

**TECHNIQUES  
IN  
MYCORRHIZAE**

**Editors  
M. J. Bukhari  
&  
B. F. Rodrigues**

**Department of Botany  
Government College of  
Arts, Science & Commerce  
Quepem Goa 403 705.**

## **Techniques in Mycorrhizae**

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## **Mass multiplication of Arbuscular Mycorrhizal (AM) fungal spores using various techniques.**

**B. F. Rodrigues**  
Department of Botany,  
Goa University, Goa 403 206.

### **I-Preparation of trap cultures.**

#### **Introduction:**

Trap cultures are necessary to obtain healthy AM fungal spores, which can be used for identification and can be used as inoculum to establish monospecific cultures. Spores directly isolated from a field soil has many drawbacks viz., a) they appear healthy but are not viable as they may be persisting in the soil for years or possibly decades, b) they lose or change appearance of their structural characters in response to root pigments, soil chemistry, temperature, moisture and microbial activity, and c) they may represent only those colonizing AM fungi with enough activity and biomass to trigger sporulation.

#### **Method:**

1. Rhizosphere soil along with the attached roots is collected by digging with a spade/shovel. A root ball is ideal.
2. Shoots are cut at the soil surface and discarded. The roots are collected and chopped into smaller fragments and mixed thoroughly with the associated soil.
3. The chopped blend is then thoroughly mixed with 1:1 (v/v) with autoclaved coarse sand.
4. After proper mixing, it is then transferred to a 15 cm plastic/earthen pot.
5. Seed the pots with plants like sorghum, onion, *Coleus sp.* etc., and keep them in a glasshouse. The plants are watered regularly and Hoaglands solution (minus P) is added as and when required.

6. After 60 days, a portion of the roots is checked for AM colonization. If colonization is observed, then the watering is stopped. The shoot portion is chopped off at the soil surface and discarded and the pots are allowed to dry. The roots are then chopped to finer pieces and mixed well in the soil. The mixture is then placed in a polyethylene bag, labeled and stored at 4°C. This is then used to isolate spores of different types, which are later used for preparation of monospecific culture.
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## **II – Establishment of monospecific culture using funnel technique.**

### **Method:**

1. Spores are extracted using the wet sieving and decantation method (Gerdemann and Nicolson, 1967) preferably from pot culture, 2-3 days before inoculation onto plant seedlings using sucrose density-gradient centrifugation.
2. After repeated washing, the spores of each target species are collected manually using a pasture pipette and stored in a watch glass (sealed in petriplate) at 4°C.
3. Spores are examined daily until the day of inoculation, and any spore/s with changed morphology (loss of contents or colour change) or parasitized are discarded.
4. Before inoculation, water is added to the spore preparation, the watch glass agitated, and any particles, hyphal fragments, or atypical spores are removed.
5. The selected spores are surface sterilized using 200-ppm streptomycin sulphate solution for 3-5 minutes and later washed with sterile water and again surface sterilized with 2% chloroamine-T solution for 3-5 minutes.
6. After surface sterilization, the spores are serially washed with sterile water for 4-5 times.
7. Funnels used in this experiment are plugged on the lower side with absorbent cotton and filled with 1:1 sand and soil mixture is added to the funnel. Top of the funnel is then wrapped with aluminium foil and sterilized in an autoclave for 45

minutes at 121°C and 15 lbs pressure. This process of sterilization is repeated for three consecutive days and cooled.

8. The surface sterilized spores are added to the funnel by making a small slit at the center of the aluminium foil. The spores are then placed about 2-3 cm below the soil and covered with soil. Over this, 3-4 sorghum seeds (surface sterilized with 0.1% mercuric chloride and washed 4-5 times with sterile distilled water) are sown and the silt in the aluminium foil is covered.
  9. The whole set of funnel is then placed in a conical flask containing sterile distilled water so that the soil in the funnel becomes wet due to the capillary action of water. The entire assembly is then kept in a glass-house for 45-50 days.
  10. Later, after 45-50 days, the plants along with soil are transferred to a 15cm pot (containing sterilized sand soil mix). About 40-50 fresh surfaced sterilized seeds of sorghum are planted and watered regularly. Hoaglands solution (minus P) is added as and when required.
  11. After another 60 days, a portion of the sorghum roots is checked for AM colonization. If colonization is observed, then the watering is stopped. The shoot portion is chopped off at the soil surface and discarded and the pots are allowed to dry. The roots are then chopped to finer pieces and mixed well in the soil. The mixture is then placed in a polyethylene bag, labeled and stored at 4°C. This is then used to isolate spores for identification of the species and/or can serve as inoculum.
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