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PATHOGENIC VARIABILITY AMONG ISOLATES OF *PHYTOPHTHORA CAPSICI* CAUSING BLACK POD DISEASE OF COCOA IN INDIA*

by

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SUMMARY

Pathogenic variability among five isolates of *Phytophthora capsici* viz., I-CP/23, I-CP/25, I-CP/27, I-CP/76 and I-CP/89 collected from five localities of Kerala state of India was studied using wounded and unwounded detached pods of 20 cocoa accessions. Mycelial discs of 5 mm diameter, cut from the periphery of seven-day-old sporulating cultures of *P. capsici* grown on carrot agar medium, were used to inoculate fully developed but unripe detached cocoa pods. Lesion size (cm^2), mycelial growth and sporulation on lesion surface were recorded after seven days of inoculation.

All the five isolates of *P. capsici* were pathogenic to wound-inoculated cocoa pods of 20 accessions. Interactions between isolate and accessions for lesion size and mycelial growth were highly significant, suggesting differential host-pathogen interactions. In general, the isolates produced less aerial mycelium on lesion surface. Studies on lesion sporulation indicated that isolates I-CP/23 and I-CP/76 produced few sporangia on Landas 356 and 365 respectively. Similarity among isolates of *P. capsici* was determined by cluster analysis based on their lesion size on 20 cocoa accessions. Two phenotypic clusters were identified. Similarly among cocoa accessions was also determined based on their reaction (lesion area) to five isolates of *P. capsici*. The cocoa accessions were grouped into five clusters.

Isolates induced low levels of infection or no infection on unwounded pods of 20 cocoa accessions tested. When cocoa pods of 20 accessions were inoculated without wounds, isolates I-CP/27, I-CP/23, I-CP/76, I-CP/25 and I-CP/89 caused infection only on 12, nine, eight, seven and four accessions respectively.

Thus, for cocoa breeding purposes, screening cocoa genotypes with two isolates of *P. capsici* may suffice in selection for resistance to *P. capsici*.

VARIABILITE PATHOGENIQUE PARMI LES ISOLATS DE *PHYTOPHTHORA CAPSICI* LEONIAN, QUI PROVOQUENT LA MALADIE DE LA POURRITURE BRUNE DU CACAOYER EN INDE

RESUME

La maladie de la pourriture brune, causée par *Phytophthora*, est la principale maladie du cacaoyer en Inde. Plusieurs espèces de *Phytophthora* ont été signalées comme causant cette maladie du cacaoyer dans différentes parties du monde. Des études antérieures sur l'espèce *Phytophthora*, associée avec le cacaoyer en Inde, ont montré que *P. capsici* Leonian cause aussi la maladie de la pourriture brune du cacaoyer, autre que *P. palmivora*. La variabilité pathogénique parmi cinq isolats de *P. capsici* collectés dans cinq endroits de l'Etat de Kerala en Inde, a été étudiée en se servant de cabosses qui présentaient ou non des lésions et qui étaient détachées de 20 accessions de cacaoyers. Des disques mycéliaux de 5 mm de diamètre, coupés à la périphérie de cultures sporulantes de sept jours de *P. capsici*, cultivée sur un milieu d'agar de carottes, ont servi à inoculer des cabosses de cacaoyers pleinement développées mais immatures. La dimension des lésions, la croissance mycéliale de surface et la sporulation sur les surfaces de cabosses, ont été déterminées après sept jours d'inoculation.

Bien que tous les cinq isolats de *P. capsici* aient été pathogéniques sur des cabosses de cacao à lésions inoculées des 20 accessions, la virulence des isolats variait à un point significatif entre et parmi les accessions. La dimension moyenne des lésions des 20 accessions de cacaoyers indiquait l'isolat I-CP/89 comme le moins pathogénique. La dimension moyenne de lésions produites par les cinq isolats de chaque accession a révélé que Landas 356 est hautement sensible et que Redaxial est le moins sensible. Mais la sensibilité de chaque accession variait à un point significatif selon les isolats.

Les isolats produisaient un faible niveau d'infection ou pas d'infection sur des cabosses de cacao non blessées parmi les accessions testées. Lorsque des cabosses de cacao des 20 accessions étaient inoculées sans lésions, les isolats I-CP/27, I-CP/23, I-CP/76, I-CP/25 et I-CP/89 ont causé des infections seulement sur 12, neuf, huit, sept et quatre accessions respectivement.

La croissance mycéliale aérienne à la surface de la lésion des cabosses inoculées variait à un point significatif selon les isolats et les accessions. Des études sur la sporulation de lésions indiquaient que les isolats I-CP/23 et I-CP/76 produisaient des sporanges sur Landas 356 et Landas 365 respectivement.

Les résultats de l'étude ont indiqué que les isolats de *P. capsici* qui existent dans l'Etat de Kerala, en Inde, manifestaient une variabilité marquée quant aux caractères pathogènes des accessions de cacaoyers testées.

VARIABILIDADE PATOGENICA ENTRE ISOLADOS DE *PHYTOPHTHORA CAPSICI* LEONIAN, CAUSADOR DA PODRIDAO PARDA EM CACAUEIROS NA INDIA

RESUMO

A podridão parda causada por *Phytophthora* é a doença mais grave que afeta os cacaueiros na India. Sabe-se que diversas espécies de *Phytophthora* causam podridão parda em cacaueiros de diferentes partes do mundo. Estudos anteriores sobre espécies de *Phytophthora* associadas com cacau na Índia revelaram que não só *P. palmivora* causa podridão parda mas também *P. capsici* Leonian. A variabilidade patogênica entre cinco localidades do estado de Kerala, India, foi estudada em frutos destacados com e sem lesões de 20 introduções. Discos miceliais de 5 mm de diâmetro retirados da periferia de culturas esporulantes, com sete dias de idade, de *P. capsici* cultivados em agar de cenoura foram usados para inocular frutos plenamente desenvolvidos mas não maduros. Sete dias após a inoculação determinaram-se o tamanho da lesão, o desenvolvimento micelial e a esporulação na superfície dos frutos.

Bem que todos os cinco isolados de *P. capsici* tenham sido patogênicos em frutos de cacau inoculados através de lesões no caso de 20 introduções recém introduzidas na India, a sua virulência variou significativamente dentro de e entre estas introduções. O tamanho médio das vinte introduções indicou que o isolado I-CP-89 foi o menos patogênico. O tamanho médio das lesões produzidas pelos cinco isolados em cada uma das introduções revelou que Landas 356 era altamente suscetível e Redaxial a menos suscetível. Entretanto, a suscetibilidade de cada introdução variou significativamente conforme o isolado.

Os isolados produziram baixos níveis de infecção ou nenhuma em frutos sem lesões das introduções testadas. No caso de inoculação de frutos sem lesão de todas as introduções, os isolades I-CP/27, I-CP/23, I-CP/76, I-CP/25 e I-CP/89 provacaram infecção somente em 12, 9, 8, 7 e 4 dessas introduções, respectivamente.

O desenvolvimento micelial aéreo na superfície da lesão provocada em frutos inoculados através de lesões variou de maneira significativa em função tanto dos isolados como das introduções. Segundo estudos de esporulação nas lesões, os isolados I-CP/23 e I-CP/76 produziram esporângios em Landas 356 e em Landas 365, respectivamente.

Assim, os resultados deste estudo indicam que os cinco isolados do fungo *P. capsici* existente no estado de Kerala, na Índia, evidenciaram variabilidade considerável das caracteristicas patogênicas nas introduções examinadas.

VARIABILIDAD PATOGENICA ENTRE AISLADOS DE *PHYTOPHTHORA CAPSICI* LEONIAN CAUSANTES DE LA ENFERMEDAD DE LA PODREDUMBRE NEGRA DE LA MAZORCA EN LA INDIA

RESUMEN

La enfermedad de la podredumbre negra de la mazorca causada por *Phytophthora* es la enfermedad principal del cacao en la India. Se ha informado que hay varias especies de *Phytophthora* que causan la enfermedad en distintas partes del mundo. Los primeros estudios sobre *Phytophthora* spp. asociados ccon el cacao en la India revelaron que *P. capsici* Leonian tambien causa la enfermedad de la podredumbre negra de la mazorca del cacao, además de *P. palmivora*. Se estudió la variabilidad patógena en cinco aislados de *P. capsici* recogidos de cinco localidades en el estado de Kerala, India, usando mazorcas destacadas lesionadas y sin lesiones de 20 accesos de cacao. Se usaron discos micélicos de 5 mm de diámetro, cortados de la periferia de cultivos esporulantes de 7 dias de *P. capsici* criados en un medio de agar zanahoria, para inocular mazorcas de cacao plenamente desarrolladas pero inmaduras. A los 7 dias de la inoculación se determinaron dimensiones de lesión, crecimiento de la superficie micélica y esporulación.

Aunque los 55 aislados de *P. capsici* eran patógenos, en la lesión de las mazorcas de cacao inoculadas, de 20 accessos, la virulencia de los aislados varió en forma significativa dentro de y entre los accesos. La dimensión media de 20 accessos de cacao indicó que el aislado IC-P/89 era el menos patógeno. La dimensión media de la lesión producida por los 5 aislados en cada acceso reveló Landas 356 altamente susceptibles y Redaxial como menos susceptible. Pero la susceptibilidad de cada acceso varió grandemente con los aislados.

Los aislados produjeron un bajo nivel de infección o ninguna infección en las mazorcas de maiz no lesionadas de los accesos probados. Cuando se inocularon mazorcas de cacao sin lesiones, los aislados I-CP/27, I-CP/23, I-CP/76, I-CP/25 e I-CP/89 causaron infección solamente en 12, 9, 8, 7 y 4 de los accesos, respectivamente.

El crecimiento mielitico aéreo en la superficie de la lesión de las mazorcas inoculadas, varió significativamente con los aislados y los accesos. Estudios de la esporulación de las lesiones indicaron que los aislados I-CP/23 e I-CP/76 produjeron esporangios en Landas 356 y Landas 365, respectivamente.

Los resultados del estudio por lo tanto indicaron que los cinco aislados de *P. capsici* existentes en el estado de Kerala, India, exhibieron una variabilidad señalada en caracteres patógenos en los accesos de cacao probados.

INTRODUCTION

Black pod disease is one of the major factors limiting yields of cocoa in Southern India where cocoa is grown as mixed crop in arecanut and coconut gardens. Different species of *Phytophthora* are known to cause black pod disease of cocoa in various parts of the world (Zentmyer, 1988 and Liyanage and Wheeler, 1989). The cocoa *Phytophthora* isolates characterized by ellipsoid or pyriform sporangia with long thin pedicels are generally referred to as *P. capsici* (Tsao and Alizadeh, 1988). *P. capsici* has been reported as the causal organism of black pod in Trinidad, Venezuela, Brazil, El Salvador, Guatemala, Central and South America and Cameroon (Zentmyer, 1988) and Jamaica (Fagan, 1988).

Recent studies on *Phytophthora* complex on black pod disease of cocoa in India revealed the occurrence of *P. capsici* on cocoa in certain localities of Kerala State (Chowdappa *et al.*, 1993). Difference in virulence among the isolates of *palmivora* causing black pod disease of cocoa is well documented (Leather, 1966, Ram and Ram, 1973 and Fagan, 1988). This inter-isolate variation was expressed as differences in rate of spread of lesions on pods, amount of mycelial growth and degree of sporulation which are all components of pathogenic virulence (Turner, 1965, Akinferon, 1971 and Fagan, 1988). In

view of these findings, identification of physiological races among the isolates of the designated *Phytophthora* spp. is considered to be an important feature for evolving resistant varieties. The objective of this study was to determine the pathogenic variability among the isolates of *P. capsici* collected from different localities of Kerala State of India and to select those isolates most appropriate for screening of cocoa accessions against black pod disease.

MATERIALS AND METHODS

Isolates

The five *P. capsici* isolates viz., I-CP/23, I-CP/25, I-CP/27, I-CP/76 and I-CP/89 were collected from Devikulam and Udumbanchola of Idukki, Ranni of Pathanamthitta and Kannara of Trichur districts of Kerala. The isolates were maintained on carrot agar medium (CA) slants at 24°C by periodical subculturing. The isolates were grown on CA for 3 days at 24 ± 1°C in dark and then incubated at 25 ± 1°C for 4 days under continuous light (two Crompton, cool daylight, 40W fluorescent lamps, 120 cm long at an intensity of 30 umol m^{-2} s^{-1}), approximately 30 cm away from the light source to induce production of sporangia.

Cocoa accessions

To study the pathogenic variability among isolates of *P. capsici*, nearly mature, detached pods of 20 cocoa accessions viz., Redaxil, Landas 356, Landas 358, Landas 364, Landas 365, Jarangan Amel × Pa7, Jarangan Amel × Na 32, Jarangan Pa7 × Na37, Pa7 × Na32, Amel × Na33, Amel × Na32, P1 × P7, P6 × P4, T85/5 × Na32, P7 × P6, T7/12, T86/2, W6/56 (T63/910), C79 and C44 were wound and surface inoculated with five isolates of *P. capsici*.

Inoculation

In the wound inoculation method, a 5 mm diameter plug was removed from the middle portion of the pod using sterile cork borer of equal diameter. A sporulating mycelial disc of similar size from 7-day-old cultures was placed in the cavity and the tissue plug was replaced and the inoculated area was covered with moist cotton wads. In the surface inoculation method, a 5 mm diameter disc of sporulating mycelial disc from 7-day-old culture was placed centrally in the furrow of surface-sterilized pod. The inoculated area was covered with cotton wad dipped in cold sterile distilled water so as to stimulate release of zoospores from sporangia on to the pod surface.

Each pod was kept in a separate polythene bag containing cotton pads dipped in sterile water. The polythene bags were sealed and incubated at 25 ± 1°C. Six pods were maintained for each accession and isolate. The length and breadth of resulting lesions were measured seven days after inoculation. The means of the elliptical lesions (less inoculum) were calculated using the formula $\pi \times L \times W$; where L and W are the half length and width respectively of the ellipse (Fagan, 1988). Each infected pod was also visually scored for the extent of mycelial growth on the lesion surface using a five-point scale (0 = nil, 1 = scanty, 2 = light, 3 = moderate, 4 = good and 5 = abundant mycelial growth). Mycelial growth index was calculated from the mean value obtained for each isolate.

The degree of sporulation was measured by scraping surface mycelium and spores from the lesion into a beaker containing 10 ml of Formalin: acetic acid: ethanol (FAA) (5 : 5 : 90) and thoroughly shaken for 5 minutes to dislodge sporangia. The sporangia present in the FAA were counted with the help of haemocytometer. Ten such samples were taken to determine the sporulation on each pod. The sporangial production per square centimetre of lesion area was calculated by dividing the number of sporangia produced on a pod by the area of the lesion on the pod.

Statistical analysis

Data were analysed using the M-Stat Computer software package. Lesion size (cm^2) of isolate-accessions combination was subjected to analysis of variance using 2 factor CRD procedure. Data on mycelial growth index were also subjected to analysis of variance after square root transformation of the data. A hierarchial classification was done using cluster analysis to determine the similarity of the isolates of *P. capsici* based on their patterns of lesion size on pods on 20 cocoa accessions, and of 20 cocoa accessions based on the pattern of reaction (lesion size) to five isolates of *P. capsici*. The cluster analysis was performed using the centroid method.

RESULTS

All the five isolates of *P. capsici* such as I-CP/23, I-CP/25, I-CP/27, I-CP/76 and I-CP/89 were pathogenic to wound inoculated pods of all cocoa accessions tested but the rate of spread of lesion varied significantly (Table 1). The interactions between isolates and accessions were highly significant (Table 2). The mean lesion size on pods of 20 cocoa accessions indicated that the isolate I-CP/89 produced smaller lesion size whereas all other isolates produced more or less equal lesion area. The lesion area on different cocoa accessions varied significantly with the isolates I-CP/23, I-CP/25, I-CP/27, I-CP/76 and I-CP/89. Isolates I-CP/23 and I-CP/25 produced smaller lesion area on Redaxil and larger lesion area on Landas 356. I-CP/27 caused lower lesion area on Landas 364 and higher lesion area on Amel × Na33. Isolate I-CP/76 induced smaller lesion area on P6 × P4 and larger lesion area Landas 356. Isolate I-CP/89 caused less lesion area on Jarangan Amel × Pa7 and more lesion area on Landas 356.

In general, all the isolates produced low aerial mycelium on the lesion surface of wound inoculated pods of 20 cocoa accessions. But amount of mycelial production varied significantly with the isolates (Table 1). The analysis of variance of mycelial production on the lesion surface indicated highly significant isolate × accession interactions (Table 2). Microscopic examination of semi-permanent slides prepared from the fungal growth on the lesion surface indicated that I-CP/23 and I-CP/76 produced 20 and 300 sporangia/cm^2 lesion area on Landas 356 and 365 respectively.

TABLE 1

Pathogenicity of five isolates of P. capsici on 20 cocoa accession by wound inoculation method (Mean value of six pods/accession)

Cocoa accessions	Lesion size (cm^2) isolates						Index of mycelial growth isolates					
	I-CP/23	I-CP/25	I-CP/27	I-CP/76	I-CP/89	Mean	I-CP/23	I-CP/25	I-CP/27	I-CP/76	I-CP/89	Mean
1 Red axil	66.09	60.07	58.30	80.65	82.88	69.60	0.70	0.70	1.22	0.70	1.22	0.94
2 Landas 364	109.88	74.01	27.99	99.81	101.02	82.54	1.58	1.22	0.70	0.70	1.22	1.08
3 Landas 356	193.06	217.78	181.60	207.08	238.75	207.65	2.34	2.34	1.58	2.34	1.22	1.72
4 Landas 358	159.07	153.20	145.28	138.53	117.34	142.68	1.22	1.58	1.22	1.22	1.58	1.36
5 Landas 365	79.20	187.54	186.74	174.69	51.48	135.93	1.22	1.22	1.58	1.22	2.03	1.45
6 Jarangan Amel × Pa7	136.26	123.63	119.40	122.18	0.54	100.40	0.70	1.22	1.22	1.58	0.70	1.08
7 Jarangan Amel × Na32	129.26	125.65	138.86	153.78	23.67	114.22	1.22	1.87	2.10	2.12	1.58	1.77
8 Jarangan Pa7 × Na37	145.28	125.96	155.59	163.12	37.98	125.58	1.22	0.70	0.70	1.87	0.70	1.03
9 Pa7 × Na32	187.05	169.96	144.30	192.92	53.40	149.52	0.70	1.25	1.25	1.20	1.22	1.12
10 Amel × Na33	170.89	177.16	191.94	190.64	147.80	175.68	1.22	0.70	2.34	1.22	0.70	1.23
11 Amel × Na32	155.19	168.70	148.69	165.97	160.78	159.86	1.22	1.22	1.22	1.22	1.22	1.22
12 P1 × P7	121.23	130.05	132.25	114.06	118.10	123.14	1.58	1.87	1.87	2.12	1.87	1.86
13 P6 × P4	82.70	133.33	134.18	67.57	56.68	94.89	1.22	1.22	1.22	0.70	1.22	1.11
14 T85/5 × Na32	145.35	131.46	114.93	131.76	78.92	120.44	1.05	0.88	1.22	1.58	1.58	1.26
15 P7 × P6	149.80	116.37	141.83	123.84	109.71	128.31	1.58	1.87	1.87	1.58	1.87	1.75
16 T7/12	100.21	122.70	146.83	100.94	107.30	115.60	1.22	1.22	1.22	1.87	2.12	1.53
17 T 86/2	157.55	164.93	155.10	153.96	108.09	147.92	1.22	0.88	1.05	1.58	1.58	1.26
18 W6/56 (T63/910)	147.00	164.16	175.11	144.34	185.83	163.29	0.88	2.03	2.34	1.87	1.87	1.79
19 CT9	129.81	103.13	127.51	112.64	113.77	117.37	1.22	1.22	0.70	1.58	1.22	1.18
20 C44	97.00	91.62	105.25	94.41	85.55	94.96	1.38	1.22	1.87	1.87	1.58	1.58
Mean	133.09	137.07	136.58	136.64	99.02		1.23	1.32	1.42	1.50	1.41	
	CD: Accession = 16.69 Isolates = 8.34 Interaction = 37.33						CD: Accession = 0.06 Isolates = 0.03 Interaction = 0.14					

Isolates were grouped into two phenotypic clusters based on lesion size by cluster analysis using centroid method (Fig. 1). Isolates such as I-CP/23, I-CP/25, I-CP/27 and I-CP/76 from Devikulam and Udumbanchola of Idukki and Ranni of Pathanamthitta districts formed the first group, whereas isolate I-CP/89 from Kannara of Trichur district is contained in the second group. On the basis of Lesion size dendrograms, cocoa accessions were grouped into five clusters (Fig. 2) as given below:

Clusters	Accessions
1	Jarangan Amel × Pa7, C79 and Redaxil. Jarangan Amel × Na32, Jarangan Pa7 × Na37, P6 × P4, T85/5 × Na32, T7/12, C44, P7 × P6.
2	P1 × P7 and Landas 358.
3	Pa7 × Na32, Landas 365, T86/2.
4	Amel × Na32, W6/56 (T 63/910).
5	Landas 356 and Landas 364.

The reaction of cocoa accessions to the isolates of *P. capsici* varied significantly (Table 1). The mean lesion size produced by five isolates of *P. capsici* revealed Landas 356 as highly susceptible and Redaxil as the least susceptible.

Isolates produced low levels of infection or no infection on unwounded pods of 20 cocoa accessions (Fig. 3). Among the five isolates of *P. capsici* only one isolate, I-CP/25, caused infection on these accessions viz., Pa7 × Na32, P6 × P4 and Amel × Na33. The accession C79 took up infection only when inoculated with I-CP/76. All the isolates except I-CP/25 caused infection on unwounded pods of Landas 364 whereas P1 × P7 was infected by all the isolates except I-CP/76.

The number of accessions susceptible to each isolate also varied. Out of the 20 accessions tested without wounds, 12 accessions were susceptible to I-CP/27 where-

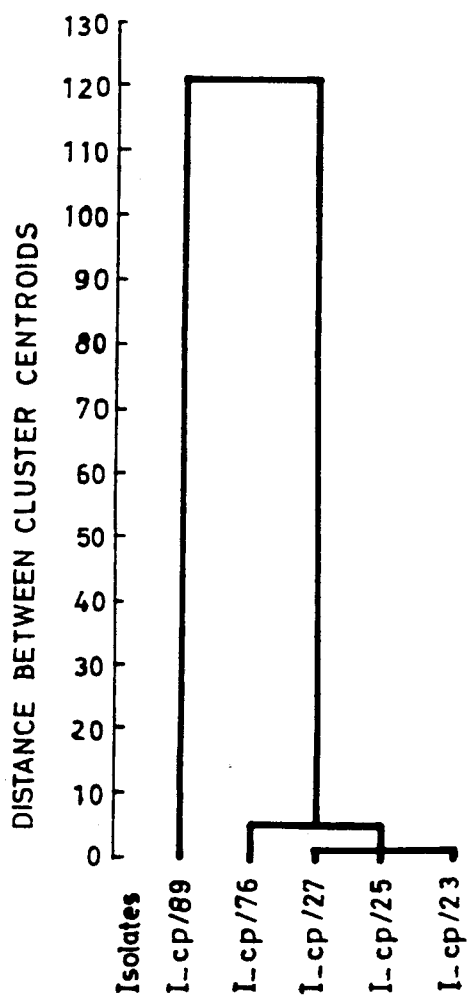

FIG. 1. Dendrogrammes showing similarity and successive clustering of isolates of *P. capsici* based on their virulence (Lesion size) on 20 cocoa accessions.

TABLE 2

Summary of analysis of variance for lesion area and mycelial growth on pods of 20 cocoa accession wound inoculated with five isolates of P. capsici

	Lesion area (cm^2)		*Mycelial growth*	
Source of variation	*df*	*MS***	*df*	*MS***
Accessions	19	16,565	19	171
Isolates	4	16,429	4	137
Accessions × Isolates	76	2,620	76	21
Error	200	544	200	4.6

** Significant P = 0.01

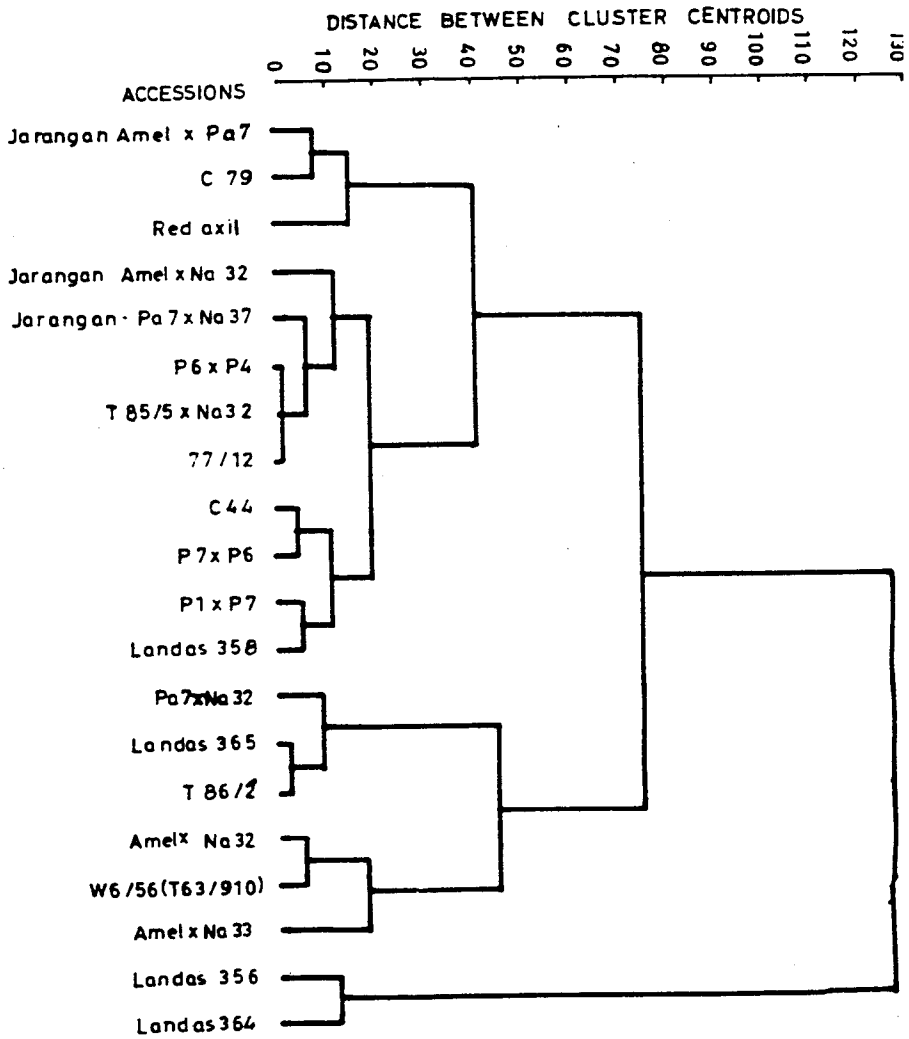

FIG. 2. Dendrogrammes showing similarity and successive clustering of cocoa accessions based on their reaction (Lesion size) to 5 isolates of *P. capsici*.

as 9, 8, 7 and 4 accessions were susceptible to I-CP/23, I-CP/76, I-CP/25 and I-CP/89 respectively (Fig. 3). The largest lesion size was observed on pods of Landas 365 and the smallest lesion size on Jarangan Amel × Na32 when inoculated with I-CP/27. The lesion size varied markedly with the accessions as well as with isolates (Fig. 3).

DISCUSSION

The concept of pathogen diversity is considered to be a common feature in host-pathogen system. A thorough insight into the pathogen diversity may lead to the development of new breeding strategies to increase durability of resistance in host-genotypes to pathogens. Virulences is one of the markers most often used today to study pathogen diversity. Differences in virulence have been used widely as phenotypic and genotypic markers (Schilder and Bergstorm, 1990, Lebda, 1981, Shattock *et al.*, 1977, Al-Kherb *et al.*, 1987, and Puhalla, 1984) to identify distinct physiological races. In the present study, the analysis of variance showed a significant isolate × accession interactions between cocoa accessions and isolates of *P. capsici* for effects on lesion area and amount of mycelial production, suggesting various isolates of *P. capsici* are differentially adopted to specific cocoa accessions, but the magnitude of specificity is not high. Similar differential interaction between the isolates of *Pyrenophora tritici-repentis* (Died) Drechs, the incitant of tan spot and wheat genotypes was reported (Schilder and Bergstorm, 1990). According to Van derplank (1982), the

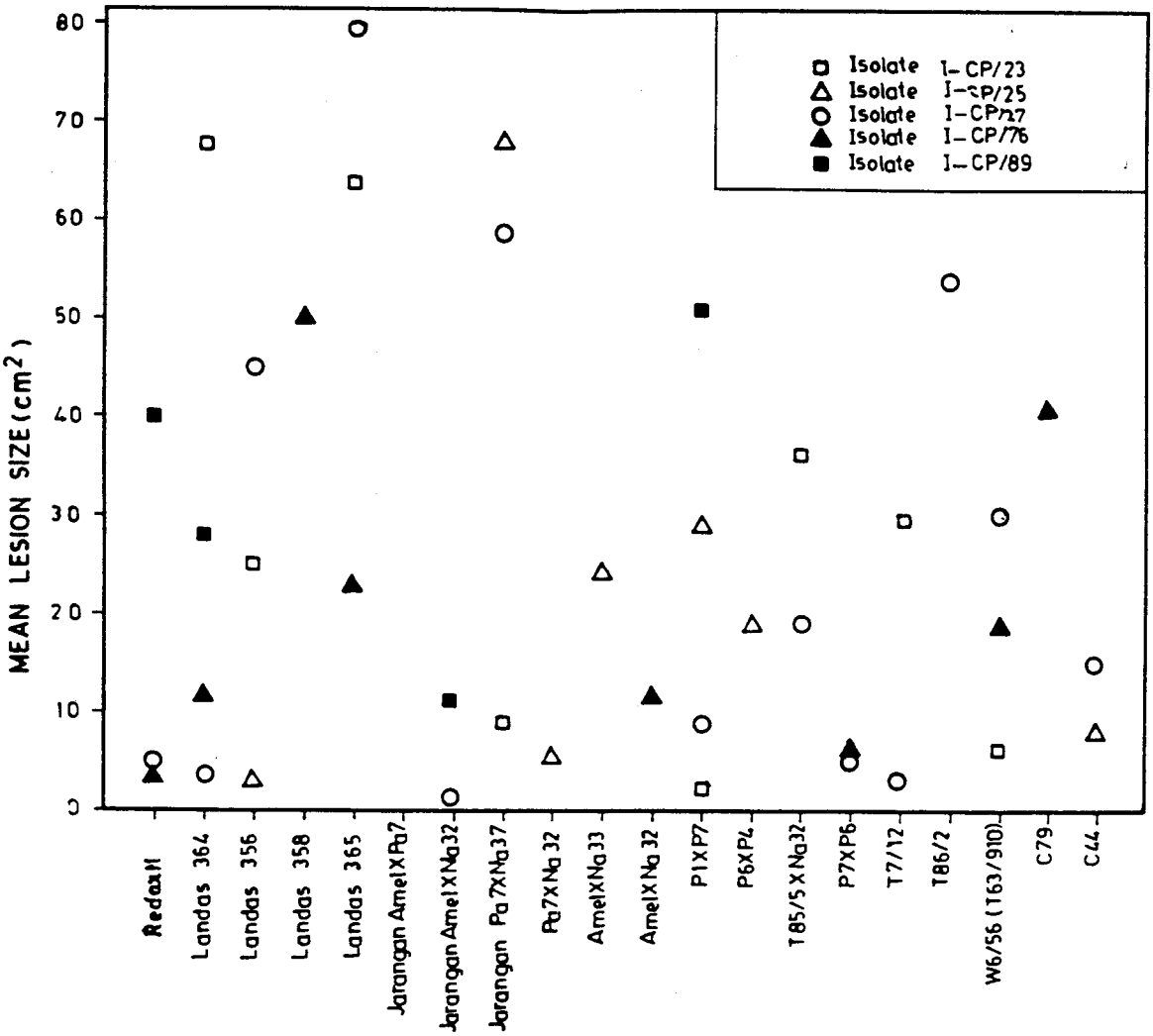

FIG. 3. Lesion area incited on 20 cocoa accessions by five isolates of *P. capsici* without injury.

specificity in host-pathogen relationship is often indicated by significant isolate × accession interactions in the analysis of variance of an experiment where a number of isolates of pathogen are tested in all possible combinations on a set of host-genotypes. Non-specificity is determined by lack of such interaction. In the present study, the isolates also showed differential reactions to different cocoa accessions when they were inoculated without injury. In this context, it should be noted that the usage of term 'virulence' to describe the quantity of disease produced in the pathosystem of *P. capsici* is appropriate since a certain degree of specificity is observed.

Cluster analysis was found to be useful in determining the similarity of the isolates in the present study and differentiated the isolates into two phenotypic clusters. The cluster analysis has been used to discern the genetic similarities among races of *Bremia lactucae* Regel, the causal agent of downy mildew of lettuce (Lebeda and Jendrulek, 1987) and among 17 isolates of *P. tritici-repentis* causing tan spot of wheat (Schilder and Bergstorm, 1990) in quantitative host-pathogen systems. Our studies on cultural, morphological and physiological characters of *P. capsici* also showed certain differences between the two groups of isolates (Chowdappa and Chandra Mohanan, unpublished). Physiological races among the isolates of *P. drechsleri* f. sp. on pigeon pea Hwang, 1992), *P. megasperma* f. sp. *Glycinea* on Soybean (Anderson and Buzzell, 1992), *P. palmivora* on rubber (Chee, 1975) and *P. meadii* on rubber (Pieries and Dantanarayana, 1975) were well documented. Cluster analysis also proved to be useful in determining the similarity of cocoa accessions based on their reaction to five isolates of *P. capsici*.

Amount of mycelial production and sporulation potential on the infected pod surface have been used as criteria in determining the virulence of *P. palmivora* isolates to different cocoa genotypes (Turner, 1965, Akinrefon, 1971 and Chandra Mohanan, 1982). According to these authors, the amount of mycelial and sporangial production was substantially higher on susceptible cocoa selections than on resistant selections. Turner (1965) further suggested comparative sporulation alone can be used as a criterion in laboratory tests for resistance. In the present study, all the five isolates of *P. capsici* produced low surface mycelium and no sporangia on lesion surface except a few sporangia by the isolates on the accession Landas 356 and Landas 365, suggesting that all the 20 cocoa accessions were moderately resistant to all the five isolates of *P. capsici*. The amount of sporulation during infection periods (June–August) will greatly influence the spread of the black pod disease. It is likely that the low degree of lesion sporulation might have been responsible for the low incidence of black

[illegible] et al., 1992), *P. capsici* on pepper, [illegible] pod disease caused by *P. capsici* in [illegible]

the 133 samples of black pod collected from different localities in south India, only five samples yielded *P. capsici* (Chowdappa and Chandra Mohanan, unpublished). These results are in agreement with previous reports of Lawrence *et al.* (1982) and Fagan (1988) in Jamaica who reported low virulence of *P. capsici* isolates compared to other *Phytophthora* spp.

The mechanisms underlying resistance in cocoa accessions to *P. capsici* isolates are not clear. Resistance in cocoa accessions was expressed as either due to rich tannins in the epicarp (Tarjot, 1972) or control of degree of sporulation by internal endocarp tissues (Thorold, 1967) or greater accumulation of phytoalexin-like substances in host tissue during host-pathogen interactions (Dagvenet and Parvais, 1981) or presence of pathogen enzymes inhibiting components in cocoa genotypes (Akinrefon, 1968) or combination of all the factors.

The amount of mycelial production and sporulation capacity did not differ widely among the isolates and should not be considered to represent true physiological races. Physiological specialization, therefore, seems to be very less in populations of *P. capsici* in the present study and rapid adaptation to particular host and loss of effective resistance appears unlikely.

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