<u>UNIT-III</u>

Mycorrhiza:

# Definition

A mycorrhiza is a symbiotic association between a green plant and a fungus. The plant makes organic molecules such as sugars by photosynthesis and supplies them to the fungus, and the fungus supplies to the plant water and mineral nutrients, such as phosphorus, taken from the soil. Mycorrhizas are located in the roots of vascular plants, but mycorrhiza-like associations also occur in bryophytes<sup>[4]</sup> and there is fossil evidence that early land plants that lacked roots formed arbuscular mycorrhizal associations.<sup>[5]</sup> Most some plant species form mycorrhizal associations, though families like <u>Brassicaceae</u> and <u>Chenopodiaceae</u> cannot. Different forms for the association are detailed in the next section. The most common is the arbuscular type that is present in 70% of plant species, including many crop plants such as wheat and rice.<sup>[6]</sup>

# Types

Mycorrhizas are commonly divided into *ectomycorrhizas* and *endomycorrhizas*. The two types are differentiated by the fact that the hyphae of ectomycorrhizal fungi do not penetrate individual <u>cells</u> within the root, while the <u>hyphae</u> of endomycorrhizal fungi penetrate the cell wall and invaginate the <u>cell membrane</u>.<sup>[7][8]</sup> Endomycorrhiza includes *arbuscular*, *ericoid*, and *orchid mycorrhiza*, while *arbutoid mycorrhizas* can be classified as *ectoendomycorrhizas*. *Monotropoid* mycorrhizas form a special category.

# Ectomycorrhiza

## <u>Ectomycorrhiza</u>

Ectomycorrhizas, or EcM, are symbiotic associations between the roots of around 10% of plant families, mostly woody plants including the <u>birch</u>, <u>dipterocarp</u>, <u>eucalyptus</u>, <u>oak</u>, <u>pine</u>, and <u>rose<sup>[9]</sup></u> families, <u>orchids</u>,<sup>[10]</sup> and fungi belonging to the <u>Basidiomycota</u>, <u>Ascomycota</u>, and <u>Zygomycota</u>. Some EcM fungi, such as many <u>Leccinum</u> and <u>Suillus</u>, are symbiotic with only one particular genus of plant, while other fungi, such as the <u>Amanita</u>, are generalists that form mycorrhizas with many different plants.<sup>[11]</sup> An individual tree may have 15 or more different fungal EcM partners at one time.<sup>[12]</sup> Thousands of ectomycorrhizal fungal species exist, hosted in over 200 genera. A recent study has conservatively estimated global ectomycorrhizal fungal species richness at approximately 7750 species, although, on the basis of estimates of knowns and unknowns in macromycete diversity, a final estimate of ECM species richness would probably be between 20,000 and 25,000.<sup>[13]</sup>

Ectomycorrhizas consist of a hyphal sheath, or mantle, covering the root tip and a <u>Hartig net</u> of hyphae surrounding the plant cells within the root <u>cortex</u>. In some cases the hyphae may also penetrate the plant cells, in which case the mycorrhiza is called an ectendomycorrhiza. Outside the root, <u>ectomycorrhizal extramatrical mycelium</u> forms an extensive network within the soil and leaf litter.

Nutrients can be shown to move between different plants through the fungal network. Carbon has been shown to move from <u>paper</u> <u>birch</u> trees into <u>Douglas-fir</u> trees thereby promoting <u>succession</u> in <u>ecosystems</u>.<sup>[14]</sup> The ectomycorrhizal fungus <u>Laccariabicolor</u> has been found to lure and kill <u>springtails</u> to obtain nitrogen, some of which may then be transferred to the mycorrhizal host plant. In a study by Klironomos and Hart, <u>Eastern White Pine</u> inoculated with *L. bicolor* was able to derive up to 25% of its nitrogen from springtails.<sup>[15][16]</sup> When compared to non-mycorrhizal fine roots, ectomycorrhizae may contain very high concentrations of trace elements, including toxic metals (cadmium, silver) or chlorine.<sup>[17]</sup>

The first genomic sequence for a representative of symbiotic fungi, the ectomycorrhizal basidiomycete *L. bicolor*, was published in 2008.<sup>[18]</sup> An expansion of several multigene families occurred in this fungus, suggesting that adaptation to symbiosis proceeded by gene duplication. Within lineage-specific genes

those coding for symbiosis-regulated secreted proteins showed an up-regulated expression in ectomycorrhizal root tips suggesting a role in the partner communication. *L. bicolor* is lacking enzymes involved in the degradation of plant cell wall components (cellulose, hemicellulose, pectins and pectates), preventing the symbiont from degrading host cells during the root colonisation. By contrast, *L. bicolor* possesses expanded multigene families associated with hydrolysis of bacterial and microfauna polysaccharides and proteins. This genome analysis revealed the dual <u>saprotrophic</u> and <u>biotrophic</u> lifestyle of the mycorrhizal fungus that enables it to grow within both soil and living plant roots.

### Arbutoid mycorrhiza

This type of mycorrhiza involves plants of the Ericaceae subfamily <u>Arbutoideae</u>. It is however different from ericoid mycorrhiza and resembles ectomycorrhiza, both functionally and in terms of the fungi involved.<sup>[citation needed]</sup> It differs from ectomycorrhiza in that some hyphae actually penetrate into the root cells, making this type of mycorrhiza an *ectendomycorrhiza*.<sup>[19]</sup>

## Endomycorrhiza

Endomycorrhizasare variable and have been further classified as arbuscular, ericoid, arbutoid, monotropoid, and orchid mycorrhizas.

Wheat is arbuscular mycorrhizal

#### Arbuscular mycorrhiza

### Main article: Arbuscular mycorrhiza

<u>Arbuscular mycorrhizas</u>, or AM (formerly known as vesicular-arbuscular mycorrhizas, or VAM), are mycorrhizas whose hyphae penetrate plant cells, producing structures that are either balloon-like (vesicles) or dichotomously branching invaginations (arbuscules) as a means of nutrient exchange. The fungal <u>hyphae</u> do not in fact penetrate the <u>protoplast</u> (i.e. the interior of the cell), but invaginate the <u>cell</u> <u>membrane</u>. The structure of the arbuscules greatly increases the contact surface area between the hypha and the cell <u>cytoplasm</u> to facilitate the transfer of nutrients between them.

Arbuscular mycorrhizas are formed only by fungi in the <u>division Glomeromycota</u>. Fossil evidence<sup>151</sup> and DNA sequence analysis<sup>[21]</sup> suggest that this mutualism appeared <u>400-460 million years ago</u>, when the first plants were colonizing land. Arbuscular mycorrhizas are found in 85% of all plant families, and occur in many crop species.<sup>[9]</sup> The hyphae of arbuscular mycorrhizal fungi produce the glycoprotein <u>glomalin</u>, which may be one of the major stores of carbon in the soil.<sup>[22]</sup> Arbuscular mycorrhizal fungi have (possibly) been asexual for many millions of years and, unusually, individuals can contain many genetically different nuclei (a phenomenon called <u>heterokaryosis</u>).<sup>[23]</sup>

#### Ericoid mycorrhiza

<u>Ericoid mycorrhizas</u> are the third of the three more ecologically important types. They have a simple intraradical (grow in cells) phase, consisting of dense coils of hyphae in the outermost layer of root cells. There is no periradical phase and the extraradical phase consists of sparse hyphae that don't extend very far into the surrounding soil. They might form sporocarps (probably in the form of small cups), but their reproductive biology is little understood.

Ericoid mycorrhizas have also been shown to have considerable <u>saprotrophic</u> capabilities, which would enable plants to receive nutrients from not-yet-decomposed materials via the decomposing actions of their <u>ericoid</u> partners.<sup>[25]</sup>

#### Orchid mycorrhiza

All <u>orchids</u> are <u>myco-heterotrophic</u> at some stage during their lifecycle and form <u>orchid mycorrhizas</u> with a range of basidiomycete fungi. [citation needed] Their hyphae penetrate into the root cells and form pelotons (coils) for nutrient exchange. [citation needed]

## Monotropoid mycorrhiza[<u>edit</u>] Main article: <u>Myco-heterotrophy</u>

This type of mycorrhiza occurs in the subfamily <u>Monotropoideae</u> of the <u>Ericaceae</u>, as well as several genera in the <u>Orchidaceae</u>. These plants are <u>heterotrophic</u> or <u>mixotrophic</u> and derive their carbon from the fungus partner. This is thus a non-mutualistic, <u>parasitic</u> type of mycorrhizal symbiosis.<sup>[citation needed]</sup>

# **Mutualist dynamics**

Nutrient exchanges and communication between a mycorrhizal fungus and plants.

Mycorrhizal fungi form a <u>mutualistic</u> relationship with the roots of most plant species. In such a relationship, both the plants themselves and those parts of the roots that host the fungi, are said to be mycorrhizal. Relatively few of the mycorrhizal relationships between plant species and fungi have been examined to date, but 95% of the plant families investigated are predominantly mycorrhizal either in the sense that most of their species associate beneficially with mycorrhizae, or are absolutely dependent on mycorrhizae. The <u>Orchidaceae</u> are notorious as a family in which the absence of the correct mycorrhizae is fatal even to germinating seeds.<sup>[26]</sup>

Recent research into <u>ectomycorrhizal</u> plants in <u>boreal forests</u> has indicated that mycorrhizal fungi and plants have a relationship that may be more complex than simply mutualistic. This relationship was noted when mycorrhizal fungi were unexpectedly found to be hoarding nitrogen from plant roots in times of nitrogen scarcity. Researchers argue that some mycorrhizae distribute nutrients based upon the environment with surrounding plants and other mycorrhizae. They go on to explain how this updated model could explain why mycorrhizae do not alleviate plant nitrogen limitation, and why plants can switch abruptly from a mixed strategy with both mycorrhizal and nonmycorrhizal roots to a purely mycorrhizal strategy as soil nitrogen availability declines.<sup>[22]</sup> It has also been suggested that evolutionary and phylogenetic relationships can explain much more variation in the strength of mycorrhizal mutualisms than ecological factors.<sup>[28]</sup>

Within mutualistic mycorrhiza, the plant gives carbohydrates (products of photosynthesis) to the fungus, while the fungus gives the plant water and minerals in exchange.

## Sugar-water/mineral exchange

In this mutualism, fungal hyphae (E) increase the surface area of the root and uptake of key nutrients while the plant supplies the fungi with fixed carbon (A=root cortex, B=root epidermis, C=arbuscle, D=vesicle, F=root hair, G=nuclei).</ref>

The mycorrhizal mutualistic association provides the fungus with relatively constant and direct access to <u>carbohydrates</u>, such as <u>glucose</u> and <u>sucrose</u>.<sup>[29]</sup> The carbohydrates are translocated from their source (usually leaves) to root tissue and on to the plant's fungal partners. In return, the plant gains the benefits of the <u>mycelium</u>'s higher absorptive capacity for water and mineral nutrients, partly because of the large surface area of fungal hyphae, which are much longer and finer than plant <u>root hairs</u>, and partly because some such fungi can mobilize soil minerals unavailable to the plants' roots. The effect is thus to improve the plant's mineral absorption capabilities.<sup>[30]</sup>

Unaided plant roots may be unable to take up <u>nutrients</u> that are chemically or physically <u>immobilised</u>; examples include <u>phosphate ions</u> and <u>micronutrients</u> such as iron. One form of such immobilization occurs in soil with high <u>clay</u> content, or soils with a strongly <u>basic pH</u>. The <u>mycelium</u> of the mycorrhizal fungus can, however, access many such nutrient sources, and make them available to the plants they colonize.<sup>[31]</sup> Thus, many plants are able to obtain phosphate, without using soil as a source. Another form of immobilisation is when nutrients are locked up in organic matter that is slow to decay, such as wood, and some mycorrhizal fungi act directly as decay organisms, mobilising the nutrients and passing some onto the host plants; for example, in some <u>dystrophic</u> forests, large amounts of phosphate and other nutrients are taken up by mycorrhizal <u>hyphae</u> acting directly on leaf litter, bypassing the need for soil

uptake.<sup>[32]</sup> <u>Inga alley cropping</u>, proposed as an alternative to <u>slash and burn</u> rainforest destruction,<sup>[33]</sup> relies upon mycorrhiza within the root system of species of <u>Inga</u> to prevent the rain from washing <u>phosphorus</u> out of the soil.<sup>[34]</sup>

In some more complex relationships, mycorrhizal fungi do not just collect immobilised soil nutrients, but connect individual plants together by <u>mycorrhizal networks</u> that transport water, carbon, and other nutrients directly from plant to plant through underground hyphal networks.<sup>[35]</sup>

<u>Suillustomentosus</u>, a <u>basidiomycete</u> fungus, produces specialized structures known as tuberculate ectomycorrhizae with its plant host <u>lodgepole pine</u> (*Pinus contorta* var. *latifolia*). These structures have been shown to host <u>nitrogen fixing bacteria</u> which contribute a significant amount of <u>nitrogen</u> and allow the pines to colonize nutrient-poor sites.<sup>[36]</sup>

## Mechanisms

The mechanisms by which mycorrhizae increase absorption include some that are physical and some that are chemical. Physically, most mycorrhizal mycelia are much smaller in diameter than the smallest root or root hair, and thus can explore soil material that roots and root hairs cannot reach, and provide a larger surface area for absorption. Chemically, the cell membrane chemistry of fungi differs from that of plants. For example, they may secrete <u>organic acids</u> that dissolve or <u>chelate</u> many ions, or release them from minerals by <u>ion exchange</u>.<sup>[32]</sup> Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils.<sup>[38]</sup>

## Disease, drought and salinity resistance and its correlation to mycorrhizae

Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soilborne <u>pathogens</u>. These associations have been found to assist in plant defense both above and belowground. Mycorrhizas have been found to excrete enzymes that are toxic to soil borne organisms such as nematodes.<sup>[39]</sup> More recent studies have shown that mycorrhizal associations result in a priming effect of plants that essentially acts as a primary immune response. When this association is formed a defense response is activated similarly to the response that occurs when the plant is under attack. As a result of this inoculation, defense responses are stronger in plants with mycorrhizal associations.<sup>[40]</sup>

AMF was also significantly correlated with soil biological fertility variables such as soil microbial communities and associated disease suppressiveness.<sup>[41]</sup> Thus, <u>ecosystem services</u> provided by AMF may depend on the soil microbiome.<sup>[41]</sup> Furthermore, AMF was significantly correlated with soil physical variable, but only with water level and not with aggregate stability.<sup>[42][43]</sup> and are also more resistant to the effects of drought.<sup>[44][45][46]</sup> The significance of arbuscular mycorrhizal fungi includes alleviation of salt stress and its beneficial effects on plant growth and productivity. Although salinity can negatively affect arbuscular mycorrhizal fungi, many reports show improved growth and performance of mycorrhizal plants under salt stress conditions <sup>[47]</sup>

## **Resistance to insects**

Research has shown that plants connected by mycorrhizal fungi can use these underground connections to produce and receive warning signals.<sup>[48][49]</sup> Specifically, when a host plant is attacked by an aphid, the plant signals surrounding connected plants of its condition. The host plant releases <u>volatile organic</u> <u>compounds</u> (VOCs) that attract the insect's predators. The plants connected by mycorrhizal fungi are also prompted to produce identical VOCs that protect the uninfected plants from being targeted by the insect.<sup>[48]</sup> Additionally, this assists the mycorrhizal fungi by preventing the plant's carbon relocation which negatively affects the fungi's growth and occurs when the plant is attacked by herbivores.<sup>[48]</sup>

# **Colonization of barren soil**

Plants grown in sterile <u>soils</u> and growth media often perform poorly without the addition of <u>spores</u> or hyphae of mycorrhizal fungi to colonise the plant roots and aid in the uptake of soil mineral nutrients.<sup>[50]</sup> The absence of mycorrhizal fungi can also slow plant growth in early succession or on degraded landscapes.<sup>[51]</sup> The introduction of alien mycorrhizal plants to nutrient-deficient ecosystems

puts indigenous non-mycorrhizal plants at a competitive disadvantage.<sup>[52]</sup> This aptitude to colonize barren soil is defined by the category <u>Oligotroph</u>.

# **Resistance to toxicity**

Fungi have been found to have a protective role for plants rooted in soils with high metal concentrations, such as <u>acidic</u> and <u>contaminated soils</u>. <u>Pine</u> trees inoculated with <u>Pisolithus tinctorius</u> planted in several contaminated sites displayed high tolerance to the prevailing contaminant, survivorship and growth.<sup>[53]</sup> One study discovered the existence of <u>Suillus luteus</u> strains with varying tolerance of <u>zinc</u>. Another study discovered that zinc-tolerant strains of <u>Suillusbovinus</u> conferred resistance to plants of <u>Pinus sylvestris</u>. This was probably due to binding of the metal to the extramatricial <u>mycelium</u> of the fungus, without affecting the exchange of beneficial substances.<sup>[52]</sup>

# Occurrence of mycorrhizal associations

At around 400 million years old, the <u>Rhynie chert</u> contains an assemblage of fossil plants preserved in sufficient detail that mycorrhizas have been observed in the stems of <u>Aglaophyton major</u>.<sup>[5]</sup>

Mycorrhizas are present in 92% of plant families studied (80% of species),<sup>[9]</sup> with <u>arbuscular</u> <u>mycorrhizas</u> being the ancestral and predominant form,<sup>[9]</sup> and the most prevalent symbiotic association found in the plant kingdom.<sup>[29]</sup> The structure of arbuscular mycorrhizas has been highly conserved since their first appearance in the fossil record,<sup>[5]</sup> with both the development of ectomycorrhizas, and the loss of mycorrhizas, <u>evolving convergently</u> on multiple occasions

## Mass production of Mycorrhizal biofertilizer

The commercial utilization of mycorrhizal fungi has become difficult because of the obligate symbiotic nature and difficulty in culturing on laboratory media. Production of AM inoculum has evolved from the original use of infested field soils to the current practice of using pot culture inoculum derived from the surface disinfected spores of single AM fungus on a host plant grown in sterilized culture medium. Several researches in different parts of the world resulted in different methods of production of AM fungal inoculum as soil based culture as well as carrier based inoculum. Root organ culture and nutrient film technique provide scope for the production of soil less culture.

As a carrier based inoculum, pot culture is widely adopted method for production. The AM inoculum was prepared by using sterilized soil and wide array of host crops were used as host. The sterilization process is a cumbersome one and scientists started using inert materials for production of AM fungi. The researchers tried use of perlite, montmorillonite clay etc., In vermiculite was tried as substrate for the replacement of soil sterilization, which resulted in the best method of inoculum production.



#### Method of production

A trench (1m x 1m x 0.3m) is formed and lined with black polythene sheet to be used as a plant growth tub.

• Mixed 50 kg of vermiculite and 5 kg of sterilized soil and packed in the trench up to a height of 20 cm

• Spread 1 kg of AM inoculum (mother culture) 2-5 cm below the surface of vermiculite

• Maize seeds surface sterilized with 5% sodium hypochlorite for 2 minutes are sown

• Applied 2 g urea, 2 g super phosphate and 1 g muriate of potash for each

trench at the time of sowing seeds. Further 10 g of urea is applied twice on 30 and 45 days after sowing for each trench

• Quality test on AM colonization in root samples is carried out on 30th and 45th day

- Stock plants are grown for 60 days (8 weeks). The inoculum is obtained by cutting all the roots of stock plants. The inoculum produced consists of a mixture of vermiculite, spores, pieces of hyphae and infected root pieces.
- Thus within 60 days 55 kg of AM inoculum could be produced from 1 sq meter area. This inoculum will be sufficient to treat 550 m2 nursery area having 11,000 seedlings.