Plant–Fungus Marriages

Ramesh Maheshwari

The roots of most land plants establish intimate associations with soil fungi. This association of plant roots with fungi is termed mycorrhiza. It is a marriage between two highly dissimilar organisms based on mutual exchange of nutrients: while the plants provide photosynthetically made carbon compounds to the fungi, the fungi in turn provide to the plants nitrogen and phosphorus scavenged from the breakdown of organic polymers in natural substrates. This mutually beneficial partnership makes possible the green cover on Earth and of all life, ultimately, dependent on plants.

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More than a century of observations have revealed that roots of majority of land plants are associated with fungi. However, it is only in recent years that the significance of this association has emerged. For example, measurements have shown that the rainforest soil is nearly devoid of soluble nutrients, since torrential rains over thousands of years have leached the nutrients away [1]. The paradox of biological luxuriance of tropical rainforests in nutrient poor soil was understood after it was discovered that the roots of trees are invariably associated with fungi. Let us examine the nature of this association in detail.

Mineral Requirements for Plant Growth

Every plant requires mineral elements for growth. A mnemonic for macronutrients required in millimolar quantities is: "See (C) Hopkins (HOPKNS) *Mighty good* (Mg) Cafe (Ca and Fe)". Of these, C (as carbon dioxide) and O (as oxygen) are obtained from air, H (as water), and phosphorus (P), potassium (K), nitrogen (N), sulphur (S), magnesium (Mg), calcium (Ca), and iron (Fe) as dissolved salts from soil water. Among these the most important

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are nitrogen (structural element for nucleic acids, proteins, ATP, chlorophyll), phosphorus (part of nucleic acids, membrane, ATP, enzymes) and potassium (required as cofactor for many enzyme reactions in cell, for protein synthesis and opening and closing of stomata). To grow a ton of wheat grain, the soil is depleted of approximately 18 kg of nitrogen, and 4 kg *each* of phosphorus and potassium. The fertility of agricultural soil diminishes every year unless fertilizers are applied to replace the lost minerals. But forests are thriving without external application of fertilizer! This paradox prompted a close look at the root system. The surprising finding is that roots of 80-95% of plants are associated with fungi – organisms comprised of microscopic, non-chlorophyllous, tube-like structures called the hyphae (*sing.* hypha).

Mycorrhiza – A Marriage Between Plants and Fungi

In one such association, common in the temperate regions, the hyphae form a sheath around the root tips (*Figure* 1) and

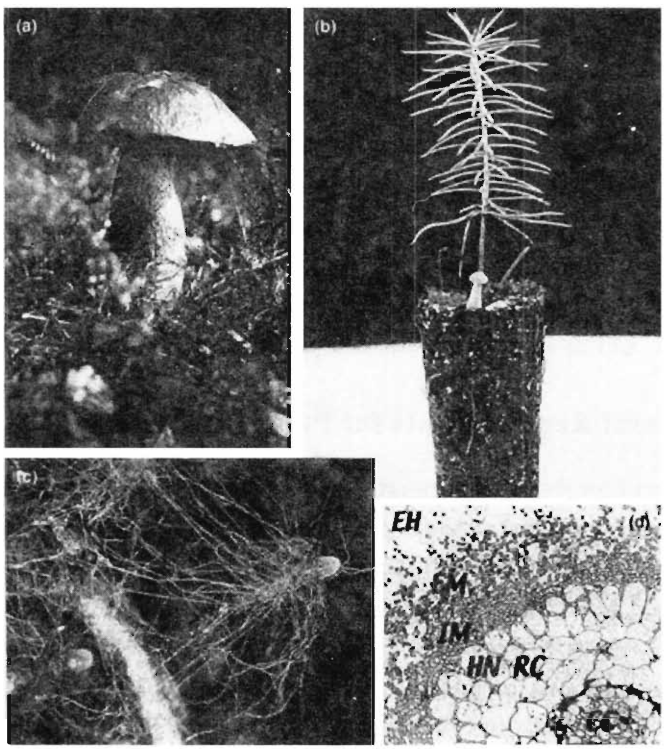

Figure 1. Mycorrhiza. (a) Boletus edulis, a mushroom fungus. (b) The fungal mycelium has developed ectomycorrhiza on the root tips of a fir seedling and has produced a spore-bearing fruiting body above ground. (c) A root hair, tubular extension of root epidermis, covered by hyphae. (d) Transverse section of mycorrhiza showing the external (EM) and internal (IM) mycelium; the fungal hyphae penetrating between the epidermal cells of the root cortex (RC) to form the Hartig net (HN). Extramatrical hyphae (EH) are extending outwardly. From F Martin et al,. New Phytol., Vol.151, pp.145-154, 2001, with permission of New Phytologist Trust.

penetrate into root epidermal cells wherein they form intracellular coils. More commonly, the hypha forms highly branched, tree-like structures called arbuscules. The hyphal coils and arbuscules are interfaces where nutrients between the photosynthetic and non-photosynthetic partners, are exchanged [2]. This intimate association of a plant and a fungus is called mycorrhiza (*pl.* mycorrhizae or mycorrhizas) – the term derived from two Greek words: *mykes* = mushroom or fungus, and *rhiza* = root; meaning 'fungus root', referring to the structure formed by the root and the associated fungus. The plant–fungus associations, referred to above, are called ectomycorrhiza and endo- or arbuscular mycorrhiza (AM), respectively. The finding of plant fossils with spores similar to that formed by the present-day mycorrhizal fungi suggests that the plant–fungus partnership evolved approximately 400 million years ago. The understanding of plant–fungus relationship has been a challenge since mycorrhiza is underground, the mycorrhizal fungi are uncultivable in laboratory media, and the individual hyphae are barely visible to the naked eye. The essential secret of this partnership has been determined only through careful experiments. Mycorrhizal fungus absorbs scarce nutrients from a large area of ground, which it supplies to the plant; affords the plant protection against water and thermal stresses, and resistance against soil-borne pathogens. The vital importance of mycorrhiza to plant life – indeed, to all forms of life that ultimately depends on plants – has taken time to be appreciated.

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In an earlier issue of *Resonance* we learned that the mycelium, which comprises the absorptive body of a fungus, can spread to an astonishingly large area in soil [3]. Experiments with plants grown in sterilized soil have shown that without the addition of spores of a mycorrhizal fungus the plants are weak, stunted and may die. The key to the paradox of luxuriance of forests is in its highly efficient, *constant* recapture of nutrients from the litter by the underground network of mycorrhizal hyphae and their delivery to the plants. It is thought that the large proportion of soil biomass consists of mycelium of mycorrhizal fungi.

Common Mycorrhizal Fungi

In due course the underground mycelium forms fruiting bodies which pop-up above the ground, enabling the reproductive spores of the fungus to be dispersed into air. The most familiar fruiting bodies are the mushrooms, puff-balls, earth stars, and bird's nest fungi (*Figure* 2) – members of the fungal phylum Basidiomycotina. These fruiting bodies are indicative of an underground symbiotic relationship between the plant roots and fungi (*Box* 1).

Role of Mycorrhizal Fungi in Nutrient Cycling

The upper layer of forest soil is a cover of litter comprising of

Figure 2 Selected postage stamps (15) featuring mushroom fungi. (Left to right),
Row 1: Amanita caesarea, Lepiota procera, Psilocybe cubensis, Lepiota procera.
Row 2: Boletus edulis, Clavaria aurea, Amanita muscaria, Armillaria mellea.
Row 3: Lactarius deliciosus, Krombholzia rufesc, Psalliota silvatica, Armilariella mellea.
Row 4: Lactarius deliciosus, Psalliota ccampestris, Lactarius deliciosus.
The scientific name of a fungus follows a binomial system of nomenclature, based on the name of the genus and species with both words italicized. The fruiting bodies which emerge above ground are formed by the aggregation, interweaving and gluing together of thousands of mycorrhizal hyphae.

Box 1.

The underground mycorrhizal network forms fruiting bodies above ground, some of which are edible. But since very few species have been successfully cultivated, the amateurs collect them from nature. Among the edible species are the white button mushroom (*Agaricus bisporus*), morel (*Morchella* spp.), saffron milk cap (*Lactarius deliciosus*), vegetable sweetbread (*Agaricus orcella*), and King Bolete (*Boletus edulis*). A beautiful mushroom having a red-cap with white to yellow spots, the 'fly-agaric' (*Amanita muscaria*) is poisonous; flies attracted to it become inebriated and die. Some countries have issued postage stamps on the edible and poisonous mushrooms (*Figure* 2). One of the mycorrhiza-forming fungi is truffle (genus *Tuber*). Its delicious fruiting bodies do not emerge above ground and are, therefore, highly prized. It produces a scent of some kind that diffuses up through soil. Although, not detected by humans; a female pig (sow) is able to detect it, most likely because it is chemically similar to the pheromone produced by a male pig. In France and Italy sows were used to sniff out the truffles (visit: *http://www.avignon-et-provence.com/provence/truffe_noire/img/truie_truffe.jpg*) but these days dogs are used for truffle 'hunting'.

fallen leaves, flowers and fruits, dead twigs, branches and trunks which the plants had made by the photosynthetic fixation of carbon dioxide from the air (*Figure* 3) and the absorption of water and minerals from soil. This organic layer of soil is rich in saprophytic fungi whose microscopic hyphae grow on dead matter.

Hitherto, it was assumed that decomposition of litter is solely by saprophytic fungi growing on dead plant tissue. However, sequence analyses of DNA extracted from mushroom fruiting bodies produced by the mycorrhizal fungi have revealed the presence of genes which encode enzymes involved in breaking down polymeric constituents. Using heterologous probes, these genes have been identified: they encode lignin peroxidases, pectinases, cellulases, hemicellulases, proteases, and phosphodiesterases which depolymerize, respectively, lignin (a polymer of phenyl-propanoid units), pectin (a family of complex polysac-

Figure 3. Basic reaction of photosynthesis in plants (symbol: ↓= taken in; ↑= given out).

$$\underset{\text{carbon dioxide}}{CO_2\downarrow} + \underset{\text{water}}{H_2O} \xrightarrow[\text{sunlight}]{\text{chlorophyll}} \underset{\text{cell material}}{(CH_2O)} + \underset{\text{oxygen}}{O_2\uparrow}$$

charides that contain 1,4-linked α-D-galacturonic acid), cellulose (a polysaccharide composed of 1,4-linked β-D-glucose residues), hemicellulose (branched polysaccharides that have a backbone composed of 1,4-linked β-D-xylose residues), protein (amino acids linked in peptide bonds), and nucleic acids (nucleotides linked together by phosphoric acid molecules). Therefore, a view has emerged that in addition to saprophytic fungi, the mycorrhizal fungi must also be involved in degradation of organic materials in the plant remains. In this process, the mineral nutrients which the plant had taken from soil as nitrate, phosphate and sulphate, and which had become chemically bonded in anabolic reactions to the polymeric constituents of the plant cell are returned to soil. The decomposition of litter releases CO_2 which the plant had taken in from air during growth.

Absorption of Phosphorus: Mycorrhizal Fungus is the Dominant Partner

In a plant, phosphorus (as orthophosphate) is absorbed from the soil solution by the root hairs by means of the transporters. A transporter is a membrane protein that catalyzes the selective uptake of molecules/ions against a concentration gradient. But in a mycorrhiza the fungus supplies much of the phosphate required by the plant – i.e. it becomes the dominant partner. This was discovered by using a compartmented pot system (*Figure* 4).

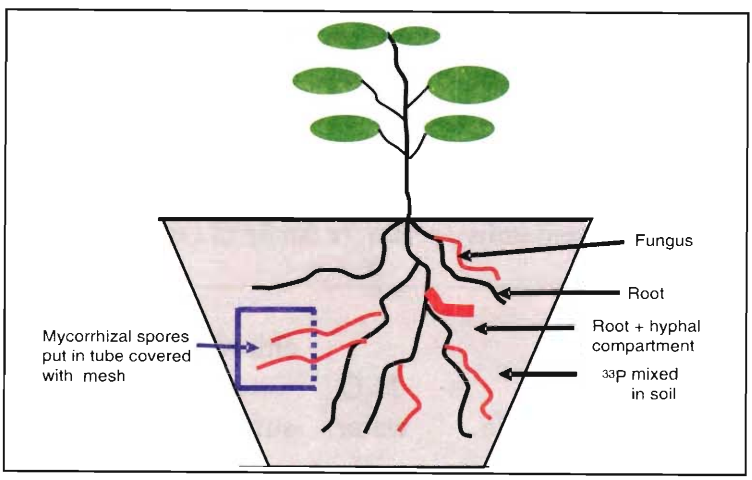


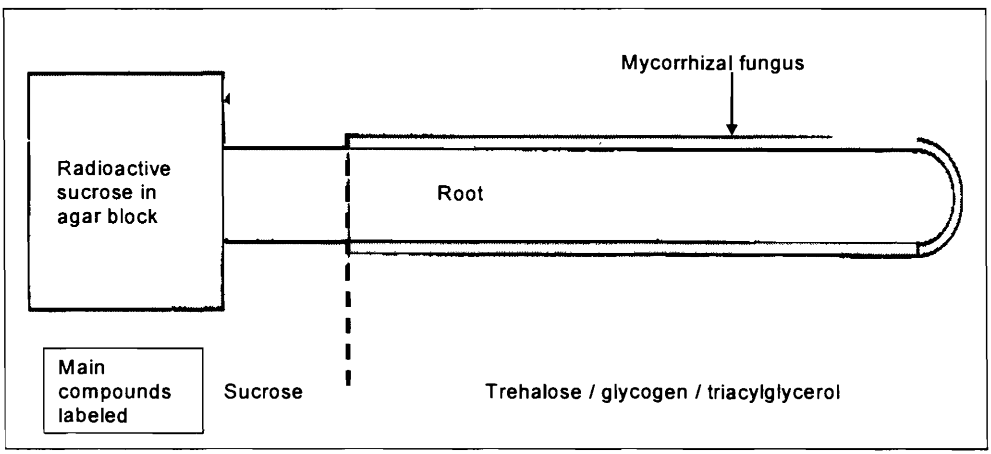
Figure 4. Diagram of an experiment using isotopically-labeled phosphorus (as $H^{33}PO_4$) to demonstrate its absorption and transfer to plant root. Modified from reference [4].

Experimental plants were grown in soil in pots into which spores of a mycorrhizal fungus had been put inside a tube capped by nylon mesh in order that the hyphae, but not the roots, could penetrate into soil in which ^{33}P (isotope)-labeled orthophosphate was mixed [4]. Thus, in this system the ^{33}P from the soil could reach the plants only via the hyphae, whereas the unlabeled P could be absorbed directly. After a period of growth, comparison of specific activity of ^{33}P in fungus-inoculated and uninoculated plants showed that in plants colonized by the fungus, the hyphae are the principal route through which phosphorus was absorbed and translocated to the plant. The results suggested that the partners must engage in a molecular dialogue – the fungus down-regulates or 'silences' the genes encoding P transporter in the plant.

Movement of Carbohydrate and Nitrogen from Plant to Fungus

The translocation of carbohydrate from the plant to the fungus was experimentally demonstrated by exposing the leaf to $^{14}CO_2$ and making an image by autoradiography. The fungus receives carbon compounds from the plant in the form of sucrose, glucose or fructose, and converts it into trehalose (a typical sugar in fungi) and triacylglycerol (lipid). This metabolic conversion may be a strategy of the fungus for maintaining a constant movement of carbohydrate towards its own direction (*Figure* 5).

Figure 5. Movement of carbohydrate from plant to fungus in mycorrhiza. Radioactive ^{14}C-labeled sucrose was applied to mycorrhizal root and parts of root with and without fungus and analyzed. The fungus converts sucrose received from root into glycogen, trehalose and mannitol (typical fungal carbohydrates) and lipid.



Unlike the symbiotic legume-root nodule bacteria, the mycorrhizal fungi do not fix atmospheric nitrogen.

Video-microscopy of stained, living hyphae suggested that the principal form in which carbon moves in hyphae is as lipid.

Unlike the symbiotic legume-root nodule bacteria, the mycorrhizal fungi do not fix atmospheric nitrogen. They obtain their nitrogen requirement from soil in inorganic forms. Inorganic nitrogen supplied as nitrate or ammonia to mycorrhizal mycelium was converted to the amino acid arginine. This serves two purposes: arginine is non-toxic; and since it has three nitrogen atoms per molecule, nitrogen can be translocated in the long hyphae in a concentrated form. The bi-directional nutrient transfer between the autotrophic plant and the heterotrophic mycorrhizal fungus is the basis of symbiotic relationship between plants and fungi.

Although mycorrhizal fungi have so far resisted attempts to grow them on artificial nutrient medium, mycorrhizal fungal mycelium can be obtained for biochemical analyses using a two-compartment Petri dish culture method. In this method slices of carrot root in one compartment are transformed with the bacterium *Agrobacterium rhizogenes*, which induces development of hairy roots. The roots which can grow continuously are inoculated with mycorrhiza spores (recovered from soil by wet sieving and decanting, or from root organ culture) and the extraradical mycelium is allowed to extend into the other compartment, separate from the roots, from where it is taken for analysis.

Interplant Nutrient Transfer

A feature of hyphae is their formation of extensive network (mycelium) by inter-hyphal fusions and branching [3]. The mycorrhizal hyphae can link with the surrounding plant roots and serve as bridges (conduits) for sharing nutrients within a plant community. The best evidence of this has come from studies on orchids. Orchids produce some of the most intricate flowers (*Figure* 6) which are fertilized only by bees and their seeds are the smallest of any flowering plant, but unlike other

Figure 6. Selected postage stamps featuring orchid flowers. (Left to right) Row1: Aerides crispum, Paphiopedilum venustum, Cymbidium bicolor. Row 2: Odontoglossum grande, Cypripedium hibridum, Cattleya sp. Row 3: Dendrobium anosmum, Vanda sanderiana, Dendrobium phalaenopsis. Orchids are partially or totally dependent on fungi for supply of water and nutrition. In Hawaii the bridal bouquet commonly comprises of orchid flowers. The fruits of the tropical, epiphytic orchid Vanilla (not shown) are the source of vanilla extract used for flavoring ice-cream.

Although plant-mycorrhizal fungus symbiosis is generally non-specific, some orchids are extremely choosy in selecting their fungal partner!

plants, their minute seeds lack an endosperm (food source). The leaves of some orchids lack chlorophyll or some crucial light-absorbing pigments; as a result they cannot fix CO_2 (photosynthesize). Some species lack not only leaves but also roots; their underground part is undifferentiated (protocorm) and is mycorrhizal. The mycorrhizal hyphae in protocorm extend outwardly and link up with the roots of surrounding photosynthetic plants and litter, drawing nutrition. In one experiment, orchid seeds packed in small bags constructed of fine nylon mesh, were placed in field conditions in different localities at varying distances from adult (flowering) non-photosynthetic orchid plants bearing mycorrhizal fungus [5]. The packets allowed the hyphae but not the roots to enter. A positive correlation between number of seeds that germinated and growth of the largest seedlings suggested that mycorrhizal fungus has a beneficial effect both on seed germination and plant growth. Molecular identification based on restriction fragment length polymorphism (RFLP) and similarity of short DNA sequences, showed that the *same* fungus had colonized the orchid seedlings at all localities in UK and Germany. Although plant–mycorrhizal fungus symbiosis is generally non-specific, some orchids are extremely choosy in selecting their fungal partner!

In an experiment simulating the ecosystem ("a microcosm experiment"), orchid seeds enclosed in mesh bags were buried in field soil to a depth of approximately 6 cm. In course of time the seedlings that grew in the bags placed close to green mycorrhizal plants gained weight, but those which were placed with aseptically grown plants lacking mycorrhiza lost weight. Direct transfer of carbon to an orchid through shared hyphae was shown by exposing the photosynthetic plant to radioactive carbon dioxide ($^{14}CO_2$) followed by autoradiography. Digital autoradiograph showed high concentrations of radiocarbon received by orchid plant from photosynthetic plant via hyphal connections [4]. This study with orchids showed that inter-plant transfer of both mineral ions and carbon compounds through shared hyphal connection can allow some species of plants to grow and survive in nature. For example, seedlings of forest trees grow and survive in deep shade, apparently nursed by the tall trees through hyphal connections. The connecting hyphae may transfer carbon compounds from a crop plant to undesired weeds.

'Cheating' in Plant–Fungus Marriages

A liaison between a plant and a fungus is not without risk. A plant may emit chemical signal(s) similar to the usual host, 'inviting' the fungus to physically link up with it (the 'interloper') and to provide it carbon compounds and nutrients drawn from the host plant to which the fungus is attached [6]. An example is a non-chlorophyllous plant known as Indian pipe or *Monotropa*, which occurs in deeply shaded forests of pine and oak trees. It will not grow if a photosynthetic tree is not close by, or if its hyphal connections are severed. It survives only because its underground part links up through a *specific* fungus to surrounding photosynthetic plants. Apparently, the non-photosynthetic plant emits some chemical signal(s), mimicking the photosynthetic plant, resulting in mycorrhizal fungal hyphae to link up with it (*Figure* 7). Some authors call the non-photosyn-

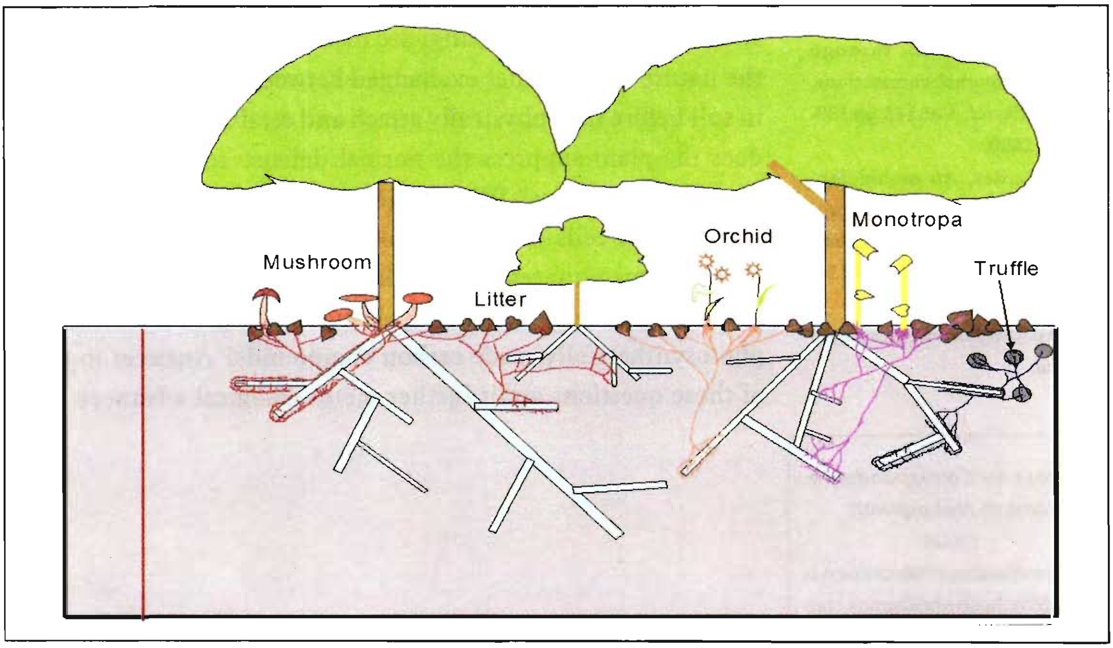


Figure 7. A diagram of plant–fungus marriages. Plants can exchange both carbon compounds and mineral nutrients via mycorrhizal bridges connecting the root system of adjacent plants. The thread-like mycelium of different species of mycorrhizal fungi is coloured. Trees with green (photosynthetic) canopy act as 'nursemaids' for plants growing in shade.

thetic plants as "cheaters" because they survive at the cost of a photosynthetic plant through shared hyphal connections [6]. The nutritional cost to the photosynthetic trees may be negligible because the trees are unlikely to be carbon-limited.

Why is Research on Mycorrhiza Important?

The association of Fungi with Plants is the general rule. Plants could not have evolved and colonized land without the Fungi. Mycorrhizal fungi allow plants to live in wasteland wherein they could not grow otherwise. Because of practical importance of mycorrhiza in agriculture and forestry, it has become a very hot area of research [7]. A lesson is that relatively little-studied species of plants and fungi offer great potential for new discoveries in biology. The discovery of inter-plant hyphal connections suggests the possibility of using selected strains of mycorrhizal fungi for transferring nitrogen from symbiotic nitrogen-fixing legume plants to non-legume plants, benefiting the latter. If mycorrhizal fungi can be grown on artificial media and their spores obtained, it will facilitate use of mycorrhizal fungi mix as biofertilizer for soil applications in agriculture. Among the questions that need answers are: How many types (species and strains) of mycorrhizal fungi are distributed in a field? What is the nature of molecules exchanged between a root and a fungus in soil before they physically attach and establish a liaison? How does the plant suppress the normal defense response against a mycorrhizal fungus? What molecular and cellular adjustments occur in the cells of the symbiotic partners so that the mycorrhizal partnership remains stable? How does the fungus assume a dominating role, inducing the host plant to give to it a share of photosynthetically made carbon compounds? Answers to some of these questions await further methodological advances.

Suggested Reading

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