Check for updates

RESEARCH ARTICLE

REVISED Cultural-morphology and molecular analysis of *Botryodiplodia theobromae*, a pathogen of coconut fruit

[version 2; peer review: 2 approved]

Osayomore Endurance Ekhorutomwen[1-3], Safa Oufensou[2], Nnamdi Ifechukwude Chidi[1], Quirico Migheli[2], Olalekan Hakeem Shittu[3]

[1]Plant Pathology Division, Nigerian Institute for Oil Palm Research, Benin City, Edo, 302115, Nigeria
[2]Department of Agricultural Sciences and Desertification Research Centre, University of Sassari, Sassari, Sardegna, 07100, Italy
[3]Department of Plant Biology and Biotechnology, University of Benin, Faculty of Life Sciences, Benin City, Edo, 300213, Nigeria

v2 **First published:** 21 May 2025, **5**:140
https://doi.org/10.12688/openreseurope.19692.1
Latest published: 12 Nov 2025, **5**:140
https://doi.org/10.12688/openreseurope.19692.2

Abstract

Background

In coconut production, less than one third of the button nuts produced in an inflorescence eventually develop into mature nuts, as a result of fruit rot and premature nut fall diseases. *Botryodiplodia theobromae* is linked to the fruit rot and premature nut fall diseases of coconut, resulting in consequential yield loss of over 60%. Furthermore, the fungus poses a concern to plants, farmers, and plant health authorities because it may survive in plant materials as an endophyte, escaping the quarantine process. This study was carried out to analyse the cultural, morphological, and molecular characters of *B. theobromae* associated with these diseases in coconut fruits.

Methods

Eight isolates of *B. theobromae* used in this study were collected from diseased coconut fruit samples (showing signs of rot and freshly fallen nuts) in two major coconut producing areas in Nigeria. Pure culture of isolates were obtained using potato dextrose agar (PDA) medium. The culture medium and microscopy were used to also examine the cultural and morphological characters of isolates. For molecular characters of isolates, DNA was extracted from each isolate and amplified with a polymerase chain reaction (PCR) using a universal primer (ITS1F/4R) and three specific primers (Lt347-F/R, Bt2aF/bR, and EF1-728F/EF2-728R). Furthermore, PCR amplicons obtained from the ITS1F/4R primers were sequenced and analyzed using bioinformatics

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 12 Nov 2025	✓ view	✓ view
version 1 21 May 2025	? view	? view

1. **Egya N. Yankey**, Council for Scientifc and Industrial Research (CSIR), Sekondi, Ghana
2. **Lellapalli Rithesh**, JC DIWAKR REDDY AGRICULTURAL COLLEGE (ANGRAU) (Ringgold ID: 363676), TADIPATRI, India

Any reports and responses or comments on the article can be found at the end of the article.

and phylogenetic methods. The genetic similarity and variation of isolates were also determined.

Results

The colony texture/color, ITS1/4 gene sequence information and phylogenetic analysis clustered the eight isolates of *B. theobromae* into five categories. The ITS1/4 sequence information using a blast search in the NCBI database, confirmed all eight fungal isolates as *B. theobromae*.

Conclusion

This study has provided a guide for proper categorization of *B. theobromae*, that is a prerequisite for early diagnosis and management of this pathogen in coconut producing areas.

Plain Language Summary
This study provides information on the identification of the pathogen causing fruit rot and premature nut fall diseases of coconut.

Keywords
Botryodiplodia theobromae, coconut, fruit rot, premature nut fall, culture, primers, bioinformatics.

This article is included in the Agriculture, Land and Farm Management gateway.

This article is included in the Horizon 2020 gateway.

Corresponding author: Osayomore Endurance Ekhorutomwen (martinsdonsteve@yahoo.com)

Author roles: Ekhorutomwen OE: Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Writing – Original Draft Preparation; **Oufensou S**: Methodology, Supervision; **Chidi NI**: Investigation, Methodology, Writing – Review & Editing; **Migheli Q**: Conceptualization, Funding Acquisition, Investigation, Methodology, Writing – Review & Editing; **Shittu OH**: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from European Union's Horizon 2020 Framework Programme under grant agreement No [862848] (999465594) Linking East and West African farming systems experience into a BELT of sustainable intensification (EWA – BELT). Also, this research has received funding from the One Planet Fellowship Program (Grant Contract Number: 406-539).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2025 Ekhorutomwen OE *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Ekhorutomwen OE, Oufensou S, Chidi NI *et al.* **Cultural-morphology and molecular analysis of *Botryodiplodia theobromae*, a pathogen of coconut fruit [version 2; peer review: 2 approved]** Open Research Europe 2025, **5**:140 https://doi.org/10.12688/openreseurope.19692.2

First published: 21 May 2025, **5**:140 https://doi.org/10.12688/openreseurope.19692.1

REVISED **Amendments from Version 1**

The manuscript underwent substantial revisions to enhance clarity, rigor, and presentation. Key statements were refined, and recent literature was cited to better substantiate the study's rationale.

In the materials and methods section, clarifications on the isolation of the test pathogen were provided to improve transparency and reproducibility.

In the results section, it was highlighted that PCR gel images were justified using markers as controls instead of water. The images were captured using a photographic imager. The primers ITS1/4 and Lt347-F/R successfully amplified DNA from all isolates; however, two out of three specific primers did not amplify DNA from all isolates. The universal primer is designed to amplify DNA from nearly all fungi, while specific primers target DNA specific to *B. theobromae*. Consequently, it is essential to sequence the amplicons from the universal primer to confirm the identity of the fungal pathogens through a BLAST search in the NCBI database. Additionally, a genetic distance table was defined, indicating compositional distance that correlates with the differences between sequences.

Appropriate statements and terminology were updated for accuracy, clarity, and consistency, with pictorial symptoms of fruit rot and nut fall included in Figure 1. Additionally, Figure 9.1 to Figure 9.8 were included that provided the phylogenetic relationship of all isolates identified. The conclusion section featured disease management strategies for the pathogen. Minor grammatical errors, formatting inconsistencies, and reference list issues were corrected, enhancing the manuscript's scientific quality and aligning it with the journal's standard.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coconut (*Cocos nucifera* L.) is a perennial plant grown in over 90 countries around the world, by an estimated 11 million farmers across 12 million hectares of land (FAO, 2014; Gurr *et al*., 2016). Coconut serves as food and a cash crop in many developing countries, providing a several foreign exchange to nations cultivating the palm (Bourke & Harwood, 2009). Coconut production in Nigeria are mostly affected by soil, climate and diseases (Ekhorutomwen *et al*., 2019). In coconut production, less than one third of the button nuts produced in an inflorescence eventually develop into mature nuts, that is, as a result of fruit rot and premature nut fall diseases (Ekhorutomwen *et al*., 2024; Rajapakse *et al*., 1995). In addition, *Botryodiplodia theobromae* (a cosmopolitan fungal pathogen) is associated with the fruit rot and premature nut fall diseases of coconut, resulting in consequential yield loss of over 60% (Dheepa *et al*., 2018; Marques *et al*., 2013; Taylor & Hyde, 2003). *B. theobromae* can occur in nature as a parasite, saprophyte, or endophyte (Alves *et al*., 2008; Machado *et al*., 2014; Slippers & Wingfield, 2007). The fungus poses a concern to plants because it may survive in plant materials as an endophyte, escaping the quarantine process (Dheepa *et al*., 2018). The fungus has a wide host range, including gymnosperms and angiosperms (Alves *et al*., 2008; Machado *et al*., 2014). Factors that contribute to the distribution of *B. theobromae* causing fruit rot and premature nut fall diseases of coconut include; host plant range determined by transmission from infected palm to susceptible healthy palm, *B. theobromae* preference for host palm, variation in transmission or infection rate, virulence of *B. theobromae*, poor agronomic practice, and climate (weather) factors such as temperature, rainfall, wind, relative humidity, etc (Ekhorutomwen *et al*., 2019).

The appearance of symptoms of fruit rot and premature nut fall diseases in coconut palms, together with the emergence of numerous *Botryodiplodia* species and other fungi species associated with coconut has called for precise and accurate identification of *B. theobromae* as the causal agent of fruit rot and premature nut fall diseases in coconut (Dheepa *et al*., 2018; Munirah *et al*., 2017). As the species covered in this article, *B. theobromae* is the recognized scientific name; it supersedes both the teleomorph *Botryosphaeria rhodina* (Berk and M.A. Curtis) Arx and the synonym *Lasiodiplodia theobromae* Pat. The information on population of plant pathogen(s) will be used for developing strategies to increase the durability of crop varietal resistance towards such pathogen(s) (Xia *et al*., 2000). Besides improving the knowledge of pathogen(s) causing diseases in coconut, accurate identification of pathogen(s) is important to establish proper control measures, quarantine programs, and to develop resistant crop varieties to pathogen(s) (Asman *et al*., 2024; Munirah *et al*., 2017; Ramachandran *et al*., 2015). In the past few years, the advancement of molecular techniques has yielded various insights about the population of pathogens (Séré *et al*., 2007). Several molecular methods have been used to identify and reveal genetic similarity or polymorphism within populations of plant pathogenic fungi (Hernández-Ceja *et al*., 2021; Kang *et al*., 2002). The genetic makeup of *B. theobromae* has been examined using a variety of molecular tools (Gunamalai *et al*., 2023; Santos *et al*., 2017). These include, but are not limited to, the use of the following genetic identifiers: the translation elongation factor gene, the tubulin gene, and the internal transcribed spacer domain.

The internal transcribed spacer and tubulin gene have been utilized to identify *B. theobromae* isolated from mango, cashew, and other tree crops (Munirah *et al*., 2017; Pandey *et al*., 2024). The pair of primers often used to amplify the internal transcribed spacer and tubulin gene in a PCR are ITS1/4 with F: 5'-TCCGTA GGTGAACCTGCGG-3' and R: 5'-TCCTC-CGCTTATTGATATGC-3' for ITS, while Bt2a/b with F: 5′-GGTAACCAAATCGGTGCTGCTTTC-3′ and R: 5′-ACCCT-CAGTGTAGTGACCCTTGGC-3′ for tubulin gene (Xie *et al*., 2016). Inter-simple sequence repeat, simple sequence repeat, and random amplified polymorphic DNA identifiers have also been used to describe *B. theobromae* obtained from Morus alba and Agave sisalana (Cardoso & Wilkinson, 2008; Xie *et al*., 2016). Ten tiny gene satellites, or simple sequence repeats, were developed by Cardoso and Wilkinson (2008) in order to genetically characterize *B. theobromae*. Shah *et al*. (2010) also developed nine tiny gene satellites and seven random

amplified polymorphic DNA identifiers, which they used to characterize *B. theobromae* isolated from the pear trees. Santos *et al*. (2017) and Jacobs *et al*. (2004) developed translation elongation factor with pair of primers: EF1-728F (5'-CATC-GAGAAGTTCGAGAA-3') and EF2-728R (5'-GGARG-TACCAGTSATCATGTT-3') for the molecular identification, sequence, and phylogenic analyses of *B. theobromae* isolated from cashew, native bark beetles, and Tomicus piniperda. Xu *et al*. (2016) developed PCR-based processes that utilize three specific primers, namely, primers Lt347 with F: 5'-AACGTAC-CTCTGTTGCTTTGGC-3' and R: 5'-TACTACGCTTGAGGGCT-GAACA-3', Np304 with F: 5'-TGAACTTCGCAGTCTGAA-3' and R: 5'-CTCCAAAGCGAGGTGTTT-3', and FaF/Bt2b with F: 5'-CATCCGCAGCGTGGGAGAACAT-3' and R: 5'ACCCTCAGTGTAGTGACCCTTGGC-3') for identification of the species belonging to the Botryosphaeriaceae family linked with stem blight disease of blueberry in China (Xu *et al*., 2016).

In this study, the universal primer (ITS1F/4R) and three species specific primers (Lt347-F/Lt347-R, Bt2aF/Bt2bR, and EF1-728F/EF2-728R) were used to amplify the DNA of *B. theobromae* isolated from diseased coconut. Furthermore, the amplification of the ITS1/4 region, together with the sequencing of the ITS1/4 region in conjunction (with other molecular identifiers), cultural and morphological characters, was employed in this investigation to identify *B. theobromae* isolated from diseased coconut fruits with signs of fruit rot and premature nut fall (Ekhorutomwen *et al*., 2024; Pandey *et al*., 2024). Hence, with economic importance of fruit rot and premature nut fall diseases of coconut, this study was carried out to analyse the cultural, morphological and molecular characters of *B. theobromae* causing these diseases in coconut fruits.

Methods

Culture media

Potato dextrose agar (PDA), Czapek Dox agar (CDA), and sodium hypochlorite were purchase from Sigma-Aldrich, St. Louis, MO, USA, and used for the study.

Study location

Two (2) locations namely; NIFOR Main Station and Coconut Garden, Isihor, in Ovia North East Local Government Area, Edo State, Nigeria, were used for this study.

Source of coconut fruits

Diseased coconut fruits showing signs of rot and freshly fallen nuts were collected from coconut plantations cultivated with the following coconut varieties {that is, green dwarf (GD), orange dwarf (OD), yellow dwarf (YD), red dwarf (RD)} in the chosen location.

Isolation of fungal from diseased coconut fruits

Diseased fruit samples (showing signs of rot and freshly fallen nuts) were taken to the laboratory with a transparent screw cap container (Phipps & Porter, 1998). Sterile knife was used to dissect diseased coconut fruit samples, and the dissected samples were surface sterilized with 200 ml 0.5% sodium hypochlorite (hypo; 3A25N). Thereafter, 1000 ml each of potato dextrose agar (PDA; ISO 9001) and Czapek's Dox agar (CDA; ISO-9000-69-5) were prepared separately, sterilized with an autoclave, and later dispensed into petri dishes. A sterile inoculating needle was used to inoculate the dissected samples into petri dishes containing PDA and CDA, and incubated at room temperature for 7 days (Dheepa *et al*., 2018; Phipps & Porter, 1998). Stock cultures of fungal isolates were obtained using the hyphal tip transfer procedure and maintained in tube slants of PDA at 10ºC (Phipps & Porter, 1998). The growth of fungal isolates on petri dishes containing PDA and CDA were used for colony description (Ashokkumar *et al*., 2018; Osagie *et al*., 2013). Colony description together with microscopy observation were used for presumptive identification of each fungal isolates as described by Commonwealth Mycological Institute (CMI) manual (Ashokkumar *et al*., 2018; Dheepa *et al*., 2018; El-Morsi & Ibrahim, 2012; Osagie *et al*., 2013; Shah *et al*., 2010). Thereafter, the fungal isolates were confirmed using molecular techniques (Ashokkumar *et al*., 2018; El-Morsi & Ibrahim, 2012; Shah *et al*., 2010).

Colonial morphological (macro and micro) characterization of fungal isolates

With the aid of a sterile cock borer, nine-millimeter discs from margins of actively growing 7-day-old culture of eight fungal isolates were plated on the center of 9 cm Petri dishes containing PDA medium. Each isolate was replicated three times. Colony growth rate was recorded by measuring colony diameter after 24 and 48 h of incubation, while colony color and texture observations were recorded after 48 h of incubation (Adeniyi *et al*., 2016; Dheepa *et al*., 2018; El-Morsi & Ibrahim, 2012; Shah *et al*., 2010). Pycnidiospores characters of each isolate *viz*., day of production, number, arrangement, size, and color were observed at weekly interval with the aid of a simple microscope.

Identification fungal isolates with molecular tools

With the use of the Fungal DNA Isolation Kit, DNA was extracted from the mycelia of the eight fungal isolates. The isolated DNA was purified and thereafter amplified using the fungal universal primer (ITS1F/4R) and three specific primers (Lt347-F/R, Bt2aF/bR, and EF1-728F/EF2-728R) in a PCR (Cardoso & Wilkinson, 2008; Hernández-Ceja *et al*., 2021; Wang *et al*., 2011; White *et al*., 1990; Xie *et al*., 2016; Xu *et al*., 2016). The DNA amplicons from PCR were cleansed with the help of a QIA-quick purification kit. The amplicons were separated with gel electrophoresis (Cardoso & Wilkinson, 2008). The purity of the extracted DNA were checked with the aid of a nanodrop spectrophotometer (Table 5). Also, the amplicons from the ITS1F/4R region were sequenced using a cycle-sequencing kit in a sequencing machine (ABI PrismTM) (Cardoso & Wilkinson, 2008). The base sequence from the ITS1F/4R region was edited and analyzed (to determine the similarities between the strands) using multiple sequence alignment, and thereafter, it was blasted using a blasting tool in the NCBI database for identification of each isolate (Cardoso & Wilkinson, 2008; Disegha & Akani, 2019).

Bioinformatics and phylogenetic analysis of fungal isolates

DNA fragments that were generated from PCR using the primer (ITS1F/R) and a genotyper were used to generate data that were

transformed into a binary character matrix by scoring the presence or absence of each nucleotide base at each locus (that is, "1" for the presence and "0" for the absence of a nucleotide base at a particular position). The generated combined data set (that is, nucleotide sequence information or codons) from the ITS1F/4R primer obtained from the DNA of the eight fungal isolates was aligned with the ClustalW algorithm, and dendrograms were also made using the same ClustalW algorithm in MEGA X (Excoffier *et al*., 2007; Kumar *et al*., 2018; Shah *et al*., 2010).

Data analysis

DNA profile that was generated using a molecular marker (ITS1F/4R) was analyzed using MEGA X (http://www.megasoftware.net) (Cardoso & Wilkinson, 2008; Kumar *et al*., 2018). While data obtained from fruit rot and premature nut fall diseases were calculated using the percentage of disease incidence (PDI) in a Microsoft Excel worksheet (Ashokkumar *et al*., 2018; Dheepa *et al*., 2018).

Results

Colonial morphological (macro and micro) characterization of *B. theobromae* isolates

All eight isolates of *B. theobromae* were coded as Bt.NGD, Bt.NOD, Bt.NYD, Bt.NRD, Bt.IGD, Bt.IOD, Bt.IYD, and Bt.IRD in this study. 等线Figure 1 and Table 4 show fungal pathogen was isolated from diseased coconut fruits showing signs of rot and fallen nuts (Phipps & Porter, 1998). The eight isolates of *B. theobromae* isolated from diseased coconut fruits with respect to their colonial morphological characters, show varying colony texture and colour on PDA after 72 h incubation. Colony growth rate of the eight isolates of *B. theobromae* were very high after 72 h of incubation, indicating their fast-growing nature (Table 1 and Figure 2). The eight fungal isolates filled the entire 9 cm diameter Petri dish surface after 48 h incubation. Three out of the eight isolates (Bt.NOD, Bt.NYD, Bt.IRD) produce brownish or reddish pigmentation (botryodiplodin) after 30 days in PDA slant (Plate 1), while five out of the eight isolates (Bt.NGD, Bt.NRD, Bt.IYD, Bt.IGD and Bt.IOD) did not show pigmentation in PDA slant. Time taken for conidiospore production varied between 63 to 70 days under culture condition (Bt.NOD and Bt.IYD produced conidiospore after 63 days of incubation, Bt.NYD, Bt.IGD and Bt.IOD produced conidiospore after 70 days of incubation, while Bt.NGD, Bt.NRD and Bt.IRD did not produce conidiospore on culture media after incubation for over 70 days (Figure 3 and Table 3). Conidiospore arrangement was clustered in Bt.NYD and Bt.IGD, scattered in Bt.NOD, Bt.IOD, and Bt.IYD. The conidiospore was light brown, initially hyaline, mostly septate, with few aseptate, and, conidiospore size (µm) in length ranges between 15.69 – 22.95, while the width ranges between 8.47 – 12.95.

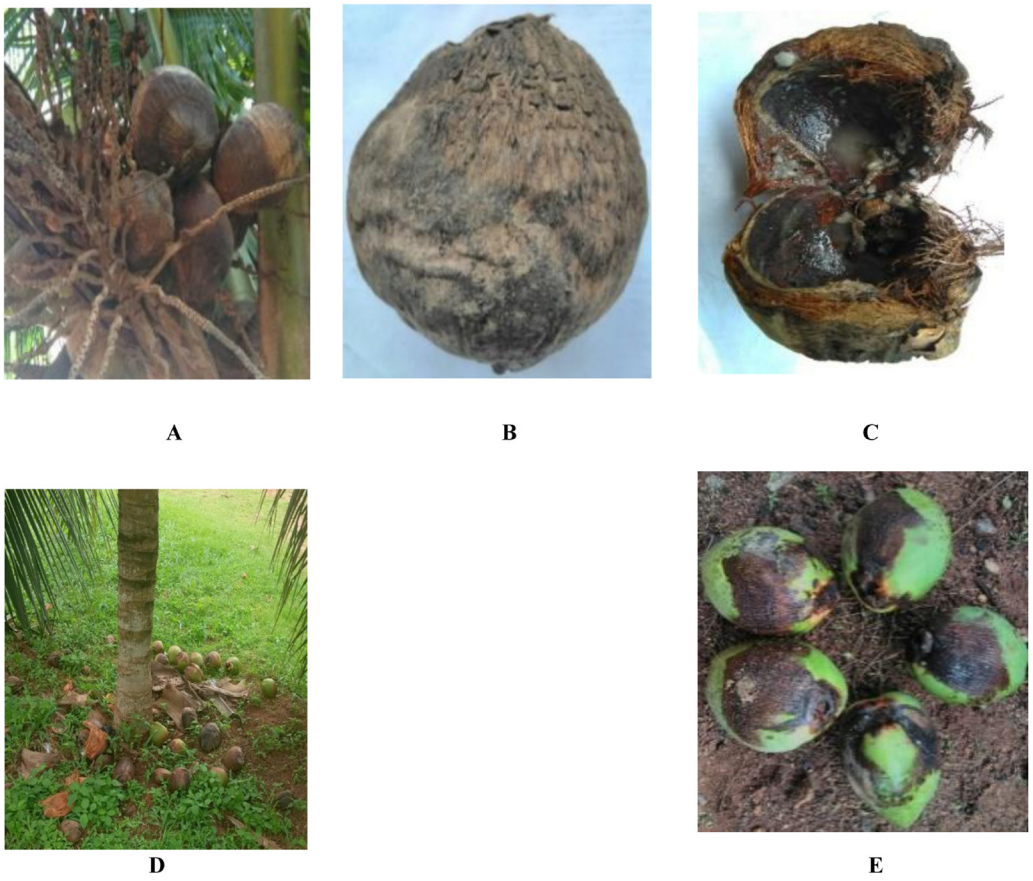

Figure 1. Signs and symptoms of coconut fruit rot and nut fall: **A** - **B**: Green dwarf coconut fruit with rot; **C**: dissection of green dwarf coconut fruit with rot; **D**: immature fallen nuts around the base of coconut palm; **E**: Cluster of fallen nuts of mature GD coconut with extensive rot.

Table 1. Colonial morphological characters of eight *Botryodiplodia theobromae* isolates.

Location	Isolates	Colony growth rate after 72 hours	Colony texture and colour on PDA after 72 hours	Brownish pigmentation in PDA slant
NIFOR Main Station	Bt.NGD Bt.NOD Bt.NYD Bt.NRD	+++ +++ +++ +++	Large, flat, uniform, dull white changing to grey Large, flat, uniform, white Large, flat, uniform, dull white changing to grey Large, raised, irregular, greyish-white	- + + -
Coconut Garden, Isihor	Bt.IGD Bt.IOD Bt.IYD Bt.IRD	+++ +++ +++ +++	Large, raised, irregular, dull white changing to grey Large, flat, uniform, greyish white Large, flat, uniform, dull white changing to grey Large, raised, irregular, dull white changing to grey	- - - +

Key: Bt (*Botryodiplodia theobromae)*, Growth rate (+++: very high), Red pigmentation (+: present; -: absent), N: NIFOR, I: Isihor, GD (green dwarf), OD (orange dwarf), YD (yellow dwarf), RD (red dwarf), PDA (potato dextrose agar)

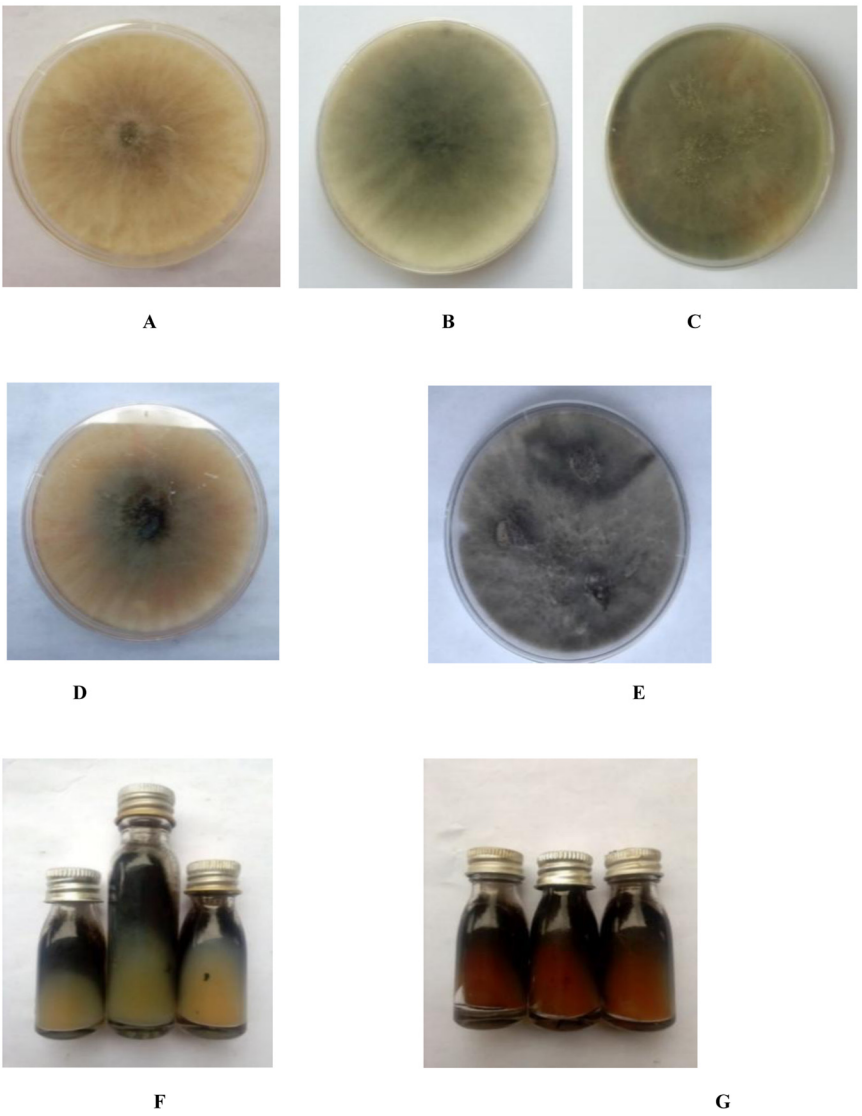

Figure 2. A-G: Isolates of *Botryodiplodia theobromae* colony on potato dextrose agar in Petri dishes and slant.



Figure 3. *Botryodiplodia theobromae* conidium and conidiospore; **A**: Conidium, **B**: Scattered conidiospore, **C**: Clustered conidiospore.

Molecular identification, bioinformatics and phylogenetic analysis of *Botryodiplodia theobromae*

The ITS1F/4R (universal primer) and Lt347-F/R (specific primer) successfully amplified the DNA of all eight isolates of *B. theobromae* in a PCR prior to diluting the DNA (Figure 4 and Figure 5). While Bt2aF/bR and EF1-728F/EF2-728R specific primers did not amplify the DNA of all eight isolates of *B. theobromae* in a PCR prior to diluting the DNA. The Bt2aF/bR primer amplified the DNA of isolates Bt.IGD, Bt.NGD, Bt.NYD, and Bt.NRD but was unable to amplify the DNA of other isolates in a PCR (Figure 6). While EF1-728F/EF2-728R amplified the DNA of isolates Bt. IGD, Bt.IYD, Bt. IRD, and Bt. NGD, it was unable to amplify the DNA of other isolates in a PCR (Figure 7). Furthermore, the sequence information of the amplified ITS1F/4R region (together with the Lt347-F/R primer) confirms the identity of each of the isolates as *B. theobromae*. The molecular analysis of the ITS1F/4R region also grouped the eight isolates of *B. theobromae* into five groups. Group 1 consists of three isolates: Bt.NYD, Bt.NGD, and Bt.IYD, with accession number MW774360.1; group 2 consists of two isolates: Bt.IGD and Bt.IRD, with accession number OM617921.1; group 3 to 5 consists of one isolate each (Bt.NOD, Bt.NRD, and Bt.IOD), with accession numbers MW282879.1, MZ502166.1, and MW478631.1 (Table 2 and Table 6). The identified isolates show 100% similarity with respect to each established accession of *B. theobromae* in the NCBI database or gene bank. Additionally, the percentage proportion of individual isolates with *B. theobromae* strains in the NCBI database shows that Group 1 isolates (representing 37.5% of the entire *B. theobromae* population) were more prevalent in both locations, followed by Group 2 isolates (25%), while Groups 3 to 5 represent 12.5%, respectively (Table 7). Isolates Bt.IOD has high level of genetic distance at site 2 (0.51896) and site 6 (0.61723) in the nucleotide sequence, isolates Bt. IYD has high level of genetic distance at site 6 (0.57171) in the nucleotide sequence, while isolate Bt.IRD also has high level of genetic distance at site 6 (0.56072) in the nucleotide sequence (Table 7). Other isolates (Bt.NOD, Bt.NRD, Bt.NYD, Bt.IGD, and Bt.NGD) have low genetic distance in the nucleotide sequence. The evolutionary history of each isolate was inferred using the ClustalW algorithm, as disclosed by Kumar *et al.* (2018). The optimal dendogram was constructed for each isolate as illustrated by Kumar *et al.* (2018), Saitou and Nei (1987), and Tamura *et al.* (2004) (Figure 8, Figure 9.1 to Figure 9.9).

Table 2. Groupings of eight *Botryodiplodia theobromae* isolates based on colony texture and colour.

Isolates	Colony texture and colour on PDA after 72 hours
Bt.NGD Bt.NYD Bt.IYD	Large, flat, uniform, dull white changing to grey
Bt.NOD	Large, flat, uniform, white
Bt.NRD	Large, raised, irregular, greyish white
Bt.IOD	Large, flat, uniform, greyish white
Bt.IGD Bt.IRD	Large, raised, irregular, dull white changing to grey

Key: Bt (*Botryodiplodia theobromae)*; GD (green dwarf), OD (orange dwarf), YD (yellow dwarf), RD (red dwarf).

Discussion

As previously mentioned, this study was conducted in two major coconut-growing locations in Edo State, Nigeria. The fungal pathogen was isolated from diseased coconut fruits showing signs of rot and fallen nuts (Phipps & Porter, 1998). The colony description of each fungal pathogen together with the microscopy observation were used for the presumptive identification of the eight isolates of *B. theobromae* (Ashokkumar *et al.*, 2018). In addition, after 72 hours, the eight isolates of *B. theobromae* exhibit different colony pigments and textures according to the colonial morphological features that were determined in the study (Adeniyi *et al.*, 2016). The colony pigments of *B. theobromae* isolates varied from white

Table 3. ***Botryodiplodia theobromae*** **conidiospore characteristics.**

Location	Isolates	Conidiospore characteristics					
		Day of production	Size in Diameter (µm)		Arrangement	Colour	Septation
			Length	Width			
NIFOR Main Station	Bt.NGD	-	-	-	-	-	-
	Bt.NOD	63	19.83	11.45	Scattered	Light brown	Septate/aseptate
	Bt.NYD	70	15.69	8.47	Clustered	Light brown	Septate
	Bt.NRD	-	-	-	-	-	-
Coconut Garden, Isihor	Bt.IGD	70	18.14	10.89	Clustered	Light brown	Septate
	Bt.IOD	70	22.13	12.33	Scattered	Light brown	Septate/aseptate
	Bt.IYD	63	22.95	12.95	Scattered	Light brown	Septate/aseptate
	Bt.IRD	-	-	-	-	-	-

Table 4. Coconut fruit population and diseases' incidence in both locations and growing seasons.

Locations	Growing seasons	No of coconut fruits sampled	No of diseased coconut fruits			PDO
				FR	PNF	
NIFOR Main Station	2021 – 2022	1922	258	13.4	110	148
	2022 – 2023	2536	419	195	224	16.5
	Grand total	**4458**	**677**	**305**	**372**	**15.2**
Coconut Garden, Isihor	2021 – 2022	2238	266	128	138	11.9
	2022 – 2023	2068	255	148	107	12.3
	Grand total	**4306**	**521**	**276**	**245**	**12.1**

Key: FR (fruit rot), PNF (Premature nut fall), PDI (percentage of disease incidence).

Table 5. Analysis of genomic DNA extracted from isolates of ***Botryodiplodia theobromae*** **using nano drop spectrophotometer.**

SAMPLES	DNA CONC (ng/µl)	A_{260}	A_{280}	$A_{260/280}$
Isolate 1	1273.3	26.446	14.395	1.837
Isolate 2	1827.0	35.539	18.291	1.943
Isolate 3	3068.0	61.360	31.041	1.980
Isolate 4	1177.3	43.546	21.387	2.036
Isolate 5	1032.5	40.651	21.985	1.849
Isolate 6	1580.2	31.604	15.661	2.020
Isolate 7	803.4	40.068	20.969	1.911
Isolate 8	1533.7	30.675	15.285	2.007

(Bt. NOD), to dull white changing to grey (Bt.NRD, Bt.NGD, Bt.NYD, and Bt.IYD), to greyish white (Bt.IOD, Bt.IRD, and Bt.IGD). The mycelia topography were either raised or flat, while the mycelia edge were either irregular or uniform. The colony pigments and textures grouped all eight isolates of *B. theobromae* into five categories (Table 2).

Table 6. Grouping of eight *Botryodiplodia theobromae* isolates based on molecular identity.

Groups	Isolates	Relationship to isolates in NCBI gene bank	Accession number	% proportion of individual strains
I	Bt.NYD Bt.NGD Bt.IYD	*Botryodiplodia theobromae* isolate BAL2	MW774360	37.5
II	Bt.IGD Bt.IRD	*Botryodiplodia theobromae* isolate LSB-1	OM617921	25
III	Bt.NOD	*Botryodiplodia theobromae* isolate MRR-030	MW282879	12.5
IV	Bt.NRD	*Botryodiplodia theobromae* isolate MKMS 2.1.2	MZ502166	12.5
V	Bt.IOD	*Botryodiplodia theobromae* isolate C18	MW478631	12.5

Table 7. Average genetic distance of eight *Botryodiplodia theobromae* from two locations.

Isolates	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Bt.NGD	0.00000						
Bt.NOD	0.00795						
Bt.NYD	0.00000	0.00996					
Bt.NRD	0.00198	0.00998	0.00198				
Bt.IGD	0.01392	0.01002	0.01793	0.00800			
Bt.IOD	0.57853	0.51896	0.57853	0.55689	0.61723		
Bt.IYD	0.00198	0.01992	0.00198	0.00000	0.00798	0.57171	
Bt.IRD	0.01408	0.00203	0.01408	0.01411	0.02434	0.56072	0.02218

Key: The genetic distance ranges from 0.00198 to 0.61723. The compositional distance correlate with the number of differences between sequences. High genetic distance: 0.5 above; Low genetic distance: 0.5 below.

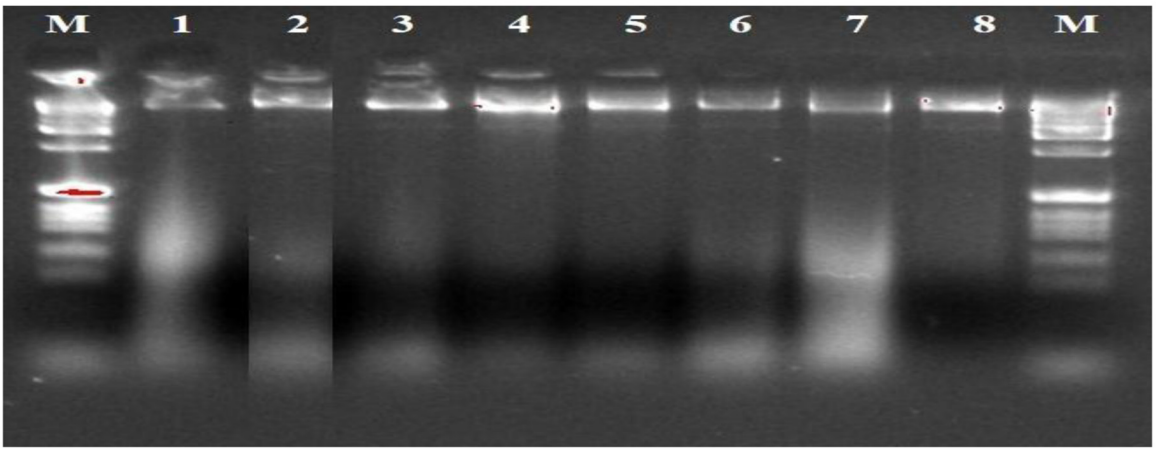

Figure 4. Gel image of genomic DNA of eight isolates.

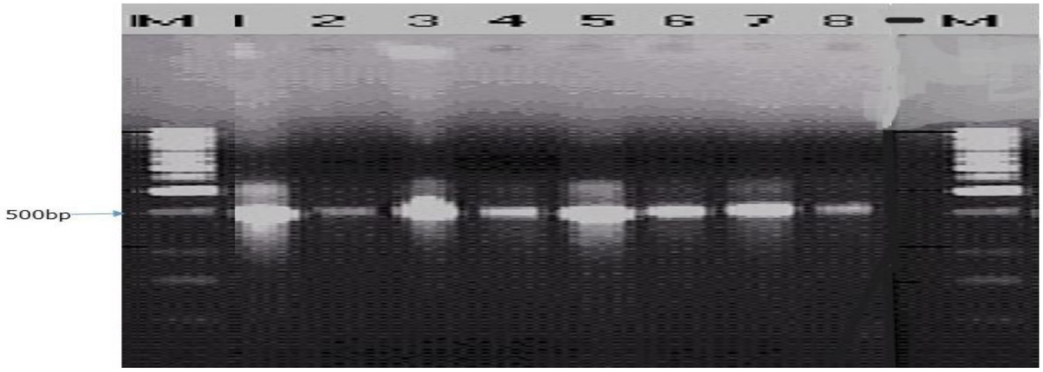

Figure 5. **Picture of DNA amplicons of eight isolates using ITS1F/4R primers.**

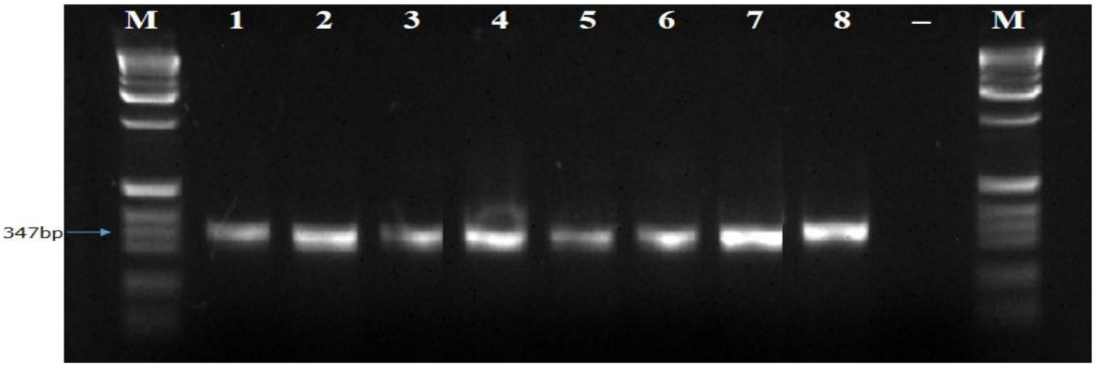

Figure 6. **Picture of DNA amplicons of test isolates using Lt347-F/R primers.**

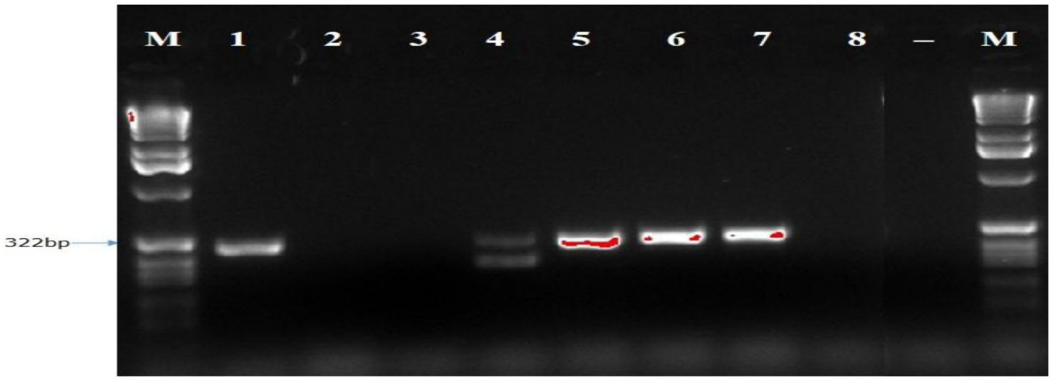

Figure 7. **Picture of DNA amplicons of test isolates using Bt2a-F/bR primers.**

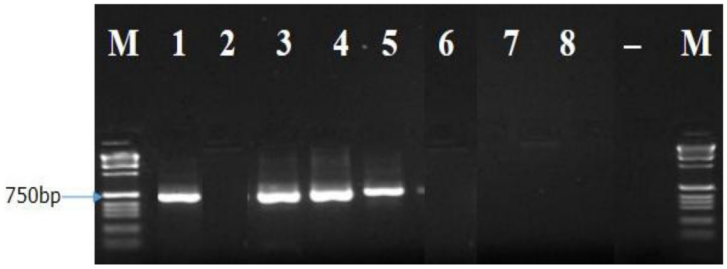

Figure 8. **Picture of DNA amplicons of test isolates using EF1-728F/EF2-728R primers.**

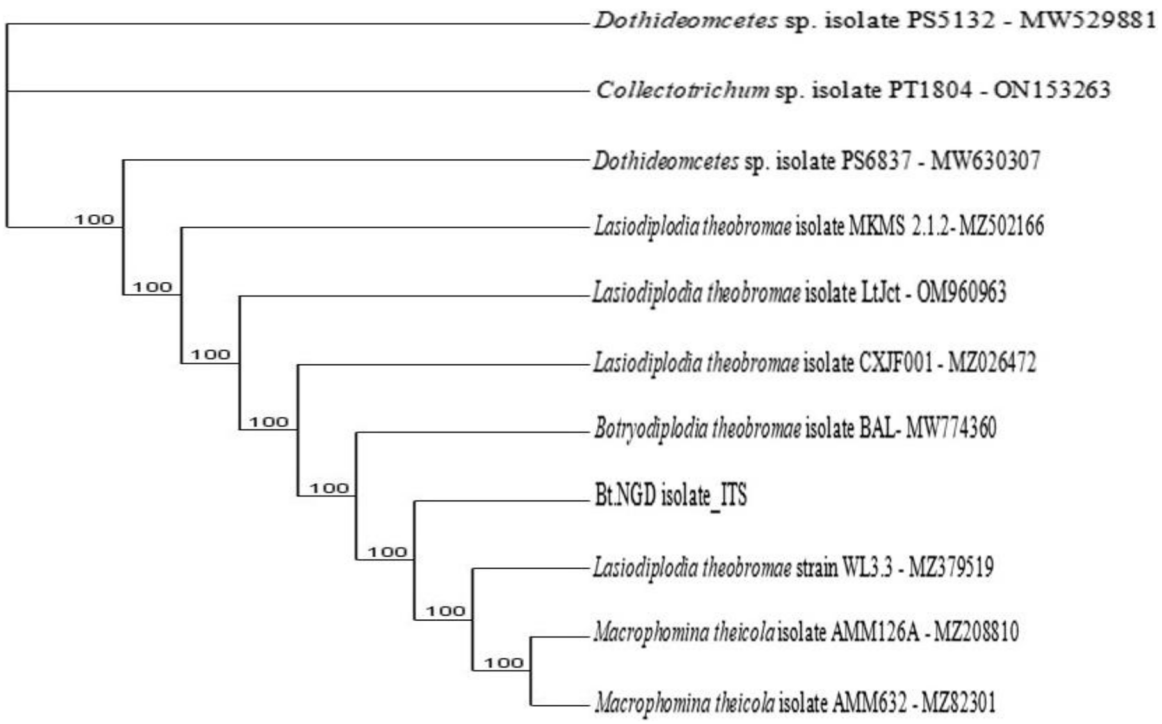

Figure 9.1. **Dendrogram of Bt.NGD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

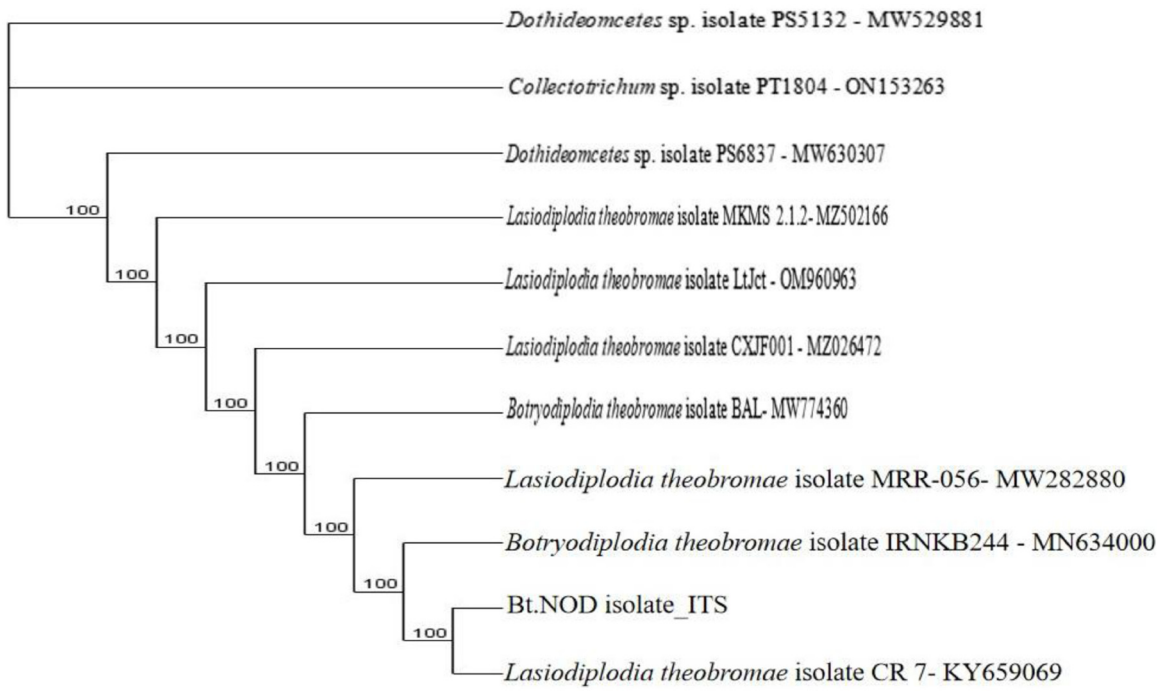

Figure 9.2. **Dendrogram of Bt.NOD using nucleotide sequence of the ITS1F/4R region NCBI data base.**

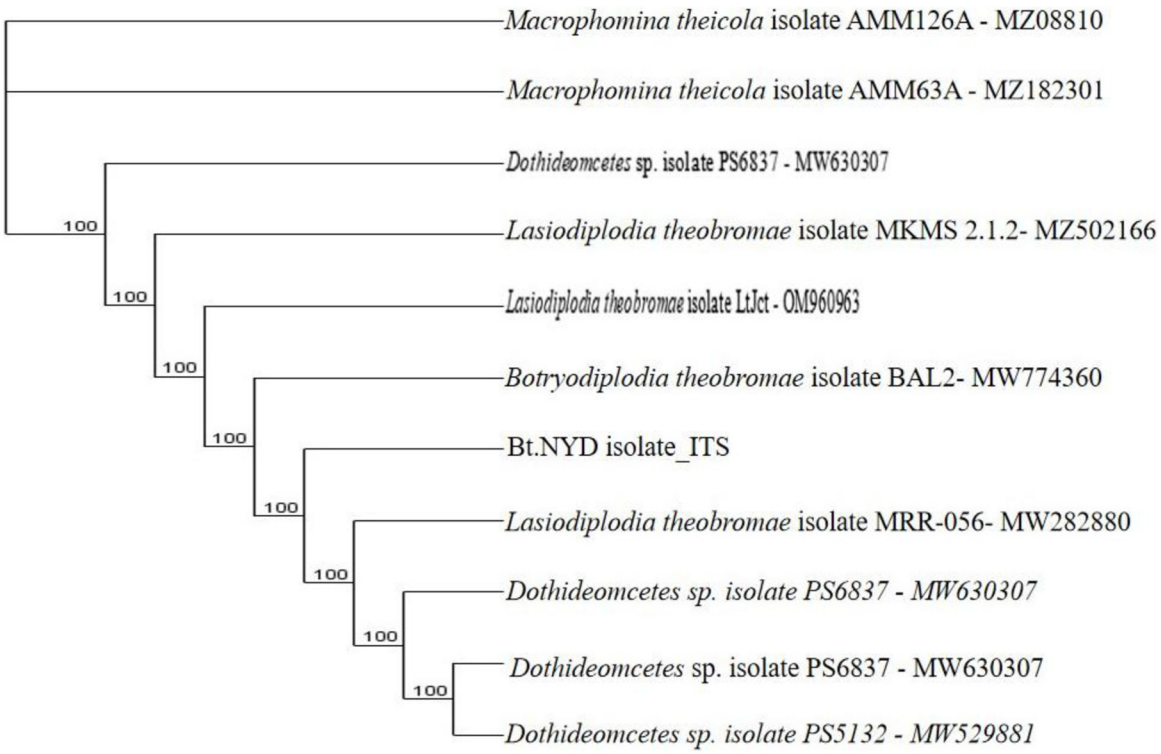

Figure 9.3. **Dendrogram of Bt.NYD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

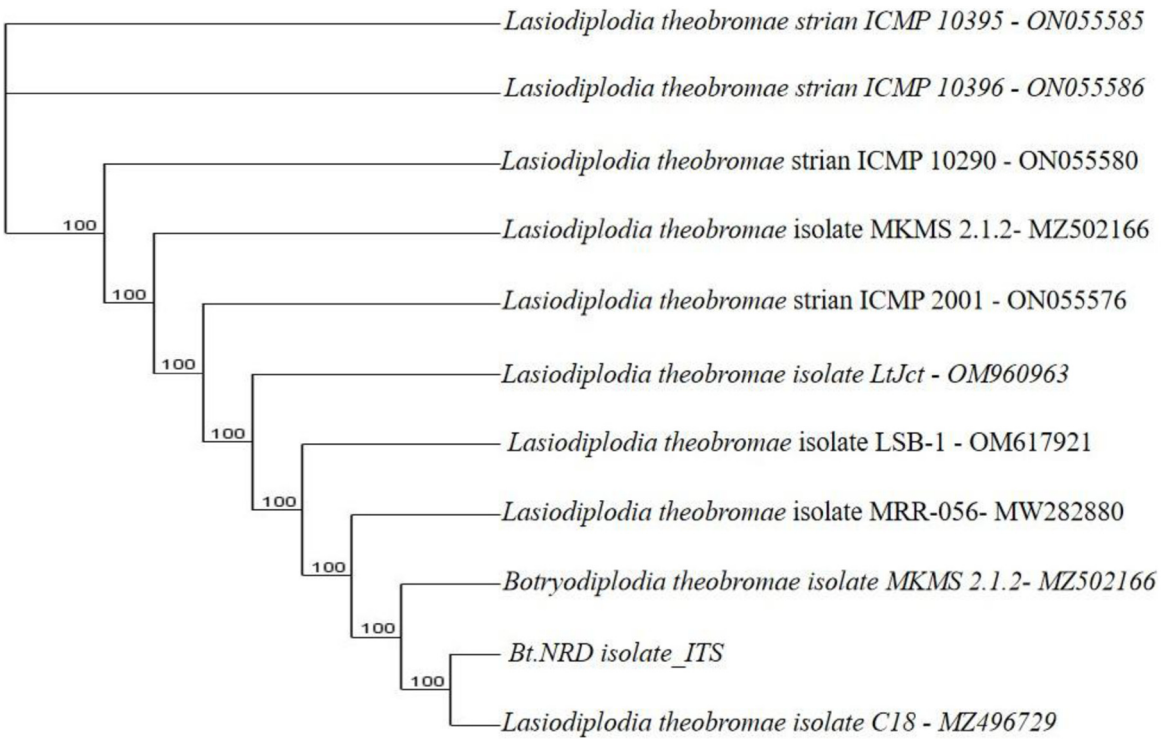

Figure 9.4. **Dendrogram of Bt.NRD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

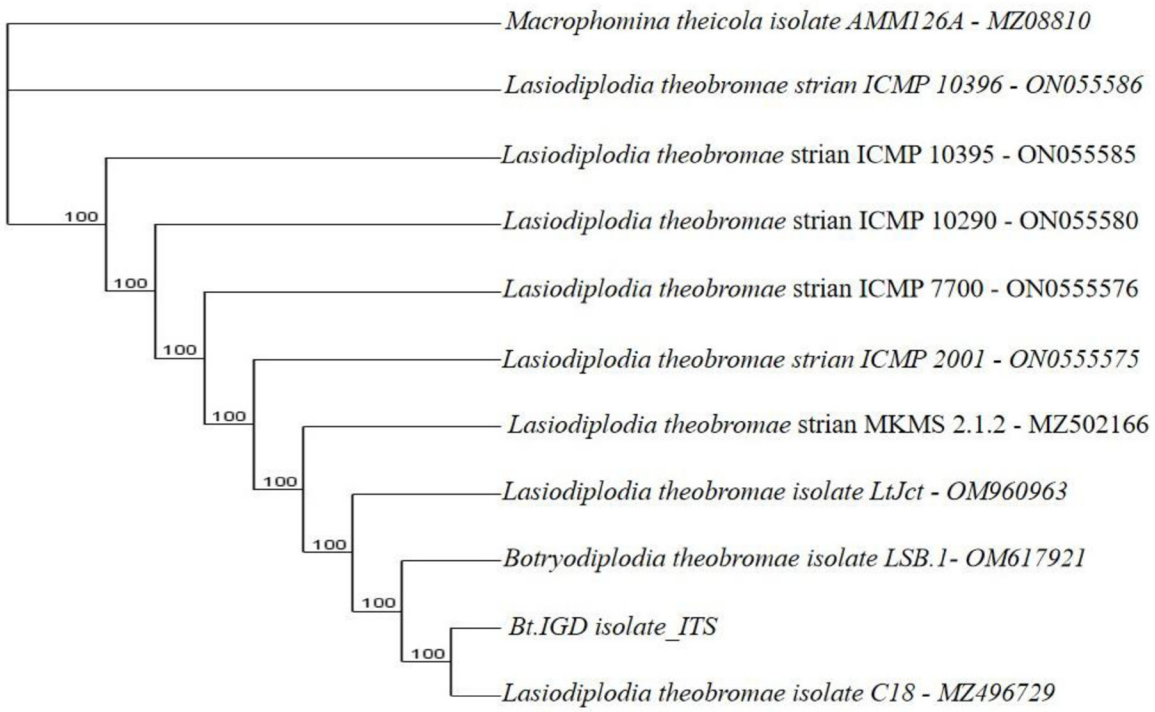

Figure 9.5. **Dendrogram of Bt.IGD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

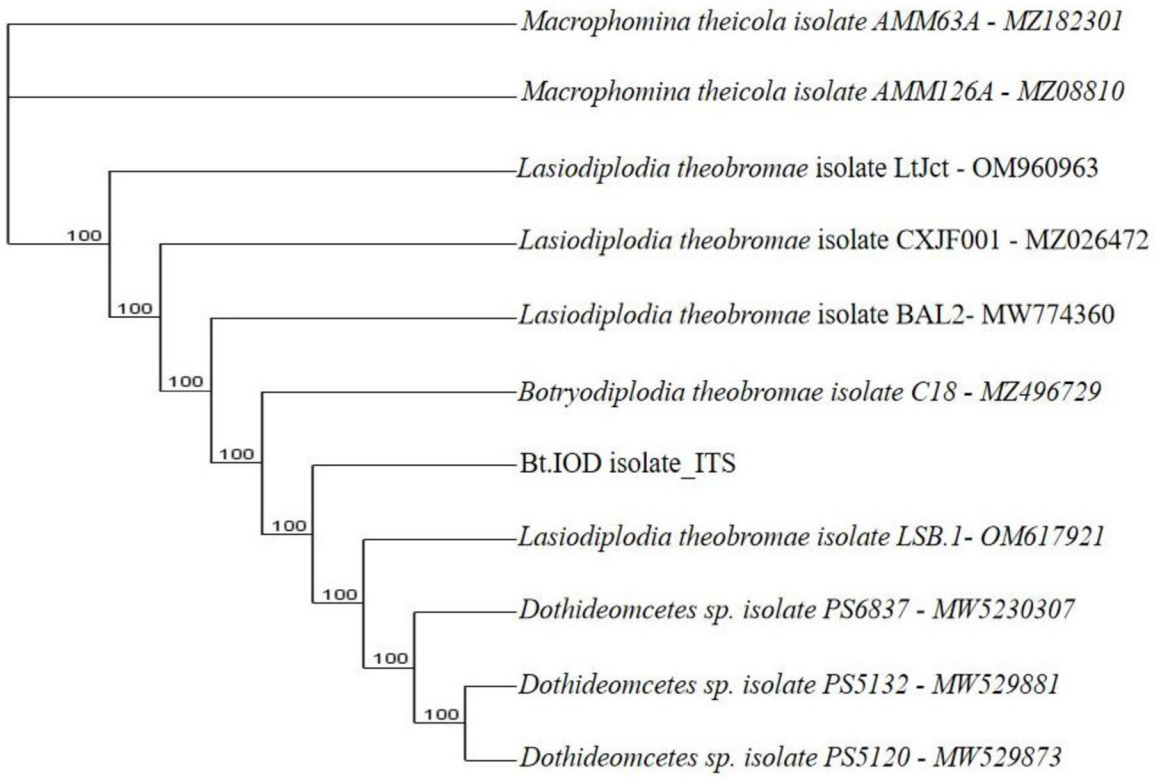

Figure 9.6. **Dendrogram of Bt.IOD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

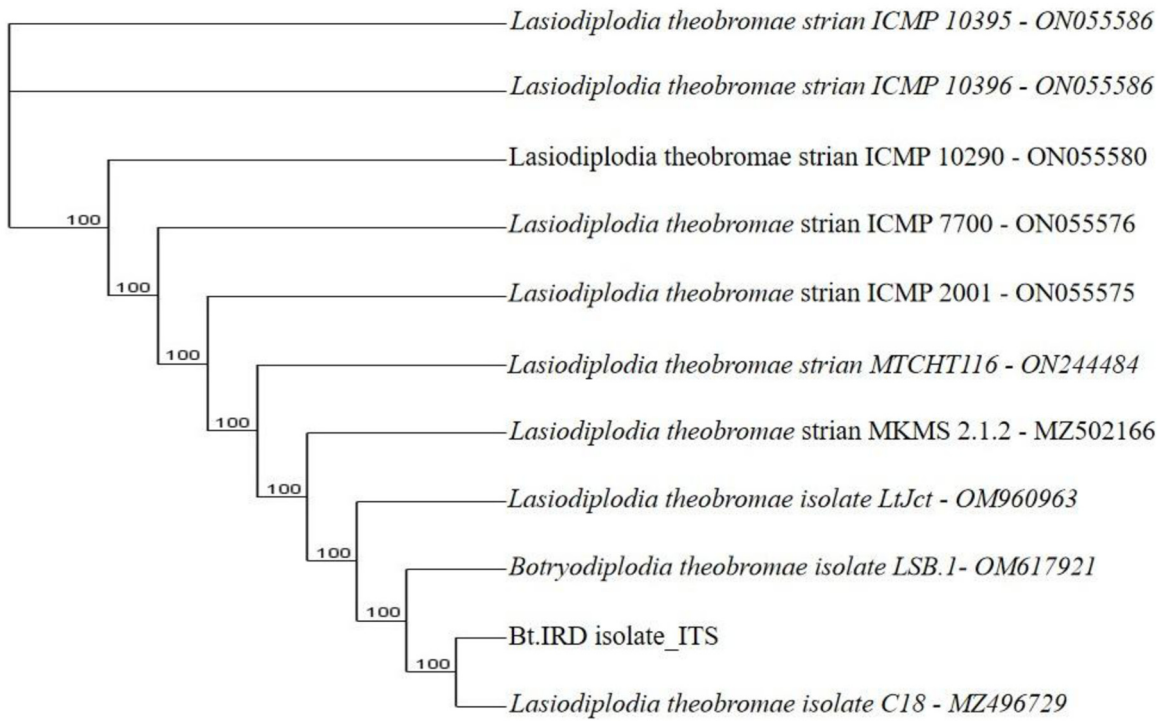

Figure 9.7. **Dendrogram of Bt.IYD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

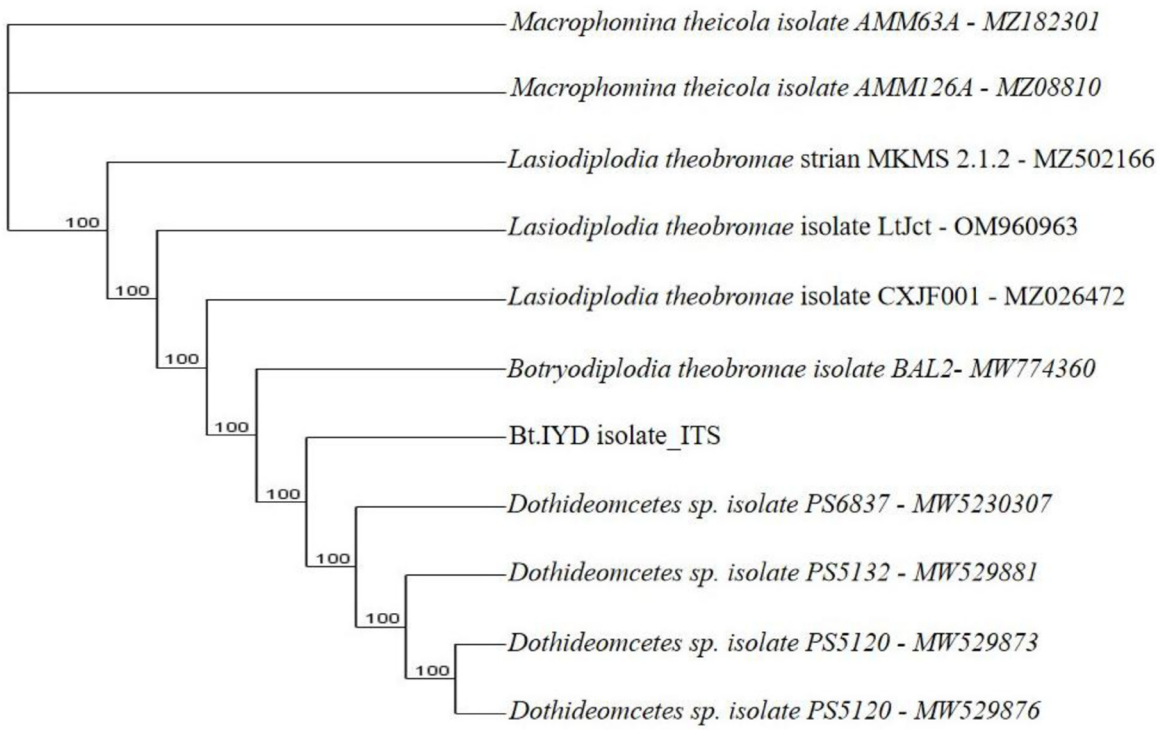

Figure 9.8. **Dendrogram of Bt.IRD using nucleotide sequence of the ITS1F/4R region in NCBI data base.**

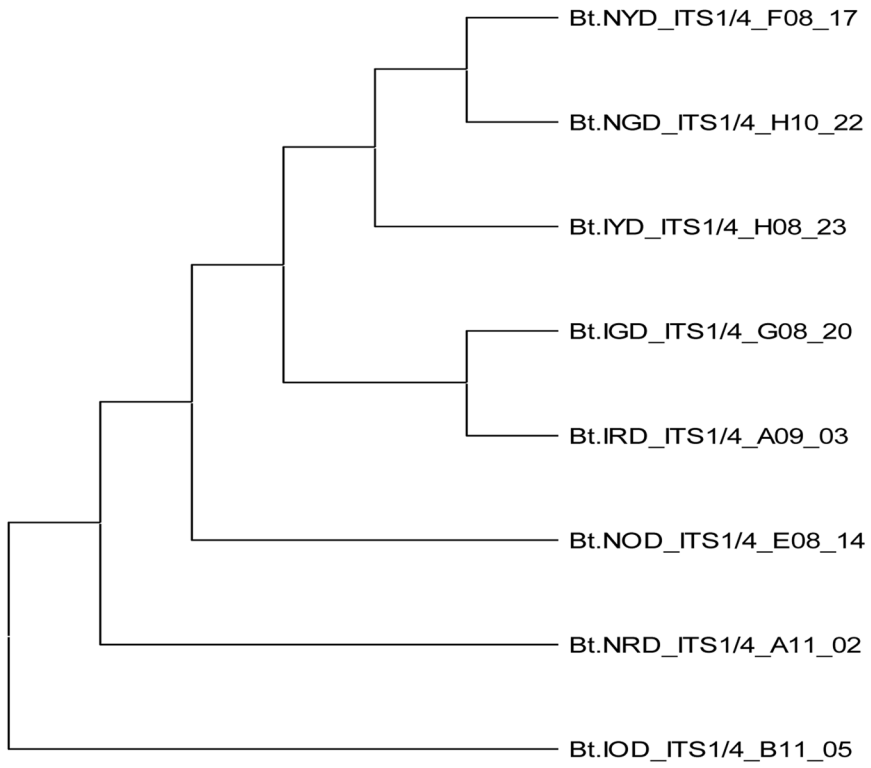

Figure 9.9. Dendrogram obtained from the combined data set of eight *Botryodiplodia theobromae* isolates.

Conidiospore arrangement were either clustered (Bt.NYD and Bt.IGD) or scattered (Bt.NOD, Bt.IOD and Bt.IYD). Conidiospore size in length ranges between 15.69 to 22.95 µm, while the width ranges between 8.47 to 12.95 µm. The conidiospore are light brown, initially hyaline, mostly septate, with few aseptate (Figure 3). Based o the numerical figures obtained from the study, isolates from Coconut Garden, Isihor, had conidiospore which numbers were greater that than that obtained from NIFOR Main Station. Studies on the colonial morphological features align with the work of Adeniyi *et al*. (2016). The colony growth rate of the eight isolates was relatively rapid after 72 hours of the incubation process, suggesting that they had grown quickly (Table 1). After being incubated in PDA for 72 hours, all eight isolates covered the area of the Petri plate. It is postulated that *B. theobromae* fastidious nature makes them to colonize susceptible host tissues easily (Ashokkumar *et al*., 2018; Dheepa *et al*., 2018).

To confirm the identity of the eight *B. theobromae* isolates using molecular technique, one universal primer (ITS1F/4R) and three specific primers (Lt347-F/R, Bt2aF/bR, EF1-728F/EF2-728R) were employed to amplify the genes of *B. theobromae* isolates. The ITS1F/4R and Lt347-F/R primers successfully amplified the DNA of all eight *B. theobromae* isolates in a PCR. But the two other specific primers (Bt2aF/bR, EF1-728F/EF2-728R) were unable to amplify the DNA of all eight *B. theobromae* isolates in a PCR. Additionally, the amplified ITS1/4 region of the eight isolates was sequenced and blasted in the NCBI database, which shows 100% similarity with *B. theobromae* (accession numbers: MW774360.1, OM617921.1, MW282879.1, MZ502166.1, and MG778942.1) in the NCBI database. Also, the ability of the specific primer (Lt347-F/R) to amplify the DNA of *B. theobromae* confirms the identity of all eight isolates examined in the study. Hence, in this study, the eight fungal isolates responsible for the fruit rot and premature nut fall diseases of coconut were identified and confirmed as *B. theobromae*. The ITS1F/4R primer was successfully utilized to amplify the ITS1/4 gene and for the quick identification of various isolates of *B. theobromae*, which is in line with different investigations (Ashokkumar *et al*., 2018; Bharanideepan, 2013; Munirah *et al*., 2017). However, Xu *et al*. (2016) demonstrated that *B. theobromae* could also be identified using a species-specific (Lt347-F/R) primer. In addition, based on ITS1/4 sequence information, Bt.NGD, Bt.NYD, and Bt.IYD isolates are genetically similar, as all three isolates have similar ITS1/4 sequence information with *B. theobromae* isolate BAL2 with accession number MW774360.1. Two isolats (Bt.NGD, Bt.NYD) from the NIFOR location and one isolate (Bt.IYD) from Isihor were grouped into Group 1. This simply suggests these isolates do coexist and have a connection in both places. Because of this, they are more common in both places. While Bt.IGD and Bt.IRD isolates are genetically similar, the two isolates have similar ITS1/4 sequence information to *B. theobromae* isolate LSB-1 with accession number OM617921.1. Both isolates were in the same location (Isihor). This helps explain why they are so common in the Isihor area. Bt.NOD, Bt.NRD, and Bt.IOD isolates have no ITS1/4 similarity with

each other. Also, they have no similarity (in terms of the ITS1/4 gene) to the other isolates mentioned previously. In addition, Bt.NOD isolate has similar ITS1/4 sequence information with *B. theobromae* isolate MRR-030 with accession number MW282879.1, Bt.NRD isolate has similar ITS1/4 sequence information with *B. theobromae* isolate MKMS 2.1.2 with accession number MZ502166.1, and Bt.IOD isolate has similar ITS1/4 sequence information with *B. theobromae* isolate C18 with accession number MG778942.1 (Table 7). The ITS1/4 sequence information and phylogenetic analysis grouped all eight isolates of *B. theobromae* into five categories (Table 7 and Figure 1). Coincidentally, the cultural and molecular techniques were able to group the eight isolates of *B. theobromae* into five groups. Group 1 consists of 3 isolates (Bt.NYD, Bt.NGD, and Bt.IYD with accession number MW774360.1); Group 2 consists of 2 isolates (Bt.IGD and Bt.IRD with accession number OM617921.1); and Groups 3 to 5 consist of 1 isolate each (Bt.NOD, Bt.NRD, and Bt.IOD with accession numbers MW282879.1, MZ502166.1, and MG778942.1, respectively). Group 1 isolates (37.5%) were more prevalent in both locations, followed by group 2 isolates (25%), while groups 3 to 5 represent 12.5%, respectively. The prevalence of Group 1 isolates in both locations indicates that these isolates are mostly responsible for the coconut fruit rot and premature nut fall diseases, followed by Group 2 isolates and Groups 3 – 5 isolates. Isolates Bt.IOD, Bt.IYD, and Bt.IRD have a high level of genetic distance at specific sites in their nucleotide sequence, while isolates Bt.NOD, Bt.NRD, Bt.NGD, Bt.IGD, and Bt.NYD have a low genetic distance in their nucleotide sequence. The substantial amount of inherited distance through genes is proportional to the extent of disparities in the nucleotide sequences in each of the three isolates, even when the substitution patterns of the nucleotide sequences are homogeneous among the lineages of each isolate.

Conclusion

Botryodiplodia theobromae causing the fruit rot and premature nut fall diseases of coconut was identified based on its cultural, morphological and molecular characteristics. Although *B. theobromae* isolates showed similarity and variation in their cultural, morphological and molecular characters. The colony colour, ITS1/4 gene sequence information and phylogenetic analysis grouped all eight isolates of *B. theobromae* into five categories, as these characters assisted in the identification and characterization of *B. theobromae*. The study has provided a guide for proper identification of *B. theobromae* required for the proper control of this pathogen in coconut producing areas. More so, coconut growers should intensify regular fungal inspections and contact expert phytopathologist for proper guidance on how to adequately control the disease's condition in coconut producing areas.

Transparency

The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

Ethics and consent

Ethical approval and consent were not required.

Data availability

Underlying data

Mendeley: Cultural-morphology and molecular analysis of *Botryodiplodia theobromae*, a pathogen of coconut fruit. Doi: 10.17632/53ns2fcgps.1 (https://data.mendeley.com/datasets/53ns2fcgps/1) (Ekhorutomwen, 2025)

This project contains the following underlying data:

- Osayomore *et al*_Manuscript.docx
- Osayomore_Unedited_Data.docx

Data are available under the terms licensed under a Creative Commons Attribution 4.0 International licence.

Accession numbers

NCBI Gene: *B. theobromae* isolated from diseased green and yellow dwarf coconut fruit [*Coco nucifera* (coconut)]. Accession number MW774360.1. https://www.ncbi.nlm.nih.gov/nuccore/MW774360.1/

NCBI Gene: *B. theobromae* isolated from diseased green and red dwarf coconut fruit [*Coco nucifera* (coconut)]. Accession number OM617921.1. https://www.ncbi.nlm.nih.gov/nuccore/OM617921.1/

NCBI Gene: *B. theobromae* isolated from diseased orange dwarf coconut fruit [*Coco nucifera* (coconut)]. Accession number MW282879.1. https://www.ncbi.nlm.nih.gov/nuccore/MW282879.1

NCBI Gene: *B. theobromae* isolated from diseased red dwarf coconut fruit [*Coco nucifera* (coconut)]. Accession number MZ502166.1. https://www.ncbi.nlm.nih.gov/nuccore/MZ502166.1

NCBI Gene: *B. theobromae* isolated from diseased orange dwarf coconut fruit [*Coco nucifera* (coconut)]. Accession number MW478631.1. https://www.ncbi.nlm.nih.gov/nuccore/MW478631.1

Authors' contributions

Osayomore Endurance Ekhorutomwen: Conceptualization, Methodology, Experimental Design, Writing of Manuscript. Oufensou S: Methodology. Nnamdi Ifechukwude Chidi: Conceptualization, Review and Editing of Manuscript. Migheli Quirico: Conceptualization, Review and Editing of Manuscript. Olalekan Hakeem Shittu: Conceptualization, Review and Editing of Manuscript.

Acknowledgements

I hereby confirm that I have received permission from those I acknowledged in this manuscript. Special thanks to the technical assistant received from Professor Virgilio Balmas, Dr. Giampiera Porqueddu and Ms. Stefania Oggiano at the Department of Agricultural Sciences and Desertification Research Centre (NRD), University of Sassari, Sassari, Italy. Also, special thanks to the growers of coconut plantations in NIFOR and Isihor Coconut Garden for their kind assistance during the field survey and sample collection.

References

Adeniyi DO, Olufolaji DB, Joseph A: **Characteristic variations in Lasiodiplodia theobromae; pathogen of inflorescence dieback of cashew in growing ecologies of Nigeria.** *Annu Res Rev Biol.* 2016; **10**(2): 1–6.
Publisher Full Text

Alves A, Crous PW, Correia A, *et al.*: **Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*.** *Fungal Divers.* 2008; **28**: 1–13.
Reference Source

Ashokkumar P, Ushamalini C, Ramjegathesh R: **Variations in morphological and molecular characterization of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl associated with coconut leaf blight.** *Madras Agric J.* 2018; **105**(1–3): 66–71.
Publisher Full Text

Asman A, Toru I, Ade R: **Effect of drought stress on dieback disease development under *Lasiodiplodia theobromae* infection in cocoa clone 'MCC 02'.** *Beverage Plant Res.* 2024; **4**(1): e034.
Publisher Full Text

Bharanideepan A: **Studies on die-back of acid lime (Lasiodiplodia theobromae) in acid lime.** M. Sc. (Ag) Thesis. Tamil Nadu Agricultural University; Coimbatore, 2013; 45.

Bourke RM, Harwood T: **Food and agriculture in Papua New Guinea**. Australian National University, 2009.
Publisher Full Text

Cardoso JE, Wilkinson MJ: **Development and characterisation of microsatellite markers for the fungus *Lasiodiplodia theobromae.*** *Summa Phytopathologica.* 2008; **34**(1): 55–57.
Publisher Full Text

Dheepa R, Goplakrishnan C, Kamalakannan A, *et al.*: **Coconut nut rot disease in India: prevalence, characterization of pathogen and standardization of inoculation techniques.** *Int J Curr Microbiol Appl Sci.* 2018; **7**(2): 2046–2057.
Publisher Full Text

Disegha GC, Akani NP: **Bioinformatic method for fungi identification.** *Journal of Biology and Genetic Research.* 2019; **5**(1): 1–14.
Reference Source

Ekhorutomwen OE, Chidi NI, Owoh EM, *et al.*: **Influence of varieties and growing seasons on the distribution of *Botryodiplodia theobromae* causing fruit rot disease of coconut.** *International Journal of Sustainable Agricultural Research.* 2024; **11**(4): 179–189.
Publisher Full Text

Ekhorutomwen EO, Udoh ME, Esiegbuya DO: **A survey report on the incidence of fruit rot and premature nut fall diseases of coconut in Badagry, Lagos State, Nigeria**. *NIFOR Annual Report*. 2019; **58**: 157–158.

Ekhorutomwen OE: **"Cultural-morphology and molecular analysis of *Botryodiplodia theobromae*, a pathogen of coconut fruit".** Mendeley Data, V1. 2025.
http://www.doi.org/10.17632/53ns2fcgps.1

El-Morsi MEA, Ibrahim IA: **Morphological, molecular and histopathological variation in different isolates of *Botryodiplodia theobromae*, the causative of leaf base rot disease of date palms in New Valley Governorate, Egypt.** *Wudpecker Journal of Agricultural Research.* 2012; **1**(6): 215–222.

Excoffier L, Laval G, Schneider S: **Arlequin (version 3.0): an integrated software package for population genetics data analysis.** *Evol Bioinform Online.* 2007; **1**: 47–50.
PubMed Abstract | **Free Full Text**

FAO: **FAOStat, Rome.** Food and Agriculture Organisation of the United Nations, 2014.
Reference Source

Gunamalai L, Duanis-Assaf D, Sharir T, *et al.*: **Comparative characterization of virulent and less-virulent *Lasiodiplodia theobromae* isolates.** *Mol Plant Microbe Interact.* 2023; **36**(8): 502–515.
PubMed Abstract | **Publisher Full Text**

Gurr GM, Johnson AC, Ash GJ, *et al.*: **Coconut lethal yellowing diseases: a phytoplasma threat to palms of global economic and social significance.** *Front Plant Sci.* 2016; **7**: 1521.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Hernández-Ceja A, Loeza-Lara PD, Espinosa-García FJ, *et al.*: ***In vitro* antifungal activity of plant extracts on pathogenic fungi of blueberry (*Vaccinium* sp.).** *Plants (Basel).* 2021; **10**(5): 852.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Jacobs K, Bergdahl DR, Wingfield MJ, *et al.*: **Leptographium wingfieldii introduced into North America and found associated with exotic Tomicus piniperda and native bark beetles.** *Mycol Res.* 2004; **108**(Pt 4): 411–418.
PubMed Abstract | **Publisher Full Text**

Kang HW, Park DS, Go SJ, *et al.*: **Fingerprinting of diverse genomes using PCR with Universal Rice Primers generated from repetitive sequence of Korean weedy rice.** *Mol Cells.* 2002; **13**(2): 281–287.
PubMed Abstract | **Publisher Full Text**

Kumar S, Stecher G, Li M, *et al.*: **MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms.** *Mol Biol Evol.* 2018; **35**(6): 1547–1549.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Machado AR, Pinho DB, Pereira OL: **Phylogeny, identification and pathogenicity of the *Botryosphaeriaceae* associated with collar and root rot of the biofuel plant *Jatropha curcas* in Brazil, with a description of new species of *Lasiodiplodia*.** *Fungal Divers.* 2014; **67**: 231–247.
Publisher Full Text

Marques MW, Lima NB, Morais MA, *et al.*: **Species of *Lasiodiplodia* associated with mango in Brazil.** *Fungal Divers.* 2013; **61**: 181–193.
Publisher Full Text

Munirah MS, Azmi AR, Yong SYC, *et al.*: **Characterization of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* causing fruit rot on pre-harvest mango in Malaysia.** *Plant Pathol Quar.* 2017; **7**(2): 202–213.
Publisher Full Text

Osagie IJ, Ojomo EE, Maduewesi AE: **Physiological studies on ten isolates of the oil palm pathogen, *Fusarium oxysporum* f. sp *elaeidis*.** *Nigerian Journal of Agriculture, Food and Environment.* 2013; **9**(1): 33–39.

Pandey KA, Sharma KH, Yadav S, *et al.*: **A new canker on *Camellia sinensis* (L.) Kuntze associated with *Diaporthe longicolla* and *Lasiodiplodia theobromae* in India.** *Physiol Mol Plant Pathol.* 2024; **134**: 102–114.
Publisher Full Text

Phipps PM, Porter DM: **Collar rot of peanut caused by *Lasiodiplodia theobromae*.** *Plant Dis.* 1998; **82**(11)**:** 1205–1209.
PubMed Abstract | **Publisher Full Text**

Rajapakse CNK, Wijesekara HTR, Norman AH: **A preliminary study on immature nut fall of coconut with reference to pest damage.** *Cocos.* 1995; **10**: 53–54.
Publisher Full Text

Ramachandran K, Uyub AM, Latiffah Z: **Molecular characterization and pathogenicity of *Erwinia* spp. associated with pineapple [*Ananas comosus* (L.) Merr] and papaya (*Carica papaya* L.).** *J Plant Prot Res.* 2015; **55**(4)**:** 396–404.
Reference Source

Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol.* 1987; **4**(4): 406–425.
PubMed Abstract | **Publisher Full Text**

Santos PHD, Carvalho BM, Aguiar KP, *et al.*: **Phylogeography and population structure analysis reveals diversity by mutations in Lasiodiplodia theobromae with distinct sources of selection.** *Genet Mol Res.* 2017; **16**(2): 1–14.
PubMed Abstract | **Publisher Full Text**

Séré Y, Onasanya A, Afolabi A, *et al.*: **Genetic diversity of the blast fungus, *Magnaporthe grisea* (Hebert) Barr, in Burkina Faso.** *Afr J Biotechnol.* 2007; **6**(22): 2568–2577.
Publisher Full Text

Shah MD, Verma KS, Singh K, *et al.*: **Morphological, pathological and molecular variability in *Botryodiplodia theobromae* (*Botryosphaeriaceae*) isolates associated with die-back and bark canker of pear trees in Punjab, India.** *Genet Mol Res.* 2010; **9**(2): 1217–1228.
PubMed Abstract

Slippers B, Wingfield MJ: ***Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact.** *Fungal Biol Rev.* 2007; **21**(2–3): 90–106.
Publisher Full Text

Tamura K, Nei M, Kumar S: **Prospects for inferring very large phylogenies by using the neighbor-joining method.** *Proc Natl Acad Sci U S A.* 2004; **101**(30): 11030–11035.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Taylor J, Hyde KD: **Micro fungi of tropical and temperate palms.** *Fungal Diversity Research Series.* 2003; **12**: 1–459.
Reference Source

Wang TY, Wang L, Zhang JH, *et al.*: **A simplified universal genomic DNA extraction protocol suitable for PCR.** *Genet Mol Res.* 2011; **10**(1): 519–525.
PubMed Abstract | **Publisher Full Text**

White TJ, Bruns T, Lee SB, *et al.*: **Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics.** In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). *PCR protocols: A guide to methods and applications.* Academic press, San Diego, 1990.
Reference Source

Xia JQ, Correll JC, Lee FN, *et al.*: **Regional population diversity of *Pyricularia grisea* in Arkansas and the influence of host selection.** *Plant Dis.* 2000; **84**(8): 877–884.
PubMed Abstract | **Publisher Full Text**

Xie HH, Wei JG, Huang RS, *et al.*: **Genetic diversity analyses of *Lasiodiplodia theobromae* on *Morus alba* and *Agave sisalana* based on RAPD and ISSR molecular markers.** *Mycology.* 2016; **7**(4): 155–164.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Xu CN, Zhang HJ, Chi FM, *et al.*: **Species-specific PCR-based assays for identification and detection of Botryosphaeriaceae species causing stem blight on blueberry in China.** *J Integr Agric.* 2016; **15**(3): 573–579.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 30 December 2025

https://doi.org/10.21956/openreseurope.23306.r64025

© **2025 Rithesh L.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✔ **Lellapalli Rithesh**
PLANT PATHOLOGY, JC DIWAKR REDDY AGRICULTURAL COLLEGE (ANGRAU) (Ringgold ID: 363676), TADIPATRI, ANDHRA PRADESH, India

Dear Editor, I am satisfied with the new version of the manuscript

Thank you

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Pathology, Mycology, Coconut, Host Pathogen Interactions

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 28 November 2025

https://doi.org/10.21956/openreseurope.23306.r64024

© **2025 Yankey E.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✔ **Egya N. Yankey**
Coconut Research Program, Council for Scientifc and Industrial Research (CSIR), Sekondi, Ghana

I am satisfied with the responses from the authors; I therefore recommend that the manuscript be accepted for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Plant Pathoolgist, Phytoplasma diseases of coconuts.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 01 October 2025

https://doi.org/10.21956/openreseurope.21300.r60137

© 2025 Rithesh L. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Lellapalli Rithesh
PLANT PATHOLOGY, JC DIWAKR REDDY AGRICULTURAL COLLEGE (ANGRAU) (Ringgold ID: 363676), TADIPATRI, ANDHRA PRADESH, India

This manuscript reports on the cultural, morphological, and molecular characterization of *Botryodiplodia theobromae* isolates obtained from diseased coconut fruits showing fruit rot and premature nut fall in two locations in Edo State, Nigeria. Eight isolates were collected from four coconut varieties. The authors examined colony growth, pigment production, conidiospore characteristics, and used one universal primer (ITS1F/4R) and three species-specific primers (Lt347-F/R, Bt2aF/bR, and EF1-728F/EF2-728R) for PCR amplification. ITS sequences were analyzed and compared to NCBI entries, grouping the isolates into five genetic clusters. The study concludes that the pathogen causing fruit rot and premature nut fall in these locations is *B. theobromae*, and that molecular and cultural characterization helps in early diagnosis and management.

Clarity and literature:
The paper is generally clear and cites many current and classical references on *B. theobromae*. However, the introduction repeats some points (host range, endophytic nature) and could be condensed. The plain language summary is very brief and could better explain why the study matters to farmers and plant health authorities. References are up to date but a few key regional or methodological papers could still be added, for example on the link between *Lasiodiplodia* genetic groups and virulence.

Study design and merit:
The design combining morphology with molecular analysis is appropriate and has academic merit. The use of multiple primers strengthens the conclusions. Including isolates from two locations and different coconut varieties adds value.

Methods and replicability:
The methods are described in detail, which is positive, but sometimes procedural minutiae (exact

volumes of disinfectants) obscure key information. It should be stated clearly which primers succeeded or failed and why only ITS data were used for phylogenetic analysis. Lane labels in the gel images should be clearer. Explaining how “site 1…site 7” in the genetic distance table was defined will help others replicate the analysis. With these clarifications, replication would be straightforward.

Statistical analysis:
No formal statistical tests were performed; the study is descriptive. This is acceptable for a diagnostic/characterization paper but could be strengthened by simple quantitative comparisons (e.g. conidiospore size or growth rate differences between groups).

Specific points to address to make the article scientifically sound

1. Condense and clarify the introduction to avoid repetition and explicitly state the research questions or hypotheses.
2. Clarify the molecular methods why only ITS data were used for tree building, and what “prior to diluting DNA” means.
3. Improve figure and table presentation combine gel images into one figure with lane labels; explain “site” numbers in the genetic distance table; add a scale bar and, if possible, bootstrap values to the dendrogram.
4. Strengthen the discussion by interpreting patterns (prevalence of groups, pigment production, genetic distance) rather than only restating results. Explain how findings might influence management strategies.
5. Polish language and spelling shorten long sentences, ensure consistent terminology (septate vs. sepatate, conidiospores vs. conidiospore).
6. Expand the plain language summary to highlight why early diagnosis matters and how farmers could benefit.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Pathology, Mycology, Coconut, Host Pathogen Interactions

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Oct 2025

OSAYOMORE EKHORUTOMWEN

I have provided the necessary information in the updated manuscript to the best of my ability.

Competing Interests: No competing interests were disclosed.

Reviewer Report 24 June 2025

https://doi.org/10.21956/openreseurope.21300.r54733

© 2025 Yankey E. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Egya N. Yankey
Coconut Research Program, Council for Scientifc and Industrial Research (CSIR), Sekondi, Ghana

The manuscript is well-written and examines an important topic that is relevant to coconut production. **While I recommend it for indexing, there are a few drawbacks to the study that must be addressed.**
1.The authors mention the fungus *Botryodiplodia theobromae* as the cause of fruit rot and nut drop. Fruit rot and nut drop are associated with several biotic and abiotic factors, including lethal yellowing diseases, Phytophthora bud rot, high temperatures, and dry spells, among others. The authors should therefore correct the statement throughout the manuscript to read that the fungus is 'associated' with the condition rather than being the cause.

2. No controls were included in the study. Samples from uninfected coconut fruits from the sampling sites should have been included in both the cultures and in the PCR analysis. The authors should clarify why this was not done.

3. In the gel pictures, there is a blank well after the eighth lane: is this an empty well or a negative control? If it is a control, kindly indicate it and state what the control test was (water?).

4. In the result section, under the molecular identification, the authors mention 'prior to diluting the DNA'. Please, what is the relevance of this statement?
5. The statement 'DNA profile' under Data analysis should read 'DNA sequences'
6. A phylogenetic tree including sequences of the fungus from other plant species will be

informative. An outgroup for rooting the tree should also be included.
7. A pictorial of the symptoms, fruit rot, and nut fall will be informative and should be included in the article.
8. The authors should add a couple of disease management strategies for the pathogen in the conclusion section.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Plant Pathoolgist, Phytoplasma diseases of coconuts.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Oct 2025

OSAYOMORE EKHORUTOMWEN

Response to comment 2: We isolated the test pathogen from diseased coconut fruit, but we didn't isolate fungi from healthy coconut fruits. Our study was not focused on the microbial community of coconut fruit.

Response to comment 3: There gel pictures only used markers as the control.

Response to comment 4. the statement has been corrected.

Response to comment 6: Requested information has been provided in the updated manuscript.

Response to comment 7. A pictorial of the symptoms, fruit rot and nut fall has been provided.

Response to comment 8. This information has been provided in the updated manuscript. Thank you so much for your review.

Competing Interests: No competing interests were disclosed.