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Spatio-temporal variations of arbuscular mycorrhizal (AM) fungi associated with *Carica papaya* L. in agro-based ecosystem of Goa, India

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Studies were carried out on spatio-temporal variations of arbuscular mycorrhizal (AM) fungi associated with *Carica papaya* L. growing in two different geographic localities in Goa, India – Western Ghats and coastal area, differing in soil characteristics and plantation status. The study recorded considerable variation in root colonization, spore density and distribution of AM fungi in the selected sites. The mean total root colonization was at a maximum in the month of July, while spore density was highest in April. The study recorded a total 33 species of AM fungi. Western Ghats recorded a relatively higher diversity of AM fungi compared to coastal area. Species richness of AM fungi was at a maximum in April and coincided with maximum mean spore density. Edaphic as well as climatic factors influenced the AM fungal parameters. The study recorded the existence of seasonality in AM fungi.

Keywords: AM fungi; *Carica papaya* L.; coastal area; root colonization; spore density; Western Ghats

Introduction

The genus *Carica* (Caricaceae) has about 48 species of which only *Carica papaya* L. (papaya) is cultivated for its edible fruits. Papaya is native to tropical America and its place of origin is assumed to be Southern Mexico and Costa Rica. It is thought to have been introduced into India during the 16th century (Reddy 2000). Interestingly, India is the world's second largest producer of papaya after Brazil with a production of up to 1500 metric tones. However, in Goa large-scale cultivation of papaya is undertaken only in government agricultural farms and by a few progressive farmers. In general, papaya cultivation is mostly confined to kitchen gardens and along the bunds due to constraints in productivity resulting from fungal and viral diseases. Therefore, at present, most of the local demand for papaya in Goa is met by importing the fruit from neighbouring states (Khade and Rodrigues 2008).

Mycorrhiza plays a key role in nutrient cycling in the ecosystem and protects the host plant against environmental stress. Under natural conditions, mycorrhizae are a normal phenomenon and AM association is the commonest mycorrhizal type. The AM fungi are obligatory biotrophic fungi that are closely associated with both host plants and soil environment. Because of its effects on plant growth and health, it is accepted that AM symbiosis can reduce chemical fertilizer and pesticide inputs. The key effects of AM symbiosis include: improved rooting and plant establishment,

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enhanced uptake of low mobile ions, increased plant tolerance to (biotic and abiotic) stresses, improvement in quality of soil structure and increased plant diversity (Zhang et al. 2003).

It is well documented that AM-mediated growth effects are primarily nutritional and are inversely related to soil fertility, especially available soil P, which affects the fungus symbiotic effectiveness (Rosalind Padma and Kandaswamy 1990; Sukhada 1992). Recent studies have confirmed these findings and demonstrated the importance of AM symbiosis to papaya in low fertility soil (Martins et al. 2000). Further studies have elucidated mycorrhizal status with respect to glucosinolate patterns (Vierheilig et al. 2000) and investigated the effect of AM fungi on leaf water potential and ethylene levels under stressed conditions (Cruz et al. 2000).

Following AM colonization, the functions of the root becomes extended in the rhizosphere due to the presence of the AM fungal external mycelium. However, we still know relatively little of the ecology of AM fungi and in particular, the mycelium network under natural conditions (Hodge 2000). In order to more fully comprehend the role of AM fungi in ecosystems, as well as their basic biology, it is important to document seasonal changes of various aspects of the life history of these fungi (Lutgen et al. 2003). Seasonality of the degree of AM colonization (Khan 1974; Read and Hodgson 1976, Van Duin et al. 1990) may modify the role of AM fungi for established perennial plants under field conditions, perhaps in relation to the efficiency of the AM fungal species (Jensen 1982; Ernst et al. 1984).

The ecology and dynamic of soil microorganisms vary both temporally and spatially (Allen and Allen 1980). Environmental factors that affect and alter soil microbial assemblage structure and function are complex. It is well known that environmental variables, such as soil pH, nutrient availability and soil moisture content, can influence the distribution and activity of soil microorganisms. The development and seasonal fluctuations in AM association has been investigated in several plant species and in several countries, including the United Kingdom (Merryweather and Fitter 1998), Portugal (Carvalho et al. 2001), Israel (He et al. 2002) and India (Muthukumar and Udaiyan 2002a). Since below-ground ecosystem as a whole is affected by AM fungi (Douds and Miller 1999) they may be useful as indicators of ecosystem change. Investigations of AM fungal dynamics can help elucidate the ecological significance of AM fungal associations in Goa, India. Recently in Goa, Khade and Rodrigues (2008) studied the ecology of AM fungi associated with papaya in agro-based ecosystem of Goa and reported seasonal variation of AM fungi. As tropical soils are exposed to changes in soil moisture variation within the year, studies are necessary to evaluate the effect of climatic and soil variables on the dynamics of AM fungi. In view of the above, the present study was undertaken, to investigate the spatial and temporal dynamics of AM fungi as well as to evaluate the effects of the climatic and edaphic variables on AM fungal dynamics in rhizosphere of papaya in agro-ecosystem of Goa, India.

Materials and methods

Study site

One-year-old, fruit bearing, female papaya plants were sampled from two geographic localities in North Goa – Valpoi (Western Ghats, N15°33′24.0″ and E 74°08′05.6″) and Old Goa (coastal area, N15°29′32.1″ and E 73°55′00.00″). The data on climatic factors were obtained from Meteorological Department, State Government of Goa. The rhizosphere soil samples were collected every three months over a period of two

years (April 2000 to January 2002). The sites selected for the study were conventionally managed farms differing in plantation status. The papaya plants at Valpoi were inter-cropped with coconut and banana and at Old Goa they were planted along the bunds of flower plots (Figure 1a). The study site also differed in soil type. At Valpoi, the soil was moderately drained, dark brown, loam to clay loam while at Old Goa the soil was well-drained, dark grey brown to dark brown gravelly sandy loam to sandy clay loam. Cultural practices such as fertilizer applications, irrigation, mulching and weeding were carried out during the course of the study. The weeds were peripheral and the species commonly encountered were grasses. Others included *Corcorus* species, *Amaranthus* species, *Mimosa pudica* L. and *Cassia tora* L. Soil samples were collected at the depth of 0–25 cm.

Collection of rhizospheric soil samples

Five healthy, fruit bearing plants available during sampling time were randomly selected at each site. While sampling, rhizosphere soil was collected along with roots. At sites where papayas were inter-cropped or present along the bunds or fence, care was taken to trace the papaya roots, which were identified by its white colour and distinct phenolic odour emitted upon crushing. Samples were collected within 0–25 cm depth at 60 cm distance from the stem of the plant and combined to give approximately 400 g moist soil per composite sample after thorough mixing. From this 100 g air-dried soil was employed for extraction of AM fungal spores, 250 g was utilized for nutrient analysis and the remaining soil was utilized for setting trap cultures. In case of roots, for each plant, two sub-samples were made. Most of the roots were employed for estimation of root colonization of AM fungi while the remaining roots were utilized for establishing trap cultures.

Samples were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed for estimation of root colonization whereas, soil samples were stored at 4°C until analyzed. Sampling procedures were carried out according to Tews and Koske (1986) except for a larger core size (15 cm in diameter) to normalize distribution of spores (St John and Koske 1988). Roots were separated from the soil by sieving through 2 mm mesh, prior to preparation of composite samples and their sub-samples.

Establishment of trap cultures

Baiting of native AM fungi were carried out by using trap cultures (Gilmore 1968) under glass house conditions. *Eleusine coracana* (L.) Gartner and *Coleus* sp. were used separately as hosts. Pots showing successful mycorrhization were maintained for a period of six months and the application of water was reduced in the final three weeks to maximize spore production (Menge 1982). At the end of six months, the plants were cut near the base and the cultures were air-dried and checked for the presence of spores. Spores isolated from pot cultures were used to verify the identification of AM fungi recovered from field soil. The cultures were deposited at the Department of Botany, Goa University, India, during June 2003.

Soil sample analysis

Standard procedures were employed for carrying out soil analysis: Soil pH (pH meter – LI 120 Elico, India), electrical conductivity (conductivity meter CM-180

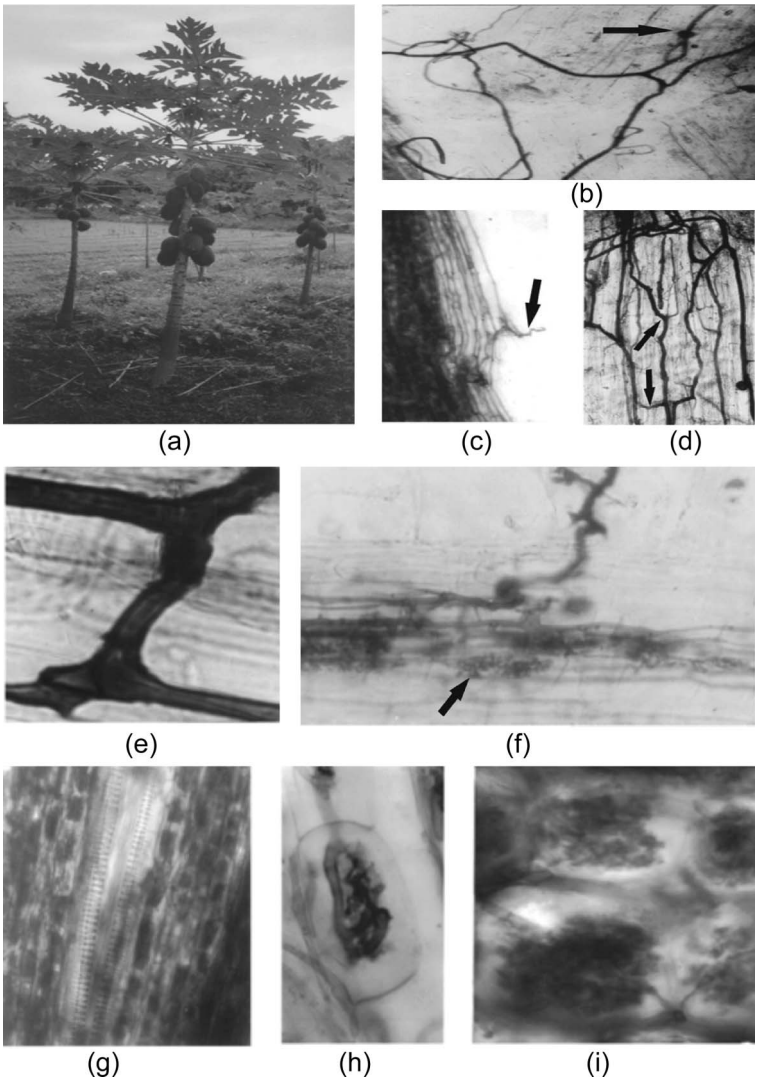

Figure 1. (a) Habit of *Carica papaya* L. (b) Spore (arrow) with extramatrical hypha (× 100). (c) Entry point of AM fungi (arrow) and hyphal colonization of AM fungi inside the root (× 100). (d) Ramification of hyphae inside the host root (× 400). (Note the H-shaped and Y-shaped connections). (e) H-shaped hyphal colonization of AM fungi (× 1000). (f) Initiation of arbuscule formation inside the host root (× 100). (Note the formation of arbuscules-arrow). (g) Arbuscular colonization of AM fungi inside the host root (× 100). (h) An arbusculate coil inside the host root cell (× 1000). (i) Mature arbuscules (× 1000). (j) Formation of vesicles inside the host root (× 1000). (k) Intraradical spores of AM fungi (× 100). (l) Intraradical spores of AM fungi (× 400). (m) Soil borne vesicles/auxiliary cells with subtending extramatrical hyphae (× 100). (n) Soil borne vesicles/auxiliary cells with subtending extramatrical hyphae (× 400). (o) Crushed soil borne spore of *Gigaspora margarita* (× 150). (p) A portion of spore of *Gigaspora margarita* with bulbous suspensor (× 150) {Note the pore at the point of attachment of bulbous suspensor to the spore}.

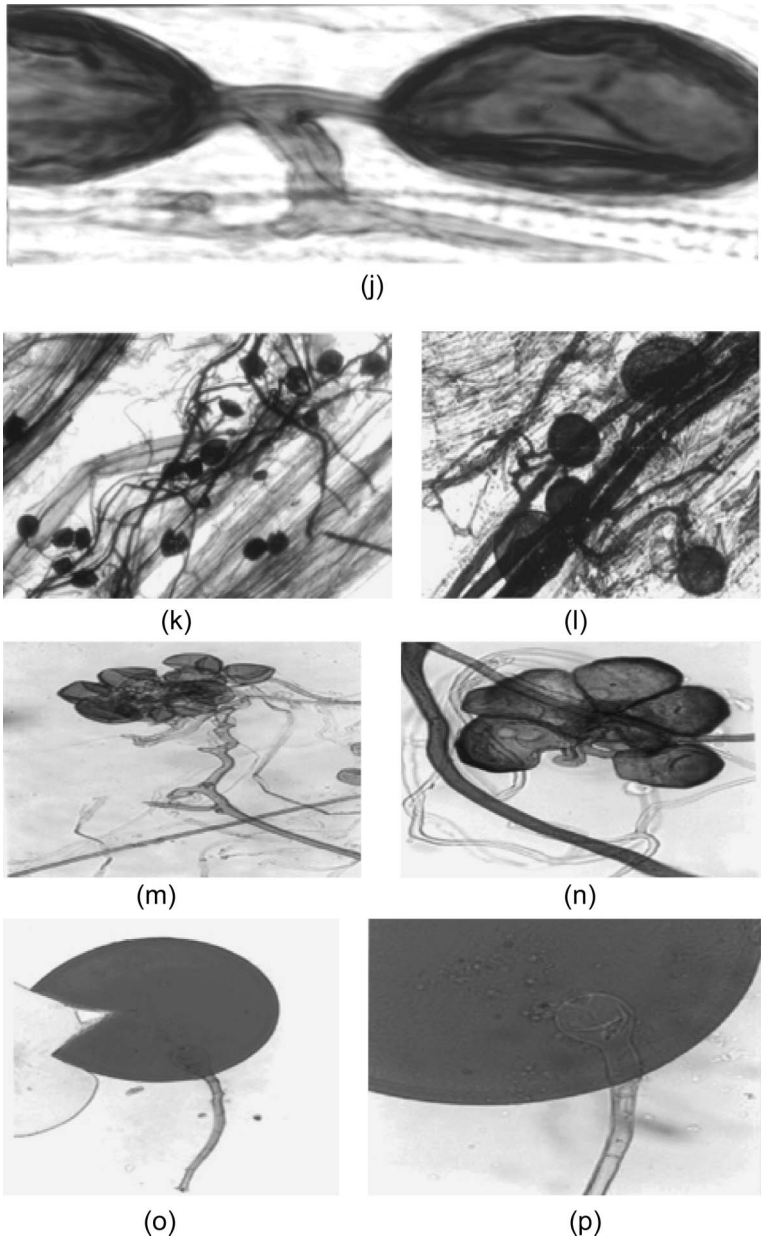

Figure 1. (*Continued*).

Elico, India) organic carbon (Walkley and Black 1934), total nitrogen (Jackson 1971) and available phosphorus (Bray and Kurtz 1945), available potassium (Hanway and Heidal 1952). For micronutrients – available zinc, copper, manganese and iron – the Lindsay and Norvel (1978) method was used.

Estimation of root colonization by AM fungi

Roots were cleared and stained in trypan blue (Phillips and Hayman 1970) and percentage total root colonization and root length colonized by hyphae, arbuscules and vesicles was estimated by magnified intersection method (McGonigle et al. 1990). The stained root bits were placed on the microscopic slides and the field of the view was moved across the slide and the presence or absence of each AM fungal structures were noted at the intersection. A total of 150 intersections were noted per samples under compound microscope at 200 × magnifications and root colonization of AM fungi was expressed in percentage. Five samples were considered per site per sampling period.

Enumeration of AM fungal spores

Spores and sporocarps of AM fungi were isolated by wet sieving and the decanting method (Gerdemann and Nicolson 1963) and quantification of spore density of AM fungi was carried out using the method described by Gaur and Adholeya (1994). Turgid healthy looking spores were counted for the study. Five replicates of field soil were considered per site per sampling period.

Identification AM fungi

Diagnostic slides with spores were prepared only using polyvinyl alcohol lactoglycerol as a mountant (Koske and Tessier 1983). Both unbroken and broken spores were observed. Spores were examined in detail using a compound microscope with magnification ranging from × 100 – × 1000. Spore morphology and wall characteristics were considered for the identification of AM fungi and these characteristics were ascertained using compound microscope, Leica WILD MP 3 and Nikon E 800. AM fungi were identified to species level using bibliographies provided by Schenck and Perez (1990). Taxonomic identification of spores was also carried out by matching the descriptions provided by International Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu).

Diversity indices

Species richness is the mean number of AM fungal species recovered from each site per sample collection.

Frequency of occurrence

Frequency of occurrence of AM fungi was calculated using the following formula (Beena et al. 2000):

$$\text{Frequency}(\%) = \frac{\text{Number of soil samples that possess spores of a particular morphotype}}{\text{Total number of soil samples screened}} \times 100$$

Statistical analysis

Data of two years were compared by Analysis of Variance for root colonization and spore density of AM fungi at two geographic localities during the study period. Prior to Multiple ANOVA, the root colonization values were arcsine transformed, while the spore density values were log transformed to fit the normal distribution pattern. Pearson's correlation coefficient was used to assess the relationship between edaphic and climatic factors and AM fungal parameters. Multiple linear regression analysis was carried out to determine the cumulative effect of climatic factors and edaphic factors on root colonization and spore density of AM fungi. Statistical analysis was carried out using mstact and WASP 1.0 packages.

Results

Climatic and edaphic factors

Average relative humidity and rainfall were higher in the first year compared to the second year (Figure 2). Relative humidity (except for July 2000) and the temperature exhibited narrow range of fluctuation during the study period (Figure 2). According to standards laid by ICAR (Indian Council for Agricultural Research) and the Agriculture Department, Goa, India, the soil pH was acidic to slightly alkaline while electrical conductivity was normal (Tables 1 and 2). The sites had high levels of organic carbon and available potassium. Available phosphorus levels were limiting and except for October and January when they were high (Table 1 and 2) in Western Ghats and the coastal area. Total nitrogen content as well as micronutrients like Fe (55.59–71.92 ppm) and Mn (78.93–100.12 mg kg^{-1}) recorded higher concentrations during October and January. Furthermore, available P, available K, Fe and Mn in Western Ghats and available P, Fe and Mn in the coastal area showed large variation over the sampling periods.

Relation between climatic and edaphic factor

At Valpoi, soil EC was significantly and negatively correlated to maximum temperature ($r = -0.762$; $p = 0.05$) and it was positively correlated to rainfall ($r = 0.891$; $p = 0.01$). Total nitrogen was significantly and positively correlated with maximum temperature ($r = 0.882$; $p = 0.05$) but, negatively correlated to rainfall ($r = -0.831$; $p = 0.05$). Whereas at Old Goa, rainfall recorded a significant positive correlation with electrical conductivity ($r = 0.878$; $p = 0.01$) and a significant negative correlation with total N ($r = -0.730$; $p = 0.05$).

Root colonization of AM fungi

All the root samples examined had AM fungal colonization. At both the sites, the root samples exhibited extramatrical hyphae with spores, numerous entry points, ramification of intraradical hyphae (Figure 1b, c, d, e), abundant arbusculate coils and arbuscules in the month of July (Figure 1f g, h, i), while vesicles were abundant during October (Figure 1j). Intraradical spores were infrequent between January and April (Figure 1k, l), whereas, soil-borne auxiliary cells occurred during April (Figure 1m, n).

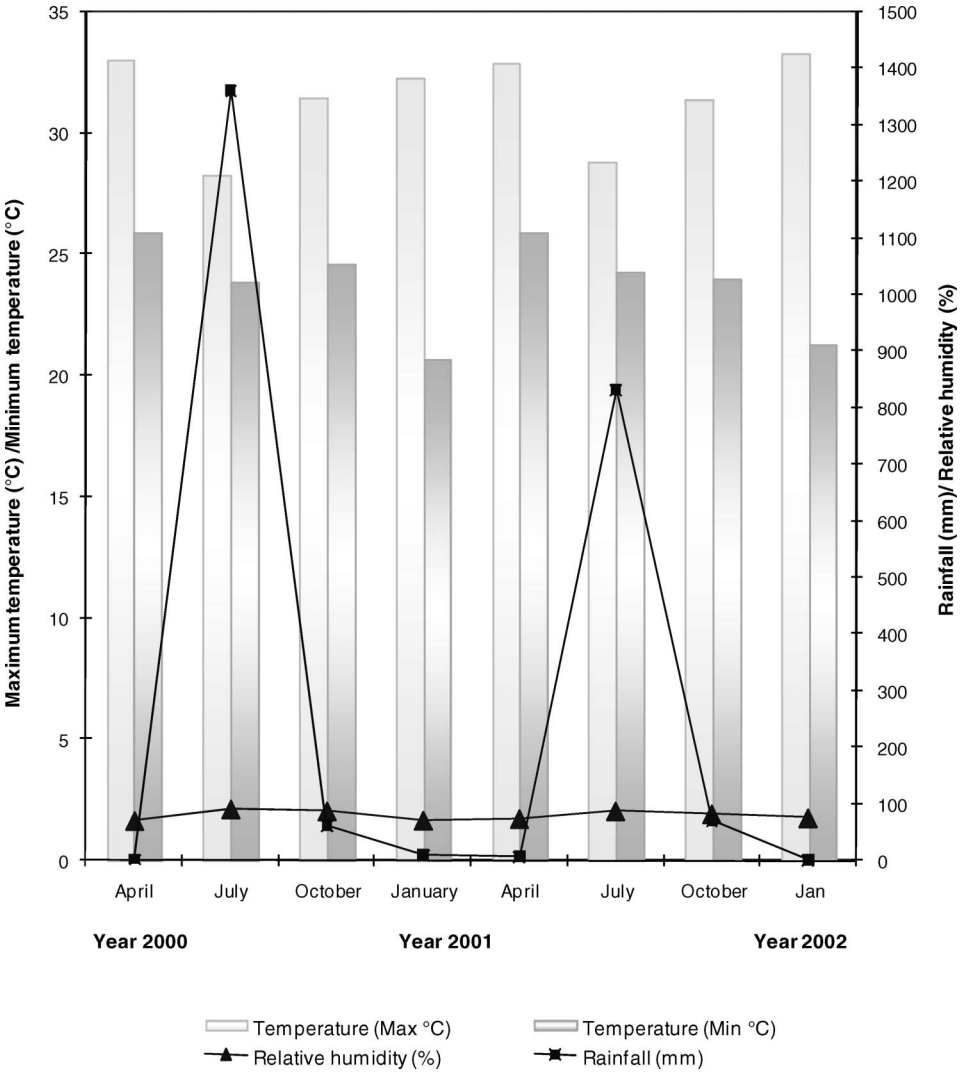

Figure 2. Climatic data recorded during the study period (April 2000–January 2002).

AM fungal colonization varied between the sites and exhibited more or less identical patterns during both the years (Figures 3 and 4). Mean hyphal colonization of AM fungi exhibited different trends during the two years. Furthermore, maximum arbuscular colonization occurred in July and maximum vesicular colonization was recorded in October at both the sites during the study period. The mean hyphal colonization during the study period ranged from 10–20%, at both the sites (Figures 3a and 4a). The mean arbuscular colonization ranged from 0–30% and 0–37% at Valpoi (Figure 3b) and Old Goa (Figure 4b), respectively. Mean vesicular colonization varied from 0–27% at Valpoi and 2–25% at Old Goa (Figure 3c and 4c). The mean total colonization of AM fungi exhibited different trends during the two years and ranged from 20–61% and 16–67% at Valpoi and Old Goa (Figure 5a and 5c), respectively.

Table 1. Soil characteristics of site Valpoi (Western Ghats).

Month and year ⇒ Factors ⇓	First year of sampling				Second year of sampling			
	April 2000	July 2000	October 2000	January 2001	April 2001	July 2001	October 2001	January 2002
pH	5.60 (0.112)	6.00 (0.237)	6.50 (0.125)	5.40 (0.229)	5.80 (0.258)	6.20 (0.178)	5.50 (0.279)	6.00 (0.193)
EC (mS cm^{-1})	0.09 (0.001)	0.17 (0.003)	0.06 (0.009)	0.05 (0.000)	0.08 (0.003)	0.12 (0.001)	0.10 (0.005)	0.09 (0.000)
Organic C (%)	0.95 (0.061)	1.89 (0.034)	1.49 (0.054)	1.43 (0.045)	0.98 (0.036)	1.83 (0.06)	1.46 (0.078)	1.03 (0.07)
Total N (%)	1.20 (0.000)	0.87 (0.001)	1.20 (0.002)	1.20 (0.002)	1.08 (0.002)	0.80 (0.000)	1.00 (0.004)	1.20 (0.004)
Available P (kg ha^{-1})	8.00 (1.891)	4.00 (1.781)	24.00 (3.943)	6.00 (1.114)	15.00 (2.560)	12.00 (2.315)	12.00 (1.671)	28.00 (3.452)
Available K (kg ha^{-1})	116.00 (4.671)	94.00 (1.116)	106.00 (1.237)	158.00 (4.985)	168.00 (3.789)	144.00 (3.418)	110.00 (1.659)	126.00 (1.381)
Cu (mg kg^{-1})	2.12 (0.012)	2.44 (0.035)	2.98 (0.041)	1.64 (0.067)	2.00 (0.089)	2.88 (0.056)	2.55 (0.034)	3.71 (0.015)
Fe (mg kg^{-1})	27.88 (0.561)	13.11 (0.653)	55.59 (0.644)	21.33 (0.534)	47.88 (0.566)	27.98 (0.233)	43.23 (0.645)	64.88 (0.823)
Zn (mg kg^{-1})	4.73 (0.004)	1.43 (0.000)	5.48 (0.004)	1.23 (0.001)	2.33 (0.001)	2.58 (0.002)	5.14 (0.002)	3.78 (0.001)
Mn (mg kg^{-1})	17.83 (1.781)	18.36 (1.563)	100.12 (2.934)	99.86 (2.564)	16.40 (1.134)	14.12 (1.161)	78.93 (2.218)	84.12 (2.156)

Values presented are mean of three readings; Values in the parenthesis indicates ± SE.

Table 2. Soil characteristics of site Old Goa (coastal area).

Month and year ⇒ Factors ⇓	First year of sampling				Second year of sampling			
	April 2000	July 2000	October 2000	January 2001	April 2001	July 2001	October 2001	January 2002
pH	5.20 (1.453)	5.60 (1.568)	5.50 (1.214)	5.50 (1.611)	5.60 (1.563)	5.60 (1.321)	5.50 (1.116)	5.50 (1.127)
EC (mS cm^{-1})	0.09 (0.003)	0.13 (0.001)	0.07 (0.004)	0.07 (0.003)	0.08 (0.003)	0.09 (0.001)	0.08 (0.003)	0.08 (0.001)
Organic C (%)	1.83 (0.034)	1.98 (0.163)	0.88 (0.171)	1.14 (0.037)	1.83 (0. 457)	1.92 (0.578)	0.90 (0.063)	1.17 (0.048)
Total N (%)	1.20 (0.001)	0.72 (0.002)	1.14 (0.001)	1.13 (0.023)	1.00 (0.000)	0.43 (0.005)	1.20 (0.020)	0.83 (0.026)
Available P (kg ha^{-1})	12.00 (1.143)	4.00 (1.467)	28.00 (2.891)	28.00 (2.884)	8.00 (1.421)	13.00 (1.327)	28.00 (2.567)	17.00 (1.897)
Available K (kg ha^{-1})	122.00 (2.610)	139.00 (2.981)	128.00 (1.658)	116.00 (2.236)	140.00 (2.461)	136.00 (1.145)	152.00 (1.136)	126.00 (1.451)
Cu (mg kg^{-1})	2.41 (0.034)	1.17 (0.091)	4.64 (0.543)	3.74 (0.612)	3.00 (0.454)	3.22 (0.422)	3.96 (0.44)	2.86 (0.167)
Fe (mg kg^{-1})	46.73 (0.451)	36.8 (0.612)	71.92 (0.567)	64.44 (0.433)	58.23 (0.671)	54.28 (0.412)	66.08 (0.533)	50.11 (0.763)
Zn (mg kg^{-1})	3.73 (0.112)	1.18 (0.134)	1.99 (0.432)	2.31 (0.432)	4.13 (0.567)	2.42 (0.234)	3.03 (0.348)	1.53 (0.221)
Mn (mg kg^{-1})	19.22 (1.346)	24.14 (1.453)	79.84 (3.567)	84.19 (3.491)	23.61 (1.453)	14.96 (1.225)	88.44 (2.544)	80.22 (2.663)

Values presented are mean of three readings; Values in the parenthesis indicates ± SE.

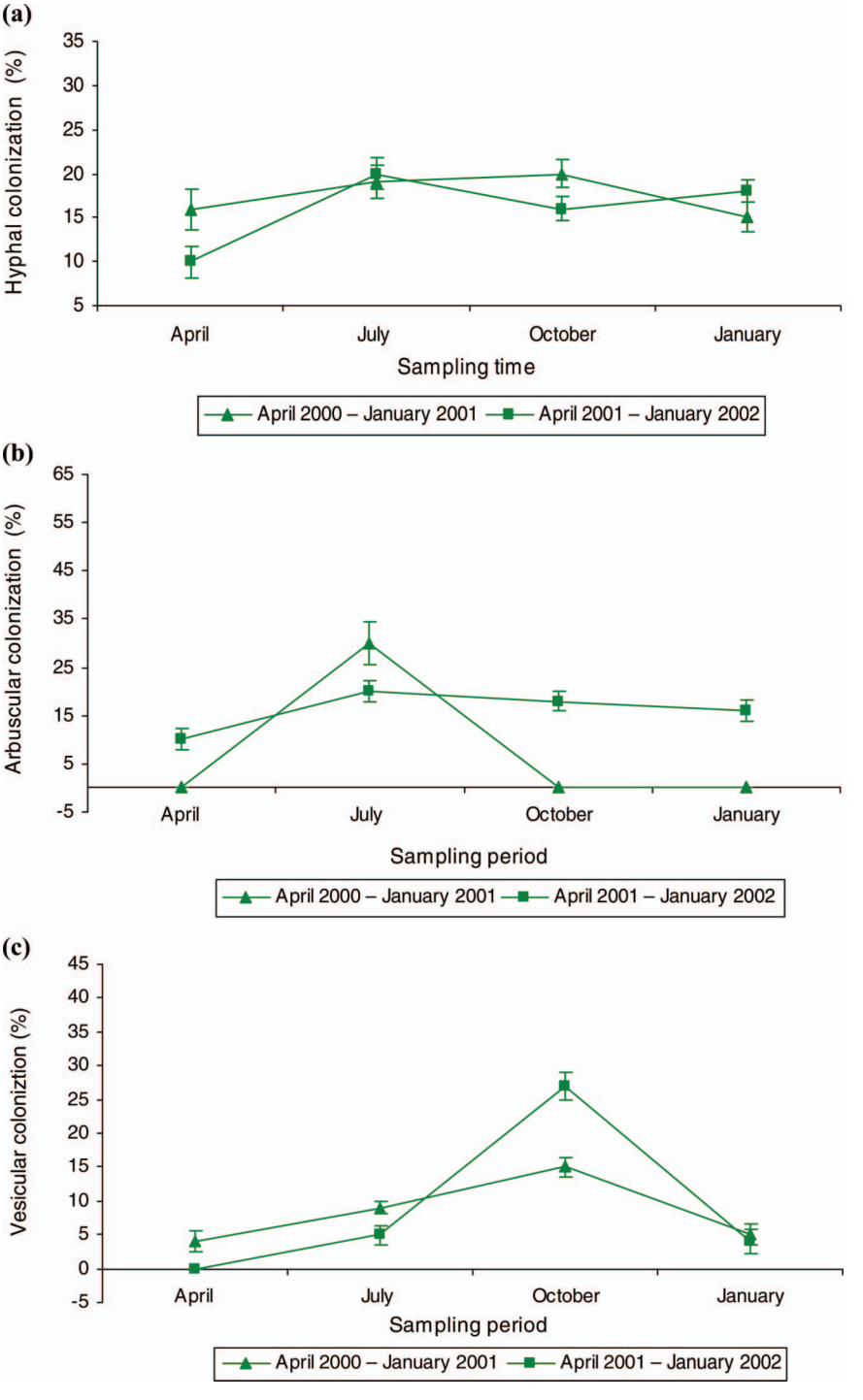

Figure 3. Seasonal variation in mean (a) hyphal, (b) arbuscular and (c) vesicular colonization of arbuscular mycorrhizal fungi in Valpoi. Error bar indicate ± SE.

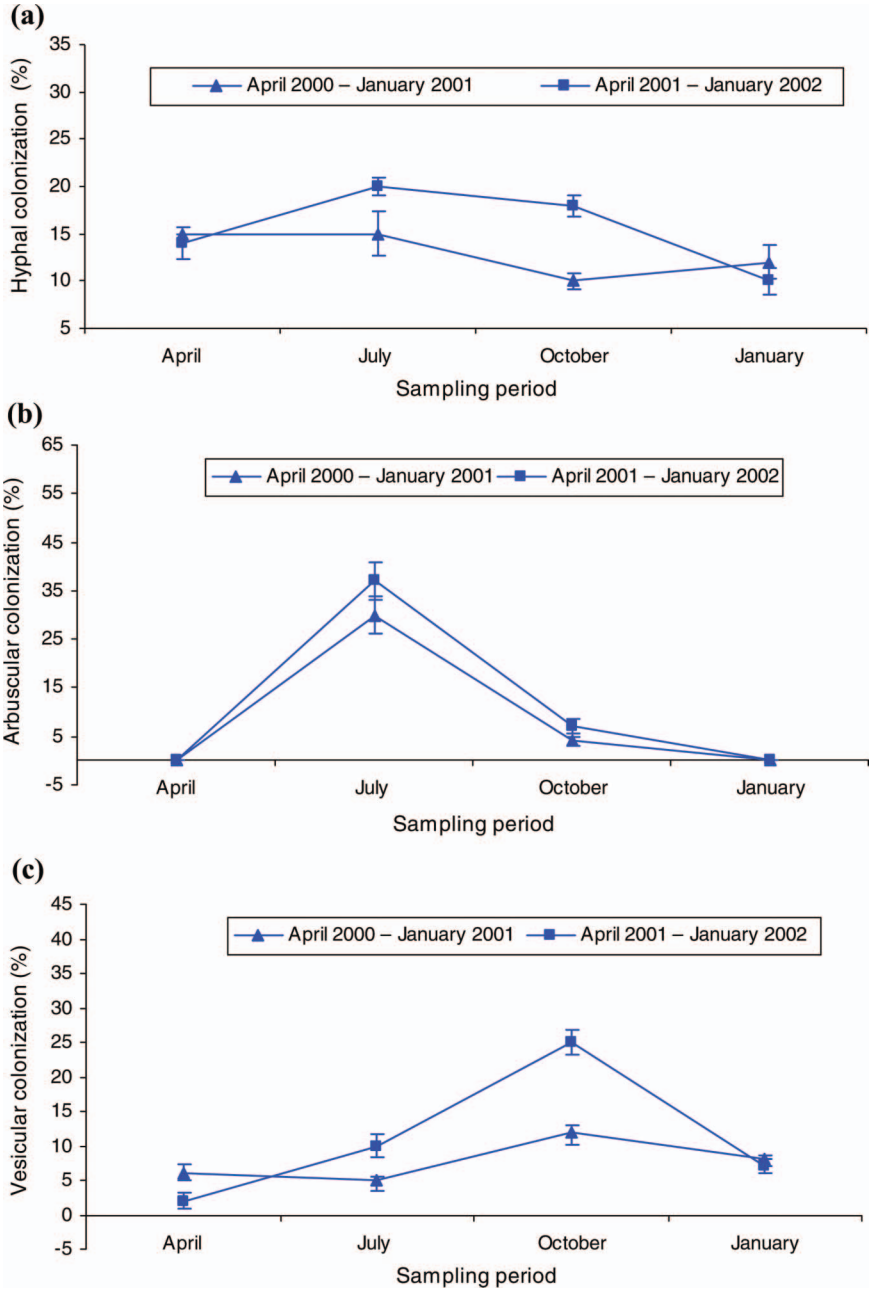

Figure 4. Seasonal variation in mean (a) hyphal, (b) arbuscular and (c) vesicular colonization of arbuscular mycorrhizal fungi in Old Goa. Error bar indicate ± SE.

The highest hyphal (25.5%), arbuscular (36.5%) and total colonization (60.5%) occurred in the coastal area while the highest vesicular colonization (21%) was recorded in Western Ghats (Figure 6a–d).

Multiple ANOVA revealed that the root colonization of AM fungi varied significantly between sites, sampling periods and different AM fungal structures

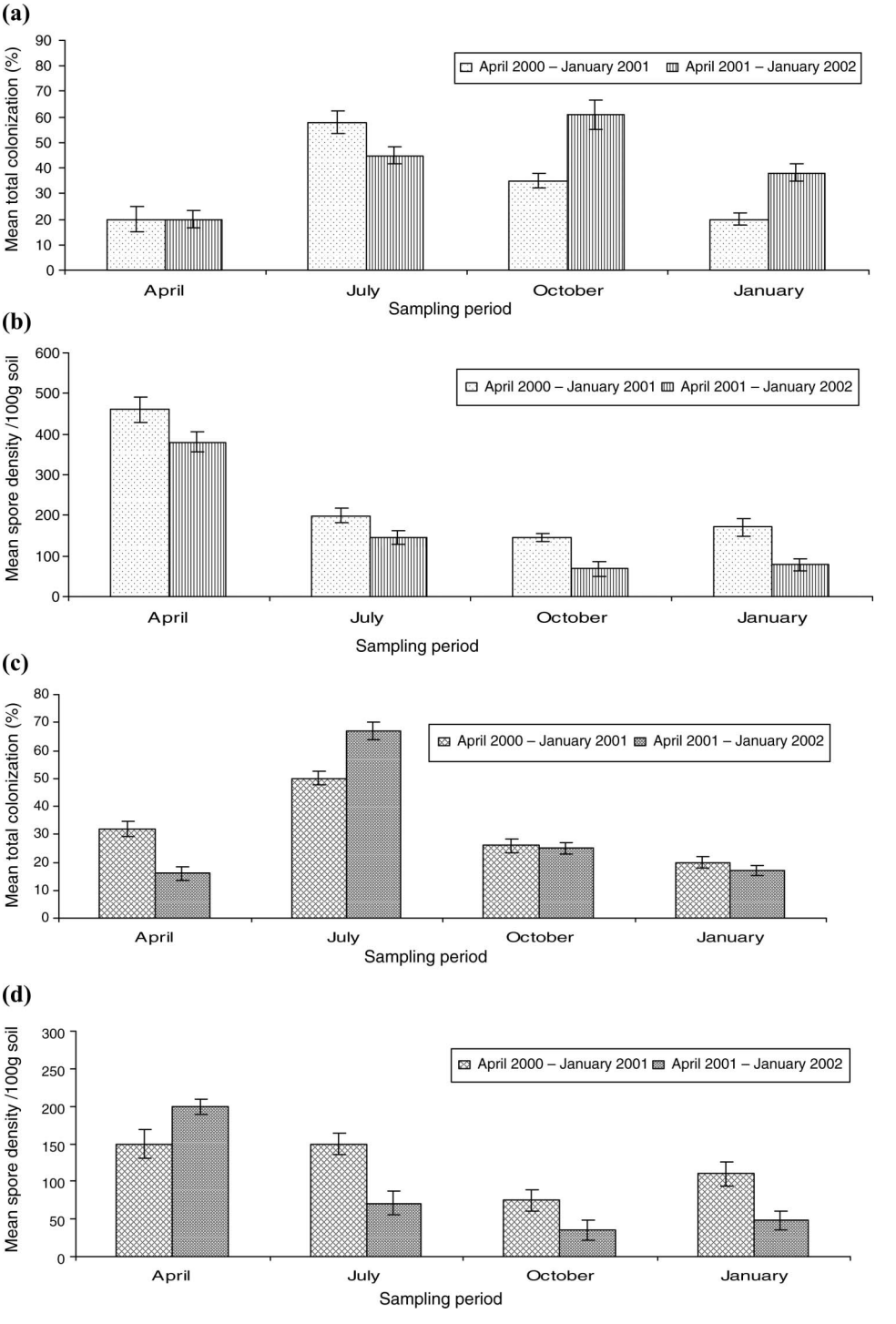

Figure 5. Seasonal variation in mean total colonization and mean spore density of arbuscular mycorrhizal in (a, b) Valpoi and (c, d) Old Goa. Error bar indicate ± SE.

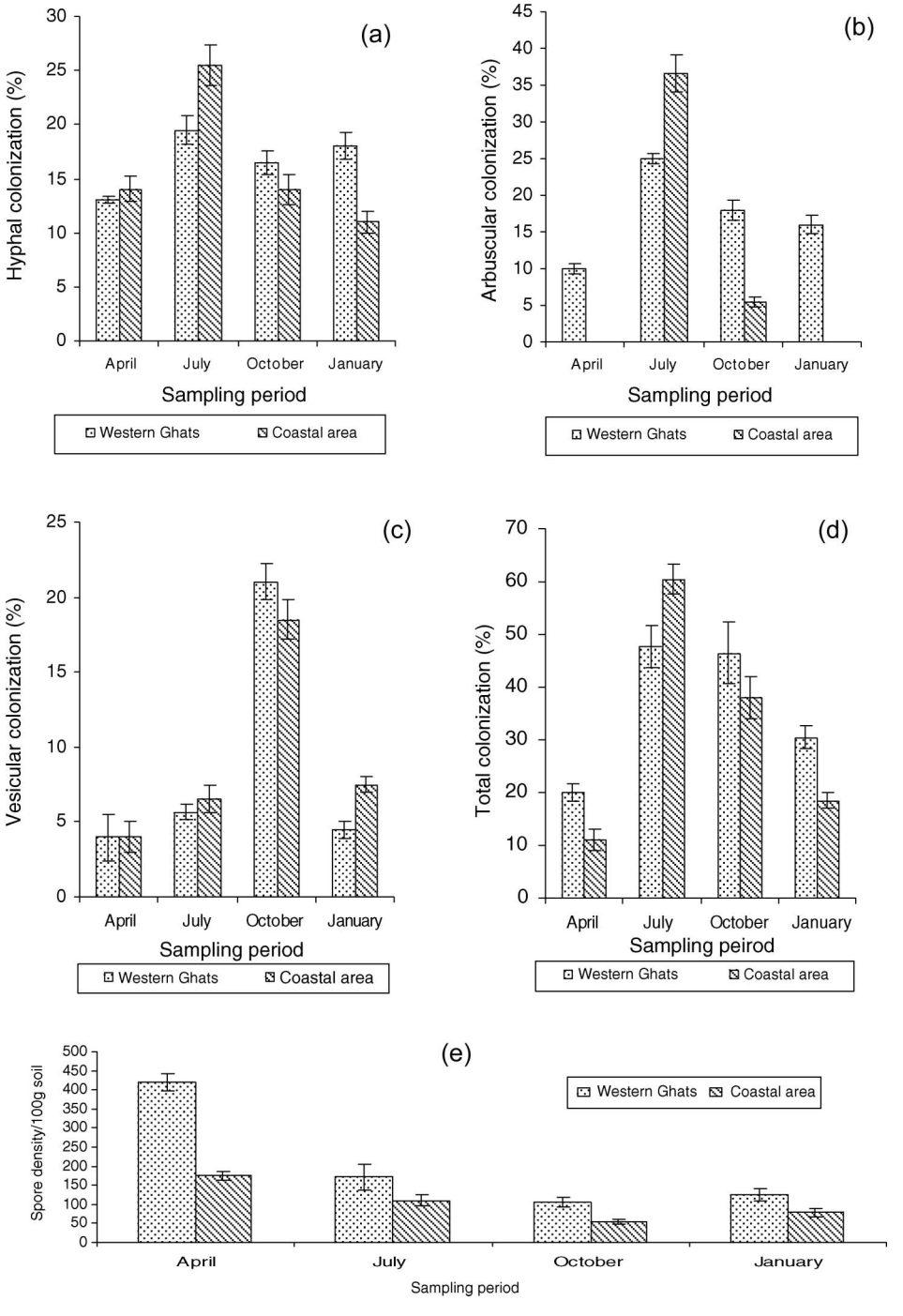

Figure 6. Seasonal variation in average hyphal, arbuscular, vesicular, total colonization (a, b, c, d) and spore density (e) of arbuscular mycorrhizal fungi in Western Ghat and the coastal area. Error bar indicate $\pm$ SE.

(Table 3). Analysis of variance also revealed AM fungal colonization varied significantly between sites and periods (Table 3). However site × year interaction was not significant (Table 3). Among the structures of AM fungi, arbuscular colonization and total colonization were positively correlated to each other ($r = 0.891$; $p = 0.01$) at Old Goa, whereas no significant correlations were recorded between AM fungal structures at Valpoi.

Spore density of AM fungi

The AM fungal spore density exhibited identical trends at Valpoi and Old Goa (Figure 5b and 5d). Spore density of AM fungi was always higher at Valpoi than Old Goa. Maximum AM fungal spore density occurred during April and thereafter drastically declined from July to October. The spore density increased from January to April. At Valpoi, the spore density varied from 68–460 spores $(100\ g)^{-1}$ soil (Figure 5b), while at Old Goa, the spore density ranged from 35–200 spores $(100\ g)^{-1}$ soil for two years (Figure 5d). The average spore density during the study period is depicted in Figure 6e. Maximum and minimum spore density occurred during April and October, respectively, in both the sites (Figure 6e).

The spore density of AM fungi varied significantly within sites ($C.D_{sites} = 0.023$; $p = 0.05$) and periods ($C.D_{years} = 0.023$; $p = 0.05$). Multiple ANOVA indicated significant interactive effect of site and period on AM fungal spore density ($C.D_{sites \times years} = 0.033$; $p = 0.05$). In general, spore density was not correlated to the extent of AM colonization. However, at Old Goa, spore density and vesicular colonization recorded significant negative correlation ($r = -0.747$; $p = 0.05$).

Relationship between climatic factors and parameters of AM fungi

Climatic factors and root colonization of AM fungi

At Valpoi, total root colonization and maximum temperature had a significant negative correlation with ($r = -0.896$; $p = 0.01$), whereas, rainfall was significantly and positively correlated to arbuscular colonization ($r = 0.773$; $p = 0.05$) and total root colonization ($r = 0.861$; $p = 0.05$). At Old Goa, arbuscular colonization was negatively correlated with maximum temperature ($r = -0.955$; $p = 0.01$). Whereas, total root colonization of AM fungi recorded a significant negative correlation with maximum temperature ($r = -0.804$; $p = 0.05$) and highly positive correlation with

Table 3. Multiple ANOVA test for root colonization of AM fungi.

Parameters	CD values
Sites	0.994*
Year of sampling	0.994*
Root colonization of AM fungi	1.217*
Sites × year of sampling	1.405
Site × root colonization	1.721*
Year × root colonization	1.721*
Site × year of sampling × root colonization	2.434

*F-test significant at 0.05 level of probability.

rainfall ($r = 0.834$; $p = 0.01$). The regression model depicting the relationship between AM parameters and climatic factors indicated that all the four climatic factors – maximum temperature, minimum temperature, rainfall and relative humidity – influenced total root colonization of AM at the two sites (Table 4).

Climatic factors and spore density of AM fungi

Although the spore density of AM fungi indicated no relation with individual climatic factors, multiple regression analysis revealed combined influence of climatic factors on AM fungi at both the study sites (Table 4).

Relationship between edaphic factors and parameters of AM fungi

Edaphic factors and root colonization of AM fungi

At Valpoi, arbuscular colonization had a significant positive correlation with electrical conductivity ($r = 0.891$; $p = 0.01$) and soil N ($r = 0.746$; $p = 0.05$). Furthermore, as total root colonization of AM fungi recorded significant positive correlation with electrical conductivity ($r = 0.746$; $p = 0.01$), it had a significant negative correlation with soil N ($r = -0.810$; $p = 0.05$). At Old Goa, hyphal colonization had significant positive correlation with pH ($r = 0.871$; $p = 0.01$), while arbuscular ($r = -0.832$; $p = 0.05$) and total colonization ($r = -0.854$) had a significant ($p = 0.05$) negative correlation with total N. Multiple regression analysis revealed the lack of combined influence of edaphic factors on AM fungal colonization at both the sites (Table 5).

Edaphic factors and spore density of AM fungi

As spore density of AM fungi and organic carbon at Valpoi were negatively correlated to each other ($r = -0.793$; $p = 0.05$) no significant correlation exhibited between spore density of AM fungi and edaphic factors at Old Goa. Multiple

Table 4. Relationships between arbuscular mycorrhizal fungi (Y) associated with *Carica papaya* L. and climatic factors (X).

Arbuscular mycorrhizal fungi	Equation†	Regression coefficient (R^2)
Mean total root colonization %		
Valpoi (Western Ghats)	$Y = 68.00 - 1.82\ T_{Max} + 0.32\ T_{Min} + 0.259\ R_a - 0.15\ R_h$	0. 877*
Old Goa (coastal area)	$Y = 41.80 - 0.76\ T_{Max} - 0.28\ T_{Min} + 1.97\ R_a - 9\ R_h$	0.804*
Mean spore density $(100\ g)^{-1}$ soil		
Valpoi (Western Ghats)	$Y = -540.61 + 19.90\ T_{Max} + 57.19\ T_{Min} + 0.2\ R_a - 16.34\ R_h$	0.916*
Old Goa (coastal area)	$Y = -578.72 + 20.54\ T_{Max} + 17.\ 28\ T_{Min} + 0.14\ R_a - 5.21\ R_h$	0.684

*Regression significant at 0.05 level of probability; †T_{Max} = maximum temperature; T_{Min} = minimum temperature; R_a = rainfall; R_h = relative humidity.

Table 5. Relationships between arbuscular mycorrhizal fungi (Y) associated with *Carica papaya* L. and edaphic factors (X).

Arbuscular mycorrhizal fungi	Equation†	Regression coefficient (R^2)
Mean total root colonization (%)		
Valpoi (Western Ghats)	Y = 22.19 − 14.60 N	0.656*
Old Goa (coastal area)	Y = 10.92 − 6.67 N	0.730*
Mean spore density $(100\ g)^{-1}$ soil		
Valpoi (Western Ghats)	Y = 41.04 − 132.84 OC − 3.08 Mn + 496 N	0.968*
Old Goa (coastal area)	–	–

*Regression significant at 0.05 level of probability; †N = total nitrogen (%), Mn, manganese (ppm), OC, organic carbon (%)

regression analysis revealed that at Valpoi, organic carbon along with manganese influenced mean spore density of AM fungi. It is evident from the value of regression coefficient that the fit of the equation for Valpoi is most appropriate. However, at site Old Goa, none of the edaphic factors contributed significantly to mean spore density of AM fungi (Table 5).

Distribution AM fungi

A total of 33 AM fungal spore morphotypes belonging to *Acaulospora*, *Cetraspora*, *Dentiscutata*, *Gigaspora*, (Figure 1 o, p) and *Glomus* and *Racocetra* were recovered from the rhizosphere of papaya. In the present study, AM fungi belonging to genus *Glomus* (18 species) were the most representative types followed by *Acaulospora* (8 species), *Racocetra* (3 species), *Gigaspora* (2 species), *Cetraspora* (1 species) and *Dentiscutata* (1 species). Five *Glomus* species that were under genus *Sclerocystis* – *Glomus clavisporum*, *G. coremioides*, *G. rubiforme*, *G. sinuosum and G. taiwanensis* – failed to appear in the pot cultures.

Maximum species of AM fungi were recovered from Valpoi (27) (Table 6) as compared to Old Goa (22) (Table 7). Fifteen species of AM fungi – *Acaulospora mellea, A. nicolsonii*, *A. scrobiculata*, *Gigaspora margarita, Glomus claroideum*, *G. constrictum*, *G. coremioides, G. fasciculatum*, *G. geosporum, G. macrocarpum, G. rubiforme*, *G. sinuosum, G. taiwanensis, Racocetra gregaria* and *Dentiscutata reticulata* – were found common to both the study sites.

Western Ghats

At Valpoi, *Acaulospora laevis*, *A. myriocarpa*, *Glomus constrictum* and *G. invermaium* occurred during the first year while, *A. mellea, A. nicolsonii, Gigaspora margarita*, *Glomus monosporum* and *G. rubiforme* were recorded only during the second year (Table 6). The frequency of occurrence of AM fungi belonging both to *Acaulospora* and *Glomus* ranged from 12.5–62.5% each, while the frequency of occurrence for *Gigaspora* species was 12.5% and ranged from 12.5–37% for *Racocetra* and 75% for *Dentiscutata*. At Valpoi, *Dentiscutata reticulata* (75%) was most the frequent species followed by *A. scrobiculata* (62.5%) and *G. claroideum* (62.5%). Species like, *A. nicolsonii*, *Gigaspora margarita*, *G. constrictum*, *G. monosporum* and *Racocetra corolloidea* were infrequent (Table 6). Species richness of AM fungi at Valpoi varied

Table 6. Distribution of arbuscular mycorrhizal fungi at site Valpoi (Western Ghats).

Arbuscular mycorrhizal fungi	First year of sampling				Second year of sampling				Frequency of occurrence (%)
	Apr 2000	Jul 2000	Oct 2000	Jan 2001	Apr 2001	Jul 2001	Oct 2001	Jan 2002	
Acaulospora foveata Trappe and Janos	+	−	−	+	+	−	−	+	50.00
Acaulospora laevis Gerd. and Trappe	+	−	+	−	+	−	−	−	37.50
Acaulospora mellea Spain and Schenck	−	−	−	−	−	−	+	+	25.00
Acaulospora myriocarpa Spain, Sieverding and Schenck	−	−	+	+	+	−	−	−	37.50
Acaulospora nicolsonii Walker, Reed and Sanders	−	−	−	−	+	−	−	−	12.50
Acaulospora scrobiculata Trappe	+	−	−	+	+	−	+	+	62.50
Gigaspora margarita Becker and Hall	−	−	−	−	−	−	+	−	12.50
Glomus clarum Nicolson and Schenck	+	−	−	+	+	−	−	+	50.00
Glomus claroideum (Smith and Schenck) Vestberg and Walker	−	−	−	+	+	+	+	+	62.50
Glomus clavisporum (Trappe) Almeida and Schenck	+	+	−	−	+	+	−	−	50.00
Glomus constrictum Trappe	−	+	−	−	−	−	−	−	12.50
Glomus coremioides (Berk. and Broome) Redecker and Morton	+	−	+	−	+	−	−	−	37.50
Glomus fasciculatum (Thaxter) Gerd. and Trappe emend. Walker and Koske	−	+	−	−	−	−	−	−	12.50
Glomus formosanum Wu and Chen	−	−	+	+	−	−	−	+	37.50
Glomus geosporum (Nicol. and Gerd.) Walker	−	−	+	−	−	−	−	+	25.00
Glomus glomerulatum Sieverding	+	−	−	+	+	−	−	+	50.00
Glomus invermaium Hall	−	−	−	−	+	−	−	−	12.50
Glomus macrocarpum Tulasne and Tulasne	−	−	−	+	+	−	−	+	37.50
Glomus monosporum Gerd. and Trappe	−	−	−	−	−	+	−	−	12.50
Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe	+	−	−	−	+	−	−	−	25.00
Glomus multicaule Gerd. and Bakshi	+	−	−	−	−	+	−	−	25.00
Glomus rubiforme (Gerd. and Trappe) Almeida and Schenck	−	−	−	−	+	−	−	+	25.00
Glomus sinuosum (Gerd. and Bakshi) Almeida and Schenck	+	−	+	−	+	−	−	−	37.50
Glomus taiwanensis (Wu and Chen) Almeida and Schenck	+	+	−	+	+	−	−	−	50.00
Racocetra corolloidea (Trappe, Gerd. and Ho) Oehl, De Souza and Sieverding	−	−	−	−	−	−	+	−	12.50
Racocetra gregaria (Schenck and Nicol.) Oehl, De Souza and Sieverding	−	−	+	−	−	+	+	−	37.50
Dentiscutata reticulata (Koske, Miller and Walker) Sieverding, De Souza and Oehl	+	+	−	+	+	−	+	+	75.00
Species richness of AM fungi	12	5	7	9	17	5	7	11	–

+ Presence of arbuscular mycorrhizal fungi; − absence of arbuscular mycorrhizal fungi.

Table 7. Distribution of arbuscular mycorrhizal fungi at site Old Goa (coastal area).

Arbuscular mycorrhizal fungi	First year of sampling				Second year of sampling				Frequency of occurrence (%)
	Apr 2000	Jul 2000	Oct 2000	Jan 2001	Apr 2001	Jul 2001	Oct 2001	Jan 2002	
Acaulospora delicata Walker, Pfeiffer and Bloss	−	−	−	−	+	−	+	+	37.50
Acaulospora mellea Spain and Schenck	−	−	−	−	+	−	+	−	25.00
Acaulospora nicolsonii Walker, Reed and Sanders	−	−	−	−	−	−	−	+	12.50
Acaulospora scrobiculata Trappe	+	−	−	+	+	−	+	+	62.50
Acaulospora spinosa Walker and Trappe	+	+	−	+	+	+	+	+	87.50
Gigaspora decipiens Hall and Abbott	−	−	+	−	−	−	−	−	12.50
Gigaspora margarita Becker and Hall	+	−	+	+	+	−	+	+	75.00
Glomus claroideum (Smith and Schenck) Vestberg and Walker	−	−	−	+	+	−	+	+	50.00
Glomus constrictum Trappe	−	−	+	−	−	−	−	−	12.50
Glomus coremioides (Berk. and Broome) Redecker and Morton	+	+	−	+	+	+	−	+	75.00
Glomus fasciculatum (Thaxter) Gerd. and Trappe emend. Walker and Koske	+	−	−	−	−	+	−	−	25.00
Glomus geosporum (Nicol. and Gerd.) Walker	+	+	+	−	+	−	−	+	62.50
Glomus macrocarpum Tulasne and Tulasne	+	−	−	−	+	−	−	−	25.00
Glomus microaggregatum Koske, Gemma and Olexia	−	−	+	−	−	−	+	−	25.00
Glomus rubiforme (Gerd. and Trappe) Almeida and Schenck	−	−	+	−	−	−	−	−	12.50
Glomus sinuosum (Gerd. and Bakshi) Almeida and Schenck	−	−	−	+	−	+	−	+	37.50
Glomus taiwanensis (Wu and Chen) Almeida and Schenck	−	+	−	−	−	−	−	−	12.50
Racocetra gregaria (Schenck and Nicol.) Oehl, De Souza and Sieverding	+	−	−	−	+	+	+	+	62.50
Cetraspora pellucida (Nicolson and Schenck) Oehl, De Souza and Sieverding	−	−	−	−	+	−	+	+	37.50
Dentiscutata reticulata (Koske, Miller and Walker) Sieverding, De Souza and Oehl	+	+	−	−	+	+	+	−	62.50
Racocetra verrucosa (Koske and C. Walker) Oehl, De Souza and Sieverding	+	−	−	−	−	−	−	−	12.50
Species richness of AM fungi	10	5	6	6	12	6	10	11	−

+ Presence of arbuscular mycorrhizal fungi; − Absence of arbuscular mycorrhizal fungi.

from 5–17 species/sampling period and exhibited similar trend for both the years (Table 6). It was at a minimum during July and steadily increased thereafter.

Coastal area

At Old Goa, *Gigaspora decipiens, Glomus constrictum*, *G. rubiforme*, *G. taiwanensis* and *Racocetra verrucosa* occurred only during the first year while *Acaulospora delicata*, *A. mellea* and *A. nicolsonii* occurred only during the second year (Table 7). Frequency of occurrence of AM fungi at Old Goa ranged from 12.5–87.5%, 12.5–75%, and 12.5–75% for *Acaulospora*, *Gigaspora*, and *Glomus*, respectively. The frequency of occurrence ranged from 12.5–62% for *Racocetra*, 37% for *Cetraspora* and 62% for *Dentiscutata*. *Acaulospora spinosa* (87.5%) was the most frequent species followed by *Gigaspora margarita* (75%) and *Glomus coremioides* (75%). Other species like *Acaulospora nicolsonii*, *Gigaspora decipiens*, *Glomus constrictum*, *G. rubiforme* and *G. taiwanensis* were infrequent (Table 7). Species richness of AM fungi ranged from 5–12 species/sampling period with maximum AM fungal species occurring in April while minimum species richness occurred during July for both the years (Table 7).

Discussion

In the present study, the soils were sufficient in nutrients due to frequent fertilizer applications. Furthermore, available P, available K, Fe and Mn in Western Ghats and available P and Fe and Mn in the coastal area showed large variation over the sampling periods, which could be due to frequency of fertilizer application. Available phosphorus was the only limiting nutrient, which can be attributed to acidic nature of soil, which decreases the availability of the phosphorus (Tandon 1994). Further, the study reported that the temperature and rainfall influenced various edaphic factors at the study sites.

The study recorded spatio-temporal variations in root colonization and spore density of AM fungi associated with papaya. Similar variations were reported by Sylvia (1986) in *Unicola paniculata* L. from fore dunes of Florida. Further, Merryweather and Fitter (1998) reported spatio-temporal variations in *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. In the recent past, Carvalho et al. (2001) also reported spatial and temporal variation of AM fungi in salt marsh plants. Similarly, Escudero and Mendoza (2005) studied seasonal variation in population attributes of AM fungi over two years in four sites of temperate grasslands of the Argentinean Flooding Pampas. Here they reported that temporal variations in spore density, spore type, AM root colonization, floristic composition and soil chemical characteristics occurred in each site and were different among sites.

It is known that AM fungal colonization is influenced by soil moisture (Lugo et al. 2003). In the present study, samplings were carried out in April (summer, dry season), July (rains, wet season), October (winter, wet season) and January (spring, dry season). Thus in wet seasons July and October, the average amount of arbuscular colonization and vesicular colonization was high, respectively. This is similar to the findings of Lugo et al. (2003) who studied root colonization patterns in grassland ecosystem. Here, summer is the wet season, while autumn and winter are dry, with occasional rain in spring. Root colonization, number of arbuscules, and vesicles reached their maximum values in summer and these values might be due to

the high metabolic activity of plants and soil moisture. The peaks of colonization observed during spring could be due to the sporadic rains that can activate fungal colonization.

In the present study, papaya plants in Westerns Ghats as well as the coastal areas recorded extramatrical hyphae with attached spores, numerous entry points, ramification of intraradical hyphae and abundant arbuscules in the month of July (monsoons). Similarly in grassland ecosystem, Lugo et al. (2003) reported abundant arbuscules, coils, vesicles and entry points in summer, the season with high moisture, temperature as well as solar radiation (wet season). Further thick walls of intercellular hyphae with projections and H connections, as well as intercellular vesicles determining the *Arum*-type, were abundant in wet seasons (Lugo et al. 2003). Thus, the present study recorded marked changes in arbuscular and vesicular colonization of AM fungi and this seasonality in the morphology of structures of AM fungi is likely to influence host nutrition. Abbott and Robson (1991) also reported increased vesicle formation at the end of growing season. Furthermore, the study recorded that the peak periods of arbuscular colonization and vesicular colonization occurring in July and October, respectively, followed a cyclic pattern (Khade and Rodrigues 2008).

Among AM fungal structures, arbuscular colonization and total colonization (consisting of hyphae, hyphal coils, arbuscules and vesicular colonization) were positively correlated in the coastal area and not in Western Ghats. Similarly, Lugo et al. (2003) reported correlations between stages of root colonization of AM fungi in grassland ecosystem. The positive correlation among arbuscules, coils, entry points and vesicles could be related to colonization by several fungi. They further reported that correlation between entry points-vesicles and entry points-arbuscules suggests Glomaceae and Acaulosporaceae families of AM fungi, which form these root colonization structures, while the entry points-coils relationship suggests Gigasporaceae because the vesicles are absent in host roots. The correlation between vesicles-arbuscules and vesicles-coils might be an expression of the symbionts' physiological condition. In fact, the presence of arbuscules and coils, which are considered the exchange structures between symbionts, along with the presence of vesicles, which are storage structures, might be due to the intense exchange activity between the symbionts. The positive correlation between coils-arbuscules could explain multiple colonization because arbuscules are the common structure among all AM fungi and coils are more frequently formed by *Gigasporaceae*.

In the present study, the spore densities of AM fungi were strongly influenced by seasons. Maximum spore density was recorded in summer (April) (Khade and Rodrigues 2008) and at a minimum during monsoons (July) for both years. Similarly, spore populations of AM fungi in agricultural crops in Ontario were reported to increase in late summer and decline in autumn (Sutton and Barron 1972). Nemec et al. (1981) reported higher spore numbers of AM fungi from November to May and low spore numbers from June to September in *Citrus* from California. Further, Escudero and Mendoza (2005) reported in their study on spatio-temporal variations in temperate grassland that the spore density was highest in summer (dry season) and lowest in winter (wet season) with intermediate values in autumn and spring.

In the present study, no significant correlation was observed between mean spore density and total root colonization. Similarly, Escudero and Mendoza (2005) in their study on spatio-temporal variations in temperate grassland reported that spore

density and AM root colonization when measured at any one time were poorly related to each other. Possible reasons for this could be: (1) Spore numbers may poorly reflect the colonization potential of the soil (Hayman and Stovold 1979; Porter 1979; Miller et al. 1985) and they are not always related to the rate and the extent of mycorrhizal formation (Abbott and Robson 1982); and (2) Poor correlation between spore density and root colonization could be because sporulation of AM fungi is dependent on a wide range of environmental factors (Muthukumar et al. 2001). However, the study recorded a significant negative relationship between spore density and vesicular colonization. During early stages of root and plant development when the nutrient demand is high, arbuscules tends to be abundant. However, sporulation tends to set in during root inactivity or senescence. Therefore, these two variables tend to be negatively related as observed by Khalil et al. (1992).

Differences in host plants and soil fertility stimulate differential sporulation by AM fungal species in the field. However, differences in climatic and edaphic factors and the host plants in the distribution of AM fungi have been so great that it is difficult to explain, which factors determine the presence and the abundance of particular AM fungi. In the present study, among the climatic factors, rainfall recorded positive correlation with arbuscular colonization (Khade and Rodrigues 2008) and total root colonization. This is in accordance with the findings of Michelini et al. (1993), who reported positive correlations of annual rainfall with hyphal colonization and total root colonization in *Citrus* across four islands of the Eastern Caribbean. Similarly, Braunberger et al. (1994) also reported that rainfall can strongly influence AM colonization and the structures associated with it. Newman et al. (1986) found a reduction of percentage and intensity of colonization in the roots of three species of Savanna grass during the dry season, which they attributed to the extended drought. A similar reduction in both percentage and intensity of root colonization was reported in the roots of sugarcane in dry season in Barbados (Chinnery et al. 1987). The study recorded that maximum temperature was negatively correlated to arbuscular colonization and total root colonization. This influence of atmospheric temperature on AM fungi may be due to its effect on soil temperature, moisture and host growth (Haugen and Smith 1992; Saito and Kato 1994).

Among the edaphic factors, electrical conductivity and soil pH had a positive correlation with arbuscular and hyphal colonization of AM fungi. These observations are contradictory to the findings of Khalil et al. (1992) that reported the absence of correlation between AM fungal structures and soil pH. However, Russel (1973) indicated that soil pH may influence the abundance of AM fungal structures possibly through its role in altering the availability of nutrients in soil solution. High levels of total nitrogen negatively influenced total colonization, which contradicts the studies of Hepper (1983) and Azizh and Habte (1989), who reported the stimulation of root colonization of AM fungi by nitrogen. In the present study, spore density of AM fungi exhibited negative relationship with organic carbon, which is in agreement with the findings of Nemec et al. (1981) in *Citrus*. High levels of Manganese recorded in the present study exhibited a negative relationship with spore density of AM fungi (Khade and Rodrigues 2008). Similarly, Sreenivasa and Bagyaraj (1988) reported enhanced root colonization and sporulation of AM fungi at sub optimal levels micronutrients, such as Zn, Fe, Cu and Mn.

Regression analysis revealed that root colonization was singly influenced by total nitrogen. Similarly, Michelini et al. (1993) also reported that only magnesium out of

the 16-site characteristics exhibited significant relationship with root colonization of AM in *Citrus* sampled from four Caribbean islands after carrying out single factor linear regression analysis. In the present study, spore density was influenced by organic carbon, manganese, electrical conductivity (EC) and total nitrogen. Similarly Nemec et al. (1981), while studying the ecology of AM fungi in *Citrus*, reported that spore numbers were influenced by soil P and other parameters like pH, organic carbon, sodium concentration that are associated with availability of phosphorus. Janardhanam et al. (1994) reported significant positive correlation between EC and spore numbers.

The present study reported the dominance of genus *Glomus* at the two study sites, which is in agreement with the findings of Nemec et al. (1981) who reported that this genus is commonly encountered in cultivated sites. However, sporocarpic species of *Glomus* that were placed earlier under genus *Sclerocystis* failed to multiply in trap cultures, which supports the findings of Muthukumar and Udaiyan (2002b). The distribution of AM fungi and their frequency of occurrence varied in the two geographic localities. The study also reported that few species of AM fungi were restricted to a particular site while few species were commonly encountered in both the sites. This is in agreement with Nemec et al. (1981) who reported common occurrence of certain AM fungal species in *Citrus* from California and Florida and the specificity of certain species to one area.

In the present study, different species of AM fungi were recovered during different times of the year and their frequency of occurrence also varied (Khade and Rodrigues 2008). Arbuscular mycorrhizal fungal development within the plant roots and the subsequent sporulation may be dependant on climatic and edaphic factors (Fitter 1985; Sanders 1990). However, the direct influence of these factors on mycorrhizal fungi is difficult to explain due to their intimate association with the host in nature. Very little is known about within-site or within-root competition among the AM fungi (Miller et al. 1985). However, the seasonal variations in spore production between co-existing endophytes and abundant spore production by one species of AM fungi were correlated with lower levels of spore production by others as a results of antagonism between the species has been reported (Gemma et al. 1989).

In the present study, few AM fungal species were most frequent as compared to the rest of AM fungi. Thus, the AM fungal species profile of the present survey closely resemble those found in heavily tilled and fertilized agricultural soils (Hamel et al. 1994) where two or three major fungal species dominate by their frequency and are accompanied by a cohort of minor ones that occur sporadically.

In the present study, it is argued that maximum species richness during April could be due to the increased number of spores whereas minimum species richness recorded during monsoons can be attributed to the low number of spores in the soil. In general, throughout the study period, Western Ghats (inter-cropped papaya plants), exhibited maximum species richness as compared to coastal areas (papaya were planted along the bunds/fence of flowers/vegetable plots). This may perhaps be because of increased root length density of plants in inter-cropping system that increases the rate of spread of AM fungi (Van Noordwijk et al. 1996, 1998). As crops differ in their ability to form associations with AM fungi, it is argued that in intercropping systems and plantations with high planting density involving various crops can be expected to increase the population as well as species richness of AM fungi.

Conclusions

High fungal diversity, relatively high percentage of root colonization and spore densities of AM fungi recorded in the present study suggest that arbuscular mycorrhizae is a well-established phenomenon in papaya from the agro-based ecosystem of Goa that exhibits variations depending on the edaphic factors and seasonal patterns in weather change. Plantation status and management practices also contribute to variations of AM fungi in the agro-based ecosystems. Furthermore, the existence of a seasonal pattern is indicated by a similarity in spore density and mycorrhizal colonization for a period of two years. In conclusion, temporal variations in spore density, spore type and AM root colonization occurred in each site and were different among sites and that contrasting seasonal and spatial niches may facilitate the maintenance of a diverse community of AM fungi in two geographic localities of Goa, India.

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