

Occurrence of *Phytophthora citrophthora* on cocoa in India*

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The natural incidence of black pod disease of cocoa caused by *Phytophthora citrophthora* is reported for the first time from India. The identity of the fungus was established on the basis of cultural and morphological characters. Isolates produced papillate, non-caducous, and highly variably shaped sporangia. None of the isolates produced chlamydospores or oospores. The cardinal temperatures were 12, 24–27, and 33°C, respectively. The isolates consistently produced symptoms similar to those observed in natural infections on both wounded and unwounded Forastero cocoa pods, and the fungus was re-isolated from the inoculated pods.

Keywords: Cocoa; Black pod disease; *Phytophthora citrophthora*; India

Black pod disease is a major disease of cocoa (*Theobroma cacao* L.) in India. Although several species of *Phytophthora* have been found to be associated with black pod disease, *P. palmivora* (Butl.) Butl., *P. capsici* Leon., *P. citrophthora* (Sm & Sm) Leon., and *P. megakarya* Brasier and Griffin have been recognized as major causative agents of black pod disease in various parts of the world (Zentmyer, 1988). Representative samples of black pod diseased cocoa tissue were collected from 133 localities of Kerala and Karnataka states, the principal cocoa-growing regions of India, during the south-west monsoon period (June–September) of 1989 and 1990. Of the 133 samples collected, 130 yielded *Phytophthora* isolates. Of these, 120 were identified as *P. palmivora* (Chowdappa and Chandra Mohanan, 1992) and five isolates as *P. capsici* (Chowdappa *et al.*, 1993). The identity of the remaining five isolates (I-CP/26), I-CP/75, I-CP/92, I-CP/93, I-CP/96) collected from Udumbanchola of Idukki, Thiruvalla of Pathanamthitta, and Thrissur and Thrissur districts of Kerala, is presented in this report.

Materials and Methods

Colony morphology was studied and radial growth rates (mm day⁻¹) determined for three replicates of isolates on carrot agar (CA) medium after 3 days of incubation in the dark at 24 ± 1°C. Sporangia were produced on CA

medium using the solid-agar medium-plate method described by Al-Hedaithy and Tsao (1979). Observations included length and breadth measurements of sporangia, presence of papilla, caducity, and sporangial shapes. Sporangial ontogeny was studied using the mycelium-agar disc-in-water method described by Al-Hedaithy and Tsao (1979). Observations on chlamydospore production were made both on CA medium grown at 24 ± 1°C in the dark and on mycelial mats submerged in sterile distilled water for 21 days at 16°C in the dark (Tsao, 1971). The mating types of the isolates were determined by directly pairing them with *P. palmivora* A₁ (P1760) and A₂ (P3760), *P. capsici* A₁ (P864) and A₂ (P575), and *P. meadii* A₁ (P643) and A₂ (P3501) on CA medium at 20°C in the dark for 14 days. The influence of temperature on vegetative growth on CA medium was determined in the dark at temperatures ranging from 6 to 36°C at intervals of 3°C. Pathogenicity was confirmed on Forastero cocoa pods following wound and surface inoculation (Brasier and Griffin, 1979).

Results and Discussion

All five *Phytophthora* isolates exhibited a similar pattern of colony growth without any characteristic pattern (Figure 1). The mean growth rate of the five isolates was 17.91 mm day⁻¹ with a range of 17.44–18.33 mm day⁻¹. Isolates produced papillate, non-caducose, highly variably shaped sporangia (Figure 2), either borne singly or in an irregular pattern (loose sympodial pattern) (Figure 3).

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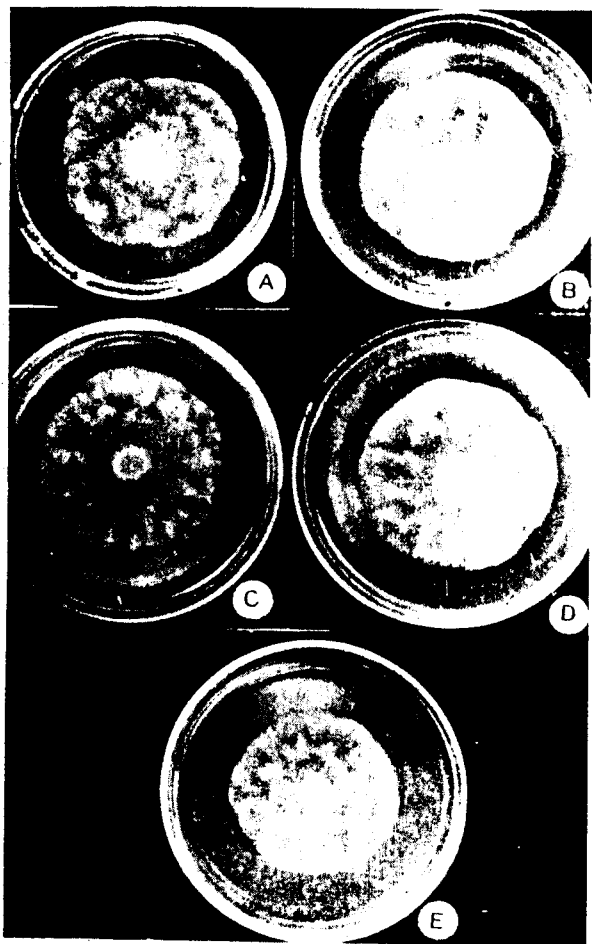


Figure 1 Colony morphology of isolates of *P. citrophthora*: A. I-CP/26; B. I-C/92; C. I-CP/93; D. I-CP/96; E. I-CP/75

In general, sporangial morphology varied from ovoid to obpyriform and ellipsoidal. These morphological characters closely resemble those reported for *P. citrophthora* (Frezzi, 1950; Vanderweyen, 1974; Kellam and Zentmyer, 1986; Mchau and Coffey, 1994). Sporangia with two papillae which was considered a characteristic feature of *P. citrophthora* (Newhook *et al.*, 1978) were seen in four per cent of the samples. The length of sporangia ranged from 54.00 to 58.75 μm and breadth from 30.00 to 32.71 μm , giving a length:breadth ratio ranging from 1.70 to 1.86 (Table 1). These sporangial dimensions fall within the range characteristic of *P. citrophthora* (Kellam and Zentmyer, 1986; Mchau and Coffey, 1994). Occasionally, characteristic swellings at the branching points of sporangiophores were noted. The isolates failed to produce chlamydo spores both on CA medium and in submerged mycelial mats. None of the isolates produced sexual structures when paired with A₁ or A₂ mating types of *P. palmivora*, *P. capsici*, or *P. meadii*. Mchau and Coffey (1994) also showed that most of the isolates assigned to *P. citrophthora* did not produce chlamydo spores or oospores. Based on the cultural and morphological characters, the isolates were identified as *P. citrophthora* (Sm &

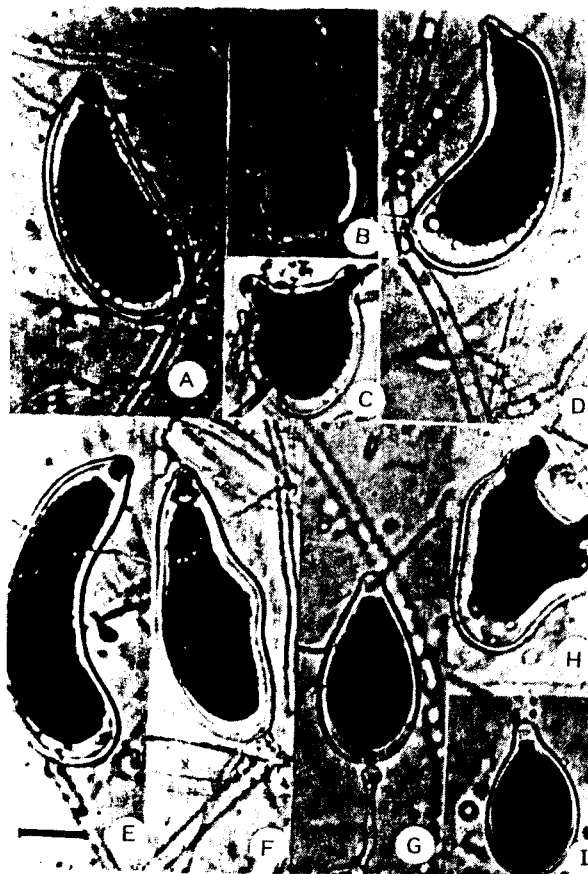


Figure 2 Sporangia of *P. citrophthora*: A. I-CP/26; B. I-CP/75; D-E. I-CP/92; F-G. I-CP/93; H-I. I-CP/96. Scale bar = 20 μm



Figure 3 Irregular arrangement of sporangia of *P. citrophthora*: A-C. I-CP/26; D-E. I-CP/75; F-G. I-CP/92; H-I. I-CP/93; J-L. I-CP/96

Sm) Leon. (New hook *et al.*, 1978; Stamps *et al.*, 1990; Mchau and Coffey, 1994).

The minimum, optimum, and maximum temperatures for the growth of the five isolates were 12, 24-27, and 33°C, respectively. The

Table 1 Sporangial measurements (μm) of *P. citrophthora* isolates on carrot agar

Isolate no.	Length*	Range	Breadth*	Range	Length:breadth ratio	Range
I-CP/26	58.50 \pm 13.00	22-95	31.83-5.43	17-37	1.82 \pm 0.49	1.00-3.55
I-CP/75	54.00 \pm 12.60	22-72	30.00 \pm 3.50	17-40	1.80 \pm 0.42	1.10-2.80
I-CP/92	57.12 \pm 18.90	20-85	32.63 \pm 4.37	20-37	1.70 \pm 0.80	1.10-2.40
I-CP/93	57.55 \pm 12.00	18-62	30.80 \pm 5.80	15-37	1.86 \pm 0.50	1.21-1.90
I-CP/96	58.75 \pm 14.00	20-80	32.71 \pm 6.20	20-42	1.70 \pm 0.56	1.30-1.80
Mean	57.18 \pm 2.89	—	31.59 \pm 1.10	—	1.77 \pm 0.004	—

*Mean of 120 sporangia \pm standard deviation

maximum growth temperature is similar to that reported by Mchau and Coffey (1994) who found that the maximum growth temperature is one of the important criteria in the characterization of *P. citrophthora*. The isolates consistently produced typical black pod symptoms on Forastero cocoa pods when inoculated with and without wounds. Visible symptoms of black pod disease appeared within 2-3 days of inoculation and covered the entire pod within 9-10 days. Symptoms produced on inoculated pods were similar to those observed in natural infections and the fungus was re-isolated from the inoculated pods. *Phytophthora citrophthora* has been reported as the causal organisms of black pod disease of cocoa only in Brazil (Kellam and Zentmyer, 1986) and Indonesia (Mchau and Coffey, 1994). This is the first report of *P. citrophthora* causing black pod disease of cocoa in India.

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