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Comparative Metagenomic Analysis of Seed Endobiome of Domesticated and Wild Finger Millet Species (*Eleusine* spp.): Unveiling Microbial Diversity and Composition

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Abstract
Domestication, which involves selective breeding, modern agricultural practices, and specific growing conditions, can influence the microbial and endophytic communities in crop plants. In this study, we examined the microbial diversity and community composition in the seeds of wild and domesticated finger millet species. We employed a metagenomic approach to investigate the seed microbial diversity and community composition of wild (*Eleusine africana*) and domesticated finger millet species (*Eleusine coracana* (L.) Gaertn) grown in the same habitat. While our findings indicated no significant change in seed endobiome diversity due to domestication, there were differences in microbial community composition between wild and domesticated species. Seeds of domesticated species had higher relative abundance of certain bacterial genera including *Helicobacter, Akkermansia, Streptococcus, Bacteroides,* and *Pseudomonas*, whereas seeds of wild species had higher relative abundance of unclassified *Streptophyta*. The seed-associated microbiota also varied among domesticated finger millet accessions. Co-occurrence network analysis revealed a strong relationship between bacteria and fungi in domesticated compared to wild species. We discuss the results obtained in the larger context of the importance of seed endobiome and how domestication processes in crop plants may have impacted the seed endobiome diversity, composition, and function compared to their wild counterparts.

Introduction

Endophytes which are non-pathogenic microorganisms that reside within plants, are particularly important in seed tissues. Seed-borne bacterial and fungal endophytes play an important role in seed development, germination, and seedling establishment [1, 2]. They enhance seed development by increasing the production of growth-promoting hormones [3]. Seed-borne endophytes, especially in monocot plants are vertically transmitted, and thus serve as an important maternal donation to the subsequent sporophytic generation of plants [4, 5]. The presence of the systemic seed fungal endophyte *Epichloë* in perennial ryegrass, tall fescue [6], and fine fescue [7] enhances germination rates besides accruing a higher biomass. Shearin et al. [8] demonstrated that seed fungal endophytes of *Phragmites australis* promoted seed germination by breaking dormancy. Seed endophytic bacteria enhance plant growth by increasing nutrient availability and inducing hormone production [9]. Bacterial seed endophytes in crops like *Oryza sativa*, rice-cut grass, finger millet, pearl millet, and maize promote plant growth by producing phytohormones and aid in nutrient acquisition [10–13]. Additionally, certain seed endophytes such as strains of *Bacillus* and *Microbacterium* harboured by switchgrass seeds hinder pathogenic fungal attack by toxin production [14]. Indeed, the positive role of seed endobiome in seed germination and seedling establishment is so critical that Ayesha et al. [4] have cautioned against the indiscriminate and routine use of systemic fungicide treatment of seeds. Considering these factors, it is clear that the seed endobiome plays a crucial role in enhancing host plant fitness.

While several studies have focussed on the impact of domestication of plants on the microbiome associated with their rhizosphere, roots, and leaves by comparing modern

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cultivars with their respective wild ancestors [15–18] only a few have addressed its effect on seed endobiome [19–22]. Johnston-Monje and Raizada [20] showed that domestication alters the seed endobiome composition of *Zea* spp. The seed microbiota of cultivated barley and wheat were more diverse than those of their respective wild species [19]. Ozkurt et al. [22] showed that in wheat, the seeds of wild variety had a higher diversity of microbiome that that in the domesticated variety. Similar increase in diversity was reported in wild species of *Vitis* compared to their domesticated species, despite being grown in the same habitat [23]. There is as yet no strong evidence suggesting that domestication substantially alters the seed microbiome diversity.

In our study we examined the seed microbiome diversity in wild and domesticated finger millet species. Finger millet, *Eleusine coracana* (L.) Gaertn (family Poaceae) is an annual allotetraploid ($2n = 4X = 36$) widely grown as a millet in the semi-arid tropics and subtropics of the world under rainfed conditions. Finger millet domestication began approximately 5000 years ago in the highlands of eastern Africa and extends from western Uganda to Ethiopia. It was introduced in India around 3000 years ago, making India the secondary center of diversity for finger millet [24]. It is the fourth most important millet after sorghum, pearl millet, and foxtail millet, and is a significant staple food grain in a number of African and Asian countries, covering 12% of the global millet production area [25].

Morphological and cytogenetic studies suggest that cultivated finger millet (*E. coracana* subsp. *coracana*) evolved from its wild progenitor, *E. coracana* subsp. *africana* ($2n = 4x = 36$, AABB). Molecular analysis reveals that *E. indica* served as the primary A genome parent, while *E. trystachya* was the secondary A genome parent for *E. coracana*. The B genome donor most probably is extinct [26]. Traits selected during domestication include changes in inflorescence size, increased seed set percentage, transition from perennial to annual growth habit, higher endosperm-to-embryo ratio, increased carbohydrate content compared to protein content, rapid germination, and larger seed size [27].

Here we analysed the seed microbiome diversity of wild and domesticated species of finger millet grown in the same habitat and ask if there is any significant differences in their microbial diversity and composition. The endobiome diversity of four wild finger millet species and one domesticated finger millet species was assessed using both culture-dependent and culture-independent metagenomic approaches. We also analysed the plant growth-promoting activities of the seed endobiome obtained from the wild and domesticated species. We discuss the results obtained in the larger context of the importance of seed endobiome and how domestication processes in crop plants may have impacted the seed endobiome diversity, composition and function compared to their wild counterparts.

Materials and Methods

Seed Material

Seeds of four wild (*E. africana, E. indica, E. trystachya,* and *E. multiflora)* consisting of eight germplasm accessions, and one domesticated finger millet species (*E. coracana*) with five accessions (Table S1), all grown in the same habitat were procured from the Departments of Crop Physiology and Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru. The seeds were viable and had not been treated with any fungicide during storage. From each of the accessions, representing the different species, a random sample of seeds ($n = 50$ seeds) were chosen for isolation of endophytes.

Isolation of Endophytes from Finger Millet Seeds

The seeds of both wild and domesticated finger millet species were cleaned through a series of sterilization steps. Initially, they were soaked in autoclaved water for 24 h. The seeds were then meticulously washed with distilled water, followed by surface sterilization using 70% (*v/v*) ethanol for 3 min, then 1% (*v/v*) sodium hypochlorite for 3 min, and finally another round of 70% (*v/v*) ethanol for 3 min. The sterilized seeds, encompassing the embryo, endosperm, and seed coat were halved and placed on various agar media to facilitate the emergence of fungal and bacterial colonies. A total of 50 individual seeds from each accession (25 each for bacteria and fungus) were used for isolation of the endophytes.

For bacterial isolation, we used Nutrient Agar (NA), Luria Broth (LB) Agar, and Trypticase Soy Agar media while for fungal isolation potato dextrose agar (PDA), Czapek Dok, and malt extract agar supplemented with streptomycin (30 µg/ml) was used. To verify the effectiveness of surface sterilization process, surface impressions of the seed segments were made on the media, and then incubated at 28 °C. The plates were regularly monitored for the emergence of endophytes (for bacteria over 3–5 days and for fungi over 7–10 days).

Since we did not get any culturable fungal endophytes from any of the seed tissue, further characterization was done only for the bacterial endophytes.

Morphological and Molecular Characterization of Bacterial Endophytes

Based on their shape/form, texture, margin, elevation, and pigmentation, the emerging bacterial endophytes were purified to represent distinct isolates [28]. Voucher numbers were

assigned to each isolate (Table S2), and deposited in the library collection at the School of Ecology and Conservation Laboratory, University of Agricultural Sciences, Bangalore, GKVK, Bengaluru, India.

The bacterial isolates were further identified through 16S rRNA sequence analysis. The bacterial DNA was extracted following the protocol described by Sambrook et al. [29], and its concentration was determined using a NanoDrop Spectrophotometer V3.2.1.

The extracted DNA was amplified using specific 16S rRNA primers (16S rRNA F: 5' GTTAGATCTTGGCTCAGGACG AACGC 3') and (16S rRNA R: 5' GATCCAGCCGCACCT TCCGATACG 3'). PCR amplifications were conducted using an Eppendorf Master cycler. The total volume of the PCR reaction mixtures was 20 µl, containing 2.0 µl of 1×PCR Taq. Buffer with $MgCl_2$ (1.5 mM), 2.0 µl of 10 mM dNTP's mix, 1.0 µl of each 16S rRNA primer (forward and reverse, 0.5 µl each), 0.3 µl of Taq DNA Polymerase (1U Genei Bengaluru), 1.0 µl of Template DNA (~50 ng/µl), and 13.7 µl of sterile distilled water. The PCR conditions included initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 58 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. After PCR amplification, the products were visualized on a 1% agarose gel with 1×TAE buffer. Sequencing of the amplified PCR products (1500 bp length) were carried out by Sanger sequencing (AgriGenome Labs Pvt. Ltd., Cochin, Kerala). The obtained sequences were compared against the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) [30]. The sequence showing the highest homology, maximum query coverage, and maximum score was used to determine the identity of the endophytic bacteria. Phylogenetic trees were constructed using the Neighbour-joining model in MEGA version 11.0 [31].

Screening of Seed Bacterial Endophytes for Plant Growth-Promoting Activities and Extracellular Enzymes

The isolates were evaluated for plant growth-promoting activity and for production of extra-cellular enzymes. The study was restricted to only bacterial isolates as we did not obtain any culturable fungal isolates. Phosphate solubilization on Pikovskaya agar medium [32] and siderophore production on CAS medium [33] were evaluated. Additionally, the isolates were evaluated for production of indole-3-acetic acid (IAA) [34], and for amylase [35], and catalase activity [36].

Total Genomic DNA Extraction from Finger Millet Seeds

Three accessions each from the wild finger millet species, *E. africana* (Accessions GE 7127, GE 7129, & GE 7130), which is the immediate ancestor of *E. corocana* [24, 37] and the domesticated species, *E. coracana* (accessions GPU 28, PR 202, & HR 911), were selected for metagenomic analysis. Seeds (0.5 g) of each of the accessions were surface sterilized as mentioned above, and then total genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method with slight modifications as described by Rajendrakumar et al. [38]. The DNA was fractioned on 1% (*w/v*) agarose, stained with ethidium bromide for visualization and quantified using Nano Drop Spectrophotometer V3.2.1.

Targeted Metagenomic Sequencing

Total genomic DNA extracted from seeds of both wild and domesticated finger millet species were analysed for bacterial and fungal diversity using the Illumina Miseq platform. Genomic DNA at a concentration of 25 ng was utilized to amplify the V3–V4 hypervariable regions of the bacterial 16S rRNA gene. The PCR reaction included KAPA HiFi Hot Start Ready Mix and a final concentration of modified 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') primers. These primers (341 F and 785 R) specifically bind to complementary sequences within the V3–V4 region of bacterial 16S rRNA genes, selectively amplifying bacterial DNA without amplifying host DNA [39]. The PCR protocol involved an initial denaturation step at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 7 min. An additional eight cycles of PCR were performed using Illumina barcoded adapters to prepare the sequencing libraries.

For fungal diversity analysis, 25 ng of DNA was used to amplify the fungal ITS2 hypervariable regions with a final concentration of ITS3 F (5-CAHCGATGAAGAACG YRG-3') and ITS4 R (5-TTCCTSCGCTTATTGATATGC-3') primers. Again, these primers specifically amplify only the internal transcribed spacer 2 (ITS2), a conserved and highly variable region within the nuclear ribosomal RNA gene in fungi [40]. The PCR conditions for fungal ITS2 amplification included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 30 s, and a final elongation at 72 °C for 7 min. Amplicons were purified using Ampure beads to remove unused primers. Library quantification was performed using the Qubit dsDNA High Sensitivity Assay Kit. Finally, sequencing was carried out using Illumina Miseq with the 2×300PE V3 sequencing kit.

The sequence reads generated in this study have been deposited in the NCBI Sequence Read Archives (SRA) database under the accession number: PRJNA984456.

Microbial Community Profiling

The raw sequences obtained were pre-processed using Trimmomatic V0.32 tool with phred score > 30. Trimming was performed by removing leading and trailing low quality or N bases (below quality 3) and scanning each read with a 4-base wide sliding window. The parameters were set for cutting when the average quality per base drops below 20, and then to drop any reads below 36 bases long [41]. The number of reads after trimming are provided in Supplementary material S1. The processed reads were further checked for quality confirmation using FastQC software (version 0.12.1). The high-quality reads that passed the FastQC assessment were imported and processed using the QIIME2 pipeline (version 2023.2) following the workflow described by Bolyen et al. [42]. The paired-end reads were denoised, merged, and chimeric sequences were removed using the DADA2 plugin in QIIME2 (qiime dada2 denoise-paired) according to the method by Callahan et al. [43] (Supplementary material S2). The resulting high-quality sequences were classified into amplicon sequence variants (ASVs) at 99% sequence similarity using pre-trained naive Bayes classifiers from the Greengenes database (13_8 version) [44] and UNITE (version 2022) for bacteria and fungi, respectively. Further, functional features of seed microbiota (bacteria and fungi) were predicted by PICRUSt2 v2.5.2 [45]. Before proceeding with further analysis, sequences belonging to the orders "Chloroplast" and "Rickettsiales" were removed from feature table.

Diversity Analysis

For diversity analysis (alpha and beta), samples were rarefied to an appropriate sampling depth: 1043 for bacteria and 2913 for fungi. This step ensures unbiased comparisons between groups by accounting for differences in sequencing depth (Fig. S1). Alpha diversity, which measures within-sample diversity, was assessed using Shannon diversity index. The independent samples t-test was employed to determine the statistical significance of alpha diversity between the two groups (wild and domesticated species).

Beta diversity was measured using weighted UniFrac distance measurements. The statistical significance between different groups was evaluated using the permutation-based ANOVA (PerMANOVA) test with 999 permutations. Principal coordinate analysis (PCoA) was performed to examine if there emerged any discernible clusters of wild and domesticated species accessions. PCoA was generated based on distance metrics and plots were generated using the QIIME2 Emperor tool to visualize the bacterial and fungal community structures.

Results

Seed Microbiome of Wild and Domesticated Finger Millet Species

Initially, we used NA, LB Agar, and Trypticase Soy Agar media for isolating bacteria; since NA supported a higher number of bacterial colonies, this was chosen for further studies for bacterial isolation. For fungal isolation, we used PDA, Czapek Dox, and Malt Extract Agar.

A total of fifteen different bacterial strains were isolated from the seeds of wild and domesticated finger millet species and identified through 16S rRNA sequencing (Table 1). However, we did not observe the emergence of fungal colonies from any of the accessions across all three media. The phylogenetic relationship of bacterial endophytes isolated from wild and domesticated finger millet species is shown in Fig. S2. Bacterial isolates from wild and domesticated finger millet species, belonged to various genera including *Paenarthrobacter* sp., *Micrococcus* sp., *Kytococcus* sp., *Deinococcus* sp., *Staphylococcus* sp., *Paenibacillus* sp, *Lysinibacillus* sp., *Serinicoccus* sp., and *Bacillus* spp. (common to both wild and domesticated species). These bacterial genera belonged to the phyla Firmicutes and with Actinobacteria belonging to the phylum Deinococcota.

Plant Growth Promotion Potential of Bacterial Endophytes

Around 26% of bacterial isolates exhibited phosphate solubilization ability and approximately 80% produced siderophores. All bacterial endophytes produced indole-3-acetic acid (IAA) and it ranged between 3.08 and 12.50 µg/ml. All tested bacterial endophytes exhibited amylase and catalase activity (Table 2).

Metagenomic Analysis of Seed Microbiome

Three wild and three domesticated finger millet accessions were selected for metagenomic analysis. The taxonomic composition of the seed microbiota was analyzed using 16S rRNA gene sequencing for bacteria and ITS2 sequencing for fungi. A total of 192 bacterial ASVs (Supplementary material S3) were identified, with cyanobacteria and proteobacteria being the predominant phyla (Fig. S3, A). At family level, both wild and domesticated species were predominated by Verrucomicrobiaceae, Streptococcaceae, and Unclassified bacteria (Fig. 1a). At the genus level, both wild and domesticated species were dominated by Streptophyta (Fig. 1b), but there were differences in microbial composition between the two groups. Domesticated species had a higher abundance

Table 1 Molecular identification of seed bacterial endophytes of wild and domesticated finger millet species using 16S rRNA

S. no	Plant species	Bacterial isolate code	Sequence length	Best blast search	Query cover (%)	Identity (%)	Homologue	Gen bank accession number
1	*E.africana* GE 7127	EA_1	903	*Bacillus subtilis* strain EA1	100	100	ON357946.1	ON778650.1
2		EA_2	894	*Bacillus velezensis* strain EA2	100	99.89	MH521182.1	ON778609.1
3	*E.africana* GE 7129	EA_3	1222	*Bacillus subtilis* strain EA3	84	85.49	MT559808.1	ON778646.1
4		EA_4	374	*Deinococcus wulumuqiensis* strain WF_1	99	100	MK828503.1	MT125900.1
5	*E.africana* GE 7130	EA_5	837	*Bacillus sp.* strain WF_2	100	98	NR_132682.1	GQ497298.1
6		EA_6	882	*Micrococcus endophyticus* strain WF_3	100	99.77	MK578827.1	MT125902.1
7	*E.indica* GE 7136	EI_1	882	*Kytococcus sp.*strain EI1	100	100	KR477809.1	ON778663.1
8	*E. tristachya* GE 7140	ET_1	861	*Staphylococcus epidermis* strain WF_4	100	100	MN938189.1	MT125903.1
9	*E.coracana* GPU28	EC_1	879	*Paenibacillus sp.* strain DF_2	100	96.71	KP189378.1	MT125904.1
10		EC_2	944	*Bacillus cereus* strain EC2	100	100	JX294518.1	ON778649.1
11	*E.coracana* PR202	EC_3	888	*Bacillus subtilis* strain EC3	100	99.89	MT081472.1	ON778664.1
12		EC_4	930	*Serinicoccus sp.* starin EC4	100	98.18	JX152778.1	ON778698.1
13		EC_5	879	*Bacillus amyloliquefaciens* DF_3	100	99.89	KF376342.1	MT125905.1
14	*E.coracana* 2888	EC_6	808	*Lysinibacillus odyssey* strain EC6	99	97.02	KC149512.1	ON778699.1
15	*E.coracana* 4596	EC_7	798	*Bacillus sp.*strain DF_1	100	100	MK775264.1	MT125899.1

Table 2 Plant growth-promoting and enzymatic activities of bacterial endophytes of finger millet seeds

Bacterial isolates	Phosphate solubilization	Siderophore production	Auxin (IAA) production (μg/ml ± SE)	Amylase	Catalase
Bacillus subtilis strain EA1	+	++	3.90 ± 0.19	++	FB
Bacillus velezensis strain EA2	+++	+	3.08 ± 0.98	+++	FB
Bacillus subtilis strain EA3	−	+	5.63 ± 0.77	++	FB
Deinococcus wulumuqiensis strain WF_1	−	+	8.15 ± 1.64	+	FB
Bacillus sp. strain WF_2	−	−	4.33 ± 0.37	+	FB
Micrococcus endophyticus strain WF_3	−	−	5.81 ± 0.29	+++	FB
Kytococcus sp. strain EI1	−	+	10.75 ± 1.07	+	FB
Staphylococcus epidermis strain WF_4	−	+++	5.65 ± 0.37	+	FB
Paenibacillus sp. strain DF_2	−	+	12.09 ± 2.48	+	FB
Bacillus cereus strain EC2	−	+	9.90 ± 0.26	+	FB
Bacillus subtilis strain EC3	++	++	6.34 ± 0.34	+++	FB
Serinicoccus sp. starin EC4	−	+	8.68 ± 0.32	+++	FB
Bacillus amyloliquefaciens DF_3	+ +	+	6.64 ± 0.26	++	FB
Lysinibacillus odyssey strain EC6	−	+	11.60 ± 3.81	+	FB
Bacillus sp. strain DF_1	−	−	12.50 ± 0.18	+++	FB

Note: Where, (−: no activity; +: < 3 mm clear zone; ++: 3–7 mm clear zone; +++: more than 7 mm clear zone around bacterial colony) and *FB*: Formation of Bubbles

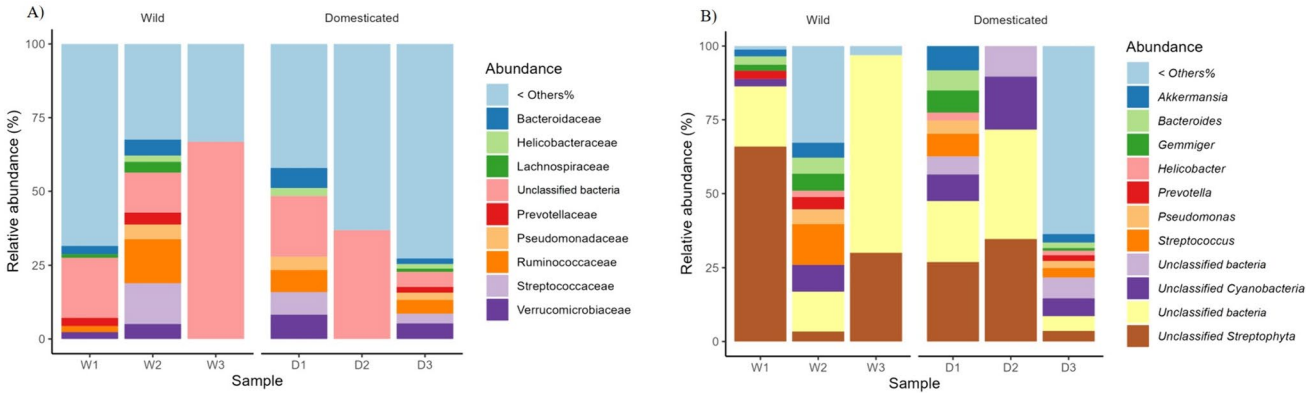

Fig. 1 Relative abundance of bacterial endophytes at family (**a**) and genus (**b**) level in wild and domesticated finger millet species. W1, W2, W3 are wild species (*E. africana*) accessions and D1, D2, D3 are domesticated species (*E. coracana*) accessions

of unclassified cyanobacteria, *Helicobacter, Akkermansia*, *Streptococcus, Bacteroides,* and *Pseudomonas*. The wild species had higher relative abundance of the unclassified *Streptophyta*. With reference to fungi, a total of 170 ASVs belonging to the phyla Ascomycota and Basidiomycota were identified (Fig. S3, B) and Supplementary material S4). At family level, both wild and domesticated species were predominated by Wallemiaceae followed by Cladosporiaceae (Fig. 2a). The genus *Wallemia* was abundant in most wild and domesticated finger millet accessions, except for accession D2 which had *Cladosporium* as the dominant genus (Fig. 2b).

Seed Microbiota Diversity Analysis

There was no significant difference in the alpha diversity between wild and domesticated finger millet species for both bacteria and fungi (Independent samples *t*-test, *p* value > 0.05) (Fig. 3). The accession D3 (HR911) and D2 (PR202) had highest, while W3 (GE 7130) and D1 (GPU 28) had lowest alpha diversity values for bacteria and fungi, respectively (Supplementary material S5). Furthermore, a principal coordinate analysis (PCoA) did not show distinct clustering of the two groups (wild and domesticated species) indicating no significant difference in beta diversity between wild and domesticated species. (Fig. 4a and b).

Co-occurrence Pattern of Microbial Communities

Co-occurrence network analysis showed more interconnected bacterial and fungal families in domesticated species compared to the wild species, indicating a higher degree of correlation among bacterial and fungal families in domesticated finger millet (Fig. S4).

Functional Profiling of Seed Microbiota

The functional predictive analysis indicates that most of the metabolic pathways are relatively abundant in the seed microbiota associated with domesticated species compared

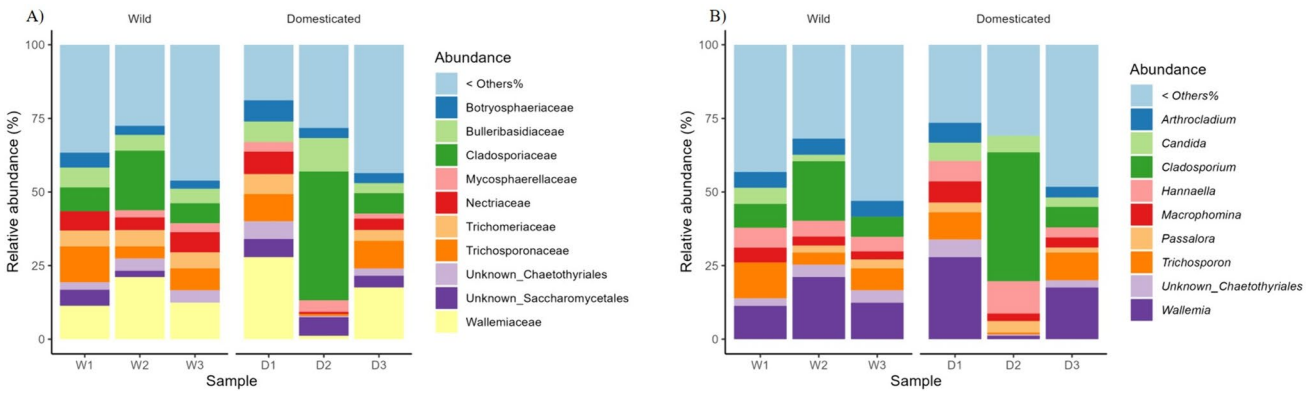

Fig. 2 Relative abundance of fungal endophytes at family (**a**) and genus (**b**) level in wild and domesticated finger millet species. W1, W2, W3 are wild species (*E. africana*) accessions and D1, D2, D3 are domesticated species (*E. coracana*) accessions

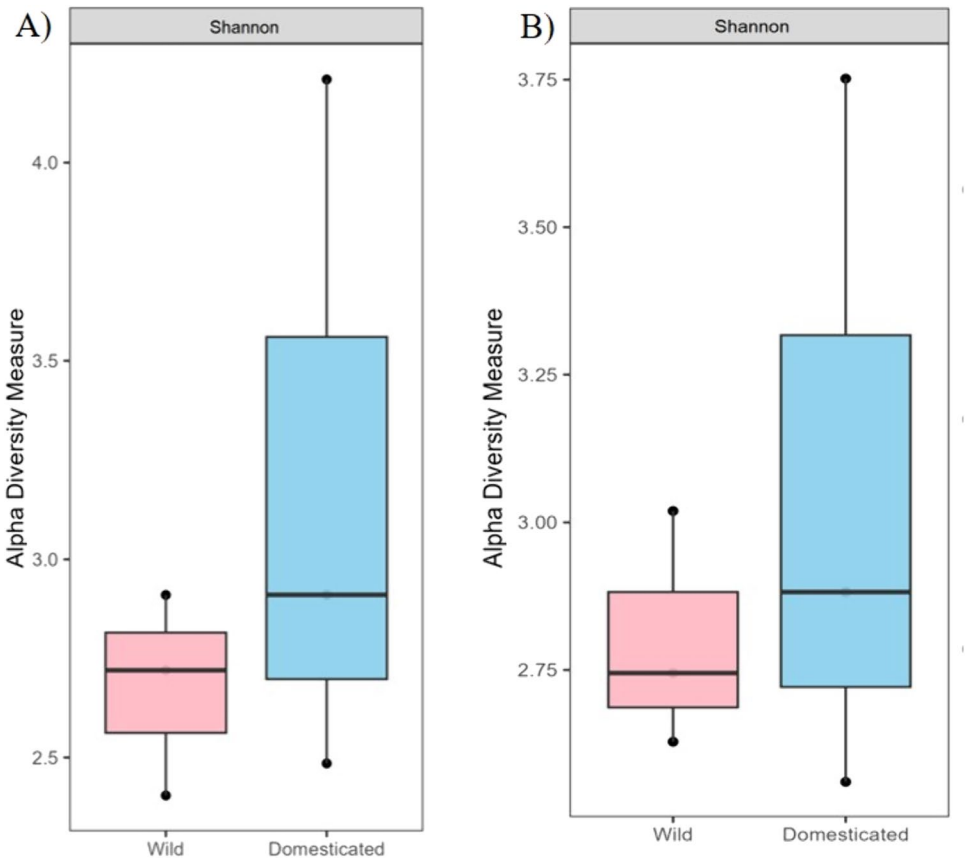

Fig. 3 Bacterial (**a**) and fungal (**b**) alpha diversity measured by Shannon diversity index. (Independent samples *t*-test, *p* value > 0.05)

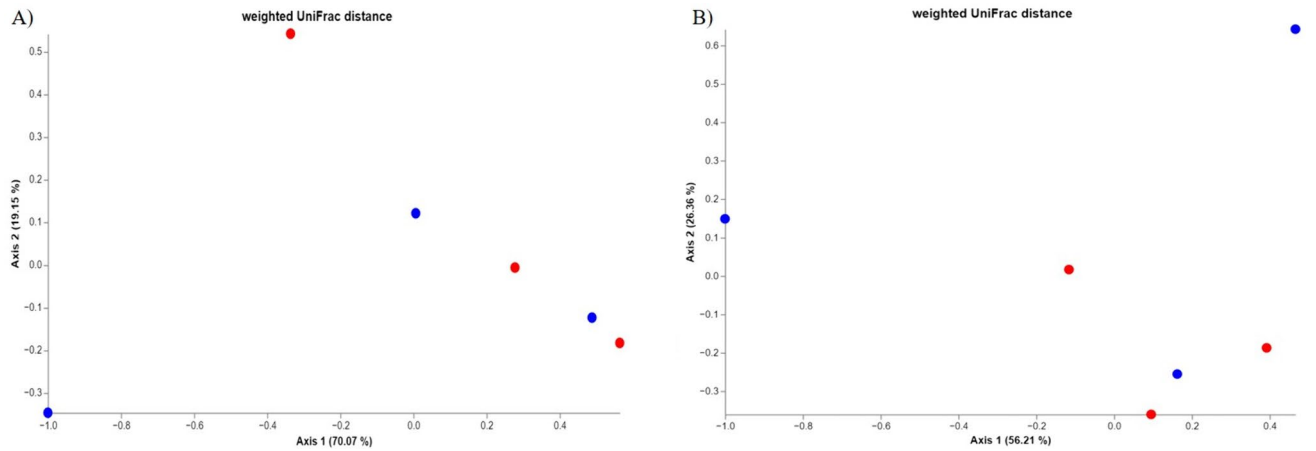

Fig. 4 Beta diversity of bacterial (**a**) and fungal (**b**) endophytes of wild and domesticated species. PCoA was plotted using Vega Editor (QIIME2). The red colour circle indicates wild accessions, and the blue colour indicates domesticated accessions

to wild species (Fig. 5a and b). Enriched pathways include those involved in respiration processes, nucleic acid, amino acid, flavin, and peptidoglycan biosynthesis. Additionally, there is a pronounced MEP (Methylerythritol Phosphate) route, which produces terpenes. Bacterial functional prediction also revealed metabolic pathways related to sucrose breakdown, starch degradation, and gluconeogenesis, which are crucial during seed germination and seedling establishment (Fig. 5a). Overall, the major metabolic pathways associated with plant growth-promoting traits are more abundant in the microbiome communities of domesticated species compared to those of wild species.

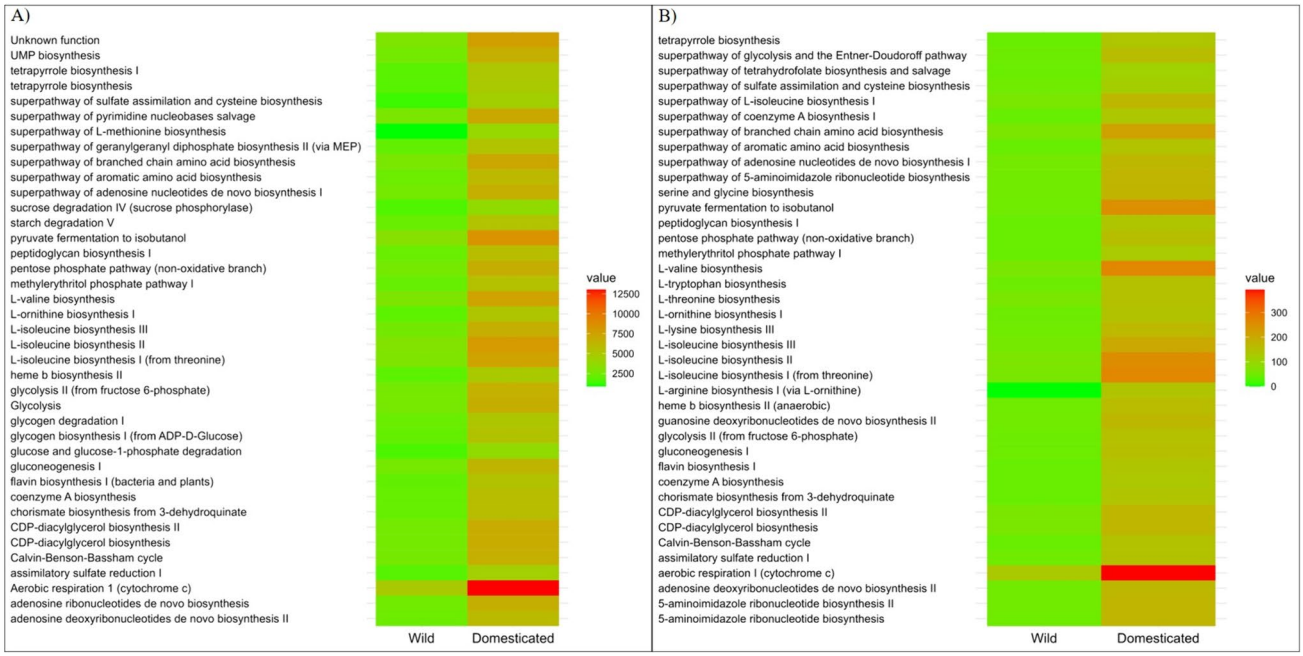

Fig. 5 Functional analysis of the metagenome depicting the major pathways enriched in seed bacterial (**a**) and fungal (**b**) endophytes of wild and domesticated finger millet species

Discussion

Our study showed no significant differences in seed microbiome diversity (alpha diversity) of wild and cultivated finger millet species. Several previous studies have reported a mixed result on the effect of domestication on seed microbiome diversity. For example, while there was no difference in diversity between wild and cultivated *Zea* species [20], the diversity was lower in cultivated (domesticated) grape vine varieties [23] and higher in cultivated barley and wheat varieties [19] compared to their respective wild counterparts. These differences can be attributed to several factors including the geographical location, growing conditions and the experimental material being evaluated.

Comparative studies involving wild and domesticated species often suffer from confounding factors, including geographical location from which the seeds are obtained and growing conditions. A study on wheat found that the root and rhizosphere samples from the same environmental conditions exhibited only a minor impact from domestication on microbiome community [18]. On the other hand, another study on wheat rhizosphere found a significant difference in bacterial communities between tall and semi-dwarf cultivars growing in the same environment [46].

Several studies have investigated the impact of domestication on the microbiome of different crops. Spor et al. [47] found that domestication did not significantly alter the diversity of wheat rhizosphere microbiome, but it did lead to significant shift in the composition of fungal communities compared to bacterial communities. On the other hand, Yue et al. [48] showed that domestication shifted the microbiome of wheat rhizospheres from fungi-dominated to bacteria-dominated in samples collected from different locations. Lu et al. [49] reported that host selection had the greatest impact on bacterial and fungal communities on soil microbes, overshadowing the effects of growth stage and sampling location. According to Abdullaeva et al. [19], higher OTU abundance and alpha diversity is present in the seed endobiome of cultivated wheat species compared to their wild counterparts from the same habitat.

These findings highlight the complexity of the relation between domestication and microbiome diversity. The studies collectively suggest that there is limited evidence for domestication driven changes (alpha diversity) in seed microbiome of crop plants though they do seem influence the composition of microbial communities in seeds [18–22] as has been obtained in our results as well (Table S4).

The higher correlation observed among bacterial and fungal taxa in domesticated species (Fig. S2) suggests that host genetics may significantly influence the assembly and structure of the microbiome [50]. Selection of certain plant traits during the process of domestication might favour the establishment of specific microbial communities which are more interconnected as an adaptive strategy to cope with the current environmental conditions. These interactions are crucial as they may influence the beneficial effect of endobiome on seed germination, seedling establishment and overall plant growth. For instance, endophytes may

mediate essential metabolic pathways which are involved in seed germination processes such as gluconeogenesis pathway, starch degradation, and sucrose degradation (Fig. 5). Additionally, they may facilitate the synthesis of aromatic amino acids (L-phenylalanine, L-tyrosine, and L-tryptophan) and certain siderophores by mediating chorismate biosynthesis.

Thus, our study reveals that there is no significant variation in the alpha diversity of the seed endobiome of wild and domesticated finger millet species. However, differences in microbial community composition were observed. Long-term studies evaluating successive generations of wild and domesticated species in diverse regions would provide insights into how crop seed endobiome diversity and community have evolved since domestication. More focussed studies would identify key factors shaping microbial communities in seeds and inform strategies to modify or regulate plant characteristics or conditions for a more beneficial microbiome.

Beyond comparing seed microbiome of wild and domesticated species, our study underscores the importance of seed microbiome as an important component of seed and plant health. While we failed to obtain any culturable fungal endophytes from the seeds of either the wild or domesticated species as also reported earlier by Mousa et al. [51], we isolated a total of 15 bacterial isolates from both the wild and domesticated species. These belonged to the genera *Paenarthrobacter, Micrococcus, Bacillus, Kytococcus, Deinococcus, Staphylococcus, Paenibacillus, Lysinibacillus, and Serinicoccus*. *Bacillus* was the most common genus in both wild and domesticated species consistent with previous reports of it being a common seed-associated endophyte [10]. The bacterial endophytes belong to the family Firmicutes and Actinobacteria. They play a significant role in seed germination by the secretion of hydrolytic enzymes such as amylase and catalase, as well as in plant growth and development through nutrient acquisition and phytohormone production (Tables 2, S3). Seed-borne endophytes play a significant role in promoting growth of several plant species. During seed development, its microbiome influences the seed's sink-drawing ability by increasing the production of growth-promoting hormones such as auxin and cytokinin [3]. In *Phragmites*, seed endophytes aid seed germination by breaking dormancy [8]. Similarly, bacterial endophytes associated with cucurbit seeds enhance nutrient acquisition and improve host plant growth [52]. In *Anadenanthera colubrina* seed endophytic bacteria boost plant growth by increasing mineral nutrient availability and inducing the production of plant hormones [9]. Bacterial endophytes of seeds of *Oryza sativa*, rice-cut grass, finger millet, pearl millet, and maize promote plant growth through phytohormone production and aiding nutrient absorption [10–13]. Additionally, certain plant growth-promoting bacterial endophytes reduce ethylene levels by synthesizing ACC deaminase further contributing to enhanced plant growth [53].

In summary, our study reinforces the importance of seed endophytes as an important constituent of the plant microbiome that play a vital role in regulating plant growth and development. The microbial composition, diversity, and assemblage structure however may be influenced by a complex set of interactions between the seed endophytes and their host plant including their domestication history and the environment in which they have been cultivated or occur. Further research is needed to understand the interaction between host genetics, microbial communities, and environmental factors in shaping seed microbiome.

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Author's Contribution M.H conceived the research idea, conducted experiments, performed data analysis, and drafted the original manuscript. K.N.N contributed valuable resources and provided critical review and editing of the manuscript. T.S.S participated in the review and editing process. R.U.S contributed significantly to the conceptualization of the study, provided supervision throughout the research process, and offered constructive feedback during the drafting and editing of the original manuscript.

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Declarations

Conflict of interests The authors declare that they have no conflict of interest in the publication.

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