Australasian Plant Pathology (2023) 52:591–593
https://doi.org/10.1007/s13313-023-00947-3

First report of *Phytophthora tropicalis* causing black pod of Cacao *(Theobroma cacao)* in India

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Received: 27 April 2023 / Accepted: 9 September 2023 / Published online: 12 October 2023

Abstract
Cacao *(Theobroma cacao)* is a tropical plant that is grown throughout India. Cacao pods exhibiting chocolate brown patched symptoms were collected. The organism was isolated and cultured on V8 Agar from the diseased samples. The pathogenicity was proved on Cacao pods by inoculating mycelium of the organism and Koch's postulates were proved by re-isolating the pathogen. The pathogen was identified as *Phytophthora tropicalis* based on cultural and morphological characters, followed by molecular investigations (ITS and COX1). This is the first report of *Phytophthora tropicalis* causing black pod of Cacao in India.

Keywords Black pod · Cacao · *Phytophthora tropicalis*

Phytophthora tropicalis

Cacao is a major plantation crop produced for chocolate production around the world. Cacao, a shade-loving crop, has been discovered to be an ideal and extremely profitable mixed crop in existing coconut and arecanut gardens. Cacao is grown on an area of 1,03,376 ha in the Indian states of Kerala, Karnataka, Andhra Pradesh, and Tamil Nadu, with a total production of 27,072 MT. Andhra Pradesh is the largest state in terms of area, with 39,714 ha and a total production of 10,903 MT (https://www.dccd.gov.in/Content.aspx?mid=20&tid=1). According to Chavez-Ramirez et al. (2021), at least six *Phytophthora* species, including *P. megakarya*, *P. palmivora*, *P. capsici*, *P. citrophora*, *P. arecae*, and *P. tropicalis*, were known to cause cacao pod rot worldwide. Till date there are only three species of *Phytophthora* were associated with pod rot of cacao in India including *P. palmivora*, *P. capsici and P. citrophora*. *Phytopthtora palmivora* is the most important pathogen infecting cacao in India and infects all areas of the plant (Chowdappa et al. 1997, 2002). The infected Cacao pods were collected from Cacao orchard in Central Horticultural Experiment Farm in Chetalli, which is located in the Western Ghats region of India. Morphological characteristics and multigene phylogenetic analyses were employed to identify species identification, and pathogenicity tests were performed.+

Symptoms observed were the emergence of a chocolate brown patch, which spreads quickly and soon occupies the entire surface of the pod. Under extreme conditions, a whitish growth composed of sporangia forms on the damaged pod surface. When there is a lot of rain and humidity, not enough sunlight, and temperatures below 21 degrees Celsius, this disease develops quickly on the pods. The minimum temperature for growth is 6 °C, optimum 27– 30 °C, and maximum 33 °C (Abad et al. 2019). Finally, the affected pods darken to a blackish colour before drying and becoming mummified (Fig. 1).

Hyphae were coenocytic, sporangiophores were umbellate and Sporangia papillate, caducous with long, often curved pedicels. Sporangia were ellipsoidal, ovoid, pyriform shapes (18–63 µm length × 13–36 µm width) (n = 25), with tapered base originated on umbellate sporangiophores. Hyphal swellings were absent. Chlamydospores were intercalary or terminal, globose or subglobose (20–33 µm diam.) (Fig. 1).

Phytophthora species have often been identified and described using morphological characteristics. But even for experts, it might be challenging to precisely identify a species because of intraspecific morphological variation. Because of this, molecular methods that study genetic sequences have been developed to help species

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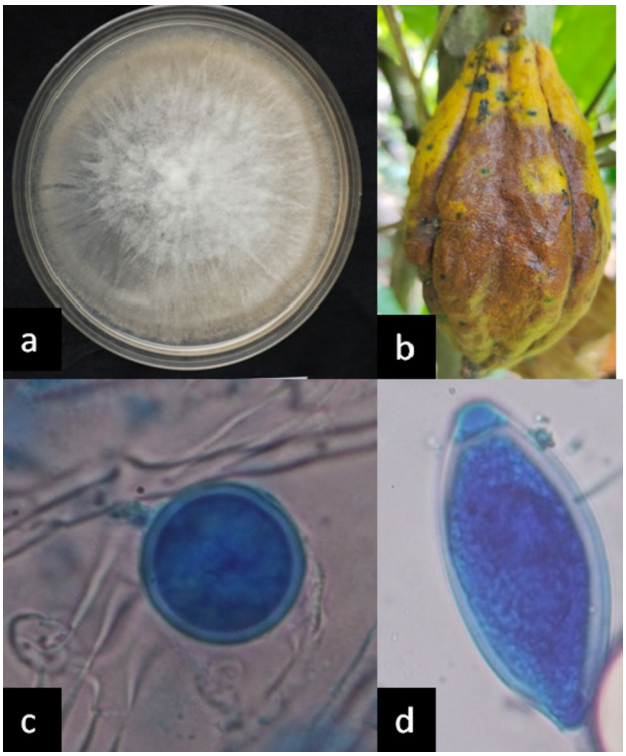

Fig. 1 *Phytophthora tropicalis* causing cacao black pod **a.** Ten days old culture on V8 media **b.** Infected cacao pod **c.** Chlamydospores **d.** Sporangia

identification and delimitation (Alvarez et al. 2016). For molecular identification, the genomic DNA was isolated from a ten-day-old culture using the CTAB method (Doyle and Doyle 1990). The mitochondrial cytochrome oxidase 1 (COX1) and ribosomal DNA internal transcribed spacer (ITS) sections were PCR-amplified using the primer sets ITS1/ITS4 (Chavez-Ramirez et al. 2021) and UCOX1/RCOX1 (Madhu et al. 2023; Martin and Tooley 2004). The purified PCR products were sequenced at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka. After the molecular study, the ITS and COX1 sequences of the isolated fungus were deposited in GenBank (Accession No: ITS: OQ874012; COX1: OQ871566). BLASTn search analysis in NCBI GenBank was used to compare the acquired sequences to those of reference strains (https://blast.ncbi.nlm.nih.gov/ Blast). Both the genes were nearly identical to the NCBI reference strains of *Phytophthora tropicalis* GU111622 and MW410939, respectively. Using the ClustalW programme, the nucleotide sequences were subjected to multiple sequence alignment. Using MEGA11 software and the maximum likelihood approach with 1000 bootstrap values, a phylogenetic tree was produced. The phylogenetic analysis indicated that the isolate (I44) clustered with the *P. tropicalis* reference strains of GenBank (Fig. 2).

The isolate was then tested for pathogenicity on the Cacao pod. It was cultured on V8 juice agar; mycelia discs were inoculated on Cacao fruit and incubated at 25 °C and 95% humidity for ten days. The organism was re-isolated from the inoculated pods exhibited similar symptoms and morphology to the earlier described one. No symptoms were observed in control. Based on the morphological characteristics and phylogeny, the pathogen was identified as *P. tropicalis.* A literature survey indicates that there was no record of *P. tropicalis* causing cacao black pod disease.

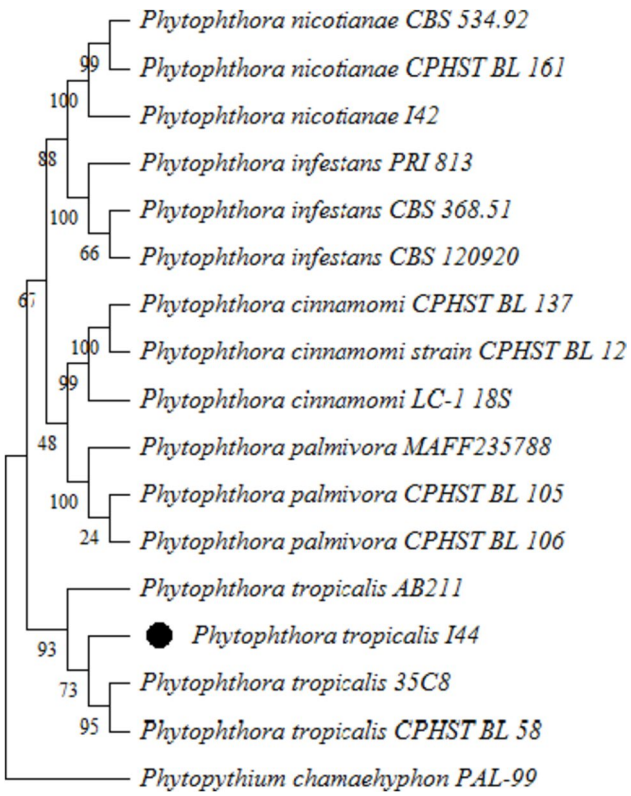

Fig. 2 Phylogenetic tree constructed based on ITS and COX 1 sequence data of isolates of *Phytophthora spp.* Sequence by MEGA with maximum likelihood method. The representative isolate was dot marked. The numbers above the nodes are the bootstrap values obtained from 1000 replicates. The tree is rooted to *Phytopythium chamaehyphon*

Fig. 3 Challenge inoculated Cacao pods showing black pod symptoms

Hence, the present study provides evidence of the first report of *P. tropicalis* as a causal organism of cacao black pod disease in India (Fig. 3).

Funding Indian Institute of Horticultural Research

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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