ARBUSCULAR MYCORRHIZAE OF GOA

- A MANUAL OF IDENTIFICATION PROTOCOLS

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IN VITRO CULTIVATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI USING ROOT ORGAN CULTURE TECHNIQUE

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Arbuscular mycorrhizal (AM) fungi colonize approximately 80% of terrestrial plants and their beneficial effects on the growth and health of plants have been recognized for some time. However, their obligate biotrophic nature has limited *in vitro* culture and large-scale production, reducing their potential use as inoculum in agricultural and horticultural practices (Plenchette *et al.,* 1996).

A natural genetic transformation of roots with the soil bacterium *Agrobacterium rhizogenes* Conn. was achieved decades ago (Riker *et al.,* 1930; Ark and Thompson, 1961) and applied in the eighties to excised roots for the cultivation of AM fungi. This technology, also known as the root organ culture technique is now receiving more and more attention to study various aspects of the symbiosis.

Various plants such as *Lycopersicon esculentum* Mill., *Trifolium pratense* L., *Trifolium repens* L., *Allium cepa* L., *Daucus carota* L., *Linum usitatissimium* L., *Tagetes pastula* L., *Solanum tuberosum* L., *Medicago truncatula* Gaertn., etc. have been used to establish monoxenic (i.e. root organ) cultures of AM fungi. Mosse and Hepper (1975) first successfully performed root organ culture by using a system based on dual culture of *Glomus mosseae* Nicolson & Gerd. spores with excised roots of *Trifolium pratense* L. (red clover). Due to their obligate symbiotic nature, less than 5% of AM fungi were successfully cultivated using the dual culture approach. The monoxenic method involved growing sterile AM fungal spores on dual culture plates with transformed *D. carota* L. (carrot) roots. *Agrobacterium rhizogenes* Conn., a gram-negative soil bacterium which induces hairy root disease of dicotyledonous plants, is used to induce hairy roots. In roots transformed with this bacterium, a segment of the bacterial DNA, named the T (transformed DNA) of the plasmid Ri (root inducing) is incorporated into the host plant cells (Chilton *et al.,* 1982). Integration and expression of this DNA in the plant genome lead to the development of hairy root phenotype and synthesis of novel low molecular weight compounds called opines (Tepfer and Tempe, 1981). Depending on the strain of *A. rhizogenes* used for the transformation, different principals opines can be found in the tissues of the hairy root such as agropine, mannopine, cucumopine or mikimopine (Dessaux *et al.,* 1992). These adventitious roots are then cultured *in vitro* on medium devoid of plant hormones, where they grow very rapidly, with a characteristic, highly branched and non-geotropic pattern. The combination of transformed carrot roots and sterile AM fungal spores can be used to produce “dual *in vitro* cultures” that provide an efficient method of producing abundant spores (>5000) and mycelia in a 9 cm petri plate (Becard and Fortin, 1988) **(Plate 13 e & f).**

Plate 13e. In vitro spore germination

Plate 13f. In vitro sporulation

Root organ culture has obvious advantages over traditional systems, permitting production of contaminant-free propagules. So far, 25 AM fungal species have been successfully cultivated in monoxenic culture (Fortin *et al.,* 2002). However, most data generated under monoxenic culture conditions have been obtained with *Glomus* and *Gigaspora* species, while *in situ* observations on *in vitro*-produced cultures of *Scutellospora* species have been seldom reported.

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 in knowledge in morphology, taxonomy, phylogeny and biochemistry fields together with some aspects of their ecology (Cranenbrouck *et al.,* 2005).

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