MYCORRHIZA NEWS

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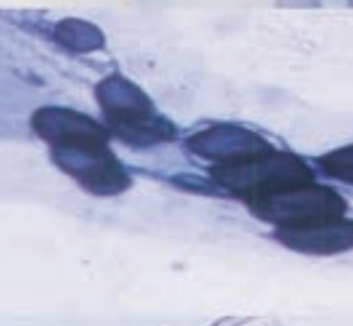

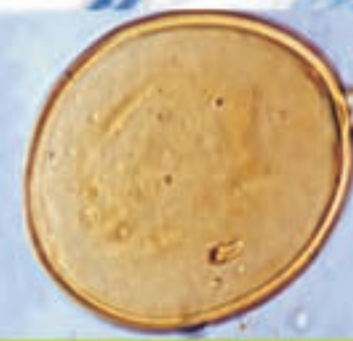

About TERI

TERI (The Energy and Resources Institute) is a dynamic and flexible organization with a global vision and a local focus. TERI's focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

TERI's Mycorrhiza Network

TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre),the CMCC (Centre for Mycorrhiza Culture Collection), 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 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

Notes on root colonization by arbuscular mycorrhizal fungi

*Sharda WK hade**

Introduction

Mycorrhizal interactions, especially Arbuscular Mycorrhizal (AM) interactions, occur in a wide variety of plants (Smith and Read 1997). For plants engaged in these interactions, the cost is in the form of organic compounds derived from photosynthesis (Fitter 1991), and the benefit provided by the AM fungi is improved uptake of water and minerals (Varma 1995), particularly phosphorous (Koide 1991). A substantial body of experimental evidence shows that root colonization by AM fungi is generally lower in nutrient-rich substrates than in nutrient-poor soils (Treseder 2004). Arbuscular mycorrhizal colonization of plants may depend on edaphic properties and environmental factors, such as precipitation and sunlight hours. Arbuscular mycorrhizal colonization is negatively correlated with total N, total P, available P and soil organic matter but positively correlated with soil pH (Lingfei, Anna, and Zhi-Wei 2005)(Kamareh, Shirvany, Matinizadeh, Etemad, and Khoshnevis 2011). However, studies that examine an array of AM fungal species from different taxonomic groups are lacking and life-history strategies for AM fungi are yet to be described (Hart and Reader 2002). A life-history strategy provides an ecological description of how an organism fulfils its life-cycle requirements (Hart and Reader 2002). A life history strategy can be described using a wide range of species traits but usually these include colonizing ability, dispersal ability, tolerance of stress and disturbance, investment into reproduction *vs* vegetative growth, and mode of reproduction (Grime 1977; Pianka 1970) (Hart and Reader 2002).

In this paper, we focus on one aspect of AM fungal life-history; namely, colonizing strategies.

Materials and methods

Samples were collected from two man-made ecosystems, *viz*., agro-ecosystem and metal contaminated ecosystem. One year fruit bearing plants of *Musa sp*. and *Carica papaya* were sampled for its feeder roots from agro-based ecosystem of Goa, India. Similarly plants from metal polluted wastelands were sampled for its feeder roots from Kanpur, Uttar Pradesh during the present study. These plants were as follows: *Calycopteris gigantea* (in flowering stage), *Cassia tora* (in flowering and fruiting stage), *Acasia auriculiformis* (in flowering stage), *Amaranthus* species (in flowering stage), *Xanthium strumarium* (in vegetative stage), *Commelina benghalensis* (in flowering stage) and *Emelia* species (in vegetative stage) with stage of development given in parenthesis. Five healthy plants were sampled per plant type. Samples were packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed. Roots were cleared and stained according to method provided by Phillips and Hayman (1970). The root samples were first washed with water and cut into 1cm bits. These root bits were cleared with 10% KOH at 15 lbs pressure in an autoclave for 15 minutes, acidified in 1N HCl and then stained in 0.05% trypan blue in lactoglycerol. The stained roots were examined under compound microscope (40X-1000X) for stages of root colonization by AM fungi. Hundred root segments for each sample were randomly selected for microscopic

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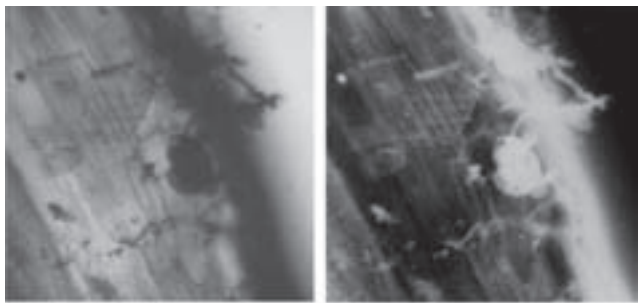
observation. For this, a single root bit was mounted in 1% glycerine on a microscopic slide and covered with 35mm cover slip. Root bit was crushed by applying slight pressure and observed under light microscope for its contents.

Results and discussion

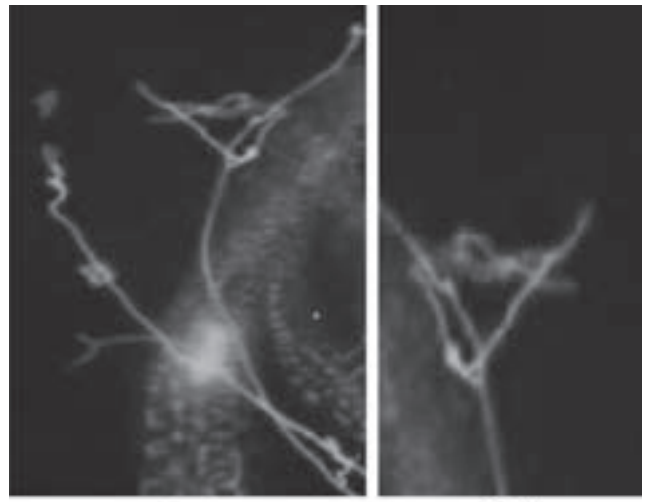
The development of AM fungi in roots can be divided into four stages (Tommerup and Briggs 1988):

1) Spore germination and hyphal growth from infective propagule of AM fungi (Plate 1): Arbuscular mycorrhizal fungal spores occur in physiologically inactive stages in soil. Their spore germinates, grows and multiplies in the presence of actively growing roots of host plants. It has been reported that, hyphae elongate 20 times more slowly in the absence of host roots than in their presence (Bècard and Piché 1989). Arbuscular mycorrhizal fungi respond to host exudates with extensive hyphal growth and branching (Giovannetti, Sbrana, Avio, Citernesi, and Logi 1993). Despite the high mycelial growth in the presence of the roots, hyphae do not always appear to exhibit "directional growth" toward the roots until they are very close to the host (Mosse and Hepper 1975). There has to be a critical distance so that the AM fungal spore can germinate and hypha can be produced towards the root surface. Warner (1980) observed the maximum root propagule distance of 2 cm for 3 propagules. But Bagyaraj (1987) showed this distance to be about 11mm, where AM fungal propagule can identify the root.
2) Growth of hyphae through soil to host roots: The mycelial system surrounding the roots is dimorphic (Mosse 1959; Nicolson 1967; Khade 1999) (Plate 2) and essentially non-septated or coenocytic. The non-septate hyphae allows a fast cytoplasmic flow in a bi-directional way, not only carrying resources from source to sink regions of the fungal colony and/or symbiotic root, but also transporting fungal organelles.



(a) (b)

Plate 1 a Spore germination and hyphal growth (× 400).
Plate 1 b Spore germination and hyphal growth (×400).
(***Note the non successful penetration of AM fungal hypahe).
(***Photographs documented from metal polluted ecosystem)



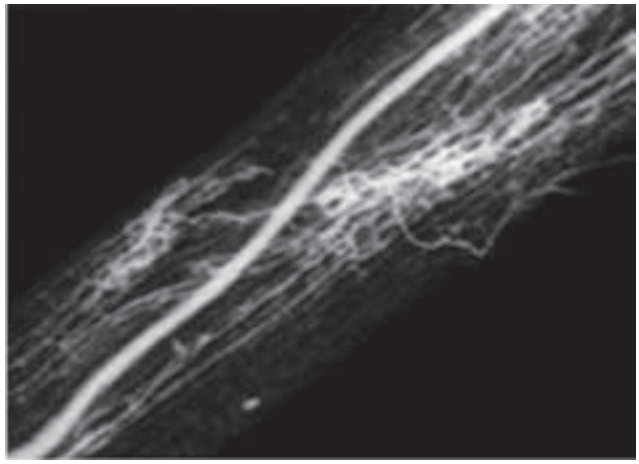
(a) (b)

Plate 2 a Dimorphic branching of AM fungal hypha (× 100).
Plate 2 b Magnified view of dimorphic branching of AM fungal hypha (× 150).
(***Photographs documented from agro-based ecosystem-Musa sp.)

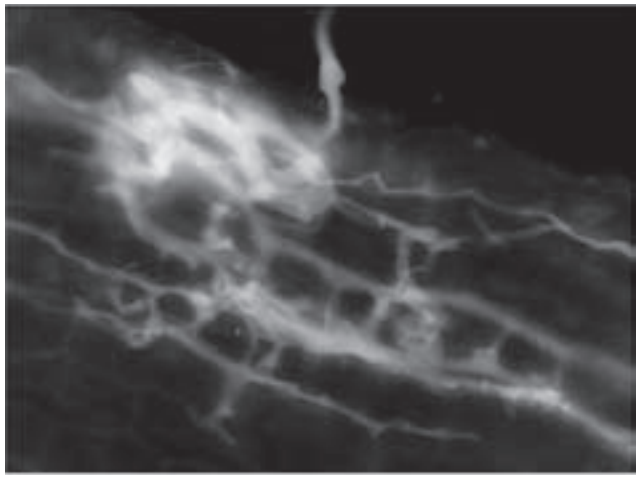
Once contact occurs, branching on the root surface takes place. The directional attraction may not be a general phenomenon, but may be more characteristic of the specific host tested (Vierheilig, Alt-Hug, Streitwolf-Engel, Mäder, and Wiemken 1998). Close observations have revealed that as the main hypha (diameter 20–30 µm) approaches a root, it puts out a characteristic fan-shaped complex of lateral branches (Giovannetti, Sbrana, Avio, Citernesi, and Logi 1993). It can be concluded that in the presence of the host, specific morphogenesis of the fungus take place, a process that the non-host plant is unable to elicit. Very recent study supports the suggestion that hyphal growth and branching are controlled by the same or a distinct regulatory signal(s) specific to the pre-infection stages (David-Schwartz, Badani, Wininger, Levy, Galili, and Kapulnik 2001). Above all, it is clear that the host plant can stimulate hyphal growth by means of different categories of signal molecule (diffusible and volatile) at several major checkpoints during fungal colonization.

3) Penetration and successful initiation of colonization in roots: Following the successful recognition events, the formation of appressoria takes place on the root epidermal cells. Penetration is characterized by localized production of wall-degrading hydrolytic enzymes

by the fungus and by the exertion of hydrostatic pressure by the hyphal tip (Bonfante and Perotto 1995). Thus, hyphae penetrates mechanically and enzymatically into the cortical cells (Kinden and Brown 1975) (Plate 3a). However, at the penetrating point the hypha always may or may not form appresorria (Abott 1982) (Plate 3b).



(a)



(b)

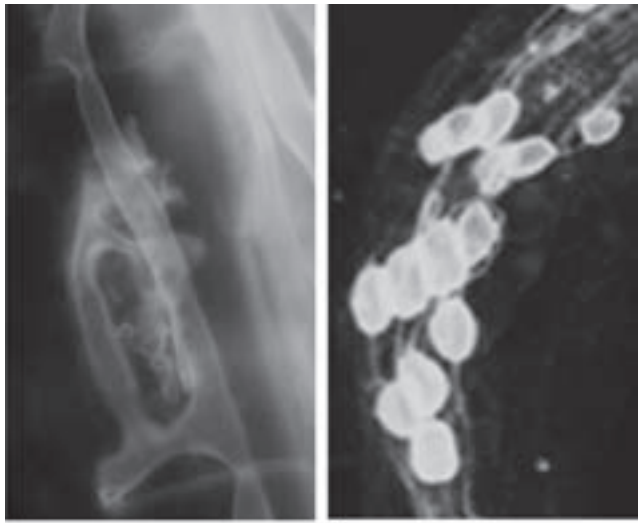
Plate 3a Successful penetration of AM fungal hyphae in the host root (× 100).
(****Note the numerous hyphal entry points inside the host root.)

Plate 3 b* Magnified view of the hyphal entry point inside the host root. Note the formation of hyphal coil at the entry point followed by subsequent ramification of hyphae inside the host root (× 1000).
(**Plate 3a photograph documented from metal polluted ecosystem; Plate 3b photograph is phase contrast version of photo published by Khade and Adholeya, 2009 from non-contaminated reference site).

4) Spread of colonization and development of internal system: As penetration and colonization of the root tissues proceeds, the host responds in a number of ways, which probably vary in different plant-fungus interactions (Smith and Read 1997). Internal colonization of the root involves the formation of intercellular hyphae, coils, and arbuscules. Arbuscules bifurcate inside the cell (Kinden and Brown 1975; Holley and Peterson 1979) (Plate 4a) and bring about nutritional transfer between two symbionts. The arbuscule is the defining specialized morphological structure hypothetically shared by all AM fungal species. Arbuscules are haustoria-like structures that are formed by profuse dichotomous hyphae branching after penetration into inner plant cortical cell walls, forming an interface between fungal tissue and the plant plasma membrane. This interface is thought to be the major site for nutrient and carbon exchanges between both partners, and it is considered to be the key structure for establishment of a functional symbiosis (Smith and Read 1997). Nevertheless, arbuscules are short-lived. In most host-fungus interactions, they degenerate within 7 to 12 days and are preferentially found in young thin roots during early stages of root colonization. However, long-lived arbuscules have also been reported in woodland plants. Thus progression of colonization requires ongoing arbuscule formation as the fungus spreads in the host roots.

Two major structural classes of AM symbiosis, *Arum* types and *Paris* types, have been identified from fungal morphological differences observed in host plant roots (Gallaud 1905). Many detailed studies have been conducted on fungal morphology of both *Arum*-type (Toth and Miller 1984; Brundrett, Piché, and Peterson 1985; Alexander, Meier, Toth, and Weber 1988; Rosewarne, Barker, and Smith 1997) and *Paris*-type colonization (Brundrett and Kendrick 1990; Cooke, Widden, and O'Halloran 1993; Whitbread, McGonigle, and Peterson 1996; Cavagnaro, Gao, Smith, and Smith 2001). Intermediate types have also been observed with both inter- and intra-cellular hyphae and arbuscules (Smith and Smith 1997). Little variation has been shown in the arbuscule formation of *Arum* type of arbuscular mycorrhiza, which consists of a single terminal arbuscule in a cortical cell apparently arising from a lateral branch of a longitudinal intercellular hypha (Gallaud 1905) and the same was recorded in the present study (Plate 4a).

Vesicles are lipid-filled sack-like structures formed within roots which develop as terminal inter-racial swelling in inter-or intra-cellular



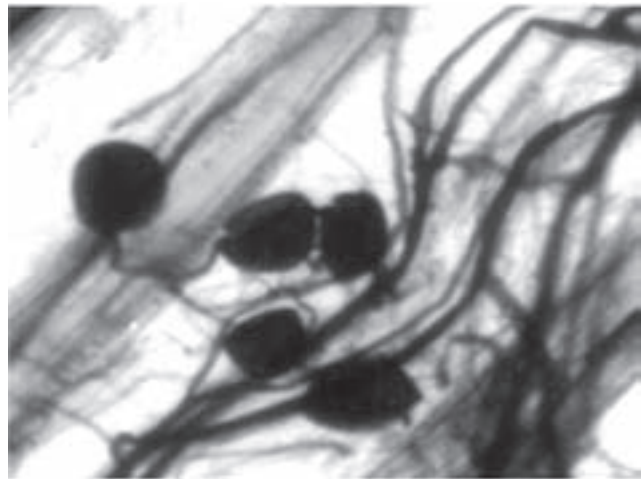
(a) (b)

Plate 4 a A single arbuscule formed inside the host root (x1000).
Plate 4 b A cluster of vesicles formed inside the host root (x 600).
(***Photographs documented from agro-based ecosystem-*Carica papaya*)

hyphae (Plate 4b). Their functions are primarily as storage organs, but they can also function as vegetative propagative structures (Biermann and Linderman 1983). Vesicles increase in numbers with the progress of root colonization.

5) Additional structures formed during the colonization of AM fungi in host roots (Khade 2003; Khade and Adholeya 2005):
   A) Auxillary cells or soil borne vesicles (Plate 5a): Arbuscular mycorrhizal fungi belonging to sub-order *Gigasporineae* do not produce vesicles inside host root but produce auxillary cells or soil borne vesicles (Khade and Adholeya 2005). Auxiliary cells are clusters of thin-walled cells which branch from extraradical hyphae of fungi in the genus *Gigaspora* and *Scutellospora* (Khade 2003; Khade and Adholeya 2005). Auxiliary cells are formed by short ramifications occurring at one or simultaneously at both sides of extraradical hyphae. Each ramification generates several branches that swell, and form clusters, which are composed of 2 to more than 20 balloon-like structures, of about 12-39 μm in diameter. Auxiliary cell surfaces have spines which are reduced to knobs as in *Gigaspora* or an almost smooth surface as in *Scutellospora* (Khade 2003; Khade and Adholeya 2005). They appear to peak at or within a short period after onset of sporulation in pot cultures (Khade 2003; Khade and Adholeya 2005). There is no evidence that auxiliary cells are functional substitutes for vesicles and available evidence speaks against an infective capacity for auxiliary cells (Biermann and Linderman 1983). It has been suggested that auxiliary cells are reminiscent of relict reproductive spores.

(a)



(b)

Plate 5 a A smooth surfaced auxillary cell of *Scutellospora* species (x 600).
Plate 5 b A cluster of spores formed inside the host root (x 300).
(***Photographs documented from agro-based ecosystem-papaya plants)

B) Intra-radical spores (Plate 5b): Spores are apparently formed when nutrients are remobilized from roots where AM fungal associations are senescing. Formation of spores completes the life cycle of AM fungi (Khade 2003). Spores are differentiated either in soil or roots (Khade 2003). Intraradical sporulation occurs abundantly in some *Glomus* species like *G. intraradices* and *G. diaphanum* (Khade 2003).

Conclusions

The life cycle of AM fungi is a plant-dependent, multiple-step process that involves recognition, signaling, and communication between the host root and the fungus. Spore germination and initial hyphal growth do not necessarily depend on the presence of the host plant (Giovannetti, Sbrana, Avio, Citernesi, and Logi 1993), but all of the subsequent processes require it (Gadkar**,** David-Schwartz, Kunik, and Kapulnik 2001). The present study recorded two stages of root colonization in soil viz., spore germination with formation of hypha (from metal polluted ecosystem) and presence of dimorphic hyphae surrounding the host roots (from banana). In the host root, numerous entry points (recorded from metal polluted ecosystem) and hyphal coil (recorded from non-contaminated reference site) with subsequent ramification of hyphae were seen. Further, in host root, the study recorded arum type of arbuscular mycorrhiza and clustered vesicles (from papaya). The study further recorded auxillary cells of *Scutellospora* species in soil (of papaya). Finally the phenomenon of intra-radical sporulation (in papaya) was also observed in host root in the present study.

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VAM–fungi formation in some water stressed plants in drier tracts of Purvanchal region

Alok Tripathi, Khalid Kafeel Khan* and N K Srivastava***

Introduction

Inoculation of crops with efficient strains of VAM fungi has been shown to improve their performance in nutritionally deficient soils in drier regions through an increased uptake of nutrients and water (Mosse, 1973, Abbott and Robson 1982, Nelson and Safir, 1982). Major portion of the Purvanchal region is dry with nutritionally deficient soils. Efficient mycorrhizal systems may improve the performance of the plants in this region. Considering that information regarding natural mycorrhizal system of the plants in the region will be helpful in formulating more efficient systems for them, the present study was undertaken. The communication reports the mycorrhizal status of 14 plants rose in the region in terms of VA-mycorrhization in the roots and AM fungal spore population in the root region.

Materials and methods

Fourteen plants *viz.*, Arandi (*Ricinus communis*), Behaya (*Ipomoea fistulosa*), Bhatkataiya (*Solanum xanthocarpum*), Dhatura (*Datura metal*), Dub (*Cynodon dactylon*), Dudhi (*Euphorbia hirta*), Gajar punna (*Croton bonplandianun*), Ganji (*Abulmoschus tuberculatus*), Gokhur (*Tribulus terrestris*), Bala (Kamraj) (*Sidaacuta*), Kansh (*Saccharum benghlense*), Latjera (*Achyranthes aspera*), Pather chotti (*Tridax procumbens*) and Rajiked (*Jatropha gossypifolea*) were included in the survey. A number of fields raising these plants were marked out at different sites spread over the region. Samples of roots of the plants with adhering soil were collected at seedling (young) and flowering (mature) stages of growth. They were washed repeatedly with sterilized distilled water and fragmented into small bits of 1 cm. The root bits were cleared in 10% KOH and stained with 0.5% Trypane blue by the method of Phillips and Hayman (1970). The stained bits were examined and the Arbuscular mycorrhization in the roots was recorded in terms of per cent root bits showing mycorrhiza formation.

The population of AM spores in the root region was estimated by extracting the spores from the root washing by sieving and decanting method of Gerdemann and Nicolson (1963). They were examined stereomicroscopically population was computed in terms of their number/50g dry soil.

Result and discussion

The data presented in the table is based on the observations made from 15 fields distributed at 5 sites (3 fields/site). They were analysed statistically by the method of Panse and Sukhatme (1985) and the minimum difference required (C.D.) for significance at 5% level has been mentioned.

The development of Arbuscular-mycorrhizal symbiosis is affected by interactions between and compatibility of its three components: the fungal endophyte, the host plant and the soil. In the present study, all the 14 plants were exposed to the same soil conditions and the same package of endophytes were available to them. However, large variations were recorded in the mycorrhizal status in terms of infections in their roots and spore population in their rhizosphere. While Dub exhibited 93% root infection, Bhatkataiya exhibited only 3% root infection. Likewise Gokhur exhibited 165 spores/50 g soil in its rhizosphere while Arandi only 11 spores/50 g soil. Plant species vary greatly in the degree of their dependence of VAM fungi which is chiefly governed by their demand for their ability to take up phosphate from soil (Hayman 1982). Thus the variation in the mycorrhizal status of different plants recorded in the present study may be safely attributed to the differences in the degree of their dependence on VA-mycorrhiza.

Many examples of lack of host specificity of AM fungi are available in literature (Berch 1987). In the present study, the indigenous AM fungi present in the soil were able to colonize all the plants and thus exhibited lack of host specificity. The present findings are in conformity with earlier reports and confirm the general belief that AM fungi find the entire potential host plants to be acceptable.

The intensity of mycorrhizal infection as well as spore population in the rhizosphere of different plants fluctuated with age of the plants. Infection level and spore number in relation to the age of the plants have been studied by a number of workers (Saif and Khan 1975; Black and Tinker, 1979; Jakobsen, 1983; and Chandra and Chatterji, 1990). Besides its innate compatibility with the symbiont many other attributes of the host, govern the development of infection and sporulation in an AM fungi symbiotic association.

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Table 1 Arbuscular Mycorrhization in the roots and spore population in the root region of different plants at seedling and flowering stages.

		Mycorrhizal Intensity (% root bits infected)		Spore Population (Number of spores/50g soil)	
Local Name of Plants	Bot. Name of Plants	Seedling	Flowering	Seedling	Flowering
Arandi	*Ricinus communis*	5	5	11	7
Behaya	*Ipomoea fistulosa*	17	20	28	37
Bhatkataiya	*Solanum xanthocarpum*	0	3	94	149
Dhatura	*Datura metal*	3	46	100	107
Dub	*Cynodon dactylon*	60	93	20	56
Dudhi	*Euphorbia hirta*	31	41	33	41
Gajarpunna	*Croton bonplandianum*	14	57	22	33
Ganji	*Abulmoschus tuberculatus*	32	45	28	36
Gokhur	*Tribulus terrestris*	11	13	100	165
Bala (Kamraj)	*Sida acuta*	10	14	19	29
Kansh	*Saccharum benghlense*	20	35	28	31
Latjera	*Achyranthes aspera*	10	36	17	32
Pather chatti	*Tridax procumbens*	21	27	19	42
Rajiked	*Jatropha gossypifolea*	0	13	50	124

Minimum difference required for significance (C.D.) at 5% level: Mycorrhizal Intensity: 3.028; Spore Population: 1.532

Amongst them the physiology and nutrient status of the plants as well as their pattern of root exudation are the prominent ones. Since these attributes are expected to change with age of the plants the extent of root infection and sporulation are bound to show variation with age (Jakobson and Nielson, 1983).

A comparison shows that a direct correlation between mycorrhizal infection and sporulation was lacking in many plants, for example, Bhatkataiya, Gokhur and Rajiked exhibited a very low level of mycorrhizal infection but a heavy sporulation. While root infection is related to the vegetative growth of the symbiont, the sporulation is related to its multiplication potential. Being divergent phases of the life the specific requirements for optimum expression of the two are expected to be different. This might have been the probable reason for the lack of a direct correlation between the order of infection and sporulation by endophyte (Kehri et al. 1987).

The findings of the present study clearly show that the soils of Purvanchal region have no scarcity of AM inoculum, however, the inoculum pool appears to be inappropriate for the plants raised in the region. Poor performance of the plants in the soils of this region is a clear indication of a lack of the efficient Arbuscular-mycorrhizal system. The results are in conformity with the hypothesis of Jenson (1982) who has stated that level of colonization of roots by the AM fungi is not directly related to the benefits offered by them.

To enable the plants to develop efficient mycorrhizal system introduction of effective stains of AM fungi in the soils of region appears to be inevitable.

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Effect of BT cotton on arbuscular mycorrhizal fungi infection under varied soil type

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Introduction

BT crops have increased rapidly worldwide in the past 11 years for economic, environmental and health benefits (Barwale et al., 2004). However, the ecological risks of BT transgenic crops were critically highlighted for potential adverse effects on agroecosystems, in particular, non-target effects on soil microorganisms (Icoz et al., 2008). Arbuscular Mycorrhizal Fungi (AMF) are important soil microorganisms providing a range of benefits to the majority of crop plants in the agro ecosystem, worthy of monitoring for non-target effects of BT transgenic crops. These BT transgenic crops may affect AMF in many ways during their life with regard to the temporal-spatial relevance between the occurrence of BT proteins and fungal symbiotic development of AMF. This may lead to an unwelcome surprise with regard to specific abundance and diversity of AMF when BT transgenic crops are planted continuously. Interactions between AMF and BT transgenic crops at individual and community level are a new urgent soil ecological issue. Some evidence about BT transgenic crop effects on AMF was revealed by different researchers. Keeping in view the above points, the research has been designed to evaluate AMF infection under both BT and non BT system and varied soil type.

Materials and methods

The study was conducted in a net-house on three different soil types at the Institute of Agricultural sciences, BHU, Varanasi (25° 19' 60 N Latitude and 83° 0' 0 E Longitude) during wet season (July to December) in 2010. BT-cotton (cvNCS-138) and its non-transgenic isoline (cvNCS-138) were grown until maturity. A no crop pot was maintained with three replications for all the three soil types.

Analysis of root sample for mycorrhiza infection

Root infection was assessed on a representative root sample taken from each plot at each harvest. Harvest roots at a depth of 15 cm were taken from plants in fixed positions and were evenly distributed over each plot. The roots from each plot sample were separated, washed free from soil and cut into l cm - 1.5 cm lengths. Root samples were stained with trypan blue (Philips and Hayman, 1970). Mycorrhizal infection of each plant was determined by estimating the per cent of root segments colonised with AM as described by Bierman and Linderman (1981). Alkaline hydrolysis of root samples with 10 % potassium hydroxide was done at 90 °C in an oven for 8-10 minutes to clear the plant cytoplasm depending upon the stiffness of the root. The roots were then washed in several changes of water and then treated with 1N hydrochloric acid for 10 minutes and ultimately stained by 0.05% tryptan blue (made in lactophenol) for about 24 hours. A minimum of 50 root fragments were examined at each time.

% root infection was obtained as follows:

$$\% \text{ root colonization} = \frac{(100 \times \text{Number of root segments infected with Arbuscular infections})}{\text{Total number of segments counted}}$$

Root samples were estimated periodically throughout the growth stages of plant. The experimental design was a factorial experiment under completely randomized block design with three replications.

Results and discussions

Root infection by AM has been presented in Table 1. The table revealed that BT-cotton affected AM during the complete life cycle. (%) Root infection decreased significantly to the extent of 10 % to 13 % in BT crop as compared to non BT counterpart and 9.5% to 15 % during the three growth stages. Although AM colonization of cotton normally progresses quickly during the first few weeks of growth reaching at a plateau of 100DAS in the range of 45% to 60% root length colonised by arsbucules. In this regard, the pattern of development of mycorrhizal colonisation in our assessment was typical and grew in a sigmoid pattern of logistic growth over 20 weeks of the assessment.

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Among the three different soils, red soil exhibited higher root infection as compared to alluvial and black soil which can be explained by the differences in available P value. AM fungi colonised both the BT and non-BT cotton cultivars equally, providing firm evidence that both the BT and non-BT cotton cultivars were equally capable of establishing mycorrhizal symbiosis. But the possibility that the gene insertions may have influenced the function of the AM symbiosis (Glandorf et al., 1997) which is reflected in the value. The lack of differences in colonisation between GM and conventional cotton that we observed corroborates that reported for GM and conventional soybean (Powell et al., 2007), but is in contrast to reports of differential mycorrhizal colonisation of GM corn (Castaldini et al., 2005 and Turrini et al., 2004).

However, if any variation in symbiotic function did occur among the cultivars, such variation was not expressed in either the level of colonisation of roots by the fungi or the growth and yield of the host.

Table 1 Root infection (%) of Cotton at different growth stages.

		Soil types(S)			
DAS	Cultivar (C)	S1	S2	S3	Mean
50	Non-Bt (V1)	35.3	19.66	21.33	24.43
	Bt (V2)	31	16.33	18.33	21.88
	Mean	33.15	17.99	19.83	
	LSD(0.05) C = 1.01 , S =1.24, C×S =1.754				
	SEm± C =0.32, S= 0.393, C×S=0.556				
100	Non-Bt (V1)	60.3	48.33	56	54.87
	Bt (V2)	54.33	44.66	51	49.99
	Mean	57.31	46.49	53.5	
	LSD(0.05) C =1.50,S=1.82,CxS=2.6				
	SEm± C=0.475, S=0.58, CxS=0.826				
150	Non-Bt (V1)	24.66	20	20.66	21.77
	Bt (V2)	22.66	17.33	17.66	19.21
	Mean	23.66	18.66	19.16	
	LSD(0.05) C =0.545 , S=0.668,CxS=0.95				
	SEm± C=0.173 ,S=0.212 ,CxS=0.301				

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Effect of *Glomus intraradices* on growth and biochemical constituents of *W. somnifera* (Ashwagandha)

Neelima Ratti and Avinash Upadhyay*

Introduction

Withania somnifera L. Dunal (Ashwagandha) is an important medicinal plant of the family Solanaceae. Its alkaloids and steroidal lactones are used in pharmaceutical industries. A green house experiment was conducted to study the influence of *Glomus intraradices* (AM fungi) inoculation on *Withania somnifera* plant. Root and shoot length, total plant dry and fresh weight, root and shoot dry and fresh weights, number of branches and leaves, chlorophyll content, protein content, kinetin content, and phosphatase enzyme activity were evaluated after 90 days growth of the plant. We observed that *Glomus intraradices* had significant effect on plant growth by increasing the value of all the parameters as compared to control plants where there was no inoculation by *G. intraradices*. Significant increase in height, number of branches, number of leaves, fresh weight and dry weight of stem, and leaf and root over untreated plants were found. *Glomus intraradices* has an effective role as bio-fertilizer on *Withania somnifera*.

Withania somnifera (Linn) Dunal, commonly known as Ashwagandha, is highly reputed in traditional Indian medicine and is one of the most extensively used plants in Ayurveda and Unani systems of medicine (Uma Devi, 1996).

Arbuscular mycorrhizal fungi develop a symbiotic relationship with higher plants and have been reported to enhance plant growth by supplying phosphate (Stoppler *et al*, 1990), nitrogen (Cliquet and Stewart, 1993), and other nutrients from the soil and translocating them to the host plant (Jensen, 1982; Smith and Gianinazzi Pearson, 1988; Raju *et al*, 1990). Mycorrhizal plants develop an extensive root system as compared to non-mycorrhizal plants, which ensures the plant of increased availability of water and nutrients, thereby helping the plant grow and develop better (Carling *et al*, 1978; Jalali and Thareja, 1985; Manjunath and Bagyaraj, 1986). Plants require large amount of P, but the available form is very low in the soil. The primary advantage of mycorrhizal hyphae in P uptake is the ability of hyphae to extend beyond the P depletion zone of the roots (Jakobsen *et al*, 1994; Jakobsen, 1995). Verma (1998) reported that mycorrhiza-treated plants showed better growth than non-mycorrhizal plants at lower level of phosphate in the soil.

Thus, the present study was done to establish *Glomus intraradices* with the root system of *W. somnifera* and to analyse the effectiveness of this mycorrhiza on vegetative growth of the plant.

Materials and methods

Plant growth conditions and methods of inoculation

Surface sterilized seeds of W. somnifera (L.) Dunal were sown in earthen tray containing sterilized soil. The tray was inoculated with 10 g of soil-based AM inoculant (*Glomus intraradices*), containing approximately 125 chlamydospores of *Glomus intraradices* at the time of sowing. Seeds sown in sterilized soil without AM fungi served as controls. Twenty seeds were sown in each tray and were grown for 30 days in glasshouse with day/night regimes of 12 h at 30 °C ± 2 °C and 45%–60% relative humidity. Germinated seedlings were transplanted in earthen pots, one plant in a pot (6 inches size) filled with autoclaved soil (pH: 8.0, EC: 0.062 ms/cm, P: 0.12%, N: 0.06%, K: 0.87%, organic C: 0.79%). The soil was collected from the experimental fields of Department of Biotechnology, Nagpur College campus where the experiment was conducted. Prior to sterilization the soil was air dried, sieved through a 20 mm sieve, and autoclaved at 121 °C for 1 h twice over a 3-day period. There were five replicates for each treatment. Plants were watered when necessary.

Roots of the seedlings were periodically checked for AM colonization by standard method of Phillips and Hayman (1970). Thirty-day-old mycorrhizal and non-mycorrhizal seedlings were transplanted in earthen pots filled with steam sterilized soil.

Effect of *G. intraradices* Schenck and Smith was studied on the plant. Plants were harvested after 90 days. Dry weight of shoot, root, and leaf was recorded after drying in an oven at 40 °C for seven days. Fresh weight of root, shoot, leaves, and height of the plants was recorded at the time of harvesting.

Analysis of phosphatase activity, protein and chlorophyll content

Assay of acid and alkaline phosphatase activity was done by the modified method of Bergmeyer (1974). Phosphatase activity was expressed as μm

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p-nitrophenol/g fresh wt/min. Protein content was estimated by the standard method of Lowery et al (1951). Chlorophyll content was estimated by the age-old method of Arnon (1949).

Extraction of crude cytokinin content

Crude cytokinin was extracted from thoroughly washed thin feeder roots of both the mycorrhizal and non-mycorrhizal plants by the method of Thiagarajan and Ahmad (1994).

Percentage root colonization and number of spores/100 g soil

The root samples were cleared using 10% KOH for 1 h at 90 °C and stained with trypan blue in lactoglycerol by standard method (Phillips and Hayman, 1970). The percentage of infected root length was evaluated by the gridline intersect method of Giovannetti and Mosseae (Giovannetti and Mosseae, 1980). Spores were isolated from the soil samples by wet sieving and decanting method (Gerdemann and Nicolson, 1963) and quantified by eelworm counting slide. Results of the experiment were analysed statistically by single factor ANOVA.

Results and discussion

It is evident from the data given in Tables (1–3) that inoculation of *Glomus intraradices* in *Withania somnifera* plants have significant effect on various physiological parameters of the plant. Plant growth, chlorophyll content, phosphatase activity, and protein content were significantly higher in the plants inoculated with *G. intraradices*. There was significant enhancement in height, number of branches, number of leaves, and root length of *W. somnifera* plants after inoculation with *Glomus intraradices*. Percent increase in height of AM inoculated plants over control was 24.94%, while it was 30% in case of root length (Table 1). Following the same trend, there was significant increase in total fresh weight and dry weight of AM inoculated *W. somnifera* plants over uninoculated (Control) plants (Table 2). Percent increase in total fresh weight of *W. somnifera* plant was 65.73% over uninoculated plants, while the increase was 61.20% in total dry weight. Fresh weight of stem, leaf, and root was 6.19, 8.26, and 3.15 g in AM inoculated plants of *W. somnifera* as compared to control plants where it was 3.74, 5.15, and 1.72 g, respectively (Table 2). Takenaga *et al* (1998) reported that AM fungi

Table 1 Effect of *G. intraradices* on growth parameters of *W. somnifera*

Sl No	Treatment	Height (cm)	No. of primary branches	No. of leaves	Root length (cm)
1	Control	34.48±2.90	1.4±0.55	32±4.95	6.0±0.38
2	AM inoculated	43.08±3.65	2.4±0.55	47±4.69	7.8±0.49
p-value at 0.05		0.0033	0.0203	0.0012	0.0002

Table 2 Effect of *G. intraradices* on fresh and dry weight of *W. somnifera* plant

Sl No	Treatment	Fresh wt (g) stem	Fresh wt (g) Leaf	Fresh wt (g) root	Total fresh wt (g)	Dry wt (g) stem	Dry wt (g) Leaf	Dry wt (g) root	Total dry wt (g)
1	Control	3.74±0.59	5.15±0.26	1.72±0.37	10.62±0.10	0.65±0.05	0.79±0.05	0.39±0.03	1.83±0.06
2	AM inoculated	6.19±0.22	8.26±0.55	3.15±0.27	17.60±0.50	1.31±0.12	1.08±0.09	0.56±0.04	2.95±0.19
p-value at 0.05		2.45E-05	2.97E-06	0.00012	1.34E-09	3.15E-06	0.0002	0.00013	1.56E-06

Table 3 Effect of *G. intraradices* on protein, kinetin content, root colonisation and spore number of *W. somnifera* plant

Sl No	Treatment	Protein content leaf (µg/mg fresh wt)	Protein content root (µg/mg fresh wt)	Alkaline phosphatise activity Root (µm p-nitrophenol/g fresh wt/min)	Acid phosphatise activity Root (µm p-nitrophenol/g fresh wt/min)	Crude kinetin content (%)	Percent root colonisation	AM spore no./100 g soil
1	Control	0.226±0.018	0.146±0.004	1.13±0.081	26.47±2.03	3.66±0.07	0.00	0.00
2	AM inoculated	0.259±0.009	0.153±0.004	1.81±0.026	31.09±1.18	5.70±0.28	63.84±2.90	188.14±10.92
p-value at 0.05		0.0077	0.0350	9.36E-08	0.00015	2.32E-07	3.28E-11	2.26E-10

Table 4 Effect of *G. intraradices* on chlorophyll content of *W. somnifera* plant

Sl No	Treatment	Chl a (mg/g fresh leaf)	Chl b (mg/g fresh leaf)	Total Chl (mg/g fresh leaf)
1	Control	0.757±0.0037	0.442±0.0126	1.212±0.0259
2	AM inoculated	0.788±0.0115	0.675±0.0046	1.482±0.0286
p-value at 0.05		0.00046	2.06E-10	2.79E-07

increased the number of leaves and flowers. Gill *et al* (2002) reported that biomass of the plant increased by mycorrhizal infection.

Acid phosphatase activity was higher than alkaline phosphatase activity in root tissue of both control as well as AM inoculated plants. Acid phosphatise activity was 17.45% higher in AM inoculated plants than the control plants. Crude kinetin content was observed to be 5.70% as compared to 3.66% in control plants (Table 3). Cytokinins have been reported to stimulate protein and chlorophyll synthesis as well as cell division and expansion in plants (Van Staden and Davey, 1979). Arbuscular mycorrhizal fungi were reported to increase cytokinin content (Allen et al, 1979). Mycorrhizal fungi improved P availability by solubilising inorganic phosphorus. Solubilization of phosphorus is mediated through release of organic acids and phosphatases (George *et al*, 1995). Mycorrhizal fungi was reported to increase both phosphatase activity and cytokinin content in cowpea roots and these biochemical changes might be responsible for improved growth of host plants (Thiagarajan and Ahmad, 1994). Mycorrhizal fungi has been reported to increase phosphatase activity in other plants also (Gianinazzi Perason and Gianinazzi, 1978). Phosphatase activity can be used as a potential indicator for the phosphorus status of the plants (Besford, 1978). Increase in acid phosphatase in the mycorrhizal plants is strongly influenced by the fungus (Dodd et al, 1987). Increased phosphatase activity was correlated with increased phosphate uptake (Capaico and Callow, 1982). It is also reported that dry weight of the shoot increased with the increase of P uptake when inoculated by *Glomus aggregatum*, *G. fasciculatum*, and *G. mosseae* (Matsubara and Sakuarai, 2000). The present data is in agreement with the above observations.

It was observed that root tissue of AM inoculated plants has prominent vesicles of AM fungi *G. intraradices.* It indicated the affinity of the fungus with the host *W. somnifera.* Percent root colonization was found to be 63.84% while number of AM spores /100 g rhizosphere soil was 188.14 (Table 3). Leaf protein level expressed as µg protein/ mg fresh weight increased in the mycorrhizal plants as compared to non-mycorrhizal plants. *G. intraradices* inoculated plants of W. somnifera exhibited higher level of protein (0.259 µg/mg) than non-mycorrhizal (0.226) plants in leaf tissue of the plant. In general, leaves of mycorrhizal as well as non-mycorrhizal plants contained high levels of protein as compared to the roots. Percent increase was 14.60% in leaf tissue and 4.79% in root tissue of AM inoculated plants over control plants (Table 3). Accumulation of protein in the mycorrhizal roots is reported in many plants and are referred to as mycorrhizins (Gianinazzi Pearson and Gianinazzi, 1989). Total chlorophyll content was found to be 22.28% higher in AM inoculated plants as compared to control plants where there was no inoculation of AM fungi (Table 4). Chlorophyll *a* was higher than chlorophyll *b*, both in control as well as inoculated plants (Table 4). Chlorophyll *a* was 4.10% higher in AM inoculated plants than in control treatment.

In conclusion, the present study indicates the potential of *Glomus intraradices* as a useful symbiont to enhance growth and other useful traits, such as protein content, phosphatase activity, and cytokinin content of W. somnifera plants, and, thus, can be exploited for use after some more confirmatory studies under field conditions.

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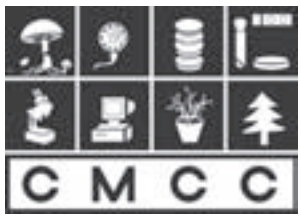

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Spores: The Solitary Morphotaxonomic signature for Arbuscular Mycorrhizal Fungi

Alok Adholeya[*] and *Chaitali Bhattacharya*[#]

During our last issue in this section we provided our readers with the vital tips of generating and handling monosporal cultures of Arbuscular Mycorrhizal Fungi (AMF). While doing so, we emphatically emphasized that one of the important steps include the scrutinization and isolation of morphologically different spores for which we require the role of an expert researcher. In order to justify our point, we wanted to provide a point of view to our readers as to why do we consider the spores of AMF as its signature and feel that appropriate selection of these miniatures is a knack.

On morphological basis, there are certain important parameters of AMF that can assist us in identifying the organism till the genus level like presence or absence of arbuscule, alignment of intraradicular hyphae, presence of auxiliary cells, etc. But these vegetative structures are not used as bonafide identification feature because of their developmental pliability and variations within different host plants. The only structure which is found to be discrete and stable is our little asexual miniatures, 'The Spores'. The spores, principally their walls, of a particular genus are found to be very specific in their structural and developmental pattern and hence, they are an important component of taxonomic description and phylogenetic analysis. Unlike other fungi, the spores formed by our AMF's are found to be very large (up to 500 µm in diameter), they can be easily trapped and the structure can be analyzed using a simple stereo zoom microscope by an amateur. A good morphotaxonomist is one who would be able to analytically evaluate the spore and mark each and every distinct point of the sample under study. As all the spores look alike for a layman, it requires an additional effort to spot the difference and hence the conclusion that the researcher has to have a decisive and critical observatory knack!

Let us share some tips that should be kept in mind while observing these little creatures to help in becoming an expert like us! Do not restrict yourself to just the spore morphology analysis as other parameters like the mode of spore formation, the sub cellular structure of the spores, the germination pattern and the color of the spores do play an important role during the process of morphotaxonomic classification. While observing varied samples, you would appreciate that the spores are present in different patterns. In most cases the spores are present outside the roots, barring a few where the spores could be inside as well. The position and production of spores could be single, in loose aggregation or in a highly organized sporocarp. Our friendly advice to the readers would be to make it a point to tabulate all the characteristic features that are witnessed while observing the spores. You would realize in the end, that the final data created from your observation will invariably assist you in identification, at least till the generic level.

Concomitant morphological and molecular analyses have led to major breakthroughs in the taxonomic organization of the phylum *Glomeromycota*. As long as the identification was based on spore morphology, spore formation and spore wall structure, the classification was very uncomplicated but filled with enigma. However, as soon as molecular phylogenetic tools became available, they were included in taxonomic analyses and soon became the drivers of the establishment of a new taxonomy with many fanciful 'new genera'. Although this article does not aim at explaining the taxonomic classification of AMF, which would be considered in our future editions, here we would provide you with the glimpses of some of the genera and its specific spore characteristics which can be noticed easily and assist amateurs in understanding the importance of spore in morphotaxonomic identification.

To begin with, in case of the genus belonging to the Glomeraceae family, it has always been seen that the layer of the spore wall is usually continuous with a

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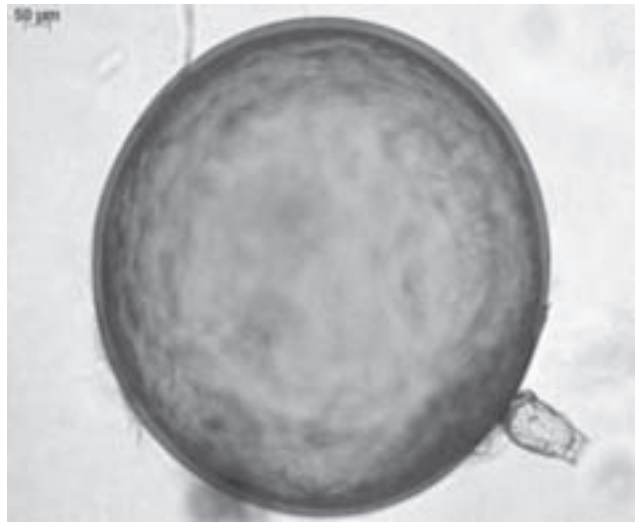
wall of the subtending hypha and the surface of spores of *Glomus* spp. may be smooth (in most species) or differently ornamented. The spores are formed either outside or less frequently within the roots as single or in aggregates, in an unorganized hyphal matrix or in a highly ordered hypha. The presence of the internal spores may be a substitute for the vesicle. The spores are normally partitioned out from the hypha by different mechanisms like amorphous plug, a septum, an inner sub layer of the laminate layer of the spore wall or thickening of all sub layers of the laminate layer of the spore wall. This group has the widest variant of phenotypes and is considered to have numerous fungi among the entire *Glomeromycota*. Hence, it is considered the most confusing one for an inexperienced person. The spores are produced blastically at the end of a sporogenous hypha although intercalarily spore formation has also been reported

The typical feature of the *Acaulosporaceae* is the presence of soporiferous saccule seen during the origin of the spore. In this case, spores are formed terminally from the neck of this saccule which ceases to grow once the spore is formed. The spores are normally sessile and mostly produced singly. The two most important genera recognized in this family include *Acaulospora* and *Entrophospora*. Spores of fungi of the genus *Acaulospora* develop laterally from the neck of a sporiferous saccule. After the saccule has become fully expanded, a spore begins to develop from the side of the subtending hypha (termed "saccule neck"). As the spore matures, the saccule loses its contents and eventually degenerates hence it is often not attached to a fully mature spore. In case of *Entrophospora*, spores develop inside the neck of a sporiferous saccule directly or at a short distance from the saccule unlike *Acaulospora*.

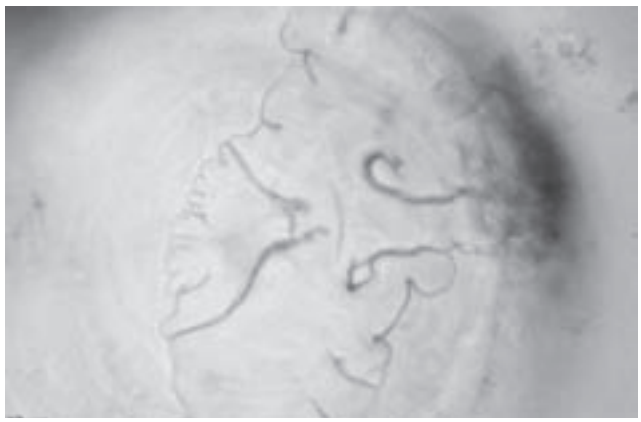
For amateurs, *Gigasporaceae* is a delight since the spores are considerably large (>200µm). The most prominent feature is the presence of a bulbous sporogenous cell formed at the end of a fertile hypha



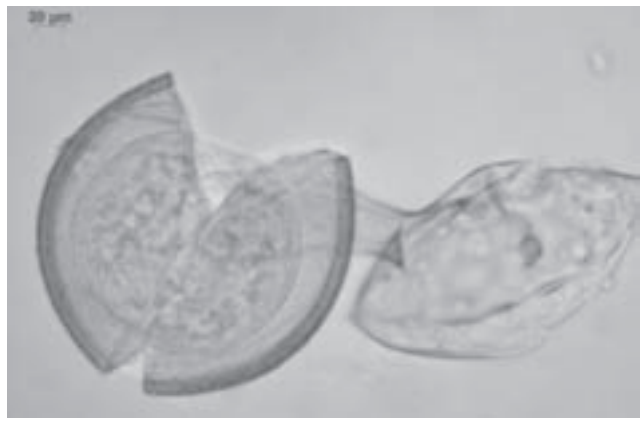
Gigaspora : Bulbous sporogenous cell

connected with mycorrhizal roots, which is partitioned from the spore by means of a plug or, more rarely, by a septum. Two prominent genera include *Gigaspora* and *Scutellospora*. Apart from spores they also form clusters of auxiliary cells. They are echinulate with spines in case of *Gigaspora* but the auxiliary cells produced by *Scutellospora* spp. are smooth or knobby. One of the unique features of *Scutellospora* is the persistent germination shield associated with the innermost flexible wall of the spore. The mycorrhizae of *Gigasporaceae* spp. consist of only arbuscules; no vesicles are produced.

Archaeosporaceae and *Paraglomaceae* are very similar to the *Acaulosporaceae* and *Glomeraceae*. The only way of confirming and determining its identity would be by molecular sequencing of the small subunit 18s ribosomal DNA using specific primers. The genus includes *Archaeospora* as in Archaeosporaceae and *Paraglomus* as in Paraglomaceae. *Archaeospora* is a dimorphic fungus, forming both acaulosporioid and glomoid spores.



Scutellospora: Germination shield



Acaulospora : Sporiferous saccule

Spores develop laterally, directly on the neck of a sporiferous saccule, and thus, they are sessile, similarly as most spores of the genus *Acaulospora*. One of the distinctive features while observing the vegetative characters is that the intraradical hyphae of the above mentioned fungi are with many coils present within and between the cortical cells and the hyphae has a patchy distribution along the roots. Similarly spores of *Paraglomus* are very similar to *Glomus* and the only visible difference is that it does not contain vesicles and their intraradical hyphae are frequently coiled within and between cortical cells unlike *Glomus* where the vesicles are very prominent and the hyphae is rarely coiled.

At the end, we are sure you would all agree to the fact that spores are indeed one of the most important components responsible for the taxonomic identification of Mycorrhiza hence they need to be analyzed with an investigative approach to reach the goal of classifying them.

Recent references

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

- *Agriculture, Ecosystems & Environment*
- *Applied Soil Ecology*
- *Ecological Modelling*
- *Field Crops Research*
- *Fungal Biology Reviews*
- *Fungal Ecology*
- *Fungal Genetics and Biology*
- *Journal of Arid Environments*
- *Journal of Hazardous Materials*
- *Pedobiologia*
- *Pedosphere*
- *Phytochemistry*
- *Scientia Horticulturae*
- *Soil and Tillage Research*
- *Soil Biology and Biochemistry*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
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