

## ULTRASTRUCTURE OF HYPHAE IN GIGASPORA ALBIDA Schenck & Smith (GLOMALES)

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**Key words:** cytology, nucleus, arbuscular mycorrhizae, endomycorrhizae, *Glomales*.

### SUMMARY

Studies were performed to describe the ultrastructure of hyphae in *Gigaspora albida*. There is an even distribution of cytoplasmic organelles with nuclei interspaced with vacuoles and polyphosphate granules in the hyphae. Evidences indicated that nuclear condition changes after germ tube formation. No clear proof of meiotic or mitotic processes were found, but elongated nuclei, presence of nucleus associated organelles, and a line of division in the central region of some of the nuclei were demonstrated.

### INTRODUCTION

Arbuscular mycorrhizal fungi are obligated symbiotic organisms which have an important role in natural agroecosystems since they constitute a key component of the rhizosphere (Lindermann, 199) and increase plant growth (Mosse & Hayman 1980). The new order *Glomales* (*Zygomycotina*), erected to include these fungi (Morton & Benny, 1990) is formed by six genera where sexual nature of the spores is not clear. Species of the genera *Gigaspora* form spores on bulbous suspensor like cells. Gerdemann & Trappe (1974), described these spores as azygospores, since they appeared to be formed on parthenogenetic gametangia. The azygosporic nature

### RESUMEN

[Ultraestructura de la hifa en *Gigaspora albida* Schenck & Smith (*Glomales*)]

Se efectuaron estudios para determinar la ultraestructura de las hifas en *Gigaspora albida*. Se observó una distribución regular de los organelos citoplasmáticos, con núcleos interespaciados con vacuolas y granos de polifosfato. Las evidencias indicaron que la condición nuclear cambia después de la formación del tubo germinativo. No se encontró ninguna prueba de procesos meióticos o mitóticos, pero se observó la presencia de núcleos alargados, una línea divisoria en la región central de algunos de éstos y organelos asociados al núcleo.

was accepted by some authors (Schenck & Pérez 1990; Morton & Benny 1990), while others considered that once the teleomorphic or anamorphic nature is not known they should be called simply spores (Walker, 1987). Tommerup & Sivasithamparam (1990), mentioned these spores as conidia. A different and smaller spore, formed by *Gigaspora decipiens* Hall & Abbott was described as zygospor by Tommerup (1988).

Understanding of reproductive events and nuclear behavior are limited in *Zygomycetes*, especially in *Glomales*. Studies of ultrastructure to clarify the processes of spore formation, hyphal development and nuclear cycle are difficult mostly due to problems with fixation, staining, and embedding of arbuscular mycorrhizal fungi



(Mosse, 1986; Maia et al. 1993a). Consequently, most of the studies have been centered in wall structure or anatomy of the endomycorrhizal interaction, which are easier to process for electron microscopy. A few papers reported the germination process on spores of some *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora* species (Gibson, 1985; Maia et al., 1994; Meier & Charvat, 1992; Mosse, 1970a, 1970b; Old et al., 1973; Sward, 1981b).

During ultrastructural studies on germ tubes of *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe; Meier & Charvat (1992), found numerous fusoid nuclei with nucleoli, elongate mitochondria, and both osmiophilic and osmiophobic lipid droplets. The multinucleate condition of spores, hyphae, auxiliary cells, vesicles and arbuscules in different species of *Glomales* have been described (Bonfante-Fasolo et al., 1981; Burggraaf & Beringer 1987; Cooke et al., 1987; Mosse 1970a; Scannerini & Bonfante-Fasolo, 1975; Sward, 1981b). However, the nature of the nuclei, whether homokaryotic or heterokaryotic needs to be determined. With TEM studies, the significance of the multinucleate state could be understood. This would demonstrate possible relationships among members of *Glomales* and the other *Zygomycetes*. Knowledge of nuclear structure in fungi has greatly increased with TEM studies even considering known methodological limitations (Beakes, 1981). The objective of this research was to describe ultrastructure of hyphae in *Gigaspora albida* Schenck & Smith. Nuclear phenomena were studied; wall and cytoplasmic organelles were described and compared with that found in other *Glomales*.

## MATERIALS AND METHODS

### Cultural procedures

An isolate of *Gigaspora albida* (GABD 927) from the International Culture Collection of VAM fungi (INVAM) was pot-cultured in a medium consisting of a mixture of 3:1 (v/v) sand and vermiculite (Liyanage, 1989), associated with Bahia grass (*Paspalum notatum* Flugge). The pot cultures received Long Ashton nutrient solution (Hewitt, 1966) every other day and were maintained in a growth room with artificial mercury light (400 to 600  $\mu\text{mol cm}^{-2} \text{sec}^{-1}$ ) of 1 h length, with a temperature range of 26°C, night and 28°C, day. Spores were isolated from pot cultures by wet sieving and decanting (Gerdemann & Nicolson 1963), followed by centrifugation in 40% (w/v) sucrose (Jenkins, 1964) and were allowed to germinate before fixation.

### Germination

Selected spores of GABD 927 were washed in water, and separated. A group was superficially disinfected with 0.5% NaOCl (10% Chlorox) for 2 min, while the other group was not disinfected. All spores were placed in petri dishes with 1% water agar + thiamine at 0.1 mg/L<sup>-1</sup> and allowed to germinate. Plates were incubated at room temperature (25°C) in the dark. Spores were observed every other day and were considered germinated with emergence of a germ tube.

### Transmission electron microscopy

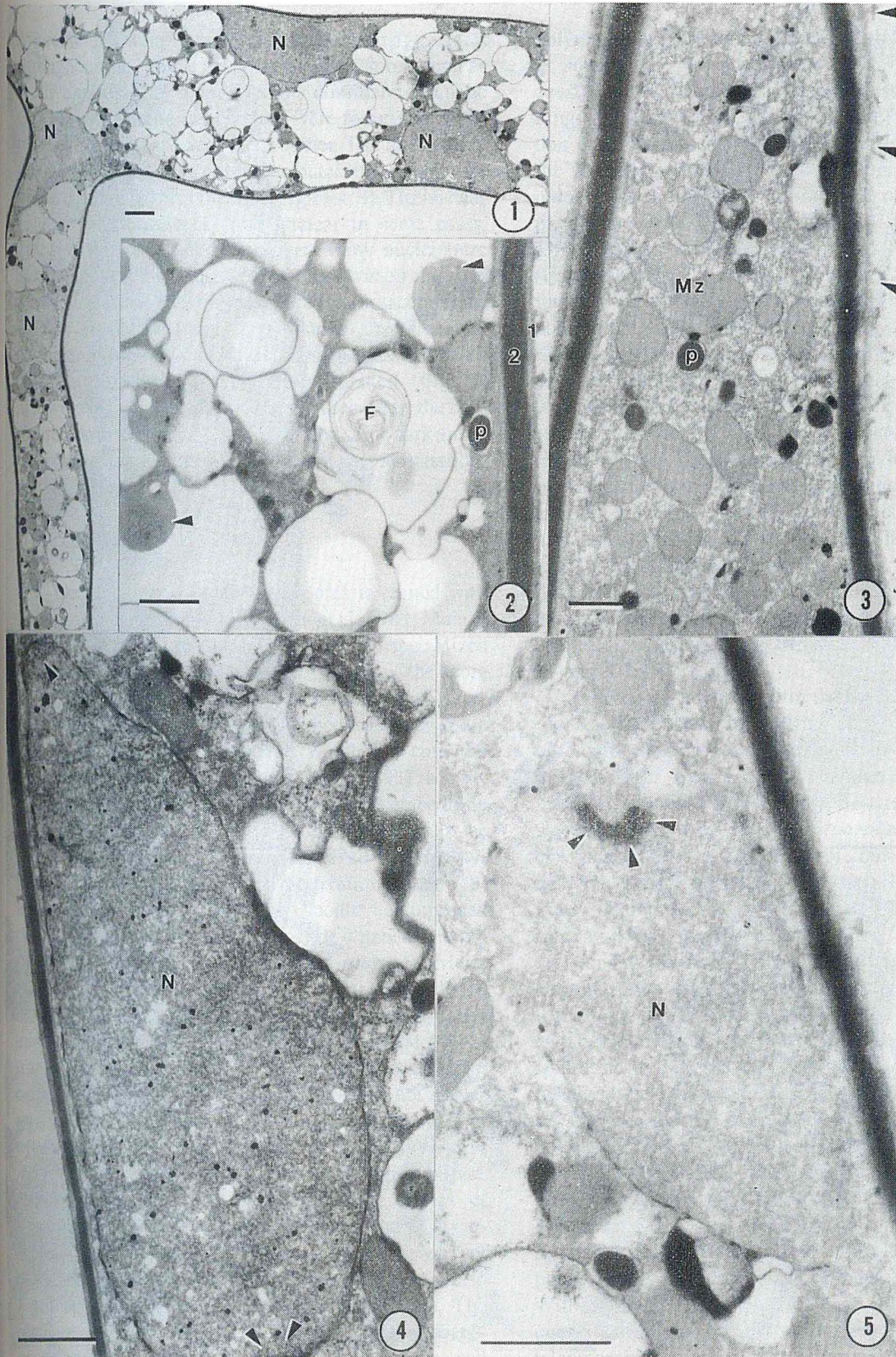
Germinating spores and hyphae were embedded in molten 2% agarose. Narrow strips of agarose containing a few germinated spores and hyphae were cut with a razor blade and processed for fixation. Fixatives used were 7.2% glutaraldehyde for 2 h at room temperature or a solution of 2% glutaraldehyde plus 2% formaldehyde, made from paraformaldehyde in 0.2 M sodium cacodylate buffer, overnight at 5°C. After postfixation in 1% osmium tetroxide and dehydration in an ethanol series followed by acetone (Benny & Aldrich 1975), specimens were embedded in ERL 4206 resin (Spurr, 1969).

Embedded material was incubated at 60°C for 48 h, for two days, for polymerization. Blocks were sectioned with a diamond knife on an LKB ultramicrotome and ultrathin sections collected on one hole formvar-coated copper grids. Sections were post-stained for 40 min in 2% (w/v) aqueous uranyl acetate followed by 5 min in Reynolds (1963) lead citrate, and observed with a JEOL-100CX electron microscope at 60 kV.

## RESULTS

The hyphal wall (Fig. 1, 2) is comparatively thinner than that reported for the spore wall, which has many laminated layers (Maia et al., 1994). The hyphal wall consisted of only two layers, the inner thicker and electron dense, the outer thinner and with adherent particles (Fig. 2). A broader view of the hyphae indicated an even distribution of cytoplasmic organelles with nuclei fairly evenly interspaced with vacuoles and polyphosphate granules (Fig. 1). The vacuoles were of various sizes and shapes, with presence of content variables (Fig. 2). Sometimes, vacuoles seemed to enclose cytoplasmic structures such as electron dense globules, myelin figures, and polyphosphate granules (Figs. 1-3).





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Mitochondria were isolated or distributed in aggregates throughout the hyphae. In some hyphal regions such as the hyphal tip, they seem to be the dominant organelle (Fig. 3). Mitochondria generally were round, but some were elongated, especially near nuclei. The nuclei were polymorphic and generally distributed throughout the hypha or localized near the wall (Figs. 1, 4, 8). The nuclear contents and envelopes were unclear in some cases, probably due to fixation problems. Many nuclei were elongated, as if they were undergoing division (Figs. 4-8). Some electron dense bands were seen adjacent to the nuclear membrane (Figs. 5, 8), and in one specimen condensed chromatin and microtubules associated with division could be vaguely distinguished (Fig. 7, arrowheads). A clear division was observed in the central region of certain nuclei (Fig. 9) and some dark areas, interpreted as condensed chromatin were seen distributed in the nuclei.

## DISCUSSION

Germination of *Gigaspora* spores is related with formation of a germinal wall (Spain et al., 1989; Maia et al., 1994). The germ tubes emerge from specialized compartments within the innermost layers of the spore wall, usually in the region near the subtending hyphae. In general many germ tubes are formed from each spore in *G. albida* (Maia et al., 1994), which is a common phenomenon in *Gigaspora* spores (Koske, 1981; Mosse, 1959). Several techniques for fixation and embedding were attempted before satisfactory results could be obtained in *G. albida* and it was observed that for hyphae the traditional gluteraldehyde fixation works well; however for spores technology should be improved (Maia et al., 1993a). Many of the same organelles found in *G. albida* were reported in *Glomus mosseae* (Meier & Charvat, 1992). Most of the vacuoles enclosed cytoplasmic structures such as polyphosphate granules and electron dense globules. Meier & Charvat (1992) interpreted the electron translucent vacuoles as being osmiophilic lipid droplets. However, they do not appear to accumulate the electron dense material reported in hyphae of some species of *Glomus* (Maia, 1991) and in *Acaulospora laevis* Gerdemann & Trappe (Mosse, 1970b). The large crystals reported in spores of *Acaulospora laevis* (Mosse, 1970b, 1970c) and *Gigaspora margarita* Becker & Hall (Sward, 1981a) were not observed in *G. albida*. Absence of such crystals was also noted in *G. mosseae* (Meier & Charvat, 1992). The pigment granules reported by Mosse (1970c) probably are different from those in *G. albida*, which are suspected of being polyphosphate granules (Gibson 1985).

Polyphosphates are found in fungi both as granules and as soluble polyphosphate (Martin et al., 1983), and constitute the primary phosphorus reserve for many fungi (Beever & Burns, 1980). Tinker (1975), suggested that polyphosphate containing vacuoles are carried by the streaming cytoplasm and continuously load and unload their contents, to balance the concentration of phosphorus in the cytoplasm. Later, within the internal mycelium, the polyphosphate disappears from the vacuoles (Cappacio & Callow, 1982), indicating it has been, transferred to the host. We found no evidences to confirm this observation. Elongated nuclei as found in hyphae of *G. albida* were also a consistent feature in hyphae of *G. mosseae* (Meier & Charvat, 1992). Such nuclei or those with blebs, may correspond to anaphase stages or can result from the flow of the cytoplasmic streaming (Beakes, 1981). The electron dense bands adjacent to the nuclear membrane, appear to be grazing sections of the spindle pole bodies, also called nucleus associated organelles or NAOs (Beakes 1981; Heath 1981a, 1981b).

In most *Zygomycetes*, the nuclear envelope remains intact during the division process and the NAOs are truly intranuclear (Beakes, 1981). In some *Mucorales*, NAOs are associated with the inner nuclear membrane (Bland & Lunney, 1975).

Dark areas distributed in the nucleus are interpreted as condensed chromatin. The phenomenon of chromatin condensation occurs normally during mitosis in most fungi (Heath, 1980). Features observed in the nuclei of *G. albida* are similar to those reported in other *Zygomycetes*, especially *Mucorales*. There, the chromosomes are not recognizable during mitosis, the nucleolus is persistent, and the NAOs are attached to the inner membrane of the nuclear envelope (Bland & Lunney, 1975).

In *Zygomycetes*, vegetative nuclear divisions are definitely mitotic (Beakes, 1981). Heath (1980) suspects that *Mucorales* may have the most primitive type of eukaryotic division. The mitotic process is simple, with spindle developing entirely from intranuclear NAOs and nuclear envelope remaining intact. *Glomales* probably have also a simple process of undergoing mitosis. Studies of meiosis are not common, particularly in *Zygomycetes* (Beakes, 1981), and in *Glomales* there is no proof that it occurs. Using morphological features, Tommerup & Sivasithamparam (1990), considered small spores formed by *G. decipiens* Hall & Abbott as zygospores. However, cytological evidence still is missing. Without evidence of synaptonemal complexes or the quantification of nucleic acids, the anamorphic or teleomorphic nature of *Glomales* spores will remain unresolved. However, recent data obtained from studies



with *Glomus versiforme* (Karsten) Bench, have shown that duplication of DNA does not occur in the dormant spores but only during the fungus hyphal phase (Bianciotto et al., 1993).

In order to demonstrate evidence of fusion or division processes in arbuscular mycorrhizal fungi, spores may need to be fixed periodically at successive stages. Also, better methods for fixation, staining and embedding of nuclear structures must be developed. Standard fixation and staining processes did not provide good enhancement of nuclear structures. Many of the fixation processes used for TEM produce artefacts or do not preserve certain specific structures (Beakes, 1981; Maia et al., 1993a). Extensive serial sectioning is necessary for obtaining a complete picture of the nucleus and its NAOs, and this is more difficult in fungi where nuclear divisions often are

very rapid (Beakes, 1981). More studies will be necessary to disclose the secret of formation of spores in *Glomales* and their sexual nature, and descriptions of nuclear condition in hyphae could be a way to understand this process.

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FIGS. 1-5. Transmission electron micrographs of developed germ tubes of *Gigaspora albida* (GABD 927). 1. General view of hypha showing distribution of nuclei (N) and other organelles. Scale bar = 1  $\mu$ m. 2. A hyphal segment with well defined 2-layered wall (1,2), containing vacuoles with apparent myelin figures (F) and others with opaque bodies (arrowhead) and polyphosphate granules (P). Scale bar = 0.5  $\mu$ m. 3. Detail of mitochondrial zone (Mz) in a hypha with many polyphosphate granules (p). Outer wall is sloughing (arrowheads). Scale bar = 0.5  $\mu$ m. 4. An elongate nucleus located near the hyphal wall, with electron dense, unidentified material (arrowhead) near its poles. Scale bar = 0.5  $\mu$ m. 5. Nucleus (N) with electron dense band (arrowheads) adjacent to the nuclear membrane, that may be nucleus associated organelles (NAOs). Scale bar = 0.5  $\mu$ m

FIGS. 6-9. Transmission electron micrographs of a hypha of *Gigaspora albida* (GABD 927). 6. An elongate nucleus (N). Scale bar = 0.5  $\mu$ m. 7. Nucleus with microtubules (arrowheads) and condensed chromatin (c). Scale bar = 0.5  $\mu$ m. 8. General aspect of a hypha with a nucleus localized near the wall. Putative NAOs are positioned adjacent to the nuclear envelope (arrowhead). Scale bar = 0.5  $\mu$ m. 9. A nucleus (N) which appears to be dividing in the central region of a hypha (arrow). Scale bar = 0.5  $\mu$ m.