

**AIM – STUDY OF THE MYCORRHIZAL
ROOT COLONIZATION AND SPORE
LOAD IN SOIL OF DIFFERENT PLANTS**

REQUIREMENTS – Tender root samples of different plants, FAA (Formalin-40%, Acetic acid-5 ml, 70 % ethanol 90 ml), 10 % KOH, Tryphan blue, lactophenol, Sieves of 100-400 micron, Filter paper, Light Microscope, Stereoscopic binocular Microscope, Microscopic slides and cover slips



PRINCIPLE

- Mycorrhiza is the product of an association between a fungus and plant root and act as bio fertilizing agent for plants
- Vesicular-arbuscular mycorrhiza (VAM) is formed by the symbiotic association between certain Phycomycetous fungi and Angiospermic plant roots with a very wide host range
- The purpose of studying the root colonization by VAM fungi to examine and confirm the intensity of mycorrhiza formation with the root system and spore load to estimate the fertility of the soil
- There are six genera of fungi belonging to Endogonaceae which have been shown to form mycorrhizal associations: *Glomus*, *Gigaspora*, *Acaulospora*, *Entrophospora*, *Sclerocystis* and *Scutellospora*



The fungus colonizes the root cortex forming a **mycelial network** and characteristic **vesicles** (bladder-like structures) and **arbuscules** (branched finger-like hyphae)

The **Chlamydospores** are formed externally in the rhizosphere soil, as well as internally in some cases in the root cortex

1. Hyphal Proliferation in the Cortex –

- ✓ Aseptate hyphae spread along the cortex in both directions from the entry point to form a colony
- ✓ Hyphae within root are initially without cross walls but later cross septa may developed



2. Visible Structures

✓ Arbuscules-

- These are complexly branched haustoria, formed within a root cortex cell
- They are formed by repeated dichotomous branching and ending in a proliferation of fine branched hyphae

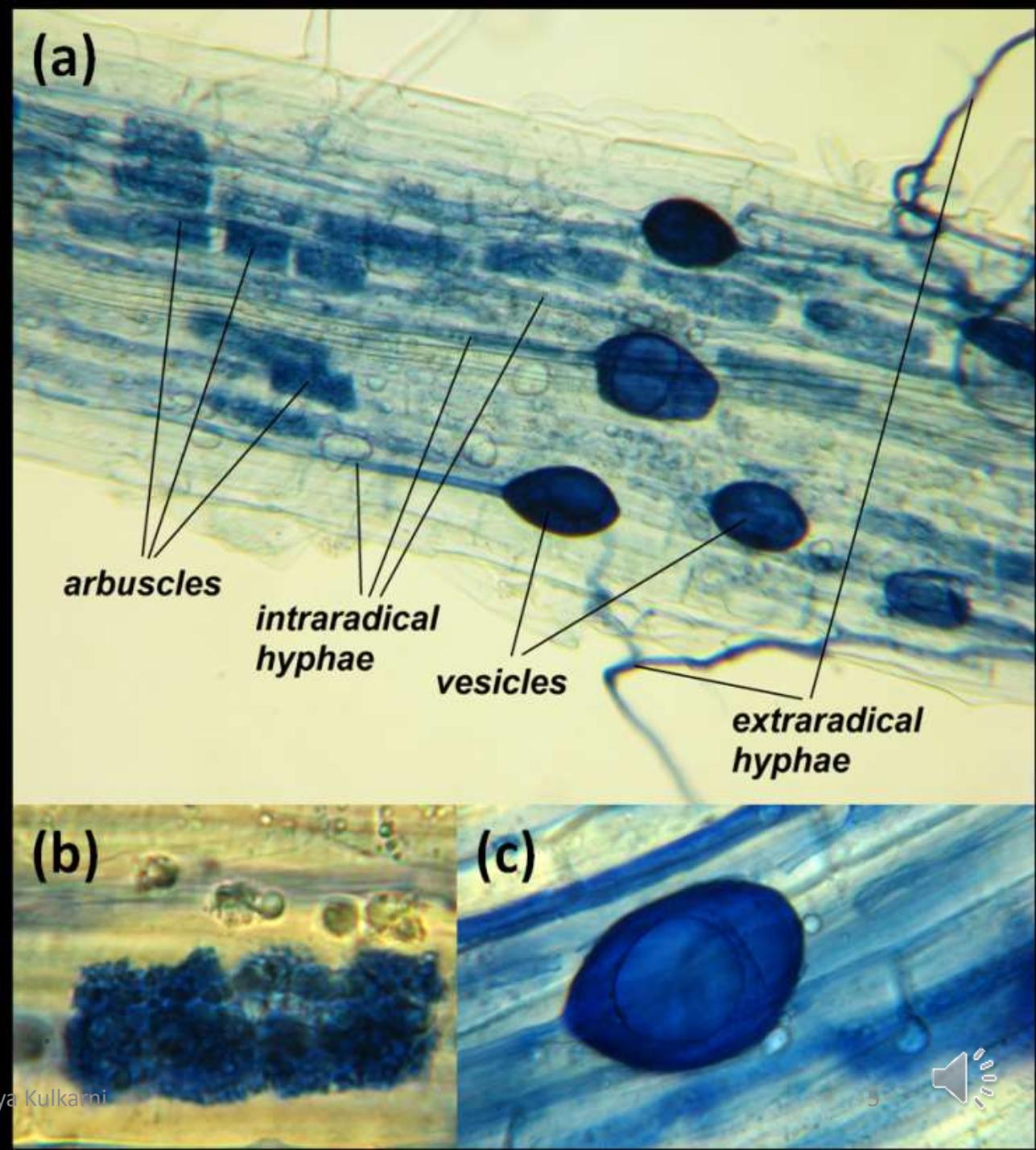
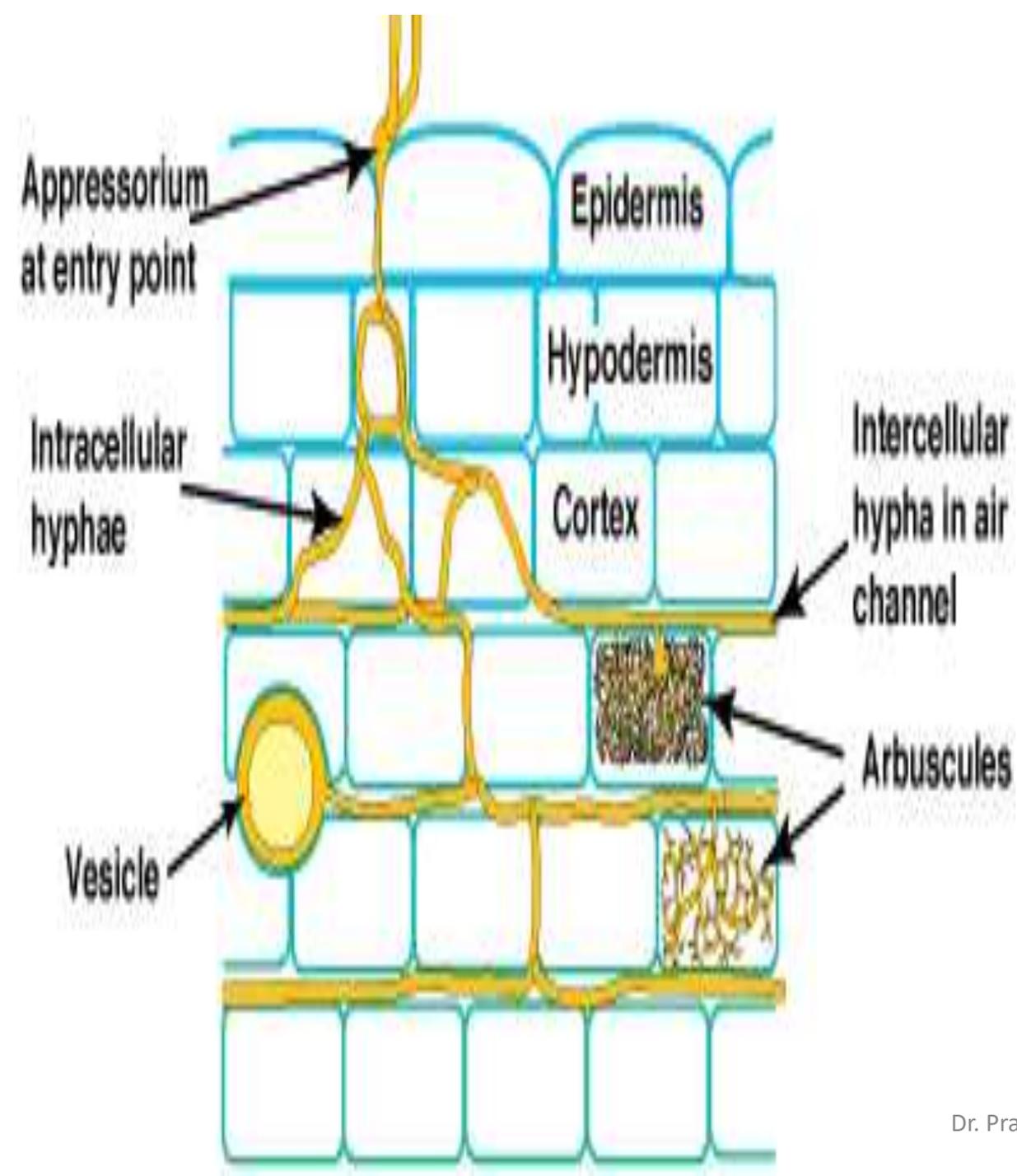
✓ Vesicles-

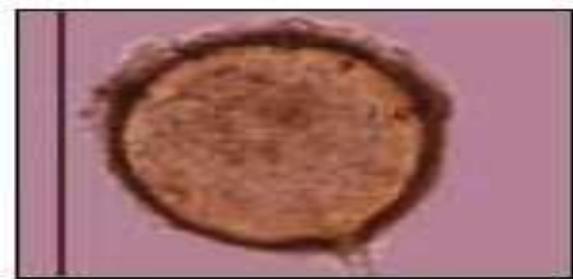
- Vesicles are hyphal swelling in the root cortex that contain lipid and cytoplasm and having a storage function
- These may be inter on intra cellular and may functions as propagules

✓ Chalmydospores-

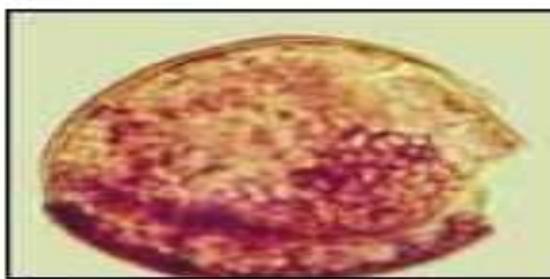
- These are swelling of one or more subtending hypha in the soil / in roots
- These usually develop with thick walls of more than one layer and can functions as propagules
- These structure contain lipids, cytoplasm and many nuclei
- They vary in their shape and size thus used for identification at genus level







Glomus mosseae



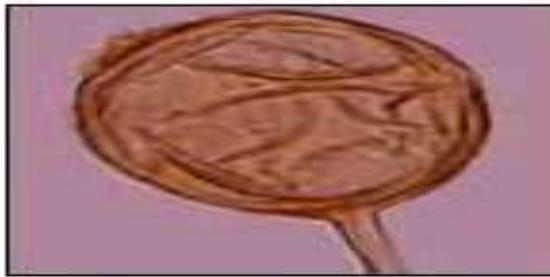
Glomus constrictum



Glomus invarmaium



Glomus caledonium



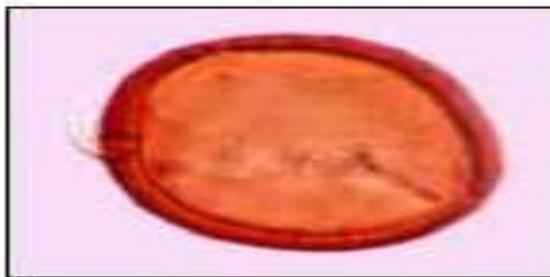
Glomus canadens



Glomus claroideum



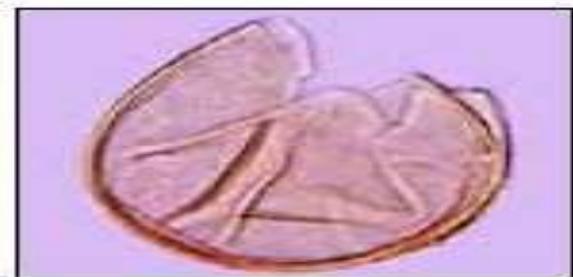
Glomus fasciculatum



Glomus occultum



Acaulospora bireticulata



Acaulospora denticulata



Acaulospora longula





Gigaspora



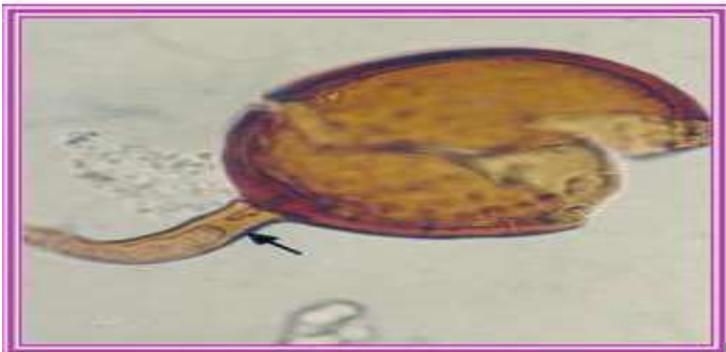
Glomus mosseae



Glomus albidum



Scutellospora



Glomus intraradices



Sclerocystis



PROCEDURE

1. Root Colonization:

- **Collect fresh tender roots of some plants, wash them thoroughly and cut them into small pieces**
- **Dip the roots bits in 10 % KOH solution and heat at 90°C for 1-2 hrs.**
- **Wash the roots bits with water for 3-4 times to remove the traces of KOH**
- **Immerse the root bits in Tryptophan blue stain (0.05% in Lactophenol) for over night (Phillips and Hyman, 1970)**



- **Pour off the stain and wash the root bits with water to remove excess stain**
- **Examine the roots bits under microscope and record the presence and absence of fungal hyphae, arbuscules, vesicles and spores**
- **The mycorrhizal colonization was determined by using following formula**

$$\% \text{ Colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$



2. Spore Load-

- Carry out Spore extraction from the soil using the Wet Sieving and Decanting Technique by Gerdemann and Nicolson (1963)
- Take 100 g of soil in a beaker and add water in the ratio of 1:10 and stir it well
- Allow the above solution to settle for few minutes
- Decant the soil suspension on ascending series of 100, 200, 300, 400 micron sieves
- Collect VAM spores from each sieve separately with some water and filter with the help of Whatman no. 1 filter paper
- Mount the filter paper on glass slide and observe under Stereoscopic microscope



- **Study the morphological characters of collected VAM spores under microscope and count the numbers/ 100 g of soil**
- **Individual spores can be picked up by a fine needle and collected in distilled water for further use**
- **Identify spores according to the manual of identification of VAM fungi by Schenek and Perez (1990)**
- **Additional spores not included in the manual were identified as per the description given in the INVAM web soil (<http://invam.caf.wvu.edu/>)**



OBSERVATION

- **Tabulate your observation in the given table:**

S.No.	Name of Plant	% Root colonization by VAM

- **Spore Load Count:**

S.No.	Soil Type	Spore Load/ 100g of Soil



- **RESULT:** Maximum and Minimum count of root colonization and Spore Load in the studies plants
- **INTERPRETATION:** Discuss the result with your teacher and interpret the results
- **PRECAUTIONS:**
 - ✓ Tender and fine roots should be collected as they have enough cortical area and are easy to macerate for getting the stain in
 - ✓ KOH treatment and washing should be done carefully
 - ✓ Remove all soil debris and sieve the soil suspension carefully as the sieves are very fine and get damaged by soil debris
 - ✓ Microscopic examination needs practice and patience



Thank you.....

