

Occurrence and Distribution of Arbuscular Mycorrhizal Fungi in Agricultural Fields of Madurai District, Tamil Nadu

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Arbuscular Mycorrhizal (AM) fungi are of considerable interest because of their ability to form symbiotic associations with various crops species. This study was carried out to find the occurrence and abundance of AM fungi in agricultural fields of Madurai district. Different AM fungal spores were isolated from the rhizosphere soil along with the roots of different crops growing in this region. Soil samples in this region exhibit varied physico-chemical parameters which correlate with Arbuscular Mycorrhizal Fungi (AMF) infection and spore count. The highest number of spore count (410±1.15) and high infection percentage (80.1±1.07 %) were found in maize grown under Mellur (MLR1) tract. The lowest number of spore count (152±1.53) and infection percentage (30±0.40 %) were observed in upland rice grown under Thirumangalam (TMQ) tract. Five AMF genera including *Glomus*, *Gigaspora*, *Scutellospora*, *Entrophospora* and *Acaulospora* were identified from the rhizosphere soil collected from the maize crop cultivated at Mellur. Genus *Acaulospora* and *Glomus* were found to be very dominant in maize crop. The results suggest that spore density in soil and root infection percentage varied from one tract to another tract and higher AM infections rates were observed in soil with low phosphorus content.

Key words: AM fungi, spore count, infection percentage, soil parameters

Arbuscular Mycorrhizal Fungi forms mutualistic symbiotic association with the roots of higher plants. In this symbiosis, the host plant provides the fungus with soluble carbon sources, at the same time the fungus enhances the uptake of certain nutrients by plants, particularly phosphate (Jayachandran and Shetty, 2003), improve fitness of plants in polluted environments (Hildebrandt *et al.*, 1999), modify root morphology (Berta *et al.*, 1990), increases resistance against soil pathogens (Orlando, 2003) and play a role in the formation of soil aggregates (Hamel *et al.*, 1997) by producing the glycoprotein Glomalin (Wright and Upadhyaya, 1998).

There are four major characteristics of AMF considered important to their potential as inoculant fungi in agriculture, horticulture and forestry. They form rapid root infections, extensive formation of hyphae in soil, efficient absorption of phosphorus and other nutrients and production of more propagules (Abbott and Robson, 1984). Therefore the above aspects suggested that the diversity and community structure of AMF were important both in sustaining the stability of the plant community and re-establishing vegetation in different types of soil.

AMF is wide spread in their distribution both among plant species and over geographical areas and associated with more than 80 per cent of the land plants (Smith *et al.*, 2003). But the major factors affecting the diversity, abundance and distribution of AMF in agricultural fields are soil pH, higher content of phosphorous, nitrogen, organic matter, water stress etc. These factors could also affect the crop production in agro ecosystems (Porras-Soriano *et al.*, 2009). Most of the field studies testing the relationship between phosphorus availability and mycorrhizal colonization suggest that increased phosphorus availability leads to decreased colonization. Wetzel and Van Der Valk (1996) found that mycorrhizal hyphae were more common in low-phosphorus North Dakota wetlands than high-phosphorus lowa wetlands.

Although AM fungi is widespread in nature and occur in almost all soils, not all plant species growing in particular area exhibit uniform root colonization and there is considerable variation among tropical plants in mycorrhizal colonization. From all these aspects it is apparent that AMF diversity in agricultural ecosystems was under risk of obliteration. Therefore it is essential to evaluate the occurrence of AMF in agro ecosystems. The objective of the present work is to investigate the occurrence and distribution of AM fungi in agricultural fields of Madurai district, Tamil Nadu, India.

Materials and Methods

Study site

The study site Madurai district lies in southern

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part of Tamil Nadu state. It has an area of 147.99 km₂, geographically lies between9.93°N to 78.12°E. It has an average elevation of 101 metres. The average annual rainfall is about 85 cm. The soil type is predominantly clay loam, red loam and black cotton types. Paddy is the major crop, followed by pulses, millet, oil seed, cotton, sugarcane and vegetables. The city is surrounded by Tirumangalam, Tiruparankundram, Mellur and Vadipatti.

Collection of soil and root samples

Rhizosphere soil and root samples were collected from different crops *viz.*, tomato, chilli, brinjal, banana, bhendi, maize, sugarcane and upland rice cultivated in and around Madurai district. The plants were uprooted with the complete root system along with rhizosphere soil. The rhizosphere soil from 20 plants of each in same field were thoroughly homogenized, sieved (2mm), air-dried and stored at 4°C until used for analysis.

Analysis of soil chemical properties

The analysis conducted to determine the soil characteristic were pH, available P and organic carbon. The pH determined by using pH meter (Richards, 1954). The available P was determined by spectrophotometer according to Olsen *et al.* (1954). The soil organic carbon (soc) content was determined by Walkley- Black (1934) chromic acid wet oxidation method. All the soil analysis was

triplicate and average values were used in the statistical analysis.

Assessment of AMF infection percentage

The root samples from tomato, chilli, brinjal, banana, bhendi, maize, sugarcane and rice plants were analyzed for AMF infection by clearing and staining method of Phillips and Hayman (1970).

Isolation and identification of AM fungal spores

The rhizosphere soils of different crops were examined for the presence of AMF spores by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and examined under a stereozoom microscope for their shape, colour and the hyphal attachment to spores.

Based on the taxonomic keys of Schenck and Perez (1990) and through INVAM web based identification (http://invam.caf.wvu.edu/cultures/ cultsearch.htm) the AMF isolates from different crops were identified.

Results and Discussion

Soil chemical analysis, Spore density and Percent colonization

Physico-chemical parameters of collected soil samples were given in Table 1. Diverse range of pH was observed. Lowest pH was recorded in VDP and TMQ (5.61 ± 0.13 and 6.12 ± 0.14), which is considerably acidic. Other samples possessed

Table 1. Sampling sites, host plants, soil type and soil physico-chemical properties of samples collected from Madurai district.

Sampling site	Standing crop at the time of sampling	Soil type	рН	SOC (%)	Available P (kg/ha)
Tiruparankundram(TDN 1)	Tomato	Red loam	6.40±0.13	0.30±0.01	6.0±0.12
Tiruparankundram (TDN 2)	Chilli	Red loam	6.80±0.16	0.46±0.01	5.6±0.13
Tiruparankundram (TDN 3)	Banana	Red loam	7.40±0.17	0.61±0.01	6.5±0.15
Mellur(MLR 1)	Maize	Red loam	7.05±0.16	0.32±0.01	4.7±0.11
Mellur(MLR 2)	Brinjal	Red loam	6.34±0.15	0.52±0.01	8.2±0.19
Mellur(MLR 3)	Bhendi	Red loam	6.21±0.14	0.28±0.01	8.9±0.21
Tirumangalam(TMQ)	Rice	Black clay	6.12±0.14	0.71±0.02	16±0.37
Vadipatti(VDP)	Sugarcane	Clay loam	5.61±0.13	0.58±0.01	13.7±0.32
SEd			0.20	0.01	0.30
CD (0.05)			0.44	0.03	0.65

Note: Values represent means of triplicate determinations \pm SE

neutral pH. Highest SOC was observed in TMQ i.e. 0.71 ± 0.02 %, lowest SOC recorded in MLR3, TDN1 and MLR1 samples. Highest available Phosphorous was found in samples of TMQ and VDP (16±0.37 and 13.7±0.32 kg/ha).

The results of the current study indicate that AMF spore count and root infection were varied among different sampling sites. Among the eight samples, highest spore count was recorded in maize grown in MLR1 block (410±1.15 spores/100g of soil), followed by chilli grown under TDN2 block (321±1.76 spores/100g of soil). Lowest spore count was

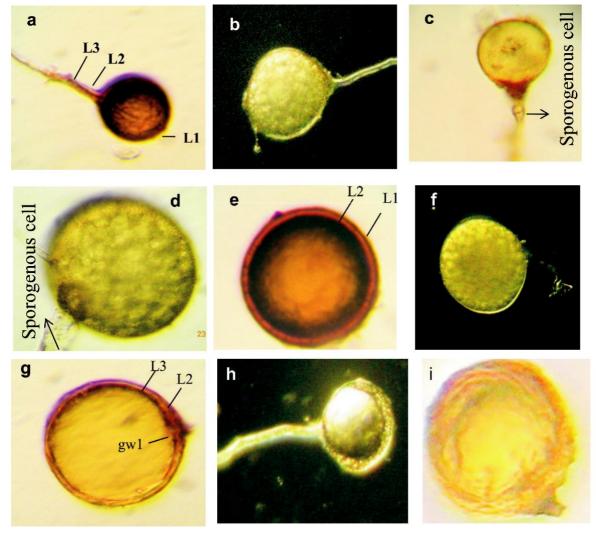
assessed from TMQ block (152±1.53 spores/100g of soil) where rice is the host plant. Of all the eight root samples, highest AMF percent colonization was observed in samples of MLR1 block (80.1±1.07 %) followed by TDN2 block (70.8±0.94 %). Lowest root colonization was detected in TMQ block (30±0.40 %) (Table 2). It is also important to observe the facts that in soil P and SOC contents are much higher in TMQ sample. It is well documented that rapid changes in soil nutrients may affect percent association and spore number of AM fungi (Abbott and Robson, 1991).

Table 2. AMF spore count and root infection percentage of samples collected from Madurai district.

Sample	Spore count number/100g of soil	Root infection		
name	number/100g of soil	percentage (%)		
TDN 1	312±1.45	70.2±0.70		
TDN 2	321±1.76	70.8±0.94		
TDN 3	291±0.88	62.5±0.83		
MLR 1	410±1.15	80.1±1.07		
MLR 2	286±1.20	60.7±0.81		
MLR 3	260±1.73	58.4±0.78		
TMQ	152±1.53	30.0±0.40		
VDP	212±0.58	50.3±0.67		
SEd	1.90	1.13		
CD (0.05)	4.02	2.39		
Note: Values represent means of triplicate determinations ± SE				

Many studies have found that the primary benefit to the plant of the mycorrhizal symbiosis is improved phosphorus nutrition (Fitter and Merryweather, 1992). If phosphorus is abundant and available, then the plant does not need to maintain the symbiosis and mycorrhizal colonization decreases. Sanders and Tinker (1973) and numerous experiments have also shown that increasing phosphate availability decreases the mycorrhization level, suggesting that AMF might play a minor role in natural ecosystems or agriculture fields with high P availability (Ryan and Graham, 2002). Similarly TMQ and VDP samples also possessed higher P content, where TMQ shows lower percent colonization and spore count among all the eight samples.

Several researchers have acknowledged the capability of monocots and other crop plants in the mass production of AMF inoculum and their host specificity (Douds *et al.*, 2005; Chaurasia and Khare, 2005). They produce enormous root biomass in short period of time compared to plants grown in natural conditions, and also the production of root



L1 - Laminae1, L2- Lamin

inae3, gw1 - germinal wall layer 1

Fig 1. AM fungal spores from maize rhizosphere soil. a. *Glomus* sp. MDU1, b. *Glomus* sp. MDU2, c. *Gigaspora* sp. MDU1, d. *Scutellospora* sp. MDU1, e. *Entrophospora* sp. MDU1, f. *Entrophospora* sp. MDU2, g. *Acaulospora* sp. MDU1, h. *Acaulospora* sp. MDU2, i. *Acaulospora* sp. MDU3

exudates is very less. Therefore we could assume that maize plant soil and root samples harbored large AMF population due to the above reasons.

Characterization of isolated AMF strains from maize

Nine AMF strains which differed in their spore colour and shape were isolated from maize crop cultivated at Mellur (MLR1) and were characterized. Based on the spore shape, spore colour and structural features as described in the INVAM website, two strains were identified as Glomus sp., one strain as Gigaspora sp., another as Scutellospora sp., two as Entrophospora sp., and three as Acaulospora sp. (Fig. 1) . These findings are in close conformity to those of Subha et al. (2008) in sweet potato. Chaurasia and Khare (2005) found three species of AM fungi i.e. Glomus aggregatum, G. fasciculatum and Sclerocystis pakistanica in the soil of Phaseolus vulgare. Sharif and Moawad (2006) also identified spores of Glomus intraradices and Glomus mosseae in potato, barley, rice, chickpea, alfalfa, wheat, barley, oat and grasses.

It is concluded from the study that the plants growing in a particular area do not exhibit a uniform spore density and root colonization. Higher AM fungal spore count and their root colonization in maize crop were observed in P deficient soil. This unique richness of AMF could be speculatively attributed to the soil nutrient content and or of host species.

References

- Abbott, L.K. and Robson, A.D. 1984. The effect of mycorrhizae on plant growth. In : VA mycorrhizae (eds., C.L. Powell and D.J. Bagyaraj). CRC uniscience series. Floroida, USA.
- Abbott, L.K. and Robson, A.D. 1991. Factors influencing the occurrence of Vesicular Arbuscular Mycorrhiza. *Agric. Ecosyst. and Environ.*, **35**:121-150.
- Berta, G., Fusconi, A., Trotta, A. and Scannerini, S. 1990. Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. *New Phytol.*, **114**: 207– 215.
- Chaurasia, B. and Khare, P.K. 2005. Hordeum vulgare: a suitable host for mass production of arbuscular mycorrhizal fungi from natural soil. *Appl. Ecol. and Environ. Res.*, **4**: 45-53.
- Douds, D.D., Nagahashi, G., Pfeffer, P.E., Kayser, W.M. and Reider, C. 2005. On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. *Canadian J. Plant Sci.*, 85: 15-21.
- Fitter, A.H. and Merryweather, J. W. 1992. Why are some plants more mycorrhizal than others? An ecological enquiry. In D. J. Read, D. H. Lewis, A. H. Fitter, and I. J. Alexander [eds.], Mycorrhizas in ecosystems, 26–36. CAB International, Wallingford, UK.
- Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal Endogene species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, **46**: 235-244.

- Hamel, C., Dalpe, Y. and Furlan, V. 1997. Indigenous populations of arbuscular mycorrhizal fungi and soil aggregate stability are major determinants of leek (*Allium porrum* L) response to inoculation with *Glomus intraradices* Schenck & Smith or *Glomus* versiforme (Karsten) Berch. Mycorrhiza, 7: 187–196.
- Hildebrandt, U., Kaldorf, M. and Bothe, H. 1999. The zinc violet and its colonization by arbuscular mycorrhizal fungi. *J. Plant Physiol.*, **154**: 709–717.
- Jayachandran, K. and Shetty, K.G. 2003. Growth response and phosphorus uptake by arbuscular mycorrhizae of wet prairie sawgrass. *Aquat. Bot.*, **76**: 281–290.
- Olsen, S.R., Cole, C.V., Watanebe, F.S. and Dean, L.A. 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. US. Dept. Agric. Cric., 939.
- Orlando, A.Q. 2003. The vesicular arbuscular mycorrhizal symbiosis A review. Afr. J. Biotechnol., 2: 539-546.
- Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesiculararbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, **55**: 138-161.
- Porras-Soriano, A., Sorano-Marintin, M.L., Porras-Piedra, A. and Azcon, P. 2009. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.*, **166**: 1350-1359.
- Richards, L.A. 1954. Diagnosis and improvements salina and alkali soils. U.S. Dep. Agr. Handbook 60, Stroudsburg, U.S.A.,
- Ryan, M.H. and Graham, J.H. 2002. Is there a role of Arbuscular mycorrhizal fungi in production agriculture? *Plant soil*, 244: 263- 271.
- Sanders, F.E. and Tinker, P.B. 1973. Phosphate flow into mycorrhizal roots. *Pesticide Science*, 4: 385-395.
- Schenck, N.C. and Perez, Y. 1990. Manual for the identification of VA mycorrhizal fungi. Synergistic Publications, Gainesville, FL., U.S.A.
- Sharif, M. and Moawad, A.M. 2006. Arbuscular Mycorrhizal Incidence and Infectivity of crops in North West Frontier Province of Pakistan. *World J. Agric. Sci.*, **2**: 123-132.
- Smith, S.E., Smith, F.A. and Jakobsen, I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.*, **133**: 16-20.
- Subha, V., Brunda Devi, K., Tilak, K.V.B.R. and Bhadraiah, B. 2008. Association of AM Fungi with sweet potato in soils of Andhra pradesh. J. Mycol. Pl. Pathol., 38: 88-90.
- Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.*, **63**: 251-263.
- Wetzel, P.R. and Van Der Valk, A. G. 1996. Vesiculararbuscular mycorrhizae in prairie pothole wetland vegetation in Iowa and North Dakota. *Canadian J. Bot.*, **74**: 883–890.
- Wright, S.F. and Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, **198**: 97–107.

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