



# **Isolation of Vesicular-Arbuscular Mycorrhizal (VAM) spores from the soil**

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## **A. Sieving method**

### **Requirements**

- Soil sample
- 500 ml beaker
- Sieves of 710  $\mu\text{m}$ , 250  $\mu\text{m}$ , 75  $\mu\text{m}$  and 45  $\mu\text{m}$ .
- Bunsen burner

# Procedure

- Take 200 ml water in 500 ml beaker.
- Heat the water to 40-50° C.
- Add 50 g of soil and mix well to form a suspension.
- Allow the heavier particles to settle down.
- Decant most of the suspension through a 710  $\mu\text{m}$  sieve to remove large organic matter and roots.

# Procedure

- Add 200 ml of water to the suspension.
- Decant the suspension through 710  $\mu\text{m}$  sieve.
- Decant this through 250  $\mu\text{m}$ , 75  $\mu\text{m}$  and 45  $\mu\text{m}$  sieves consequently.
- Collect the residue on the 45  $\mu\text{m}$  sieve.
- Wash the residues well with water and collect the spore.

## **B. Floatation method**

### **Requirements**

- Soil sample
- Sucrose solutions (20, 40 and 60 %)
- Blender
- Fine sieve
- Centrifuge
- Centrifuge tube (50 ml)

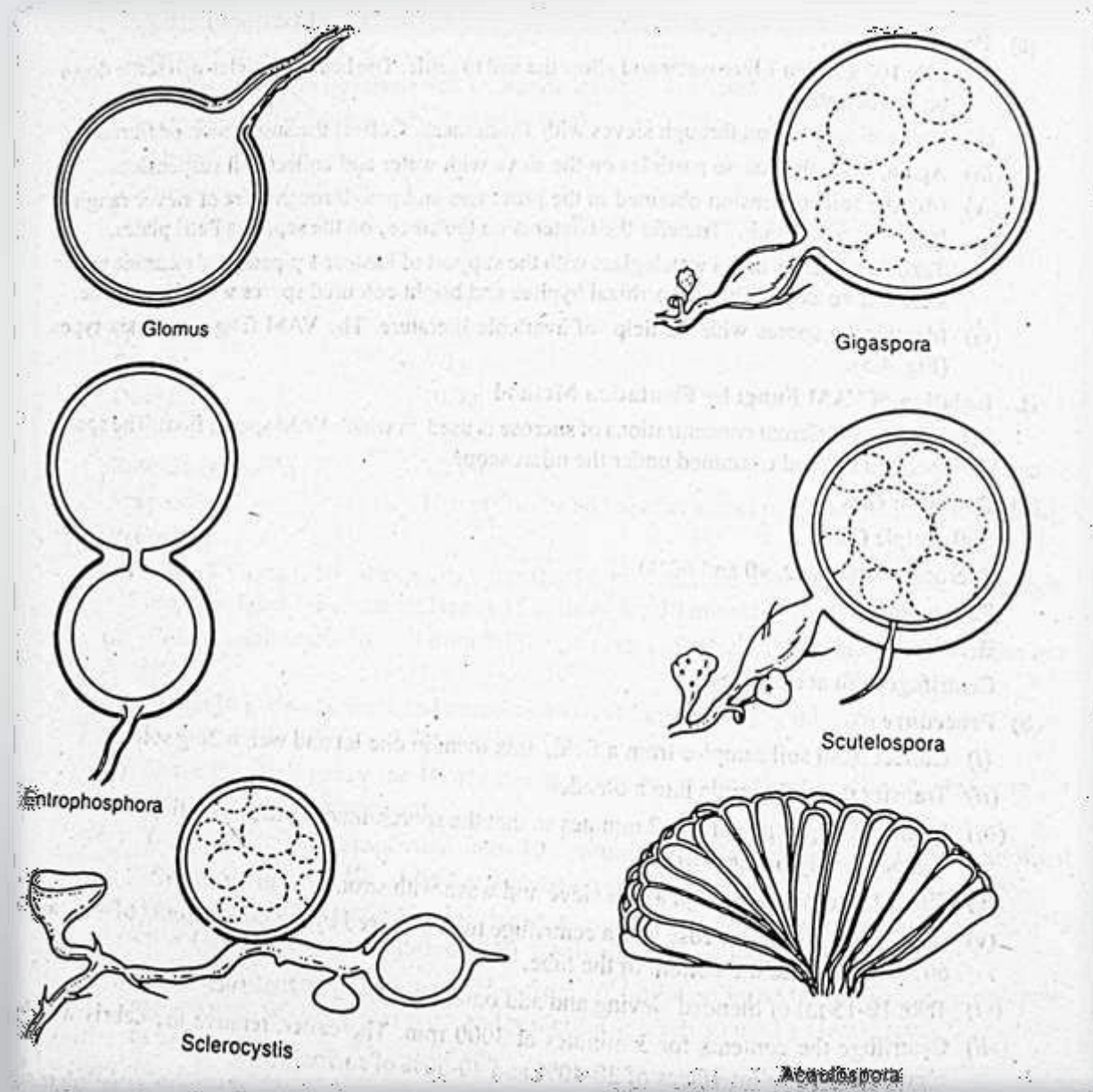
# Procedure

- Collect fresh soil samples from the field, mix them well and weigh 20 g soil.
- Transfer the soil into a blender.
- Blend it at high speed for 1-2 minutes so that the spores attached to the soil particles or roots may become free.
- Filter the contents through a fine sieve and wash with strong stream of water.
- Pour 10 ml of 20% sucrose into a centrifuge tube followed by the same amount of 40% and 60% sucrose into the bottom of the tube.

# Procedure

- Take 10-15 ml of blended sieving and add onto the surface of 20% sucrose layer.
- Centrifuge the contents for 3 minutes at 3000 rpm. Thereafter, remove the debris which accumulate at the interfaces of 20-40% and 40-60% of sucrose.
- Gently wash the spores present on fine sieve with a strong stream of water so that sucrose should be removed.
- Collect the spores and observe under microscope.

# Characterisation of Spores





# Mass Production : Problems and prospects

- Being obligate symbionts AM fungi could be mass produced only in the presence of living roots.
- Since AM fungal associations are universal and have been reported in almost all terrestrial plants, these can be reproduced on a wide range of host plants.
- There are several techniques reported for mass production of AM inoculum.

## a. *In vivo* culture

- AM fungi are grown on roots of green house plants and chopped mycorrhizal roots, often mixed with growth media containing hyphae and spores, are used as source of inoculum.
- Soil could be replaced by inert substances such as **vermiculite, perlite, sand or a mixture of these** for crude inoculum production.

# Mass production of VAM



Mass production of VAM inside  
Bricklined tank



Mass production of VAM inside  
a cement tank

# Method of production



**1. Tank for mass multiplication of AM**



**2. Sprinkling of water in tank with vermiculite**



**3. Making of furrows to sow maize seeds**



# Method of production



**4. Sowing the seeds in furrows**



**5. View of the maize sown AM pit**



**6. Vermiculite contained raised AM infected maize plants**

## **b. *In vitro*/ axenic culture techniques**

- **i) Solution culture**
- **ii) Aeroponic culture**
- **iii) Root organ culture**

## **i) Solution culture**

- Involves growing infected roots in aqueous medium enriched with mineral nutrients required for the growth of the roots under controlled biotic and abiotic conditions.

## **ii) Aeroponic culture**

Involves applying a fine mist of nutrient solutions to colonized roots for AM fungal inoculum production.



### **iii) Root organ culture**

- Use of a modified agar medium (MS rooting medium)/ liquid medium for creation of increased amount of roots from callus tissue and these roots are infected by AM spores or by surface sterilized root bits obtained from mycorrhizal plant.

<b>Crop</b>	<b>Mycorrhiza species</b>
Barley, maize, wheat	<i>Glomus spp.</i>
Bean	<i>Asaulospora morrowiae, Glomus, Gigaspora</i>
Peanut	<i>Glomus fasciculatum, Sclerocystis dussi</i>
Pea	<i>Glomus intraradices</i>
Cotton	<i>Glomus sp., Sclerocystis sinuosa</i>
Tomato, potato	<i>Gigaspora margarita, Glomus spp., Acaulospora sp.</i>
Black pepper	<i>Entrophosphora colombiana, Scutellospora sp.</i>
Cardamom	<i>Glomus fasciculautm</i>
Citrus	<i>Glomus faciculatum, G. mosseae</i>
Marigold	<i>Glomus mosseae</i>

# Benefits of mycorrhiza

- Produce more vigorous and healthy plants.
- Increase plant establishment and survival at seedling or transplanting.
- Enhance flowering and fruiting.
- Increase yields and crop quality.
- Improve drought tolerance, allowing watering reduction.

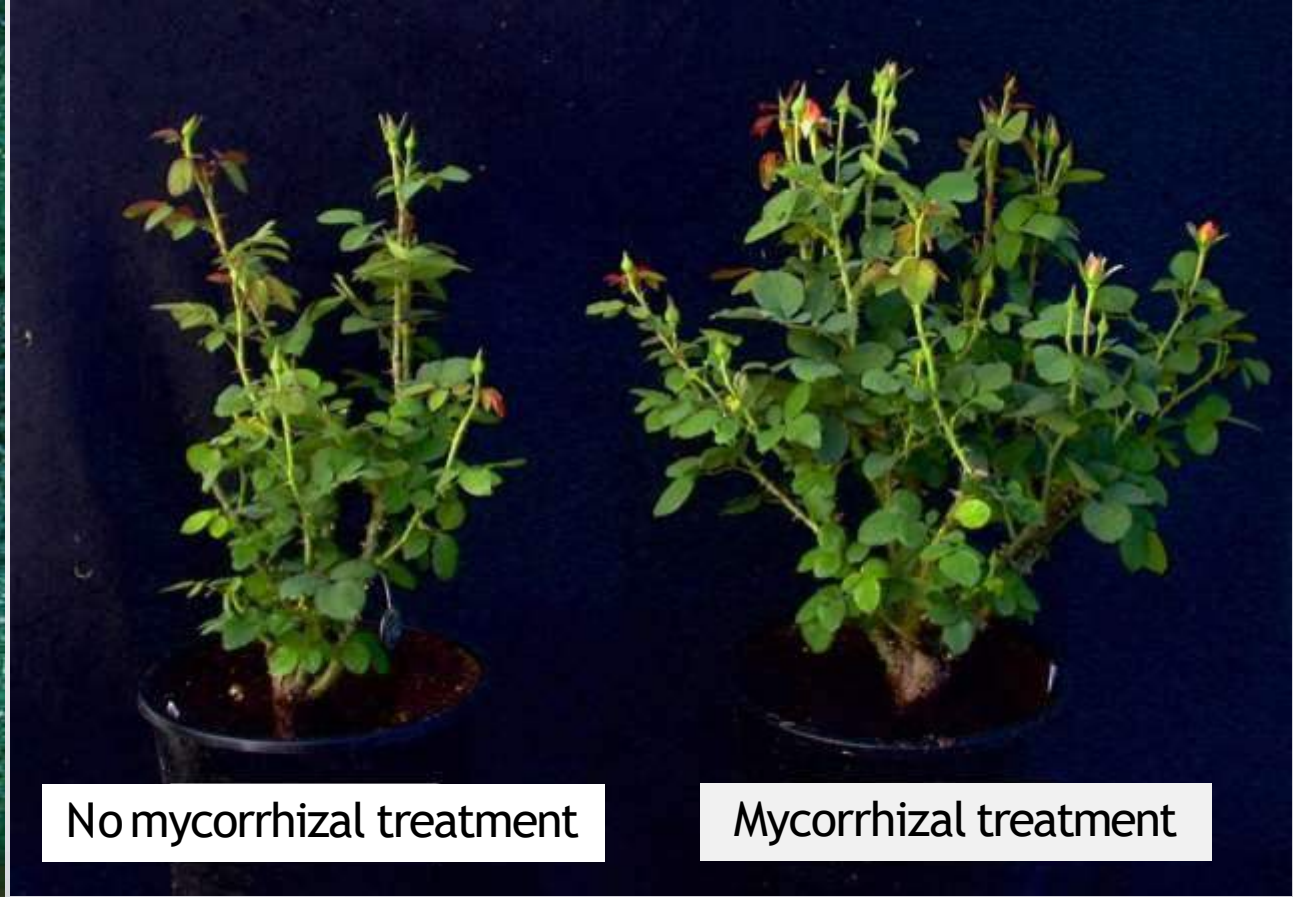
# Benefits of mycorrhiza

- Optimize fertilizers use, especially Phosphorus.
- Increase tolerance to soil salinity.
- Reduce disease occurrence.
- Contribute to maintain soil quality and nutrient cycling.
- Contribute to control soil erosion.



No mycorrhizal treatment

Mycorrhizal treatment



No mycorrhizal treatment

Mycorrhizal treatment



# Application of VAM fungi

- **Nursery application**

100 g bulk inoculum is sufficient for one m<sup>2</sup>. The inoculum should be applied a 2-3 cm below the soil at the time of sowing. The seeds/cuttings should be sown/planted above the VAM inoculum to cause infection.

# Application of VAM fungi

- **For polythene bag raised crops**

5 to 10 g bulk inoculum is sufficient for each packet. Mix 10 kg of inoculum with 1000 kg of sand potting mixture and pack the potting mixture in polythene bag before sowing.

# Application of VAM fungi

- **For out-planting**

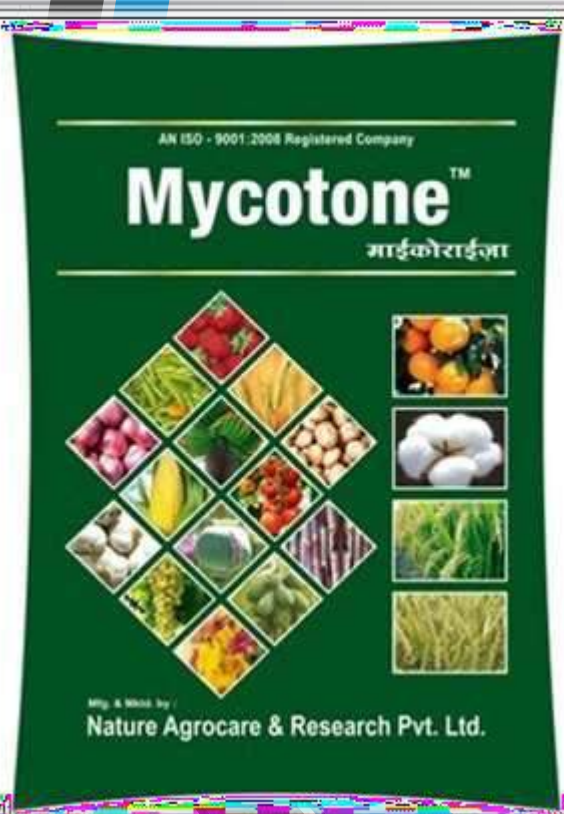
20 g of VAM inoculum is required per seedling. Apply inoculum at the time of planting.

- **For existing trees**

200 g VAM inoculum is required for inoculating one tree. Apply inoculum near the root surface at the time of fertilizer application.



# Few mycorrhizal products available commercially



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