Tropical and Subtropical Agroecosystems

ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH VARIETIES OF *Carica papaya* L. IN TROPICAL AGRO-BASED ECOSYSTEM OF GOA, INDIA

[MICORRIZAS ARBUSCULARES ASOCIADAS CON VARIEDADES DE *Carica papaya* L. EN ECOSISTEMAS AGRÍCOLAS TROPICALES DE GOA, INDIA]

Sharda W. Khade[1*] and B.F. Rodrigues

Department of Botany, Goa University, Taleigao Plateau, Goa (403 206) India.
[1]Current address: Darshan Apts, II[nd] Floor, Vidhyanagar Colony, Carenzalem Post, Miramar, Panaji, Goa 403 002, India.
Email: sharda_khade @yahoo.com Tel: 91 (0832)2462978.
**Corresponding author.*

SUMMARY

The occurrence of arbuscular mycorrhizal (AM) fungi was investigated in six varieties of *Carica papaya* L in tropical agrobased ecosystem of Goa, India. All the varieties selected for the survey (CO-1, Coorg honeydew, CO-2, Sunrise solo, Washington and Local) were colonized with AM fungi. The root colonization ranged from 26 to 77 %, while the spore density ranged from 100 to 236 spores per 100 g of rhizosphere soil. Total nitrogen, available potassium and organic carbon content of the rhizosphere soil along with root colonization and spore density of AM fungi varied significantly in different varieties of papaya. Available phosphorus and available potassium recorded significant negative correlation with spore density of AM fungi. However organic carbon, available phosphorus and total nitrogen recorded significant positive correlation with root colonization of AM fungi. Thirteen species of AM fungi belonging to the genera *Acaulospora*, *Glomus*, *Gigaspora* and *Destiscutata* were found to be associated with the varieties of papaya considered.

Key words: Arbuscular mycorrhizal fungi; edaphic factors; root colonization; spore density.

RESUMEN

Se investigó la ocurrencia de mycorriza arbusculares en seis variedades de *Carica papaya* L. en sistemas ecosistemas agrícolas tropicales de Goa, India. Todas la variedades estudiadas (CO-1, Coorg honeydew, CO-2, Sunrise solo, Washington y Local) estuvieron colonizadas por las micorrizas arbusculares. La colonización de la raíz fue de 26 a 77%, mientras que la densidad de esporas fluctuó de 100 a 236 esporas por 100g de suelo de rizosfera. El nitrógeno total, potasio disponible y carbono orgánico del suelo de rizosfera en conjunto con la colonización de la raíz y densidad de esporas fluctuó de manera significativa entre variedades. El fósforo y postasio disponibles, así como el nitrógeno total estuvieron positivamente correlacioneados con la colonización de la raíz. Trece especies de micorrizas arbusculares pertenencientes a los géneros *Acaulospora*, *Glomus*, *Gigaspora* y *Destiscutata* fueron encontrados asociados en las variedades de papaya estudiadas.

Palabras clave: Hongos; micorrizas arbusculares; factores edáficos; colonización de raíz; densidad de esporas.

INTRODUCTION

Agriculture creates an artificial ecosystem that requires constant human intervention, whereas in natural ecosystem, the internal regulation of function is product of plant biodiversity through flows of energy and nutrients and this form of control is progressively lost under agricultural intensification (Swift and Anderson, 1993). In fact modern agriculture implies simplification of the structure of the environment over vast areas, replacing nature's diversity with a small number of cultivated plants and domesticated animals (Altieri, 1999).

A great effort of focusing on the biological systems and the agro-ecosystem as a whole is needed to better understand the complex processes and interactions governing the stability of agricultural land. However, the question remains as to what aspects of the plant soil interface merit extensive investigations that will contribute to the large scale problem of agriculture stability. O' Neill, *et al.* (1991) presented a convincing argument that '*Mycorrhizal Research*' is one such area deserving extensive investigation for sustainable agriculture, primarily because mycorrhizal fungi are a crucial link between roots and soil. Undoubtedly, an improved understanding and management of the symbiosis of the plants with AM fungi in agro-

ecosystems ultimately has a large social and environmental impact, particularly in low input sustainable agriculture and in tropical agro-ecosystems (Singh & Adholeya, 2002). It is certainly of great importance to India, where nearly half of the districts have been classified as being highly deficient in plant available phosphorus (Pingali *et al.,* 1997).

Arbuscular mycorrhizal fungi are common root colonizing fungi that form symbiotic association with higher plants (Mosse, 1972; Hayman, 1982; Fitter 1985). Although these fungi are obligate symbionts they are not host specific and one species may be found to be associated with various plants in the same locality. Also, one host plant can support mixed populations of AM fungal species (Hass & Menge, 1990). The intrinsic interest in the study of AM fungi is due to their beneficial role in the growth of the plants (Venkataraman *et al.,* 1990). However, prior to exploiting the bio-fertilizer potential of these organisms, it is necessary to examine the occurrence and distribution of AM fungi.

Papaya (*Carica papaya* L.) is a member of the *Caricaceae*, a small dicotyledonous family consisting of six genera of herbaceous, shrubby or arborescent plants. It is now the only species belonging to the genus *Carica*. The domesticated papayas appear to have originated from a small-fruited ancestor in Central America. Papaya is by far the best known and economically most important species of the family. Many cultivars are grown in many tropical and subtropical countries for their edible, vitamin-rich fruits and to a lesser extent also for their milky latex. The different proteinases, present in the latex obtained from green unripe fruits, have a broad spectrum of activity and are therefore widely used in the food and pharmaceutical industries (Van Droogenbroeck *et al.*, 2002). Papayas are believed to have been introduced into India during the 16th century (Reddy, 2000). Interestingly, India is one of the largest producers with production of 700,000 ton of papaya in the year 2007 (FAO, 2007). Papaya is a polygamous plant and has many sex forms. The three basic sex types are (Ram, 1993): staminate or male, hermaphrodite and pistillate or female. In India, a large number of varieties are found for cultivation. This variability is seen due open pollination and indiscriminate multiplication using open pollinated seeds. In papaya there are two basic types of varieties. Those varieties, that produce only female and male plants are said to be *dioecious* and those that produce female and hermaphrodite plants are called *gynodioecious*. Some of the important gynodioeciuos varieties are Coorg Honey Dew, Sunrise Solo, CO-7 and Surya. The varieties like CO-1, CO-2, Washington and Pusa Dwarf come under dioecious types (Reddy, 2000). The local variety found in Goa is gynodioecious one.

Incidence of AM association in papaya has been reported by Bhattarai *et al.* (1989), Ravi *et al.* (1995) and Khade *et al.* (2002). In the present paper, an effort was made to study the AM association in papaya in agro-based ecosystem of Goa, India. Hence the present study was under taken to survey the rhizosphere soil and roots of papaya varieties for AM association. This survey intended to examine the root colonization characteristics, spore density pattern and the AM fungal species in different varieties of papaya. The study also addresses the relationship between edaphic factors, root colonization and spore density of AM fungi.

MATERIAL AND METHODS

Study site

The State of Goa lies between 14^{o} N - 16^{o} N latitudes and 73^{o} E – 75^{o} E longitudes. The total area available for utilization in the state is 361,113 ha of which total cropped area is 171,356 ha. 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. Other fruit crops like papaya (*Carica papaya* L.), jackfruit (*Artocarpus hetrophyllus* L.), pine-apple (*Ananas cosmosus* L.), are also cultivated among which papaya is the most popular one. Six varieties of papaya were sampled during December 1999 from Collem region (15^{o} 29′32.1″ N; 74^{o}13′59.5″ E) located in South Goa, India (Figure 1).

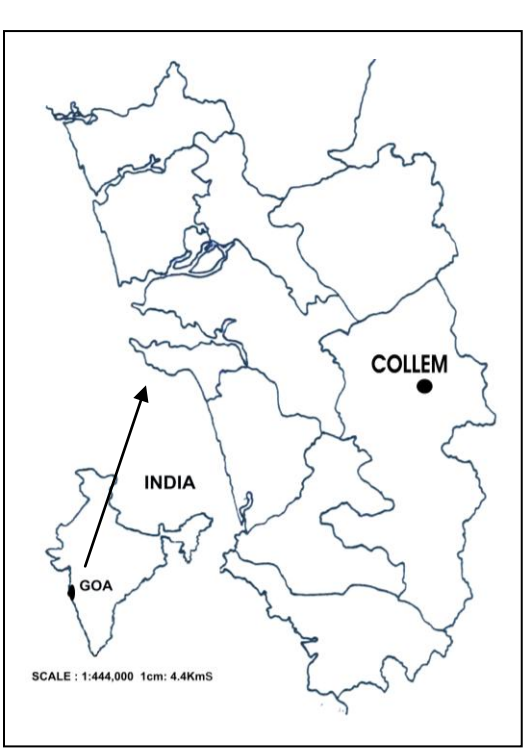

Figure 1. Map of Goa showing the study site.

The maximum and minimum temperatures recorded during this period were 32.6°C and 20.7°C respectively with relative humidity ranging from 56 to 73%. The soil was dark brown to reddish brown, well drained gravelly with silt clay loam texture and medium water holding capacity.

Plantation status and management regime

Two year old, fruit bearing, papaya plants were sampled from papaya plantation (approximately 10-12 acres) during the study (Figure 2A). The plantations comprised of six varieties distributed randomly over a field site. Papaya plants were widely spaced i.e. planted at a distance of 2.5 x 2.5 m in a row (each variety / row) and intercropped with pineapple that were planted on the sides in 3-4 m deep trenches (Figure 2A). Pineapples were planted in dual rows with 60 cm spacing between them (Figure 2A). Pineapple crop did not interfere with papaya plants since it thrived in over flowing water that accumulated in the trenches during irrigation of papayas. In the present study, papaya plantations were managed semi-conventionally. Inorganic fertilizer (19:19:19) was applied in six split doses per year at an interval of two months. Papaya plants received 250 g N, 250 g P_2O_5 and 50 g K_2O per plant per year. A single dose of vermi-compost (2 kg plant^{-1}) rich in organic content was applied prior to monsoons (May). Plantations were irrigated twice a week all the year round except during monsoons.

Sample Collection

Six varieties of papaya *viz*., CO-1, Coorg Honey Dew, CO-2, Sunrise Solo, Washington and Local were selected for the study. Five healthy plants were randomly sampled per variety. Three sub-samples were collected per variety. While sampling, care was taken to trace the papaya roots, which were identified by its white colour and distinct phenolic odor emitted upon crushing. Samples were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed whereas soil samples were stored at 4°C until analyzed. Sampling procedures were carried out according to Tews and Koske (1986) except that the core size was bigger (15 cm in diameter). This was carried out to avoid non normal distribution of spores found in counts from small core samples (St. John & Koske, 1988). In all, for six varieties, ninety soil cores (15 per each of the 6 varieties) were sampled in 30 papaya plants (5 per each of the 6 varieties). Three random soil cores were collected from within 60 cm of each plant/ variety, each at the depth of 0-25cm. These three soil cores were combined to give approximately 400g moist soil of composite sample after thorough mixing. From this 100g 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 per variety, 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.

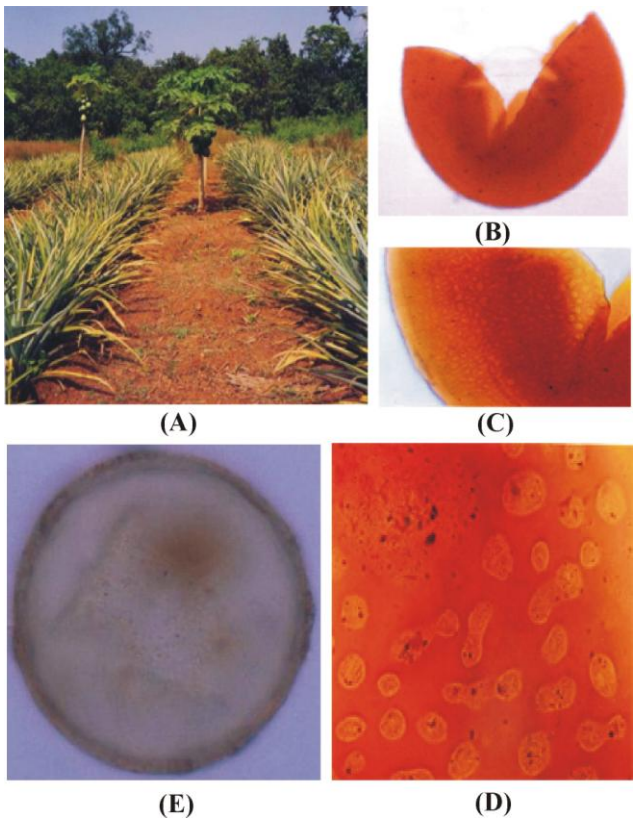

Figure 2.
A) Habit of *Carica papaya* L.
B) A crushed spore of *Acaulospora foveata* (x 300)
C) Portion of spore wall of *Acaulospora foveata* showing the pits (x 400)
D) Spore wall surface of *Acaulospora foveata* showing large irregular pits (x1000).
E) A spore of *Acaulospora spinosa* (x 400).

Establishment of pot cultures

Baiting of native AM fungi were carried out by using open pot cultures (Gilmore, 1968). For establishing pot cultures, 50g of rhizosphere soil along with root bits of papaya was 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

and placed over the soil sand mixture and covered with 2 cm of soil. The pots with *Eleusine coracana* (L.) were maintained under glass house conditions and watered adequately. Cuttings of *Coleus* sp. were also used as host plants for baiting the native AM fungi**.** Similarly, *Coleus* plants were maintained under glass house conditions and watered adequately. Five pot cultures were maintained per variety. The roots of host plants were checked for AM colonization after 45 days. Pots showing successful mycorrhization were maintained for period of six months and application of water was reduced at final three weeks to maximize spore production (Menge, 1982). At the end of 6 months the plants were cut near the base, the cultures were air-dried and checked for the presence of spores. Spores isolated from pot cultures were used for identification of AM fungi.

Soil sample analysis

Five rhizosphere soil samples per variety 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 & Heidel, 1952) using Flame Photometer (Systronic 3292). Available zinc, copper, manganese and iron were quantified by DTPA-$CaCl_2$- TEA method (Lindsay & Norvell, 1978) using Atomic Absorption Spectrophotometer (AAS 4139).

Processing of roots and estimation of root colonization

Roots were cleared and stained according to method provided by Phillips and Hayman (1970). Feeder roots of papayas were considered for the study. The root samples were first washed with water and cut into 1-cm bits. These root bits were cleared with 10% KOH at 15 lbs pressure in an autoclave for 15 minutes, acidified in 1N HCl and then stained in 0.05% trypan blue in lactoglycerol. The stained roots were examined under compound microscope (40X-400X) for the presence of hyphae, arbuscules and or vesicles. Hundred root segments for each sample were randomly selected for microscopic observation and estimation of the degree of colonization was carried out using slide method (Giovannetti and Mosse, 1980). A single root bit was mounted in 1% glycerine on a microscopic slide and covered with 35mm cover slip. Root bit was crushed by applying slight pressure and observed under light microscope, Leica WILD MP 3 and Nikon E800. A segment was considered mycorrhizal when it showed the presence of hyphae and/ arbuscules and / vesicles. Total root colonization by AM fungi was expressed in percent.

Extraction, quantification and identification of AM fungi

Wet sieving and decanting method (Gerdemann and Nicolson, 1963) was employed for extraction of AM fungal spores and sporocarps from the rhizosphere soil sampled. The steps are as follows: Hundred grams of rhizosphere soil was suspended in 1000ml of tap water. The mixture was stirred for 10-15 seconds and the coarse particles were allowed to settle in water for 1-2 minutes. The soil water mixture was decanted through sieves arranged in descending order of mesh pore size (500µm- 37µm). The above two steps were repeated twice to see that the majority of spores are extracted from the rhizosphere soil. Debris from each sieve was collected separately in beakers. Debris were filtered thorough Whatman no 1 filter paper. The filter paper was placed on petri-plate and care was taken to see that it remains moist. The contents of the filter paper were examined for the spores and sporocarps under stereo-microscope (Leica MS 5). Spore density of AM fungi was quantified using Gaur and Adholeya (1994) method. Spore density was expressed as total number of spores recorded/100g rhizosphere soil. Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in polyvinyl alcohol lactoglycerol (Koske & Tessier, 1983). Spore morphology and wall characteristics were considered for the identification of AM fungi. These characteristics were ascertained using compound microscope, Leica WILD MP3 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 (1993ab), Bentivenga and Morton (1995), Walker and Vestberg (1998), Redecker *et al.* (2000) and Morton and Redecker (2001). Taxonomic identification of spores was also carried out by matching the descriptions provided by International Collection of Vesicular AM fungi (http:// invam.caf.wvu.edu). Names and epithets of AM fungi were followed according to recommendations of Walker and Trappe (1993).

Diversity indices

Species richness per variety is mean number of AM fungal species associated with each variety (Beena *et al.*, 2000).

Frequency of occurrence

Frequency (%) of occurrence of AM fungi was calculated dividing the number of soil samples that possess spores of particular species with the total number of soil samples screened, and multiplied by 100 (Beena *et al*., 2000).

Statistical analysis

Data was statistically analyzed using MSTAC package. Pearson's correlation test was performed to assess the relationship between root colonization, spore density and edaphic factors. Data of six varieties were compared by analysis of variance for root colonization and spore density of AM fungi and the edaphic factors. Prior to ANOVA, organic carbon and total nitrogen values were square root transformed to fit the normal distribution pattern.

RESULTS

Soil characteristics

Data on rhizosphere soil analysis is represented in Table 1. The pH of the soil was acidic (5.7) to near neutral (6.9) and electrical conductivity ranged from normal (0.06 m mhos/cm) to satisfactory (0.29 mmhos/cm) thereby indicating no likelihood of salinity problems affecting plant growth. Organic carbon content of the soil was high (1.35% to 2.88%) while, total nitrogen levels (0.19% to 0.32%) were limiting. The available phosphorus levels (17.03 Kg/ Ha to 23.93 Kg/Ha) were optimum for papaya however, available potassium levels were high(140 Kg/Ha to 180 Kg/Ha). Micronutrients viz., copper (2.04 ppm to 2.87ppm) and Manganese (40.22 ppm to 48.33 ppm) were present in high levels. Zinc levels were ranging from medium (0.72 ppm) to high (0.94ppm) and Iron (10.31ppm to 12.48 ppm) levels were intermediate in the rhizosphere soil of papaya. The levels of soil parameters are according to Indian agricultural soil standards. Analysis of variance revealed that total nitrogen, available potassium and organic carbon content of the rhizosphere soil varied significantly in different varieties of papaya. However, pH, electrical conductivity, available soil phosphorus and micronutrients in the rhizosphere soil exhibited no significant variation within the varieties (Table 1).

Table 1. Comparative account of edaphic factors in six varieties of *Carica papaya* L.

Edaphic factors	[ϕ] Variety						[Ŧ] C. D (*P*=0.05)
	CO - 1	Coorg Honey Dew	CO-2	Sunrise Solo	Washington	Local	
pH	5.80 (0.220)	5.80 (0.197)	6.90 (0.269)	5.70 (0.222)	5.80 (0.227)	6.90 (0.261)	NS
E.C (m mhos/ cm)	0.07 (0.002)	0.07 (0.000)	0.10 (0.004)	0.07 (0.002)	0.06 (0.001)	0.29 (0.008)	NS
[Ŧ]Organic carbon (%)	2.88 (0.058)	1.35 (0.062)	1.39 (0.053)	1.41 (0.021)	1.40 (0.100)	2.18 (0.140)	0.040
[Ŧ]Total N (%)	0.28 (0.001)	0.20 (0.001)	0.20 (0.003)	0.19 (0.001)	0.23 (0.002)	0.32 (0.002)	0.003
Available P (Kg/ Ha)	23.93 (3.775)	17.03 (1.309)	21.34 (1.147)	20.64 (2.179)	17.96 (1.775)	20.04 (2.224)	NS
[Ŧ]Available K (Kg/ Ha)	180.00 (5.098)	108.00 (1.430)	180.00 (1.677)	148.00 (2.591)	140.00 (2.174)	140.00 (1.821)	6.591
Cu (ug g^{-1})	2.63 (0.085)	2.87 (0.068)	2.04 (0.172)	3.24 (0.408)	2.640 (0.090)	2.04 (0.120)	NS
Fe (ug g^{-1})	11.34 (0.440)	13.11 (0.450)	10.31 (0.390)	12.48 (0.360)	11.45 (0.050)	10.69 (0.440)	NS
Zn (ug g^{-1})	0.94 (0.000)	0.95 (0.000)	0.75 (0.040)	0.90 (0.001)	0.84 (0.036)	0.72 (0.044)	NS
Mn (ug g^{-1})	46.77 (1.395)	47.37 (2.242)	42.17 (1.626)	48.33 (2.164)	48.02 (1.847)	40.22 (2.132)	NS

[ϕ]Values presented are mean of five readings; Values in the parenthesis indicates ± 1S E.

[Ŧ] F test significant at 0.05 level of probability; NS- F test not significant at 0.05 level of probability.

Root colonization and spore density of AM fungi

All the varieties selected for the study exhibited AM association (Table 2). Root colonization of AM fungi was characterized by the presence of hyphae, hyphal coils, arbuscules and vesicles. Hyphal and vesicular colonization was pre-dominant, whereas arbuscular colonization was recorded in CO-1 and Local. The highest mean total root colonization was recorded in CO-1 (77%) followed by Local (73%), whereas the lowest mean total root colonization was observed in Coorg Honey Dew (26%). Analysis of variance revealed that mean total root colonization of AM fungi and varied significantly in six varieties of papaya (Table 2). The maximum spore density (236 spores/ 100g rhizosphere soil) was recorded in Coorg Honey Dew whereas the minimum spore density (100 spores/ 100g rhizosphere soil) was recorded in CO-1. Mean spore density of AM fungi exhibited significant variation, in the rhizosphere soil of papaya varieties (Table 2).

Table 2. Arbuscular mycorrhizal association in six varieties of *Carica papaya* L. ϕ Φ

Variety	Type*	Total root colonization (%)	Spore density /100g rhizosphere soil
CO-1	H, A, V	77.00 (3.654)	100.00 (4.941)
Coorg Honey Dew	H,V	26.00 (2.192)	236.00 (4.25)
CO-2	H, V	46 .00 (4.04)	108.00 (5.080)
Sunrise Solo	H, V	40 .00 (2.349)	123.00 (4.215)
Washington	H, V	36.00 (2.282)	166.00 (5.533)
Local	H, A, V	73.00 (1.92)	132.00 (6.390)
C.D (P = 0.05)	-	0.621	13.287

ϕ Values presented are mean of five readings. Values in the parenthesis indicate ± 1S E.
Φ F- test significant at 0.05 level of probability.
* Type of root colonization: H – Hyphal colonization; A- Arbuscular colonization; V- Vesicular colonization

Relation between AM fungi and edaphic factors

In the present study, no significant correlation was recorded between mean total root colonization and mean spore density of AM fungi (Table 3). Among the 10 rhizosphere edaphic parameters analyzed, four parameters exhibited significant correlation with AM fungi. Mean total root colonization levels of AM fungi exhibited significant positive correlation with total nitrogen, available phosphorus, and organic carbon content of the rhizosphere soil (Table 3). Whereas, mean spore density of AM fungi exhibited significant negative correlation with available phosphorus and available potassium (Table 3). However, micronutrients *viz.*, copper, zinc, iron, manganese and other edaphic factors like pH, and electrical conductivity exhibited no significant correlation with mean total root colonization or mean spore density of AM fungi (Table 3).

Distribution of AM fungi

Data on diversity of AM fungi and their frequency of occurrence in the rhizosphere soil of papaya is recorded in Table 4. A total of 13 species of AM fungi belonging to four genera viz., *Acaulospora* (3), *Gigaspora* (1), *Glomus* (8), and *Destiscutata* (1) were recovered from the rhizosphere soil of six varieties of papaya with species number given in parenthesis. The identification of these fungi was confirmed with AM fungi recovered from pot cultures. Species of *Glomus sinuosum* and *Glomus taiwanensis* failed to produce new sporocarps in pot cultures whereas, the remaining fungal species were recovered from the original samples as well as from pot cultures. Although, the present study reported a, comparatively higher number of *Glomus* species, pooled data indicated that *Acaulospora scrobiculata* (100%) was most frequently encountered AM fungal species followed by *Acaulospora spinosa* (Figure 2E) (83%). However, *Acaulospora foveata* (33%) (Figure 2 B-D) exhibited comparatively low frequency of occurrence whereas, *Gigaspora margarita* and *Destiscutata recticulata* recorded a frequency of 66.66% and 50% respectively. Frequency of occurrence for *Glomus* species ranged from 16.66 to 50% (Table 4). It is evident from Table 4 that species richness of AM fungi varied from 4 species per variety (CO-2) to 9 species per variety (CO-1).

Table 3. Pearson's correlation coefficient (r) between arbuscular mycorrhizal fungal parameters and edaphic factors in six varieties of *Carica papaya* L.

Parameters	Mean spore density/ 100 g rhizosphere soil	Mean total root colonization (%)
Mean spore density/ 100g rhizosphere soil	1.000	-0.693
Mean total root colonization (%)	-0.693	1.000
pH	-0.238	0.221
Electrical conductivity (m mhos cm^{-1})	-0.693	0.612
Organic carbon (%)	-0.498	0.915**
Total N (%)	-0.289	0.854*
Available P (Kg Ha^{-1})	-0.886**	0.746*
Available K (Kg Ha^{-1})	-0.927**	0.662
Copper (ug g^{-1})	-0.219	0.524
Iron (ug g^{-1})	0.010	-0.485
Zinc (ug g^{-1})	-0.149	-0.27
Manganese (ug g^{-1})	0.713	-0.448

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

Table 4. Distribution of arbuscular mycorrhizal fungi in six varieties of *Carica papaya* L.

Arbuscular mycorrhizal fungi	* Varieties of *Carica papaya* L.						Frequency of occurrence (%)
	1	2	3	4	5	6	
Acaulospora foveata Trappe & Janos	-	-	-	+	+	+	50.00
Acaulospora scrobiculata Trappe	+	+	+	+	+	+	100.00
Acaulospora spinosa Walker & Trappe	+	+	+	+	-	+	83.33
Gigaspora margarita Becker & Hall	+	-	-	+	+	+	66.66
Glomus claroideum (Smith & Schenck) Vestberg & Walker	+	+	+	-	-	-	50.00
Glomus constrictum Trappe	-	-	-	-	+	-	16.66
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	+	-	+	-	-	+	50.00
Glomus geosporum (Nicol. & Gerd.) Walker	-	-	-	-	+	-	16.66
Glomus heterosporum Smith & Schenck	-	-	-	-	+	-	16.66
Glomus macrocarpum Tulasne & Tulasne	+	+	-	-	-	+	50.00
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck	+	-	-	+	-	+	50.00
Glomus taiwanensis (Wu & Chen) Almeida & Schenck	+	-	-	+	-	+	50.00
Destiscutata reticulata (Koske, D.D. Mill. & C. Walker) Sieverding, De Souza & Oehl	+	+	-	-	+	-	50.00
Species richness	9	5	4	6	7	8	-

* 1-CO-1, 2 -Coorg Honey Dew, 3-CO-2, 4-Sunrise Solo, 5- Washington, 6-Local.
+ = Presence of arbuscular mycorrhizal fungi. - = Absence of arbuscular mycorrhizal fungi.

DISCUSSION

In general, nutrient (both macronutrients and micronutrients) levels in the rhizosphere soil of six papaya varieties were ranging from medium to high except nitrogen which can be attributed to application of fertilizers at regular intervals. At the study site, papaya plants were planted on a slope with pineapple planted along the deep trenches on either side to prevent water logging. Thus low levels of nitrogen recorded in the present study could be due factors such as leaching (loss of N from the root zone with drainage water) or run off (loss of N with surface flow during over irrigation) (Tandon, 1994).

The present investigation supplements the initial efforts of Bhattarai *et al.* (1989), Ravi *et al.* (1995) and Khade *et al.* (2002) to study AM association in *Carica papaya* L. Bhattarai *et al.* (1989) reported 51% root colonization and a high spore density of 350 spores/50g rhizosphere soil in papaya sampled from Nepal while, Ravi *et al.* (1995) reported a relatively low colonization of AM fungi (15%) in three year old local variety of papaya sampled from Vamban area

(Tamil Nadu). Similarly, Khade *et al.* (2002) reported high root colonization (78%) and a high spore density of 240 spores/50g rhizosphere soil in papaya sampled from Quepem, South Goa. In another study on spatial –temporal variations of AM fungi in papaya plantations, Khade and Rodrigues (2008 a) reported 40% root colonization and 280 spores/ 100g soil from Collem. In the present study, the degree of root colonization by native AM fungi varied significantly in six papaya varieties and this variation can be attributed to the differential preference of the AM fungi towards the varieties. These results support the findings of various authors who reported that root colonization of AM fungi is genetically controlled (Kesva Rao *et al*., 1990; Mercy *et al*., 1990; Raju *et al*., 1990; Karangiannidis *et al*., 1997). Karangiannidis and Velemis (2000) also reported significant differences between apple and peach varieties as regard to their abilities to form AM association. They reported that the peach trees of Naoussa variety and apple trees of Mutsu variety exhibited minimum root colonization by AM fungi. It is also apparent from the present study that the spore density of AM fungi varied significantly in varieties of papaya which is in agreement with the findings of Karangiannidis *et al*. (1997) who reported similar results in four grape vine rootstocks. On studies carried out by Akond *et al*. (2008) on AM fungi associated with fifteen widely cultivated vegetable crops, fourteen out of the fifteen crops reported developed AM colonization in their root tissues with a range of 7% to 98% and housed AM spores, with a density range of 37-259 spores per 100g air dried soil; the spore density in the soil did not have any effect on symbiotic colonization in root tissues, although these two parameters of AM fungi varied significantly within the selected vegetable crops. In the present study, the absence of correlation recorded between root colonization and spore density of AM fungi can be attributed to the fact that spore numbers may poorly reflect the colonization potential of the soil (Miller *et al.*, 1985) and they are not always related to the rate and the extent of mycorrhizal formation (Abbott & Robson, 1982). Our studies are contradictory to the findings of Wu *et al.* (2006), who reported significant positive correlation between root colonization and spore density of AM fungi in *Citrus*

Generally, the beneficial effects of AM fungi has been observed under sub-optimal nutrient status of soil (Wani & Lee, 1995) and maximum root colonization and spore density of AM fungi occurs in low soil fertility (Hayman, 1970). In the present study pH of the soil was acidic (except for local variety) and did not vary significantly within six varieties of papaya. Neither, it recorded significant correlation with spore density or root colonization of AM fungi, which is in agreement with the findings of Akond *et al*. (2008). However our studies are contradictory to the fact that acidic soils commonly have poorer structure, lower water and root penetration, less heterotrophic microorganisms and more toxic ions than those with basic pH (Hoyt *et al.* 1967). These factors associated with the previous ones harm plant growth. Thus, plants become more susceptible and responsive to mycorrhizal colonization (Carrenho *et al*., 2007).

In the present study, the available soil P levels were optimum and did not significantly vary within the rhizosphere of the six papaya varieties. Although, the fluctuation range of soil P was narrow, it was seen to be positively correlated with root colonization and negatively correlated with spore density. Literature exists on P fertilization reducing as well as increasing root colonization by AM fungi (Wani and Lee, 1995) indicating that the initial soil fertility level mediates the response (Gavito and Miller, 1998). Tropical soils are usually low in phosphorous and moreover, due to chemical fixation of phosphate fertilizers their efficiency of utilization by plants is of the order of 10-15% (Gaur, 1982). Thus, the positive correlation between mean total root colonization and available soil P in the present study suggest that adding fertilizers to the soils that are low in P can increase the root colonization possibly through a direct effect on AM fungi (Bolan *et al.*, 1984). The negative correlation between mean spore density and available soil P recorded in the present study is in agreement with the findings of Nemec *et al.*, (1981) and Khade and Rodrigues (2008 a), who reported similar findings while studying the distribution and ecology of AM fungi in *Citrus* nurseries and spatio-temporal variations of AM fungi in papaya plantations. Contradictory to this, Wu *et al.* (2006) reported that the AM colonization was significantly positively correlated with spore density and negatively correlated with soil available phosphorus content, indicating that higher spore density and lower soil available phosphorus content could accelerate the colonization on *Citrus* roots. However, Wu *et al.* (2006) further reported that the spore density was significantly negatively correlated with soil available phosphorus content, suggesting that soil available phosphorus could inhibit the increase of spore density and these reports are in agreement with our findings on papaya varieties. Nitrogen plays an important role in influencing the mycorrhizal formation and functions mainly through changes in soil pH and thereby suppressing or stimulating root colonization and spore production of AM fungi (Slyvia and Neal, 1990). In the present study, total nitrogen content in the rhizosphere soil was low and exhibited a significant positive correlation with total root colonization when available P levels were optimum for papaya. Bevege (1971) in a field experiment also reported that in *Araucaria cunninghamii*, AM colonization increased

with addition of nitrogen at intermediate levels of P. Similarly, Khade and Rodrigues (2008a) recorded significant positive correlation between N and total root colonization in their studies on spatio-temporal variations of AM fungi in papaya plantations from Goa, India. In the present study high levels of available potassium negatively influenced spore density of AM fungi associated with the rhizosphere soil of papaya varieties. These findings are contradictory to Nemec *et al*. (1981), who reported the absence of correlation between the two *Citrus* nurseries of California. Many studies have evaluated the influence of organic matter on arbuscular mycorrhizae (St John *et al.,* 1983; Joner & Jakobsen, 1995; Douds *et al.*, 1997; Gaur & Adholeya, 2002; Gryndler *et al.*, 2002) with very different results, indicating variable responses on plants and fungi. In the present study, organic carbon content of the soil positively influenced the root colonization by AM fungi there by emphasizing the importance of organic matter in this process (Hepper and Warner, 1983). However results obtained in the present study are contradictory to the findings of Nappi *et al*. (1985) who reported high root colonization of AM fungi in vineyards with low organic matter. Further, our studies do not support the view that microbial activity probably is intensified after the addition of organic matter, increasing the concentration of nutrients in the soil (Beyer *et al*. 1999) which may reduce the internal growth of AM fungi (Carrenho *et al*., 2007).

The identification of indigenous AM fungi is a fundamental requirement to understand biodiversity and essential for monitoring changes in natural, managed or disturbed ecosystems. Diversity in AM fungi can be explored at this level by studying spore characteristics, ultra-structural features and colonization patterns in different agricultural crops, including different varieties of the same crop. The AM fungi are abundant and ecologically very important in the tropics (Khanam, 2007). The present study brings out the enumeration of various species of AM fungi were associated with six varieties of papaya. A total of 13 species of AM fungi belonging to four genera viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Dentiscutata* were recovered from the rhizosphere soil of six varieties of papaya. Similarly, 15 species of AM fungi were reported to be associated with papaya plantations of Collem and they belonged to above mentioned four genera's (Khade and Rodrigues, 2008a). In the present study, some species were quite common while others were encountered infrequently in the rhizosphere soil of papayas; AM fungi belonging to *Glomus* were the most representative type which could be attributed to the fact that the distribution of this genus is world wide and that *Glomus* species apparently are the most common of AM fungi found in cultivated soils (Nemec *et al.,* 1981, Khade and Rodrigues, 2008a, 2008b). Similarly Blaszokowski (1993), and Talukdar and Germida (1993) reported prevalence of *Glomus species* in agriculturally used soils in contrast to rich AM communities containing *Gigaspora* species, *Scutellospora* species and *Acaulospora* species in uncultivated soils. Also maximum number of *Glomus* species were found to be associated with forest tree species (Khade and Rodrigues, 2003a), medicinal herbs (Bukhari *et al.,* 2003), tubers (Khade and Rodrigues, 2003b), bananas (Khade and Rodrigues, 2004) and papayas (Khade and Rodrigues, 2008a, 2008b) from Goa, India. The range of species richness of AM fungi recorded in the present study (four to nine species per variety) is higher than reported by Bukhari *et al.,* (2003) and Jaiswal *et al.,* (2003) who reported two to six species per plant in medicinal herbs and two to four species per plant in mangrove ecosystem of Goa respectively. However, in another study on spatial–temporal variations of AM fungi in papaya plantations, Khade and Rodrigues (2008) reported 15 species of AM fungi from Collem.

Arbuscular mycorrhizal fungi recorded in this survey (if not all) were largely sporulating types since propagules in the soil or root pieces eventually produced spores after pot culturing under glass house conditions with susceptible host. However, an 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 AM 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).

CONCLUSIONS

The symbiotic association between AM fungi and the roots of plants is widespread in the natural environment. The present study documents the association of AM fungi in six varieties of papaya in tropical agro-based ecosystem of Goa, India for the first time and emphasizes the fact that, this symbiosis is controlled by various edaphic factors. The study also enumerates species associated with papaya varieties from agro-based ecosystem of Goa, India. Further steps need to be undertaken to study the effects of that management practices like tillage, use of inorganic fertilizers, crop rotations with fallow, irrigation and rationalized use of pesticides on AM fungi in order to maximize their beneficial effects. Also, further research should be targeted towards understanding the

functional ecology of AM fungi in agro-based papaya plantations.

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