4 Life Cycle of *Glomus* Species in Monoxenic Culture

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1 Introduction

With respect to the Glomeromycota taxonomy, the genus *Glomus* includes close to 110 described species, making this genus the most important of the order Glomerales (Schüßler et al. 2001). As a consequence, a large number of research investigations on AM fungi are based on *Glomus* species isolates. Even though a limited number of species are cultivated under monoxenic culture, a huge amount of knowledge has already been generated to draw a reliable picture of their life cycle.

The potential of this technology, although still in its infancy, has already influenced and stimulated research investigations notably in colony growth kinetics and sporulation (Fortin et al. 2002). With respect to the *Glomus* species life cycle, this review is subdivided into four major sections: (1) the spore germination, (2) the pre-symbiotic stage, (3) the host root connection, and (4) the symbiotic stage.

2 Life Cycle

Obligate biotrophism triggered the acquisition by arbuscular mycorrhizal (AM) fungi of ingenious morphologies enabling them to survive in the absence of host plants, to adapt to a variety of plant host partners, and to allow synergy with other AM fungal species. For a given monoxenic culture, all phases of the fungal life cycle simultaneously exist and interact with each other. The knowledge we have about the *Glomus* spp. life cycle,

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originating from either in vivo or in vitro systems, remains somehow fragmented between intraradical (root colonization) and extraradical phases (mycelium and sporulation). Methodologically, investigations using in vivo technology are preferentially oriented towards the intraradical phase, with essentially imprecise spore counts as available extraradical data. With the in vitro system, both the intra- and the extraradical phases can be studied, with access to repetitive and non-destructive measurements.

Existing descriptions of AM fungal life cycles are based on morphological observations done either with agar-coated glass slides buried in soil (Powell 1976), with surface-sterilized spores grown in sterile substrate (Garriock et al. 1989) or with monoxenic cultures (Strullu et al. 1997). In the latter case, the proposed life cycles are based on the germination potential of fungal propagules (Strullu and Romand 1986, 1987), linked to species description (Chabot et al. 1992; de Souza and Berbara 1999; Pawlowska et al. 1999; Karandashov et al. 2000; Declerck et al. 2000), nuclei mycelium behaviour (Bago et al. 1999a), and nutritive and environmental conditions (Bago et al. 1996, 1999b; Hildebrandt et al. 2002).

3
AM Fungi Propagule Germination Stage

The AM fungi propagules found capable to germinate, to differentiate mycelium, and to complete the fungal life cycle are the spores, either from inside roots or isolated from the rhizosphere, and the intraradical vesicles, either isolated from the roots or still embedded within tissues of colonized roots. AM fungi hyphae from the peridium and the intraradical mycelium also are suspected to have a regeneration power. As germinating fungal propagules originate, most of the time, from non-sterile substrate, this section is based on data usually obtained from surface sterilized material, and not necessarily from monoxenic cultures.

The long-term survival of AM fungi spores in soil has been associated to their thick wall architecture and their capacity to easily fall into dormancy (Tommerup 1983). Factors involved in AM fungal spore dormancy have been attributed to ageing, physiological status, and harvesting time (Hepper and Smith 1976; Tommerup 1983; Hardie 1984). Breaking dormancy can be achieved by stratification at 4 °C (Camprubi et al. 1990; Juge et al. 2002). A 50% decrease in polyamines content was registered during spore cold treatment, and polyamine treatment did not affect the spore germination potential (El-Ghachtouli et al. 1996).

Glomus spore germination and germ tube elongation are usually not affected by the plant host (Schreiner and Koide 1993; Logi et al. 1998; Giovannetti and Sbrana 1998). *Glomus* spores may take between a few days

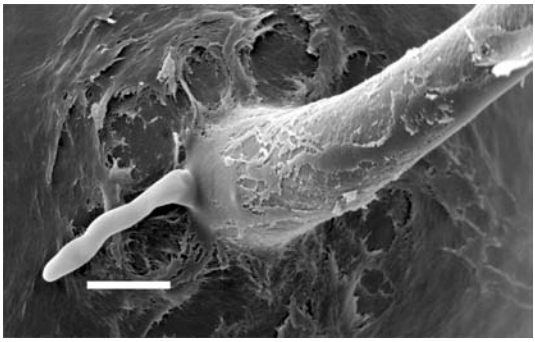

Fig. 1. Scanning electron microscope: spore germination through the subtending hyphal wall, *G. intraradices* spore (*bar* = 7 µm)

up to 6 months to germinate. Germination rates may remain as low as 2–10%.

Germination usually proceeds by the forcing of the inner spore wall through the lumen of the subtending hyphae (Gilmore 1968; Meier and Charvat 1992; de Souza and Berbara 1999), directly through the spore wall (Tommerup and Kidby 1980), or the subtending hyphal wall (Giovannetti et al. 1991; Fig. 1). Spore germination gives rise either to a straight, thick-walled hyphae (de Souza and Berbara 1999) or to stunted hyphae, depending on the spore physiological status (Juge et al. 2002).

Hydration and metabolism activation are prerequisites for AM fungi spore germination (Tommerup 1984). Inhibitors of protein synthesis and of RNA and mtDNA are known to prevent germination (Hepper 1979; Beilby and Kidby 1982; Beilby 1983). Multiple nuclei in quiescent spores (Cooke et al. 1987; Meier and Charvat 1992) and active nuclei replication and DNA synthesis were observed at germination (Bianciotto and Bonfante 1993). Quiescent spore lipid content reaches 40–60% of their biomass (Sancholle et al. 2001). During germination, de novo synthesis of sterols, diacylglycerol, phospholipids and free fatty acids occurs (Beilby and Kidby 1980; Gaspar et al. 1994; Sancholle et al. 2001) whereas triacyl glycerides are consumed (Gaspar et al. 1994).

Extreme dry or wet environmental conditions inhibited germination (Siqueira et al. 1985). Neutral pH usually supported or promoted germination (Green et al. 1976; Tommerup 1983; Pons et al. 1984; Gunasekaran et al. 1987) whereas acidity had an inhibitory effect (Siqueira et al. 1985). Optimum germination temperatures varied in the range 20–30 °C (Daniels and Trappe 1980; Sheik and Sanders 1988). Oxygen tension over 5% (LeTacon et al. 1983) promoted spore germination, whereas 5% CO_2 had no effect. Non-sterile soil filtrates and soil extract agar improved spore germination (Daniels and Trappe 1980; Gunasekaran et al. 1987). Flavonoid compounds (Tsai and Phillips 1991; Leu and Chang 1993; Poulin et al. 1997), low-P

media (Pons et al. 1984), low glucose concentration, D-galacturonic acid (Siqueira and Hubbell 1984), 50% sucrose (Vilarino and Sainz 1997), low xyloglucan concentration (Garcia-Garrida et al. 1999) and thiamin (Hepper and Smith 1976) increased germination rates. The bacteria *Paenibacillus validus*, antagonistic towards soil-borne fungal pathogens (Hildebrandt et al. 2002), *Streptomyces* species (Tylka et al. 1991), bacterial contaminants (Mayo et al. 1986) and nitrogen-fixing bacteria (Tilak et al. 1990) all stimulated germination. Lower mineral content medium (water-agar pH 6.0) favours germination, while high mineral content or rich media inhibit it (Budi et al. 1999).

In addition to spores, several other AM fungi propagules have the potential to germinate. The germination of isolated intraradical vesicles was clearly demonstrated by Strullu et al. (1997), Diop et al. (1994), and Declerck et al. (1998). Such germination occurred through the lumen of their subtending hypha attachment (Declerck et al. 1998), the germ tubes generating runner and ramified hyphae similar to those of AM fungi spore. To date, no systematic investigation has been conducted on factors influencing their germination. Among other fungal structures capable of re-growth are hyphae from the peridium of *G. mosseae* sporocarps which have the capability to elongate and differentiate vesicle-like structures (VLS; Fig. 2; Budi et al.

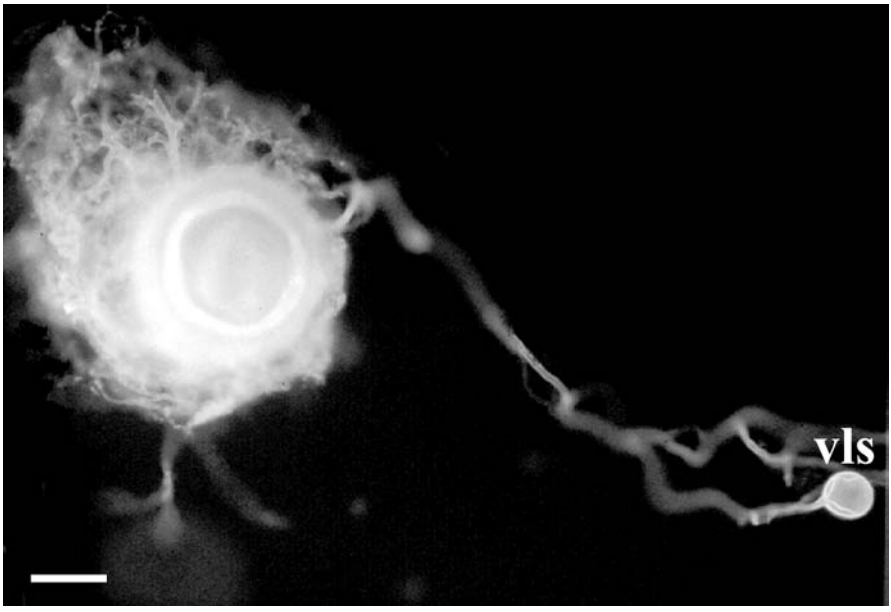

Fig. 2. Dissecting microscope: pre-symbiotic hyphae emerging from *G. mosseae* peridium with vesicle-like structure (*VLS*), UV fluorescence (*bar* = 40 µm)

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