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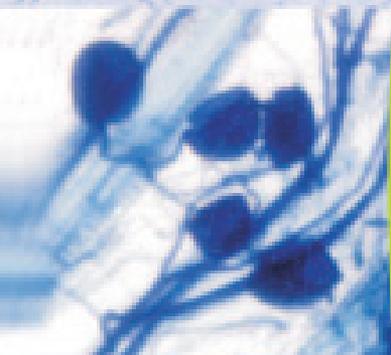
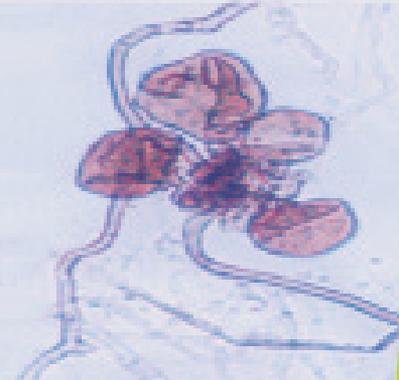
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Mycorrhiza News

The *Mycorrhiza News* provides a forum for the dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers compiled from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.



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RESEARCH FINDING PAPERS

A Commercially Available Fabric Whitener Serves as a Stain for Detection of Arbuscular Mycorrhizal Fungi in Roots

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Introduction

Arbuscular mycorrhiza (AM) is the symbiotic association between fungi in the phylum Glomeromycota (Schüßler, Schwarzott, and Walker 2001) and various terrestrial plants. In general, AM fungi colonize cryptogenic and other plants and provide them with mineral nutrients, especially phosphate, and in turn, receive the photosynthates (Smith and Read 1997).

The detection of AM fungi in roots is done by several methods (Vierheilig, Schweiger, and Brundrett 2005). Several stains are reported to be suspected carcinogen (Vierheilig, Coughlan, Wyss *et al.* 1998). Moreover, stains such as ink are also reported to be efficient for observing AM fungi (Das and Kayang 2008; Vierheilig, Coughlan, Wyss *et al.* 1998).

However, there is no report of fabric whitener as a stain for detection of AM fungal colonization in roots. Therefore, we report the use of fabric whitener as a staining agent for discerning the structures of AM fungi in root.

Materials and Methods

Sampling of Plant from Natural Habitat and Trap Culture

Three plant samples of *Scoparia dulcis* L. were collected from the bank of a river in Tripura. For the root of trap culture, *Paspalum notatum* Flüggé was used as trap plant soil and root samples from

rubber plantations in Amtali, Tripura, were used as a source inoculum. The one part of source inoculum for rubber plantation was mixed with two parts of sand. The mixture was placed in a 2 kg polyethylene pot. The seeds of *P. notatum* were placed on the pot for proliferation of mycorrhizal spores and root colonization. Five plants of *P. notatum* were harvested after three months for root colonization study.

Root Processing and Staining

The root samples from the plant *S. dulcis* and *P. notatum* were washed in tap water. After washing, these were cut into ~1 cm pieces and treated with 10% NaOH. The sample was heated for 1 hour at 90°C. Then, these were stained in a mixture of 5 ml of 5% acetic acid and 2 ml of Ujala supreme, fabric whitener (manufactured by Jyothi Laboratories Ltd). Then, it was mounted in lactoglycerol and photographs were taken in Magnus Olympus Light Microscope. The colonization was quantified using magnified intersection method (McGonigle *et al.* 1990).

Results and Discussion

The mycorrhizal structures, such as hyphae, vesicles, and arbuscules were observed in *S. dulcis* and *P. notatum* (Figure 1). The structure such as arbuscules was lightly stained in *S. dulcis* than in *P. notatum*. The maximum colonization was recorded in *S. dulcis* when compared to trap plant, *P. notatum*.

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The number of vesicles recorded in trap plants was high when compared to *S. dulcis* (Table 1).

Table 1: Mycorrhizal colonization of plants stained with fabric whitener

Plants	Percentage of arbuscules	Percentage of vesicles	Percentage of hyphae
<i>Scoparia dulcis</i>	25.90 ± 6.29	6.50 ± 4.15	78.85 ± 8.18
<i>Paspalumnotatum</i>	15.87 ± 3.99	11.05 ± 3.90	75.83 ± 9.11

The staining in *S. dulcis* was light and this was due to the concentration of staining mixture. However, the

increase in concentration during staining of the trap plants improved the staining quality of root. This also led to the good contrast and clarity of the structures for observation.

The availability and cost effectiveness of this fabric whitener makes the stain perfect for detection of mycorrhizal colonization. However, stain such as ink reported earlier by Das and Kayang (2008) was least available. The use of this fabric whitener in clothes may be less harmful and could be used as a stain for observing mycorrhizal fungi in root. The other brands of fabric whitener may also give the same result, but they need to be tested.

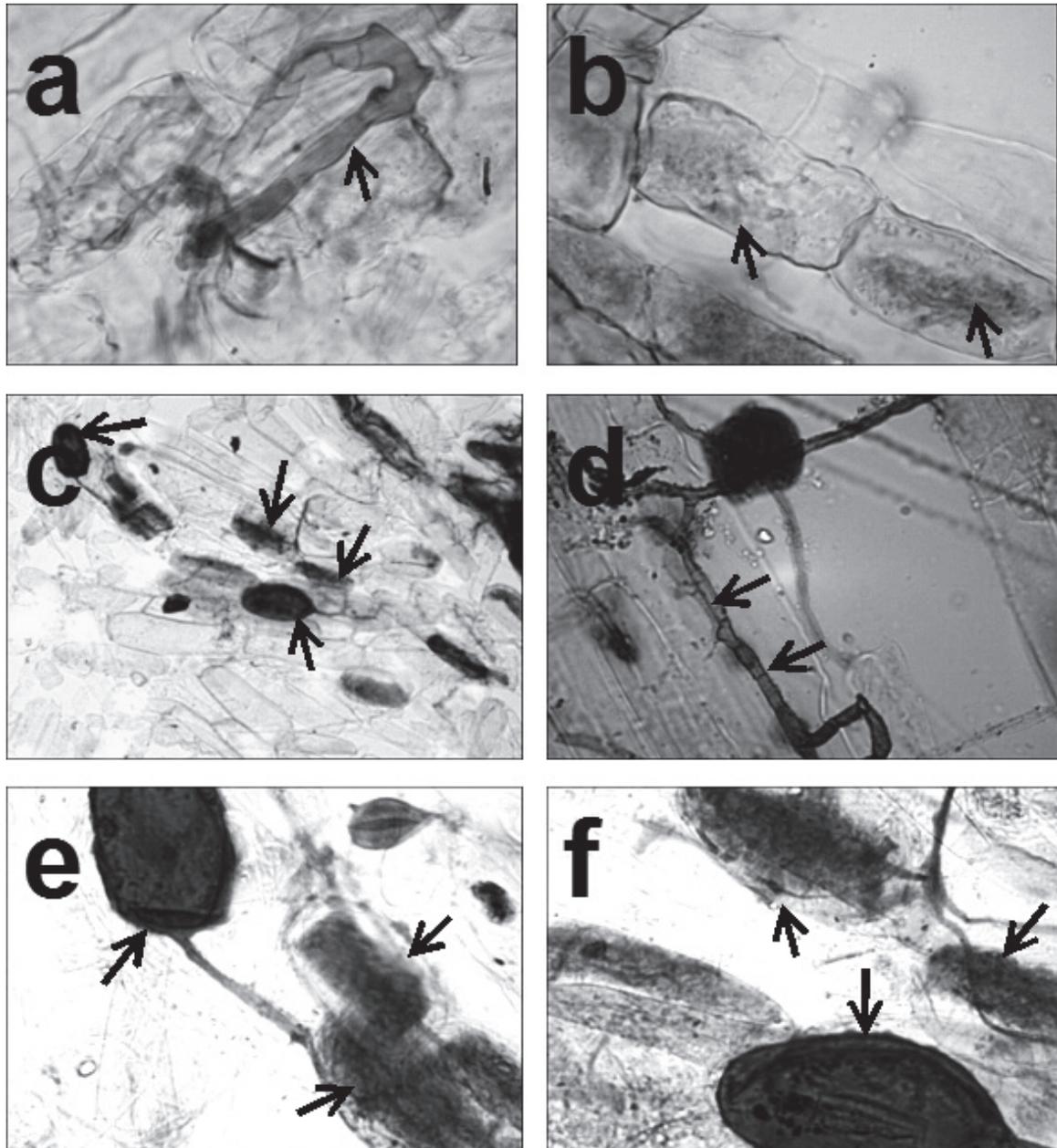


Figure 1: Mycorrhizal colonization of plants: (a) Intracellular hyphae in *S. dulcis* (400×), (b) Arbuscules in the root of *S. dulcis* (400×), (c) Arbuscules and vesicles in *P. notatum* (100×), (d) Hyphae in *P. notatum* (400×), (e and f) Arbuscules and vesicles in *P. notatum* (400×).

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Species Diversity and Population Density of Arbuscular Mycorrhizal Fungi Associated with *Carthamus tinctorius* L. Rhizosphere Soils of Telangana, India

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Abstract

Rhizosphere soil samples of *Carthamus tinctorius* L. from different locations of Telangana, India, were screened for the association and species diversity of arbuscular mycorrhizal fungi (AMF). A total of 42 AM fungal species were recorded representing six genera, which include *Acaulospora*, *Glomus*, *Gigaspora*, *Scutellospora*, *Funneliformis*, and *Entrophospora*. The results indicate that AM fungal spore abundance and diversity are high in the areas studied with *Glomus*, the most common and widely distributed genus among the Glomeromycotan genera represented by 16 species suggesting its predominance in all the soils surveyed. Shannon-Weaver index of diversity (H') was highest (3.353) in Adilabad and lowest (3.045) in Sangareddy soils. AM spore density and total number of species exhibited a significantly negative correlation with the moisture content ($r = -0.970^{**}$, $p < 0.01$, and $r = -0.828^*$, $p < 0.05$, respectively), between spore density and available P in the soil ($r = -0.888^{**}$, $p < 0.01$) and spore density and total number of species showed significantly negative correlation with available potassium ($r = -0.888^{**}$, $r = -0.906^{**}$, $p < 0.01$, respectively). Spore density and species richness showed no correlation with soil factors pH, electron conductivity, organic carbon, and available N. *Glomus fasciculatum* was found to be dominant species with highest relative spore density. It will be beneficial to isolate indigenous AMF associated with the host plants and use them as bioinoculant to improve plant health and soil quality.

Key words: Arbuscular mycorrhizal fungi, Diversity, *Glomus*

Introduction

AMF are major components of the soil microbial community belonging to phylum Glomeromycota (Schüßler *et al.* 2001). In natural and agricultural ecosystems, associations between plants and AMF are common (Smith and Read 2008). Mycorrhizal fungi are benefitted with carbohydrate substrates from plants and in turn, the plants are provided with nutrients

especially immobile nutrients, such as phosphate compounds from soil solution through hyphal network of the fungi apart from increased absorptive surface area of the roots (Loyanachan 2000). Any disturbance in their relationship may cause changes in terms of decreased population status and diversity of these mycorrhizal fungi.

Distribution, abundance, and functioning of AMF are primarily based upon the root colonization and spore count, which further depend upon many environmental factors (Daniels 1984). Since AMF diversity is measured mainly based on the morphological characteristics of the spores, it does not necessarily reflect the structure of the community (Morton *et al.* 1995). Nevertheless, diversity data are of special interest in order to know how abundant and frequent species are in a given ecosystem. These species are more feasible to obtain in single cultures and test them to promote better plant growth and nutrition as well as reduce the use of chemical fertilizers. Therefore, it is necessary to study the effect of agricultural management on the mycorrhizal activity and diversity in order to optimize the effect of this symbiosis. Numerous studies have indicated that AMF are ubiquitous for the majority of agricultural crops. The impact of AM in tropical agriculture will be greater, and the beneficial effects are dependent on their population density as well as species composition in the field soils.

Safflower (*Carthamus tinctorius* L.) is a multipurpose crop mainly grown in India for its much-valued edible oil. Safflower produces oil rich in polyunsaturated fatty acids (linoleic acid 78%), which play an important role in reducing blood cholesterol level and is considered as a healthy cooking medium. As of now, information available on AM fungal association with safflower is scanty. Therefore, the objective of this investigation was to study the spore density and species diversity of AMF in the rhizosphere soils of safflower cultivated in the agricultural fields of Kohir, Zaheerabad, Tandur, Bainsa, Baser, Adilabad, and Sangareddy in Telangana, India. The rhizosphere soil samples of

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safflower were collected every 30 days interval from the date of seed sowing to harvest and were examined for the mycorrhizal association.

This paper is aimed to study the occurrence, taxonomically identify the existing mycorrhizae, distribution of spore density and species richness (SR) of AMF, analyse the species diversity of indigenous AMF in the rhizosphere soils of safflower collected from the field sites of different areas of Telangana, India.

Materials and Methods

Soil Sampling at Different Field Sites

Rhizosphere soil samples were collected from safflower cultivated agricultural field sites located at Kohir, Zaheerabad, Tandur, Bainsa, Baser, Adilabad, Sangareddy in Telangana, India, during 2012 and 2013. Soil samples were collected randomly from three individuals of each safflower plant per field site on 30, 60, 90, and 120 days interval after seed sowing.

The rhizosphere soil samples were taken with the help of widger by lifting gently a block of soil with the plant roots intact. Later, the root system along with the soil around was carefully uprooted and placed in sterile polythene bag. These soil samples were carefully ground, mixed, and made into a composite sample. Each composite sample representing one field site was a mixture of rhizosphere soil of three plants. The soil samples were quickly transported to the laboratory and moisture content was determined immediately. These soils were stored at 4°C until further analysis was performed. The analysis included AMF spore count, determination of species numbers, and soil physico-chemical factors.

Trap Cultures

Studies of AMF species diversity rely on the extraction and identification of fungal spores because they are quantifiable and identifiable to a species level (Morton *et al.* 1995). The establishment of successive trap cultures had been successful in detecting non-sporulating AMF and can provide more complete representations of species present in the soil (Stutz and Morton 1996) revealing higher SR of AMF compared to field soil. So, the trap cultures were established by mixing field soil with sterile sand in 1:1 (Stutz and Morton 1996) ratio using sorghum as host plant. After four months of growth period under greenhouse conditions, 50 gm of soil was sampled to extract spores.

Analysis of Soil Physico-Chemical Factors

Composite soil samples were analysed for physico-chemical characteristics like soil texture, pH, moisture content, electrical conductivity, organic carbon (OC),

available P, N, K. Moisture content of the soil samples was determined immediately after bringing to the laboratory by oven drying method.

Soil texture was determined by feel method (Ghosh *et al.* 1983), soil pH by glass electrode method (1:2.5 soil/water suspension) using pH meter. Electrical conductivity was measured in 1:5 soil suspension (electron conductivity bridge method), OC by rapid titration method (Walkley and Black 1934), available nitrogen by micro-kjeldahl method and available K using flame photometer (Jackson 1973), P by Olsen's method (Olsen *et al.* 1954).

Extraction and Estimation of AM Fungal Spore Population

AM fungal communities occurring in the original soil samples or produced in the trap cultures were studied by spore extraction from soil. Hence, 100 g of composite soil sample was wet sieved and decanted following the methodology proposed by Gerdemann and Nicholson (1963) for extraction of extramatrical chlamydospores, azygospores, and zygozospores produced by the mycorrhizal fungi followed by sucrose density gradient centrifugation technique (Daniels and Skipper 1982). The procedure included passage of soil suspension through 710, 450, 250, 106, 75, and 45 µm sieves. The residues recovered from each of the sieves were layered on to a water-sucrose solution (70% [wt/vol]) gradient and centrifuged for 2 min (Walker *et al.* 1982). The resulting supernatant was then passed through the 32 µm sieve, washed with tap water, and transferred to new petri plates. Spores, spore clusters, and sporocarps recovered from all these sieves were collected into petri plates over a nylon cloth, examined, and counted by using stereo-binocular microscope. For taxonomic identification, fungal spores were picked under stereo-binocular microscope with a small syringe with fine needle and subsequently mounted on slides in a drop of Polyvinyl-lactic acid glycerol (PVLG) (Omar *et al.* 1979) mixed in 1:1 (v/v) with Melzer's reagent (Morton 1988). Only healthy looking spores were mounted. The spores were examined under a compound microscope at a magnification of up to ×400 for identification up to species level. Only 70–85% of the spores mounted on the slides could be identified to the species level; the rest consisting mostly old and decaying spores with missing clear features were discarded.

Identification of AM Fungal Spores

AM fungal spores were counted and identified for samples taken directly from the field sites and the spores of samples obtained from 'AMF trap cultures' that were inoculated with field samples. The AM fungal spore specimens were identified up to species level using the standard key (Schenck and

Perez 1990) and by comparison with morphological description of spores presented with reference culture information available in the web page (<http://invam.caf.wvu.edu/fungi/taxonomy/species/D.htm>) of the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM 2013). In most cases, identification of the specimens was made by the observations of morphological features of the spores obtained after wet sieving and decanting. These observations were corroborated with the observations of freshly formed AMF species in trap cultures.

AMF Diversity Indices

The abundance of spores of all AM fungal species together was determined for each sample and expressed as spore density (SD) (the number of AMF spores) per 10 gm soil for each field site sample. SR was measured as a number of identified AMF species present in a particular field site. The more the number of species present the 'richer' is the sample. To determine the differences in the structure of AMF communities of different sampling sites, the following parameters were calculated: isolation frequency (IF) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all the sampling sites to the total number of sampling sites and it reflects the distribution status. Relative abundance (RA) was calculated as the percentage of spore number of an individual species (genus) to the total number of all identified species (genus). The importance value (IV) was used to calculate the dominance of AMF species based on IF and RA and was calculated as $IV = (IF + RA) / 2$. An $IV \geq 50\%$ indicates that a genus or species is dominant, $10\% < IV < 50\%$ applies to common genera or

species; an $IV \leq 10\%$ indicates that a genus or species is rare (Chen *et al.* 2012).

Simpson Dominance index (D) is a measure of diversity, which takes into account both SR and an evenness of abundance among the species present. Simpson's index (D) was calculated using the formula $D = \sum (n_i / N)^2$ as per Simpson (1949). Simpson Diversity index was calculated as $1 - D$. Shannon-Weaver (Shannon-Wiener) index of Diversity (H') as per Shannon and Wiener (1963) was calculated by the formula, $H' = - \sum P_i \ln P_i$. Where $P_i = n_i / N$, where n_i is the spore density of an individual species and N is the total spore density of all species (Magurran 1988) per sampling site. The dominant AM fungal species were determined according to RA ($RA > 5\%$) and IF ($IF > 50\%$) as per Li *et al.* (2007).

Statistical Analysis

Ecological measures such as spore density, SR, RA, IF, Simpson's index of dominance, Shannon-Wiener index of diversity were used to describe the structure of AMF communities. The formulae used to calculate these parameters are summarized in Table 1. The dominant AMF species were determined according to RA ($RA > 5\%$) and IF ($IF > 50\%$) (Li *et al.* 2007). Spore density to some extent reflects the biomass of AMF species, RA reveals the sporulation ability of different AMF species, and IF indicates the extent of distribution of AMF species in an ecosystem. Shannon-Wiener index of diversity reflects the index of diversity of AMF. Correlation analysis was carried out with SPSS software package (version 20.0). The relationship between AM fungal spore density and total number of species and soil parameters was determined by Pearson's correlation analysis.

Table 1: Diversity measures used to describe AM fungal communities

Spore density (SD)	The number spores in 10 gm soil
Species richness (SR)	Number of identified AMF species per soil sample/sampling site
Relative abundance (RA)	$\frac{\text{Spore number of a particular species (genus)} \times 100}{\text{Total number of identified spore density}}$
Isolation frequency (IF)	$\frac{\text{The number of sampling sites in which AMF species occurred} \times 100}{\text{The total number of sampling sites}}$
Simpson's index of dominance (D)	$D = \sum (n_i / N)^2$
Shannon-Wiener index of diversity (H')	$H' = - \sum P_i \ln P_i$

P_i is the RA of each identified species per sampling site and calculated by the following formula:

$P_i = n_i / N$, where n_i is the spore density of a species and N is the spore density of all species per sampling site

Results and Discussion

Soil Analyses

The physico-chemical factors of rhizosphere soils supporting the growth of safflower are presented in Table 2. The investigated soils were of sandy clay to clayey type. The soil pH recorded in the seven areas varied between 6.7 (neutral) to 7.5 (slightly alkaline), which favoured the maximum AM fungal population density and high AM fungal species. Moisture content of these soil samples varied between 21–28%. Our results showed that the soils with low moisture content supported high spore numbers. This can be explained by the fact that mycorrhizal spores and their efficiency

to promote plant growth are affected indirectly by anaerobiosis (Safir and Duniway 1984). Electrical conductivity ranged between normal (0.28 dSm^{-1}) to satisfactory (0.98 dSm^{-1}) levels indicating that salinity effects were mostly negligible. The soils were optimum in nutrient levels, particularly with OC levels varying from 0.3 to 0.5% (low-medium), available N between 46 and 120 kg/Ac (low-high), levels of K were | 145–369 kg/Ac (moderate to high), and interestingly available phosphorus was 5–29 kg/Ac (low to medium). The crop is grown in Rabi season and experiences high temperatures, low moisture, and fewer nutrients being a dry season.

Table 2: The physico-chemical characteristics of *Carthamus tinctorius* rhizosphere soils of Kohir, Zaheerabad, Tandur, Bainsa, Baser, Adilabad, Sangareddy field sites of Telangana

Soil factors	Kohir	Zaheerabad	Tandur	Bainsa	Baser	Adilabad	Sangareddy
Colour	Greyish black	Brownish black	Black	Greyish black	Grey	Black	Brownish black
Texture	S clay	S clay	S clay	S clay	Clay	S clay	S clay
Moisture (%)	22	25	24	24	25	21	28
pH	7.0	6.7	7.2	6.8	7.4	7.2	7.5
EC (dSm^{-1})	0.59	0.41	0.28	0.38	0.51	0.43	0.98
OC (%)	0.4	0.3	0.5	0.3	0.5	0.3	0.4
Available N (kg/Ac)	52	46	93	103	98	93	120
Available P (kg/Ac)	8	15	6	11	9	5	29
Available K (kg/Ac)	145	151	184	235	235	145	369

S clay–Sandy clay, EC–Electron conductivity, OC–Organic carbon, N–Nitrogen, P–Phosphorus, K–Potassium

Table 3: AM fungal species isolated from *Carthamus tinctorius* rhizosphere soils of Kohir, Zaheerabad, Tandur, Bainsa, Baser, Adilabad, Sangareddy field sites of Telangana

S.No.	AM fungal species	Koh	Zah	Tan	Bain	Basr	Adb	SR
	Total number of species->	33	31	32	26	27	34	24
1	<i>Acaulospora alpina</i> (Oehl & Sieverding)	+	+	+	-	-	+	-
2	<i>A. bireticulata</i> (Rothwell & Trappe)	+	+	+	+	+	+	+
3	<i>A. delicata</i> (Walker, Pfeiffer, & Boss)	+	+	+	+	+	+	+
4	<i>A. denticulata</i> (Sieverding & Toro)	+	+	+	-	-	+	+
5	<i>A. elegans</i> (Trappe & Gerdemann)	+	+	+	+	+	+	+
6	<i>A. foveata</i> (Trappe & Janos)	+	+	+	-	-	+	-
7	<i>A. gerdemanii</i> (Sieverding & Tom)	+	+	+	+	+	+	+
8	<i>A. laevis</i> (Gerdemann & Trappe)	+	+	+	+	+	+	+
9	<i>A. mellea</i> (Spain & Schenck)	+	+	+	+	-	+	+

10	<i>A. morrowiae</i> (Spain & Schenck)	+	+	+	+	+	+	-
11	<i>A. myriocarpa</i> (Spain, Sieverd, & Schenck)	+	+	+	+	+	+	+
12	<i>A. nicolsonii</i> (Walker, Read, & Sanders)	-	-	+	+	+	+	+
13	<i>A. scrobiculata</i> (Trappe)	+	+	+	+	+	+	-
14	<i>Glomus aggregatum</i> (Schenck & Smith)	+	+	+	+	+	+	+
15	<i>G. ambisporum</i> (Smith & Schenck)	+	+	+	+	+	+	+
16	<i>G. australe</i> (Berkeley) (Berch)	+	-	-	-	-	-	-
17	<i>G. clarioides</i> (Schenck & Smith)	-	-	-	+	+	+	-
18	<i>G. clavisorum</i> (Trappe) (Almeida & Schenck)	-	-	-	-	-	-	+
19	<i>G. constrictum</i> (Trappe)	+	+	+	+	+	+	-
20	<i>G. diaphanum</i> (Morton & Walker)	-	+	-	-	+	+	-
21	<i>G. epigeum</i> (Daniels & Trappe)	+	+	+	+	-	+	+
22	<i>G. fasciculatum</i> (Thaxter)	+	+	+	+	+	+	+
23	<i>G. intraradices</i> (Schenck & Smith)	-	+	+	+	+	-	-
24	<i>G. manihotis</i> (Howeler, Sieverd, & Schenck)	+	+	+	+	+	+	+
25	<i>G. microaggregatum</i> (Koske, Gemma & Olexia)	-	+	-	-	+	+	+
26	<i>G. multicaule</i> (Gerd. & Bakshi)	+	+	+	-	+	+	-
27	<i>G. multisubstensum</i> (Mukerji, Bhattacharjee, & Tewari)	+	+	+	-	-	-	+
28	<i>G. reticulatum</i> (Bhattacharjee & Mukerji)	+	+	+	+	+	+	+
29	<i>G. rubiforme</i> (Gerd. & Trappe) (Almeida & Schenck)	-	-	+	+	-	-	-
30	<i>Gigaspora gigantea</i> (Nicol. & Gerd.) (Gerd. & Trappe)	+	+	-	-	-	-	-
31	<i>Gig. margarita</i> (Becker & Hall)	+	+	+	+	+	+	+
32	<i>Gig. rami sporophora</i> (Spain, Sieverding, & Schenck)	+	+	+	-	-	+	-
33	<i>Scutellospora calospora</i> (Nicol. & Gerd.)	+	+	+	+	+	+	+
34	<i>S. microcarpa</i> (Iqbal & Parveen)	+	-	-	-	+	+	+
35	<i>S. nigra</i> (Redhead) (Walker & Sanders)	+	+	-	-	-	+	-
36	<i>Scutellospora sp1</i> (Walker & Sanders)	+	-	-	-	-	+	-
37	<i>Funneliformis caledonius</i> (Nicolson & Gerd.) (Walker & Schüssler)	+	-	-	-	-	-	-
38	<i>F. geosporum</i> (Nicolson & Gerd.) (Walker)	+	+	+	+	+	+	+
39	<i>F. mosseae</i> (Nicol. & Gerd.) (Gerd. & Trappe)	+	+	+	+	+	+	+
40	<i>Entrophospora Schenkii</i> (Sieverding & Toro)	+	-	+	+	+	-	-
41	<i>Entrophospora sp.</i>	-	-	+	-	-	-	+
42	Unidentified genus/sp1	-	-	+	+	+	+	-

Koh- Kohir, Zah- Zaheerabad, Tan- Tandur, Bain- Bainsa, Basr- Baser, Adb- Adilabad, SR- Sangareddy

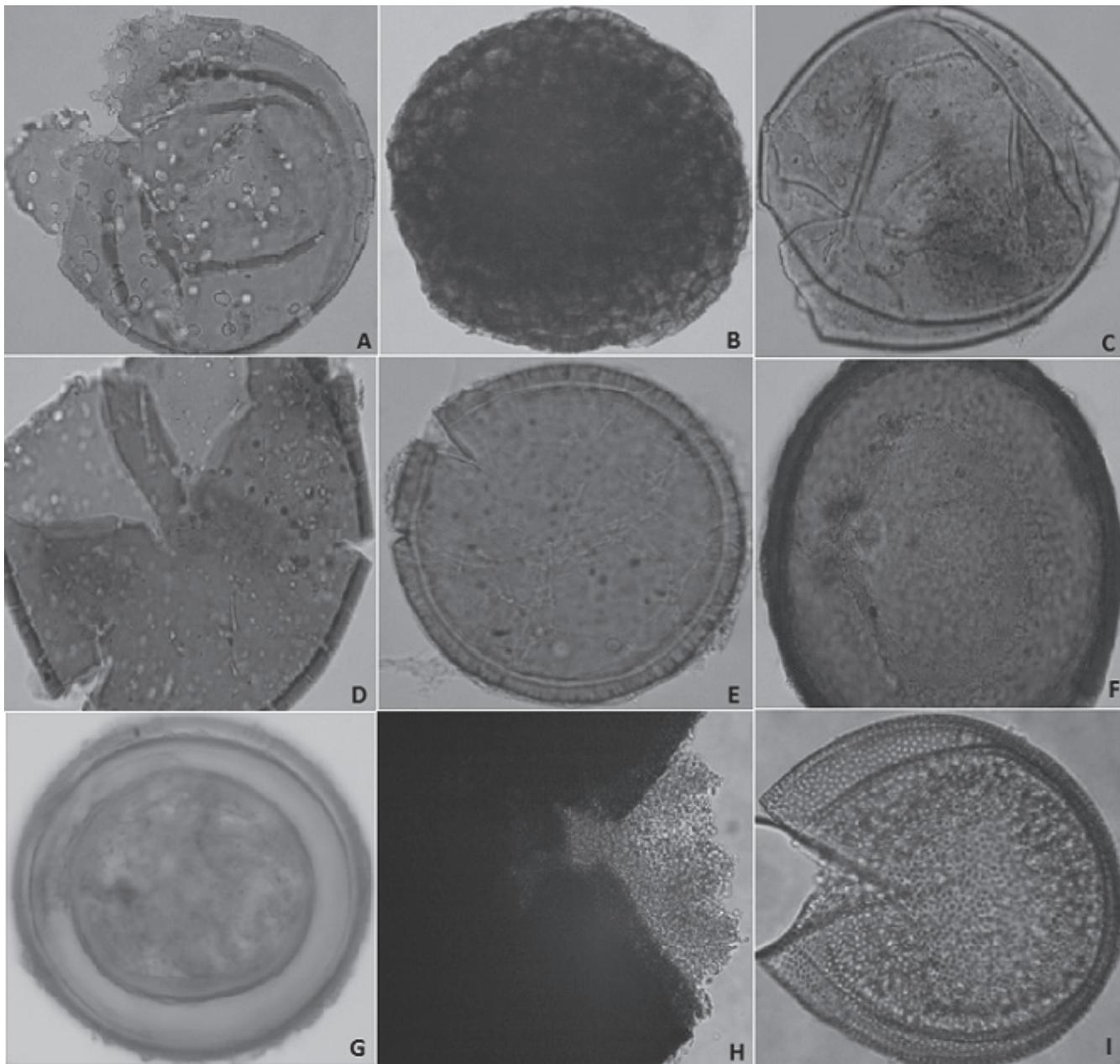


Figure 1: (A) *A. alpina*, (B) *A. bireticulata*, (C) *A. delicata*, (D) *A. foveata*, (E) *A. gerdemaniai*, (F) *A. mellea*, (G) *A. morrowiae*, (H) *A. myriocarpa*, (I) *A. scrobiculata*

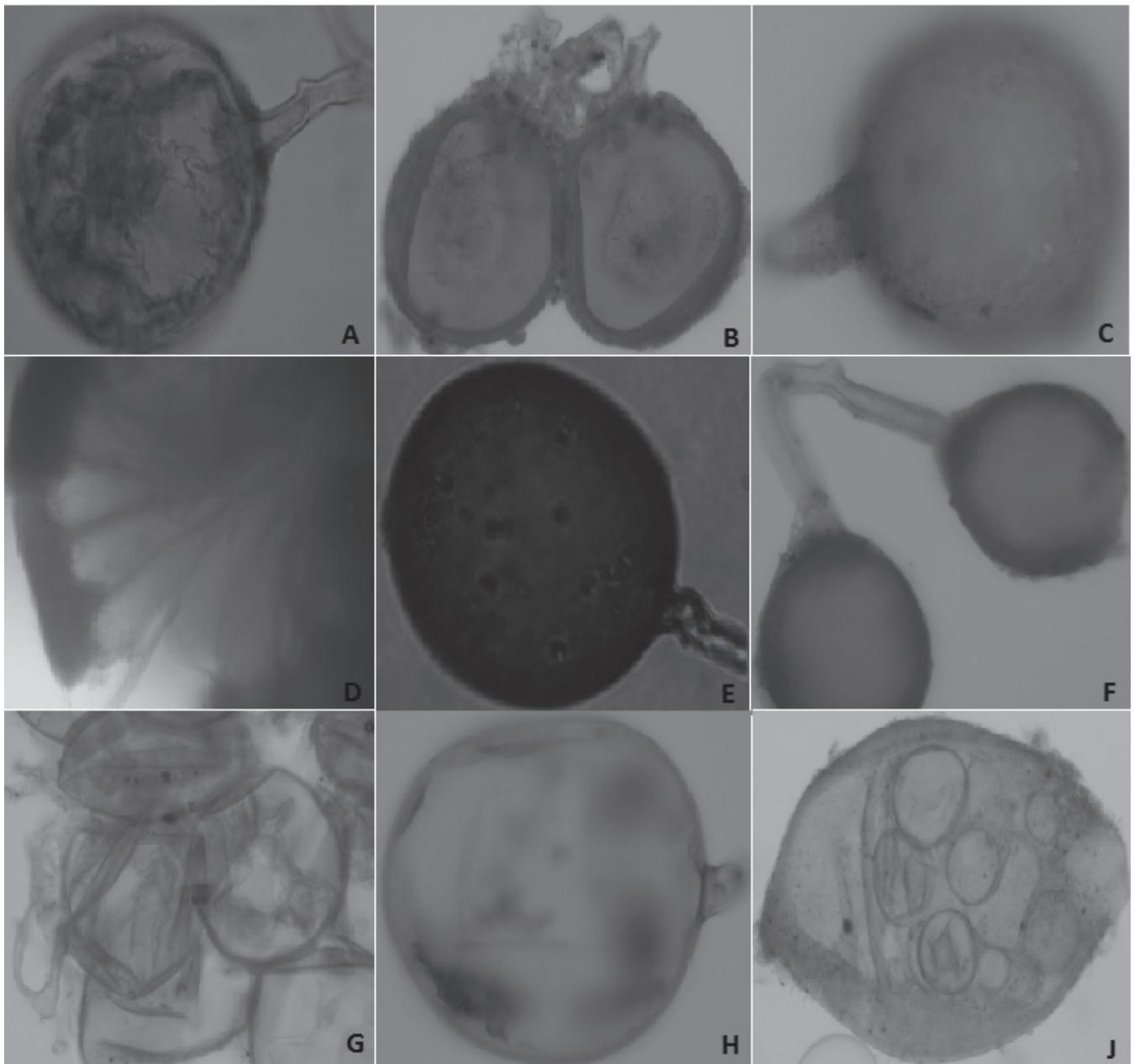


Figure 2: (A) *G. aggregatum*, (B) *G. ambisporum*, (C) *G. australe*, (D) *G. clavisporum*, (E) *G. constrictum*, (F) *G. diaphanum*, (G) *G. fasciculatum*, (H) *G. manihotis*, (I) *G. microaggregatum*

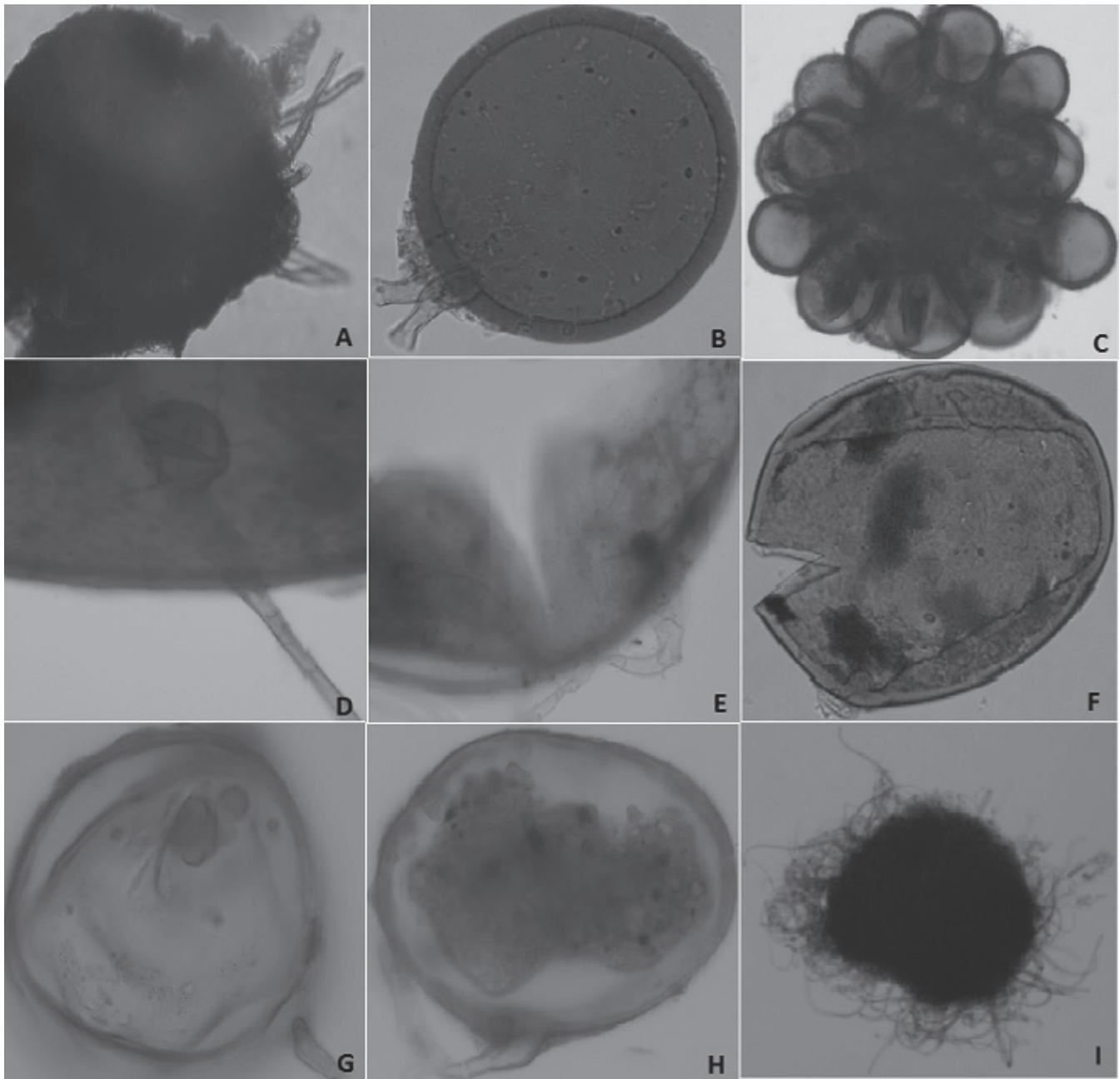


Figure 3: (A) *Glomus heterosporum*, (B) *G. multisubstensum*, (C) *G. rubiforme*, (D) *Gigaspora gigantea*, (E) *Gig. rami sporopora*, (F) *Scutellospora calospora*, (G) *Funneliformis geosporum*, (H) *F. mosseae*, (I) Unidentified genus/species.

Table 4: Number of AM fungal spores identified per species, RA, IF and IV of AMF in the *Carthamus tinctorius* rhizosphere soils of Kohir, Zaheerabad, Tandur, Bainsa, Baser, Adilabad, Sangareddy field sites of Telangana

Field sites ---->	Koh	Zah	Tan	Bains	Basr	Adb	SR	SD	IF	RA	IV
	(AM fungal spores per 10 gm soil)								(%)	(%)	(%)
spore density --->	334	303	299	310	303	355	231	2135			
A. alpine	8	6	10	-	-	8	-	32	57.1	1.5	29.3
A. bireticulata	23	16	20	28	12	24	14	137	100	6.42	53.2

<i>A. delicata</i>	13	14	20	14	10	18	18	107	100	5.01	52.5
<i>A. denticulata</i>	4	8	8	-	-	8	11	39	71.4	1.83	36.6
<i>A. elegans</i>	8	18	12	16	12	16	16	98	100	4.59	52.3
<i>A. gerdemanii</i>	10	5	8	15	15	4	6	63	100	2.95	51.5
<i>A. foveata</i>	20	22	18	-	-	21	-	81	57.1	3.79	30.5
<i>A. laevis</i>	14	19	22	23	20	9	13	120	100	5.62	52.8
<i>A. mellea</i>	15	14	9	11	-	6	2	57	85.7	2.67	44.2
<i>A. morrowiae</i>	10	12	10	6	18	3	-	59	85.7	2.76	44.2
<i>A. myriocarpa</i>	8	11	5	19	10	6	10	69	100	3.23	51.6
<i>A. nicolsonii</i>	-	-	8	15	14	4	11	52	71.4	2.44	36.9
<i>A. scrobiculata</i>	7	4	8	6	14	4	-	43	85.7	2.01	43.9
<i>Glomus aggregatum</i>	6	4	8	16	10	14	6	64	100	3.00	51.5
<i>G. ambisporum</i>	11	6	6	10	8	6	11	58	100	2.72	51.4
<i>G. australe</i>	5	-	-	-	-	-	-	5	14.3	0.23	7.3
<i>G. clarioides</i>	-	-	-	1	15	13	-	29	42.9	1.36	22.1
<i>G. clavisporum</i>	2	-	-	-	-	-	4	6	28.6	0.28	14.3
<i>G. constrictum</i>	4	16	6	12	10	18	-	66	85.7	3.09	44.4
<i>G. diaphanum</i>	-	9	-	-	7	16	-	32	42.9	1.50	22.2
<i>G. epigeum</i>	10	6	4	18	-	14	8	60	85.7	2.81	44.3
<i>G. fasciculatum</i>	35	20	16	21	28	22	12	154	100	7.21	53.6
<i>G. intraradices</i>	-	6	6	8	4	-	-	24	57.1	1.12	29.1
<i>G. manihotis</i>	6	2	2	5	8	8	14	45	100	2.11	51.1
<i>G. microaggregatum</i>	-	2	-	-	2	13	4	21	57.1	0.98	29.1
<i>G. multicaule</i>	6	4	8	-	4	4	-	26	71.4	1.22	36.3
<i>G. multisubstensum</i>	9	3	5	-	-	-	8	25	57.1	1.17	29.2
<i>G. reticulatum</i>	11	6	8	15	4	13	2	59	100	2.76	51.4
<i>G. rubiforme</i>	3	-	5	1	-	-	-	9	42.9	0.42	21.6
<i>Gigaspora gigantea</i>	3	2	-	-	-	-	-	5	28.6	0.23	14.4
<i>Gig. margarita</i>	6	4	5	8	6	4	10	43	100	2.01	51.0
<i>Gig. rami sporopora</i>	4	8	1	-	-	3	-	16	57.1	0.75	28.9

Scutellosporacalospora	4	8	6	10	4	9	8	49	100	2.30	51.2
S. microcarpa	6	-	-	-	6	9	5	26	57.1	1.22	29.2
S. nigra	6	8	-	-	-	9	-	23	42.8	1.08	22.0
Scutellospora sp.	8	-	-	-	-	10	-	18	28.6	0.84	14.7
Funneliformiscaledonius	4	-	-	-	-	-	-	4	14.3	0.19	7.2
F. geosporum	10	18	12	16	19	11	10	96	100	4.50	52.3
F. mosseae	29	22	31	10	19	12	22	145	100	6.79	53.4
Entrophosporaschenkii	6	-	2	2	8	-	-	18	57.1	0.84	29.0
Entrophospora sp.	-	-	6	-	-	-	6	12	28.6	0.56	14.6
Unidentifiedgenus/sp1	-	-	4	4	16	16	-	40	71.4	1.87	36.7

Koh-Kohir, Zah-Zaheerabad, Tan-Tandur, Bains-Bainsa, Basr-Baser, Adb-Adilabad, SRy-Sangareddy, SD-Spore density, IF-Isolation frequency, IV-Importance value

Table 5: Diversity measurement of AMF community

S.No.	Factors	Kohir	Zah	Tandur	Bainsa	Baser	Adilabad	SR
1	Spore density SD	334	303	299	310	303	355	231
2	Av Pop size	9.54	9.77	9.34	11.92	11.22	11.06	9.63
3	Total no. of species	33	31	32	26	27	34	24
5	Simpson dominance index	0.084	0.085	0.087	0.096	0.09	0.072	0.097
6	Simpson diversity index	0.916	0.915	0.913	0.904	0.911	0.928	0.090
7	Shannon index	3.33	3.23	3.251	3.078	3.149	3.353	3.045

Zah-Zaheerabad, SR-Sangareddy, spore density-per 10 gm soil

AMF Spore Density in the Soil

The physico-chemical factors associated with the rhizosphere soils of safflower supported good amount of AM fungal population density (Table 4). The rhizosphere soils of field sites from different areas revealed the presence of high AM fungal spore population. This study confirms the formation of mycorrhizal association in safflower grown in semi-arid tropical soils. A total of 2,135 spores of AMF were retrieved from the present soil samples of Telangana, India. The association of good amount of AM fungal spore density indicates that the soil factors, such as nutrient status, soil type, soil pH, moisture content, OC seems to greatly influence AM spore number.

The average spore density of different field sites ranged from 231 to 355 spores per 10 gm soil. The soils of Adilabad field sites harboured maximum

number of spores (355 per 10 gm soil), followed by Kohir (334), Bainsa (310), Zaheerabad and Baser (303), and Tandur (299) and minimum in Sangareddy soils (231). The high spore densities in the rhizosphere soils of our study could be due to the prevalent dry environment in Rabi season throughout the crop growth period. Our results are similar to the findings of Cardoso *et al.* (2003) who reported that AMF spore density is influenced by temperature and high sporulation may be the result of stress in the area. In the present study, sampling was carried out in the Rabi season (dry season) when increased spore densities can be anticipated (Verbruggen and Kiers 2010, Abdelhalim *et al.* 2013).

A total of 42 AM fungal species were identified on the basis of morphological criteria (Table 3) representing six genera, which include *Acaulospora*,

Glomus, *Gigaspora*, *Scutellospora*, *Funnelformis*, and *Entrophospora*. *Acaulospora* was represented by 13 species, *Glomus* by 16, *Gigaspora* and *Funnelformis* by 3 spp. each, *Scutellospora* by 4 spp., *Entrophospora* by 1 sp. and one genus/species was unidentified. The isolated 42 AM fungal species from the present soils were *A. alpina* (Fig. 1A), *A. bireticulata* (Fig. 1B), *A. delicata* (Fig. 1C), *A. denticulata*, *A. elegans*, *A. foveata* (Fig. 1D), *A. gerdemanii* (Fig. 1E), *A. laevis*, *A. mellea* (Fig. 1F), *A. morrowiae* (Fig. 1G), *A. myriocarpa* (Fig. 1H), *A. nicolsonii*, *A. scrobiculata* (Fig. 1I), *Glomus aggregatum* (Fig. 2A), *G. ambisporum* (Fig. 2B), *G. australe* (Fig. 2C), *G. clarioides*, *G. clavisorum* (Fig. 2D), *G. constrictum* (Fig. 2E), *G. diaphanum* (Fig. 2F), *G. epigeum*, *G. fasciculatum* (Fig. 2G), *G. flavisorum*, *G. heterosporum* (Fig. 3A), *G. intraradices*, *G. manihotis* (Fig. 2H), *G. microaggregatum* (Fig. 2I), *G. multicaule*, *G. multisubstansum* (Fig. 3B), *G. reticulatum*, *G. rubiforme* (Fig. 3C), *Gigaspora gigantean* (Fig. 3D), *Gig. margarita*, *Gig. rami sporopora* (Fig. 3E), *Scutellospora calospora* (Fig. 3F), *S. microcarpa*, *S. nigra*, *Scutellospora* sp., *Funnelformis caledonius*, *F. geosporum* (Fig. 3G), *F. mosseae* (Fig. 3H), *Entrophospora Schenkii*, *Entrophospora* sp., Unidentified genus/sp1 (Fig. 3I).

In the present study, a total of 42 AM fungal species (Morphospecies) detected in seven different field sites is surprisingly a large number from about nearly 250 AMF species that have so far been described worldwide for the phylum Glomeromycota (Oehl *et al.* 2003). Pearson correlation analysis showed a significant correlation between spore density and total number of species ($r = 0.787^*$, $p < 0.05$).

In the present investigated soils the genus *Glomus* was the most dominant and widely distributed among the Glomeromycotan genera represented by 16 spp. suggesting thereby, its predominance (Abdelhalim *et al.* 2013; Hindumathi and Reddy 2011; Snoeck *et al.* 2010, Janaki Rani and Manoharachary 1994), followed by *Acaulospora* by 13 spp. Moreover, *Glomus* species have the potential to produce a relatively high number of spores with in a very short period of time (Oehl *et al.* 2009).

The highest species diversity of 34 species was recorded in the rhizosphere soils of Adilabad, followed by 33, 32, 31, 27, 26 spp. from Kohir, Tandur, Zaheerabad, Baser and Bainsa, respectively and lowest species diversity of 24 spp was recorded in Sangareddy soils (Table 3).

The RA of AMF ranged from 0.19 to 7.21%, IF from 14.28 to 100% and IV from 7.24 to 53.61% (Table 4). Based on the RA and IF, *G. fasciculatum* was observed to be the most predominant species with high spore density (SD) of 154 spores and RA 7.21%, followed by *F. mosseae* (145), *A. bireticulata* (137) and *A. laevis* (120) and RA 6.79%, 6.42% and

5.62%, respectively. These findings are in accordance with earlier reports (Karthikeyan and Selvaraj 2009; Kavitha and Nelson 2013). Lowest SD of *Funnelformis caledonius* (4 spores) with RA 0.32% was encountered in the present soils.

However, *G. aggregatum*, *G. ambisporum*, *G. fasciculatum*, *G. heterosporum*, *G. reticulatum*, *G. manihotis*, *F. geosporum*, *F. mosseae*, of Glomales, *A. bireticulata*, *A. delicata*, *A. elegans*, *A. gerdemanii*, *A. laevis*, *A. myriocarpa* of Acaulosporaceae, *Gigaspora margarita*, and *Scutellospora calospora* of Gigasporaceae contributed to greater IF of 100%. The reason for higher frequency of occurrence of AM fungal species could be attributed to the favourable soil environmental conditions of the sampling sites (Kavitha and Nelson 2013).

The IV used to evaluate the dominance of AMF based on IF and RA is presented in Table 4. The AM fungal species *A. bireticulata*, *A. delicata*, *A. elegans*, *A. gerdemanii*, *A. myriocarpa*, *G. ambisporum*, *G. aggregatum*, *G. fasciculatum*, *G. heterosporum*, *G. manihotis*, *G. reticulatum*, *Gigaspora margarita*, *Scutellospora calospora*, *F. geosporum*, *F. mosseae*, and showed $IV \geq 50\%$ indicating that these species were dominant.

Diversity Index

Furthermore, the AMF diversity expressed by the Shannon–Wiener/Shannon–Weaver index of diversity (H') and Simpson index of dominance (D) showed greater diversity (Table 5). Shannon–Weaver index of diversity (H') was highest (3.353) in Adilabad and lowest (3.045) in Sangareddy soils. Simpson dominance index (D) was highest (0.096) in soils of Bainsa and lowest in Adilabad (0.072). Simpson diversity index was highest (0.928) in Adilabad soils showing high diversity and lowest (0.090) in Sangareddy soils showing low diversity.

High diversity index value suggests that a higher species of indigenous AMF exists. A high H' value suggests a stable and ancient site, while a low H' value could suggest a site affected by agricultural management. The low index of dominance (0.072) (Table 5) for AMF in the field site of Adilabad indicates shared dominance of many AM fungal species while a higher value of 0.096 indicates dominance by a few species of AMF in Sangareddy soils.

Species dominance was shared by a maximum of 34 AMF out of a total 42 species in Adilabad soil as revealed by higher percentage frequency of occurrence of the species which accounts for more than 78% of the total species. However, species dominance of 24 AMF species represent 57% of the total species occurred in the field sites of Sangareddy.

In case of general diversity indices the result was in contrast to the indices of dominance, a value of 0.928 (Adilabad) and 0.090 (Sangareddy) suggesting a greater diversity of AM fungal species in the Adilabad than in Sangareddy soils. There was a decline of around 22% AMF species diversity in Sangareddy soils. This is in confirmity with the higher number of AMF species and spores in Adilabad than in Sangareddy soils.

Relationship between Soil Physico-Chemical Characters and AM Fungal Spore

AM spore density and total number of species exhibited a significantly negative correlation with the moisture content ($r = -0.970^{**}$, $p < 0.01$ & $r = -0.828^*$, $p < 0.05$, respectively). Reid and Bowen (1979) reported an increase in soil moisture content found to have negative effect on spore number. More number of AM fungal propagules were retrieved from soils with low P in confirmity with earlier results (Janaki Rani and Manoharachary 1994). A significantly negative correlation was observed between spore density and available P in the soil ($r = -0.888^{**}$, $p < 0.01$) similar to our earlier reports (Hindumathi and Reddy 2011). This can be attributed to the fact, that available soil P suppresses AM spore density. Significantly negative correlation of spore density and total number of species with available K ($r = -0.888^{**}$, & $r = -0.906^{**}$, $p < 0.05$, respectively) clearly indicates that increase in their levels inhibits mycorrhizal development. Spore density and SR showed no correlation with soil factors pH, EC, OC, and available N.

Results of the present study indicate that AMF abundance and diversity are high in the areas studied with *Glomus* as the most common and widely distributed genus in all the soils surveyed. The genus *Glomus* is represented by more number of species compared to other genera contributing the most to the biodiversity index. It can be concluded that moisture content, available P, available K might be the factors for influencing AMF spore abundance and diversity.

The present study provides the first information on a diversity of AMF in safflower from different areas of Telangana state, India.

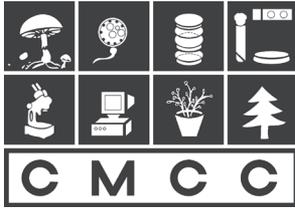
Acknowledgements

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Morphotaxonomy of *Funnelformis coronatum*/*Glomus coronatum* (accession-CMCC/AM-1504)

Kiran Sunar^a, Priya Ahuja^a, Maunata Ghorui^a, and Alok Adholeya^{b*}

Nomenclature and taxonomy of Arbuscular Mycorrhiza Fungi (AMF) is largely based on distinct morphological features that are unique and generally confined to one genus or taxa. Though some general features, such as spore colour, shape, and ornamentation, are common in almost all the species, some specific taxonomic characters such as spore colour, attachment of the subtending hyphae to the spore base, nature of spore wall layers, formation of occlusions, and type of spore bases play a key role in designating the status of genus and species to a particular AMF fungus (Gerdemann and Trappe 1974; Schenck and Pérez 1990; Walker and Sanders 1986). On the basis of taxonomic inputs received with the advent of new technologies for studying spore morphologies, the present classification of AMF now consists of three classes—Archaeosporomycetes, Glomeromycetes, and Paraglomeromycetes, which is further divided into five orders—Archaeosporales, Diversisporales, Gigasporales, Glomerales, and Paraglomerales—and further down to fourteen families, twenty nine genera, and approximately more than three hundred species (Palenzuela *et al.* 2008; Walker and Schüßler 2004). In continuation to our previous articles, wherein we have been making efforts to describe specific genera of AMF present in our germplasm bank, in this current article, we would like to describe a very unique species of AMF available in Centre for Mycorrhizal Culture Collection Bank with the accession number CMCC/AM-1504.

Monosporal Establishment

The isolate designated as CMCC/AM-1504 was isolated from the trap raised from the soil collected

from Rajkot district of Gujarat with wild grass as host. This district falls in North Saurashtra agro-climatic zone that has shallow, medium black soil. The soil was light grey in colour and mostly sandy clay loam in texture with a neutral to alkaline pH. When tested for the nutritional status of the original soil sample, it was observed that the available potassium content was adequate while available phosphorous and nitrogen were comparatively less. Trap cultures were raised for proliferation of indigenous mycorrhiza in pot conditions with a suitable host for a period of three months. After adequate growth cycle of the host plant, spores propagated in trap culture were checked by wet sieving and decanting method (Gerdeman and Nicholson 1963) and were categorized on the basis of their morphology. In the next step, all the healthy spores were grouped according to their size, structure, and colour. Morphologically similar types of spores were grouped or isolated for obtaining pure single species culture of AMF. Voucher specimen of all the potential monosporal was prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., were carried out under compound microscope (10×, 40×, and 100×) after mounting in Polyvinyl Lacto glycerol and Polyvinyl Lacto glycerol: Melzer's reagents (1:1). Selected healthy single AMF spores were used to raise monospecific cultures that were inoculated to pre-germinated seed of a suitable host. After a successful growth period of three to six months, the host roots were evaluated for colonization and sporulation. Cultures showing colonized roots and spores were considered as successful cultures for raising monosporals and were considered to be pure when the spores isolated from them were

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morphotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monospores (Figure 1).

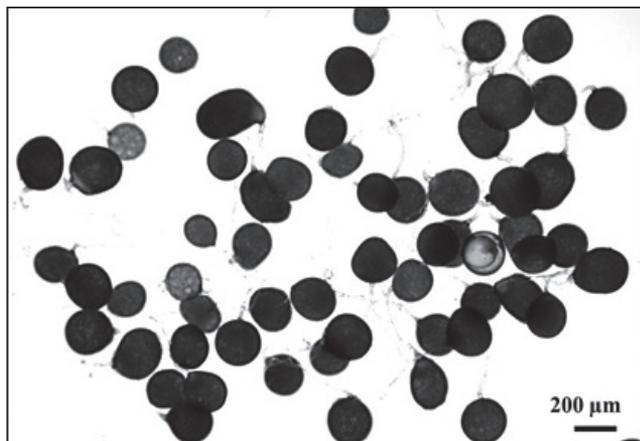


Figure 1: Identical spores obtained from monospore culture of isolate CMCC/AM-1504

In case of CMCC/AM-1504, the fully sporulation state was attained only after two consecutive cycles of the host plant.

A detailed morphotaxonomic description of this accession has been presented as adopted by various workers for identification.

Spore Morphology and Shape

Spores isolated from the monospore cultures were borne singly or in small clusters of three or four in the extraradical region; they were devoid of sporocarps and each of them were suspended with a single subtending hyphae. The colour of spores ranged from yellowish brown while young to dark orange brown at maturity. The shape of the spores varied from globose to sub-globose. Scanning electron micrograph of the spore surface revealed an outer smooth wall surface in mature spores with no any prominent pits. Each spore appeared globose suspended with a distinct funnel-shaped subtending hyphae (Figure 2).

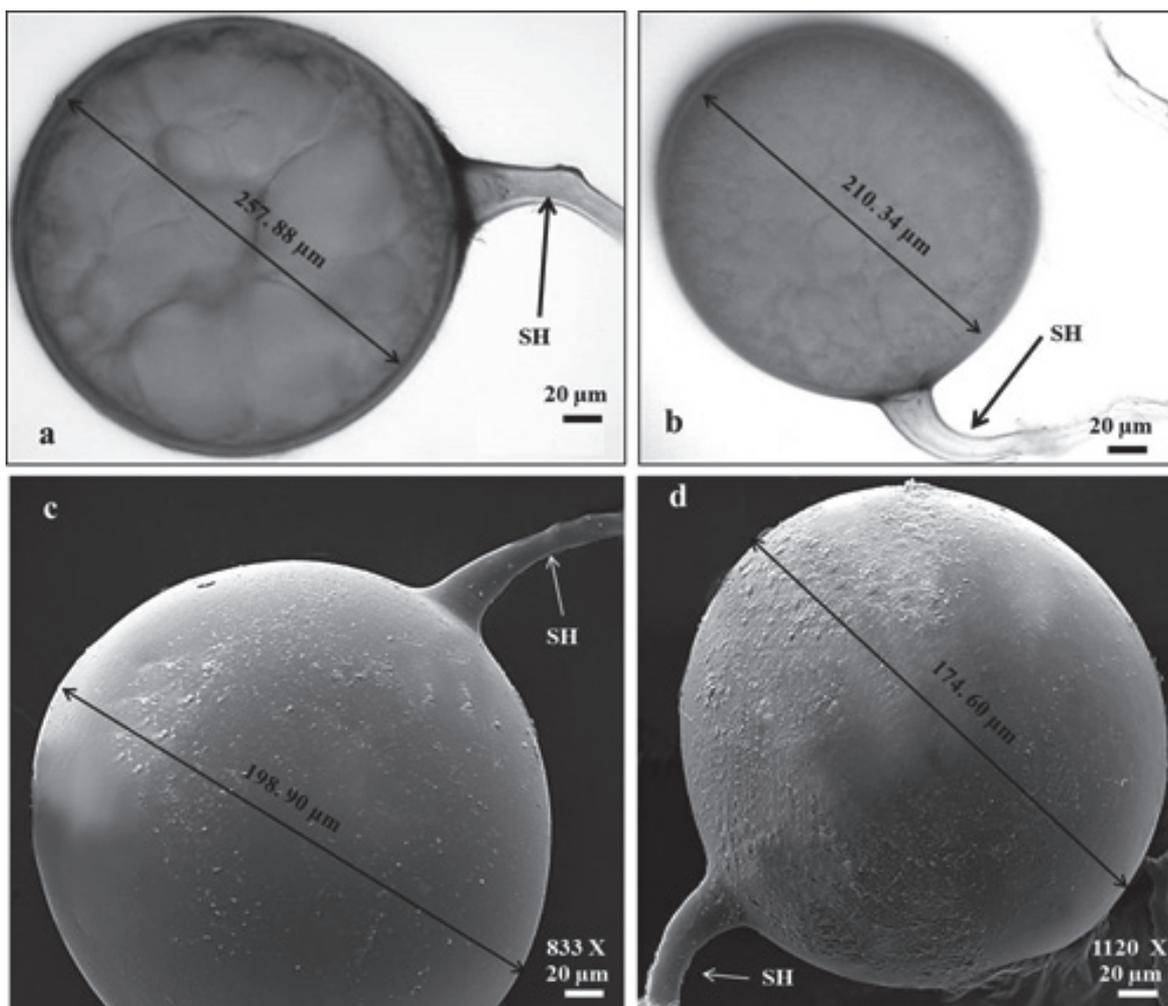


Figure 2: Compound and scanning electron micrograph (SEM) of spores of CMCC/AM 1504 showing globose spore structure and a single spore with funnel-shaped subtending hyphae. Spore mounted in Melzer's reagent: (a) and PVLG: Melzer's reagents, (b) SEM images of the spores (c and d).

The average spore diameter ranged from 180 (291.86) to 390 μm (Figure 3).

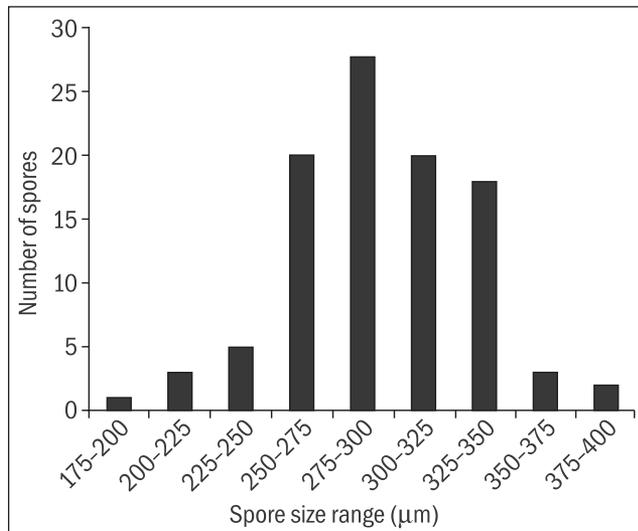


Figure 3: Analysis of spore diameters of 50 healthy spores obtained from one-year old monosporal culture

Subcellular Structure of Spore

Mature spores are composed of the following subcellular structures and wall layers:

The spore consists of two wall layers.

SWL-1 (Evanescent): The first layer of the spore designated as (SWL-1) is the evanescent layer that is hyaline in colour with a thickness of 2.7–4.2 μm and is continuous with the outer wall of the subtending hyphae. This layer is somewhat roughened. As the name suggests, this layer deteriorates and gets sloughed off with the ageing of the spore and thus is rarely present in the spore wall of the mature specimen. This spore wall layer is flexible in nature and is stained lightly in Melzer’s reagent appearing pinkish red in juvenile spores (Figure 4).

SWL-2 (Laminated): The inner wall layer designated as SWL-2 is smooth, laminated permanent wall layer that is brownish-orange in colour with a thickness of 4.3–7.2 μm and is continuous with the inner wall of the subtending hyphae. This wall layer is a conglomerate of very thin sub-layers that tightly

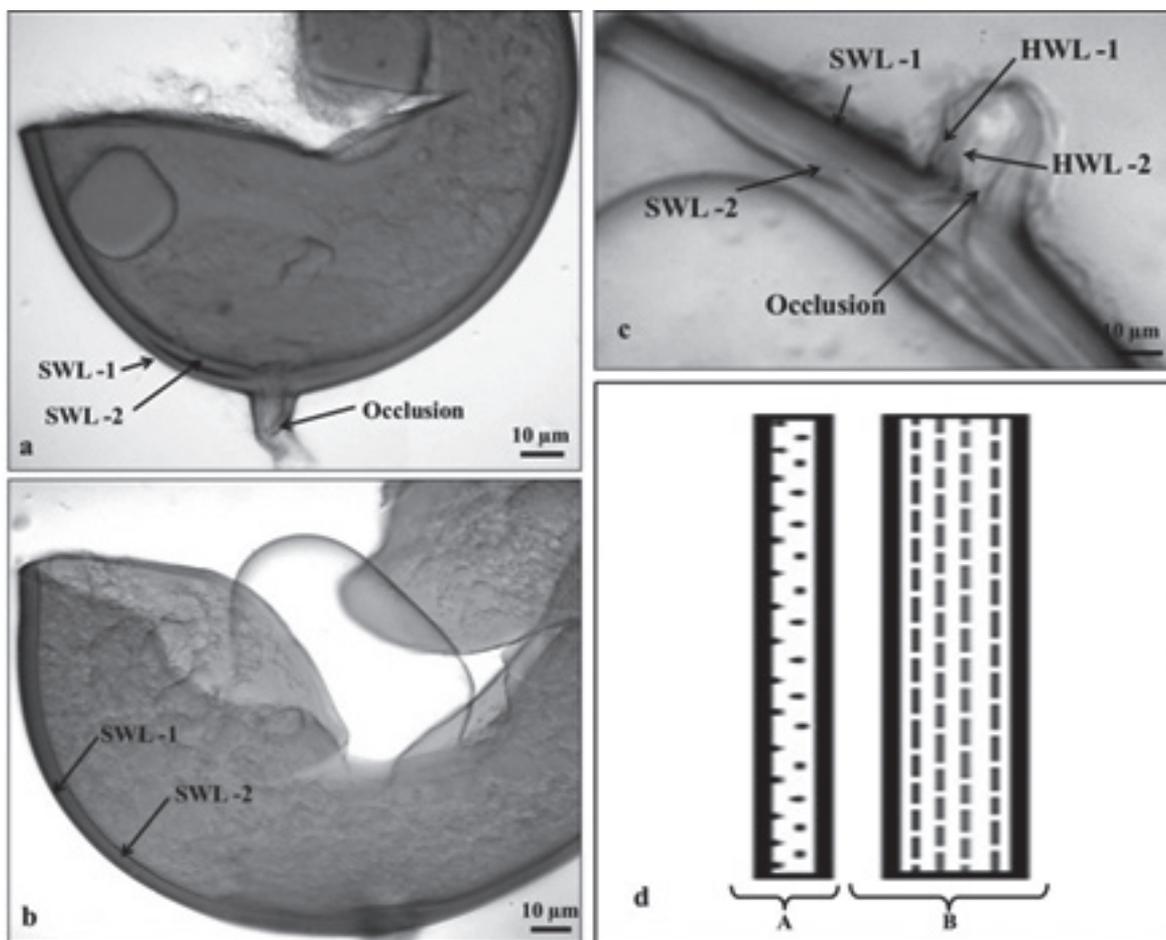


Figure 4: Compound microscopic images of spore wall layers of CMCC-AM 1504 after mounting in PVLG: Melzer’s reagent: (a) and PVLG (b and c). The outer layer SWL-1 is evanescent while the inner wall layer SWL-2 is laminated. HWL-1 and HWL-2 are hyphal wall layers of the subtending hyphae, respectively (c). Murograph (Walker 1983) of the mature spore showing outer evanescent and inner laminated layer (d)

adhered to each other. This layer stains reddish-brown in both young and mature spores (Figure 4).

Subtending Hyphae

All the spores show intact, straight or recurved, distinctly funnel-shaped subtending hyphae. The width of the subtending hyphae at the point of attachment at the spore base ranges from 20.25 to 25.75 μm . The hyphal wall of the subtending hyphae is brownish-orange in colour and is incessant with the two wall layers of the spore. Both the wall layers of the spores (SWL-1 and SWL-2) are continuous with the wall layers of the subtending hyphae. Layer two (HWL-2) is colourless and is generally the only layer that remains adhered to the mature spore. The average width of HWL-1 is around 2.4 μm and that of HWL-2 is 3.5 μm (Figures 5 and 6).

Occlusion

Most of the spores in low resolution appear to have an open pore, but higher magnification of the spore base reveals a thin curved septum, which is on an average 30–50 μm away from the point of attachment (Figure 5a and b). In some cases, the recurved septum is very difficult to detect and forms just 8–10 μm away from the point of attachment (Figure 5c). The septum is continuous with the innermost sub-layers of the laminated spore wall layer.

Mycorrhiza

Mycorrhiza structures, such as arbuscules, extra and intraradical hypha, and vesicles observed in *Sorghum bicolor* roots, are deeply stained in ink vinegar. Root colonization assays showed the presence of both extraradical and intraradical hyphae.

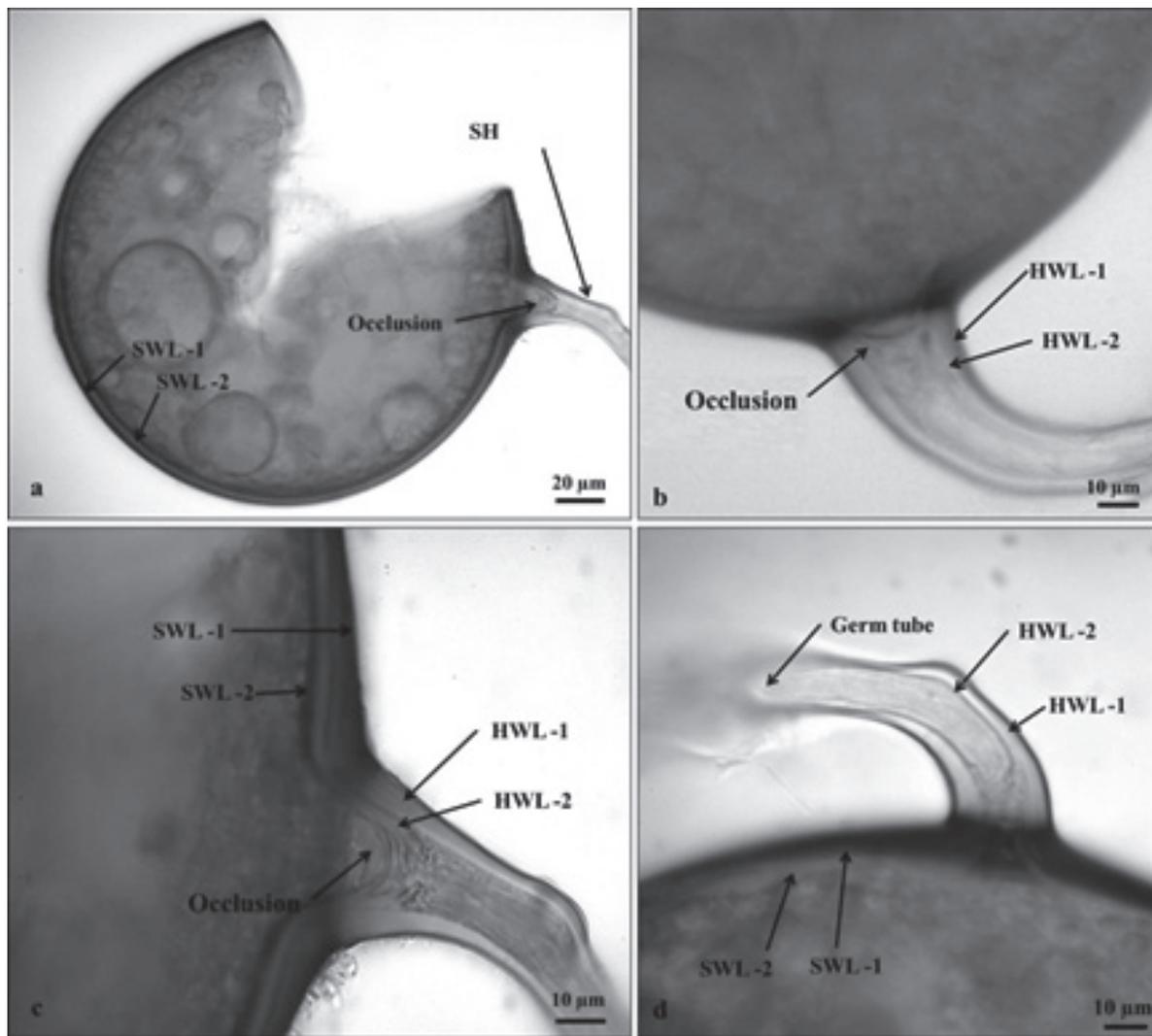


Figure 5: Compound microscopic images of spore of CMCC-AM 1504 showing funnel-shaped subtending hyphae after mounting it with PVLG: Melzer's reagent (a) and PVLG (b and c). The recurved septum is continuous with the innermost layer of the laminated wall layer of the spore (c). Hyphal layers HWL-1 and HWL-2 are in continuum with the spore wall layers SWL-1 and SWL-2, respectively (c and d).

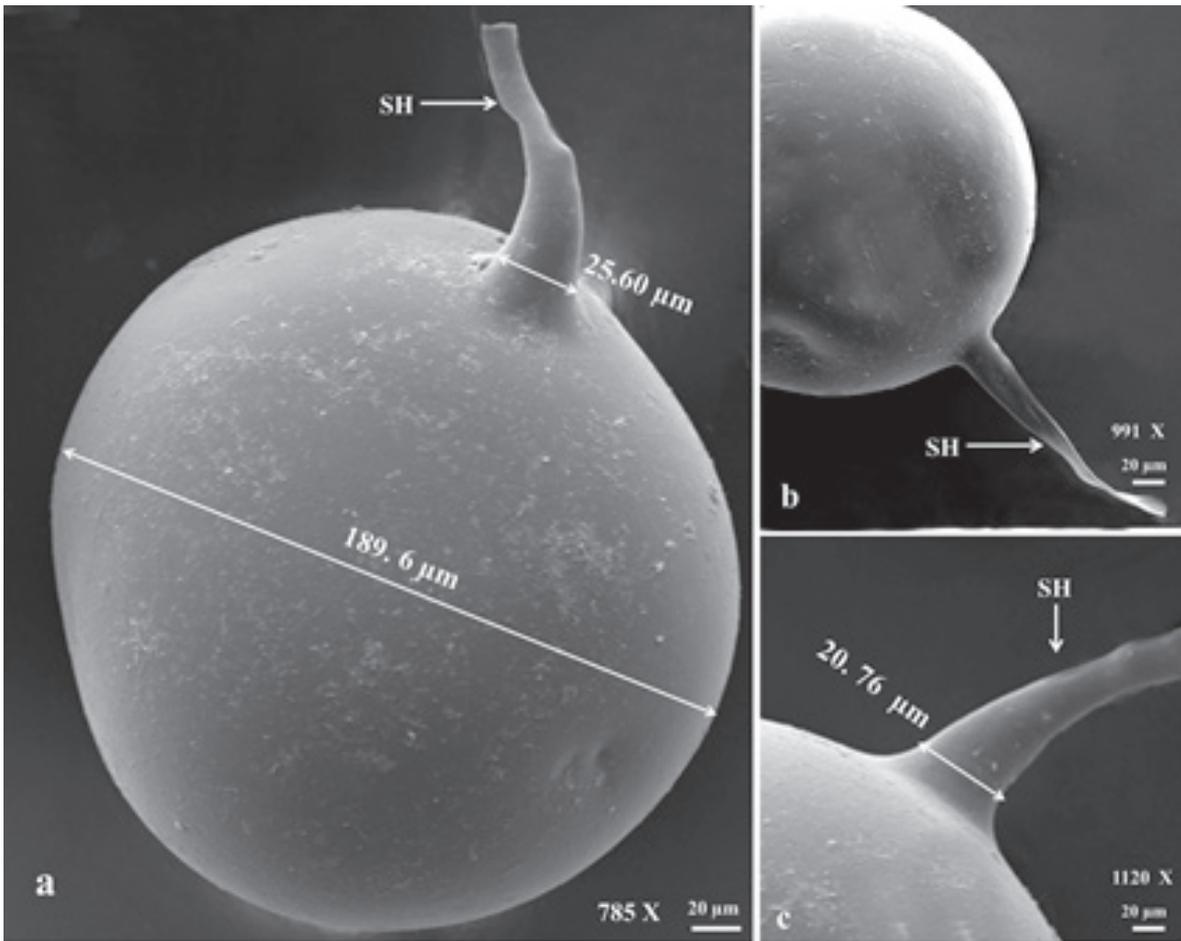


Figure 6: SEMs of spores of CMCC-AM 1504 showing funnel-shaped subtending hyphae attached to a globose spore. The point of attachment at the spore base is wide and tapers towards the end assuming a typical funnel shape (average width -20.25 to 25.75 μm)

During the early stages of mycorrhiza development, numerous branched external hyphae are seen that bear thin-walled deeply stained extra metrical vesicles. Intraradical hyphae show coiling at the entry points and later on continue to grow parallel with the root axis. Arbuscules are abundantly formed within the cortical regions and are thin walled and profusely branched (Figure 7). During the later stage of mycorrhiza development, abundant extraradical spores are formed singly.

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-1504, many distinguishing features regarding the family, genera, and the species could be derived. The following features were taken into consideration for characterization and identification:

- Globose, asexual spores produced singly with layered spore walls.
- Spore wall layer is composed of outer evanescent mucilaginous layer and a smooth inner laminated

layer that is continuous with the subtending hyphal wall.

- Spores are of varying shapes and sizes ranging from globose to sub-globose.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular arbuscules.

All these features suggest that the culture CMCC-AM-1504 belongs to the family Glomeraceae (Walker and Schüßler, 2004).

Some of the unique morphotaxonomic features of the accession are as under:

- Dark orange-brown, globose, asexual spores produced singly with layered spore walls, spores are of varying shapes and sizes ranging from globose to sub-globose, sizes ranging from 180 (291.86) to 390 μm.
- Spore wall layer is composed of outer evanescent mucilaginous layer and a smooth inner laminated layer that is continuous with the subtending hyphal wall.

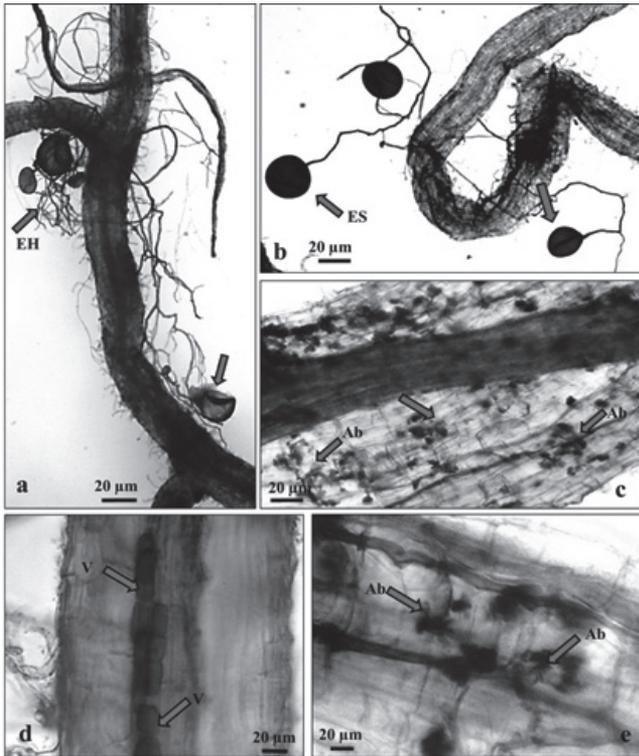


Figure 7: Compound microscopic images of roots of *Sorghum bicolor* stained in ink vinegar observed for root colonization by CMCC-AM 1504 showing extraradical and intraradical hypha bearing extra metrical vesicles (a and b) and abundant arbuscules in the cortical cells (c), magnified view (100×) of solitary intercalary vesicles (d) and finely branched arbuscules (e).

- Presence of intact, straight or recurved, distinctly funnel-shaped subtending hyphae. The width of the subtending hyphae at the point of attachment at the spore base ranges from 20.25 to 25.75 µm.
- Funnel-shaped subtending hyphae with a thin curved septum, which is on an average 30–50 µm away from the point of attachment.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular arbuscules.

The taxonomic feature of the accession CMCC/AM-1504 matches the characters of *Glomus coronatum* Giovan., the species originally described by Giovannetti *et al.* (1991) isolated from *Anacyclus radiatus* growing in maritime sand dunes located near Follonica, Italy. The genus *Glomus* under Glomeromycota underwent a revision by Walker and Schüßler (2004), who proposed a new generic name for *Glomus mosseae* as *Funneliformis mosseae* C. Walker and Schüßler gen. nov. (*Funneliformis mosseae* is the generic type)

The following were the characteristic features considered for this revision:

- Coloured spores formed in the soil or substratum

in sporocarps or ectocarpic spores singly or in loose clusters in the substratum.

- Spores often with a distinct funnel-shaped spore base.
- Spore wall normally containing two to three wall layers/components.
- Outer wall layer usually hyaline while inner wall layers are laminated and coloured.
- Spores normally occluded by a septum in the subtending hypha distal to the spore base.

On the basis of the morphological features described in this study, the accession CMCC/AM-1504 matches the characters of *Funneliformis coronatum* (Giovann.) C. Walker & A. Schüßler comb. nov. belonging to the family Glomeraceae. *Funneliformis coronatum* has many morphotaxonomic characters similar to *Funneliformis mosseae* such as the spore shape, diameter, funnel-shaped spore base but the feature that differentiates the two are: *Funneliformis coronatum* is comparatively darker than *Funneliformis mosseae*. The spore wall of *Funneliformis coronatum* has two sub-layers while *Funneliformis mosseae* has three sub-layers. *Funneliformis mosseae* is widely distributed in the world while *Funneliformis coronatum* is mainly restricted to the warmer regions.

Systematic Classification

Glomeromycota

Glomeromycetes

Glomerales

Glomeraceae

Glomus coronatum/*Funneliformis coronatum*

The revision in the taxonomy and nomenclature in Glomeromycota was mainly influenced by the molecular phylogenetic data that were derived through the gene sequences of the conserved regions. Morphologically similar taxa and groups have now been designated as specific genus and species largely based on molecular information. However all the revised genera differ distinctively in their morphological characters with each other and therefore morphotaxonomy of AMF still plays an important role in characterization and identification. Sequence analyses of rDNA regions have often confirmed the morphologically defined species, and the molecular data have characterized new genera and families in AM taxonomy. It is therefore advised to our distinguished readers to kindly correlate their morphotaxonomic studies with molecular phylogenetic results.

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3–6 April 2016

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Tel.: 00 33 1 70 94 65 08
E-mail: inscription@lepublicsysteme.fr, info@ecfg13.org
Website: <http://www.ecfg13.org/events.php?IDManif=896&IDModule=71&IDRub=1694>

Orlando, **Florida**
13–17 July 2016

The Allied Genetics Conference (TAGC)

The Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998
Tel.: (301) 634-7300
E-mail: society@genetics-gsa.org
Website: <http://www.genetics2016.org/>

Helsinki, **Finland**
1–5 August 2016

8th International Association for Lichenology Symposium (IAL8)

E-mail: ial8@helsinki.fi
Website: www.ial8.luomus.fi

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