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ccastro@uady.mx
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ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH *Carica papaya* L. IN AGRO-BASED ECOSYSTEM OF GOA, INDIA

[ECOLOGÍA DE MICRORRIZA ARBUSCULAR ASOCIADO CON *Carica papaya* L. EN AGROECOSYSTEMAS DE GOA, INDIA]

Khade Sharda W.* and B. F. Rodrigues

*Department of Botany, Goa University, Taleigao Plateau, Goa (403 206) India. Current address: Darshan Apts, II[nd] Floor, Vidhyanagar Colony, Miramar, Carenzalem Post. Panaji, Goa 403 001, India. Email- sharda_khade @yahoo.com *Corresponding author.*

SUMMARY

In the present investigation, arbuscular mycorrhizal (AM) association in mono-culture plantation of *Carica papaya* L. was studied. Similar root colonization and spore density patterns of AM fungi were recorded for two consecutive years. The mean total colonization was lowest in April and highest in July, whereas spore density was minimum in October and maximum in April. Pearson's correlation revealed that root colonization and spore density were influenced by climatic as well as edaphic factors. Species richness of AM fungi varied from 5 to 9 species per sampling period. The present study recorded a total of 18 AM fungal species belonging to four genera: *Acaulospora, Glomus, Gigaspora,* and *Scutellospora*. *Glomus claroideum* was the most frequently occurring species and was recovered throughout the study period. Arbuscular mycorrhiza are well established in *Carica papaya*,exhibiting variations depending on edaphic factors and seasonal patterns in the weather.

Key words: Arbuscular mycorrhizal fungi, *Acaulospora*, climatic factors, edaphic factors, *Glomus*, *Gigaspora*, root colonization, spore density, *Scutellospora.*

RESUMEN

En el presente trabajo se estudio la asociación de AM en monocultivo de *Carica papaya* por un período de dos años. Se encontró un patrón similar para ambos años en cuanto a colonización de las raíces y densidad de esporas. La colonización fue menor en abril y mayor en julio, mientras que la densidad de esporas fue menor en octubre y máxima en abril. Se encontró asociación entre la colonización de la raíz y densidad de esporas con factores edáficos y climáticos. Se encontró en cinco y nueve especies de AM en cada período de muestreo. Se registraron en total 18 AM pertenecientes a cuatro generos: *Acaulospora, Glomus, Gigaspora,* y *Scutellospora*. *Glomus claroideum* fue la especie encontrada con mayor frecuencia y fue recuperada durante todo el período de muestreo. Se concluyó que AM es un fenómeno bien establecido para *Carica papaya* y exhibe variaciones asociadas con factores edáficos y patrones estacionales del clima.

Palabras clave: Micorrizas arbusculares, *Acaulospora*, factores climáticos, factores edáficos, *Glomus*, *Gigaspora*, colonización de raíz, densidad de esporas, *Scutellospora.*

INTRODUCTION

The State of Goa lies Western Ghats which extend from the Tapti river (Gujarat) in the North, down to the peninsular tip of South India. The land elevation ranges from the sea level to 1022m. Out of the total cropped area, 58.18% (99,692 ha) is under horticultural crop cultivation (Khade, 2003). The most important horticultural crop is cashew, followed by coconut, mango and banana. Papaya (*Carica papaya* L.), is also cultivated, being one of the most popular tropical fruits.

Carica papaya is valued for its rich nutrient content and therapeutic properties. India is the second largest producer of papaya in the world after Brazil, with production up to 1.5 million ton. However in Goa, papaya cultivation is mostly confined to kitchen garden and along the bunds due to constraints in productivity resulting from fungal and viral diseases. Therefore at present, most of the demand for papaya is met by importing the fruit from neighbouring states.

The wide spread symbiotic arbuscular mycorrhizal association is gaining importance due to the fact that they help in plant productivity. In general, mycorrhiza represents a mutualistic partnership involving carbon and nutrient exchange between symbionts (Ruotsalainen *et al.,* 2002). Investment in the nutrient uptake via mycorrhizal symbiosis implies a

considerable carbon cost for the host plant (Douds *et al.,* 1988; Jones *et al.,* 1991; Eissenstat *et al.,*1993; Smith and Read, 1997) which, however, leads to improved nutrient acquisition, largely due to increased absorption surface provided by the fungal hyphae (Koide, 1991). Tuomi *et al.,* (2001) proposed that plants might maximize their growth by optimizing mycorrhizal colonization in their roots. Thus, optimal mycorrhizal colonization may change if the availability of soil nutrients and photosynthetic energy fluctuates (Fitter, 1991; Tuomi *et al.,* (2001).

Arbuscular mycorrhizal (AM) symbiosis is characterized by short life cycles of arbuscules (Alexander *et al.,* 1989), rapid colonization of new roots and appearance of vesicles in the oldest colonization units (Smith and Read, 1997). Seasonal shifts in AM colonization have been found which could indicate that the benefit of mycorrhizal symbiosis for the plant changes during the season (Fitter, 1986; Fitter, 1991; Lapointe and Molard, 1997). Earlier workers have conducted studies on seasonal variation of AM fungi in fruit crops like *Citrus* (Nemec *et al.,* 1981) strawberry and raspberry (Mason, 1964). The present study was undertaken as an initial step in understanding the ecology of AM fungi associated with monoculture plantation of *Carica papaya* L. in tropical agro-based ecosystem of Goa, India.

MATERIAL AND METHODS

Study site.

One-year-old, fruit bearing, *Carica papaya* L. plants were sampled from Kodar (N 15^0 29' 32.1" and 73^0 55' 20.00"), Goa. The soil was well-drained, strong brown, gravelly clay loam. The study was conducted for two years (April 2000 to January 2002. Cultural practices *viz.,* fertilizer applications, irrigation, mulching and weeding, were carried out during the course of the study. Inorganic fertilizer (19:19:19 N:P:K) was applied in six split doses per year at an interval of two months. Papaya plants received 250g N, 250g P_2O_5 and 50g K_2O per plant per year. Plants were irrigated twice a week round the year except in monsoons. *Mulching* **(Plate 1A)** was carried out prior to monsoons while weeding was carried out throughout the year except during monsoons (July-September).

Weeds were restricted to periphery of the plot as the plants were mulched at the base and did not interfere with sample collection Therefore during sampling, mulches at the base were first cleared and then the samples were collected. As *Carica papaya* L. produces numerous feeder roots mostly near the soil surface, sampling was at the depth of 0-25 cm throughout the study period, where feeder roots and rhizosphere soil surrounding them were collected.

Collection of samples.

Five healthy, fruit bearing plants were randomly selected per sampling period. Rhizosphere soil was collected along with roots. Samples were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed whereas soil samples were stored at 4^oC until analyzed. Sampling procedures were carried out according to Tews and Koske (1986), with a core size of 15 cm in diameter, to avoid non-normal distribution of spores recorded in counts from small core samples (St. John and Koske, 1988).

Samples were collected four times a year at an interval of two months for a period of two years. Three random soil cores were collected from within 60 cm of each plant, and combined to give approximately 1200 g moist soil composite sample after thorough mixing. From this, 100 g air dried soil was employed for extraction of AM fungal spores, 250g was utilized for nutrient analysis and the remaining soil was utilized for setting open pot 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 remaining were utilized for establishing pot cultures.

Establishment of pot cultures.

For establishing pot cultures, 50 g of rhizosphere soil of papaya along with the roots were mixed with equal quantity of sterilized sand and placed in 12.5 cm diameter pots. Seeds of *Eleusine coracana* (L.) Gartner were sterilized with 0.1% $HgCl_2$, washed thoroughly with distilled water, placed over the soil sand mixture and covered with 2 cm of soil. Cuttings of *Coleus* sp. were also used as host plants for baiting the native arbuscular mycorrhizal fungi**.** Pots were watered daily to its field capacity, with distilled water, Hoaglands nutrient (no phosphate) solution was added every 15 days. Five pot cultures were maintained per sampling period. The roots of host species were checked for AM colonization after 45 days. Pots showing successful mycorrhization were maintained for a period of six months, and application of water was reduced at final three weeks to maximize spore production (Menge, 1982). At the end of six months, plants were cut near the base and cultures were air-dried and checked for the presence of spores. Spores isolated from pot cultures by wet sieving and decanting method (Gerdemann and Nicolson, 1963) were used to verify the identification of AM fungi.

Soil sample analysis.

Rhizosphere soil samples were employed for analysis of 10 edaphic factors. Soil pH was measured in 1:2 soil water suspension using pH meter (LI 120 Elico, India). Electrical conductivity was measured at room temperature in 1:5 soil suspension using conductivity meter (CM-180 Elico, India). Standard soil analysis techniques viz., Walkley and Black's rapid titration method (1934), micro-Kjeldahl method (Jackson, 1971) and Bray and Kurtz method (1945) were employed for determination of organic carbon, total nitrogen and available phosphorus respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel, 1952) using flame photometer (Systronic 3292). Available zinc, copper, manganese and iron were quantified by DTPA-$CaCl_2$- TEA method (Lindsay and Norvell, 1978) using Atomic Absorption Spectrophotometer (AAS 4139).

Estimation of root colonization AM fungi.

Roots were cleared in 10% KOH, acidified in 1N HCl and stained in 0.05% trypan blue in lactoglycerol (Phillips and Hayman, 1970). Percent total root colonization and root length colonized by hyphae, arbuscules and vesicles was estimated by grid line intersection method (Mc Gonigle *et al*., 1990). Hundred and fifty intersections were examined per samples under compound microscope at 200X magnifications and root colonization of AM fungi was expressed in percentage.

Quantification of spore density of AM fungi.

Spores and sporocarps of AM fungi were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963) and quantification of spore density of AM fungi was carried out using method described by Gaur and Adholeya (1994). Only turgid healthy looking spores were considered for the study.

Identification of AM fungi.

For identification purpose, spores isolated from field samples and from pot cultures were used. Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in polyvinyl alcohol lactoglycerol (PVLG) (Koske and Tessier, 1983). Spore morphology and wall characteristics were considered for the identification of AM fungi using compound microscope, Leica WILD MP 3 and Nikon E 800. Arbuscular mycorrhizal fungi were identified to species level using bibliographies provided by Almeida and Schenck (1990), Morton and Benny (1990), Schenck and Perez, (1990), Wu (1993), Bentivenga and Morton (1995), Walker and Vestberg (1998), Redecker *et al.,* (2000). 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). Names and epithets of AM fungi were followed according to recommendations of Walker and Trappe (1993). Voucher specimen slides were deposited in Department of Botany, Goa University, India.

Diversity indices and frequency of occurrence.

Species richness is mean number of AM fungal species recovered from particular site per sample collection. Frequency of occurrence of AM fungi was calculated using the following formula (Beena et al., 2000). Frequency (%) = Number of soil samples containing spores of particular species x 100 / Total number of soil samples screened

Statistical analysis

Pearson's correlation test was performed to assess the relationship between edaphic and climatic factors and arbuscular mycorrhizal fungal parameters. Data of spore density was subjected to log transformations while data on root colonization was subjected to arcsine transformations. Statistical analysis was carried out using mstac package.

RESULTS

Relation between climatic and edaphic factors.

Pearson correlation analysis showed that among the edaphic factors, electrical conductivity recorded significant negative correlation with maximum temperature values (r= -0.899; P = 0.01) and positive correlation with rainfall (r = 0.976; P = 0.01). While, organic carbon recorded significant negative and positive correlation with maximum temperature (r = -0.771; P = 0.05) and rainfall (r = 0.844; P= 0.01) respectively. Similarly, zinc also recorded a significant negative and positive correlation with maximum temperature (r= -0.991; P = 0.01) and rainfall (r = 0.926; P= 0.01) respectively. While iron, recorded significant negative correlation with minimum temperature (r = -0.708; P = 0.05)**.**

Root colonization of AM fungi.

All the root samples of *C. papaya* exhibited the presence of arbuscular mycorrhizal colonization during the study. Different morphological characteristics were evident during the study, *viz.*, extraradical hyphae, intraradical hyphae (Plate 1 B), arbuscules (Plate 1 C), vesicles (Plate 1 D),

external/soil borne vesicles (Plate 2 E, F) and intraradical spores (Plate 2 G, H). Approximately similar pattern of root colonization was evident during two years of study period. Maximum mean arbuscular colonization was recorded in July, while maximum mean vesicular colonization was recorded in October (Fig.1b and Fig.1c) for two consecutive periods. The mean hyphal colonization during the study period ranged from 20% to 30% (Fig.1a), whereas the mean arbuscular mycorrhizal colonization ranged from 0% to 60% (Fig.1b). The mean vesicular colonization varied from 0% to 39% (Fig. 1c). The mean total colonization was lowest in April and highest in July (Fig. 2a). Pearson's correlation analysis revealed that only arbuscular and vesicular colonization recorded a significant negative correlation with each other (r= -0.819; *P*= 0.01).

Spore density of AM fungi.

Maximum spore density of arbuscular mycorrhizal fungi was recorded in April and there after it drastically declined from July to October. Spore density exhibited increasing trend from January to April. Mean spore density ranged from 54 spores/100 g soil (October 2001) to 392 spores/100 g soil (April 2000) for a period of two years (Fig. 2). Pearson's correlation analysis revealed significant negative correlation between spore density and hyphal colonization (r = -0.789; *P*=0.05) of arbuscular mycorrhizal fungi.

Relationship between climatic factors and parameters of AM fungi.

Hyphal colonization recorded highly significant negative and positive correlation with maximum temperature and rainfall, respectively. Hyphal colonization also exhibited significant positive correlation with relative humidity (Table 1). While vesicular colonization recorded significant negative correlation with rainfall. However, regression analysis did not reveal the cumulative effect of climatic factors on root colonization of arbuscular mycorrhizal fungi. Further more, Pearson's correlation analysis revealed that, spore density of arbuscular mycorrhizal fungi recorded no significant correlation with climatic factors (Table 1).

Relationship between edaphic factors and parameters of AM fungi.

Arbuscular colonization recorded highly significant positive correlation with electrical conductivity, organic carbon and zinc: whereas, vesicular colonization recorded highly significant negative correlation with organic carbon and positive colonization with manganese. Vesicular colonization also recorded significant negative correlation with electrical conductivity, available potassium and zinc (Table 2). Spore density of arbuscular mycorrhizal fungi exhibited significant negative correlation with manganese (Table 2).

Distribution arbuscular mycorrhizal fungi.

Comparative account of number AM fungal species belonging to different genera encountered during the study is presented in Table 3. Maximum numbers of *Glomus* species were recovered as compared to other genera of AM fungi. The present study recorded a total of 18 AM fungal species belonging to four genera: *Acaulospora* (Plate 2 I, J), *Glomus, Gigaspora,* and *Scutellospora*. Four species were recovered in the first year: *Acaulospora laevis, Gigaspora decipiens, Gigaspora margarita, Glomus clavisporum,* while species like *Acaulospora mellea, Acaulospora nicolsonii, Glomus geosporum, Glomus microcarpum, Glomus taiwanensis and Scutellospora gregaria* were recorded only during the second year of sampling (Table 3). The frequency of occurrence ranged from 12.50% to 75%, 12.50% to 25%, 12.50 to 100% and 12.50% to 62% for *Acaulospora, Gigaspora, Glomus* and *Scutellospora* species, respectively. *Glomus claroideum* was the most frequently occurring species (100%) followed by *Acaulospora scrobiculata* (75%), *Glomus sinuosum* (75%) and *Scutellospora gregaria* (62.5%) (Table 3). Infrequently occurring AM species were *Acaulospora laevis, Acaulospora mellea, Gigaspora margarita* and *Glomus clavisporum* each recording 12.5% frequency. Species richness of arbuscular mycorrhizal fungi ranged from 5 to 8 species/sampling period (Fig.2c).

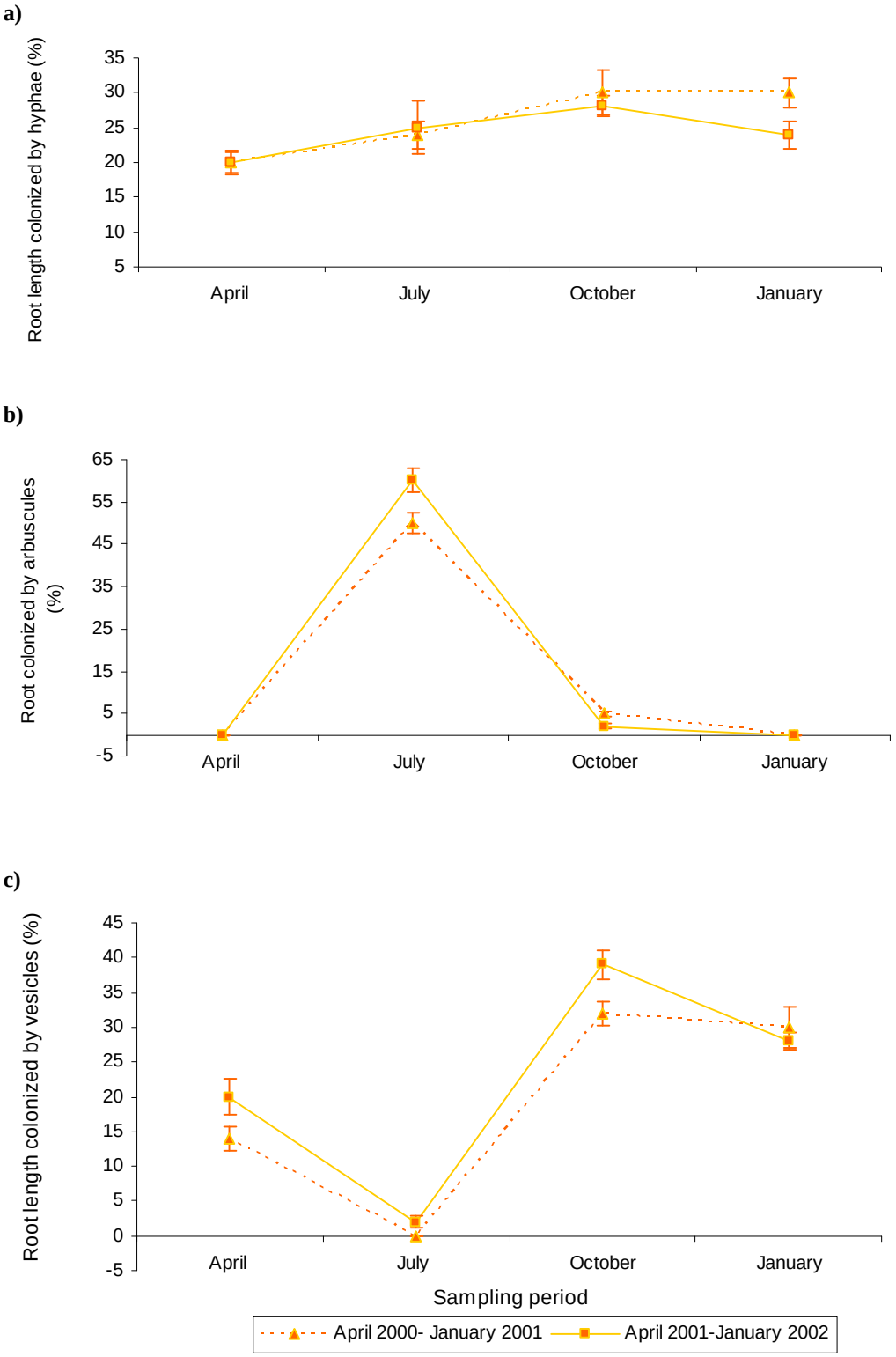
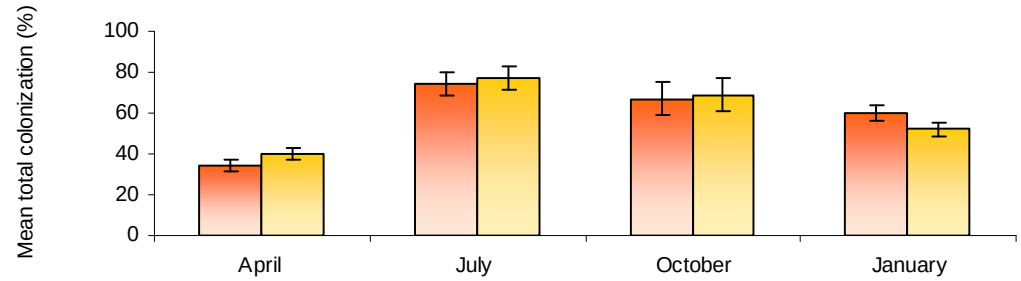
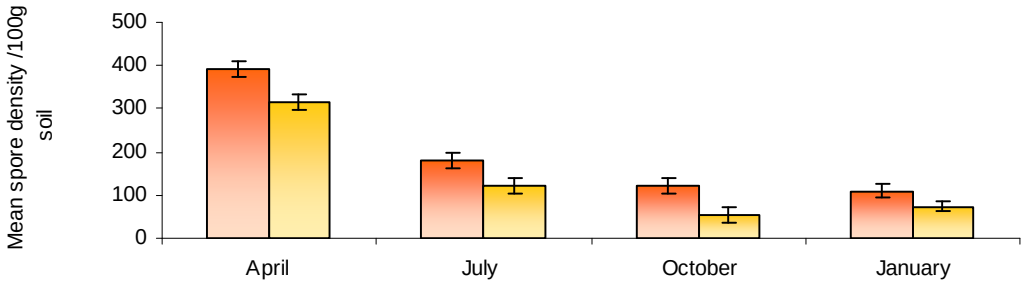


Figure 1: Seasonal variation in *Carica papaya* root length colonized by arbuscular mycorrhizal fungal structures: hyphae, arbuscles andvesicles. Error bar indicate 1 ± SE

a)



b)



c)

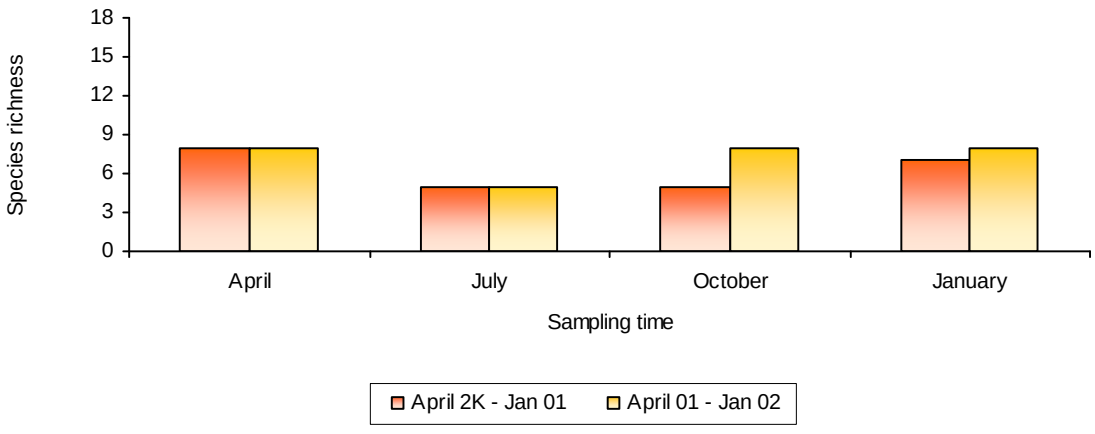
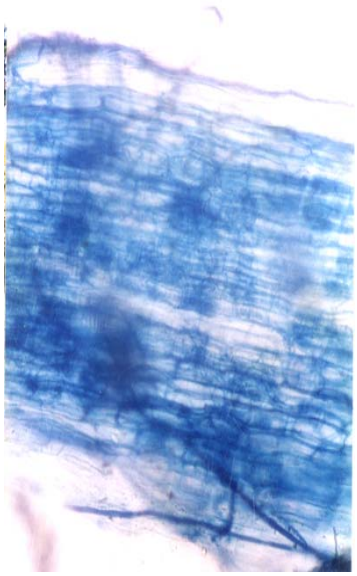
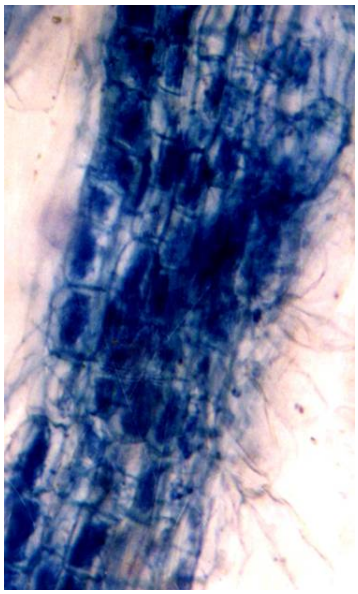


Figure 2: Seasonal variations in a) Total root colonization, b) Spore density and, c) Species richness of arbuscular mycorrhizal fungi in *Carica papaya*. Error bar indicate 1 ± SE

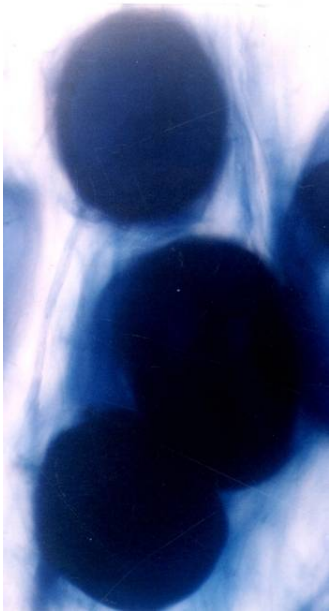
(A)



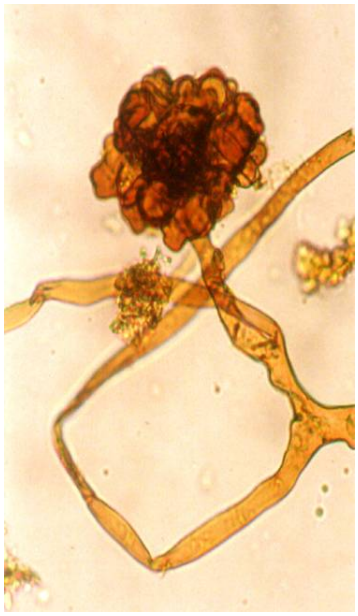
(B)



(C)



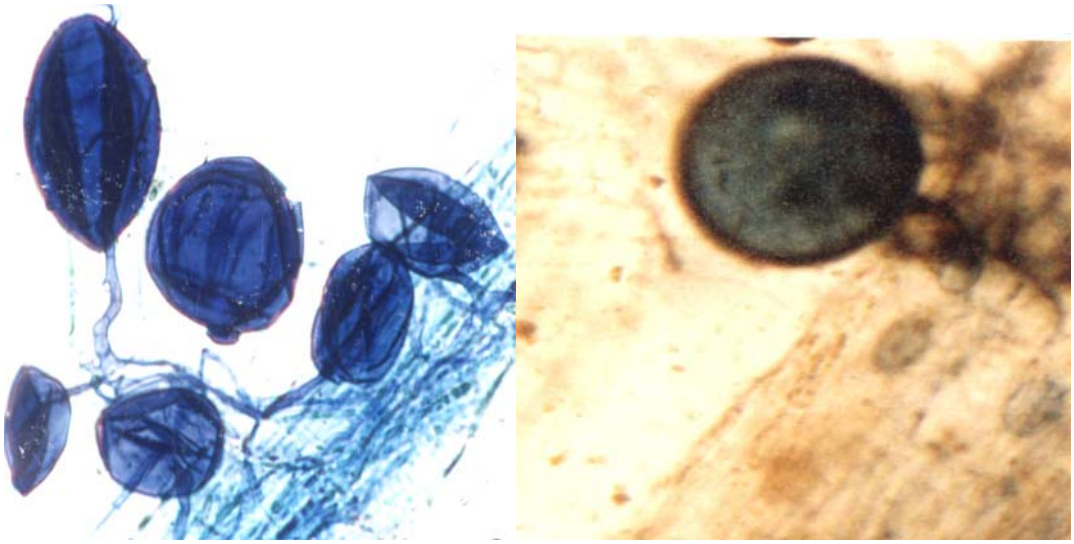
(D)



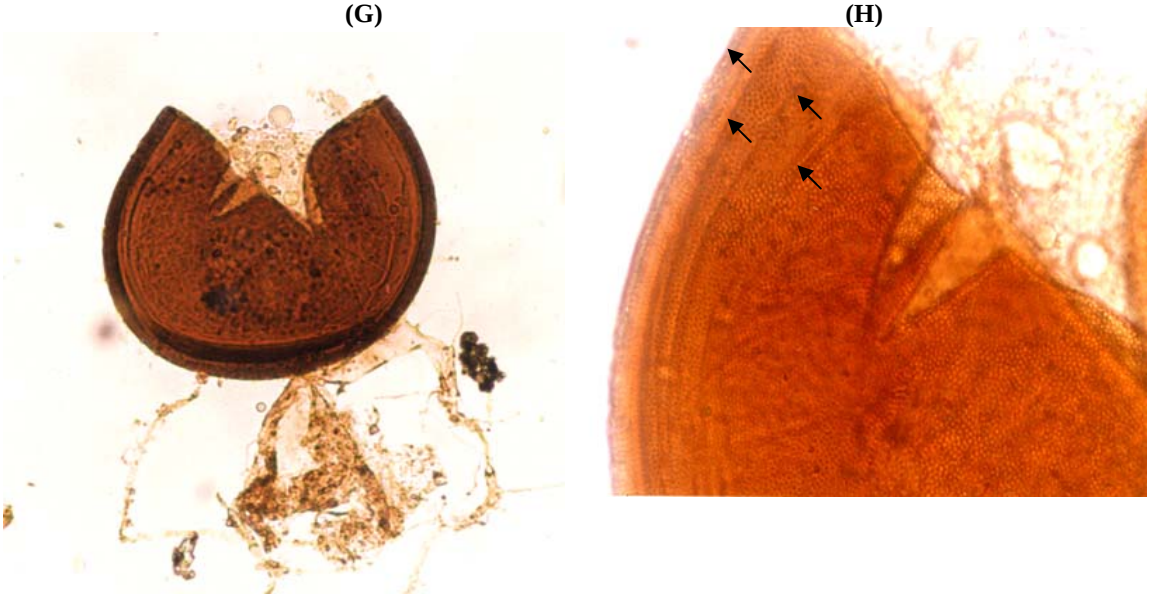
(E)

(F)

Plate 1:A) Habit (mulched at the base during monsoons), B) Hyphal colonization of AM fungi (x100) (*Note the longitudinal ramification of AM hyphae), C) Mature arbuscules inside the host cells (x 100) D) Mature vesicles inside the host cells (x 1000), E) Soil borne vesicle of *Scutellospora* sp. (x150), F) Soil borne vesicle of *Scutellospora* sp. (x150)



(G) **(H)**



(I) **(J)**

Plate 2:

G) Intraradical spores in clusters (x 400), H) Single intraradical spore (x100), I) A portion of spore of *Acaulospora scrobiculata* Trappe attached to degenerated saccule (x 400), J) Crushed spore of *Acaulospora scrobiculata* Trappe (x 1000) (* Note the wall layers and pitted ornamentation).

Table 1. Correlation coefficient (r) between climatic factors and arbuscular mycorrhizal fungi.

Climatic factors	Parameters of arbuscular mycorrhizal fungi				
	Mean root colonization (%)				Spore density /100 g soil
	Hyphal	Arbuscular	Vesicular	Total	
Temperature Max (^{0}c)	-0.936**	-0.175	0.651	0.015	0.268
Min (^{0}C)	0.108	-0.554	-0.321	-0.169	0.688
Rainfall (mm)	0.927**	-0.079	-0.804*	-0.153	-0.093
Relative humidity (%)	0.734*	0.308	-0.359	0.165	-0.416

*Correlation significant at 0.05 level of probability. ** Correlation significant at 0.01 level of probability.

Table 2. Pearson's Correlation coefficient (r) between edaphic factors and arbuscular mycorrhizal fungi.

Edaphic Factors	Parameters of arbuscular mycorrhizal fungi				
	Mean root colonization (%)				Mean spore density/100g Soil
	Hyphal	Arbuscular	Vesicular	Total	
Ph	0.003	-0.645	0.485	-0.124	0.128
E.C (m mhos/cm)	0.020	0.909**	-0.766*	-0.203	-0.199
Organic C (%)	-0.467	0.861**	-0.932**	-0.286	0.342
Total N (%)	0.512	0.263	-0.037	0.170	-0.353
Available P (Kg/ Ha)	0.631	-0.348	0.407	-0.404	-0.358
Available K (Kg/ Ha)	-0.455	0.621	-0.711*	-0.323	0.239
Cu (ppm)	0.460	-0.415	0.456	-0.223	-0.571
Fe (ppm)	0.412	0.483	0.414	-0.254	-0.217
Zn (ppm)	0.073	0.958**	-0.791*	-0.231	-0.197
Mn (ppm)	0.821*	-0.482	0.858**	0.515	-0.724*

*Correlation significant at 0.05 level of probability. ** Correlation significant at 0.01 level of probability.

DISCUSSION

In the present study, sampling was carried out twice in the dry season (January and April) and twice in the wet season (July and October). Seasonal variation was recorded in arbuscular mycorrhizal fungi associated with *C. papaya* and supports the finding of Mason (1964) and Nemec *et al.,* (1981) who reported seasonal variation in fruit crops. The marked changes in arbuscular and vesicular colonization of AM fungi and seasonality in the morphology of structures of AM fungi found here, are likely to influence host nutrition. The arbuscules are responsible for nutrient transfer to the host plant (Cox and Tinker, 1976) and therefore changes in arbuscular colonization over the study period may indicate functional symbiosis. Peak periods of arbuscular colonization and vesicular colonization occurring in July and October, respectively, followed a cyclic pattern. Similarly, Alexander *et al.* (1988) have reported that arbuscule formation follows a cyclic pattern where it ceases at the end of growing season when vesicle formation increases.

Spore densities of arbuscular mycorrhizal fungi were strongly influenced by seasons. Maximum spore density was recorded in summer (April) for both the years, in agreement with Mason (1964), who reported large numbers of spores associated with raspberry during two continuous summers and attributed it to decrease in spore germination of arbuscular mycorrhizal fungi due to absence of young roots. The low spore density recorded in the rhizosphere of *C. papaya* in July for two continuous years suggests that spore germination occurred when plant roots were growing actively and corroborates with the study of Mason (1964) on barley. In the present study, further decrease in spore densities during October corroborates findings of Giovannetti, (1985) who reported that spore densities declined over winter, but spores persisted as they were recovered through out the year. General increase in spore density recorded in the present study from January to April may be related to the cessation of growth of many of the roots and their subsequent death (Mason, 1964).

Table 3. Arbuscular mycorrhizal fungi associated with *Carica papaya* L.

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 laevis Gerd. & Trappe	-	+	-	-	-	-	-	-	12.50
Acaulospora mellea Spain & Schenck	-	-	-	-	-	-	-	+	12.50
Acaulospora nicolsonii Walker, Reed & Sanders	-	-	-	-	+	+	+	-	37.50
Acaulospora scrobiculata Trappe	+	+	-	+	-	+	+	+	75.00
Acaulospora spinosa Walker & Trappe	+	-	-	-	+	-	+	-	37.50
Gigaspora decipiens Hall & Abbott	+	-	-	+	-	-	-	-	25.00
Gigaspora margarita Becker & Hall	+	-	-	-	-	-	-	-	12.50
Glomus claroideum (Smith & Schenck) Vestberg & Walker	+	+	+	+	+	+	+	+	100.00
Glomus clavisporum (Trappe) Almeida & Schenck	-	-	+	-	-	-	-	-	12.50
Glomus coremioides (Berk. & Broome) Redecker & Morton	+	+	+	+	+	+	-	-	75.00
Glomus geosporum (Nicol. & Gerd.) Walker	-	-	-	-	-	-	+	+	25.00
Glomus macrocarpum Tulasne & Tulasne	+	-	-	+	+	-	-	+	50.00
Glomus microcarpum Tulasne & Tulasne	-	-	-	-	+	-	+	-	25.00
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck	+	+	+	+	-	+	-	+	75.00
Glomus taiwanensis (Wu & Chen) Almeida & Schenck	-	-	-	-	+	-	-	-	12.50
Scutellospora gregaria (Schenck & Nicol.) Walker & Sanders	-	-	-	-	-	-	+	+	25.00
Scutellospora nigra (Redhead)Walkers &Sanders	-	-	+	+	+	-	+	+	62.50
Scutellospora verrucosa (Koske & Walker) Walker & Sanders	-	-	-	-	-	-	-	+	12.50

+ and – indicates the presence and absence respectively.

In the present study, among climatic factors, rainfall, relative humidity and maximum temperature recorded significant correlation with hyphal colonization and vesicular colonization. Michelini *et al.* (1993) also reported positive correlations of annual rainfall with hyphal colonization and total root colonization in *Citrus* across four islands of Eastern Caribbean. 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). However, correlation between spore density and climatic factors was not found.

Among edaphic factors, electrical conductivity recorded positive correlation between arbuscular colonization and negative correlation with vesicular colonization. Electrical conductivity provides a measure of concentration of soluble salts in soil solution. It is argued that positive relation between electrical conductivity and AM colonization indicate

active AM-mediated uptake of nutrients while negative correlation may indicate passive stage of vesicular symbiosis. Further, the negative relationship between available potassium and vesicular colonization recorded in the present study is contradictory to the findings of Daniels and Trappe (1980), who reported no correlation between the two. In the present study, organic carbon exhibited positive correlation with arbuscular colonization and negative correlation with vesicular colonization. Similarly, Gianinazzi- Pearson *et al.,* (1981) attributed negative relationship between organic matter and fungal colonization to increased hydrolysis of organic phosphorus from phytates. Among the four micronutrients analyzed during the study, zinc and manganese influenced the root colonization of AM fungi. Earlier works have reported varied response of micronutrients on arbuscular mycorrhizal fungi. Hepper (1979) indicated that zinc and manganese inhibited spore germination while Ojala *et al.,* (1978) reported greater mycorrhizal response in soil with low levels of Zn, Cu, Fe and Mn.

Spore density was influenced by electrical conductivity. Conflicting reports are available on the nature of relationship between electrical conductivity and spore density. Baby and Manibhusanrao (1992) reported the absence of correlation between the two, whereas Janardhanam *et al.*,(1994) found significant positive correlation between E.C and spore numbers.

In the present study arbuscular mycorrhizal fungi belonging to the genera *Acaulospora, Gigaspora, Glomus* and *Scutellospora* were associated with *C.* papaya. Species belonging to *Glomus* were the most representative type. Nemec *et al.* (1981) also reported *Glomus* species as apparently the most common arbuscular mycorrhizal fungi found in cultivated soil. The study reported relatively large number of arbuscular mycorrhizal fungi associated with *C. papaya* as compared to the species reported by Mason (1964) in seasonal studies on strawberry, raspberry and barley. Nemec *et al.* (1981) also reported relatively few number species in their study on ecology of arbuscular mycorrhizal fungi in *Citrus* from California and Florida.

Open pot culture technique was not successful for sporocarpic species of *Glomus* that were placed earlier under genus *Sclerocystis*, as they failed to produce new sporocarps. This is accordance with the findings of Muthkumar and Udaiyan (2002). A likely reason for failure in production of new spores by certain species of arbuscular mycorrhizal fungi may perhaps be due to the fact that spores may have not been viable even though they appeared so (Miller *et al.,* 1985), or the spores may have extended dormancy or their quiescence was not broken in the conditions and time span used (Tommerup, 1983). The findings of Khade and Rodrigues are contradictory (2003) to above findings who reported growing sporocarp of *Glomus clavisporum* (Trappe) Almeida & Schenck in rhizosphere of tubers of *Gloriosa superba* L. from Taleigao plateau, Goa, India.

The species diversity and distribution of arbuscular mycorrhizal fungi are usually considered to be more influenced by the environmental, physical and nutritional status of the soil than the identity of their plant partner (Anderson *et al.,* 1984; Koske, 1987; Allen *et al.,* 1995). Several species of arbuscular mycorrhizal fungi were recovered here during the first year only, possible because *C. papaya* plants selected for survey had completed their first season of growth on the study sites. Thus, fungal species available to the plants were those already colonizing the roots or those present at the planting location. Therefore, previous crops and concurrent weed species, (where the plots were maintained fallow prior to planting of papaya plants) must have determined the local population of the endophytes when the papaya plants were planted. Schenck and Kinloch (1980) also reported that species composition of arbuscular mycorrhizal fungi change with both time and crop. Thus, the species reported during the second year of sampling in the present study, might more likely reflect the associations with *C. papaya*. Species of arbuscular mycorrhizal fungi were recovered during different times of the year. It is known that several arbuscular mycorrhizal fungi can occur within a single root (Abbott and Robson 1984; Abbott and Robson 1991), suggesting a possibility of inter-specific competition between them. Species richness of arbuscular mycorrhizal fungi was not constant throughout the study and varied with sampling periods.

CONCLUSION

The present study brings out the fact that arbuscular mycorrhiza is a well established phenomenon in *C. papaya* that exhibit variations depending on the edaphic factors and seasonal patterns in the weather. The study also highlights the fact that the existence of a seasonal pattern of arbuscular mycorrhizal fungi is indicated by a similarity in spore density, mycorrhizal colonization and arbuscular mycorrhizal structures of the two years.

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