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Distribution and Diversity of AM Fungi in Selected Tree Species Growing in Semi-arid Forest of Gujarat, India

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ABSTRACT

The diversity and community structure of arbuscular mycorrhizal (AM) fungi from four forests (Kapadvanj, Satlasana, Vijaynagar, and Danta) belonging to the semi-arid region of Gujarat, India were investigated. 59 species of AM fungi belonging to six genera *Acaulospora* (11), *Entrophospora* (1), *Gigaspora* (5), *Glomus* (35), *Pacispora* (1) and *Scutellospora* (6) were recovered. The most dominant genus was *Glomus* in all selected sites, followed by *Acaulospora*. Weak and non-significant correlation was found between spore density and percent root colonization; percent root colonization and species richness, whereas positive correlation was found between spore density and species richness. Although lower value of Sorenson's coefficient of similarity revealed less overlapping in species composition of AM fungi but general diversity and distribution of AM fungi showed little variation in all selected sites. We found strong positive correlation between isolation frequency and relative abundance of AM fungal species. Spore density and colonization by AM fungi highly varied in all selected sites and weak, non-significant correlation existed between them. Spore density, percent root colonization and species richness in Vijaynagar were significantly higher compared to Kapadvanj, Satlasana and Danta. Seventeen tree species representing 14 genera and 13 families were examined for AM fungal association from semi-arid forest of Gujarat. All plant species were potentially colonized by AM fungi. Large variation was found in spore density (44-1529/50 g dried soil), percent colonization (19.02-81.72%) and number of species recovered (8-22) in selected plants. We suggest that the high diversity of AM fungi was present in selected semi-arid forests of Gujarat, India and the plants growing in the semi-arid environment are highly dependent on AM fungal colonization.

Keywords: Arbuscular mycorrhiza, Diversity, Gujarat, Root colonization, Spore density, Species richness**,** Semi-arid forest, Trees

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are obligate symbionts associated with the roots of host plants in which energy moves primarily from host to fungus and inorganic resources from fungus to plants. AM fungi are the major component of rhizosphere soil in natural ecosystems. It has been estimated that about 80% of the terrestrial plant families in the world are associated with AM fungi (Giovanneti and Sbrana 1998). AM fungi are known to improve plant growth by improved nutrient uptake of their host plant (Farzaneh *et al*. 2011) particularly phosphorus (Smith *et al*. 2003) and improved water status (Zhu *et al*. 2012) even in drought condition (Khalvati *et al*. 2005). This ability of AM fungi makes them an important component of semi-arid and arid ecosystems (Allen and Allen 1992). They are also known to improve growth of host plants in drought stress (Song 2005), saline stress (Evelin *et al*. 2009) and heavy metal stress (Cicatelli *et al*. 2010).

Gujarat is situated on the western coast of India and lies between 20^0 07' N - 24^0 43' N latitude and $68^0$10' E - 74^0 29' E longitude. According to Gujarat Forest Statistics (2021-22) Geographical area of Gujarat state is 1,96,244 km^2 in that 21876.45 km^2 covered by forest, which constitutes 11.15% of total geographic area of the state. In Gujarat 90,520 (46.18 %) and 62,180 (31.72 %) km^2 area belongs to semi-arid and arid regions respectively. Scarcity of water is a serious problem in arid and semi-arid regions. Beneficial functions provided by AM fungi to plants include improved overall growth of plant (Chandarana and Jasrai

2011), well developed root system (Bhattacharya *et al*., 2002), enhanced phytohormone activity (Kaldorf and Ludwig-Muller, 2000), better water relation (Sheng *et al*., 2008) and decreased resistance to hydraulic conductivity (Graham and Syvertsen, 1984). AM fungi help to reduce growth of plant pathogenic fungi (Kapoor and Mukerji, 1998), restoration and re-establishment of vegetation in degraded ecosystems (Zhang *et al*., 2011; Zhang *et al*., 2012) and maintain plant biodiversity and ecosystem functioning (van der Heijden *et al*., 1998). AM fungi play an important role in the re-establishment of the vegetation in disturbed arid ecosystem (Azcon-Aguilar *et al*., 2003) and accelerate plant establishment in semi-arid sites (Caravaca *et al*., 2003).

Previous studies have also confirmed that AM fungi are very common in arid and semi-arid area (Zhao and Zhao, 2007; Diallo *et al*., 1999; Mutabaruka *et al*., 2002; Uhlmann *et al*., 2004; Tao and Zhiwei, 2005; Yang *et al*., 2010). Various authors have reported that AM fungal association is quite common in plants growing in arid and semi-arid area in India (Chandarana and Jasrai, 2016: Kamalvanshi *et al*., 2011; Panwar and Tarafdar, 2006a, Panwar and Tarafdar, 2006b). AM fungal associations have also been investigated in other ecosystems in India (Beena *et al*., 2000; Khade and Rodrigues, 2010; Muthukumar and Udaiyan, 2000; Singh *et al*., 2003). But no reports have been found on AM fungal association from plants growing in semi-arid forests of Gujarat. In this context the present study was carried out to determine (1) the biodiversity of AM fungi in semi-arid forest of Gujarat, (2) association of AM fungi with trees growing in semi-arid regions of Gujarat.

MATERIALS AND METHODS

Study sites

The study was undertaken in four different forests belonging to semi-arid region of Gujarat: Kapadvanj, Satlasana, Vijaynagar and Danta. The soil and root sample from selected trees were collected from Kapadvanj Taluka belonging to Attarsumba forest range in Kheda District; Satlasana Taluka, Vijaynagar Talukaand Danta Taluka belonging to Dharoi range in Mehsana District; Dholvani range in Sabarkantha District and Danta-West range in Banaskantha District. The site characteristics are presented in **Table 1**. The natural disturbance was noted in all selected sites (Kapadvanj, Satlasana, Danta and Vijaynagar) due to the soil erosion in forest. Forest fire is also responsible for soil degradation and disturbance in Satlasana and Danta. Moreover, quarrying in Danta is also major factor of disturbance and degradation. Interference of human activities such as deforestation, collection of forest products and grazing were also noted in all selected forests.

Table 1: Characteristics of study sites

Characteristics	Site			
	Kapadvanj	**Satlasana**	**Vijaynagar**	**Danta**
Forest range	*Atarsumba*	Dharoi	Dholvani	Danta-West
Location	23.02°N 73.07°E	24.02° N 72.79° E	23.99^0 N 73.27^0 E	24.19^0 N 72.77^0 E
Forest Type	*Tropical dry deciduous, ravine*	Tropical dry deciduous thorny scrub, hilly	Tropical dry mixed deciduous, hilly	Tropical dry deciduous thorn, hilly
Annual Rainfall (mm)	932	754	834	864
Average Temperature				
Summer ^{O}C	33.51	33.61	33.72	33.55
Winter ^{O}C	20.60	19.83	20.05	19.02

Sampling

Seventeen tree species belonging to 13 families were investigated. In each tree species 5-7 individuals were randomly selected for sampling of soil and roots. After removing surface soil, the soil and root samples were collected to a depth of 10 – 30 cm during April – May 2011 (dry season), making sure that roots were connected to sampled plants. Minimum 50 m distance was kept between two samples. Soil samples were placed in polythene bags for transport. Soil samples were air-dried and stored at room temperature before use. Roots were immediately fixed in FAA solution (Zhao *et al*., 2001).

Soil analysis

The samples were sieved through 2 mm sieve to remove larger soil particles and analyzed. pH was measured by pH meter (Chemiline, CL-110) in 1:2 soil water suspension. Electrical conductivity (EC) was calculated using conductivity meter (Equiptronics, EQ-660A) at room temperature in 1:2 soil suspension (Aery, 2010). Rapid titration method (Walkley and Black, 1934) was employed for soil organic carbon (SOC). Available phosphorus (Bray and Kurtz, 1945) and available potassium (Toth and Prince, 1949) were also quantified.

Isolation and identification of arbuscular mycorrhizal fungal spores

AM fungal spores were isolated from 50 g dried soil sample by wet sieving and decanting technique (Gerdemann and Nicholson, 1963) followed by centrifugation in 60% sucrose and enumerated. Isolated spores were mounted in PVLG (Koske and Tessier, 1983) and stained in mixture (1:1 v/v) of PVLG and Melzer'sreagent (Brundrett *et al*., 1994) and observed under microscope (Lawrence and Mayo, LM-52-1804). AM fungi were identified based on spore morphology and wall characteristics (Schenck and Perez, 1990; Morton and Benny, 1990, Oehl and Sieverding, 2004). Taxonomic positions of identified AM fungi were confirmed by matching the descriptions provided on the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (https://invam.ku.edu) and *Endogone* and *Complexipes* species deposited in the Department of Plant pathology, University of Agriculture in Szczecin, Poland (http://www.zor.zut.edu.pl/Glo meromycota/) and original description provided by AMF phylogeny website (http://www.amf-phylogeny.com).

Table 2: Diversity measures used to describe AMF communities

Spore density (SD)	:	The number of spores per 50 g soil
Species Richness (SR)	:	The number of identified AMF species per soil sample
Isolation frequency (IF)	:	$\text{IF} = \frac{number\ of\ soil\ samples\ where\ a\ species(genus)occurred}{total\ number\ of\ soil\ samples} \times 100$
Relative abundance (RA)	:	$\text{RA} = \frac{spore\ numbers\ of\ a\ species(genus)}{total\ numbers\ of\ spores\ (N)} \times 100$
Shannon-Wiener index of diversity (H')	:	$H' = -\sum P_i \ln P_i$
Evenness (E)	:	$E = \frac{H'}{H'max}$
Simpson's index of dominance (D)	:	$1-\sum P_i^2$
Sorenson's coefficient (C_S)	:	$Cs = 2j/(a + b)$

$P_i = n_i/N$, Where n_i is the spore numbers of a species (genus) and N total numbers of spores. H'_{max} is the maximal of H' and calculated by H' = ln S, Where S is the total number of identified species per sampling site. a and b are the total number of species per sampling site and j is the total number of common species at all site.

Diversity Studies

Ecological measures of diversity used to describe structure of AMF community in selected sites include spore density (SD), species richness (SR), isolation frequency (IF) and relative abundance (RA) (Beena *et al*., 2000), Shannon diversity index, evenness (Shannon and Weaver, 1949) and Simpson's diversity index (Simpson, 1951) and Sorenson's coefficient (**Table 2**). Spore density reflected the biomass of AMF species (genus) to some extent. RA is defined as the percentage of spore numbers of a species (genus), which indicates the ability of sporulation of different AMF species (genus). IF indicated the extent of distribution of given AMF species (genus) in an ecosystem. We considered the dominant AMF species according to IF (IF ≥ 30%) and RA (RA ≥ 3%). Shannon-Weiner diversity index shows general diversity of AMF in selected sites. Simpson's diversity index shows the dominance of AMF species in selected sites. Sorenson's coefficient was used to compare similarity existing in general structure of AMF communities.

Root Colonization

The root colonization (Sylvia, 1994) and percentage colonization by the symbiont were determined by the method described by McGonigle *et al*., (1990).

Data Analysis

Mean and standard error was calculated from data obtained. Differences in the five rhizospheric edaphic factors pH, electrical conductivity (EC), soil organic carbon (SOC), available phosphorus (AP) and available potassium (AK) between selected siteswere analyzed by one-way analysis of variance (ANOVA). One-way ANOVA was also performed to find out the differences in the mean spore count and mean percent root colonization and species richness in the selected sites. The mean spore density, species richness and mean mycorrhizal colonization for each site were calculated by averaging spore numbers, species richness and colonization of all the samples collected from each sites. Difference was considered significant when P≤0.05. Pearson correlation coefficient was carried out to assess the relationship between percent root colonization, spore density and species richness. All statistical analysis was carried outby SPSS software (IBM SPSS Statistics 20).

RESULTS

Physico-chemical properties

The physico-chemical properties of studied sites having pH slightly acidic to slightly alkaline ranged from 6.89-7.56 and electrical conductivity ranged from 0.22-0.37 (**Table 3**). The sites had high level of soil organic carbon (ranged 1.15% - 1.87%) and available potassium (ranged 405.81-450.43 kg/ha) (**Table 3**). The AP ranged between 16.33-79.31 kg/ha and was very low in Vijaynagar in contrast to level of AP in other selected sites. SOC were significantly higher in Vijaynagar than other selected sites. Although no significant difference was found for AP in Kapadvanj, Satlasana and Danta but there was a remarkable difference in the mean value (**Table 3**). The mean value for AP was quite higher in Danta than Kapadvanj and Satlasana. No significant difference was found in AK in all selected sites.

Table 3: Soil characteristics of studied sites

Parameters	Site			
	Kapadvanj	**Satlasana**	**Vijaynagar**	**Danta**
pH	*7.56±0.04*	7.48±*0.06*	6.89±*0.05*	7.50±*0.06*
ECm mhos/cm	0.37±*0.02*	0.35±*0.01*	0.22±*0.02*	0.34±*0.02*
SOC %	1.23±*0.04*	1.15±*0.04*	1.87±*0.10*	1.20±*0.04*
AP kg/ha	68.54±*296*	62.24±*3.43*	16.33±*1.92*	79.31±*3.38*
AK kg/ha	450.43±*7.38*	447.55±*8.93*	405.81±*26.62*	417.62±*10.13*
Sand %	86.83	85.91	72.00	79.23
Silt %	11.65	13.11	25.03	18.64
Clay %	1.52	0.98	2.97	2.13
Textural Class	Sand	Loamy Sand	Loamy Sand	Loamy Sand

EC, Electrical conductivity; SOC, Soil organic carbon; AP, Available phosphorous; AK, Available potassium

The diversity of AM fungi

Diversity of AM fungi found in all selected sites is depicted in **Table 4**. A total of 59 taxa representing 6 genera of AM fungi were identified. From that, 11 species belonged to *Acaulospora*, 1 to *Entrophospora*, 5 to *Gigaspora*, 35 to *Glomus*, 1 to *Pacispora* and 6 to *Scutellospora*. In the identified AMF species 34 species were recorded from Kapadvanj, 35, 30, and 24 from Satlasana, Vijaynagar and Danta respectively. 34 species of AMF noted in Kapadvanj include *Acaulospora* (5), *Gigaspora* (5), *Glomus* (20) and *Scutellospora* (4). In Satlasana, 35 AM fungal species belonged to 5 genera *Acaulospora* (5), *Entrophospora* (1), *Gigaspora* (3), *Glomus* (23) and *Scutellospora* (3). In Vijaynagar 30 species of AM fungi belonging to 3 genera *Acaulospora* (8), *Glomus* (21) and *Pacispora* (1) were noted. Whereas in Danta 24 species were recorded belonging to 3 genera *Acaulospora* (4), *Glomus* (18) and *Scutellospora* (2). Only 8 species, 1 belonging to *Acaulospora* and 7 to *Glomus* were common at all selected sites.

IF and RA of all AM fungal species are shown in **Table 4**. Based on IF and RA *Glomus* was the most dominant genus in all selected sites followed by *Acaulospora*. *Gigaspora* and *Scutellospora* belonging to *Gigasporaceae* were quite common in Kapadvanj and Satlasana in contrast to their abscence in Vijaynagar and *Scutellospora* was present in Danta with very low IF and RA. Whereas *Entrophospora* was present only in Satlasana and *Pacispora* in Vijaynagar. The result of IF and RA indicated, 1 dominant species in Kapadvanj (*Glomus fasciculatum*), 3 dominant species in Satlasana (*Glomus fasciculatum*, *Glomus aggregatum*, and *Glomus etunicatum*), 5 species in Vijaynagar (*Acaulospora scrobiculata*, *Glomus fasciculatum*, *Glomus etunicatum*, *Glomus aggregatum*, and *Acaulospora spinosa*) and 3 species in Danta (*Glomus fasciculatum*, *Acaulospora scrobiculata*, and *Glomus constrictum*). The IF of AM fungi ranged between 3.57-32.14 at Kapadvanj, 3.45-65.52 at Satlasana, 4.76-76.19 at Vijaynagar and 4.76-57.14 at Danta. The RA of AM fungi varied between 0.14-22.45 (Kapadvanj), 0.05-29.78 (Satlasana), 0.02-19.06 (Vijaynagar) and 0.06-35.41 (Danta). It was noted that there is a significant positive correlation between IF and RA of AM fungi in all selected sites Kapadvanj ($r = 0.737$, $P<0.01$), Satlasana ($r = 0.945$, $P<0.01$), Vijaynagar ($r = 0.858$, $P<0.01$) and Danta ($r = 0.892$, $P<0.01$).

Table 4: List of isolated AM fungi, its isolation frequency (%) and relative abundance (%)

Sp. No.	Arbuscular Mycorrhizal Fungi	Kapadvanj		Satlasana		Vijaynagar		Danta	
		IF	RA	IF	RA	IF	RA	IF	RA
	***Acaulospora* Gerd. and Trappe**	**53.57**	**7.61**	**41.38**	**7.36**	**90.48**	**30.89**	**50**	**14.66**
1	*Acaulospora cavernata* Błaszk.	10.71	1.96	6.90	0.81	4.76	0.77	-	-
2	*Acaulospora delicata* C. Walker, C.M. Pfeiff. and Bloss	-	-	-	-	9.52	0.84	9.52	0.78
3	*Acaulospora denticulate* Sieverd. And S. Toro	-	-	-	-	4.76	0.26	-	-
4	*Acaulospora lacunosa* J.B. Morton	-	-	3.45	0.25	-	-	4.76	0.19
5	*Acaulospora longula* Spain and N.C. Schenck	-	-	-	-	4.76	0.42	-	-
6	*Acaulospora myriocarpa* Spain, Sieverd. and N.C. Schenck	7.14	1.03	-	-	-	-	-	-
7	*Acaulospora paulinae* Błaszk.	-	-	-	-	23.81	1.96	14.29	2.47
8	*Acaulospora rehmii* Sieverd. and S. Toro	10.71	1.33	-	-	-	-	-	-
9	*Acaulospora scrobiculata* Trappe	21.43	2.30	24.14	4.35	76.19	19.06	38.10	7.39
10	*Acaulospora spinosa* C. Walker and Trappe	7.14	0.99	10.34	1.20	42.86	6.84	-	-
11	*Acaulospora undulate* Sieverd.	-	-	6.90	0.75	4.76	0.73	-	-
	***Entrophospora* R.N. Ames and R.W. Schneid.**	**-**	**-**	**3.45**	**0.22**	**-**	**-**	**-**	**-**

Sp. No.	Arbuscular Mycorrhizal Fungi	Kapadvanj		Satlasana		Vijaynagar		Danta	
		IF	RA	IF	RA	IF	RA	IF	RA
12	*Entrophospora infrequens* (I.R. Hall) R.N. Ames and R.W. Schneid.	-	-	3.45	0.22	-	-	-	-
	***Gigaspora* Gerd. and Trappe**	**35.71**	**2.27**	**20.69**	**1.64**	**-**	**-**	**-**	**-**
13	*Gigaspora albida* N.C. Schenckand G.S. Sm.	10.71	0.53	-	-	-	-	-	-
14	*Gigaspora candida* Bhattacharjee, Mukerji, J.P. Tewariand Skoropad	3.57	0.25	3.45	0.20	-	-	-	-
15	*Gigaspora decipiens* I.R. Hall and L.K. Abbott	3.57	0.34	13.79	1.29	-	-	-	-
16	*Gigaspora gigantea* (T.H. Nicolson andGerd.) Gerd. and Trappe	14.29	0.62	3.45	0.14	-	-	-	-
17	*Gigaspora margarita* W.N. Becker and I.R. Hall	7.14	0.54	-	-	-	-	-	-
	***Glomus* Tul. and C. Tul.**	**100**	**85.67**	**100**	**89.04**	**100**	**65.64**	**100**	**83.53**
18	*Glomus aggregatum* N.C. Schenck and G.S. Sm.	21.43	6.83	44.83	15.82	47.62	13.74	14.29	6.09
19	*Glomus ambisporum* G.S. Sm. and N.C. Schenck	14.29	6.93	10.34	2.78	-	-	9.52	2.81
20	*Glomus aurantium* Błaszk., Blanke, Renker and Buscot	14.29	2.42	-	-	-	-	-	-
21	*Glomus austral* (Berk.) S.M. Berch	10.71	2.15	6.90	0.96	4.76	1.54	-	-
22	*Glomus boreale* (Thaxt.) Trappe and Gerd.	-	-	-	-	19.05	3.82	-	-
23	*Glomus caledonium* (T.H. Nicolson and Gerd.) Trappe and Gerd.	-	-	-	-	4.76	0.19	-	-
24	*Glomus claroideum* N.C. Schenck and G.S. Sm.	-	-	3.45	0.20	-	-	-	-
25	*Glomus clarum* T.H. Nicolson and N.C. Schenck	-	-	10.34	0.60	-	-	-	-
26	*Glomus clavisporum* (Trappe) R.T. Almeida and N.C. Schenck	10.71	0.14	6.90	0.35	9.52	0.04	4.76	0.06
27	*Glomus constrictum* Trappe	25	3.03	24.14	3.34	47.62	1.89	38.10	5.56
28	*Glomus coronatum* Giovann.	-	-	-	-	4.76	0.15	-	-
29	*Glomus deserticola* Trappe, Bloss and J.A. Menge	7.14	2.33	20.69	4.14	4.76	0.44	4.76	1.64
30	*Glomus etunicatum* W.N. Becker and Gerd.	17.86	8.51	37.93	14.93	47.62	7.07	4.76	1.17
31	*Glomus fasciculatum* (Thaxt.) Gerd. and Trappe	32.14	22.45	65.52	29.78	47.62	16.11	57.14	35.41
32	*Glomus fecundisporum* N.C. Schenck and G.S. Sm.	-	-	-	-	-	-	9.52	1.22
33	*Glomus formosanum* C.G. Wu and Z.C. Chen	-	-	13.79	3.39	4.76	0.15	-	-
34	*Glomus geosporum* (T.H. Nicolson and Gerd.) C. Walker	-	-	10.34	0.83	28.57	2.87	14.29	1.67
35	*Glomus globiferum* Koske and C. Walker	7.14	1.85	-	-	-	-	4.76	0.86

Sp. No.	Arbuscular Mycorrhizal Fungi	Kapadvanj		Satlasana		Vijaynagar		Danta	
		IF	RA	IF	RA	IF	RA	IF	RA
36	*Glomus halonatum* S.L. Rose and Trappe	-	-	3.45	0.27	-	-	-	-
37	*Glomus heterosporum* G.S. Sm. and N.C. Schenck	14.29	14.92	3.45	0.57	28.57	9.41	28.57	8.50
38	*Glomus intraradices* N.C. Schenck and G.S. Sm.	10.71	8.54	10.34	3.71	-	-	19.05	8.56
39	*Glomus leptotichum* N.C. Schenck and G.S. Sm.	14.29	1.45	-	-	-	-	-	-
40	*Glomus macrocarpum* Tul. and C. Tul.	-	-	6.90	0.94	23.81	1.57	9.52	2.14
41	*Glomus maculosum* D.D. Mill. and C. Walker	-	-	3.45	1.07	-	-	-	-
42	*Glomus magnicaule* I.R. Hall	3.57	0.49	-	-	-	-	-	-
43	*Glomus microcarpum* Tul. and C. Tul.	3.57	1.41	10.34	3.93	9.52	3.66	-	-
44	*Glomus mosseae* (T.H. Nicolson and Gerd.) Gerd. and Trappe	-	-	-	-	23.81	1.11	19.05	1.45
45	*Glomus multicaule* Gerd. and B.K. Bakshi	-	-	-	-	4.76	0.35	4.76	1.45
46	*Glomus multisubstensum* Mukerji, Bhattacharjee and J.P. Tewari	-	-	3.45	0.40	9.52	0.24	-	-
47	*Glomus pansihalos* S.M. Berch and Koske	3.57	0.22	-	-	9.52	1.14	4.76	1.14
48	*Glomus reticulatum* Bhattacharjee and Mukerji	3.57	0.88	3.45	0.13	-	-	-	-
49	*Glomus rubiforme* (Gerd. and Trappe) R.T. Almeida and N.C. Schenck	17.86	054	6.90	0.18	-	-	14.29	1.31
50	*Glomus sinuosum* (Gerd. and B.K. Bakshi) R.T. Almeida and N.C. Schenck	7.14	0.19	20.69	0.43	9.52	0.12	-	-
51	*Glomus tortuosum* N.C. Schenck and G.S. Sm.	7.14	0.40	10.34	0.30	4.76	0.02	-	-
52	*Glomus versiforme* (P. Karst.) S.M. Berch	-	-	-	-	-	-	14.29	5.67
	***Pacispora* Oehl and Sieverd.**	**-**	**-**	**-**	**-**	**28.57**	**3.47**	**-**	**-**
53	*Pacispora dominikii* (Błaszk.) Sieverd. And Oehl	-	-	-	-			-	-
	***Scutellospora* C. Walker and F.E. Sanders**	**46.43**	**4.45**	**20.69**	**1.73**	**-**	**-**	**10**	**1.81**
54	*Scutellospora biornata* Spain, Sieverd. and S. Toro	21.43	1.53	10.34	0.67	-	-	-	-
55	*Scutellospora erythropus* (Koske and C. Walker) C. Walker and F.E. Sanders	7.14	0.23	-	-	-	-	-	-
56	*Scutellospora fulgida* Koske and C. Walker	28.57	2.42	10.34	1.01	-	-	9.52	1.39
57	*Scutellospora heterogama* (T.H. Nicolson and Gerd.) C. Walker and F.E. Sanders	-	-	-	-	-	-	4.76	1.08
58	Scutellospora *reticulata* (Koske, D.D. Mill. and C. Walker) C. Walker and F.E. Sanders	3.57	0.26	-	-	-	-	-	-
59	*Scutellospora verrucosa* (Koske D.D. and C. Walker) C. Walker and F.E. Sanders	-	-	3.45	0.04	-	-	-	-

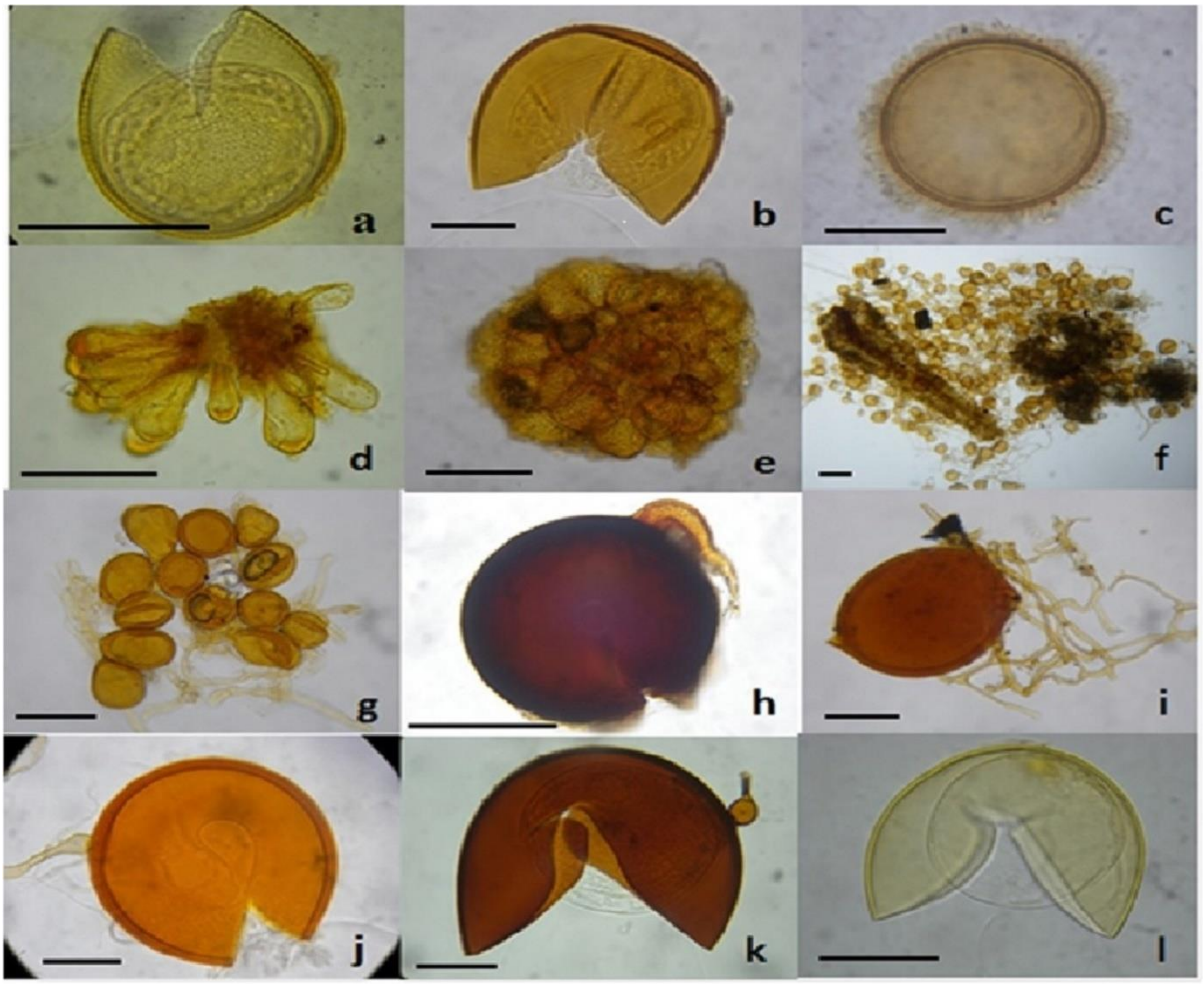

Figure 1: Some Arbuscular mycorrhizal fungi isolated from semi-arid forest of Gujarat. a, Crushed spore of *Acaulospora scrobiculata* showing pitted wall, inner germination wall and Cicatrix; b, Crushed spore of *Acaulospora tuberculata* showing inner germination wall and Cicatrix; c, Spore of *Acaulospora spinosa* with densly organized spines; d, Sporocarp of *Glomus clavisporum*; e, Sporocarp of *Glomus sinuosum* showing thick walled peridium; f, Sporocarp of *Glomus intraradices*; g, Sporocarp of *Glomus fasciculatum*; h, Spore of *Glomus constrictum*showing constricted hyphae; i, Spore of *Glomus multicauli* showing multiple hyphal attachment at opposite side; j, Crushed spore of *Gigaspora decipies*; k, Crushed spore of *Scutellospora biornata* showing inner germination wall, germination shield and bulbous suspensor; l, crushed spore of *Scutellospora fulgida* showing inner flexible wall. Scale Bar = 100 µm

Spore density, root colonization, species richness and AM fungal distribution

The details of spore density, percent root colonization and species richness of AM fungi is given in **Table 5**. The spore density of AM fungi in 50 g of dried soil sample in Kapadvanj ranged from 18-556. AM fungal spore density ranged from 70-681 in Satlasana, 406-2026 in Vijaynagar and 16-398 in Danta. Percent root colonization by AM fungi in all selected sites ranged from 12.9-86.67% in Kapadvanj, 10.71-93.33% in Satlasana, 60-93.33% in Vijaynagar and 16.67-52.17% in Danta. Species richness in all selected sites varied between 2-6 in Kapadvanj, 3-9 in Satlasana, 2-10 in Vijaynagar and 2-6 in Danta. In Vijaynagar mean spore density (1185±101), mean root colonization (75.87±1.84%) and mean species richness (5.95±0.44) were significantly higher than other selected sites. Whereas no significant difference was found in mean spore density in Kapadvanj (218±26), Satlasana (286±34) and Danta (171±24). No significant difference was found in mean root colonization in Kapadvanj (50.28±3.58), Satlasana (47.33±4.28) and Danta (32.74±2.61).

Table 5: MSD, MRC and SR from selected sites

	Site			
	Kapadvanj	**Satlasana**	**Vijaynagar**	**Danta**
MSD 50 gm^{-1}	218±26	286±34	1185±101	171±24
SD range	18-556	70-681	406-2026	16-398
MRC (%)	50.28±3.58	47.33±4.28	75.87±1.84	32.74±2.61
RC range	12.9-86.67	10.71-93.33	60-93.33	16.67-52.17
SR	4.04±0.22	4.38±0.28	5.95±0.44	3.57±0.21
SR range	2-7	3-9	2-10	2-6

MSD, Mean spore density; MRC, Mean root colonization; SR, Species richness

Although no significant difference was found in mean spore density and mean root colonization in Kapadvanj, Satlasana, and Danta but there was remarkable difference in the mean value of spore density and percent root colonization. Mean root colonization and mean spore density was noticeably lower in Danta. Mean species richness was also not significantly different in Kapadvanj (4.04±0.22), Satlasana (4.38±0.28) and Danta (3.57±0.21). Correlation analysis revealed that the weak and non-significant correlation existing between AM fungal spore density and percent root colonization in all selected sites Kapadvanj (r = 0.253, P>0.05), Satlasana (r = -0.110, P>0.05), Vijaynagar (r = 0.135, P>0.05) and Danta (r = -0.182, P>0.05). Significant positive correlation was found in selected sites Kapadvanj (r = 0.476, P< 0.05), Satlasana (r = 0.721, P>0.01), Vijaynagar (r = 0.612, P>0.01) and Danta (r = 0.551, P<0.01) between spore density and species richness of AM fungi. Weak and non-significant correlation was also noted between percent root colonization and species richness of AM fungi in selected sites Kapadvanj (r = -0.145, P>0.05), Satlasana (r = -0.273, P>0.05), Vijaynagar (r = -0.140, P>0.05) and Danta (r = 0.277, P>0.05).

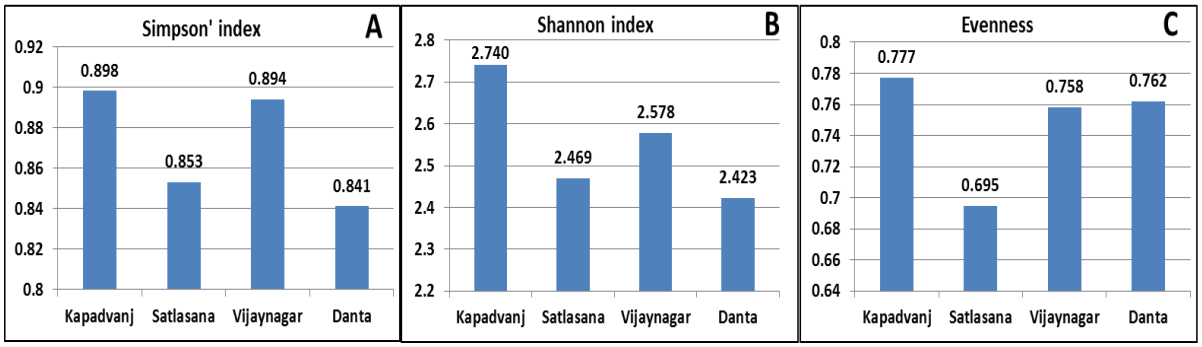

Figure 2: Diversity indices for AM fungi in selected trees at the study sites. A, Simpson's index; B, Shannon-weiner index; C, Evenness

The community analysis of AM Fungi by diversity indices revealed less variation in all selected sites. General diversity (Shannon-Weiner index) was quite higher in Kapadvanj (2.740) followed by Vijaynagar (2.578), Satlasana (2.469) and Danta (2.423). The higher value of evenness in Kapadvanj (0.777) indicates that AM fungal species more evenly distributed than Danta (0.762), Vjaynagar (0.758) and Taranga (0.695). The index of dominance (Simpson's index) was also higher in Kapadvanj (0.898) followed by Vijaynagar (0.894), Satlasana (0.853) and lowest in Danta (0.841) which shows dominance of few AM species in Kapadvanj and Vijaynagar. The Sorenson's coefficient of similarity between selected sites varied between 0.47-0.67 (**Table 6**). The Sorenson's coefficient (C_s) of similarity was highest between Kapadvanj and Satlasana (0.67) and between Satlasana and Vijaynagar (0.59) and lowest between Kapadvanj and Vijaynagar (0.47) and Kapadvanj and Danta (0.48).

Table 6: Sorenson's coefficient (CS) of similarity for AM fungi among selected sites

Site	C_S
Kapadvanj – Satlasana	0.67
Kapadvanj – Vijaynagar	0.47
Kapadvanj – Danta	0.48
Satlasana – Vijaynagar	0.59
Satlasana – Danta	0.51
Vijaynagar – Danta	0.56

The soil and roots collected from rhizosphere of 17 species of trees representing 14 genera and 13 families were examined for AM fungal associations (**Table 7**). The spore density of AM fungi in selected trees varied from 44-1529 per 50 g dried soil. The maximum spore density was found in *Zizyphus glabrata* (1529±113) followed by *Diospyros melanoxylon* (1269±105), *Azadirachta indica* (932±366) and *Madhuca indica* (800±241) and least number of spores was found in *Moringa oleifera* (44±12). The percent root colonization by AM fungi among selected trees varied from 19.02-81.71 percent. The highest colonization was found in *Commiphora wightii* (81.72±3.98), followed by *Diospyrosmel anoxylon* (78.54±3.80), *Zizyphus glabrata* (76.67±6.24) and *Madhuca indica* (67.50±8.32) and lowest colonization was found in *Holoptelea integrifolia* (19.02±1.66).

Table 7: Distribution, spore density, root colonization and species richness of AM fungi in selected plants

Host Plant	AM fungi	SD	RC
Annonaceae			
Miliusa tomentosa (Roxb.) Sinclair	2 4 7 9 11 15 18 19 24 27 29 30 31 32 37 49 51 52 54 56 57	212±39	48.04±7.70
Apocynaceae			
Wrightia tinctoria R.Br.	7 8 9 10 18 19 26 27 30 31 34 35 38 40 44 48 49 51 56 58	226±47	51.59±4.87
Wrightia tomentosa R. and S.	1 9 1618 19 25 27 29 30 31 37 42 54 56	158±37	62.36±9.06
Bignoniaceae			
Tecomella undulata (Sw.) Seem.	1 8 9 18 19 20 26 27 30 31 37 38 39 49 50 54 55 56	298±42	61.79±4.10
Bombacaceae			
Bombax ceiba L.	10 11 15 16 17 18 20 21 26 27 29 30 31 37 38 43 48 49 50 51 54 56	238±65	28.73±4.04
Burceraceae			
Commiphora wightii (Arn.) Bhandari	10 14 15 18 27 30 31 33 38 40 41 43 50 56	388±55	81.72±3.98
Ebenaceae			
Diospyros melanoxylon Roxb.	1 3 5 9 10 11 18 22 27 28 30 31 34 43 44 45 46 47 50 51 53	1269±105	78.54±3.80
Diospyros montana L.	9 10 18 19 25 31 34 38 43 49 50 51 54 59	166±37	43.81±3.91
Meliaceae			
Azadirachta indica A. Juss	2 7 9 18 23 27 30 31 33 34 37 38 40 43 44 46 50 53	932±366	63.33±5.16
Melia azadirachta L.	16 9 13 18 21 27 30 31 33 34 35 37 56	464±92	44.45±6.11
Moringaceae			
Moringa concanensis Nimmo	4 6 9 14 16 18 19 27 30 31 36 37 39 49 52 54	146±33	38.18±5.80

Host Plant	AM fungi	SD	RC
Moringa oleifera Lam.	7 9 13 16 18 19 26 31 37 44 51	44±12	43.33±4.55
Rhamnaceae			
Zizyphus glabrata Heyne ex Roth	7 9 10 18 22 27 30 31 34 40 43 44 53	1529±113	76.67±6.24
Sapotaceae			
Madhuca indica J.F. Gmel.	1 9 10 17 21 27 37 47 49 50	800±241	67.50±8.32
Sterculiaceae			
Sterculia urens Roxb.	1 9 10 18 21 24 26 27 30 31 33 34 35 38 40 45 46 47 50	339±74	26.03±3.31
Ulmaceae			
Holoptelea integrifolia (Roxb.) Planch	7 9 18 20 27 29 30 31 38 39 40 47 49 50 56	178±21	19.02±1.66
Verbinaceae			
Vitex negundo L.	9 12 18 26 27 29 30 31 34 37 49	277±76	45.58±11.45

The numbers correspond to the species of AM fungi (Table 4) associated with the selected tree species

The number of AM fungal species associated with selected trees highly varied from 8-22. The maximum number of species was recovered from *Bombax ceiba* (22), *Diospyros melanoxylon* (21), *Miliusa tomentosa* (21), *Wrightia tinctoria* (20) and *Sterculia urens* (19) and least number of species was recorded in *Moringa oleifera* (8). It was noted that the plant species, belonging to the same genera or family shows varied degree of colonization and spore density when collected from different sites or plant community (data not showed). The AM fungal species showed no host specificity in present study. Moreover, the same plant species was found to be associated with different AM fungal species when collected from different sites or different plant community.

DISCUSSION

In the present study, the soil of all the selected sites showed that the rhizosphere soil was nutrient rich and nutrients are readily available in all selected sites except available phosphorous in Vijaynagar. A high number of AM fungal species (59) were found in semi-arid forest of Gujarat, India. The diversity of AM fungi was greater than that in jhum fallow and natural forest soils (44) of Arunachal Pradesh, northeastern India (Singh *et al*., 2003) and in the Western Ghats region (35), Southern India (Muthukumar and Udaiyan, 2000), in the hot-dry valley (43) of the Jinsha River, southwest, China (Zhao and Zhao, 2007), semi-arid region (44) of Namibia (Uhlmann *et al*., 2004). The possible reasons for the high diversity of AM fungi in semi-arid regions, could be their preference for arid and hot habitat (Stutz and Morton, 1996; Stutz *et al*., 2000), preference of different host plants (Martínez-García and Pugnaire, 2011; Yang *et al*., 2012) and physical and chemical properties of soil (Martínez-García and Pugnaire, 2011).

The present study confirms that *Glomus* is the most dominant genus in semi-arid regions of Gujarat, India. Soil samples of sites were nearly neutral to slightly alkaline and genus *Glomus* was dominant in this soil type and it is reported that *Glomus* is common in neutral and slightly alkaline soils (Mukerji *et al*., 2002). The dominance of genus *Glomus* was recorded by various workers in different arid and semi-arid areas (Pande and Tarafdar, 2004; Uhlmann *et al*., 2004; Zhao and Zhao, 2007; Kamalvanshi *et al*., 2011). Moreover, dominance of genus *Glomus* was also recorded in India by different workers in various ecosystems like coastal sand dunes of the west-coast of India (Beena *et al*., 2000), jhum fallow and natural forest soils of Arunachal Pradesh, north-eastern India (Singh *et al*., 2003), in Thar desert (Panwar and Tarafdar, 2006a), arid zones of Rajasthan (Panwar and Tarafdar, 2006b) and in the Western Ghats region, Southern India (Muthukumar and Udaiyan, 2000). The possible reason for this dominance of genus *Glomus* is the ability of its spores to germinate in varied temperature and pH (Wang *et al.*, 1997). Dominance of genus *Glomus* may also be due to the availability of moisture and time

taken for maturity. *Glomus* species possess small spore in size and attain early maturity as compared to family *Gigasporaceae* in semi-arid areas where moisture is retained for short period of time (Boddington and Dodd, 2000; Tao *et al*., 2004).

In present study, it was noted that the IF and RA of genus *Acaulospora* was quite higher in Vijaynagar than the other selected sites. The possible reason for the dominance of *Acaulospora* in Vijaynagar is the slight soil acidity (6.6-7.2) whereas soils from other selected sites were neutral to basic in nature Kapadvanj (7.1-7.9), Satlasana (7-7.9) and Danta (7.3-7.9). This is in accordance with the study that members belonging to genus *Acaulospora* prefer acidic soil (Abbott and Robson, 1991; Charoenpakdee *et al*., 2010). Moreover, thegenus *Gigaspora* and *Scutellospora* were common in Kapadvanj and Satlasana in contrast to that of *Scutellospora* present in Danta with very low IF and RA, and its absence in Vijaynagar. The soil of Kapadvanj and Satlasana was sandy whereas soil of Danta and Vijaynagar was sandy loam, which is an agreement with the study that members belong to genus *Gigaspora* and *Scutellospora* normally prefer sandy soils (Lee and Koske, 1994). The study recorded that there was a significant positive correlation between isolation frequency and relative abundance at all selected sites, and it shows that the AM fungal species producing more spores have wider distribution, while species producing less spores having small geographic ranges. Similarly Zhao and Zhao (2007) also reported significant positive correlation between IF and RA in the hot dry valley of the Jinsha River, southwest, China.

In the present study, AM fungi exhibited little or no host specificity. Various studies also confirm that AM fungi lack host specificity (Mohammad *et al*., 2003; Muthukumar and Udaiyan, 2000; Panwar and Tarafdar, 2006a; Panwar and Tarafdar, 2006b; Radhika and Rodrigues, 2010). It could be due to the fact that the community composition of AM fungi was affected by environmental factors and vegetation (Brundrett, 1991). This study showed that there was a lower Shannon-Weiner index in Danta than other selected sites and it could be due to the less number of species present in Danta.

However no considerable difference was found in evenness and Simpson's index in all selected sites, indicating a stable and a diverse AMF community. There was a considerable difference found in Sorenson's coefficient of similarity (ranged, C_S= 0.47-0.67) between selected sites, indicating that community of AM fungiis quite different among selected sites. Öpik *et al*. (2006) studied the AM fungal composition at global level and observed different distribution pattern in AM fungal composition, e.g. some species distributed globaly while others were limited to a few ecosystem only. A similar result was found in present study; among 59 species of AM fungi only 8 species of AM fungi were common in all selected sites. This could be due to the reason that AM fungal species composition is dependent on plant community existing in an ecosystem (van der Heigden *et al*., 1998), environmental factor (Brundrett, 1991), and disturbance (Zhang *et al*., 2004; Das and Kayang, 2009).

The spore density, percent root colonization and species richness are significantly higher in Vijaynagar than in other selected sites. Although no significant difference was found in spore density and percent root colonization but considerable difference was noted in mean value of spore density and percent root colonization. Mean spore density and mean percent root colonization were lower in Danta than Kapadvanj and Satlasana. The less spore density and percent root colonization with an increase in soil AP was observed in the study can be attributed to the fact that, AP inhibits AM root colonization as well as their density (Datta and Kulkarni, 2012). Phosphate deficiency can increase root exudation by a potential host plant, which may be correlated with the degree of AM fungal formation (Elias and Safir, 1987). Earlier it was also observed that very high phosphorus levels resulted in aborted AM fungus penetration of roots by a reaction at the root periphery and their illustrations suggest that this process occurred within exodermal short cells (Amijee *et al.,* 1989). More availability of organic carbon also could be the reason of high spore density and percent root colonization in Vijaynagar. Earlier Khanam *et al*. (2006) reported positive correlation between spore density and organic carbon; percent root colonization and organic carbon.

The spore density was highly variable in selected sites (18-2026 per 50 g dried soil). The similar result was observed by Zhao and Zhao (2007) in the hot-dry valley of the Jinshariver and Radhika and Rodrigues (2010) in selected medicinal plants of Western Ghats, Goa region. The mean spore density observed in all selected sites was quite higher (170-1185 per site per 50 g dried soil) in comparison to jhum fallow and natural forest soils of Arunachal Pradesh, north eastern India (Singh *et al*., 2003) and semi-arid region of Namibia (Uhlmann *et al*., 2004). The number of spores in different plants also varied in selected trees (44-1529 per 50 g dried soil). A similar result was also noted by Shi *et al*. (2006) in species belongs to *Meliaceae* family and Zhao *et al*. (2001) in plants collected from the tropical rain forest of Xishuangbanna, southwest China.

The large variation in spore population may be due to different species of AM fungi have different ability of sporulation (Bever *et al*., 1996). This can be attributed to several reasons, interspecific competition between AM fungi (Brundrett and Kendrik, 1990), competition between AM fungi and environmental factors (Gemma and Koske, 1988), Seasonality (Fontenla *et al*., 1998), host dependence (Bever *et al*., 1996), metabolic pathway of host plants (Lugo and Cabello, 2002) and soil physico-chemical properties (Bhardwaj *et al*., 1997). Also sporulation rate is quite higher in senescing roots (Sutton and Barron, 1972) and when root activity is interrupted by a long dry season (Janos, 1980).

The variation in percentage of colonization in selected sites (10.71-93.33 percent) and forest trees (19.02-81.71 percent) were recorded in present study. The result of the present study is in accordance with various reports (Sharma *et al*., 1986; Raghupathy and Mahadevan, 1993; Muthukumar and Udaiyan, 2000; Dhar and Mridha, 2006). The large variation in root colonization in natural ecosystems and in host plant is greatly affected by plant life forms (Muthukumar and Udaiyan, 2000), seasonal variation in development of host plants (Sutton and Barron, 1972), soil types and quality (Raman and Gopinathan, 1992), edaphic and climatic factors (Abbott and Robson, 1991; Khade and Rodrigues, 2010), variable host susceptibility (Mehrotra, 1998) and ability of the AM fungi to produce hydrolytic enzymes (Gianinazzi-Pearson, 1994). The involvement of intercellular or intracellular mycorrhizal associations or association of more than one mycorrhizal fungus with single host tree might be attributed to their physiological, ecological, and genetical variability (Sharma *et al*. 1986).

Weak and non-significant correlation was found between percent root colonization and spore density in all selected sites contradicts several previous reports (Muthukumar and Udaiyan, 2000; Muthukumar *et al*., 2003) in which highly significant positive correlation found between percent root colonization and spore count. However, this observation is consistent with other study in nature soils (Brundrett, 1991; Shi *et al*., 2006). Present study indicates that there was a positive correlation existing between spore count and species richness of AM fungi in other sites. This result is in accordance with Zhao and Zhao (2007) who found positive correlation between spore density and species richness in the hot-dry valley of the Jinsha River, southwest China. Rosendahl and Stykenbrock (2004) studied the community structure of AM fungi by using LSU r DNA and found that non-sporulating AM fungal species might be dominant in undisturbed soil, and moderate disturbance of soil could favor the spread and growth of fast sporulating AM fungi. Therefore, it is possible that these disturbed systems favored sporulating species in hot and semi-arid climate. Such fast growing, sporulating species have better chance to survive. Whereas, lack of noticeable relationship between percent root colonization and species richness found in the present study is in contrast with the report of Shi *et al*. (2006). These findings suggest that the detailed study on the relation between spore density, percent root colonization and species richness in the natural ecosystems need further investigation.

The forest sites selected for the study belong to semi-arid regions of Gujarat, India. The ecosystem is characterized by high temperature and drought stress throughout the year. These conditions are limiting factors for plant growth and establishment. In present study, all the 17 species of trees representing 14 genera and 13 families surveyed were colonized by AM fungi. Similar situation have been reported in drought influenced

ecosystems (Mukerji and Kapoor, 1986; Stutz *et al*., 2000). In contrast to that Zhao *et al*. (2001) found 56% of plants were mycorrhizal in humid tropical rain forest; Onguene and Kuyper (2001) demonstrated that 79% plants were arbuscular mycorrhizal in the rain forest of south Camerroon; Tawaraya *et al*. (2003) found 77% of tree species grown in peat swamp forests of central Kalimanthan, Indonesia were arbuscular mycorrhizal. Comparison of AM fungal colonization intensity in roots of different ecosystems suggests that the plants growing in semi-arid or arid regions where temperature is high and water is limiting might be more dependent on AM fungal association.

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