Emergence of Germ Tube from Germination Shield in *Scutellospora verrucosa* Walker & Sanders

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Abstract

A germ tube emergence from the germ tube initial of germination shield was recorded in a spore of *S. verrucosa* isolated from *Neragamia alata* W & A (Meliaceae). The germ tube was found to be present in the inner germinal walls of the spore and it penetrated the outer wall. The germ tube emergence from germination shield can be used as a marker to determine the systematic position of *Scutellospora* genera.

Key words- Germ tube**,** *S. verrucosa*, germination shield, germ tube initial

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Arbuscular mycorrhizal (AM) fungi form a monophyletic group of obligate plant symbiotic fungi that belong to phylum Glomeromycota (Schussler et al 2001). The origin of AM fungi, based on molecular clock dating, is estimated to have occurred around 600 million years ago (Redecker et al 2000). Moreover, fossil evidence demonstrated the presence of arbuscular mycorrhizal-like fungi in the first Bryophyte-like land plants in the early Devonian, approximately 400 million years ago (Remy et al 1994; Taylor et al 1995). It is estimated that more than 80 per cent of vascular plants today live in symbiotic association with AM fungi (Smith and Read 1997).

Among the 14 AM fungal genera, *Scutellospora* accounts for approximately 17% of the described species (De Souza 2000). The genus *Scutellospora* belongs to the family Gigasporaceae, order Diversisporales and was erected following dichotomy of the genus *Gigaspora sensu* Gerd. & Trappe (Walker and Sanders 1986). All the *Scutellospora* species produce large spores (120–640 µm in diam) with multi-layered walls and a prominent germination shield. The germination shield is a specialized structure that distinguishes *Scutellospora* from the closely related genus *Gigaspora* Gerd. & Trappe (Walker and Sanders 1986) and the rest of the members of the Glomeromycota. The 'germination orb' that is known to occur in species of *Acaulospora* Gerd. and Trappe (Diversisporales, Acaulosporaceae) and *Pacispora* Oehl and Sieverd. (Glomerales, Glomeraceae) (Spain 1992; Oehl and Sieverding 2004) is more delicate, usually smaller, less complex and clearly distinguishable from the germination shield produced by *Scutellospora* species.

The complex mode of germination via the germination shield is known to occur exclusively in the genus *Scutellospora* in the lower Devonian Rhynie chert suggesting that major diversification with the phylum Glomeromycota occurred before the Early Devonian (Dotzler et al 2006). The present study reports the emergence of germ tube from germ tube initial in the germination shield of *Scutellospora verrucosa.*

Materials and Methods

The spore with prominent germ tube arising from the germ tube initial of the germination shield was isolated along with other AM fungal spores from the rhizosphere soil of *Naregamia alata* W. & A. (Meliaceae) growing wild at Uguem in South Goa region of Western Ghats. The South Goa district has an area of 1966 sq km situated between the latitudinal parallels of 15°29'32" N and 14°53'57" N and longitudinal parallels of 73°46'21" E and 74°20'11" E. The climate is tropical, warm and humid with laterite, lateritic, or clayey-loamy soil. Mean temperature ranges from 20-35C with avg. rainfall of 2500 mm. Plant identification was confirmed using the floras (Rao 1985; Mathew 1991).

A modified method of wet sieving and decanting technique (Muthukumar et al 1996) was employed to isolate the AM spores. Spores were mounted in poly vinyl-lacto glycerol (PVLG) (Koske and Tessier 1983), examined under Olympus BX 41 compound microscope and identified based on spore

morphology and wall characteristics using various bibliographies (Schenck and Perez 1990; Redecker et al 2000; Morton and Redecker 2001). Photomicrography was carried out using Olympus DP12 digital camera. Identification of spores was carried out by matching the descriptions provided by the International culture collection of Vesicular Arbuscular Mycorrhizal fungi (INVAM) (http: //invam.caf. wvu.edu.) and Glomeromycota phylogeny http://www.lrz-muenchen.de/~schuessler/amphylo/.

Results and Discussion

Original description of *S. verrucosa* is given by Walker and Sanders (1986) and we restrict our discussion only to a brief characterization of the germination shield. In *S. verrucosa*, the germination shield is hyaline to pale brown, ovoid in outline and occurs in the inner germinal wall. Its length is approximately 1.5 times the breadth. Margins of the shield have many convolutions that appear as deep warts when viewed from the side (Fig. 1 a & b).

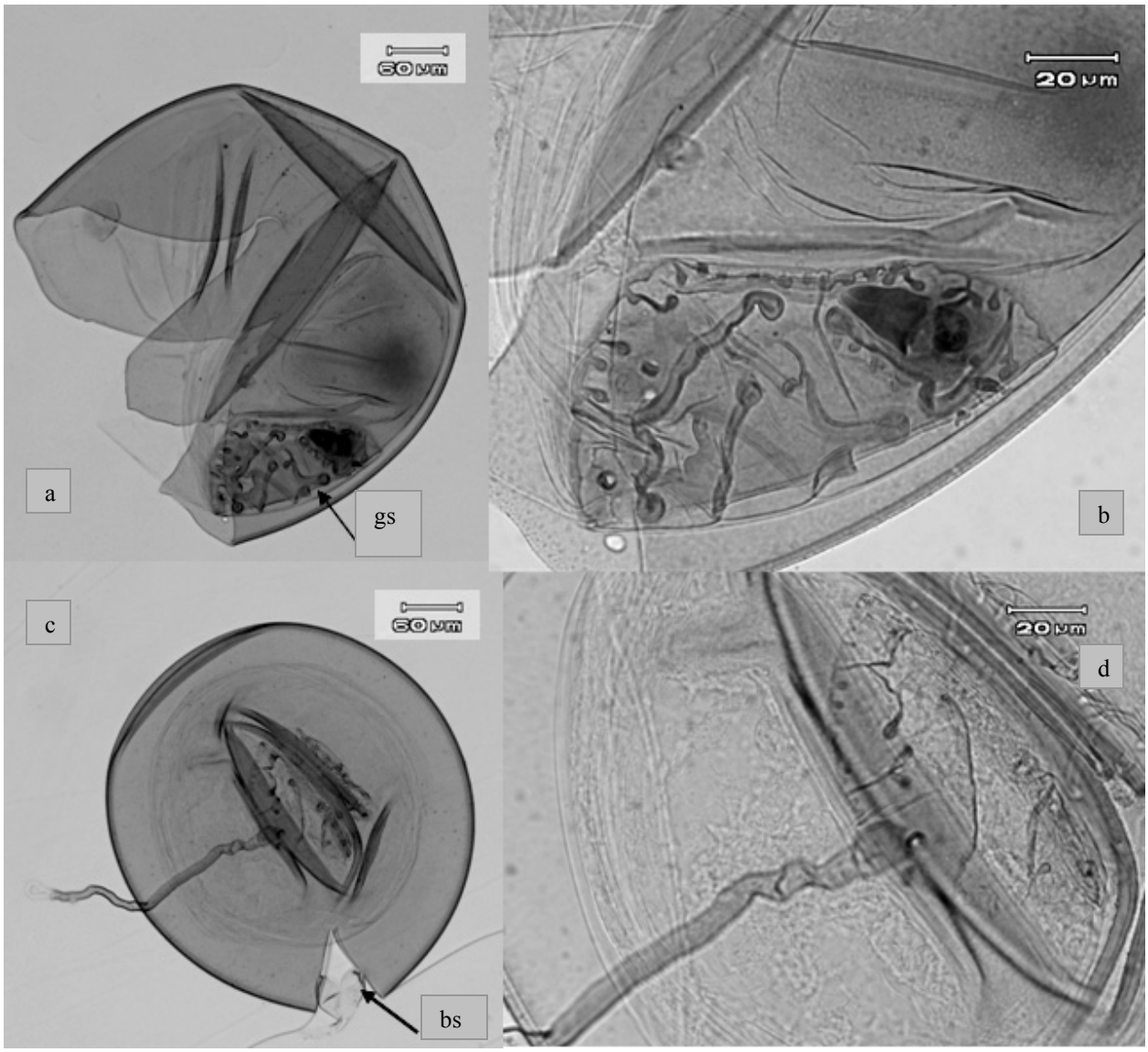

Figure 1. a = Broken spore of *S. verrucosa* with germination shield (gs); b = inner most wall layer with germionation shield (x 400); c = broken spore of *S. verrucosa* with germination shield and germ tube (gt) and bulbous suspensor (bs x 100); germ tube (x 400)

In the present study, germ tube (150 x 5.8 µm) emergence from germination shield through germ tube initials in *S. verrucosa* was observed. Germ tube is formed from the germination shield (101.5 x 72.5 µm) present in the inner flexible wall and then penetrates the outer wall group (Fig. 1 c & d). A distinct hyaline bulbous suspensor (bs) was also observed in the spore (Fig. 1 c).

Earlier, Walker and Sanders (1986) described the mode of formation of germination shield and germ tube formation in *Gigaspora calospora* (presently described as *Scutellospora calospora*). According to them, initially a hole appears in the wall of the inner wall group, through which the cell plasma membrane protrudes. The extruding membrane then folds back on itself to form the characteristic 'wishbone' formation. The plasma membrane extrudes and extends, sandwiched between inner and outer wall groups. Two lobes of germination shield are formed due to growth and development which meet and a germ tube initial is formed in each lobe from which germ tube is known to emerge.

Dotzler et al (2006) described Glomeromycotan spores from partially degraded axes of the Rhynie chert land plant *Asteroxylon mackiei* Kidst. and W. H. Lang that displayed a complex mode of germination involving the formation of a germination shield and placed it under a new genus *Scutellosporites* to designate a fossil taxon.

The present observation is the first report on the emergence of germ tube from germ tube initials in *S. verrucosa*. As germination shield represents a complex structure that consistently occurs between distinct layers of the spore wall, and this feature is regarded as derived within the Glomeromycota (Bentivenga and Morton 1996). The prominent germination shield and germ tube emergence from germ tube initial described in the present study corresponds to germination shield produced by Gigasporaceae genus *Scutellospora* as a diagnostic marker to determine the systematic position.

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