Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2015, 5(3):13-19

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Diversity of Arbuscular Mycorrhizal fungi (AMF) in the rhizosphere of sugarcane

N. Suresh and R. Nelson

PG and Research Department of Botany, Government Arts College, Ariyalur, Tamil Nadu, S. India

---

ABSTRACT

The present study deals with the diversity and distribution of AMF in the rhizosphere soil of sugarcane in different sites in Ariyalur, Perambalur and Thanjavur districts of Tamil Nadu. S. India. The results revealed variation in AM spore population, root colonization and number of AM fungal species in different sampling season. Higher spore population and number of AM fungal species was recorded in the rainy season (August to October), minimum were recorded in the winter season (November to February) and lowest were recorded in the summer season (March to May). Altogether twenty five different AM fungal species belonging to four genera viz., Glomus, Gigaspora, Acaulospora *and* Scutellospora *were characterized. They showed variable distribution pattern. It was observed that* Glomus *was the most dominant genus in the rhizospheric soil of sugarcane.* Glomus aggregatum, G. *mosseae* and *G.* fasciculatum *were identified in all the sampling seasons.*

Key words: AM colonization, Sugarcane, seasonal variation

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with most economically important plants [1,2]. These fungi improve plant growth under low fertility conditions, confer tolerance to some plant pathogens, improve water balance of the plants, and contribute to the formation of soil structure and also help plants to become established in new areas [3]. AM colonization varies with change of season. Seasonal effects also influence the establishment of plants under field conditions, depending on the efficiency of the indigenous AM fungi [4]. Information on seasonal variation of spore count and root colonization of AM fungi is useful for timely inoculation of suitable species. So, the present was aimed at investigating natural AM fungal spore diversity in the rhizosphere soil and its colonization in sugarcane as a function of seasonal variation at different sites in Ariyalur, Perambalur and Thanjavur districts of Tamil Nadu, S. India.

MATERIALS AND METHODS

Location of Sampling Sites

The study sites were located in three districts of Tamil Nadu in India, which included 1.Ariyalur: (Vellaur, Sokkanathapuram, Thamaraikulam, Keelarayampuram and Unjini) 2.Perambalur: (Neikuppai, Puthur, Nallarikkai, Kolappadi and Kadur) and 3. Thanjavur (Villangudi, Villiyanallur, Semmangudi, Okkakudi and Anaikudi). Ariyalur

is located at Latitude 11°0812'.09"N, Longitude 79°04'33"E, Altitude 83m. Annual rainfall is 954mm. Ariyalur is located in between Thanjavur and Perambalur. Geographically Perambalur district is located between 78° 52' 59.85"E longitude and 11°14'00.59"N latitude. There is a marked diurnal temperature differences: The temperature can be as below as 20 °C in June and high as 34°- 42 °C in March. The annual rain fall is 861 mm. Soil is mainly black coloured, sandy clay loam. Thanjavur is situated beside the mighty River Cauveri, Thanjavur is geographically located in between 10.8°N and 79.15°E. Thanjavur has a tropical climatic condition. During summer the average temperature of the city rises to 36.6 °C, while during the winter season, it averages to 30.2 °C, and it at experiences heavy rain of about 111.37 cm during the rainy season.

Collection of root and soil samples

At different seasons the soil samples and fine roots were collected in polythene bags from each plant. About 200-250 gm of soil samples from rhizosphere of each plant at a depth of 15-30 cm was collected in polythene bags. These samples were mixed to from a composite sample and then brought to laboratory and used for the isolation of AM fungal spores, mycorrhizal quantification, root colonization and stored at 5-10 °C. The fine roots in sample were removed, rinsed with tap water and fixed in formalin: acetic acid: alcohol (FAA), and used for the determination of root colonization. The soil samples were then air dried in the shade at laboratory temperature for spore counting.

Isolation of AM fungi from soil

'Wet-Sieving and Decanting technique' was used for isolation of AM fungi spores [5]. For this, sieves of different sizes i.e. 150µm, 120µm, 90µm, 63µm, and 45µm wereused. Soil sample (50gm) was thoroughly mixed in 500ml water in a beaker using magnetic stirrer and allowed to settle. The sieves were placed in the following order 150µm, 120µm, 90µm, 63µm and 45µm from top to bottom. Thewater of the beaker was decanted on a series of sieves, on which spores were trapped and then they were washed with running tap water. The trapped spores were transferred to Whatman No. 1 filter paper by repeated washing with water. Spores were picked using a needle under stereo binocular microscope. The spores were mounted on glycerol for further observation.

Mycorrhizal quantification

Quantitative estimation of AM fungi spores was done by modified Grid line intersect method [6]. In this method, the filter paper was divided into many small compartments by a ball point pen and each compartment was numbered. The total numbers of spores were counted under stereo binocular microscope by using counter.

Determination of Root Colonization

Freshly collected root samples were washed gently to free from soil particles. Roots were treated with 10% KOH solution for 30 min in a hot bath. The treated roots were washed with water and treated with 2% HCl solution. Acidified root samples were stained with 0.05 % trypan blue in lactic acid for 10-15 min in a hot bath. The roots were destained with lactic acid and observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope. Fungal structures are stained and can be easily recognized [7]. The mycorrhizal colonization was determined by using the following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Identification of AM fungi

The main structures of AM fungi, the spores were used for identification. Following morphological criteria *viz*., colour, size, shape, wall structure, bulbous suspensor, the number and arrangement of spores in the sporocarps were used for AM fungi identification. The AM fungal spores were identified using the identification manual of Walker, Sheneck, Perez and Mukerji [8,9,10].

Statistical Analysis

Ecological measures of diversity used to describe the structure of AMF communities included spore density, species richness, relative abundance, isolation frequency, Shannon-Wiener index of diversity, Simpson's index of dominance. The formula used to calculate these parameters is given in the Table - 1. Spore density reflects the biomass of AMF species, at least to some extent. Relative abundance was defined as the percentage of spore number of a species, which indicated the sporulation ability of different species of AMF. Isolation frequency was defined as the percentage of soil samples in which a species occurred, which revealed extent of distribution of given AMF

species in an ecosystem. The degree of diversity was reflected by Shannon-Wiener index of diversity. The Pearson correlation coefficient was employed to determine the relationship between Spore density and Species Richness, Relative abundance and Isolation frequency.

RESULTS AND DISCUSSION

The rate of AM fungal colonization in roots and endo-mycorrhizal spore diversity in the rhizosphere of sugarcane showed wide range of variation in different seasons and different sites. Maximum abundance of AM spores was recorded during the rainy season followed by winter season and spore counts were minimum in summer season. A total of 12,129 spores of AMF belonging to 25 AM fungal species were wet sieved from soil sample collected from ten different agro ecosystems. Of the isolated AM spores, *Glomus* was represented by 16 species forming the predominant genus followed by *Acaulospora*, *Gigaspora*, and *Scutellospora*, which were represented by three species each. The identified species were *Glomus aggregatum, G. fasciculatum, G. mosseae, G. macrocarpum, G. ambisporum, G. fulvum, G. multisubstatum, G. maculosum, G. geosporum, G. scintillans, G. deserticola, G. constrictum, G. clarium, G. palvinatum, G. reticulatum, G. flavisporum, Aculospora bireticulata, A. spinosa, A. elegans, Gigaspora margarita, Gi. candida, Gi. decipiens, Scutellospora nigra, S. minuta, S. calospora* (Table -2). The results of the present study supports the findings by Trimurtulu and John [11] and Lakshman *et al.*, [12] who also reported the dominance of the *Glomus*. Sharma *et al.* also reported the dominance of the *Glomus* [13]. They described that the wider adaptation of this taxon in varied soil conditions may be attributed to the sporulation pattern of *Glomus*. *Glomus* species are considered as cosmopolitan fungi in many ecosystems [14]. They dominate habitats in cold, temperate and tropical regions. They usually occur in neutral and slightly alkaline soil [15]. Other genera were less common in the present study, with only a few examples of species, such as *Gigaspora* and *Scutellospora*. There were only a small number of species present in the Gigasporaceae. Often, *Gigaspora* species predominate in sandy soils such as dunes [16]. *Acaulospora* species are frequently associated with acidic soil [17]. It is possible that spores of *Gigaspora* and *Scutellospora* took longer time to form and mature. The members of Gigasporaceae typically established an extensive mycelium in soil and produced fewer spores than members of the Acaulosporaceae and Glomaceae [18, 19].

Table – 1 Diversity measures used to describe AM communities

Spore density (SD)	The number spores in 100gm soil
Species richness (SR)	Number of identified AMF species per soil sample
Relative abundance (RA)	$\frac{\text{Spore number of a species (genus)}}{\text{Total number of identified spore samples}} \times 100$
IF (Isolation Frequency)	$\frac{\text{The number soil samples in which AMF species occurred}}{\text{The total number of soil samples}} \times 100$
Simpson's index of dominance	$D= \sum (n_{i/} N)^2$
Shannon –Wiener index of Diversity	$H' = -\sum P\ i\ In\ P\ i$
P is the relative abundance of each identified species per sampling site and calculated by the following formula,	

Climatic seasons seem to be more influential on the distribution and abundance of mycorrhizal spores. Spore counts steadily increased from July reaching a maximum value in October (rainy season). After November, spore abundance decreased steadily in winter and attained its minimum in March and May (summer) (Fig. -1 & Table - 3). The results of the present study coincides with the observations made by earlier authors who have reported that the spore population varied during rainy, winter and summer seasons. Spore populations were observed to be maximum in rainy season when compared to winter and summer seasons [20, 21].

Table - 4 reveals that the percentage of AMF colonization of sugarcane root varied greatly among different study sites. Highest infection rate of 90% was observed at Kolappady and Neikuppai and lowest infection rate of 30% was recorded at Semmangudi. AM root colonization, was higher in winter season followed by the rainy season. The colonization was minimum in summer season. The highest colonization was observed in samples collected from Perambalur District followed by the samples collected from Ariyalur District. Lowest colonization was observed in

samples collected from Thanjavur District (Table -4). The result of the present study supports the findings of Gemma and Koske [22].

Table – 2 Relative abundance (RA) and Isolation frequency of AM in Rhizosphere soil of Sugarcane

Name of the Organisms	Relative Abundance (RA)%	Isolation Frequency (IF) %
1.*Glomus aggregatum*	10.47	80
2.*Glomus fasciculatum*	9.72	70
3.*Glomus mosseae*	8.72	60
4.*Glomus mcrocarpum*	3.74	40
5.*Glomus ambisporum*	3.99	30
6.*Glomus fulvum*	2.74	60
7.*Glomus multisubstatum*	4.23	40
8.*Glomus maculosum*	3.24	20
9.*Glomus geosporum*	2.49	10
10 *Glomus scintillans*	4.73	60
11.*Glomus deserticola*	2.74	50
12.*Glomus constrictum*	2.99	30
13.*Glomus clarium*	3.24	40
14.*Glomus palvinatum*	5.23	60
15.*Glomus reticulatum*	2.74	20
16.*Glomus falicsculatum*	2.99	10
17.*Aculospora bireticulata*	3.49	60
18.*Aculospora spinosa*	1.99	50
19.*Aculospora elegans*	2.49	10
20.*Gigaspora margarita*	2.24	70
21.*Gigaspora candida*	1.74	40
22.*Gigaspora decipiens*	1.99	40
23.*Scutellospora nigra*	2.99	60
24.*Scutellospora minuta*	3.99	20
25.*Scutellospora calospora*	2.49	10

Table - 3 The Seasonal variation of spore density

Sites	Rainy Season (August-October)	Winter Season (November-February)	Summer Season (March-May)
Thanjavur(dt)			
1.Vilangudi	401	292	133
2.Semmangudi	348	267	189
3.Anaikkudi	237	161	146
4.Okkakudi	318	198	111
5.Villiyanallur	299	197	109
Perambalur(dt)			
6.Kolappady	539	332	207
7.Nallarikkai	336	220	248
8.Kadur	290	211	132
9.Neikuppai	496	331	222
10.Puthur	382	234	185
Ariyalur(dt)			
11.Vellur	428	289	199
12.Sokkanathapuram	309	199	156
13.Keelarayampuram	497	239	225
14.Thamaraikulam	502	309	221
15.Nagulkuzhi	401	273	211
Total Spores	12,129		

Table -4 Percentage of root colonization of AMF in roots of Sugarcane

Study sites	% of AM Colonization in Roots	No. of vesicles per Roots	No. of Arbuscles per Roots
Thanjavur(dt)			
1.Vilangudi	40	6	2
2.Semmangudi	30	4	1
3.Anaikkudi	50	3	2
4.Okkakudi	40	4	1
5.Villiyanallur	40	5	2
Perambalur(dt)			
6.Kolappady	90	8	4
7.Nallarikkai	60	4	1
8.Kadur	50	3	2
9.Neikuppai	90	9	4
10.Puthur	80	7	1
Ariyalur(dt)			
11.Vellur	60	6	1
12.Sokkanathapuram	80	4	1
13.Keelarayampuram	70	5	2
14.Thamaraikulam	70	7	3
15.Nagulkuzhi	70	8	3

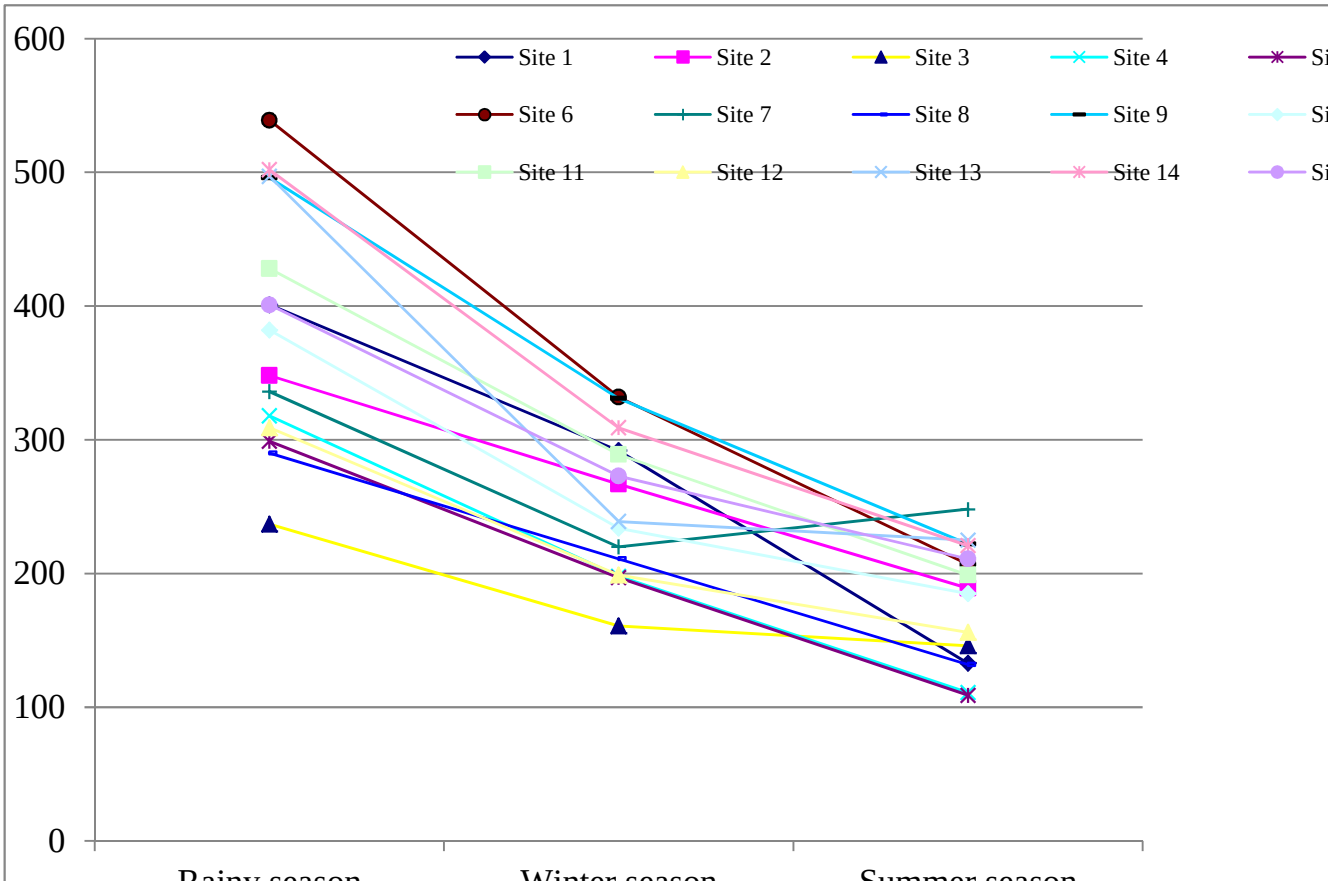
Table - 5 Diversity measurement of AMF community

Sites	Spore density (SD)/100 of soil	Species Richness (SR)	Shannon-Wiener index of diversity (H')	Simpson's of dominance (D)
S1	610	4	0.448	0.4484
S2	570	5	0.274	0.2735
S3	499	3	0.343	0.3425
S4	502	4	0.336	0.3363
S5	526	5	0.155	0.1553
S6	742	11	0.492	0.4915
S7	592	7	0.369	0.3688
S8	488	5	0.336	0.3358
S9	731	9	0.147	0.1472
S10	680	8	0.273	0.2731
S11	599	5	0.426	0.4261
S12	636	4	0.199	0.1986
S13	703	8	0.233	0.2334
S14	721	8	0.183	0.1825
S15	706	10	0.227	0.2268

In this investigation the species richness was maximum at Kolappady (SR 11 %, SD 742/100g soil) followed by Nagulkuzhi (SR 10 %, SD 706/100g soil). Spore density and species richness were found to be minimum in Anaikkudi (SR 3 %, SD 499/100g soil) (Table -5). Based on relative abundance and isolation frequency, it was observed that *Glomus aggregatum* was most dominant (10.47 % of RA) followed by *Glomus fasciculatum* (9.72 % of RA) and *Glomus mossae* (8.72 % of RA). However, *Glomus aggregatum* contributed to greater isolation frequency (80%) and was widely distributed followed by *Glomus fasciculatum* (70%). Furthermore, Shannon-Wiener index of diversity (H') and Simpson's index of dominance (D) showed greater diversity. Species diversity was calculated using these two indices. The Shannon–Weiner diversity index ranged from 00.147 to 00.492. The

highest occurred in S6 (H'=00.492) and the lowest in S9 (H'=00.147). Similarly, the Simpson's index ranged from 0.1825 to 0.4915, the highest was in S6 and the lowest was in S14 (Table - 5).

Fig. – 1 The Seasonal variation of spore density Rainy Season, Winter Season & Summer Season



Site1-Vilangudi 2.Semmangudi 3.Anaikkudi 4.Okkakudi 5.Villiyanallur 6.Kolappady 7.Nallarikkai 8.Kadur 9.Neikuppai 10.Puthur 11.Vellur 12.Sokkanathapuram 13.Keelarayampuram 14.Thamaraikulam 15.Nagulkuzhi

CONCLUSION

The present study shows good association of AM fungi in sugarcane plants in the agro-ecosystem. It also emphasizes the fact, that this symbiosis is controlled by various edaphic factors. Higher levels of fungal root colonization is an indication of better fungal root contact which is a prerequisite for increased benefits of AM symbiosis and better adaptation to present soils. Rainy seasons should be preferable (spore density was higher) for AM fungal inoculation as compared to summer season. The present study results showing increasing spore density (Rainy season) and root colonization with increase in winter season. *Glomus* sp was dominant in all sites. The sugarcane plant offers the possibility of using AM fungi as a potential bio- fertilizer for enhancement of crop growth as well as productivity.

Acknowledgements
Authors are highly thankful to Dr. T. Kavitha, Assistant Professor. PG & Research Department of Microbiology, J.J. College of Arts & Science, Pudukkottai for helping in identification AM fungi.

REFERENCES

[1] Gerdemann, J.W. *Ann Rev Phytopathol*, **1968**, 6, 397-418.
[2] Thapar G.R, Dunisway J.M, *Am Phytopathol Soc* USA, **1992,** 77- 80.

[3] Panwar J and Vyas A**,** *Mycorrhiza News*, **2002**, 14: 14-16.
[4] Giovannetti M. and Nicolson T.H, *Trans. Br Mycol Soc* **1983**, 80, 552-557.
[5] Gerdemann J.W, Nicolson Y.H, *Br Mycol Soc*, **1963**, 46, 235-244.
[6] Adholeya A, Gaur A, *Mycorrhiza News*, **1994**, 6, 10-11.
[7] Philips J.M, Hayman D.S, *Br Mycol Soc*, **1970**, 55 (1), 158-161.
[8] Hart, M.M. & R.J. Reader, *New Phytologist*, **2002**, 153, 335- 344.
[9] Schenck NC, Perez Y, *Manual for identification of VAM fungi*, 3rd Ed.Synergistic Publication Gainesville, Florida, U. S .A. **1990**.
[10] Mukerji KG, Mathur B, Chamola B P and Chitralekha P (Eds), *Advances in Botany*, APH Corporation, New Delhi, India, **1996,** pp 213-221.
[11] Trimurtulu N, Johri B.N, *J Mycol Plant Pathol*, **1998,** 28:236-239.
[12] Lakshman, H.C, National Conference, **1999,** 97(1), 105-110.
[13] Sharma S.K, Sharma G.D, Mishra R.R, *Acta Bot Ind*, **1986**, 14, 87-92.
[14] Sýkorová Z, Ineichen K, Wiemken A, Redecker D, *Mycorrhiza.*, **2007**, 18, 1-14.
[15] Mukerji KG, Manoharachary C, Chamola, BP *Techniques in mycorrhizal studies.* Kluwer Academic Publishers, Dordrecht, Boston, London, **2002**.
[16] Lee PJ, Koske RE, *Mycol Res* **1994,** 98, 453–357.
[17] Abbott AD, Robson, *Agric Ecosys Environ*, **1991**, 35, 121-150.
[18] Hepper, C.M, *Isolation and culture of VAM fungi*. In: *VA Mycorrhizae*. Powell, C.L & Bagyaraj D.J. (Eds), CRT Press, Florida, USA, **1984**, pp 95-112.
[19] Piotrowski J. S., Denich T., Klironomos J. N., Graham J. M. and Rillig M. C, *New Phytol***, 2004,** 164, 365–373.
[20] Sangeeta Kaushal**,** *J Mycol Plant Pathol* **2000,** 30(3), 386-388.
[21] Ragupathy S, Mahadevan A, *Mycorrhiza* **1993**, 33, 123-136.
[22] Gemma J.N, R.E. Koske, *Mycologia*, **1988**.80, 211-216.