



RESEARCH ARTICLE

# Deciphering the effects of the culturable endophytic fungal community of maize under varied agronomic practices

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## Abstract

We used culture-dependent methods to isolate endophytic fungi from various tissues of maize (*Zea mays* L.), including leaves, cobs and roots. The study assessed the diversity of endophytic fungal communities in maize cultivated under long-term conservation agriculture practices in northern India, specifically conservative tillage (CT) and reduced tillage (RT). Endophytes were identified using ITS-rDNA sequencing to determine their distribution patterns within asymptomatic maize tissues. Leaf tissues harboured endophytic genera such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Sarocladium*. Premature cobs contained *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Kalmusia*, *Mucor*, *Penicillium* and *Sarocladium*. Root tissue of maize also contained important genera like *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium* and *Purpureocillium*. Across both tillage systems, leaf tissues consistently yielded *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*. Root tissues under both tillage systems frequently harboured *Aspergillus* and *Penicillium*. *Kalmusia*, *Mucor*, *Penicillium* and *Sarocladium* were observed exclusively under the RT system. The findings reveal distinct distribution patterns shaping the maize mycobiome under varying agronomic conditions.

**Keywords:** conventional tillage; distribution patterns; fungal endophytes; mycobiome; reduced tillage

## Introduction

Maize (*Zea mays* L.) stands as an important commercial crop and its cultivation has occurred to various hot and temperate regions worldwide. Its economic importance stems from its role as a food, feed and industrial raw material. In the realm of crop plants, a distinctive microbial community is intrinsically associated, residing both within and outside their living tissues. Microbes inherently inhabiting their plant-based niches have been deemed the plant's "second genome" (1), capable of influencing traits beyond the potential of the plant's own genome. Over time, the plant microbiome and its host have forged intricate eco-evolutionary connections. Microbial communities are recognized for their differential effects on plants—whether beneficial, neutral or pathogenic. De Bary (1886) introduced the term 'endophyte' to describe microorganisms inhabiting internal, symptomless plant tissues (2). Fungal endophytes are ubiquitous in asymptomatic photosynthetic parts of all significant land plant lineages. These cryptic symbionts, though common, vary in diversity and potential host compatibility (3). Many fungal endophytes exhibit the ability to shift between pathogenic and commensal or mutualistic lifestyles based on environmental conditions and host factors (4–6). Some endophytes enhance host response to abiotic stresses and confer plants, enhancing their responses to various stresses both abiotic (7) and biotic, including pathogens (8, 9) and herbivores (10).

Endophytes were first isolated and cultured from *Lolium temulentum* seeds (11). The wide array of host plants spanning different ecological niches exhibits striking species diversity of non-systemic endophytic fungi, garnering substantial attention (12). Fungal endophytes, broadly known to be beneficial, can also manifest harmful tendencies. The study demonstrated that the host genotype primarily influences the composition of the phyllosphere microbiome. In other cases, geographic location has a significant impact on community composition. Endophytic fungi have brought in attention from diverse researchers due to the various benefits they bestow upon their associated hosts, including the production of bioactive secondary metabolites, growth promotion, improved yield, enhanced host tolerance to both biotic and abiotic stresses and disease resistance as a pivotal trait in sustainable agricultural systems (13, 14). Endophytes emerge as defensive bioresources with eco-friendly applicability, offering potential for efficient and targeted crop improvement strategies while minimizing environmental pollution.

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interested in endophytic fungi because of the benefits they provide to their related hosts, such as increased yield, growth promotion and the generation of beneficial secondary metabolites (13, 14). Disease resistance is a crucial characteristic in sustainable agricultural systems, as is increased host tolerance to biotic and abiotic stresses (13, 14). Endophytes emerge as defensive bioresources with eco-friendly applicability, offering potential for efficient and targeted crop improvement strategies while minimizing environmental pollution.

For example, *Penicillium citrinum* and *Aspergillus terreus* enhance biomass production in sunflower and mitigate disease effects (15). These endophytes produce secondary metabolites utilized by the host defense responses and enabling survival under severely infected conditions (16). Additionally, *Curvularia* sp. enhances the thermotolerance of the host plant against abiotic heat stress (17). Interestingly, certain insect species prefer endophyte-free grass over endophyte-infected grass (18). While plants can resorb nutrients from senescent leaves before they fall off, components bound to plant tissues appear to remain largely inaccessible (19). Endophytes may contribute to the breakdown of these tissue-bound components, releasing essential elements like nitrogen and phosphorus that are chemically bonded to lignocellulose (19). Maize seeds colonized by filamentous fungi, primarily *Fusarium* sp. and *Aspergillus* sp., represent a major source of hydrolytic enzymes (20). Microbial enzymes from maize-associated fungi are valued in agriculture and industry due to their high stability (21).

Several studies have revealed previously unexplored fungal lineages and diversity associated with various plant species (22, 23). Presenting the possibility of isolating useful endophytes with potential applications across various sectors has a view to commercial utilization. Research has shown that endophytes may include pathogenic species in latent forms that do not exhibit symptoms in the healthy tissues of their host plants. Some of these endophytes have the capacity to infect hosts different from the ones they reside in (6).

For instance, the association of endophytic fungi, such as in tall fescue (a member of the Poaceae family), has significantly improved its physiological functioning, leading to higher rates of photosynthesis, increased biomass accumulation in the form of fresh weight, greater tiller production during regrowth and reduced leaf roll percentage during drying cycles compared to endophyte-free tall fescue (24). Endophytes can enhance water-use efficiency, improving plant survival under variable conditions. Microbial populations in the rhizosphere may control when plants flower by influencing nitrogen uptake rates and the conversion of tryptophan into the phytohormone indole acetic acid (IAA) (25). The alterations induced by the microbiome in plant physiological functions represent a promising approach. Operated by the secondary genome consisting of a microbial community, this approach could reduce the inputs of fertilizers and pesticides without altering the genetic makeup of plants.

Environmental factors such as temperature, humidity, radiation and rainfall strongly influence the assembly of phyllosphere and endosphere communities. Thus, climate change has directly altered the complex processes by which the plant microbiome assembles on plant tissues. The impact of climate change on microbial communities within crop ecosystems has significantly altered those assembled on the surface compared to those residing inside tissues. Moreover, the crop season influences the microbial population found in plant tissues. Early in the growing season, the soil microbiome significantly impacts the leaf

microbiota, but as the season progresses, the leaf microbiota begins to differentiate, becoming less diverse and transitioning to a higher percentage of leaf-specific taxa on bean, soybean and canola (26). The composition of the phyllosphere microbiome is influenced by various factors, with certain factors being dynamic (e.g., radiation, temperature, humidity, pollution and nutrient availability) and others being more stable (e.g., host genotype and location) (27).

Seasonally assembled plant-associated communities often display year-to-year stability (28). However, communities of other perennial plants exhibit significant annual variations and high seasonal variability (29). Environmental conditions that influence the endophytic community can increase diversity endophytic populations, affecting metabolite production. Variations in crop season conditions influence the endophytic community by increasing the number and diversity of those associated with plants. Plants can extend their adaptive capabilities to the environment by using their roots to interact with mycorrhizal fungi and other related microbes (30). Arbuscular mycorrhizae, mycoparasites and nematophagous fungi were particularly favored in no-till soils, whereas fungal saprotrophs and plant pathogens predominated in tilled soils. Traditional tillage and fertilizer management drove communities toward competitors with rapid growth. Copiotrophic bacteria (Proteobacteria, Acidobacteria and Actinobacteria) and *Fusarium* species thrived in the presence of conventional tillage and fertilizers and extensive research has demonstrated that tillage intensity and residue management markedly alter microbial community structure, biomass and functional attributes (31–33). Reduced tillage, agricultural residue retention and crop rotation have been extensively studied for their effects on microbial biomass, activity and composition (34). Conventional tillage with residue removal promoted complete fungal predominance, but zero tillage without residue retention produced incredibly low microflora populations. These findings, along with previous research on soil quality, root rot, nematode populations and yield, affirm that zero tillage combined with residue removal is unsustainable (35). Instead, zero tillage should be coupled with an adequate level of residue retention. The long-term advantage of zero tillage with residue retention lies in fostering the development of pathogen antagonists and predators, creating a more hostile ecosystem and achieving a new ecological balance. It is prudent to adopt agricultural practices that preserve and restore microbial functional diversity, as this is safer than practices that destroy it. The deeper understanding of the functional relationships among microorganisms is needed to determine the effects of diversity on ecosystem functioning and stability (36). To enhance the variety of recovered fungi for further development as biological control agents against plant diseases, the examination of cultivable maize-associated fungi in this study was more extensive than in previous research. The host benefits from these fungi in a number of ways, such as increased yield, growth promotion, tolerance to biotic and abiotic challenges, disease resistance and the generation of beneficial secondary metabolites (37, 38). According to Parkinson (1994), filamentous fungi play a vital role in nutrient cycling in agro-ecosystems and the decomposition of organic matter (such as lignin degradation) (39). This study focused on characterising the maize-associated mycobiome across selected regions of India and examining how biotic factors (tissue specificity) and abiotic factors (conventional and reduced tillage) influence its composition. The emphasis was largely on the significance of abiotic and biotic variables in shaping the maize mycobiome cultivated for an extended period in a conservation agriculture system. By

conducting an extensive culture based survey, this study aimed to recover a broad range of endophytic fungi that may serve as candidates for future biocontrol applications against maize diseases.

## Materials and Methods

### Sampling sites and peculiarities of field conditions

Samples were collected from two major maize-growing regions of India, namely Patna, Bihar (25° 36' 45.63" N and 85° 9' 31.95" E) and New Delhi (28° 63' 3.31" N, 77.1° 5.25" E), for mycobiome analysis. The hybrid maize variety PMH-1 was cultivated at both two sampling sites. The crop was sampled during the VT-R1 growth stages. A total of 48 tissue samples were collected from CT fields and 48 from RT fields. During the sampling period, the plants progressed through distinct stages. Sampling was conducted at the R4 stage (dough phase), when kernels reached approximately 50 % maximum dry weight. New Delhi and Patna share a dry-winter humid subtropical climate (Cwa classification).

### Isolation and preservation

Bulk samples obtained from the test fields were aseptically collected from healthy plant tissues, including leaves, cobs and roots, as well as from bulk soil. Each contained about 12 represented samples from C T and RT fields. Aseptically collected asymptomatic plant tissues were transported to the laboratory in a refrigerated container maintained at 4–6 °C. Subsequently, a sterilized Falcon tube was filled with 120 mg of each cumulative sample separately. External microorganisms were eliminated by surface sterilization using a sequential process of 2–3 % NaOCl (2 min) and 70 % ethanol (30 sec). Sterile samples were homogenized by mortar and pestle. The tissues were treated with 10 mL of sterile phosphate-buffered saline (PBS, containing per litre: NaCl 8 g; KCl 0.2 g; Na<sub>2</sub>HPO<sub>4</sub> 1.44 g; KH<sub>2</sub>PO<sub>4</sub> 0.24 g; pH adjusted to 7.4) with 0.1 % Tween-20 (PBS-T). This composition is reducing the surface tension and penetration into the samples. After treatments of tissues released an inoculum of endophytic fungus, was used to prepare an aliquot from which serial dilutions of the solution up to 10<sup>-9</sup> were made. The sample extract was suspended in 9 mL of saline and utilized as a starting point for gradient of dilutions, followed by using the spread plate and pour plate techniques.

The aliquot (50 µL) was then plated onto the enriched media. Inoculated plates were then incubated at 28 °C ± 2 °C for 15 days, with regular checks for fungal growth. Isolates were preserved via lyophilization on sterile paper discs and stored at 18 °C and 4 °C for long-term maintenance. The colonisation rate of endophytic fungi from various locations was determined using the formula: total number of typical maize segments colonized by endophyte divided by the total number of segments incubated (40).

### Genomic DNA isolation using CTAB approach

The CTAB approach was employed to isolate genomic DNA from endophytic fungi, with slight modifications. Pure fungal mycelium was cultivated on 80 mL of potato dextrose broth (PDB) for five days at 28 °C ± 2 °C in an incubator shaker with a 120-rpm rotation. To prevent DNA shearing, the mycelium was filtered, dried and homogenized thoroughly using a sterile mortar and pestle. The ground mycelium was mixed with lysis buffer (TrisHCl-0.788 g/100 mL, EDTA-1.861 g/100 mL, SDS-3 %, 2-mercaptoethanol-1 %, pH-8) and incubated for 1 hr in a water bath at 65 °C. An equal volume

of Tris-saturated phenol (pH 7.9–8.1) was added, followed by centrifugation at 12000 rpm for 15 min at 40 °C. Subsequently, a phenol, chloroform and isoamyl alcohol solution (25:24:1) was added in an equal volume to a new tube after retrieving the aqueous phase. The tube was then centrifuged as previously described. The supernatant was treated with 1/2 volume of chloroform x 1/10 sodium acetate (pH 5.2) and centrifuged at 1000 rpm. The resulting supernatant was mixed with approximately 500 µL of 70 % ice-cold alcohol, kept at 4 °C for 20 min and then centrifuged once more at 12000 rpm. The resulting supernatant was carefully discarded and the genomic DNA pellets were air dried in a sterile laminar air flow before being dissolved in 50 µL of sterile nuclear-free water. Subsequently, DNA samples were purified and their integrity was verified through gel electrophoresis before being stored at 4 °C for subsequent use in the analysis of the endophytic community related to maize.

### Sequencing and molecular identification

The identification of these fungal colonies involved both molecular and morphological methods. In parallel, pure cultures of endophytic fungi were allowed to grow until they reached the sporulation stage. These colonies were utilized for more in-depth morphological and microscopic studies. The internal transcribed spacer region of ribosomal DNA (ITS-rDNA) was amplified using ITS1-ITS4 primer pairs (41) with an Eppendorf Master Gradient Thermocycler (Eppendorf, Germany). The PCR reaction mixture (25 µL) included 1 µL of DNA material, 12.5 µL of ready-mix (Promega PCR Master mix), 1.0 µL of forward and reverse primers (20 pM each) and 9.5 µL of nuclease-free water. PCR involved an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 40 sec, primer annealing at 53 °C for 30 sec, extension for 1 min at 72 °C and a final extension for 10 min at 72 °C. PCR products were resolved via gel electrophoresis on a 1.5 % agarose gel (Tris borate EDTA) for 40 min at 80 V, using a 100 bp ladder (Promega) as a reference marker. Amplified bands were visualized using Gel imaging equipment under UV light. Sequencing was done using an ABI3730xl DNA analyzer. Sequences were assembled, validated and corrected using Codon Code Aligner v. 3.7.1 (Codon Code Corporation). These sequences were then queried against the NCBI BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for species identification based on the best hits (97 % sequence similarity).

### Analysis of data

To assess species richness and evenness within the maize mycobiome across different crop seasons and tissues, the Shannon-Weiner Diversity Index (H') (42) was utilized. Species accumulation curves were computed using EstimateS v.9.1.0 (43) (<http://viceroy.eeb.uconn.edu/EstimateS>) and compared to ACE and Chao1 curves. Permutational analysis of variance (PERMANOVA, 9999 permutations, using Bray-Curtis dissimilarities) was conducted on the endophyte assemblages across cultivation seasons and tissues (roots vs. aerial tissues) using R software (<http://www.r-project.org/>), along with correspondence analysis using the (PAST 4.10) Paleontological Statistics Software Package. An indicator species test (44) was performed to identify species specific to each season. Indicator species, the most distinctive species within each group closely associated with it and found in most samples from that group, were determined. Hierarchical clustering of different samples (tissues and tillage system) based on the similarity index (Bray-

Curtis) was performed using the UPGMA algorithm to confirm the patterns of mycobiome assemblage in tissues and subsets derived from tissues under various tillage systems.

## Results

### Isolation and diversity analysis

Fungal colonies were isolated from a total of eighty-eight plates containing different dilutions ( $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ) of maize tissues. The analysis revealed that endophytic fungal isolates associated with maize crops were most abundant in Bihar (56.48 %), followed by Delhi (43.51 %). The endophytic population diversity of maize varied with crop seasons, showing that kharif (monsoon) season maize had less population diversity compared to rabi (winter) season maize crop. The Shannon–Wiener diversity values were higher for Bihar ( $H' = 3.117$ ) than Delhi ( $H' = 2.103$ ), indicating greater fungal diversity at the rabi site. These values reflect higher species diversity in the Bihar maize system. Furthermore, the diversity index values for endophytic fungi associated with different maize tissues, such as leaves, cobs and roots, were estimated to be 2.520, 2.220 and 2.054 respectively. Thus, diversity decreased from leaf to cob to root. The study also demonstrated that both crop seasons and cultivation practices, such as conservative tillage and reduced tillage in selected locations of India, had dilutive effects on endophytic fungal assemblages in maize, including cropping systems (Supplementary Fig. 5).

Moreover, cultivation practices (conservative tillage and reduced tillage) had a lesser impact on the assemblages of endophytic fungi compared to crop seasons. The major genera of endophytic fungi associated with maize tissues, including *Aspergillus*, *Cladosporium*, *Alternaria*, *Sarocladium*, *Penicillium* and *Fusarium*, were found to be genetically different. These genera were consistently present in maize tissues (leaf, cob and root) regardless of sampling locations, cropping seasons and cultivation practices related to maize-based cropping systems. This study highlighted the strong association of *Aspergillus*, *Cladosporium*, *Alternaria*, *Sarocladium*, *Penicillium* and *Fusarium* with maize tissues, overriding the effects of geographical, seasonal and tillage parameters during maize cultivation.

### Molecular identification

The cultivable endophytic fungal isolates were subjected to molecular identification using ITS-rDNA barcoding. Cultures that developed without any contamination were selected for further growth in liquid media to harvest mycelia without sporulation. These mycelia were then used for molecular identification by amplifying the ITS regions of rDNA (ITS1, 5.8S and ITS4), which serve as the primary DNA barcode region for endophytic fungi taxa. The consensus sequences of the ITS regions of all isolates were analysed using BLASTn (Basic Local Alignment Search Tool). The results showed a 99–100 % similarity with reference sequences from the GenBank database, providing a high probability of species identification. The sequences obtained from the isolates were submitted to GenBank (NCBI) and detailed information about the isolates of endophytic fungi, including their geographical location, season, maize tissues of origin and accession numbers, was documented in Supplementary Table 1.

The identified endophytic fungal species were phylogenetically dissimilar. The majority belonged to the division

Ascomycota (96.12 %), with Eurotiomycetes (46.81 %), Sordariomycetes (25.20 %) and Dothideomycetes (24.09 %) as the predominant classes within this division. Additionally, a smaller portion of the identified species belonged to the division Zygomycota (3.87 %), specifically zygomycetes (3.87 %). The predominant orders of endophytic fungi were Eurotiales and Hypocreales, encompassing more than 50 % of fungal isolates from different parts of the maize plant (Supplementary Fig. 6). Common genera, such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Curvularia*, *Alternaria*, *Fusarium*, *Kalmusia*, *Mucor*, *Purpureocillium* and *Sarocladium* sp., were closely associated within the maize based niche and were distributed across various maize tissues.

### Innate association of maize mycobiome

Data from conservative agricultural systems following conventional tillage and reduced tillage during two major crop seasons were utilized to compare the fungal species assemblages of the mycobiome community on maize. This study identified a distinct microbial assemblage pattern using tissues obtained from maize, broadly classified into three groups: the aerial parts (leaves and cobs) and the subterranean parts (roots). Plant samples were collected within the two seasons, from plants grown under conservative tillage, as well as from plants grown under reduced tillage. Various abiotic and biotic parameters were selected to understand the influence of mycobiome on maize, including crop season and tillage practices, as well as tissue specificity for the association of endophytes.

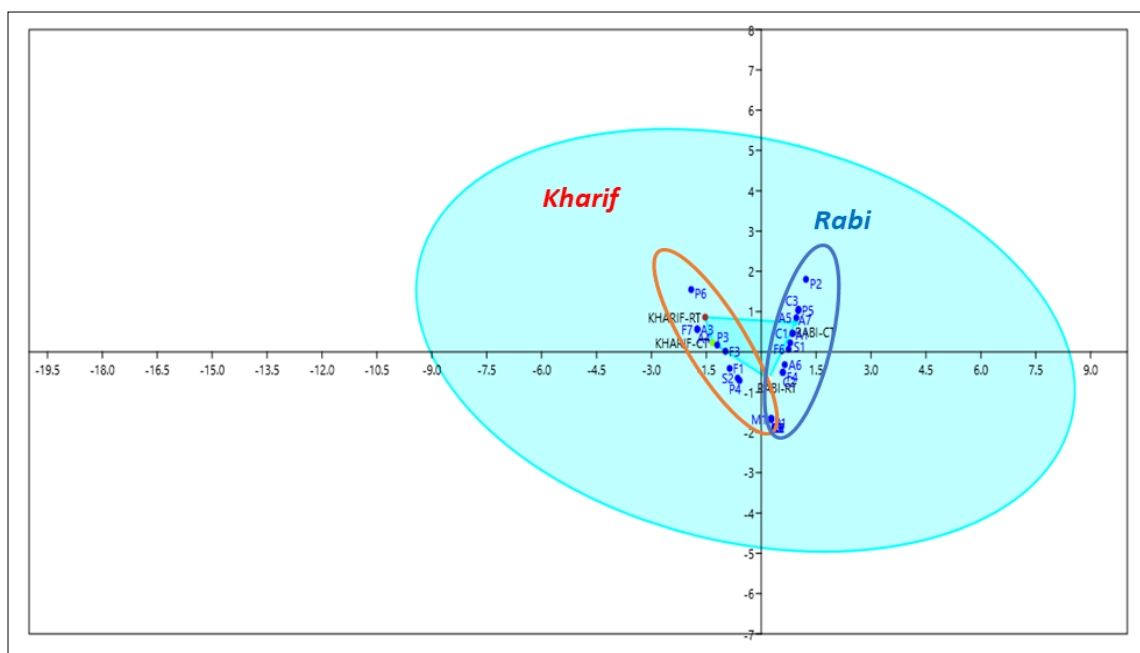
### Crop season strongly influences mycobiome distribution patterns

Crop season had a marked effect on the composition of maize-associated endophytic fungi. PERMANOVA ( $p = 0.0001$ ,  $F = 13.04$ ) and correspondence analysis confirmed significant seasonal shifts in community structure. The overlap between fungal communities from the two seasons is almost negligible (Fig. 1). The relative abundance (%) of the most predominant endophytic fungal taxa across the season also significantly varied from one season to another for the identified endophytic fungi associated with maize (Fig. 2). Nine genera; *Aspergillus*, *Alternaria*, *Mucor*, *Cladosporium*, *Fusarium*, *Kalmusia*, *Penicillium*, *Purpureocillium* and *Sarocladium* were common to both seasons and collectively represented the core cultivable mycobiome (Table 1). While a significantly smaller number of fungal genera, such as *Aspergillus*, *Penicillium* and *Fusarium*, were common to both crop seasons, fungi from a wide range of taxa were identified in maize. Nine fungal genera were found in maize during the *Rabi* (winter) season, but only four were described in it grown during the *Kharif* (monsoon) season. *Aspergillus* (27.77 %), *Alternaria* (10.68 %), *Cladosporium* (21.79 %), *Curvularia* (2.13 %), *Fusarium* (10.25 %), *Kalmusia* (2.56 %), *Mucor* (5.98 %), *Penicillium* (11.53 %) and *Sarocladium* (7.26 %) were the most common genera in *Rabi* season (Fig. 2). Of the four fungal genera found during the *Kharif* season, *Aspergillus* (19.41 %), *Fusarium* (28.15 %), *Penicillium* (36.49 %) and *Purpureocillium* (15.53 %) were found to be the most prevalent (Fig. 2). These genera are associated with the maize niche and form a mycobiome over most of the traditional maize season. Despite the fact that the mycobiome community barcode-based identification revealed notable differences between fungal isolates below the genus level of taxonomy, some of the species identified by the indicator species study were known to exist in that form during the maize crop seasons. *Aspergillus terreus* (indicator value = 1.00 and

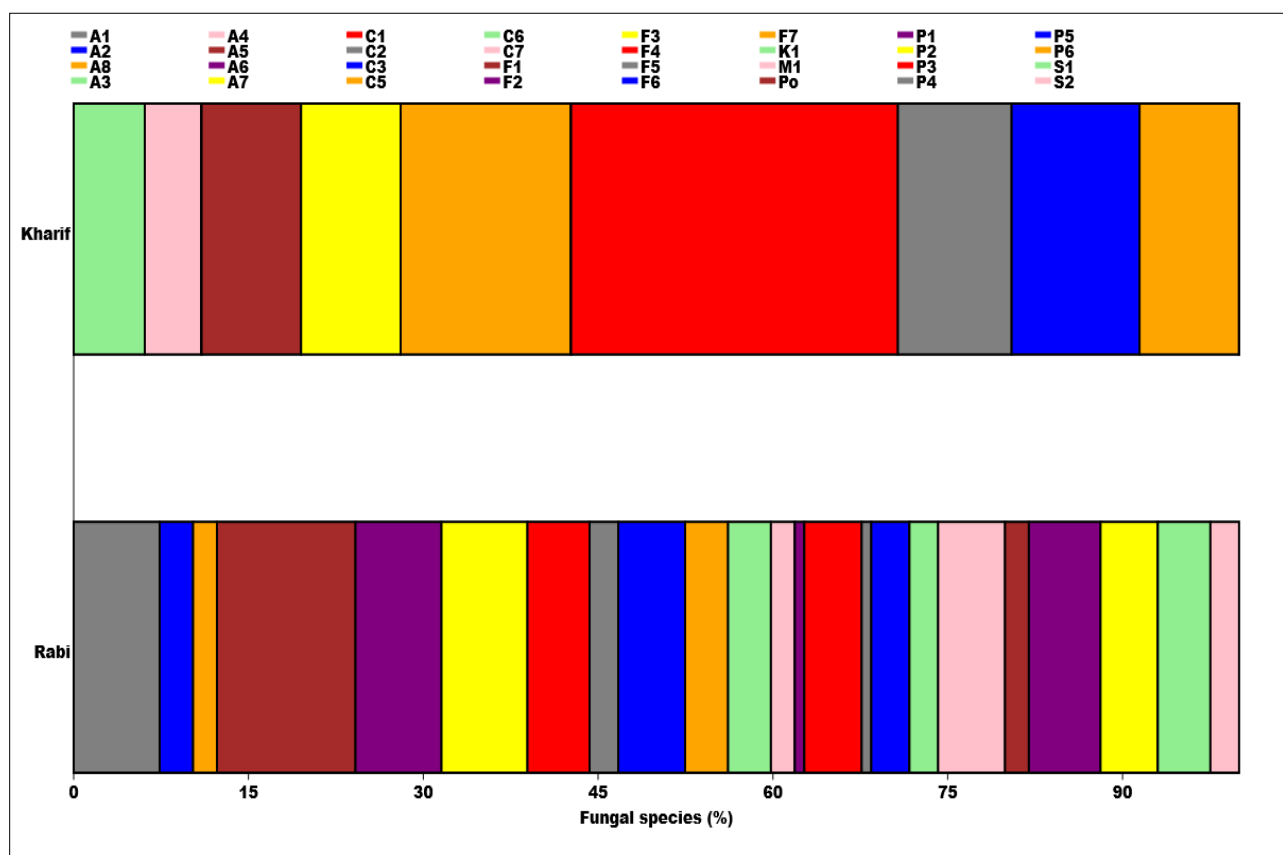
**Table 1.** Endophytic fungal isolates obtained from different tissues of maize plants under different tillage system across crop seasons

| Fungal species                        | Microhabitat | GenBank accession | Tillage | Season |
|---------------------------------------|--------------|-------------------|---------|--------|
| <i>Alternaria alternata</i>           | Leaf         | OQ859990          | RT      | Rabi   |
| <i>Alternaria alternata</i>           | Cob          | OQ860003          | RT      | Rabi   |
| <i>Alternaria alternata</i>           | Cob          | OQ860006          | CT      | Rabi   |
| <i>Alternaria alternata</i>           | Leaf         | OQ860007          | CT      | Rabi   |
| <i>Alternaria alternata</i>           | Leaf         | OQ860009          | CT      | Rabi   |
| <i>Alternaria alternata</i>           | Cob          | OQ860016          | RT      | Rabi   |
| <i>Alternaria</i> sp.                 | Leaf         | OQ860020          | RT      | Rabi   |
| <i>Alternaria tenuissima</i>          | Cob          | OQ863201          | RT      | Rabi   |
| <i>Aspergillus flavus</i>             | Root         | OQ732719          | CT      | Kharif |
| <i>Aspergillus fumigatus</i>          | Root         | OQ732717          | CT      | Kharif |
| <i>Aspergillus niger</i>              | Leaf         | OQ859993          | RT      | Rabi   |
| <i>Aspergillus niger</i>              | Leaf         | OQ859994          | RT      | Rabi   |
| <i>Aspergillus niger</i>              | Cob          | OQ818860          | CT      | Rabi   |
| <i>Aspergillus niger</i>              | Leaf         | OQ860000          | CT      | Rabi   |
| <i>Aspergillus niger</i>              | Leaf         | OQ860010          | CT      | Rabi   |
| <i>Aspergillus niger</i>              | Root         | OQ860012          | CT      | Rabi   |
| <i>Aspergillus niger</i>              | Root         | OQ860025          | RT      | Rabi   |
| <i>Aspergillus</i> sp.                | Root         | OQ863202          | CT      | Rabi   |
| <i>Aspergillus terreus</i>            | Cob          | OQ859985          | CT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ859986          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ859987          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Cob          | OQ859988          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Cob          | OQ859989          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Leaf         | OQ859995          | CT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ860013          | CT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ860015          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ860023          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ860024          | RT      | Rabi   |
| <i>Aspergillus terreus</i>            | Root         | OQ860026          | RT      | Rabi   |
| <i>Cladosporium cladosporioides</i>   | Leaf         | OQ859984          | CT      | Rabi   |
| <i>Cladosporium cladosporioides</i>   | Leaf         | OQ859991          | RT      | Rabi   |
| <i>Cladosporium oxysporum</i>         | Cob          | OQ859980          | CT      | Rabi   |
| <i>Cladosporium</i> sp.               | Leaf         | OQ859983          | CT      | Rabi   |
| <i>Cladosporium</i> sp.               | Root         | OQ860022          | RT      | Rabi   |
| <i>Cladosporium tenuissimum</i>       | Leaf         | OQ859982          | RT      | Rabi   |
| <i>Cladosporium tenuissimum</i>       | Leaf         | OQ859997          | RT      | Rabi   |
| <i>Cladosporium xanthochromaticum</i> | Leaf         | OQ859992          | RT      | Rabi   |
| <i>Curvularia lunata</i>              | Root         | OQ860014          | RT      | Rabi   |
| <i>Fusarium equiseti</i>              | Cob          | OQ732710          | CT      | Kharif |
| <i>Fusarium equiseti</i>              | Leaf         | OQ732714          | CT      | Kharif |
| <i>Fusarium equiseti</i>              | Leaf         | OQ732715          | CT      | Kharif |
| <i>Fusarium equiseti</i>              | Cob          | OQ732724          | RT      | Kharif |
| <i>Fusarium neocosmosporillum</i>     | Root         | OQ731788          | CT      | Rabi   |
| <i>Fusarium proliferatum</i>          | Leaf         | OQ860004          | RT      | Rabi   |
| <i>Fusarium verticillioides</i>       | Leaf         | OQ732729          | CT      | Kharif |
| <i>Fusarium fujikuroi</i>             | Cob          | OQ860017          | RT      | Rabi   |
| <i>Fusarium incarnatum</i>            | Leaf         | OQ732727          | RT      | Kharif |
| <i>Fusarium incarnatum</i>            | Leaf         | OQ732725          | RT      | Kharif |
| <i>Fusarium odoratissimum</i>         | Leaf         | OQ859981          | RT      | Rabi   |
| <i>Fusarium proliferatum</i>          | Leaf         | OQ860008          | CT      | Rabi   |
| <i>Kalmusia italic</i>                | Cob          | OQ860018          | RT      | Rabi   |
| <i>Mucor fragilis</i>                 | Cob          | OQ859999          | RT      | Rabi   |
| <i>Penicillium sumatrense</i>         | Cob          | OQ859979          | CT      | Rabi   |
| <i>Penicillium chermesinum</i>        | Cob          | OQ860002          | RT      | Rabi   |
| <i>Penicillium chermesinum</i>        | Cob          | OQ860019          | RT      | Rabi   |
| <i>Penicillium expansum</i>           | Leaf         | OQ860011          | CT      | Rabi   |
| <i>Penicillium oxalicum</i>           | Cob          | OQ732709          | CT      | Kharif |
| <i>Penicillium oxalicum</i>           | Cob          | OQ732711          | CT      | Kharif |
| <i>Penicillium oxalicum</i>           | Cob          | OQ732712          | CT      | Kharif |
| <i>Penicillium oxalicum</i>           | Leaf         | OQ732716          | CT      | Kharif |
| <i>Penicillium oxalicum</i>           | Root         | OQ732718          | CT      | Kharif |
| <i>Penicillium oxalicum</i>           | Cob          | OQ732722          | RT      | Kharif |
| <i>Penicillium oxalicum</i>           | Cob          | OQ732723          | RT      | Kharif |
| <i>Penicillium oxalicum</i>           | Leaf         | OQ732726          | RT      | Kharif |
| <i>Penicillium raperi</i>             | Root         | OQ732720          | CT      | Kharif |
| <i>Penicillium</i> sp.                | Leaf         | OQ860021          | RT      | Rabi   |
| <i>Penicillium</i> sp.                | Leaf         | OQ732713          | CT      | Kharif |
| <i>Penicillium</i> sp.                | Root         | OQ732721          | RT      | Kharif |
| <i>Purpureocillium lilacinum</i>      | Root         | OQ732728          | RT      | Kharif |
| <i>Sarocladium implicatum</i>         | Leaf         | OQ860005          | CT      | Rabi   |
| <i>Sarocladium strictum</i>           | Cob          | OQ859998          | RT      | Rabi   |

Rabi: winter; Kharif: monsoon season; RT: reduced tillage; CT: Conservation tillage



**Fig. 1.** Comparison of microbial assemblages in maize plants by effect of crop season and cultivation practises using correspondence analysis. The most common genera of fungi to be used for crop season-based studies were *Alternaria* [*Alternaria alternata* (A1), *Alternaria* spp. (A2), *Alternaria tenuissima* (A8)], *Aspergillus* [*Aspergillus flavus* (A3), *Aspergillus fumigatus* (A4), *Aspergillus niger* (A5), *Aspergillus* spp. (A6) and *Aspergillus terreus* (A7)], *Cladosporium* [*Cladosporium cladosporioides* (C1), *Cladosporium oxysporum* (C2), *Cladosporium* spp. (C3), *Cladosporium tenuissimum* (C5) and *Cladosporium xanthochromaticum* (C6)] *Curvularia* [*Curvularia lunata* (C7)]; *Fusarium* [*Fusarium equiseti* (F1), *Fusarium fujikuroi* (F2), *Fusarium incarnatum* (F3), *Fusarium neocosmosporillum* (F4), *Fusarium odoratissimum* (F5), *Fusarium proliferatum* (F6) and *Fusarium verticillioides* (F7)] *Kalmusia* [*Kalmusia italic* (K1)]; *Mucor* [*Mucor fragilis* (M1)]; *Penicillium* [*Penicillium sumatrense* (Po), *Penicillium chermesinum* (P1), *Penicillium expansum* (P2), *Penicillium oxalicum* (P3), *Penicillium raperi* (P4), *Penicillium* spp. (P5)]; *Purpureocillium* [*Purpureocillium lilacinum* (P6)] *Sarocladium* [*Sarocladium implectum* (S1), *Sarocladium strictum* (S2)].



**Fig. 2.** The relative abundance of the most predominant endophytic fungal taxa across crop season of maize. PMH-1 was the hybrid maize variety that was cultivated at the two sampling sites. Relative abundances of fungal genera *Aspergillus* (19.41 %), *Fusarium* (28.15 %), *Penicillium* (36.49 %) and *Purpureocillium* (15.53 %) are in Kharif and more close to each genera of endophytic fungi [A3:8.74 %, A4:10.68 %, F1:8.74 %, F3:9.71 %, F7:9.71 %, P3:21.36 %, P4:5.83 %, P5:9.71 %, P6:15.53 %] and endophytic fungi such as *Aspergillus* (27.77 %), *Alternaria* (10.68 %), *Cladosporium* (21.79 %), *Curvularia* (2.13 %), *Fusarium* (10.25 %), *Kalmusia* (2.56 %), *Mucor* (5.98 %), *Penicillium* (11.53 %) and *Sarocladium* (7.26 %) are in Rabi season and more close to each genera of endophytic fungi as [A1:7.69 %, A2:2.99 %, A5:12.39 %, A6:7.69 %, A7:7.69 %, C1:5.55 %, C2:2.56 %, C3:5.98 %, C5:3.84 %, C6:8.4 %, C7:2.13 %, F2:0.85 %, F4:5.12 %, F5:0.85 %, F6:3.41 %, K1:2.56 %, M1:5.98 %, P1:6.41 %, P2:5.12 %, S1:4.70 %, S2:2.56 %].

probability = 0.003) and *Aspergillus niger* (indicator value = 0.833 and probability = 0.017) were the more categorized indicator species for the rabi season. *Penicillium oxalicum* was the more broadly classified indicator species for the kharif season (indicator value = 0.833 and probability = 0.026). For their respective crop seasons, these three species were thought to be important indicator species. In summary, *Aspergillus* spp. is an indicator species for maize during the rabi season, whereas *Penicillium* spp. is associated with maize during the kharif season. The observation of the fungal isolate led to the conclusion that one of the most significant variables capable of ensuring the mycobiome pattern in the maize-based niche is the crop season.

### Plant tissues influence mycobiome distribution patterns

Plant tissues and their influence on the distribution pattern of the mycobiome were examined in detail. Tissue-specific differences in mycobiome structure were examined across leaf, cob and root samples. Each tissue of the maize plant leaf, cob and root harboured endophytic fungi, showcasing a highly diverse taxonomic and fungal genera distribution pattern as well as predominance within the maize niches.

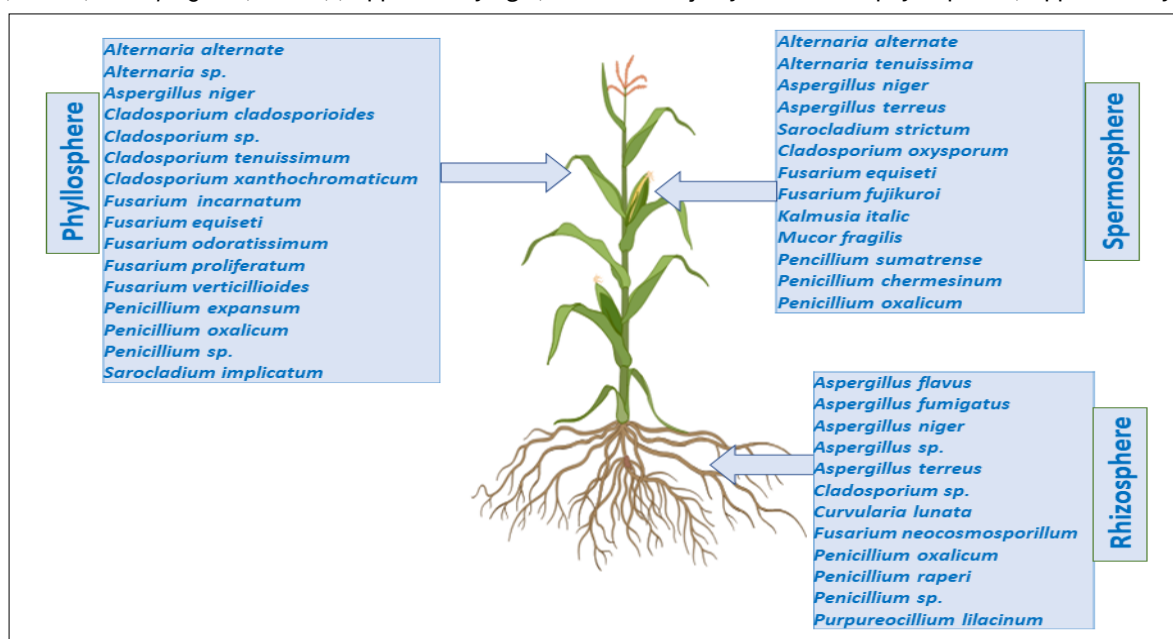
A total of nine genera were isolated across maize tissues (Fig. 3, Supplementary Tables 1–3). These fungal species were confirmed not only through morphological studies but also through molecular barcode-based approaches. Leaf tissues contributed 35.18 % of all recovered isolates, reflecting the high endophyte load of the phyllosphere. Majorly six genera strongly associated with the phyllosphere possessed the abundance, as follows: *Alternaria* (11.40 %), *Aspergillus* (11.40 %), *Cladosporium* (28.07 %), *Fusarium* (298.2 %), *Penicillium* (14.04 %) and *Sarocladium* (5.26 %) (Supplementary Fig. 1). Premature cobs supported eight genera of endophytic fungi (Supplementary Fig. 1). The cob community was dominated by *Aspergillus* (31.18 %) and *Penicillium* (26.88 %), with contributions from *Alternaria*, *Kalmusia*, *Mucor*, *Sarocladium*, *Cladosporium* and *Fusarium*. Cob tissues accounted for 28.97 % of the total isolates, representing the spermosphere-associated mycobiome. Root tissues harboured six predominant genera, dominated by *Penicillium* (32.11 %) and *Aspergillus* (29.36 %) (Supplementary Fig. 1).

Isolates from the rhizosphere region were categorised into as many as six different fungal genera using the DNA barcode of fungi. approximately 32.51 % of isolates were in the share of identified endophytic fungal genera associated, especially with the tissue portion of the rhizosphere of plants in long-term conservative agricultural systems.

The fungal communities were found to be significantly different when comparing aerial tissues to the roots, as shown by correspondence analysis (Supplementary Fig. 2) and PERMANOVA analysis ( $p$ -value = 0.0030,  $F = 8.90$ ). The tissues were primarily classified into aerial (leaf and cob) and subterranean (root) tissues. About six genera: *Alternaria*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Sarocladium* and *Penicillium*-predominated in the aerial section. Only four: *Aspergillus*, *Fusarium*, *Cladosporium* and *Penicillium* were adapted to all over maize niches, encompassing the phyllosphere, spermosphere and rhizosphere. These four genera formed the core cultivable mycobiome across tissues and environmental conditions.

### Varied distribution of the maize mycobiome under the influence of cultivation practices

Conventional tillage (CT) practice involves the transformation of deep soil tilt through ploughing. Reduced tillage (RT), which retains more surface residues, was compared with CT to evaluate impacts on fungal assemblages. Planting the subsequent crop with RT ensures a residue cover ranging from 15 % to 30 %. RT specifically aims to minimize soil disturbance or maintain surface residues, promoting soil, environmental and economic sustainability. The mycobiome assemblages within maize were significantly affected by tillage practices, as indicated by the PERMANOVA analysis ( $p$ -value = 0.0050). However, the impact of this factor was relatively minimal ( $F = 3.11$ ). Correspondence analysis based on endophytic species showed that fungal communities associated with tissues from both RT and CT maize fields had relatively limited overlap (Supplementary Fig. 4). Notably, common genera of endophytic fungi composing mycobiome were found in leaf samples of maize from both CT and RT-practiced fields, including *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*, accounting for the majority of the maize phyllosphere (Supplementary Fig. 3).



**Fig. 3.** Fungal species isolated from maize niches as areal (leaf and cob) and subterranean (root) tissues. PMH-1 is the hybrid maize variety that was cultivated at the two sampling sites (Bihar and Delhi, India). Briefly, the structure of the mycobiome is composed of the following genera of endophytic fungi: *Aspergillus*, *Alternaria*, *Mucor*, *Cladosporium*, *Fusarium*, *Kalmusia*, *Penicillium*, *Purpureocillium* and *Sarocladium*.

The phyllosphere of the maize mycobiome community was divided into subsets based on tillage practices followed in the field. In CT fields, the phyllosphere community was dominated by *Fusarium* (34.55 %), followed by *Aspergillus* and *Penicillium* (16.36 % each). Furthermore, cob samples associated with the mycobiome community from both CT and RT systems show that certain common fungal genera, *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*, are more dominant. Endophytic fungi structured under the CT system are *Alternaria* (8.33 %), *Aspergillus* (36.11 %), *Cladosporium* (13.89 %), *Fusarium* (5.56 %) and *Penicillium* (36.11 %), while *Alternaria* (19.61 %), *Aspergillus* (19.61 %), *Fusarium* (9.80 %), *Kalmusia* (11.76 %), *Mucor* (13.73 %), *Penicillium* (15.69 %) and *Sarocladium* (9.80 %) are dominated under the RT system (Supplementary Fig. 3). The RT system displayed a more diverse mycobiome and maintained more genera of endophytic fungi such as *Kalmusia*, *Mucor*, *Penicillium* and *Sarocladium* compared to the CT system. The underground root of maize was dominated the mycobiome community in both CT and RT systems. It included significant genera, *Penicillium* and *Aspergillus* (Supplementary Fig. 3). Endophytic fungi associated with the root system of maize under the CT system are *Aspergillus* (49.09 %), *Fusarium* (21.82 %) and *Penicillium* (29.09 %), while those under the RT system are *Aspergillus* (36.96 %), *Cladosporium* (21.74 %), *Curvularia* (10.87 %), *Penicillium* (17.39 %) and *Purpureocillium* (13.04 %). Some isolates from root samples in the RT system showed potential for harboring inoculum and virulence channels of foliar pathogens, potentially contributing to disease development. This sheds light on agricultural debris and its role as a primary source for disease progression. In contrast, the CT system, involving deep ploughing, did not encounter foliar pathogens like *Curvularia lunata* in the root regions through the culture-dependent method of identifying the fungal community. *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Cladosporium* and *Sarocladium* are genera of endophytic mycobiome community adopted in maize niches irrespective to tillage practice that was followed in field (Supplementary Table 1–3).

#### Patterns of mycobiome flexibility in maize under the impact of biotic and abiotic variables

By the influence of biotic factors as tissues alone, the cultivable component of the mycobiome linked with maize has demonstrated similarity from cob and leaf together and root area as another cluster (Fig. 4). When comparing the effects of biotic (organisms) and abiotic (tillage) factors in the maize-based niche, mycobiome assembly patterns have evidently been persisting to a greater extent as

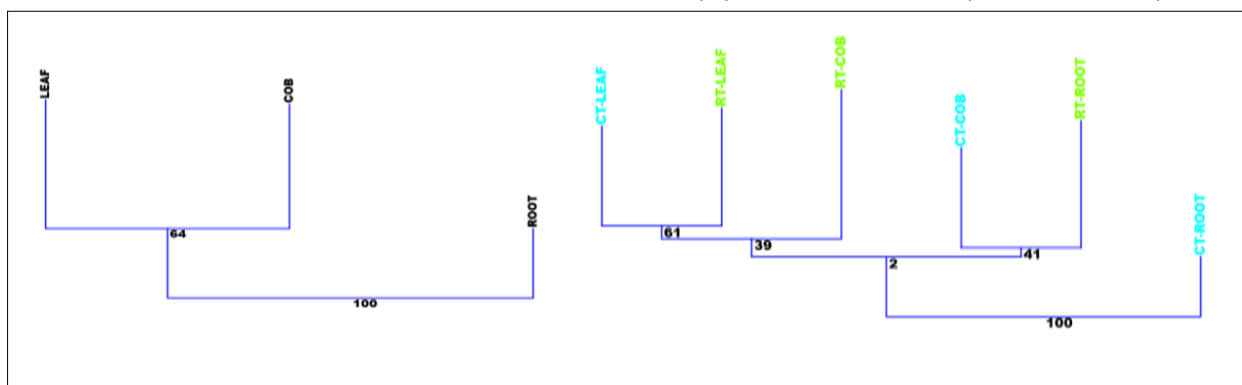
endophytic fungi from the leaf and cob region from CT and RT have clustered together (Fig. 4). In the natural world, including the agroecosystem, the assemblage of microbes in plants including maize in our study has ties to preset patterns that may be formulated as the course of evolution for plant-microbial interaction over the course of biological time scales. This study also demonstrated that microbial assemblages have both flexible and inflexible components. The patterns of microbes found in agriculturally significant crops like maize have pushed for increased levels of flexibility in agricultural practices, which has led to the development of methods for determining the microbial community in an agro ecosystem as a useful way to increase production and productivity.

#### Discussion

By 2050, the demand for food production is projected to surge by up to 60 % compared to current levels, catering to the estimated 10 billion people on earth (45). Despite fungal pathogens exerting a dominant influence on maize, this study underscores the significant diversity of fungi that can coexist as endogenous microorganisms within maize plant. Notably, for certain species, this study marks the first record of their isolation as maize endophytes. In a rapidly advancing world driven by technological progress, the persistently rising demand for food and periodic food crises worldwide poses significant challenges to food productivity. This study was predicated on the hypothesis that plants might harbor a highly diversified community of endophytic fungi with potential efficacy in plant protection. Plant samples from entirely healthy tissues were carefully selected for this study. The conscious decision to employ a culture-dependent technique over cultivation-independent metagenomics approaches was made with the goal of comprehending biologically intricate patterns within the crop system. Additionally, it aimed to leverage the potential of endophytic fungi to control diseases in maize crops in the future. A total of 297 isolates generated from primary dilutions of leaf, cob and root samples, yielded 71 representative endophytic isolates (Supplementary Table 1).

Among these, 32 were from conventional tillage and 39 from reduced tillage systems. These isolates spanned various species, totaling 71, which were considered for molecular identification.

Our findings show strong dominance of Ascomycota across all tissues, consistent with reports of Ascomycete prevalence in cereal endophyte communities. Tissue-specific richness (16 species in leaves,



**Fig. 4.** Hierarchical clustering of different samples (tissues and tillage systems) was analysed based on the similarity index (Bray-Curtis) with the algorithm paired grouping (UPGMA). The samples of aerial (leaf and cob) and subterranean (root) tissues of the PMH-1 hybrid maize variety, that was cultivated at the two sampling sites, namely Bihar and Delhi, India. Clustering patterns of aerial tissue-associated mycobiome community showed more affinity to similarity of structure in niche than those from subterranean samples-associated mycobiome community.

13 in cobs, 12 in roots) reflects known differences in microenvironmental conditions and entry pathways, including rhizosphere-mediated colonization. These species belonged mainly to Ascomycota (96.12 %) and Zygomycota (3.87 %), with Ascomycota further classified into Eurotiomycetes, Sordariomycetes, or Dothideomycetes. The interplay of plant exudates in the rhizosphere initiates microbial interactions, facilitating the entry of endophytes into the plant's roots. Endophytes eventually establish colonies within the plant's tissues, travelling through the stem, leaves and overall plant endosphere (46). As for diversity, *Aspergillus* and *Penicillium* were found to be prevalent across all maize tissues. Previous studies have also reported the presence of various fungal species in maize, such as *Acremonium*, *Botryodiplodia* sp. and different *Fusarium* species, often isolated from roots or other plant parts (47–51). Our investigation into the mycobiome in relation to plant tissues revealed a distribution pattern mirroring most of the endophyte species. Furthermore, we found that multiple factors, including plant species, genotype, agricultural practices and environmental conditions, possess the potential to influence the structure and diversity of endophytic communities. For instance, the level of nitrogen fertilization was observed to impact the structure and abundance of endophytic microbial populations associated with sweet sorghum plants (52). Similarly, studies have shown the influence of soil tillage and agricultural residue management on the structure and functions of soil microbial communities (31, 32). The endophytic population within a given plant species is susceptible to variation based on changing climatic conditions within the same locality and it can also exhibit distinct characteristics from one region to another. Key elements such as temperature, elevation, latitude and rainfall play pivotal roles in influencing endophytes. The interplay of these factors can alter the composition of endophytes in plants. Climate change-induced erratic rainfall may manifest in excessive or limited precipitation (53). Comparing juvenile leaves to fully developed leaves of teak (*Tectona grandis* L.) and rain tree (*Samanea saman* Merr.), it was observed that the latter gave rise to a higher number of species with increased colonization frequency, especially during the rainy season (54). Our study on the maize-based niche revealed that both the abundance and composition of dominant endophytes varied with seasons and displayed specific associations with plant tissues. Moreover, under different agricultural practices such as conventional tillage and reduced tillage, the mycobiome association with maize tissues underwent significant alterations. To our knowledge, this is the first research delving into the biological exploration of endophytic fungal microorganisms in maize, particularly focusing on their distribution patterns under the influence of agricultural practices like conventional tillage and reduced tillage, within this northern region of India. The significance of an organic approach in agricultural food crops, emphasizing the reduction of chemical usage to mitigate their detrimental effects on the environment and human health, reinforces the importance of endophyte research. Leveraging microbial resources as biocontrol agents to prevent plant diseases enhance growth and increase production and productivity presents a viable alternative. All isolated species may harbour the potential to serve as novel biological control agents. However, exploring species not yet recognized as plant pathogens could hold promise in combating maize diseases. These species constitute a reservoir for ecologically sustainable management strategies.

## Conclusion

This study demonstrates that maize hosts a substantial diversity of cultivable endophytic fungi in addition to the commonly encountered fungal species. For some species, this marks the first instance of their isolation as maize endophytes. However, it's important to note that there are notable regional and seasonal variations in microbial populations. The microbial communities exhibit a high degree of organization based on the host organ from which they were isolated, with the aerial sections differing from the roots. Enhanced characterization of fungal distribution and diversity within the maize ecosystem will contribute to the development of microorganisms that can be employed in integrated disease management strategies. Through the sampling of plant tissues from various parts of the plant and across different seasons, this study has revealed that the communities in the cob and leaf regions display more overlap compared to the root area. This study also established a cultivable endophyte collection that can be evaluated for future biocontrol potential against maize diseases.

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## Authors' contributions

WV conducted the experiments at two locations, namely ICAR-IARI, New Delhi, and Patna, Bihar and was responsible for data generation and preparation of the first draft. RG contributed to the conceptualization of the study and supervised the experiments. AK was involved in conceptualization, supervision of the experiments, and finalisation of the manuscript draft. NS carried out the laboratory experiments and performed data analysis. All authors read and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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