MYCORRHIZA NETWORK

MYCORRHIZA NEWS

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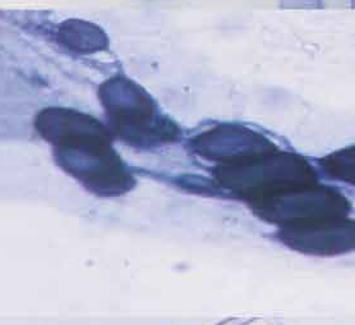

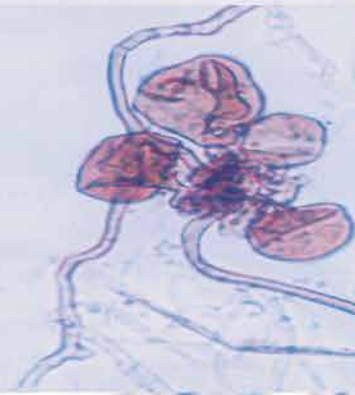

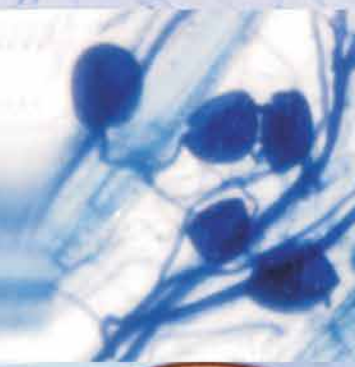

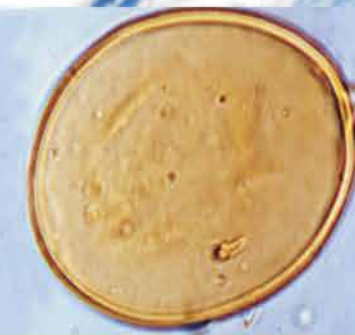

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Mycorrhiza News

The *Mycorrhiza News* provides a forum for 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 complied 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

Arbuscular Mycorrhizal Status in Turmeric (*Curcuma longa* L.) from Assam, India

S C Dutta and B Neog*

Introduction

Plant roots are the ecological niches of many microorganisms which are limited to the soil. Mycorrhizal fungi are symbiotic association between plant roots and soil fungi which develop extensive hyphal network in roots of 80 per cent of the terrestrial flowering plants (Smith and Read 1997). Arbuscular mycorrhizae (AM) are the most common subgroup of endomycorrhizae which on colonization, increase the growth and yield of crop plants including turmeric (Sumathi *et al*. 2011). The greatest effect of AM symbiosis on the host plant occurs when it is phosphorus deficient by up taking it through the mutualistic relation (Koide 1992). It has been observed by many researchers that AM Fungi (AMF) are an important component of soil microbial mass and regulate several essential biological processes to increase the growth and production of many agricultural crops (Mosse 1973; Gredemenn 1975; Sumathi *et al*. 2011).

In this present investigation, the AM fungal status of major spice crop turmeric (*Curcuma longa* L.) is assessed in the crop fields of Assam.

Study Area

Assam, the second largest state of North East India and situated between 24º 2′–27º 6′N latitude and 89º 8′–96 ºE longitude, covers an area of 78,438 sq. km. Five administrative districts of Assam, i.e., Dibrugarh, Jorhat, Sonitpur, Lakhimpur, and Kamrup, have been selected for the present investigation as cultivation of turmeric is done in a large and commercial scale in these regions. Dibrugarh lies between 27° 5' 38" N to 27° 42' 30" N latitude and 94° 33' 46" E to 95° 29' 8" E longitude. Jorhat is located at 26.75° N 94.22° E. It has an average elevation of 116 m (381 ft). Sonitpur lies between 92° 16' to 93° 43' E longitudes and 26° 30' to 27° 01' N latitudes. Lakhimpur lies between 26.48' and 27.53' N latitudes and 93°42' and 94.20' E longitudes. Kamrup is situated between 25.46' and 26.49' N latitude and between 90.48' and 91.50' E longitude.

Sample Collection

A total number of five sampling sites, each from representative districts, were selected. Root, rhizome, and rhizospheric soil samples (8–15 cm depth) were collected at random from the fields during spring 2012 after the maturity of the turmeric plants. Roots of turmeric were washed carefully to clear the attached soil particles and cut into 1-cm long segments and immediately stored in Formalin, Acetic Acid, Alcohol (FAA) solution in the field itself for further analysis of total root colonization. One part of soil samples from the rhizospheric region was stored at 4 ºC for further analysis and the other part was air dried in shade for spore counting.

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Root Colonization by AMF

Staining of the root segments was done by Trypan blue method (Philips and Hayman 1970). Segments were cleared with distilled water and immerged in 10 per cent KOH and heated to 90 °C for 15–30 min. followed by 1 per cent HCl. They were again washed in distilled water and placed in alkaline 3 per cent H_2O_2 to make the root bits colourless. Staining was done by placing the roots in 0.05 per cent Trypan blue in lacto phenol for 15–30 min. The excess stain was removed by applying clear lacto phenol. Finally, root segments were mounted on glass slides with Polyvinyl Alcohol Lactoglycerol (PVLG) and observed under a compound microscope and quantified for AMF infection according to Giovannetti and Mosse (1980).

Isolation of Zygospores and Identification

The AM spores and fungi distributed in the soil samples were collected in triplicate for each sample by the wet sieving and decanting techniques followed by floating-centrifugation in 50 per cent sucrose (Dalpe 1993). The spores were collected on grid-pattern filter paper and counted using a stereomicroscope. Spores were carefully extracted with needles brushes and mounted on slides in PVLG reagent (Schenck and Perez 1990). Identification of the spores was done by observing the diagnostic characteristics of the spores such as, spore wall stratification, colour, size of the spore, and type of hyphal attachment of the spore under a compound microscope by using the manual of Schenck and Perez (1990) and as per the description given in the INVAM website (http://invam.caf.wvu.edu/).

Analysis of Soil Physico-chemical Properties

The pH of the soil was measured using an automatic glass electrode pH meter in 1:2.5 soil: distilled water suspensions after 4 h of incubation. Soil moisture content was determined gravimetrically after drying at 105 °C until a constant weight was obtained. The organic C was determined by potassium dichromate oxidation (Walkley and Black 1934). Available nitrogen was estimated by alkaline permanganate method developed by Subbiah and Asija (1956). Available phosphorus was extracted and measured using Bray No. 1 method (Bray and Kurtz 1945) and Jackson (1973) method, respectively. The estimation of potassium was carried out by the Flame photometer method of Jackson (1973).

Statistical Analysis

The data obtained was subjected to analysis of variance and comparison of means was performed through Tukey's Test with a probability of 0.05. The values obtained were analyzed using SPSS version 13.

Results

The edaphic features of different field's soils' (rhizosphere) turmeric grown areas are given in Table 1. The soil physico-chemical properties varied considerably among the five districts particularly with reference to organic carbon (0.74–2.38 per cent), available phosphorus (21.28–23.87 kg/ha), a available potassium level (105–143 kg/ha) and a nitrogen content (282–467 kg/ha). All the study sites were found to be acidic with pH ranging 4.91–5.23.

Table 1: Physico-chemical characteristics of rhizospheric soil of *Curcuma longa* L.

Study sites	pH	Moisture (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Organic carbon (%)
Dibrugarh	4.89 ±0.27	19.89 ±0.12 a	426.93 ±3.60 a	21.65 ±0.46 ca	122.01 ±4.8 a	2.15 ±0.20 a
Jorhat	4.89 ±0.28 a	19.94 ±0.31 a	321.67 ±36.01 b	22.18 ±0.76 cba	123.31 ±11.3 ca	1.74 ±0.15 b
Sonitpur	4.73 ±0.25 a	19.72 ±0.43 a	422.08 ±46.50 a	21.66 ±0.39 c	115.33 ±5.8 a	1.65 ±0.13 fcb
Lakhimpur	4.67 ±0.47 a	19.38 ±0.41 a	446.53 ±24.00 ca	23.17 ±0.50 b	119.95 ±7.5 a	2.02 ±0.09 eba
Kamrup	5.20 ±0.24 a	18.56 ±0.84 b	373.19 ±38.20 da	22.46 ±0.57 cba	109.28 ±3.20 ba	0.98 ±0.15 d

Data are the means of five individual observations at each study site. Values followed by similar letters are not significantly different from each other at P = 0.05 according to Tukey's test.

Table 2: Colonization, spore density, and species richness of AM fungi associated with *Curcuma longa* of Assam (India)

Study sites	Root colonization (%)	No of spores/100g soil	AM sp. associated with turmeric
Dibrugarh	37.8 ±8.20 a	36.20 ±8.19 a	*Glomus* and *Gigaspora* sp.
Jorhat	37.2 ±8.30 a	42.60 ±5.77 a	*Glomus* and *Gigaspora* sp.
Sonitpur	43.0 ±6.00 da	36.40 ±8.40 a	*Glomus* and *Acaulospora* sp.
Lakhimpur	29.2 ±4.10 ca	16.60 ±3.80 b	*Glomus* and *Gigaspora* sp
Kamrup	39.6 ±5.30 a	48.40 ±8.60 a	*Glomus* and *Acaulospora* sp.

Data are the means of five individual observations at each study site. Values followed by similar letters are not significantly different from each other at P = 0.05 according to Tukey's test.

An attempt was made to observe the occurrence of different AMF in turmeric plant in turmeric grown areas of Assam. During the study, the diversity of AM fungi and the percent root colonization in turmeric roots were evaluated. It is found to vary quantitatively from cultivated field to field. A total of five morpho species were found belonging to three genera *Glomus, Gigaspora,* and *Acaulospora*. The number of AM spores ranged from 12 to 59 per 100 g of soil. All the study cites were found to be colonized by AM fungi in almost all the plants. The range of colonization is 24–52 per cent in the study sites (Table 2).

Discussion

Different researchers have studied the association of AM fungi in different crops and concluded that a possible pattern of AM fungal community can be found out for a defined crop in specific agro-systems. Hence, this attempt to study the pattern of AM fungal occurrence and diversity for turmeric cultivation of different turmeric grown areas of Assam (India) is preliminary work to interpret the possibility of AM fungal colonization and abundance of fungal spores with their species richness.

In the present study, the root colonization of specific turmeric grown areas was found to be low, but the percentage of root fragments which were colonized by AM fungi revealed the presence of the active fungal association in the soil. Though the growth period of turmeric allowed sufficient time for the establishment of the hyphal network, agricultural management practices, and the wide range of host fungal and environmental conditions could explain the relative low level of mycorrhizal colonization. Root colonization, spore density, and formation of vesicles and arbuscules varied in different turmeric grown areas indicating the variability of AM susceptibility within the plant species. The result of the present study was not deviating from the findings of Khade *et al.* (2007) on root colonization and spore density of *Curcuma longa* L.

Statistical analysis showed that variations in the percentage of root colonization and the spore density of the respective rhizospheric soils were significant. Correlation analysis showed neither the soil pH nor the soil available phosphorus played any significant role in the root colonization of AM fungi. Moreover, there was no significant correlation between the percentage of root colonization and spore density of AMF in all the turmeric fields. The varied root colonization and spore and species density may be due to the changes in soil edaphic factors (Hayman 1982) or the suitability of AM fungal species to a specific plant of a particular geographic region. The adjoining plant communities also influence the AM fungal flora of soil (Bagyaraj *et al.* 1980). The study showed that all the fields were having acidic soil. As phosphate content of rhizospheric soil shows significant variance due to various factors such as pH, moisture, rhizoexudates, and PGPR influence (Richardson 2001), there might be considerable variation in root colonization in the same plant growing in different geographical areas (Chethan *et al.* 2008). Morita and Konshi (1989) observed that root colonization by this fungal association was two times higher in plants grown in lightly fertilized soil than the heavily fertilized fields. The input of chemical fertilizers along with other organic fertilizers by the farmers over the years might be another reason for the depletion in the root colonization and spore density associated with the turmeric plants of the surveyed fields. Moreover, the low spore density observed in the study may be due to the insertion of less pressure by the warmer climate of Assam for sporulation of AM fungi (Hetrick *et al.* 1983). It is evident from the isolation of AM fungal

species associated with *Curcuma longa* L. that *Glomus* sp. was found to be dominant in all the turmeric fields. A number of previous authors have reported the ubiquitous nature of *Glomus* sp. in different host plants (Gerdemann 1968; Hayman 1982; Powell and Bagyaraj 1984; Jha *et al.* 1994).

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Selection of Efficient VAM Species for Marigold Cultivation and its Effect on Growth, Yield, and Quality Parameters of two Marigold Varieties

Vijetha, Chaya P Patil, and B S Kulkarni

Introduction

In the present paper, the authors have reported the effect of 12 different species of mycorrhizal inoculation on the germination and growth of marigold raised in pot with soil as the media. This experiment was conducted to know whether all the species of mycorrhizal inoculation would be beneficial for plant growth. Vesicular–arbuscular mycorrhizal (VAM) fungi are obligate parasites that form symbiotic relationships with plant roots. VAM fungi are associated with increased phosphorus uptake, growth, and with increased drought resistance due to a fungal-hyphae-extended nutrient uptake zone around roots of many species (Hayman, 1980). Different species of VAM have different growth promotional effects on particular plant species (Patil and Patil 2008). Thus, when considering VAM inoculation, it is important to examine several different species of VAM for their effect on plant growth.

Marigold (*Tagetes erecta* L.*)*, which occupies a prominent place in ornamental horticulture, is a commercially exploited flower crop originated from Mexico and belong to the family Asteraceae. Free-flowering habit, short duration to produce marketable flowers, wide spectrum of attractive colours, shape, size, and good keeping quality make marigold an attractive option for flower growers. Apart from its significance in ornamental horticulture, it is valued for its other properties also. The aromatic oil extracted from *Tagetes minuta*, commonly known as 'Tagetes oil', possesses larvicidal properties and is used as a fly repellent. The carotenoids present in *Tagetes* are the major sources of pigments used in poultry industry as a feed additive to intensify the yellow colour of egg yolks and broiler skin (Scott *et al.* 1968). The principal pigment present in the flower is xanthophyll. The xanthophyll lutein accounts for more than 80–90 per cent and is present in the form of esters of palmitic and myristic acids (Alam *et al.* 1968).

Materials and Method

The present investigation was carried out as a pot-experiment in Department of Agriculture Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi, Gokak taluk, Belgaum, Karnataka during the period from November 2012–February 2013. The details of the materials used and the methods adopted during the investigation are presented below.

Planting Material

The marigold seeds used for the experiment were collected from two sources, a local variety obtained from a farmer in Murgod, Belgaum, and a second variety named Double Orange from Namdhari Seed Company Pvt Ltd.

VAM species used for the experiment

- Control
- *Glomus bagyarajii*
- *Glomus fasiculatum*
- *Glomus intraradices*
- *Glomus leptotichum*
- *Glomus monosporum*
- *Glomus monihofis*
- *Glomus mosseae*
- *Entrophospora*
- *Aculospora laevis*
- *Sclerocystis dusii*
- *Gigaspora gigantia*
- *Gigaspora margarita*

The marigold seeds of two varieties were sown in poly bags with 12 VAM species and a control. Mycorrhiza was placed in the poly bag and then soil was added to it. Marigold seeds were placed over the soil and were finally covered with soil. The poly bags were watered regularly. Weeding was done as per the requirement.

Inoculation with AM Fungus

Culture of 12 vasicular arbuscular mycorrhizal fungi (VAM) inoculums was obtained from Department of Agricultural Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi. The inoculum was multiplied in a sterilized potting mixture using maize (*Zea mays*) as host plant in the shade house of Microbiology Department. The inoculum used consisted of sand and soil in 1:1 ratio, and root

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segments of maize comprising hyphae, vesicles, arbuscules, and chlamydospores of 12 AM fungus. Five gram of inoculum was applied per poly bag before sowing.

Results and Discussion

A mutualistic symbiotic association exists between the endomycorrhizal fungi (AM fungi) and the roots of the horticultural crops (host plants). The AM fungal symbiosis starts with the penetration through the radicle and finally to the roots of the germinating seedlings. The local variety of marigold seeds were inoculated with 12 different AM fungal inoculums in the germinating media. The media constituents help in the early germination of the seeds. The germination per cent and vigour was influenced by different VA mycorrhizal fungi on the Local variety of marigold (Plates 1, 2, 3, and 4). Least number of days taken for germination was recorded by *Gigaspora* margarita (5 days) followed by *Glomus fasciculatum*, *Glomus monihofis*, *Entrophospora,* and *Gigaspora gigantia* (6 days). The highest germination percent was recorded with *Gigaspora margarita*, *Glomus bagyarajii*, *Glomus monihofis*, *Entrophospora*, and *Gigaspora gigantia* (93.33 per cent).

In Double Orange variety of Marigold, days taken for germination, germination per cent and vigour were also influenced by different VA mycorrhizal fungi existing on it. Least number of days taken for germination was recorded by *Gigaspora margarita* (5 days) followed by *Sclerocystisdusii*, *Entrophospora*, *Glomus monosporum*, *Glomus leptotichum*, and *Glomus fasciculatum* (6 days). The highest germination percent was recorded with *Gigaspora gigantia* (100 per cent) followed by *Glomus monihofis* (93.33 per cent) when compared to control (Tables 1 and 2). The significant enhancement in germination was also noticed in VAM inoculated seeds when compared to uninoculated control (Plates 5, 6, 7, and 8). Increased germination due to VAM inoculation is also reported in papaya (Duragannavar *et al*. 2004), and in jamun (Devachandra 2006).

Enhancement in germination could be due to the fact that soon after sowing of seeds they start imbibing water resulting in triphsic increase in seed fresh weight (Hartmann *et al*. 1997). Another important characteristic of seeds during imbibition is that they become 'leaky'. Several compounds including amino acids, organic acids, inorganic ions, sugars, phenolic compounds, and proteins leak out from the imbibing seeds (Simon, 1984). These solutes/leaked compounds may help VAM fungal propagules to germinate early. The time gap between the first phase and the last phase of germination may help the AM fungi to germinate and establish contacts as soon as radicles emerge out. Hence, AM fungi help better seed germination by mutualistic symbiosis with roots of the plant and competing with the pathogens for space and

Table 1: Effect of VAM species on days taken for germination and germination per cent in Local variety of marigold

Treatments	Days taken for germination	Germination per cent
T_1- Control	7	86.33
T_{2-} *Glomus bagyarajii*	8	93.63
T_{3-} *Glomus fasiculatum*	6	66.66
T_{4-} *Glomus intraradices*	8	86.66
T_{5-} *Glomus leptotichum*	8	73.33
T_{6-} *Glomus monosporum*	8	66.66
T_{7-} *Glomus monihofis*	6	93.33
T_{8-} *Glomus mosseae*	7	86.66
T_{9-} *Entrophospora*	6	93.33
T_{10-} *Aculospora laevis*	8	80
T_{11-} *Sclerocystis dusii*	7	80
T_{12-} *Gigaspora gigantia*	6	93.33
T_{13-} *Gigaspora margarita*	5	93.33
S Em ±	0.06	0.21
CD @1%	0.23	0.82

Table 2: Effect of VAM species on days taken for germination and germination per cent in Double Orange variety of marigold

Treatments	Days taken for germination	Germination per cent
T_1- Control	9	80.03
T_{2-} *Glomus bagyarajii*	9	73.33
T_{3-} *Glomus fasiculatum*	6	80
T_{4-} *Glomus intraradices*	8	86.66
T_{5-} *Glomus leptotichum*	6	80
T_{6-} *Glomus monosporum*	6	73.33
T_{7-} *Glomus monihofis*	8	93.33
T_{8-} *Glomus mosseae*	5	73.33
T_{9-} *Entrophospora*	6	86.66
T_{10-} *Aculospora laevis*	6	73.33
T_{11-} *Sclerocystis dusii*	9	66.66
T_{12-} *Gigaspora gigantia*	7	100
T_{13-} *Gigaspora margarita*	5	80
S Em ±	0.06	0.01
CD @1%	0.23	0.04

nutrition. The differences observed in the efficacy of germination by the different AM fungal species could be attributed to the leached solutes leaked from the appropriate host. Thus, leachet released might play a prominent role in early propagation of AM fungal mycelium, thereby contributing to improved efficiency and early seed germination (Cruz *et al*. 2003).

These plant exudates enhance the hyphal growth as the fungus approach the vicinity of the roots. However, these promoting effects appear to be host specific.

In the Local variety (Table 3), highest shoot height was recorded with *Glomus mosseae* (41.90 cm) as compared to control (21.63 cm). Highest root length was recorded with *Glomusmonosporum* (36 cm) as compared to control (15.13 cm). Highest number of roots was recorded with *Glomus mosseae* (66.33) followed by *Acaulospora laevis* (51.33), and least with *Glomus bagyarajii* (18.33). Highest root volume was recorded with *Glomus leptotichum* (3.97 ml) followed by *Glomus monihofis* (3.50 ml), *Glomus mosseae* (2.83 ml), and *Aculospora laevis* (3.77 ml) as compared to control (0.43 ml). Highest fresh root weight was recorded with *Glomus leptotichum* (5.40 mg) and least with control (0.15 mg). Highest fresh shoot weight was recorded with *Glomus mosseae* (9.44 mg) and least with the control (0.83 mg). Highest root to shoot ratio was recorded with *Glomus monihofis* (0.81) which was on par with *Glomus leptotichum* (0.63). The least root to shoot weight was recorded with both *Gigaspora margarita* and *Sclerocystis dusii* (0.15).

In the Double Orange variety (Table 4), the highest shoot height was recorded with *Entrophospora* (36 cm) on par with all the other treatments. The least shoot height was recorded with control (22.83 cm). Highest root length was recorded with *Glomus fasciculatum* (36.33 cm) and the least in control (15 cm). Higher number of roots was recorded with *Entrophospora* (58.33) which was on par with all the other treatment other than control (22.33). Highest root volume was recorded with *Entrophospora* (5.33 ml) which was on par with *Glomus leptotichum* (4.83 ml), *Aculospora laevis* (4.33 ml), *Glomus intraradices* (4.17 ml), *Glomusfasciculatum* (3.17 ml), and *Glomusmosseae* (3.50 ml). The least root volume was recorded with control (0.67 ml). Highest fresh root weight was recorded with *Entrophospora* (5.78 mg) and least with control (0.51 mg). Highest fresh shoot weight was recorded with *Glomus mosseae* (8.33 mg) and the least with control (3.33 mg). Highest root to shoot ratio was recorded with *Entrophospora* (0.72) and the least with *Gigaspora gigantia* (0.20).

In the present investigation on marigold, the morphological and anatomical changes were evident with respect to shoot parameters such as height of shoot and number of leaves, as well as to root

Table 3: Effect of 12 species of VAM on growth of Marigold Local variety

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves	No. of roots	Root volume (ml)	Fresh root weight (mg)	Fresh shoot weight (mg)	Root: Shoot ratio
T_1- Control	21.63	15.13	10.67	26.33	0.43	0.15	0.83	0.40
T_2- *Glomus bagyarajii*	22	24.93	8.33	18.33	1.10	0.62	2.68	0.22
T_3- *Glomus fasiculatum*	24.63	15.37	10	28.33	1.13	0.71	2.93	0.24
T_4- *Glomus intraradices*	30.50	22.43	11.33	28.33	2	0.97	5.27	0.46
T_5- *Glomus leptotichum*	35.67	28.33	11.67	44.33	3.97	5.40	8.12	0.65
T_6- *Glomus monosporum*	27.50	36	10	41.33	2.17	1.82	3.69	0.45
T_7- *Glomus monihofis*	31.50	28.33	10.50	33.33	3.50	4.45	5.47	0.81
T_8- *Glomus mosseae*	41.90	33.17	12.67	66.33	2.83	4.39	9.44	0.46
T_9- *Entrophospora*	36.67	22	12	30.67	2.50	1.98	7.36	0.27
T_{10}- *Aculospora laevis*	34.50	30.83	11.67	51.33	3.77	3.60	8.13	0.41
T_{11}- *Sclerocystis dusii*	30.83	18.30	9.67	25.67	1.83	0.78	5.29	0.15
T_{12}- *Gigaspora gigantia*	31.67	15.67	10.33	33.33	1.23	0.97	6.27	0.16
T_{13}- *Gigaspora margarita*	27	20	11	24	0.83	0.64	4.25	0.15
S Em ±	2.99	2.91	1.55	5.34	0.31	0.05	0.08	0.06
CD @1%	11.76	11.43	NS	20.98	1.21	0.19	0.32	0.23

Table 4: Effect of 12 species of VA Mycorrhizae on growth of Marigold Double Orange variety

Treatments	Shoot length (cm)	Root length (cm)	No. of roots	No. of leaves	Root volume (ml)	Fresh root weight (mg)	Fresh shoot weight (mg)	Root : Shoot ratio
T_1- Control	22.83	15	22.33	12	0.67	0.51	3.33	0.28
T_2- *Glomus bagyarajii*	25.17	20.83	42.67	11.33	1.17	0.89	3.66	0.24
T_3- *Glomus fasiculatum*	29.33	36.33	44.67	12	3.17	2.76	8.33	0.33
T_4- *Glomus intraradices*	30.33	24.83	t.67	12.67	4.17	3.20	7.66	0.42
T_5- *Glomus leptotichum*	34	33	55.33	11.33	4.83	3.65	6.66	0.55
T_6- *Glomus monosporum*	29	27.17	41.67	10.67	1.83	1.88	5.33	0.35
T_7- *Glomus monihofis*	29.17	28.17	50	13.67	1.83	1.47	5.97	0.24
T_8- *Glomus mosseae*	31.83	32.67	52.67	12	3.50	2.88	8.33	0.35
T_9- *Entrophospora*	36	32.33	58.33	12	5.33	5.78	7.97	0.72
T_{10}- *Aculospora laevis*	33.33	35	48.67	12.67	4.33	2.91	6.33	0.46
T_{11}- *Sclerocystis dusii*	35.33	29	39.33	11.33	1.83	2	6.33	0.32
T_{12}- *Gigaspora gigantia*	31	21.50	45.33	11.33	1.33	1.14	5.66	0.20
T_{13}- *Gigaspora margarita*	31.33	26.33	39	10.67	2.33	1.59	5.33	0.30
S Em ±	2.76	2.90	5.69	1.03	0.59	0.19	0.01	0.03
CD @1%	10.84	11.40	22.37	NS	2.33	0.73	0.06	0.14

parameters such as number of roots, length of longest lateral roots, root volume, fresh root weight, fresh shoot weight, and root to shoot ratio (Tables 3 and 4).

Modification in the root geometry and morphology might have a morphogenetic effect mediated by Indole Acetic Acid (IAA) and gibberellins (Allen *et al*. 1980). Hooker and Artkinson (1992) concluded that root morphogenesis can be modified by AM fungal metabolism or by hormones independent of external nutrients supplied. Hence, the root morphogenetic growth (i.e., root geometry) and the promising effect of AM fungi observed in the present investigation and from the literature (Slankis 1957) could also be attributed to the phytohormones such as gibberellins and auxins, and vitamins produced by the AM fungi. Further, it is well documented that infection of plant roots by AM fungi has beneficial effects on vegetative parameters and biomass production (fresh weight) of host plants (Duragannavar 2005).

The increase in these parameters may be attributed to the synthesis of hormones and growth factors by AM fungi, leading to increased cell multiplication and cell division resulting in overall increase in plant height and number of leaves.

Percent root colonization was higher with all the VAM-inoculated seedling as compared to control in both the varieties (Table 5). In Local variety, maximum per cent root of root colonization was recorded with Gigaspora margarita, Sclerocystis dusii, Glomus mosseae, and Glomus monihofis (99 per cent) which was on par with Gigaspora gigantia, Aculospora laevis, Glomus leptotichum (98 per cent), and Entrophospora (97 per cent), as compared to the control (55 per cent). Whereas in Double Orange variety, the maximum per cent of root colonization was recorded with Glomus mosseae (92 per cent) which was on par with Glomus mosseae (92 per cent), Gigaspora gigantia (91 per cent), and Glomus monihofis (90 per cent). The least root colonization was recorded with control (46 per cent).

The highest root infection/colonization (Plate 9) and increased root geometry in *Entrophospora, Glomus mosseae, Aculospora laevis*, *Glomus leptotichum*, *Aculospora laevis,* and Sclerocystis dussii inoculated marigold seedlings have direct effect on germination and development of sound and healthy rhizosphere that would contribute to improved growth resulting in improved nutrient uptake, thus most suited for use to grow marigold. Similar results were also reported by Hooker *et al.* (1992); Venkat (2004); Santosh (2004).

Table 5: Effect of VAM inoculation on root colonization

Treatments	Percent root colonization	
	Local	Double Orange
T_1- Control	55	46
T_2- *Glomus bagyarajii*	81	69
T_3- *Glomus fasiculatum*	86	74
T_4- *Glomus intraradices*	93	78
T_5- *Glomus leptotichum*	98	81
T_6- *Glomus monosporum*	95.66	85
T_7- *Glomus monihofis*	99	90
T_8- *Glomus mosseae*	99	92
T_9- *Entrophospora*	97	87
T_{10}- *Aculospora laevis*	98	89
T_{11}- *Sclerocystis dusii*	99	90
T_{12}- *Gigaspora gigantia*	98	91
T_{13}- *Gigaspora margarita*	99	79
S Em ±	0.58	0.57
C D @ 1%	2.29	2.26

Plate 1: Effect of 12 different VAM on growth of marigold Local variety

Plate 2: Effect of different host preferred VAM on growth of marigold Local variety

Con- control, G. mono- G. *monospora*, Entro- *Entrophospora*, A.l- *Aculospora laeives*, S.d- *Sclerocystis dussii*

Plate 3: Effect of 12 VAM Fungi on shoot and root growth of marigold Local variety

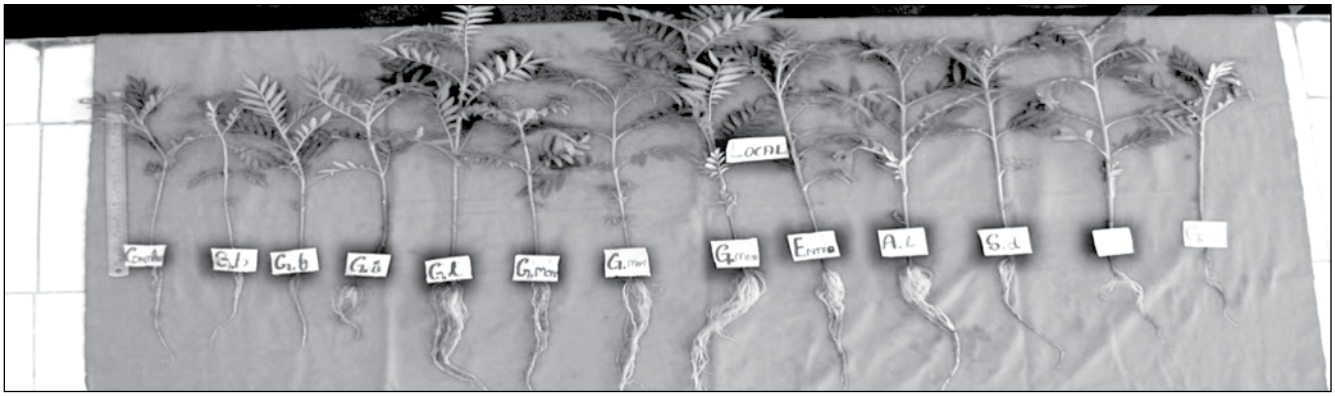
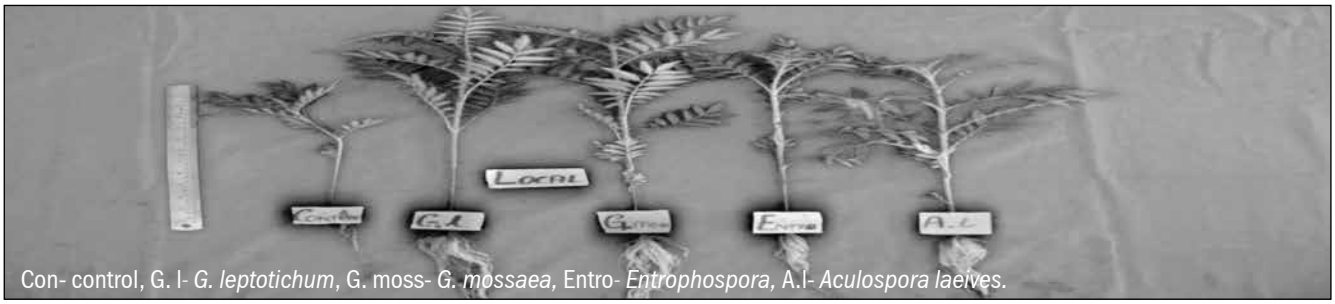


Plate 4: Effect of host preferred VAM on root and shoot growth of marigold Local varviety



Con- control, G. l- *G. leptotichum*, G. moss- *G. mossaea,* Entro- *Entrophospora,* A.l- *Aculospora laeives.*

Plate 5: Effect of 12 VAM Fungi on shoot and root growth of marigold Double Orange variety

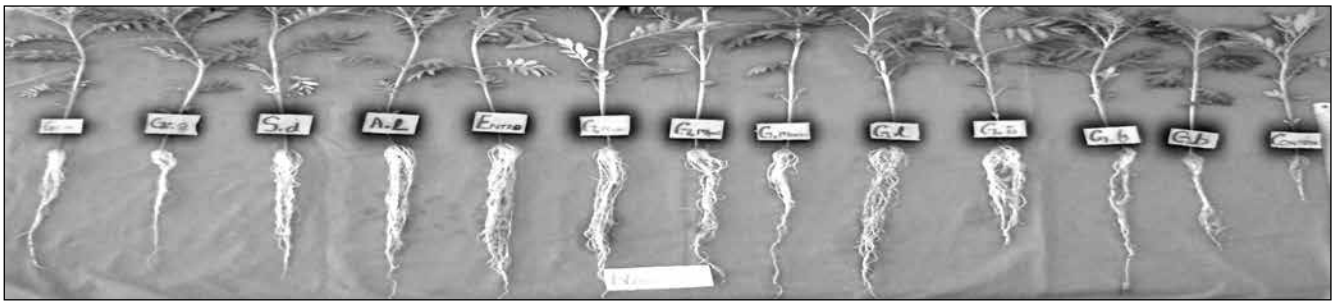
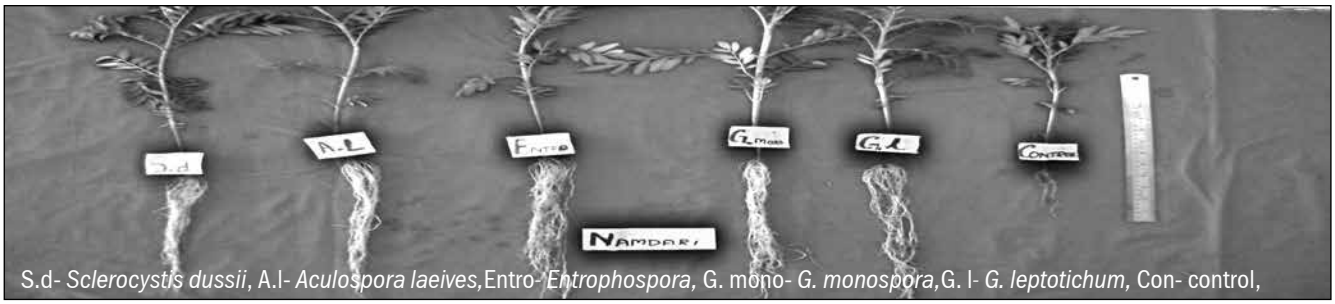


Plate 6: Effect of host preferred VAM on shoot and root growth of marigold Double Orange variety



S.d- *Sclerocystis dussii*, A.l- *Aculospora laeives,*Entro- *Entrophospora,* G. mono- *G. monospora,*G. l- *G. leptotichum,* Con- control,

Plate 7: Effect of 12 different VAM on growth of marigold Double Orange variety

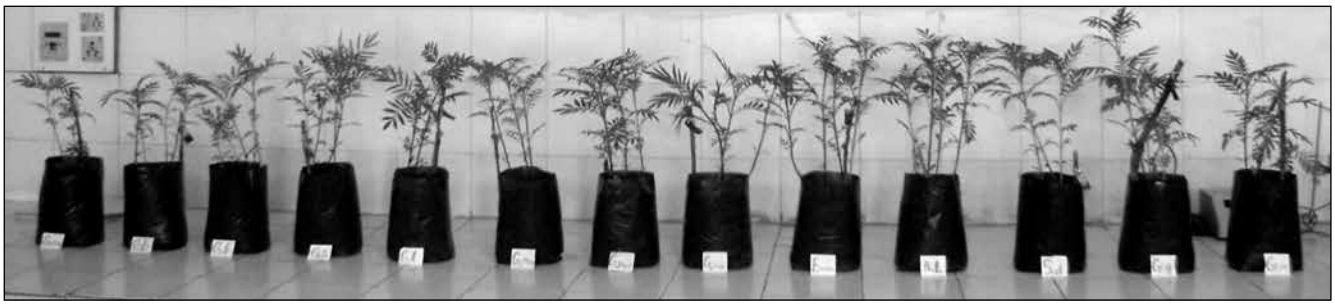
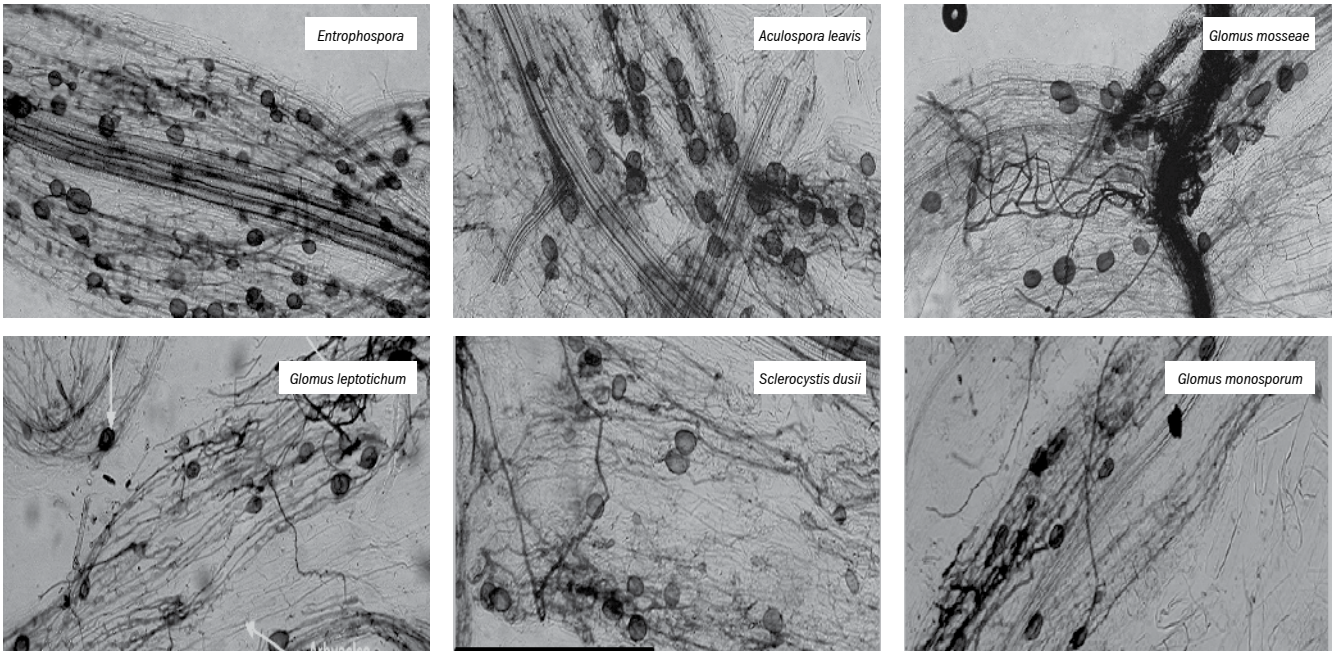


Plate 8: Effect of host preferred VAM on growth of marigold Double Orange variety

Con- control, Entro- *Entrophospora,* A.l- *Aculospora laeives,* S.d- *Sclerocystis dussii,* Gi.g- *G. gigantia*

Plate 9: Root Staining of marigold inoculated with host preferred VAM Fungi



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Morphotaxonomy of *Acaulospora kentinensis* (accession CMCC/AM-2502)

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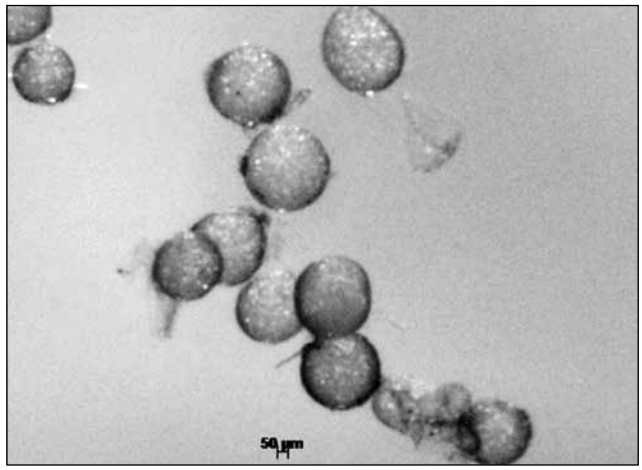
Taxonomy of Arbuscular Mycorrhiza Fungi (AMF) belonging to Glomeromycota was largely based on morphological characters such as spore morphology, structure, size and spore wall until the last decades (Morton & Benny 1990, Schenck & Pérez 1990, Walker & Sanders 1986, Gerdemann & Trappe 1974). With the advent of reliable molecular tools, they were soon included in taxonomic analysis of these fungi and became one of the main approaches for characterization and identification of various groups of these unique microorganisms (Simon *et al.* 1992, Schüßler *et al.* 2001, Morton & Redecker 2001). Initially, spore formation characteristics such as gigasporoid, entrophosporoid, scutellosporoid, acaulosporoid, glomoid, and radial-glomoid were considered. On the basis of these characteristics, the AMF were organized in three families, viz. Glomeraceae, Gigasporaceae, and Acaulosporaceae, and six genera, *Glomus, Gigaspora, Sclerocystis, Scutellospora, Entrophospora,* and *Acaulospora* within one order *Glomerales* of the phylum *Zygomycota* (Morton & Benny 1990). However, in the present-day taxonomic studies, a simultaneous morphological characterization followed by molecular analyses has led to major revisions in the AMF taxonomic organization (Oehl *et al.* 2011). In view of diverse morphological features among different genera in the order Glomerales, morphotaxonomic characterization still plays an important role in the characterization of AMF in the preliminary stages of identification. In this article, we therefore would like to present a study conducted to characterize an interesting and precious AMF available in the Centre for Mycorrhizal Culture Collection (CMCC) Bank with the accession number CMCC/AM 2502 which have been isolated, purified and maintained as pure culture in our germplasm bank.

Isolation and purification of this culture were most challenging since it took 12–18 months to get a desired sporulation count (30–50 spores/g soil) from a single monospore that was used to initiate the culture *in situ*. The spores were collected, cleaned, and observed for a detailed morphotaxonomic analysis, which is described in the following sections:

Figure 1: Spores isolated from monosporal culture of CMCC/AM-2502



Spore Morphology and Shape

The spores are acaulosporoid, sessile, and are present individually in the soil extraradically devoid of sporocarp. The spore initiates with the formation of a sporiferous saccule at the top of sporogenous hyphae continuous with the mycorrhizal hyphae. The spore develops from the subtending hyphae

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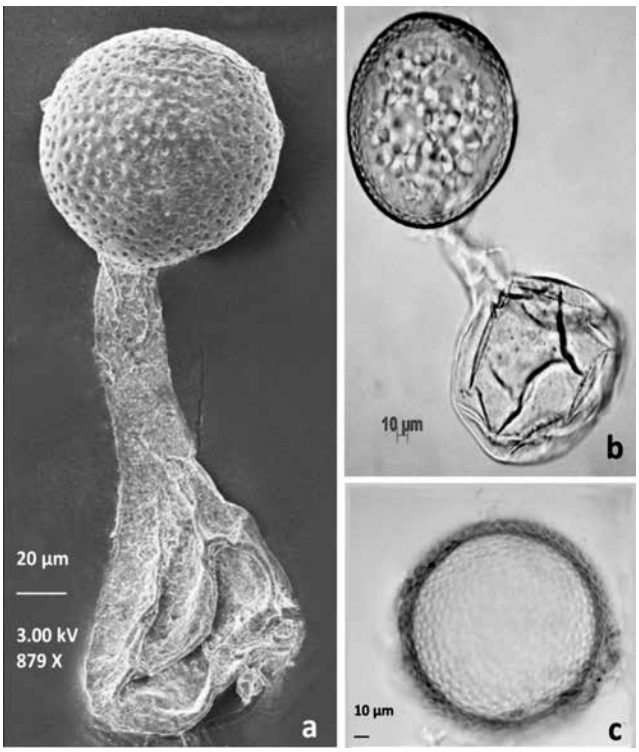
which are termed as "saccule neck". Once the spores complete differentiation, the spores cease expansion and at maturity, the saccule detaches itself from them (Figures 1 and 2).

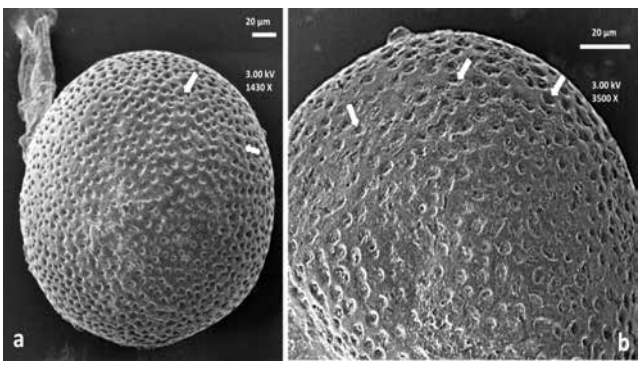
Figure 2: Scanning electron micrograph (a) compound microscopic images (b & c) of spores of CMCC/AM-2502 showing globose spores with sporiferous saccule



Spore Surface Ornamentation

Scanning electron micrograph of the spores and wall surface revealed that the outermost wall is ornamented with circular perforations commonly observed as pitted structures. The perforations were distinct when the spores were still attached to the saccule (Figure 3).

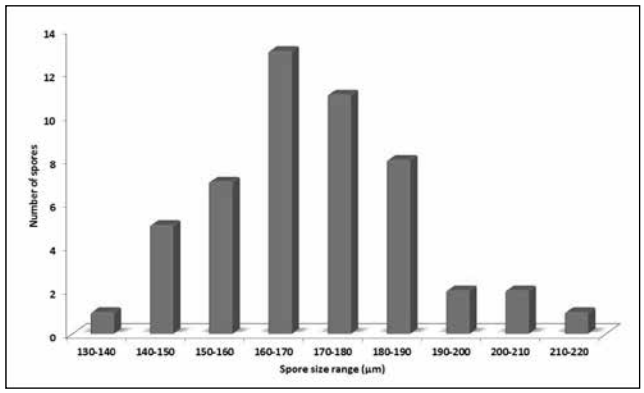
Figure 3: Scanning electron micrograph of spores of CMCC/AM-2502 showing globose spores with pitted structures on the spore surface



Spore Size and Diameter

The spores are pale yellow or sub-hyaline to yellow in colour—globose to subglobose—sometimes ovoid in shape. The diameters of the spores were found to lie between 130–220 µm when an average of 50 spores was evaluated (Figure 4).

Figure 4: Analysis of spore diameter of 50 healthy spores obtained from one-year old culture



Subcellular Structure of Spore

The matured spore is composed of the following wall layers:

Layer 1 (L1-Evanescent): Hyaline, continuous with the wall of the saccule neck. This layer is present only in the spores attached with the saccules and gets sloughed off on detachment or collapse of the saccules.

Layer 2 (L2-Laminated): Sub-hyaline to yellow in colour. This inner layer is rigid and ornamented with pits; pits are circular to sub-circular in shape. On maturity, when the saccule neck detaches from the spore, it leaves a scar on the spore surface which is termed Cicatrix and appears like a ridge (Figures 5 and 6).

Germinal Walls

There are two hyaline inner flexible germinal walls (GW1 and GW2), which are readily seen when the spore is broken.

GW1: Consists of two layers which are usually indistinguishable and tends to be adherent (Figures 5 and 6).

GW2: Bi-layered, usually adherent, L1 tends to become dislodged and float away with applied pressure whereas L2 is, amorphous. L2 is non-reactive in young spores and gradually becomes reactive in mature spores.

Reaction with Melzer's reagent

Stains pinkish red to a reddish-purple or pastel red with Melzer's reagent the inner germinal wall is more or less distinct and appear tightly adherent (Figure 5a).

Reaction with PVLG

Stains yellowish to golden brown in colour, appears plastic type in Polyvinyl Lacto Glycerol (PVLG) (Figure 5b).

Figure 5: Spore wall layers seen after Melzer's reaction (a) PVLG treatment (b) showing distinctly stained spore wall layers and Germinal layers (GW)

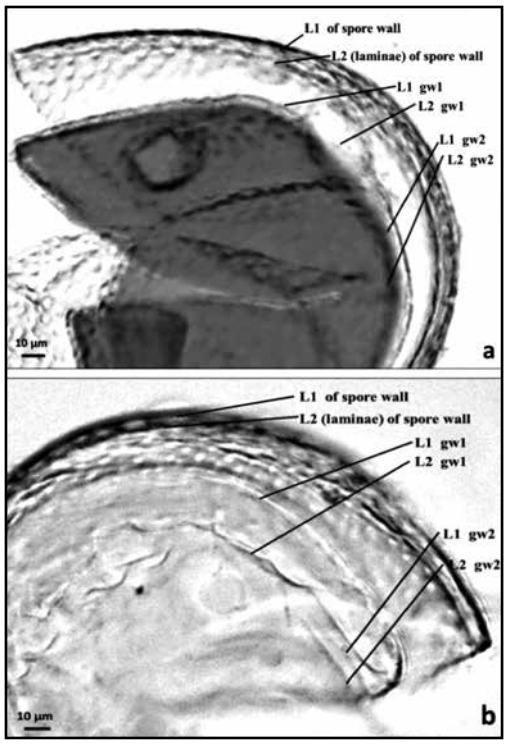
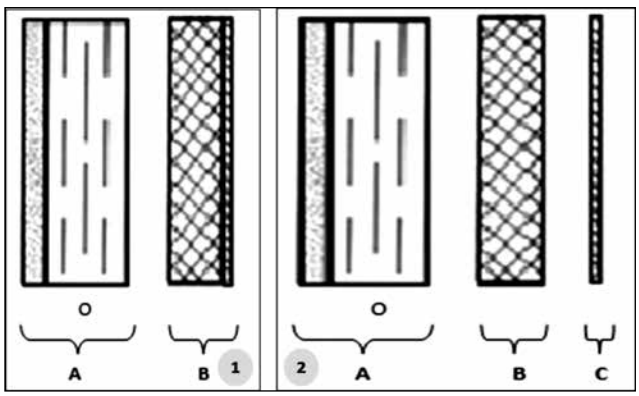


Figure 6: Murograph (Walker 1983) of *Acaulospora kentinensis* where spore wall are shown by dots, laminated wall with broken lines and inner germinal layers by designed lines. Muronym = A(EL) B(CA) when the inner laminated wall is intact (1) or A(EL) B(C) C(A) when the inner germinal wall layer is separated (2)



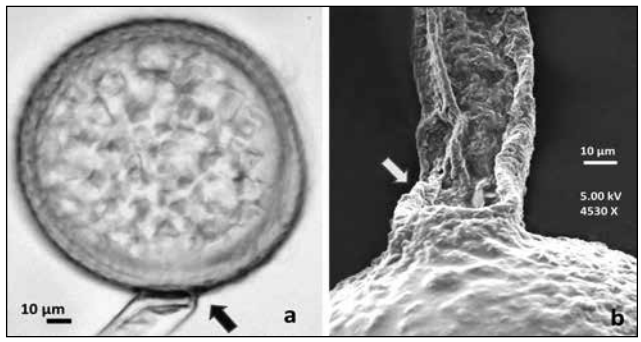
Sporiferous Saccule

The saccuole is subglobose in shape, hyaline in colour which is due to the hydrolyzation or disintegration of the contents as reported by other workers. The saccule wall is continued with the layer 1 of the spore wall. It is hyaline layer and has surface smooth. The diameter varies from 200–350 µm with neck tapering towards the end. It usually collapses at maturity and gets detached from the mature spores.

Cicatrix

A circular, ovoid scar, indicating the point of attachment between the spore and the saccule, consists of a slightly raised tapering collar comprising closely packed tubercles surrounding an unornamented depression (Figure 7).

Figure 7: Compound microscopic (a) scanning electron micrograph (b) spore showing circular cicatrix (arrows)



Conclusions and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-2502, many distinguishing features regarding the family, genera and the species could be derived. The following features were taken into consideration for the characterization:

- The spores are single, sessile, without a stem or pedicel, hyaline when young and golden yellow when mature and are acaulosporiod.
- Spores formed on or within the neck of a sporiferous saccule.
- Bi-layered spore wall, an outer bilayered flexible hyaline wall (GW1), and an inner bilayered hyaline wall (GW2). The inner layer of the spore wall is ornamented with beads (granular excrescences).

All these above features suggest that this isolate belongs to the family—Acaulosporaceae (Gerd. & Trappe, http://invam.wvu.edu/the-fungi/classification/acaulosporaceae)

Other unique identifying features of this accession are as follows:

- Spores acaulosporiod, development of the spores from saccule neck. After maturity, the saccule remains loosely attached to the spore.
- Spores distinctly larger up to 210 µm in diameter, spores are ornamented with pits. The wall layer shows a distinct bi-layered spore wall and bi-layered germinal wall layer. The inner germinal wall layer stains pinkish to reddish with Mwlzer's reagent.
- Most of the spores were found to have circular to ovoid cicatrix.

This accession CMCC/AM 2502 matches the features of the genus *Acaulospora* (Gerd. & Trappe 1974). The type species of this is designated as *A. laevis*. Due to very unique features such as presence of pitted spore wall, bigger spore size, this accession is found to have a close resemblance with ***Acaulospora kentinensis*** (WU & Liu) Kaonongbua, Morton & Bever comb. nov. MycoBank MB515731; Basionym: *Entrophospora kentinensis* WU & Liu, 1995; Synonym: *Kuklospora kentinensis* (WU & Liu) Sieverd & Oehl, 2006.

Systematic Classification

Glomeromycota
Glomeromycetes
Glomerales
Acaulosporaceae
Acaulaspora kentinensis

Spores of AMF have been described using a wide array of distinguishing features such as "walls", "groups", "components" that are unique among members of the Kingdom Fungi. These structures were known to be taxonomically important because they are highly more or less conserved and phenotypically stable in most of the environment. However, these features are not stable taxonomic markers for species identification. With the advent of molecular tools, morphotaxonomic characterization is now considered a preliminary step towards species identification. It is therefore advised to our distinguished readers to kindly correlate their morphotaxonomic studies with the molecular data.

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