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Metagenomic Exploration of Endophytic Fungal Diversity in Germinating Groundnut Seed

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ABSTRACT: Groundnut (*Arachis hypogaea* L) a oil seed legume crop has been widely cultivated and its adaptability and commercialisation is closely related to its mycobiota. Nevertheless, although these beneficial microorganisms participate in seed formation, the metabolism of nutrients, disease immunity and resistance to stress, there is hardly any attention given to this microbiota diversity because culture-dependent approaches are limited. In this study, a high-throughput metagenomic sequencing using ITS [internal transcribed space] region was employed for analyzing the endophytic fungal community during groundnut seed germination. The seeds after collected from Acharapakkam, Tamil Nadu, India, were surface sterilized and germinated in the laboratory and subjected to Illumina MiSeq sequencing. A total of 197 fungal species in which (72.91%) belong to the Ascomycota were assigned to taxonomy using QIIME2-based genomics processing and compared to the UNITE database. The proportion of yeast-like as opposed to filamentous taxa such as *Candida*, *Pichia*, *Wickerhamiella* and *Kodamaea* against *Aspergillus*, *Talaromyces* and *Fusarium* were more prevalent in this study. Also some rare and curious taxa, under phylum Basidiomycota such as *Tylospora asterophora*, *Sterigmatomyces halophilus* and *Basidiobolus* have brought to light the secret diversity dwelling in groundnut tissues in metagenomic study. Functional annotation presented high diversity of ecological roles, comprising commensalism, mycoparasitism, saprotrophic and opportunistic pathogenicity. This study integrates a metagenomic approach with traditional fungal microbiological research to gain a comprehensive understanding of beneficial fungal populations. This study finds that germinating groundnut seeds have a changing and functional diverse mycobiome that confer resilient and early growth of the plant.

INTRODUCTION

Groundnut (*Arachis hypogaea*) is also known as peanut, in tamil name it is called as nilakadalai or verkadalai. It is self pollinating oilseed- legume crop grown across worldwide in tropical and subtropical regions from the family fabaceae(1). It has numerous health benefits including vitamins and minerals, but it is an incomplete protein source. In groundnuts, endophytic fungi have applications to foster environmental farming, improve soil health, boost endurance to stress and disease, and accelerate growth. Their beneficial works make them an essential instrument to aid in groundnut safety and productivity, especially beneath harsh conditions of nature. Fungal endophytes play a significant role in the groundnut lifecycle by aiding in seed germination, bolstering seedling vigor, promoting disease resistance, and enhancing biocontrol and phytohormone functions. By integrating both traditional culturing techniques and modern metagenomics, we are now better informed of the structural and operational diversity of microbial communities in plants, especially endophytes(2). Unfortunately, culture-based isolation techniques are limited in selecting media and the viability of microbes outside their native habitat. As a result, only 1–10% of the total microbial diversity is recovered from the endophytic community(3). On the other hand, metagenomic techniques, like high-throughput sequencing of ITS or 18S rRNA genes, offer a thorough picture, encompassing all culturable and non-culturable fungal taxa linked to germinated seed(4).

The integration of both approaches is effective in identifying latent microbial relationships concerning the groundnut seeds which have germinated(5).Meanwhile Metagenomics helps in identifying the fundamentally essential or scarce fungal taxa that are non-culturable in labs but play vital roles in ecosystem functions, while culture-based techniques help in identifying strains that have proven bioactive properties, like cellulase production or pathogen suppression.

Metagenomic profiling of germinated peanut seeds not only reveals a dynamic and diverse mycobiome that includes supportive and hidden pathogenic species (6), but also aids researchers in tracking microbial machinations during development. This is critical in bioengineering microbial bioinoculants or seed treatments aimed at enhancing emergence alongside the disease resistance. In addition , a practical metagenome, for example, genes linked to antifungal activity or metabolite biosynthesis, provides information about the impact of endophytes on host physiology during the early developmental stages(7).

So, understanding the relationship between metagenomics and the classical methods of fungal isolation goes beyond methods of work: it is vital in formulating the complete picture of the groundnut seed–endophyte system. This is because the initial culture-based studies support direct application in biocontrol or biofertilizer development. However, metagenomics uncovers the wider ecological systems and functional capabilities of the seed microbiome. However, the two approaches should be combined because it is essential in the identification of elite fungal consortia combined for seed treatment, enhancement of germination, and sustainable peanut cultivation under biotic and abiotic stress.

MATERIALS AND METHODS

Groundnut seed samples were collected from the farm field from Acharapakkam, near Pallipettai, Tamil Nadu, India. The seeds obtained were carefully washed to free them from soil particles adhering to the outer surface, and preserved in sterile containers. For the growth of germinating seeds were then placed in a petriplate immersed in cotton with distilled water. The germinated seeds for endophytic fungi analysis were surface sterilized by standard protocols in order to get rid of the external microorganisms. The internal Tissues of the germinated seeds which is grown(stem,leaf,root,cotyledon) were harvested for metagenomic DNA extraction after they were sterilized. DNA from germinating seeds, was extracted as per the manufacturer's instructions (DNA extraction kit). High throughput sequencing of endophytic fungal communities was conducted to uncover and describe the diversity of endophytic fungi in distinct tissues of the groundnut plant.

DNA EXTRACTION AND ITS REGION

Extraction of DNA in germinating seed samples were extracted in a CTAB extraction (Cleansorb-Funakoshi, Tokyo, Japan), a method while cetyltrimethylammonium bromide (CTAB) method, it was successful in extracting high quality DNA from germinated seed sample. The quality of the purified DNA was assessed by 1.5% agarose gel electrophoresis after extraction. The DNA was quantified using the qubit DNA BR assay kit (Invitrogen, Carlsbad, CA). The isolated DNA samples were then normalized to the same concentration, pooled together into a mix and stored. Fungal communities were analysed by PCR amplification of the internal transcribed spacer 2 (ITS2) with the primers ITS3 (5' GCATCGATGAAGAACGCAGC 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3').

PREPARING AND SEQUENCING AN NGS LIBRARY

We purified the PCR products after successfully amplifying the targeted DNA regions (for example, ITS2 for fungi) and used them to prepare the Next-Generation Sequencing (NGS) library. We used a standard two-step PCR protocol to prepare the library. In the second PCR step, we added sample-specific index barcodes and adapters that work with Illumina. We first used the Qubit DNA BR Assay (Invitrogen, Carlsbad, CA) to measure the amount of purified amplicons and the Agilent 4200 TapeStation system to check their quality. The final library was made by combining samples that had the same number of moles of each. We used the Illumina MiSeq platform (Illumina, San Diego, CA) to sequence the pooled library. The sequencing chemistry we used was paired-end (2 × 250 bp). This sequencing method makes sure that the ITS2 region is well-covered and that the reads are of high quality. This lets accurately identify endophytic fungal communities that are linked to plant samples. Before the downstream bioinformatics analysis, the raw reads were filtered for quality.

DATA ANALYSIS AND FUNGAL IDENTIFICATION

We sequenced the amplified Internal Transcribed Spacer (ITS) region from the extracted DNA using high-throughput platforms to make a profile of the fungal community. Using specific primers (like ITS1/ITS4), the ITS region, which is often

used as a DNA barcode for fungi, was amplified through PCR (Polymerase Chain Reaction). QIIME2 (Quantitative Insights Into Microbial Ecology 2) is a popular tool for microbiome and mycobiome studies that was used to process and analyze the raw sequencing data. The first step were to demultiplex, check the quality, and denoise the amplicon using DADA2 or Deblur. These programs fixed mistakes in the sequencing and found Amplicon Sequence Variants (ASVs). We found and got rid of chimeric sequences, which are mistakes that happen during PCR, to avoid giving the wrong taxonomic name. Also, sequence truncation was done based on quality score thresholds to keep high-confidence reads for later analysis. Scientists used a Naïve Bayes classifier trained on the UNITE fungal ITS database to identify and classify the ASVs. This method allowed them to pinpoint fungal taxa from phylum to species level. This thorough approach helped them to map out the variety of endophytic fungi found in the groundnut plant samples.

FUNGAL DIVERSITY

QIIME2 played a role in sequence processing and taxonomic classification for fungal diversity analysis. The process grouped sequences into Operational Taxonomic Units (OTUs) with 97% similarity. To ensure reliability, the analysis included OTUs with sequence reads between 10 and 100.

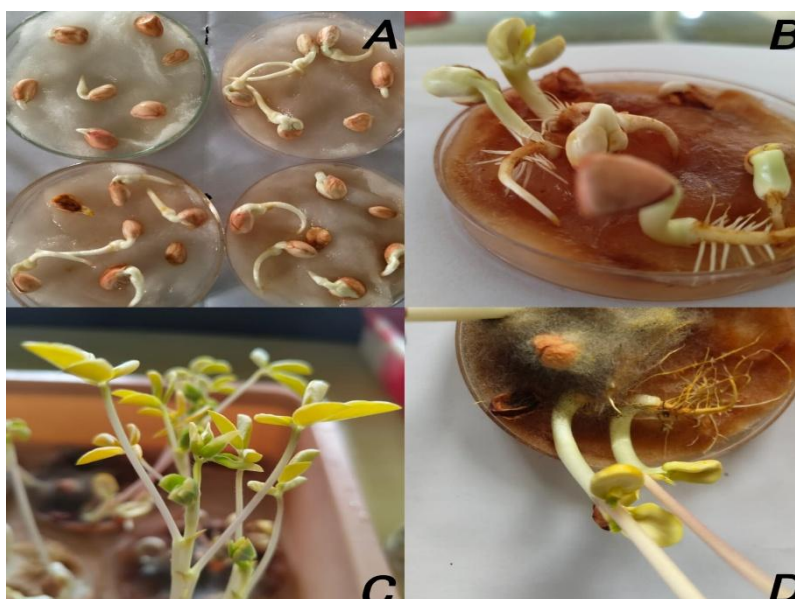
FUNCTIONAL ANNOTATION

We carried out functional annotation to evaluate the ecological roles and biological traits of the identified fungal taxa. Also predicted functional roles based on taxonomic identity, which included groupings like saprotrophic, pathogenic, or symbiotic fungi. Cellularity determination helped distinguish unicellular yeasts from multicellular filamentous fungi. We also categorized fungi as microfungi or macrofungi based on their shape and structure. Later then we figured out the mode of sexual reproduction (teleomorphic or anamorphic) from taxonomic lineage, microscopic characterisation and database references, which gave insights into how these fungi reproduce.

RESULTS

Groundnut seeds were germinated for 10-15 days under controlled conditions in the laboratory until the plant grew to a viable size for examination. The growth process proceeds a specific timeline, during the seed germination of various stages (Fig 1). In the first 1-3 days, the root emerges from the seed. Later 4-8 days of time period the stem begins to grow. From the days of 9-12 the leaf had formed and finally from day 12-15 the plant starts to develop cotyledons and complete their growth development.

Fig1: Growth of the Germinated seeds (A-Root, B-Stem, C-Leaf, D-Cotyledon)



Fungal diversity

The endophytic fungal diversity was further characterized by metagenomic sequencing of the seedlings. We obtained a total of 197 species of fungi and grouped them at the level of genus or species. The NCBI database has obtained the relevant sequence information that will be submitted regarding these taxa, and a differentiated accession ID has been assigned to each of them (TABLE1).

TABLE 1: Identified Genera/Species with NCBI Accession ID

S.no	Genera /species	NCBI ACCESSION ID
	ROOT	
1	<i>Rhizopus arrhizus</i>	PV893169
2	<i>Trichosporon asahii</i>	PV893170
3	<i>Hannaella taiwanensis</i>	PV893171
4	<i>Filobasidium sp</i>	PV893172
5	<i>Malassezia sp</i>	PV893173
6	<i>Malassezia slooffiae</i>	PV893174
7	<i>Jamniaea rosea</i>	PV893175
8	<i>Sterigmatomyces halophilus</i>	PV893176
9	<i>Basidiobolus pelinellus</i>	PV893177
10	<i>Wickerhamiella versatilis</i>	PV893178
11	<i>Wickerhamiella pararugosa</i>	PV893179
12	<i>Starmerella etchellsii</i>	PV893180
13	<i>Diutina ranongensis</i>	PV893181
14	<i>Candida tropicalis</i>	PV893182
15	<i>Candida phangngaensis</i>	PV893183
16	<i>Candida baemuloni</i>	PV893184
17	<i>Candida glabrata</i>	PV893185
18	<i>Saturnispora silvae</i>	PV893186
19	<i>Saturnispora diversa</i>	PV893187
20	<i>Pichia mandshurica</i>	PV893188
21	<i>Pichia kudriavzevii</i>	PV893189
22	<i>Pichia cactophila</i>	PV893190
23	<i>Cyberlindnera jadinii</i>	PV893191
24	<i>Kodamaea ohmeri</i>	PV893192
25	<i>Geotrichum silvicola</i>	PV893193
26	<i>Geotrichum candidum</i>	PV893194
27	<i>Aspergillus pseudogracilis</i>	PV893195
28	<i>Aspergillus aculeatus</i>	PV893196
29	<i>Ampelomyces quisqualis</i>	PV893197
	STEM	
30	<i>Rhizopus arrhizus</i>	PV817758
31	<i>Trichosporon asahii</i>	PV817759
32	<i>Hannaella taiwanensis</i>	PV817760
33	<i>Hannaella oryzae</i>	PV817761
34	<i>Dioszegia zsoltyi</i>	PV817762
35	<i>Sterigmatomyces halophilus</i>	PV817763
36	<i>Pleurotus columbinus</i>	PV817764
37	<i>Wickerhamiella versatilis</i>	PV817765
38	<i>Wickerhamiella pararugosa</i>	PV817766
39	<i>Wickerhamiella brachini</i>	PV817767

40	<i>Starmerella etchellsii</i>	PV817768
41	<i>Diutina ranongensis</i>	PV817769
42	<i>Candida tropicalis</i>	PV817770
43	<i>Candida saopaulonensis</i>	PV817771
44	<i>Candida phangngaensis</i>	PV817772
45	<i>Saturnispora silvae</i>	PV817773
46	<i>Pichia terricola</i>	PV817774
47	<i>Pichia mandshurica</i>	PV817775
48	<i>Pichia kudriavzevii</i>	PV817776
49	<i>Pichia cactophila</i>	PV817777
50	<i>Dekkera sp.</i>	PV817778
51	<i>Kodamaea obmeri</i>	PV817779
52	<i>Geotrichum silvicola</i>	PV817780
53	<i>Geotrichum candidum</i>	PV817781
54	<i>Geotrichum candidum</i>	PV817782
	COTYLEDON	
55	<i>Rhizopus arrhizus</i>	PV701997
56	<i>Hannaella oryzae</i>	PV701998
57	<i>Malassezia slooffiae</i>	PV701999
58	<i>Malassezia japonica</i>	PV702000
59	<i>Jamniaea pallidilutea</i>	PV702001
60	<i>Sterigmatomyces halophilus</i>	PV702002
61	<i>Starmerella etchellsii</i>	PV702003
62	<i>Blastobotrys attinorum</i>	PV702004
63	<i>Candida tropicalis</i>	PV702005
64	<i>Pichia kudriavzevii</i>	PV702006
65	<i>Kodamaea obmeri</i>	PV702007
66	<i>Geotrichum silvicola</i>	PV702008
67	<i>Geotrichum candidum</i>	PV702009
68	<i>Talaromyces purgamentorum</i>	PV702010
69	<i>Talaromyces marneffei</i>	PV826838
70	<i>Aspergillus fumigatus</i>	PV826839
71	<i>Xenodidymella clematidis</i>	PV826840
	LEAF	
72	<i>Rhizopus arrhizus</i>	PV826841
73	<i>Ustilago sparsa</i>	PV826843
74	<i>Cutaneotrichosporon jirovecii</i>	PV826844
75	<i>Saitozyma paraflava</i>	PV826845
76	<i>Saitozyma flava</i>	PV826846
77	<i>Carlososaea hoberbergiae</i>	PV826847
78	<i>Papiliotrema terrestris</i>	PV826848
79	<i>Papiliotrema fusca</i>	PV826849
80	<i>Vishniacozyma victoriae</i>	PV826850
81	<i>Vishniacozyma insularis</i>	PV826851
82	<i>Hannaella sinensis</i>	PV826852
83	<i>Hannaella luteola</i>	PV826853
84	<i>Rhodospiridiobolus fluvialis</i>	PV826854
85	<i>Malassezia slooffiae</i>	PV826855

86	<i>Malassezia restricta</i>	PV826856
87	<i>Malassezia arunalokei</i>	PV826858
88	<i>Tilletia trachypogonis</i>	PV826859
89	<i>Tilletia setariae</i>	PV826860
90	<i>Phragmotenium oryzae</i>	PV826862
91	<i>Hasegawazyma lactose</i>	PV826863
92	<i>Erythrobasidium proteacearum</i>	PV826864
93	<i>Symmetrospora marina</i>	PV826865
94	<i>Cystobasidium slooffiae</i>	PV826866
95	<i>Sterigmatomyces halophilus</i>	PV826867
96	<i>Peniophora molesta</i>	PV826868
97	<i>Peniophora gilbertsonii</i>	PV826869
98	<i>Fomes meliae</i>	PV826870
99	<i>Irpex lacerates</i>	PV826871
100	<i>Ceriporia viridans</i>	PV826872
101	<i>Auricularia Australiana</i>	PV826873
102	<i>Tylospora asterophora</i>	PV826874
103	<i>Schizophyllum commune</i>	PV826875
104	<i>Coprinellus radians</i>	PV826876
105	<i>Pleurotus pulmonarius</i>	PV826877
106	<i>Xylaria hypoxylon</i>	PV826878
107	<i>Ascotricha lusitanica</i>	PV826879
108	<i>Microdochium paspali</i>	PV826880
109	<i>Hypoxylon sporistriataticum</i>	PV826881
110	<i>Hypoxylon notatum</i>	PV826882
111	<i>Hypoxylon lignicola</i>	PV826883
112	<i>Hypoxylon hypomiltum</i>	PV826884
113	<i>Hypoxylon addis</i>	PV826885
114	<i>Daldinia eschscholtzii</i>	PV826886
115	<i>Peroneutypa polyporae</i>	PV826887
116	<i>Sordaria fimicola</i>	PV826888
117	<i>Neurospora crassa</i>	PV826889
118	<i>Cladorrhinum flexuosum</i>	PV826890
119	<i>Fusarium protoensiforme</i>	PV826891
120	<i>Clonostachys miodochialis</i>	PV826892
121	<i>Plectosphaerella cucumerina</i>	PV826893
122	<i>Pestalotiopsis montellicoides</i>	PV826894
123	<i>Nigrospora zimmermanii</i>	PV826895
124	<i>Wickerhamiella versatilis</i>	PV826896
125	<i>Wickerhamiella pararugosa</i>	PV826897
126	<i>Starmerella etchellsii</i>	PV826898
127	<i>Starmerella apicola</i>	PV826899
128	<i>Hyphopichia burtonii</i>	PV826900
129	<i>Candida tropicalis</i>	PV826901
130	<i>Candida sake</i>	PV826903
131	<i>Candida phangngaensis</i>	PV826904
132	<i>Candida parapsilosis</i>	PV826905
133	<i>Candida inconspicua</i>	PV826906

134	<i>Kluyveromyces marxianus</i>	PV826907
135	<i>Kazachstania humilis</i>	PV826908
136	<i>Saturnispora diversa</i>	PV826910
137	<i>Pichia terricola</i>	PV826911
138	<i>Pichia mandschurica</i>	PV826912
139	<i>Pichia kudriavzevii</i>	PV826913
140	<i>Pichia kluyveri</i>	PV826914
141	<i>Pichia barkeri</i>	PV826915
142	<i>Kodamaea ohmeri</i>	PV826916
143	<i>Clavispora lusitaniae</i>	PV826917
144	<i>Geotrichum silvicola</i>	PV826918
145	<i>Geotrichum phurueaensis</i>	PV826919
146	<i>Geotrichum candidum</i>	PV826920
147	<i>Geotrichum candidum</i>	PV826921
148	<i>Yamadazyma triangularis</i>	PV826922
149	<i>Meyerozyma guilliermondii</i>	PV826923
150	<i>Debaryomyces prosopidis</i>	PV826924
151	<i>Sclerotinia nivalis</i>	PV826925
152	<i>Phialocephala fluminis</i>	PV826926
153	<i>Micarea myriocarpa</i>	PV826927
154	<i>Talaromyces purpureogenus</i>	PV826928
155	<i>Talaromyces purgamentorum</i>	PV826929
156	<i>Penicillium georgiense</i>	PV826930
157	<i>Aspergillus salinarum</i>	PV826931
158	<i>Aspergillus ruber</i>	PV826932
159	<i>Aspergillus puniceus</i>	PV826933
160	<i>Aspergillus pulvericola</i>	PV826934
161	<i>Aspergillus pseudogracilis</i>	PV826935
162	<i>Aspergillus occultus</i>	PV826936
163	<i>Aspergillus japonicus</i>	PV826937
164	<i>Aspergillus halophilicus</i>	PV826938
165	<i>Aspergillus fumigatus</i>	PV826939
166	<i>Aspergillus flavus</i>	PV826940
167	<i>Aspergillus clavatorphorus</i>	PV826941
168	<i>Exophiala jeanselmei</i>	PV826942
169	<i>Cladophialophora eucalypti</i>	PV826943
170	<i>Zeloasperisporium eucalyptorum</i>	PV826944
171	<i>Ochroconis mirabilis</i>	PV826945
172	<i>Torula chromolaenae</i>	PV826946
173	<i>Aurantiascoma nebelii</i>	PV826947
174	<i>Currularia lunata</i>	PV826948
175	<i>Alternaria celosiicola</i>	PV826949
176	<i>Septoriella oudemansii</i>	PV826950
177	<i>Phaeosphaeria musae</i>	PV826951
178	<i>Ophiosphaerella agrostidis</i>	PV826952
179	<i>Leptospora thailandica</i>	PV826953
180	<i>Edenia gomezpompae</i>	PV826954
181	<i>Periconia macrospinosa</i>	PV826955

182	<i>Periconia epilithographicola</i>	PV826956
183	<i>Paramonodictys paeoniae</i>	PV826957
184	<i>Neopyrenochaeta annellidica</i>	PV826958
185	<i>Neomassarina chromolaenae</i>	PV826959
186	<i>Pseudolophiostoma mangiferae</i>	PV826960
187	<i>Pseudopithomyces chartarum</i>	PV826961
188	<i>Kalmusia erioi</i>	PV826962
189	<i>Xenodidymella clematidis</i>	PV826963
190	<i>Hazslinszkyomyces sp</i>	PV826838
191	<i>Catenulostroma hermannusense</i>	PV826839
192	<i>Chaetothyria guttulata</i>	PV826840
193	<i>Pseudocercospora bakeri</i>	PV826841
194	<i>Passalora natrassii</i>	PV826842
195	<i>Ramichloridium eucleae</i>	PV826843
196	<i>Ramichloridium endophyticum</i>	PV826844
197	<i>Neodeightonia phoenicum</i>	PV826846

These results are visually supported by the bar graph (fig 2), where the relative proportions of each phylum level are clearly depicted. The dominance of Ascomycota suggests their potential functional importance in plant health, endophytic colonization, and nutrient cycling in the studied agroecological niche. Basidiomycota are in moderate which modify and degrade. The less abundant species phyla mucoromycota were present which encompass the pathogens, mycorrhizal fungi and known for the metabolite production. In 197 species leaf has 126 species, Root has 29 species, Stem has 25 species, Cotyledons has 17 species, whereas leaf is the highest number of species followed by Root, Stem, Cotyledon.

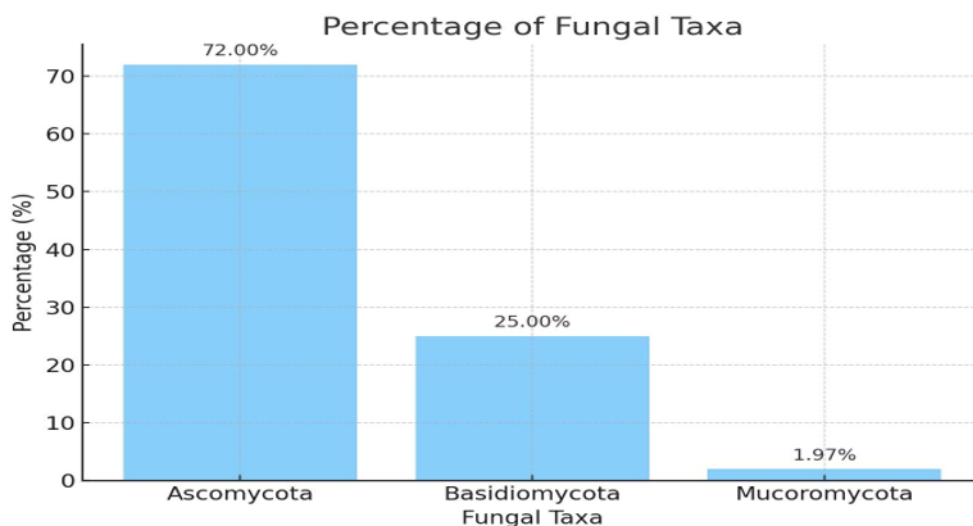


FIGURE 2: Percentage of fungal amplicon sequence variants (ASVs) at phylum level.

A total of 197 fungal species were analyzed from groundnut plant samples. These sequences were classified into three major fungal phyla: Ascomycota, Basidiomycota, and Mucoromycota (TABLE 2).

- Ascomycota was the dominant phylum, comprising 72.91% (144 species) of the total fungal taxa. This indicates a strong prevalence of filamentous ascomycetes and endophytic ascomycetous fungi within the groundnut-associated microbiome.
- Basidiomycota accounted for 25.12% (49 species), representing mostly yeast-like and saprotrophic fungi, which are common in phyllosphere and rhizosphere environments.

- Mucoromycota was the least represented, making up only 1.97% (4 species), and primarily included fast-growing saprotrophs like *Rhizopus arrhizus*.

TABLE 2: Assigned trophic roles, cellularity, micro/Macro classification and mode of reproduction for the identified genera/species

Taxa	Phylum	Trophic Roles	Uni/Multicellular	Micro/Macro Fungi	Mode of Reproduction
ROOT					
<i>Rhizopus arrhizus</i>	Mucoromycota	Saprotrophic	Multicellular	Microfungi	Asexual (sporangiospores), sexual (zygospores)
<i>Trichosporon asahii</i>	Basidiomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (blastospores), sexual (basidiospores)
<i>Hannaella taiwanensis</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Filobasidium sp</i>	Basidiomycota	Saprotrophic/Endophytic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Malassezia sp</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores rare)
<i>Malassezia slooffiae</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Jamnia rosea</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Sterigmatomyces halophilus</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Basidiobolus heliothys</i>	Basidiomycota	Mycorrhizal/Saprotrophic	Multicellular	Microfungi	Sexual (basidiospores)
<i>Wickerhamiella versatilis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Wickerhamiella parvulus</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Starmerella etchellsii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Diatrya ranongensis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Candida tropicalis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida phangngaensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida haemuloni</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida glabrata</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Saturnispora silvae</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Saturnispora diversa</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia mandshurica</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia kudriavzevii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia cactophila</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Cyberlindnera jadinii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kodamaea ohmeri</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Geotrichum silvicola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Aspergillus pseudogracilis</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus aculeatus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Ampelomyces quisqualis</i>	Ascomycota	Mycoparasitic	Multicellular	Microfungi	Asexual (pycnidia), sexual (rare/unknown)
STEM					
<i>Rhizopus arrhizus</i>	Mucoromycota	Saprotrophic	Multicellular	Microfungi	Asexual (sporangiospores), sexual (zygospores)

<i>Trichosporon asabii</i>	Basidiomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (blastoconidia), sexual (basidiospores)
<i>Hannaella taiwanensis</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Hannaella oryzae</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Dioszegia zsolzii</i>	Basidiomycota	Endophytic/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Sterigmatomyces halophilus</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Pleurotus columbinus</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Asexual (spores), sexual (basidiospores)
<i>Wickerhamiella versatilis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascos)
<i>Wickerhamiella pararugosa</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Wickerhamiella brachini</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Starmerella etchellsii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Diutina ranongensis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida tropicalis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida saopaulonensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida phangngaensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Saturnispora silvae</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia terricola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia mandshurica</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Pichia kudriavzevii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia cactophila</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Dekkera sp.</i>	Ascomycota	Fermentative/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kodamaea ohmeri</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Geotrichum silvicola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
COTYLEDON					
<i>Rhizopus arrhizus</i>	Mucoromycota	Saprotrophic	Multicellular	Microfungi	Asexual (sporangiospores), sexual (zygospores)
<i>Hannaella oryzae</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Malassezia slooffiae</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Malassezia japonica</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Jaminalia pallidilutea</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Sterigmatomyces halophilus</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Starmerella etchellsii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Blastobotrys attinorum</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida tropicalis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Pichia kudriavzevii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kodamaea obmeri</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Geotrichum silvicola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Talaromyces purgamentorum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Talaromyces marneffei</i>	Ascomycota	Pathogenic (dimorphic)	Dimorphic	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus fumigatus</i>	Ascomycota	Opportunistic Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Xenodidymella clematidis</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
LEAF					
<i>Rhizopus arrhizus</i>	Mucoromycota	Saprotrophic	Multicellular	Microfungi	Asexual (sporangiospores), sexual (zygospores)
<i>Wallemia sebi</i>	Basidiomycota	Xerophilic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (basidiospores)
<i>Ustilago sparsa</i>	Basidiomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (teliospores), asexual (sporidia)
<i>Cutaneotrichosporon jirovecii</i>	Basidiomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Saitozyma paraflava</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Saitozyma flava</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Carlosroa hoberbergiae</i>	Basidiomycota	Endophytic/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)

<i>Papiliotrema terrestris</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Papiliotrema fusca</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Vishniacozyma victoriae</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Vishniacozyma insularis</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Hannaella sinensis</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Hannaella luteola</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Rhodosporidiobolus fluvialis</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Malassezia slooffiae</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Malassezia restricta</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Malassezia cuniculi</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Malassezia arunalokei</i>	Basidiomycota	Commensal/Pathogenic	Unicellular	Microfungi	Asexual (budding), sexual (rare)
<i>Tilletia trachypogonis</i>	Basidiomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (teliospores, basidiospores)
<i>Tilletia setariae</i>	Basidiomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (teliospores, basidiospores)
<i>Jaminalia pallidilutea</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Phragmotenium oryzicola</i>	Basidiomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (basidiospores)
<i>Haseganazyma lactosa</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Erythrobasidium proteacearum</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Symmetrospora marina</i>	Basidiomycota	Saprotrophic (marine)	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Cystobasidium slooffiae</i>	Basidiomycota	Commensal/Endophytic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Sterigmatomyces halophilus</i>	Basidiomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (basidiospores)
<i>Peniophora molesta</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Peniophora gilbertsonii</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Fomes meliae</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores)
<i>Irpex lacerates</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Ceriporia viridans</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores)
<i>Auricularia australiana</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Tylospora asterophora</i>	Basidiomycota	Mycorrhizal	Multicellular	Microfungi	Sexual (basidiospores)
<i>Schizophyllum commune</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Coprinellus radians</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores)
<i>Pleurotus pulmonarius</i>	Basidiomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (basidiospores), asexual (conidia)
<i>Xylaria hypoxylon</i>	Ascomycota	Saprotrophic/Endophytic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Ascotricha lusitanica</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Sexual (ascospores), asexual (conidia)

<i>Microdochium paspali</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Hypoxylon sporistriataticum</i>	Ascomycota	Saprotrophic/Endophytic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Hypoxylon notatum</i>	Ascomycota	Saprotrophic/Endophytic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Hypoxylon lignicola</i>	Ascomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Hypoxylon hypomiltum</i>	Ascomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Hypoxylon addis</i>	Ascomycota	Saprotrophic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Daldinia eschscholtzii</i>	Ascomycota	Saprotrophic/Endophytic	Multicellular	Macrofungi	Sexual (ascospores), asexual (conidia)
<i>Peroneutypa polysporae</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Sexual (ascospores), asexual (conidia)
<i>Sordaria fimicola</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Sexual (ascospores), asexual (perithecia)
<i>Neurospora crassa</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Sexual (ascospores), asexual (conidia)
<i>Cladorrhinum flexuosum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Fusarium protoensiforme</i>	Ascomycota	Plant Pathogen/Saprotroph	Multicellular	Microfungi	Asexual (macro/microconidia), sexual (ascospores)
<i>Clonostachys miodochialis</i>	Ascomycota	Mycoparasitic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Plectosphaerella cucumerina</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Pestalotiopsis montelicoides</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Nigrospora zimmermanii</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Wickerhamiella versatilis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Wickerhamiella pararugosa</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Starmerella etchellsii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Starmerella apicola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Hyphopichia burtonii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida tropicalis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida saopaulonensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida sake</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida phangngaensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida parapsilosis</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Candida inconspicua</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kluyveromyces marxianus</i>	Ascomycota	Fermentative/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kazachstania humilis</i>	Ascomycota	Fermentative/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Saturnispora silvae</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Saturnispora diversa</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia terricola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia mandshurica</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia kudriavzevii</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia kluyveri</i>	Ascomycota	Fermentative/Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Pichia barkeri</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Kodamaea obmeri</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Clavispora lusitaniae</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)

<i>Geotrichum silvicola</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum phurueaensis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Geotrichum candidum</i>	Ascomycota	Saprotrophic/Pathogenic	Unicellular	Microfungi	Asexual (arthroconidia), sexual (ascospores)
<i>Yamadazyma triangularis</i>	Ascomycota	Saprotrophic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Meyerozyma guilliermondii</i>	Ascomycota	Opportunistic Pathogen	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Debaryomyces prosopidis</i>	Ascomycota	Saprotrophic/Endophytic	Unicellular	Microfungi	Asexual (budding), sexual (ascospores)
<i>Sclerotinia nivalis</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (ascospores), asexual (sclerotia/conidia)
<i>Phialocephala fluminis</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Micarea myriocarpa</i>	Ascomycota	Lichenized	Multicellular	Microfungi	Sexual (ascospores), asexual (conidia)
<i>Talaromyces purpureogenus</i>	Ascomycota	Saprotrophic/Endophytic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Talaromyces purgamentorum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Penicillium georgiense</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus salinarum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus ruber</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus puniceus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus pulvericola</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus pseudogracilis</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)

<i>Aspergillus occultus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus japonicus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus halophilicus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus fumigatus</i>	Ascomycota	Opportunistic Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus flavus</i>	Ascomycota	Opportunistic Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Aspergillus clavatorphorus</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Exophiala jeanselmei</i>	Ascomycota	Opportunistic Pathogen	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Cladophialophora eucalypti</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Zeloasperisporium eucalyptorum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), sexual (ascospores)
<i>Ochroconis mirabilis</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia), Sexual (ascospores)
<i>Torula chromolaenae</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia)
<i>Aurantiascoma nephelii</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Curvularia lunata</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia), Sexual (ascospores)
<i>Alternaria celosicola</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia)
<i>Septoriella oudemansii</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (ascospores)
<i>Phaeosphaeria musae</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (ascospores)
<i>Ophiosphaerella agrostidis</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Sexual (ascospores)
<i>Leptospora thailandica</i>	Ascomycota	Endophytic/Saprotrophic	Multicellular	Microfungi	Sexual (ascospores)
<i>Edenia gomezpompae</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia), Sexual (ascospores)
<i>Periconia macrospinosa</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia)

<i>Periconia epilithobigraphicola</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia)
<i>Paramonodictys paeoniae</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia)
<i>Neopyrenochaeta annellidica</i>	Ascomycota	Endophytic/Pathogenic	Multicellular	Microfungi	Asexual (conidia)
<i>Neomassarina chromolaenae</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Pseudolophiostoma mangiferae</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Pseudophthomyces chartarum</i>	Ascomycota	Saprotrophic	Multicellular	Microfungi	Asexual (conidia)
<i>Kalmusia erioi</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Xenodidymella clematidis</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Hazslinszkyomyces sp</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Catenulostroma hermanusense</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia)
<i>Chaetothyria guttulata</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia)
<i>Pseudocercospora bakeri</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia)
<i>Passalora natrassii</i>	Ascomycota	Plant Pathogen	Multicellular	Microfungi	Asexual (conidia)
<i>Ramichloridium enclae</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia)
<i>Ramichloridium endophyticum</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Asexual (conidia)
<i>Pseudorobillarda phragmitis</i>	Ascomycota	Endophytic	Multicellular	Microfungi	Sexual (ascospores)
<i>Neodeightonia phoenicum</i>	Ascomycota	Endophytic/Pathogenic	Multicellular	Microfungi	Asexual (conidia), Sexual (ascospores)

DISCUSSION

Fungal taxonomy and biodiversity

In this study, we examined groundnut (*Arachis hypogaea*) plant samples collected from Acharapakkam near Pallipettai for their endophytic fungal diversity using a metagenomics approach. This study uses detailed metagenomic sequencing to show a much wider range of fungi inside germinating seeds, including some that can't be grown in labs and rare types like *Xenodidymella clematidis* and *Micarea myriocarpa*. This is different from earlier groundnut microbiome studies, which used culture-based or

simpler methods (8,9). In this study the fungal species from laboratory growth are less compared to metagenomics in which 197 fungal species are found from major groups. Ascomycota was the most common, and yeast-like fungi such as *Candida*, *Pichia*, and *Wickerhamiella* played a big part, which wasn't often mentioned before. We highlight how these fungi help control the immune system (*Aspergillus fumigatus*), (*Ampelomyces quisqualis*), and make iron-grabbing molecules (*Rhizopus arrhizus*). This helps us understand more about how seeds germinate in an eco-friendly way (10,11,12). This work matters because it can help create new bio-based products to treat seeds for farming that can handle climate change. It does this by giving us a first look at the fungi linked to groundnuts. Future research should focus on co-culture tests to examine the interactions between microbes in mapping tissues to identify colonization trends and supporting gene labels with techniques that examine RNA or proteins. By utilizing their natural microbes to combat disease, aid in seed germination, and improve plant growth, groundnuts can be grown sustainably and this research contributes to our understanding of fungal diversity (13,14,15).

FUNCTIONAL ANNOTATION

Interestingly, *A. fumigatus* can encode clusters of genes involved in the biosynthesis of gliotoxins, which are important for avoiding the immune system and allowing the organism to survive inside the host(16). However, *Rhizopus arrhizus*, a member of the Order Mucorales, has genes that produce siderophores, such as heme oxygenase and rhizoferrin, which are essential for iron uptake in rhizospheres with low nutrient levels. This fungus can colonize and thrive in a range of environmental conditions because of its life cycle, which includes both asexual sporangiospores and sexual zygospores(17). Furthermore, these fungi mainly reproduce by budding and have altered mitochondrial genomes (18).

Their existence as endophytes implies a latent or opportunistic lifestyle that is influenced by host conditions, notwithstanding their potential for pathogenicity. In contrast, mycoparasitic fungi such as *Ampelomyces quisqualis* express β -1,3-glucanases and serine proteases, allowing destruction of other fungi, contributing to microbial balance within the plant microbiome (19). Additional gene annotation identified common domains associated with signal transduction (MAP kinases, GPCRs), antioxidant activity (superoxide dismutases), and secondary metabolite biosynthesis, indicating diverse functions in competition, mutualism, or plant defense modification. According to Rodriguez et al. (20), these functional characteristics are essential for endophytic colonization, host adaptation, and potentially promoting plant growth in stressful environments.

CONCLUSION

The study gives an insight into a large fungal variation on groundnut plant samples with the endophytic fungal diversity dominated by yeast-like genera: *Candida*, *Wickerhamiella*, *Pichia*, *Kodamaea*. These taxa, especially *Wickerhamiella versatilis* and *Candida tropicalis*, tend to associate more often than filamentous fungi such as *Aspergillus*, *Talaromyces*, and *Fusarium*; hence the proposition that yeasts may have gained adaptation to internal plant environments. Newly recorded and quite rare taxa under phylum basidiomycota such as *Carlososaea hoberbergiae*, *Tylospora asterophora*, *Peniophora gilbertsonii* and *Aurantiascoma nebelii* further bring to light the concealed fungal biodiversity in groundnut tissues. Comparatively, it seems that the yeast forms occupy the endophytic niche because they can invade plant tissues without causing any obvious harm, whereas filamentous fungi may be doing something more specialized or opportunistic. These variations point to multiple functions of plant-microbe associations, such as stress tolerance, nutrient cycling, and disease resistance. The results add to the current knowledge on groundnut endophytes while setting a firm platform for future research.

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