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Endophytic fungi in agriculture: Diversity, functions, and prospects for bioprotection

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Abstract Endophytic fungi, residing within plant tissues without causing harm, play a pivotal role in agricultural ecosystems by enhancing plant health and resilience. This review explores the diverse taxa of endophytic fungi and their multifaceted functions, including promotion of plant growth, enhancement of nutrient uptake, and facilitation of stress tolerance against biotic and abiotic challenges. Through symbiotic interactions, these fungi can induce systemic resistance in plants, offering a natural alternative to chemical pesticides for the management of plant pathogens and pests. Recent advances in molecular techniques have unveiled the complex diversity of endophytic communities, revealing their ecological significance and potential for biotechnological applications. The integration of endophytic fungi into sustainable agricultural practices holds promise for bioprotection, contributing to food security while minimizing environmental impacts. This review discusses various strategies for harnessing the beneficial properties of endophytic fungi, including inoculation protocols and the selection of compatible plant-fungal combinations. Furthermore, it addresses the challenges and prospects for future research, emphasizing the need for a greater understanding of the interactions between endophytes, host plants, and environmental factors. By leveraging the potential of endophytic fungi, agriculture can transition towards more resilient, sustainable, and environmentally friendly practices, paving the way for innovative solutions in crop management and protection.

Keywords: Endophytic fungi, Bioprotection, Plant growth promotion, Sustainable agriculture, Stress tolerance

Introduction to endophytic fungi

Endophytic fungi inhabit plant tissues without effecting apparent disease symptoms, maintaining a mutualistic symbiosis throughout the host's life cycle. The association positively influences host physiology by producing growth hormones, synthesizing essential nutrients, secreting antifungal and antibacterial metabolites, mitigating abiotic stress effects, and augmenting tolerance to drought, heat, and heavy metals. Consequently, endophytic fungi constitute a critical foundation of sustainable agricultural systems and an

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alternative to agrochemicals (Bhardwaj *et al.,* 2023). These organisms demonstrate extensive diversity; a single host may house hundreds of endophytic species spanning nearly every known fungal lineage. A profound understanding of their interactions with host plants and agroecosystems remains underdeveloped, partly owing to limited culture-based isolation and incomplete characterization. Molecular identification of internal transcribed spacers (ITS) rDNA sequences facilitates inventorying and selection of potential biocontrol candidates from endophytic fungal collections (Wen *et al.,* 2022). Soil hosts a massive and diverse cultivable microbial population; it serves as a substantial microbial reservoir from which plant-growth-promoting microorganisms originate. *In vitro* studies can assess inhibition of mycelial growth among phytopathogens, suggesting that endophytic fungi may function as efficient biocontrol agents. Inoculation of soil or seed with fungal endophytes enhances host nutrient uptake and promotes growth through symbiosis.

Diversity of endophytic fungi

Endophytic fungi occur within healthy plant tissues across diverse terrestrial biomes, including various families spread among eight functional ecological groups. They maintain symbiotic relationships with root, shoot, and leaf tissues (Bhardwaj *et al.,* 2023). The taxonomic composition of endophytes varies among host species, locations, seasons, plant ages, and tissues, with their abundance, richness, and diversity linked to host specificity. Endophytic fungi are widely distributed among host species and geographical regions and have been recorded in virtually all major plant groups, from liverworts and mosses to angiosperms. Cluster analysis supports the host generalism of endophytes, although some low-host-range taxa are present, particularly among Xylariaceae, which show marked specificity for woody hosts among four different plant species in the Mediterranean basin. These associations imply implications regarding the evolutionary pathways of endophytism and the origin of fungal lifestyles as well as a role for locality and transmission patterns in shaping evolution and the distribution of fungal endophytes.

Taxonomy and classification

The term "endophyte" refers to organisms, often fungi or bacteria, present inside plant tissues without causing apparent harm (Bhardwaj *et al.,* 2023). Endophytic fungi inhabit a broad range of host plants and are widely dispersed across geographical locations (Grabka *et al.,* 2022). Taxonomically, they are ubiquitous across fungal phyla but predominantly belong to Ascomycota and

Basidiomycota, which encompass the majority of identified species. Following spore germination, these fungi can colonize plant roots and other organs, remaining symptomless throughout their residence. Typologically, they serve as latent pathogens, saprophytes, woody endophytes, or biotrophs on living or dead tissues. This diversity across taxonomy, geography, and host selection underscores the ecological complexity of endophytic fungi, motivating further study of their functional roles.

Geographical distribution

Endophytic fungi colonize symbiotically inside plant tissues without causing any apparent symptom of infection (Bhardwaj *et al.,* 2023) and likely exist in combination with their host plants (Grabka *et al.,* 2022). On the basis of different functions and roles, endophytic fungi are categorized into many types, such as Class 1, which include clavicipitaceous endophytic fungi; Class 2, which include nonclavicipitaceous endophytic fungi and possess less prominent widths of plant association; Class 3, which include a wider range of colonization strategy but harbour restricted colonization in different plants; and Class 4, which include dark septate endophytes. Endophytic fungi can be found in all of the plant species in every one of the vital biomes on Earth; therefore, these endophytic fungi are almost exclusively associated with plant species. Endophytic fungi are found in roots and leaves of several species of trees viz. *Fagus sylvatica, Populus tremuloides, Arabidopsis thaliana, Plantago lanceolata, Pinus monticola, Pinus contorta* and *Cistus creticus* in diverse environments. Several endophytic fungi species have a cosmopolitan distribution; others usually are widespread within a tropical or temperate kind of climate. For example, cottonwoods on the western coast of America have 13 endophytes species of which only 1 species was shared with the eastern population which was about 3000 km distant.

Host plant associations

Endophytic fungi are an integral component of the plant microecosystem, inhabiting the internal plant tissues of both wild and cultivated species without causing apparent harm (Grabka *et al.,* 2022). Taxonomically, fungal endophytes span 12 phyla and 294 families. Their geographic and host range is extensive, with the richest species diversity reported in tropical rainforests; however, dry and saline environments also harbour a significant number of unknown fungal species (Bhardwaj *et al.,* 2023). Host-plant associations vary from strictly host-specific to non-specific, colonizing one or multiple plant species. Fungal endophytes colonize the inner rhizome, root, crown, rhizosphere, and occasionally the root tissue of host plants, influencing physiological and ecological characteristics. Some endophyte species are vital for the survival of specific plants, such as those providing heat tolerances to the grass *Dichanthelium lanuginosum*. Communities exhibit asymmetrical specialization, with most host plants associating with a narrow range of fungal endophytes. High colonization rates, especially in grasses from remote high plateaus, enable endemic species to withstand short growth seasons under low temperatures.

Functions of endophytic fungi

Endophytic fungi reside within healthy plant tissues without causing symptoms of infection, existing as unknown endobionts, harmless endophytes, latent pathogens, or mutualists (Bhardwaj *et al.,* 2023). The abundance of these fungi modulates the diversity and structure of plant-associated fungal communities, and they are proposed to promote plant propagation and growth, increase productivity, biomass yield, and enhance tolerance and resistance to biotic and abiotic stress. Additionally, endophytic fungi optimize nutrient uptake, regulate host metabolism, and synthesize a broad spectrum of bioactive metabolites with diverse biological activities. These attributes lay a firm foundation for harnessing endophytic fungi as bioprotectants to boost agricultural production.

Plant growth promotion

Endophytic fungi are widespread microorganisms that inhabit plants during at least one phase of their life cycle without producing symptoms or acute infection, often functioning as biological control agents against herbivores and pathogens. The number of endophytic fungi increases with decreasing latitude, and this pattern is associated with increasing plant diversity and density. Some endophytic fungi adapt to extreme environmental conditions and can promote plant growth and resistance. In *Colobanthus quitensis*, extremophilic endophytic fungi significantly attenuate the effects of UV-B radiation, enhance photosynthetic activity, and improve drought tolerance (Zenteno-Alegría *et al.,* 2024). Plant-associated microbes reduce metal bioavailability, decreasing its uptake and accumulation, thereby more effectively protecting plants such as *Lycopersicon esculentum* from the combined effects of heavy metal toxicity and water stress. Some extremophilic endophytic fungi, while protecting plants and their fruits from both phytopathogenic fungi and fruit-spoiling microorganisms, also act as biocontrollers against saline stress. Endophytic fungi often persist in the organs of host plants, affecting product quality and potentially human health. Quantifying these risks and benefits is crucial for future applications (Grabka *et al.,* 2022).

Disease resistance mechanisms

Endophytic fungi are known to trigger a series of mechanisms in plants that lead to bioprotection against a broad spectrum of biotic and abiotic disorders (Fontana *et al.,* 2021). Several classes of endophytes carrying out biological control against plant diseases are also candidates for genetic modulation and interaction with the host. They may offer increased tolerance and and abiotic stresses when colonizing the host plant and overall promote survival and growth in stressful environments. In return, secondary metabolites produced by the endophytes provide biological activity and may be desirable products themselves. Competition among fungal species is commonplace and may entail mycoparasitism, resource competition, or antimicrobial substance synthesis (Bhardwaj *et al.,* 2023). For instance, *Trichoderma* species frequently attack pathogenic fungi such as *Rhizoctonia solani*, thereby reducing their harmful effects on the plant. Similarly, the non-pathogenic endophyte *Clonostachys rosea* inhibits *B. cinerea* and and *Phytophthora* species. Plant association mediates microbial conflict by selecting its microbial communities and shaping the outcome of interspecific interactions. Some plants preferentially recruit beneficial endophytes through root exudates, which potentially enhance recruitment of compatible species and simultaneously restrict entry of detrimental fungi. Spatial segregation within the host further alleviates competition by restricting inhabitants to specific niches such as the vascular system and intercellular gaps. Such insights are key to the design of biocontrol protocols and ultimately to successful management of plant diseases.

Stress tolerance

Endophytic fungi are well recognized for their ability to enhance plants' tolerance of abiotic stresses such as salinity, drought, and extreme temperatures. Abiotic stress is a major hazard to food production and, in some of world's most important grain crops; the fungi influence tolerance of the plant to these stresses (Redman *et al.,* 2021). Because endophytes are vertically transmitted, their effects are retained in successive generations. The fungi also generally increase biomass and reduce water consumption. Endophytic fungi can confer salinity tolerance on plants from even non-saline locations and consistently improve growth after salt exposure. Some endophytic fungi provide commercial-scale tolerance to drought and temperature extremes, with good efficacy in field conditions, which greatly enhances yield and quality.

Isolation and identification techniques

Endophytes normally cannot be observed by the naked eye due to their small size and hidden location inside plants. However, isolation and identification of these fungi can be achieved through the classical isolation method and molecular systematics (Nursyazwani Maadon *et al.,* 2018). The classical isolation method involves sterilizing the plant material and transferring small pieces from the inner part of leaves, stems, or roots to culture medium. Plates are incubated and monitored periodically to determine the intensity of endophyte colonization. To obtain pure cultures of the isolated fungi, each mycelium growing on the margin of expanding colonies is transferred to new culture plates at a regular interval until contamination by other fungi or bacteria is absent (Cheng *et al.,* 2023). Endophytic fungi identification is usually based on physiological and biochemical characteristics or PCR followed by sequencing of specific DNA regions. PCR prior to sequencing is often conducted to amplify parts of some ribosomal regions, such as ITS1, 5.8S, and ITS2; D1 and D2 domains of 28S (LSU) ribosomal RNA gene; and small subunit of the 18S (SSU) rRNA gene.

Culture-based methods

Culture-based approaches have been extensively employed for isolating and identifying endophytic fungi that colonize the roots of healthy plants, facilitating surveys and functional studies (Chen *et al.,* 2020). Detailed examination of stem internal tissues and leaves allows for the isolation of culturable endophytes, while the advent of advanced molecular methods overcomes previous constraints associated with culture dependency (Wijayawardene *et al.,* 2021). Therefore, the isolation of endophytic fungi from diverse plant organs often depends on a combination of culture- and DNA-based techniques.

Molecular techniques

Cultivation of endophytic fungi from surface-sterilized plant tissues on semi-selective media is a conventional procedure used for morphological identification. However, the diversity of culturable endophytes is often underestimated, particularly when host and geographic-specific fungal groups dominate the environment. Molecular methods contribute to circumvent this limitation (Ponchon *et al.,* 2022) and are used extensively to study complex plant–endophyte–pathogen interactions (Paplomatas, 2004). Amplification of the internal transcribed spacer (ITS) sequence of endophyte rDNA enables the screening of endophytic colonies by polymerase chain reaction (PCR) as an initial step in the identification process. Although amplicon length is variable—and not always sufficient for species identification—PCR fingerprinting methods (e.g., denaturing-gradient gel electrophoresis) can differentiate closely related isolates. The nucleotide sequences of the PCR products are compared with those in nucleotide databases, such as GenBank. Allele-specific markers such as microsatellites, which allow reliable separation of fungal species, will allow a higher degree of resolution. Often-endophytic fungi, such as endophytic insect-pathogenic *Metarhizium* isolates, can be distinguished from closely related saprophytic or entomopathogenic strains using PCR procedures that target toxin-biosynthesis genes times while glutamin synthetase, a housekeeping gene conserved in all fungal species, provides a positive amplification control.

Early barcoding experiments that focused on arbuscular mycorrhizal fungi (AMF) already provided some insights into the design of appropriate molecular markers (Sharma *et al.,* 2010). Different sets of conserved primers help distinguish between rDNA regions (i.e., the small and large subunit [SSU and LSU, respectively] or the internal transcribed spacer [ITS]). Because of the frequent overlap of the sequence haplotypes among related species, it is difficult to define species boundaries for AMF using present molecular methods. Identifying new marker genes that differentiate genetic variation among intra- and interspecies remains a priority. The limitation of the molecular methods can be overcome by a combination of quantitative and qualitative data achieved by both molecular and microscopy methods. Recent investigations that revised the classification of family Gigasporaceae (Glomeromycota) based on molecular characters and morphological data refuted the earlier classification of Gigasporaceae. For the construction of a robust evolutionary tree, conserved datasets of molecular and morphological traits should have equal consideration.

The development and synthesis of molecular barcodes (unique short sequence tags that offer a higher degree of specificity than primers) for large-scale survey and detection of potentially antagonistic endophytes will represent a significant step forward. Available culturing techniques, genome sequencing strategies (e.g., the 1 KFG project), and improved bioinformatics tools will allow the rapid development of a complex scheme of molecular probes that target key metabolic signals predicting active biocontrol activity. The rapid identification of potential biocontrol agents at the field level will then become a possibility through genomic and synthetic biology approaches, which will significantly accelerate bioprotection strategies.

Endophytic fungi and soil health

Endophytic fungi interact widely with not only their compatible host plants but also structures of the plants (roots, leaves, and stem) via the rhizospheric, phyllospheric, and endospheric areas and participate in the formation of symbiotic assemblages with hosts and other groups of soil microbes. As a result, besides the usual benefits of growth promoters, disease resistors, and stress tolerators to host plants, endophytic fungi help simplify soil nutrient cycling in an agricultural ecosystem. Hence, the maintenance and regulation of the healthy soil ecosystem play a vital role in diverting the plant of an ecosystem towards agricultural intensification, and they still require systematic exploration and exploitation. Furthermore, plant–microbe symbiosis plays an important role in sustainable agriculture, not only by restoring nitrogen and carbon sequestration, maintaining soil organic carbon, and regulating greenhouse gases but also by enhancing the productivity of agriculture. However, the endophytic fungi in a particular rhizospheric zone solely contribute to balanced soil health, community structuring, and resource potential for sustainable agriculture. Nowadays, sustainable agriculture depends primarily on plant–microbe interaction(s)/associations due to their properties and benefits. The soil acts as the primary habitat of the plant, where it gains its nutrient requirements.

Soil microbial interactions

A diverse community of microorganisms, including endophytic fungi, unnerves the phyllosphere and mediates interspecific interactions in soils. Soil microbes help regulate the carbon cycle, break down organic matter (OM), decompose plant residues and mitigate the effects of abiotic stresses when associated with plant roots at the soil interface (Grabka *et al.,* 2022). Organic matter is a rich source of nutrients for microorganisms and plants and rich in C–P groups absorbed by clay minerals. The microbial compartment of organic matter content increases the microbial count, enzyme activity, genomic activity, earthworm activity and aggregate stability (Fontana *et al.,* 2021). These complex groups of organisms determine soil biosphere anthropogenically and govern soil thickness and soil composition (Bhardwaj *et al.,* 2023). They manage nutrient cycling including nitrogen and phosphorus cycling. The soil carbon accumulated in this layer is sensitive to climate conditions and landscape history. Together, soil microbes form an intricate network that affects plant growth, increases mineralization rate and enhance tolerance to environmental stresses.

The active layer represents the horizon between the soil and the atmosphere and defines soil inheritance. Roots grow laterally in the top 2 cm of the organic matter at the soil surface and facilitate the cycling of limited nutrients. The biogeochemical cycling of nutrient is clustered around the active layer as opposed to the subsoil and provides vital resources to plants. Organic matter influences the nutrient supply of active layer with nutrients imparted by roots. Roots produce organic acids which are associated with active layer to contribute to the available pool of nutrients. The active layer microbial communities influence soil chemistry and composition by inter alia nutrient cycling and respiration. Active layer habitats are known to be sensitive to environmental changes. Understanding how these active layer microbial groups interact with the environment is critical to assess the impact of climate change. Soil microbes comprise wide variety secondary metabolites that influence the growth of microorganisms in the rhizosphere and plant-pathogen interactions. Plant-associated microbial communities have been observed to regulate plant growth and health. The bacterial and fungal rhizosphere communities influence pathogen behaviour through positive and/or negative interactions determines the composition of the plant-endophytic and the soil communities and plant–soil feedback. Soil microbes may act antagonistically by direct pathogen suppression. Endophytic fungi that have beneficial associations with plants widely used to promote green agricultural systems. They decompose potentially hazardous xenobiotics and other harmful elements and use industrial wastes, sewage sludge and agro-chemicals to restore forest fire-damaged plants. These microbes improve short-term soil nutrient availability and mediate the interaction of fungal endophytes with plant diseases through secondary metabolite biosynthesis.

Nutrient cycling

Interactions between bacterial and fungal taxa are often pivotal in the ecological balance of soil communities, influencing soil health through nutrient cycling, organic matter formation, and plant defense mechanisms. Fungal endophytes are linked to plant growth promotion and soil health; a given endophyte can produce enzymes that hydrolyze complex organic substrates, releasing nitrogen, phosphate, and other elements to the detriment of pathogens. Nutrient cycling critically contributes to the supply of macronutrients (such as phosphorus, calcium, and nitrate) and micronutrients (such as zinc, copper, and manganese). Details regarding bacteria–fungi interactions remain relatively scarce. More than 90% of soil phosphorus exists in insoluble forms and is unavailable to plants. However, the phosphate-solubilizing activity of fungal endophytes from AMF-infected tissues, together with phosphate solubilization by bacteria, can increase available P for plant uptake. Interspecific synergy between these organisms appears to accomplish greater nutrient utilization efficiently. Endophytes with the ability to solubilize Zn and/or K may represent an alternative means to reduce the use of inorganic fertilizers. Endophytic colonization with bacteria and fungi can also induce an enhancement in growth of Amaranthus tricolor and *Zea mays* (Gowtham *et al.,* 2024).

Characterizing soil fungal communities is essential to understand bioprotection and biogeochemical processes. In the USA, samples obtained from a typical agricultural crop field with early and late rotation management underwent DNA extraction and sequence metabolomics. A total of 9007 fungal operational taxonomic units (OTUs) were obtained at 97% sequence similarity, with the dominant guilds identified as 61% saprotrophs and 23% pathotrophs. Early rotation field sites contained significantly more saprotrophs, while late rotation sites had more pathotrophs, suggesting an agricultural system implemented in early rotation fields that harbours a fungal community with a greater association with soil health, nutrient cycling, and higher rates of organic matter degradation (Grabka *et al.,* 2022). Other studies have similarly explored the fungal communities of agroecosystems, brownfields, and arctic regions.

Bioprotection strategies using endophytic fungi

Fungal endophytes are a major group of micro-organisms that share an intimate symbiotic relationship with plants. These fungi have the ability to synthesize various bioactive metabolites and play an important role in improving plant protection by enhancing soil quality, enabling plants to overcome biotic and abiotic stresses. Consequently, there is a reduction in the use of chemical fertilizers and pesticides (Gowtham *et al.,* 2024). Biotic stress as a result of microbial pathogens, pests and nematodes poses one of the greatest obstacles to plant growth and development. This stress increases the production of reactive oxygen species in plants, which affects many molecular and physiological processes and ultimately reduces crop productivity. Fungal endophytes occupy the internal tissues of plants without causing any apparent damage and provide a potential solution to some of the current challenges faced by conventional farming. They also help reduce the impact of fungal diseases on crops by producing antimicrobial compounds, activating plant defense genes, synthesizing bioactive metabolites and generating hormonal molecules. These processes decrease the prevalence and virulence of pathogenic fungi, enabling plants to better withstand fungal infection and minimizing the requirement for chemical fungicides. Endophytic fungi have significant potential to be harnessed for the biological control of phytopathogens and the production of beneficial bioactive compounds in plants. Biologically active compound-producing and plant growth-promoting endophytes have attracted considerable interest as potential sources of new drugs and environmentally friendly crop protectors (Bhardwaj *et al.,* 2023). While the genus *Trichoderma* has been extensively studied, many other promising endophytic fungal groups remain underexplored. Molecular identification and biochemical characterization of these microbes are necessary to elucidate the mechanisms underlying their antagonistic activity. The future of biological control using fungal endophytes in sustainable agriculture is encouraging, offering opportunities to reduce chemical pesticide use enhance crop health and increase yields. Continued research is focused on developing genetically optimized strains and improving soil health and nutrient cycling, thereby contributing to more sustainable farming methods.

Biocontrol agents

Endophytes include bacteria, fungi, and actinobacteria, and among them, fungal endophytes are the most common and diverse. Fungal endophytes inhabit most plants and form interdependent relationships that can span the entire plant life cycle. They play a notable role in improving plant health and have been studied extensively to understand their influence on both biotic and abiotic stresses (Bhardwaj *et al.,* 2023). Endophytic fungi, or fungal endophytes, are microorganisms dwelling asymptomatically inside plant tissues throughout a significant portion of their life cycle (Fontana *et al.,* 2021). These microorganisms inhabit a wide range of environments and have displayed remarkable potential in agricultural biotechnology and medicine. These fungi establish symbiotic associations with host plants, colonizing almost every plant species globally and residing in diverse plant tissues, including roots, stems, leaves, flowers, fruits, and seeds. Enhanced plant growth, yield, and resistance to stress factors such as drought, salinity, and extreme temperatures are among the many beneficial functions associated with fungal endophytes. Recent research indicates that endophytic fungi can serve as effective biocontrol agents against an array of plant pathogens and pests, functioning as biofertilizers suitable for sustainable agriculture. Several studies have highlighted their application as biocontrol agents to safeguard crops including cereals, fruits, and vegetables. Many endophytic fungi have been commercialized as biocontrol agents, with their application in field conditions consistently yielding substantial crop protection. Notably, although species of the genus *Trichoderma* have been extensively investigated, other promising endophytic fungal genera remain underexplored. Extensive molecular identification and characterization of the bioactive compounds produced by these fungi are essential to elucidate their antagonistic mechanisms and to harness their full potential in sustainable agricultural practices.

Biofertilizers

Among the benefits provided by endophytic fungi are those classified under biofertilization, organic fertilizers that supply nutrients to plants and maintain soil fertility without direct application of chemicals to the crops. The use of biofertilizers is widespread in conventional agriculture but more important in sustainable and ecological agriculture, in which genetically modified organisms are not recommended, and enhancing natural structures to assist appropriate root growth is essential. Some native endophytic species may become promising candidates for such uses. Biofertilization is important primarily in micropropagation, where laboratory (*in vitro*) and greenhouse stages require biofertilization due to the lack of microorganisms that assist nutrition, an essential requirement upon transfer from the micropropagation stage to soil.

Case studies in agriculture

Endophytic fungi occur in every crop species examined, and their coexistence with hosts between harvest and consumption draws growing interest (Bhardwaj *et al.,* 2023). An extended review illustrates their positive roles with cereals, fruit trees, and vegetables, supporting field performance and offering practical examples of their beneficial presence (Grabka *et al.,* 2022). Several recent studies demonstrate the potential of endophytic fungi to improve plant productivity. A survey of field maize reveals 10 species colonizing stem and root tissues, with only *Aspergillus* sp. exhibiting pathogenic effects. Endophyte-inoculated wheat shows enhancements in harvest index, maturity time, and yield parameters relative to uninoculated controls. Additionally, inoculation with *Penicillium oxalicum*, a convenient biofertilizer, significantly increases plant height, branch radius, and effective branch number compared to uninoculated wheat.

Cereal crops

The importance of a sustainable agricultural approach to fulfil the food security demands of an exponentially increasing human population is well known. The over-usage of synthetic chemicals for managing various biotic stresses in modern agricultural practices has an adverse impact on health and the environment, and a constant search for alternatives is underway. The bioprotective potential of the diverse group of microbes known as endophytic fungi is increasingly being realized in this context. Endophytic fungi colonize tissue intracellularly without harming the host plant. The fungal community associated within plants belongs to diverse taxonomic groups and has shown biopesticidal and biostimulant properties and antagonistic activity against a broad range of phytopathogens (Grabka *et al.,* 2022). Genera such as *Alternaria, Aspergillus, Chaetomium, Cladosporium, Fusarium, Penicillium,* and *Trichoderma* dominated isolates from several crops and frequently produced beneficial secondary metabolites and enzymes that suppressed other microbes (Bhardwaj *et al.,* 2023). Endophytes affect agroecosystems directly by influencing plant fitness or indirectly by modulating the resident soil microbial community. Their biocontrol ability along with their capability to enhance the production of many physiologically important compounds as elicited by biotic and abiotic stress pressure make them highly prospective for sustainable agricultural production.

Fruit trees

Fruit trees support the world's food supply and have importance in tropical and temperate fruit production by providing domestic food products. Fungal endophytes have been studied in fruit trees for their bioprotective potential. The culture-based diversity of fungal endophytes in date palm (*Phoenix dactylifera* L.) revealed the dominance of the genera *Alternaria, Aspergillus, Chaetomium, Fusarium,* and *Penicillium,* distributed mainly in the tissues of roots and leaves (Fontana *et al.,* 2021). The endophytic colonization of *Arachis hypogea* by the entomopathogenic *Beauveria bassiana* was studied under greenhouse and field conditions. The ability of *B. bassiana* to colonize *A. hypogea* plants was dependent on soil sterilization. This fungus provided control of the insect attack by significantly increasing plant growth and seed production under inoculation and herbivore pressure. The results encourage a greater search for new endophytes that can be exploited as bioprotectants in crops such as tree nuts with the aim of declining synthetic pesticides and increasing sustainable agriculture (Grabka *et al.,* 2022). Eighteen endophytic fungi belonging to *Aspergillus, Colletotrichum, Curvularia, Fusarium, Nigrospora, Penicillium,* and *Alternaria* were isolated from *Citrus reticulata* var. *Ponkan*. The isolates were tested for their antifungal activities against seven pathogens. *Aspergillus flavus* strain GF3 exhibited the most potent antifungal activity against the *Phyllosticta citricarpa* (the causal agent of Citrus Black Spot—CBS), *Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides*, and *Fusarium oxysporum* (Bhardwaj *et al.,* 2023).

Vegetable production

Vegetable crops serve as essential sources of vitamins, minerals, dietary fiber, antioxidants, and many natural nutrients. The productivity and cultivated area of vegetables have continuously increased throughout recent decades (Bódalo *et al.,* 2023). The abundance and diversity of endophytic fungi within vegetables depends upon the geographical location, soil parameters, physiology of the host, and crop management (Fontana *et al.,* 2021). The soil in which vegetables are grown is rich in organic material, an important source of inoculum for infection by endophytic fungi. These microorganisms can also reduce biotic and abiotic stresses in these crops, enhance the nutrient content of the plants, increase mineral uptake, and improve plant photosynthetic efficiency and biomass accumulation.

Challenges in utilizing endophytic fungi

Endophytic fungi colonize the internal tissues of host plants without causing external symptoms of infection (Gowtham *et al.,* 2024). Regulatory approval processes for bio-formulated products remain lengthy, hindering development. The productivity and quality of many economically important crops are severely affected by biotic and abiotic stresses, limiting their yield. Although extensive research has been undertaken to suppress these constraints using synthetic agrochemicals, they exhibit detrimental impacts on the environment and human health. Consequently, alternative approaches have become necessary to address agricultural challenges without harming the environment. Utilization of endophytic fungi as biocontrol agents or biostimulants presents a valuable alternative; however, large-scale commercial use is restricted due to limited knowledge of specific host–endophyte interactions and their regulatory mechanisms. The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* can colonize the roots of some cultivated crop plants and provide additional protection against relevant soil-feeding insects. Implementing microbial inoculants in the field also encounters particular difficulties, including abiotic factors that are difficult to control and the challenge of transferring promising controlled-environment trials to field crops. Despite challenges, fungal endophytes may play a key role in reducing the use of toxic agrochemicals or increasing plant production to meet global demands.

Regulatory hurdles

Since 1929, when the term "endophytes" was first used (Giehl *et al.,* 2023), the concept of fungal endophytes has broadened to apply to every fungus found inside plants, from the rhizosphere to living tissues, from photosynthetic shoot tissues to nonphotosynthetic roots, bulbs, and rhizomes. Fungal endophytes can penetrate and colonize living healthy tissues of both nonvascular and vascular plants (Torres-Mendoza *et al.,* 2020). Several commensal and mutualistic endophytic fungal species are known to promote plant growth, improve resistance to pathogens, or enhance tolerance to abiotic stress in a wide variety of hosts and climates. Endophytic fungi have been recognized for roles such as biocontrol, biofertilizers, and stress tolerance, among others, in various crops.

Field application issues

The successful application of endophytic fungi in the field is hindered by regulatory and field implementation challenges. Regulatory frameworks in many countries lack specific provisions for bioproducts based on endophytic fungi, introducing considerable uncertainty for the agricultural sector. In some regions, bioproducts are inappropriately classified under legislation designed for synthetic substances, as is the case with biostimulants. Even in instances where an appropriate regulatory framework exists, such as for biocontrol products, the extensive testing and costs required to prove the effectiveness of endophytic fungi discourage many farmers and companies from pursuing their development and commercialization despite the advantageous properties of these microorganisms (Fontana *et al.,* 2021). Field application of microorganisms entails additional difficulties, although bioproducts incorporating endophytic fungi can address many of the limitations associated with free microorganisms. The use of living microorganisms requires large quantities, and the endophyte must compete with the indigenous microbiota present in the soil. Consequently, the survival and persistence of the microorganisms may be affected, sometimes limiting their effectiveness. Microorganisms that are not compatible with the culture may also change the composition of the indigenous microbial community, potentially resulting in environmental imbalances. Furthermore, microorganisms can be rendered ineffective by adverse weather conditions such as extreme temperatures, ultraviolet radiation, or drought, which reduces their viability and consequently their protection capacity on the crop (Grabka *et al.,* 2022).

Future prospects for research

Endophytic fungi offer diverse potential for more sustainable crop production, but bioprotection still faces fundamental challenges. Advances in "omics" approaches show promise through revealing intricate molecular and physiological fungal–plant interactions, as well as uncovering novel gene clusters and bioactive compounds. Engineering beneficial microbiomes further enhances prospects for sustainable crop protection, pointing to beneficial endophytes as core components in governing plant–pathogen interactions (Grabka *et al.,* 2022). Synthetic biology tools enable the design and engineering of new fungal strains with targeted traits, which can stimulate the biosynthesis of biologically active substances that suppress specific pathogens (Bhardwaj *et al.,* 2023). For the foreseeable future, the greatest benefits derive from naturally occurring fungi, but the emerging ability to tailor strains for particular objectives signals a fundamental change in the management and protection of global food production. Future research should also clarify the antagonistic behavior of these fungi to optimize their use for the natural management of phytopathogens (Fontana *et al.,* 2021).

Genomic studies

Genomic approaches provide essential insights into endophytic fungal communities and their unresolved bioprotective mechanisms. Fields of large-scale, sky-island endophytic communities can be monitored by periodic sequencing to detect emerging pathogens in their incipient phases (Grabka *et al.,* 2022). With advances in DNA sequencing, several endophytic fungi from crops have been sequenced. Endophytes with particular traits can be "print-selected" for open-field application or integrated into artificial communities in a customized fashion. Pan-genome sequencing aids the search for biosynthetic gene clusters associated with known bioactive compounds (Doty *et al.,* 2022). Pan-genomes also reveal metabolic pathways that strain selection for biocontrol should prioritize (Bhardwaj *et al.,* 2023). Development of more robust agents stems from missing resistances to known chemical fungicides, sufficient spatial distribution, and consideration of the antagonistic effect of one endophyte on another.

Synthetic biology approaches

The burgeoning field of synthetic biology seeks to design and construct endophytic fungi with enhanced bioprotection capabilities. One straightforward strategy is heterologous expression of biocontrol and growth-promoting genes in well-characterized endophytes isolated from target crops; for example, the E. coleosporum strain from banana could be genetically engineered for this purpose (Gowtham *et al.,* 2024). Synthetic constructs genuinely afford the prospect of custom-tailored endophytes optimized for particular agricultural environments (Bhardwaj *et al.,* 2023). Genome-editing technologies such as CRISPR-Cas9 have, however, so far largely focused on single-gene modifications, whereas the complex, multi-gene expression networks underlying bioprotection remain opaque. A superior alternative that has yet to be pursued in the fungal context involves genome refactoring. Here a multi-gene locus is re-synthesized, enabling extensive transcriptome engineering by editing promoter sites without hampering gene function. The genomes of numerous bioprotective fungi have now been determined, facilitating the identification of appropriate candidates for the required level of control.

Ethical considerations in bioprotection

Contemporary agriculturally focused research has demonstrated the efficacy of endophytic fungi as sources of natural bioactive metabolites that suppress growth and infection by phytopathogens. Nonetheless, attempts to utilize such metabolites often face challenges related to chemical instability under environmental stresses. The ability of endophytic fungi to enhance growth, nutrient uptake, and stress resistance also positions them as promising biocontrol agents, offering sustainable alternatives to chemical pesticides and fertilizers. In parallel with measures to establish biodiversity protocols, the application of bioprotection strategies using endophytic fungi should address critical issues of intellectual property rights; benefit sharing, data citation rights, access to research methodologies and techniques, and the judicious exploitation of genomic and biotechnological resources. Integrating comprehensive policies that safeguard biological resources and promote equitable collaborations will facilitate the broader adoption of endophytic fungi in sustainable agriculture (Bhardwaj *et al.,* 2023).

Biodiversity conservation

Biodiversity loss occurs at both regional and global scales, with first victims often localized rare and endemic species (Fontana *et al.,* 2021).

Endophytic fungi represent a microbial group with high potential to compensate for biological losses (Bhardwaj *et al.,* 2023). Fungal endophytes may contribute to bioprotection of both plant diversity and virus-vector insect diversity, as strengthened plant defence is likely to cascade across trophic levels (Grabka *et al.,* 2022). Without a global strategy to reduce or stop the loss of biological diversity, the degradation will negatively impact human wellbeing (e.g., food security). An obligation to reduce the loss of biodiversity is the recently adopted international treaty on access and benefit sharing of genetic material. Since endophytes are producers of the bioactive molecules, world fungal endophytes should therefore be given strong impetus from bioprospection.

Intellectual property rights

In the pursuit of bioprotection strategies, it is crucial to address ethical issues, including biodiversity conservation and intellectual property rights, which the scientific community must handle as it advances in these fields. The emerging technologies around bioprotection have tremendous potential to increase security and reduce the environmental impact of food production. Yet as ecological and financially beneficial as such tools may be, access to them is unequal, and their widespread use could uphold or exacerbate existing inequalities. Intellectual property rights directly affect the access, dissemination, and development of any such technology. Therefore, the systems of ownership around bioprotection research could shape the technology's distribution and also its creation. Endophytic fungi, microorganisms living inside plants, constitute one of the most diverse and ubiquitous groups in terrestrial plants. Their presence can increase plant growth, resistance to diseases, and tolerance to abiotic stress such as drought or high temperatures. The use of endophytic fungi as an agricultural inoculant represents an effective strategy to protect plants from diseases while diminishing the use of agrochemicals. Additionally, endophytic fungi interact with soil microorganisms and participate in nutrient cycling. Advances in the study of symbionts of endophytic fungi, evolution, and synthetic biology offer tools for combating pathogens, promoting growth, and improving crops' physiological capacities. Patent applications covering endophytic fungi have risen over twenty years in many sectors, although not in agriculture; one reason is the difficulties of translating laboratory results into the field for practical application (Torres-Mendoza *et al.,* 2020; Bhardwaj *et al.,* 2023).

Conclusion

Biotic and abiotic stresses reduce crop yields and demand substantial pesticide application, while abiotic stresses like temperature extremes and soil salinization restrict agricultural land use. Although many crop-protection products exist, the endophyte-plant-microbiome triad represents a rich alternative source of novel compounds relevant for agriculture, for which skilled fungal taxonomists remain indispensible (Fontana *et al.,* 2021). Endophytic fungi are present in every major lineage of land plants, yet the majority remain unknown and underexplored. Although common taxa might be more readily grown in culture, some endophytes may serve as sources of new biologically active metabolites. Fungal microbiomes evolve as natural extensions of hosts, allowing symbiont populations to increase as defenses activate; nevertheless, many fundamental questions about fungal endophytes remain unanswered (Grabka *et al.,* 2022). Characterization and deployment of endophytic fungal assemblages present promising avenues to increase agricultural efficiency and sustainability for a growing population (Bhardwaj *et al.,* 2023).

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Bhardwaj, M., Kailoo, S., Khan, R. T., Khan, S. S. and Rasool, S. (2023). Harnessing fungal endophytes for natural management: a biocontrol perspective. Frontiers in Microbiology, 14:1280258.

Bódalo, A., Borrego, R., Garrido, C., Bolivar-Anillo, H. J., Cantoral, J. M., Vela-Delgado, M. D., González-Rodríguez, V. E. and Carbú, M. (2023). In vitro studies of endophytic bacteria isolated from ginger (*Zingiber officinale*) as potential plant-growth-promoting and biocontrol agents against Botrytis cinerea and Colletotrichum acutatum. Plants (Basel), 12:4032.

Chen, J., Akutse, K. S., Saqib, H. S. A., Wu, X., Yang, F., Xia, X., Wang, L., Goettel, M. S., You, M. and Gurr, G. M. (2020). Fungal endophyte communities of crucifer crops are seasonally dynamic and structured by plant identity, plant tissue and environmental factors. Frontiers in Microbiology, 11:1519.

Cheng, C. Y., Zhang, M. Y., Niu, Y. C., Zhang, M., Geng, Y. H. and Deng, H. (2023). Comparison of fungal genera isolated from cucumber plants and rhizosphere soil by using various cultural media. Journal of Fungi (Basel), 9:934.

Doty, S. L., Joubert, P. M., Firrincieli, A., Sher, A. W., Tournay, R., Kill, C., Parikh, S. S. and Okubara, P. (2022). Potential biocontrol activities of Populus endophytes against several plant pathogens using different inhibitory mechanisms. Pathogens, 12:13.

Fontana, D. C., de Paula, S., Torres, A. G., de Souza, V. H. M., Pascholati, S. F., Schmidt, D. and Dourado Neto, D. (2021). Endophytic fungi: biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens, 10:570.

Giehl, A., Dos Santos, A. A., Cadamuro, R. D., Tadioto, V., Guterres, I. Z., Prá Zuchi, I. D., Minussi, G. D. A., Fongaro, G., Silva, I. T. and Alves, S. L. Jr. (2023). Biochemical and biotechnological insights into fungus–plant interactions for enhanced sustainable agricultural and industrial processes. Plants (Basel), 12:2688.

Gowtham, H. G., Hema, P., Murali, M., Shilpa, N., Nataraj, K., Basavaraj, G. L., Singh, S. B., Aiyaz, M., Udayashankar, A. C. and Amruthesh, K. N. (2024). Fungal endophytes as mitigators against biotic and abiotic stresses in crop plants. Journal of Fungi (Basel), 10:116.

Grabka, R., d'Entremont, T. W., Adams, S. J., Walker, A. K., Tanney, J. B., Abbasi, P. A. and Ali, S. (2022). Fungal endophytes and their role in agricultural plant protection against pests and pathogens. Plants (Basel), 11:384.

Nursyazwani Maadon, S., Ahmad Wakid, S., Izni Zainudin, I., Syahani Rusli, L., Syahril Mohd Zan, M., Hasan, N. A., Abu Shah, N. A. and Rosseleena Rohani, E. (2018). Isolation and identification of endophytic fungi from UiTM Reserve Forest, Negeri Sembilan. Journal of Sustainability Science and Management, 13:111-119.

Paplomatas, E. J. (2004). Molecular diagnostics for soilborne fungal pathogens. Journal of Plant Pathology, 86:257-261.

Ponchon, M., Reineke, A., Massot, M., Bidochka, M. J., Thiéry, D. and Papura, D. (2022). Three methods assessing the association of the endophytic entomopathogenic fungus Metarhizium robertsii with non-grafted grapevine Vitis vinifera. Microorganisms, 10:2437.

Redman, R. S., Kim, Y. O., Cho, S., Mercer, M., Rienstra, M., Manglona, R., Biaggi, T., Zhou, X. G., Chilvers, M., Gray, Z. and Rodriguez, R. J. (2021). A symbiotic approach to generating stress tolerant crops. Microorganisms, 9:920.

Sharma, D. K., Jha, D. K. and Pandey, R. R. (2010). Molecular approaches in arbuscular mycorrhizal research: a review. African Journal of Microbiology Research, 4:1113-1120.

Torres-Mendoza, D., Ortega, H. E. and Cubilla-Rios, L. (2020). Patents on endophytic fungi related to secondary metabolites and biotransformation applications. Journal of Fungi (Basel), 6:58.

Wen, J., Okyere, S.K., Wang, S., Wang, J., Xie, L., Ran, Y. and Hu, Y. (2022). Endophytic fungi: an effective alternative source of plant-derived bioactive compounds for pharmacological studies. Journal of Fungi (Basel), 8:205.

Wijayawardene, N. N., Bahram, M., Sánchez-Castro, I., Dai, D. Q., Ariyawansa, K. G. S. U., Jayalal, U., Suwannarach, N. and Tedersoo, L. (2021). Current insight into culture-dependent and culture-independent methods in discovering ascomycetous taxa. Journal of Fungi (Basel), 7:703.

Zenteno-Alegría, C. O., Yarzábal Rodríguez, L. A., Ciancas Jiménez, J., Álvarez Gutiérrez, P. E., Gunde-Cimerman, N. and Batista-García, R. A. (2024). Fungi beyond limits: the agricultural promise of extremophiles. Microbial Biotechnology, 17:e14439.

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