

Research Paper :

Diversity of AM fungi in different farm soils

SUCHITRA RAKESH, K. KUMUTHA AND D. BALACHANDAR

Accepted : February, 2010

See end of the article for authors' affiliations

Correspondence to :

K.KUMUTHA

Department of
Agricultural
Microbiology, Centre of
Plant Molecular Biology,
Tamil Nadu Agricultural
University,
COIMBATORE (T.N.)
INDIA

ABSTRACT

The present investigation was focused on assessment of AM fungal diversity in different agricultural soils. Soil samples were collected from three different agricultural soils. All the three soil samples were analysed for physico-chemical characteristics (standard method) and diversity of AM fungi. By considering spore wall layers, shape and colour of the spore, 8 different species was observed morphologically in eastern block soil (sandy clay loam) namely, *Glomus clarum*, *Glomus fasciculatum*, *Gigaspora decipens*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus viscosum*, *Scutellospora* sp. and *Glomus geosporum*. Six different AM species were identified from millet breeding station sample (clay loam) as, *Glomus mosseae*, *Gigaspora margarita*, *Glomus fasciculatum*, *Scutellospora* sp. and *Glomus geosporum*. From dryland sample (sandy clay loam), six species identified are *Acaulospora* sp., *Glomus mosseae*, *Scutellospora* sp., *Gigaspora gigantea* and *Glomus albidum*.

Key words : Diversity, Assessment, AM species, Physico-chemical, Agricultural soils

Arbuscular mycorrhizal (AM) fungi form associations with the majority of terrestrial plant species and improve the growth and health of individual plants (Smith and Read, 1997). AM fungi are thought to be the oldest group of organisms living in symbiosis with land plants (Blackwell, 2000; Redecker *et al.*, 2000). The fungi involved are in 19 genera and around 210 species belonging to the newly established phylum Glomeromycota (Schubler *et al.*, 2001). AM fungi have been shown to have a pivotal role in plant community ecology by altering plant productivity and diversity (Klironomos *et al.*, 2000), changing the course of succession (Gange *et al.*, 1999), and affecting plant competition (Hartnett and Wilson, 1999). Until recently, AM fungal species were generally assumed functionally similar, so there was little focus on AM fungal diversity (van der Heijden *et al.*, 1998) and ecosystem variability and productivity (Hart and Klironomos, 2002) since these are directly influenced by AM fungi diversity, making an accurate assessment of species richness and community composition is crucial to understand the role of AM fungi in ecosystem functioning.

The Vesicular–arbuscular forms are the most common and widely occurring mycorrhizal associations. In fact, it has been suggested that up to 25,000 plant species have potential to form vesicular arbuscular mycorrhiza with representatives largely from crop plants, herbs and tropical trees. These mycorrhizas are agriculturally important and have greater economic significance. These mutualistic associations may result in enhanced survival, nutrient acquisition, reproduction and growth for the component organisms (Smith and Read,

1997). The fungus invades plant root tissues and in general, gains a supply of carbon from the host, vegetative structures of these fungi (*i.e.* mycorrhizae and mycelium in the soil) occur largely under ground and are difficult to be tracked and identified. Identification of biological species in the AM fungi was mostly investigated based on the morphological and developmental characteristics of fungal spores (Morton and Benny, 1990). More than 150 AMF species are described based on their spore morphology (Walker and Trappe, 1993).

MATERIALS AND METHODS

Garden land and dryland field soil samples were collected from three different locations *viz.*, Eastern block, Millet breeding station and dryland having sandy loam and clay loam texture at Tamil Nadu Agricultural University, Coimbatore. From each soil, samples were collected from the top layer at 15-20 cm depth at 3 locations. Samples were pooled together and by quartering method, representative samples were made. Finally these three samples were used for analysis.

Extraction of AM fungal spores:

Spores of AM fungi associated with the respective soil samples were isolated by wet sieving and decantation with slight modifications as described by Gerdemann and Nicolson (1963).

Physicochemical characteristics of the collected soil samples:

Collected soil samples were first analysed for their

physico-chemical characteristics such as pH, EC and available nutrients. The pH and EC were measured as per the standard method (Jackson, 1973). Available nitrogen content was estimated using alkaline permanganate method (Subbiah and Asija, 1956) and Olsen's extractant method was followed (Olsen *et al.*, 1954) for estimating available phosphorus. Neutral normal ammonium acetate was used to estimate available potassium content (Standford and English, 1949).

Quantification of extracted spores:

Sievings from each sieve were collected separately in to small beakers and transferred again into the fine sieve. The sievings from fine sieve was collected using a fine get of water into a griddled filter paper funnel so as to remove the excess water. The filter paper was griddled at one half of the sheet and it was folded in such a way that the marked portion was the receiving surface during filtration. After the filtration the filter paper was removed gently and spreaded on a bigger size Petriplate with spores and other debris and observed under stereozoom microscope. Presence of spores were counted by moving the Petriplate and the total number of spores were calculated corresponding to the weight of soil taken for analysis.

Morphological identification of AM spores:

For identification spores were mounted on glass slides in PVLG (Polyvinyl alcohol lactoglycerol) and PVLG + Melzer's reagent (1:1,V/V). The slides were examined

at 45X magnification under phase contrast microscope and then identified to genus/ species level by using current taxonomic criteria (Schenck and Perez, 1987; Sharda, 2008). Macrocharacteristics such as size, shape and colour of the spores, nature of the subtending hyphae and also the microcharacteristics *viz.*, spore wall layers, wall ornamentation etc, were used for identification of spores as per the keys of Schenck and Perez (1987). The identified spores were designated and tabulated.

RESULTS AND DISCUSSION

Physico-chemical characteristics of the soil indicated the variation of soil properties. pH of all the three soil samples was found slightly alkaline and EC was observed normal. All the three soils were low in available N status (227 to 260 kg/ ha) and high in K status (639 to 993 kg/ ha). Variations in available P status was observed. Eastern block soil is having very high P status, followed by middle P status in Millet breeding station and low level of P was observed in dryland soils (10.8 kg/ ha).

The AM spore count results in the three soils showed the existence of variation with reference to the nutrient status, especially the available P content of the dryland soil, harbour maximum number of AM fungal spores followed by millet breeding station soil and comparatively eastern block soils hold minimum number of AM spores (135 spores / 100 g soil).

Morphological analysis of the collected spores revealed the presence of eight different types in alluvial soil. Out of eight species observed, six species belonged

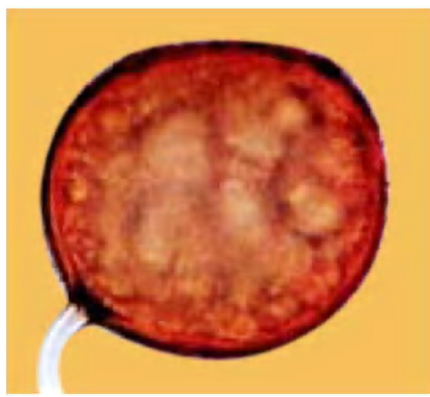
Table 1 : Physico-chemical properties of the collected soil samples

Sr. No.	Soil samples	pH	EC (dSm ⁻¹)	Texture	Available nutrient status (kg ha ⁻¹)			No. of spores / 100g of soil sample
					N	P ₂ O ₅	K ₂ O	
1.	EB	8.66	0.46	SCI	227	34.4	639	135
2.	DL	8.29	0.50	SCI	260	10.8	715	150
3.	MBS	8.47	0.37	CI	241	11.8	993	145

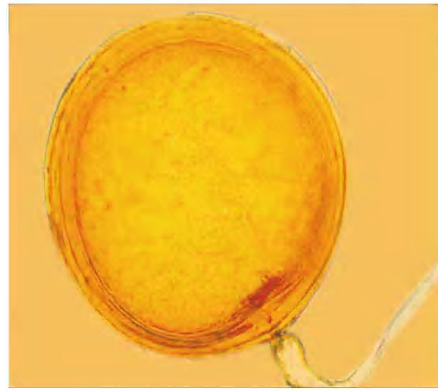
EB – Eastern block, DL- Dryland , MBS-Millet Breeding station SCI – Sandy Clay Loam, CI – Clay loam

Table 2 : Morphological identification of AM spores in different soil types

AM spores identified	Soil types		
	Dryland soil	Millet breeding station soil	Eastern block soil sample
1.	<i>Acaulospora</i> sp.	<i>Glomus mosseae</i>	<i>Glomus clarum</i>
2.	<i>Glomus mosseae</i>	<i>Gigaspora margarita</i>	<i>Glomus fasciculatum</i>
3.	<i>Scutellospora</i> sp.	<i>Glomus fasciculatum</i>	<i>Gigaspora decipens</i>
4.	<i>Gigaspora gigantea</i>	<i>Scutellospora</i> sp.	<i>Glomus etunicatum</i>
5.	<i>Glomus albidum</i>	<i>Glomus geosporum</i>	<i>Glomus mosseae</i>
6.	-	-	<i>Glomus viscosum</i>
7.	-	-	<i>Scutellospora</i> sp.
8.	-	-	<i>Glomus geosporum</i>



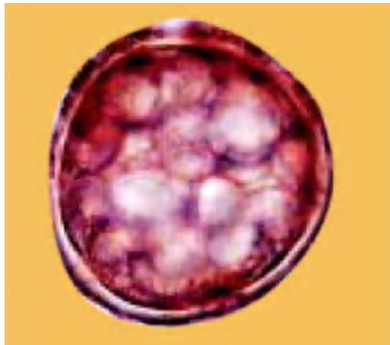
Glomus etunicatum



Gigaspora decipens



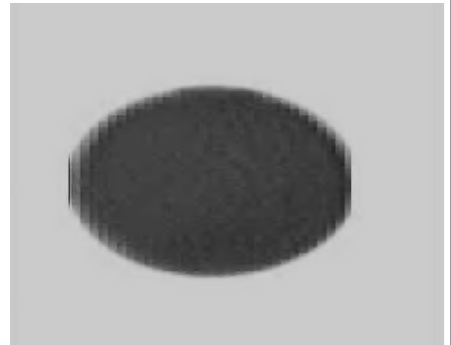
Glomus fasciculatum



Gloms clarum



Gloms mosseae



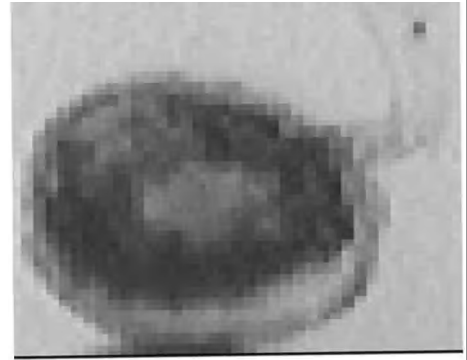
Scutellospora sp.



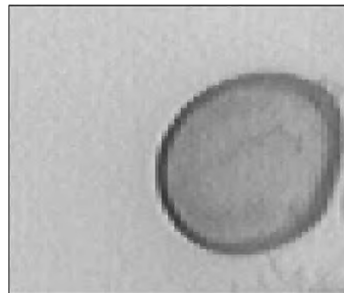
Glomus sp.



Glomus viscosum

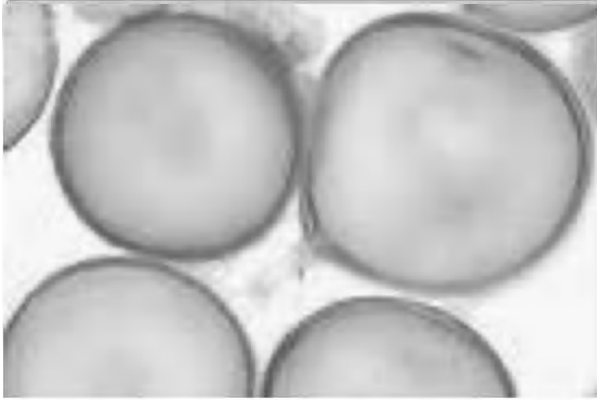


Glomus geosporum



Glomus sp.

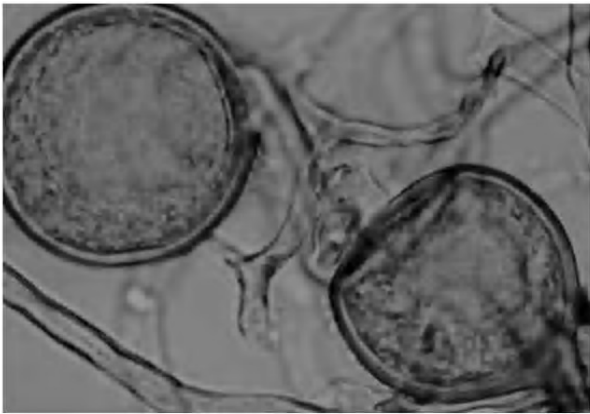
Plate 1 : Diversity of AM spores in eastern block soils



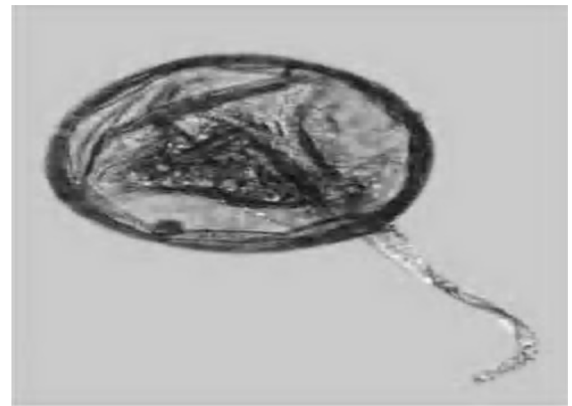
Glomus geosporum



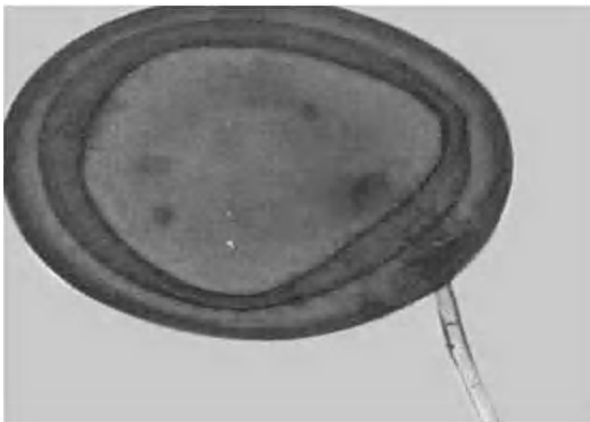
Scutellopora sp.



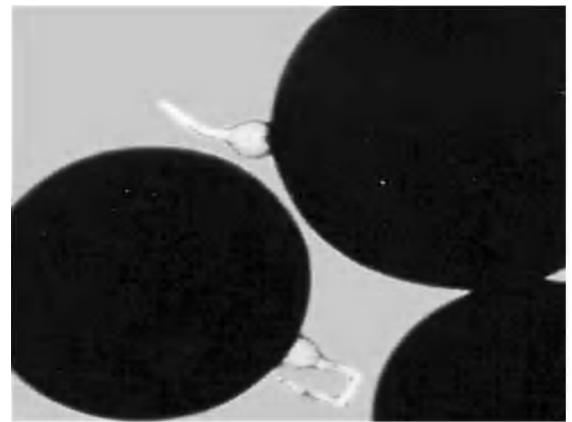
Glomus fasciculatum



Glomus mosseae

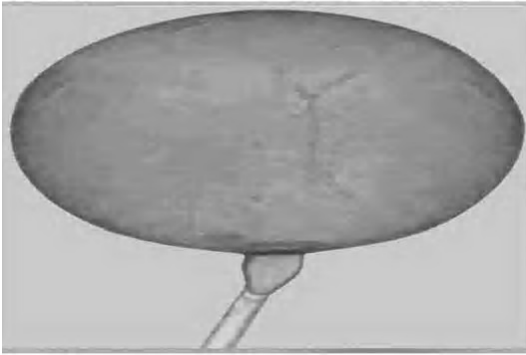


Scutellopora sp.

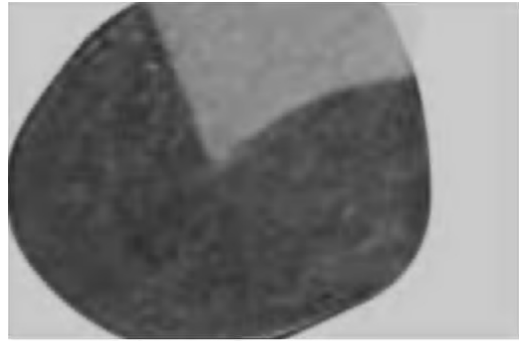


Scutellopora nigra

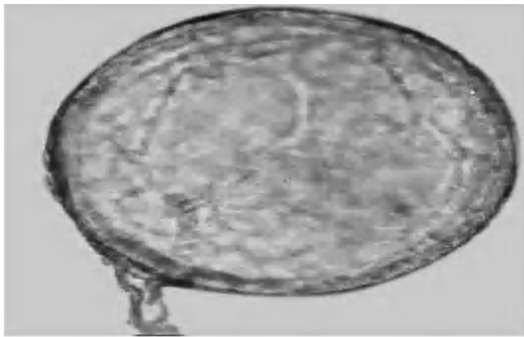
Plate 2 : Diversity of AM spores in millet breeding station soils



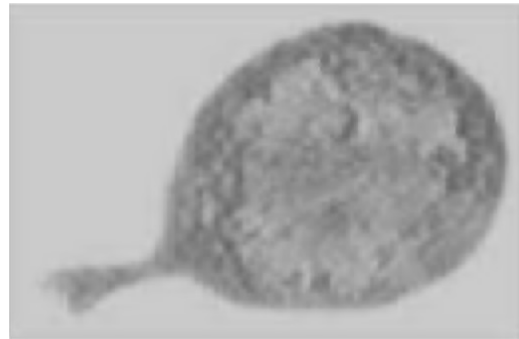
Glomus mosseae



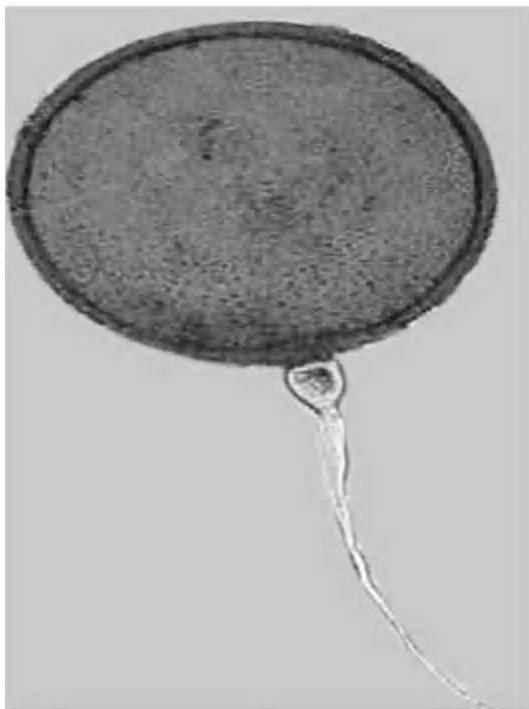
Acaulospora sp.



Glomus sp.



Glomus albidum



Gigaspora gigantea



Scutellospora sp.

Plate 3 : Diversity of AM spores in dryland soils

Table 3: Diversity of AM spores in different soil types

Sr. No.	Species identified	EB	MBS	DS
1.	<i>Glomus clarum</i>	+	-	-
2.	<i>Glomus fasciculatum</i>	+	+	-
3.	<i>Glomus etunicatum</i>	+	-	-
4.	<i>Glomus mosseae</i>	+	+	+
5.	<i>Glomus viscosum</i>	+	-	-
6.	<i>Glomus geosporum</i>	+	+	-
7.	<i>Glomus albidum</i>	-	-	+
8.	<i>Gigaspora decipens</i>	+	-	-
9.	<i>Gigaspora margarita</i>	-	+	-
10.	<i>Gigaspora gigantea</i>	-	-	+
11.	<i>Scutellospora</i> sp.	+	+	+
12.	<i>Acaulospora</i> sp.	-	-	+

EB- Eastern block

MBS- Millet breeding station

DL- Dryland

to the genus *Glomus*, while one in *Scutellospora* and *Gigaspora* each, In red soil, five species were identified, out of which three belonged to *Glomus*, one in each of *Scutellospora* and *Gigaspora*.

In dryland soils, six species comprising of four genera were identified as *Acaulospora* sp., *Glomus mosseae*, *G. albidum*, *Scutellospora* sp., *S. nigra* and *Gigaspora gigantea*. By analyzing the occurrence of AM spores, it showed the prevalence of mainly two types of spores viz., *Glomus mosseae* and *Scutellospora* sp. in all the three soil types. The genus *Glomus* was predominant, followed by *Scutellospora* and *Gigaspora*. In dryland soils the genus *Acaulospora* was present, which was not common in other soil types.

Several workers reported the wider occurrence of *Glomus* in several soils. Hanumantha Gowda (1996) reported that *Glomus* was found to be predominant than any other AM spores identified in different location soils. This suggested that *Glomus* is highly adaptable to various ecological niches and can make association with plants under wide range of soil properties. *Scutellospora* was reported to be common in petridophytes of western ghats of southern India (Muthukumar and Udaiyan, 2002). Occurrence of certain AM species have been linked with soil factors. Predominance of *G. mosseae* with fine textured soils and high pH soils as well as *A. laevis* with coarse textured soils (Kendrick and Berch, 1985). Occurrence of other species may be explained by the fact that changes in soil fertility, cultural practices (Schenck and Sequeira, 1987). Environmental factors (Abbott *et al.*, 1992) as well as with the cropping pattern followed in that particular soil.

By analyzing the overall results, it was quite apparent

that the genus *Acaulospora* was unique in dryland, where mainly dryland crops are grown and the garden land soil (millet soil and eastern block soils) had higher association of *Glomus* and *Scutellospora*. Predominance of *Acaulospora* in dry soils may be due to their adaptation to moisture and other stresses in that soils. This results may be exploited further to develop efficient strains of AMF towards different cropping patterns of these three farm soils.

Conclusion:

Upon morphological examination of AM spores, it was observed that eastern block samples (sandy clay loam) had six species of *Glomus*, one in *Gigaspora* and *Scutellospora* each. In millet breeding station (clay loam) sample also, occurrence of the three genera were reported. In dryland (sandy clay loam) soils, the genus *Acaulospora* was reported as unique than other samples. Sandy clay loam soils are having more number of AM genera as well as the species compared to the clay loam soils.

Acknowledgement:

The authors are grateful to Dr. T. Muthukumar (Dept. of Botany, Bharathiar University, Coimbatore) for their unfailing help and kind co-operation for his assistance in carrying out morphological identification.

Authors' affiliations:

SUCHITRA RAKESH AND D. BALACHANDAR,
Department of Agricultural Microbiology, Centre of Plant
Molecular Biology, Tamil Nadu Agricultural University,
COIMBATORE (T.N.) INDIA

REFERENCES

- Abbott, L.K.**, Robson, A.D. and Gazey, C. (1992). Selection of inoculant vesicular arbuscular mycorrhizal fungi. In: *Methods in Microbiology*. Academic Press Ltd., London, **24**: 1-21.
- Blackwell, M.** (2000). Terrestrial life-fungal from the start? *Sci.*, **289**:1884-1885.
- Gange, A.C.**, Bower, E., Stagg, P.G., Aplin, D.M., Gillam, A.E. and Bracken, M. (1999). A comparison of visualization techniques for recording Arbuscular Mycorrhizal colonization. *New Phytol.*, **142**: 123-132.
- Gerdemann, J.V.** and Nicolson, T.H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.*, **46**:235-244.
- Hanumantha Gowda, M.** (1996). Screening of VA mycorrhizae and *Azotobacter* for mulberry cultivation. Ph.D. Thesis, University of Mysore (Karnataka).

- Hart, M.M.** and Klironomos, J.N. (2002). *Diversity of arbuscular mycorrhizal fungi and ecosystem functioning*. Springer, Berlin Heidelberg, New York, pp. 225-242.
- Hartnett, D.C.** and Wilson, G.W.T. (1999). Mycorrhizae influence plant community structure and diversity in tall grass prairie. *Ecol.*, **80**: 1187-1195.
- Jackson, M.L.** (1973). *Soil Chemical Analysis*. Prentice Hall of India Private Ltd., New Delhi, pp. 56-70.
- Kendrick, W.B.** and Berch, S.M. (1985). Mycorrhizae: applications in agriculture and forestry. In: *Comprehensive Biotechnology*. Pergoan Press, Oxford, **13**: 109-152.
- Klironomos, J.N.**, McCune, J., Hart, M. and Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol. Lett.*, **3**: 137-141.
- Morton, J.B.** and Benny, B.L. (1990). Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*), A new order *Glomales*, two new suborders, *Glomineae* and *Gigasporineae* and two new families, *Acaulosporaceae* and *Gigasporaceae* with and emendation of *Glomales*. *Mycotoxon*, **37**: 471-491.
- Muthukumar, T.** and Udaiyan, K. (2002). Arbuscular mycorrhizal fungal composition in semi-arid soils of Western Ghats, Southern India. *Curr. Sci.*, **82**: 624-628.
- Olsen, S.R.**, Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Cir.*, 939.
- Redecker, D.**, Morton, J.B. and Bruns, T.D. (2000). Ancestral lineages of arbuscular mycorrhizal fungi (*Glomales*). *Molec. Phylogenet. Evol.*, **14**: 276-284.
- Schenck, N.C.** and Perez, Y. (1987). *Manual of the identification of VA-mycorrhizal fungi*. University of Florida, Gainesville, FL. pp. 245.
- Schenck, N.C.** and Sequeira, J.O. (1987). Ecology of VA mycorrhizal fungi in temperate agroecosystem. In: *Mycorrhizae in the Next Decade: Practical Applications and Research Priorities*. Proc. Of 7th NACOM (Eds DM Sylvia, LLHung and JH Graham). pp.2-4.
- Schubler, A.**, Schwarzott, D. and Walker, C. (2001). A new fungal phylum, glomeromycota: Phylogeny and evolution. *Mycol. Res.*, **105** (12): 1413-1421.
- Sharda, W.K.** (2008). Taxonomy of arbuscular mycorrhizal fungi. *Mycorrhiza News*, **20**(1).
- Smith, S.E.** and Read, D.J. (1997). *Mycorrhizal Symbiosis*. Academic Press, Inc San Diego California. ISBN 0-12-652840-3.
- Stanford, S.** and English, L. (1949). Use of flame photometer in rapid soil tests of K. *Canadian J. Agron.*, **41**: 446-447.
- Subbiah, B.V.** and Asija, G.L. (1956). A rapid procedure for estimation of available nitrogen in soils. *Curr. Sci.*, **25**: 259-260.
- Van der Heijden, M.G.A.**, Boller, T., Wiemken, A. and Sanders, J.A. (1998). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, **79** : 2082-2091.
- Walker, C.** and Trappe, J.M. (1993). Names and epithets in the *Glomales* and *Endogonales*. *Mycol. Res.*, **97** : 339-344.

