

Mycorrhizal Symbiosis: Ecological Functions, Sustainable Agricultural Global Applications, and Future Prospects

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Abstract

Mycorrhizal symbiosis represents one of the most widespread and ecologically significant mutualistic associations in terrestrial ecosystems, involving the interaction between plant roots and soil fungi. This relationship enhances the acquisition of essential nutrients, particularly phosphorus and nitrogen, while improving water uptake, soil structure, and plant tolerance to environmental stresses. Beyond its role in plant nutrition, mycorrhizal symbiosis contributes to ecosystem functioning by influencing nutrient cycling, soil biodiversity, carbon sequestration, and vegetation dynamics. In agricultural systems, the application of mycorrhizal fungi has gained increasing attention as a sustainable strategy to improve crop productivity while reducing dependence on synthetic fertilizers and other agrochemicals. Numerous studies have demonstrated that mycorrhizal associations can enhance plant growth, increase resistance to pathogens, and improve resilience to drought, salinity, and other abiotic stresses. These benefits support the development of environmentally responsible farming practices that align with global goals for sustainable agriculture and food security. Despite considerable progress in understanding mycorrhizal interactions, several challenges remain, including variability in field performance, host specificity, and the influence of environmental and management factors on symbiotic effectiveness. Future research should focus on optimizing inoculation technologies, exploring fungal diversity, and integrating mycorrhizal applications into diverse agricultural systems. Advancements in molecular biology, microbial ecology, and biotechnology are expected to provide new insights into the mechanisms governing plant–fungus interactions and facilitate the development of innovative approaches for sustainable land management. This review examines the ecological functions of mycorrhizal symbiosis, evaluates its applications in sustainable agriculture, and discusses future prospects for enhancing ecosystem resilience and agricultural productivity.

Keywords: Mycorrhizal symbiosis, Sustainable agriculture, Plant-fungus interactions, Nutrient cycling, Soil health, Ecosystem resilience, Biofertilizers

Introduction

Mycorrhizal symbiosis is one of the most important mutualistic associations in terrestrial ecosystems, involving a close relationship between plant roots and specialized soil fungi. Among the various forms of mycorrhizae, ectomycorrhizae play a crucial role in the growth, survival, and productivity of many forest tree species, particularly conifers and several broad-leaved trees. The ecological significance of ectomycorrhizal associations has been recognized for decades, as these symbiotic relationships enhance nutrient acquisition, water uptake, and overall plant fitness while contributing to ecosystem stability and forest sustainability (Harley & Smith, 1983; Smith & Read, 2008). Many forest trees exhibit a high degree of dependence on ectomycorrhizal fungi for successful establishment and development. In natural and managed forests, seedlings lacking appropriate mycorrhizal partners often show reduced growth, poor nutrient status, and lower survival rates. Consequently, inoculation with suitable ectomycorrhizal fungi during nursery production has become an important practice for improving seedling quality and post-transplant performance (Smith & Read, 2008). The fungal hyphae associated with ectomycorrhizal roots extend far beyond the rhizosphere, creating an extensive absorptive network that increases the plant's capacity to access water and nutrients unavailable to non-mycorrhizal roots. This enhanced absorptive surface is particularly important for the

acquisition of relatively immobile nutrients such as phosphorus and certain micronutrients (van der Heijden et al., 2015). In addition to improving nutrient uptake, ectomycorrhizal fungi contribute to several physiological and ecological processes that benefit host plants. These fungi enhance water relations, improve tolerance to drought and temperature stress, and provide protection against certain soil-borne pathogens and root-feeding organisms (Averill et al., 2019). Mycorrhizal associations also influence carbon cycling by facilitating the transfer of photosynthetically derived carbon from plants to fungal networks, thereby contributing to soil organic matter formation and ecosystem carbon storage (Frey, 2019; Prasad 2021a). Through these functions, ectomycorrhizal fungi play a vital role in maintaining forest productivity and resilience under changing environmental conditions.

The term *mycorrhiza*, derived from the Greek words *mykes* (fungus) and *rhiza* (root), refers to the symbiotic association between fungi and plant roots. Traditionally, mycorrhizae have been classified into ectomycorrhizae and endomycorrhizae based on their structural characteristics. In ectomycorrhizae, fungal hyphae form a dense mantle around root tips and penetrate the root cortex intercellularly, creating a structure known as the Hartig net. In contrast, endomycorrhizal fungi colonize root cells internally without forming an external sheath (Prasad et al., 2005;

Prasad, 2010; Brundrett & Tedersoo, 2018). Ectomycorrhizal fungi are predominantly members of the phyla Basidiomycota and Ascomycota and are associated with numerous temperate, subtropical, and tropical tree species. Research on ectomycorrhizae in India began during the mid-twentieth century with reports describing mycorrhizal associations in several coniferous species, including *Abies spectabilis*, *Cedrus deodara*, *Pinus roxburghii*, and *Taxus baccata* (Bakshi, 1957; Chaudhuri, 1945). These early investigations laid the foundation for subsequent studies on the diversity, ecology, and practical applications of ectomycorrhizal fungi in Indian forestry. Recent research has further highlighted the importance of native ectomycorrhizal fungi in afforestation, ecosystem restoration, and climate-resilient forest management (Tedersoo et al., 2020). Morphologically, ectomycorrhizal roots differ markedly from non-colonized roots. Colonized roots typically exhibit reduced root hairs and become enveloped by a fungal mantle. Hyphae extending from this mantle explore the surrounding soil, while internal hyphae form the Hartig net between cortical cells, creating an efficient interface for nutrient exchange between the plant and fungus (Brundrett & Tedersoo, 2018). Through this symbiotic exchange, plants supply carbohydrates and other organic compounds to the fungus, while the fungus provides mineral nutrients, water, and protection against biotic and abiotic stresses. Ectomycorrhizal associations are widespread in forest ecosystems and are particularly common among tree genera such as *Quercus* (oak), *Fagus* (beech), *Betula* (birch), *Pinus* (pine), and *Picea* (spruce). These relationships support nutrient cycling, enhance biodiversity, and contribute to forest ecosystem functioning. Given the increasing pressures of climate change, land degradation, and declining soil fertility, understanding and managing ectomycorrhizal symbioses have become essential components of sustainable forestry and ecosystem conservation. Continued research into the diversity, functioning, and application of ectomycorrhizal fungi will provide valuable insights for improving forest health, productivity, and resilience in the future.

Occurrence and Distribution of Ectomycorrhizae

Ectomycorrhizae are specialized symbiotic structures formed through coordinated interactions between plant roots and compatible soil fungi. This mutualism creates a distinct root organ that enables nutrient and carbon exchange between the host plant and fungal partner. Ectomycorrhizal (ECM) symbioses are ecologically important, occurring in about 2–3% of vascular plant species worldwide, representing nearly 10% of terrestrial plant families, and dominating many temperate, boreal, and some tropical forests (Smith & Read, 2008; Brundrett & Tedersoo, 2018). Ectomycorrhizal associations are especially common in woody plants, including members of the families Pinaceae, Fagaceae, Betulaceae, Salicaceae, Juglandaceae, Myrtaceae, and Dipterocarpaceae. Major ectomycorrhizal tree genera include *Pinus*, *Picea*, *Larix*, *Abies*, *Quercus*, *Fagus*, *Betula*, *Salix*, *Populus*, and *Eucalyptus* (Tedersoo et al., 2020; Prasad 2021b; Prasad & Rajasekaran, 2024). Some genera, such as *Alnus*, *Casuarina*, *Cupressus*, *Juniperus*, *Tilia*, and *Ulmus*, can form both ectomycorrhizal and arbuscular mycorrhizal associations depending on environmental conditions, soil properties, and host developmental stage (Brundrett & Tedersoo, 2018). More than 6,000 fungal species are recognized as ectomycorrhizal partners worldwide, most of them belonging to the phyla Basidiomycota and Ascomycota (Tedersoo & Bahram, 2019). Prominent ectomycorrhizal genera

include *Boletus*, *Suillus*, *Russula*, *Cortinarius*, *Hebeloma*, *Laccaria*, *Pisolithus*, *Rhizopogon*, *Thelephora*, and *Scleroderma*. Several ascomycetous fungi, including *Cenococcum geophilum* and truffles (*Tuber* spp.), also form ectomycorrhizal associations with forest trees (Martin et al., 2016). This high fungal diversity supports ecosystem functioning, nutrient cycling, and host adaptation across a wide range of environments.

Ectomycorrhizal symbiosis begins when fungal spores or hyphae respond to chemical signals released by plant roots. Root exudates direct fungal growth toward the root surface, where the fungus colonizes feeder roots and forms a mantle around the root tip. The hyphae then penetrate the intercellular spaces of the root cortex to form the Hartig net, the main interface for nutrient and carbon exchange between the two partners and a defining feature of ectomycorrhizal associations (Smith & Read, 2008; Martin et al., 2016). Formation of the Hartig net causes structural and physiological changes in root cortical cells (Figure 1). These changes increase the surface area available for nutrient transfer and enhance metabolic activity at the plant–fungus interface. Studies show that transfer-cell-like structures may develop in cortical tissues, promoting efficient movement of mineral nutrients, water, and carbohydrates between the host and fungal symbiont (Genre et al., 2020). Through this interface, fungi receive photosynthetically derived carbon, while plants gain improved access to soil nutrients and water. Most ectomycorrhizal fungi reproduce through fruiting bodies that appear seasonally under favorable conditions. Although sporocarp production is often periodic, spores can remain viable in soil for long periods, creating a persistent inoculum reservoir. Spore germination is influenced by root exudates, soil microorganisms, temperature, moisture, and nutrient availability (Tedersoo & Bahram, 2019). In hypogeous fungi such as truffles and *Rhizopogon*, dispersal often depends on small mammals and other forest animals that consume the fruiting bodies and later disperse spores in their feces, aiding fungal spread and forest regeneration (Peay et al., 2016). Ectomycorrhizal colonization often causes substantial changes in root morphology. Colonized roots typically become shorter, thicker, and more highly branched than non-mycorrhizal roots, while root hairs are reduced or absent. Root systems may exhibit simple, bifurcate, coralloid, nodular, or multiforked branching patterns depending on the fungal species involved. The color of ectomycorrhizal root tips varies widely and is largely determined by the fungal mantle, which may appear white, yellow, orange, red, brown, or black (Agerer, 2006). These morphological characteristics are frequently used for field identification of ectomycorrhizal associations. Functional differences among ectomycorrhizal fungi can significantly influence host performance. Certain fungal species produce extensive rhizomorph networks that facilitate long-distance transport of water and nutrients, thereby enhancing drought tolerance and nutrient acquisition under stressful environmental conditions (Lilleskov et al., 2019). Other species synthesize phytohormones such as auxins and cytokinins, which regulate root architecture, seedling growth, and biomass allocation patterns. Additionally, many ectomycorrhizal fungi produce antimicrobial compounds that suppress soil-borne pathogens and contribute to the development of beneficial microbial communities in the rhizosphere (Genre et al., 2020).

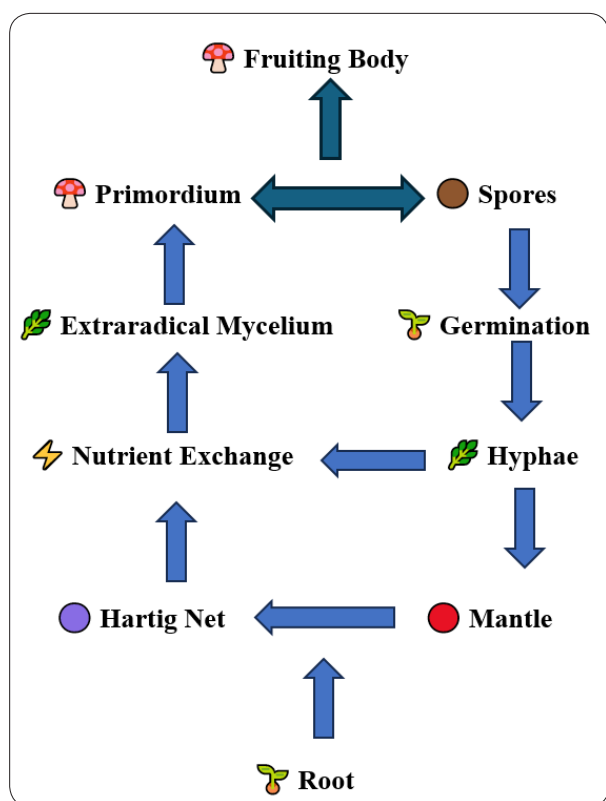


Figure 1: Systematic representation of in vitro Ectomycorrhizal Life cycle

The ecological importance of ectomycorrhizal fungi has been documented in numerous forest ecosystems worldwide, including those of India. Early studies reported ectomycorrhizal associations in species such as *Pinus roxburghii*, *Cedrus deodara*, *Abies pindrow*, and *Picea smithiana*. Subsequent investigations have identified diverse ectomycorrhizal fungi associated with Himalayan pine forests, including species of *Scleroderma*, *Rhizopogon*, *Suillus*, *Astraeus*, *Amanita*, *Laccaria*, *Lactarius*, *Boletus*, and *Thelephora* (Sharma & Singh, 1990; Prasad et al., 2017). The distribution and composition of these fungal communities vary with altitude, climate, vegetation type, and soil properties, reflecting the dynamic nature of ectomycorrhizal ecosystems. Overall, the widespread occurrence and remarkable diversity of ectomycorrhizal associations underscore their critical role in forest ecology. By enhancing nutrient uptake, improving stress tolerance, protecting against pathogens, and supporting ecosystem processes, ectomycorrhizal fungi contribute substantially to forest productivity, biodiversity conservation, and sustainable forest management under changing environmental conditions.

Occurrence and Distribution of Endomycorrhiza

Endomycorrhizae, commonly referred to as arbuscular mycorrhizae (AM), represent the most widespread type of mycorrhizal association in terrestrial ecosystems. These symbiotic relationships are formed between the roots of plants and fungi belonging primarily to the phylum Glomeromycota. Unlike ectomycorrhizae, endomycorrhizal fungi penetrate the cortical cells of plant roots, where they develop specialized structures known as arbuscules and vesicles. Arbuscules function as sites of nutrient exchange between the host plant and the fungus, whereas vesicles serve as storage organs for lipids and other nutrients (Smith & Read, 2008; Brundrett & Tedersoo, 2018). Arbuscular mycorrhizal (AM) associations are estimated to occur in approximately 70–80% of all terrestrial plant species, including a wide range of agricultural crops, grasses, herbs, shrubs, and tropical trees (Genre et al.,

2020). Fossil evidence indicates that these symbiotic relationships originated more than 400 million years ago and played a critical role in the colonization of land by early plants (Rich et al., 2021). Today, AM fungi are found in diverse ecosystems, ranging from tropical rainforests and temperate woodlands to grasslands, deserts, wetlands, and agricultural lands, demonstrating their remarkable ecological adaptability. The distribution of endomycorrhizae is influenced by environmental conditions, soil properties, host plant species, and land management practices. Arbuscular mycorrhizal fungi are particularly abundant in grasslands and agricultural soils, where they contribute significantly to nutrient cycling and plant productivity. Most economically important crop species, including cereals, legumes, oilseeds, vegetables, and fruit crops, establish symbiotic associations with AM fungi. Examples include wheat (*Triticum aestivum*), maize (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), sorghum (*Sorghum bicolor*), and sunflower (*Helianthus annuus*) (Prasad & Rajak, 2002; Begum et al., 2019). However, certain plant families, such as Brassicaceae, Chenopodiaceae, and Caryophyllaceae, generally do not form arbuscular mycorrhizal associations or exhibit only limited colonization. Globally, more than 340 species of arbuscular mycorrhizal fungi have been described, although molecular studies suggest that actual diversity is considerably higher (Öpik et al., 2022). These fungi belong to several genera, including *Rhizophagus*, *Funnelformis*, *Claroideoglossum*, *Gigaspora*, *Acaulospora*, and *Scutellospora*. The distribution and abundance of these taxa vary according to climatic conditions, soil fertility, vegetation type, and ecological succession. Tropical and subtropical regions generally support a high diversity of AM fungi because of favorable temperature and moisture conditions that promote fungal growth and root colonization.

The establishment of endomycorrhizal symbiosis begins with chemical communication between plant roots and fungal propagules in the soil. Plant roots release signaling compounds, particularly strigolactones, which stimulate spore germination and hyphal branching. In response, fungi produce signaling molecules known as Myc factors that initiate root colonization (Lanfranco et al., 2018). Following successful recognition, fungal hyphae penetrate the root epidermis and colonize cortical tissues, where arbuscules develop within living plant cells (Figure 2). These highly branched structures create an extensive surface area for the exchange of phosphorus, nitrogen, sulfur, and micronutrients from the fungus to the plant, while carbon compounds produced through photosynthesis are transferred from the plant to the fungal partner. Endomycorrhizal fungi play a crucial role in enhancing nutrient acquisition, particularly phosphorus uptake. Because phosphorus is relatively immobile in soil, fungal hyphae extend beyond the nutrient depletion zone surrounding roots and access phosphorus reserves unavailable to plant roots alone (van der Heijden et al., 2015). In addition to improving nutrient acquisition, AM fungi enhance water absorption, improve soil aggregation, increase resistance to root pathogens, and promote plant tolerance to environmental stresses such as drought, salinity, heavy metal contamination, and temperature extremes (Begum et al., 2019; Genre et al., 2020). The occurrence of endomycorrhizae is strongly influenced by soil characteristics. Moderate levels of soil phosphorus generally favor mycorrhizal development, whereas excessive phosphorus fertilization can suppress fungal colonization and reduce symbiotic efficiency. Soil pH, moisture availability, organic matter content, and microbial community composition also affect the abundance and diversity of AM fungi (Berruti et al., 2016). Agricultural practices such as intensive tillage, monocropping, and excessive agrochemical application may reduce mycorrhizal populations, whereas conservation agriculture, crop rotation, and organic farming systems often

promote their establishment and activity. In India, arbuscular mycorrhizal fungi are widely distributed across agricultural, forest, and grassland ecosystems. Numerous studies have documented AM fungal associations in economically important crops, medicinal plants, fruit trees, and forest species. Genera such as *Glomus* (currently reclassified into several genera including *Rhizophagus* and *Funnelliformis*), *Acaulospora*, *Gigaspora*, and *Scutellospora* are frequently reported from Indian soils (Prasad & Rajak, 1999; Prasad et al., 2017). These fungi contribute significantly to soil fertility, sustainable crop production, and ecosystem restoration efforts in diverse agroecological regions of the country.

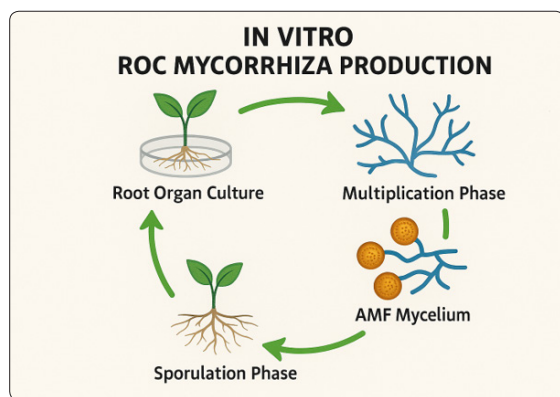


Figure 2: Systematic representation of in vitro ROC (Root Organ Culture) Mycorrhiza Life cycle

Recent advances in molecular biology and high-throughput sequencing technologies have revealed previously unknown patterns of endomycorrhizal diversity and distribution. Studies indicate that AM fungal communities are structured by both environmental factors and host plant identity, with important implications for ecosystem functioning and resilience under climate change scenarios (Prasad & Rajak, 2001; Opik et al., 2022). As global concerns regarding soil degradation, food security, and environmental sustainability continue to increase, understanding the ecology and distribution of endomycorrhizal fungi has become essential for developing sustainable agricultural and land-management strategies. Overall, endomycorrhizae represent a fundamental component of terrestrial ecosystems. Their widespread occurrence across plant taxa and habitats highlights their ecological importance in nutrient cycling, soil health maintenance, plant productivity, and ecosystem stability. Continued research on the diversity, distribution, and functional roles of endomycorrhizal fungi will contribute to sustainable agriculture, biodiversity conservation, and climate-resilient ecosystem management.

Ectomycorrhizae: Characterization and Identification

The characterization and identification of ectomycorrhizae (ECM) have been central areas of mycorrhizal research for nearly a century. Early studies relied primarily on morphological observations of colonized root systems, focusing on visible features such as root branching patterns, mantle color, texture, and overall root morphology. Initial classification systems developed during the early twentieth century provided a foundation for understanding ectomycorrhizal diversity, although their application was often limited by the absence of reliable methods for identifying the fungal symbionts involved (Melin, 1927; Smith & Read, 2008). One of the earliest attempts to classify ectomycorrhizae was based on gross morphological characteristics of pine roots, which allowed researchers to distinguish different ectomycorrhizal types according to external appearance. Subsequent studies incorporated anatomical features such as mantle structure, hyphal arrangement,

and Hartig net development, providing greater taxonomic resolution (Agerer, 2006). Although these early classification systems were useful for describing ectomycorrhizal diversity, they often lacked sufficient specificity for accurate identification because many unrelated fungal species can produce similar root morphologies. During the mid-twentieth century, researchers expanded the characterization of ectomycorrhizae across various host species. Detailed descriptions were produced for ectomycorrhizal associations occurring on *Salix* (willow), *Pinus radiata*, and *Eucalyptus* species, among others. These investigations documented substantial variation in root morphology, mantle organization, and branching patterns. However, in many cases, the fungal partners responsible for the observed ectomycorrhizae could not be conclusively identified because fruiting bodies were absent or difficult to associate directly with colonized roots (Marx, 1975; Rambelli, 1966). As a result, ectomycorrhizal identification remained largely descriptive rather than taxonomically precise.

A major advancement in ectomycorrhizal research occurred with the development of standardized morphological and anatomical classification systems. The comprehensive framework proposed by Reinhard Agerer emphasized the integration of macroscopic and microscopic characteristics, including mantle color, surface texture, hyphal structures, rhizomorph formation, branching patterns, and Hartig net architecture (Agerer, 1987–2008). This approach enabled more consistent descriptions of ectomycorrhizal types and facilitated comparisons among studies conducted in different geographical regions and forest ecosystems.

Morphological characterization remains an important component of ectomycorrhizal identification. Key diagnostic features include root tip shape, branching pattern, mantle thickness, color, surface ornamentation, emanating hyphae, and the presence or absence of rhizomorphs. Microscopic examination further reveals details of mantle anatomy, hyphal arrangement, cystidia, and Hartig net development, all of which contribute valuable taxonomic information (Agerer, 2006). Nevertheless, morphological identification alone can be challenging because environmental conditions, host species, and developmental stage may influence ectomycorrhizal appearance. The introduction of molecular techniques has revolutionized the identification of ectomycorrhizal fungi. DNA-based approaches, particularly sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA, have become the standard method for fungal identification. These techniques allow direct identification of fungal symbionts from colonized root tips without requiring the presence of fruiting bodies (Köljal et al., 2013). Molecular methods have revealed that a single forest ecosystem may harbor hundreds of ectomycorrhizal fungal species, many of which were previously unknown or difficult to distinguish using morphology alone.

Recent advances in high-throughput sequencing, environmental DNA (eDNA) analysis, and metabarcoding have further enhanced the study of ectomycorrhizal communities. These technologies enable researchers to assess fungal diversity, community composition, and ecological interactions at unprecedented scales. Metabarcoding studies have demonstrated that ectomycorrhizal fungal assemblages vary according to host species, soil characteristics, climatic conditions, and geographic location (Tedesso et al., 2020). Such approaches have significantly improved understanding of fungal biodiversity and ecosystem functioning. In addition to morphological and molecular methods, physiological and biochemical techniques are increasingly used to characterize ectomycorrhizal fungi. Stable isotope analysis, enzymatic profiling, and metabolomic studies provide insights into nutrient acquisition strategies, carbon allocation patterns,

and ecological functions of different fungal taxa (Martin et al., 2016). These complementary approaches contribute to a more comprehensive understanding of ectomycorrhizal diversity and functionality. Despite substantial progress, challenges remain in the characterization and identification of ectomycorrhizae. Many fungal species exhibit considerable morphological plasticity, and numerous taxa remain poorly described or undiscovered. Furthermore, molecular databases continue to expand as new species are identified from previously unexplored ecosystems. Future research integrating morphological, anatomical, molecular, and ecological data is expected to improve taxonomic resolution and enhance understanding of ectomycorrhizal symbioses across diverse forest ecosystems. Overall, the evolution of ectomycorrhizal identification methods from simple morphological observations to advanced molecular techniques has transformed the field of mycorrhizal biology. Modern approaches now provide accurate and reliable tools for studying ectomycorrhizal diversity, ecology, and function, thereby supporting sustainable forest management, biodiversity conservation, and ecosystem restoration initiatives.

Criteria for the Characterization and Identification of Ectomycorrhizae

The accurate characterization and identification of ectomycorrhizae (ECM) are essential for understanding fungal diversity, host specificity, ecological functions, and applications in forestry and ecosystem restoration. Modern identification approaches integrate morphological, anatomical, cultural, biochemical, and molecular characteristics to achieve reliable taxonomic resolution. While traditional methods based on root morphology and fungal sporocarps remain valuable, contemporary studies increasingly employ microscopic and DNA-based techniques to confirm fungal identity (Agerer, 2006; Smith & Read, 2008; Kõljalg et al., 2013).

Morphological Characteristics of Ectomycorrhizae

Morphological examination of ectomycorrhizal root tips is often the first step in identification. Colonized roots may exhibit a variety of branching patterns, including simple, bifurcate, monopodial-pinnate, coralloid, pyramidal, racemose, or irregularly branched forms. Individual root tips may be elongated, club-shaped, cylindrical, or sessile depending on the fungal species involved (Agerer, 2006). Color is another important diagnostic feature and may range from white, cream, yellow, orange, and brown to dark black. The surface appearance of ectomycorrhizal roots may be smooth, cottony, tomentose, velvety, woolly, or covered with abundant emanating hyphae. These characteristics are frequently used in field identification and preliminary classification of ectomycorrhizal types (Brundrett et al., 1996).

Mantle and Hartig Net Characteristics

The fungal mantle, which surrounds the root tip, provides important taxonomic information. Mantles may exhibit different structural organizations, including pseudoparenchymatous, prosenchymatous, plectenchymatous, palisade-like, or multilayered arrangements. Mantle thickness varies among fungal species and may be influenced by environmental factors such as soil nutrient status, moisture, and host physiology (Agerer, 2006). Mantle ornamentation also serves as a useful diagnostic feature. Specialized structures may include emanating hyphae, cystidia, setae, rhizomorphs, hyphal strands, and dendroid branching patterns. The Hartig net, formed by fungal hyphae between cortical cells, represents the principal interface for nutrient exchange between the host and fungus. The extent and depth of Hartig net penetration into root cortical tissues are often species-specific and contribute to ectomycorrhizal identification (Martin et al., 2016).

Association Between Roots and Fungal Sporocarps

The identification of fungal sporocarps associated with colonized

roots provides valuable evidence for establishing host–fungus relationships. Field observations combined with molecular analysis can link fruiting bodies to specific ectomycorrhizal root tips. Historically, such associations were based primarily on spatial proximity; however, DNA sequencing now allows direct confirmation of fungal identity from both root tissues and sporocarps (Kõljalg et al., 2013). This approach has significantly improved the accuracy of ectomycorrhizal fungal identification.

Taxonomic Characteristics of Fungal Sporocarps

Macroscopic and microscopic features of fungal fruiting bodies remain important taxonomic criteria. Diagnostic characteristics include:

- Pileus (cap) color, shape, texture, and size
- Gill attachment and arrangement
- Presence or absence of annulus and volva
- Spore print color
- Spore size, shape, wall thickness, and ornamentation
- Cap morphology (convex, conical, campanulate, umbonate, funnel-shaped, or flat)
- Margin characteristics (straight, incurved, rolled, striate, or lobed)
- Surface texture (smooth, glabrous, fibrillose, scaly, velvety, or tomentose)

These characteristics continue to play a critical role in fungal taxonomy and complement molecular identification methods (Largent, 1986; Agerer, 2006).

Cultural Characteristics

Isolation and cultivation of ectomycorrhizal fungi under controlled laboratory conditions provide additional information for species identification. Characteristics such as colony morphology, pigmentation, growth rate, hyphal structure, and nutrient requirements can assist in differentiating fungal taxa. Pure-culture synthesis experiments, in which fungal isolates are inoculated onto host seedlings, remain an important method for confirming specific host–fungus compatibility and mycorrhiza formation (Marx, 1975; Molina & Trappe, 1982).

Molecular Identification Techniques

Molecular methods have become the standard approach for ectomycorrhizal identification. Sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA is widely recognized as the primary fungal barcode marker. Additional genomic regions, including LSU, SSU, and protein-coding genes, are increasingly used for phylogenetic studies (Kõljalg et al., 2013). High-throughput sequencing, environmental DNA (eDNA), metabarcoding, and metagenomic analyses now enable rapid assessment of ectomycorrhizal diversity directly from environmental samples. These approaches have revealed substantial hidden diversity in forest ecosystems and have transformed the study of fungal ecology and taxonomy (Tedersoo et al., 2020).

Chemical and Histochemical Tests

Chemical reagents have traditionally been used to aid fungal identification by producing characteristic color reactions in fungal tissues. Reagents commonly employed include:

- Melzer's reagent
- Potassium hydroxide (KOH)
- Ammonium hydroxide (NH₄OH)
- Ferrous sulfate (FeSO₄)
- Formalin
- Phenol
- Aniline dyes

These reactions may assist in distinguishing closely related fungal taxa and continue to be used in conjunction with morphological and molecular analyses (Largent, 1986).

Fluorescence Characteristics

Certain fungal tissues exhibit distinctive fluorescence when exposed to ultraviolet (UV) light. Fluorescence microscopy can facilitate the visualization of fungal structures, including hyphae, rhizomorphs, and mantle tissues. Modern fluorescent staining techniques have further improved the detection and study of ectomycorrhizal colonization patterns within roots (Genre et al., 2020).

Microscopic Characteristics

Microscopic examination remains one of the most informative approaches for ectomycorrhizal characterization. Important features include:

- Hyphal diameter and wall thickness
- Hyphal orientation and branching patterns
- Presence or absence of clamp connections
- Septation patterns
- Rhizomorph structure and anatomy
- Cystidia and specialized hyphal structures
- Mantle organization
- Hartig net architecture

These anatomical traits are frequently species-specific and contribute substantially to accurate identification (Agerer, 2006). The identification of ectomycorrhizae has evolved from simple morphological observations to highly sophisticated integrative approaches combining morphology, anatomy, culture-based studies, biochemical analyses, microscopy, and molecular techniques. While traditional diagnostic characteristics remain valuable, DNA-based methods now provide the highest level of accuracy and have significantly expanded understanding of ectomycorrhizal diversity. The integration of classical and modern approaches offers the most reliable framework for characterizing ectomycorrhizal fungi and investigating their ecological and functional roles in forest ecosystems.

Physiology, Biochemistry, and *In Vitro* Synthesis of Ectomycorrhizae

Physiology and Biochemistry of Ectomycorrhizal Fungi

Ectomycorrhizal (ECM) fungi are highly specialized symbiotic organisms that form mutualistic associations with the roots of numerous forest trees. These fungi can be isolated from ectomycorrhizal root tips, spores, rhizomorphs, and fungal sporocarps and are commonly maintained under laboratory conditions using nutrient media such as Modified Melin–Norkrans (MMN) medium, which remains the standard medium for culturing many ectomycorrhizal species (Marx, 1969; Smith & Read, 2008). Although some ECM fungi can grow on general fungal media such as potato dextrose agar (PDA), MMN medium provides nutrients better suited to their physiological requirements. Ectomycorrhizal fungi exhibit unique nutritional adaptations resulting from their symbiotic lifestyle. Unlike saprotrophic fungi, most ECM species possess a reduced capacity to degrade complex plant polymers such as cellulose and lignin. Consequently, they rely heavily on their host plants for carbon compounds derived from photosynthesis (Martin et al., 2016). The host transfers a significant proportion of its fixed carbon to the fungal partner in the form of sugars and other organic compounds, while the fungus supplies mineral nutrients and water to the plant. Carbohydrates play a central role in ectomycorrhizal metabolism. Many ECM fungi efficiently utilize simple sugars such as glucose, fructose, and sucrose, whereas utilization of more complex carbohydrates varies among species. Earlier physiological studies demonstrated

that numerous ectomycorrhizal fungi grow well on glucose and other readily available carbon sources but show limited ability to metabolize cellulose directly (Smith & Read, 2008). This dependence on host-derived carbon contributes to the ecological specificity of ectomycorrhizal associations and influences fungal distribution within forest soils. In addition to carbon, ectomycorrhizal fungi require a range of vitamins, amino acids, and mineral nutrients for growth and development. Nitrogen metabolism is particularly important because many ECM fungi can access organic nitrogen pools unavailable to plants, including amino acids, peptides, and proteins present in forest soils. Through enzymatic activity, these fungi mobilize nutrients and transfer them to host plants, thereby enhancing nutrient acquisition under nutrient-poor conditions (Lindahl & Tunlid, 2015). Recent genomic and transcriptomic studies have provided new insights into the biochemical mechanisms underlying ectomycorrhizal symbiosis. Comparative genome analyses reveal that ECM fungi possess specialized gene repertoires associated with nutrient transport, signaling, stress tolerance, and symbiotic development. During root colonization, extensive molecular communication occurs between the host plant and fungal symbiont, involving signaling molecules, phytohormones, transport proteins, and transcriptional regulators that coordinate the establishment and maintenance of the symbiosis (Martin et al., 2016; Genre et al., 2020). Ectomycorrhizal fungi also contribute to plant health through the production of bioactive compounds. Many species synthesize phytohormones such as auxins, cytokinins, and gibberellins that influence root architecture and plant growth. Additionally, certain ECM fungi produce antimicrobial metabolites that suppress soil-borne pathogens and enhance host resistance against diseases (Bonfante & Genre, 2010). These physiological and biochemical functions contribute significantly to forest ecosystem productivity and resilience.

In Vitro Synthesis of Ectomycorrhizae

The *in vitro* synthesis of ectomycorrhizae has been fundamental to understanding the biology, specificity, and functioning of ectomycorrhizal associations. Controlled synthesis experiments provide direct evidence of a fungus's ability to form ectomycorrhizae with a particular host species and remain one of the most reliable methods for confirming symbiotic compatibility (Molina & Trappe, 1982). The first successful synthesis of ectomycorrhizae under laboratory conditions was achieved by Elias Melin during the early twentieth century. Using seedlings of *Picea abies*, *Pinus sylvestris*, and *Larix decidua*, Melin demonstrated that inoculation with compatible fungal isolates resulted in the formation of characteristic ectomycorrhizal structures (Melin, 1921, 1936). These pioneering studies established the experimental foundation for modern ectomycorrhizal research. Early synthesis experiments commonly employed sterile sand as a growth substrate because of its relatively inert nature. However, sand possesses several limitations, including low water-holding capacity and poor nutrient retention. To overcome these constraints, researchers introduced alternative substrates such as vermiculite and peat-based mixtures. Vermiculite proved particularly useful because of its sterility, favorable moisture retention properties, and minimal interference with fungal growth (Hacskeylo, 1953). Subsequently, the incorporation of finely ground sphagnum peat moss improved pH stability and nutrient availability, creating more favorable conditions for ectomycorrhizal development (Marx & Zak, 1965).

Numerous studies have successfully synthesized ectomycorrhizae using different host–fungus combinations. Experimental work involving *Thelephora terrestris*, *Pisolithus tinctorius*, *Laccaria laccata*, *Lactarius sanguifluus*, *Suillus bovinus*, and *Piloderma croceum* demonstrated substantial variation in ectomycorrhizal

morphology and physiological performance among fungal species (Fortin et al., 1980; Nylund & Unestam, 1982). These studies revealed that while some ectomycorrhizal structures are strongly influenced by fungal identity, host species can also affect the morphology and functioning of the resulting symbiosis.

One of the most extensively studied ectomycorrhizal fungi is *Pisolithus tinctorius* (currently classified within the *Pisolithus* complex). This fungus exhibits broad host compatibility, rapid colonization ability, and high tolerance to environmental stress. Consequently, it has been widely used in forestry nurseries and reforestation programs around the world (Marx, 1991). Inoculation with *Pisolithus* species often enhances seedling growth, nutrient uptake, drought tolerance, and survival following transplantation. Modern *in vitro* synthesis studies increasingly incorporate advanced microscopy, molecular biology, and genomic approaches to investigate symbiotic development. Confocal microscopy and transcriptomic analyses now allow researchers to monitor fungal colonization, nutrient exchange, and gene expression during the earliest stages of ectomycorrhizal formation (Genre et al., 2020). These technologies have significantly expanded understanding of the molecular mechanisms governing host recognition, fungal colonization, and nutrient transfer. Although numerous ectomycorrhizal fungi have been successfully synthesized under laboratory conditions, only a limited number have been developed for large-scale commercial application. Challenges remain regarding host specificity, environmental adaptability, inoculum production, and field performance. Future research should focus on identifying highly effective fungal strains, optimizing inoculation technologies, and evaluating long-term ecological benefits in forestry and ecosystem restoration programs. Overall, *in vitro* synthesis has played a critical role in advancing ectomycorrhizal research. By providing controlled experimental systems for studying plant–fungus interactions, these approaches continue to improve our understanding of ectomycorrhizal physiology, ecology, and practical applications in sustainable forest management.

Nutrient Uptake in Ectomycorrhizal Associations **Nutrient Uptake and Mineral Nutrition**

One of the most significant ecological functions of ectomycorrhizal (ECM) fungi is their ability to enhance nutrient acquisition by host plants. The beneficial effects of ectomycorrhizal symbiosis on plant nutrition have been recognized for nearly a century, and extensive research has demonstrated that ECM fungi improve the uptake of both macro- and micronutrients, thereby promoting plant growth, survival, and productivity (Smith & Read, 2008; van der Heijden et al., 2015). Early investigations revealed that ectomycorrhizal seedlings frequently exhibit greater biomass accumulation and higher nutrient concentrations than non-mycorrhizal plants grown under similar environmental conditions. Classical studies on pine species showed that mycorrhizal seedlings absorbed substantially greater amounts of phosphorus (P), nitrogen (N), and potassium (K), resulting in enhanced growth and vigor (Hatch, 1937). These findings established the foundation for understanding the nutritional benefits of ectomycorrhizal symbiosis and stimulated further research into the mechanisms underlying nutrient acquisition.

Phosphorus is generally regarded as the nutrient most strongly influenced by ectomycorrhizal associations. In many forest soils, phosphorus availability is limited because phosphate ions are relatively immobile and often bound to soil minerals or organic matter. Ectomycorrhizal fungi overcome these limitations through the development of extensive extraradical hyphal networks that explore soil volumes beyond the depletion zone surrounding plant roots. These hyphae increase the absorptive surface area of the root system and facilitate the acquisition of phosphorus from

otherwise inaccessible soil microsites (Smith & Read, 2008; Genre et al., 2020). In addition to physical exploration of the soil, many ectomycorrhizal fungi produce extracellular enzymes and organic acids capable of mobilizing nutrients from complex organic and inorganic compounds. These biochemical processes enhance the availability of phosphorus and other nutrients, particularly in nutrient-poor forest ecosystems where organic matter serves as a major nutrient reservoir (Lindahl & Tunlid, 2015). Consequently, ectomycorrhizal fungi play a crucial role in nutrient cycling and ecosystem functioning. Nitrogen uptake is another important benefit associated with ectomycorrhizal colonization. Forest soils often contain large quantities of nitrogen in organic forms that are not directly accessible to plants. Many ECM fungi possess enzymatic capabilities that enable the decomposition of organic nitrogen compounds, including amino acids, peptides, and proteins. The liberated nitrogen is absorbed by fungal hyphae and subsequently transferred to the host plant, thereby improving plant nutrition and growth under nitrogen-limited conditions (Averill et al., 2019). Recent studies indicate that ectomycorrhizal fungi contribute significantly to nitrogen retention and cycling in forest ecosystems, influencing both plant productivity and soil carbon dynamics. Ectomycorrhizal fungi also facilitate the uptake of potassium, calcium, magnesium, sulfur, and numerous micronutrients. Research has demonstrated enhanced absorption of calcium and magnesium in several conifer species colonized by ECM fungi (Smith & Read, 2008). Similarly, improved acquisition of essential trace elements such as zinc (Zn), copper (Cu), manganese (Mn), boron (B), molybdenum (Mo), and iron (Fe) has been reported in mycorrhizal seedlings compared with non-mycorrhizal controls (van der Heijden et al., 2015). The uptake efficiency of these nutrients depends on fungal species, host genotype, soil conditions, and environmental factors.

The relationship between soil fertility and ectomycorrhizal colonization has been extensively investigated. Numerous studies have reported an inverse relationship between nutrient availability and the degree of mycorrhizal infection. High levels of readily available phosphorus or nitrogen often reduce fungal colonization because plants become less dependent on fungal-mediated nutrient acquisition (Smith & Smith, 2011). Excessive fertilizer application may therefore suppress ectomycorrhizal development and alter fungal community composition, potentially reducing long-term ecosystem sustainability. Environmental conditions strongly influence nutrient transfer within ectomycorrhizal associations. Factors such as soil pH, moisture, temperature, organic matter content, and nutrient availability affect both fungal activity and nutrient uptake efficiency. Nitrogen deposition resulting from anthropogenic activities has received particular attention because excessive nitrogen inputs can alter carbohydrate allocation patterns, reduce ectomycorrhizal diversity, and modify nutrient cycling processes within forest ecosystems (Lilleskov et al., 2019). The nutritional benefits provided by ectomycorrhizal fungi have important implications for forestry, ecosystem restoration, and sustainable land management. In nursery production systems, inoculation with effective ectomycorrhizal fungi often improves seedling growth, nutrient status, and field establishment. Enhanced nutrient acquisition contributes to greater stress tolerance, increased biomass production, and improved survival following transplantation (Marx, 1991). Consequently, ectomycorrhizal inoculation has become an important component of reforestation and afforestation programs worldwide. Recent advances in molecular biology, isotope tracing, and genomic analyses have significantly improved understanding of nutrient exchange mechanisms between plants and ectomycorrhizal fungi. These studies reveal complex networks of nutrient transporters and signaling pathways that regulate nutrient acquisition, allocation, and transfer between symbiotic partners (Martin et al., 2016). Such

knowledge is expected to facilitate the development of innovative approaches for improving nutrient-use efficiency and promoting sustainable forest management under changing environmental conditions. Overall, ectomycorrhizal fungi represent a critical component of plant nutrition in forest ecosystems. Through extensive soil exploration, nutrient mobilization, and efficient transfer of mineral elements to host plants, these fungi enhance nutrient uptake, support plant growth, and contribute substantially to ecosystem productivity and resilience.

Inoculum and Inoculation of Ectomycorrhizal Fungi Types of Inoculums and Their Application

The establishment of ectomycorrhizal (ECM) symbiosis in forestry nurseries and field plantations depends largely on the type and quality of fungal inoculum used. Different inoculum sources vary in effectiveness, cost, ease of application, and ecological suitability. The three principal types of ectomycorrhizal inoculum include soil-based inoculum, spore-based inoculum, and pure culture (mycelial) inoculum (Smith & Read, 2008; Marx, 1991). Soil inoculum, obtained from the rhizosphere of healthy ectomycorrhizal host trees, has historically been one of the most widely used methods, particularly in developing regions. This inoculum consists of forest soil containing native ectomycorrhizal propagules such as fungal hyphae, spores, and infected root fragments. In nursery systems, small proportions of such soil are mixed with sterilized or partially sterilized growth media to introduce beneficial fungi into the planting substrate. Although this method is inexpensive and relatively simple, it lacks specificity and may introduce unwanted microorganisms along with beneficial fungi (Mikola, 1970; Brundrett et al., 1996). Spore-based inoculation represents another commonly used approach for ectomycorrhizal establishment. In this method, spores or macerated fruiting bodies of ectomycorrhizal fungi are applied directly to seeds, seedlings, or soil. Spore suspensions are typically prepared by blending fresh sporocarps in sterile water and applying the resulting suspension to nursery beds or irrigation systems. This technique has been successfully used to enhance growth of pine species in several regions, including Australia, South Africa, and North America (Marx et al., 1984; Theodorou & Bowen, 1970). However, the effectiveness of spore inoculum depends on spore viability, environmental conditions, and the availability of fruiting bodies, which may vary seasonally. Additionally, accurate assessment of spore viability remains a challenge in many cases. Pure culture or mycelial inoculum is considered the most controlled and reliable method for ectomycorrhizal inoculation. In this approach, fungal isolates are obtained from ectomycorrhizal root tips or sporocarps and cultured under sterile laboratory conditions on nutrient media such as Modified Melin–Norkrans (MMN) medium. The resulting mycelium is then used to inoculate seedlings in sterile or controlled substrates, allowing precise host–fungus pairing (Molina & Trappe, 1982; Marx, 1991). This method provides greater consistency and reproducibility compared with soil or spore inoculation techniques.

Development of Pure Culture Inoculum

Early efforts to develop vegetative inoculum of ectomycorrhizal fungi were pioneered by Ernst Moser, who demonstrated the production of fungal inoculum using liquid culture systems and sterile carrier materials such as peat moss (Moser, 1958). These foundational studies established the feasibility of producing viable ectomycorrhizal inoculum under controlled conditions. Subsequent refinements included the use of inert substrates such as vermiculite and peat-based mixtures to improve water retention, sterility, and fungal growth performance (Hacskeylo, 1953; Marx & Zak, 1965). These improvements allowed large-scale production of inoculum suitable for nursery application. Later research demonstrated that inoculation with selected ectomycorrhizal fungi, particularly

Pisolithus and *Laccaria species*, could significantly enhance seedling growth and survival under field conditions (Marx, 1991).

Nursery and Field Applications

The development of effective inoculation techniques has had major implications for forestry and ecosystem restoration. Mycorrhizal inoculation in nurseries has been widely adopted to improve the quality of planting stock used in afforestation and reforestation programs. Inoculated seedlings generally exhibit improved nutrient uptake, greater resistance to drought and pathogens, and higher survival rates after transplantation (Smith & Read, 2008). Field studies have demonstrated that ectomycorrhizal inoculation can significantly enhance plant establishment in degraded and disturbed environments, including mine spoils, eroded soils, and nutrient-deficient substrates. Improved performance of inoculated seedlings has been reported in a variety of restoration contexts, highlighting the practical importance of ectomycorrhizal biotechnology in sustainable land management (Brundrett et al., 1996; van der Heijden et al., 2015).

Limitations and Future Prospects

Despite considerable success in experimental and operational settings, several challenges remain in the large-scale application of ectomycorrhizal inoculum. These include variability in field performance, competition with native fungal communities, difficulty in maintaining inoculum viability, and limited understanding of long-term ecological impacts. Additionally, host specificity and environmental compatibility must be carefully considered when selecting fungal strains for inoculation programs. Recent advances in molecular biology, microbial ecology, and biotechnology have improved the selection and monitoring of effective ectomycorrhizal strains. High-throughput sequencing and genomic tools now allow more precise characterization of fungal communities and their functional traits, enabling better selection of inoculants suited to specific environmental conditions (Tedersoo et al., 2020). These developments are expected to enhance the efficiency and predictability of ectomycorrhizal inoculation in future forestry and ecological restoration programs.

Climatic and Environmental Factors Influencing Ectomycorrhizal Infection

Climatic Factors Affecting Ectomycorrhizal Development

The establishment and intensity of ectomycorrhizal (ECM) colonization are strongly influenced by environmental and climatic conditions, particularly light availability, temperature, soil moisture, and soil chemistry. Among these factors, light intensity plays an indirect but important role because it regulates photosynthetic activity and, consequently, the supply of carbohydrates to plant roots. Since ectomycorrhizal fungi are entirely dependent on host-derived carbon, reduced photosynthesis under low light conditions can limit carbohydrate allocation to roots and suppress fungal colonization. Conversely, adequate light enhances photosynthetic rates, increasing the availability of soluble sugars required for sustaining symbiotic fungal growth and activity (Smith & Read, 2008; van der Heijden et al., 2015).

In nursery systems, excessive shading may reduce seedling vigor and indirectly decrease ectomycorrhizal infection due to limited carbon availability. However, moderate shading is sometimes applied to protect young seedlings from excessive solar radiation and heat stress. Therefore, maintaining an optimal balance of light exposure is essential for promoting both seedling growth and successful mycorrhizal establishment.

Temperature and Moisture Requirements

Ectomycorrhizal fungi generally exhibit optimal growth within moderate temperature ranges, typically between 15°C and 25°C,

although this range may vary among species and ecological conditions. Temperatures outside this range can reduce fungal metabolic activity, hyphal growth, and colonization efficiency. Soil moisture is equally important, as both drought stress and waterlogging can negatively affect fungal development and symbiotic functioning. Adequate but well-drained soil moisture conditions are therefore essential for maintaining active ectomycorrhizal associations (Brundrett et al., 1996).

Soil Chemistry and Nutrient Availability

Soil pH is a key factor regulating ectomycorrhizal distribution and performance. Most ectomycorrhizal fungi thrive in slightly acidic soils, typically within the pH range of approximately 4.5 to 6.0. Acidic conditions favor nutrient solubility and support fungal enzyme activity, whereas alkaline conditions may restrict fungal growth and reduce symbiotic efficiency. Soil nutrient status also plays a major regulatory role in ECM development. High concentrations of readily available nitrogen or phosphorus often suppress mycorrhizal colonization because plants become less dependent on fungal nutrient acquisition under fertile conditions (Smith & Smith, 2011). Excessive application of chemical fertilizers in nursery soils can therefore have a negative impact on ectomycorrhizal development. High nutrient availability reduces plant carbon allocation to fungal partners, resulting in reduced colonization intensity. For optimal mycorrhizal formation, fertilizer inputs should be carefully managed and maintained at moderate levels to avoid disrupting symbiotic balance.

Root Exudates and Biochemical Interactions

Root exudates, including soluble carbohydrates, amino acids, organic acids, and signaling compounds, play a central role in stimulating ectomycorrhizal fungal growth and colonization. These compounds act as chemical signals that attract fungal hyphae and initiate symbiotic interactions. In return, fungi respond by producing signaling molecules that facilitate root penetration and the establishment of the Hartig net structure (Genre et al., 2020). Vitamins such as thiamine and other growth-promoting factors are also required by many ectomycorrhizal fungi for optimal development. The availability of these compounds in the rhizosphere is influenced by both plant physiology and soil microbial activity, highlighting the complex biochemical interactions that regulate symbiosis.

Soil Microbial Interactions and Competition

Ectomycorrhizal fungi exist within a highly competitive soil microbial environment. They interact with a wide range of bacteria, saprotrophic fungi, and other soil microorganisms. Competitive interactions can influence fungal establishment and colonization success. In some cases, saprotrophic fungi may inhibit ectomycorrhizal development through competition for nutrients and space, while beneficial bacterial communities may enhance symbiosis by improving nutrient availability and root health (Lindahl & Tunlid, 2015).

Nursery Management Practices and Mycorrhizal Development
Nursery management practices significantly influence ectomycorrhizal formation. Excessive irrigation, poor drainage, and over-fertilization can suppress fungal colonization, while well-balanced watering regimes and minimal chemical disturbance generally support healthy symbiotic development. Soil sterilization or the use of biocides may initially reduce microbial populations, including beneficial fungi; however, ectomycorrhizal colonization can be re-established following inoculation or natural recolonization under suitable environmental conditions (Brundrett et al., 1996). Modern nursery practices increasingly emphasize the integration of controlled mycorrhizal inoculation, reduced chemical inputs,

and optimized environmental conditions to enhance seedling quality and field performance. These approaches are particularly important in forestry and ecological restoration programs where ectomycorrhizal associations are essential for plant survival and ecosystem recovery. Climatic and environmental factors exert a strong influence on ectomycorrhizal infection and functioning. Light intensity, temperature, soil moisture, nutrient availability, and soil chemistry collectively regulate the establishment and efficiency of plant–fungus symbiosis. Understanding these factors is essential for optimizing nursery practices, improving forest regeneration, and promoting sustainable ecosystem management under changing environmental conditions.

Resistance to Plant Diseases in Ectomycorrhizal Associations Protective Role Against Root Pathogens

Ectomycorrhizal (ECM) symbioses contribute significantly to plant health by enhancing resistance against a wide range of soil-borne pathogens. Many important root-infecting fungi and oomycetes, including *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Fusarium* species, primarily attack young, actively growing cortical tissues of feeder roots, leading to tissue necrosis, reduced nutrient uptake, and impaired plant growth. However, ectomycorrhizal colonization often reduces the severity and incidence of such infections by forming a physical and biochemical barrier around root tissues (Smith & Read, 2008; van der Heijden et al., 2015). A well-developed ectomycorrhizal mantle acts as a protective sheath around the root surface, limiting direct pathogen access to cortical cells. In addition to this physical barrier, ectomycorrhizal fungi can alter root exudation patterns and modify the rhizosphere environment, thereby reducing pathogen establishment and colonization. Consequently, mycorrhizal roots are generally less susceptible to infection compared with non-mycorrhizal feeder roots growing under similar environmental conditions.

Antibiosis and Antifungal Compounds

Several ectomycorrhizal fungi produce secondary metabolites with antimicrobial properties that contribute to pathogen suppression. Species belonging to genera such as *Lactarius*, *Cortinarius*, and *Hygrophorus* have been reported to synthesize antibiotic compounds with inhibitory effects on soil-borne pathogens, including *Rhizoctonia solani*, *Pythium debaryanum*, and *Fusarium oxysporum*. In contrast, some ectomycorrhizal genera, such as *Russula*, exhibit limited or no detectable antibiotic production, indicating that disease suppression mechanisms vary among fungal taxa (Lindahl & Tunlid, 2015). Although antimicrobial activity has been well documented under laboratory conditions, the extent to which these compounds are produced and function effectively in natural soil environments remains an active area of research. In situ expression of antibiotic metabolites is influenced by host–fungus interactions, soil conditions, and microbial community dynamics.

Volatile Organic Compounds and Induced Resistance

Some ectomycorrhizal fungi are capable of producing volatile organic compounds (VOCs) with fungistatic properties. For example, certain species within the *Boletus* complex have been shown to produce compounds such as alcohols and organic acids with inhibitory effects on microbial growth in vitro. Additionally, colonization of host roots by ectomycorrhizal fungi can stimulate the accumulation of plant-derived defense-related metabolites, including terpenoids and sesquiterpenes (Bonfante & Genre, 2010). In mycorrhizal plants, elevated levels of defensive secondary metabolites have been associated with enhanced resistance to pathogen attack. These compounds may act directly against pathogens or function indirectly by strengthening plant defense responses. Studies have demonstrated that ectomycorrhizal colonization can significantly increase the concentration of

terpenoid compounds in roots, thereby contributing to improved disease resistance compared with non-mycorrhizal plants.

Induced Systemic Resistance and Plant Defense Activation

Beyond direct antifungal effects, ectomycorrhizal fungi can trigger induced systemic resistance (ISR) in host plants. This process involves the activation of plant defense signaling pathways that enhance the plant's ability to respond to subsequent pathogen attacks. ISR is typically associated with the modulation of jasmonic acid and ethylene signaling pathways, leading to a heightened state of defense readiness throughout the plant system (Pieterse et al., 2014). Through ISR, ectomycorrhizal fungi indirectly reduce disease severity by priming host plants for faster and stronger defensive responses. This mechanism is increasingly recognized as a key component of biological disease control in forest and agricultural ecosystems.

Ecological and Practical Significance

The disease-suppressive effects of ectomycorrhizal associations have important implications for forestry, nursery management, and ecosystem restoration. Inoculation of seedlings with effective ectomycorrhizal fungi can reduce mortality caused by soil-borne pathogens and improve plant establishment in both natural and managed ecosystems. These benefits are particularly important in reforestation programs and degraded land restoration, where pathogen pressure is often high and environmental conditions are unfavourable for seedling survival. Modern research highlights that disease resistance in ectomycorrhizal systems is not attributed to a single mechanism but rather results from a combination of physical barriers, biochemical antagonism, rhizosphere modification, and host immune activation. Understanding these integrated mechanisms is essential for improving the application of ectomycorrhizal fungi in sustainable plant protection strategies.

Ectomycorrhizae: Growth and Development of Forest Seedlings

Importance of Ectomycorrhizae in Forest Establishment

The requirement of many forest tree species for ectomycorrhizal (ECM) symbiosis became evident during early afforestation trials, when plantations of exotic conifers repeatedly failed due to the absence of compatible mycorrhizal fungi in the soil. Successful establishment of species such as pine and oak was often achieved only after the introduction of appropriate ectomycorrhizal fungi, highlighting their essential role in seedling survival and growth (Smith & Read, 2008; Brundrett et al., 1996). These observations led to the recognition that ectomycorrhizae are not merely beneficial but often critical for the establishment of many forest tree species, particularly in nutrient-poor or previously non-forested soils. Early field studies demonstrated that afforestation efforts in grasslands and degraded ecosystems were strongly dependent on the presence of compatible mycorrhizal fungi. Without these symbionts, seedlings frequently exhibited poor growth, nutrient deficiency symptoms, and high mortality rates. Conversely, when appropriate ectomycorrhizal associations were established, significant improvements in plant survival and biomass accumulation were observed across a range of forest species (Marx, 1991).

Development of Mycorrhizal Inoculation Techniques

The concept of enhancing forest seedling performance through controlled ectomycorrhizal inoculation was first systematically developed by Ernst Moser, who emphasized the importance of introducing ecologically adapted fungal species into nursery systems (Moser, 1958). His pioneering work demonstrated that seedlings inoculated with selected ectomycorrhizal fungi exhibited improved survival and growth after transplantation compared with non-inoculated controls. Subsequent research in different

regions of the world validated and expanded these findings. Studies conducted in Australia, Argentina, and the United States showed that nursery inoculation with compatible ectomycorrhizal fungi significantly enhanced field performance of conifer seedlings (Marx & Cordell, 1989; Molina, 1982; Theodorou & Bowen, 1970). These developments established the scientific foundation for modern mycorrhizal forestry practices.

Nursery Inoculation and Containerized Seedling Production

Advances in nursery technology have enabled the large-scale production of ectomycorrhizal seedlings using both bare-root and containerized systems. Controlled inoculation with pure cultures of ectomycorrhizal fungi allows for precise selection of fungal strains with desirable ecological and physiological traits, such as drought tolerance, nutrient efficiency, and pathogen resistance (Brundrett et al., 1996; Smith & Read, 2008). Containerized seedling production has become particularly important in modern forestry due to its ability to produce uniform, high-quality planting stock. Inoculation of container-grown seedlings with fungi such as *Laccaria laccata* and *Pisolithus* species has been widely reported to improve root development, nutrient uptake, and post-planting survival (Marx, 1991; Smith & Read, 2008). For example, experimental inoculation of Douglas fir seedlings with multiple isolates of ectomycorrhizal fungi has demonstrated significant improvements in growth performance and stress tolerance under field conditions. Similarly, studies on Sitka spruce and other commercially important conifers have shown that pre-inoculation with ectomycorrhizal fungi enhances establishment success and early growth following transplantation (van der Heijden et al., 2015). These findings underscore the practical importance of ectomycorrhizal biotechnology in commercial forestry and ecological restoration.

Field Performance and Ecological Applications

Field trials across diverse ecological regions have consistently shown that ectomycorrhizal inoculation improves seedling performance in degraded soils, mine spoils, and reforestation sites. Enhanced nutrient acquisition, improved water relations, and increased resistance to environmental stress are among the key factors contributing to improved survival rates of inoculated seedlings (Marx, 1991). In addition to growth promotion, ectomycorrhizal fungi contribute to ecosystem restoration by facilitating nutrient cycling and improving soil structure. Their ability to establish symbiotic networks in challenging environments makes them valuable tools in afforestation programs aimed at rehabilitating degraded landscapes and restoring forest ecosystems.

Ectomycorrhizae in Nursery Systems

Introduction and Role of "Mother Seedlings"

Ectomycorrhizal (ECM) fungi can be introduced into nursery systems through several inoculation strategies, one of which involves the use of pre-inoculated "mother seedlings." In this approach, seedlings that already possess well-developed ectomycorrhizal associations are planted within or adjacent to transplant or nursery beds. The fungal symbionts then spread naturally through hyphal networks to nearby non-mycorrhizal seedlings, facilitating the establishment of ectomycorrhizal infection in the surrounding plants (Brundrett et al., 1996; Smith & Read, 2008). This method is particularly useful in situations where soil inoculum transport is impractical due to long distances, high cost, or the risk of reduced fungal viability during handling. Compared with bulk soil transfer, the use of mother seedlings provides a more biologically active and ecologically stable source of inoculum, while minimizing contamination with unwanted soil organisms.

Advantages Over Soil-Based Inoculation

The mother-seedling technique offers several advantages over traditional soil inoculation methods. Soil inoculum, although widely used, may lose effectiveness during transport and often introduces a complex and uncontrolled microbial community into nursery systems. In contrast, mother seedlings provide a continuous and self-propagating source of ectomycorrhizal fungi, allowing gradual and sustained colonization of surrounding seedlings.

This approach is especially relevant for large-scale nursery operations and reforestation programs, where maintaining inoculum viability and ensuring consistent colonization are critical challenges. The method also reduces dependence on external inoculum sources and supports the establishment of locally adapted fungal communities, which are often better suited to regional environmental conditions (Brundrett et al., 1996; van der Heijden et al., 2015).

Application in Pine Species and Tropical Forestry

The use of ectomycorrhizal mother seedlings has been explored in several pine species, particularly in tropical and subtropical forestry contexts. Species such as *Pinus merkusii* have shown strong potential for growth in high-rainfall, low-elevation tropical environments when associated with appropriate ectomycorrhizal fungi. In forestry development programs, such species are considered promising due to their favorable growth characteristics, including high mean annual increment, desirable wood properties, and adaptability to plantation systems. In regions such as South and Southeast Asia, the introduction of suitable pine species for plantation forestry often requires careful consideration of compatible ectomycorrhizal fungi. Because ectomycorrhizal specificity can vary among host and fungal species, successful establishment depends on either the presence of native compatible fungi or deliberate inoculation using locally adapted inoculum sources (Smith & Read, 2008).

Use of Local Inoculum and Field Trials

Where introduction of pre-inoculated planting stock is not feasible, local ectomycorrhizal inoculum sources can be utilized to initiate symbiosis. These may include forest soil, native ectomycorrhizal fruiting bodies, or nursery-based inoculum derived from indigenous fungal communities. Once ectomycorrhizal formation is confirmed, field trials and experimental plantations can be established to evaluate growth performance, survival rates, and host–fungus compatibility under local environmental conditions. Such trials are essential for determining the ecological suitability and economic viability of introducing new tree species into non-native or marginal environments. They also contribute to identifying host-specific or broadly compatible ectomycorrhizal fungi that can be used in operational forestry programs (Marx, 1991; Tedersoo et al., 2020).

Ecological and Silvicultural Significance

The use of ectomycorrhizal inoculation techniques in nurseries represents an important component of modern silviculture. By improving seedling quality, enhancing nutrient uptake, and increasing resistance to environmental stress, ectomycorrhizal fungi contribute directly to successful forest establishment and productivity. The mother-seedling approach, in particular, offers a biologically efficient and cost-effective strategy for introducing beneficial fungi into nursery systems, especially in large-scale afforestation projects. As forestry systems increasingly focus on sustainability and ecosystem-based management, the integration

of ectomycorrhizal biotechnology into nursery practices is expected to play a growing role in improving plantation success and supporting forest restoration initiatives.

Current Advances and Future Prospects of Ectomycorrhizal Research

Integrating Modern Molecular and Ecological Approaches

Recent advances in ectomycorrhizal (ECM) research have significantly expanded understanding of plant–fungus interactions through the integration of molecular biology, genomics, and microbial ecology. High-throughput DNA sequencing technologies, including metabarcoding and metagenomics, have enabled detailed characterization of ectomycorrhizal fungal communities in natural and managed ecosystems. These approaches have revealed a much higher diversity of ECM fungi than previously recognized and have improved the ability to link specific fungal taxa with ecological functions such as nutrient acquisition, stress tolerance, and host specificity (Tedersoo et al., 2020; Nguyen et al., 2016). Meta-transcriptomic and genome-resolved studies have further clarified the molecular mechanisms underlying symbiosis formation. These investigations show that ectomycorrhizal fungi possess specialized gene sets involved in nutrient transport, carbon metabolism, and host signaling. Comparative genomics has also demonstrated that many ECM fungi have evolved reduced plant cell wall–degrading enzyme repertoires, reflecting their transition from saprotrophic to symbiotic lifestyles (Martin et al., 2016). Such findings have deepened understanding of the evolutionary adaptations that support mutualistic interactions between plants and fungi.

Advances in Inoculum Development and Application Technologies

The development of standardized and commercially viable ectomycorrhizal inoculum formulations has significantly improved the practical application of mycorrhizal biotechnology in forestry. Modern inoculum systems include liquid cultures, alginate beads, encapsulated formulations, and carrier-based products designed to enhance shelf life, viability, and ease of application in nursery and field conditions (Brundrett et al., 1996; Smith & Read, 2008). Improved delivery systems, including seed coating, root dip treatments, and substrate incorporation methods, have increased inoculation efficiency and uniformity in large-scale nursery production. These technologies have made it possible to integrate ectomycorrhizal inoculation into operational forestry practices, particularly in containerized seedling production systems used for afforestation and reforestation programs.

Field Performance and Ecological Considerations

Despite significant progress in inoculum technology, the long-term field performance of introduced ectomycorrhizal fungi remains variable. One of the key challenges is maintaining compatibility between introduced fungal strains and native soil microbial communities. In some cases, introduced fungi may fail to persist due to competition with indigenous ectomycorrhizal species or inability to adapt to local environmental conditions (van der Heijden et al., 2015). Environmental factors such as soil pH, temperature fluctuations, moisture availability, and nutrient status also strongly influence the persistence and effectiveness of inoculated fungi. Additionally, changing climatic conditions are expected to alter fungal distribution patterns and host–fungus interactions, potentially affecting the success of ectomycorrhizal-based forestry practices in the future. Recent ecological studies emphasize the importance of using locally adapted or regionally compatible fungal strains to improve inoculation success. This “ecological matching” approach aims to align fungal traits with specific environmental conditions and host species requirements, thereby enhancing long-term stability and ecosystem integration (Bahram et al., 2018).

Future Directions in Ectomycorrhizal Research

Future research in ectomycorrhizal biology is expected to focus on functional trait-based selection of fungal inoculants, allowing researchers to identify strains with specific adaptive advantages such as drought tolerance, nutrient efficiency, and pathogen resistance. Advances in functional genomics and systems biology will further enable prediction of fungal performance under diverse environmental conditions. Another promising direction is the use of synthetic microbial communities, where multiple beneficial fungi and bacteria are combined to enhance plant growth and resilience. This approach recognizes that plant health in natural ecosystems is often influenced by complex microbial interactions rather than single symbiotic partners. In addition, climate change research is increasingly focusing on how ectomycorrhizal networks respond to elevated temperatures, altered precipitation regimes, and increased atmospheric nitrogen deposition. Understanding these responses is critical for predicting forest ecosystem resilience and carbon cycling dynamics under future climate scenarios (Terrer et al., 2019). Ectomycorrhizal research has entered a highly advanced phase characterized by molecular precision, ecological integration, and technological innovation. The combination of genomic tools, improved inoculum technologies, and ecological insights has greatly enhanced the potential for applying ectomycorrhizal fungi in sustainable forestry and ecosystem restoration. However, successful long-term application depends on addressing challenges related to environmental variability, fungal persistence, and climate change adaptation. Continued interdisciplinary research will be essential for fully realizing the ecological and economic benefits of ectomycorrhizal symbiosis. Ectomycorrhizal (ECM) symbioses play a central role in improving plant nutritional status, stress tolerance, and disease resistance in forest ecosystems. By enhancing the uptake of essential nutrients—particularly nitrogen and phosphorus—ectomycorrhizal fungi contribute to improved plant vigor and overall physiological performance. In many forest tree species, including *Pinus* spp., ECM associations have been shown to significantly increase survival and growth compared with non-mycorrhizal seedlings, especially under nutrient-poor or environmentally stressful conditions (Smith & Read, 2008; van der Heijden et al., 2015).

Disease Resistance and Rhizosphere Protection

One of the most important functional benefits of ectomycorrhizal symbiosis is increased resistance to soil-borne pathogens. For example, in pine species, mycorrhizal roots often show reduced susceptibility to *Phytophthora cinnamomi*, whereas non-mycorrhizal roots are highly vulnerable to infection and tissue necrosis. This enhanced resistance is attributed to multiple interacting mechanisms, including the physical barrier provided by the fungal mantle, changes in root exudation patterns, and the establishment of a distinct microbial community in the rhizosphere of mycorrhizal roots. The microbial assemblage associated with ectomycorrhizal roots differs significantly from that of non-mycorrhizal roots, suggesting that disease suppression is partly mediated through shifts in rhizosphere microbiome structure. These microbial interactions remain an important and active area of research, particularly in understanding how ECM fungi influence pathogen suppression and soil health at the ecosystem level (Lindahl & Tunlid, 2015).

Stress Tolerance During Nursery Handling and Field Establishment
Ectomycorrhizal colonization also improves seedling tolerance to environmental stress during critical stages such as nursery handling, transportation, and field transplantation. Non-mycorrhizal seedlings are generally more susceptible to desiccation during lifting, storage, and transport due to reduced water uptake efficiency. In contrast, mycorrhizal seedlings maintain improved hydraulic conductivity and root-soil contact, resulting in better survival during transplant

shock. In field conditions, non-mycorrhizal feeder roots located near the soil surface are more vulnerable to drought stress and frost damage. ECM fungi enhance the functional rooting depth and improve access to soil moisture, thereby increasing resilience to both drought and temperature extremes. These physiological advantages are particularly important in reforestation programs and degraded land restoration, where environmental conditions are often harsh and unpredictable.

Research Gaps and Future Directions

Despite substantial progress in understanding ectomycorrhizal functioning, several research gaps remain. One key area requiring further investigation is the role of the rhizosphere microbiome associated with ectomycorrhizal roots. Although it is known that ECM fungi influence microbial community composition, the specific functional interactions between fungi, bacteria, and other soil microorganisms in disease suppression and nutrient cycling are not yet fully understood. Another important research need is the long-term field performance of inoculated ectomycorrhizal fungi under varying environmental and climatic conditions. Many inoculation studies demonstrate early growth benefits, but the persistence and ecological stability of introduced fungi over time remain uncertain. Understanding how native and introduced ECM fungi interact over multiple growing seasons is essential for improving inoculation strategies in forestry practice. Climate change also introduces new uncertainties, including altered precipitation patterns, increased temperature stress, and shifting host–fungus compatibility. Future research must address how ectomycorrhizal networks respond to these changes and how they influence forest resilience and carbon cycling at larger spatial scales. Advances in molecular ecology, functional genomics, and soil microbiome analysis are expected to play a major role in addressing these questions. Integrating these approaches will help identify functionally important fungal strains and optimize their application in sustainable forestry and ecosystem restoration. There is a growing recognition among botanists, agronomists, horticulturists, and foresters of the critical importance of ectomycorrhizal symbiosis in plant productivity and ecosystem functioning. The integration of ectomycorrhizal biotechnology into nursery management and forest restoration practices represents a significant advancement toward sustainable land-use systems. Continued interdisciplinary research will further enhance the application of these symbioses in improving plant health, ecosystem resilience, and environmental sustainability.

Current Advances and Future Prospects in Ectomycorrhizal Research

Recent progress in ectomycorrhizal (ECM) research has been driven by advances in molecular biology, high-throughput sequencing technologies, and microbial ecology, enabling a more refined understanding of fungal diversity, community assembly, and functional roles in forest ecosystems. Metagenomic and amplicon-based sequencing approaches have significantly improved the resolution of ECM community profiling, allowing researchers to identify both culturable and previously cryptic fungal taxa and to link specific fungal lineages with host performance and environmental conditions (Tedersoo et al., 2020). These developments have strengthened the selection process for functionally efficient fungal strains with potential application in forest restoration and commercial forestry. In parallel, controlled inoculation experiments and field trials have demonstrated that ECM fungi can enhance seedling establishment by improving nutrient acquisition, particularly nitrogen and phosphorus uptake, as well as increasing tolerance to abiotic stresses such as drought and soil acidity. However, inoculation success remains highly context-dependent, influenced by soil properties, host specificity, and competition with native fungal communities.

Foundational synthesis work on mycorrhizal functioning has emphasized that symbiotic outcomes are strongly regulated by environmental gradients and plant–fungal compatibility, which must be considered when designing inoculation strategies (Smith & Read, 2008; van der Heijden et al., 2015).

Recent developments in inoculum formulation, including carrier-based products, liquid inoculants, and controlled-release systems, have improved the scalability of ECM application in forestry practices. Despite these advances, challenges persist in ensuring long-term persistence of introduced fungi, maintaining functional stability under field conditions, and predicting performance under climate change scenarios. Emerging research suggests that integrating multi-omics approaches (metagenomics, transcriptomics, and metabolomics) with long-term ecological monitoring will be essential for identifying robust fungal–host combinations and improving predictive models for inoculation success. Overall, ectomycorrhizal inoculation represents a scientifically supported and increasingly practical tool for enhancing forest productivity and resilience. Future research is expected to focus on climate-adaptive inoculant development, synthetic community design, and ecosystem-scale functional assessments to support sustainable forest management under changing environmental conditions.

Conclusion

Ectomycorrhizal (ECM) symbiosis plays a crucial role in enhancing plant nutritional status, particularly through improved uptake of phosphorus, nitrogen, and micronutrients, which in turn contributes to increased plant vigor and resistance to a range of biotic and abiotic stresses. One of the most significant functional benefits of ECM associations is their capacity to reduce the severity of root infections caused by soilborne pathogens. For instance, in conifer systems such as *Pinus* spp., ECM-colonized roots have been shown to exhibit markedly lower infection rates by pathogens such as *Phytophthora cinnamomi* compared with non-mycorrhizal roots, suggesting a strong protective effect mediated by the fungal symbiosis. This enhanced disease resistance is widely attributed to multiple interacting mechanisms, including physical barriers created by the fungal mantle, competitive exclusion of pathogens in the rhizosphere, and shifts in root-associated microbial communities. Emerging evidence supports the hypothesis that ECM roots harbor a distinct microbial assemblage in both the rhizosphere and fungal sheath compared with non-mycorrhizal roots, which may contribute to pathogen suppression through microbial antagonism and induced systemic resistance. However, the precise composition, functional roles, and stability of these microbial communities remain insufficiently understood and represent an important area for future investigation.

ECM symbiosis also improves plant resilience during nursery handling and field transplantation. Mycorrhizal seedlings generally exhibit greater tolerance to desiccation stress during lifting, storage, transportation, and planting operations due to improved water uptake efficiency and enhanced root system stability. In contrast, non-mycorrhizal seedlings are more vulnerable to moisture loss and physiological stress during these stages, often resulting in reduced survival rates after out planting. Furthermore, ECM-colonized plants show greater resistance to drought and frost damage in field conditions, particularly in upper soil layers where uncolonized fine roots are more exposed to environmental fluctuations and physical injury. Despite these well-documented benefits, several critical research gaps remain. Further studies are needed to better understand the long-term stability of introduced ECM fungi under field conditions and their interaction with native fungal communities. In addition, there is a need to investigate

host specificity and compatibility across different tree species and genotypes, particularly under variable soil and climatic conditions. Advanced multi-omics approaches, combined with long-term ecological monitoring, are required to elucidate the functional mechanisms underlying pathogen suppression and stress tolerance in ECM-associated plants. Moreover, climate change impacts on ECM community structure and function remain poorly understood and require urgent attention to support adaptive forest management strategies. Overall, there is growing recognition among botanists, foresters, horticulturists, and agricultural scientists of the ecological and practical importance of ectomycorrhizal associations. Their application in forestry and restoration ecology offers a promising strategy for improving seedling survival, enhancing ecosystem resilience, and supporting sustainable land management practices under changing environmental conditions.

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Conflict of Interest

The authors declares that there are no conflicts of interest related to this publication.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability

The data and information supporting the findings of this study are available from the authors upon reasonable request.

References

1. Agerer, R. (2006). Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress*, 5(2), 67–107.
2. Agerer, R. (Ed.). (1987–2008). *Colour atlas of ectomycorrhizae* (1–13 series). Einhorn-Verlag.
3. Averill, Christopher, C., Turner, B. L., & Finzi, A. C. (2019). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), 543–545.
4. Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Polme, S., Sunagawa, S., Ryberg, M., & Bork, P. (2018). Structure and function of the global topsoil microbiome. *Nature*, 560(7717), 233–237.
5. Bakshi, B. K. (1957). Mycorrhiza in Indian forest trees. *Indian Forester*, 83, 36–45.
6. Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Frontiers in Plant Science*, 10, 1068.
7. Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Frontiers in Microbiology*, 6, 1559.
8. Bonfante, P., & Genre, A. (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications*, 1, 48.
9. Brundrett, M. C., & Leho Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New*

- Phytologist, 220(4), 1108–1115.
10. Brundrett, M. C., & Leho Tedersoo. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, 220(4), 1108–1115.
 11. Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. ACIAR.
 12. Chaudhuri, H. (1945). Studies on mycorrhizal association in Himalayan conifers. *Indian Forest Records, New Series, Botany*, 1, 1–18.
 13. Christopher Averill, C., Turner, B. L., & Finzi, A. C. (2019). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), 543–545.
 14. Fortin, J. A., Piché, Y., & Lalonde, M. (1980). Technique for the observation of early stages of ectomycorrhiza formation. *Canadian Journal of Botany*, 58(3), 361–365.
 15. Frey, S. D. (2019). Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics*, 50, 237–259.
 16. Genre, A., Lanfranco, L., Perotto, S., & Bonfante, P. (2020). Unique and common traits in mycorrhizal symbioses. *Nature Reviews Microbiology*, 18(11), 649–660.
 17. Hacskeylo, E. (1953). Pure culture synthesis of ectotrophic mycorrhizae. *Botanical Gazette*, 115(2), 143–151.
 18. Harey, J. L., & Smith, S. E. (1983). *Mycorrhizal symbiosis*. Academic Press.
 19. Hatch, A. B. (1937). The physiological relations of mycorrhizal fungi and forest trees. *Black Rock Forest Bulletin*, 6, 1–69.
 20. Koljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, 22(21), 5271–5277.
 21. Lanfranco, L., Fiorilli, V., & Gutjahr, C. (2018). Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. *New Phytologist*, 220(4), 1031–1046.
 22. Largent, D. L. (1986). How to identify mushrooms to genus I: Macroscopic features (2nd ed.). Mad River Press.
 23. Leho Tedersoo, L., & Bahram, M. (2019). Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biological Reviews*, 94(5), 1857–1880.
 24. Leho Tedersoo, L., Bahram, M., Zobel, M., & Wilson, S. D. (2020). How mycorrhizal associations drive plant population and community biology. *Science*, 367(6480), eaba1223.
 25. Leho Tedersoo, L., Bahram, M., Zobel, M., & Wilson, S. D. (2020). How mycorrhizal associations drive plant population and community biology. *Science*, 367(6480), eaba1223.
 26. Lilleskov, E. A., Kuyper, T. W., Bidartondo, M. I., & Hobbie, E. A. (2019). Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities. *Fungal Ecology*, 39, 66–76.
 27. Lindahl, B. D., & Tunlid, A. (2015). Ectomycorrhizal fungi as decomposers in forest soils. *New Phytologist*, 205(4), 1443–1447.
 28. Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, 14(12), 760–773.
 29. Marx, D. H. (1969). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, 59, 153–163.
 30. Marx, D. H. (1975). Development and structure of ectomycorrhizae in forest trees. *Forest Science*, 21(2), 95–102.
 31. Marx, D. H. (1991). The practical significance of ectomycorrhizae in forest establishment. In D. L. Keister & P. B. Cregan (Eds.), *The rhizosphere and plant growth* (pp. 54–90). Springer.
 32. Marx, D. H., & Cordell, C. E. (1989). The use of specific ectomycorrhizal fungi to improve reforestation practices. *Forest Ecology and Management*, 28, 101–117.
 33. Marx, D. H., & Zak, B. (1965). Effect of pH and substrate on ectomycorrhizal formation. *Forest Science*, 11(1), 66–75.
 34. Melin, E. (1921). Untersuchungen über die Larix-Mykorrhiza. *Svensk Botanisk Tidskrift*, 15, 192–203.
 35. Melin, E. (1927). Studies on the mycorrhizal relationships of forest trees. *Svensk Botanisk Tidskrift*, 21, 177–313.
 36. Mikola, P. (1970). Mycorrhizal inoculation in forest nurseries. *Communicationes Instituti Forestalis Fenniae*, 69, 1–20.
 37. Molina, R. (1982). Ectomycorrhizal inoculation of containerized seedlings. *Canadian Journal of Forest Research*, 12(3), 456–462.
 38. Molina, R., & Trappe, J. M. (1982). Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. *Forest Science*, 28(3), 423–458.
 39. Nguyen, N. H., Bruns, T. D., & Peay, K. G. (2016). The importance of fungi in forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 47, 1–21.
 40. Nylund, J. E., & Unestam, T. (1982). Structure and physiology of ectomycorrhizae formed by *Piloderma croceum* and Norway spruce. *New Phytologist*, 91(1), 63–79.
 41. Opik, M., Davison, J., Moora, M., & Zobel, M. (2022). DNA-based detection and identification of arbuscular mycorrhizal fungi: Current status and future directions. *New Phytologist*, 235(4), 1350–1364.
 42. Peay, K. G., Kennedy, P. G., & Talbot, J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology*, 14(7), 434–447.
 43. Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, 52, 347–375.
 44. Prasad, K. & Rajasekaran, S. (2024). Mycorrhizae Fungi: A Dynamic Microsymbiont for Sustainable Agriculture for Dramatically Increasing Population. In: 9Th Asian PGPR India Chapter Beneficial Microbes for Sustainable Agriculture, Ponmurugan P, Anita A, Reedy MS (eds.), Excel India Publisher, New Delhi pp.431-446.
 45. Prasad, K. (2010). Ectomycorrhizal Symbiosis: Possibilities and Prospects. In: Progress in Mycology, M. K. Rao and G. Kovices (eds.), Springer Science, The Netherlands and Scientific Publisher, Jodhpur, India pp. 290-308.
 46. Prasad, K. (2021a). Glycoprotein Producing AM Fungi lifecycle and Potential Role in Agricultural Plant Lifespan and Global Environmental Changes for Sustainable Green Technology. *Journal of Ecology and Natural Resources*. 5 (2): 1-18.
 47. Prasad, K. (2021b). Arbuscular mycorrhizal fungi and plant collaborations influence ecology and environmental changes for global sustainable development. *Journal of Ecology and Natural Resources* 5(1): 1-16.
 48. Prasad, K. & Rajak, R. C. (2001). Microbes and Wasteland Management: Challenge ahead. In: A.K. Roy and S.K. Verma (ed.) *Management of Wasteland to protect environment*. Scientific Publishers (India) Jodhpur, India. 27-39.
 50. Prasad, K. & Rajak, R. C. (2002). Management of Plant pests through biological entities : Possibilities and Prospects. In: P. C. Trivedi (ed.) *Plant Pest Management*, Aavishkar Publishers, Distributors, Jaipur, India. 75-99.
 51. Prasad, K. & Rajak, R. C. (1999). Recent advances in Mycorrhizal Taxonomy Morphological and Molecular criteria. In: R. C. Rajak (eds.). *Microbial Biotechnology for sustainable Development and Productivity*. Vol. I Scientific Publication (India), Jodhpur, 62-72.
 52. Prasad, K., Pandey, A. K. & Rajak, R.C. 2005. Ectomycorrhizal diversity and its role in the production of forest plants. In: *Frontiers in Plant Sciences*, K. G. Mukherjee, K. V. B. R. Tilak, S. M. Reddy, L

- .V. Gangawane, P. Prakash and I. K. Kunwar (eds.), I. K. International Pvt. Ltd. New Delhi, India, pp.617-642.
53. Prasad, R., Bhola, D., Tuteja, N., & Varma, A. (2017). Arbuscular mycorrhizal fungi and sustainable agriculture. In A. Varma, R. Prasad, & N. Tuteja (Eds.), *Mycorrhiza—Function, Diversity, State of the Art* (pp. 523–548). Springer.
 54. Prasad, R., Bhola, D., Tuteja, N., & Varma, A. (2017). Ectomycorrhizal fungi and their role in forest ecosystems. In A. Varma, R. Prasad, & N. Tuteja (Eds.), *Mycorrhiza—Function, Diversity, State of the Art* (pp. 239–266). Springer.
 55. Rambelli, A. (1966). Studies on ectomycorrhizae of *Pinus radiata* seedlings. *Allionia*, 12, 45–58.
 56. Rich, M. K., Nouri, E., Courty, P. E., & Reinhardt, D. (2021). Diet of arbuscular mycorrhizal fungi: Bread and butter? *Trends in Plant Science*, 26(7), 652–660.
 57. Sharma, J. R., & Singh, S. (1990). Ectomycorrhizal fungi associated with *Pinus roxburghii* in the northwestern Himalayas. *Indian Forester*, 116(8), 621–628.
 58. Smith, F. A., & Smith, S. E. (2011). Roles of arbuscular and ectomycorrhizas in plant nutrition. *Annual Review of Plant Biology*, 62, 227–250.
 59. Smith, F. A., & Smith, S. E. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology*, 62, 227–250.
 60. Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed.). Academic Press.
 61. Tedersoo, L., Bahram, M., & Zobel, M. (2020). How mycorrhizal associations drive plant population and community biology. *Nature Communications*, 11, 1–10.
 62. Tedersoo, L., Bahram, M., Zobel, M., & Wilson, S. D. (2020). Mycorrhizal fungi and forest ecosystem functioning. *Science*, 367(6480), eaba1223.
 63. Terrer, C., Phillips, R. P., Hungate, B. A., Rosende, J., Reich, P. B., & others. (2019). A trade-off between plant and ecosystem carbon storage under elevated CO₂. *Nature*, 580, 1–6.
 64. Theodorou, C., & Bowen, G. D. (1970). Mycorrhizal inoculation of pine seedlings in Australia. *Australian Forest Research*, 4, 27–38.
 65. van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, 205(4), 1406–1423.