Chapter 1
Fungal Spore Germination and Pre-symbiotic Mycelial Growth – Physiological and Genetic Aspects

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Abstract Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, living symbiotically in the roots of most land plants. They form spores in the soil, which are able to germinate and grow, but are unable to complete their life cycle without establishing a functional symbiosis with a host plant. In this chapter, results of recent studies providing new insights into the main developmental switches occurring in the fungal organism, from the relief of spore dormancy to the development of germlings and growth arrest in the absence of the host, are reviewed. The knowledge of environmental, cytological, biochemical and molecular events involved in early stages of AMF life cycle may reveal how these obligate symbionts compensate for the lack of host-regulated spore germination, possibly representing a strong selective disadvantage. Diverse scientific approaches showed multiple survival strategies, active during pre-symbiotic mycelial growth, contributing to the survival of AM fungal individuals and populations.

Keywords Arbuscular mycorrhizal fungi • Spore dormancy • AMF life cycle • Spore germination • Pre-symbiotic growth • Germling growth arrest • Host signals • Survival strategies • Ancient asexuals • Gene expression

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1 Introduction

Arbuscular mycorrhizal (AM) fungi (AMF) are obligate biotrophs, which live symbiotically in the roots of about 80% of plant species. Most AMF form spores in the soil which are able to germinate and grow from a quiescent-like state in response to different edaphic and environmental conditions, but are unable to produce extensive mycelia and to complete their life cycle without establishing a functional symbiosis with a host plant (Mosse 1959; Hepper and Smith 1976). The key developmental switches occurring in the fungal organism, from the germination of an individual spore to the formation of an extensive hyphal network in the soil, involve a sequence of morphogenetic events represented by: spore germination and pre-symbiotic mycelial growth, differential hyphal branching pattern in the presence of host roots, appressorium formation, root colonization, arbuscule development, extraradical mycelial growth and spore production (Giovannetti 2000).

The lack of host-regulated spore germination, contrary to what happens with many pathogenic biotrophic fungi, could have represented a strong selective disadvantage. Nevertheless, AMF are considered evolutionary successful "living fossils", having survived and evolved for 460 millions years, their ancestral nature having been shown by diverse fossil records and DNA sequence data (Simon et al. 1993; Remy et al. 1994; Phipps and Taylor 1996; Redecker et al. 2000a, b). Their persistence indicates that they must have evolved efficient strategies to overcome the lack of spore germination regulation and to allow the survival of individuals and populations (Logi et al. 1998; Giovannetti et al. 2000; Giovannetti 2002).

The aim of this chapter is to review recent developments which contributed to our understanding of cellular and molecular events involved in the early stages of the life cycle of AMF, from relieving spore dormancy and triggering spore germination to germling growth and growth arrest in the absence of the host.

2 Spore Dormancy

The phenomenon of spore dormancy has concerned researchers since Godfrey's early studies on spore germination (Godfrey 1957). As early as 1959, Barbara Mosse suggested the storage of collected spores on damp filter paper at 5°C for 6 weeks in order to obtain the regular germination of resting spores of an *Endogone* sp. (presumably *Glomus mosseae*) (Mosse 1959). Eighty percent of spores treated in this way germinated within 3–4 days. The problem of erratic spore germination has been mentioned in many reports, and in 1983 Tommerup gave a clear-cut definition of spore dormancy, making a distinction between dormancy and quiescence (Tommerup 1983a). A dormant spore was defined as one failing to germinate when exposed to physical and chemical conditions which support germination of apparently identical spores, defined as quiescent spores. Differences in cytoplasmic organization between young and old resting spores were described in *Acaulospora laevis* and in *Glomus* species: in dormant spores the oil globules enlarged at the expense of the cytoplasm, which was restricted to small interstitial spaces (Mosse 1970a, b;

Meier and Charvat 1992; Maia and Kimbrough 1998). A fine network of cytoplasmic material interlaced between large lipid droplets was also described by Sward (1981a) in dormant spores of *Gigaspora margarita*.

The relief of dormancy by storage was reported by many authors. Hepper and Smith (1976) found that spores of *G. mosseae* from freshly harvested sporocarps germinated slowly compared to spores detached from sporocarps and stored at 6°C for 5 weeks. The same results were obtained with a North American isolate of *G. mosseae*, which showed a marked difference in germinability between freshly isolated and 10°C-stored spores (Daniels and Graham 1976). Diverse species of the genus *Glomus* exhibited spore dormancy, such as *Glomus intraradices, Glomus clarum, Glomus caledonium, Glomus monosporum* (Hepper 1979; Tommerup 1983b; Louis and Lim 1988; Douds and Schenck 1991; Juge et al. 2002). Other species, such as *Glomus coronatum*, showed erratic germination even after cold treatments lasting 1 year (Giovannetti et al. 1991).

A marked dormancy was shown by spores of *A. laevis*, which germinated after 6 months storage in two different experimental conditions (Tommerup 1983a; Gazey et al. 1993). Other species within the genus *Acaulospora* exhibited the same behaviour: in a laboratory experiment only a small proportion of spores stored for 2 months germinated, while most spores germinated well after storage for 4–6 months (Gazey et al. 1993). Similarly, *Acaulospora longula* showed complete relief of dormancy after 8 weeks storage at 23°C in soil (Douds and Schenck 1991).

Not all the species and genera of AMF show spore dormancy. Spores of *Gigaspora gigantea* collected throughout the year from sand dunes did not show any dormancy, and were able to germinate as early as 1 day after incubation, either when they had been surface sterilized or not (Koske 1981a), while newly formed spores showed a period of endogenous dormancy (Gemma and Koske 1988). Germ tubes of *G. margarita* emerged after 72 h incubation on water agar or within 3–5 days on agar media without any storage treatment (Sward 1981c; Siqueira et al. 1982). Similarly, spores of *Scutellospora fulgida* and *Scutellospora persica* did not possess any dormancy, showing mycelial growth and the formation of auxiliary cells after 2 weeks in the dark at 24°C (Turrini et al. 2008).

Propagule dormancy may contribute to the survival of AMF in adverse environments, but despite many different experimental reports on spore dormancy of many species of AMF a complete understanding of the phenomenon has not been obtained. We still do not know whether dormancy is more species or genus than isolate correlated, because experiments have often been carried out on different isolates. Moreover, no studies have been performed on the molecular bases of dormancy: we ignore whether it may be affected by the presence of compounds in young spores, which inhibit germination, or by the occurrence of compounds in mature, old spores, which enhance germination.

3 Triggers for Spore Germination

The molecular signals which relieve spore dormancy and activate the cell cycle still remain unknown, though different environmental conditions triggering the initiation of germination in genera and species of AMF have been investigated. In fact,

resting spores of many AM fungal species germinate both in soil and in agar under adequate physical, chemical and microbiological conditions.

Many germination factors have been identified which play important roles in growth activation of quiescent spores. Although complex interactions among different factors probably play the most important role in spore germination in nature, many investigators have studied germination factors such as pH, temperature, moisture, mineral and organic nutrients, host plants, and microorganisms as if they were independent triggers, and as such they will be considered here.

3.1 pH

Differences in spore germination among species and genera are often related to the environment where the endophytes live and to which they are ecologically adapted (Sylvia and Williams 1992; Clark 1997). For example, spore population surveys from different sites showed that *A. laevis* is the predominant AM fungus in low pH soils (Abbott and Robson 1977), or even the only species in soils at pH < 4.9 (Nicolson and Schenck 1979). Also data from INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, http://invam.caf.wvu.edu/) collection showed that 88.5% of *Acaulospora* isolates live at soil pH < 6.0 (Morton et al. 1993). Accordingly, results obtained in experimental conditions demonstrated that spore germination of *A. laevis* is strongly regulated by soil pH, being optimum between 4 and 5, decreasing at pH 6, and declining to less than 10% between pH 6.5 and 8 (Hepper 1984b). Similar results were exhibited by *Gigaspora coralloidea* and *Gigaspora heterogama* isolated from acidic soils, which germinated best at pH from 4 to 6 (Green et al. 1976). Other authors reported that *G. margarita* is less sensitive to acidic conditions than *G. mosseae* (Siqueira et al. 1984).

An isolate of *G. mosseae*, collected from a wheat field, showed a pH optimum for spore germination between 6 and 9 in water or in soil extract agar, and was not able to form germ tubes at pH 4 and 5 (Green et al. 1976). Another strain of the same species, isolated from an agricultural soil, failed to germinate at pH 4.5 (Mosse and Hepper 1975). Other species of the genus *Glomus* germinated best at pH ranging from 6 to 8 and were capable of producing root infection and multiplying in very alkaline soils (Daniels and Trappe 1980; Giovannetti 1983; Douds 1997).

The thorough surveys of Sieverding (1991) confirmed that *G. mosseae* does not occur in natural tropical soils with pH < 5.5. Thus, it may be that edaphic factors related to the environment from which the different species of glomeromycotan fungi were originally isolated play an important role in spore germination and presymbiotic hyphal growth (Giovannetti and Gianinazzi-Pearson 1994). However, it may also be that the optimal pH values attributed to each species are actually characteristic of the isolates used in each experiment and cannot be applied to all the isolates of the species. Each isolate originating from a specific environment could in fact represent an ecotype adapted to peculiar soil characteristics. This could apply in particular to *G. mosseae*, which has been shown to occur in

55 different countries throughout all continents and biomes (Avio et al. 2009). Different geographic isolates of the same species should be used to obtain new evidence on this point.

3.2 Temperature

The germination of AM fungal spores is greatly affected by temperature and the limits for germination exhibited by different species have been ascribed to their fundamental dissimilarity. Tommerup (1983b) reported that three fungal species isolated from the same source possessed different temperature limits for germination: *A. laevis* germinated best at temperature ranges of 15–25°C, *Gigaspora calospora* at 10–30°C, and *G. caledonium* at 10–25°C.

Some studies have suggested that many differences among glomeromycotan fungi in temperature ranges affecting spore germination reflect the differences in the environments from which the fungi were isolated. Accordingly, two Florida isolates of *G. coralloidea* and *G. heterogama* germinated best at 34°C, while *G. mosseae*, isolated from more northern latitudes, showed maximum germination at 20°C and failed to germinate at 34°C (Schenck et al. 1975). Also, an isolate of *Glomus epigaeum* from cool climates showed maximum germination at 22°C (Daniels and Trappe 1980). Most rapid germination of spores of *G. gigantea* was obtained at 30°C, whereas no germination occurred at 15°C, and only 6% spores germinated at 35°C (Koske 1981a).

It is interesting to note that the lethal exposure times to 60°C for *G. caledonium* and *A. laevis* spores were 5 min and 1 min, respectively (Tommerup and Kidby 1980). Viability of *G. intraradices* and *G. mosseae* was nil beyond 60°C, and that of *Glomus deserticola* beyond 54°C (Nemec 1987). Interestingly, an isolate of *G. intraradices* showed a great tolerance to 45°C for up to 24 h (Bendavid-Val et al. 1997).

Temperature optima for germination may be related to the environment to which each endophyte is indigenous. The demonstration of this requires the germination of different strains of the same species isolated from geographical areas with very dissimilar climates.

3.3 Moisture

Soil water content can have variable effects upon spore germination of species and genera of AMF. *G. margarita* spores germinated independently of soil water content, while germination of *G. intraradices, G. mosseae* and *A. longula* was strongly inhibited by matric potentials between −0.50 and −2.20 MPa (Douds and Schenck 1991). Other authors reported that spore germination of *G. epigaeum* and *G. gigantea* was increased at soil moisture near field capacity or above (Daniels and Trappe 1980; Koske 1981a). Three other *Glomus* species, *Glomus macrocarpum, G. clarum* and

G. etunicatum, showed tolerance to soil drying, maximum germination occurring at matric potential of −0.01 MPa (Sylvia and Schenck 1983). *G. epigaeum* spores germinated well when soil moisture content ranged from field capacity to soil saturation, and no germination was observed below −3.4 MPa (Daniels and Trappe 1980), while *G. gigantea* showed delayed germination at −1.0 Mpa (Koske 1981a).

As noted above for pH and temperature, differences in spore germination of AM fungal species and genera are often related to the moisture conditions of the environment to which they are ecologically adapted. No general conclusions can be made without knowing germination responses of several isolates of a species, each from environments with widely different moisture regimes, to different soil matric potentials. Moreover, it is probable that soil wetting and drying cycles are the most important factors affecting survival, germination and thus infectivity of AMF in nature; in particular in Mediterranean climates where glomeromycotan spores survive the hot and dry summers to colonize young emerging plants during the following seasons (Braunberger et al. 1996).

3.4 Mineral and Organic Nutrients

The germination of AM fungal spores is inconsistently affected by mineral nutrient content of soil. *G. gigantea* spores germinated at the same rate regardless of phosphorus concentrations (5–500 ppm) in sand plates (Koske 1981a). Germination of *G. mosseae* and *G. caledonium* spores was not affected by phosphorus concentrations in agar up to 30 mM, while above this level germination was reduced by 56% or more (Hepper 1983). Similar results were obtained with *G. margarita*, whose spores germinated well up to 16 mM phosphate solution (Tawaraya et al. 1996a) and with *G. epigaeum* spores, whose germination was not influenced by increasing levels of NH_4NO_3 and K_2SO_4, up to 200 ppm (Daniels and Trappe 1980). However, when phosphorus was added to soil, spore germination of different species of AMF decreased with soil P increments (De Miranda and Harris 1994). Investigations on the role of inorganic sulphur-containing compounds on the growth of *G. caledonium* mycelium showed that it was stimulated by the presence of thiosulphate, metabisulphite, sulphite and sulphate in the medium (Hepper 1984b).

Some inorganic ions completely inhibit spore germination of AMF. Hepper and Smith (1976) found that the inhibitory effects of different agar media on germination of *G. mosseae* spores was due to Mn and Zn. Toxicity of heavy metals such as Cu, Mn and Zn also affected spore germination of *G. caledonium* (Hepper 1979).

Germination and hyphal growth of different AM fungal species, evaluated in acidic soils with varying Al saturation, showed that most species of *Gigaspora* and *Scutellospora* were more tolerant than *Glomus* species (Bartolome-Esteban and Schenck 1994). However, no generalization is possible, since different isolates within a species showed varying responses to heavy metals (Gildon and Tinker 1981; Weissenhorn et al. 1993).

Studies on the effects of salinity on AM fungal spores showed inhibition of germination and hyphal growth by increasing concentrations of NaCl (Hirrel 1981; Estaun 1989; Juniper and Abbott 1993, 2006; McMillen et al. 1998). However, Koske et al. (1996) reported on the ability of *G. gigantea* spores to retain germinability upon exposure to natural conditions of immersion in sea water and stressed the importance of this feature for the dispersal of the species in coastal waters.

A range of organic substrates such as glucose, fructose, sucrose, L-arabinose and aspartic, succinic, malic, pyruvic acids, reduced germination and germ tube growth of *G. mosseae* spores (Siqueira et al. 1982). Accordingly, their germination was inhibited by excess nutrients, such as those provided by Potato Dextrose or Nutrient Broth Agars (Daniels and Graham 1976). However, hyphal growth of *G. mosseae* was stimulated by tartaric acid (Mosse 1959), and peptone, yeast extract, thiamine, cystine, glycine and lysine showed great growth promoting effects on *G. caledonium* hyphae (Hepper 1979; Hepper and Jakobsen 1983). Recent findings also reported that a growth stimulant isolated from the brown alga *Laminaria japonica* increased hyphal growth of *G. margarita* sterile spores germinated in vitro (Kuwada et al. 2006).

3.5 Host/Non Host Plants

Early germination trials showed that AMF are able to germinate in axenic culture in the absence of the host (Godfrey 1957; Mosse 1959; Hepper and Smith 1976; Powell 1976; Koske 1981a). Thus, host-derived signals do not represent essential factors for spore germination of AMF. Accordingly, the presence of growing host roots did not trigger the relief of spore dormancy in different AM fungal species (Tommerup 1983a). Nevertheless, host roots, crude or purified root exudates and compounds derived by their fractioning positively affected spore germination and germling growth in different experimental conditions, depending on both plant and AM fungal species (Graham 1982; Bécard and Piché 1989; Gianinazzi-Pearson et al. 1989; Nair et al. 1991; Tsai and Phillips 1991; Giovannetti et al. 1993a, 1994, 1996; Suriyapperuma and Koske 1995; Tawaraya et al. 1996b; Buée et al. 2000; Nagahashi and Douds 2000; Scervino et al. 2006). The key compounds exuded from host roots able to induce hyphal branching in AMF, strigolactones, stimulated spore germination in *Gigaspora rosea, G. intraradices* and *Glomus claroideum*, and increased mitochondrial density and respiration in *G. intraradices* (Tamasloukht et al. 2003; Besserer et al. 2006). Modulation of AM fungal spore germination was also reported in the presence of exudates of mycorrhizal and non mycorrhizal host roots and of their differential flavonoid components, which showed species-specific effects (Scervino et al. 2005a, b). Detailed information on fungal responses to host-derived signals is given later in this volume (Chapters 2 and 4).

It is interesting to note that transgenic plants may or may not affect AMF life cycle, since the experimental works showed different results depending on the type of genetic modification and gene product expressed. Elfstrand et al. (2005) reported that the constitutive 35S-driven expression of *Mtchit* 3-3, a class III chitinase gene

in *Medicago truncatula* root-organ cultures, was associated with stimulation of spore germination of *G. intraradices* and *Glomus constrictum*, suggesting that the *Mtchit* 3-3 gene product might directly act on the walls of AM fungal spores. Actually, one gene belonging to class III chitinases was specifically induced in mycorrhizal *M. truncatula* (Salzer et al. 2000; Bonanomi et al. 2001).

Root exudates of both non host and ectomycorrhizal plants often showed no effects on spore germination (Daniels and Trappe 1980; Azcón and Ocampo 1984; El-Atrach et al. 1989; Gianinazzi-Pearson et al. 1989). Nevertheless, contradictory fungal behaviours were reported, in in vitro and in vivo experiments (Ocampo et al. 1980; Glenn et al. 1985; Parra-Garcia et al. 1992; Schreiner and Koide 1993a, b; Giovannetti and Sbrana 1998). The release of inhibitory compounds by non-hosts was reported by different authors (Vierheilig et al. 2000; Roberts and Anderson 2001; Oba et al. 2002; Bainard et al. 2009), and a heat-labile factor able to reduce *G. gigantea* and *G. intraradices* germination and growth was detected in root exudates of a non mycorrhizal tomato mutant (David-Schwartz et al. 2001, 2003; Gadkar et al. 2003).

3.6 Microorganisms

Although several species of AMF germinate well in axenic culture, some growth stimulation by soil and rhizosphere microorganisms has been reported (Mosse 1959; Watrud et al. 1978; Daniels and Trappe 1980; Azcón-Aguilar et al. 1986; Azcón 1987, 1989; Gryndler et al. 2000; Scervino et al. 2008; Pivato et al. 2009). The mechanisms of such activity remain unknown. Many laboratory experiments indicated that different bacterial isolates may affect spore germination and hyphal extension. For example, *Streptomyces orientalis* stimulated germination of *G. mosseae* (Mugnier and Mosse 1987), diverse field isolates of *Streptomyces* spp. increased germination of *G. margarita* by production of volatile compounds (Carpenter-Boggs et al. 1995; Tylka et al. 1991), and *Klebsiella pneumoniae* increased hyphal extension in *G. deserticola* germlings (Will and Sylvia 1990).

Differential effects of factors released by *Bacillus subtilis*, *Mesorhizobium mediterraneum* and a PGPR strain on *G. mosseae* and *G. rosea* spore germination and growth was reported by Requena et al. (1999). *G. mosseae* spore germination was not affected by bacteria, whereas a fungistatic effect was evidenced in *G. rosea* when challenged with a strain of *B. subtilis*, although such strain was able to induce hyphal growth enhancement in *G. mosseae*.

Several saprophytic fungi isolated from *G. mosseae* sporocarps decreased or did not affect germination of *G. mosseae* spores on water agar (Fracchia et al. 1998). By contrast, the soil fungus *Trichoderma* spp. enhanced the development of mycelium from germinating spores of *G. mosseae* (Calvet et al. 1992). A recent study reported the increase of *G. rosea* hyphal length in the presence of exudates of *Drechslera* sp., a common fungal endophyte isolated by the inner cortical cells of the grass *Lolium multiflorum* (Scervino et al. 2009).

Gram-positive bacteria (*Paenibacillus* spp. and *Bacillus* spp.) were found associated or attached to fungal hyphae (Artursson and Jansson 2003), and among them *Paenibacillus validus* induced the production of new spores of *G. intraradices* grown in plates in dual culture in the absence of the host (Hildebrandt et al. 2002, 2006).

Different taxa of microbes are associated with spores collected from the field, which may remain contaminated even after surface disinfestation procedures (Mayo et al. 1986; Ames et al. 1989; Walley and Germida 1996). Investigations on the role played by such resident microbial populations are very interesting. For example, spore-associated bacteria, including *Pseudomonas* and *Corynebacterium*, enhanced germination of *Glomus versiforme* spores in vitro, confirming that this fungal species germinates best under non-sterile conditions (Mayo et al. 1986). Other bacteria were intimately associated with the outer spore wall of *G. clarum* (Walley and Germida 1996), or embedded in the electron-dense spore wall of *Glomus* species (Filippi et al. 1998; Maia and Kimbrough 1998), confirming previous reports on the occurrence of chitin-decomposing microorganisms in washed, healthy spores of *G. macrocarpum* (Ames et al. 1989). Recent PCR-DGGE analyses showed that bacterial species associated with spores of *Glomus geosporum* and *G. constrictum* belonged to taxonomic groups known to degrade biopolymers (*Cellvibrio, Chondromyces, Flexibacter, Lysobacter*, and *Pseudomonas*) (Roesti et al. 2005), suggesting that such microbes, being able to digest the outer walls of AMF, mainly composed of chitin, may aid spore germination.

In the family *Gigasporaceae*, spores originating from different geographic areas were shown to harbour intracellular symbionts belonging to β-proteobacteria (Bianciotto et al. 2000, 2003), which could possibly affect germination, since an isolate of *G. margarita*, cured of its endobacteria, showed delayed germling growth (Lumini et al. 2007). Actually, previous results showed that germination frequency of *G. decipiens* spores was significantly enhanced by diverse intracellular strains of *Burkholderia vietnamiensis*, but not by *Burkholderia pseudomallei* (Levy et al. 2003).

4 Modes of Spore Germination

Glomeromycotan fungi germinate in different ways depending on the genus. Spores of most *Glomus* species germinate by regrowth from the end of hyphal attachments (Godfrey 1957; Mosse 1959). Many germ tubes may emerge from the old subtending hypha, as in *G. clarum*, or a single one, as in *G. mosseae* and in *G. caledonium*. Some *Glomus* species, such as *Glomus viscosum*, germinate after forming a balloon-shaped swelling at the broken end of the subtending hypha (Godfrey 1957; Walker et al. 1995). By contrast, germ tubes of *Gigaspora*, *Scutellospora* and *Acaulospora* species emerge directly through the spore wall. Though, different germination structures can be formed depending on the genus. A simple structure is produced by *Gigaspora* spores, which germinate after a papillate layer has formed in the inner

part of the spore wall. Light and electron microscopy studies of this mode of germination were performed in *G. margarita* (Becker and Hall 1976; Sward 1981b, c). In other genera inner (or germinal) walls are involved in germination, with the formation of specialised structures usually on the outer surface of the innermost wall. Species of *Scutellospora* develop germination shields (Walker and Sanders 1986) whose morphology has been recently used to taxonomically revise the family *Gigasporaceae* (Oehl et al. 2008). A different structure, described in some species of *Acaulospora* and *Kuklospora*, was termed "germination orb" (Spain 1992) since it differs morphologically from *Scutellospora* shields, while persisting after germination. The cellular events leading to spore germination in *A. laevis* were monitored at the ultrastructural level, by means of sequential sampling of spores incubated in conditions allowing germination (Mosse 1970a, b). Germination structures were described as dense peripheral compartments, containing cytoplasm and many nuclei, from which germ tubes arose and pushed through the outer layers of the spore wall. Some *Pacispora* species are known to develop *Glomus*-like spores with germination structures morphologically similar to *Scutellospora* shields, but differing from them, since they are delicate, deteriorating over time and therefore difficult to discern (Walker et al. 2004; da Silva et al. 2008). Distinctive simpler germination structures occur in spores of *Archaeospora trappei* and some *Ambispora* species (Spain 2003; Spain et al. 2006; Goto et al. 2008).

Multiple germination can be defined as the abilily of fungal spores to germinate several times by producing successive germ tubes when those formed previously are severed from the parent spores (Koske 1981b). This capacity was described in spores of a *Glomus* sp. (Mosse 1959), and later studied in *G. gigantea*, whose spores were able to germinate up to ten times over a period of 50 days, after their germ tubes had been severed (Koske 1981b). Multiple germination may be considered an additional strategy to increase the probability of successful infection of a host root by germinating spores of AMF.

5 Development of Pre-symbiotic Mycelium

After germination, hyphae generally follow a forward, linear growth, with a strong apical dominance and regular, right-angled branches. Hyphae are thick-walled, aseptate, about 5–10 μm wide, and contain many nuclei (Fig. 1a, b).

Cytoplasm and nuclei can be easily observed migrating along two directions in hyphae originating from spores during germination (Mosse 1959). Ultrastructural studies confirmed these early observations, obtaining clear evidence of a swirling motion of the cytoplasm, and suggested redistribution of spore cytoplasm into the germ tube (Sward 1981c). Two-photon fluorescence microscopy and video-enhanced microscopy allowed the detection of nuclei moving along hyphae originating from germinated spores of *G. rosea* and *G. caledonium*, respectively (Bago et al. 1998; Logi et al. 1998). Such movement could be a microtubules (MT)-dependent process, since in *G. mosseae* germlings nuclei were always detected in close association

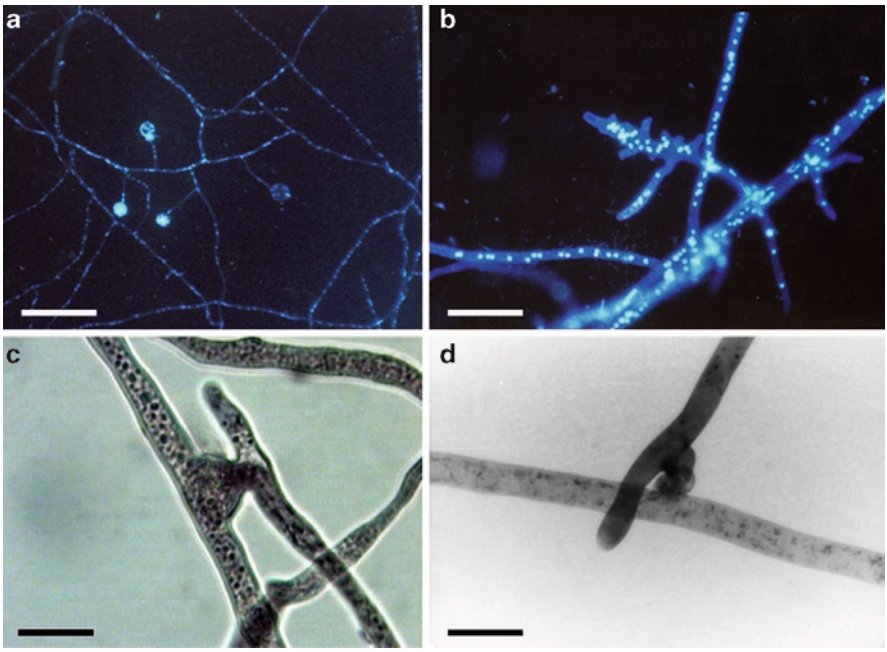

Fig. 1 Micrographs showing differential stainings of mycelium originated by *Glomus mosseae* spores growing in the absence of the host. (**a**, **b**) DAPI-stained mycelium showing nuclear distribution along hyphae and in secondary spores. Scale bars = 130 and 40 μm, respectively; (**c**) Haematoxylin-stained anastomosing hyphae showing protoplasm continuity in the hyphal bridge. Scale bar = 13 μm; (**d**) Succinate dehydrogenase localisation and Trypan blue staining of an incompatible interaction between hyphae belonging to geographically different isolates. Scale bar = 10 μm

with MT, as visualised by indirect immunofluorescence microscopy (Astrom et al. 1994), confirming previous observations on the growth of *G. margarita* germ-tubes (Sward 1981c).

The elongating germ tubes give rise to a mycelial network whose extension is highly variable between individuals. Even when growing in the most suitable media, hyphal growth of AMF is poor. For example, mycelial length in *G. caledonium* reached 30–50 mm after 10–15 days growth on water agar, and the mean growth rate of the mycelium during the early phase was 1.97 ± 0.39 μm/min (Logi et al. 1998). Accordingly, hyphal growth rate in *G. mosseae* growing in the absence of root factors ranged between 1.65 and 2.7 μm/min (Mosse 1959; Giovannetti et al. 1993b). New hyphae of *G. clarum* extended up to 8 mm after 10 days incubation (Louis and Lim 1988). Hyphal length of *G. margarita* after 9 days growth ranged between 18 and 25 mm (Bécard and Piché 1989; Gianinazzi-Pearson et al. 1989), while that of *G. gigantea* reached 54.4 cm after 15 days growth in vitro (Douds et al. 1996).

Studies on transgenic plants designed to constitutively express the insecticidal toxin from *Bacillus thuringiensis* reported diverse effects on hyphal growth of *G. mosseae* germinated sporocarps, which was lower in the presence of *Bt* corn 176

than in the presence of *Bt* 11 or non-transgenic plants. By contrast, hyphal length of *G. mosseae* did not show differences when grown in soil samples containing *Bt* and non-*Bt* plant residues (Turrini et al. 2004a; Castaldini et al. 2005). Root exudates of aubergine plants transformed to express the antimicrobial *Dm*-AMP1 defensin from *Dahlia merckii* did not affect hyphal growth of *G. mosseae*, as compared with non transgenic plants (Turrini et al. 2004b).

Fungal hyphae expanding from the primary mycelium or from branches meet frequently and often fuse, by means of hyphal fusions (anastomoses), when growing on agar or on membranes (Fig. 1c). The occurrence of anastomosis in AMF was mentioned by some authors who did not report any quantitative data on the frequency of hyphal fusions in the different species or on the cytological events involved (Godfrey 1957; Mosse 1959; Tommerup 1988). In 1999 for the first time anastomoses between living hyphae of individually germinated spores of AMF were monitored via a combination of time-lapse and video-enhanced light microscopy, image analysis, and epifluorescence microscopy (Giovannetti et al. 1999) The percentage of contacts leading to anastomosis ranged from 35% to 69% in hyphae from the same germling and from 34% to 90% in hyphae from different germlings of the same isolate of *G. mosseae, G. caledonium, G. intraradices*. By contrast, no anastomoses were detected between hyphae from the same or different germlings of *G. rosea* and *Scutellospora castanea*. Such differential behaviour of AM fungal species belonging to *Glomeraceae* and *Gigasporaceae* families was later confirmed by other authors (de Souza and Declerck 2003; de la Providencia et al. 2005).

Spatiotemporal studies made it possible to monitor anastomosis formation: complete fusion of hyphal walls and the establishment of cytoplasmic flow in the fusion bridge took about 35 min after a hyphal tip showed directed growth towards another hypha, both in *G. caledonium* and in *G. mosseae* mycelia. Protoplasmic continuity, the distinctive mark of true anastomoses, was evidenced by SDH activity in hyphal bridges, where cellular organelles moved at the speed of 1.8 μm/s (Giovannetti et al. 1999). Nuclear migration through fusion bridges suggested that genetic exchange could occur by means of anastomosis between hyphae derived from genetically different individuals. Accordingly, other studies demonstrated that geographically and genetically different *G. mosseae* isolates were unable to fuse (Giovannetti et al. 2003) (Fig. 1d), while genetic exchange occurred, by means of anastomosis, between genetically distinct isolates of one population of *G. intraradices* from the same field (Croll et al. 2009). Such nuclear exchange may represent a fundamental mechanism allowing the maintenance of genetic diversity in AMF, hitherto regarded as ancient asexuals.

6 Biochemical Changes During Germination and Pre-symbiotic Growth

The germination of AM fungal spores is characterized by increased activity of the cytoplasm, involving essential biochemical changes for the switching from a metabolically quiescent state to active metabolism.

Early studies on biochemical events that take place during germination and growth of germlings in *G. caledonium* reported that kinetics of radioactive leucine and uracil incorporation was suggestive of RNA and protein synthesis being operative by 35 min after imbibition (Beilby and Kidby 1982). The response of ungerminated and pregerminated spores to inhibitors of nucleic acid synthesis suggested that the synthesis of mRNA, unnecessary for germination of *G. caledonium* spores, was required for germling growth, and that mitochondrial DNA was synthesized during germination and hyphal growth (Hepper 1979; Beilby 1983). However, production of detectable amounts of ribosomal and mRNAs during imbibition and cold storage was shown in ungerminated spores of *G. rosea* (Franken et al. 1997). Other authors were not able to demonstrate the occurrence of DNA synthesis in vitro, during and after germination of *G. margarita* spores, by using cell cycle inhibitors or direct labelling of nuclear DNA (Burggraaf and Beringer 1989). By contrast, the capability of DNA replication was reported to occur in a small nuclear population of germlings of the same species (Bianciotto and Bonfante 1993). More evidences of DNA replication and transcription during germination and early stage of fungal growth are reported in Section 7.

Protein synthesis was demonstrated to be essential for spore germination and germling growth by studying the effects of the protein synthesis inhibitor cycloheximide (Hepper 1979) and later confirmed by using radioactive leucine and the same metabolic inhibitor (Beilby 1983). The early report, based on ^{14}C labeled acetate, that amino acid biosinthetic pathway were operating within 35 min of imbibition in *G. caledonium* (Beilby and Kidby 1982), has been recently confirmed by ^{15}N labeling experiments and gene expression studies, which showed the ability of *G. intraradices* and *G. mosseae* to synthesize aminoacids from endogenous reserves (Breuninger et al. 2004; Gachomo et al. 2009).

A net synthesis of lipids was observed during germination and germ-tube growth of *G. caledonium* spores, with an increase of free fatty acids and polar lipids and a decrease in neutral lipids (Beilby and Kidby 1980). Total lipid content increased from 45% of dry weight in ungerminated spores to 55% and 75% of dry weight in 7 and 14 days old germinated spores, respectively. However, in other experiments, using ^{13}C-labeled substrates and nuclear magnetic resonance spectroscopy, no detectable labeling of lipids was reported (Bago et al. 1999a), suggesting the lack of lipid biosynthesis in *G. intraradices* germinating spores. Later experiments, using ^{13}C labeled glycerol or ^{14}C acetate furtherly supported this hypothesis (Bago et al. 2002b; Trépanier et al. 2005). On the other hand, the occurrence of labeled 18- and 20-carbon fatty acids but not of 16-carbon fatty acids in germinating spores of *G. intraradices* and *G. rosea*, suggested that germlings could elongate and desaturate palmitic acid even in the absence of fatty acid synthase activity (Trépanier et al. 2005).

Other biosynthetic abilities of AMF during spore germination and germling growth have been demonstrated in *G. caledonium* and *G. intraradices*, which were able to synthesize sterols (Beilby and Kidby 1980; Fontaine et al. 2001a, b), as confirmed by the use of sterol biosynthesis inhibitors (Zocco et al. 2008).

The biosynthesis of polyamines, important regulators of fungal growth and differentation (Walters 1995), was studied in *G. mosseae* and *G. rosea* in order to

assess the effects on AMF of polyamine biosynthesis inhibitors used to control plant disease. An increase in polyamines levels was observed after germination in *G. mosseae*, although enhanced germling growth in the presence of exogenous putrescine and spermidine suggested a low, growth limiting level of their endogenous concentrations (El Gachtouli et al. 1996). Interestingly, polyamine biosynthesis seems to occur only via the ornithine decarboxylase in *G. mosseae*, while in *G. rosea* the alternative pathway using arginine decarboxylase was active (Sannazzaro et al. 2004).

As for carbohydrate metabolism, cytochemical studies and isozyme staining performed on spores or germ tubes showed the occurrence of many enzymes of central metabolic pathways such as glycolysis, tricarboxylic acid cycle (TCA), pentose phosphate pathway and gluconeogenesis (Macdonald and Lewis 1978; Hepper et al. 1986; Saito 1995), many of which were operative 35 min after hydration. A rapid increase in spore ATP concentration after 45 min was evidence of the presence of an active respiratory system in *G. caledonium* germlings (Beilby and Kidby 1982).

A thorough survey of biochemical potentiality of germinating spores of *G. intraradices* was performed by using ^{13}C-labeled substrates and nuclear magnetic resonance spectroscopy. The labeling patterns observed were consistent with significant carbon fluxes via various pathways, confirming that gluconeogenesis, TCA, glycolysis, and pentose phosphate pathway are operational in germlings, and supporting the important role played by glyoxylate cycle and non-photosynthetic one-carbon metabolism during germination (Bago et al. 1999a).

Since triacylglycerols (TAG) and free fatty acids may represent a large proportion of AMF spores' weight (Beilby and Kidby 1980; Gaspar et al. 1994), their degradation is central to the process of spores germination and germling growth. Actually, the breakdown of TAG was assessed 5 days after germination in *G. versiforme* spores, probably by an active lipase (Gaspar et al. 1994, 1997).

Ultrastructural data on the movement and disappearance of lipid globules in hyphae originating from germinating spores (Maia and Kimbrough 1998; Bago et al. 2002a, b) support the hypothesis that storage lipids are used to provide precursors for anabolism through glyoxylate pathway and gluconeogenesis, and to fuel respiratory chains by β-oxidation and TCA, as confirmed by labeling experiments (Bago et al. 1999a; Lammers et al. 2001). Such hypothesis was also confirmed by the detection of isocitrate lyase and malate synthase genes involved in the glyoxylate cycle and of an acyl CoA dehydrogenase involved in fatty acid β-oxidation in *G. intraradices* and *G. rosea* spores (Lammers et al. 2001; Bago et al. 2002b).

Interestingly, trehalose was detected in spores of *G. etunicatum* in small quantities, decreasing during the early germination stage, suggesting its role as a source of energy before the start of lipid breakdown (Bécard et al. 1991).

Electron microscope observations of membrane-bound crystals in spores of *G. margarita* and *A. laevis* suggested the occurrence of protein storage material, observed in different stages of apparent breakdown in *G. margarita* germlings (Bonfante et al. 1994; Mosse 1970b; Sward 1981a, b). Native and denatured

protein profiles of *G. mosseae* showed the presence of bands whose intensity decreased during spore germination, supporting the hypothesis of the existence of storage proteins in AM fungal spores (Avio and Giovannetti 1998; Samra et al. 1996). In addition to storage proteins, spores may utilize N stored in the form of aminoacids, especially asparagine, which was present in high concentration in quiescent spores of *G. intraradices* and *G. caledonium* (Beilby and Kidby 1982; Gachomo et al. 2009).

Transmembrane electric potential differences and ion fluxes in AM fungal hyphae showed a generally weak polarization of germ tubes growing in the absence of host derived signals, confirming a basal metabolic activity with low ATP consumption (Berbara et al. 1995; Ayling et al. 2000; Ramos et al. 2008).

In summary, AMF spores possess a large pool of enzymes allowing them to germinate and grow. Though, in the absence of host roots germling growth is arrested, even before depletion of spore reserves (see Section 8), while a boost of metabolism, primarily an increase of respiration (Tamasloukht et al. 2003; Bücking et al. 2008), occurs in the presence of root exudates. Interestingly, analyses of electric potential differences and H^+ ion flux profile in AM fungal hyphae showed a strong influence of host derived signals, which induced ion fluxes enhancement depending on the specific hyphal domains, suggesting a differential activation and distribution of electrogenic H^+-pump isoforms through plasma membrane (Ayling et al. 2000; Ramos et al. 2008).

7 Cytological and Genetic Changes During Germination and Pre-symbiotic Growth

Early evidence of cell cycle activation in AMF growing in the absence of the host was reported by Mosse, who described the development of dense regions containing normal cytoplasm and many dividing nuclei in spores of *A. laevis* prior to germination (Mosse 1970a). Also, Sward (1981b) observed a large number of nuclei with highly condensed chromatin and prominent nucleoli in *G. margarita* spores after 24 h of incubation on water agar. Cytological studies showed that nuclei from quiescent spores of *G. versiforme* were in the GO/G1 phase, whereas nuclei from mycorrhizal roots were in the synthetic and G2/M phases (Bianciotto et al. 1995). Mitotic spindles were also detected in germinated spores of *G. mosseae* by tubulin immunostaining, confirming the occurrence of DNA replication during pre-symbiotic growth (Requena et al. 2000). In the latter work, the gene *Gm*TOR2, encoding a protein with high homology to the C terminus of *Saccharomyces cerevisiae* TOR2 (controlling cell cycle), was characterised. Under treatment with the anti-inflammatory drug rapamycin, which interferes with TOR2 by arresting *S. cerevisiae* cell cycle in G1 phase, *G. mosseae* spore germination was unaffected, whereas hyphal growth decreased, suggesting that nuclear replication in the pre-symbiotic stage is only necessary for hyphal growth (Requena et al. 2000).

EST sequencing from germinated spores of *G. intraradices* and *G. rosea* revealed putative homologues to cell cycle and meiosis-specific genes from other fungi, such as chromatin assembly factor, ubiquitin-encoding genes (Stommel et al. 2001) and *Neurospora crassa* NDT80, known to control exit from pachytene phase of meiosis (Jun et al. 2002). Furthermore, a putative gene involved in the biosynthesis of new nucleotides was detected in germinated spores of *G. intraradices* (Jun et al. 2002).

The occurrence of nuclear division was inferred in non symbiotic mycelium by using image analysis counts of the number of nuclei (Bécard and Pfeffer 1993), which decreased from 2,000 to 800 in individual spores during the early days of germination, suggesting the migration of nuclei from spores to hyphae. This was confirmed by data on the occurrence of cytoskeletal components, both microtubules and microfilaments, in the mycelium originating from germinating spores of *G. mosseae* and *G. caledonium* (Astrom et al. 1994; Logi et al. 1998). The presence of such components is consistent with the role of cytoskeleton in the migration of nuclei and cellular organelles during active growth. Expression of β-tubulins in germinating AM fungal spores (Franken et al. 1997; Butehorn et al. 1999) was confirmed by the detection of sequences putatively encoding other cytoskeletal proteins, such as α-tubulin, β-actin, dynein and actin-related protein, possibly involved in nuclear and nutrient movements, in *G. intraradices* germinated spores (Jun et al. 2002). Recently, full-length β-tubulin gene has been sequenced from *G. gigantea* and *G. clarum*, showing some peculiar traits compared to fungi other than glomeromycota (Msiska and Morton 2009).

Nuclear division in *G. rosea* hyphae was also detected in the presence of host root exudates or of the synthetic strigolactone GR24, which induced an accumulation of nuclei in the apical area of treated hyphae (Buée et al. 2000; Besserer et al. 2008).

Early experiments showed that inhibitors of mRNA translation hindered AM fungal spore germination (Hepper 1979; Beilby 1983). Accordingly, differential display analysis of *G. rosea* did not show changes in RNA accumulation patterns during hyphal development, suggesting that in this phase proteins are produced only by translating transcripts synthesized prior and during spore germination (Franken et al. 2000).

Many expressed genes detected in germinating AM fungal spores showed homology to those encoding for proteins involved in translation, protein processing, primary metabolism and transport processes (Franken et al. 1997; Lammers et al. 2001; Stommel et al. 2001; Jun et al. 2002; Bago et al. 2002a, 2003). The identification of genes putatively codifying for several enzymes involved in carbon metabolism and lipid breakdown often confirmed biochemical data.

An interesting gene, *G. mosseae Gm*GIN1, was highly and specifically expressed in non symbiotic mycelium, whereas it was silenced during the symbiosis, both in the intraradical structures and the extraradical mycelium (Requena et al. 2002). Interestingly, several genes with homology to the N-terminus of *Gm*GIN1, sequenced from *Magnaporthe grisea, N. crassa, Gibberella zeae* and *Aspergillus nidulans*, encode for a family of proteins playing an essential role in polarized growth, septal formation and hyphal morphological changes in the phytopathogenic

fungus *Ustilago maydis* and in the ectomycorrhizal fungus *Suillus bovinus* (Gorfer et al. 2001; Weinzierl et al. 2002).

A 14-3-3 protein encoding gene, known to be involved in modulation of cell ion pumps and channels, was detected in *G. intraradices* mycelium (Porcel et al. 2006). This finding suggests an important role of this gene in controlling the activity of P-type H^+-ATPases, detected in *G. intraradices* and *G. mosseae* (Requena et al. 2003; Corradi and Sanders 2006), which are responsible of the maintenance of hyphal ionic gradient during polarized growth (Ramos et al. 2008).

Interestingly, a sequence showing strong similarity to an endonuclease involved in lateral transfer of an rDNA intron has been detected in *G. intraradices* germinated spores, suggesting the occurrence of lateral gene transfer during nuclear exchange between anastomosing hyphae belonging to genetically different AMF (Jun et al. 2002; Croll et al. 2009).

Induction of genes encoding for putative pyruvate carboxylase and mitochondrial ADP/ATP translocase, involved in respiration enhancement activity, has been observed in *G. rosea* and *G. intraradices* during early responses to host root factors, before hyphal branching (Tamasloukht et al. 2003, 2007). The expression of the former gene could explain the stimulatory effects exerted by CO_2 on AM fungal growth (Bécard and Piché 1989), whereas the expression of the latter gene could be necessary for the delivery of large quantity of ATP produced at high respiration rates (Requena et al. 2003). Activation of such genes and oxygen consumption were induced by host root exudates after 0.5–3 h, when no morphological change in hyphal growth pattern was detectable yet. On the contrary, no differences in the expression of key metabolic genes during the first 48 h after strigolactone analogue GR24 treatment were observed in *G. rosea*, which showed strong enhancement in transcript levels after 2 days of incubation, independently of GR24 treatment (Besserer et al. 2008). These findings suggest that other unknown signal molecules may be active and that strigolactone-induced mitochondrial activity is due to post-translational regulation of key enzymes (Delano-Frier and Tejeda-Sartorius 2008; Rani et al. 2008). The need of host-derived signals for developmental stages following spore germination can be inferred by results obtained with the *pmi* mutants of *Solanum lycopersicum*, which are regularly colonised by extraradical mycelium and mycorrhizal roots but are not susceptible to colonisation by hyphal germlings (David-Schwartz et al. 2001, 2003).

AM fungal spores germinating in the absence of host-derived factors constitutively release unknown compounds which are perceived as signals by host plants and are able to elicit recognition responses, such as a transient cytoplasmic calcium induction in soybean cells (Navazio et al. 2007) and the accumulation of starch in *Lotus japonicus* roots (Gutjahr et al. 2009). Ca^{2+}-mediated signaling was also suggested by expression of genes involved in Ca^{2+}-mediated signal transduction in *M. truncatula* roots in the presence of a diffusible factor released by *G. mosseae* (Weidmann et al. 2004). Previous studies had reported the release of a diffusible signal by *G. mosseae, G. rosea, G. gigantea, G. margarita* and *G. intraradices* growing in the presence of host plants (Chabaud et al. 2002; Kosuta et al. 2003). The perception of such signals by *M. truncatula* induced root expression of the early nodulin gene

*Mt*ENOD11, which was related, both spatially and temporally, with the appearance of hyphal branching enhancement. Moreover, factors released by *G. margarita* and *G. intraradices* mycelium growing nearby *M. truncatula* plant roots were able to induce lateral root formation (Olah et al. 2005) and those released by *G. intraradices* branching hyphae elicited root calcium-spiking responses (Kosuta et al. 2008). No information is still available on the chemical nature of AM fungal factor(s).

Although many studies reported germling growth improvement by different microorganisms, little is known about the molecular mechanisms of such phenomenon. Changes in AM fungal gene expression in response to the perception of microbial derived factors were detected by Requena et al. (1999) during co-culture of *G. mosseae* with a strain of the rhizobacterium *B. subtilis*, inducing mycelial growth increases. In particular, down-regulation of the putative gene *Gm*FOX2, encoding a protein involved in long-chain fatty acids catabolism, was evidenced. It is not known which is the signaling pattern between bacteria and fungi, although it has been hypothesized that an increase in fungal cAMP, due to the perception of flavonoid/estrogen bacterial signals, could be responsible for the glucose repression stage that down-regulates *Gm*FOX2 (Requena et al. 1999).

8 Growth Arrest in the Absence of the Host

Although spores of AMF are able to germinate in vitro in response to different edaphic and environmental conditions, they are not capable of extensive independent hyphal growth, and, in the absence of the host, germlings cease growth within 8–20 days (Mosse 1959; Daniels and Graham 1976; Beilby and Kidby 1980; Koske 1981a; Hepper 1984b; Bécard and Piché 1989; Giovannetti et al. 1993b; Schreiner and Koide 1993b; Logi et al. 1998) (Fig. 2).

Microchambers allowing continuous observation of living mycelium over a period of several hours, showed that when no host-derived signals from the surrounding environment were perceived by *G. caledonium* and *G. rosea* germlings, hyphae entered a state of developmental arrest. Cytoplasm, nuclei and cellular organelles were retracted from the tips and from peripheral hyphae and retraction septa were produced, separating viable from empty hyphal segments (Logi et al. 1998). In vivo two-photon microscopy, carried out on *G. rosea* germlings, showed differences in the organization and distribution of nuclei between actively growing hyphae and those undergoing septation (Bago et al. 1998, 1999b). Protoplasmic flow rate, measured in actively growing germlings on the basis of the movement of cell particles – nuclei, small vacuoles, mitochondria, fat droplets, tiny organelles – ranged from 2.98 to 4.27 μm/s in living hyphae of *G. caledonium* (Giovannetti et al. 2000). Microchambers and two-photon microscopy studies revealed that neither protoplasm streaming nor nuclear movements occurred in protoplasm-retracting hyphae and that progressively enlarged vacuoles led to the formation of empty areas where a cross wall was eventually formed (Bago et al. 1998, 1999b; Giovannetti et al. 2000) (Fig. 3a, b).

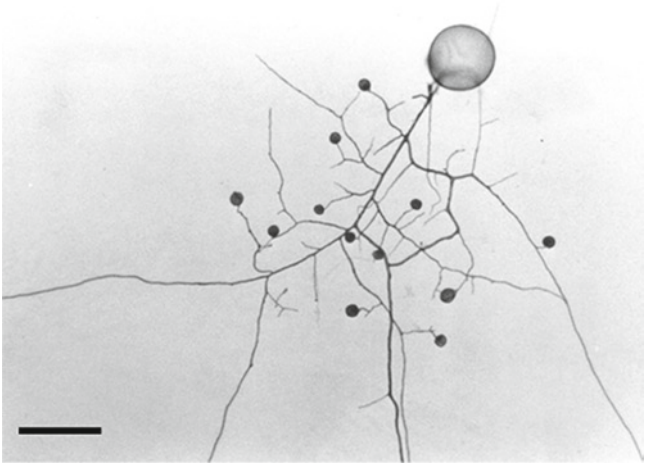

Fig. 2 Micrograph showing the limited growth of a *Glomus mosseae* spore in the absence of host derived signals. Scale bar = 240 μm

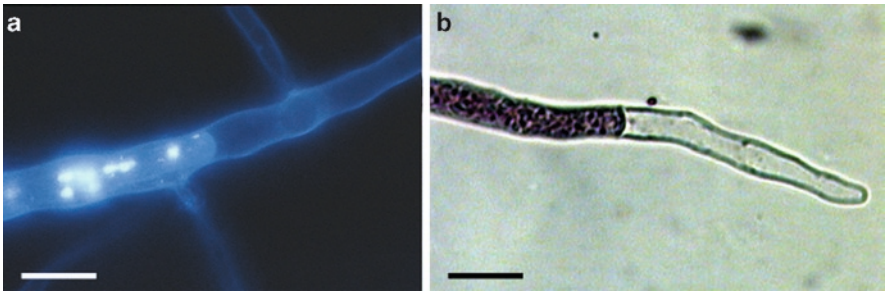

Fig. 3 Micrographs showing protoplasm retraction during growth arrest in hyphae originating from *Glomus mosseae* spores. (**a**) DAPI staining, evidencing nuclar occurrence in retracting protoplasm. Scale bar = 7 μm; (**b**) Haematoxylin staining showing a viable hyphal compartment below a septum isolating the empty hyphal tip. Scale bar = 10 μm

Metabolic activity was still detectable in *G. caledonium* 6-month-old hyphae proximal to the mother spore, which was able to retain infectivity, suggesting that such resource reallocation is functional to long-term maintenance of viability, allowing survival of fungal propagules in the absence of host plants (Logi et al. 1998; Giovannetti et al. 2000).

The reasons for such behaviour have been investigated with the aim of determining whether vital metabolic pathways may be blocked. The main results have been considered earlier in this chapter (see Section 6), and they indicate that germinating spores do possess the metabolic machinery for hyphal growth and that spore reserves are not totally depleted during germling growth (Hepper 1979; Beilby and Kidby 1980; Koske 1981b). Germinating AM fungal spores showed low respiratory

activity and reduced resource utilization, allowing limited biosynthesis, whereas higher respiration rates and use of C sources, sustaining growth and morphogenesis, were detected after the perception of host root factors. Respiratory metabolism seems a suitable control target for non symbiotic growth arrest, which has been suggested to represent a strategic mechanism preventing spore reserves consumption in the absence of host-regulated germination.

9 Concluding Remarks

Several survival strategies are supposed to have affected the evolutionary history of AMF, allowing them to overcome their obligate biotrophic status. The first survival strategy is represented by the wide host range – ~80% of land plant species –, which increases the possibility of individually germinated spores to come into contact and colonise host roots: such strategy, relying wholly on chance, appears a weak explanation for 460 million years continued existence. A second evolutionary mechanism allows the survival of spores germinated in the absence of host roots by mycelial growth arrest, which is accompanied by peripheral protoplasm withdrawal and resource reallocation towards mother spores, functional to retaining long-term colonisation ability. Challenges remain concerning factors triggering the onset of growth arrest and the molecular mechanisms involved. Further energy-saving mechanisms allow the unequivocal discrimination of host from non host roots, since AM fungal hyphae undergo a biochemical switch and a distinctive pattern of hyphal morphogenesis only after perceiving host-derived signals. Recently, we obtained data on the ability of AMF germlings to plug into a compatible mycorrhizal mycelium by means of anastomoses, thus gaining access to plant-derived carbon before undergoing growth arrest, enhancing their survival chances. The ability of AM fungal mycelium to form anastomosis and to discriminate self from nonself may represent a fundamental additional survival strategy. These strategies may compensate for the lack of host-regulated spore germination, an apparently inconsistent behaviour for obligate symbionts, and contribute to the survival of individuals and populations of AMF.

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