# Diversity, community composition and phylogeny of culturable fungal endophytes of Dioscorea bulbifera L.

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## Abstract

The origin of endophytic symbiosis dates long back in evolutionary history. Over the period of evolution, they have established a special relationship with their host plants. The research nowadays is focused on understanding the ecology, evolution and bioactive potential of endophytes. The current study is aimed to describe the biodiversity of culturable fungal endophytes associated with *Dioscorea bulbifera* L. Overall, the endophytic fungal taxa belonged to two phyla, four classes, ten orders, twenty-five genera and thirty-eight species showing abundant diversity. The class Dothidiomycetes was reported to be the richest and most abundant in terms of species richness. Diversity indices demonstrated significant diversity of fungal endophytes within and between the different tissues. Moreover, fungal isolates such as, *Acrocalymma medicaginis*, *Curvularia lycoperscii, Talaromyces macrospora, Fusarium laceratum, Paecilomyces formosus* and *Microascus cirrosus* obtained in this study have not been reported earlier as endophytes from any plant. The endophytic fungal isolates, *Nigrospora oryzae, Flavodon flavus* and *Talaromyces sp.*, have been reported first time as endophytes from *D. bulbifera* L. Since, the relevance of endophyte-plant interactions is still indistinct therefore, deeper understanding of these interactions at molecular and genetic level is required to be done.

## Introduction

Plants can no longer be considered as standalone entities nowadays. Indeed, they harbour a huge diversity of microbes residing inside or outside them. Endophytes are the microorganisms that live in the internal tissues of the plants without causing immediate overt effects (Bacon and White 2000; Tiwari and Bae 2022). They are integral part of plant biology that can serve as important component of plant micro-ecosystems (Christian et al. 2016). They have characteristics that are important for ecology and evolution of the plant. They have been isolated from several plants of economic and medicinal interest. Among various groups of endophytes harboured by the plants, fungal endophytes constitute a highly beneficial group. They can be considered as a hidden or unexplored components of fungal diversity (Rodriguez and Redman 2008). They are critical for the survival of the host plant and its fitness (Ahmad et al. 2022). Although, the interactions between plant and endophytic fungi are symbiotic but they may turn out to be pathogenic (latent) or saprophytic during certain phases of the plant growth or under certain environmental conditions such as senescence, stress (Xia et al. 2019).

The exciting findings in plants with nutritional or medicinal importance have encouraged scientific community to explore the role of fungi associated with them. In addition, the striking species diversity of fungal endophytes isolated from host plants growing in diverse ecological niches has drawn more importance of researchers (Duan et al. 2019). They have potential applications in various fields of industry, agriculture and medicine. The quest for the discovery of new compounds from fungal endophytes has become a fascinating area of research nowadays. The molecular crosstalk triggered by the endophytes with the host plants produces untapped bioactive metabolites with profitable outcomes (Dhayanithy et al. 2019). Interestingly, a plethora of metabolites produced by them have wide range of therapeutic applications (Duan et al. 2019). Their biocontrol potential against bacterial, fungal, insect and nematode pathogens endows protection to the host plants against various diseases (Potshangbam et al. 2017; Gupta et al. 2022). Besides, fungal endophytes also boost sustainable agriculture thereby, reducing usage of chemical fertilisers and pesticides (Khan et al. 2017; Kaul et al. 2017). They are considered as potential bio-stimulants that could facilitate commercial bioprospecting of secondary metabolites (Das et al. 2022).

The members of the family Dioscoreacea have great ethnopharmacological and pharmacological potential. The multifaceted therapeutic applications of its members can be attributed to their diverse phytochemical composition. *Dioscorea bulbifera* L. is a unique herb among 600 species of the family Dioscoreaceae. It is a perennial, creeping and climbing vine with non-spiny stem, widely distributed in tropical and temperate regions of Asia, Africa, and America (Kumar et al. 2012). The remedial potential of this herb against diabetes, tumour, cancer, dyslipidaemia, HIV, cardiovascular, reproductive disorders, viral, parasitic, bacterial and fungal infections has been reported (Ghosh et al. 2013; Ghosh et al. 2015; Guan et al. 2017; Bhat et al. 2019; Kundu et al. 2021). The unique phytochemistry of its leaves, bulbils, flowers and tubers make it an immensely important medicinal plant (Galani et al. 2017). The main phytoconstituents include flavonoids (Gao et al. 2002), diterpenoids (Murray et al. 1984; Komoroi 1997; Teponno et al. 2006, 2007) and steroidal saponins (Li and Zhao 1999; Teponno et al. 2006). Although, clerodane diterpenoids have been reported as primary and characteristic constituent of this plant (Komori 1997) but the activity of *D. bulbifera* L. is attributed mainly to the action of various steroidal saponins (Ghosh et al. 2015). Therefore, there is a possibility that the endophytic fungi residing inside it may be producing similar bioactive metabolites as produce by it.

*D. bulbifera* has been less explored for its endophytic community composition. Literature analysis demonstrated that only a few reports are available on the isolation of fungal endophytes from it. Li et al (2008) conducted a study to isolate endophytic Streptomycetes from pharmaceutical plants in tropical rainforests of Xishuangbanna, Yunnan province. Out of forty-one isolated strains, three were obtained from *D. bulbifera* L. In another study, endophytic actinomycetes were isolated from traditional medicinal plants of Sichuan region of China which included *D. bulbifera* (Peng et al. 2015). In addition, a novel endophytic actinobacteria, *Streptomyces dioscori* was isolated from the bulbils of *D. bulbifera* (Wang et al. 2018). Although, many workers have reported the pharmacological importance of the plant, but information about their endophytic community composition is seldom available. The current study is aimed to provide an overview of the diversity and distribution of culturable endophytic fungi from various tissues of *D. bulbifera* L. The data presented here will add information on the existing literature of fungal endophytes obtained from *D. bulbifera* L. and interactions between them. Therefore, it is worthwhile to explore endophytic communities associated with it.

## Materials And Methods

# Composition of liquid and solid media

Liquid and solid media were prepared in distilled water with pH of 6.8 ± 2 and autoclaved at 121°C for 21 min. The composition of 1 L of each media included the following ingredients 1) Potato dextrose broth (PDB): 200 g potatoes, 20 g dextrose; 2) Potato dextrose agar (PDA) 200 g potatoes, 20 g dextrose, 15 g agar; 3) Czapek dox agar (CDA): 30 g sucrose, 2 g sodium nitrate, 1g dipotassium phosphate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 0.01 g ferrous sulphate; 4) Glycerine asparagine agar (GAA): 1 g L-Asparagine, 1 g dipotassium phosphate, 1 ml trace salt solution; 20 g agar; 5) Muller Hinton agar (MHA): 300 g HM infusion B, 17.5 g casein, 1.5 g starch, 17 agar; 6) Nutrient broth (NB): 5 g peptone, 5 g sodium chloride, 1.5 g HM peptone B, 1.5 g yeast extract; 7) Nutrient agar (NA): 5 g peptone, 5 g sodium chloride, 1.5 g HM peptone B, 1.5 g yeast extract, 15 g agar; 8) Water agar (WA): 15 g agar. The antibiotic, chloramphenicol at a concentration of 1 mg/L was added (except for MHA, NA and NB) to avoid the growth of bacteria, if any.

## Study Site And Sampling

The young, healthy and asymptomatic plants were randomly selected at the collection site located at Dhok Banyad (Lattitude 33.0493° N and 74.4907° E longitude; altitude 2077 feet above mean sea level), tehsil Beri Patan in district Rajouri of Jammu and Kashmir (UT), India (Fig. 1). The girth of the selected plants was approximately 2 cm. The sampling was done in summer season (September-October). The healthy samples including bulbil, leaf, stem and tuber (Fig. 2) were collected, kept in sterile zip lock bags, immediately bought to lab and stored in refrigerator at 4 C till further processing. The plant samples were identified by Dr Harish Chander, Department of Botany, University of Jammu (J&K), India.

## Isolation Of Fungal Endophytes

Different media were employed for the isolation of culturable fungal endophytes from the selected plant. Surface sterilization and isolation of fungal endophytes was done by Direct inoculation method (Sharma et al. 2018) with slight modifications. Healthy plant tissues were washed gently with mild detergent under running tap water to remove dust and debris. The samples were processed further under aseptic conditions. Surface sterilization technique involved washing of plant material twice in distilled water for about 1 min followed by treatment with 70% ethanol for 1 min. Further, washing of various tissues was done with sodium hypochlorite (0.5%) for different time intervals, depending on the type of tissue. Leaves were treated with 0.5% sodium hypochlorite for 30 sec while stem, bulbils and roots were treated with 0.5% sodium hypochlorite for 1 min. Again, the samples were treated with 70% ethanol for 1 min. Each step was followed by washing with autoclaved distilled water for 2 min. Surface sterilized plant parts were dried with sterile filter paper under aseptic conditions. The outer hard coat of bulbils and tuber samples was peeled with sterile scalpel. The samples were further chopped into small segments with sterile scalpel (5–10 mm) and placed on different media for the isolation of fungal endophytes.

Schultz imprinting method (Schulz et al. 1993) was followed to determine the efficacy of surface sterilisation technique in removing epiphytes. Surface sterilized explants were imprinted on one end of inoculated plates and observed for the growth, if any. In another control, the last soaking distilled water (0.1 ml) was poured and spreaded on petri plates containing specific medium. The plates were observed for growth, if any to ensure stringent surface sterilisation conditions. The inoculated plates were stored at 28 C and monitored at regular intervals for the growth of fungal endophytes or any microbial growth contamination. Every time a single mycelium developed from explants; it was sub-cultured. The isolated fungal endophytes were purified and stored on slants at 4 ºC for further use. The purity of the endophytic fungal isolates was checked using hyphal tip method as followed by Duan et al (2019).

## Characterisation Of Endophytic Fungal Isolates

The initial characterisation of endophytic fungal isolates involved observation of morphological characteristics. Total genomic DNA was isolated from fungal endophytes grown in potato dextrose broth (Himedia) at 28°C for 7 days using cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof 1984) with slight modifications. The quality and quantity the isolated gDNA were checked using 0.7% agarose (Sigma-Aldrich) gel electrophoresis stained with ethidium bromide against the standard λ DNA. Gel documentation was done using gel documentation unit (BioRad, Germany). DNA purification was done by giving RNase treatment to the isolated gDNA. The quantification of gDNA was done using Nanodrop (NanoDrop, 800, Thermo Scientific). The PCR amplification of nuclear ribosomal internal transcribed spacer (ITS) region for sequencing was carried out in a Thermal Cycler (Eppendorf MasterCycler) using universal primers ITS-1 (5 -TCCGTAGGTGAACCTGCGG-3 ) and ITS-4 (5 -TCCTCCGCTTATTGATATGC-3 ) (White et al. 1990). The primers amplified ITS1-5.8S-ITS2 regions of rDNA. PCR reaction was run for 25 µl reaction mixture and it comprised of 12.5 µl PCR grade water (Sigma), 2.5 µl Taq buffer (10X); 2 µl $MgCl_2$ (2 mM); 2.5 µl dNTPs (2.5 mM each); 2 µl of each primer (1 pM/µl); 0.5 µl (2.5 U/µl) of Taq polymerase (ThermoFisher, Bangalore, India) along with 1 µl (50 ng/µl) of template DNA. The thermal cycler was programmed with initial denaturation temperature at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec and extension at 72°C for 1 min. The final extension was done at 72°C for 10 min. The PCR product was electrophoresed using 1.2% agarose gel stained with ethidium bromide. The amplified product was sent for Sanger sequencing at Sci Genome, Cochin. The results of sequencing were obtained in FASTA format. Basic local alignment search tool (BLAST) was used to find out sequence identity with sequences available in the database http://blast.ncbi.nlm.nih.gov/Blast.cgi; http://www.ncbi.nlm.nih.gov/genbank/). The ITS gene sequence data of identified endophytic fungi was submitted in National Centre for Biotechnology Information (NCBI), GenBank (http://www.ncbi.nlm.nih.gov/genbank/submit) to obtain accession numbers.

Evolutionary analysis was conducted using the software MEGA-X (Tamura et al. 2013) and the evolutionary history of the fungal endophytes was inferred using the Neighbor-Joining method (Saitou and Nei 1987). Bootstrap values, represented the percentage of replicate trees (500 replicates) in which the associated taxa clustered together were shown next to the branches (Felsenstein 1985).

# Data analysis

Data were processed using Microsoft Access v. 2010 and diversity analysis was carried out with Microsoft Excel v. 2010. The colonization frequency (CF%) of endophytic fungal isolates was calculated (Petrini and Fisher 1988; Hata and Futai 1995) and expressed in percentage. Colonization rate demonstrate the degree of colonisation of each endophytic fungal isolates in different tissues of the host plant

$CF\ (\%) = (N/N_t) \times 100$

where N = Total number of explants yielding ≥ 1 endophytic fungal isolate and

$N_t$ = Total number of segments of plant tissue inoculated.
Similarly, colonisation and isolation rate of endophytic fungal isolates in different tissues was calculated following the method of Kumar and Hyde (2004).
$CR = (T/T_E) \times 100$

where T = Total number of segments of plant tissues colonized by endophytic fungal isolates and $T_E$ = Total number of explants inoculated
The isolation rate (IR) was calculated to demonstrate the richness of endophytic fungal isolates and the degree of multiple colonization in different tissues of the host plant.
$IR = (T_E/T_F) \times 100$

where T = Total number of endophytic fungal isolates obtained from plant tissues and

$T_E$ = Total number of explants inoculated
The similarity of endophytic fungal assemblages among different tissues was estimated using the similarity indices (Osono and Mori 2004) as given below:

Sorensen's index of similarity (QS) = 2a/ (2a + b + c + d + e)
where 'a' is the number of species common in endophytic population of stem, leaf, tuber and bulbil

while b, c, d and e are the number of species specified to stem, leaf, tuber and bulbils respectively.

Jaccard's index of similarity (JS) = a/(a + b + c + d + e)
where 'a' is the number of species common in endophytic population of stem, leaf, tuber and bulbil

while b, c, d and e are the number of species specified to stem, leaf, tuber and bulbils respectively.

Similarly, the diversity of endophytic fungal assemblages among different tissues was estimated using the diversity indices such as, Shannon diversity index (H'), Simpson's index (D) and Simpson's diversity index (1-D) as given below (Simpson 1951; Suryanarayanan and Kumaresan 2000; Tao et al. 2008; Kusari et al. 2013).

Simpson's index of dominance (D) = $\sum (n/N)^2$

where n = the total number of endophytic fungal isolates of a particular species and

N = the total number of endophytic fungal isolates of all species.

Simpson's diversity index = 1-D

Shannon-Wiener index (H') = $\sum (p_i \ln p_i)$

where $p_i = n/N$
It is important to note that the natural log makes all of the terms of the summation negative for H', therefore the inverse of the sum is taken.

Other diversity indices such as, species richness among the endophytic fungal isolates was determined by calculating the Menhinick's index ($D_{mn}$) using the formula given below (Whittaker 1977).

Menhinick's species richness ($D_{mn}$) = $S/(N)^{1/2}$

where S = total number of species

The fungal dominance referred as Camargo's index was determined as inverse of species richness. A species can be defined as dominant if $p_i > 1/D_{mn}$, where 'p' is the relative abundance of a species and 'i' defined as the number of competing species present in the community. Species evenness was also calculated as shown below (Kusari et al. 2013).

Camargo's index = $1/D_{mn}$

where $D_{mn}$ = Species richness

Species evenness (E) = H'/ln S.

## Results

# Isolation of fungal endophytes

In the current study, a total of 187 endophytic fungal isolates were obtained from 1600 surface sterilised explants. A huge variation in the number of endophytic fungal isolates residing inside various tissues of the selected plant was also observed (Table 1). Bulbils were found to inhabit maximum (38%) number of fungal endophytes followed by other tissues such as tuber (28%), stem (23%) and leaf (11%). The variation was depicted in the form of statistical data. Calculation of the colonisation rate revealed that bulbils had maximum colonisation rate of 16.5% followed by 12.25% for tuber, 9.5% for stem and 5% for leaf. Similarly, isolation rate was found to be maximum in bulbils (17.75%).

Table 1
Colonisation and isolation rate of endophytic fungal isolates of *D. bulbifera* L.

| Tissues | Segments incubated (A) | Segments infected (B) | Total isolates (C) | Endophytic species | Total CR (B/C) | Total IR (C/A) |
|---|---|---|---|---|---|---|
| **Stem** | 400 | 38 | 43 | 9 | 0.095 | 0.107 |
| **Leaf** | 400 | 20 | 20 | 11 | 0.05 | 0.05 |
| **Bulbils** | 400 | 66 | 71 | 24 | 0.165 | 0.177 |
| **Tuber** | 400 | 49 | 52 | 20 | 0.122 | 0.13 |
| **Total** | 1600 | 173 | 187 | 64 | - | - |
| where IR: Isolation rate; CR: Colonisation rate | | | | | | |

## Characterisation Of Fungal Endophytes

The endophytic fungal isolates were characterized based on sequence analysis of ITS regions. These sequences were compared with the corresponding sequences of reference fungal taxa available in the database. The best matching sequences obtained in the GenBank database for endophytic fungal isolates have been summarized below (Table 2). The percentage similarity for most of the sequences of endophytic fungal isolates obtained after BLAST analysis ranged between 99–100% except for the two endophytic fungal isolates TD10 and TD10Z which it was found to be 97%. The confirmed sequences were submitted to the GenBank to retrieve accession numbers. Overall, the endophytic fungal taxa belonged to twenty-five genera and thirty-eight species.

Table 2
Molecular characterisation of endophytic fungal isolates of *D. bulbifera* L.

| S. no | Accession No. | Isolation source | Closely related species | Query coverage | % Similarity | E-value | Identified fungus | Gen Bank accession number |
|---|---|---|---|---|---|---|---|---|
| 1. | AS1 | Stem | KP638340 *Bipolaris sorokiniana* | 100 | 100 | 0 | *Bipolaris sorokiniana* | MK075008 |
| 2. | AS2 | Stem | MG515225 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK075010 |
| 3. | AS3 | Stem | KY785009 *Fusarium incarnatum* | 100 | 100 | 0 | *Fusarium incarnatum* | MK189484 |
| 4. | AS7 | Stem | KX685659 *Curvularia lunata* | 100 | 100 | 0 | *Curvularia lunata* | MK075011 |
| 5. | AS17 | Stem | KU360629 *Diaporthe longicolla* | 100 | 99 | 0 | *Diaporthe longicolla* | MK075019 |
| 6. | ASW3 | Stem | KP638340 *Bipolaris sorokiniana* | 100 | 100 | 0 | *Bipolaris sorokiniana* | MK075017 |
| 7. | ASW7 | Stem | KX894661 *Aspergillus protuberus* | 100 | 100 | 0 | *Aspergillus protuberus* | MK075007 |
| 8. | ASW13 | Stem | KY211035 *Fusarium chlamydosporum* | 100 | 100 | 0 | *Fusarium chlamydosporum* | MK075009 |
| 9. | AL12 | Leaf | KP998485 *Phyllosticta capitalensis* | 100 | 99 | 0 | *Phyllosticta capitalensis* | MK075018 |
| 10. | ALW1 | Leaf | KX685658 *Curvularia lunata* | 100 | 100 | 0 | *Curvularia lunata* | MK075020 |
| 11. | ALW4 | Leaf | MG515225 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK075016 |
| 12. | ALG8 | Leaf | KC662170 *Colletotrichum gloeosporioides* | 100 | 100 | 0 | *Colletotrichum gleosporioides* | MK075012 |
| 13. | ALG9 | Leaf | JX625169 *Colletotrichum destructivum* | 100 | 100 | 0 | *Colletotrichum destructivum* | MK189485 |
| 14. | AR1 | Tuber | KY910884 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK075015 |
| 15. | AR9(3) | Tuber | MG980071 *Sarocladium implicatum* | 100 | 99 | 0 | *Sarocladium implicatum* | MK075022 |
| 16. | ARW1 | Tuber | KF751072 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK075013 |
| 17. | ARW2 | Tuber | KP686180 *Clonostachys pseudochroleuca* | 100 | 100 | 0 | *Clonostachys pseudochrolencha* | MK075014 |
| 18. | R9(3) | Tuber | KT310923 *Sarocladium implicatum* | 100 | 99 | 0 | *Sarocladium implicatum* | MK075021 |
| 19. | BD3B | Bubils | MF171065 *Cladosporium cladosporioides* | 100 | 100 | 0 | *Cladosporium cladosporioides* | MK189486 |
| 20. | BD18 | Bulbils | MH010905 *Curvularia specifera* | 99 | 100 | 0 | *Curvularia specifera* | MK119226 |
| 21. | BD2B | Bulbils | KY785009 *Fusarium incarnatum* | 100 | 100 | 0 | *Fusarium incarnatum* | MK119227 |
| 22. | BD19 | Bulbils | MG543710 *Fusarium proliferatum* | 100 | 100 | 0 | *Fusarium proliferatum* | MK119228 |
| 23. | TD4Z | Tuber | MH027197 *Epicoccum nigrum* | 99 | 99 | 0 | *Epicoccum nigrum* | MK992489 |
| 24. | TD8 | Tuber | MH027197 *Epicoccum nigrum* | 100 | 99 | 0 | *Epicoccum nigrum* | MK992772 |
| 25. | TD10Z | Tuber | DQ018090 *Dictyosporium hepatasporum* | 100 | 97 | 0 | *Dictyosporium hepatosporum* | MK119232 |
| 26. | H22 | Bulbils | MG735754 *Fusarium proliferatum* | 100 | 100 | 0 | *Fusarium proliferatum* | MK119229 |
| 27. | G2 | Bulbils | KY785009 *Fusarium incarnatum* | 100 | 100 | 0 | *Fusarium incarnatum* | MK119230 |
| 28. | G4 | Bulbils | JX391935 *Fusarium laceratum* | 99 | 100 | 0 | *Fusarium laceratum* | MK119231 |
| 29. | G8 | Bulbils | MH161266 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK992776 |
| 30. | BD4 | Bulbils | MG679518 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK119214 |

| S. no | Accession No. | Isolation source | Closely related species | Query coverage | % Similarity | E-value | Identified fungus | Gen Bank accession number |
|---|---|---|---|---|---|---|---|---|
| **31.** | TD2 | Tuber | KU939069 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK119215 |
| **32.** | TD5 | Tuber | MG272269 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK119216 |
| **33.** | IF | Tuber | KY432816 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK994106 |
| **34.** | WAN | Tuber | KY290716 *Fusarium chlamydosporum* | 100 | 100 | 0 | *Fusarium chlamydosporum* | MK119217 |
| **35.** | G3 | Bulbils | KU939069 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK119218 |
| | G9 | Bulbils | KY910878 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK119219 |
| **37.** | H1B | Bulbils | KX442659 *Curvularia lunata* | 100 | 100 | 0 | *Curvularia lunata* | MK119222 |
| **38.** | TD3 | Tuber | FJ755236 *Phomopsis longicolla* | 100 | 99 | 0 | *Phomopsis longicolla* | MK119220 |
| **39.** | TD1 | Tuber | KR047085 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK119221 |
| **40.** | Q1 | Tuber | MF663687 *Talaromyces flavus* | 100 | 99 | 0 | *Talaromyces flavus* | MK994104 |
| **41.** | BD12 | Bulbils | KU752196 *Fusarium equiseti* | 100 | 100 | 0 | *Fusarium equiseti* | MK248617 |
| **42.** | G16 | Bulbils | MG923826 *Botryosphaeria dothidea* | 100 | 100 | 0 | *Botryosphaeria dothidea* | MK119223 |
| **43.** | BD5 | Bulbils | FJ755236 *Phomopsis longicolla* | 100 | 99 | 0 | *Phomopsis longicolla* | MK119224 |
| **44.** | H15A | Bulbils | KP170621 *Acrocalymma medicaginis* | 100 | 100 | 0 | *Acrocalymma medicaginis* | MK119225 |
| **45.** | TD11 | Tuber | LT899791 *Talaromyces angelicus* | 100 | 99 | 0 | *Talaromyces angelicus* | MK994099 |
| **46.** | L2 | Leaf | JX677940 *Talaromyces flavus* | 100 | 99 | 0 | *Talaromyces flavus* | |
| **47.** | TD10 | Tuber | JN418791 *Xylaria sp.* | 98 | 97 | 0 | *Xylaria sp.* | MK205002 |
| **48** | Z2 | Leaf | MF5809671 *Nigrospora oryzae* | 100 | 100 | 0 | *Nigrospora oryzae* | MK205003 |
| **49** | N1 | Bulbils | MG708106 *Parathyridaria percutanea* | 100 | 99 | 0 | *Parathyridarea percutanea* | MK205004 |
| **50** | L1 | Leaf | MG199583 *Chaetomium globosum* | 100 | 100 | 0 | *Chaetomium globosum* | MK205005 |
| **51** | G1 | Bulbils | G576111 *Epicoccum sorghinum* | 100 | 100 | 0 | *Epicoccum sorghinum* | MK248637 |
| **52** | GT1Z | Bulbils | KX011505 *Talaromyces macrospores* | 99 | 100 | 0 | *Talaromyces macrospora* | MK992870 |
| **53** | BD20Z | Bulbils | MG708106 *Parathyridaria percutanea* | 100 | 99 | 0 | *Parathyridarea percutanea* | MK205006 |
| **54** | BD14 | Bulbils | KY883347 *Curvularia lycopersicii* | 100 | 100 | 0 | *Curvularia lycopersicii* | MK205007 |
| **55** | BD1B | Bulbils | KY523047 *Clonostachys rosea* | 100 | 99 | 0 | *Clonostachys rosea* | MK205008 |
| **56** | N4 | Bulbils | G708106 *Parathyridaria percutanea* | 100 | 99 | 0 | *Parathyridaria percutanea* | MK205009 |
| **57** | T1 | Tuber | MH038177 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK205010 |
| **58** | ZB | Leaf | KX272862 *Microascus cirrosus* | 100 | 100 | 0 | *Microascus cirrosus* | MK205011 |
| **59** | TD9Z | Tuber | MG272269 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK205013 |
| **60** | WAT | Tuber | MG272269 *Fusarium solani* | 100 | 100 | 0 | *Fusarium solani* | MK205014 |
| **61** | TD7A | Tuber | MF135528 *Talaromyces purpureogenus* | 100 | 100 | 0 | *Talaromyces purpureogenus* | MK249801 |
| **62** | Z1 | Leaf | NR_153472 *Nigrospora guilinensis* | 100 | 100 | 0 | *Nigrospora guilinensis* | MK994103 |

| S. no | Accession No. | Isolation source | Closely related species | Query coverage | % Similarity | E-value | Identified fungus | Gen Bank accession number |
|---|---|---|---|---|---|---|---|---|
| **63** | N2 | Bulbils | KX681615 *Fusarium cf. incarnatum* | 100 | 100 | 0 | *Fusarium incarnatum* | MK992899 |
| **64** | Z3 | Leaf | KY283787 *Dichotomopilus funicola* | 100 | 99 | 0 | *Dichotomopilus funicola* | MK205012 |
| **65** | ZA | Leaf | KM277979 *Paecilomyces formosus* | 97 | 99 | 0 | *Paecilomyces formosus* | MK205015 |
| **67** | BD17 | Bulbils | MH345962 *Alternaria tenuissima* | 100 | 100 | 0 | *Alternaria tenuissima* | MK994100 |
| **68** | AS31 | Stem | FJ478126 *Flavodon flavus* | 100 | 100 | 0 | *Flavodon flavus* | MK075023 |

## Phylogenetic Analysis

A phylogenetic tree was constructed using Neighbor-Joining method (Fig. 3) that depicted evolutionary relationship among taxa of endophytic fungal isolates with the reference taxa obtained from Gen Bank. Bootstrap values were computed and shown next to the branches in the phylogenetic tree indicating the stability of tree/subtree and the bootstrap analysis was done for 500 replicates. It can be demonstrated that the endophytic fungal taxa could be included in the phylum Ascomycota and Basidiomycota (Table 3). The phylum Ascomycota was dominant representing almost 97% of the endophytic fungal taxa whereas Basidiomycota was represented by 3% only. The endophytic fungal taxa of the phylum Ascomycota included three classes viz. Sordariomycetes, Dothidiomycetes and Eurotiomycetes. The class Sordariomycetes was represented by the orders Hypocreales, Xylariales, Sordariales, Glomerellales, Microascales and Diaporthales. Other classes, such as Dothidiomycetes was represented by order Pleosporales and Botryosphaeriales whereas Eurotiomycetes was represented by order Eurotiales (Fig. 4). Similarly, the phylum Basidiomycota corresponded to class Agaricomycetes with order Polyporales. The class Dothidiomycetes was reported to be the richest and most abundant in terms of species richness followed by Sordariomycetes and Eurotiomycetes.

Table 3
Species richness of various orders of endophytic taxa obtained from *D. bulbifera* L.

| Phylum | Classes | Orders | Number of species obtained | Species richness |
|---|---|---|---|---|
| **Ascomycota** | Sordariomycetes | Hypocreales | 9 | 65.8 |
| | | Diaporthales | 1 | 7.31 |
| | | Glomerellales | 2 | 14.67 |
| | | Sordiales | 2 | 14.67 |
| | | Xylariales | 3 | 21.94 |
| | | Microascales | 1 | 14.67 |
| | Dothidiomycetes | Pleosporales | 10 | 73.15 |
| | | Botryosphaeriales | 2 | 14.67 |
| | Eurotiomycetes | Eurotiales | 7 | 51.20 |
| **Basidiomycota** | Agaricomycetes | Polyporales | 1 | 7.31 |

In the evolutionary tree, grouping of the endophytic fungal isolates belonging to Ascomycetes was done ranging from group I-group VIII (Fig. 3). The endophytic fungal isolates formed clade with closely repeated species with bootstrap values of 100% or slightly lesser. *Morchella angusticeps* KM587905 was laid down separately as an outgroup belonging to the phylum Basidiomycota.

Sequencing analysis of cultured fungal ITS type depicted that group I (Pleosporales) included isolates BD14, BD17, BD18, AS7, ASW3, G1, H15A, N1and TD10Z that belonged to the identified species of Pleosporales (77–100% bootstrap value). Group II (Botryosporiales) included AL12 and and G16 that formed clade with *Phyllosticta capitalensis* and *Botryosphaerea dothidea* respectively with 100% bootstrap values.

In group III (Eurotiales), AW7, ZA and BD3B formed separate clades with *Aspergillus protuberus* and *Paecilomyces formosus* respectively with 100% bootstrap values. Similarly, L2, TD11, GT1Z belonged to different species of the genus *Talaromyces*. Group IV (Xylariales) was comprised of Z1 and Z2 that belonged to *Nigrospora sp.* and TD10A that belonged to *Xylaria spp.* (with 100% bootstrap value).

The sequences of L1 and Z3 matched with species of Sordiales (with 100% boot strap values) and formed a strongly supported group V. Likewise, sequences of ALG8 and ALG9 formed clade with *Colletotrichum gleosporiodes* and *Colletotrichum destructivum* (with 100% boot strap value) respectively and were grouped into group VI (Glomerellales).

Group VII (Hypocreales) comprised of endophytic fungal isolates, ARW2, BD1B, AR9(3), G4, AS2, ASW13, AS3 that belonged to different species of the order Hypocreales. Similarly, a strongly supported group VIII (Diaporthales) was formed by AS17 and TD3 with 99–100% bootstrap value.

## Fungal Diversity

The number of fungal taxa obtained from various tissues of *D. bulbifera* L. ranged from 7–15 (Fig. 5). The colonisation frequency of the fungal endophytes has been tabulated below (Table 4). Among various endophytic fungal isolates obtained from bulbils *F. equiseti* (BD14) was found to have maximum colonisation frequency followed by *F. incarnatum* (N2). The endophytic fungal isolate *C. lunata* (ALW1), obtained from stem tissue was found to have maximum colonisation frequency. Similarly, among endophytic fungal isolates obtained from leaves, *C. gleospoiriodes* (ALG8) was found to have maximum colonisation frequency. In the tuber tissue, the endophytic fungal isolate *F. equiseti* (TD1) was found to have maximum colonisation frequency.

Table 4
Colonization frequency of endophytic fungal isolates obtained from *D. bulbifera* L.

| Phylogenetic group (genus) | Accession no. | Identified fungus | Tissue colonized by endophytic fungal isolates | | | | Total no. of isolates obtained | No. of plant segments positive for endophytes (A) | Total no. of segments incubated (B) | Colonization frequency (CF) CF = A/B*100 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stem | Leaf | Bulbils | Tuber | | | | |
| **ASCOMYCETES** | | | | | | | | | | |
| **Hypocreales** | | | | | | | | | | |
| *Fusarium* | ALW4 | *Fusarium equiseti* | - | 3 | - | - | 37 | 3 | 400 | 0.75 |
| | BD4 | *Fusarium equiseti* | - | - | 9 | - | | 9 | 400 | 2.25 |
| | BD12 | *Fusarium equiseti* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | G3 | *Fusarium equiseti* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | G8 | *Fusarium equiseti* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | G9 | *Fusarium equiseti* | - | - | 3 | - | | 3 | 400 | 0.75 |
| | AS2 | *Fusarium equiseti* | 4 | - | - | - | | 4 | 400 | 1 |
| | TD1 | *Fusarium equiseti* | - | - | - | 10 | | 7 | 400 | 1.75 |
| | TD2 | *Fusarium equiseti* | - | - | - | 2 | | 2 | 400 | 0.5 |
| | BD2B | *Fusarium incarnatum* | - | - | 3 | - | 6 | 3 | 400 | 0.75 |
| | G2 | *Fusarium incarnatum* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | AS3 | *Fusarium incarnatum* | 1 | - | - | - | | 1 | 400 | 0.25 |
| | N2 | *Fusarium incarnatum* | - | - | 7 | - | 6 | 7 | 400 | 1.50 |
| | WAN | *Fusarium chlamydosporum* | - | - | - | 3 | 3 | 3 | 400 | 0.75 |
| | G4 | *Fusarium laceratum* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |
| | BD19 | *Fusarium proliferatum* | - | - | 1 | - | 3 | 1 | 400 | 0.25 |
| | H22 | *Fusarium proliferatum* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | AR1 | *Fusarium solani* | - | - | - | 3 | 18 | 3 | 400 | 0.75 |
| | ARW1 | *Fusarium solani* | - | - | - | 2 | | 2 | 400 | 0.5 |
| | TD5 | *Fusarium solani* | - | - | - | 4 | | 4 | 400 | 1 |
| | T1 | *Fusarium solani* | - | - | - | 5 | | 5 | 400 | 1.25 |
| | TD9Z | *Fusarium solani* | - | - | - | 2 | | 2 | 400 | 0.5 |
| | WAT | *Fusarium solani* | - | - | - | 2 | | 2 | 400 | 0.5 |
| | IF | *Fusarium solani* | - | - | - | 1 | 1 | 1 | 400 | 0.25 |
| Clonostachys | BD1B | *Clonostachys rosea* | - | - | 9 | - | 10 | 6 | 400 | 1.5 |
| | ARW2 | *Clonostachys pseudochroleuca* | - | - | - | 1 | | 1 | 400 | 0.25 |
| Sarocladium | AR93 | *Sarocladium implicatum* | - | - | - | 3 | 5 | 3 | 400 | 0.75 |
| | R93 | *Sarocladium implicatum* | - | - | - | 2 | | 2 | 400 | 0.5 |
| **Diaporthales** | | | | | | | | | | |
| *Phomopsis* | TD3 | *Phomopsis longicolla* | - | - | | 2 | 8 | 2 | 400 | 0.5 |
| | *BD5* | *Phomopsis longicolla* | - | - | 5 | - | | 4 | 400 | 1 |
| | *AS17* | *Phomopsis/Diaporthe longicolla* | 1 | - | - | - | | 1 | 400 | 0.25 |

| Phylogenetic group (genus) | Accession no. | Identified fungus | Tissue colonized by endophytic fungal isolates | | | | Total no. of isolates obtained | No. of plant segments positive for endophytes (A) | Total no. of segments incubated (B) | Colonization frequency (CF) CF = A/B*100 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stem | Leaf | Bulbils | Tuber | | | | |
| **Xylariales** | | | | | | | | | | |
| *Xylaria* | TD10 | *Xylaria sp.* | - | - | - | 2 | 2 | 2 | 400 | 0.5 |
| *Nigrospora* | Z2 | *Nigrospora oryzae* | - | 3 | - | - | 4 | 3 | 400 | 0.75 |
| | Z1 | *Nigrospora sphaerica* | - | 1 | - | - | | 1 | 400 | 0.25 |
| **Microascales** | | | | | | | | | | |
| *Microascus* | ZB | *Microascus cirrosus* | - | 1 | - | - | 1 | 1 | 400 | 0.25 |
| Glomerellales | | | | | | | | | | |
| *Colletotrichum* | ALG8 | *Colletotrichum gleosporioides* | - | 4 | - | - | 4 | 4 | 400 | 1 |
| | ALG9 | *Colletotrichum destructivum* | - | 2 | - | - | 2 | 2 | 400 | 0.5 |
| **Sordariales** | | | | | | | | | | |
| Chaetomium/ *Dichotomopilus* | L1 | *Chaetomium globosum* | - | 1 | - | - | 2 | 1 | 400 | 0.25 |
| | *Z3* | *Dichotomopilus funicola* | - | 1 | - | - | | 1 | 400 | 0.25 |
| **Pleosporales** | | | | | | | | | | |
| *Alternaria* | BD17 | *Alternaria tenuissimea* | - | - | 4 | - | 4 | 4 | 400 | 1 |
| *Dictyosporium* | TD10Z | *Dictyosporium hepatosporum* | - | - | - | *1* | *1* | *1* | 400 | 0.25 |
| *Bipolaris* | AS1 | *Bipolaris sorokiniana* | 6 | - | - | - | 14 | 6 | 400 | 1.5 |
| | ASW3 | *Bipolaris sorokiniana* | 8 | - | - | - | | 5 | 400 | 1.25 |
| *Curvularia* | BD18 | *Curvularia specifera* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |
| | BD14 | *Curvularia lycopersicii* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |
| | ALW1 | *Curvularia lunata* | 9 | - | - | - | 18 | 8 | 400 | 2 |
| | H1B | *Curvularia lunata* | - | - | 1 | - | | 1 | 400 | 0.25 |
| | AS7 | *Curvularia lunata* | 8 | - | - | - | | 7 | 400 | 1.75 |
| *Acrocalymma* | H15A | *Acrocalymma medicaginis* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |
| *Epicoccum* | *TD4Z* | *Epicoccum nigrum* | - | - | - | 2 | 4 | 2 | 400 | 0.5 |
| | *TD8* | *Epicoccum nigrum* | - | - | - | 2 | | 2 | 400 | 0.5 |
| | *G1* | *Epicoccum sorghinum* | - | - | - | - | | | 400 | |
| *Parathyridaria* | *N1* | *Parathyridaria percutanea* | - | - | 1 | - | 5 | 1 | 400 | 0.25 |
| | *N4* | *Parathyridaria percutanea* | - | - | 2 | - | | 2 | 400 | 0.5 |
| | *BD20Z* | *Parathyridaria percutanea* | - | - | 2 | - | | 2 | 400 | 0.5 |
| **Botryosphaeriales** | | | | | | | | | | |
| *Phyllosticta* | *AL12* | *Phyllosticta capitalensis* | - | 2 | - | - | 2 | 2 | 400 | 0.5 |
| *Botryosphaeria* | *G16* | *Botryosphaeria dothidea* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |

| Phylogenetic group (genus) | Accession no. | Identified fungus | Tissue colonized by endophytic fungal isolates | | | | Total no. of isolates obtained | No. of plant segments positive for endophytes (A) | Total no. of segments incubated (B) | Colonization frequency (CF) CF = A/B*100 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stem | Leaf | Bulbils | Tuber | | | | |
| **Eurotiales** | | | | | | | | | | |
| *Aspergillus* | ASW7 | *Aspergillus protuberus* | *4* | - | - | - | 4 | 4 | 400 | 1 |
| *Paecilomyces* | *ZA* | *Paecilomyces formosus* | - | 1 | - | - | 1 | 1 | 400 | 0.25 |
| *Cladosporium* | BD3B | *Cladosporium cladosporioides* | - | - | *2* | - | 2 | 2 | 400 | 0.5 |
| *Penicillium/ Talaromyces* | L2 | *Talaromyces flavus* | - | *1* | - | - | 2 | 1 | 400 | 0.25 |
| | *Q1* | *Talaromyces flavus* | - | - | - | 1 | | 1 | 400 | 0.25 |
| | TD11 | *Talaromyces pinophilus* | - | - | - | 2 | 2 | 2 | 400 | 0.5 |
| | TD7A | *Talaromyces purpureogenus* | - | - | - | 1 | 1 | 1 | 400 | 0.25 |
| | GT1Z | *Talaromyces microsporus* | - | - | 2 | - | 2 | 2 | 400 | 0.5 |
| **BASIDIOMYCETES** | | | | | | | | | | |
| **Polyporales** | | | | | | | | | | |
| *Flavodon* | AS31 | *Flavodon flavus* | 2 | - | - | - | 2 | 2 | 400 | 0.5 |

Moreover, certain taxa such as, *Curvularia, Phomopsis, Aspergillus* and *Flavodon w*ere specific to stem tissue; *Dichotomopilus, Nigrospora, Chaetomium, Phyllosticta, Microascus, Paecilomyces* and *Colletotrichum* were specific to leaf tissue; *Sarocladium, Dictyosporum* and *Xylaria* were specific to tubers; *Cladosporium, Botryosphaeria, Acrocalymma, Parathyridaria* and *Alternaria* were specific to tubers bulbils. Furthermore, the taxa *Curvularia* was isolated from both stem and bulbils; *Talaromyces* from both leaf and tuber; *Clonostachys, Epicoccum* and *Phomopsis* were specific to tuber and bulbils. Only *Fusarium* taxa was isolated from all tissues studied (Table 4).

Biodiversity of the endophytic fungal isolates obtained was calculated using diversity indices (Table 5). The Shannon index revealed a higher consistency of endophytic fungal isolates in the bulbils compared to other tissues. Furthermore, the Simpson's index clearly revealed that the leaves harboured highly diverse fungal endophytes compared to other tissues. Similarly, Margalef's index revealed that bulbils had high taxonomic richness compared to the leaves, tuber and stem. The similarity indices for endophytic fungal taxa associated with various tissues tested demonstrated significant diversity of fungal endophytes within and between the different tissues (Table 5).

Table 5
Diversity indices of endophytic taxa colonizing various tissues of *D. bulbifera* L.

| Indices | Leaf | Stem | Bulbil | Tuber |
|---|---|---|---|---|
| **Simpson's index of dominance (D)** | 0.011 | 0.05 | 0.160 | 0.080 |
| **Simpson's diversity index (1-D)** | 0.989 | 0.95 | 0.839 | 0.920 |
| **Menhinick's richness ($D_{mn}$)** | 0.951 | 0.512 | 1.17 | 0.805 |
| **Camargo's index ($1/D_{mn}$)** | 1.05 | 1.709 | 0.855 | 1.24 |
| **Shannon-Wiener index (H')** | 0.239 | 0.337 | 0.366 | 0.357 |
| **Species evenness (E)** | 0.093 | 0.158 | 0.132 | 0.148 |
| **Jaccard's Index** | 0.058 | | | |
| **Sorenson's index** | 0.030 | | | |

## Discussion

Endophytic fungi have garnered immense importance in various fields of biotechnology (Kusari and Spiteller 2011). For the isolation of endophytes with desirable traits, certain strategies are employed (Strobel et al. 2002). For instance, a detailed investigation of a particular ecosystem, its plant

species, their relationship with the environment, soil composition etc before selection of a plant would result in the isolation of diverse and potential endophytes (Kusari and Spiteller 2011). Plants with medicinal importance are preferably used for the isolation of endophytes (Aly et al. 2011; Dhayanithy et al. 2019; Tyagi et al. 2021; Keshri et al. 2021; Kapoor et al. 2022). Since, *D. bulbifera* L. has been less explored for its endophytic community, therefore, the current study was conducted to explore of fungal endobiome of *D. bulbifera* L. for diversity, community composition and phylogenetic analysis. A few workers have isolated fungal endophytes from *D. bulbifera* L., but hardly any report is available on diversity analysis. Seasonal variation among endophytic fungi associated with *D. bulbifera* was done by Suradkar et al (2014). Ahmed et al (2012) isolated 70 fungal isolates from 270 explants of *D. bulbifera* L. Furthermore, other studies on endophytes of *D. bulbifera* L. include actinomycetes and bacteria (Peng et al. 2015).

The current study reported maximum colonisation in the bulbils followed by other tissues which is in contrast to previous studies reporting maximum colonisation of endophytic fungal isolates of *D. bulbifera* L. in the root tissue (Ahmed et al. 2012). The differences in fungal assembly depend on certain physiological and environmental factors (Gond et al. 2012). The structure and substrates of the bulbils can influence infection of the endophytic fungi and increase their colonization frequency. The study conducted by Kaur et al (2015) reported that peculiar anatomy of leaves harbour myriad of endophytic fungi. The leaf provides large surface area which aids in capturing of inoculum due to its natural stomatal opening, hydathodes, and glandular entry points (Rajini et al. 2020). Also, it has been noted that the hairy surface helps with accumulation of endophytic inoculum and easy access to internal tissues. However, our study reported that leaf tissue possessed lesser number of endophytes which does not corroborate previous studies (Kumar and Hyde 2004; Katoch et al. 2017; Sharma et al. 2018). This might be due to the shorter life time of leaves as compared with the other tissues (Sadeghi et al. 2019).

The current study recorded that endophytic fungal taxa of *D. bulbifera* L. belonged to 2 phyla, 4 classes, 10 orders, 25 genera and 38 species showing abundant diversity. Except for one fungal species belonging to Basidiomycota, all the fungal endophytes isolated in this study belonged to the phylum Ascomycota. The predominance of Ascomycota reported in this study is typical of fungal endophytes which implies that they have co-evolved with plants (Ramos-Garza et al. 2016). Ascomycota covering approximately 8% of the Earth's landmasses and is one of the most diverse and ubiquitous phyla of eukaryotes (Katoch et al. 2017). In our study endophytic community belonging to Sordariomycetes, Dothidiomycetes, Eurotiomycetes and Agaricomycetes was reported. Majority of the endophytic fungal isolates, obtained in our study belonged to the class, Sordariomycetes and Dothideomycetes of the phylum Ascomycota. According to various research groups fungal endophytic community of terrestrial plants mainly belong to the class Sordariomycetes, Dothideomycetes and Pezizomycetes fungi (Suryanarayanan et al, 2011). Therefore, our results were consistent with the previous study that Sordariomycetes and Dothideomycetes were the main groups of endophytic fungi from other plants (Zhang et al. 2014; Chen et al. 2019) and contradictory to studies conducted to other groups (Katoch et al. 2017; Xiang et al. 2018; Alomia et al, 2022). Among four different classes of endophytic fungal isolates species richness was abundant in the class Dothidiomycetes which is in lines with previous report (Sadeghi et al. 2019).

Some of the endophytic fungal isolates obtained in our study have already been reported as endophytes either from various species of *Dioscorea* or other medicinal plants (Kusari et al. 2013; Xiang et al. 2018; Bhattacharya et al. 2019; Chowdhary and Kaushik 2019; Duan et al. 2019; Gong et al. 2019; Manoharan et al. 2019; Suleiman et al. 2019; Gupta et al. 2022). However, to the best of our knowledge the endophytic fungal isolates, *A. medicaginis*, *C. lycoperscii, T. macrospora, F. laceratum, P. formosus* and *M. cirrosus* have not been earlier reported as endophytes while *N. oryzae, F. flavus, Talaromyces sp*., have been reported first time as endophytes from *D. bulbifera* L.

Although, reports on other species of *Acrocalymma* such as *A. vagum, A. fici*, isolated as endophytes are available but so far hardly any report is available where *A. medicaginis* has been reported as an endophyte (Jin et al. 2018; Maulana et al. 2018; Chen et al. 2019; Liu and Wei 2019; Radiastuti et al. 2019; Huang et al. 2020). The genus *Acrocalymma* was first introduced for a single novel species *Acrocalymma medicaginis* causing root and crown rot of *Medicago sativa* (Alcorn and Irwin 1987; Nikandrow 1990). Transition of fungal lifestyles from parasitic to mutualistic or vice versa has been reported in the literature. Apart from physiological stress on the host plant, it is influenced by certain parameters *viz*. genetic factors of both partners, imbalance in nutrient exchange and environmental variations (Kogel et al. 2006; Rodriguez and Redman 2008). *A. medicaginis* was described as a sexual morph of *Massarina walkeri* (Shoemaker et al. 1991). Although, there are only eight described species of Acrocalymma, more than 200 ITS sequences have been deposited from these species in GenBank. Endophytic strains of *Acrocalymma vagum* have the highest number of reported sequences, followed by *A. medicaginis* (Mortimer et al. 2021).

In addition, *Paecilomyces formosus* (Khan et al. 2012; Bilal et al. 2017) and *Microascus cirrosus* (Chaudhary and Arya 2012) that are known human pathogens have also been reported as endophytes in this study. *Flavodon flavus*, belonging to the phylum Basidiomycetes has previously been reported as endophytes from other plants (Brum et al. 2012; Katoch et al. 2014). Likewise, *N. oryzae* and *Talaromyces sp.* have not been earlier reported as endophytes from *D. bulbifera* L.

Based on colonisation frequency (%), it can be depicted that *Fusarium* was the most prevalent species. Higher colonisation frequency of *F. equiseti* (BD4) isolated from bulbils, *C. lunata* (ALW1), obtained from stem tissue and *C. gleospoiriodes* (ALG8) isolated from leaf tissue was found. Also, the endophytic fungal isolate *F. equiseti* (TD1) was found to have maximum colonisation frequency in the tuber tissue. It can be inferred from the data for colonization frequency that different endophytic fungi inhabit different plant tissues and to different extent. These microbiotas might have adapted to different tissue microenvironments, thereby, resulting in tissue specificity. The variation in assemblage of endophytic fungal isolates inside various tissues of the host plant can be attributed to certain factors such as the physiology of the tissue, its chemical composition and anatomy etc (Gond et

al. 2012; Sharma et al. 2018). However, certain other factors such as soil conditions, climate and the dynamics of soil microflora also play a vital role in the colonization of endophytes in the plant tissues (Qin et al. 2009; Xu et al. 2010).

The genus *Fusarium* has been isolated as endophyte from innumerable plants genera and it shows tremendous variability in terms of genetics, biology, ecology etc (Toghueo 2020). Due to their unique biosynthetic ability, they are capable of colonising diverse hosts. Therefore, any species belonging to *Fusarium* genus, can be isolated as an endophyte. It is considered as an important group among fungi (Stepien et al. 2018). In the current study, *Fusarium* was found to be the dominant genera. Similar to the current study, various species of this genera have been reported to be in abundance in root/tuber and aerial tissues (Tan et al. 2018). On contrary, the study conducted by Hamzah et al (2018) reported that endophytic fungal isolates of the genera *Pestalotiopsis* are dominant followed by *Alternaria* and *Cladosporium.* Moreover, the study conducted by Gupta et al (2022) is in contrast to our study. It reported *Fusarium oxysporum* as most frequently isolated fungal endophyte from *Zingiber officinale*, whereas our study reported *Fusarium equiseti* as most frequently isolated fungal isolate.

*Colletotrichum* is considered as an important and widespread genus. Some of its species are serious plant pathogens, and some are frequent endophytes (Rodrigues and Petrini 1997; Farr and Rossman 2013). Although, there have been numerous studies on the genus *Colletotrichum*, but understanding of endophytic species is very limited (Vieira et al. 2014). Previously, several endophytic species of *Colletotrichum* have been reported from a single host. Tao et al (2013) recorded, 17 *Colletotrichum* species from *Bletilla ochracea*, out of which 7 new species were reported. Similarly, Zheng et al (2022) isolated 13 *Colletotrichum spp*. from aquatic plants in South China. Recently, fungal endophytic community analysis revealed predominance of the genera *Colletotrichum* in *Hevea spp.* (Amaral et al. 2022). Tan et al (2018) isolated *C. gleosporiodes* from leaf tissue of *Dysosma versipellis* in abundance, which is in consensus with our study. Similarly, Prasher and Kumar (2021) reported *Colletotrichum gloeosporioides* to be the dominant species from leaf tissue of *Dillenia indica.* In contrast, Mao et al (2021) reported that members of the genus *Phyllosticta* isolated from *Eucalyptus excerta* were the primary isolates, with high colonization frequency.

The genus *Curvularia* is cosmopolitan and ubiquitous. It is comprised of species associated with plant and human as pathogens (Khiralla et al. 2019). However, its species have also been isolated as endophytes from various plants (Khiralla et al. 2016; Sharma et al. 2018; Kalimuthu et al. 2022). A previous study conducted by Kharwar et al (2011) reported endophytic *Curvularia lunata* isolated from leaf tissue of *Adenocalymma alliaceum* as dominant species which well corroborates the present study.

The diversity indices of endophytic taxa colonizing various tissues of *D. bulbifera* L. varied. The value of the Evenness index was higher in stem tissue. High Simpson index was observed in leaves which is well supported by previous reports (Prasher and Kumar 2022). Species richness of the endophytic fungal isolates was higher in bulbil tissues which is in contrast to earlier findings (Sahani and Hemalatha 2018; Bhattacharya et al. 2020).

Overall, the current study provides insight into the diversity of culturable endophytic fungal population associated with *D. bulbifera* L. The endophytic fungal colonization rate, diversity, and community composition is highly affected by host species, tissue types, and abiotic factors. Although this is the first work on community composition of fungal endophytes, more research is required to identify the functional and ecological significance of these fungal endophytes. Further, exploration of non-culturable fungal endophytes can be done using Next-generation sequencing and diverse omics techniques might provide us improved understanding of complex microbiome compositions and their interactions with host plants. Moreover, systematic bio guided investigations are required to further explore the potential of these endophytes and their bioactive metabolites. Investigation of potential metabolites produced by these organisms may lead to the discovery of novel natural products.

# Declarations


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### Author contribution

S. Sharma and S. Kaul were the primary contributors to study concept and design. All authors contributed to material preparation and data collection. Molecular lab work, diversity and phylogenetic analyses were performed by S. Sharma. The first draft of the manuscript was written by S. Sharma, which was then developed by changes, edits, comments, and suggestions from S. Kaul and M.K. Dhar. All authors read and approved the final manuscript.

### Data availability

The sequence data generated in this study has been deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov) under the accession numbers given in the text.

### Ethics approval

Not applicable

### Consent for publication

Informed consent was obtained from all the individual participants included in the study

### Declaration of interest statement

The authors declare that they have no conflict of interest.

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## Figures


**Fig. 1** Map of collection site

**Figure 1**

Map of collection site

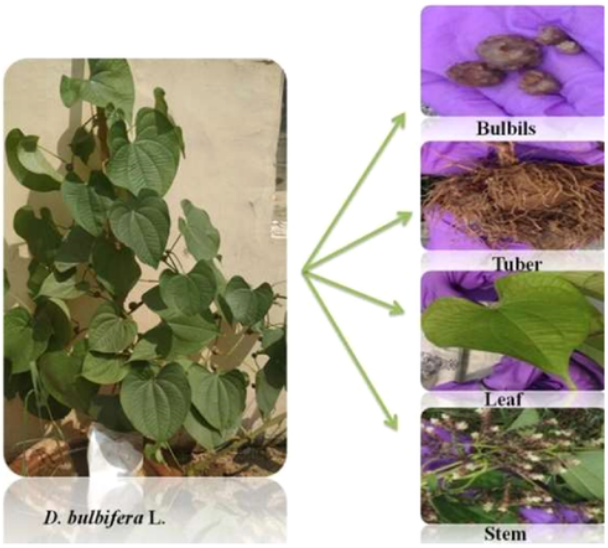


**Fig. 2** Plant material used for the isolation of fungal endophytes

Figure 2

Plant material used for the isolation of fungal endophytes

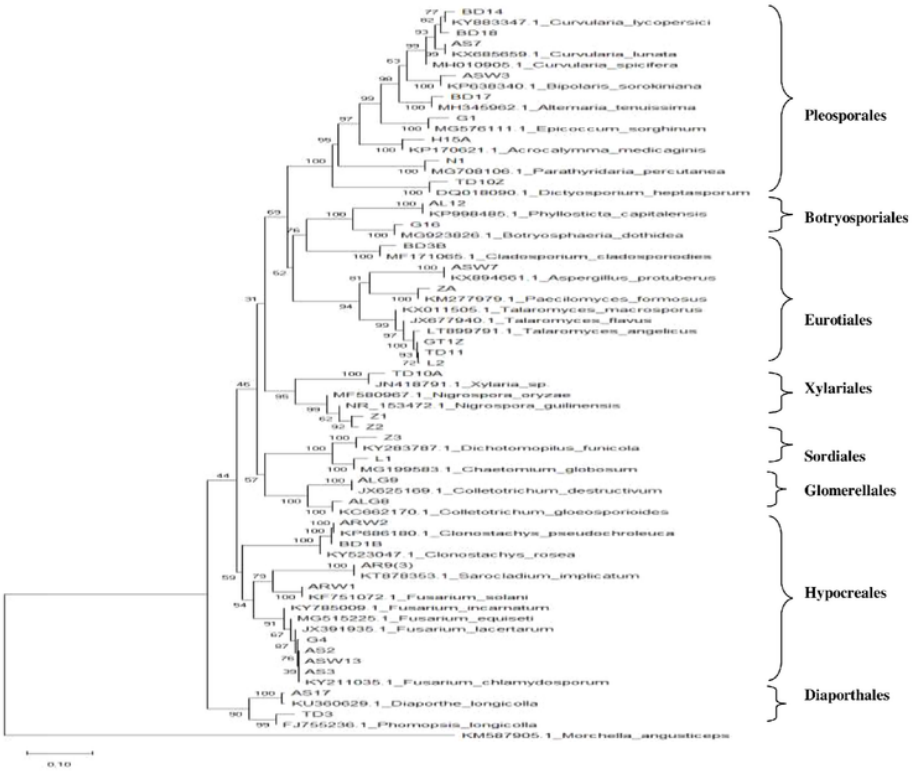


**Fig. 3** Phylogenetic tree of endophytic fungi isolated from various tissues of *D. bulbifera* L.

Figure 3

Phylogenetic tree of endophytic fungi isolated from various tissues of *D. bulbifera* L.

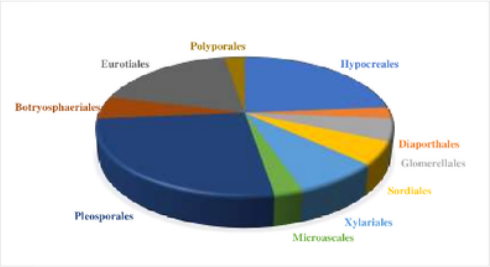


**Fig. 4** Composition of endophytic fungi of *D. bulbifera* L. at the order level

**Figure 4**

Composition of endophytic fungi of *D. bulbifera* L. at the order level

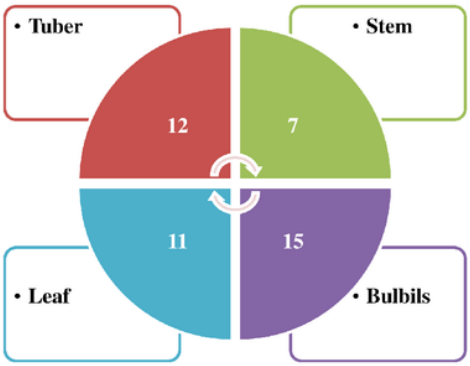


**Fig. 5** Endophytic fungal taxa obtained from various tissues of *D. bulbifera* L.

**Figure 5**

Endophytic fungal taxa obtained from various tissues of *D. bulbifera* L.