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Monoxenic Culture of AM fungus *Glomus clarum* using Ri T-DNA transformed roots

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Abstract:

In the present study indigenous isolate of *Glomus clarum* was successfully grown in monoxenic culture system using Ri T-DNA transformed chicory roots (*Cichorium intybus* L.). Surface-sterilized spores isolated from pure culture were used as a source of fungal inoculum for monoxenic culture. Spore germination was carried out on MSR medium. Various developmental features such as spore germination, development of extra-radical mycelium and sporulation in *G. clarum* as observed under *in vitro* conditions were recorded.

Introduction:

Arbuscular mycorrhizae (AM) are obligate symbionts and around 80% of plants are colonized by these fungi which belong to the phylum *Glomeromycota.* The co-existence of various AM fungal isolates in the soil results in healthy plant growth and helps in seedling establishment; nutrient cycling; conservation of soil structure; resistance to drought, temperature and salinity; and control of plant pathogens. Since AM fungi are obligate biotrophs, they are unable to complete their life cycle in the absence of a host plant (Azcón-Aguilar *et al*., 1998). After contact with a suitable host, inter- and/or intracellular colonization of the root cortex occurs (Bonfante-Fasolo, 1984 and 1987; Smith and Smith, 1997) allowing the completion of the AM fungal life cycle. Due to their obligate biotrophic nature, the *in vitro* culture and large-scale production of these fungi has been limited there by reducing their potential for use as inoculum in agricultural and horticultural practices (Plenchette *et al*., 1996). With the advancement in research on AM fungi, novel tools have been developed for growing these fungi under *in vitro* conditions one of them being root organ culture.

In vitro culture of AM fungi was achieved for the first time in the early 1960s

(Mosse, 1962). In this pioneering work, the Ri T-DNA-transformed *Daucus carota* L. (carrot) roots were used as the host by the wild type strain of *Agrobacterium rhizogenes* containing a plasmid that induces a hairy root phenotype. The establishment of *in vitro* root-organ cultures has greatly influenced our understanding of the AM symbiosis. Although the *in vitro* system is artificial, it allows non destructive, morphological and physiological investigations of the AM symbiosis. Moreover, it is possible to increase its similarity to a natural system by allowing the development of the AM symbiosis in a nutrient environment which is closer to that of the mineral soil. This technique permits production of pure, viable, contamination free and abundant propagules in a smaller space. Therefore *in vitro* dual culture of Ri T-DNA transformed roots and AM fungi has proved to be a powerful tool for the study of these symbionts.

The present work was aimed to study the establishment of *in vitro* culture of an indigenous isolate of *Glomus clarum* (Nicolson and Schenck, 1979) using Ri T-DNA transformed chicory roots (*Cichorium intybus* L.) as the host.

Materials and Methods:

Inoculum preparation

Indigenous isolate of *Glomus clarum* (Nicolson and Schenck, 1979) was propagated in green house using soil-sand (1:1) pot cultures with *Solenostemon scutellariodes* (L.) as host.

Glomus clarum spores were extracted from soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Isolated spores were twice disinfected for 10 minutes in 2% chloramine T with 1-2 added drops of tween 20, followed by a 10 minute bath in an antibiotic solution (streptomycin sulfate 0.02% w/v and gentamycin sulfate 0.01% w/v) (Bécard and Fortin, 1988). The two disinfection steps were followed by triple rinsing with sterile deionized water. Surface-sterilized spores isolated from pure culture were then used as a source of fungal inoculum for monoxenic culture.

Culture medium

The Modified Strullu-Romand (MSR) medium (Declerck *et al*., 1996; Diop 1995; Strullu and Romand, 1986) was used for both routine maintenance of the transformed roots and establishment of the dual culture.

Root culture

Ri T-DNA transformed chicory roots (*Cichorium intybus* L.) were routinely cultured on Modified Strullu-Romand (MSR) medium and the Petri plates were incubated in an inverted position in the dark at 27^{o}C.

Dual culture

For the establishment of mycorrhizal symbiosis, an actively growing transformed *Chicory* (*Cichorium intybus* L.) root with several lateral branches was placed in the vicinity of the germinated spore and the Petri plates were incubated in an inverted position in the dark at 27^{o}C.

Development of Mycorrhizal symbiosis

Extra-radical hyphal growth and sporulation were monitored non-destructively, using Olympus research microscope BX 41 (100X–1000X). The roots were also cleared with 10% KOH and stained with trypan blue as described by Phillips and Hayman (1970) to observe the AM fungal colonization *in vitro*.

Results and Discussion:

Spore germination

Spore germination was achieved 3 to 5 days after incubation (Fig. 2 a-b). The hyphal length was measured after every second day for a period of 6 days (Fig.1).

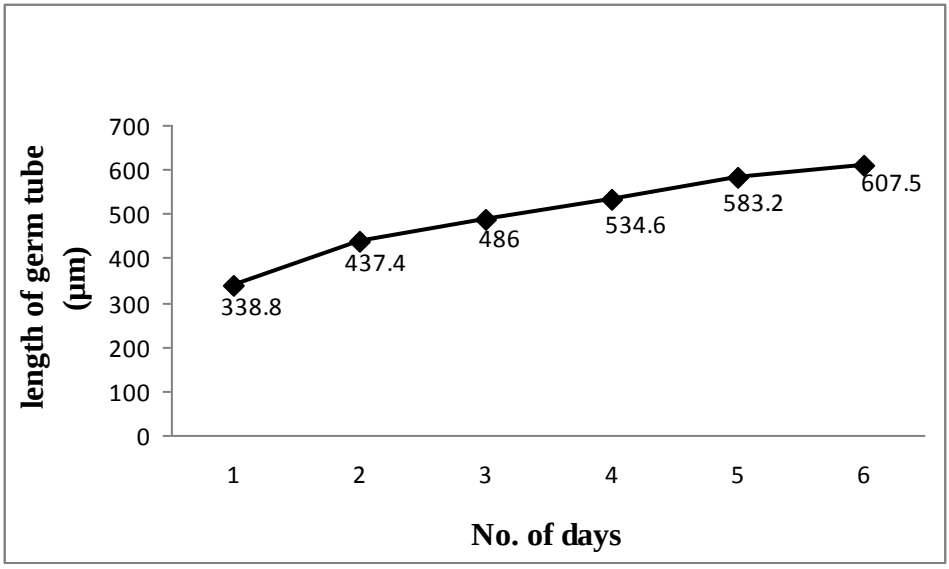

Fig.1: Germ tube growth in *in vitro* grown spores of *Glomus clarum*.

Dual culture

Initial contact between the hypha and the transformed root was observed on the tenth day after germination. Hyphal penetration into the growing roots was observed under light microscope (Fig. 2 c-d). Intra-radical vesicles of *G. clarum* were also observed in the transformed roots of chicory (Fig. 2 e-f). Sporulation was observed after four months of hyphal penetration (Fig. 3 a-d). Upon sub-culturing the colonized transformed root fragments from the starter/mother culture, sporulation was achieved after 20 days of culturing.

The success achieved by using root organ culture technique in cultivation of AM fungi *in vitro* is not only restricted to the study of the symbiotic interactions, but also permits the increase of knowledge in the morphology, taxonomy, phylogeny and biochemistry fields together with some aspects of their ecology (Cranenbrouck *et al*., 2005). This study describes the successful establishment of dual culture between *G. clarum* and transformed chicory roots on the MSR medium. Following infection, considerable internal root colonization and extensive proliferation of extra-radical mycelium was observed. Spores were produced in abundance, and therefore a standard method for dual culture of *G. clarum* along with transformed chicory roots and completion of life cycle of the AM fungus has been established.

The use of roots organ culture in studies on AM fungi may contribute greatly to a better understanding of the events that occur during the pre- and post

colonization phase (Becard and Fortin, 1988), possibly filling some gaps in the taxonomy of these fungi. In conclusion, the dual culture of *G. clarum* along with transformed chicory roots on the MSR medium permits mass production of pure, viable, contamination free and abundant AM fungal inoculum in a small space.

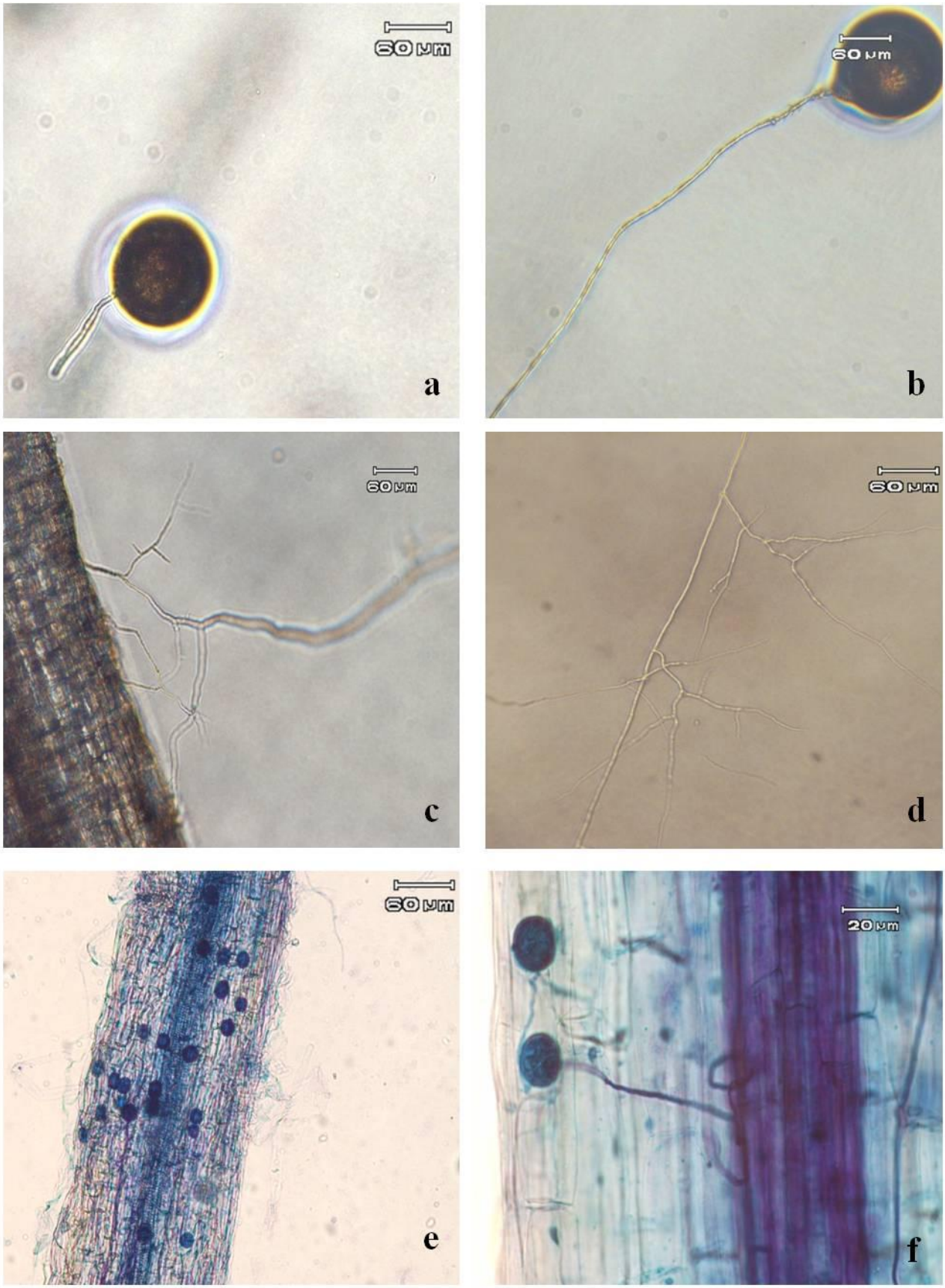

Fig. 2 a-b: spore of *Glomus clarum* showing germ tube growth in *in vitro*. **c-d:** *in vitro* culture of *G. clarum* in association with excised Ri T-DNA transformed chicory root. **e-f:** transformed chicory root showing intraradical vesicles of *G. clarum*.

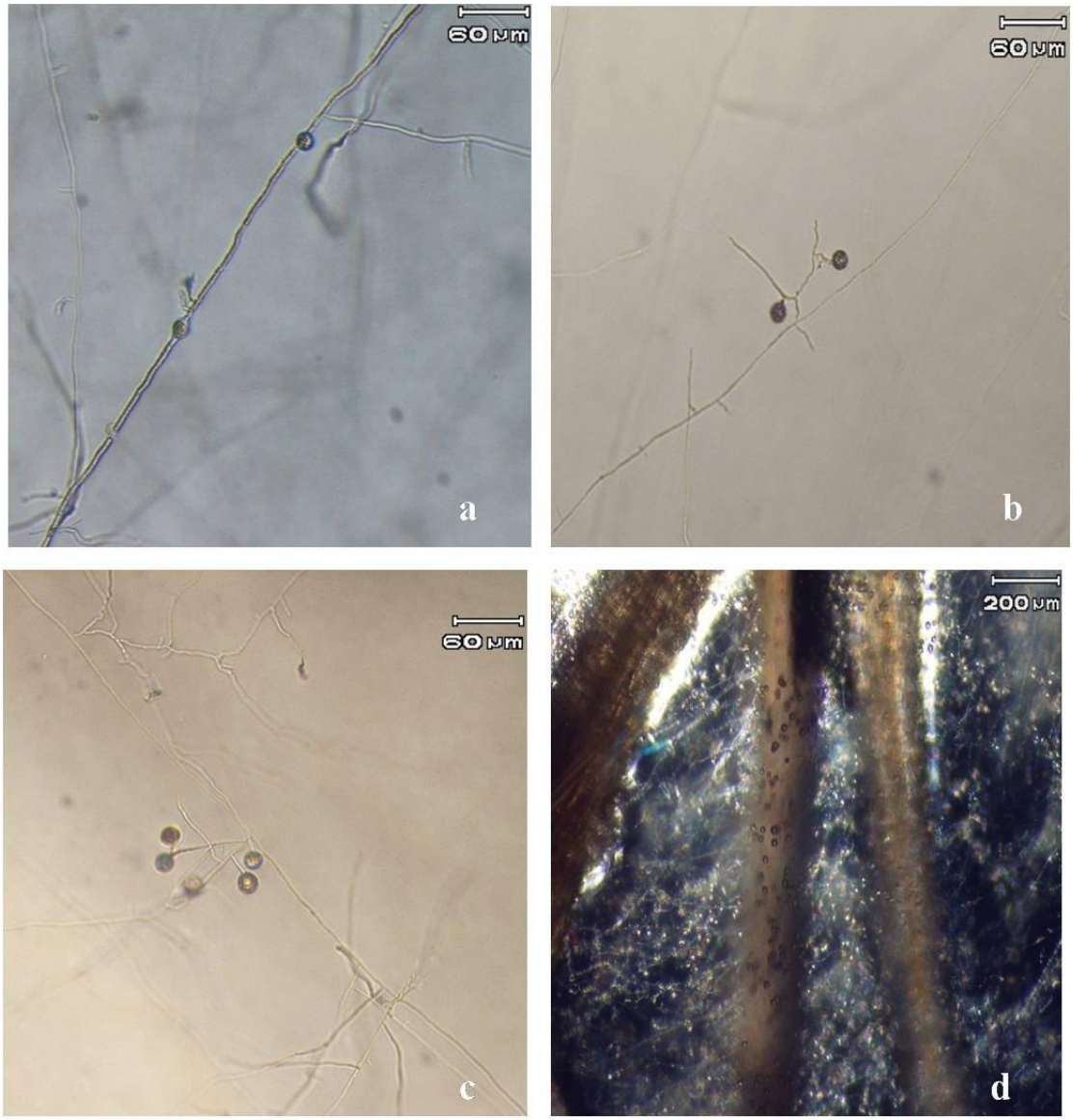

Fig. 3 a-d: Development of extra-radical mycelium and sporulation in *G. clarum* in *in vitro* culture.

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