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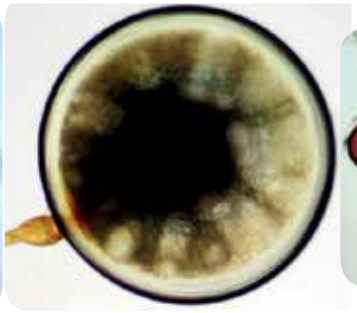
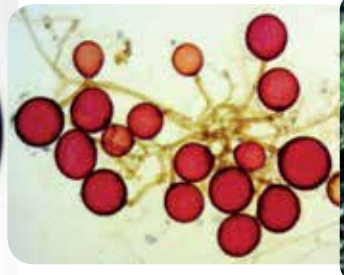

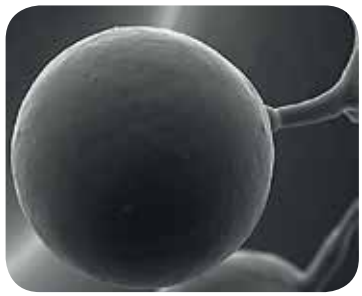

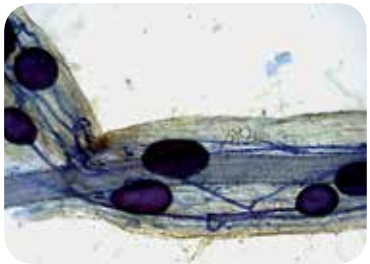

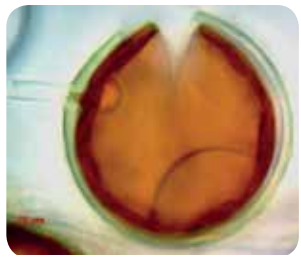


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TERI's **Mycorrhiza Network**

TERI's Mycorrhiza Network is primarily responsible for establishing the Mycorrhiza Information Centre (MIC), the Centre for Mycorrhiza Culture Collection (CMCC), and publishing *Mycorrhiza News*. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

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 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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Lone *vis-a-vis* Synergistic Inoculation Effect of Arbuscular Mycorrhizal Fungi and Non-Mycorrhizal Bioinoculants on Morpho-Metric Indices in *Putranjiva roxburghii* Wall. (Putranjivaceae), a Medicinal-cum-Industrial Crop Plant

Vipin Parkash[a] and Ankur Jyoti Saikia[b]

Putranjiva roxburghii Wall. Syn. *Drypetes roxburghii* (Wall.) Hurusawa is also known as 'Indian Amulet Plant' and belongs to the family Putranjivaceae (erstwhile included in Euphorbiaceae); the plant is well known for its medicinal properties (Minj *et al.* 2016). It is an evergreen tree with drooping branches characterized by a corky bark, coriaceous leaves, small stipules along with small dioecious flowers, and apex pointed narrow-based drupes (fruits) (Kumar 2012). It is pan-Asian in distribution, widely grown in Thailand, Nepal, Bangladesh, India, China, Myanmar, and Sri Lanka (Phuphathanaphong and Chayamarit 2006). Accepted as a highly medicinal plant (Khare 2007), it has been a source of several phytochemicals. Six triterpenoids have been isolated from the bark of *P. roxburghii*, viz.—putranjivanonol, putranjic acid (Garg and Mitra 1968), friedelin, putranjivadione, friedelanol, and roxburgholone (Sengupta and Mukherjee 1968). Again, Roxburghonic acid (a friedelane triterpenoid keto acid) and *putraflavone* (a biflavonoid) were isolated from the alcoholic extract of *P. roxburghii* leaves (Garg and Mitra 1971a and b). In conjunction with the medicinal properties, this plant has great commercial importance for its valued wood and for production of bio-oil (Subramanian *et al.* 2015).

On the other hand, the plant population in its natural habitat has become rare because of large-scale destruction of trees for its wood, short (seed) viability, and low (seed) germination. Keeping its biological and economic importance in view, it is important to conserve these trees (Ishii and Kambou 2007). Rejuvenation of wild plant populations using *ex-situ* raised individuals is considered a suitable means of reducing the risk of threatened species (Bowes 1999).

Arbuscular Mycorrhizal (AM) fungi are beneficial microorganisms that form symbiotic association with the roots of higher plants and can be utilized as biofertilizers both in forestry and agro-cropping systems (Parkash *et al.* 2005; Bagyaraj 2015). They play an important role in improving plant growth, nutrient uptake, especially phosphorus (Mukerji and Dixon 1992; Gill *et al.* 2002; Khan and Uniyal 1992), and provide stress tolerance and disease resistance to plants (Mehrotra *et al.* 1995). The combined and synergistic effect of AM fungi along with other microbial bioinoculants (e.g., *Trichoderma* sp., etc.) on seedlings of forestry plant species (e.g., *Eucalyptus saligna* Sm., *Rauvolfia serpentina* Benth., etc.) has been tested by researchers (Parkash *et al.* 2011a and b). Although dual inoculation had good growth response, triple inoculation had more pronounced and significant response on growth and development.

Plant growth analysis is considered to be a standard approach to study plant growth and productivity (Wilson 1981). Growth and yield are functions of a large number of metabolic processes, which are affected by environmental and genetic factors (Hokmalipour *et al.* 2011). Plant growth is influenced by presence of bacteria and fungi, and

a Forest Pathology Division, Forest Research Institute (Indian Council of Forestry Research & Education Autonomous Council under the Ministry of Environment, Forests and Climate Change, Government of India), Dehradun 248 195, Uttarakhand, India. Email: bhardwajvpnpark@rediffmail.com

b Soil Microbiology Laboratory - I, Silviculture & Forest Management Division, Rain Forest Research Institute (Indian Council of Forestry Research and Education, Autonomous Council under the Ministry of Environment, Forests and Climate Change, Government of India), Deovan, Jorhat 785 001, Assam, India. Email: ankurj.saikia05@gmail.com

their interactions are particularly common in the rhizospheres of plants with high relative densities of microbes (Berg and Smalla 2009). Although selection preference of bio-inoculation has been reported on some medicinal as well as biodiesel plant species, there are no reports of bio-inoculation effect on morpho-metrics in *P. roxburghii*. So, the objective of this study is to ascertain the effect of AM fungal inocula in lone and mixed inoculations (with non-mycorrhizal bioinoculants) on morpho-metric parameters of *P. roxburghii*.

Materials and Method

Experimental design

One-year-old seedlings of *P. roxburghii* Wall. were taken for the present study. The experiment was conducted in the nursery of Rain Forest Research Institute located at a distance of 10 km east of Jorhat City on NH-37 (on Jorhat–Tinsukia Highway, Assam, India (26°46′53″N 94°17′29″E, 107 masl). The annual average precipitation is 500 mm and the annual average temperature 26 °C. The experiment was conducted using nursery soil in a ratio of 1:1:2 of sand, field yield manure, and soil. The experiment was designed in randomized block design with five replications of each treatment. The seedlings were treated with bacteria (i.e., *Pseudomonas putida* Trevisan [T_B]), fungus (i.e., *Trichoderma harzianum* Rifai [T_F]) and mycorrhiza (i.e., *Funneliformis mosseae* [T H Nicolson and Gerd.] C Walker and A Schüßler [T_M]), and mixed consortium of bacteria, mycorrhiza, and fungus (T_{MBF}) and were analysed at 0, 30, 60, and 120 days after inoculation (DAI) for morpho-metric indices. The bio-agents utilized were available at the Soil Microbiology Laboratory – I of Rain Forest Research Institute (Jorhat, Assam).

Growth parameter calculation

Observations on height and diameter of the newly emergent shoot were recorded to see the AM inoculation effect on seedlings until 120 DAI. The biovolume index (Bi) of the seedlings was calculated using standard formula (Hatchell 1985).

Statistical analyses

Standard error of means and coefficient of variance were calculated for all physico-chemical growth and yield data (Gupta 2001). MS Excel software 2007 was utilized for computation and transformation of data.

Results and Discussion

Increase in height and diameter (in centimetre) of the test plant seedlings shoot along with Bi are shown in Table 1 and Figure 1. At 30 DAI, maximum increase of height and diameter was observed in T_{BMF} (24) and T_M (2.82), while lowest values were observed in T_B (0.80) and T_{BMF} (0.10), respectively. Again, at 60 DAI, T_{BMF} exhibited the highest increase in height (5.8) and lowest in diameter (0.2), while lowest values with respect to increase in height (1.07) and highest value for diameter (0.37) were exhibited by T_B and T_M, respectively. An effect at par with the result of 60 DAI is also expressed by the treatments at 90 DAI. On 120 DAI, T_{BMF} exhibited highest increase in height

Table 1 Growth response of *Putranjiva roxburghii* seedlings at 30, 60, 90, and 120 DAI using different microbial inoculants

DAI	Treatment/ AM strain	Increase in height (cm)*	Increase in diameter (cm)*	Biovolume index (B_i)*
30	Control	0.87 ± 0.008	0.19 ± 0.023	33.06 ± 0.089
	T_B	0.80 ± 0.020	0.22 ± 0.023	44.58 ± 0.32
	T_M	1.17 ± 0.023	0.24 ± 0.026	59.11 ± 0.33
	T_F	2.23 ± 0.027	0.16 ± 0.021	56.10 ± 0.27
	T_{BMF}	2.82 ± 0.023	0.10 ± 0.017	83.60 ± 0.23
60	Control	1.12 ± 0.008	0.24 ± 0.026	40.19 ± 0.37
	T_B	1.07 ± 0.011	0.29 ± 0.034	54.23 ± 0.21
	T_M	1.77 ± 0.023	0.37 ± 0.017	75.47 ± 0.25
	T_F	4.65 ± 0.046	0.34 ± 0.024	76.81 ± 0.23
	T_{BMF}	5.8 ± 0.38	0.2 ± 0.021	105.12 ± 0.80
90	Control	1.12 ± 0.029	0.24 ± 0.029	47.85 ± 0.29
	T_B	1.06 ± 0.013	0.29 ± 0.031	64.30 ± 0.31
	T_M	1.76 ± 0.021	0.37 ± 0.023	93.12 ± 0.53
	T_F	4.65 ± 0.047	0.33 ± 0.021	100.25 ± 0.76
	T_{BMF}	5.8 ± 0.41	0.2 ± 0.019	128.96 ± 0.32
120	Control	2.93 ± 0.027	1.24 ± 0.044	86.66 ± 0.23
	T_B	7.54 ± 0.083	1.23 ± 0.037	124.81 ± 0.47
	T_M	8.63 ± 0.037	1.2 ± 0.029	170.03 ± 0.91
	T_F	9.03 ± 0.026	1.2 ± 0.017	183.25 ± 1.04
	T_{BMF}	9.9 ± 0.057	1.3 ± 0.023	232.05 ± 1.57
Coefficient of Variance		0.82	0.89	0.56

±: Standard error of mean; *: Average of five replications; T_B: *Pseudomonas putida*; T_F: *Trichoderma harzianum*; T_M: *Funneliformis mosseae*; T_{MBF}: mixed consortium of *P. putida*, *T. harzianum*, and *F. mosseae*

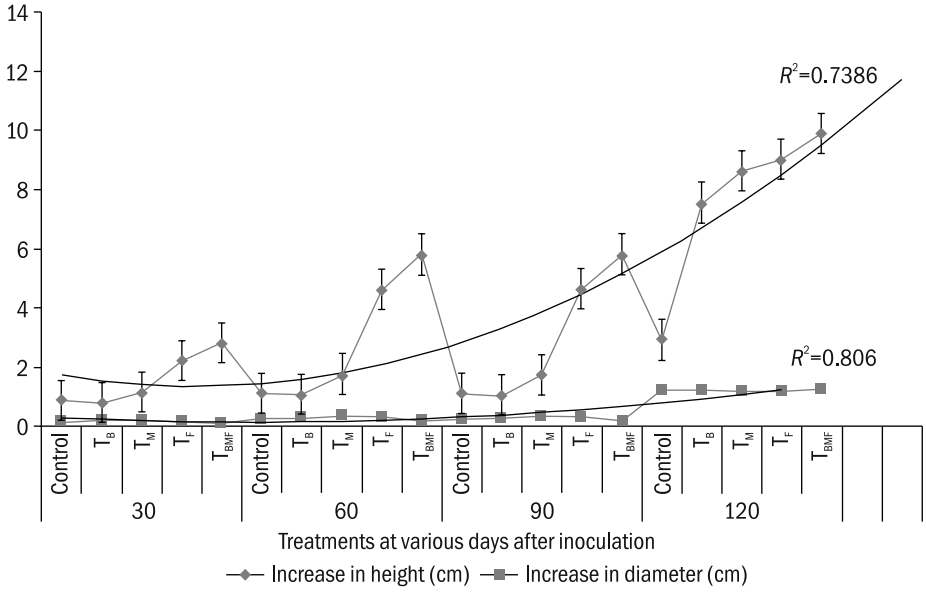


Figure 1 Growth response of *Putranjiva roxburghii* seedlings at 30, 60, 90, and 120 DAI

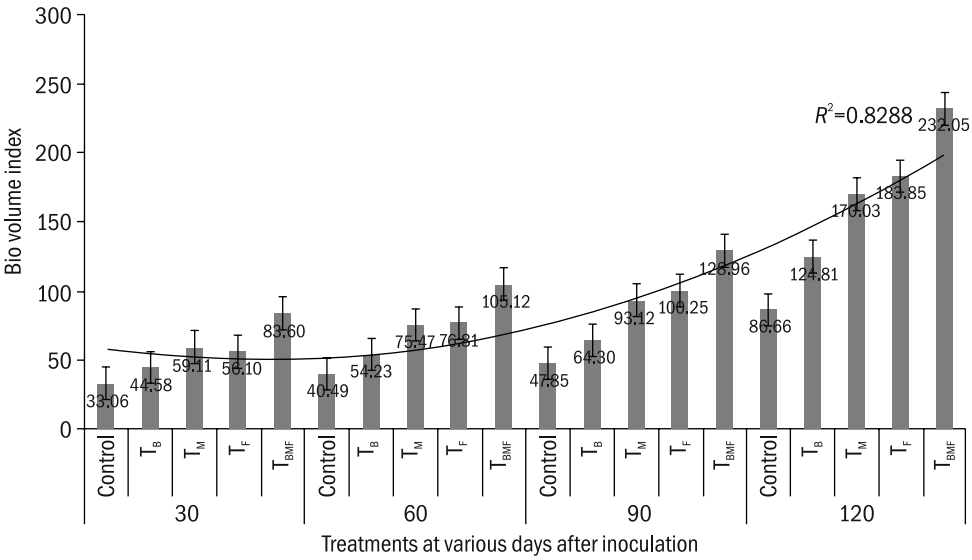
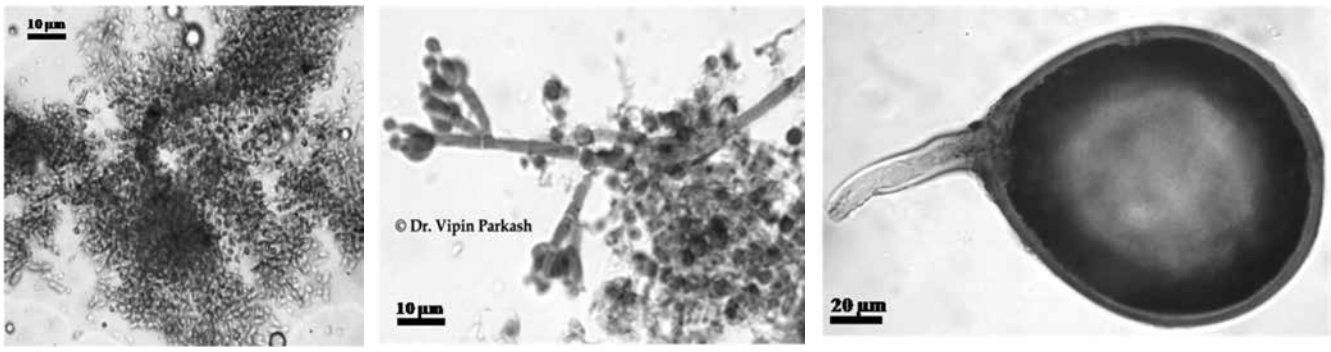


Figure 2 Effect on Biovolume indices (Bi) of *Putranjiva roxburghii* seedlings at 30, 60, 90, and 120 DAI



(A) **(B)** **(C)**

Figure 3 (**A**) *Pseudomonas putida*; (**B**) *Trichoderma harzianum*; (**C**) *Funneliformis mosseae*

(9.9) and diameter (1.3) while the non-inoculated seedlings showed lowest values (2.93, 1.24).

With regard to the Bi of the seedlings (Table 1 and Figure 2), it is an appreciable fact that at all the DAIs (i.e., 30, 60, 90, and 120), maximum values (83.60, 105.12, 128.96, and 232.05) were exhibited by T_{BMF} while lowest values (33.06, 40.19, 47.85, and 86.66) were observed in the non-inoculated seedlings.

All bioinoculants when applied alone showed promising results on growth and development of seedlings, but when these are applied mixed together, then their effects profound substantially (Parkash *et al*. 2017). It has been reported that plant growth promoting rhizobacteria (inclusive of *Pseudomonas putida*) influence the root colonization, penetration, and nutrient translocation activity of AM fungi (Azcon-Aguilar and Barea 1979). Again, synergistic interaction of AM fungi with saprobic fungus *Trichoderma* has also resulted in enhanced growth responses in host plants (Parkash and Aggarwal 2011). Through this study, AM fungal inclusive synergistic inoculation proves to promote growth parameters and Bi of the seedlings in comparison to the non-inoculated treatments. Out of the other inoculation treatments, the synergistic effect of bacterial, non-mycorrhizal fungal, and AM fungal strains together proved to be the best treatment.

Acknowledgements

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Isolation and Characterization of Arbuscular Mycorrhizal Fungi and Their Role in Plants Growing Under Harsh Environments

A N Ganeshamurthy[a], Komal Sharma[b], Debasis Mitra[b], T K Radha[a], and T R Rupa[a]*

Soil microorganisms, such as arbuscular mycorrhizal fungi (AMFs or AM fungi) represent a key link between plants and soil mineral nutrients. Vesicular arbuscular mycorrhizas are produced by aseptate mycelial fungi and are so-called because of their characteristic structures, namely vesicles and arbuscules found in roots with type of infection. Mycorrhizas are found in bryophytes, pteridophytes, gymnosperms, excluding the pinaceae that have ecto mycorrhiza, and in some woody families of angiosperms. Mycorrhiza has a symbiotic association between fungus and roots of a vascular plant. In a mycorrhizal association, the fungus colonizes the host plants roots, either intracellular as in AMF, or extracellular as in ectomycorrhizal fungi. Mycorrhizal symbiosis is both a functional and structural association between fungi and roots of plants belonging to division Glomeromycota, Ascomycota, and Basdiomycota (Schüßler *et al.* 2001). The mycorrhizae may be ectotrophic or endotrophic according to the major area of fungal colonization in the roots. Mycorrhizal plants were found to maintain higher drought tolerance (Augé 2001) and to have better access to phosphorus than non-mycorrhizal ones (Neumann and George 2004). AMF are obligate symbionts belonging to the phylum Glomeromycota (Schüßler *et al.* 2001), which form mutualistic symbioses with about 80% of land plant species, including several agricultural crops. They provide the host plant with mineral nutrients and water, in exchange for photosynthetic products (Smith and Read 2008). The AMF mycelium that emerges from the root system can acquire nutrients from soil volumes that are inaccessible to roots (Smith *et al.* 2000). Furthermore, fungal hyphae are much thinner than roots and are therefore able to penetrate smaller pores (Allen 2011). Thus, AMFs can alleviate the limitation in plant growth caused by an inadequate nutrient supply (Nouri *et al.* 2014). In most cases, mycorrhizae benefit their host plants by improving nutrient uptake, such as Phosphorus (P), Nitrogen (N), and micronutrients (Clark and Zeto 2000; Ward *et al.* 2001). AMF also provide their host plants with protection against abiotic stresses (Augé 2001) as well as biotic stress (Khaosaad *et al.* 2007). Certain plants grow under very harsh environments, such as cracks and crevices of rocks, on walls of buildings, on rocky terrain, on very dry conditions in certain pockets, etc. We believe that these plants survive with the assistance of certain microbial association assisting the plants to overcome such harsh abiotic stresses. One such possibility is the plant–micorrhizal association to overcome both drought and nutritional constraints. Hence, this study was undertaken to examine the mycorrhizal existence and their isolation from such harsh environments.

Materials and Methods

Sampling sites

The root and the rhizospheric soil samples were collected under asceptic conditions as per the method of mentioned by Schenck (1982) from *Albizia saman, Pongamia pinnata, Alstonia scholaris, Ficus racemosa, Acacia concinna, Azadirachta indica, Tamarindus indica, Vachellia nilotica, Schefflera actinophylla*, and *F. religiosa* plants that grow in very harsh situations, such as soil covered with hard, compact crust, cracks of rocks, and similar situations in four districts of Karnataka, *viz.*, Bengaluru, Bengaluru Rural, Chickballapur, and Kolar.

Collection of soil and root samples

Rhizospheric soil was sampled by making a soil cross-section of around 30 cm diameter and a depth of 30 cm, 20 cm away from the stem of the trees to have an access to the roots. Soils collected represent vertical cross-sections of the root zones. Twenty samples were collected from different harsh rhizospheric soils. In each sample, around 1 kg of the soil with their root systems were collected. This sampling method was chosen because the shallow root system did not allow the soil cross-section to avoid destruction of natural ecosystem. The samples were brought to the laboratory and the fine roots in each sample was removed, rinsed with tap water, and stored for future experiments. The soil samples were air dried in shade, crushed, and sieved at laboratory temperature for microbial isolation and spore counting. Soil and root samples were kept separately in

[a] ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka 560 089, India
* Corresponding author, Email: angmurthy@gmail.com
[b] Graphic Era University, Dehradun, Uttarakhand 248 002, India

plastic bags for further studies. The harsh conditions from where the soil samples were collected are shown in Figure 1.

Figure 1 Site representing a harsh environment rhizospheric soil

Isolation of mycorrhizal spores

AM fungal spores occurring in rhizospheric soil sample was isolated by using wet sieving and decanting technique (Gerdemann and Nicolson 1963). A 100 g dried rhizospheric soil sample was weighed and mixed with 500 mL water in a beaker. The suspension was kept undisturbed for 5 minutes for the sedimentation of heavier particles. After 5 minutes, the suspension was carefully decanted through the sieves stack that was arranged in a descending order of 710, 250, 75, and 45 µm mesh size. Each level of the sieve was washed with water until it appeared that all possible material had passed through. The retained material on the sieve was re-suspended in water and the process was repeated for two or three times until a clear solution was visible. The AM fungal spores in the bottom sieves, *viz*., 75 and 45 µm were transferred into the 15 mL polypropylene tube with distilled water.

Observation of spores

The spores collected through sieving technique were washed into glass Petri dishes and examined under a sterio binocular microscope (Stemi DV4, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). Healthy spores were picked by using a disposable Pasteur pipette, and spores were mounted with cover slip on slides for identification purpose.

Morphological identification of isolates

The AM fungal spore isolates were identified based on spore morphology, as described by Schenck and Perez (1990) and different taxonomic keys proposed by different workers. The characteristics that were considered for spore identification were spore morphology; size, shape, and peridium of spore; sporocarps colour; wall ornamentation; subtending hyphae; and mode of attachment.

Estimation of glomalin content

Total glomalin content of soil sample was estimated by pressure cooker method as described by Wright and Upadhyaya (1996). One gram of soil was taken in an autoclavable centrifuge tube and 8 mL of 50 mM sodium citrate was added. The mixture was then autoclaved for 60 minutes at 121 °C. After autoclaving, the tubes were centrifuged at 7,000 rpm for 15 minutes to sediment soil particles. The supernatant containing the extract was collected and stored at 4 °C. This process was repeated for two more times and the extracts were pooled and stored. The protein content in the extract was quantified using Bradford assay protocol using bovine serum albumin as the standard. The glomalin content was read by spectrophotometer at 595 nm and expressed as milligram per gram of soil.

Trap culturing of AMF by using finger millet

The single spore culture method did not work, so we resorted to use of trap cultures as a practical approach. Production of spores was achieved by using Finger Millet (*Eleusine coracana)* in arka fermented cocopeat (soil less growth culture medium developed by IIHR). For each of the 20 rhizospheric soil samples, two replicates were prepared. Pots were filled with an autoclaved plant growth substrate (Coco-Pith) with 1% Arka Microbial Consortium mixed in each pot, which helped in plant growth. To trap the mycorrhizal spores, 50 g of each soil sample and 10 g of crushed root samples were mixed with upper layer on the surface of the growth substrate in the pots. Finger millet seeds were sown in each pot to trap the mycorrhizal inoculums. After 45 days, the root samples from each pot were taken for the evaluation of AM fungal root colonization.

To determine the AM fungal infection and colonization within root of finger millet (*E. coracana*), a combined method of Phillip and Hayman (1970) and Koske and Gemma (1989) was used. Percentage of root colonization was determined using the method described by McGonigle *et al.* (1990). Freshly collected secondary and tertiary root samples were gently washed to remove cocopeat that was attached to the root surfaces, submerged in 10% KOH solution and autoclaved for 15 minutes at 121 °C. The KOH solution was decanted and the treated roots were rinsed with tap water for 3–4 times until no brown colour appeared in the rinsed water. The treated root samples were further immersed in 2% HCl solution for 5 minutes. Without rinsing with water, HCl was decanted and the root samples were stained with 0.05% trypan blue in lactoglycerol (400 mL lactic acid + 400 mL glycerol + 100 mL water) and autoclaved for 15 minutes at 121 °C. After autoclaving, the stained solution was decanted and the roots were destained with lactoglycerol solution to remove the excess of stains and used for microscopic observations. The slide was prepared by keeping 10 segments of stained root on a clean glass slide and observed under compound microscope (OLYMPUS TH4-200 microscope, Olympus®, Tokyo, Japan). The

AM infection was detected by the presence of spore or hyphae in the root cortex. Percentage of AM root colonization was calculated using the formula:

Percentage of colonization = *Number of root segments colonized* ÷ *Total number of root segments* × *100*

Response of AMF inoculum on onion

After trap culturing of AM fungi, the inoculum from finger millet was used to evaluate its plant growth response on onion. Quadruplicate pots were filled with autoclaved Cocopeat + Inoculum (1:1). The onion seeds were sown in each pot. After 45 days, AM fungal root colonization in onion and vigour index of plants was observed. Evaluation of AM fungal root colonization in onion was estimated using the same protocol as mentioned above for finger millet.

For calculating the vigour index, the germination rate of plant was determined during the growth of plant. After 45 days, five plants along with their roots were taken from each treatment. The shoot and root length of treatments along with control was noted for calculation of vigour index. The vigour index was calculated by using the formula:

Vigor index = *(Mean root length + Mean shoot length)* × *(Percentage of germination)*

Result and Discussion

AM fungal spores were present in all twenty samples collected from rhizospheric soils of plants growing under harsh conditions. Colonization of plant roots by AMF under such harsh environment shows that these AMF are helping plants survive under such environments.

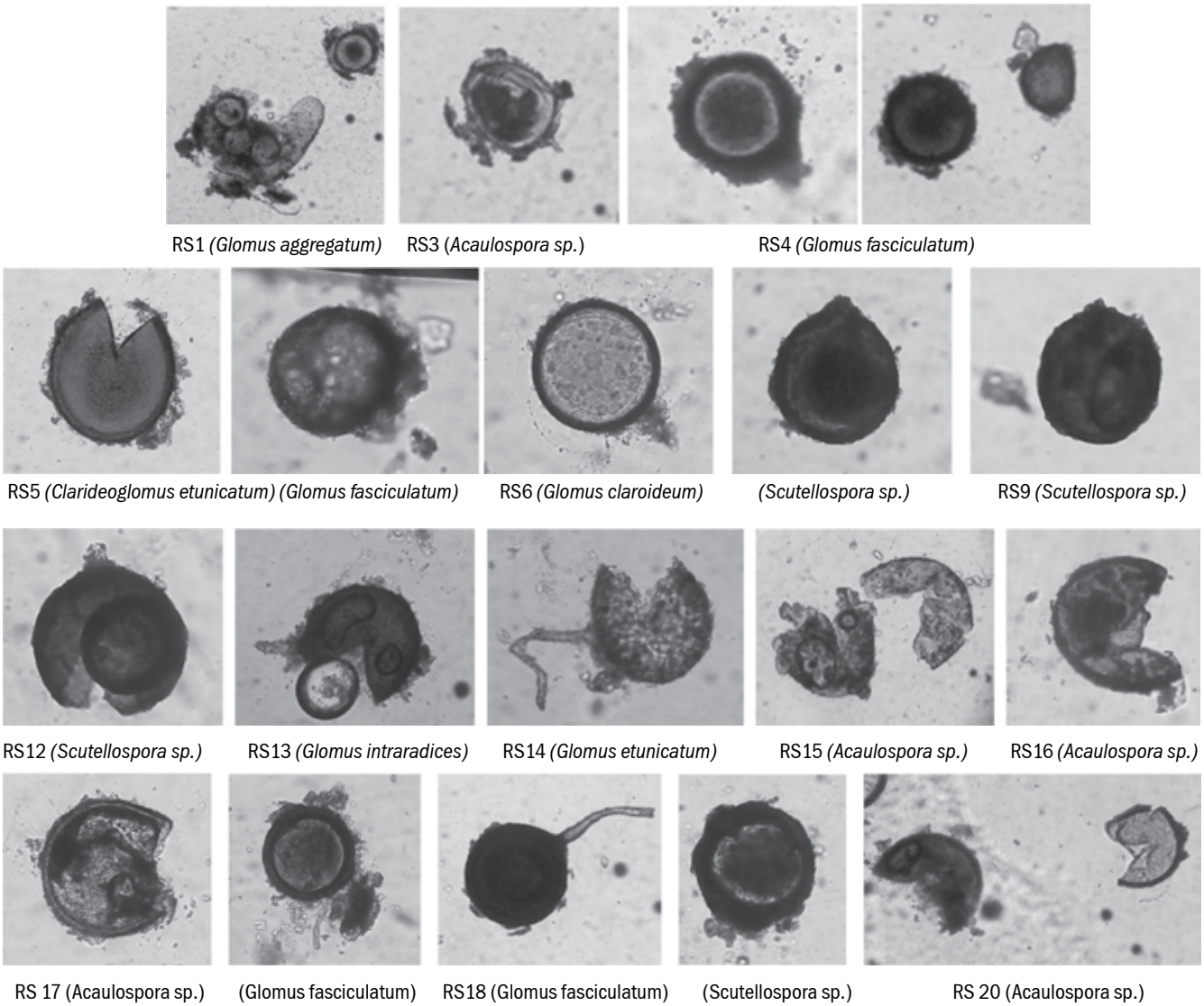

Figure 2 Morphological identification of AMF spores

Morphological identification of cultured AMF spores

The AM fungal spore isolates were identified based on spore morphology, size, shape, and peridium of spore, sporocarps colour, wall ornamentation, subtending hyphae, and mode of attachment, as described by Schenck and Perez (1990). Spores from 14 out of 20 isolates could be identified. The remaining six samples could not be identified because of the unclear images of spore. In these 14 samples, the identified AM species are *Acaulospora sp.*, *Glomus aggregatum*, *Rhizophagus fasciculatus*, *Clarideoglomus (Glomus) etunicatum*, *G. claroideum*, *Scutellospora sp.*, *G. intraradices*, and *G. etunicatum* (Figure 2).

Evaluation of AM fungal colonization in finger millet

The root colonization percentage varied with samples (Figure 3). Maximum rate of colonization was recorded in sample RS 2 and RS 7 and the minimum was seen in sample RS 16 and RS 20. Variability in percentage of infection may be due to differences in the ability of AM fungi to cause infection and preferences of AM to infect finger millet. The images of roots colonization of fourteen samples are shown in Figure 4.

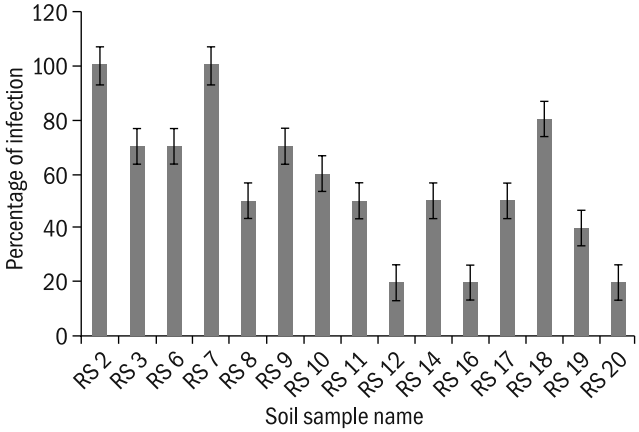

Figure 3 Roots infection rate in finger millet

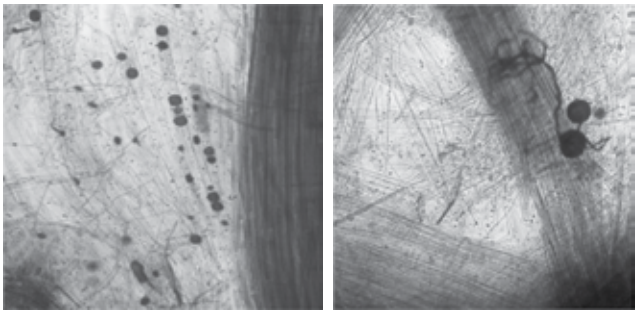

Figure 4 Colonization of roots of finger millet

Table 1 Glomalin concentration in the collected soil samples

Sl. No.	Sample ID	Glomalin (mg/mL)
1	RS 1	0.1261
2	RS 2	0.3232
3	RS 3	0.0709
4	RS 4	0.2404
5	RS 5	0.0709
6	RS 6	0.2601
7	RS 7	0.0748
8	RS 8	0.0985
9	RS 9	0.1143
10	RS 10	0.1103
11	RS 11	0.1537
12	RS 12	0.1734
13	RS 13	0.1379
14	RS 14	0.2798
15	RS 15	0.1182
16	RS 16	0.1064
17	RS 17	0.1064
18	RS 18	0.3232
19	RS 19	0.2049
20	RS 20	0.1103

Glomalin quantification from soil sample

Glomalin is the glycoprotein produced by AM fungi to bind soil particles and improve soil aggregate stability. Quantified glomalin helps in selecting the AM species for their intended use in agriculture. Hence, glomalin was estimated from soil samples collected in this study (Table 1). The glomalin content in soil varied from 0.0709–3,232 (mg/mL). Since each soil had multiple species of AMF, a relationship between glomalin production and species of AMF could not be established.

Response of AMF inoculum on onion

In trap culturing, 14 samples showed the positive results of root colonization. Out of which, 10 samples were further used for analysis of plant growth promotion of AM fungi using onion as a test crop. The inoculum of AM from trap culture, which was

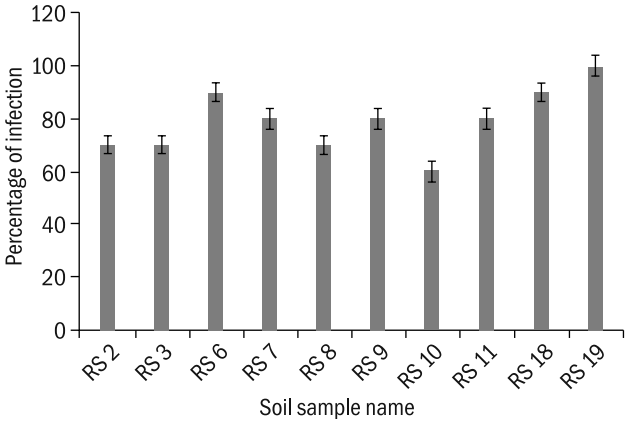

Figure 5 Percentage of infection of roots of onion

selected for plant growth analysis, was chosen on the basis of their percentage of infection in roots, quality of root colonization, and also on the basis of high AM hyphal infection. For the analysis of plant growth promotion by AM fungi, pots sown with onion seeds were maintained in glasshouse for 45 days. After 45 days, the evaluation of AM fungal colonization and percentage of infection in onion was done by staining the roots. The percentage of infection of onion roots is shown in Figure 5. Photographs showing the level of infection are presented in Figure 6. From the results of percentage of infection, the maximum rate of colonization was seen in sample RS 19 and the minimum rate of colonization was seen in sample RS 10.

The Vigour Index was also calculated for each of these 10 treatments with all four replicates. The calculated mean root length, mean shoot length, percentage of germination, and Vigour Index are presented in Table 2.

The maximum shoot length as compared to control was observed in treatment RS 18 followed by RS 9 and RS 6, but in case of root length, each treatment recorded greater root length as compared to control. The maximum root length was seen in sample RS 19 followed by RS 10, RS 2, RS 11, RS 7, RS 8, RS 6, RS 9, RS 18, and RS 3. The maximum vigour values were recorded in treatments RS 2 and RS 6.

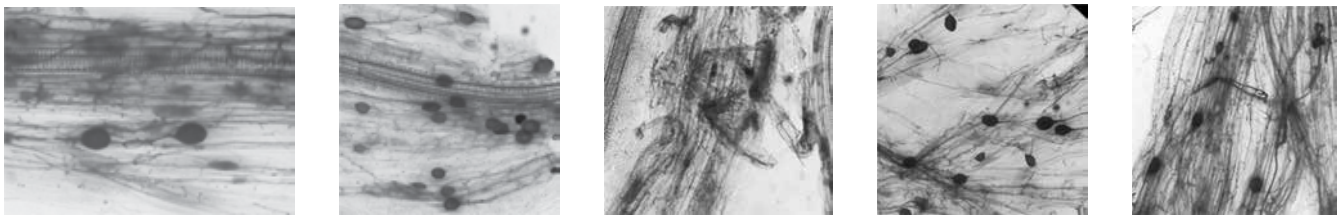

Figure 6 AMF root colonization in onion

Table 2 Vigour index of different treatments

Sl. No.	Treatments	Shoot Length (cm)	Root Length (cm)	Percentage of Germination	Vigour Index
1	Control	20.37	07.10	20%	549.4
2	RS 2	19.99	14.34	80%	2,746.4
3	RS 3	19.94	11.88	80%	2,545.6
4	RS 6	22.14	12.59	80%	2,778.4
5	RS 7	20.98	13.61	60%	2,075.4
6	RS 8	20.44	12.93	75%	2,502.8
7	RS 9	22.52	12.36	45%	1,569.6
8	RS 10	20.50	14.40	65%	2,268.5
9	RS 11	21.38	14.26	65%	2,316.6
10	RS 18	23.15	12.09	60%	2,114.4
11	RS 19	21.92	16.66	65%	2,507.7
SEM	00.71	01.07			
CD	02.07	03.11			

Conclusion

The study revealed that plants (onion) growing in harsh environments have mycorrhizal association. The identified AM species are *Acaulospora sp., G. aggregatum, G. fasciculatum, Clarideoglomus (Glomus) etunicatum, G. claroideum, Scutellospora sp., G. intraradices*, and *G. etunicatum*. The production of glomalin by AMF in these soils showed that there is scope for further detailed studies on these isolates of AMF.

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Morpho-taxonomy of *Scutellospora heterogama* (Accession CMCC/AM-2201)

*Aditi Pandit, Varsha, and Alok Adholeya**

Mycorrhiza is association of the plant roots with the soil fungi. More than 90% of terrestrial plants are found with this type of root-colonizing fungi. This association is mutualistic where the fungi receive 20% of the plant's carbon and the plant in return gets minerals. The arbuscular mycorrhiza fungi (AMF) is the most common type of mycorrhiza, found in 80% of plant species (Strack *et al*. 2003). More than 150 species of the Zygomycota includes the Glomales (Morton and Benny 1990). Morphological feature is one of the methods to describe different taxonomic levels of this microorganism. Across different families and genera, various morphological characters are shown by the AMF and this approach helps mycorrhizologists in classification and identification of AMF species. The identification includes features such as spore size, structure, number of wall layers, hyphal attachments, hyphae size, reaction with reagents, etc. (Souza 2015). So far, new technologies and advancement in microscopy and staining procedure have greatly aided towards identifying specific characters and developing reliable taxonomic keys for species identification. This recent issue will focus on the morphological study of arbuscular mycorrhizal fungus that was collected from Centre for Mycorrhizal Culture Collection Bank, TERI, with the accession number CMCC/AM-2201.

Monosporal Establishment

The isolate designated as CMCC/AM-2201 was isolated from the trap raised from the soil that was collected from natural consortia The trap culture of this accession was raised for proliferation of indigenous mycorrhiza in pot conditions with Sorghum as a host plant under greenhouse conditions for the period of 3–6 months. After adequate sporulation growth, trap sample was analysed by wet sieving and decanting method (Gerdemann and Nicolson 1963) for categorizing on the basis of spore density, diversity, size, and colour. During wet sieving, the soil sample was allowed to pass through a series of sieves of 60, 100, and 300 British Standard Size, respectively. Morphologically, similar and healthy spores were isolated for obtaining pure single species culture. Voucher specimens of all the healthy spores were prepared to investigate the morpho-taxonomic characters. Specimens were mounted in two agents, namely polyvinyl lacto glycerol (PVLG) and polyvinyl lacto glycerol: Melzer's reagents. These specimens were analysed for spore and its different features, such as number of wall layers, hyphal length, and attachment, under a compound microscope. Also for root colonization estimation, the host roots were screened for colonization with the AMF. Monosporal cultures with good spore density and colonization percentage were considered as pure and successful ones (Figure 1).

Spore Morphology and Shape

Spores that were isolated from the monosporals were developed blastically from a bulbous sporogenous cell formed at the end of a hypha connected with mycorrhizal roots (Walker and Sanders 1986). Most of the spores were dark orange-brown to red-brown in colour. The shape of the spores varied from globose to sub-globose and occasionally to oblong or ellipsoid. Scanning electron micrograph (SEM) of the spores

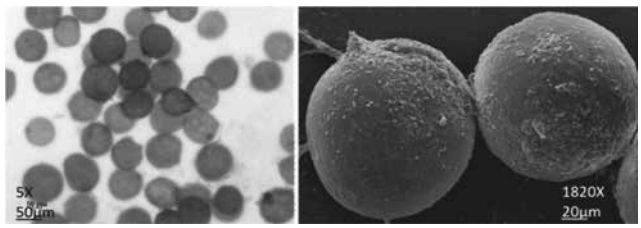

Figure 1 Compound microscopic images (5×) and scanning electron micrographs (SEMs) of CMCC/AM-2201 showing spore with small subtending hyphae

Centre for Mycorrhizal Research, Biotechnology and Management of Bioresources, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi 110 003, India
* Corresponding author, Email: aloka@teri.res.in

showed that the outer surface of the spores is slightly rough. The outer wall of the spores is mucilaginous and appears rough with organic debris sticking to it (Figure 2). The average diameters of the spores were found to be in the range of 90–175 µm (Figure 3).

Subcellular Structure of the Spore

Spores of this accession show less reaction with PVLG but are highly reactive with polyvinyl lacto glycerol: Melzer's reagents. However, mature spores are composed of the following subcellular structures and wall layers (Figure 4):

Spore wall layer 1 (L1)

L1 is an outermost rigid and permanent layer. It is usually pale brown in colour and shows less reaction with the reagents. When mounted in PVLG, warts are often invisible after several months of storage, while this effect is variable.

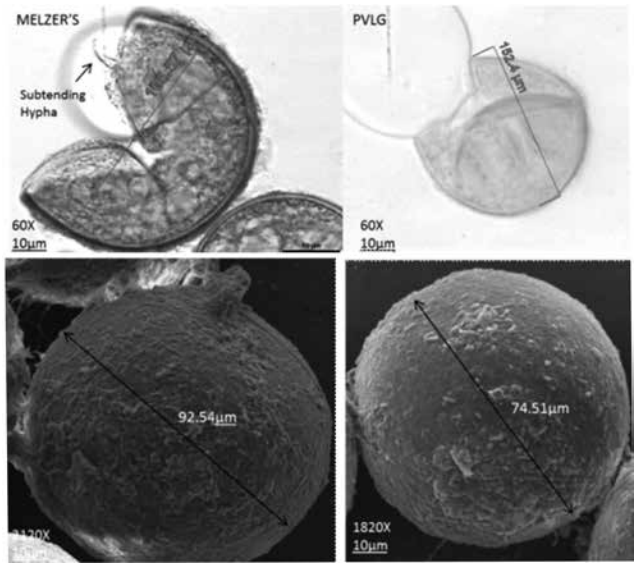

Figure 2 Compound microscopic images (40×) and SEM of mature spores of accession CMCC/AM-2201 showing globose- to sub-globose-shaped spores with single subtending hyphae. SEM images reveal slightly rough outer layer of the spore

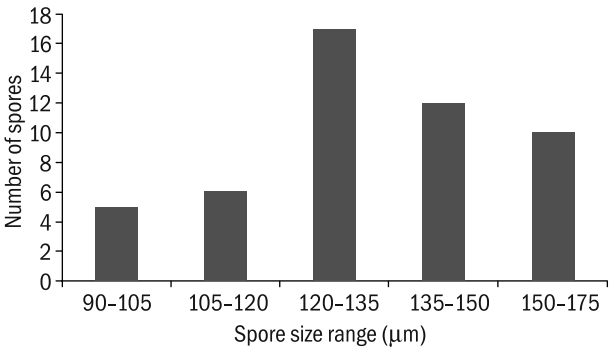

Figure 3 Analysis of spore diameter of 50 healthy and cleaned spores obtained from 1-year-old monosporal culture of accession CMCC/AM-2201

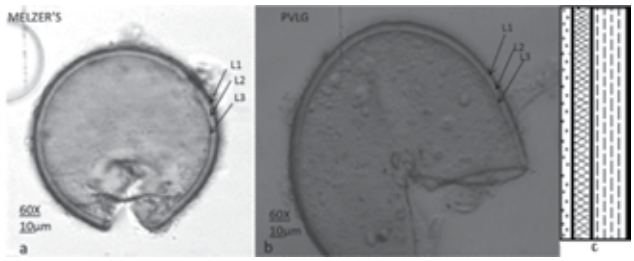

Figure 4 Compound microscopic images of spore wall layers of CMCC/AM-2201 after mounting in PVLG: Melzer's and PVLG reagent (a and b). Murograph (Walker 1983) of the mature spore showing three distinct wall layers (c).

Spore wall layer 2 (L2)

This layer consists of orange-brown to red-brown sub-layers. This layer is often laminated and is 4.0–8.0 µm thick in mature spores. This layer stains dark red-brown in Melzer's reagent.

Spore wall layer 3 (L3)

It is the innermost, very thin, hyaline, and flexible layer. It is less than 1 µm thick and can be seen in crushed spores.

Germinal Walls: Two bilayered flexible hyaline inner walls (GW1 and GW2). They are formed completely separate from the spore wall.

GW1: This wall layer consists of two layers (L1 and L2) that are tightly adhering to each other. L1 is less thick as compared to L2. These are so thin that they appear as one layer.

GW2: It consists of two layers (L1 and L2) that tightly adhere. Both of these layers also are thin enough to appear as one layer in PVLG. However, only L2 differentially stains a pinkish-purple to slightly darker purple in Melzer's reagent (Figure 5).

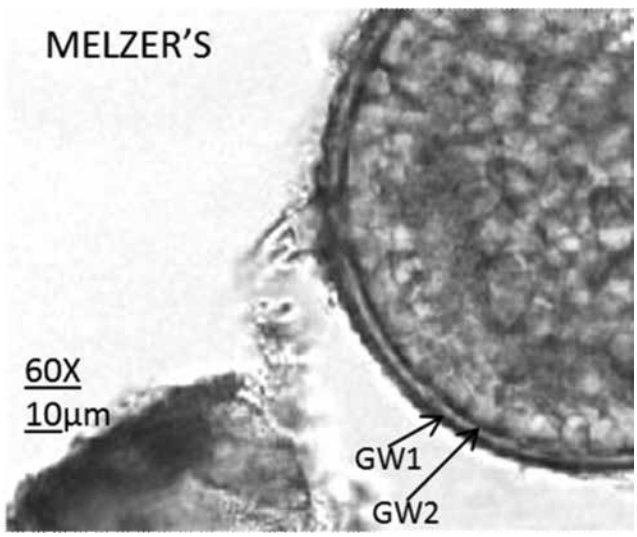

Figure 5 Compound microscopic images of spore germinal wall layers of CMCC/AM-2201 after mounting in PVLG: Melzer's

Subtending Hyphae

The subtending hyphae is intact and width ranges from 6.23–(7.38)–10.56 µm. It has two layers (L1 and L2) but only L2 is readily discernible at the level of the compound microscope (Figure 6).

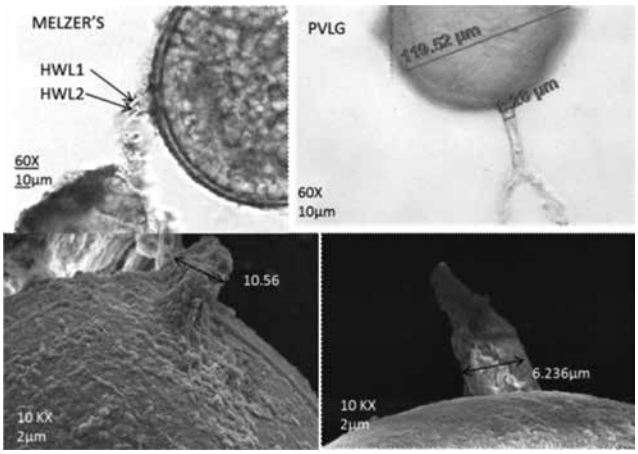

Figure 6 Compound microscopic images of spore of CMCC/AM-2201 showing subtending hyphae after mounting in PVLG: Melzer's and PVLG along with Hyphal wall layers (L1 and L2).

Mycorrhiza

After 3–6 months, the roots host Sorghum were checked for AMF colonization. The roots were processed using ink-vinegar (Phillips and Hayman 1970). Root colonization estimation showed presence of intra-radical hyphae, vesicles, and spores. Abundant arbuscules were found through the root cortex. Extra-radical hyphae were abundant and were usually seen bearing spores bearing or in a group of two to three (Cannon and Kirk 2007) (Figure 7).

Conclusion and Classification Level

On the basis of above morpho-taxonomic analysis of the accession CMCC/AM-2201, many distinguishing features regarding the family, genera, and the species could be derived (Morton and Benny 1990). The following features were taken into consideration for characterization and identification:

- Globose, asexual spores produced singly with three-layered spore walls.
- Spore wall layer is composed of outer hyaline layer followed by one inner fine sub-layer followed by a third layer that is flexible.
- Spores are of varying shapes and sizes ranging from globose to sub-sub-globose.
- Formation of both intra-radical and extra-radical hyphae and abundant vesicles and intra-cellular arbuscules.

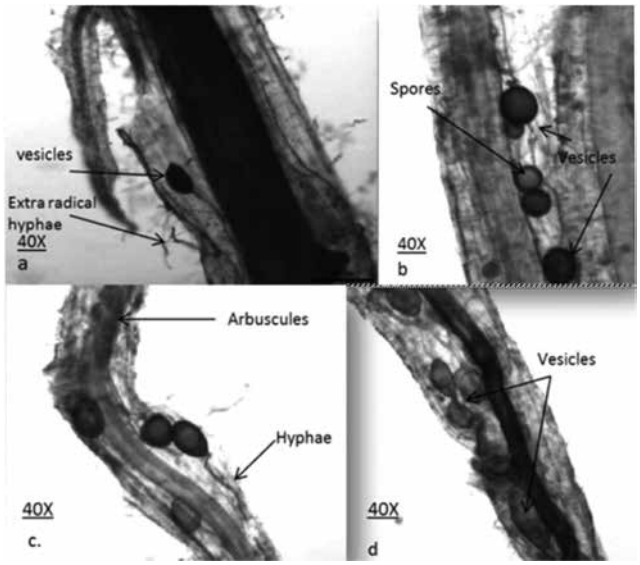

Figure 7 Compound microscopic images (10x) of roots of *Sorghum bicolor* stained in ink vinegar and observed for root colonization by CMCC/AM-2201 showing extra-radical hypha (a), arbuscules (b), and abundant vesicles in the cortical cells (c). Spores in the extra-radical hyphae are also seen in small clusters (d)

All these features suggest that the culture CMCC/AM-2201 belongs to the family *Scutellospora heterogama*.

Following are some of the unique morpho-taxonomic features of the accession:

- From dark orange-brown to red-brown in colour globose to sub-globose, asexual spores produced singly with layered spore walls; spores are of varying shapes and sizes ranging from globose to sub-globose. Size ranges 90–(130)–180 µm.
- Spore wall layer is composed of outer hyaline layer, inner fine layer, a third flexible innermost layer that is continuous with the subtending germinal wall.
- Formation of both intra-radical and extra-radical hyphae and abundant vesicles and intracellular arbuscules.

The taxonomic feature of the accession CMCC/AM-2201 matches the characters of *Scutellospora heterogama* (Walker 1983).

Systematic classification

Glomeromycota
Glomeromycetes
Diversisporales
Gigasporaceae
Scutellospora
Scutellospora heterogama

Mostly study focusses on ecology, taxonomy, phylogeny, genetics, molecular, and functional properties of the fungi.

Morpho-taxonomy of an organism is one of the conventional methods that help in comparison with existing species. Study of features, such as spore morphology, wall layers, hyphal attachment, reaction with reagents, help to know the AMF species and genera but sometimes it cannot distinguish the organism at species level. So, other modern and alternative methods were used for species identification, which includes fatty acid methyl ester profiles and molecular characterisation (sequencing of ITS region of 18s rDNA) (Schüßler and Walker 2010).

Acknowledgements

We acknowledge the contribution of Awadhesh Ram for maintenance of AMF cultures under greenhouse conditions. Technical assistance provided by Chandrakant Tripathi during scanning electron microscopy of AMF spores is also thankfully acknowledged.

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Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Applied Soil Ecology*
- *Biotechnology Reports*
- *Biological Conservation*
- *Ecotoxicology and Environmental Safety*
- *Soil Biology and Biochemistry*
- *Flora*
- *Pedobiologia*
- *Science of the Total Environment*
- *Energy Procedia*
- *Plant Physiology and Biochemistry*
- *Saudi Journal of Biological Sciences*
- *Environmental and Experimental Botany*
- *Chemosphere*
- *Scientia Horticulturae*
- *Mycoscience*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Sun X*, Shi J, Ding G. 2017	**Combined effects of arbuscular mycorrhiza and drought stress on plant growth and mortality of forage sorghum** *Applied Soil Ecology* **119**: 384–391 [*Institute for Forest Resources & Environment of Guizhou, Guizhou University, Guiyang, 550025, China]
Oyewole BO*, Olawuyi OJ, Odebode AC, Abiala MA. 2017	**Influence of arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea** *Biotechnology Reports* **14**: 8–15 [*National Horticultural Research Institute, Ibadan, Nigeria]
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