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Introduction

The importance of mycorrhirzae in providing nutrition to most vascular plants and maintaining the health of ecosystem has been demonstrated in last parts of the 20th century (Marks and Kozlowski, 1973; Sanders et al. 1975; and Trappe and Fogel, 1977). Further, rangeland productivity along with cropland and environmental protection correlated with different mycorrhizal aspects has been dealt by Doug et al. (2008), Boomsma and Vyn (2008), Shi et al. (2007), Turjaman et al. (2006), and Bharadwaj et al. (2007). Reddy and Goud (1989) and Reddy and Bias (1990) studied in detail the mycorrhizal correlation with the physical and biological factors. Each natural soil and weed species harbour different indigenous Arbuscular Mycorrhizal (AM) fungus population. The mycorrhizal fungus translocate colonization nutrient to the host plant. The host provides photosynthates to the mycorrhizal fungi. The mycorrhizal mycelium thus serves as highly efficient extensions of the root system. About 80 per cent of the world's plant species belong to the families that are typically mycorrhizal, the great majority of these form Vesicular Arbuscular (VA) endosymbionts.

Material and Methods

Different undisturbed areas were selected in and around Kaziranga biosphere reserve for collection of weed flora and soil samples. Different weed flora were screened for their effectiveness towards mycorrhizal dependency. Four species, namely, *Ageratum conyzoides, Spiranthes acmella, Comellina benghalensis,* and *Cassia tora* evaluated for VAM infection rate in the roots. Spore population and pH of rhizosphere soil of each weed sample was recorded. Five replicas of each soil sample as well as weed are taken into consideration for evaluation of different perimeters. We followed Phillips and Hayman (1970) for rapid assessment of infection rate while techniques of Gerdemann and Nicolson (1963) were followed for spore collection.

Discussion and Conclusion

In the recent years, Kaziranga National Park in Assam is threatened by the invasion of some exotic weeds and annual flood problems. Another problem faced by this world heritage site is the pollution problems due to installation of an oil refinery at upstream. In future, effluents such as phenolic compounds, oil, and suspended solids may create a serious impact on this biosphere.

Among the plants screened, *Ageratum conyzoides* revealed the maximum AM fungal colonization followed by *Comellina benghalensis*, *Spiranthes acmella*, and *Cassia tora* (Table 1). These plants thrive well under the stress climatic conditions prevailing in this region and grown in both fallow agricultural and waste lands. Table 1 also explains variation in different ranges within Kaziranga. Both vesicles and arbuscles are

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present in the infective root bits. It was also observed that more the infection rate of endosymbiont, better the area coverage by that particular weed. Moreover, spore population of endobionts is also highest where infection rate is maximum. This way we can suggest that by cross inoculating weeds like *Ageratum conyzoides*, *Comellina benghalensis*, and *Spiranthes acmella* in natural site of Kaziranga, degraded ecosystem can be restored. These endobionts may be effective to control invasion of weeds like *Parthenium*, *Mimosa*, and restore ecosystem in Kaziranga biosphere reserve.

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Arbuscular mycorrhiza fungal diversity in the open land adjacent to rubber plantation in Tripura, Northeast India

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Abstract

We have examined Arbuscular Mycorrhizal (AM) fungal colonization in plants and compared AM fungal diversity in the open land and rubber plantation of Tripura, Northeast India. The AM hyphal colonization was highest observed in the roots of *Cassia tora* and lowest in *Colocassia* sp. Four and eight AM fungal species were extracted from the soil samples of rubber plantation and open land, respectively. *Glomus*, *Funneliformis*, and *Ambispora* were isolated from the soil samples. *Glomus multiculae* was highly abundant species isolated from both the sites. Diversity was more in open land than rubber plantation. The result reveals that the studied rubber plantation is poor in AM fungal diversity.

Key words: arbuscular mycorrhizal fungal colonization, diversity, open land, rubber plantation

Introduction

Arbuscular Mycorrhizal (AM) fungi play a very important role in natural ecosystems and in agroecosystems, which are considered to be the most generally distributed and significant symbiotic association in nature (Brachmann and Parniske, 2006). AM fungi increase plant nutrient uptake ability, particularly for phosphorus and zinc in nutrient-poor soils (Scheneiger and Jakobsen, 2000). AM fungal symbiosis presents resistance to the plant against abiotic stresses, such as drought, salinity, metal toxicity, and environmental stresses. Moreover, AM fungal colonization increases plants' tolerance to pathogens; thus acting as a biocontrol agent (Azcon-Aguilar and Barea, 1996; Chhabra, Bhatnagar, and Sharma, 1992). The edaphic factors or soil nutrient status are claimed to be implicated in the patterns and timing of the development of AM fungi (Mullen and Schmidt, 1993).

The practices such as crop rotation, fertilization, and tillage affect the composition and diversity of AM fungal communities as well as spore and mycelium densities and extent of infection in roots in temperate and tropical agroecosystems (Jansa, Mozafar, Anken *et al.* 2002; Oehl, Sieverding, Ineichen *et al.* 2003). Therefore, modern agroforestry and agricultural planting systems respect biological factors like mycorrhizal fungi as inevitable components of useful plants grown in monocultures (Sieverding, 1991).

Hevea brasiliensis Muell. Arg. is commonly known as a rubber plant belonging to the family Euphorbiaceae. It is the main tree species exploited in the world for the production of natural rubber (Compagnon and D'Auzac, 1986). The demand for this raw material is steadily increasing because it has particular properties of elasticity, stiffness, and resistance to heating that are superior to synthetic rubber (Delabarre and Serier, 1995).

However, study of AM diversity in the open land adjacent to rubber plantation gives the chance to focus on the comparison of AM fungal communities at these sites.

Materials and Methods

Study sites and sample collection

The soil samples of rubber plantation site of Tripura, i.e., Takmachara (30-year-old plantation) and its adjacent open land (Takmachara) were collected for analysis of root colonization of plants, AM fungal spore, and soil physico-chemical properties. The soil samples at depths of approximately 10-20 cm were randomly chosen from five points in rubber plants and also from neighbouring open land. The soil samples were made into composite, labelled, and placed in plastic bags for further analysis. A total of 10 dominant plants were examined for root colonization. Three roots samples of each plant were collected for root colonization study. Out of which, six plants were found growing in rubber plantation and four were found in open land. Diodella sarmentosa (Sw.) Bacigalupo & Cabral ex Borhidi, Lindernia crustacea (L.) Muell., Urena lobata L. and Cassia tora L. were found growing in the open land adjacent to rubber plantation. In rubber plantation, plants such as U. lobata, C. tora, Oplismenus burmannii (Retz.) P. Beauv., Eupatorium odoratum L., Colocassia sp. and Hevea brasiliensis Muell. Arg. were found.

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Table 1: Soil physicochemical and AM fungal spore density of rubber plantation and open land

| Sites | рН | Electrical conductivity (cS cm ⁻¹) | Organic carbon (%) | Available nitrogen (kg/Ha) | Available phosphorus (kg/Ha) | Available potassium (kg/Ha) | Available calcium (kg/Ha) | Spore density/ 100g soil |
|----------------------|------|--|-----------------------|----------------------------------|------------------------------------|-----------------------------------|---------------------------------|--------------------------------|
| Rubber plantation | 4.52 | 128.00 | 1.40 | 349.09 | 14.77 | 160.39 | 120.43 | 780.00 |
| Open land | 5.08 | 99.00 | 1.99 | 344.99 | 9.26 | 176.91 | 382.67 | 1,516.00 |

Soil analysis

From fresh soil, pH and electrical conductivity were measured, 10 g of soil was dissolved in 50 ml of distilled water and stirred for 20 min. This solution was kept overnight and then the soil pH and electrical conductivity was measured using a digital meter. From other soil samples that were air dried, ground to fine powder and sieved, the soil chemical properties were determined. The Organic Carbon was determined by using Walkley-Black (1934) method. The soil available Nitrogen was estimated following Black (1982) method. Available Phosphorus, Potassium, and Calcium of soil were determined using the method of Jackson (1978).

Preparation of roots and assessment of AM fungi

Three root samples from each species were collected and washed thoroughly with tap water for several times and cut into pieces of approximately one cm long in size. Then the roots were cleared and stained (Das and Kayang, 2008). Root segments were mounted on slide and examined for mycorrhizal structures under the microscope. The estimation of AM fungal colonization was done by the magnified intersection method (McGonigle, Miller, Evans et al. 1990).

AM fungal spore isolation and identification

AM fungal spores in soil were isolated using the modified wet sieving and decanting method. The 25g soil of each sample was suspended in water and passed through a 35 µm sieve. The residues on the sieve were then filtered by filter paper. The spores were then picked up with needle from that filter paper in one drop of polyvinyl alcohol-lactoglycerol under a dissecting microscope (Koske and Tessier, 1983) for enumeration and identification. The sporocarps and spore clusters were regarded as one unit. Taxonomic evaluations were carried out based on morphological characteristics using identification keys of International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi and AMF phylogeny.

Statistical analysis

Standard errors of means and Relative Abundance (RA) were calculated.

 $RA = \frac{\text{Spore numbers of a species (genus) x 100\%}}{\text{The total number of identified spore samples}}$

Diversity indices were also calculated according to Hammer, Harper, Ryan et al. (2001).

Results

Soil characteristics

The soil samples from both the sites were acidic and the electrical conductivity of rubber plantation was higher than open land. Organic Carbon and available Potassium was high in open land than rubber plantation. In contrast, available Nitrogen was high in rubber plantation than open land. Available Phosphorus revealed higher concentration in rubber plantation than open land. But the amount of available Calcium was considerably high in open land than rubber plantation. The soil properties were depicted in Table 1.

Mycorrhizal colonization

The mycorrhizal structural colonization is presented in Table 2. The structures such as arbuscules, vesicles, and hyphae were observed in the roots of plants from rubber plantation and open land (Plate 1). Arbuscules colonization was highest observed in E. odoratum and lowest was noticed in D. sarmentosa. The vesicles were maximum in U. lobata in the open land and least was observed in L. crustacea. The arbuscular mycorrhizal hyphal colonization was highest observed in C. tora and lowest in Colocassia sp.

AM fungal diversity

The spore density was higher in open land than rubber plantation (Table 1). The relative abundance of AM fungal species was presented in Table 3. A total of four species from rubber plantation and eight species from open land were isolated. There were four species, namely, Glomus multiculae, Glomus sp 1, Glomus sp 2, and *Glomus* sp 3 were similar in both the samples. Glomus sp 1 was highly abundant and Glomus sp 2 showed lowest abundance in rubber plantation. In case of open land, G. multiculae expressed highest abundance; whereas lowest abundance was

Table 2: Mycorrhizal colonization in plants growing in open land and rubber plantation

| Plants | Family | Site | % RLA | % RLV | % RLH |
|----------------------|------------------|------|--------------|------------------|--------------|
| Hevea brasiliensis | Euphorbiaceae | R | 7.82 ± 1.88 | 30.79 ± 4.76 | 92.59 ± 2.25 |
| Diodella sarmentosa | Rubiaceae | 0 | 1.44 ± 1.25 | 15.31 ± 3.06 | 59.01 ± 5.30 |
| Lindernia crustacea | Scrophulariaceae | 0 | 2.97 ± 1.27 | 8.64 ± 2.19 | 33.24 ± 3.08 |
| Urena lobata | Malvaceae | 0 | 13.97 ± 1.75 | 34.64 ± 1.48 | 91.97 ± 2.13 |
| | | R | 10.81 ± 3.46 | 26.98 ± 5.53 | 49.23 ± 6.61 |
| Cassia tora | Fabaceae | 0 | 5.21 ± 1.89 | 17.30 ± 3.06 | 43.30 ± 6.41 |
| | | R | 32.58 ± 1.93 | 10.18 ± 1.06 | 98.78 ± 0.53 |
| Oplismenus burmannii | Poaceae | R | 23.10 ± 5.28 | 10.04 ± 3.23 | 39.17 ± 5.65 |
| Eupatorium odoratum | Asteraceae | R | 32.60 ± 3.27 | 13.68 ± 2.75 | 80.84 ± 3.41 |
| Colocassia sp. | Araceae | R | 0.0 | 0.0 | 14.26 ± 3.70 |

R-Rubber plantation; O-Open land

%RLA, %RLV, and % RLH are per cent root length with arbuscules, vesicles, and hyphae, respectively.



Plate 1: Arbuscular mycorrhizal colonization in roots (a) Root segment showing vesicles in *Diodella sarmentosa* (x100) (b) Vesicle in *Hevea brasiliensis* (x400) (c) Root portion showing hyphae in *Urena lobata* (x400) (d) Arbuscular mycorrhizal colonization in *Cassia tora* (x100) (e) Arbuscules in *Oplismenus burmanii* (x400) (f) Vesicles in *Urena lobata* (x400) (g) Arbuscules in *Cassia tora* (x100) (h) Hyphae in *Lindernia crustacea* (x400) (i) Extraradical hyphae in *Eupatorium odoratum* (x100) (j) Intracellular hyphae in *Eupatorium odoratum* (x400).

Table 3: Relative abundance of AM fungal species isolated from

 rubber plantation and open land

| AM fungi | Rubber plantation | Open land |
|--------------------------|-------------------|-----------|
| Ambispora sp 1 | 0.00 | 1.52 |
| Funneliformis mosseae | 0.00 | 1.52 |
| Glomus clavisporum | 0.00 | 1.52 |
| G. macrocarpum | 0.00 | 4.55 |
| G. multiculae | 32.00 | 50.00 |
| Glomus sp 1 | 36.00 | 19.70 |
| Glomus sp 2 | 14.67 | 16.67 |
| Glomus sp 3 | 17.33 | 4.55 |
| | 100.00 | 100.00 |

Table 4: Diversity indices of AM fungal species isolated from

 rubber plantation and open land

| Diversity indices | Rubber plantation | Open land |
|-------------------|-------------------|-----------|
| Таха | 4.0 | 8.0 |
| Dominance | 0.289 | 0.349 |
| Shannon | 1.321 | 1.455 |
| Simpson | 0.711 | 0.651 |
| Evenness | 0.937 | 0.535 |

represented by three species, i.e., *Ambispora* sp 1, *Funneliformis mosseae*, and *G. clavisporum* (Plate 2).

Diversity index illustrated that Dominance and Shannon indices were high in open land although Simpson and Evenness were high in rubber plantation (Table 4).



Plate 2: AM fungal species (a) *Glomus* sp 1 (x400) (b) *Glomus* sp 2 (x400) (c) *Glomus* sp 3 (x400) (d) *Glomus* macrocarpum (x400) (e) *Glomus* multiculae (x400) (f) *Funneliformis* mosseae (x400) (g) *Glomus* clavisporum (x400) (h) *Ambispora* sp 1 (x400).

Discussion

Under natural conditions, plants sustain a considerable diversity of fungal root endophytes (Vandenkoornhuyse Baldauf, Leyval et al., 2002). These associations usually include multiple concurrent symbionts, which can result in joint antagonistic and/ or synergistic effects (Larimer, Bever, and Clay, 2010). AM fungi are among the most important beneficial fungal root endophytes, and they are known to colonize the roots of the majority of land plants. The average spore density of Takmachara rubber plantation was 195 per 25 g of soil and for open land was 379 per 25 g of soil. The spore density was significantly higher in open land than rubber plantation. Therefore, it was completely unknown, whether changes of native fungal communities occur naturally with time or if and how management induced changes in AM fungal communities (Sieverding, 1991). The poor soils together with the high turnover of organic matter make it reasonable to suspect an important ecological role of AM fungal symbioses in the nutrient cycling system of natural and agricultural ecosystems (Denslow, Vitousek, and Schultz, 1987). Glomus was highly abundant in both the sites. The dominance of Glomus from northeast region of India was also reported earlier by Das and Kayang (2009). Certain species of Glomales are adapted to acidic soils and generally, dominate the AM fungal community; they may be dominant due to their high competitiveness and reproductive capability (Sieverding, 1991).

According to Deka, Philip, Vinod et al. (1998) AM infection in the roots of five-year-old rubber plantation ranged between 68 to 88 per cent on surface layers in different treatments which resembles with this study. In Kerala, Nair and Girija (1988) recorded highest VAM infection in rubber (71 per cent) compared to other tree crops of economic importance. Reduction in mycorrhizal colonization of rubber plantation site plants might be due to harmful components present in rubber plants. The toxicity of some metals may be so high that plant growth is retarded before large quantities of an element can be translocated (Haghiri, 1973). Zhao, Wang, and Dou (2009) reported that adverse effects of rubber crumb on turf grass growth might be attributed to some toxic substances releasing from crumb rubber. Miguel, Fowler, and Sollars (2002) reported that rubber exhibits high levels of sulfur, zinc, and other heavy metals. Improvement in mycorrhizal colonization per cent in C. tora to certain extend under rubber plantation may be due to the resistance of this species against toxic substances present in rubber plant.

In this present investigation, soil samples from both the sites were acidic but the soil sample of rubber plantation was highly acidic and the spore density of this site was also less than the open land. Soil pH has a great relevance for plant growth as it influences nutrient mobilization as well as availability (Marschner, 1995). Johnson (1991) and Mohammad, Hamad, and Malkawi (2003) found that spore production increased with soil pH and organic carbon. This study also revealed the same tendency. Coughlan, Dalpé, Lapointe *et al.* (2000) inferred that the tendency for colonization levels to increase with pH was due to the greater ability of the taxa present to colonize host roots. Some AM fungal species produce more spores than others, with environmental factors significantly influencing spore reproduction (Saif and Khan, 1975).

Conclusion

In this study, the open land adjacent to the rubber plantation showed more spore density and diversity of AM fungi was comparatively higher than the rubber plantation. However, mycorrhizal colonization in plants was found to be high in rubber plantation.

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Morphotaxonomy of Rhizophagus irregularis (accession CMCC/AM-1102)

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Morphotaxonomic characterization plays an important role in describing microorganisms at different taxonomic levels. This approach has been very well explored in case of AMF taxonomy and has helped Mycorrhizologists to classify them on the basis of some very distinct and identifying features. Simple features, such as spore size, structure, wall layers, hyphal attachments, wall ornamentations, etc., are of great significance when it comes to the nomenclature of these unique microorganisms. With reference to our previously published articles, in this current issue we shall take up the morphotaxonomic study of one of the interesting and precious arbuscular mycorrhizal fungus available in the Centre for Mycorrhizal Culture Collection Bank with the accession number CMCC/AM-1102.

Figure 1: Scanning electron micrograph of CMCC/AM-1102 showing characteristic irregular types of spores (a-470 X; b-876 X)



This culture has been isolated and raised from the soils of Karimnagar district of Telangana, India. Initially the soil samples were analysed for AMF spore density, types, spores of varying sizes, colour, and diversity by suspending the soil samples in water and allowing them to pass through a series of sieves of 60, 100, and 300 British Standard Size (BSS), respectively. The sieving from each fraction was critically observed with the help of a stereo-zoom microscope.

In the next step, all the healthy spores were categorized according to their size, structure, and colour. Similar types of spores were grouped and

were used to obtain pure single species culture of arbuscular mycorrhizal fungi. Voucher specimen of this potential monosporal was prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., was done under a compound microscope (under 10X, 40X, and 100X magnification) after mounting in Polyvinly Lacto glycerol and Polyvinly Lacto glycerol: Melzer's reagents (1:1). Selected healthy single AMF spores were used to raise monospecific cultures which 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 are morophotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals.

Figure 2: Spores isolated from monosporal culture of CMCC/AM-1102



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In case of CMCC/AM-1102, the pure monosporal culture was established after six months of initiation from single spore. Good sporulation and colonization up to 80 per cent were recorded only after six months. Data presented in Table 1 shows sporulation rate of this culture after consecutive cycles of one and two years, respectively.

 Table 1: Spore count of accession CMCC/AM-1102 at different time intervals

| Activity | Date/Time interval | Spore count/g |
|--|--------------------|---------------|
| Initiation | 20-07-2012 | single |
| First check for colonization/sporulation | March 2013 | 3-7 |
| First cycle | October 2013 | 5-8 |
| Second cycle | April 2014 | 6-8 |

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

Spore Morphology and Shape

Spore isolated from the cultures were borne singly or in loose aggregates. All the spores were devoid of any sporocarp. Interestingly, spores were found to be of varying shapes and sizes which varied from globose, oval, oblong to irregular spores with deep wall depressions and apical cap-like swellings of their outermost wall layer 1 (Figures 1 and 2). Juvenile spores were hyaline and yellow when mature.

Spore Size and Diameter

This culture was found to have spores of varying shapes and sizes, therefore an average of 100 spores were evaluated. The diameters of most of the spores were found to lie between $91.43-250.18 \mu m$ (Figure 3).

Figure 3: Analysis of spore diameter of 100 healthy spores obtained from one year old culture



Spore Wall and Wall Layer

The other workers have reported the presence of three wall layers L1, L2, and L3. The first layer is only present in the juvenile stage (shows no reaction in Melzer's reagent), while L2 and L3 are formed sequentially in both the spore wall and in the wall of the subtending hypha of mature spores. In our study also, the mature spores were found to have two distinct wall layers (Figures 4 and 5).

Outer Layer (L1)—Evanescent layer is hyaline and somewhat appears granular in mature spores and clearly visible in PVLG and Melzer's reagent (Melzre's: PVLG-1:1). Thickness ranges from 1.5 to 5 µm.

Inner Layer (L2)—Laminated layer is a continuous and permanent layer, hyaline in juvenile and yellow colour when mature in PVLG. The thickness of this layer is found to range between $2.5-5.8 \mu m$ and stains a dark red-brown colour in Melzer's reagent.

Figure 4: Spore wall layers seen after Melzer's reaction (a&b); Murograph (Walker 1983) of Rhizophagus irregularis where evanescent walls are shown by dots, laminated wall with broken lines. Muronym= A (EL) (c) Figure 5: Spore and spore wall layers seen after PVLG reaction (a&b)





Subtending Hypha and Occlusion

All the spores show an intact, cylindrical to slightly flared subtending hyphae arising from the inner layer and measures about $10.42-18.5 \mu m$ at the base. The subtending hypha also contains two wall layers where the inner layer is continuous with the spore wall (L2). The thickness of the hyphal wall varies from $3.15-4.5 \mu m$. The hypha is hyaline in colour but changes to yellow on reaction to Melzer's reagent. A thin recurved septum (from inner lamina of L3 spore wall layer) is seen which is either plugged or open (Figures 6 and 7).

Figure 6: Spore showing subtending hypha after reaction with PVLG (a) and Melzer's reagent (b) Figure 7: Scanning Electron Micrograph of spores showing subtending hyphal attachment with the spore and the flared base





Mycorrhizae

Root colonization assay of the host plant after six months of inoculation showed the presence of both the types of intraradical and extraradical hyphae. Staining with 3 per cent ink vinegar showed the presence of arbuscules, vesicles, and hyphae in cortical cells. Intercalary arbuscules were dense and compact (Figures 8 and 9). Spores seen as dense clusters within roots, there is a higher proportion of irregularly-shaped spores with varying shapes and sizes.

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-1102, many distinguishing features regarding the family, genera, and the species could be derived. The following features were taken into consideration for characterization:

 Asexual spores produced in aggregates in an unorganized hyphal matrix in abundance with spore wall layers continuous with the subtending hypha. **Figure 8**: Root colonization study showing coiling at the entry point (a) and arbuscules and intercalary hypha (b) **Figure 9**: Root colonization study showing intercalary vesicles (a) and thick walled hyphae (b)



- Wall layer containing outer evanescent mucilaginous layer and a rigid inner layer that is continuous with the subtending hyphal wall;
- Spores of varying shapes and sizes ranging from globose, oval, oblong to irregular;
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intracellular arbuscules.

All these above features suggest that this culture belongs to the family **Glomeraceae**.

Some of the unique morphotaxonomic characters of this accession are as follows:

- Frequent formation of irregular spores with deep wall depressions and apical cap-like swellings of the outermost wall layer 1;
- Production of spores mainly in oblong aggregates containing spores arising either from the tip of or intercalary along dichotomously branched hyphae continuous with mycorrhizal extraradical hyphae;
- Intense reactivity of the laminate innermost spore wall layer in Melzer's reagent;
- Tendency to form spores mainly inside roots of its host plant.

This culture resembles the type genera *Rhizophagus* populinus (Dangeard 1896). However due to the presence of varying irregular shaped asexual spores, the accession CMCC/AM-1102 matches all the identifying characters of *Rhizophagus irregularis* (Błaszk., Wubet, Renker and Buscot) C. Walker and A. Schüßler comb. nov. (Synonym \equiv *Glomus irregulare* Błaszk., Wubet, Renker and Buscot, Mycotaxon 106: 252, 2008)

Systematic Classification

Glomeromycota Glomeromycetes Glomerales Glomeraceae *Rhizophagus irregularis*

Morphotaxonomic characterization and its limitations to the species identification has always been a matter of great dispute among the research groups. Studies based on rDNA sequence have often confirmed the morphologically defined species and the molecular data have erected new genera and families, revealing a considerable unknown AM diversity. It is therefore advised to our distinguished readers to kindly correlate their morphotaxonomic studies with the molecular data.

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