

Omics technologies for understanding the plant–fungal endophyte interactions: crop improvement for future security

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ABSTRACT

Fungi are a unique group of microbes that exist in all of the world's niches having different habitats and environments. They are known to interact with various species present on the earth, including plants. Fungi interact with different niches of plants such as roots (rhizosphere), tissue, and internal spaces (endophytic) and surfaces (epiphytic). The fungi that inhabit the plants are specifically known as endophytic fungi. These plant–fungal interactions play a significant role for the host, such as protecting the plants from biotic and abiotic factors known as major growth constraints. Fungal endophytes also supply several nutrients and plant growth hormones, which help in plant growth enhancement. Additionally, nitrogen fixation, solubilization of minerals, production of phytohormones, antibiotics, secondary metabolites, hydrogen cyanide proteolytic enzymes, and activation of plant responses to stresses are the various mechanisms of endophytic fungi. Endophytic fungi have been known for various biotechnological applications that were unraveled through exploring biodiversity and understanding the interaction of plant–fungal endophyte. There are many unexplored species and interaction studies that need to be explored, and for the study, omics tools are recognized as the finest approach. In the present review, different omics tools that could be used for a deeper understanding of plant–fungal endophyte interactions and endophytic fungi biodiversity have been detailed.

1. INTRODUCTION

Plants have co-existed with various beneficial microbes that interact in different regions, i.e., rhizospheric, epiphytic, and endophytic regions.

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All types of microbes interacting in the different regions show different proximity, and they tend to benefit from plant growth and development [1,2]. Among the three regions of interaction, endophytic microbial interaction has the closest proximity to plants. The endophytic microbial community resides inside the plant's healthy tissues and establishes itself at particular sites, including leaf, seed, roots, and stem, without causing any damage to the host plant. Endophytic microbes could be transmitted to the plants either vertically or horizontally, and different groups of microbes could be inhibited [3,4]. The endophytic microbes intrude the plant structure through root hair development, root injuries, and tissue damage, and different microbes, including bacteria, fungi, and archaea, enter the plants [5].

Endophytic fungi play a very important role in plant growth promotion, as they help in the protection and growth of plants. The

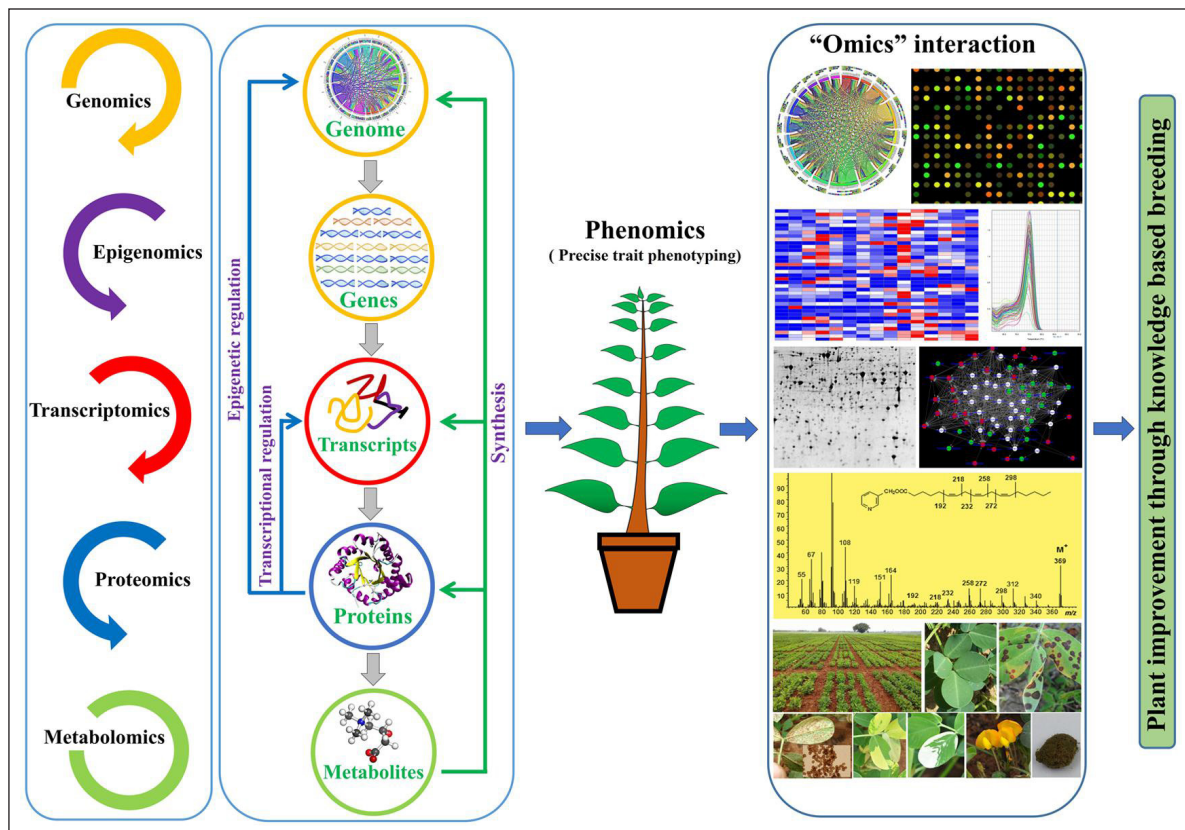


Figure 1: Different omics tools for better understanding of plant–fungal interactions. Adapted from Kumar et al. [120].

fungal endophytes protect the plants from both biotic and abiotic stresses from which a huge amount of damage has been reported [6,7]. Plant protection from biotic factors such as microbial pathogens and pests is achieved by preventing entry. If pathogens enter the plant structure, endophytic fungi prevent further establishment of pathogens by limiting the available resources such as nutrients or by producing antibiotics and hydrogen cyanide, which inhibit the growth of the pathogens [8,9]. Under abiotic stress such as drought, salinity, extreme temperature, heavy metals, nutrient-depleted environment, and extreme pH, plants' chlorophyll pigmentation, carbon oxide, soil fertility, and photosynthetic rate decrease, and metal accumulation and reactive oxygen species increase, which help the endophytic fungi in alleviating all such problems. Endophytic fungi alleviate abiotic stress by activating the plant stress response system and producing various anti-stress agents, which mitigate the plant stress effects [10].

Apart from mitigating abiotic and biotic stress, endophytic fungi supply the soluble form of nutrients and several phytohormones through various mechanisms such as solubilization of potassium and phosphorus, and production of phytohormones such as auxin, which promote plant production and growth [11]. Endophytic fungi are also a source of various secondary metabolites, which also enhance plant growth [12,13]. All these mechanisms that protect and promote plants have been exhibited by the diversity of fungal endophytes but despite being diverse many species are unknown. The biodiversity study of fungal endophytes is very important for further exploration as they hold a plethora of biotechnological applications in the field of agriculture, environment, and industry, and it has attracted the attention of many ecologists, chemists, and taxonomists [14-16].

To have in-depth knowledge of all the benefits of the fungal endophytes, the very first step is culturing, and a huge number of the endophytic fungi are unexplored as they cannot be cultured on the plate. The isolation or culturing of microbes is very important for the study and exploration applications and biodiversity [17]. The omics tool is a promising approach with which the fungal diversity and interaction of plants and fungi could be explored, and these applications could be studied well for future applications and ease. Different omics approaches such as genomics, transcriptomics, metagenomics, metatranscriptomics, proteomics, metaproteomics, and metabolomics [Figure 1] have been known [17,18]. In the present review, the need and omics approaches for understanding the plant–fungal endophyte interaction have been discussed in detail.

2. NEED FOR OMICS TOOLS

Access to large-scale omics datasets has revolutionized the field of biology and led to the emergence of systematic approaches to advance the understanding of biological processes [19]. In recent years, omics approaches have walked into people's vision. The technology with higher sensitivity and an extensive range of applications is thus favored by the scientific community [20]. The need for omics approaches has been realized in a range of research areas such as system microbiology, microbiome analysis, genotype–phenotype interactions, food and nutrition, disease biology, and natural product discovery [21-25]. The omics technology emerged from integrative analysis, ergo, related to the ability to identify a large number of biomolecules and the ability to resolve the dynamics of the ecosystem in establishing their interactions. The advent of this highly uniform technology to understand the complexity

of interactions between plants and microbial communities at the molecular level, coupled with the rapid development of the computational tools that are needed to sort and analyze such data, has revolutionized the science of plant–microbial interactions. The efficient functioning of a biological system needs a synergistic interaction between different components, and the integration of omics datasets with rigorous statistical analysis provides information about the complete system [18,26].

The omics techniques are of major importance in unraveling the complexity of the interactions between endophytic fungal communities and their host plants, thereby providing valuable insights into the mechanisms at the molecular level and promising applications of these relationships. The majority of the discoveries in the area of mutualistic relations between fungal endophytes and plants have been completely based on traditional scientific approaches, but the integration of omics is recent to this field [17]. Multi-omics approaches could be applied to study plant–endophyte interactions. Biochemical, physiological, and molecular investigations have well-evidenced the benefits imparted to the host plants by the associated endophytes, especially in terms of growth promotion of their host, enhancing stress resistance and metabolic capabilities; and further knowledge of the complexity of these mechanisms could be gained by adopting a multi-omics approach.

Genomics provides a view of the entire genomic-level information of endophytic fungal communities, helping researchers to find novel genes coding for potent metabolic compounds. Moreover, it also provides insights into the existing biodiversity of endophytes, phylogenetic lineage, evolution, and eco-physiological information [27]. Metagenomics is a significant technique that allows the direct analysis of the entire genome within environmental samples. Transcriptomics and proteomics impart knowledge about the profiling of gene expression and proteomes assembled by endophytic fungal communities, respectively. Furthermore, multi-omics technologies, when coupled with the metabolomics study, can progressively resolve aspects of the relationships between endophyte infection, accumulation of the metabolites, and stress alleviation in host plants [28]. Thus, none of the omics approaches is complete.

The integration of the data generated from metagenomics with metatranscriptomics and metaproteomics can help in detailing the intricacies involved in the establishment of endophytism. Similarly, data interpretation from transcriptomics or proteomics is incomplete in the absence of information from genomics. The use of combinatorial omics tools can help in resolving the enigma existing in the endophyte–host relationship [29]. Implementing multiple omics is thus a novel approach to understanding the multiple functions of endophytic fungi and how they interact with their host plants, and it can further encourage researchers to explore potential strains of fungal endophytes and their bioactive compounds [8].

3. OMICS APPROACH FOR UNDERSTANDING THE PLANT–FUNGAL ENDOPHYTE INTERACTION

3.1. Genomics

There is a lack of knowledge regarding the fundamental physiological features of the fungal endophyte and host interaction; thus, it's very important to dig deeper for knowledge [30]. Genome analysis has been a new tool to steadily look into endophytic fungi and plant interactions and to reveal essential attributes such as mineral acquisition, nitrogen fixation, and phytohormone production [31,32]. A genetic characteristic that affects colonizing favoritism and many other bioactivities, both

directly and indirectly, has been identified through whole-genome research of endophytic microbiomes. Genomics helps identify desired genes that are involved in the production of antibiotics, the endophytic secretory system, insertion elements, resistance to antibiotics, surface attachment, transport systems, and other metabolic pathways that help promote plant development. These studies have advanced our understanding of endophyte ecology and evolution. Gene acyl homoserine lactone synthases, hyperadherence factors, hydrolases, and fusaric acid resistance proteins demonstrate the endophytic bacteria's (*Pantoea ananatis*) biotechnological potentials [33]. Endophytic members of the fungal order are of great interest since they have the ability to promote plant growth and resist stress. It has been suggested that the genome sequence of *Piriformospora indica* could belong to a plant probiotic [34].

Since the development of high-throughput genome sequencing technology, there has been a noticeable increase in the number of whole-genome sequencing research [35]. The genetic landscape of endophytic fungi has been clarified through the use of high-throughput sequencing technologies such as next-generation sequencing (NGS) systems from Illumina, PacBio, and Oxford Nanopore [36]. Fungal endophytes can produce secondary metabolites, and their genetic makeup and host–environment adaptations can all be understood through whole-genome sequencing [37]. It is possible to find conserved genes, distinctive traits, putative virulence factors, and symbiotic genes by comparing the genomes of various endophytic fungi. The genome of *Alternaria* sp. is 34.70 Mb in size [38]. Apart from the phytopathogenic *Alternaria* species, endophytic species are known to produce numerous secondary metabolites as well. These metabolites have unexpected benefits as medicines due to their anti-inflammatory, anti-microbial, antiviral, and anti-carcinogenic mechanisms of action. Furthermore, the genomes of *Hypoxylon pulvicidum* (41.44 Mb) and *Hypoxylon* sp. (45.30 Mb) have been sequenced [39,40]. The endophytic fungi namely, *Pestalotiopsis fici* has been reported to secrete various secondary metabolites such as chloropestolides, chloropupekananin, chloropupekanolides, chloropupekanone, pestalodiols, and pestalofones. These secondary metabolites exhibit various biological activities such as inhibition of HIV-1 replication, prevention of tumor cytotoxicity, and acting as antifungal agents. The complete genome of *P. fici* reveals a high concentration of enzymes that are active on carbohydrates, especially pectinase, and a notable number of genes that are involved in the synthesis of secondary metabolites. Its capacity to generate naturally occurring compounds with a wide range of biological functions is indicated by the presence of genes involved in the production of secondary metabolites [41]. Based on antiSMASH 4.0, a study identified 65 gene clusters in the endophytic fungal strain *Calcarisporium arbuscula* NRRL 3705 that code to produce secondary metabolites and also identified the gene cluster responsible for aurovertin production. Furthermore, the researchers hypothesized several gene clusters related to the synthesis of mycotoxins, including alternariol, citrinin, aflatoxin, isoflavipucine, and destruxin. Additionally, it was found that twenty-three of the sixty-five gene clusters contained genes that encoded for the synthesis of polyketide synthases (PKS), whereas the other twelve gene clusters contained genes encoding for the synthesis of non-ribosomal peptide synthases (NRPS). Moreover, fragments per kilobase of exon per million mapped fragments (FPKM)-based RNA sequencing was used to evaluate gene expression using reference genes such as *gpdA*, *tubC*, and *actA* as housekeeping genes [42]. The various roles that fungal endophytes play in plant ecosystems and agriculture are becoming better understood through genetic research. They emphasize how these fungi could be used in a variety of industries, including agriculture and medicine.

3.2. Metagenomics

One of the fundamental components of genomic research that reveals the genome, or DNA identity and integrity, of endophytic microorganisms from plant samples is metagenomics [43]. Metagenomic techniques have high importance for the study of fungal endophytes due to the fact that metagenomics provides a reliable tool for investigation into the diversity, potential development, and ecological functions of these fungi in host plants and habitats to which they are related [44]. Metagenomics makes the study of fungal diversity in plant tissues and other environmental materials feasible. It gives the count and type of fungal species, giving an insight into the composition of fungal endophyte communities. Technologies based on a high-throughput metagenomic approach provide detailed insights about the morphology, physiology, and dynamics of the microbiome. Fungal endophytic strains have been screened using these methods. It has given scientists a variety of tools for the rapid and economic analysis of DNA sequences from environmental samples [30].

By employing the mNGS technique, the complicated properties of bacteria in plants can be studied without the necessity of culture. In addition, a number of endophytic bacteria and fungi from sorghum and oak plants have been examined using Illumina and Roche's technique of 454 pyrosequencing [45,46]. Shotgun metagenome sequencing analysis is performed on various bioinformatics platforms, MG-RAST and PiCRUST, along with accompanying functional pipelines that help show both the taxonomy and potential functional genes implicated in plant growth promotion [47,48].

As an initial step, ITS region sequencing was used for analyzing the fungal communities of samples, but it was restricted to a few samples. These were usually culture-driven analyses. Furthermore, the identity of a fungal endophyte isolated from the medicinal tree, namely *Aquilaria malaccensis*, was determined using internal transcribed spacer region sequencing, and a detailed analysis of it was presented [49]. The new procedure for the ARISA technique has been introduced to identify the diversity of fungal species present in the environment. This specifically looked into the lengths of ITS1 rDNA for the various fungal species [50]. The first publication using NGS to analyze the ITS region of a residential endophytic species in a South African host plant was published [51]. Illumina fungal sequencing was used in another study to enable the creation of the network, providing the occurrence and co-occurrence of symbiosis [52]. Endophytic fungus populations in the leaves of *Pleioblastus amarus*, *Bambusa rigida*, and *Phyllostachys edulis* were surveyed using robust ITS sequences from the Illumina MiSeq™ high-throughput. The most prominent enriched endophytic fungus species were *Cladosporium*, *Trichomerium*, unclassified_p_Ascmycota, *Sporobolomyces*, and *Camptophora* [53].

A metagenomics method allows for opening up the hidden potential of uncultured microbial communities, or endophytes, beyond the information that is obtainable from the genomes of particular taxa [54]. The method entails sampling every individual's DNA and determining the genetic content. Based on the metagenomic procedure, Langa-lomba et al. provided information on the fungal microbiota of two Somontano Vineyards under the PDO, which are located in Huesca, Spain. Its findings showed a unique mycobiota of the inner wood (and, to a lesser extent, of other organs of plants) composed of microorganisms that have been frequently mentioned in related previous research whose priming effect the grape plant promotes, which is variable depending on the genotype under consideration, the management strategy used, or the crop age [55]. A study by Pais et al. [56] reveals that metagenomic analysis has led to the discovery of

hidden relationships between fungal density, plant disease variability, and genetic distance in *Cornus florida* (Cornaceae), which implies that there is an association between the phylogenetic patterns among the fungi involved in the plant disease.

Both metagenomics and computational methods have now become important molecular approaches for determining the functional genes of fungal endophytes from their host plants. One of the chosen model plants used to study the relationship of fungal endophytes with different host plant tissues with diverse levels of biological strength is *Ephedra sinica* [8]. Considering the multitude of endophytic members isolated from the genera *Talaromyces*, *Aporospora*, and *Aspergillus* all from the same plant root, a particular and prolific prevalence of endophytic fungal strains of the genus *Phyllosticta* has been reported from the roots and stem of *Ephedra sinica* [57].

3.3. Transcriptomics

The field of transcriptomics is one of the subfields of molecular biology that focuses on the transcriptome of an organism—all RNA molecules produced by the genome at a time [58]. Transcriptomics focuses on the quantification of gene expression at the RNA level, including the production of all types of RNA molecules, including messenger RNA (mRNA), short noncoding RNA (ncRNA), and small RNA. A common technique in transcriptomics is RNA-Seq [58]. The entire fungal endophyte transcriptome is scrawled using RNA sequencing (RNA-Seq). It provides researchers with data on unique transcripts, alternative splicing, and gene expression levels in different conditions to help better interpret the dynamic changes in gene expression during the interaction with the host. The other approach for transcriptome analysis is microarray technology [59]. Microarrays allow researchers to simultaneously gauge the expression levels of hundreds of genes. Through transcriptomics, genes that are activated or deactivated in response to external stimuli or specific situations can be traced. The interpretation of gene expression pattern changes within different biological conditions that heavily rely on differential gene expression analysis [59].

It has been found that transcriptomics is an applicable approach for studying microbial communities associated with various plants [60,61]. Transcriptomics helps in understanding the mechanisms of adaptation of microbial communities in environments that are changing by comparing the transcriptomes of interacting species groups. While most of the research based on the genome and metagenome of endophytes lists the presence or absence of some specific genes, understanding endophytic phenomena requires an understanding of the expression of certain genes in different microenvironments. A thorough analysis of the symbioticity of bacterial and host plant genes, which are differentially expressed genes (DEGs), would shed light on the basic aspects and mechanisms of their mutualistic functions.

By comparing the transcriptomes of endophyte-free and endophyte-infected plants, the identification of the underlying mechanisms of endophyte-mediated disease resistance and plant growth promotion abilities can be simplified. Comparative studies in contrast to differentially expressed patterns of endophytes in the host plant and outside can be of use to find the aspects of interaction involved in maintaining linkage. Moreover, research may likewise focus on the differential expression of numerous host plant genes under different conditions, whether or not endophytes are present. Suppression subtractive hybridization (SSH), microarray analysis, and SOLiD-SAGE-like approaches are effectively incorporated into differential expression analysis [30,62]. Transcriptomics that rely on genomes offers critical support for successful genome use. The elucidation of

Table 1: Transcriptomics studies unveiled the benefits of endophytic fungi to their host plants.

S. No.	Endophyte	Host Plant	Benefits	References
1.	<i>Piriformospora indica</i>	<i>Hordeum vulgare</i>	Induction of systemic disease resistance	Molitor et al. [63]
2.	<i>Fusarium verticillioides</i>	<i>Zea mays</i>	Decrease in the negative effects of phytopathogens	Jonkers et al. [64]
3.	<i>Colletotrichum tropicale</i>	<i>Theobroma cacao</i>	Alterations in the physiology, metabolism, and morphology of the host; resistance to infections and herbivores	Mejia et al. [65]
4.	<i>Epichloë festucae</i>	<i>Lolium perenne</i> L. cv Samson	Modifications to the host's development, specifically in the areas of trichome production and cell wall biogenesis; resistance to fungal infections and dehydration	Dupont et al. [66]
5.	<i>Piriformospora indica</i>	<i>Hordeum vulgare</i>	Salt stress tolerance	Ghaffari et al. [67]
6.	<i>Epichloë coenophiala</i>	<i>Lolium arundinaceum</i>	Resistance to disease and responses to abiotic stress	Dinkins et al. [68]
7.	<i>Chaetomium cupreum</i>	<i>Eucalyptus globulus</i>	Tolerance to heavy metals; intricate control of auxin metabolism and biosynthesis to promote plant growth	Ortiz et al. [69]
8.	<i>Exophiala pisciphila</i>	<i>Zea mays</i>	Tolerance to heavy metals by the remodeled host cell walls	Shen et al. [70]
9.	<i>Pestalotiopsis</i> sp. strain 9143	<i>Platycladus orientalis</i>	Expose growth regulation and sulfur exchange in the initial stages of symbiotic interaction	Shaffer et al. [71]
10.	<i>Trichoderma virens</i>	<i>Zea mays</i>	Development as a biocontrol agent	Malinich et al. [72]
11.	<i>Alternaria burnsii</i> NCIM 1409	<i>Nothapodytes nimmoniana</i>	Insights into the camptothecin biosynthesis ability	Natarajan et al. [73]
12.	<i>Aspergillus oryzae</i> YRA3	<i>Atractylis carduus</i>	A probable biological agent to control <i>Rhizoctonia</i> root rot of sorghum	Rashad et al. [74]

the endophytic lifestyle of symbionts is thus enabled by the integrated approach of genome and transcriptome analysis. One of the early transcriptome-based studies focused on the genes associated with the pyrimidine metabolism of *Epulorhiza* sp., an endophyte isolated from the roots of *Anoectochilus roxburghii* [17]. It has been observed that the grass *Festuca rubra* that has the endophytic fungus *Epichloë festucae* integrated with it demonstrates very different antifungal gene expression patterns [62]. This highlights the fact that endophyte–grass symbiosis is marked by molecular interactions and alterations in gene expression [Table 1].

3.4. Metatranscriptomics

Metatranscriptomics is an emerging field that pertains to the pattern characterization of gene expression by sequencing the expressed gene in the microbial community and has led to the discovery of many unknown plant–pathogen interactions [75]. In metatranscriptome due to the dominance of rRNA a strong community profile is generated for organisms belonging to different domains of life. This technique has been applied to study diversified niches such as oceans, soil, and rhizosphere of plants [76-79]. The simpler definition for metatranscriptome could be the total group of RNA content or that gives an overall picture of the gene expression of a microbial community. The primary aim of metatranscriptomics is to go in-depth detailing of the active metabolic pathways performed in the environment. The transcriptomes of an individual whether in the community or pure culture are dominated by the ribosomal RNA and this dominance in 16S and 23S subunits of prokaryotes can be visualized using the agarose gels.

This advanced RNA sequencing technique employed on the collected samples from numerous ecosystems helps in the study of microbial diversity, plant–microbe interactions, isolation of plant growth-promoting microbes, and also identification of potential biological control agents [80]. The neighboring plant species share their mycorrhizal symbionts despite being associated with different mycorrhizal fungi such as ericoid, ectomycorrhizal, and arbuscular mycorrhizal fungi. It is the composition of the plant community that helps in forming the silhouettes of fungal community. Also these fungal communities undergo seasonal changes that may differ between consecutive years, and therefore making the process of their development acyclic. This gives an understanding that the different taxa, their existing correlation, and their interaction with the associated plant community cannot be explained with the assessment of parameters within a set period of time, and here the use of metatranscriptomes and RNA-based methods could aid better in the analysis of their responses better [81].

A study was conducted in Argentina for the characterization of four different fungi involved in the grapevine trunk diseases locally known as “Hoja de malvón” affecting grapes. Here a metatranscriptomic approach is used for the different aspects of the process of characterization from the construction of molecular marker to kmer count evaluation. As a result, a number of microorganisms with a negative association with the disease pathogen were identified as potential biological control agents. In another microbiome study conducted on mummified peach fruits in Korea, an amalgamation of metagenomics and metatranscriptomics was used. The results showed the co-inhabitation of fungi and bacteria, and by combining the DNA shotgun and RNA sequencing the diversity of microbial community was increased [82]. A metatranscriptomic comparison of interactions between endophytic and pathogenic *Fusarium* with the *Arabidopsis* plant revealed plant transcriptional plasticity at the early level of infection and therefore giving an understanding of gene

regulation to different responses generated by fungal endophytes and pathogen complexes [83]. The metatranscriptomic analyses of grapes revealed the complex interaction dynamics of its microbiome and also highlighted the differences in expressed functional genes of filamentous and yeast fungi during noble rot and gray rot [84].

3.5. Proteomics

The real workhorses living in the cell and reacting to the surrounding environment with a denotation of the active state of the cell are proteins. A large number of research efforts have been invested in the understanding of plant–microbe interaction. Proteomics is used to get an insight into the plant defense mechanisms performed during conditions of biotic stress [85]. Proteomics is defined as the study of proteins extensively describing their abundance, modification, and binding nature with associated networks. In simpler terms, it is the study of different proteins that are expressed by an organism [86]. Proteomics is compatible with working alongside other functional genomics such as transcriptomics and metabolomics. The use of mass spectrometry in recent years has become dominant in the field of proteomic evaluations [30]. It has brought a certain kind of revolution to the fields of research, agriculture, and clinical trials. The different kinds of techniques that are being used in proteomics are gel based (fluorescent two-dimensional difference gel electrophoresis, two-dimensional gel electrophoresis) and gel free (multidimensional protein identification technology, isotope-coded affinity tags, isobaric tagged for relative and absolute quantitation, mass spectrophotometry, and MALDI-TOF) [75].

The advancement in bioinformatics tools and techniques has made functional identification studies in proteomics more accessible and easier. There are several database and search algorithms such as SEQUEST, Mascot, PeptideProphet, ProteinProphet, and DBParser that aid in proteomic analysis [87-90]. A larger percentage of crop losses every year are caused by fungi though the bacteria result in most number of plant diseases. Proteomics have been used to describe the plant–fungi interactions related to biotrophic and necrotrophic fungi groups. It could provide a better understanding of their relationship at both quantitative and qualitative levels and enhance knowledge of management strategies. Though a lot of consideration is given to plant and fungi associations with the help of proteomics, there is still a need to devolve more into solving the constitutional queries. The mutually beneficial relationship shared among the plants and fungi helps in the in-depth adjustment of plant metabolism and the regulation of different molecular mechanisms. Also, many of these mechanisms are not able to be characterized in a proper manner [91].

Endophytic fungi reside inside the living cells of the host plant. They do not sporulate and are in a mutualistic relationship with the host plant. They are an important form of bioresource. Endophytic fungi have abundant benefits and applications in the fields of agriculture, industry, and medicine. With the help of protein profiling, one gets an overall snapshot of pathogens' conversion to endophytes. In the last five decades, plant–fungus interaction has been a keen topic of interest for researchers, although the information related to sequences in public databases is limited. This gives an opportunity for proteomics to be associated with the comparative analysis of plants. Also, in modern times, fungal biology is also facing challenges in understanding the function, expression, and regulation of the whole protein content coding by the fungal genes. And this understanding is vital for assessing plant–fungal interactions, fungal colonization, and pathogenicity. The use of proteomics in this regard plays a vital role in mapping the changes in plant–microbe interaction studies [92].

A study was undertaken to conduct a comprehensive proteomics analysis of arbuscular mycorrhizal fungi-modulated proteins both at the root and shoot/leaves levels. The results concluded the presence of several protein transporters helping in mineral uptake, along with the ribosomal translational apparatus playing a big role in the systemic reprogramming of translation [93]. An experiment was conducted using transcriptomics and proteomics techniques on endophyte-inoculated and free plants to evaluate the effect of fungal endophyte *Gilmaniella* sp. AL12 on *Atractylodes lancea* plant's metabolism and other related regulatory processes at both translational and transcriptional levels. It was concluded that the fungal endophyte weakens the immune response of the host plant, their interaction increased the biomass and sesquiterpenoid content, increased photosynthesis, expanded the TCA and glycolysis cycle, and enhanced metabolic flux [94]. Entomopathogenic fungi such as *Beauveria bassiana*, *Lecanicillium* cf. *psalliotae*, and *Lecanicillium dimorphum* may act as endophytes colonizing the palm tree tissue. The proteomic techniques were used to study the interaction of these fungi with the date palm tree *Phoenix dactylifera* L. at the molecular level. An endophytic colonization of these fungi modulating plant defense responses, energy metabolism, and possibility of modulating cell division-related proteins in the in vitro palms was suggested [95].

Piriformospora indica is known to promote growth and help in the survival of a diverse number of plants in times of abiotic stress, especially during drought. The barley plant leaves exposed to moisture stress were inoculated with *P. indica* and its response was characterized using proteomics and metabolomics. The other conclusion drawn from the interaction was that the colonization of roots by the fungal endophyte increased the activity of photosystem and electron transfer chain, and in addition to this promotion it accumulated protective proteins pertaining to functions such as energy modulation, photorespiration, autophagy, primary metabolism, and transporters. In another study related to *Piriformospora indica*, quantitative proteomic analysis of the fungus and its interaction with the host plant and rhizospheric bacteria was performed for the reflection of associated, hidden proteins and enzymes. Also a protocol was described for better extraction of the cellular proteins from the fungus. The protocol included 2D gel electrophoresis after the interaction with *Azotobacter chroococcum* in the axenic culture [96]. *Serendipita indica*-colonized barley plants were analyzed for proteomic analysis, and it was revealed that the fungus improved photosynthesis in barley plant, *Hordeum vulgare* L. under salt stress conditions and provided the protein profiling of the functional proteins [97].

3.6. Metaproteomics

Metaproteomics revolves around the functional identification of the expressed metagenome functional expression and also includes the interpretation of the associated metabolic functions that occur in a community during the sample collection. It is also referred to as whole-community proteomics. Metaproteomics plays a significant role in the functional identification of new genes expressed in stress conditions and also in the genomic diversity of microbiome complex in a particular environment [98,99]. The extraction of total protein from the microenvironment can be done using either direct or indirect lysis. Through direct lysis the total protein content can be extracted directly from the plant endosphere in a natural or stressed condition. Also, the analysis of protein fingerprints can be done to study the potential of endophytes and the effect of their metabolite production. In the case of indirect lysis, the extraction of total protein content is carried out from already isolated endophytes in a stressed environment.

The 2D gel electrophoresis is used for obtaining protein fingerprints that can be further used for the analysis of the role of endophytes in stressful conditions [98,96]. Metaproteomics uses the high-end performance of mass spectrophotometers for the exclusive protein characterization expressed by a microbial community in a given sample [30]. It quantifies the peptides and proteins by spot intensity analysis on the gels and in liquid chromatography by protein tagging. But for measuring the relative abundance signal intensity/spectra counts may be used [100-102]. A study was conducted to evaluate the relationship of symbiotic microbiota with *Dermatocarpon miniatum*'s entire lichen thallus by using the metaproteomic analysis. The result showcased the identification of 138 proteins after the use of SDS-PAGE, LC/MS analysis, and Mascot search in UniRef100 and Swiss-Prot databases. Both the proteins from lichens and fungi were associated with different microbial communities, and they also gave extended input to the fungal and algal associations [103].

3.7. Metabolomics

In many biological domains, the advent of high-throughput technology known as “omics” has proven to be tremendously advantageous [104]. Metabolomics has surfaced as a potentially valuable technique for phenotypic characterization in dynamic contexts [105]. The goal of a field of study known as “metabolomics” is to identify and quantify each and every metabolite that exists in a given organism [106]. Small molecules (less than 1 kDa) that are either products or intermediates in metabolic processes are commonly referred to as metabolites. The understanding of the interactions between plant microbes has grown significantly in the past few years, but the chemical communication that results in priming is still not widely understood [107]. As a supplement to existing “omics” techniques, metabolomics provides the ability to characterize changes to the metabolomes of interacting species through a variety of sophisticated bioanalytical techniques combined with chemometrics and bioinformatics tools [108]. Gaining more insight into the separate metabolisms of the plant and its endophyte, as well as the metabolic interplay supporting the interactome, can be facilitated by metabolomics [109].

Metabolomic analysis frequently entails a number of processes, including sample preparation, measurement, and data processing [110]. In metabolomics, quenching is a technique used to stop metabolite turnover, especially during sample preparation and collection. This mechanism is quite efficient in processing most key metabolites, such as sugars, amino acids, organic acids, and carbohydrates. Secondary metabolites, which are a group of metabolites derived from three families, including phenolics, alkaloids, terpenes, and steroids, typically have a much slower turnover rate and are more chemically stable than primary metabolites, which must be quenched during sample preparation [111]. These subsequent types of metabolites are sometimes of higher interest to traditional medicine. Samples are extracted after quenching, and this procedure typically uses a range of organic or inorganic solvents, such as methanol, ethanol, ethyl acetate, or hexane, depending on the target metabolites [112]. Identification of metabolites is as important a step in metabolomics as extraction and quantification. There are two different but complementary ways to accomplish this: untargeted and targeted [113].

Metabolites that have been isolated from fungi can be characterized using a variety of methods [114]. Sample preparation, data collection, data mining and analysis, statistical modeling, signature biomarkers, and biochemical interpretation are all included in an adaptable metabolomics flowchart [115]. Various techniques have been employed by scientists for conducting metabolite profiling [116]. Several detection techniques are commonly used to separate and

identify compounds, including liquid chromatography–ultraviolet and visible spectrum or diode array detection [LC-UV (DAD)], liquid chromatography–mass spectrometry (LC-MS), LC-MS/MS, various forms of high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) imaging, gas–liquid chromatography, and liquid chromatography/time of flight–mass spectrometry (LC-TOF/MS) [28,117-119]. The spatial metabolome may be utilized based on desorption electrospray ionization–imaging mass spectrometry (DESI-IMS), matrix-assisted laser desorption/ionization-IMS (MALDI-IMS), as well as airflow-assisted desorption electrospray ionization mass spectrometry imaging (AFADESI-MSI) when traditional MS is unable to reliably detect spatial–temporal occurrences of metabolites [28].

4. CONCLUSION

Fungi, the very important part of Eukarya domain, have been known since a long time and are found in various niches. This category of organism plays various significant roles in the functioning of various organisms, including plants. Several fungi are associated with the internal tissues of plants, which play a vital role in their survivability. After a long period of research, endophytic fungi are known to have a wide range of applications in various industries, and several species have been recognized as industrially important. However, after a long study, a huge number of fungi are still unknown because they cannot be cultured, so omics tools are a solution with the help of which unknown fungal species could be researched and their interactions could be studied. In future, several omics tools could be used to study the fungal diversity residing inside the plants, and their applications could be explored.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

10. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

11. PUBLISHER'S NOTE

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