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RESEARCH FINDING PAPERS

Cymbopogon winterianus Jowitt ex Bor (Citronella Grass) as a Promissive and Promotive Host for Mass Inoculum Production of Arbuscular Mycorrhizal Spores

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Introduction

Mycorrhizal fungi are described as the symbiotic association between plants and fungi; it is highly evolved non-pathogenic symbiotic association between roots of the vascular plants that colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivated crop (Miller and Bever 1999). The partners in this association are members of the fungal kingdom (Glomeromycota, Ascomycetes, and Basidiomycetes, but not protoctistan fungi such as Oomycetes) and most vascular plants (Harley and Smith 1983). Amongst different types of mycorrhizal fungi, arbuscular mycorrhizal (AM) fungi are an important asexually reproducing soil microbial community that forms symbiotic relationships with a wide range of hosts, nearly 90 percent of the flowering plants (Brundrett 1991), apart from being the most ancestral as well as the commonest type of mycorrhizal symbiosis (Brundrett 2002). AM colonization is further characterized by structures formed by the aseptate, obligately symbiotic fungi of the phylum Glomeromycota (Schüßler et al. 2001) termed 'vesicle', which act as storage or reproductive organs within or between cortical cells and 'arbuscule' that provide a large surface contact area between host and fungus (Manoharachary and Kunwar 2015).

In spite of the multi-faceted role of AM fungal species/strains, lack in mass production techniques

of AM fungal propagules (owing to obligate nature of this biotroph as well as absence of a suitable host plant) serves as a hindrance to fundamental research as well as for application purposes. Notwithstanding the diverse methods of AM fungi inoculum (Mosse and Thompson 1981; Jarstfer *et al.* 1988, Tiwari and Adholeya 2003), the method most common in practice is the traditional pot-culture method (Parkash and Aggarwal 2012) where the fungi are usually maintained and multiply in conjunction with roots of suitable host plant, preferably a monocot host. The present study serves to propose and prospect the role of *Cymbopogon winterianus* Jowitt ex Bor in lieu of *Zea mays* L. as a promissive and promotive host plant for mass multiplication of AM fungal propagules/spores.

Materials and Methods

Isolation, Quantification, and Root Colonization of AM Spores

Isolation of AM spores was done by using wet sieving and decanting technique of Gerdemann and Nicolson (1963) and Singh and Tiwari (2001). Sieves of different sizes, that is, 150 μ m, 120 μ m, 90 μ m, 63 μ m, and 45 μ m were used. About 150 μ m sieves were used for collecting plant debris in the soil. In a small plastic container with a capacity of about 1500 ml, 100 g of soil was mixed in water. The soil was thoroughly mixed with water and allowed to settle overnight. The water

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was decanted on a series of sieves in the following order 150 μ m, 120 μ m, 90 μ m, 63 μ m, and 45 μ m from top to bottom on which spores were trapped. The trapped spores were then transferred to Whatman filter paper no. 1 by repeated washing with water. The spores were picked by hypodermic needle under stereo-binocular microscope and mounted in polyvinyl lactic acid.

Trap and Starter Cultures

The mycorrhizal inoculum production was done by using 'soil funnel technique'. Single dominant and efficient AM spore/s was/were mass produced in this technique. The best host was selected for starter culture of inoculum production, that is, sorghum (Sorghum vulgare Pers.) and wheat (Triticum aestivum L.). In this technique, earthen funnels (chillams) were taken and germination of seeds was made possible in such a way that the roots of the seedlings must touch the inoculum of AM fungi. The seedlings were raised up to 30 days in the earthen funnels containing sterilized sand:soil (40:120 g). The experiment was repeated again and AM spores were collected by wet sieving and decanting technique of Gerdemann and Nicolson (1963) and Singh and Tiwari (2001) after 45-60 days. These final spores of strains (EM, and EM₂) were used for mass multiplication by using different hosts in bigger earthen pots for further study.

Pot Culture

Mass multiplication of efficient dominant AM spore/s was carried out by using different hosts and substrates in pots. For pot cultures of mycorrhizal spore production, two best hosts were selected, that is, maize, (Zea mays L.) and citronella grass (Cymbopogon winterianus Jowitt ex Bor) for different seasons. The substrate used for pot cultures was sand:soil (1:3) by gram ratio in sterilized conditions. The pot cultures were maintained for several days on different hosts. The pots were given Hoagland's solution, except Monopotassium phosphate (KH₂PO₄) solution once in a fortnight. The soil containing mycorrhizal spores, mycelium, and colonized roots in pot cultures were used to inoculate seedlings for further growth experiments and to prepare other pot cultures for further use.

Results and Discussion

The dominant and efficient strains, that is, Glomus species (EM₁) and Acaulospora species (EM₂) from collected rhizospheric samples were isolated and trapped on hosts like wheat (Triticum aestivum L.) and jowar (Sorghum vulgare Pers.) in a tray. The trapped culture was then mass produced in deep depots and earthen pots for obtaining starter culture on wheat and jowar. The starter culture was then recycled and reseeded thrice and pot cultured again on above mentioned hosts. The pot culture was recycled again and reseeded thrice (see Plate 1). Mass production (average of five replicates) of two strains of AM fungi on two monocot hosts, that is, Cymbopogon winterianus Jowitt ex Bor and Zea mays L. (pot cultures after first cycle) at 45 days and 90 days interval are shown in Table 1, Plate 2 and Figures 1 and 2. After 45 days, the host C. winterianus showed maximum

Table1 Mass production of two strains of AM on Cymbopogon winterianus and Zea mays (Pot Cultures after first cycle)

			After 45 days*	:			After 90 days	*		
SI.No.	Hosts	Treat- ments	Height (cm)	Temperature (°C)	Spore no. (50 g/ 500 mL)	Colonization (%)	Height (cm)	Temperature (°C)	Spore no. (50 g/ 500 mL)	Colonization (%)
	Cymbopogon- winterianus	EM1	11.2 ± 0.14	19.17 ± 0.23	123 ± 1.63	86.3 ± 0.92	25.53 ± 0.36	24.1 ± 0	136 ± 1.7	90.5 ± 0.04
	Jowitt ex Bor	EM₂	11.73 ± 0.54	19.23 ± 0.12	122 ± 1.41	90.5 ± 1.08	23.93 ± 0.16	24.73 ± 0.17	161 ± 1.7	99.6 ± 0.43
		С	7.6 ± 0.51	19 ± 0	17 ± 3.39	72.7 ± 1.68	11 ± 0.08	24.5 ± 0	111 ± 1.63	72.17 ± 7.19
	Zea mays L.	EM_1	12.5 ± 0.08	18.7 ± 0.16	111 ± 0.47	60.2 ± 0.21	42.87 ± 0.03	23.97 ± 0.23	143 ± 1.24	77.07 ± 1.46
		EM₂	12.43 ± 0.124	20.37 ± 0.16	118 ± 0.81	60.07 ± 0.09	43.95 ± 0.04	24.47 ± 0.29	137 ± 2.05	71.17 ± 1.02
		С	9.77 ± 0.16	19.03 ± 0.047	11 ± 0.81	19.7 ± 0.29	35.57 ± 0.05	24.17 ± 0.31	112 ± 2.5	59.77 ± 1.27

± SEM (Standard error of mean); * Average of five replications; EM, = Glomus species, EM, = Acaulospora species; C = Control

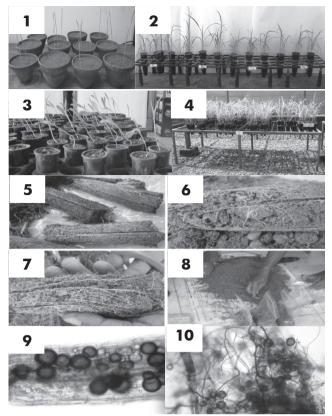


Plate I: 1-4: Mass production of AM fungi on different monocot hosts; 5-8: Harvesting of AM fungal inoculum after 70-90 days (red circle shows AM infection on rootin image 6); 9-10: Mass spore production (intra- and extra-radical spores in roots of *Cymbopogon winterianus*).

height of plants (11.73 cm), temperature (19.23°C) and colonization intensity in roots (90.5 per cent) with inoculation of EM₂ (Acaulospora sp.). But spore number was maximum (123 per 50g/500mL) with inoculation of EM, (Glomus sp.). The same host after 90 days showed maximum height (25.53 cm) with inoculation of EM, (Glomus sp.), however maximum temperature (24.73°C), colonization intensity in roots (90.5 per cent) and maximum spore number (161.3 per 50 g/500 mL) was seen with the inoculation of EM_{a} (*Acaulospora* sp.). The same study in case of Z. mays after 45 days showed maximum height of plants (12.5 cm), spore numbers (110.67 per 50 g/500 mL), and colonization intensity in root (60.2 per cent) with inoculation of EM, (Glomus sp.), except temperature (20.37°C) which was highest in plants inoculated with EM, (Acaulospora sp.). After 90 days, the host Z. mays showed maximum height (43.95 cm) and temperature (25.47°C) with inoculation of EM₂ (Acaulospora sp.); however, maximum spore number (142.67 per 50 g/500 mL) and colonization intensity (77.07 per cent) was seen in the case of plants inoculated with EM, (Glomus sp.). In case of C. winterianus, EM, (Acaulospora sp.) strain showed the best result of spore production in comparison to EM_1 (*Glomus* sp.) and control whereas in case of Z. mays, EM_1 (*Glomus* sp.) strain showed the best result of spore production in comparison to EM_2 (Acaulospora sp.) and control. There was an increase in spore number and root colonization intensity (in per cent) with AM fungal strains inoculation in comparison to control treatment. However, the AM spore production and root colonization were more in case of C. winterianus than Z. mays as host in general, irrespective of AM strain.

Mass inoculum production of AM fungi with efficient strains in the field for cultivated crops has usually met with little success because of the inability to culture the fungus axenically. Also, mass production of AM fungi is difficult because these are obligate symbionts (Parkash and Aggarwal 2012). Large-scale production of AM inoculum for field inoculation is technically feasible through pot culture using an appropriate host (Wood 1985). Soils of low fertility have proved particularly effective in supporting inoculum production.

In this study, *Cymbopogon winterianus* was a good host for mass production among all the AM fungal species under the growth conditions as it had good spore numbers and colonization than *Zea mays* and was selected for further mass multiplication. In general, monocots are the best hosts for AM spore production (Sundra-Babu *et al.* 2001). Sieverding and Leihner (1984) have reported that graminaceous and leguminous crops generally tend to increase AM population whereas mycotrophic plants decrease the population of AM fungi.

Al-Raddad (1995) used five crops for inoculation with *Glomus mosseae* and crops were grown for 10 weeks to assess mycorrhizal infection and sporulation. For all hosts, the percentage of the root length infected by the AM fungus increased rapidly up to 10 weeks after sowing. The highest spore number was achieved in the rhizosphere of barley plants, followed by chickpea and beans. The type of the crop as well as the harvest date greatly influenced the spore population and the extent of root colonization by *G. mosseae* (Al-Raddad 1995).

Sreenivasa and Bagyaraj (1987) screened seven hosts, that is, *Panicum maximum, Chrysopogon fulvus, Themeda triandra, Chloris gayana, Brachiaria brizantha, Passpalum scrobiculatum*, and *Eleusine coracana* to find a suitable host for mass production of the AM fungus. *Chloris gayana* (Rhode grass) was found to be the best host with the highest percentage of mycorrhizal colonization (94 per cent), sporulation (547 spores per 50 ml substrate), and inoculum potential (1.65×10^7 per g).

When both soil and spore inoculum at a similar range of spore densities were used to inoculate Sudan grass roots, then they were infected more rapidly and subsequent spore production was greater with soil inoculum than with spore inoculum (Ferguson 1981). Many hosts, which show great susceptibility to AM infection, are Bahia grass, Rhode grass, Guinea grass, Coleus, and Clover (Mehrotra 1992).

Sporulation of vesicular AM fungi can be influenced by inoculum dosage (Ferguson 1981), temperature (Furlan and Forting 1973), and soil fertility (Menge *et al.* 1978). The host plant may also stimulate selectively or limit sporulation of certain AM fungal species suggesting varied affinities between hosts and symbionts. Therefore, in production of AM spore inoculum, the influence of the host plant should be considered (Barbara *et al.* 1986).

Again, type of root system determines the extent of interaction between AM fungi and host; apart from the supply of carbohydrates to the fungal partner. The presence of more number and fine nature of fibrous roots facilitates the entry of AM fungus (Al-Raddad 1995). In the present context, maximum fibrousity in the roots of Citronella grass has promoted more AM fungal density as well as colonization in comparison to maize.

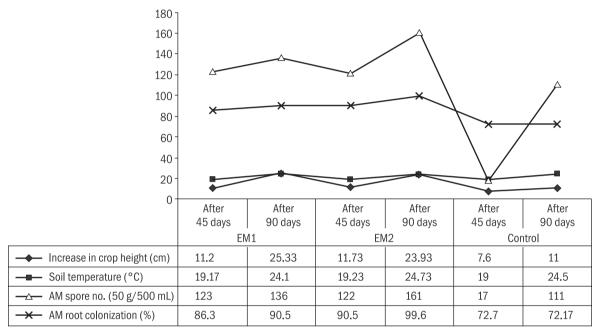


Figure 1 Mass production of AM fungi on Cymbopogon winterianus Jowitt ex Bor.

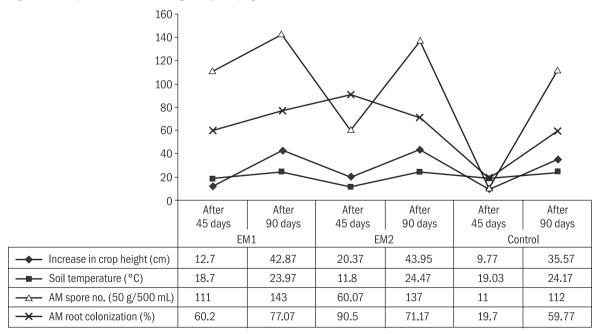


Figure 2 Mass production of AM fungi on Zea mays L

Acknowledgement

The senior author, Vipin Parkash is thankful to Indian Council of Forestry Research and Education, Dehradun, for financial assistance in the project no. RFRI-12/2008-09/SFM.

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Biodiversity of Arbuscular Mycorrhizal Fungi in India: A Review

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Introduction

Arbuscular mycorrhizal (AM) fungi eminently show symbiosis in terrestrial ecosystems, associating with about 80 per cent of plant families worldwide. They are recognized as an extremely important component of most terrestrial ecosystems, helping plant establishment by enhancing plant nutrient acquisition; improving soil quality; increasing resistance to environmental stresses; and also plaving an important role in plant biodiversity, productivity, and ecosystem variability. According to the current taxonomy, AM fungi belong to the phylum Glomeromycota, including 4 families and 12 genera. Till date, only fewer than 200 species of AM fungi have been described (Redecker and Philipp 2006), although numerous studies on AM fungal diversity in different ecosystems worldwide have shown that AM fungi distribution globally (Treseder and Cross 2006). India is situated north of the equator between 66°E to 98°E and 8°N to 36°N. It is bordered by Nepal, China, and Bhutan in north; Bangladesh and Myanmar in the east; the Bay of Bengal in the south east; Indian Ocean in the south; the Arabian Sea in the west; and Pakistan in the northwest. With only 2.4 per cent of world's land area, India accounts for 7-8 per cent of recorded species of the world (Goval and Arora 2009). The country is home to hills, deserts, forests, rivers, mountains, plateaus, and seashores. India is divided into five physiographic regions, that is, northern mountains, Indo-Gangetic plains, peninsular plateau, deserts, and coastal plains. Studies on AM fungal biodiversity in India began in the 1970s (Manoharachary et al. 2005). Bakshiin (1974) was the first to publish an account of 14 spore types in India: Glomus macrocarpum, Gl. macrocarpum Tul. and Tul. var. geosporum, Gl. mosseae, Glomus sp., etc. Thereafter, more and more research work on AM fungi within India was carried out. This review aims to summarize the advances in AM fungal biodiversity, including species diversity, habitat diversity, and host diversity within India.

AM Fungal Species Diversity

A total of 120 AM fungi species within 11 genera have been reported in the rhizosphere of different plants in various environments of India (Table 1). The most common and widely distributed genera is *Glomus* (61 species) followed by *Acaulospora* (20 species) and *Scutellospora* (12 species). Table 1 AM fungi reported in India

Table 1 AM fungi reported in India		
VAM fungi	References	
Acaulospora appendicula	Pawar and Kakde 2012	
Acaulospora bireticulata	Mahesh and Selvaraj 2009	
Acaulospora delicata	Sundar <i>et al.</i> 2011	
Acaulospora dilatata	Srivastava et al. 2012	
Acaulospora elegans	Selvaraj and Hoon 2004	
Acaulospora foveata	Rao <i>et al.</i> 2012	
Acaulospora gerdemanii	Srivastava <i>et al.</i> 2012	
Acaulospora lacunosa	Kumar et al. 2012	
Acaulospora laevis	Gaur and Kaushik 2012	
Acaulospora longula	Singh et al. 2012	
Acaulospora mellea	Rao <i>et al.</i> 2012	
Acaulospora morrowae	Sundar et al. 2011	
Acaulospora nicolsonii	Chauhan <i>et al.</i> 2013	
Acaulospora rehmi	Tripathi and Khare 2012	
Acaulospora scorbiculata	Sastry and Johri 1999	
Acaulospora spinosa	Khade and Rodrigues 2009	
Acaulospora sporocarp	Singh et al. 2012	
Acaulospora trappei	Kumar et al. 2012	
Acaulospora tuberculata	Tripathi and Khare 2012	
Acaulospora terricola	Swarupa et al. 2004	
Acaulospora undulata	Tripathi and Khare 2012	
Desticutata reticulate	Khade and Rodrigues 2009	
Endogene gigantea	Bakshi 1974	
Enthrospora columbiana	Mehrotra and Prakash 2006	
Enthrospora infrequens	Rao <i>et al.</i> 2012	
Gigaspora albida	Mahesh and Selvaraj 2009	
Gigaspora calospora	Bakshi 1974	
Gigaspora candida	Rao <i>et al.</i> 2012	
Gigaspora decipens	Sundar et al. 2011	
Gigaspora gigantea	Pawar and Kadke 2012	
Gigaspora gilmorei	Bhattacharjee and Sharma 2011	
Gigaspora margarita	Mahesh and Selvaraj 2009	
Gigaspora nigra	Kumar et al. 2012	
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Table 1: AM fungi reported in India

/AM fungi	References
Gigaspora reticulata	Rao et al. 2012
Gigaspora rosea	Mahesh and Selvaraj 2009
Gigaspora verrucosa	Rao et al. 2012
Glomus aggregatum	Ganesan <i>et al.</i> 1991
Glomus albidum	Singh and Adholeya 2013
Glomus ambisporum	Tripathi and Khare 2012
Glomus auranitum	Bakshi 1974
Glomus australe	Selvaraj and Hoon 2004
Glomus boreale	Pawar and Kakde 2012
Glomus bagyarajii	Mehrota 1997
Glomus caledonium	Kumari 2011
Glomus citricolum	Mahesh and Selvaraj 2009
Glomus claroideum	Khade and Rodrigues 2009
Glomus clarum	Selvaraj et al. 2011
Glomus constrictum	Mahesh and Selvara 2009
Glomus convolutum	Tripathi and Khare 2012
Glomus coronatum	Gogoi and Singh 2011
Glomus delhiense	Mukherji <i>et al.</i> 1983
Glomus deserticola	Mahesh and Selvaraj 2009
Glomus diaphanum	Singh et al. 2012
Glomus etunicatum	Muthukumar and Tamilselvi 2010
Glomus facundisporum	Bhattacharjee and Sharma 2011
Glomus fasciculatum	Shivaputra et al. 2004
Glomus fistulosum	Singh 2002
Glomus fuegianum	Kumar et al. 2012
Glomus fulvum	Sundar et al. 2011
Glomus geosporum	Bakshi 1974
Glomus glomerulatum	Srivastava et al. 2012
Glomus heterosporum	Khade and Rodrigues 2009
Glomus hyderabadnesis	Swarupa et al. 2004
Glomus hoi	Gogoi ans Singh 2011
Glomus indica	Muthukumar and Tamilselvi 2010
Glomus intraradices	Sastry and Johri 1999
Glomus invermayanum	Bhatacharjee and Sharma 2011
Glomus lepotichum	Bhatacharjee and Sharma 2011
Glomus macrocarpum	Bakshi 1974
Glomus margarita	Selvaraj et al. 2011
Glomus microaggregatum	Mehrotra and Prakash 2006

Table 1: AM fungi reported in India

Table 1: AM fungi reported in In VAM fungi	dia References
Glomus minuta	Srivastava et al. 2012
Glomus monosporum	Singh <i>et al.</i> 2011
Glomus mosseae	Banerjee et al. 2013
Glomus multicaule	Gerdemann and Bakshii 1976
Glomus multicaulis	Mahesh and Selvaraj 2009
Glomus multisubstensum	Mukherji <i>et al.</i> 1983
Glomus occultum	Gogoi and Singh 2011
Glomus palladium	Kumar et al. 2012
Glomus pansihalos	Kumar et al. 2012
Glomus postulatum	Tripathi and Khare 2012
Glomus radiatum	Pawar and Kakde 2012
Glomus reticulatum	Bhatacharjee et al. 1982
Glomus rubiformis	Mehrotra and Prakash 2006
Glomus scintillans	Kumar <i>et al.</i> 2012
Glomus segmentatum	Pawar and Kakde 2012
Glomus sinuosum	Khade and Rodrigues 2009
Glomus sterilum	Mehrotra and Baijal 1992
Glomus taiwanensis	Khade and Rodrigues 2009
Glomus tenebrosum	Tripathi and Khare 2012
Glomus tortuosum	Mehrotra and Prakash 2006
Glomus versiforme	Gogoi and Singh 2011
Glomus vesiculiferum	Singh 2002
Glomus viscosum	Singh 2002
Glomus xanthium	Gogoi and Singh 2011
Sclerocystis biornata	Bharacharjee and Sharma 2011
Sclerocystis ceremoides	Bakshi 1974
Sclerocystis clavispora	Singh <i>et al.</i> 2012
Sclerocystis coccogena	Singh et al. 2012
Sclerocystis coralloidea	Selvaraj and Hoon 2004
Sclerocystis dussi	Selvaraj and Hoon 2004
Sclerocystis microcarpus	Rao <i>et al.</i> 2012
Sclerocystis pachycaulis	Sastry and Johri 1999
Sclerocystis pakistanica	Tripathi and Khare 2012
Sclerocystis rubiformis	Singh et al. 2012
Sclerocystis sinuosa	Gerdmann and Bakshii 1976
Scutellospora auriglobosa	Pawaar and Kakde 2012
Scutellospora biornata	Bhatacharjee and Sharma 2011
Scutellospora calospora	Mehrotra and Prakash 2006
Scutellospora erythropa	Mahesh and Selvaraj 2009
	Contd

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Table 1: AM fungi reported in India

VAM fungi	References
Scutellospora fulgida	Pawaar and Kakde 2012
Scutellospora gregaria	Rao et al. 2012
Scutellospora heterogama	Mahesh and Selvaraj 2009
Scutellospora nigra	Mahesh and Selvaraj 2009
Scutellospora pellucida	Muthukumar and Tamilselvi 2010
Scutellospora scutata	Srivastava et al. 2012
Scutellospora sinosa	Tripathi and Khare 2012
Scutellospora weresubiae	Rao et al. 2012

Abbreviations: VAM: Vesicular- arbuscular mycorrhiza Note: The scientific names have been amended according to the Internet information (http://www.lrzmuenchen.de/~schuessler/ amphylo)

AM Fungal Habitat Diversity

It has been shown that besides farmlands (Muthukumar and Tamilselvi 2010; Bhattacharjee and Sharma 2011; Singh and Adholeva 2013); fruit lands(Shivaputra et al. 2004; Khade and Rodrigues 2009; Srivastava et al. 2012); vegetable lands (Mehrotra and Baijal 1992; Singh 2002; Srivastava et al. 2012); grasslands (Muthukumar and Udaiyan 1995; Chaurasia and Khare 1999; Dhillion and Gardsjord 2004); AM fungi also occur in tropical rain forests (Raja et al. 1991;Rao et al. 2012; Tripathi and Khare 2012), coastal tropical forests (Raghupathy and Mahadevan 1991), and natural reserves (Bakshi 1974; Ganeshan et al. 1991; Gerdemann and Bakshii 1976; Banerjee et al. 2013). AM fungi occurred in all kinds of landforms all over India, such as mountains(Gaur and Kaushik 2011; Kumar et al. 2012; Gaur and Kaushik 2012), plateaus (Sastry and Johri 1999;Khade and Rodrigues 2009), hills (Gopinathan et al. 1991; Sundar et al. 2011; Pawaar and Kakde 2012), deserts (Talukdar and Thakuria 2001; Mehrotra and Prakash 2006), and also in arid and semi-arid areas of Rajasthan (Neeraj and Verma 1991; Ganesan et al. 1991; Mohan and Verma 1995). However, AM fungal biodiversity differs from different soil climatic zones, varying with various environmental factors (Zhang et al. 1999).

In recent years, VAM fungal diversity in polluted environment received more attraction such as degraded grasslands (Muthukumar and Udaiyan 1995; Chaurasia and Khare 1999; Wath and Deoatre 1999), sewage irrigated soils (Mehrotra and Prakash 2006; Selvaraj and Mahesh 2008), heavy metal polluted sites (Swaminathan and Verma 1979; Raman and Sambadan 1998; Sastry and Johri 1999), mining disturbed soil (Rodrigues 1995; Jamaluddin and Chandra 1997; Tarafdar and Rao 1997; Rao and Gupta 1999; Mehrotra and Prakash 2006), tannery effluents (Raman and Sambadan 1998; Selvaraj and Mahesh 2008), and water-logged sites(Rattan *et al.* 1999).

AM Fungal Host Diversity

AM fungi are ubiquitous in natural ecosystems, associating with about 80 per cent of terrestrial plant species worldwide (Smith and Read 1997). Most plant phyla including bryophyte, pteridophyte, gymnosperm, and angiosperm form mycorrhizal association with fungi. Only a few families, such as Cyperaceae, Brassicaceae, Carvophyllaceae, and Amaranthaceae are assumed never to form mycorrhizal association or they do so rarely (Hirsch and Kapulink 1998). India ranks tenth in world and fourth in Asia in terms of plant diversity with over 45,500 plant species, representing nearly 11 per cent of the world's known floral diversity. According to Botanical Survey of India (2012), India's share of crops is 44 per cent as compared to world average of 11 per cent and has 23.39 per cent of its geographical area under forest and tree cover. Indian researchers are doing researches on the mycorrhizal status of plant species in terrestrial ecosystems and have examined a number of plant species belonging to different families. The diversity of AM fungi in agricultural fields was reported on Leucaena leucocephala from Bangalore (Nalini et al. 1986); ornamentals and cultivated plants at Allahabad and adjoining areas (Kehri et al. 1987); crop fields of Konkan and Solapur (Dalal and Hippalgaonkar 1995), tea plantation at Nilgiris, Tamil Nadu (Kumaran and Santhanakrishnan 1995); pearl millet, maize, wheat, pigeonpea, and chick pea in Gwalior (Singh and Pandva 1995); and different agro-climatic regions of India (Singh and Adholeya 2002). These plants include food crops, economic crops, vegetables, fruits, ornamental plants, medicinal plants, trees, fiber crops, etc., as shown in Table 2. Interestingly, even some forest trees such as *Eucalyptus* are considered to be ectomycorrhizal and have been found to form VAM colonization.

Table 2 Plants species reported to be AM associated in India

Food crops (Godse *et al.* 1976; Swaminathan and Verma 1979; Pravathi *et al.* 2013; Vijayalakshmi and Rao 1988; Ammani *et al.* 1988; Sulochna and Manoharachary 1989; Edathil *et al.* 1994; Harikumar 1995; Raman and Sambadan 1998; Hasan 2002; Caglar and Akgun 2006; Muthukumar and Tamilselvi 2010; Hindumathi and Reddy 2011; Bhattacharjee and Sharma 2011; Singh and Adholeya 2013) Cajanus cajan Cicer arietinum Eleusine coracana Glycine max. Hordeum vulgare Lens culinaris Oryza sativa (L.) Pennisetum typhoides Phaseolus mungo Phaseolus aureus Sesamum indicum Sorghum bicolour Triticum aestivum Vigna radiate (L.) Zea mays

Contd...

Table 2 Plants species reported to be Economic crops (Singh 2002; Kumari 2011; Gogoi and Singh 2011; Singh et al. 2012)	A AM associated in India Arachis hypogaea Carthamus tinctorius L. Coffee Cucrcuma longa Indigofera tinctoria Piper longum Pistachio khinjuck Pistachio terebinthus Queensland arrowroot Saccharum barberi	Tab
Fruits (Girija and Nair 1998; Mohandas 1990; Pandey and Singh 1990; Rani and Bhaduria 2001; Shivaputra <i>et al.</i> 2004, Khade and Rodrigues 2009)	Carica papaya Citrullus lanatus Cucumis melo L. Litchi chinensis Mangifera indica Musa sp. Papaya c.v. Sunset solo Persea americana Pistachio vera Prunus persica Royle delicious Strawberry	For Uda 199 and
Vegetables (Sulochana and Manoharachary 1989; Mehrotra and Baijal 1992, Edathil <i>et al.</i> 1994; Harikumar 1995; Vijayakumar <i>et al.</i> 2000; Srivastava <i>et al.</i> 2012)	Allium sativum Benincasa hispida Coccinia indica Cucumis melo L. Cucurbita maxima Kaempferia galangal Lagenaria vulgaris Luffa acutangula (L.) Lycopersicon esculentum Momordica charantia L. Pisum sativum Prosopsis cineraria Solanum lycopersicum Solanum melongena Solanum tuberosum L. Trichosanthes anguina L. Trigonella foenum-graecum	For Ger and and Uni 200 Tha and Rac
Medicinal plants (Gupta <i>et al.</i> 2000; Rani and Bhaduria 2001; Mahesh and Selvaraj 2009; Gaur and Kaushik 2011; Sundar <i>et al.</i> 2011;Kumari 2011, Selvaraj <i>et al.</i> 2011; Pawaar and Kakde 2012; Gaur and Kaushik 2012; Chauhan <i>et al.</i> 2013)	Aegle marmelos Correa. Andrographis paniculata Asclepias currasavica. Asparagus racemous willd. Bersama engleriana Bidens pilosa Boerhaavia diffusa Borreria ocymoides Breynia retusa Bryophyllum pinnatum Canscora perfoliata	

Catharanthus roseus L.

Chonemorphafragrans

Cyclea peltata (Lann.)

Cymbopogon winterianus

Centella asiatica

Clitoria biflora Dalz

Dioscorea bulbifera

Eclipta prostrata

Emblica officinalis

Heracleum rigens

Hydrocotyle javanica

Indigofera aspalathoides

Justicia

Crotolaria pallid

Table 2 Plants species reported to be AM associated in India

Forage grasses (Muthukumar and Udaiyan 1995; Chaurasia and Khare 1999; Wath and Deotare 1999; Dhillion and Gardsjord 2004)

Forest and trees (Bakshi 1974; Gerdemann and Bakshii 1976; Mukherji and Bhattacharjee 1983; Ganeshan *et* al. 1991; Bhat *et al.* 1994; Uniyal and Uniyal 2000; Talukdar and Thakuria 2001; Tamuli and Boruah 2002; Thangaswamy and Hoon 2004; Jagadish and Rao 2012; Tripathi and Khare 2012; Rao *et al.* 2012; Banerjee *et al.* 2013)

Indigofera tinctoria Naravellia zeylanica Ocimum gratisimum Ocimum spp. Odendia carbonaisis Peperomia pellucida Phyllanthus amarus Premna obtusifolia Prosopsis cineraria Ricinus communis Scoparia dulcis Shorea robusta Sida acuta Siegesbeckia orientalis Sisymbrium irio Solanum varium Stevia rebaudiana Bertoni.

Crotalaria juncea Pennisetum pedicellatum Pennisetum purpureum Rhynchosia minima Trigonella foenum-graecum

Acacia melanoxylon Acacia nilotica Acacia Senegal Acacia tortilis Albizia amara Albizia odoratissima Alnus nepalensis Aquillaria agalocha Araucaria cunninghamii

Azadirachta indica Bambusa balcooa Bridelia retusa spreng. Cassia fistula Celtis Australia Dalbergia paniculata Dalbergia sissoo Dendrocalamus strictus Diospyros peregrine Drynaria quericifolia Eucalyptus camaldulensis Eucalyptus tereticornis Excoecaria agailocha Fraxinus udhei Gmelina arborea Grewia optiva Hardwickia binata Macaranga peltata Maerua arenaria Mangrove Mesua ferrea Osmunda regalis Prosopis juliflora Schrebera swietenioides Tecomella undulata Tectona grandis Terminalia alata Terminalia myrocarpa Toona ciliata Typha elephantina Walsura robusta Xylia xylocarpa Zanthoxylum rhetsa

Table 2 Plants species reported to be AM associated in India

Ornamentals and flowering plants (Brundrett 1991; Ganeshan *et al.* 1991; Singh 2002; Tripathi and Khare 2012; Kumar *et al.* 2012)

Weeds and grasses (Kehri and Chandra 1989; Rathi and Singh 1990; Raman and Sambadan 1998; Kanakdurga et al. 1990; Muthukumar and Udaiyan 1995; Kapoor et al. 1996; Chaurasia and Khare 1999; Sastry and Johri 1999; Rattan et al. 1999; Mehrotra and Prakash 2006; Selvaraj and Mahesh 2008; Maggirwar et al. 2014) Amarvllis belladonna Aster amellus Catharanthus roseus Crysanthemum Leucanthemum Dahlia variabilis Geranium pelargonium Gladiolus grandiflorus Hibiscus rosa sinensis Hydrangea paniculata Jacobinea carnea Jasminum sambac Lagerstroemia speciosa Lilium rubescens Michelia champaca Nerium indicum Pithecellobium dulce Rhododendron anthopogan Rhododendron arboretum Rhododendron barbatum Rhododendron campanulatum Rhododendron lepidotum Rosa indica Salvia splendens Senecio cineraria Tagetes patula Zizypus jujube Ageratum convzoides

Cassiatora Chloris virgata Coronopus didymus Cyanodon dactylon Dactyloctenium aegyptium Desmodiu Eclipta alba Eragrostiella bifara Eragrostis spectabilis Elusine indica Heteropogon contortus Hydrocotyle asiatica Mimosa pudica Peperomia pellucid Portulaca oleracea Seena occidentalis Solanum khasianum Sisymbrium irio Tectona grandis

Conclusion

Indian mycorrhizasts have done a considerable amount of work on the occurrence of AM fungi in fields with plants located at different geographical regions of the country. These plants include food crops, economic crops, vegetables, fruits, ornamental plants, medical plants, wild weeds, trees, etc., as listed in Table 2. The studies indicate that the genus *Glomus* is ubiquitous in various ecosystems in India. The distribution of other genera, that is, *Entrophospora*, *Gigaspora*, *Sclerocystis*, and *Scutellospora* is limited, indicating greater adaptability of Glomus to varied soil conditions in India. A commendable work has been reported about AM associations in forest trees, cereals, millets, pulse crops, vegetables, fruits, sugar crops, food crops, fodder crops, medicinal plants, economic crops etc.; however, the work is restricted only to few species. Till date a total of 120 AM fungi species within 11 genera have been reported from India, as shown in Table 1. Considering the vast majority of crops and fruit production in the country, the information on the AM association appears to be far from complete and will go a long way to boost the crops and fruit production after knowing the beneficial effects of AM on host plant. Research advances are needed to improve our understanding of the physiology of mycorrhizae subjected to adverse physical and chemical conditions. Effective molecular tools have been developed to identify the AM fungi and a new monophyletic phylum, the Glomeromycota, for AM fungi has been established based on small subunit ribosomal ribonucleic acid gene sequences. It is therefore important and urgent for Indian mycorrhizasts to use these molecular tools in future studies on the studying the biodiversity and ecology of AM fungi in India.

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Morphotaxonomy of *Glomus intraradices/Rhizophagus intraradices* (accession CMCC/AM-1106)

Kiran Sunar, Priya Ahuja, Aditi Pandit, and Alok Adholeya*

Arbuscular mycorrhizal fungi (AMF) are known as very important component of soil micro-flora and interact with other microorganisms in the rhizosphere and influence soil microbial populations and other soil constituents. These are known to be cosmopolitan in nature and are found distributed in environment ranging from tropic to temperate as well as artic regions. AMF are present in the soil, in form of chlamydospores and have been recovered from the soils from a variety of habitats. India is known for its diverse ecological zones, and AMF population in its different forests and agricultural lands are unique and diverse. Some of the genera are cosmopolitan in nature and are found in almost every part of the country. Most of the research work on AMF is carried out mainly on ecology, taxonomy, phylogeny, genetics, and functional symbiosis. Initially, identification of morphological features was widely used to characterize AMF. Some of the important information about the genera or species to which the AMF belongs is derived from spore morphology, wall layers, hyphal attachment etc. However, it has been observed that many species that belong to the genus of Glomus, share some common morphological characters. In such cases, it becomes necessary to critically examine every morphological character to correctly identify the species on the basis of morphology.

In continuation to the previous series of articles regarding the morphotaxonomic studies of our successfully isolated and established AMF species, the study of an extremely well-known genus that has been successfully maintained in Centre for Mycorrhizal Culture Collection (CMCC) of TERI with the accession number CMCC/AM-1106 has been taken up in this article.

The researchers have enlisted a detailed morphological description of this accession that

was critically observed to reach to a level of morphotaxonomic identification.

Monosporal Establishment

This culture has been isolated and raised from the soils of Mukteshwar, Uttarakhand. In order to capture the mycorrhizal diversity of varying shapes and sizes, the soil sample was suspended in water and was passed through sieves of 60, 100, and 300 British Standard Size mesh sizes. The sieving from each group was collected separately and critically observed with the help of a stereo-zoom microscope. Morphologically similar looking and healthy spores were isolated. About 50 monosporals were initiated in order to obtain pure single species culture of AMF. The morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., was done on visible basis under compound microscope after preparing voucher specimens. Two kinds of mounting agents, polyvinyl lacto glycerol (PVLG) and Melzer's reagent were used for slide preparation. Monosporal are raised using single spores along with solitary pre-germinated seed of a suitable host plant grown in micro tips. These cultures are then evaluated for their success rate after an interval of three months and beyond. The culture is considered to be pure when the spores isolated from them are morophotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals. Initially, it took almost 10 months for accession CMCC/AM-1106 to reach to a high spore count and good percentage colonization state.

Spore Morphology and Shape

Spores isolated from the monosporal cultures were borne singly or in small clusters of four to eight in the

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extraradical region; they were devoid of sporocarps and each of them were suspended with a single prominent subtending hyphae. The colour of spores ranged from white, pale cream to yellow brown and sometimes light brown. The shape of the spores varied from globose to sub-globose. Scanning electron micrograph (SEM) of the spore surface revealed an outer smooth wall surface in mature spores with no any prominent pits. Each spore appeared globose suspended with distinct cylindrical subtending hyphae (Figure 1). The average spore diameter ranged from $110-(120)-150 \mu m$ (Figures 2 and 3).

Subcellular Structure of the Spore

Spores of this accession showed golden yellow colour in PVLG and golden yellow to golden red in Melzer's: PVLG. The outer layer stains pinkish red in juvenile spores.

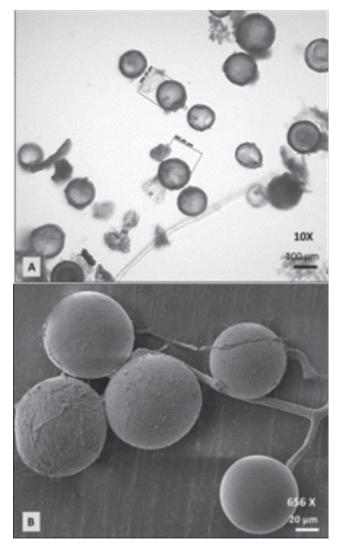


Figure 1: Compound microscopic images (10×) and scanning electron micrographs of CMCC/AM-1106 showing spores formed in small clusters with cylindrical subtending hypha

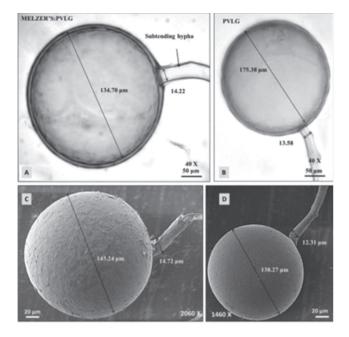


Figure 2: Compound microscopic images (40×) and scanning electron micrographs of CMCC/AM-1106 showing single globose spores with single subtending hyphae. SEM images reveal a smooth outer surface

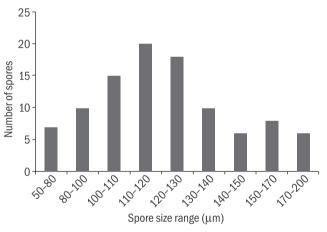


Figure 3: Analysis of spore diameter of 100 healthy spores obtained from six-month-old culture of the accession CMCC/AM-1106

The mature spore under microscope shows following subcellular structures and wall layers (Figure 4):

Spore wall is composed of three distinct wall layers (L1, L2, and L3). L1 is the outermost hyaline layer present only in the juvenile spores. L2 is hyaline and L3 is the only layer that reacts in Melzer's reagent and appear golden brown to golden red in mature spores.

Spore Wall Layer 1(L1): The outermost layer designated as L1 is hyaline and mucilaginous and stains light pink to purple in Melzer's reagent in young spores. This layer completely disappears or degrades from the mature spores. L1 in young

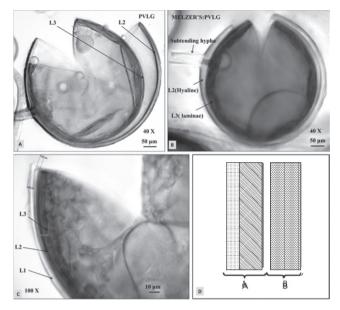


Figure 4: Compound microscopic images (40×) of CMCC/AM-1106 showing single globose spores with single subtending hyphae and distinct wall layers after mounting in PVLG (A) and Melzer's: PVLG (B). Murogram (Walker 1983) of composite wall layers of the accession CMCC/AM- 1106

spores appears granular due to accumulation of organic debris. This layer is also present in the subtending hypha of young spores.

- Spore Wall Layer 2 (L2): The second inner wall layer designated as L2 lies adherent to the outer layer L1. It appears hyaline, white to cream white in Melzer's reagent. This layer also tends to disintegrate with age in mature spores. The average thickness of this layer is 1.2- 3.5 μm.
- Spore Wall Layer 3 (L3): The only permanent and laminated layer of this accession designated as L3 lies adherent to L1 and L2 in young spores. L3 consists of sublayers or laminae that remain adhered to each other and tends to separate with the applied pressure. This layer is the only prominent layer that stains golden brown or yellow in Melzer's reagent. The thickness varies from 2.5 to 6.5 µm in mature spores. L3 is continuous with the subtending hyphae.

Subtending Hyphae and Occlusion

Most of the spores are attached to long and cylindrical subtending hyphae. The inner wall (L3) of the spore is always continuous with the inner wall (SWL3) of the subtending hyphae and is typically constricted at the point of attachment. The average thickness lies between 12.5 and 17.5 μ m (Figures 5c and 5d). In a young spore, all the three layers are continuous with the subtending hypha. The inner wall of the subtending

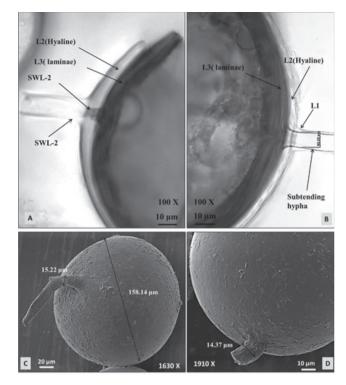


Figure 5: Compound microscopic images (100×) and SEM of CMCC/AM-1106 showing single globose spores with single subtending hyphae and distinct wall layers after mounting in Melzer's: PVLG. Both L2 and L3 extend further to subtending hyphae. There is a slight constriction of the subtending hyphae at the point of attachment

hyphae reacts slightly with Melzer's reagent and appears brownish in mature spores. The innermost sub layer L3 of the spore wall does not extend further into the subtending hyphae and usually forms an occlusion that plugs all the contents of the spore from the hyphal contents (Figures 5a and 5b).

Mycorrhizae

The accession CMCC/AM-1106 is found to be one of the most rapidly colonizing organisms. *In situ* grown *Sorghum* bicolour roots show abundant colonization where the hyphae, arbuscules, and vesicles stain dark in ink: vinegar stain. Vesicles are numerous and run parallel to the root cortical cells. The intraradical vesicle develops into spores (Figure 6).

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-1106, many distinguishing features regarding the family, genera, and the species could be derived. The following key features of this accession were noted:

 Asexual spores produced mostly in loose aggregates in abundance with spore size ranging

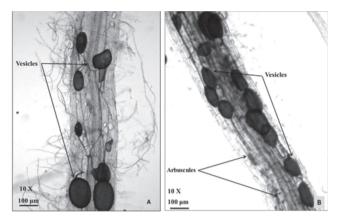


Figure 6: Compound microscopic images (10×) of roots of Sorghum bicolour colonized by CMCC/AM-1106 after three months of growth period. The vesicles are stained dark in ink: vinegar stain and runs parallel to the root epidermis. The vesicles later develop into intraradical spores.

from globose to sub globose and sometimes oval. Spore colour pale white to light yellow.

- Wall layer containing outer evanescent mucilaginous layer, a hyaline second and laminated inner layer. All the spore walls are continuous with the subtending hyphal wall.
- 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 in loose aggregates in abundance. Spore colour pale white to light yellow.
- Spores of similar shapes and sizes ranging from globose to sub-globose as well as oval with average spore size ranging from 100 to 200 µm in diameter.
- Young spores have smooth and mucilaginous outer layer, a hyaline second layer and a laminated inner wall layer. All the wall layers are continuous with the wall layers of the subtending hypha.
- Spores are suspended with a cylindrical subtending hypha that arises from the inner layer. There is a constriction at the point of attachment. Subtending hypha also consists of three wall layers and all are continuous to the spore wall layers. The inner wall layer L3 is darker than the other two wall layers.
- The innermost sub-layer of L3 of the spore wall forms a bridging structure and resembles a septum.

 Vesicles are numerous and run parallel to the root cortical cells. The intraradical vesicle develops into spores.

The described accession CMCC/AM-1106 resembles all the characters of previously characterized species of *Glomus intraradices* Schenck and Smith 1982. The type species first described was isolated from Florida, USA. *G. intraradices* is one of the most commonly occurring *Glomus* species worldwide. The isolate CMCC/ AM-1106 was originally isolated from the soils of Mukteshwar, Uttarakhand.

The current taxonomic name of *G. intraradices* is *Rhizophagus intraradices* (Schenck and Smith 1982) Walker and Schüßler comb. nov., 2010.

Systematic Classification

Glomeromycota Glomeromycetes Glomerales Glomeraceae Glomus intraradices/Rhizophagus intraradices

Most of the research work on AMF is mainly focussed on taxonomy, phylogeny, ecology, genetics, and functional properties. Initially, identification of morphological features was widely used to characterize AMF. Information about spore morphology, wall layers, hyphal attachment, etc., revealed important information about the genera and species to which the AMF belongs. However in recent times, other characterization techniques such as biochemical characterization through generation of fatty acid methyl ester profiles (FAME) and molecular characterization through sequencing of ITS region of 18s rDNA has become popular. The application of molecular tools to the identification of AMF in the field, particularly in plant roots, has revealed a hidden diversity with many detected sequences that cannot be related to known taxa. As morphological identification of AMF species based on spore and hyphal morphology is highly imprecise and the spore bank in the soil does not reflect the distribution of species in roots, the molecular identification of the fungi in plant remains the most realistic approach. Therefore, it is advised that the readers correlate their taxonomic observations with molecular data.

Acknowledgements

The authors acknowledge the contribution of Awadhesh Ram for maintenance of AMF cultures. Technical assistance provided by Chandrakant Tripathi and Yeshpal Bhardwaj during scanning electron microscopy of AMF spores is also thankfully acknowledged.

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FORTHCOMING EVENTS CONFERENCES, CONGRESSES, SEMINARS, SYMPOSIUMS, AND WORKSHOPS

Helsinki, Finland 1–5 August 2016	Eighth International Association for Lichenology Symposium (IAL8) Email: ial8@helsinki.fi Website: www/ial8.luomus.fi
Sao Paulo, Brazil 24–26 August 2016	Environmental Microbiology Conference OMICS International Conferences, 2360 Corporate Circle, Suite 400 Henderson, NV 89074-7722, USA
	<i>Tel.</i> : +1-888-843-8169, +1-650-268-9744 <i>Fax</i> : +1-650-618-1417 , +1-650-618-1414 <i>Email</i> : environmentalmicrobiology@microbiologyconferences.com
Thessaloniki, Greece 4–8 September 2016	Ninth International Conference on Integrated Fruit Production 50A Stadiou Str. 555 35 Pilea, Thessaloniki, Greece
	Tel.: +30 2310 247743, +30 2310 247734 Fax: +30 2310 247746 Email: info@globalevents.org, admin@iobc-greece2016.com, secretariat@iobc-greece2016.com Website: https://www.iobc-wprs.org
Estonia, Tartu 7–9 September 2016	Integrated Control in Oilseed Crops 2016: Meeting of the Working Group on Integrated Control in Oilseed Crops Estonian University of Life Sciences, Friedrich Reinhold Kreutzwaldi 1, 51014 Tartu, Estonia Dr Barbara Barratt, AgResearch Invermay, Private Bag, 50034 Mosgiel, New Zealand
	<i>Tel.</i> : +64 3 489 9059 <i>Fax</i> : +64 3 489 3739 <i>Email</i> : barbara.barratt@agresearch.co.nz
San Antonio, Texas, USA 12–14 September 2016	International Conference on Mycology OMICS International Conferences, 2360 Corporate Circle, Suite 400 Henderson, NV 89074-7722, USA
	<i>Tel.</i> : +1-888-843-8169 <i>Fax</i> : +1-650-618-1417 <i>Email</i> : mycology@insightconferences.com <i>Website</i> : http://mycology.conferenceseries.com/conference-brochure.php
Berlin, Germany 12–15 September 2016	XIV Meeting of the IOBC-WPRS Working Group "Biological Control of Fungal and Bacterial Plant Pathogens": Biocontrol and Microbial Ecology
	Mandy Wagner <i>Tel.</i> : +49 3641 31 16-160 <i>Email</i> : registrierung@conventus.de <i>Website</i> : www.iobc-wprsBerlin2016.de
Las Vegas, USA 19–21 September 2016	Fourth Annual Conference on Applied Microbiology OMICS International Conferences, 2360 Corporate Circle, Suite 400 Henderson, NV 89074-7722, USA
	<i>Tel.</i> : +1-888-843-8169 <i>Fax</i> : +1-650-618-1417 <i>Email</i> : appliedmicrobe@microbiologyconferences.com <i>Website</i> : http://microbiology.omicsgroup.com/america/
New Delhi , India 19–21 September 2016	Eleventh World Congress on Biotechnology OMICS International Conferences, 2360 Corporate Circle, Suite 400 Henderson, NV 89074-7722, USA
	<i>Tel.</i> : +1-888-843-8169 <i>Fax</i> : +1-650-618-1417 <i>Email</i> : biotechnology@omicsgroup.com <i>Website:</i> http://www.biotechnologycongress.com/india/conference-brochure.php
Florida, USA 25–30 September 2016	ICE 2016, XXV International Congress of Entomology, Orlando, Florida, USA Entomological Society of America, 3 Park Place, Suite 307, Annapolis, MD 21401-3722, USA
	<i>Tel.</i> : +1 301-731-4535 <i>Fax</i> : +1 301-731-4538 <i>Email</i> : info@ice2016orlando.org <i>Website</i> : http://ice2016orlando.org

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Printed and published by Dr Ajay Mathur on behalf of The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi – 110003, and *printed at* Multiplexus (India), C-440, DSIDC, Narela Industrial Park, Narela, Delhi – 110040.