Short Communication

Modified Strullu and Romand (MSR) Medium Devoid of Sucrose Promotes Higher *in vitro* Germination in *Rhizophagus irregularis*

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts belonging to the phylum Glomeromycota (Schüßler et al 2001), that cannot complete their life cycles without establishing a functional symbiosis with a host plant. Spore germination ability has considerable impact on different host plant species (Pawlowska et al 1999) and capacity of the fungi to complete its life cycle relies on the production of adequate spores. Though the potential of AMF's plant growth promotion and biocontrol efficacy is well recognized, culturing and mass multiplication still remain a constraint in their popularization. A primary pre-requisite for germination of AM propagules (spores and root fragments) is hydro-metabolism activation (Dalpe et al 2005). The time required for spore germination of different genera may range from 2 to 90 d. Attempts to determine requirements for spore germination and germ tube growth on artificial media have met with variable success, probably due to variation in methodology, fungal species and the culture conditions employed.

In pre-symbiotic phase AM spores germinate and develop germtubes which grow using the reserve materials from propagules. Some components of the medium may interfere with attempts at synthesizing symbioses, either by directly inhibiting the fungus or by rendering the root less capable of supporting mycorrhiza formation. Many factors such as pH, temperature, moisture, mineral and organic nutrients (Clark 1997), substrate (Maia and Yano-Melo 2001) and flavanols (Bécard and Piché 1992) have been identified as playing important roles in initiating germination and germtube growth. However, systematic information about effects of sucrose concentration in the substrate on AMF spore germination is limited (Carr et al 1985; Dalpe et al 2005). The presence of various carbohydrates in the medium may result in the blocking of receptor/recognition sites on hyphae or host cell walls, which could prevent germ tubes and hyphae from locating host roots (Allen 1992). Anatomical changes in excised *Solanum lycopersicum L.* (tomato) root, in relation to the sucrose concentration in the culture medium, were also observed by Street and McGregor (1952). The purpose of this study was to evaluate the effect of sucrose on spore germination and germ- tube growth in *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) Walker & Schuessler.

Rhizophagus irregularis was originally isolated from rhizosphere of mangrove plant *Rhizophora apiculata* Blume from Sal estuary (Goa, India) and propagated using sieving in pot cultures with sterile sand as substrate and *Solenostemon scutellarioides* (L.) Codd (Coleus) as host. Subsequently, pure culture of *R. irregularis* was raised and maintained in a polyhouse using the same host. Identification was done based on spore morphological characteristics and the relevant literature (Blaszkowski et al 2008; Stockinger et al 2009).

Spores of *R. irregularis* were isolated from pure culture by wet sieving and decanting method (Gerdemann and Nicolson 1963) and selected spores were stored at 5C before sterilization. After surface sterilization, using micropipette, they were transferred to Petri plates containing a solution of 2% (w/v) streptomycin sulphate and stored overnight. Modified Strullu and Romand (MSR) medium (Declerck et al 1998) solidified with 5% clarigel, with and without sucrose was used as substrate.

An experimental design with 30 replicates was employed for two treatments. A single spore was inoculated in each Petri plate and incubated in an inverted position in dark at 26C. Germination was assessed every 4d up to 26d. Spores were considered to have germinated if a germtube was visible. Observation on hyphal growth was carried out after every 12 h. Pearson's correlation coefficient analysis was performed using WASP (Web Based Agricultural package) 2.0 ($P \leqslant 0.05$).

The present study is the first report on an inhibitory effect of sucrose on germtube growth of *R. irregularis* using MSR medium. Germinating spores produced germtubes that grew through the subtending hyphae. In the present study, *in vitro* germination in MSR medium without sucrose was observed 38h after inoculation and recorded 90% germination whereas in

MSR medium with sucrose germination was recorded after 60 h with 75% germination after 26 d (Fig. 1). Differences in spore germination rate were observed with significantly greater germination rate being observed in MSR medium without sucrose (r = 90; $P \leqslant 0.05$) (Table 1). De Souza and Berbara (1999) reported germination in *G. clarum* Nicol. & Schenck after 7 d. Dalpe et al (2005) reported germination rate as low as 10-12% in *G. intraradices* Schenck & Smith in MSR medium with sucrose. Sucrose is known to prolong formation of appresorium in *Gigaspora margarita* Becker & Hall leading to lower germination in MSR medium with sucrose (Bécard and Fortin 1988). Carr et al (1985) found that concentrations of sucrose exceeding $5gL^{-1}$ in the medium reduced growth of hyphae of *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe in root organ culture medium. MacDonald and Lewis (1978) implied that AM fungi possess enzymes for carbon metabolism while according to Sancholle et al (2001) AM fungal germination is known to depend on availability of spore reserves and not on the nutrients present in substrate. This suggests AM fungal spores are affected by the exogenous sucrose. Lower germination rate in MSR with sucrose might also be attributed to nutrient toxicity in the substrate (Clark 1997).

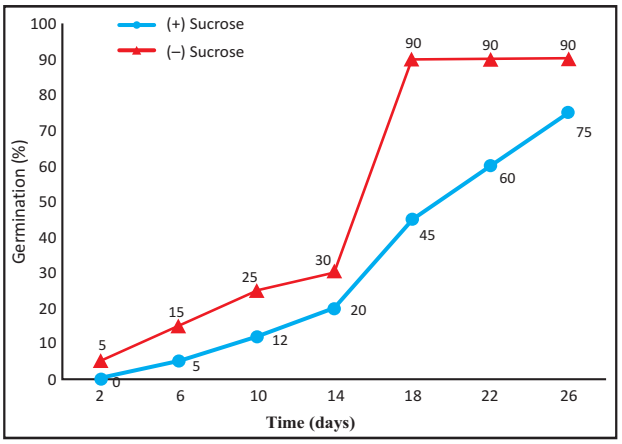

Figure 1. Percent germination in *R. irregularis* spores growing on MSR medium with and without sucrose (after 26 d)

Germ tube length was significantly greater in MSR medium without sucrose. Average width of germ tube was higher in MSR medium without sucrose and was non-significant (Table 1). The hyphae were longer and had fewer branches in MSR medium without sucrose, and were shorter with more branching in MSR medium with sucrose (Fig. 2). Although sucrose is one of the nutrients exchanged during symbiotic phase (Smith and Read 2008), higher concentration of sucrose is known to produce inhibitory effect on hyphal growth resulting in decreased hyphal length. Siqueira et al (1982) observed that in *Gigaspora margarita* low concentration (4g L^{-1}) of sucrose favoured germtube growth while concentrations above 4g L^{-1} reduced germ

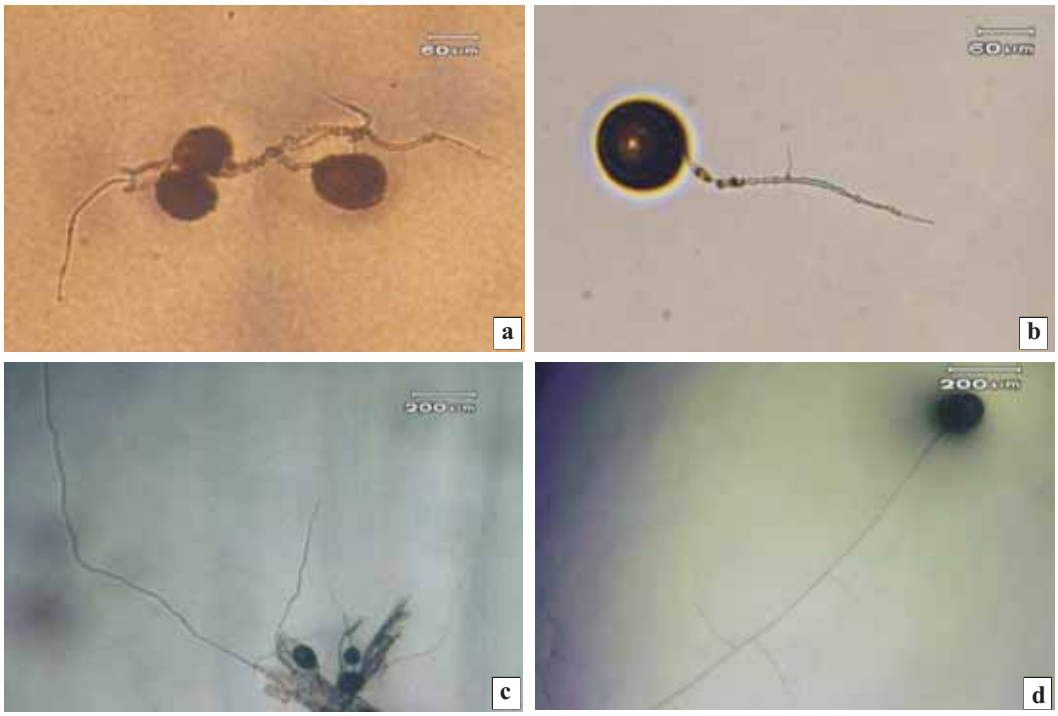

Figure 2. a & b = hyphal growth on modified MSR supplemented with sucrose; c & d = hyphal growth on modified MSR without the addition of sucrose

Table 1. Length and width of germtube and number of hyphal branches in *R. irregularis* growing in MSR media with and without sucrose (after 26 d)

Substrate	Germ tube growth		
	Length (μm)*	Width (μm)*	No. of branches*
MSR medium (+ sucrose)	354.2 a	9.8 a	3a
MSR medium (- sucrose)	658.4b	10.0a	1.7b

*The means followed by different letters in a column are significantly different at P⩽0.05 (n=30).

tube growth. The different methods and techniques used to date *in vitro* germination studies involving AM fungi have usually been carried out using MSR medium with sucrose. However, the present study recommends the use of MSR medium minus sucrose to enable more rapid germination.

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