

Mycorrhiza types Isolation Characterization screening & mass production

Mycorrhiza

Introduction, isolation, characterization, screening, mass production, advantages and application.

Presented by:

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MYCORRHIZA



- The word mycorrhiza was coined by the German scientist **Albert Bernhard Frank** in 1885.
- The word mycorrhiza is derived from the **Greek** words – '**mukes**' meaning fungus and '**rhiza**' meaning roots.
- Mycorrhiza (fungus-root) can be defined as a **symbiotic** association between **fungi** and **plant roots**.

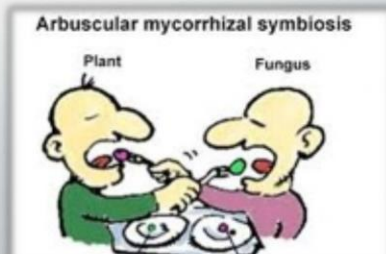
Mycorrhiza

Importance

- **95%** of all the world's plant species form mycorrhizal relationships with fungi and that in the majority of cases the plant would not survive without them.
- Present in 95% of plants (83% Dicots, 79% Monocots and 100% Gymnosperms).
- Brassicaceae, Cyperaceae, and Juncaceae- do not have mycorrhizal associations (10-20%).
- The **Orchidaceae** are notorious as a family in which the absence of the correct mycorrhizae is fatal even to germinating seeds

Importance

- Mycorrhizae have existed for a very long time and can be demonstrated in the fossilized roots of some of the earliest land plants.
- Some scientists have suggested that plants were only able to move on to land when they had developed mycorrhizal relationships with fungi.



Classification of mycorrhiza

Based on tropic level by A.B. Frank

- Ectotropic Mycorrhiza
- Endotropic Mycorrhiza

Based on morphological and anatomical feature

- Ectomycorrhiza
- Endomycorrhiza
- Ectendomycorrhiza

Broadly classified into (Mark)

- Ectomycorrhiza (EcM)
- Endomycorrhiza (AM / VAM)
- Ectendomycorrhiza
- Monotropoid mycorrhiza
- Arbutoid mycorrhiza
- Orchid mycorrhiza
- Ericoid mycorrhiza

Ectomycorrhiza or ectotrophic mycorrhiza (EcM)

- **Ectomycorrhizas**, or EM, are typically formed between the roots of around 10% of plant families, mostly woody plants including the birch, dipterocarp, eucalyptus, oak, pine, deodar and rose families, orchids, and fungi belonging to the **Basidiomycota**, **Ascomycota** and **Zygomycota**.
- Commonly associated with **trans temperate forest trees**.

- Ectomycorrhizal fungi form a **sheath** or **mantle** around the root, and hyphae emanate through the soil increasing the surface area.
- The fungus grows within the root cell wall but never penetrates the cell interior.
- It grows between the cells of the cortex to form **Hartig net**.
- The **Hartig net** present outside the endodermis and meristematic zones is the **site** for **nutrient exchange**.
- **Colonization** of root tips induces marked **changes** in the **host root morphology**.

Fungus forming ectomycorrhizae

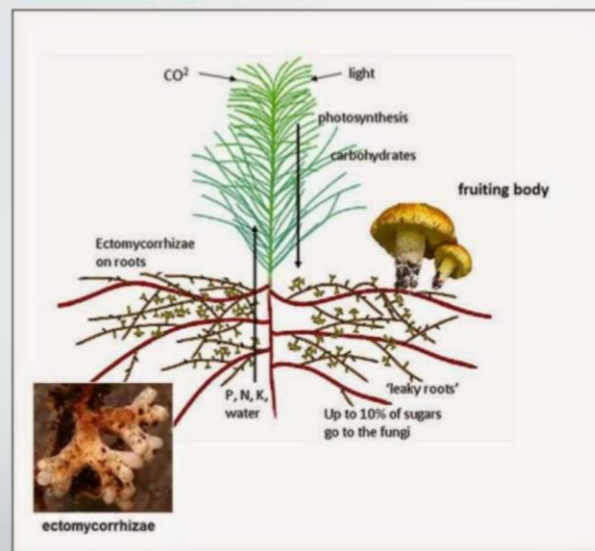
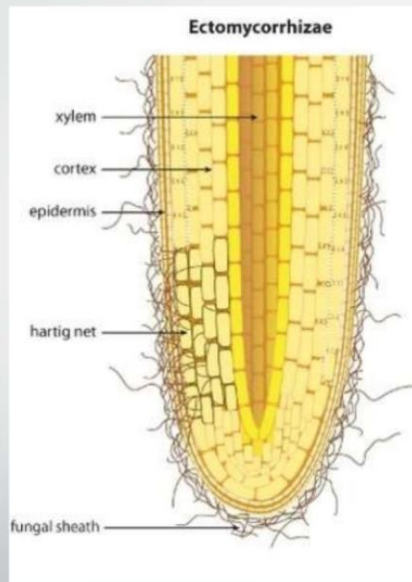
- *Amanita muscaria*
- *Boletus variegatus*
- *Paxillus involutus*
- *Rhizopogon vinicolor*
- *Entomoloma*
- *Sclerodendran*



Amanita muscaria



Entomoloma



Ectomycorrhiza

Advantages of ectomycorrhiza

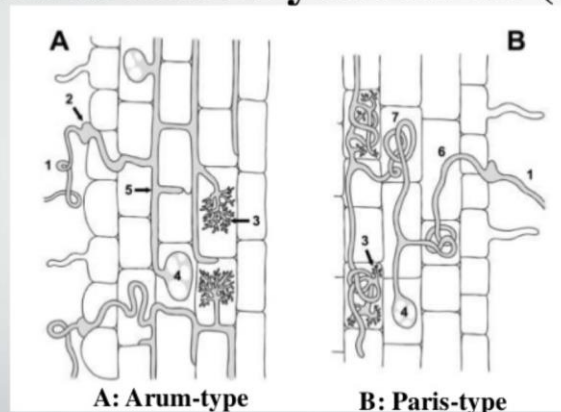
- Extensive multibranching hyphae increases the water holding capacity of plants.
- Increase the tolerance to drought, high soil temperature, organic and inorganic soil toxins, extremes of soil acidity to sulphur and aluminium.
- Deter infection of feeder roots by some rot pathogens.
- Enhance the uptake of many nutrients. (P, Cu, Zn through Hartig net)
- Disease control through barrier effect, competitive exclusion.
- Play a key role in afforestation.

Endomycorrhiza or endotrophic mycorrhiza

- **Arbuscular mycorrhizae** (often called AM) are the most common and widespread of all mycorrhizae and are found in as many as 85%-90% of the world's plant species.
- Commonly associated with agricultural, horticulture crops in addition to tropical trees.

- The external hyphal mantle or sheath is absent or scanty. The fungal hyphae enters inside the root cortex and penetrates the cortical cells.
- This is not a destructive parasitic association but endomycorrhiza are present at certain times as a part of normal root development.
- AM fungi penetrate the cell walls of root cells.
- They grow between the cell wall and cell membrane forming **arbuscules**.
- **VAM** fungi produce **vesicles** for lipid storage.

Two main types of root colonization in arbuscular mycorrhizae (AM).



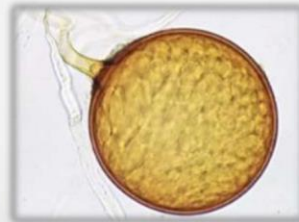
1: extraradical hyphae; 2: appressorium/hyphopodium; 3: arbusculum; 4: vesiculum; 5: intercellular hyphae; 6: intracellular hyphae; 7: hyphal coils.

Two main types of root colonization in arbuscular mycorrhizae (AM).

- In the **Arum-type** the fungal hyphae grow **intercellularly** and well-developed arbuscules are formed on branches entering the neighboring cells.
- In the **Paris-type** the hyphae grow **intracellularly**, develop hyphal coils in some cortical cells and smaller arbuscules develop on these coils. Both the fungal and the plant partner influence the type developed

Fungi forming endomycorrhizae

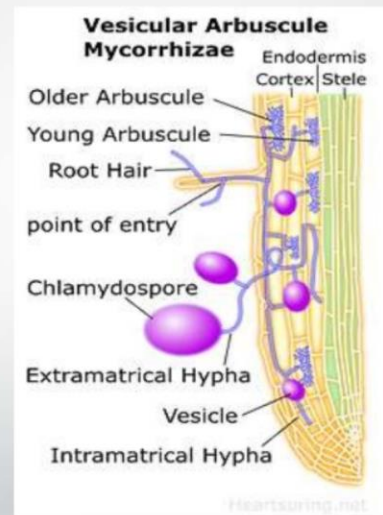
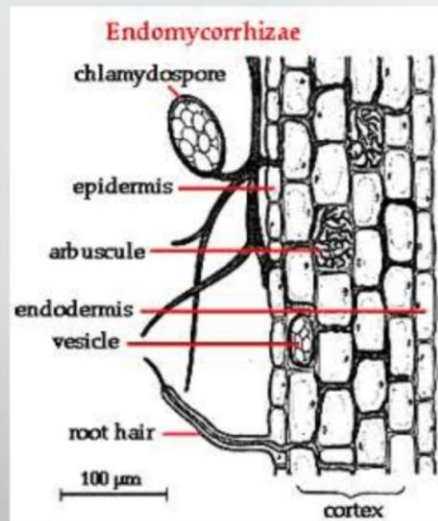
- *Endogone*
- *Glomus*
- *Sclerocystis*
- *Acaulospora*
- *Gigaspora*
- *Enterophthora*
- *Scutellispora*



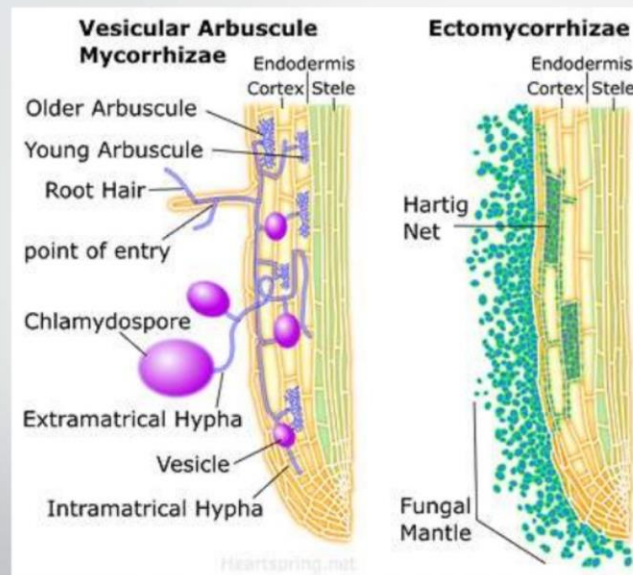
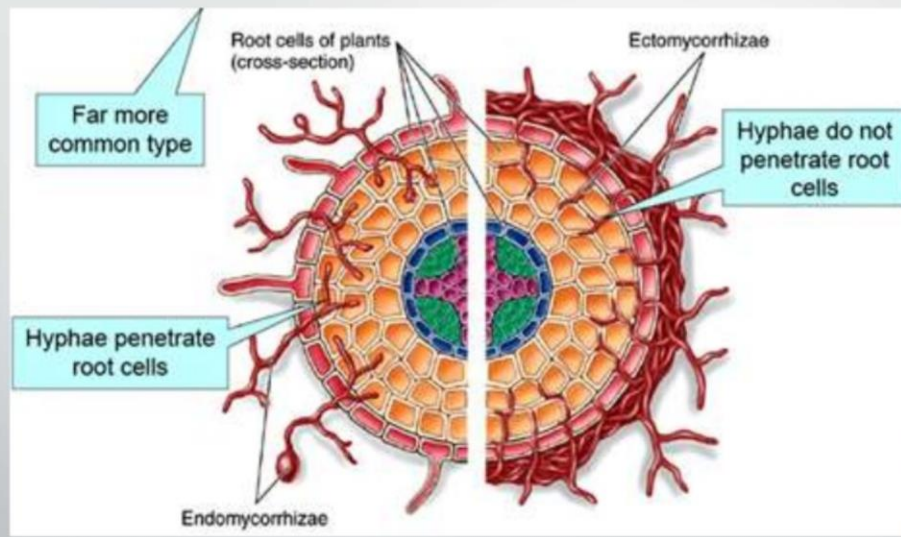
Glomus



Gigaspora



Endomycorrhizae	Ectomycorrhizae
Generally fungi produce its typical structures, vesicles and arbuscules inside the root system.	Fungi produce majority of its structure outside the root system.
Commonly associated with agricultural, horticultural and tropical trees.	Commonly associated with trans temperate forest tree roots.
Have a loose network of hyphae in the soil and an extensive growth within the cortex cells of the plants.	Form a complete mantle or sheath over the surface of the root and hyphae grows out into the soil.
Cannot be cultured on artificial media.	Can be cultured on artificial media.
Doesn't cause morphological changes in roots.	Cause morphological changes in roots.



Ectendomycorrhiza

- They share the features of both ecto- and endomycorrhiza.
- They have less developed hyphal mantle.
- The hyphae within the host penetrate its cells and grow within.
- These are found in both angiosperms and gymnosperms.
- Fungus associated are Ascomycetes.
- Hosts are *Eucalyptus*, *Salix*, *Alnus* etc.

Monotropoid mycorrhiza

- The family Monotropaceae, which includes achlorophyllous plants, develop the association.
- These plants entirely depend upon the fungus for carbon and energy.
- Sheath, inter- and intracellular hyphae and peg-like are present.



Monotropa sp

Arbutoid mycorrhiza

- These are found in the family Ericaceae.
- The fungi penetrate into the cortical cells forming extensive coils of hyphae.
- The mycosymbionts are Basidiomycetes.
- Sheath, inter- and coiled intercellular hyphae are present.

Orchid mycorrhiza

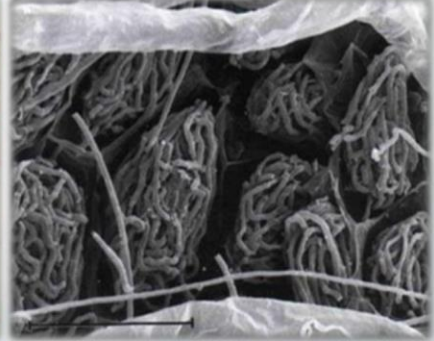
- At some point of time all Orchids are infected by orchidaceous mycorrhiza – **Basidiomycota**.
- Orchids **germinate only after infection by mycorrhiza**. Ex: *Rhizoctonia sp.*
- Within cells, hyphae form coils called **pelotons** which greatly increase the interfacial surface area between orchid and fungus.



Cymbidium orchid



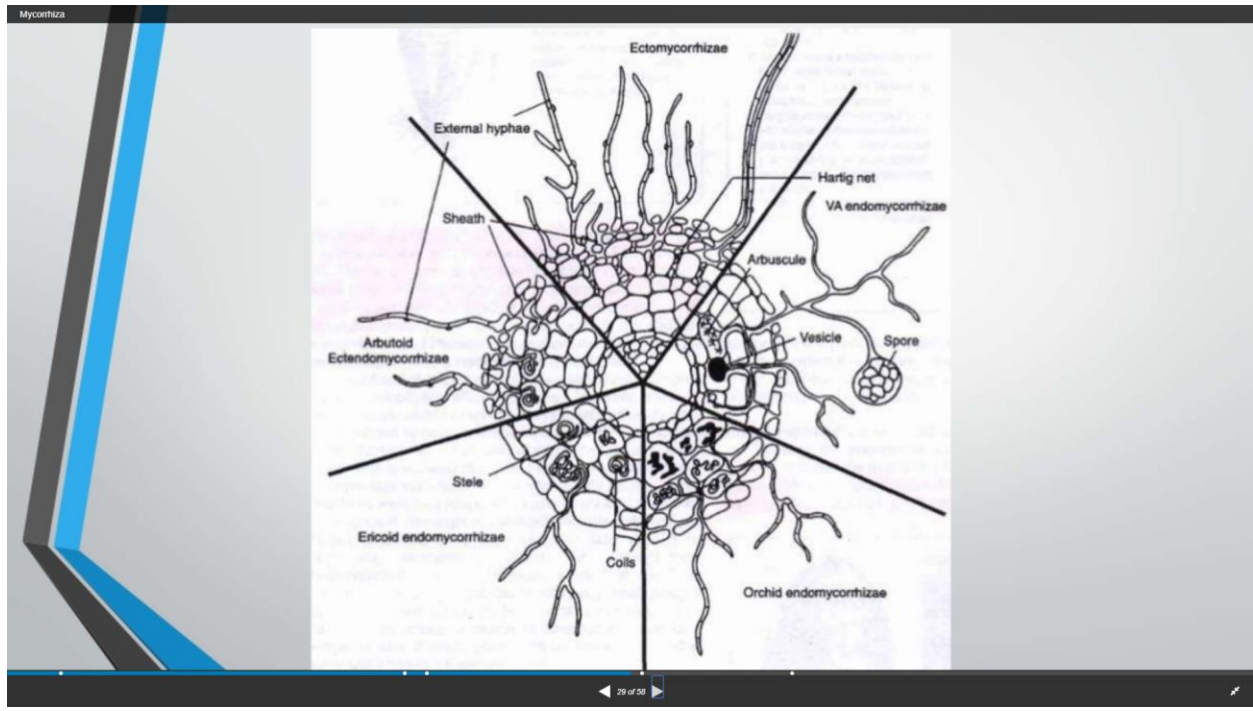
Orchid pelotons stained red in a light micrograph of sectioned tissue.



Scanning electronmicrograph of orchid pelotons

Ericoid mycorrhiza

- This type occurs in the family Ericaceae.
- These plants have fine roots and the fungal members of **ascomycetes** like *Pezizella*, *Clavaria* forms the association in the outer region of cortical layer of roots.
- The ericoid fungal hyphae form a **loose network** over the hair root surface the hyphae can also penetrate the epidermal cells, often at several points in each cell and coiled hyphae fill the cell.
- Up to 80% of root volume can be fungal tissue and it is through these coils that nutrient exchange is thought to occur



Mycorrhiza	Host range	Types of relationship
Ectomycorrhiza	Gymnosperms and Angiosperms	Sheath, intercellular hyphae
Endomycorrhiza (VAM)	All groups of plant kingdom	Coiled intracellular hyphae, vesicle and arbuscules present
Ectendomycorrhiza	Gymnosperms and Angiosperms	Sheath optional, inter and intracellular hyphae
Monotropoid mycorrhiza	Very restricted, Monotropaceae	Sheath, inter and coiled intracellular hyphae
Arbutoid mycorrhiza	Very restricted, Ericales	Sheath, inter and coiled intracellular hyphae
Orchid mycorrhiza,	Restricted, Orchidaceae	Only coiled intracellular hyphae
Ericoid mycorrhiza	Very restricted, Ericales	No sheath, no intercellular hyphae, long,

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

A. Sieving method

Requirements

- Soil sample
- 500 ml beaker
- Sieves of 710 μm , 250 μm , 75 μm and 45 μm .
- 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 μm sieve to remove large organic matter and roots.

Procedure

- Add 200 ml of water to the suspension.
- Decant the suspension through 710 μm sieve.
- Decant this through 250 μm , 75 μm and 45 μm sieves consequently.
- Collect the residue on the 45 μm sieve.
- Wash the residues well with water and collect the spore.

B. Flootation 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.

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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.

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