Current Journal of Applied Science and Technology

38(6): 1-8, 2019; Article no.CJAST.53673
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Morphological and Molecular Characterization of Endophytic Fungi Associated with Cocoa (*Theobroma cacao* L.) in India

M. Chaithra[1,2], S. Vanitha[1*], A. Ramanathan[1], V. Jegadeeshwari[3], V. Rajesh[4], V. Hegde[5] and E. Apshara[6]

[1]Department of Plant Pathology, TNAU, Coimbatore, TN, India.
[2]Department of Plant Pathology, CPCRI, RS, Vittal, Karnataka, India.
[3]Department of Spices and Plantation Crops, TNAU, India.
[4]Department of Plant Biotechnology, CPMB&B, TNAU, India.
[5]Division of Plant Protection, CPCRI, Kasaragod, Kerala, India.
[6]Department of Horticulture, CPCRI, RS, Vittal, Karnataka, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MC, SV and VH designed the study. Author MC performed the laboratory experiments and produced the manuscript. Performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors VJ and EA provided samples to carry out the experiment. Authors AR and VR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/v38i630447
Editor(s):
(1) Dr. Bishun Deo Prasad, Assistant Professor, Department of Molecular Biology and Genetic Engineering, Bihar Agricultural College, Bihar Agricultural University, India.
Reviewers:
(1) M. M. V. Baig, Yeshwant Mahavidyalay, India.
(2) Adeji Alaba Olaitan, Cocoa Research Institute of Nigeria, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53673

Original Research Article

Received 28 October 2019
Accepted 03 January 2020
Published 13 January 2020

ABSTRACT

Aim: To identify cocoa associated endophytic fungi through morphological and molecular techniques.
Place and Duration of Study: Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu from April 2018 to November 2019.
Methodology: Endophytic fungal isolates were isolated from different parts of cocoa using Petrini method. Isolated endophytic fungal strains were grown in Malt extract broth and total genomic DNA

**Corresponding author: E-mail: vanitha1969@yahoo.com;*

was isolated and amplified using universal primers ITS1F and ITS4R. Amplified rDNA was visualized and documented.

Results: Morphological and molecular characterization of six endophytic fungi revealed that they are from four different taxa *viz.*, *Lasiodiplodia pseudotheobromae* PAK-7, *Arthrinium rasikravindrae* P11, *Arthrinium rasikravindrae* P12, *Diaporthe* sp. Vef-3, *Lasiodiplodia theobromae* TN-R-3, *Colletotrichum* sp. TN-9-2 belonging to four different families *viz.,* Botryosphaeriaceae, Apiosporaceae, Diaporthaceae and Glomorellaceae under Phylum Ascomycota.

Conclusion: The present study indicates the distribution and diversity of fungal endophytes in different plant parts of the cocoa tree in south India.

Keywords: Cocoa; endophytic fungi; ITS.

1. INTRODUCTION

Endophytes are a diverse group of microbes living inside plants without any external symptoms. They influence plant health by the production of phytohormones and antimicrobial compounds. They also compete with plant pathogens for ecological niche and nutrients. The major group of endophytic fungi belongs to Ascomycetes and anamorphic fungi [1]. Basidiomycetes and zygomycetes are rarely encountered. These fungi have been identified in nearly 3,00,000 plant species and they dwell in all plant parts (leaf, flower, petiole, root, fruit) [2].There existed a co-evolution between endophyte and its host which lead to low virulence [3]. The interaction between endophyte and the host ranges from symbiotic, mutualistic to pathogenic [4,5] depending on the natural environmental condition and plant health [6]. Cocoa (*Theobroma cacao* L.) is an introduced tropical rainforest crop grown as an intercrop or mixed crop within a coconut, arecanut or oil palm plantations in India. In India Cocoa is being cultivated in the States of Kerala, Karnataka, Andhra Pradesh and Tamil Nadu in an area of 78,000 ha with a total production of 16,050 MT. The average productivity of cocoa in Indian is 475 kg/ha [7]. The present study was aimed to identify the endophytic fungi of cocoa both from morphologically and molecularly using PCR-based molecular techniques which helps to know endophytic fungal diversity in the healthy cocoa tree.

2. MATERIALS AND METHODS

2.1 Sample Collection

During September 2017 asymptomatic plant from a disease-prone area was located and marked. Different plant parts were excised with sterile knife and samples were carried to the lab in a zip-lock cover and kept at 4℃ until further use (Table 1).

2.2 Isolation of Endophytic Fungi

Isolation of endophytic fungi from cocoa was done by following Petrini method [8]. Sterilization involved washing of collected plant samples in running tap water followed by drying. Washed plant parts *viz*, stem, leaf, petiole and roots were excised into small bits with sterile scalpel and surface sterilized with 4% NaOCl for 30 sec-1 min followed by two times rinse in sterile distilled water. A second wash was given with 70% ethanol for 1-2 min. the surface sterilization efficiency was checked by plating last wash water on malt extract agar which served as a control. Plant parts were properly dried before placing on MEA and incubated at 27±2℃ for 7-14 days depending on the growth of the endophyte.

2.3 Morphological Characterization

Initial identification of fungal endophytes was done based on morphological observations. A loop full of mycelium was taken on a cavity slide and observed under the Stereo Binocular Phase-contrast microscope (LEICA DM2000 LED) for the presence of structures like mycelium (color & septation) sexual and asexual structures/spores. Images were captured and analyzed using an image analyzer.

2.4 ITS-PCR and Sequencing

Molecular characterization was done following ITS-PCR and sequencing of the amplified DNA fragment. Cultures were grown in MEB ((Malt extract broth) for 7 days at 27±2℃ inside a BOD incubator. Approximately 200 mg of freeze-dried (-20℃) mycelium was macerated with 1-2 ml of CTAB buffer (10%CTAB- 10 ml, 1M Tris base-5 ml, 5 M EDTA-2 ml (pH-8), mercaptoethanol-1 ml and distilled water-18 ml) warmed at 60℃. Transferred 700µl of macerate into a eppendorf tube (2 ml) and warmed at 65℃ for 10 min. Added 750 µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) and inverted it to form an emulsion.

Table 1. Geographical distribution and isolation sources of endophytic fungi

Sl.no	Isolate	Intercropping system	Place of collection	Plant part
1.	Pak-7	Coconut and cocoa	Palakad, Kerala	Petiole
2.	P11	Coconut and cocoa	Palakad, Kerala	Petiole
3.	P12	Coconut and cocoa	Palakad, Kerala	Petiole
4.	VEF-3	Arecanut and cocoa	CPCRI,RS, Vittal, Karnataka	Petiole
5.	TN-R-3	Coconut and cocoa	TNAU, Coimbatore, TN	Root
6.	TN-9-2	Coconut and cocoa	TNAU, Coimbatore, TN	Root

Centrifuged the content @10000 rpm for 10 min. The supernatant was transferred into a sterile eppendorf tube and 0.5 vol of 5 M NaCl (150 µl) and 2 vol (600 µl) of ice-cold Isopropanol was added and incubated @ -20℃ for 12h/overnight. Later tubes were inverted 2-3 times and centrifuged @13000 rpm at 4℃ for 10min. supernatant was discarded and the pellet was washed with 70% ethanol. After proper drying pellet was suspended in 50 µL of TE buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA). The quantity of DNA was checked by electrophoresis in 0.8% agarose (HiMedia Pvt Ltd. India) gel supplemented with 2 µl of ethidium bromide for 45 min at 90 V in 1X TAE buffer (Tris base -4.84 g, acetic acid-1.09 ml, EDTA-0.292 g, distilled water-100 ml, pH-8.1—8.2) and visualized under UV transilluminator.

Before PCR the concentration of genomic DNA was adjusted to 200 ng. Later ITS-PCR was carried out using internal transcribed spacer primers ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') with a final reaction mixture concentration of 55 µl (smart primer - 20 µl, ITS1F- 4 µl, ITS4R- 4 µl, d_2H_20- 8 µl and DNA-8 µl). Amplification was carried out in Eppendorf thermal cycler with an initial denaturation at 95℃ for 3 min, denaturation at 95℃ for 1min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min followed by a final extension at 72℃ for 5 min with 35 cycles. The PCR amplified products were visualized in 1.2% agarose gel and documented. The amplified product was sent to AgriGenome Lab Pvt Ltd for purification and sequencing.

3. RESULTS AND DISCUSSION

The current study was carried out to find the associated endophytic fungi in healthy cocoa trees in a disease-prone area. Isolated endophytic fungi were morphologically and molecularly characterized following microscopic observations and rDNA sequence blast in NCBI database USA.

3.1 Morphological Characterization

Morphological characterization is one of the oldest and reliable methods for the identification of any microorganisms. In the present investigation, cocoa endophytic fungi were characterized morphologically by studying mycelial and spore characters. Isolate PAK-7 (*Lasiodiplodia pseudotheobromae)* and TN-R-3 (*Lasiodiplodia theobromae*) produced colored and septate mycelium without any sexual or asexual structures. Similarly, morphological characterization of endophytic and saprobic isolates of *Lasiodiplodia pseudotheobromae* NI173 was studied where it produced brown colored conidia with 19–25×12–15 µm size [9].

The genus *Arthrinium* is widespread and occurs as a saprobe on a different range of substrate [10]. It is a plant pathogen reported to cause diseases like kernel blight of barley [11], damping-off of wheat [12]. Its endophytic nature has been reported in lichens, marine algae plant tissues [13,14,15]. Hanada et al. [16] reported the endophytic nature of *Arthrinium sp* in a cocoa tree. In the present study *Arthrinium rasikravindrae* isolates P11 and *Arthrinium rasikravindrae* P12 produced olive green colored lenticular single-celled conidia with septate olive green colored mycelium. The present finding is in agreement with Singh et al. [17] where he isolated *Arthrinium rasikravindrii* sp. nov. from the soil and it produced dark brown lenticular conidia with hyaline equatorial germ slits together with balloon-shaped, anomalous conidia [18].

The Isolate TN-9-2 (*Colletotrichum* sp.) produced hyaline dumble shaped single-celled conidia this is in agreement with Rojas et al. [19]. The endophytic nature of *Diaporthe* sp., *Colletotrichum* sp and *Lasiodiplodia* sp in cocoa was reported by Rubini et al. [20]. Ding et al. [21] studied the morphological characters of endophytic *Diaporthe* sp. isolated from *Camptotheca acuminate* from China. It produced

a gray-colored colony with branched and septate mycelium. This finding matching with that of the present study where isolate VEF-3 (*Diaporthe* sp.) produced light brown to dark-colored septate mycelium with few alpha conidia in MEA medium.

3.2 Molecular Characterization and Phylogeny

3.2.1 ITS-PCR and sequencing

Isolated and amplified rDNA from endophytic fungi were subjected to PCR analysis. The DNA band size ranged from 336-457bp (Fig 2). ITS rDNA sequence analysis of PAK-7 and TN-R-3 showed cent percent similarity with *Lasiodiplodia pseudotheobromae* and *Lasiodiplodia theobromae* respectively (Table 2). Similarly de Silva et al. [9] did molecular characterization of *Lasiodiplodia pseudotheobromae* isolated from Magnolia forest plants using combined ITS, tef1 and tub2 genes.

The endophytic *C. gloeosporioides* strains from asymptomatic leaves, anthracnose lesions on leaves and fruits of *Theobroma cacao* (cacao) were subjected to multilocus phylogeny to distinguish host-associated pathogens from asymptomatic cocoa plant parts [22]. Endophytic Isolate TN-9-2 from the present investigation produced dumble shaped hyaline conidia which is the characteristic feature of genera *Colletotrichum.* The sequencing result showed that it matched with that of *Colletotrichum* sp. voucher UOM 1290T with 100% identity (Table 2). Lima et al. [23] identified *C. gloeosporioides*, *C. boninense*, and *C. simmondsii* in Brazilian pepper tree using combined morphological and PCR taxon-specific primer with CaInt/ITS4, CgInt/ITS4, and Col1/ITS4, which amplify specific bands in *C. acutatum, C. gloeosporioides lato sensu, and Colletotrichum boninensis*, respectively.

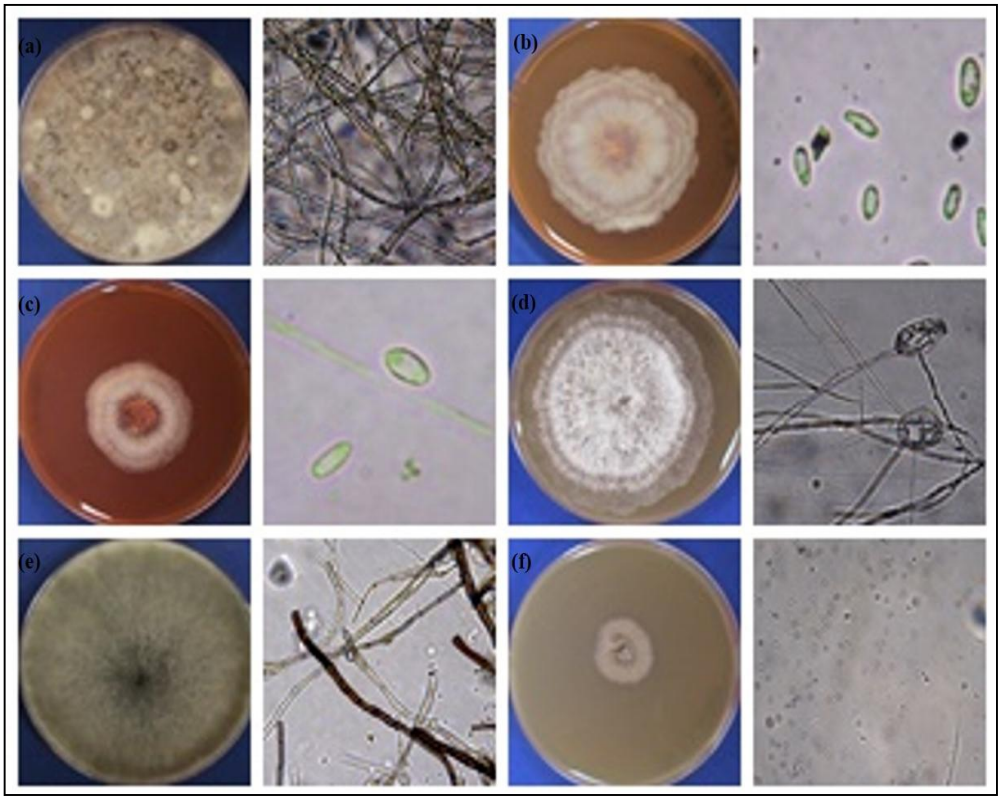

Fig. 1. Macroscopic and microscopic observations on endophytic fungal isolates of cocoa. a-PAK-7 (*Lasiodiplodia pseudotheobromae*), b- c *Arthrinium rasikravindrae* P11 and P12 isolates conidia, d- Vef-3 (*Diaporthe* sp.) melanized mycelium, e- TN-R-3 (*Lasiodiplodia theobromae*) melanized mycelium, f- TN-9-2 (*Colletotrichum* sp.) conidia

Table 2. A comparison on morphological characters of different endophytic fungal isolates of cocoa

Sl. no	Isolate	Organism	Culture color	Margin	Mycelium	Spore
1	PAK-7	*Lasiodiplodia pseudotheobromae*	Lichen Green	Entire	Light brown color	Sterile
2	P11	*Arthrinium rasikravindrae*	Pinkish white	Irregular/Wavy	Olive green color	Olive green color conidia
3	P12	*Arthrinium rasikravindrae*	Pinkish white	Fringed	Olive green color	Olive green color conidia
4	Vef-3	*Diaporthe* sp.	Chalk white	Fringed	Light brown to dark brown	Colorless Alpha conidia
5	TN-R-3	*Lasiodiplodia theobromae*	Ash gray	Irregular	Chocolate brown and septate	Sterile
6	TN-9-2	*Colletotrichum* sp.	Ash gray	Irregular	Hyaline	Dumble shaped single-celled hyaline conidia

Table 3. Molecular characterization of endophytic fungal isolates of cocoa, based on NCBI sequence blast result

Sl. no	Isolate	Accession number	Closest match	Query coverage (%)	Percent identity
1.	Pak-7	MN418007	*Lasiodiplodia pseudotheobromae*	100	100
2.	P11	MN414159	*Arthrinium rasikravindrae*	100	100
3.	P12	MN413149	*Arthrinium rasikravindrae*	100	100
4.	VEF-3	MN420881	*Diaporthe* sp.	100	100
5.	TN-R-3	MN400974	*Lasiodiplodia theobromae*	100	100
6.	TN-9-2	MN412519	*Colletotrichum* sp	100	100

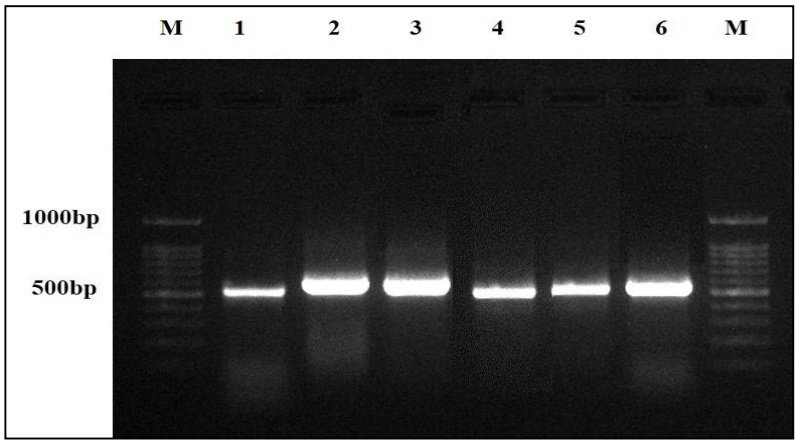

Fig. 2. Molecular characterization of the endophytic fungi of cocoa using ITS1F and ITS4R primers: M-1.5kb Marker, 1- PAK-7 (*Lasiodiplodia pseudotheobromae*), 2- P11 (*Arthrinium rasikravindrae*), 3- P12 (*Arthrinium rasikravindrae*), 4- Vef-3 (*Diaporthe* sp.), 5 -TN-R-3 (*Lasiodiplodia theobromae*), 6 -TN-9-2 (*Colletotrichum* sp.)

Hanada et al. [16] reported the endophytic nature of *Arthrinium sp* in a cocoa tree. In the present study endophytic isolates, P11 and P12 were isolated from cocoa and they were molecularly characterized using ITS1F and ITS4R primers. The sequence result showed 100% identity with

Arthrinium rasikavindii (Table 2). Ghasemi et al. [24] identified endophytic *Arthrinium arundinis* from oak trees in Arasbaran forest based on morphological characters and ITS-rDNA and Tub region sequence data.

Many reports are suggesting the endophytic nature of *Diaporthe* sp. In cocoa as well as in the forest tree. In the present study endophytic fungal isolate Vef-3 sequence data matched with taxa *Diaporthe* sp. with 100% similarity.

3.2.2 Phylogenetic analysis

The phylogenetic tree is used to represent evolutionary relationships between organisms which are having ancestors in common. In the present study evolutionary tree was constructed using MEGA7 software with four clades representing four different genera *Lasiodiplodia, Diaporthe, Arthrinium,* and *Colletotrichum* belonging to the same Phylum Ascomycota representing four different families (Fig. 3). The

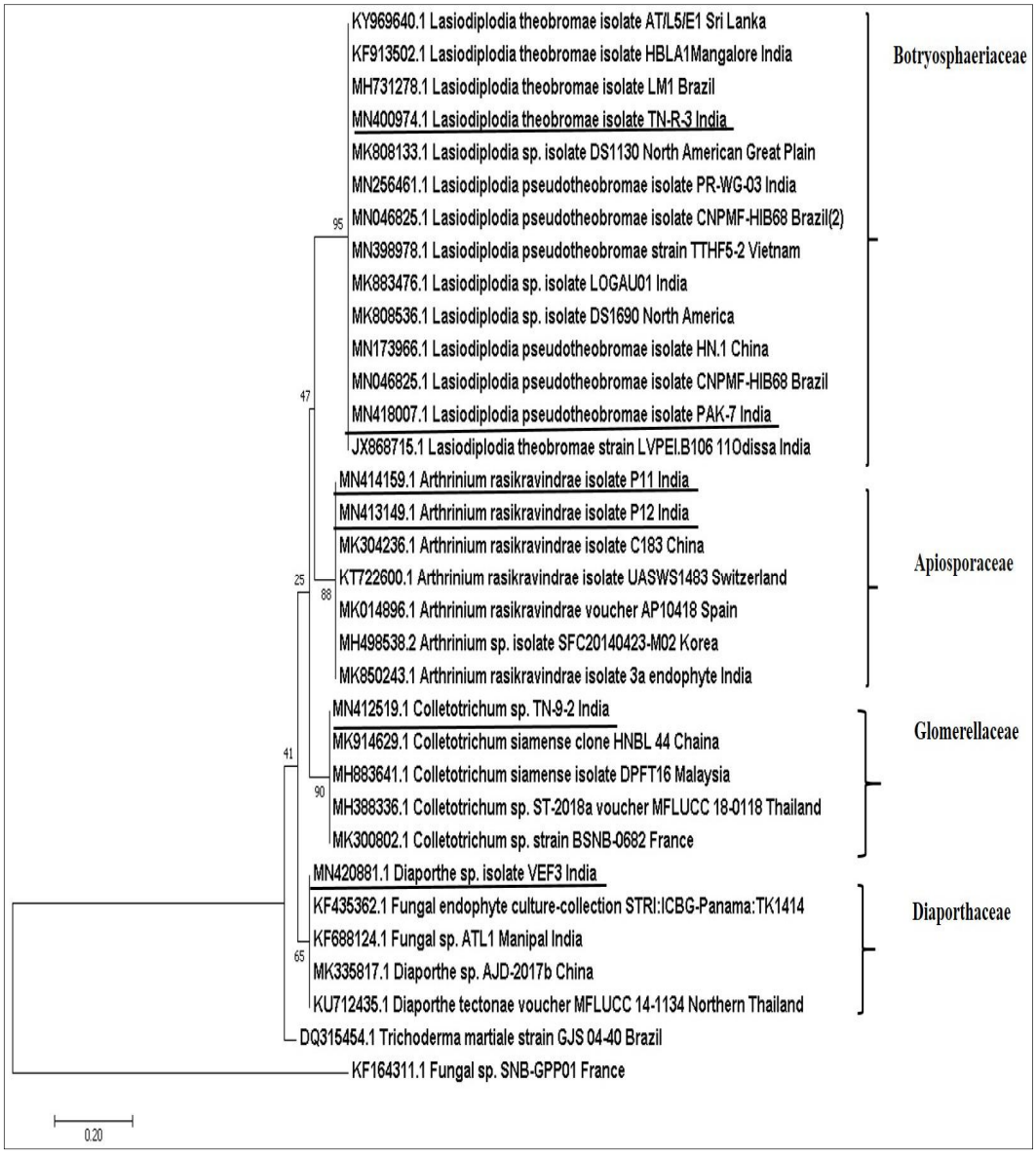

Fig. 3. Phylogenetic relationship between six endophytic fungal isolates of *Theobroma cacao* based on ITS rDNA sequence by Neighbour-joining (NJ) method following the bootstrap test (1000 replication).The evolutionary distances were computed using the Maximum Composite Likelihood method following Gamma distribution using MEGA7 Software

genus *Lasiodiodiplodia* was from Botryosphaeriaceae, *Arthrinium* from Apiosporaceae, *Colletotrichum* from Glomerellaceae and *Diaporthe* from Diaporthacea. The first clade consisted of two sequences of *Lasiodiodiplodia* which clustered with twelve sequences from NCBI database (MN046825, MN173966, MK808536, MK883476, MN398978, MN256461, MK808133, MH731278, KY969640, KF913502, KF164311, JX868715) with 95% bootstrap value. The second clade comprised of the genus Arthrinium, which consisted of two sequences of endophytic fungi of cocoa which clustered with five NCBI database sequences (MK304236, KT722600, MK014896, MH498538o, and MK850243) with 88% bootstrap value. The third clade comprised of the *Colletotrichum* genus which consisted of one sequence of endophytic fungi that clustered with four sequences from the NCBI database (MK914629, MH883641, MH388336 and MK300802) with 90% bootstrap value. The fourth clade comprised the genus *Diaporthe* with one endophytic fungal sequence which clustered with four NCBI database sequences (KF435362, KF688124, MK335817 and KU712435) with bootstrap value 65%.

In the present investigation, all six isolated endophytic fungal taxa belonged to the phylum Ascomycota. This is in agreement with the findings of Evans et al.[25] and Crozier et al. [23] where they isolated and characterized fungal community inhabiting in root, stem and branches of cocoa. The studies on the fungal endophytic community in Cocoa are not well known so efforts may be taken to characterize these microbes to characterize it both morphologically and molecularly.

4. CONCLUSION

Studies on endophytes are a new field of research that gives us a way to understand the diversity of the endophytic community in a plant. The present study aims to identify endophytic fungal isolates of cocoa based on morphological and molecular characters. A total of six endophytic fungal isolates were studied and they are all from the same phylum Ascomycota, belonging to different families. Isolates *Lasiodiplodia pseudotheobromae* PAK-7 and *Lasiodiplodia theobromae* TN-R-3 are from Botryosphaeriaceae, *Arthrinium rasikravindrae* P11 and *Arthrinium rasikravindrae* P12 were from Apiosporaceae, *Diaporthe* sp Vef-3 was from Diaporthaceae and *Colletotrichum* sp. TN-9-2 was from Glomorellaceae which indicated the fungal diversity in different plant parts of cocoa. For better understanding further studies may be carried out to confirm the endophytic nature of asymptomatic fungal pathogens as well as antagonistic microbes in a cocoa tree.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arnold AE. Understanding the diversity of foliar fungal endophytes: Progress, challenges and frontiers. Fungal Biol. Rev. 2007;21:51–66.
2. Yua J, Wub Y, Heb Z, Lib M, Zhub K, Gaoa B. Diversity and antifungal activity of endophytic fungi associated with *Camellia oleifera*. Mycobio. 2018;46:2:85–91.
3. Sieber TN. Endophytic fungi in forest trees: Are they mutualists? Fungal Biol Rev. 2007;21:75–89.
4. Usuki F, Narisawa K. A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira* and a nonmycorrhizal plant, Chinese cabbage. Mycologia. 2007;99: 175–184.
5. Tellenbach C, Gru¨nig CR, Sieber TN. Negative effects on survival and performance of norway spruce seedlings colonized by dark septate root endophytes are primarily isolate dependent. Environ Microbiol. 2011;13:2508–2517.
6. Schulz B, Boyle C. The endophytic continuum. Mycol Res. 2005;109:661–686.
7. Directorate of Cashewnut & Cocoa Development (DCCD). Ministry of Agriculture and Farmers welfare. Governament of India; 2019.
8. Petrini O. Taxonomy of endophytic fungi of aerial plant tissues. In: Microbiology of the phylosphere, Edited by NJ Fokkenna, J Van Den Heuvel. Cambridge University Press, Cambridge; 1986.
9. De Silva NI, Phillips AJL, Liu J, Lumyong S, Hyde KD. Phylogeny and morphology of *Lasiodiplodia* species associated with Magnolia forest plants. Scientific Reports. 2019;9:14355.
10. Agut M, Calvo MA. *In vitro* conidial germination in *Arthrinium aureum* and *Arthrinium phaeospermum*. Myco-pathologia. 2004;157:363–367.

11. Martínez-Cano C, Grey WE, Sands DC. First report of *Arthrinium arundinis* causing kernel blight on barley. Plant Dis. 1992;76:1077.
12. Mavragani DC, Abdellatif L, McConkey B, et al. First report of damping-off of durum wheat caused by *Arthrinium sacchari* in the semi-arid Saskatchewan fields. Plant Dis. 2007;91:469.
DOI: 10.1094 /PDIS-91-4-0469A
13. Ramos HP, Braun GH, Pupo MT, Said S. Antimicrobial activity from endophytic fungi *Arthrinium* state of *Apiospora montagnei* Sacc. and *Papulaspora immersa*. Braz. Arch. Biol. Technol. 2010;53(3):629-632.
14. Suryanarayanan TS, Venkatachalam A, Thirunavukkarasu N, Ravishankar JP, Doble M, Geetha, V. Internal mycobiota of marine macroalgae from the Tamil nadu coast: Distribution, diversity and bio-technological potential. Bot Mar. 2010; 53(5):457-468.
15. He Y, Zhang Z. Diversity of organism in the *Usnea longissima* lichen. Afr. J. Microbiol. Res. 2012;6(22):4797-4804.
16. Hanada RE, Pomella AWV, Costa HS, Bezerra JL, Loguercio LL, Pereira JO. Endophytic fungal diversity in *Theobroma cacao* (cacao) and *T. grandiflorum* (cupuaçu) trees and their potential for growth promotion and biocontrol of black-pod disease. Fungal Biol. 2010:114(11-12):901-910.
17. Singh SM, Yadav LS, Singh PN, Hepat R, Sharma R, Singh SK. Arthrinium rasikravindrii sp. nov. from Svalbard, Norway. Mycotaxon. 2012;122:449–460.
18. Sharma R, Kulkarni G, Sonawane MS, Shouche YS. A new endophytic species of *Arthrinium* (Apiosporaceae) from *Jatropha podagrica*. Mycoscience. 2014;55(2):118-123.19.
19. Rojas EI, Rehner SA, Samuels GJ, et al. *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panamá: Multi-locus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia. 2010;102(6):1318–1338.
20. Rubini MR, Silva-Ribeiro RT, Pomella AW, Maki CS, Araújo WL, Dos Santos DR, Azevedo JL. Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. Int J Biol Sci. 2005;1(1):24-33.
21. Ding X, Liu K, Deng B, Chen W, Li W, Liu F. Isolation and characterization of endophytic fungi from *Camptotheca acuminata*. World J Microbiol Biotechnol. 2013;29(10):1831-1838.
22. Croziera J, Thomasa SE, Aimeb MC, Evansa HC, Holmesa KA. Molecular characterization of fungal endophytic morphospecies isolated from stems and pods of *Theobroma cacao*. Plant Pathol. 2006;55:783–791.
23. Lima JS, Figueiredo JG, Gomes RG, Stringari D, Goulin EH, Adamoski D, Glienke C. Genetic diversity of *Colletotrichum* spp. an endophytic fungi in a medicinal plant. Brazilian pepper tree. ISRN Microbial. 2012;215716:1-7.
24. Ghasemi ES, Arzanlou M, Babai AA, Narmani A. Morphological and molecular characterization of endophytic fungi from oak trees in Arasbaran forests. J App Res Plant Prot. 2019;8(1):1-17.
25. Evans HC, Holmes KA, Thomas SE. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycol Prog. 2003;2(2):149–160.

© 2019 Chaithra et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53673