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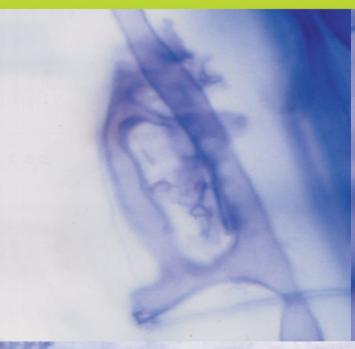

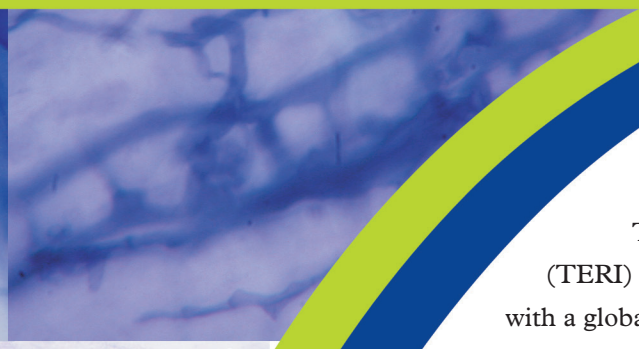

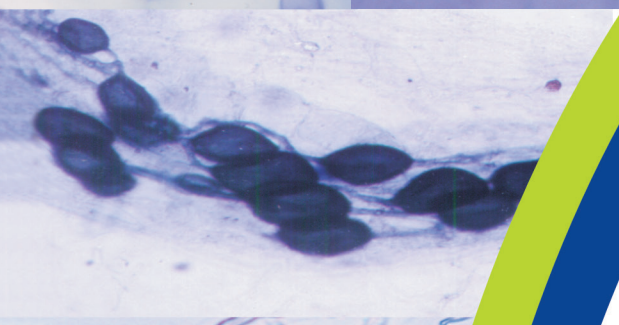

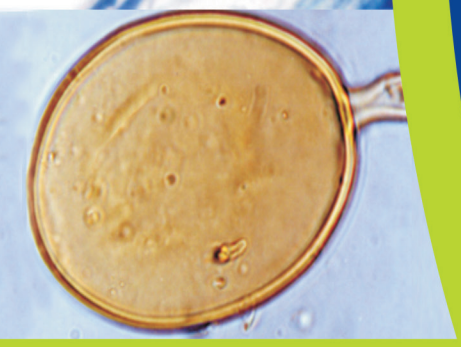

About TERI

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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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Research finding papers

Influence of AM Fungi on Growth and Biochemical Constituents of Wheat (*Triticum aestivum* L.)

S L Khapke[a], R K Aher[a], and B A Patil[b]*

Introduction

Wheat (*Triticum aestivum* L.) is the second most important cereal preceded by rice. It is eaten in various forms by more than one thousand million human beings in the world. In India, it is the second most important staple food crop, which contains a high percentage of carbohydrates and proteins.

The Arbuscular Mycorrhizal Fungal (AMF) association is known to improve plant growth through better uptake of nutrients and tolerance to drought and salinity (Harley and Smith 1997). The mycorrhizal mutualistic symbiosis between plant roots and soil fungi is known to play a significant role in uptake of Phosphorus (P) by plants (Barrow and Rocadri 1977). Among mycorrhiza, AM fungi, a fungal biofertilizer, is the most promising; it is extensively used to enhance the growth in different crop plants (Marwaha 1995; Inchal and Lakshman 2006). AMF biofertilizer is a natural product carrying living microorganisms derived from the plant root or cultivated soil. As such, no harmful effect on soil fertility or plant growth is generally discernible (Sen 2005). The beneficial effects of AMF have been reported in many leguminous plants and other crop plants (Mamatha and Bagyaraj 2001). AMF helps in plant nutrition, disease resistance, and provides an alternative to chemical fertilizers particularly in land reclamation, habitat restoration, and sustainable agriculture. The AM association is seen in most grasses, cereals, and millets (Ammani 1989). There is more work on the incidence of AMF in relation to crop plants (Bagyaraj *et al*. 1979; Bagyaraj and Verma 1995), cereal crops (Ammani *et al*. 1985), vegetable plants (Creighton *et al.* 1986), and cash crops. Most horticultural plants are colonized by AMF whose presence can enhance the growth of the host plant (Ortas and Verma 2008). The increase in stomatal behaviour and photosynthesis of host plants along with increase in the chlorophyll concentration due to inoculation of the AM fungi in different plant species is well documented (Allen *et al*. 1981; Panwar 1991).

The present study was undertaken to find out the effect of the AMF species *Glomus mosseae* (GM) on the growth performance of wheat (*Triticum aestivum* L.) plant.

Materials and Methods

Plant material and AM inoculum

A pot experiment was carried out in the Department of Botany, New Arts, Commerce and Science College, Ahmednagar, Maharashtra, during 2009–10 to study the effect of AM inoculation *Glomus mosseae* (GM) on *Triticum aestivum* L. Soil-based culture of AMF, *Glomus mosseae,* was multiplied in *jowar* roots.

The seeds of *Triticum aestivum* L. var. 2496 used as an *experimental* plant were obtained from Mahatma Phule Krishi Vidyapeeth Rahuri, Ahmednagar.

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Uniform and healthy seeds of wheat cultivar were selected. They were surface sterilized with 0.1 per cent $HgCL_2$ and washed with distilled water three times. Then, the seeds were soaked in distilled water for four hours. Thereafter, 15 well-imbibed seeds were sown in each pot.

Experimental design

Pots of 30×30 cm size were filled with 15 kg of sterilized sandy loam soil. Pots were arranged in completely randomized block design. The experiment was replicated thrice.

For AMF (*Glomus mosseae*), four treatments, such as 25g, 50g, 75g, and 100g of mixed inoculum were placed just below 5cm in experimental pots (containing 250–300 spores/100g inoculum). Control was maintained without application of AMF. Plants were irrigated once in two days. Observations were recorded at seedling and anthesis stages after the AMF inoculation.

Morphological parameters

Shoot length, root length, number of leaves, dry weight of root, dry weight of shoot, girth of stem, and the percentage of root colonization were estimated by the Phillips and Hayman (1970) method. Biochemical parameters, such as total chlorophyll were determined by Amon's (1949) method. The amount of chlorophyll was expressed in mg/g fresh weight.

Results and Discussion

Plant samples were taken at seedling and anthesis stages for estimating growth and biochemical parameters as well as AMF root colonization. In this study, it was observed that plants inoculated with AMF (GM) had grown taller than the non-inoculated plants. The growth characters, such as root length, shoot length, and stem girth were found to be increased due to the influence of AMF (GM) (Table 1). Root and shoot lengths significantly increased in plants at seedling and anthesis stages inoculated with 75g *Glomus mosseae* as compared to control. The increased root length (10.70cm) and shoot length (22.44cm) were observed in 75g *Glomus mosseae* inoculated plants as compared to control. Similar observations were made by Chiramel *et al.* (2006). They showed that plants inoculated with *Glomus leptotrichum*, *G. etunicatum*, and *G. mosseae* showed higher plant height, root length, and shoot length as compared to non-inoculated control plants. Stem girth was also significantly more (1.46cm) in plants inoculated with 75g *Glomus mosseae* compared to control plants. Role of Vesicular–Arbuscular Mycorrhiza (VAM) fungi in improving the stem girth and plant biomass is well documented in tropical plantation crops, such as citrus and mango (Manjunath *et al.* 2001).

The growth character, such as number of leaves per plant was found to be increased due to the influence of AMF (GM) fungi. It is clear from the results (Table 2) that all the GM inoculated plants showed higher leaf number as compared to control plants at seedling and anthesis stages. The results were supported by the work of Boby and Bagyaraj (2003), who found the enhanced growth of *Coleus forskohlii* with the dual inoculation of AM fungi and Plant Growth Promoting Rhizobacteria (PGPR) organisms. The results (Table 2) revealed that all the GM inoculated plants showed more root and shoot dry weights as compared to control plants. An increase in dry weight of root and shoot (4.70g and 10.32g, respectively) was observed in 75g GM inoculated plants as compared to control plants during anthesis stage. The treatment of *Glomus fasciculatum* recorded good shoot and dry weights and similar results were shown in soybean and cowpea when inoculated with AMF (Gupta *et al.* 1999).

Table 1: Effect of AMF (GM) on morphological parameters of *Triticum aestivum L*[*]

Treatment	Root length (cm)		Shoot length (cm)		Stem girth (cm)	
	Seedling stage	Anthesis stage	Seedling stage	Anthesis stage	Seedling stage	Anthesis stage
C	2.11	4.20	7.12	10.53	0.22	0.52
25	3.53	5.24	8.36	11.70	0.78	1.23
50	4.50	6.23	9.21	14.50	0.80	1.32
75	5.10	10.70	10.34	22.44	0.96	1.46
100	4.68	10.30	9.50	21.62	1.02	1.45

[*]Values are mean ±SD of triplicates; statistically significant ($P < 0.05$) compared to control
AMF: Arbuscular Mycorrhizal Fungi; GM: *Glomous mosseae*; C: Control

Table 2: Effect of AMF (GM) on morphological parameters of *Triticum aestivum* L*

Treatment	Number of leaves (per plant)		Dry weight of roots (g)		Dry weight of shoot (g)	
	Seedling stage	Anthesis stage	Seedling stage	Anthesis stage	Seedling stage	Anthesis stage
C	04	06	0.26	0.92	1.78	4.92
25	04	07	0.64	1.50	1.98	5.60
50	05	07	1.12	2.34	3.02	7.64
75	06	08	1.76	4.70	4.76	10.32
100	05	08	1.90	4.62	4.98	9.80

*Values are mean ±SD of triplicates; statistically significant ($P < 0.05$) compared to control
AMF: Arbuscular Mycorrhizal Fungi; GM: *Glomous mosseae*; C: Control

Table 3: Effect of AMF (GM) on AM root colonization and total chlorophyll content in *Triticum aestivum* L*

Treatment	AM colonization in root (%)		Total chlorophyll content mg/gm fresh wt.	
	Seedling stage	Anthesis stage	Seedling stage	Anthesis stage
C	–	30.33	0.672	2.856
25	10	45.66	0.784	3.324
50	12	55	0.844	3.638
75	14.33	70.66	1.246	5.183
100	19.66	73	0.848	3.652

*Values are mean ±SD of triplicates; statistically significant ($P < 0.05$) compared to control
AMF: Arbuscular Mycorrhizal Fungi; GM: *Glomous mosseae*; C: Control

The data on percentage increase of mycorrhizal colonization due to inoculation of AMF (GM) is given in Table 3. The percentage root colonization due to AMF (GM) was higher (78 per cent) as compared to control plants (30.33 per cent) during anthesis stage. This root colonization by AM showed significantly improved phosphorous uptake per unit root length due to the enhancement of the total root surface by hyphal growth (Li *et al.* 1991). Significantly higher amounts of total chlorophyll (1.246 mg/g and 5.183 mg/g fresh wt.) were observed in AM (75g GM) inoculated wheat plant during seedling and anthesis stages as compared to non-inoculated control as shown in Table 3. Similar result was observed by Allen *et al.* (1981) in *Bouteloua gracilis*.

Hence, it can be concluded from the present investigation that inoculation of *Glomus mosseae* was found to be more promising to induce the growth of wheat plant. The study also shows that AM fungi help wheat perform better. The strategy of using AM fungal species as a biofertilizer will help to improve the growth and yield of wheat plant.

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Screening of Different Hosts and Substrates for Inocula Production of Arbuscular Mycorrhizal Fungi

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Introduction

Arbuscular mycorrhizal (AM) fungi colonize the roots of majority of crop plants, forming a symbiosis that potentially enhances nutrient uptake, disease resistance, water relations, and soil aggregation. The fungus takes up fixed carbon from the apoplast of the root cortex (Shachar-Hill *et al.,* 1995). The extraradical phase of the fungus acts as an extension of root system for the uptake of mineral nutrients, especially immobile nutrients such as P, Cu, and Zn. Inoculation with effective isolate of AM fungi is one way of ensuring the potential benefits of symbiosis for crop production. There are instances when inoculation with AM fungi is necessary or desirable. An AM fungus available as inoculum may be more effective on a crop in comparison to the effectiveness of the indigenous AM fungal community. Different workers analysed the efficacy of different hosts and substrates for the inoculum production of mycorrhizal fungi (Nehra *et al.,* 2003; Singh and Jamaludin, 2006; Reddy *et al.,* 2006).

To get maximum agricultural benefit, inoculation of soil with the suitable type of AM fungi is necessary. However, the obligate biotrophic nature of AM fungi is a major obstacle for large-scale inocula production. Organic wastes are rich in nutrients and their positive influence on AM root colonization have been reported by many workers (Gaur and Adholeya, 2002; Gyndler *et al.*, 2005; Douds *et al.*, 2010; Tanwar *et al.*, 2013). Sugarcane is grown in Yamunanagar, Kurukshetra, Ambala, and Karnal districts of Haryana. One tonne of sugarcane produces approximately 33kg of sugarcane bagasse, i.e., the residue left after extracting juice. Sugarcane ash is also an organic waste from sugar industry. Thus, these organic wastes can be used for the multiplication of AM fungi. The other important factor is the physiology of host plant which influences spore production (Simpson and Draft, 1990). Selection of appropriate trap plant for the production of AM inocula also plays an important role in AM fungal propagation. Hosts selected in present investigation are barley, onion, and sesbania. Onion is a member of family *liliaceae*, barley from *poaceae,* and sesbania from *leguminosae*. All the members of these three families are ideal trap plants for mycorrhizal colonization because of their extensive root system (Fusconi *et al.*, 2005; Perner *et al.*, 2007). Keeping in view the above information, in the present investigation a pot experiment was conducted for the evaluation of three hosts, i.e., barley, sesbania/dhaincha, and onion and two substrates, i.e., sugarmill ash and sugarmill bagasse for mass culture of two AM fungi, i.e., *Acaulospora laevis* and *Funneliformis mossseae* in polyhouse conditions.

Materials and Methods

Soil characteristics

The experimental soil was collected from Botanical Garden of Botany Department, Kurukshetra University and passed through a sieve of 2 mm and soil characteristics were as follows: sand—64.3%, silt—21.80%, clay—3.90%, starting pH—6.5, electrical conductivity—0.25dsm^{-1}, total nitrogen—0.042%, available phosphorus—0.017%, and organic carbon—0.06%.

Experimental design

The experiment was 3×2×6 factorial design employing three types of hosts, two types of substrates, and six different concentrations of sugarcane bagasse (0, 50, 75, 100, 125, and 150g) and sugarcane ash (0, 10, 20, 30, 40, 50g). Each treatment was replicated three times and total 72 pots were there for this experimental setup.

Selection of nurse plants

Three trap plants namely barley, sesbania/dhaincha, and onion were selected for mass multiplication of *A. laevis* and *F. mossseae*.

Selection of substrates

Two waste substrates, i.e., sugarmill ash and sugarmill bagasse with traditional substrate, i.e., sand: soil (1:3), were selected to find out the most suitable substrate for mass culture of *A. laevis* and *F. mossseae*. All the substrate material was autoclaved at 121°C for 2 hours prior to use.

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Selection of AM endophytes

For mass culture, biodiversity of AM fungi associated with onion, barley, and sesbania was studied and spores of *A. laevis* and *F. mossseae* were isolated from the rhizosphere of onion, sesbania, and barley by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) as these two AM fungal species were found to be dominant one.

Production of starter inoculum

Starter inoculum of *A. laevis* and *F. mossseae* was raised by funnel technique (Menge and Timmer, 1982) using maize as a host for a period of three months.

Experimental setup

Pots (25×25 cm) were filled with sieved and autoclaved sand: soil (1:3) mixture. Different concentrations of sugarcane ash and bagasse were added and mixed thoroughly. In control pots, only sand: soil (1:3) was added. Then 10% inoculum each AM fungus, i.e., *A. laevis* and *F. mossseae* (colonized root pieces of maize along with soil containing 300–400 AM spores/100g of soil) raised from funnel technique was added to these pots. Seeds of different host plants like onion, sesbania, and barley were surface sterilized with 0.5% (v/v) sodium hypochlorite for 10 min and subsequently washed with sterilized distilled water. Seeds of barley and sesbania were sown in pots while the seeds of onion were sown in a shallow tray containing soil: sand (3:1) for germination and after germination, single seedling was transplanted to each pot for mass culturing. Plants were watered regularly to maintain the moisture at approximately 60% water-holding capacity of soil and supplied with 50ml Hoagland's solution (Hoagland and Arnon 1950) after every 15 days during the experiment.

Harvest and analysis

Vegetative growth response was assessed after 75 days of planting by uprooting the whole plant. Plant height was recorded followed by washing of plants under running tap water. Roots and shoots were separated and kept in oven at 70°C to dry until a constant weight was obtained and then their dry weight was recorded. AM spore quantification and percent mycorrhizal colonization were determined by the methods of Gerdeman and Nicolson (1963) and Phillips and Hayman (1970), respectively.

Data analysis

The data was analysed by applying DMRT (Duncan's Multiple Range Test) and mean differences followed by different superscripts in a row are significant at $P \geq 0.05$.

Results and Discussion

The results revealed that mass culturing of the selected AM fungi, i.e., *G. mosseae* and *A. laevis* showed different results with three hosts and two substrates.

Regarding *A. laevis*, when barley was used a host and ash as a substrates, maximum AM spore number was found (38.33 ± 2.51) at 10g amount of ash while mycorrhizal root colonization was maximum at 40g (77.77 ± 3.85) of ash followed by 50g (75.55 ± 10.1), and 10g (73.33 ± 6.67). Dry root and shoot weight were found to be maximum at 10g conc., i.e., 0.28 ± 0.03 and 2.78 ± 0.23 (Table 1).

When sesbania was used as a trap plant with ash as a substrate, AM spore number was recorded maximum (36 ± 9.16) at 10g of ash and mycorrhizal association (86.66 ± 0.00) at 50g of ash followed by 10 g of ash (82.22 ± 3.84). In similar way, when onion was used a host plant and ash as a substrate, AM spore number (48 ± 2.51) and mycorrhizal root colonization (85.53 ± 3.85) were found maximum at 10g and 20g of ash, respectively. Root and shoot dry weight also followed similar trend (Table 1).

From the above results, it can be concluded that onion was proved to be the best host with sugarcane ash as a substrate. Another substrate sugarcane bagasse was also used with selected hosts for mass culturing of *A. laevis*. When barley was used as trap plant, all the studied parameters showed better response. Mycorrhizal spore count and percent root colonization were maximum at 100g of bagasse (60.33 ± 1.15 and 100 ± 0). Plant height, shoot dry weight, and root dry weight were maximum at 75g, 50g, and 75g of bagasse, respectively (Table 2).

When sesbania was used as stock plant with sugarcane bagasse as a substrate, the maximum mycorrhizal spore population (27.66 ± 3.51) and mycorrhizal root colonization (62.22 ± 3.84) were found at 50g and 100g of sugarcane bagasse (Table 2). Other recorded growth parameters, i.e., plant height, shoot dry weight, and root dry weight were found to be maximum at 150g, 125g, and 150g, of sugarcane bagasse, respectively.

Effect of sugarcane bagasse on the growth of *A. laevis* with onion as a trap plant showed maximum percentage root colonization (93.33 ± 0.00) and AM spore population (47.00 ± 3.60) at 100g of sugarcane bagasse while plant height, shoot dry weight, and root dry weight were maximum at 75g, 50g, and 50g of sugarcane bagasse, respectively (Table 2). It is envisaged from above results that among three hosts with sugarcane bagasse, barley was found to be the best host for rapid and mass culturing of *A. laevis*.

Among two substrates, sugarcane bagasse was found to be the best suitable substrate for inoculum

production of *A. laevis* as it increased AM spore count (60.33 ± 1.15) and root colonization (100 ± 0.00) to maximum with barley as host plant.

Similar substrates and hosts were screened for mass multiplication of *F. mosseae* (Tables 3 and 4). When sugarcane ash was used as substrate with barley as a trap plant, mycorrhizal spore population (24.33 ± 4.04), and percentage root colonization (33.33 ± 0.00) were maximum at 20g and 10g (Table 3). Other growth parameters like plant height and shoot biomass were also found maximum at 10g concentration of ash.

In case of sesbania, maximum spore density (22 ± 2.64) and AM root colonization (62.22 ± 3.84) were observed at 10g of sugarcane ash. Root dry weight was also found to be maximum at this concentration, while shoot dry weight (3.37 ± 1.39) was maximum at 40g of ash. With regard to third trap plant, i.e., onion, AM spore population (86.66 ± 1.52) was maximum at 50g amount of ash while root colonization (100 ± 0.00) was found to be maximum at 10g of sugarcane ash. Other growth parameters like plant height, shoot dry weight, and root dry weight were also observed to be maximum at 10g of ash.

When sugarcane bagasse was used as substrate with these host plant in different concentrations, it was observed that all treated plants showed positive response with respect to different parameters (Table 4). When barley was used as trap plant, maximum spore density (30.33 ± 4.50) and root colonization (72.22 ± 3.84) were found at 150g and 75g of sugarcane bagasse, respectively. While other growth parameters like plant height (58.86 ± 1.72), shoot biomass (4.79 ± 1.09), and root biomass (0.80 ± 0.17) were found maximum at 150g, 75g, and 50g of sugarcane bagasse, respectively. With sesbania as host plant, maximum AM spore population (42.00 ± 6.08) was found at 100g of sugarcane bagasse while AM root colonization was observed to be maximum (77.77 ± 3.85) at 50g and 125g of substrate used. Maximum plant height (53.80 ± 5.62), shoot dry weight (4.47 ± 0.38), and root dry weight (1.31 ± 0.21) were found at 75g, 125g, and 150g of sugarcane bagasse.

When onion was used as host plant with sugarcane bagasse as substrate, maximum AM spore abundance (65.66 ± 8.14) and mycorrhizal root colonization (100 ± 0.00) were found at 100 g of sugarcane bagasse. Root colonization was also found 100% at 75g of sugarcane bagasse while plant height, shoot biomass, and root dry matter were found to be maximum at 100g, 150g, and 50g of sugarcane bagasse, respectively. From the above results, it is clear that, among two substrates screened, sugarcane ash was proved to be suitable substrate with onion as best trap plant for mass culturing of *G. mosseae* as it increased the spore density and root colonization to maximum. Onion was found suitable host plant for mass culturing of *G. mosseae* with both the substrates used as 100% AM colonization was found in this plant. Present results revealed that there was varied percentage root colonization raised in soil–sand mixture with different amount of substrates used. This might be due to the soil factor and amount of substrate mixed which affected the number of vesicles per root and ultimately AM spore population. As in the present investigation, addition of substrates enhanced the mycorrhizal colonization and spore population with different trap plants. Muthukumar and Udaiyan (2002) also reported an enhancement in the AM spore population when they used compost as a substrate. The positive effect of organic matter on AM growth could be an effect of higher humidity since organic matter has a beneficial effect on soil structure and water-holding capacity. The added organic matter could also increase the soil porosity. In the present investigation, the increased biomass of the trap plants may be due to the better arbuscular mycorrhizal growth, which in turn improved root architecture for better nutrient and water uptake. Increase in growth parameters of trap plants used was observed in all the concentration of substrate used as compared to control plants.

Results of current study varied with different host plant and different concentration of substrates used. In case of mass multiplication of *G. mosseae* by using different host plants and sugarcane ash as substrate, maximum AM growth was found at 10g amount of substrate used but in case of bagasse as a substrate, maximum spore count, and root colonization was observed at 100g of substrate and onion as host plant.

Chaurasia and Khare (2005) found barley to be a suitable host for mass culture of AM fungi in soil–sand based medium. *Hordeum vulgare* L. was also proved to be best host with sugarcane bagasse as a substrate for mass culture of the *A. laevis* in the present investigation as it showed maximum spore number and root colonization. Several workers found onion as the best host for rapid and mass culturing of AM fungi (Nehra *et al.*, 2003; Sheela and Sundaram, 2003; Kaushish, 2008). Similar were the results in the current study when mass culture of *A. laevis* with onion as trap plant and sugarcane ash as substrate was done. It has been also documented that the area of host protoplast during Vesicular Arbuscular Mycorrhiza (VAM) infection is more in monocots than dicots (Toth *et al.*, 1990).

It is well known that AM fungus symbiosis is a complex system and extent of endophyte-host interaction depends on the type of root system and supply of carbohydrates to the fungal partners

(Struble and Skipper, 1988). Addition of any organic substances into soil may enhance moisture retention in the soil and increases the AM fungal population (Douds *et al.*, 2008).

Different host plants also affect AM fungal community. It has also been reported that trap cultures, using trap plants grown, soil diluted with sterile sand, are most commonly used to isolate AM fungi. Pot-culturing method usually results in the isolation of more species than other methods (An *et al.*, 1990). Similar basic medium was used in the present investigation. In the current study, variations in AM spore number and root colonization were observed with different trap plants. This might be due to variation in host plant root type and morphology, carbon biomass, nutrient, and endogenous hormonal level. These factors might be expected to influence the richness of AM fungi isolated from soil in trap cultures (Brundrett *et al.*, 1999). The best trap plant may vary in different ecosystem. Chaurasia and Khare (2005) reported that AM fungal colonization, spore formation, and production depend upon the type of host as well as the duration of infection of these symbionts. This might be the reason for different rates of mycorrhizal colonization and sporulation with different trap plants in the present investigation. It has been observed that monocots with rapidly developing fibrous root system can be considered as ideal trap plants for producing AM spores. Present results are also in accordance with this as onion was found to be the best host for inoculum production of *A. laevis* with sugarcane ash as substrate. Results of the current investigation also envisaged that either host plants favour the association of particular AM species or AM fungi may show some preference for the host plant. The production of large number of spores of *F. mosseae* than *A. laevis* showed that host plant influences these two AM fungal genera.

Conclusion

A standard method of screening and certification of effectiveness should be considered before distribution at commercial level which will prevent the distribution of low quality inoculum. However, one of the main tasks for producers and researchers is to raise awareness in the public about potentials of mycorrhizal technology for sustainable plant production and soil conservation.

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Table 1: Efficacy of different hosts and sugarcane ash as substrate for mass culturing of *A. levis*

		Barley					Sesbania/Dhaincha					Onion				
Sl. No.	Amount of sugarcane ash (g)	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW
1	0	*34.76 ± 1.46^{c}	12.66 ±2.08^{d}	17.77 ±3.85^{c}	2.68 ±0.34^{a}	0.17 ± 0.05^{a}	33.56 ±1.28^{a}	19.33 ± 3.05^{b}	28.88 ± 3.85^{d}	4.90 ± 0.40^{b}	1.38 ± 0.15^{b}	61.5 ±3.00^{b}	13.33 ± 1.52^{d}	44.44 ± 3.84^{e}	2.72 ± 0.54^{c}	1.05 ± 0.23^{b}
2	10	39.66 ± 5.76^{b}	38.33 ±2.51^{a}	73.33 ±6.67^{a}	2.78 ±0.23^{a}	0.28 ± 0.03^{a}	36.86 ±0.55^{a}	36.00 ± 9.16^{a}	82.22 ± 3.84^{b}	4.64 ± 0.31^{b}	1.30 ± 0.16^{b}	66.2 ±4.98^{a}	48.00 ± 2.51^{a}	68.88 ± 3.85^{c}	10.79 ± 0.53^{a}	1.34 ± 0.15^{b}
3	20	32.36 ± 1.74^{c}	16.66 ±2.08^{c}	71.10 ±7.70^{a}	2.62 ±0.25^{a}	0.27 ± 0.04^{a}	37.66 ±1.92^{a}	23.00 ± 2.30^{b}	48.88 ± 3.85^{c}	4.93 ± 0.16^{b}	1.93 ± 0.07^{a}	49.4 ±4.50^{c}	31.66 ± 4.04^{b}	85.53 ± 3.85^{a}	9.60 ± 0.54^{a}	1.65 ± 0.21^{a}
4	30	46.66 ± 3.93^{a}	25.66 ±3.51^{b}	28.88 ±3.85^{b}	2.79 ±0.20^{a}	0.13 ± 0.06^{a}	34.3 ± 0.92^{a}	22.6 ± 3.05^{b}	75.55 ± 3.85^{b}	4.77 ± 0.28^{b}	1.32 ± 0.26^{b}	61.4 ±5.58^{b}	18.00 ± 2.00^{c}	73.33 ± 0.00^{b}	5.75 ± 0.53^{b}	1.24 ± 0.15^{b}
5	40	43.96 ± 3.21^{a}	27.00 ± 3.00^{b}	77.77 ±3.85^{a}	2.20 ±0.17^{a}	0.18 ± 0.06^{a}	34.50 ±2.11^{a}	21.00 ±1.00^{b}	79.99 ± 6.66^{b}	4.38 ± 0.49^{b}	1.22 ± 0.23^{b}	52.5 ±1.00^{c}	17.66 ± 3.05^{c}	60.00 ± 0.00^{d}	8.99 ± 0.36^{a}	1.00 ± 0.09^{b}
6	50	42.43 ± 3.34^{a}	18.33 ±3.05^{c}	75.55 ±10.18^{a}	2.57 ±0.25^{a}	0.23 ± 0.05^{a}	33.36 ±1.10^{a}	22.00 ±1.73^{b}	86.66 ± 0.00^{a}	6.57 ± 0.41^{a}	1.46 ± 0.19^{b}	52.4 ±1.15^{c}	18.00 ± 2.00^{c}	51.10 ± 3.85^{d}	2.72 ± 0.46^{c}	1.18 ± 0.15^{b}

* Each value is mean of three replicates ± Standard Deviation PH: Plant height (cm) SC: Mycorrhizal spore count/10 g of soil
MRC: % mycorrhizal root colonization SDW: Shoot dry weight (g) RDW: Root dry weight (g)
Data was analysed by DMRT and different superscript symbols (a,b,c,d) within a row represent significant difference at P ≥ 0.05

Table 2: Efficacy of different hosts and sugarcane bagasse as substrate for mass culturing of *A. laevis*

		Barley					Sesbania/Dhaincha					Onion				
Sl. No.	Sugarcane bagasse (g)	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW
1	0	*30.03 ±1.00^{c}	12.66 ±1.52^{d}	13.33 ±0.00^{c}	0.68 ± 0.10^{c}	0.33 ± 0.05^{c}	43.16 ±2.73^{a}	13.33 ± 1.05^{d}	26.66 ± 6.66^{d}	4.19 ± 0.49^{b}	1.02 ± 0.05^{d}	61.50 ±3.00^{a}	19.33 ±2.08^{b}	75.55 ± 10.18^{b}	2.72 ±0.54^{b}	1.05 ±0.23^{a}
2	50	36.40 ±1.05^{b}	24.00 ±3.00^{c}	24.44 ±3.84^{b}	2.54 ± 0.42^{a}	0.48 ± 0.04^{c}	46.46 ±2.80^{a}	27.66 ± 3.51^{a}	42.22 ± 3.84^{c}	4.55 ± 0.20^{b}	1.66 ± 0.09^{c}	66.20 ±6.08^{a}	22.33 ±1.52^{b}	77.77 ± 10.18^{b}	6.76 ±0.46^{a}	1.24 ±0.36^{a}
3	75	43.00 ±4.87^{a}	14.00 ±3.00^{d}	20.00 ±0.00^{b}	1.75 ± 0.34^{b}	0.85 ± 0.09^{a}	45.26 ±7.69^{a}	17.33 ± 1.52^{c}	53.33 ± 0.00^{b}	4.44 ± 0.24^{b}	0.92 ± 0.09^{d}	67.96 ±1.50^{a}	21.00 ±3.60^{b}	79.99 ± 6.66^{b}	6.05 ±0.10^{a}	0.67 ±0.20^{a}
4	100	37.60 ±0.80^{b}	60.33 ±1.15^{a}	100 ±0.00^{a}	1.51 ± 0.37^{b}	0.49 ± 0.05^{c}	48.23 ±1.88^{a}	21.33 ± 3.21^{ab}	62.22 ± 3.84^{a}	4.90 ± 0.13^{b}	1.66 ± 0.07^{c}	66.73 ±5.01^{a}	47.00 ±3.60^{a}	93.33 ± 0.00^{a}	5.78 ±0.33^{a}	1.10 ±0.22^{a}
5	125	32.73 ±0.86^{c}	31.00 ±2.00^{b}	28.88 ±3.85^{b}	1.60 ± 0.22^{b}	0.63 ± 0.09^{b}	49.76 ±1.58^{a}	15.66 ± 2.51^{c}	51.10 ± 3.85^{b}	6.76 ± 0.21^{a}	2.39 ± 0.40^{b}	64.16 ±14.43^{a}	20.00 ±1.51^{b}	71.10 ± 3.85^{b}	2.96 ±0.90^{b}	0.97 ±0.15^{a}
6	150	31.70 ±0.90^{c}	14.33 ±1.15^{d}	24.44 ±3.85^{b}	1.34 ± 0.23^{b}	0.78 ± 0.22^{a}	50.23 ±1.42^{a}	17.33 ± 3.21^{c}	48.88 ± 3.85^{b}	4.75 ± 0.16^{b}	3.43 ± 0.39^{a}	45.83 ±0.57^{b}	20.00 ±2.64^{b}	77.77 ± 3.85^{b}	1.80 ±0.26^{b}	0.51 ±0.30^{a}

* Each value is mean of three replicates ± Standard Deviation PH: Plant height (cm) SC: Mycorrhizal spore count/10 g of soil
MRC: % mycorrhizal root colonization SDW: Shoot dry weight (g) RDW: Root dry weight (g)
Data was analysed by DMRT and different superscript symbols (a,b,c,d) within a row represent significant difference at P ≥ 0.05

Table 3: Efficacy of different hosts and sugarcane ash as substrate for mass culturing of *F. mosseae*

		Barley					Sesbania/Dhaincha					Onion				
Sl. No.	Sugarcane ash (g)	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW
1	0	*31.6 ± 0.88^{b}	13.66 ± 1.15^{c}	19.99 ± 4.66^{c}	2.17 ± 0.22^{a}	0.47 ± 0.10^{a}	12.63 ± 1.00^{c}	12.66 ± 1.52^{b}	44.44 ± 3.84^{c}	0.71 ± 0.49^{c}	0.29 ± 0.12^{c}	49.63 ±5.70^{b}	9.00 ± 2.64^{c}	95.55 ±7.70^{a}	1.44 ± 0.10^{c}	19.95 ±3.88^{c}
2	10	36.46 ± 2.70^{a}	21.00 ±0.00^{b}	33.33 ± 0.00^{a}	2.99 ± 0.12^{a}	0.63 ± 0.16^{a}	39.20 ± 3.45^{b}	22.00 ± 2.64^{a}	62.22 ± 3.84^{a}	3.21 ± 0.79^{a}	1.37 ± 0.53^{a}	62.20 ±4.20^{a}	20.00 ±3.00^{b}	100 ± 0.00^{a}	5.99 ± 0.33^{a}	26.25 ±0.52^{a}
3	20	33.93 ± 1.15^{b}	24.33 ±4.04^{a}	24.44 ± 2.84^{b}	2.25 ± 0.26^{a}	0.76 ± 0.34^{a}	22.5 ± 4.69^{c}	15.33 ± 2.08^{b}	46.66 ± 0.00^{c}	2.10 ± 1.10^{b}	0.42 ± 0.14^{c}	46.50 ±3.29^{b}	26.66 ±5.13^{b}	98.88 ±3.85^{a}	1.87 ± 0.08^{c}	21.76 ±0.22^{b}
4	30	36.10 ± 1.95^{a}	16.00 ±3.00^{c}	17.77 ± 3.85^{c}	2.49 ± 0.40^{a}	0.58 ± 0.16^{a}	38.00 ± 3.60^{b}	21.00 ± 2.00^{a}	55.55 ± 3.85^{b}	2.33 ± 1.04^{b}	0.78 ± 0.25^{b}	61.6 ±10.53^{a}	19.33 ±2.59^{b}	99.99 ±6.66^{a}	1.43 ± 0.28^{c}	22.70 ±1.01^{b}
5	40	30.43 ± 0.80^{b}	15.00 ±0.00^{c}	20.00± 0.00^{c}	2.42± 0.04^{a}	0.74± 0.32^{a}	35.3± 2.80b	21.66± 1.15^{a}	57.77± 3.85^{b}	3.37± 1.39^{a}	0.78± 0.18^{b}	58.86 ±4.35^{a}	26.00 ±4.00^{b}	94.44 ±7.69^{a}	1.47± 0.53c	19.12 ±0.51^{b}
6	50	31.7 ± 1.45^{b}	13.33 ±4.93^{c}	26.66 ± 0.00^{b}	2.58 ± 0.41^{a}	0.84 ± 0.07^{a}	45.50 ± 4.68a	20.00 ± 0.00^{a}	54.44 ± 3.84^{b}	3.14 ± 0.70^{a}	0.88 ± 0.13^{b}	59.56 ±8.48^{a}	86.66 ±1.52^{a}	97.77 ±10.18^{a}	2.55 ± 0.30^{b}	19.50 ±0.43^{a}

* Each value is mean of three replicates ± Standard Deviation MRC: % Mycorrhizal Root Colonization PH: Plant height (cm)
SC: Mycorrhizal spore count/10g of soil SDW: Shoot dry weight (g) RDW: Root dry weight (g)
Data was analysed by DMRT and different superscript symbols (a,b,c,d) within a row represent significant difference at $P \geq 0.05$

Table 4: Efficacy of different hosts and sugarcane bagasse as substrate for mass culturing of *F. mosseae*

		Barley					Sesbania/Dhaincha					Onion				
Sl. No.	Sugarcane bagasse (g)	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW	PH	SC	MRC	SDW	RDW
1	0	33.96 ±3.07^{c}	12 ± 2.64^{c}	17.77 ±3.85^{d}	2.67 ±0..84^{b}	0.41 ±0.16^{b}	26.36 ± 1.92^{d}	10.00 ±1.00^{c}	13.33 ±0.00^{e}	0.59 ±0.06^{c}	0.20 ±0.13^{c}	57.96 ±7.30^{a}	21.33 ±3.21^{d}	82.22 ±3.04^{d}	2.46 ± 0.40^{b}	12.40 ±0.45^{c}
2	50	34.63 ±0.80^{c}	23 ± 2.00^{b}	33.33 ±0.00^{c}	4.66 ±0.88^{a}	0.80 ±0.17^{a}	37.23 ± 2.74^{c}	16.33 ±4.16^{b}	77.77 ±3.85^{a}	2.23 ±0.54^{b}	0.70 ±0.19^{b}	61.10 ±7.20^{a}	36.33 ±6.11^{b}	88.88 ±3.85^{c}	2.61 ± 0.66^{b}	19.74 ±0.55^{a}
3	75	36.26 ±2.59^{c}	16 ± 2.35^{b}	72.22 ±3.84^{a}	4.79 ±1.09^{a}	0.78 ±0.13^{a}	53.80 ± 5.62^{a}	15.00 ±6.55^{b}	33.33 ±0.00^{d}	2.93 ±0.21^{b}	0.73 ±0.20^{b}	48.50 ±3.70^{b}	37.00 ±7.21^{b}	100 ± 0.00^{a}	2.29 ±0.24^{b}	16.87 ±0.36^{b}
4	100	36.23 ±1.73^{c}	19.66 ±4.16^{b}	20 ±0.00^{d}	3.97 ±0.88^{a}	0.60 ±0.14^{a}	43.70 ± 3.14^{b}	42.00 ±6.08^{a}	66.66 ±0.00^{b}	4.25 ±0.50^{a}	0.91 ±0.07^{b}	61.33 ±1.95^{a}	65.66 ±8.14^{a}	100 ± 0.00^{a}	5.73 ±0.15^{a}	5.32 ±1.00^{d}
5	125	46.66 ±3.54^{b}	22.33 ±4.72^{b}	57.77 ±3.85^{b}	4.01 ±0.09^{a}	0.69 ±0.30^{a}	47.70 ± 6.00^{b}	13.33 ±2.51^{b}	77.47 ±3.05^{a}	4.47 ±0.38^{a}	0.82 ±0.07^{b}	62.56 ±0.83^{a}	28.3 ±4.01^{c}	91.10 ±3.85^{c}	2.58 ±0.34^{b}	17.12 ±0.61^{b}
6	150	58.86 ±1.42^{a}	30.33 ±4.50^{a}	60 ±0.00^{b}	3.86 ±0.45^{a}	0.73 ±0.26^{a}	41.90 ± 2.16^{b}	15.00 ±2.00^{b}	44.41 ±3.84^{c}	4.03 ±0.41^{a}	1.31 ±0.21^{a}	58.30 ±1.20^{a}	38.3 ±5.40^{b}	93.33 ±0.00^{b}	5.76 ±0.09^{a}	3.09 ±0.20^{e}

* Each value is mean of three replicates ± Standard Deviation MRC: % Mycorrhizal Root Colonization PH: Plant height (cm)
SC: Mycorrhizal spore count/10g of soil SDW: Shoot dry weight (g) RDW: Root dry weight (g)
Data was analysed by DMRT and different superscript symbols (a,b,c,d) within a row represent significant difference at $P \geq 0.05$

Morphotaxonomy of *Claroideoglomus etunicatum* (accession–CMCC/AM–1206)

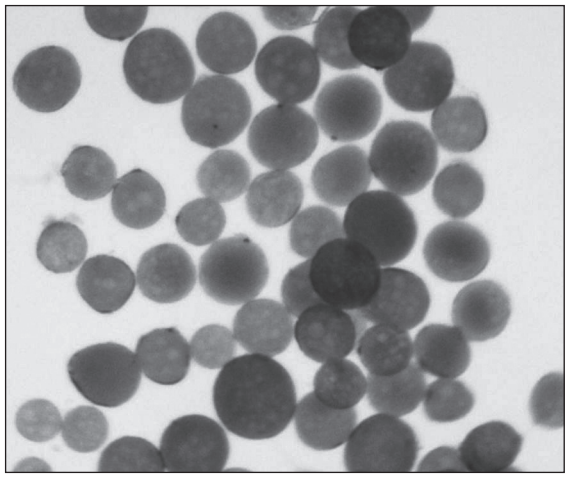
Kiran Sunar[1], Maunata Ghorui[2], and Alok Adholeya[#]*

Morphotaxonimical characterization, which is considered as the only tool for preliminary characterization of any given taxa, is mostly based on visible characters. In case of describing an Arbuscular Mycorrhizal Fungus (AMF) species, characters, such as spore structure, shape, size, wall layers, surface ornamentations, hyphal attachments, type of spore formations, and the reaction of wall layers towards different dyes play an important role. To this end, we have once again taken up the morphotaxonomic characterization of one of our unique AMF species available in the Centre for Mycorrhizal Culture Collection Bank with the accession number CMCC/AM–1206.

This culture has been isolated and raised from the soil of Jaisalmer district of Rajasthan, India. Initially, the soil samples were analysed for AMF spore density, types, spores of varying sizes, colour, and diversity; for this, the soil samples were suspended in water and passed 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 used to obtain pure single species culture of AMF. Voucher specimen of this potential monosporal was prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., was done under compound microscope (10X, 40X, and 100X) after mounting in (Polyvinyl Lacto glycerol and Polyvinyl Lacto glycerol: Melzer's reagents,1:1). Selected healthy single AMF spore were used to raise monospecific cultures, which were inoculated to pre-germinated seed of a suitable host. After a successful growth period of 3–6 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 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.

In the case of CMCC/AM–1206, the pure monosporal culture was established after six months of initiation from single spore. Good sporulation and colonization up to 80–90% were recorded only after six months. The spores were collected after one complete growth cycle of the host; they were observed for a detailed morphotaxonomic analysis as described in the following sections.

Figure 1: Spores isolated from monosporal culture of CMCC/AM–1206



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

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Spore Morphology and Shape

Spores isolated from the cultures were generally found to be borne singly or in loose aggregates and were devoid of any sporocarp. They were found to be of varying shapes and sizes most of which were oval to gloobose spores. Young juvenile spores were hyaline and yellow to brownish when mature (Figures 1, 2, and 3).

Figure 2: Compound microscopic images of the spores of accession CMCC/AM–1206, after mounting in Polyvinyl Lacto glycerol showing a single subtending hyphae (a) and single spore mounted in Polyvinyl Lacto glycerol: Melzer's reagent showing brown red-stained inner wall layer (b)

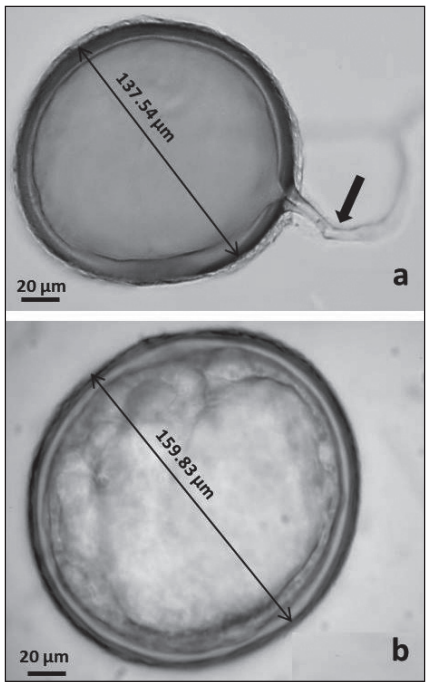
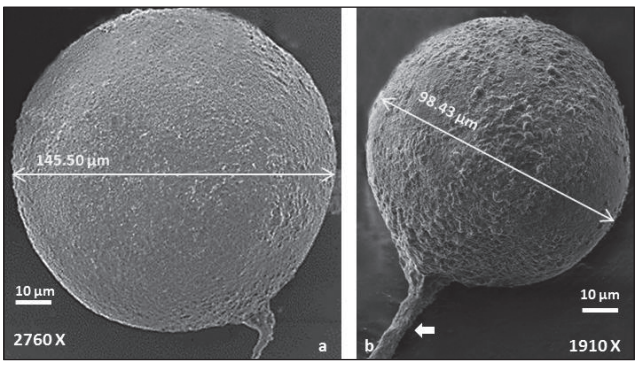


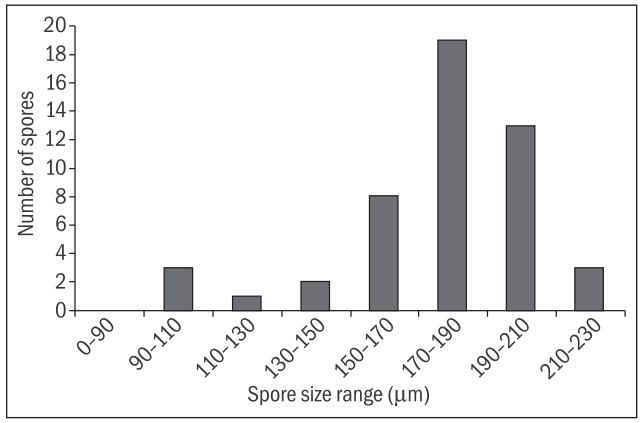
Figure 3: Scanning electron micrograph of spores of CMCC/AM–1206 showing a smooth inner wall (a) and (b) the warty outer wall which is mucilaginous layer



Spore size and diameter

The majority of mature spores of this accession were gloobose and oval and were of varying diameter. The average diameter of 50 spores was evaluated and it was found that the spore size ranged from 90 to 250 µm and the average spore diameter was found to lie between 170 and 190 µm (Figure 4).

Figure 4: Analysis of 50 healthy spores of CMCC/AM–1206 obtained from one year culture



Spore wall and wall layers

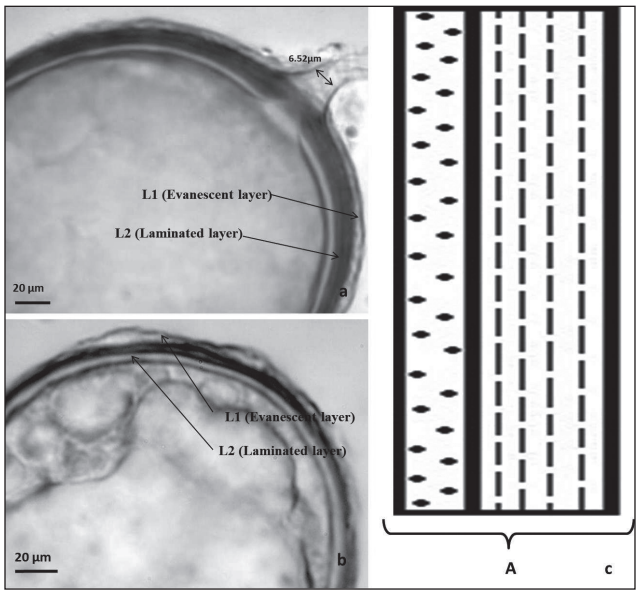
The spore consists of two wall layers (L1 and L2) that gradually differentiate consecutively as spores develop. **Layer 1 (L1):** This is the evanescent outermost mucilaginous layer which is formed in young juvenile spores and also along the subtending hyphal wall. This layer degrades or sloughs off as the spore matures and at maturity, it may be absent or present in patches. Developmentally, this wall layer is the first layer to be formed in the juvenile spores. Generally, organic materials get accumulated on the wall surface resulting in no reaction in Melzer's reagent. In some young spores, this is stained pink with Melzer's reagent. The average thickness of this layer lies between 0.5 and 2.5 µm. **Layer 2 (L2):** The inner layer also known as laminated wall layer is initiated as a single layer. The subsequent adherent sub-layers are formed as the spore matures. All the sub-layers possess the same phenotypic properties. Stains light brown to red brown in Melzer's and the average thickness lies between 4.0 and 6.5 µm in mature spores (Figure 5).

Subtending Hypha and Occlusion

Subtending hypha

The majority of spores at maturity show cylindrical to slightly flared subtending hyphae which arises from the inner layer. The subtending hypha also consists of two wall layers (L1 and L2) which are continuous to the wall layer of the spores. L1 is the outer evanescent layer present at the origin of attachment and is usually present in hyphae of juvenile spores whereas L2 is the inner layer that is continuous with the L2 of the spore wall from the point of attachment of the spores (Figures 6 and 7).

Figure 5: Compound microscopic images of spore wall layers of CMCC/AM–1206 after mounting in PVLG: Melzer's (a) and PVLG (b) the outer and inner layers are designated as L1 and L2, respectively. Murograph (Walker 1983) of the mature spore showing evanescent layer in dots and inner laminated layers in broken lines (c)



Occlusion

The innermost sub-layer of L2 layer of the spore wall in most of the spores forms a bridging structure and resembles a septum [Figure 6 (a) and (b)].

Figure 6: Compound microscopic images of spores showing the subtending hyphal attachment which is continuous with the inner wall layer L2 (a). A small occlusion resembling a bridging structure is also observed (b)

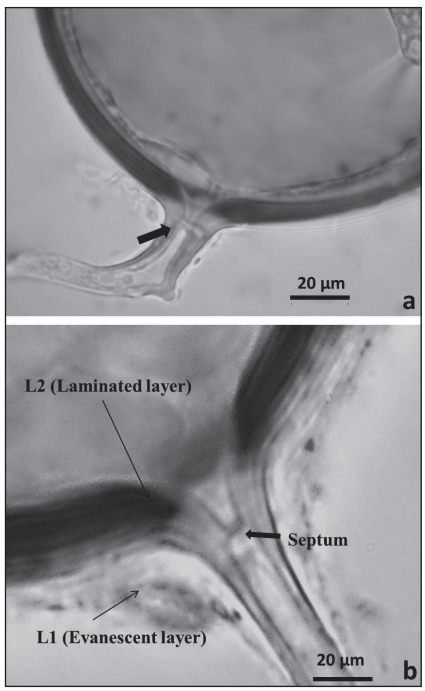
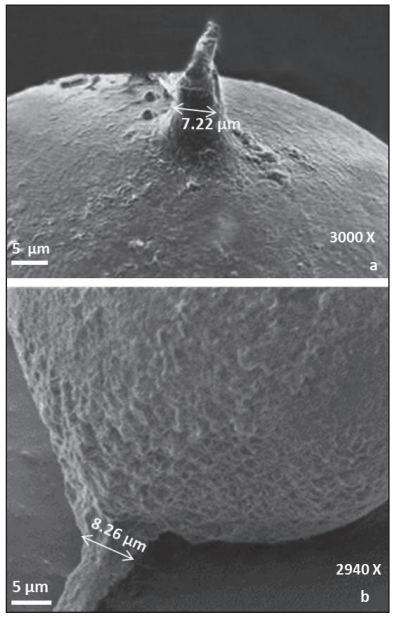


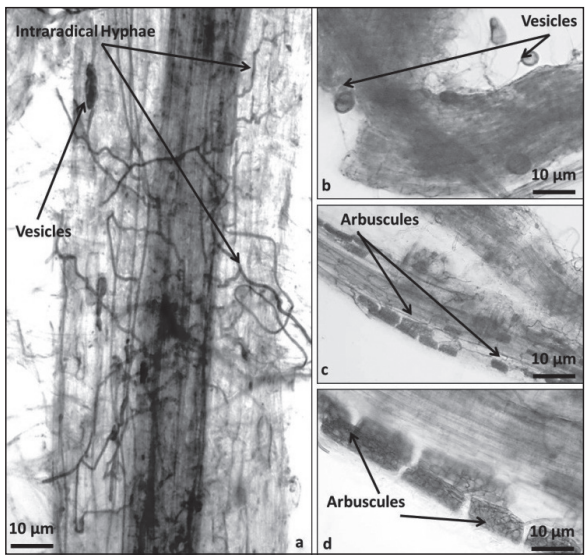
Figure 7: Scanning electron micrograph of mature spores showing the point of attachment with a flared base



Mycorrhizae

Root colonization assay of sorghum roots after three months of inoculation showed profuse colonization with numerous arbuscules and vesicles. Both intraradical and extra radical types of hyphae are observed. The hypha forms long infection units which are interconnected with each other by perpendicular branches ("H" or "h" type of connections). The hyphae are stained dark in ink vinegar. Vesicles are gloobose to sub-gloobose and are formed mostly towards the end of the third month of infection (Figure 8).

Figure 8: Compound microscopic images of colonized roots of sorghum after six weeks of inoculation with CMCC/AM–1206 showing intensive colonization of the cortical cells with branched hyphae (a) vesicles and abundant arbuscules (b, c, and d)



Conclusion and Classification Level

On the basis of the above morphotaxonomic analysis of the accession CMCC/AM–1206, 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 singly or in loose aggregates in abundance with spore wall layers continuous with the subtending hypha.
- Wall layer containing outer evanescent mucilaginous layer and a laminated inner layer that is continuous with the subtending hyphal wall.
- Spores of varying shapes and sizes ranging from globose to sub-globose as well as oval. There is formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular arbuscules.

All these features suggest that the culture CMCC/AM–1206 belongs to the family **Glomeraceae.**

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

- Frequent production of asexual spores produced singly or in loose aggregates in abundance.
- Spores were of varying shapes and sizes ranging from globose to sub-globose as well as oval with average spore size ranging from 90 to 250 μm in diameter.
- A majority of spores at maturity show cylindrical to slightly flared subtending hyphae which arises from the inner layer. The subtending hypha also consists of two wall layers (L1 and L2) which are continuous to the wall layer of the spores.
- The spore wall layers are continuous with the subtending hypha. The innermost sub-layer of L2 layer of the spore wall forms a bridging structure and resembles a septum.

The culture resembles all the characters of previously characterized species of *Glomus etunicatum* W.N. Becker and Gerd. The type of *G. etunicatum* was previously selected from spores isolated from *Onion* (Baker and Gerdemann 1977). *G. etunicatum* is one of the most commonly occurring AMF in the world. The specimen of *G. etunicatum* accession CMCC/AM–1206 characterized in this present study is originally isolated from the soil of Jaisalmer district of Rajasthan, India. The morphological characters fully match the characters as described by Becker and Gerdemann (1977) and Stürmer and Morton (1997) but differ in one way that the outer layer of our specimen did not stain in Melzer's reagent. Our specimen matches with *G. etunicatum* earlier described from Poland by Blaszkowski (1990) where they have also reported that the outer layer did not stain with Melzer's reagent.

The current taxonomic name of *G. etunicatum* is *Claroideoglomus etunicatum* (WN Becker and Gerd) C Walker and A Schüßler 2010.

Systematic Classification

Glomeromycota
Glomeromycetes
Glomerales
Glomeraceae
Glomus etunicatum* / *Claroideoglomus etunicatum

Morphotaxonomic characterization for species identification has got its own limitations. With many unique characters having tendency to vary from host to host and different geographic locations, the identities of similar species become quite tough if they are solely based on visible taxonomic scores. This limitation has been successfully overcome with the advent of molecular methods for identification of organisms. Sequence analyses of rDNA regions have often confirmed the morphologically defined species and the molecular data have characterized new genera and families in AM taxonomy. It is, therefore, advised to our distinguished readers to kindly correlate their morphotaxonomic studies with the molecular data.

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