

## VARIATION IN PATHOGENICITY AMONG THE ISOLATES OF *COLLETOTRICHUM GLOESPORIODES* ON COCOA IN INDIA

by

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### SUMMARY

*Colletotrichum* disease of cocoa (*Theobroma cacao* L.) occurs in most of the cocoa growing regions in India. Symptomatology of the disease reveals that besides pod rot there are three kinds of foliar symptoms, viz., blight, shot hole and irregular spot.

The leaves, pods and seedlings showing various symptoms of the disease were collected from 101 cocoa gardens including nurseries in the states of Kerala, Karnataka and Tamil Nadu. Isolation of the pathogen from infected plant parts invariably yielded *Colletotrichum gloeosporioides* Penz. A total of 312 isolates of the pathogen were collected, of which 198 were from leaves, 82 from pods, and 32 from seedlings. Based on the colour of the colony when grown on potato dextrose agar medium the isolates under each symptom group were classified into light (gray), dark (olive gray to black) and white types. Disease index was calculated by inoculating 18-day-old cocoa seedlings. Of the 312 isolates, 299 were pathogenic, of which 210 incited blight, 77 incited shot hole and only 12 isolates incited irregular spot. The most virulent isolate among blight, shot hole and irregular spot group was found in Kanyakumari, Cannanore and Chikmagalur districts respectively. The study further showed that most of the isolates of *C. gloeosporioides* were pathogenic and among them leaf blight group of isolates were predominant and wide-spread. Inoculation trials also revealed a great variation in pathogenicity among the isolates under each symptom group. In general isolates of light type were found to be most virulent.

From the results it may be concluded that while screening for disease resistance it is desirable to select light type of isolates covering a wide range of collections.

### VARIATION DE PATHOGENICITE ENTRE LES ISOLATS DE *COLLETOTRICHUM GLOESPORIODES* SUR LE CACAOYER EN INDE

#### RESUME

L'antracnose à *Colletotrichum* (*Colletotrichum gloeosporioides*) du cacaoyer (*Theobroma cacao* L.) se produit dans la plupart des régions cacaocoles de l'Inde. La symptomatologie de la maladie révèle qu'outre la pourriture de la cabosse, se présentent trois genres de symptômes foliaires, à savoir: le flétrissement, les trous des feuilles et les taches irrégulières.

Les feuilles, cabosses et plantules révélant les symptômes de la maladie ont été collectées à partir de 101 cacaoyères, y compris des pépinières dans les Etats de Kerala, Karnataka et Tamil Nadu. Des isolats de pathogènes provenant de parties infectées de plants contenaient toujours du *Colletotrichum gloeosporioides* Penz. Un total de 312 isolats du pathogène fut rassemblé, dont 196 provenaient des feuilles, 80 des cabosses et 23 des plantules. Sur la base de la couleur de la colonie, lorsqu'elle était cultivée sur un milieu d'agar à dextrose, de pomme de terre, les isolats, dans chaque groupe de symptômes, étaient classés en clair (gris), foncé (gris olive à noir) et blanc. L'index des maladies a été calculé en inoculant des plantules de cacaoyers de 18 jours. Sur les 312 isolats, 299 étaient pathogènes, dont 210 provoquaient le flétrissement des feuilles, 77 provoquaient les trous des feuilles; 12 isolats seulement provoquaient des taches foliaires irrégulières. L'isolat le plus virulent du flétrissement foliaire, des perforations de feuilles et du groupe des taches irrégulières, a été trouvé dans les districts de Kanyakumary, Cannanore et Chikmagalur, respectivement. Les essais d'inoculation révélèrent aussi une forte variation de pathogénicité entre les isolats dans chaque groupe de symptômes. En général, les isolats du type clair étaient les plus virulents, suivis par les types foncés et blancs. Par ailleurs, au sein de chaque groupe, des variations significatives se manifestaient entre les isolats.

L'on pense que, tout en criblant la résistance à la maladie, il vaut mieux sélectionner les types clairs d'isolats, mettant en jeu une vaste gamme de collections.

### VARIAÇÃO DE PATOGENIA ENTRE ISOLADOS DE *COLLETOTRICHUM GLOESPORIODES* EM CACAU NA ÍNDIA

#### RESUMO

A doença *Colletotrichum* (*Colletotrichum gloeosporioides*) do cacau (*Theobroma cacao* L.) ocorre na maioria das regiões de cultivo de cacau na Índia. A sintomatologia da enfermidade revela que, além da podridão do fruto, há tres tipos de sintomas foliares: mildiu, orifícios (shot hole) e manchas irregulares.

Folhas, frutos e plântulas, apresentando os sintomas da moléstia, foram coletadas de 101 jardins cacaueiros, incluindo os viveiros dos estados de Kerala, Karnataka e Tamil Nadu. Isolados de patógeno das partes da planta afetada, invariavelmente produziram *Colletotrichum gloeosporioides* Penz. Num total de 312 isolados de patógenos coletados, 196 eram de folhas, 80 de frutos e 23 de mudas. Baseando-nos na cor da colônia mantida em agar de dextrose de batata, classificamos os isolados em tres tipos: claro (cinza), escuro (cinza-oliva a negro) e branco. O índice de doença foi calculado pela inoculação de mudas de cacau de 18 dias de idade. Dos 312 isolados, 299 eram patogênicos, dos quais 210 provocaram mildiu foliar, 77 provocaram "shot hole" e apenas 12 provocaram máculas irregulares nas folhas. Os isolados mais virulentos, nos tres grupos de sintomas, foram encontrados em Kanyakumary, Cannanore e Chikmagalur. Em geral, os isolados do tipo claro eram os mais virulentos, seguidos dos tipos escuro e branco. Dentro de cada grupo houve variação significativa entre os isolados.

Considera-se que, ao procurar classificar a resistência à doença, convém selecionar tipos claros de isolados incluindo uma ampla gama de coleções.

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RESUMEN

La enfermedad del cacao por *colletotrichum* (*Colletotrichum gloeosporioides*) se presenta en la mayoría de las regiones cacaoteras de la India. La sintomatología de la enfermedad revela que además de la podredumbre negra de la mazorca hay tres tipos de síntomas foliares como son el marchitado, el agujero del brote y la mancha irregular.

Las hojas, mazorcas y plántulas que muestran los síntomas de la enfermedad fueron recogidas de 101 fincas de cacao incluyendo los viveros, de los estados de Kerala, Karnataka y Tamil Nadu. Los aislamientos del patógeno de las partes infectadas de las plantas dieron invariablemente *Colletotrichum gloeosporioides* Penz. Se recogieron un total de 312 aislados del patógeno, de los cuales 196 eran de hojas, 80 mazorcas y 23 de plántulas. Basados en el color de la colonia al mantenerla en un medio de agar dextrosa de patata, los aislados de cada grupo de síntomas se clasificaron en: ligero (gris), oscuro (gris oliva a negro) y blanco. El índice de la enfermedad se calculó inoculando plántulas de cacao de 18 días de edad. De los 312 aislados, 299 eran patogénicos, de los cuales 210 indujeron marchitamiento, 77 incitaron agujeros del brote y solo 12 aislados produjeron manchas irregulares de la hoja. El aislado más virulento de marchitamiento de la hoja, agujero del brote y mancha irregular como grupo se encontró en Kanyakumary, Cannanore y Chikmagalur respectivamente. Las pruebas de inoculación también revelaron una gran variedad en la patogenicidad entre los aislados de cada grupo por síntomas. En general los aislados del tipo ligero se encontró que eran los más virulentos seguidos por los tipos oscuro y blanco. Dentro de cada grupo hubo también una variación importante entre los aislados.

Se piensa que al hacer la selección para resistencia a la enfermedad, es mejor seleccionar tipos ligeros de aislados que afecten a una gran gama de colecciones.

INTRODUCTION

IN India, cocoa (*Theobroma cacao* L.) is cultivated along the west coast mainly in all the districts of Kerala, Dakshina Kannada district of Karnataka and Kanyakumari district of Tamil Nadu. From the available information the area under cocoa cultivation is estimated to be around 20,000 ha in these three states. It has proved to be a suitable and profitable mixed crop in arecanut and coconut gardens.

Among the many important fungal diseases that affect this crop, *Phytophthora palmivora* (Butl.) Butl., *Colletotrichum gloeosporioides* Penz., *Botryodiplodia theobromae* Pat. are considered as the major pathogens in this region (Ramakrishnan and Thankappan, 1965; Nambiar and Nair, 1972; Reddy and Chandra Mohanan, 1976; Sarma and Nambiar, 1976). Diseases of cocoa caused by *P. palmivora* and *C. gloeosporioides* are wide spread in India (Chandra Mohanan and Kaveriappa, 1982). Though *C. gloeosporioides* has been reported on cocoa from various countries, no attempt has been made earlier to compare the isolates from different localities (Briton-Jones, 1934). The detailed studies on the symptomatology of *Colletotrichum* diseases of cocoa in India have revealed that besides pod rot, there are three distinct types of foliar symptoms, viz., blight, shot hole and irregular spot (Chandra Mohanan and Kaveriappa, 1983). The present paper deals with the variation in pathogenicity of different isolates of *C. gloeosporioides* collected from major cocoa growing areas of India.

MATERIALS AND METHODS

*Sample collection*

As Kerala is the major cocoa growing state in this country, samples were collected from all the districts in this state. In Karnataka six districts viz., Dakshina Kannada, Kodagu, Tumkur, Bangalore, Shimoga and Chikmagalur were covered. In Tamil Nadu cocoa is mainly cultivated in Kanyakumari district and hence samples were collected only from this district. Infected leaves, pods, seedlings in nurseries and self-sown seedlings were collected at random from each sampling site. A total of 101 gardens including nurseries were randomly selected in the present survey.

*Isolation*

Samples showing disease symptoms were examined under a microscope and whenever *Colletotrichum* sp. was found associated with the lesions, isolations were made on potato dextrose agar (PDA) medium supplemented with 100 units/ml streptomycin. The plates were incubated at room temperature which ranged from 20–32°C in different localities. From the resulting cultures single spore isolations were made. These sub-cultures were compared with each other and grouped on the basis of colour, general appearance of the colony and aerial mycelium. One representative culture from among the cultures of similar types from each garden was selected and thus a total of 312 isolates were used for pathogenicity tests.

*Pathogenicity tests*

*Raising cocoa seedlings:* Ripe cocoa pods of 'Forestero' variety were collected from a single cultivar. The beans from the middle portion of the pod were scooped out. The testa of the beans were removed and then kept for germination on a tray lined with moist blotting paper. The beans were covered with moist blotting paper and incubated for 48 hours at room temperature (29 ± 1°C). These pregerminated beans were sown in polythene bags containing a soil mixture of sand, loam and farm yard manure (1 : 1 : 1) and watered daily. The seedlings of uniform size were selected for inoculation. Age of the seedlings was calculated from the date of sowing of pre-germinated beans.

*Preparation of inoculum:* Freshly isolated 5-day-old cultures maintained on oat meal agar (OMA) slants were used for preparation of inoculum to preclude the possible loss of pathogenicity. About 4 ml of tap water was added to each slant and shaken well for 5 min. on a mechanical shaker to dislodge the conidia. The suspension containing conidia and mycelial bits prepared from each culture was used as inoculum.

*Inoculation:* Eighteen-day-old seedlings were selected for inoculation. Both the surface of leaves were sprayed with the inoculum until dripping wet. After inoculation each seedling was covered with polythene bag containing two pieces of cotton pads moistened with 50 ml sterile distilled water (SDW) to maintain high humidity and transferred to moist chamber. Thirty seedlings were inoculated with each isolate. Seedlings sprayed with SDW served as control.

TABLE 2  
Relationship between colony colour and disease index

	Disease index		
	White	Dark	Light
<b>I. Leaf blight</b>			
No. of isolates studied	15	20	175
Range	0.19-6.38	1.66-18.27	1.66-50.56
Mean	2.49	7.12	6.66
<b>II. Shot hole</b>			
No. of isolates studied	10	5	62
Range	16.66-21.77	16.66-44.35	16.66-82.45
Mean	17.64	28.59	32.15
<b>III. Irregular spot</b>			
No. of isolates studied	—	—	12
Range	—	—	1.95-55.65
Mean	—	—	22.95

pathogenic (Table 2). In the shot hole group also the difference in disease index between light and white types was significant.

#### DISCUSSION

A number of *Colletotrichum* species have been reported to infect cocoa from different parts of the world. They are, *C. theobromae*, *C. luxificum* and *C. cradwickii* from West Indies; *C. incarnatum* from Camerouns and Sri Lanka (Briton-Jones, 1934), *C. fructi-theobromae* from Brazil and *C. theobromicolum* from Congo (Thorold, 1975); *C. gloeosporioides* from Bahia, Brazil (Ram *et al.*, 1972), Costa Rica (Diaz and New Hall, 1966) and Ghana (Dakwa and Danquah, 1978). In India, earlier *C. gloeosporioides* has been reported to cause blight, shot hole and irregular spot symptoms on leaves (Sharma and Nambiar, 1976; Chandra Mohanan and Kaveriappa, 1983) and pod rots (Reddy and Chandra Mohanan, 1976). In all our isolations only *C. gloeosporioides* was found to be associated with foliar symptoms and pod rot. In the present study based on the type of symptoms on leaves caused by different isolates under artificial inoculation they could be grouped into leaf blight, shot hole and irregular spot. Survey records also show the occurrence of these three types of symptoms in nature. However, the pod isolates, when inoculated on seedlings produced either shot hole or blight symptoms. Since none of the pod isolates when inoculated on seedlings caused irregular leaf spots and the

cocoa plants showing irregular leaf spot symptoms were reported to be confined to a limited area in Karnataka state (Chandra Mohanan and Kaveriappa, 1983) it may be said that this group of isolates is not predominant in nature. The degree of infection among isolates of each symptom group and also the degree of infection among the isolates of each culture group varied. In the studies on pathogenicity of different species of *Colletotrichum* on tomato fruit, significant differences in virulence were observed among isolates. Among the different species of *Colletotrichum* studied for variability, *C. gloeosporioides* was found to show greater variability (Batson and Roy, 1982). Genetically, *Glomerella cingulata*, the perfect stage of *C. gloeosporioides* is known to be a variable fungus (Wheeler, 1954). From the observations on such variation in pathogenicity among the isolates of *G. cingulata* var. *Orbicularis*. Jenkins *et al.* (1964) suggest that the species has a relatively broad base of genes for pathogenicity.

In the leaf blight and shot hole groups the difference in disease index between light and white types was statistically significant. Such studies could not be undertaken with irregular spot isolates as very few isolates were available for study in this group. The studies on cultural characters and virulence of the isolates have revealed that the light types were highly virulent and the white types the least. In *C. falcatum* also a high degree of virulence among light type isolate was reported (Abbott, 1938; Chona and Srivastava, 1960). In *C. lini* the less frequent white types were reported to be less pathogenic (Zarzycka, 1976). In the present study among the 299 pathogenic isolates 210 were found to belong to light culture group. The highly virulent nature of the light type of isolates, perhaps helps them to perpetuate and predominate under natural conditions. Therefore while screening cocoa germplasm for resistance to *C. gloeosporioides* disease it is better to give more importance to light type of isolates. Since the isolates of light type also showed considerable variation in virulence it is desirable to select the more virulent type of isolate of this group, while screening the germplasm/cultivars. *C. falcatum* also has been reported to show considerable variation in the virulence of the different isolates of light group (Chona and Srivastava, 1960). Such significant variations among isolates within each group may be the reason for certain differences in experimental results reported by research workers from different localities.

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**Moist chamber:** It was built with areca palm leaves on top and four sides (to reduce the possible influence of direct sunlight and high temperature on disease development). Gunny cloth was spread on the floor of the chamber before transferring the inoculated seedlings. The chamber was sprayed from inside with water at 10.00, 12.00, 14.30 and 17.00 hours to maintain high humidity. The polythene covers were removed from the seedlings 72 hours after inoculation. The experiment was arranged in randomised block design with three replications of ten seedlings per replication.

**Rating:** Disease severity was assessed 8 days after inoculation. The severity of shot hole and irregular spot was assessed by counting the number of spots per seedling. The severity was rated on a five-point-scale as follows and infection index was calculated.

No. of spots	Rating
None	0
1-10	1
11-25	2
26-50	3
51-75	4
More than 75 spots	5

For leaf blight symptoms the infection index was calculated by using five point rating scale based on the per cent leaf area infected as follows:

Per cent leaf area infected	Rating
0	0
1-10	1
10-25	2
25-50	3
50-75	4
75-100	5

The infection index was calculated by using the following formula (McKinney, 1923; Reddy *et al.*, 1978).

$$\text{Infection index} = \frac{\text{Sum of individual ratings} \times 100}{\text{Total no. of plants OR No. of leaves observed} \times \text{Max. disease ratings (5)}}$$

The pathogenic isolates were grouped on the basis of the symptoms produced on the seedlings, viz., blight, shot hole and irregular spot. The isolates of each group were further classified into white, dark and light types on the basis of the colour of the colony on PDA.

The disease index of the isolates of white, dark and light types under each symptom group was statistically analysed to find out significant differences if any, among them.

### Isolation

The microscopic examination of the samples collected from various gardens showed the presence of conidia on lesions. In certain cases acervuli with setae were also observed. Conidia were oblong to cylindrical, hyaline single celled with both ends rounded or one end tapering. The setae whenever observed were dark brown to black and septate. All the isolates were identified as *Colletotrichum gloeosporioides* Penz. Identification was confirmed by sending representative cultures to CMI, England.

### Pathogenicity

Of the 312 isolates collected and tested, 299 infected cocoa seedlings under artificial inoculation. Of the 299 pathogenic isolates, 196 isolates were the collections from cocoa leaves, 80 from pods and 23 were from seedlings. Of the 299 isolates, 210 incited leaf blight symptoms, 77 shot hole symptoms and only 12 isolates incited irregular leaf spot symptoms under artificial inoculation. Of the 80 pod isolates 70 produced leaf blight and 10 produced shot hole symptoms and none resulted in irregular leaf spot symptoms (Table 1).

The disease index varied significantly among the isolates of leaf blight, shot hole and irregular leaf spot. The disease index of the most virulent isolate of leaf blight group was 50.56%. This isolate was found in the collections of Kulasekharam area of Kanyakumari district. Isolate collected from Kasaragod, Cannanore district was the most virulent among the shot hole isolates with a mean disease index of 82.45%. Similarly among the isolates of irregular spot symptoms, that collected from Kalkare, Chikmagalur district was found to be the most virulent (disease index = 55.65%).

Among the pod isolates collected from Kulasekharam area was the most virulent with a mean disease index of 39.91%. This isolate produced leaf blight symptoms on seedlings. Maximum number of pod isolates incited leaf blight symptoms and none showed irregular spots on seedlings.

### Relationship between disease index and colony colour

The disease index of the isolates of white, dark and light types under each symptom group when statistically analysed was found to vary significantly. In leaf blight group the difference in the disease index between white and light and white and dark types was significant. But the difference in disease index between light and dark type was not significant. White type isolates were least

TABLE I  
Grouping of *Colletotrichum gloeosporioides* isolates

State	Symptom groups on the basis of artificial inoculation															Colletotrichum isolates Total of three groups %							
	I. Leaf blight					II. Shot hole					III. Irregular leaf spot												
	Source of isolates					Source of isolates					Source of isolates												
	Leaf a b	Pod a b	Seedling a b	Total	%	Leaf a b	Pod a b	Seedling a b	Total	%	Leaf a b	Pod a b	Seedling a b	Total	%								
Kerala	55	55	42	42	3	3	100	72	30	77	4	10	5	13	39	28	0	0	0	0	0	139	46
Karnataka	67	73	20	22	5	5	92	70	18	67	2	7	7	26	27	21	12	9	0	0	0	131	44
Tamil Nadu	10	56	8	44	0	0	18	62	4	36	4	36	3	27	11	38	0	0	0	0	0	29	10
Total	132	63	70	33	8	4	210	70	52	68	10	13	15	19	77	26	12	4	0	0	0	299	—

a—No. of isolates

b—Percentages