Journal of Plantation Crops 21 (Supplement) : 129-133 (1993)

MORPHOLOGICAL VARIABILITY AMONG ISOLATES OF *PHYTOPHTHORA PALMIVORA* CAUSING BLACK POD DISEASE OF COCOA IN INDIA

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ABSTRACT

Morphological studies were conducted on 120 isolates of *Phytophthora palmivora* isolated from various localities in Kerala and Karnataka states. The colonies of the isolates of *P. palmivora* are either stellate or smooth combed with sharply defined edge. Aerial mycelium was spares, being more or less absent from the peripheral areas of the colony. The size and shape of sporangia varied greatly among different isolates and even within a single isolate of *P. palmivora* .

INTRODUCTION

Several species of *Phytophthora* are known to cause black pod disease of cocoa (*Theobroma cacao L.*) Kaosiri *et al.* 1978; Brasier and Griffin 1979; Zentmyer *et al.*, 1981 Kellam and Zentmyer, 1986; Liyanage and Wheeler, 1989). The group of *Phytophthora palmivora* isolates which produce *caducous* sporangia with characteristic ovoid shape and a short pedicel occurring on tropical tree crops (Chee, 1974) has been generally referred to as *P. palmivora* MF1 (morphological form 1) (Griffin 1979; Brasier and Griffin, 1979). *P. palmivora* is the main cause of black pod disease in most of the cocoa producing countries except Brazil, Nigeria and Cameroon (Zentmyer, 1983). Differences in sporangial characters among the isolates of *P. palmivora* have been reported from various parts of the world (Waterhouse, 1974; Brasier and Griffin, 1979). This paper deals with the morphological variability among the isolates of *P. palmivora* collected from major cocoa growing areas of the country.

MATERIALS AND METHODS

In the course of the studies on *Phytophthora* species associated with cocoa in India during 1989 to 1991, 120 isolates of *P. palmivora* Butl. causing black pod disease of cocoa have been collected from Kerala (94 isolates) and Karnataka (26 isolates)

Colony characteristics of the isolates were studied by inoculating 90 mm plates containing 15 ml of carrot agar medium (CA) with 5 mm diameter discs cut from the advancing margin of 3 day old cultures. The cultures were incubated in the dark at 24±1°C for 5 days.

The characteristics of sporangia were studied on CA as well as on cocoa pods. The 3-day-old dark grown CA cultures at room temperature 25±1°C under continuous light (two crompton cool-day-light, 40 W fluorescent lamps, 120 cm long at an intensity of 30 µm mol $m^{-2}s^{-1}$, suspended 30 cm above the cultures). Three cocoa accessions such as NC 29, NC 39 and NC 43 were tested with one of the isolates (I-CP/109) to study variation in sporangia as affected by cocoa accessions. After four days of incubation, the morphology of sporangia mounted in lactophenol cotton blue was studied. Fifty sporangia were measured for each isolate. A submerged culture method described by Tsao (1971) was used to produce chlamydospores of isolates of *P. palmivora*. 25 ml of carrot broth (prepared by steaming 200 g sliced carrot in 1000 ml of distilled water) taken in a 250 ml conical flask was inoculated with four mycelial discs each of 5 mm diameter from a 3-day-old culture. After incubation for 7 days at 24°C, the carrot broth was replaced aseptically by 10ml of sterile distilled water and submerged culture was further incubated for 30 days at 16°C in the dark. Fifty chlamydospores were measured for each isolate.

RESULTS AND DISCUSSION

On the basis of growth rates, the 120 isolates of *P. palmivora* on CA were classified into 8 groups. From 120 isolates the data on representative isolate from each group are presented in Table I. All isolates grew well on CA and the colony morphology of isolates was similar to that reported by Brasier and Griffin (1979) for *P palmivora* MF1. The colonies were either smooth-combed or stellate with sharply defined edge. Aerial mycelium was sparse, being more or less absent from peripheral areas of the colony. The growth rate of the isolates ranged from 8.7 to 15.41 mm/day.

The typical sporangium of *P. palmivora* produced on CA and on cocoa pods is shown in Fig. 1. The sporangia of the isolates were either ellipsoidal, near spherical to ovate or elongated with round bases (Fig. 2). Similar variation in size and shape of sporangia has been reported by earlier workers in *P. palmivora* (Waterhouse, 1974, Brasier and Griffin, 1979; Fagan, 1988). On CA, the sporangial dimensions of the isolates ranged from 39-45 x 25-29 µm (Table II). On cocoa pods, the range of sporangial dimensions of these isolates varied from 31-48 x 22-27 µm (Table III).

These sporangial dimensions varied slightly from those reported earlier for *P. palmivora* by Waterhouse (1974) (35-60 x 20-40µm), Brasier and Griffin (1979) (41-45 x 23-26 µm) and Fagan (1988) (41-59 x 23-31 µm). The average L/B ratios of the isolates ranged from 1.37 to 1.60 on CA (Table II) while on cocoa pods, L/B ratios ranged from 1.34 to 1.84 (Table III). A wide range of L/B ratios has been reported by various workers for isolates of *P. palmivora.* The L/B ratio reported by Fagan (1988) was 1.69, 1.3 to 1.4 by Zentmyer (1988), 1.4 to 1.6 by Waterhouse (1974) and 1.2 to 1.8 by Brasier and Griffin (1979).

In the present study the pedicel length of the isolates of *P. palmivora* ranged from 2.7 to 3.9 µm on CA (Table II) whereas, it varied from 2.46 to 4.3 µm on cocoa pods (Table III). Our data on sporangial pedicel lengths are in accordance

Table I. Origin of isolates of *P. palmivora* used in the present study

Isolate No.	Geographic origin	Cropping system
I-CP/14	Thodupuzha, Idukki (Dist.)	Coconut and Cocoa
I-CP/17	Thodupuzha, Idukki (Dist.)	Coconut and Cocoa
I-CP/18	Thodupuzha, Idukki (Dist.)	Coconut and Cocoa
I-CP/40	Thottackad, Kottayam (Dist.)	Coconut and Cocoa
I-CP/63	Thamarassery, Kozhikode (Dist.)	Arecanut and Cocoa
I-CP/98	Thrissoor, Thrissoor (Dist.)	Arecanut and Cocoa
I-CP/129	Subramanya, Dakshina Kannada (Dist.)	Arecanut and Cocoa
I-CP/109	Wythiri, Wyanad (Dist.)	Cocoa under Forest

Table II. Growth rate and sporangial characteristics of isolates * of P. palmivora produced in carrot agar plates

Isolate No.	Growth rate (mm/day)	Length (L) (µm)	Breadth (B) (µm)	L/B ratio	Pedicel length (µm)
I-CP/14	14.65±0.26	40.68±1.17	25.22±0.71	1.60±0.04	3.90±0.38
I-CP/17	8.7±0.08	40.93±1.91	27.81±0.69	1.41±0.04	3.43±0.36
I-CP/18	10.30±0.10	41+2.30	27.50±1.41	1.48±0.51	3.49±0.48
I-CP/40	12.12±0.12	41.52±1.05	27.18±0.73	1.59±0.03	3.50±0.41
I-CP/63	11.20±0.35	39±1.71	27.50±1.00	1.38±0.08	2.96±0.24
I-Cp/98	13.11±0.14	45.22±1.50	29.88±0.69	1.49±0.04	2.70±0.24
I-CP/129	15.41±1.23	40±1.25	28.57±1.00	1.37±0.04	3.21±0.43
I-CP/109	9.45±0.07	43.12±1.21	27.08±0.77	1.55±0.05	2.90±0.22

* Average size (µm) of 50 sporangia ± standard deviations.

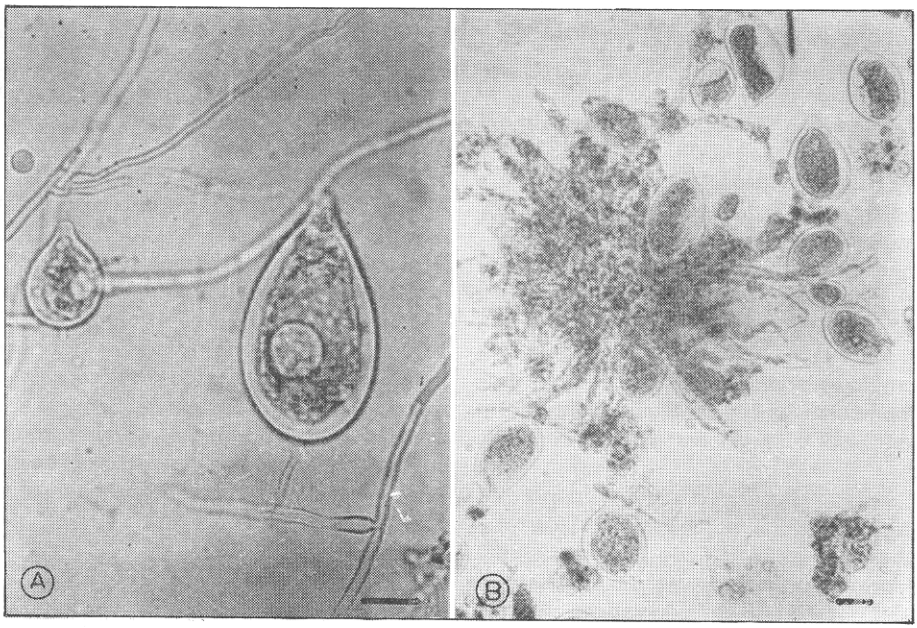

Fig.1 Photo micrograph showing sporangia of of *P. palmivora*
A. Sporangia produced on carrot agar, B. Sporangia on cocoa pods
Both figures share the same scale bar, which represents 10 μm

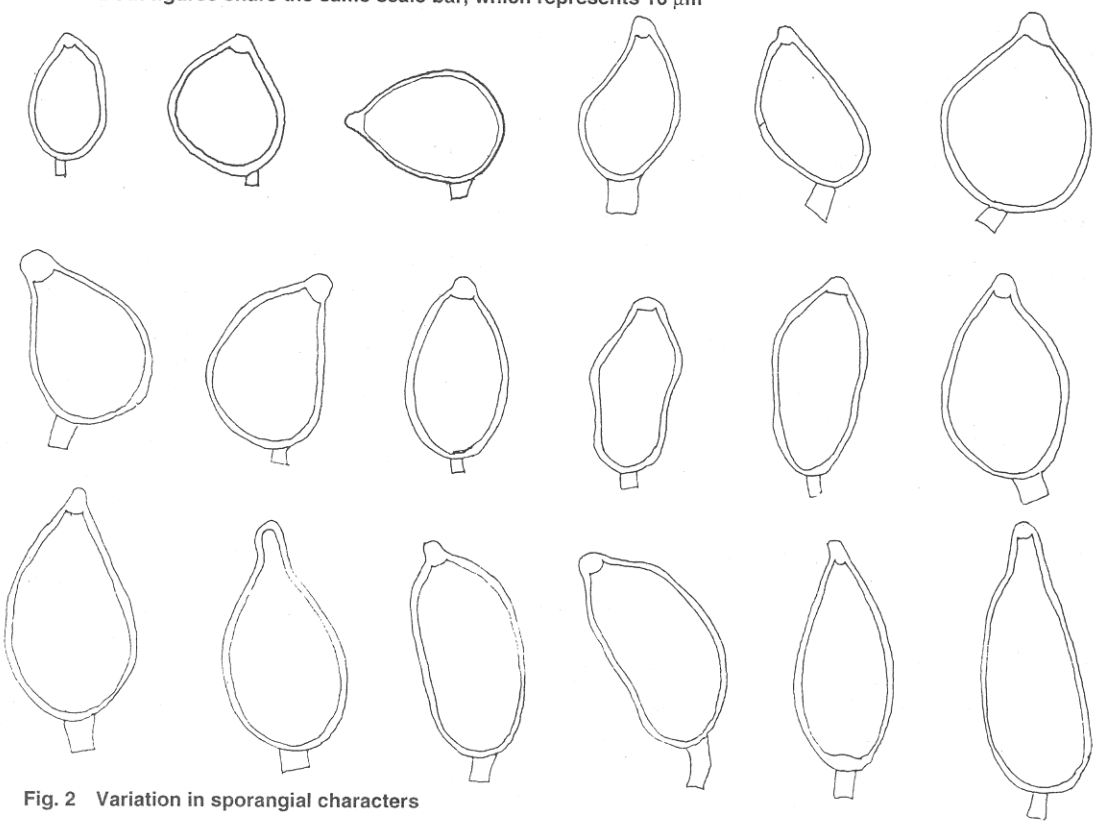

Fig. 2 Variation in sporangial characters

with findings of Brasier and Griffin (1979) for isolates of *P. palmivora* (2-5 µm).

The sporangial size also varied with the cocoa accessions such as NC 29, NC 39 and NC 43 when inoculated with isolate I-CP/109. The sizes varied from 40-55 to 24-28 µm (Table IV). The L/B ratios varied from 1.65 to 2.27. The pedicel length ranged from 3.54 to 4.2 µm. This indicates that the same isolate may show morphological variation on different cocoa accessions. Similarly, occurrence of unusually large sporangia on some infected cocoa pods as rare phenomenon was reported also in the Jamaican isolates of *P. palmivora* (Fagan, 1988).

All isolates produced chlamydospores on submerged mycelial units grown in carrot broth in the dark at 16°C. No differences in chlamydospore morphology between isolates were noted. The chlamydospores were produced singly in terminal or intercalary position on vegetative hyphae. They were globose, thin or thick walled. The mean diameter of chlamydospores in the isolates of *P. palmivora* varied from 34.80 to 35.52 µm (range 20-45 µm). Similarly, Brasier and griffin (1979) reported that differences in chlamydospore morphology among isolates were not evident from distinguishing the forms within morphological forms eg. the mean diameter of chlamydospores in the three isolates of *P. palmivora* is 31.6 µm (range 23.8 - 40.8 µm).

From the foregoing account, it could be inferred that the differences between isolates of *P. palmivora* from different parts of the world probably is due to differences in media and environmental conditions (Brasier and Griffin 1979). From these observations it appears that, while sporangial character is probably characteristic of a given isolate, such wide variation exists within the morphological form that this character may not have any diagnostic value for separating forms within morphological form of *P. palmivora*.

ACKNOWLEDGEMENT

We thank Dr. K.K.N. Nambiar, Head, Division of Crop Protection for critical comments offered on the manuscript. We are also thankful to Mr. Moosa, Technical Assistant, Plant pathology, CPCRI, Kasaragod, for excellent assistance in photo micrography.

Table III. Sporangial characters of isolates* of *P. palmivora* produced on coca pods (Landas 365)

Isolate No.	Legnth (L) (µm)	Breadth (B) (µm)	L/B ratio	Pedicel length(µm)
I-CP/14	40.18±3.1	27.84±1.61	1.44±0.24	2.66±0.64
I-CP/17	48.25±5.41	26.12±1.20	1.84±0.58	4.31±0.97
I-CP/18	31.37±1.16	23.56±0.98	1.34±0.15	3.26±0.63
I-CP/40	38.56±2.5	25.43±1.12	1.51±0.21	3.57±0.59
I-CP/63	37.5±1.97	25.62±0.95	1.46±0.13	2.46±0.41
I-Cp/98	36.37±2.41	22.31±0.84	1.63±0.21	3.62±1.14
I-CP/129	35.25±1.61	22.96±0.99	1.53±0.15	3.90±0.76
I-CP/109	39.87±1.80	23.75±0.73	1.67±0.16	3.33±0.42

* average size (µm) of 50 sporangia ± standard deviations

Table IV. Sporangial characters* of isolate I-CP/109 produced on different cocoa accesssions

Cocoa accession	Legnth (L) (µm)	Breadth (B) (µm)	L/B ratio	Pedicel length(µm)
NC 29 (P6 x P4)	51.84±4.29	28.87±0.99	1.79±0.33	3.70±0.55
NC 39 (T7/12)	55.61±2.53	24.44±0.87	2.27±0.32	3.54±0.81
NC 43 (W6/56(T63/910)	40.06±1.93	24.56±1.11	1.65±0.21	4.2±1.23

*average size (µm) of 50 sporangia ± standard deviations

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DISCUSSION

M. ANANDARAJ : *Phytophthora palmivora* complex from cocoa has been found to belong to several different species based on morphological characters of asexual and seasonal spore structures. Why have you confined only to sporangial characters?

P. CHOWDAPPA : In our studies, several species of *Phytophthora* causing black pod disease of cocoa were delineated based on morphological characters, physiological parameters and biochemical evidencesuch protein electroohoresis and restriction digestion patterns of DNA. However, the present paper deals with morphological variations among isolates of *P. Palmivora* only.

C. THOMAS JOHN : Based on your studies is there any recommendation for control of black pod disease of cocoa in India?

P. CHOWDAPPA : The present study deals with morphological characters among isolates of *P. palmivora*, and not on field control