

Glomus bagyarajii Sp. Nov., A New Species of Glomaceae (Glomales, Zygomycetes) from India

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ABSTRACT

A new species of the arbuscular mycorrhizal fungus, Glomus, isolated from contaminated pot cultures of other glomalean fungi is described. The fungus, named G. bagyarajii, produces abundant bright yellow to yellow brown spores, (36-)100.8(-125) μm , with four wall layers in two groups. Group A consists of an outer, hyaline evanescent wall layer, (0.5-)3.0 (-3.5) μm thick and an inner yellow to yellow brown laminated layer, (1.6-)3.6 (-4.0) μm thick. Group B consists of an outer hyaline unit wall layer, (0.5-)1.0(-1.6) μm thick and an inner hyaline, membranous wall layer, (0.25)0.5(-1.0) μm thick. Reaction of wall layers to Melzers's reagent is negative. Subtending hypha hyaline to pale yellow, cylindrical or funnel-shaped (0.7-)10.8(-14.0) μm wide at the spore base. Germination is by regrowth of the subtending hypha.

INTRODUCTION

Glomalean taxonomy is still in the formative stages of exploration and documentation of fungal diversity because very few areas of the world have been extensively sampled for indigenous species (Morton, 1993). Only few *arbuscular mycorrhizal* (AM) species have been described from India (Gerdemann and Bakshi, 1976; Mukherji *et al.*, 1983).

Description of AM fungi is mainly based on the morphological characteristics of spores and sporocarps formed in soil (Morton, 1988; Walker, 1992). Spore wall characteristics are now recognized as the most important criteria in delimiting AM fungal species (Walker, 1983; Morton, 1988; Mehrotra and Baijal, 1994), but are dependent on the clarification of spore development (Walker 1992) and reactions to different mountants and fixatives (Spain, 1990). Studies on the changes in wall characters with spore or sporocarp development may improve definition of these characters (Giovannetti *et al.*, 1991); Franke and Morton, 1994). In addition, it has been found that anomalies in interpretation of spore characteristics can be avoided if colour, wall and germination characteristics are studied first in water and then in other mountants (Spain, 1990; Walker, 1992). Ultrastructural studies of spore

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morphology and the process of spore reproduction may be used to clarify the observations made with light microscope (Maia *et al.*, 1994). Inclusion of certain key characteristics of intraradical structures of arbuscular mycorrhiza, as an additional criteria to identify AM fungi, has also been suggested (Morton and Bentivenga, 1994), but phenotypic features of such structures have been found to be affected by host species (Brundrett and Kendrick, 1990).

The aim of this paper is to describe an unreported species of *Glomus* which produces abundant soil-borne spores in pot cultures. The species was observed as a frequent contaminant in pot cultures of AM fungi isolated from revegetated coal mine spoils.

MATERIALS AND METHODS

Pot Culture

Spores of the undescribed species of *Glomus* were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963) from contaminated pot cultures of AM fungi isolated from revegetated coal mine spoils. Few morphologically identical spores of the fungus were selected, surface-sterilized and placed singly onto the roots of sterile corn (*Zea mays* L.) seedlings. Inoculated seedlings were regularly observed for spore germination and infection of roots under a dissecting microscope. Infected seedlings were transplanted to autoclaved pots containing sterilized sandy-loam soil and sand mixture (1:1 v/v) as the growth medium. Pots were kept in a greenhouse at 25-30°C with 13 hr photoperiod. Plants were watered daily according to the requirement. Pot cultures were harvested sequentially from 4th to 24th week after transplanting to study the life cycle of the fungus and its spore development.

Measurement of mycorrhizal structures

Pot cultures were finally harvested after 24 weeks. Roots were cleared in 10% (w/v) KOH solution at 90°C and stained with 0.05% (w/v) acid fuchsin in lacto-glycerol. Stained root pieces were examined under light microscope for measurement of mycorrhizal structures. Spores and sporocarps, extracted from soil were mounted in water, polyvinyl alcohol/lactic acid/glycerol (PVLG, Koske and Tessier, 1983), mixture of PVLG and Melzer's reagent (1:1 v/v) and mixture of PVLG and cotton-blue. Spore colour was examined under a dissecting microscope on freshly collected spores immersed in water. Spore and wall dimensions were measured on at least 100 spores mounted in water and PVLG. Classification, wall descriptions and terminology follow those suggested by Morton and Benny (1990), Walker (1983, 1986), Berch and Koske (1986), Morton (1986) and Berch (1987). Spelling of scientific names are those suggested by Almeida (1989).

Voucher specimens have been deposited in the Department of Botany, University of Allahabad, Allahabad and Centre for Mycorrhizal Culture Collection (CMCC), New Delhi, India.

Germination Studies

Spores of the undescribed AM fungal species, kept at 4°C for two months, were surface-sterilized with 0.5% sodium hypochlorite for 3 min, followed by 200 ppm solution of streptomycin and washed three times in sterile distilled water. Spores were then transferred to 1% water agar plates and incubated at 24°C. Germinated spores were stained with cotton blue and observed with light microscope.

Scanning Electron Microscopy

Selected spores of the AM fungus were fixed in 3.0% (w/v) glutaraldehyde in 0.02M phosphate buffer (pH 6) at 4°C for 24 hr, dehydrated in graded ethanol series, mounted on an aluminium stub with colloidal silver paint, coated with gold and examined using a Leica stereoscan 430 scanning electron microscope.

RESULTS AND DISCUSSION

Species Description

Glomus bagyarajii Mehrotra, sp. Nov. (Figs. 1-8)

Sporocarpia hypogea, sub-globose vel irregularia, (160-320(-480) X (200-) 400(-640) µm. Peridium destitutum. Sporae singulae in solum, luteolo vel albolutea, globosae, (36-) 100.8 (-125.2) µm dia, sub-globosae vel irregulares, (56-) 108 (-126) X (49-) 90 (-104.4) µm efformatae.

Tunica sporae e stratis quatuor in turris duabus. Turra externa (Group A) e stratis duabus (strata 1 & 2); uno evanescenti, hyalino, (0.5-) 3.0 (-3.5) µm crasso; secundo laminato, fulvo ad aureobruneam, (1.6-) 3.6 (-4.0) µm crasso; turra interior (Group B) e stratis duabus (strata 3 & 4); tertio rigido, hyalino, (0.5-) 1.0 (-1.6) µm crasso; quarto membranaceo, hyalino, (0.25-) 0.5(-1.0) µm crasso. Hyphae sustentantes rectae vel curvatae, cylindricae vel infundibuliformis, (7.0-) 10.8 (-14.0) µm dia. Formans arbusculare mycorrhizae.

Sporocarpis hypogeous, sub-globose to irregular, (160-) 320(-480) X (200-) 400(-640) µm. Peridium absent. Chlamydo spores formed singly in soil, yellow to yellow brown in transmitted light, globose, (36-) 100.8(-125.2) µm dia, sub-globose to irregular-shaped, (56-) 108 (-126) X (49-) 90 (-104.4) µm. Spore lumen content with many small lipid globules.

Spore wall structure of four layers in 2 groups (A&B). Group A: wall layer 1, evanescent, hyaline, (0.5-) 3.0 (-3.5) µm thick, closely adherent to wall layer 2 in young spores, but separates in mature spores; wall layer 2, laminated, yellow to yellow brown, with 3-4 laminae, (1.6-) 3.6(-4.0) µm

thick, generally thicker near the point of attachment. Group B: wall layer 3 unit, hyaline, (0.5-) 0.1(-1.6) μm thick; wall layer 4 membranous, hyaline, (0.25-) 0.5(-1.0) μm thick.

Subtending hypha hyaline to pale yellow, straight or recurved, cylindrical or funnel-shaped, (7.0-) 10.8 (-14.0) μm wide at the spore base. Wall of subtending hypha 1-1.5 μm thick, continuous with spore layers 2 and 3.

Pore at the spore base, 2-3.6 μm wide, occluded by curved septum or by the innermost membranous wall layer. Reaction of wall layers to Melzer's reagent negative. Wall layer 3, 3 & 4 can be easily detected when stained with cotton-blue.

Distribution and habitat

To date, *G. bagyarajii*, is known from India. Origin of spores of *G. bagyarajii* is unknown. Chemical properties of the soil were pH-7.7, available P- 4.5 mg/kg, OC-0.88%, K-25.8 mg/kg.

Mycorrhizal associations

G. bagyarajii formed arbuscular mycorrhizal associations in pot cultures with *Zea mays* L., *Lonicera leucoccephala* (Lamk.) de Wit and *Cinchona citriaris* L. This species also formed intraradical spores.

Etymology

Named after D. J. Bagyaraj in recognition of his contributions to the field of mycorrhizal research.

Specimens examined

Holotype - India, Department of Botany, University of Allahabad, Allahabad. From pot cultures (Culture No. 55-16) on *Zea mays* L. Isotype: India, Deposited in Centre for Mycorrhizal Culture Collection (CMCC), Tata Energy Research Institute, New Delhi, India (Culture No. AM-1019).

Mycorrhizal Formation and Morphology

One month after transplanting, mycorrhizal infection was 55% in corn plants. Intraradical hyphae were hyaline, (3.5-)5.4(-7.2) μm . Spores formed inside the roots were globose, subglobose to irregular-shaped, (72-) 108 X 42 (-57.6) μm . Extraradical hyphae were hyaline to pale yellow, (3.5-) 7.2(-10.8) μm wide. Two months after transplanting, sporocarps were produced in pot cultures (Fig. 1). Spores in sporocarps were observed in three stages of development: (i) very young spores were hyaline, with two wall layers; wall layer 1, evanescent, roughened, 0.25-0.5 μm thick (Fig. 3) and wall layer 2 unit, smooth, 0.5-1.0 μm thick, (ii) young spores were pale yellow to yellow, with three wall layers; wall layer 1 hyaline, evanescent, 0.5-2.0 μm thick; wall layer 2 laminate, 1.6-3.0 μm thick and wall layer 3 unit, 0.25-0.5 μm thick, which may

Figures 1-3. Scanning electron micrographs of *Glomus bagyarajii*.

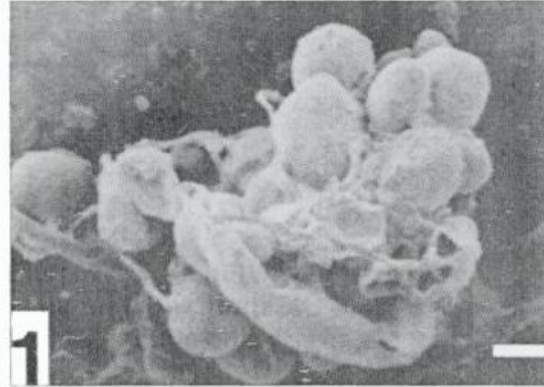


Figure 1. A sporocarp.
Scale bar = 54 μ m.

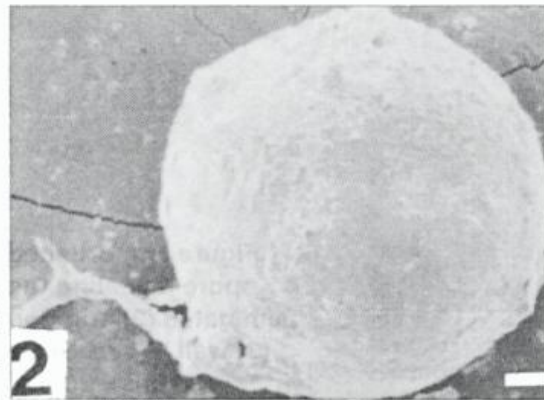


Figure 2. A spore with funnel-shaped hyphal attachment.
Scale bar = 26 μ m.

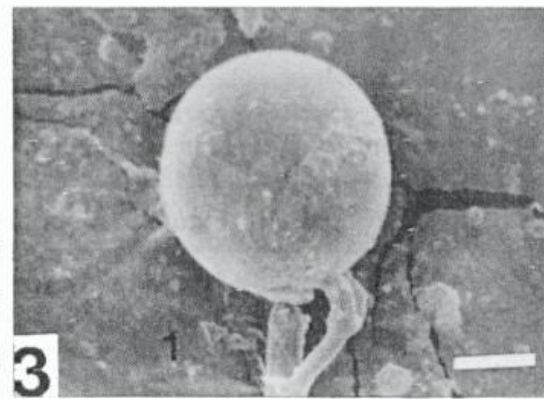


Figure 3. A young spore with sloughed off wall layer (arrow). Note the roughness of the evanescent layer.
Scale bar = 26 μ m.

sometimes appear as membranous layer (Fig.5); (iii) mature spores were yellow to yellow brown, with four distinct wall layers; wall layer 1 evanescent, 2.5-3.0 μ m thick (Fig. 4); wall layer 2 laminated, 3-3.6 μ m thick; wall layer 3 unit, 0.5-1.0 μ m thick; wall layer 4 membranous, 0.25-0.5 μ m thick (Fig 6 & 8). In *Gigaspora* spp., where direct spore

Figures 4-8. Light micrographs of the spore wall structure of *G. bagyarajii*.

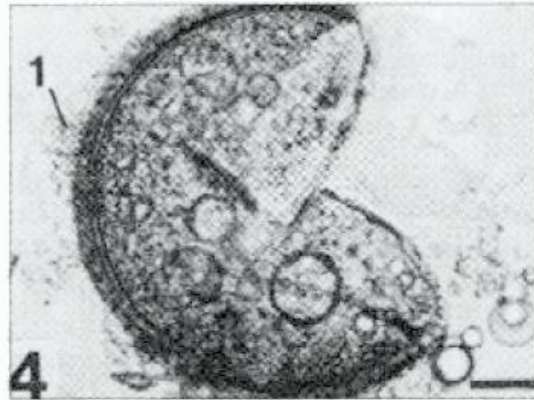


Figure 4. A mature spore with remnants of the outermost wall layer (1). Scale bar = 30 μ m.



Figure 5. A crushed spore revealing the laminated (2) and unit (3) wall layers. Scale bar = 20 μ m.

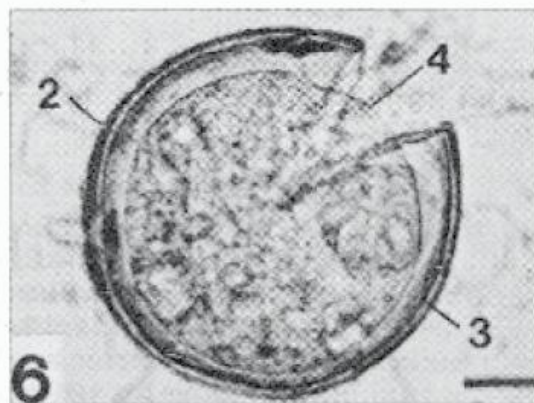


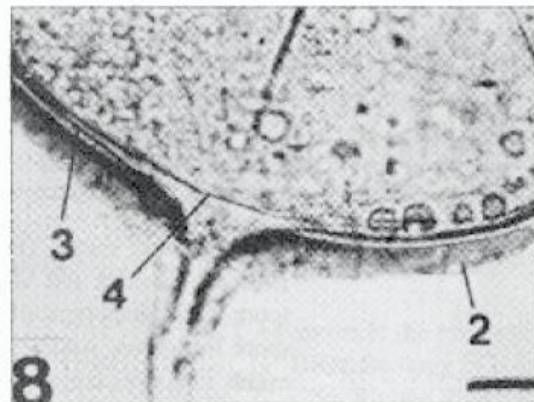
Figure 6. A spore with laminated (2), unit (3) and membranous (4) wall layers. Scale bar = 20 μ m.

germination like *Glomus* spp. occurs, spore development proceeds in a linear sequence divided into 3 stages: stage 1 - initial spore expansion coupled with differentiation of two thin layers of almost equal thickness; stage 2 - laminae added to the innermost layer; stage 3 - papillate layer differentiated on the inner surface of the laminae followed by the

Figure 7. A ruptured spore wall with evanescent, laminated and unit wall layers. Scale bar = 20 μ m.



Figure 8. A mature spore with laminated (2), unit (3) and membranous (4) wall layers. Note the occlusion of the pore at the point of hyphal attachment.



emergence of germ tube (Bentivenga and Morton, 1995). From the present study, it appears that the wall differentiation in *Glomus* spp. also takes place in three stages of spore development.

Very young spores of *G. bagyarajii* were hyaline, as has also been observed for spores of *G. ambisporum* Smith & Schenck (Smith & Schenck, 1985), *G. coronatum* Giovannetti, Avio & Salutini (Giovannetti *et al.*, 1991), *G. intraradix* Schenck & Smith (Chabot *et al.*, 1992) and *Entrophospora kentinensis* (Wu *et al.*, 1995). This indicates that most *Glomus* spp., if not all (e.g., *G. fasciculatum*, Walker & Koske, 1987), follow the same sequence of change in spore colour during early stages of spores development. It appears that the age of the spore is of decisive significance to the value of spore colour as a character for identification of AM fungal species.

Germination in *G. bagyarajii* occurred by the regrowth of the subtending hypha, as has also been reported in most *Glomus* spp. (Sequeira *et al.*, 1985); Meier and Charvat, 1992; Giovannetti *et al.*, 1991).

Ultrastructural study of *G. bagyarajii* spores showed that: (i) shape of the

subtending hypha near the spore base is either cylindrical or funnel-shaped; (ii) the outermost evanescent wall layer is roughened; and (iii) no ornamentation is present on the spore surface.

Spores of *G. bagyarajii* can be distinguished from other *Glomus* spp. by their bright yellow to yellow brown colour. Young spores of *G. bagyarajii* resemble those formed by *G. fasciculatum* (Thaxter) Gerd & Trappe emend. Walker & Koske in having yellow spores and 3 wall layers. Spores of *G. bagyarajii*, however, possess an outermost evanescent wall layer and the wall layers do not stain Melzer's reagent. In addition, mature spores of *G. bagyarajii* possess 4 wall layers. Young spores of *G. bagyarajii* might also be confused with those of *G. stunicatum*, particularly when the innermost wall layer in the former is closely adherent to the laminated layer. However, the two species can be readily distinguished by the presence of 4 wall layers in mature spores of *G. bagyarajii*. The spore colour and wall characteristics of *G. bagyarajii* are almost similar to that of *G. manihoti* Howeler, Sieverding & Schenck, but differs in having: (i) sporocarp; (ii) smaller spore diameter; and (iii) innermost membranous wall layer.

This and other studies (Franke and Morton, 1994; Giovannetti et al., 1991; Bentivenga and Morton, 1995) have shown that within a composite spore wall, phenotypically distinct layers are formed during the process of spore formation. Additional studies on the morphological and ontogenetic events in spores are required for improving the use of wall characteristics in identification of AM fungi.

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