Ajit Varma · Ram Prasad
Narendra Tuteja *Editors*

Mycorrhiza - Function, Diversity, State of the Art

Fourth Edition

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Foreword

In the first half of the nineteenth century, Justus von Liebig popularized the "Law of the Minimum", stating that plant growth is controlled by the scarcest resource—being the "limiting factor"—rather than the total amount of resources available. Although sunlight and temperature may be such limiting factors, the term mostly refers to mineral nutrients. In view of the recognition of this principle, Liebig highlighted that trace minerals—next to major nutrients—are essential for plant growth and may often be a limiting factor for plant development and even survival. This is particularly critical under conditions where micronutrients are in low supply or even immobilized and, hence, cannot be taken up by the plant roots alone.

Fungi generally inhabit the rhizosphere of vascular plants, where they live off organic material. They access their carbon and nutrient source *via* an extensive network of hyphae with a very large surface area, aiding the decomposition of the organic material in the root zone. Mycorrhizal fungi form a symbiotic association within the plants' rhizosphere. This may occur—mostly as a mutualistic association—either intracellular (arbuscular mycorrhizal fungi) or extracellular (ectomycorrhizal fungi). Due to their large reactive surface area, mycorrhizal fungi are attributed with a significant function in biogeochemical cycles. They play an important role in agricultural and natural ecosystems, where they increase production in the former and sustainability of the ecosystem in the latter case, particularly under low nutrient conditions (Pate and Beard 1984). The extent to which plants depend on mycorrhizae varies, but most plants studies so far show augmented development with mycorrhizal associations. Some orchid species are even facultatively myco-heterotrophic and form a parasitic relationship with mycorrhizal fungi for part of their life cycle. Other myco-heterotrophic plants are totally dependent on mycorrhizal associations, in which case the relationship becomes entirely parasitic in favour of the plant. On example is the ghost plant (*Monotropa uniflora*), which is an herbaceous perennial plant devoid of chlorophyll (Yang and Pfister 2006). Due to its lack of photosynthetic capacity, it is myco-heterotroph on mycorrhizal fungi associated with certain trees (mostly beech trees), providing the energy for the fungus and ultimately also for its parasite.

In symbiotic plant associations with mycorrhizae, the benefit for the fungus is generally attributed to the access to photosynthetically produced carbohydrates, translocated from the plant's leaves to the roots and its fungal associate. The plant—on the other hand—benefits from the high absorptive capacity of the large surface area of the hyphal system (the mycelium) for mineral elements and even water. The fungal hyphae are much finer and longer than the plants roots, allowing access and direct contact to a larger volume of soil. This improves access to mineral nutrients, particularly also micronutrients, which often can be a limiting factor for plant growth, as the "Law of the Minimum" suggests. The mycelium of micorrhizal fungi can even mobilize nutrients that are physically or chemically immobilized and, hence, cannot be taken up by the plant roots alone. Heavy clay soils are prone to immobilization of certain micronutrients and even phosphate. Under such conditions, mycorrhizal–plant associations may be essential for the survival of the plant. Consequently, one may argue that the plant–mycorrhizal symbiosis is next to the plant–rhizobial symbiosis (performing symbiotic nitrogen fixation) the most important symbiotic system for sustainability and productivity of terrestrial plant systems.

Plant–mycorrhizal associations are found under most environmental conditions on the planet and in a vast number of combinations with varying associated partners—multiple or singular. Consequently, we are still far from understanding the details of the various combinations of relationships of plants with micorrhizae formed in different habitats.

This volume is the fourth in a series of books on mycorrhizae, which on the one hand acknowledges the vastness of the research area and on the other hand provides a condensed insight into the most recent discoveries in the field. The book picks up the points outlined above and exemplarily elucidates them in various settings and environmental conditions. It mainly focuses on natural ecosystems while spanning the bridge to agricultural systems where it is called for. Keeping to a holistic approach, the various chapters explain how recent research results on plant–mycorrhizal associations in combination with new information on mycorrhizal and rhizobial symbionts can help refining existing and new concepts on how such symbiotic systems can augment ecosystem sustainability and vigour. In order to outline the basis for progress in the field, the first chapter of this volume puts mycorrizal research in a historical perspective. The following four chapters employ various angles to focus on ways how the plant–micorrhizal system can generate enhanced nutrient uptake and how bacteria can provide additional benefits. Chapters 6, 7, 9 and 10 cover the role of the symbiosis in essentially undisturbed and disturbed ecosystems, including the role in early succession. The signalling processes in the establishment of rhizobial and mycorrhizal symbiotic endophytes are discussed in Chap. 8, while several specific adaptations of fungi, e.g. Truffle (Chap. 11), and plants, e.g. Grapevine (Chap. 13), or specific environmental conditions, e.g. wetlands (Chap. 14), or hypoxic conditions (Chap. 16) are covered in the second half of the book. Climate change and the question how arbuscular mycorrhizal fungi are affected by this recent, global phenomenon is the topic of

Chap. 15. The final two chapters introduce a new symbiont *Piriformospora indica* (*Serendipita indica*) and outline its large-scale cultivation in a bioreactor.

With the intensive research in the area continuing, this volume presents the current state of the art. As such, it is a valuable reference and basis for future investigations. With more scientific progress in the field, we can look forward to enhanced insights into this important and exciting symbiotic system.

Alexander P. Hansen

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Preface

We have already published mycorrhiza volumes in the years 1991, 1992, 1995, 1999, and 2008. Since then, a lot of new research findings and approaches have been published in literature. The present volume will emphasize the current perspectives of mycorrhiza around the globe. Springer-verlag, Heidelberg, Germany, has invited us to compile three new volumes in quick succession (2017) highlighting the work on mycorrhiza after 2008. Mycorrhizas have been an essential stabilizing factor in the terrestrial ecosystems for centuries. These fungi aid in the productivity of plants via the formation of dynamic associations with plant roots. The symbiotic associations formed are an important subject to evaluate numerous opportunities using modern tools of biotechnology. The possibilities of genetically manipulating these associations have led to optimization of plant productivity in ecosystems with minimal risk of environmental damage. The resourceful management of mycorrhizal associations has the potential to favor the sustainable production of quality foods while ensuring environmental quality for future generations.

The unique associations formed by these fungi have sparked a vast array of interest in mycorrhizal studies. Recent developments in the study of mycorrhizas have encouraged us to present a new book on progress in this field. The fourth edition of the mycorrhiza book gives exemplary insight into the advancements in mycorrhizal studies. It is hoped that this new edition will interest readers in the latest results of mycorrhiza research and also encourage young researchers to prove the challenging field of mycorrhizal studies.

This volume consists of 18 chapters covering the diverse mycorrhizal associations by 46 subject specialists.

We are grateful to the many people who helped us to bring this volume to light. We wish to thank Drs. Jutta Lindenborn, Isabel Ullmann, Man-Thi Tran, and Hanna Hensler-Fritton Springer Heidelberg for generous assistance and patience in continuing the volume. Finally, special thanks go to our families, immediate and extended, not forgetting those who have passed away, for their support or their incentives in putting everything together. Editors in particular are very thankful to Dr. Ashok K. Chauhan, Founder President of the Ritnand Balved Education

Foundation (an umbrella organization of Amity Institutions), New Delhi, for the kind support and constant encouragement received. Special thanks are due to my esteemed faculty colleagues and dear student Ms Diksha Bhola and other technical staff.

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Chapter 1
Introduction to Mycorrhiza: Historical Development

Ram Prasad, Diksha Bhola, Khalid Akdi, Cristina Cruz, Sairam KVSS, Narendra Tuteja, and Ajit Varma

Abstract Arbuscular mycorrhizal fungi (AMF) are vital component of natural ecosystem, being gifted to form symbiont with plant roots. AM fungi have mutualistic relationships with more than 80% of terrestrial plant species. Because of wide range of relationships with host plants, it becomes difficult to identify the species on the morphological bases as the spores are to be extracted from the soil. In spite of their abundance and wide range of relationship with plant species, AMF have shown low species diversity. AMF have high functional diversity because different combinations of host plants and AMF have different effects on the numerous aspects of symbiosis. Recent fossil evidence has dated the appearance of arbuscular mycorrhizae back to 460 million years, preexisting vascular plants. These studies benefit the paleoecological importance of mycorrhizae and enhancement to our understanding of the evolution of mutualisms.

1.1 Introduction

Arbuscular mycorrhiza (AM) association is the most widespread plant–fungal symbiosis on earth, in the majority of extant land plants, and can be traced back to the Early Devonian, more than 400 million years ago, which corresponds to the

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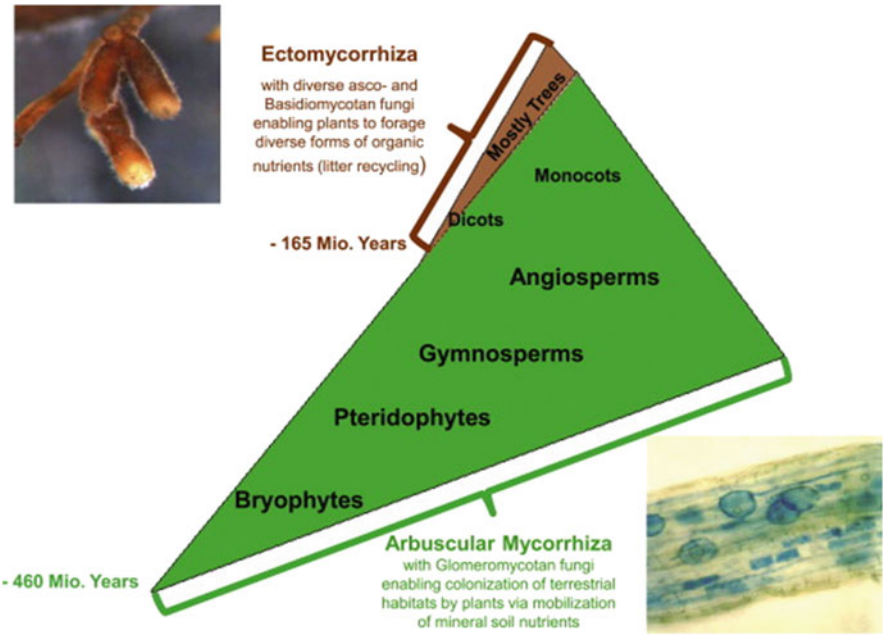

Fig. 1.1 Summary of the natural historical development of arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) symbioses in relation to the ecological radiation of terrestrial plants (Reprinted with permission from Buscot 2015)

onset of the colonization of terrestrial habitats by plants (Fig. 1.1) (Remy et al. 1994; Schwendemann et al. 2011; Buscot 2015). Presently, the majority of land plants have mutually beneficial symbiotic relationship with a fungal partner in which the plant normally receives soil nutrients in exchange for photosynthates. However, this understanding is based almost exclusively on investigations of the AM associations of higher plants, whereas virtually nothing is known about their functionality in the "lower," more evolutionarily ancient land plant clades (liverworts, clubmosses, and ferns). There is evidence that AM is an evolutionary ancient partnership that commenced with the earliest land plants, but the absence of information on the functioning of the symbiosis in lower plants is a major gap in our understanding of its significance in driving colonization of land plant, and the costs–benefits of plant–fungal co-evolution in lower plants are mysterious.

1.2 Structure of Mycorrhizal Fungi

Frank (1885) was probably the first to identify the extensive nature of associations between plant roots and mycorrhizal fungi (Frank and Trappe 2005). Four major mycorrhizal types have been described based on their structure and function,

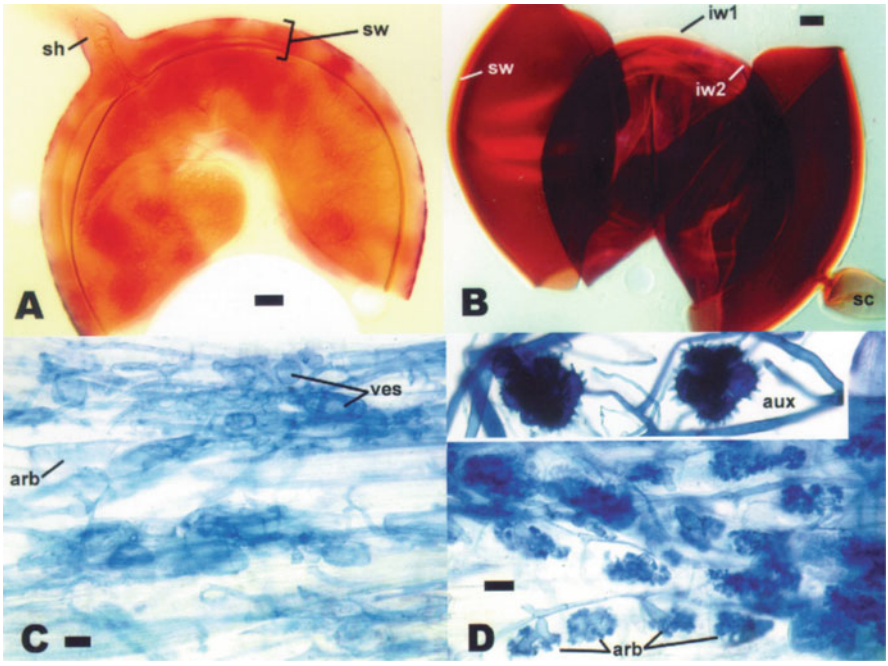

Fig. 1.2 Examples of spores and colonization by arbuscular mycorrhizal fungi: (**a**) Subcellular structure of a *Glomus clarum* spore broken and mounted in Melzer's reagent. (**b**) Subcellular structure of a *Scutellospora pellucida* spore broken and mounted in Melzer's reagent. (**c**) Typical mycorrhizae of *Acaulospora morrowiae* stained in 0.05% trypan blue. (**d**) Typical mycorrhizae of *Gigaspora rosea* stained in 0.05% trypan blue; *inset* shows auxiliary cells. Abbreviations: *sw* spore wall; *sh* subtending hyphae; *sc* sporogenous cell; *iw1* first inner wall; *iw2* second flexible inner wall; *arb* arbuscule; *ves* vesicle; *aux* auxiliary cells. *Scale bar* = 20 μm (Reprinted with permission from Bever et al. 2001)

namely, arbuscular mycorrhiza (AM), ectomycorrhiza (EM), orchid mycorrhiza, and ericoid mycorrhiza (Fig. 1.2 and Table 1.1).

Today, despite the large number of plant species forming AM associations worldwide, two types of arbuscular mycorrhizal (AM) associations, the Arum type and the Paris type, have been recognized based on morphological characteristics of the colonization process. In the Arum type, the fungal symbiont spread in the root cortex via intercellular hyphae. Short side branches penetrate the cortex cells and produce arbuscules. The Arum type (linear) is commonly described in fast-growing root systems of crop plants. In the Paris type (coiled), the hyphae grow intracellular coils and spread directly from cell to cell inside the cortex. Co-occurrence of Arum- and Paris-type morphology of AM is found in cucumber and tomato (Kubota et al. 2005).

Arbuscules are relatively short-lived, at least in the Arum-type mycorrhiza, and the hyphae are comparatively long-lived (Holley and Peterson 1979; Smith and Dickson 1991). The arbuscules gradually degenerate, while the plant cell remains

Table 1.1 Numbers of plant and fungal species forming arbuscular mycorrhizal, ectomycorrhizal, orchid mycorrhizal, or ericoid mycorrhizal associations (van der Heijden et al. 2015)

Mycorrhizal type	Major groups of plants	Number of plant species hosting mycorrhizal fungi	Fungal identity	Total estimated number of fungal taxa
Arbuscular mycorrhiza	Most herbs, grasses and many trees, many hornworts and liverworts	200,000	Glomeromycota	300–1600
Ectomycorrhiza	Pinaceae and Angiosperms (mostly shrubs and trees, mostly temperate), some liverworts	6000	Basidiomycota and Ascomycota	20,000
Orchid mycorrhiza	Orchids	20,000–35,000	Basidiomycota	25,000
Ericoid mycorrhiza	Members of the Ericaceae, some liverworts	3900	Mainly Ascomycota, some Basidiomycota	>150
Nonmycorrhizal plant species	Brassicaceae, Crassulaceae, Orobanchaceae, Proteaceae, etc.	51,500	–	0

alive, which is a difference related to many plant pathogenic fungi which cause plant cell death. For rapidly growing crop species, the formation of arbuscules may take 2–3 days and the whole arbuscular cycle may take around 1 week.

Mycorrhizal fungi live inside the cortex of plant roots, on the surface of the root, or around the epidermal cells of the root. The hyphae of these fungi also grow out from the roots into the soil where they forage for nutrients that are limiting to plant growth, especially nitrates and phosphates, but organically bound nutrients are also acquired by some mycorrhizal types (e.g., EM and ericoid mycorrhizal fungi) (Read and Perez-Moreno 2003). These nutrients as well as other benefits are then delivered to their host plants in return for carbohydrates (Smith and Read 2008). Consequently, the mycorrhizal symbiosis exerts a strong influence on plant growth and fitness.

1.3 Taxonomy of Arbuscular Mycorrhizal Fungi

Taxonomy identifies and describes names, generating tools for taxonomic identification of fungi (species lists, descriptions of formally delimited species, and identification keys, among others). Arbuscular mycorrhizal fungi (AMF) are characterized by the development of branching structures called arbuscules inside the cortical cells of roots (Fig. 1.2). Arbuscules increase the contact area between plant root and fungus

and are thought to be the primary sites of exchange of the plant's carbon for the fungus's phosphorus. One suborder of these fungi, Glomineae, also forms vesicles, or sack-like reservoirs, within plant cortical cells (Fig. 1.2c). The presence of the fungi (members of the Glomeromycota) within the root assists the uptake of nutrients and water and may also have a protective effect against soil pathogens.

AMF are believed to propagate via infective hyphae, hyphal fragments, or asexual spores (Fig. 1.3). A general life history begins with colonization of a root and the development of arbuscules from branch hyphae within the root. Hyphae may extend from one infected root to another or from an infected root to the root of another plant. Spores form in the root cortex or in the soil. These spores may be dormant for a period, but they will eventually germinate and colonize another root. Spores may be dispersed away from the site in which they were formed. Viable spores are generally short-lived (exceptions: some spores of *Acaulospora* species), and viability is limited by dormancy, susceptibility to pathogens, and some

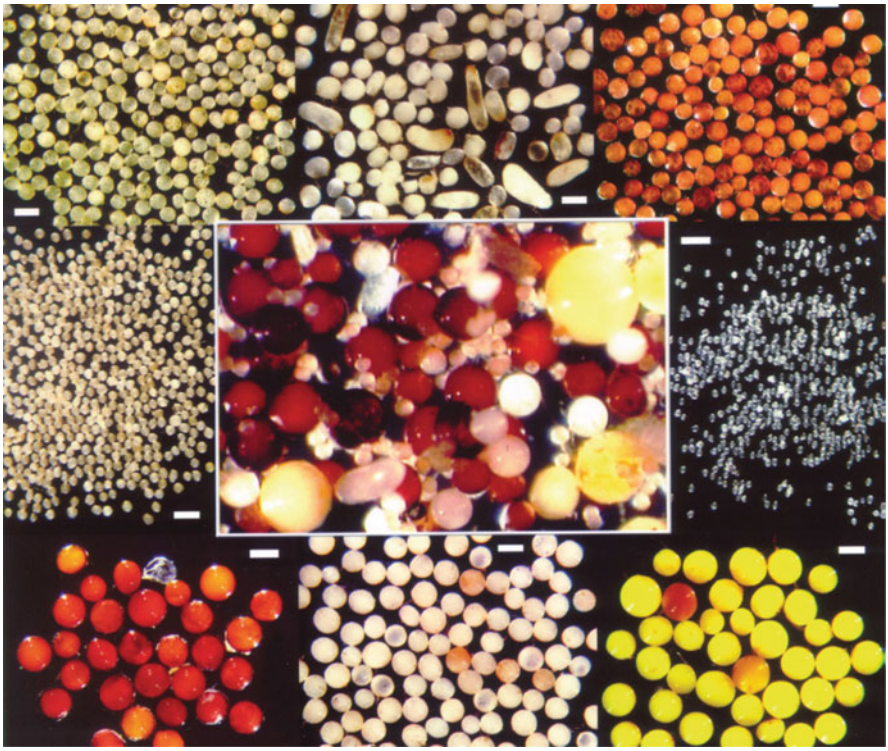

Fig. 1.3 Spores of arbuscular mycorrhizal fungi: the central picture is a collective spore from nine species of AM fungi. Around this central photo, we have arranged pictures of individual species. Starting in the *upper left* corner and moving clockwise around the central photo, these species are *Scutellospora calospora, S. pellucida, S. heterogama, Archaeospora trappei, Gigaspora gigantea, Gi. rosea, Acaulospora colossica*, and *Ac. morrowiae*. *Scale bar* = 200 μm (Reprinted with permission from Bever et al. 2001)

supplementary factors. Although the morphology and architecture of external hyphae and internal mycorrhizal structures can differ between families of AM fungi (e.g., Fig. 1.2c, d), there are few differences between species to species within the same genus. Therefore, taxonomy of these fungi is based on the discrete characters of the spore subcellular structure, which can vary from simple to very complex for a single multinucleate cell (e.g., Fig. 1.2a, b; Morton 1988; Morton and Bentivenga 1994). On the basis of characteristic features of spore wall and spore ontogeny, AMF are grouped into genera that include approximately 145 species described to date. Undoubtedly, the majority of AM fungal species remain undefined. The International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University, for example, currently maintain approximately 40 isolates that do not belong to currently described species (Morton et al. 1993). For more information on this collection, see the INVAM Web site at http://invam.caf.wvu.edu.

1.4 Conclusion and Future Prospects

A major development have been through in the arena of mycorrhizal research after the discovery that mycorrhizal associations are abundant and important for plant nutrition. Currently, more than 100 years later, the ecological function of symbiosis is much better implicit, the biodiversity and evolution of this symbiosis is no longer a black box, genomes of a widespread array of mycorrhizal fungi have been sequenced, and molecular and nanoscale level interactions establishing the symbiosis are starting to be revealed.

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Chapter 2
Mobilization of Micronutrients by Mycorrhizal Fungi

Priyanku Teotia, Manoj Kumar, Ram Prasad, Vivek Kumar, Narendra Tuteja, and Ajit Varma

Abstract Mycorrhizal fungus constitutes heterogeneous fungal taxa embracing an array of plant species. This group is found allied with the roots of beyond 90% of the plant species in this world. There is a range of mycorrhizal associations, among which arbuscular and ectotrophic mycorrhizal interactions are of high biological and economic significance. This chapter gives details about habitation, host range, and structural components of these mycorrhizal groups, along with a meticulous discussion on the mineral absorption, mechanisms involved in different absorption pathways. In addition to enhancement of mineral nutrient uptake by plants in soil, several mycorrhizal fungi execute an important task in mobilizing mineral nutrients from inaccessible organic substrate, mineral particles, and rock surfaces. Mycorrhizal fungi adopt various methods to achieve the purpose effectively, like greater area of absorption for the roots of plant, liberation of biochemical compounds, and consortium with different microbes. Furthermore, mycorrhizal fungi also provide an imperative C sink in soil other than mobilizing nutrients, consequently playing an important role in the cycling of these mineral elements. The role of every partner in a mycorrhizal association is to be exposed by the application of molecular and genetic tools, coupled with high-throughput sequencing and advanced microscopy. The signaling pathways between plants and fungi have recently been elucidated, and recognition of a range of novel nutrient transporters has unveiled a number of cellular processes which are fundamental to the mycorrhizal symbiosis. Various transporters, particularly proton-coupled phosphate transporters, have been documented on both the fungal and plant membranes which contribute to transmission of phosphate from fungi to plants. Even though much work has been formerly done on several aspects, such as symbioses, the extent to which these are functionally essential in agriculture remains uncertain. It is a vital need to spotlight on the questions, whose answers will offer novel perspectives on mycorrhizal utility.

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2.1 Introduction

Even the modest things are vital to the world particularly in relation to getting plants established. Under natural environmental condition, plants remain in close association with soil microorganisms called mycorrhizal fungi. The mycorrhizal fungi inhabit plant roots and extend the root system into the adjoining soil. Unexpected quantities of mycorrhizal filaments are found available in healthy soil. An extremely small section of soil associated with dynamically growing plants may be full of numerous fungal filaments. The affiliation is favorable for the reason that the plants have the benefit of improved uptake of water and mineral nutrient, resistance against diseases, greater survival, and enhanced growth.

The term "mycorrhiza" was coined by the German scientist, A. B. Frank, about a century ago. Factually, word "Mycorrhiza" stands for fungus root; however, it is a symbiotic association existing among a group of soil fungi and the roots of higher plant (Habte 2000). This is a mutualistic organization that depicts the bidirectional interaction and exchange of resources across the mycorrhizal interface. In this association, the mycorrhizal fungus provides the host plant with mineral nutrients, like phosphate and nitrogen, and amplifies the abiotic stress tolerance against conditions of drought, salinity, and heavy metal and biotic stress resistance from various root pathogens, and in return, the host plant transports about 4–20% of its photosynthetic product, i.e., carbon compound to the mycorrhizal fungus (Wright et al. 1998). Records of fossil study indicate that mycorrhizal association commenced about 400–450 million years back, and these mycorrhizal interactions played a significant role in colonization of land by the plants (Smith and Read 2008). Even though mycorrhiza came to light nearly 100 years back, their significance in enhancing plant productivity did not get appropriate credit until past 50 years, until molecular biology got highly developed and gave an insight into the mode of action of mycorrhizal fungi. Presently, numerous scientists around the globe are engaged in study of the mycorrhizal interactions, and any research on plant productivity can hardly be considered as complete, without inclusion of mycorrhizal associations (Habte 2000). About 90% of the identified land plant species formulate mycorrhizal relationship with the ubiquitous fungi in soil (Bonfante and Genre 2010). In dissimilarity with the reciprocal beneficial mycorrhizal association, several mycoheterotrophic plants, nearly 400 species from diverse plant families of bryophytes, pteridophytes, and angiosperms, rely on mycorrhizal fungi to fulfill their carbon need. Such plants lose their photosynthetic efficacy and become parasitic on mycorrhizal fungi coupled with adjacent autotrophic plant (Bücking et al. 2012).

In the following chapter, the main prominence is given to mutually beneficial ectotrophic and arbuscular mycorrhizal associations, since they have great ecological and economic implications (Marschner and Dell 1994). In environmentally sustainable agriculture, arbuscular mycorrhizal fungi can be regarded as "biofertilizer and bioprotector" owing to their capability to colonize and facilitate ample variety of food and cash crops. In contrast, ectomycorrhizal fungi colonize a

smaller number of plant species and operate as symbiotic cohort of trees and shrubs; these play a foremost role in the forest ecosystem (Finlay 2008) and could be a fundamental element in phytoremediation as well as revegetation purposes (Bücking 2011; Giri et al. 2005).

2.2 Occurrence and Host Specificity of Mycorrhizal Fungi

AM fungi in general belong to six genera from the class azygosporous zygomycetes. While ectomycorrhizal fungi largely belong to the class basidiomycetes, a few belong to the class zygosporic zygomycetes and ascomycetes. AM are remarkably proficient in mobilizing the inorganic phosphorus (P) and thus prevail well in temperate and arid climates where P is frequently a limiting factor. The AM associations subsist in a large variety of tropical and temperate tree species, as they are not much specific in forming association with the host plant species (Bücking et al. 2002). These associations are known to rarely exist in members of the plants belonging to families Amaranthaceae, Pinaceae, Betulaceae, Brassicaceae, Chenopodiaceae, Cyperaceae, Juncaceae, Proteaceae, and Polygonaceae. As compared to AM, ectomycorrhizal fungi are more efficient in captivating N, and these are more frequent in boreal zone as well as in the temperate zone with high humidity, as the occurrence of low temperature with high humidity promotes the accretion of organic matter, reduced pH, and less N availability (Kilpeläinena et al. 2016). Ectomycorrhizal fungi interact with reasonably lesser section of all plant species, probably just about 3%; however, this 3% embodies almost all of trees of the temperate and boreal forests (especially the plant species of family Fagaceae and Pinaceae); therefore, it can be said that the majority of the forests (in terms of land area) on the surface of earth are dependent on ectomycorrhizal fungi (Habte 2000; Smith and Read 2008; Bonfante and Genre 2010).

2.3 Variation in Structure Among Mycorrhizal Fungi

Mycorrhizal fungi form a variety of associations with the plants; among these, endomycorrhizal association of the arbuscular (AM) type and ectomycorrhizal (ECM) associations have a greater economic and ecological importance. Arbuscular mycorrhizal (AM) and ECM associations differ in their structural aspects as well as the plant and fungal species these embrace (Fig. 2.1 and Table 2.1).

In the ECM, the fungal hyphae infringe the cortex section in the root of the host plant but do not penetrate the cortical cells. The ECM forms hyphal network around cortical cells of the root; this hyphal network is known as the “Hartig Net.” In addition to Hartig net, the ECM also forms a thick layer of hyphal mat on the surface of roots, known as sheath or mantle; this sheath covers feeder roots. Thus,

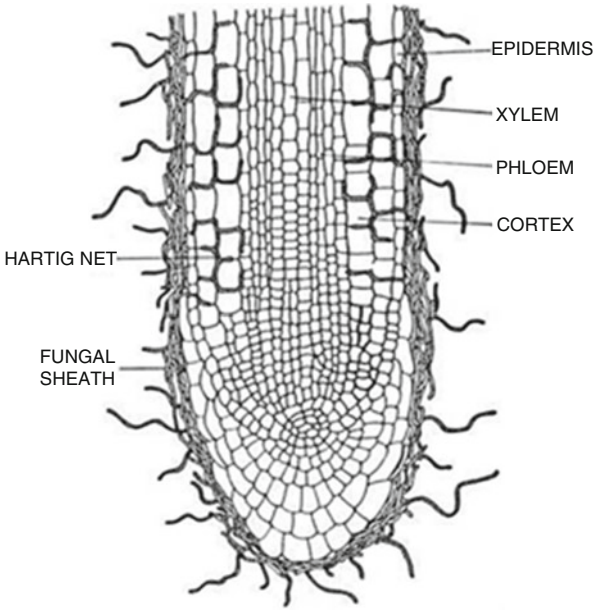

Fig. 2.1 Ectomycorrhizal fungi showing its structure in an infecting root (Source: http://www.biologydiscussion.com/plants/absorption-of-mineral/absorption-of-mineral-salts-by-higher-plant-with-diagram/22764)

Table 2.1 Comparison between AM and ECM associations (Modified after Bücking et al. 2012)

Features	AM fungi association	Ectomycorrhizal fungi association
Transport of nutrients to plant	Specifically important for phosphorus transport, also contribute to nitrogen transport	Specifically important for nitrogen Transport but also have significant Contribution in P transport
Occurrence of fungi	Mainly in warm and dry climates where phosphorus availability is low	Climates with low temperature and high humidity, where nitrogen availability is low
Plant host range	Associates with a very wide range of hosts	Associates with comparatively lower portion of plant species
Type of fungal nutrition	Obligate biotrophophic fungi	Facultative saprotrophytic fungi
Structural elements in fungi	Arbuscules, ERM, and vesicles in some types	Mantle, Hartig net, and ERM
Fungal mode of penetration in host plant	Both inter- and extracellular penetration	Only intercellular penetration
Pathway of nutrient uptake	Both plant and mycorrhizal pathway	Mainly mycorrhizal pathway

they are also known as "sheathing mycorrhiza." Infectivity of the host plants by ectomycorrhizal fungi generally leads to modification in the feeder roots that can be observed by naked eyes (Genre 2010). The feeder roots inhabited by fungi are thicker, show more branching, and are differently colored as compared to uncolonized roots. Usually, the ectomycorrhizas initiate in between the fine roots

and dikaryotic mycelia, which are formed by the union of two different monokaryotic hyphae that germinate from spores. The distinctive fungal sheath or mantle which is composed of aggregated hyphae appends to the surface of roots. This mycelium is correlated to the extramatrical hyphae to facilitate exploration of the substrate; moreover, these are accountable for mineral nutrition mobilization and uptake of water in the symbiotic tissues (Fig. 2.2). "Hartig Net" in the inner zone of the mantle forms an interface where exchange of metabolites takes place. The root cells bounded by fungal hyphae are living; the fungal hyphae are apoplastic but can colonize the epidermal cells as in angiosperms or cortical cell as in gymnosperms (Barker et al. 1998).

In AM or in the fungi, hyphae enter into the cortical cells of the roots and either may produce balloon-like, membrane-bound organelles of diverse shapes, outside or inside the cortical cells, called vesicles, or may constitute finely divided dichotomously branched hyphal invaginations called arbuscules. These structures are supposed to be the site for the exchange of materials among the host plant and fungi. Vesicles, on the other hand, have twin function, they commonly act as storage structure, and lately after they are aged, they function as reproductive structures. The characteristic features of the VA mycorrhizas are vesicles and arbuscules together with large spores. Vesicles are mostly invisible in these types of mycorrhizal associations; therefore, several scientists recommend the use of the term AM, more favorable over the designation vesicular–arbuscular (VA) mycorrhiza. Both AM fungi and ECM fungi expand their hyphae from the root into soil (extraradical hyphae), which are responsible for mobilization of nutrients from soil into the roots (Fig. 2.2).

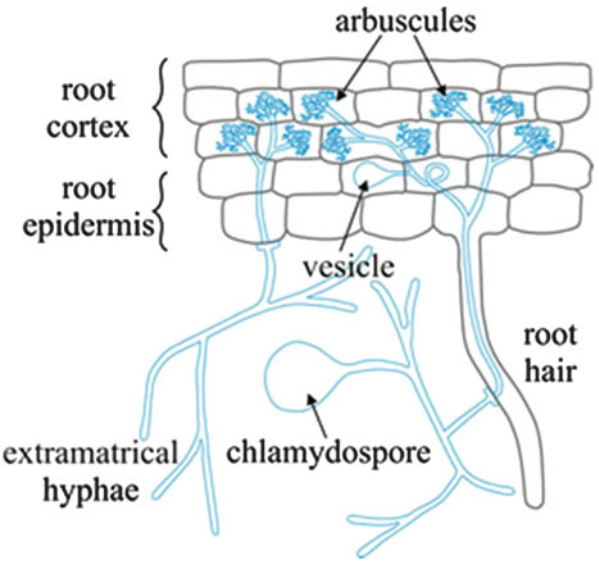

Fig. 2.2 AM fungi showing its structures in infecting root (Adopted from: © http://www.davidmoore.org.uk/assets/mostly_mycology/diane_howarth/am.htm)

2.4 Nutrient Uptake Pathways in Mycorrhizal Roots

There are two pathways *via* which the plants take up nutrients from the soil (Smith et al. 2011). The intake could be either by "plant pathway" that comprises of unmediated uptake of the nutrients by the epidermal cells of the root hairs from the soil or the nutrient uptake by plants can take place through the "mycorrhizal pathway," which consists of nutrient uptake by the extraradical mycelium of its fungal associate which further transfers the nutrients to "Hartig net" in the ECM association, or else to the intraradical mycelium in the AM association, and ultimately to the plant from the interfacial apoplast (Harrison et al. 2002). The nutrient uptake from the soil by means of plant pathway, on the other hand, is consistently constrained by the reduced mobility of nutrients in the soil (Bücking and Kafle 2015). AM and ECM roots diverge in their structural aspects, and this dissimilarity has correlation with their slightly diverse method of nutrient uptake in the AM and ECM plants (Fig. 2.3 and Table 2.2).

The AM roots do not create the fungal sheath and therefore can apparently exploit both the pathways for uptake of nutrient from soil (Bücking et al. 2012). The AM symbionts show a collective mode of the nutrient uptake, which has also been suggested previously (Bücking and Kafle 2015). This led to the supposition that the nutrient uptake through the mycorrhizal pathway can be evaded while availability of nutrient in the soil is excess. Moreover, the plants associated with mycorrhiza do not always show a affirmative growth response. However, such notion has become contentious now (Smith and Read 1997; Smith et al. 2009, 2011), and it has been established that the mycorrhizal pathway can direct the entire P uptake as well as that the factual role of mycorrhizal pathway toward complete P uptake can be "veiled" (Smith et al. 2003; Nagy et al. 2009). The transporters in plants, which are concerned with P uptake by means of plant pathway, are downregulated in reaction to AM symbiosis (Harley and Smith 1983; Chiou et al. 2001; Grunwald et al. 2009); at the same time, mycorrhizal transporters that are particularly involved in the P uptake from mycorrhizal interface are upregulated (Xu et al. 2007; Paszkowski et al. 2002). The total amount of P uptake by common investment of the pathways also depends on plant and the fungal species. Zhang et al. (2015) confirmed that *Rhizophagus irregularis* was more proficient in P absorption as compared to *Acaulospora longula* and *Gigaspora margarita* in *Lotus japonicus*. Grunwald et al. (2009) have established that the *Glomus intraradices* species has the utmost capability to repress the expression of P transporters in plants within the plant pathway; at the same time, *G. mosseae* showed the slightest outcome. This evidence also supports the concept that the positive input of mycorrhizal pathway to the nutrient accessibility is reliant on the effectiveness with which the AM associates interact along with exchange of nutrient across the mycorrhizal interface (Bücking et al. 2012). The suppression of the plant pathway by AM fungi can result in growth reductions in mycorrhizal plants, once the mycorrhizal pathway is unable to reimburse the reduced uptake by the plant pathway (Smith and Smith 2011). The assumption is that the AM fungus can induce the downregulation of the transporters

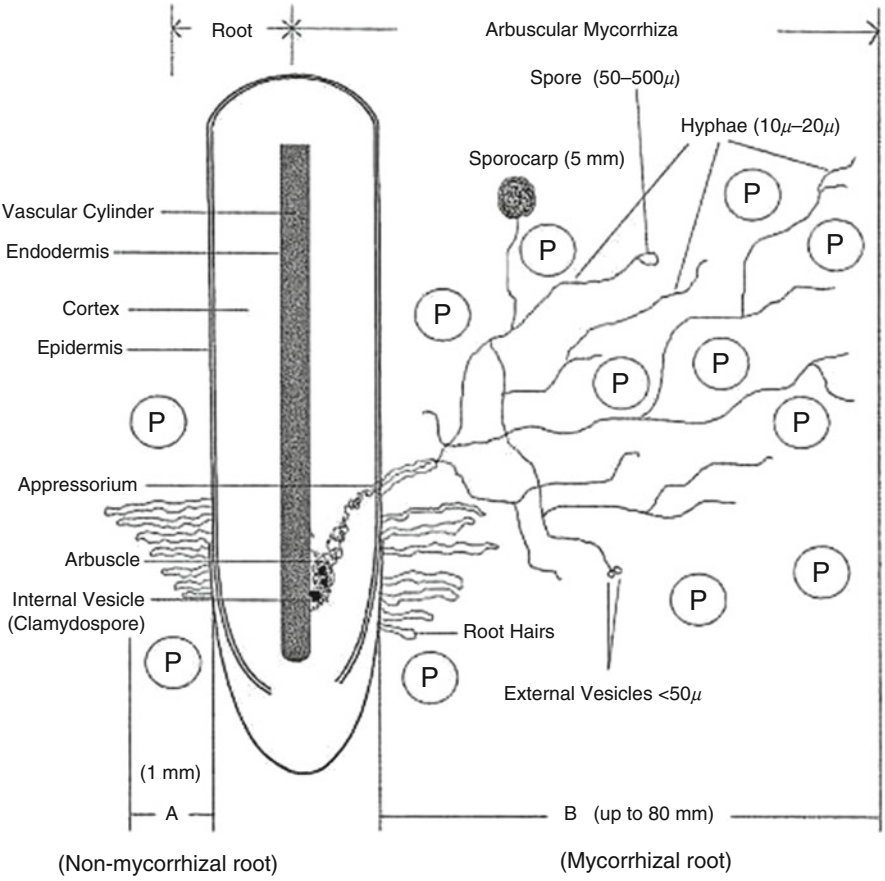

Fig. 2.3 Nutrient uptake pathways in non-mycorrhizal and mycorrhizal roots (Adapted from: Available from: http://dx.doi.org/10.5772/52570)

Table 2.2 Transportation of various nutrients by AM fungi and ectomycorrhizal fungi

Nutrient transported	AM fungi	Ectomycorrhizal fungi
P	+	+
NH_4^+	+	+
Co	+	+
NO_3	–	+
K	+	+
Ca	+	–
SO_4^+	+	–
Cu	+	–
Zn	+	–
Fe	+	+
Mn	+	+
Mg	+	+

of plant pathway to augment its C accessibility. The higher reliance on mycorrhizal pathway for the nutrient acquirement has shown to stimulate the C circulation to root system of the plant (Nielsen et al. 1998; Postma and Lynch 2011).

Of the tree species associated with ectomycorrhiza, most parts of their root surface consist of the region that is nonfunctional in nutrient uptake and the regions that are actively responsible for nutrient acquisition like non-mycorrhizal white or the ECM roots that stand for merely 2 or 16% of the entire root length, correspondingly (Taylor and Peterson 2002). In this condition, the role of the fungal mantle or the sheath that surrounds the root tips is mainly essential (Taylor and Peterson 2005). In a situation, when the fungal layer restricts the infusion of nutrient ions through it, the root tissue lying beneath the fungal mantle would be separated from the nutrient solution in the soil, and such roots will be exclusively reliant upon mycorrhizal pathway for the nutrient acquirement. The ECM fungal species and its structural and functional properties of the mantle are responsible for determining whether the fungal sheath would present an apoplastic barrier or not. Taylor and Peterson (2005) carried out research in relation to the evaluation of permeability of the *Pinus banksiana/Hebeloma cylindrosporum* fungal mantle to the berberine and the radioactive sulfate ions. They established that the fungal mantle was absolutely impervious to the tracer dye. Above an exposure period of 24 h to sulfate ions, the fungal mantle still demonstrated to be impermeable. Such outcomes revealed that plant can exceedingly depend on the fungal partner for the supply of mineral nutrients, because there is modest amount of plant tissue which has the ability of nutrient absorption from exterior of fungal mantle. Some other fungi have been shown to release hydrophobins during ECM development (Coelho et al. 2010). Hydrophobin is a diminutive hydrophobic protein that is accountable for clasping of the fungal hyphae to a surface; moreover, it may add to the impermeability of water in the fungal sheath (Unestam 1991; Unestam and Sun 1995). Therefore, only 2% of root surface of the pines is non-mycorrhizal, as well as ERM of the ECM fungus can signify nearly 99% of the nutrient-exchange interface along the length of the roots in pine (Rousseau et al. 1992), ECM-associated tree species like pine are thought to be greatly dependent on their fungal cohort (Ouahmane et al. 2009; Brundrett 2002), and it may be established that the mycorrhizal pathway plays a more significant role for nutrient acquisition in the ECM root systems as compared to the AM root systems (Bücking et al. 2012).

2.5 Possible Mechanisms of Nutrient Acquisition by Mycorrhizal Fungi

Mycorrhizal fungi are capable of absorbing and transporting almost all the 15 essential macro- and micronutrients vital for growth of the plant. Mycorrhizal fungi ooze out strong chemical compounds into the soil that mobilize firm or rock-bound nutrients such as phosphorous, iron, and other “tightly arrested” mineral nutrients

in the soil. The entire process of dissolution and transportation of nutrients is of great importance in providing nutrition to the plant, and this requires the consideration of high levels of fertility by the non-mycorrhizal plants for maintaining their health. Mycorrhizal fungi create an elaborate web of hyphae that confines and absorbs nutrients restoring the nutritional assets in soils. In the non-mycorrhizal situation, much of this fertility is exhausted or mislaid from the soil system. Mycorrhizal interactions may directly influence the growth of the host plant through the improvement in nutritional attainment by the fungal associate or obliquely by altering the transpiration rates and constitution of the rhizospheric microflora (Marschner and Dell 1994), mobilization of nutrient from the organic substrates (Finlay 2008), by improving the fertilizer use efficacy (Jeff et al. 2005), or by advantageous alliance with other soil microbes (Finlay 2008).

The two key steps in nutrient absorption from the soil and release of the nutrients through mycorrhizal association involve:

(1) Mobilization and acquisition by the fungal mycelia
(2) Transportation of absorbed nutrients across the fungal–root interface

2.5.1 Mobilization and Absorption of Nutrients

In addition to the hyphae that are in the direct touch with the surface of the root, every mycorrhizal fungi also builds up extramatrical mycelium that extends from surface of infected root into the adjacent soil. Both the fungi, arbuscular mycorrhizal (AM) and ECM, manufacture huge quantity of the extramatrical mycelium. Among these, arbuscular mycorrhizal mycelium extends up to many centimeters from the surface of the infected root while ECM mycelium most likely spreads up to some meters (Goltapeh et al. 2008). In both cases, the mycelium stretches adequately afar from the nutrient depletion zone for inaccessible and bound mineral nutrients around each root; moreover, it also exhibits an intricate structure that provides it with an efficient nutrient gathering network (Schachtman et al. 1998; Bücking and Heyser 2001; Goltapeh et al. 2008). One of the components of mycorrhiza is the extramatrical mycelium that competently exhumes bulk soil for sparse nutrients plus transports obtained nutrients to the fungal–root interface where nutrients are transferred to the host plant (Bücking and Kafle 2015). Many ectomycorrhizal fungi spread extramatrical mycelium in the form of a dispersed mat of the individual hyphae forming intricate linear multi-hyphal arrangement recognized as rhizomorphs. The hyphae that are at the center of rhizomorphs are devoid of cell wall and measure about 35 μm in diameter; these play an important role in the transport of photosynthetic assimilates and inorganic mineral nutrients (Table 2.2). Conversely, diffused hyphae in the disperse mat that grow in front of the arbuscular mycorrhizas that measure nearly diameter 1–5 μm in diameter make available a widespread surface area for the absorption of nutrient from the soil. At the same time, hyphae with larger diameter of up to 10 μm are liable for an