



RESEARCH ARTICLE

Spectrum of fungal endophytes in banana species of Nagaland, India

Bendangsenla^{1*}, Susanta Banik¹, N Tiameren Ao¹, Damitre Lytan², Kavi Sumi³, E Lireni Kikon¹,
Pezangulie Chakruno³ & Hijam Shila Devi²

¹Department of Plant Pathology, School of Agricultural Sciences, Medziphema Campus, Nagaland University, Medziphema 797 106, Nagaland, India

²Department of Entomology, School of Agricultural Sciences, Medziphema Campus, Nagaland University, Medziphema 797 106, Nagaland, India

³School of Agricultural Sciences, Medziphema Campus, Nagaland University, Medziphema 797 106, Nagaland, India

*Correspondence email - bensen1144@gmail.com

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Abstract

Fungal endophytes represent microbial communities and are found in different plant tissues that include leaves and roots and exhibit a wide range of beneficial interactions with its host plant. Nagaland, a Northeast state of India consists of vast number of wild bananas growing throughout the hilly terrain of the state. However, the association of this wild banana with the microbe community has not been studied and poorly understood. In this study, focus was made to isolate fungal endophytes from healthy leaves and roots of wild and cultivated banana from four districts of Nagaland during 2021–24. Altogether, 281 fungal endophytes were isolated and out of these, 246 isolates were obtained from wild banana and 35 isolates from cultivated banana plant. A total of 135 isolates belonging to 15 genera were identified, with 14 genera under Ascomycota and one as Mucoromycota. The identified isolates belonged to the genera *Penicillium*, *Trichoderma*, *Fusarium*, *Colletotrichum*, *Diaporthe*, *Apiospora*, *Aspergillus*, *Pestalotiopsis*, *Mucor*, *Phomopsis*, *Botrytis*, *Helminthosporium*, *Alternaria*, *Beauveria* and *Cladosporium*. Molecular identification using ITS 1 and 4 primers was also carried out for some selected isolates (24 nos.). Relative abundance (Ra) showed that among the identified genera, the highest Ra was in *Diaporthe* sp. with 5.39 %. A diverse group of fungal endophyte genera was found which could be due to the geographical location of collection sites, rich diversity of banana present in this region. Moreover, with limited information on endophytic fungi inhabiting banana species of Nagaland, this study gives a novel understanding of the unexplored community of fungi.

Keywords: banana; fungal endophytes; identification; Nagaland; Northeast India

Introduction

The study of endophytes from tropical plants have gained significant recognition. Endophytes are recognised as diverse and contribute promising sources of biologically active novel compounds (1). Endophytes are microbes that lives inside the healthy plant tissues without causing any harm or symptoms to the host plant (2). Endophytic fungi are also called as fungi that lives in healthy, functioning root tissues at the ‘moment’ of sample collection (3). In a previous study this concept was supported that fungi and bacteria can be detected at a specific ‘moment’ within the healthy tissues of host plant. Endophytes are gaining an increase in commercial and scientific interest as they benefit their host plants by protecting them against diseases and insect pest caused by various plant pathogenic fungi, bacteria, nematodes, etc. (5, 6) and are also known to produce growth hormones, fix atmospheric nitrogen, cause phosphate solubilisation and improve overall growth of the host plant (7, 8).

There have been very few studies that have been conducted on the endophytes of *Musa* sp. and most have been restricted to cultivated plants and very rarely on the wild *Musa* sp. (9). Some workers have studied endophytic fungi from

Musa sp. (wild banana) which have been recorded and documented (10–12). Wild banana (*Musa* sp.) is native to Southeast Asia and is the ancestor of edible banana and the highest species diversity is found in this region (9). In India, the majority of wild *Musa* species can be found in low rainfall zones of deciduous woods, wet evergreen forests and some tropical rain forests. In addition to areas of Southeast India, the Andaman and Nicobar Islands, the Northeastern Indian Himalayas, which include parts of Nagaland, Assam, Arunachal Pradesh and Meghalaya, are home to tropical rain forests. In this study, the fungal endophytes from leaves and roots of wild and some cultivated bananas were isolated and identified. Many reports have stated that plant leaves and roots foster a wide range of fungi. Studies on fungal endophytes of *Musa* sp. isolated from leaves were conducted in Australia and Hongkong (9, 10) and from roots were conducted from Malaysia (11) however, the occurrence of fungal endophytes in *Musa* sp. in Nagaland, India has not been documented. Therefore, the aim of this study was to isolate and identify the fungal endophytes of wild and cultivated banana. This study was primarily prompted by the desire to determine whether the fungal endophytes found in one location differed than the ones found in other location site. Lastly, because no such work has ever been conducted on the banana species of Nagaland.

Materials and Methods

Sample collection

The sample collection for isolation of fungal endophytes from the banana species of Nagaland was carried out in four districts (17 locations) of Nagaland; Chumoukedima, Kohima, Peren and Mokokchung during 2021–24 (Fig. 1, Table 1). A total of 12 wild banana and 1 cultivated banana plants were randomly selected from each district. Twelve samples each from the leaves and roots of wild banana and 1 sample each from the leaf and root of cultivated banana was collected from the 4 districts of Nagaland. Altogether, 48 leaves and roots samples of wild banana and 4 leaves and roots samples of cultivated banana were collected from the 4 districts of Nagaland.

Isolation of endophytes from leaves

The leaf sample from banana plant was washed with distilled water and air dried and then 1 cm segment of the leaf was cut using a sterilised scalpel. The cut bits of banana leaf were sterilised in sodium hypochlorite 2 % solution for 3 min, followed by rinsing in sterilised distilled water for 1 min and blot drying with filter paper (sterilised) to eliminate excessive water (12).

Once the leaf bits were completely dried, imprinting procedure was done by pressing the sterilised leaf bits onto the top of already poured potato dextrose agar (PDA) to affirm the effectiveness of the surface sterilisation method and to affirm that isolation of only endophytic fungi is carried out. If there is no growth of fungi on the impression plate, then surface sterilisation method used was successful in eliminating the fungi on the surface or epiphytes (13). The blot dried leaf bits were then placed onto plates with PDA and incubation was done at $25 \pm 1^\circ\text{C}$. A total of four leaf bits were placed on to a plate poured with PDA. The PDA plates with leaf bits were kept for incubation for 1–4 days or up to the growth of mycelium were observed from the leaf bits. Three replications were maintained for each sample. The mycelium that was arising from the leaf bits were sub-cultured onto new plates of PDA.

Isolation of endophytes from roots

The roots were collected by digging the soil on all sides of the banana plant. The root samples were placed in plastic bag in accordance with their specific location. The roots were cleaned with flowing running water to eliminate the soil that was adhered to them. Surface sterilisation method was carried out in order to isolate

Table 1. Sites of collection of banana samples for isolation of endophytes

District	Collection site	Longitude	Latitude	Altitude (msl)
Chumoukedima	Patkai Christian College (PCC)	93.8003° E	25.8017° N	248
	Ruzaphema (RZP)	93.787385° E	25.714404° N	486
	Medziphema (MDZ)	93.8816° E	25.7594° N	489
	Kukidolong (KKD)	93.8172° E	25.7698° N	275
	Viswema Village (VSM)	94.1450° E	25.5615° N	1679
	Dzulakie (DLK)	93.956° E	25.621° N	1817
Kohima	Kiruphe, Basa Village (KBV)	94.008138° E	25.738138° N	1595
	Sechu Zubza (SCZ)	94.0353° E	25.7099° N	1081
	Lerie Colony (LCZ)	94.108362° E	25.649241° N	1456
	Kohima village (KHV)	94.1086° E	25.6751° N	1489
Peren	Sector – B, Old Jalukie (OJL)	93.4306° E	25.3415° N	503
	Mhainamtsi, Jalukie (MJL)	93.7027° E	25.6327° N	1117
	Punglwa (PGW)	93.8418° E	25.6792° N	709
Mokokchung	Chuchuyimlang (CCY)	94.4599° E	26.4064° N	1137
	Yisemyong (YSY)	94.632567° E	26.438266° N	1117
	Changtongya (CTY)	94.6827° E	26.5345° N	820
	Alichen (ALC)	94.4555° E	26.2693° N	1197

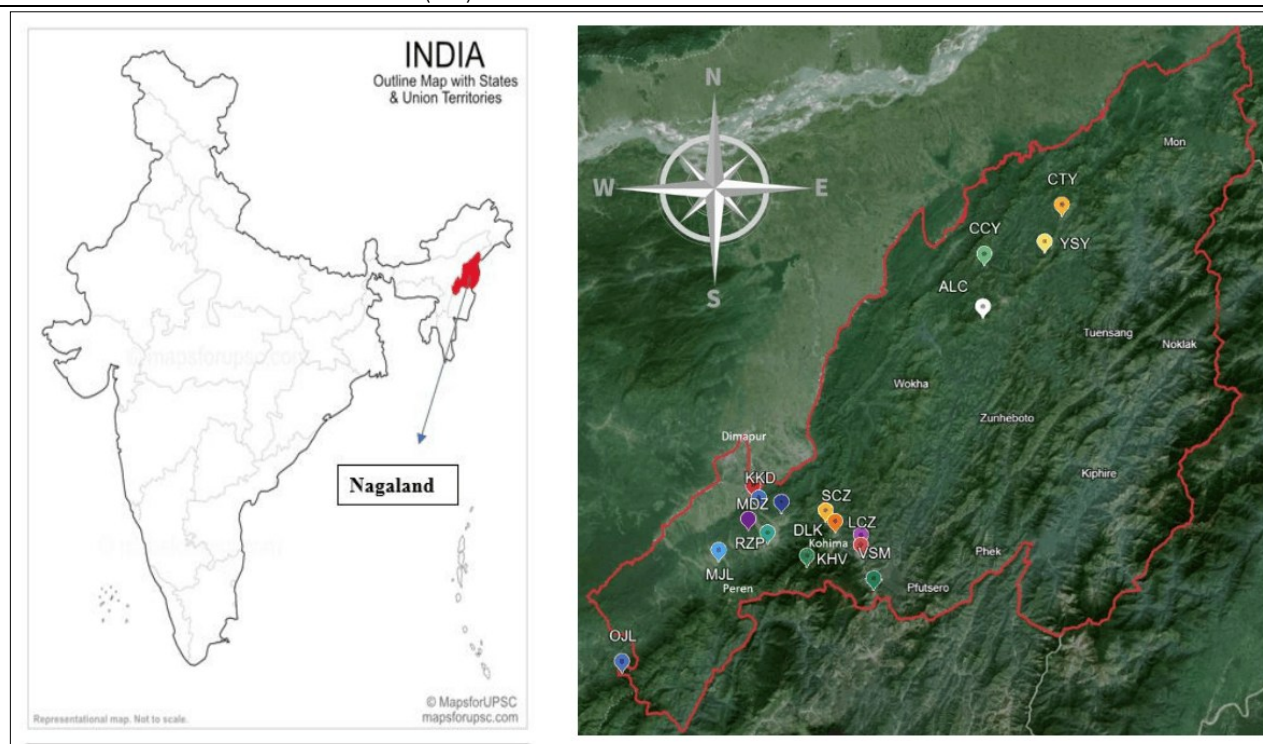


Fig. 1. Mapping of collection sites from the four districts of Nagaland [Source: Google Earth].

endophytic fungi. The roots were cut into tiny pieces (between 2.0 and 3.0 cm), immersed in 70 % ethanol for 30 sec, 1 % sodium hypochlorite for 3 min and 95 % ethanol for 5 min. Finally, the roots were washed three times with sterile distilled water. After drying with sterile filter paper, the root fragments were sliced into smaller pieces (1.0–1.5 cm) and placed onto PDA (11). Three replications were maintained for each sample.

On the PDA plate, four root fragments were positioned. The root fragments were imprinted on PDA plate prior to plating in order to identify any epiphytes present on the root pieces (9). Fungal growth was monitored daily while the plates were incubated at $27 \pm 1^\circ\text{C}$ to monitor for any fungal growth from the fragmented roots. On fresh PDA plates, mycelial growths from the roots were sub-cultured.

Morphological characterisation and identification of the fungal endophytes

The pure culture isolated from the leaves and roots were observed under microscope to study the morphological characters, photomicrographs of each isolate and measurements of the conidia were also recorded. The sporulating isolates were identified up to genus level and some to species level wherever possible. Nonsporulating isolates were classified into morphospecies on the ground of colony surface texture, pigmentation of hypha, exudes produced and growth rate (13).

Molecular characterisation and identification of the fungal endophytes

Molecular characterisation of some selected fungal endophytes was carried out based on its performance *in vitro*. The fungal endophytes were grown on potato dextrose broth (PDB) of 100 mL containing 10 μL of Tween 80 and incubated at shaker incubator for 7 days at 28°C . The mycelium was sieved through a sterile Whatman filter paper No. 1 and the excess broth was drained out. Mycelium weighing about 5–10 g was taken and grounded with the help of a mortar and pestle in liquid nitrogen (DNA extraction kit, HiMedia).

The DNA barcode marker for fungi identification is considered to be the internal transcribed spacer (ITS) region and so, the region was used in the present investigation (14). DNA extraction for the selected fungal endophytes was carried out using HiMedia DNA extraction kit. Following that, polymerase chain reaction (PCR) amplification was also carried out using the HiMedia PCR amplification kit and PCR was performed in Thermal cycler (Bio-Era, Model ADEPT, India). Polymerase chain reaction amplification was performed in a total volume of 50 μL that consist of 2 μL of template DNA, 10X assay buffer, 1 μL of Forward Primer, 1 μL of Reverse Primer, 5 μL of 25mM MgCl_2 , 5 μL of 2.5 mM dNTP mix, 0.5 μL of Taq DNA polymerase and 24.5 μL of molecular biology grade water. The universal primers used for amplification of the extracted DNA were ITS1 (Forward) (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (Reverse) (5'-TCCTCCGCTTATTGATATGC-3') (15). The PCR cycles was performed based on HiMedia PCR kit procedure. The initial denaturation was done at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. Final extension was done at 72°C for 1 min and 10 sec.

Electrophoresis was carried out for detection of the PCR product by using agarose gel at 1.0 %. 100 bp ladder was used as a molecular size standard marker. With the help of the

electrophoresis, the PCR products were distinguished (at 75 V for 60 min) using agarose gel with 1x Tris acetate EDTA buffer. Ethidium bromide ($0.5 \mu\text{g mL}^{-1}$) was used to stain the gel before pouring. The stained gel was observed and the figure was captured with the help of a gel documentation system.

Polymerase chain reaction products of 18S rRNA gene was purified and sequenced. The sequences were lined up with the help of BioEdit Sequence Alignment Editor Version 7.0.5 software to achieve consensus sequences. Comparison of the consensus sequences were done with other DNA sequences in GenBank using BLAST (basic local alignment search tool) in NCBI (National Centre for Biotechnology Information). Isolates identification was done on the basis of the highest agreement of the BLAST search. Phylogenetic tree was constructed using the MEGA11 software. The nucleotide sequences of ITS 18S rRNA gene were deposited in NCBI GenBank for acquiring the accession number.

Relative abundance

Relative abundance (Ra) was also calculated by the available number of endophytes that were obtained from all the leaves and roots samples collected from the four districts.

The relative abundance (p_i) is the proportional representation of a species in a community or sample of a community. The relative abundance (p_i) of each species is expressed as

$$P_i = \frac{n_i \times 100}{N}$$

where n_i is the number of individuals of the same species and N is the total number of individuals for all species (16).

Results and Discussion

Collection of samples for isolation of fungal endophytes

An exploration was conducted in Nagaland, India where four districts (Dimapur, Kohima, Peren and Chumoukedima districts) with different locations (17) were selected and sample collection from wild banana (12 samples each from healthy leaf and roots) and cultivated banana (1 sample each from healthy leaf and roots) was carried out.

Isolation and identification of fungal endophytes

Overall colonisation

To characterise and identify the fungal endophytes, pure cultures were used. The isolates were identified based on their macroscopic and microscopic characters (17, 18). The key objective of identifying the isolates morphologically is to categorise the isolated fungal endophytes in accordance to their related morphological traits. The isolated fungal endophytes were examined as belonging to the same category or genus if their morphological traits paralleled the morphological descriptions formerly reported (19).

A total of 281 fungal endophytes was isolated from the leaves and roots of healthy banana plants and out of these, 246 isolates were obtained from the wild banana and 35 isolates from cultivated banana species of the 4 districts of Nagaland. Fungal mycelium was not observed on the imprint plates used in the surface sterilisation which indicates the effectiveness of the surface sterilisation technique for isolating the fungal endophytes from leaves and roots. The results shows that all the fungal isolates

isolated from the leaves and roots were endophytes, as epiphytic fungi that inhibits the surface of the leaves and roots could not grow after surface sterilisation (20).

In total, 166 isolates (59.07 %) were acquired from leaf samples and 115 isolates (40.93 %) from root samples. In this study, a greater number of fungal endophytes were isolated from leaf samples as compared to root samples and previous studies have also shown that endophytes may exhibit tissue specificity (13). Variation in the assemblages of endophytes in distinct tissue types maybe a reflection of tissue preferences of individual dominating taxa (21, 22) and may also indicate their scope for exploiting and surviving within a specific substrate (23).

A total of 135 isolates (48.02 %) of fungi were identified based on morphological characteristics of the total 281 isolates that belonged to 15 genera of which 119 isolates (88.15 %) were from the wild and only 16 isolates (11.85 %) were from cultivated banana. Out of the 15 genera, 14 belonged to Ascomycota Phylum and 1 belonged to Mucoromycota.

The morphologically identified fungal endophytes belonged to the genera *Penicillium*, *Trichoderma*, *Fusarium*, *Colletotrichum*, *Diaporthe*, *Apiospora*, *Aspergillus*, *Pestalotiopsis*, *Mucor*, *Phomopsis*, *Botrytis*, *Helminthosporium*, *Alternaria*, *Beauveria* and *Cladosporium*. Out of the 15 genera that were identified, with the exception of *Beauveria* and *Cladosporium*, which were found only from the cultivated banana species, the rest of the 12 genera viz., *Penicillium*, *Trichoderma*, *Fusarium*, *Colletotrichum*, *Diaporthe*, *Apiospora*, *Aspergillus*, *Pestalotiopsis*, *Alternaria*, *Mucor*, *Phomopsis*, *Botrytis* and *Helminthosporium*, the majority of all the identified species were found from the wild banana plants. Genera like *Trichoderma*, *Apiospora*, *Pestalotiopsis*, *Mucor*, *Phomopsis*, *Botrytis* and *Helminthosporium* were only found in the wild banana plants (Fig. 2).

Earlier reports state that 61 fungal taxa from 7500 samples of *Musa acuminata* (wild) were isolated from 5 locations (10). It was found that *Colletotrichum gloeosporioides*, *Colletotrichum musae* and sterile mycelia were common fungal endophytes isolated from wild *Musa acuminata* in all the collection sites in Thailand. However, the fungal endophytes were found to differ in all the 5 locations. A previous study reported that fungal endophytes from the healthy roots, pseudostems, corms and leaves of banana plant cv (21). Manzano affected with *Fusarium* wilt and 143 isolates with 11 genera were isolated and some of which are *Fusarium* sp., *Colletotrichum* sp., *Phomopsis* sp., *Cladosporium* sp., *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., mycelia sterilia etc. Out of these 143 fungal endophytes were isolated, 45.46 % were from leaf sample, 20.28 % from root sample, 18.18 % from pseudostem and 16.08 % from corm. A total of 139 fungal endophytes were isolated and identified belonging to 40 fungal taxa and 14 genera from 15 varieties of banana and some of which are *Arthrinium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Verticillium*, mycelium sterile etc (24). *Pestalotiopsis thaeae*, *P. eugeniae* and *P. oxyanthi* were isolated and identified from the leaves of banana as endophytes (12). These studies shows that *Musa* sp. host a wide range of fungal endophytes taxa.

Penicillium capsulatum, *P. citrinum*, *P. thomii* and *P. variculosum* were reported as endophytes isolated from various parts of banana plant (25). *Penicillium* sp. are often detailed as endophytic fungi in various recorded studies from several types of

plants and have been isolated from divergent plant parts (26).

Site effect

The most common isolated fungal endophyte isolates varied from one district to another. Banana samples from Mokokchung district were found to be frequently colonised by *Penicillium* sp. and *Diaporthe* sp. *Trichoderma* sp. was found to be common in Chumoukedima and Peren district, but it was not observed in Mokokchung district. This may be due to environmental conditions in which the banana was found as Chumoukedima and Peren districts are located at a lower elevation ranging between 200–1000 msl where the climate is warmer with high humidity which may attribute to the distribution of *Trichoderma* sp. in these locations, whereas Mokokchung district is located at an altitude above 1000 msl and comparatively cooler with lower humidity (Tables 1 and 2). *Colletotrichum* sp. was found to be most common in Kohima district. *Apiospora* sp. was commonly found only in Chumoukedima and Kohima district. Fungal endophyte species like *Botrytis* sp. was found only in Chumoukedima district, *Helminthosporium* sp., *Alternaria* sp. and *Beauveria* sp. were found only in Kohima district and *Cladosporium* sp. was found only in Peren district.

The endophyte species abundance also varied according to the site within each location. There are a number of studies that shows that site specific factors may influence the level of infection (27). The variation is presumably a reflection of range of environmental conditions of the sample collection sites. This differences in environmental factors includes temperature, rainfall, humidity and potential inoculum sources (10). Other factors such as the elevation of location sites may also contribute in the distribution of the fungal endophyte species. For example, Mokokchung district is characterised by sub-montane terrain featuring valleys, foothills and mid-slope mountains with a warm and sub-tropical climate with an average temperature of 10–15 °C in winter and 28– 30 °C in summer with annual rainfall of 1600–2500 mm. Whereas, Chumoukedima district has an average elevation of 221 msl with hot and humid summer, mild winter and heavy rainfall during monsoon. The maximum temperature during summer is about 34 °C and during winter is about 20 °C. Kohima district, the capital of Nagaland is a mountainous terrain that sits at an elevation of 1261–1444 m and has a moderate, humid subtropical climate with pleasant summers with temperatures ranging from 26–32 °C while winters are cold with occasional frost and light snowfall at higher altitudes.

Samples collected and isolated from all the 4 districts gave high number of sterile mycelia. The predominance of sterile mycelia observed here (37.37 %) with 105 isolates aligns with the high rates for *Musa acuminata* in South China, suggesting that a significant portion of the banana endophyte community may be difficult to sporulate under standard culture conditions (28).

Molecular identification and characterisation of the fungal endophytes

Molecular characterisation was carried out for some selected isolates on the basis of their performance in *in vitro* plant growth promotional test and the antagonistic effects of the isolated fungal endophytes against the *Fusarium* wilt disease of banana (21). For species confirmation, sequencing of the ITS regions were done. The ITS regions were amplified using ITS1 as forward and ITS4 as reverse primers. Molecular identification of the selected fungal

Table 2. Distribution of fungal endophyte isolates Identified based on location (district wise)

Sl. No.	Identified isolates	District	Wild (Leaves)	Wild (Roots)	Cultivated (Leaves)	Cultivated (Roots)	Sub total	Total
1.	<i>Penicillium</i> sp.	Dimapur	6	-	-	1	7	29
		Kohima	1	4	-	-	5	
		Peren	1	3	-	-	4	
		Mokokchung	7	4	1	1	13	
2.	<i>Trichoderma</i> sp.	Dimapur	2	3	-	-	5	11
		Kohima	-	1	-	-	1	
		Peren	-	5	-	-	5	
		Mokokchung	-	-	-	-	0	
3.	<i>Fusarium</i> sp.	Dimapur	4	-	-	-	3	16
		Kohima	2	1	-	1	4	
		Peren	-	2	1	-	3	
		Mokokchung	-	5	-	-	5	
4.	<i>Colletotrichum</i> sp.	Dimapur	1	-	2	-	3	16
		Kohima	10	-	-	-	10	
		Peren	-	-	-	-	0	
		Mokokchung	2	1	-	-	3	
5.	<i>Diaporthe</i> sp.	Dimapur	5	-	-	-	5	30
		Kohima	-	9	-	-	9	
		Peren	1	-	-	-	1	
		Mokokchung	6	8	1	-	15	
6.	<i>Apiospora</i> sp.	Dimapur	1	-	-	-	1	6
		Kohima	5	-	-	-	5	
		Peren	-	-	-	-	0	
		Mokokchung	-	-	-	-	0	
7.	<i>Aspergillus</i> sp.	Dimapur	1	2	-	-	3	6
		Kohima	-	1	1	-	2	
		Peren	-	-	-	-	0	
		Mokokchung	1	-	-	-	1	
8.	<i>Pestalotiopsis</i> sp.	Dimapur	-	-	-	-	0	6
		Kohima	2	-	-	-	2	
		Peren	3	-	-	-	3	
		Mokokchung	1	-	-	-	1	
9.	<i>Mucor</i> sp.	Dimapur	-	-	-	-	0	3
		Kohima	-	-	-	-	0	
		Peren	-	2	-	-	2	
		Mokokchung	-	1	-	-	1	
10.	<i>Phomopsis</i> sp.	Dimapur	-	-	-	-	0	2
		Kohima	-	1	-	-	1	
		Peren	-	-	-	-	0	
		Mokokchung	-	1	-	-	1	
11.	<i>Botrytis</i> sp.	Dimapur	-	-	-	1	1	1
12.	<i>Helminthosporium</i> sp.	Kohima	1	-	-	-	1	1
13.	<i>Alternaria</i> sp.	Kohima	2	-	4	-	6	6
14.	<i>Beauveria felina</i>	Kohima	-	-	-	1	1	1
15.	<i>Cladosporium tenuissimum</i>	Peren	-	-	1	-	1	1
		Total	65	54	11	5	135	135

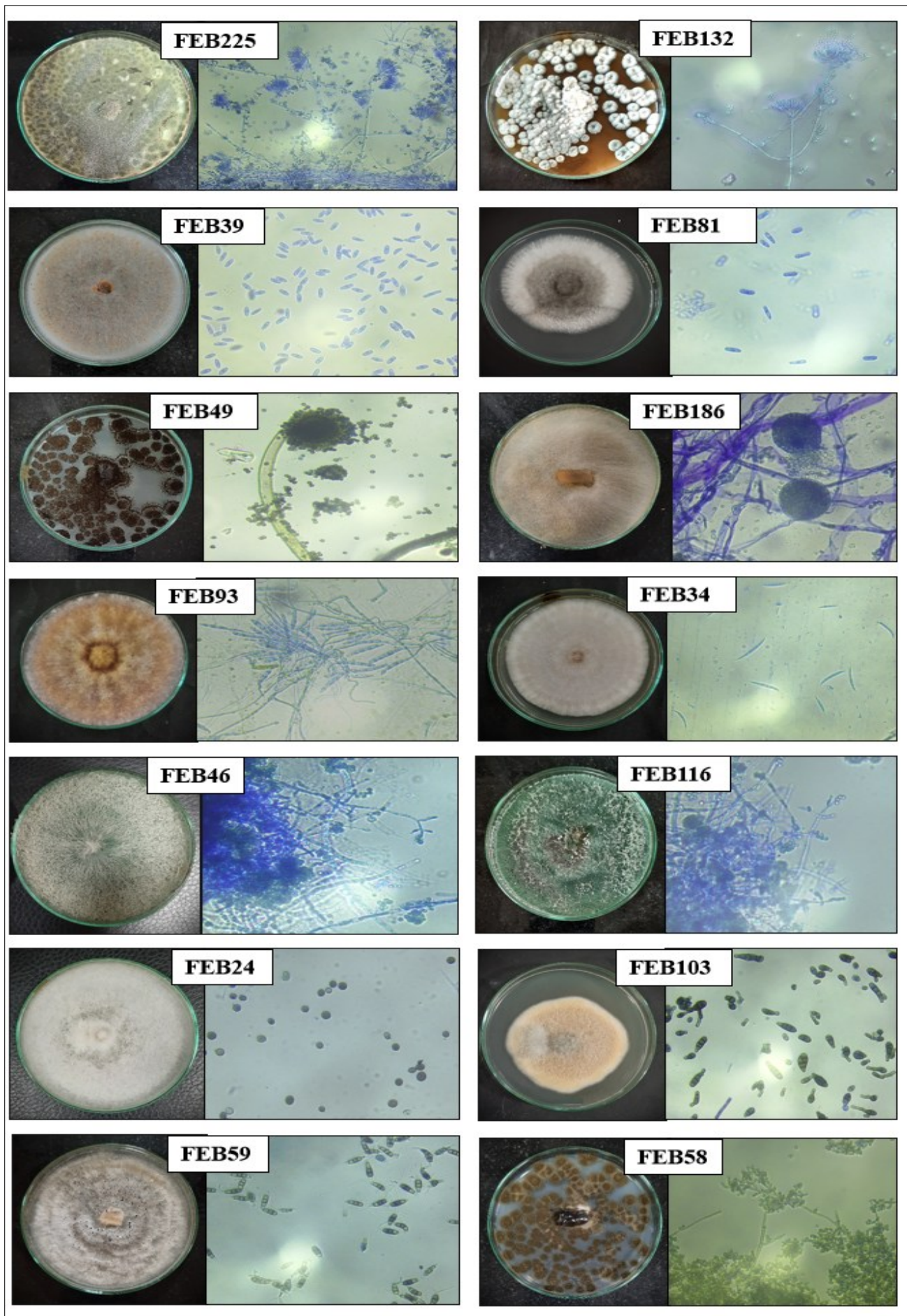


Fig. 2. Some of the isolated and identified fungal endophytes of banana as observed in Petri plates and under microscope (40x). FEB225 and FEB132 (*Penicillium* sp.), FEB39 and FEB81 (*Colletotrichum* sp.), FEB49 (*Aspergillus niger*), FEB186 (*Mucor circinelloides*), FEB93 and FEB34 (*Fusarium* sp.), FEB46 and FEB116 (*Trichoderma* sp.), FEB24 (*Apiospora* sp.), FEB103 (*Alternaria* sp.), FEB59 (*Pestalotiopsis* sp.), FEB58 (*Cladosporium tenuissimum*). *FEB: Fungal endophytes of banana.

endophytes isolate species revealed a diverse number of genera.

Out of the 24 isolates that were identified, 22 belonged to Phylum Ascomycota and 2 belongs to Phylum Mucoromycota. The nucleotide sequences were submitted to GenBank and the accession numbers were acquired for all the identified isolates and phylogenetic tree of the identified isolates were prepared using MEGA11 software and neighbour joining method.

The isolates that were identified through molecular characterisation are *Apiospora longistroma* (FEB75), *Colletotrichum horii* (FEB83), *Cladosporium tenuissimum* (FEB178), *Colletotrichum gloeosporioides* (FEB269), *Mucor circinelloides* (FEB186), *Mucor circinelloides* (FEB251), *Trichoderma asperellum* (FEB46), *Diaporthe phaseolorum* (FEB27), *Diaporthe sp.* (FEB129), *Beauveria felina* (FEB143), *Aspergillus clavatonanicus* (FEB51), *Penicillium citrinum* (FEB187), *Phomopsis sp.* (FEB254), *Colletotrichum fructicola* (FEB65), *Colletotrichum gloeosporioides* (FEB68), *Aspergillus versicolor* (FEB23), *Diaporthe chromolaenae* (FEB249), *Trichoderma asperellum* (FEB116), *Apiospora hydei* (FEB80), *Colletotrichum kahawae* (FEB81), *Diaporthe fructicola* (FEB115), *Fusarium haematococcum* (FEB3), *Trichoderma hamatum* (FEB5) and *Fusarium solani* (FEB9) (Fig. 3, Table 3).

The molecular ecology of fungi is identified using the nuclear ribosomal ITS region and it is most widely used DNA sequence and fungal DNA barcode. It assists in finding out the genus to species of fungi (14). Several workers have also identified fungal endophytes from banana and various other crops. *T. asperellum* and *T. virens* were identified as endophytes isolated from the roots of banana plants through molecular and amplified fragment length polymorphism (AFLP) identification (29). Overall 31 isolates of fungal endophytes were identified from the roots of wild banana (*Musa acuminata*) through molecular characterisation and some of the identified isolates were *Fusarium proliferatum*, *F. oxysporum*, *F. solani*, *Trichoderma atroviride*, *Rhizoctonia solani* etc (11).

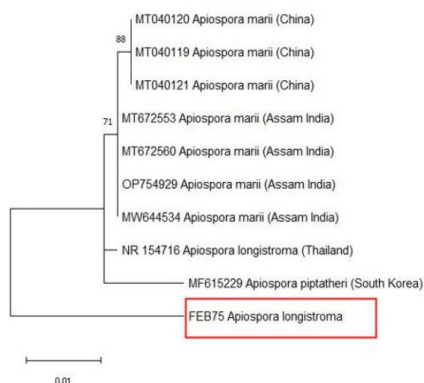
Endophytes like *Colletotrichum*, *Fusarium*, *Pestalotiopsis*, *Penicillium* and *Aspergillus* were identified through molecular characterisation using ITS1 and ITS4 primers from banana leaves (12). Fungal endophytes were identified from *Musa paradisiaca* (plantain banana) using ITS1 and ITS4 primers and identified 9 isolates, some of which are *Cladosporium cladosporioides*, *Fusarium chlamydosporium*, *Fusarium keratoplasticum*, *Fusarium solani* etc (30). Studies on endophytic *Apiospora* species from *Wurfbainia villosa* and grasses in Guangdong and Yunnan provinces was done in China and 199 strains of *Apiospora* sp. were identified through molecular identification using ITS1 and ITS4 primers, the large subunit nuclear rDNA and the partial elongation factor 1- α and d β -tubulin to give a clarified phylogenetic affinity of the genus *Apiospora* and its various species (31). Among many genera of fungi, *Fusarium* has been widely documented to be an endophyte from several plants including banana (10, 32, 33). Isolation and characterisation of endophytic *Fusarium* sp. from the wild bananas of Pandenglang, Indonesia was carried out and identified four *Fusarium* species complex through ITS sequencing and *F. solani* species complex was one among them (34).

Beauveria felina, which is an entomopathogen was also identified as a fungal endophyte in this present study. The isolate molecularly identified as *B. felina* in the current study showed 100 % similarity with *B. felina* that were isolated and identified in other regions of the world (Fig. 3). It is a first report of *Beauveria felina* as an endophyte from banana plants or any other plant in the world (35). The fungus *Beauveria felina* is often classified as one of the so called good biocontrol agents (36). *Beauveria felina* has been found to effectively control fall army worm *Spodoptera frugiperda*, an invasive pest on maize in India (37). However, no information is available about the growth of this entomopathogenic fungus in the presence of other endophytic fungi, which are usually found in plant tissues or its role in plant growth promotion and disease

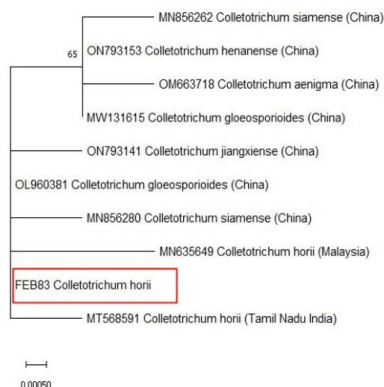
Table 3. The ITS sequence-based identification of the endophytic fungal isolates of banana

Sl. No.	Fungal isolates	GenBank accession No.	Homolog sequence	Sequence identity %	Closest accession number in the database	Isolation source		
						Plant part	Wild/Cultivated	Location
1	FEB75	PP726886	<i>Apiospora longistroma</i>	95.83 %	NR_154716	Leaves	Wild	Kohima
2	FEB83	PP405942	<i>Colletotrichum horii</i>	99.83 %	MT568591	Leaves	Wild	Kohima
3	FEB178	PP729470	<i>Cladosporium tenuissimum</i>	100.00 %	OQ629133	Leaves	Cultivated	Peren
4	FEB269	PP726650	<i>Colletotrichum gloeosporioides</i>	100.00 %	MZ823561	Roots	Wild	Mokokchung
5	FEB186	PP729469	<i>Mucor circinelloides</i>	100.00 %	MH854642	Roots	Wild	Peren
6	FEB251	PP729471	<i>Mucor circinelloides</i>	100.00 %	MH854642	Roots	Wild	Mokokchung
7	FEB46	PP729472	<i>Trichoderma asperellum</i>	99.83 %	KT358889	Roots	Wild	Chumoukedima
8	FEB27	PP766971	<i>Diaporthe phaseolorum</i>	99.46 %	KX815357	Leaves	Wild	Chumoukedima
9	FEB129	PP726704	<i>Diaporthe sp.</i>	99.46 %	ON322885	Roots	Wild	Kohima
10	FEB143	PP715981	<i>Beauveria felina</i>	100.00 %	MH856642	Roots	Cultivated	Kohima
11	FEB51	PP726706	<i>Aspergillus clavatonanicus</i>	100 %	DQ355025	Roots	Cultivated	Chumoukedima
12	FEB187	PP726707	<i>Penicillium citrinum</i>	100 %	OR354745	Roots	Wild	Peren
13	FEB254	PP726708	<i>Phomopsis sp.</i>	99.83 %	MN486556	Roots	Wild	Mokokchung
14	FEB65	PP726709	<i>Colletotrichum fructicola</i>	100.00 %	LC776011	Leaves	Wild	Kohima
15	FEB68	-	<i>Colletotrichum gloeosporioides</i>	89.87 %	MF380748	Leaves	Wild	Kohima
16	FEB23	PP726885	<i>Aspergillus versicolor</i>	100 %	MK027304	Leaves	Wild	Chumoukedima
17	FEB249	PP726710	<i>Diaporthe chromolaenae</i>	100.00 %	MT214362	Roots	Wild	Mokokchung
18	FEB116	PP729473	<i>Trichoderma asperellum</i>	99.84 %	LC075715	Roots	Wild	Kohima
19	FEB80	PP726725	<i>Apiospora hydei</i>	100.00 %	KY 494717	Leaves	Wild	Kohima
20	FEB81	PP726729	<i>Colletotrichum kahawae</i>	100 %	MN856281	Leaves	Wild	Kohima
21	FEB115	PP726730	<i>Diaporthe fructicola</i>	100 %	PP542170	Roots	Wild	Kohima
22	FEB3	PP726881	<i>Fusarium haematococcum</i>	90.40 %	JN088237	Leaves	Wild	Chumoukedima
23	FEB5	PP726711	<i>Trichoderma hamatum</i>	100 %	MN264503	Leaves	Wild	Chumoukedima
24	FEB9	PP729474	<i>Fusarium solani</i>	99.59 %	MG827183	Leaves	Wild	Chumoukedima

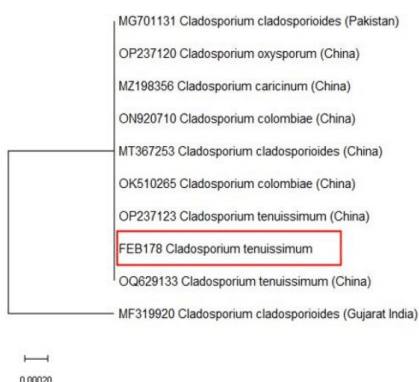
*FEB: Fungal endophytes of banana.



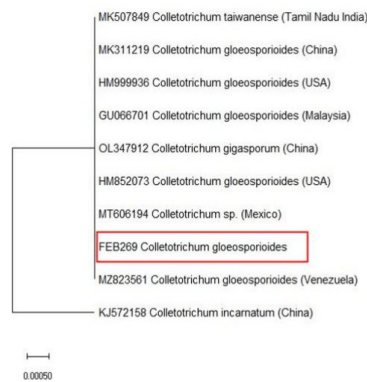
a. FEB75 *Apiospora longistroma*



b. FEB83 *Colletotrichum horii*



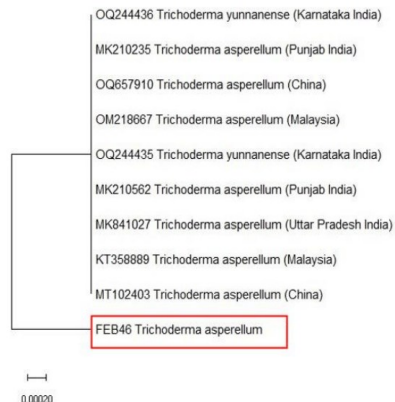
c. FEB178 *Cladosporium tenuissimum*



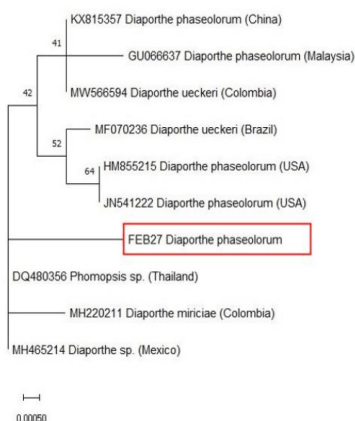
d. FEB269 *Colletotrichum gloeosporioides*



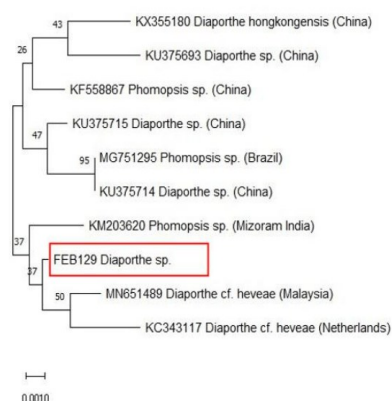
e. FEB186 and FEB251 *Mucor circinelloides*



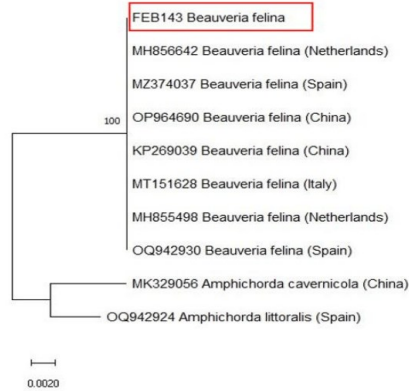
f. FEB46 *Trichoderma asperellum*



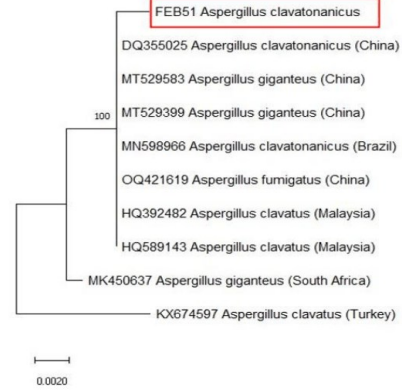
g. FEB27 *Diaporthe phaseolorum*



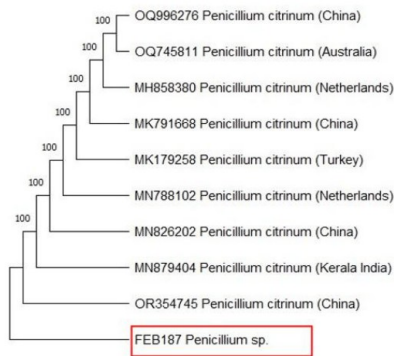
h. FEB129 *Diaporthe* sp.



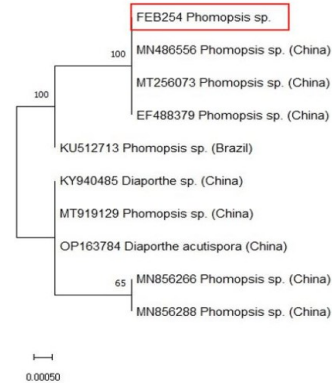
i. FEB143 *Beauveria felina*



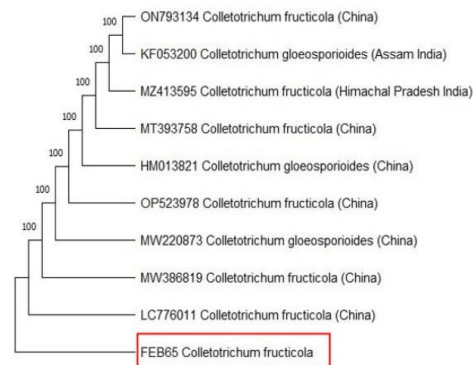
j. FEB51 *Aspergillus clavatonanicus*



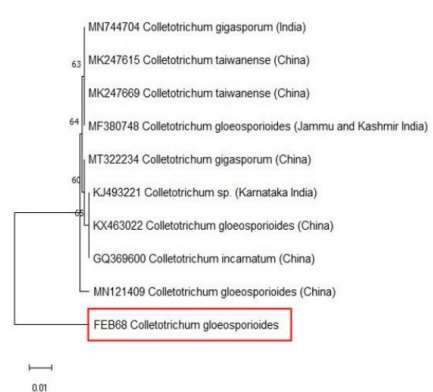
k. FEB187 *Penicillium* sp.



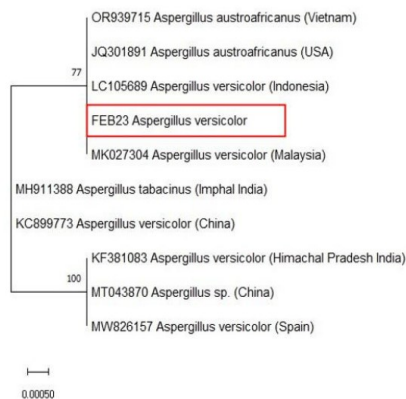
l. FEB254 *Phomopsis* sp.



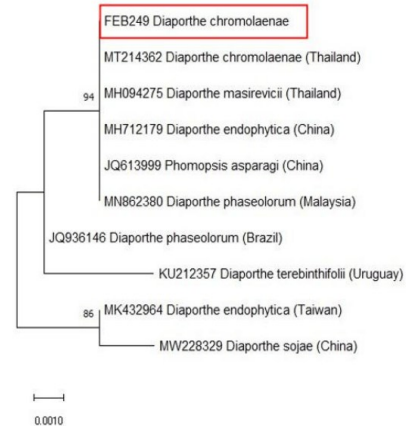
m. FEB65 *Colletotrichum fructicola*



n. FEB68 *Colletotrichum gloeosporioides*



o. FEB23 *Aspergillus versicolor*



p. FEB249 *Diaporthe chromolaenae*

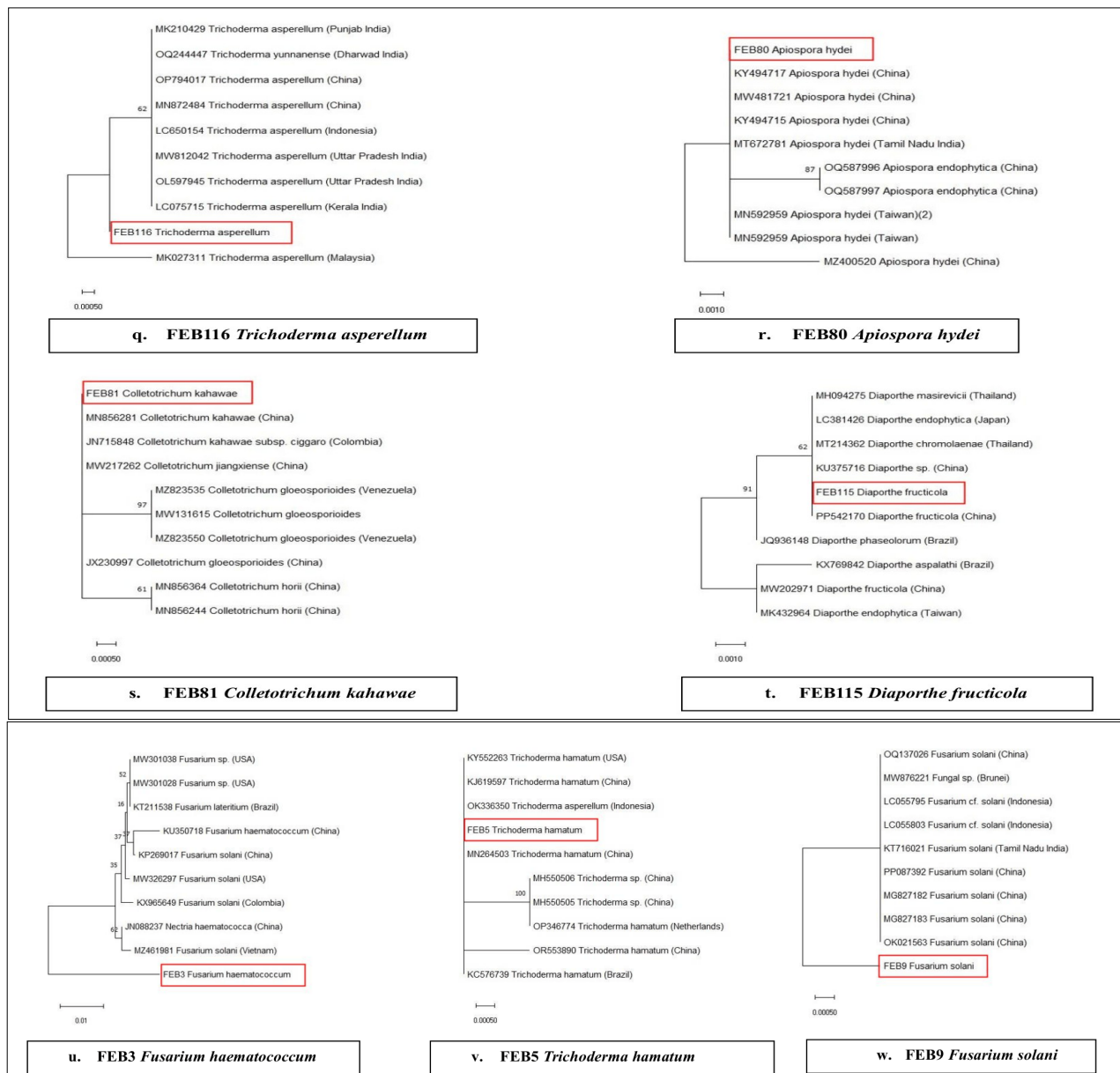


Fig. 3. Phylogenetic analysis of ITS sequences of fungal isolate FEB75, FEB83, FEB178, FEB269, FEB186, FEB251, FEB46, FEB27, FEB129, FEB143, FEB51, FEB187, FEB254, FEB65, FEB68, FEB23, FEB249, FEB116, FEB80, FEB81, FEB115, FEB3, FEB5 and FEB9 with reference sequences retrieved from NCBI (National Center for Biotechnology Information). The analysis was implemented in MEGA 11 using the neighbor-joining method. The number given over branches indicate bootstrap coefficient. *FEB: Fungal endophytes of banana.

suppression (38).

Relative abundance (Ra)

Relative abundance (Ra) was calculated for all the identified endophytes based on their genus, from the samples collected from the 4 districts. Overall, mycelia sterilia was found to be maximum in all the districts with Peren districts leading, followed by Kohima district with a percentage of 12.81 % and 9.61 %, respectively. Among the identified genera, the Ra ranged from 0–5.39 %, with the highest Ra observed in *Diaporthe* sp. with 5.39 % from Mokokchung district which was followed by *Penicillium* sp. with 4.63 %, also from Mokokchung district and *Colletotrichum* sp. with 3.56 % from the samples collected from Kohima district (Table 4). Genera like *Penicillium*, *Fusarium* and *Diaporthe* were found in all the four districts. However, some genera like *Botrytis*, *Helminthosporium*, *Beauveria* and *Cladosporium* were found only in one district, which may indicate that these genera are not commonly found in the banana plant ecosystem.

A total of 163 fungal endophytes were isolated from 200 samples of banana leaf (*Musa acuminata*), some of which belonged to *Alternaria tenuis* (7.9 %), *Uncinula* sp. (1.8 %), *Penicillium* sp. (1.8 %), *Aspergillus* sp. (1.2 %), *Cladosporium* sp. (0.6 %) and sterile mycelium (6.7 %). They also isolated and identified 68 fungal endophytes from 100 root samples and some of which are *Aspergillus* sp. (31 %), *Paecilomyces* sp. (16 %), *Penicillium* sp. (15 %), *Fusarium* sp. (10 %) and sterile mycelium (10 %) (39).

Conclusion

Generally, healthy tissues of plants are known to have considerable diversity of endophytes in contrast with infected plants. This study shows that the leaves and roots of wild and cultivated banana foster a diverse range of fungal endophytes. The current work which was based on collection, isolation and identification serves as a foundation for the study of fungal endophytes associated with wild and cultivated banana of

Table 4. Morphotypes and relative abundance (Ra) of the fungal endophytes isolated from the four districts of Nagaland

Sl. No.	Identified isolates	Phylum	Class	Order	Plant parts	Wild or cultivated banana or both	Relative abundance (Ra in %)					Total isolates
							District wise				Mokokchung	
							Chumoukedima	Kohima	Peren			
1.	<i>Penicillium</i> sp.	Ascomycota	Eurotiomycetes	Eurotiales	l, r	both	2.49	1.78	1.42	4.63	29	
2.	<i>Trichoderma</i> sp.	Ascomycota	Sordariomycetes	Hypocreales	l, r	wild	1.78	0.36	1.78	0	11	
3.	<i>Fusarium</i> sp.	Ascomycota	Sordariomycetes	Hypocreales	l, r	both	1.07	1.42	1.07	1.78	16	
4.	<i>Colletotrichum</i> sp.	Ascomycota	Sordariomycetes	Glomerellales	l	both	1.07	3.56	0	1.07	16	
5.	<i>Diaporthe</i> sp.	Ascomycota	Sordariomycetes	Diaporthales	l, r	both	1.78	3.20	0.36	5.39	30	
6.	<i>Apiospora</i> sp.	Ascomycota	Sordariomycetes	Xylariales	l	wild	0.36	1.78	0	0	6	
7.	<i>Aspergillus</i> sp.	Ascomycota	Eurotiomycetes	Eurotiales	l, r	both	1.07	0.71	0	0.36	6	
8.	<i>Pestalotiopsis</i> sp.	Ascomycota	Sordariomycetes	Amphisphaeriales	l	wild	0	0.71	1.07	0.36	6	
9.	<i>Mucor</i> sp.	Mucoromycota	Mucoromycetes	<i>Mucorales</i>	r	wild	0	0	0.71	0.36	3	
10.	<i>Phomopsis</i> sp.	Ascomycota	Sordariomycetes	Diaporthales	r	wild	0	0.36	0	0.36	2	
11.	<i>Botrytis</i> sp.	Ascomycota	Leotiomycetes	Helotiales	r	cultivated	0.36	0	0	0	1	
12.	<i>Helminthosporium</i> sp.	Ascomycota	Dothideomycetes	Pleosporales	l	wild	0	0.36	0	0	1	
13.	<i>Alternaria</i> sp.	Ascomycota	Dothideomycetes	Pleosporales	l	both	0	2.14	0	0	6	
14.	<i>Beauveria felina</i>	Ascomycota	Sordariomycetes	Hypocreales	r	cultivated	0	0.36	0	0	1	
15.	<i>Cladosporium tenuissimum</i>	Ascomycota	Dothideomycetes	Capnodiales	l	cultivated	0	0.36	0	0	1	
16.	<i>Mycelia sterilia</i>	Ascomycota/ Basidiomycota	Indeterminate	Indeterminate	l, r	both	7.12	9.61	12.81	7.83	105	
17.	Non identified isolates	-	-	-	-	-	-	-	-	-	41	
Total											281	

*l: leaf; r: roots.

Nagaland. A total of 135 isolates were identified through morphological characterisation and out of these, 24 isolates were identified based on molecular characterisation. A new species not yet reported elsewhere as a fungal endophyte was isolated in this present study. Future studies need to be employed for broader sampling strategies and ecological analysis to investigate fungal endophyte diversity using diversity indices and other useful statistical approaches. Such studies will provide a robust analysis of the richness of species present in this part of the region. The isolated fungal endophytes could be tested for their role in plant growth and development and its ability in suppressing important plant pathogens needs to be thoroughly studied which will play a significant role in managing the diseases and also assist in the overall growth and development of the host plant.

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Authors' contributions

B, SB, NTA and HSD carried out the conceptualisation, research and methodology. B and DL carried out the data analysis. B, KS and ELK carried out the sample collections and laboratory works. B, SB and PC carried out the writing and drafting of the article. All authors read and approved the final 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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