (Fig. 1). However, in Chorlem area, the mean root colonization and spore density of AM fungi exhibited a weak negative correlation ( $r = -0.45$ ,  $P > 0.05$ ,  $n = 4$ ) with highest root colonization and spore density recorded in *Pityrogramma calomelanos* (75%) and *Christella dentata* (116 spore 100 g<sup>-1</sup> rhizosphere soil) respectively (Fig. 2). The host species significantly influenced root colonization ( $r$ ) and spore density ( $s$ ) in Mollem ( $C.D_r = 0.092$  &  $C.D_s = 5.40$ ;  $P < 0.05$ ) as well as Chorlem ( $C.D_r = 0.589$  &  $C.D_s = 5.83$ ;  $P < 0.05$ ) area.

**Table 2.** Type of colonization by AM fungi in pteridophytes.

Name of pteridophytes	Type of colonization			Type of propagules	
	Hyphal colonization	Arbuscular colonization	Vesicular colonization	Presence of spores	Presence of sporocarps
<i>Sellaginella</i> sp.	+	–	+	+	+
<i>Lygodium flexuosum</i>	+	–	+	+	+
<i>Lindsaea heterophylla</i>	+	+	+	+	–
<i>Pteris vittata</i>	+	–	+	+	+
<i>Adiantum lunulatum</i>	+	–	+	+	+
<i>Athyrium hohenackeranum</i>	+	–	+	+	–
<i>Blechnum orientale</i>	+	–	+	+	–
<i>Gleichenia dichotoma</i>	+	–	+	+	–
<i>Pityrogramma calomelanos</i>	+	–	+	+	+
<i>Christella dentata</i>	+	–	+	+	+

+ = present; – = absent.

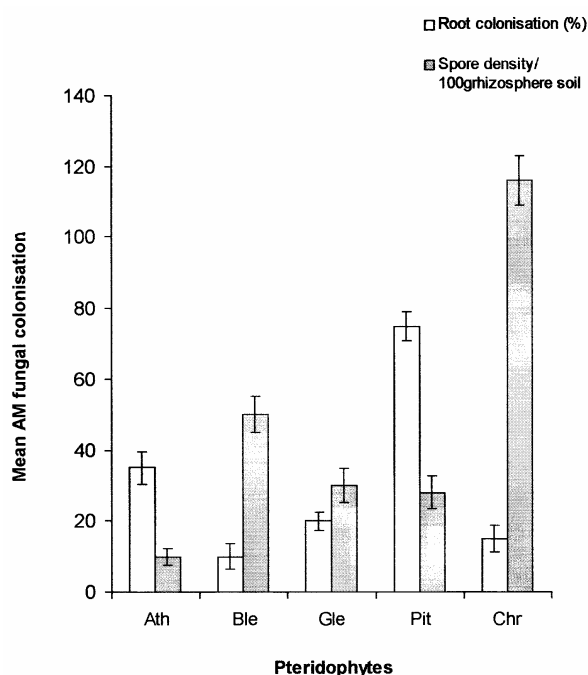


**Fig. 1.** An account of AM fungal association in pteridophytes from Mollem area. (Sel – *Sellaginella* sp., Lyg – *Lygodium flexuosum*, Li – *Lindsaea heterophylla*, Pte – *Pteris vittata*, Adi – *Adiantum lunulatum*; Error bar indicates  $\pm 1$  S.D.)

During the course of study, a total of 17 AM fungal species belonging to five genera namely *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocystis* and *Scutellospora* were recorded. *Glomus* was most dominant among all the genera (Table 3). Out of the ten pteridophyte species studied, sporocarpic species of AM fungi were recorded in six pteridophytes (Tables 2 & 3). The species composition of AM fungi showed variability in the rhizosphere soil of the pteridophytes selected for study. In Mollem area the species richness of AM fungi ranged from 2-3 species/pteridophyte whereas in Chorlem area it ranged from 2-5 species/pteridophyte (Table 3).

## Discussion

The present study is the first report on arbuscular mycorrhizal status of pteridophytes from western ghat region of Goa. Our study recorded the absence of correlation between root colonization and spore density of AM fungi which is in accordance with earlier report on AM fungal association in pteridophytes from western ghats, Southern India (Muthukumar & Udaiyan 2000). A possible reason for the lack of correlation may be due



**Fig. 2.** An account of AM fungal association in pteridophytes from Chorlem area. (Ath – *Athyrium hohenackerianum*, Moore, Ble – *Blechnum orientale*, Gle – *Gleichenia dichotoma*, Pit – *Pityrogramma calomelanos*, Chr – *Christella dentata*; Error bar indicates  $\pm 1$  S.D.)

to the fact that sporulation of AM fungi is dependent on wide range of environmental factors (Muthukumar *et al.* 2001).

The spore density range recorded in our study is much higher (10-155 spore  $100\text{ g}^{-1}$  rhizosphere soil) than reported by Muthukumar & Udaiyan (2000) in the rhizosphere soil of pteridophytes from western ghats, Southern India (2.35-39.52 spore  $100\text{ g}^{-1}$  rhizosphere soil). Also, our study recorded the presence of 17 AM fungal species belonging to five genera, which differs from their findings that reported the presence of 8 AM fungi belonging to four genera. Comparatively higher spore density and recovery of relatively higher number of AM fungi from the rhizosphere of pteridophytes, in the present study may be attributed to low host specificity of AM fungi and varying eco-edaphic factors along the different regions of the western ghats.

In our study the composition of AM fungi varied in the rhizosphere soil of the pteridophytes from the same area and is in agreement with the findings of Zhao & Zhao (1999).

The present work, mainly documents AM fungal status of terricolous (soil) pteridophytes and

**Table 3.** Diversity of AM fungi associated with pteridophytes.

AM fungal species	Sel	Lyg	Lin	Pte	Adi	Ath	Ble	Gle	Pit	Chr
<i>Acaulospora foveata</i> Trappe & Janos	–	–	–	–	+	–	–	+	–	+
<i>Glomus claroideum</i> Schenck & Smith emend. Walker & Vestberg	–	–	–	+	–	–	+	–	–	–
<i>Glomus etunicatum</i> Becker & Gerdemann	+	–	–	–	–	–	+	–	–	–
<i>Glomus fasciculatum</i> (Thaxter) Gerdemann & Trappe emend. Walker & Koske	–	–	+	–	–	+	–	–	–	–
<i>Glomus formosanum</i> Wu & Chen	+	–	–	–	–	–	–	–	–	–
<i>Glomus hoi</i> Berch & Trappe	–	–	+	–	–	–	–	–	–	–
<i>Glomus macrocarpum</i> (Tul.) Tul. Var. <i>geospora</i> Nicolson & Gerdemann	–	–	–	+	–	–	–	–	–	–
<i>Glomus multicaulis</i> Gerdemann & Bakshi	–	–	–	–	–	–	+	–	–	–
<i>Glomus</i> sp.	–	–	–	–	–	–	–	–	+	–
<i>Gigaspora albida</i> Schenck & Smith	–	–	–	–	–	–	–	–	+	–
<i>Gigaspora decipiens</i> Hall & Abbott	–	–	–	–	–	–	–	–	+	–
<i>Gigaspora margarita</i> Becker & Hall	+	–	–	–	–	–	–	–	+	–
<i>Sclerocystis rubiformis</i> Wu & Chen	–	+	–	–	+	–	–	+	–	–
<i>Sclerocystis sinuosa</i> Iqbal & Bushra	–	–	–	+	–	–	–	–	–	–
<i>Sclerocystis taiwanensis</i> Wu & Chen	–	+	–	–	+	–	–	–	–	+
<i>Scutellospora gregaria</i> (Schenck & Nicolson) Walker & Sanders	–	–	–	–	+	–	–	+	+	+
<i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders	–	–	–	–	–	+	–	–	–	–
Species richness of AM fungi	3	2	2	2	3	2	3	3	5	3

Sel – *Sellaginella* sp., Lyg – *Lygodium flexuosum*, Li – *Lindsaea heterophylla*, Pte – *Pteris vittata*, Adi – *Adiantum lunulatum*; Ath – *Athyrium hohenackeranum*. Moore, Ble – *Blechnum orientale*, Gle – *Gleichenia dichotoma*, Pit – *Pityrogramma calomelanos*, Chr – *Christella dentata*.

further studies on arbuscular mycorrhizal association with the epiphytic and other lithophytic pteridophytes in this region needs to be undertaken. Such studies would enable us to better understand the role of arbuscular mycorrhizal fungi in this fascinating group of plant kingdom.

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