Cey. J. Sci. (Bio. Sci.) 38 (2): 57-66, 2009

A PHENETIC ANALYSIS OF *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES FROM SELECTED HOST PLANTS

P.L.T. Gunawardhana[1]*, A.M.W.K. Senevirathna[2], N.K.B. Adikaram[1] and D.M.D. Yakandawala[1]

[1]Department of Botany, University of Peradeniya, Sri Lanka
[2]Rubber Research Institute, Darton Field, Agalawatta, Sri Lanka
Accepted 19 December 2009

ABSTRACT

Colletotrichum gloeosporioides is a ubiquitous fungus which infects a wide variety of plants in tropical, sub-tropical and temperate regions. This fungus accounts for substantial economic losses through out the world via both preharvest and postharvest diseases. A phenetic analysis of *C. gloeosporioides* isolated from *Capsicum frutescens, Carica papaya, Mangifera indica, Persea americana, Ficus religiosa* and *Hevea brasiliensis* was carried out to identify sub-specific populations. A total of 40 isolates from these six host species were used. The overall similarity among different isolates of *C. gloeosporioides* was determined using culture, conidial and appressorial characteristics. According to the resulting phenogram, fungal isolates had divided into two distinct groups at the initial stage separating *C. papaya* isolates from the rest of the isolates. The subsequent branching has lead to separation of *C. gloeosporioides* isolates of different hosts into distinct groups. A high degree of similarity was observed among the isolates obtained from *C. frutescens, H. brasiliensis* and *F. religiosa*. Similarly, isolates of *P. americana* and *M. indica* appear to be morphologically more similar to each other. Further, the study confirms the cross infection potential of some *C. gloeosporioides* isolates and the presence of host specific populations

Key Words: anthracnose, phenetic analysis, cross inoculation

INTRODUCTION

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. (teleomorph *Glomerella cingulata* (Stonem.) Spauld. & von Schrenk.) is a ubiquitous fungus which infects a wide variety of plants (Sutton, 1992) in tropical, sub-tropical and temperate regions. The pathogen can cause a number of diseases, anthracnose, leaf spot and seedling blight in cereals, legumes, ornamentals, vegetables and fruits. The most significant damage of this fungus occurs upon its attack on fruiting stage, leading to the incidence of anthracnose (Bailey *et al.*, 1992).

The taxonomy of *C. gloeosporioides* has gained much attention due to the broad host range that this fungus exhibits. Since the plant pathogens are often named after the host plant, there are 17 acknowledged generic synonyms for *Colletotrichum* while there are about 600 synonyms for *C. gloeosporioides*. Von Arx in 1957 changed this system to a more systematic form via using morphological traits of different fungi. As a result Sutton in 1992, reduced the number of *Colletotrichum* species to nine; *C. gloeosporioides*, *C. crassipes*, *C. lini*, *C. destructivum*, *C fuscum*, *C. fusarioides*, *C. phyllachoroides*, *C. paludosum*, *C. atramentarium*, *C. graminicola* and *C. dematium*.

Morphological, growth, physiological and molecular differences have been used for taxonomic studies of *Colletotrichum* (Hong *et al*., 2008). The morphological characters which can be used for taxonomic purposes are limited to cultural, conidial and appressorial characters (Gunell and Gubler, 1992). Physiological characters that are commonly used for this pathogen are growth rate, virulence, germ-tube elongation and rate of appressorium formation.

Polymorphism in ribosomal RNA (rDNA) and mitochondrial DNA (mtDNA) has been used to assess the variability among populations of *C. gloeosporioides* that infect tropical fruits. These studies revealed that *C. gloeosporioides* isolates of *Persea americana* vary in rDNA and mtDNA banding patterns while isolates of *Mangifera indica* exhibit a similar rDNA and mtDNA banding patterns, independent from their

*Corresponding author's email: pubudug83@gmail.com

geographic origin (Mills *et al*., 1992b). In addition, arbitrarily primed PCR (AP-PCR) technique can also be used for taxonomic studies of this fungus (Freeman *et al.*, 1995).

Further, *C. gloeosporioides* isolates of different hosts consist of the cross inoculation capacity (Alahakoon *et al*., 1994). *P. americana*, *M. indica* and *Nephelium lappaceum* were most susceptible while *Garcinia mangostana* and *Syzygium samarangense* were least susceptible to *C. gloeosporioides* isolates of other crops. Although, variation on pathogenicity of C. *gloeosporioides* on different fruits has been observed through laboratory experiments (Freeman and Shabi, 1996) no field experiments on intact fruits have been carried out.

Therefore, a phenetic analysis of *C. gloeosporioides* isolates from different host plants was carried out with the objectives of identifying sub-specific levels or geographic populations whereas a cross inoculation study was done to distinguish cross infection potential of different isolates of *C. gloeosporioides*.

MATERIALS AND METHODS

Isolation and growth of *Colletotrichum*:

Similar varieties of each of *Capsicum frutescens, Carica papaya*, *M. indica* and *P. americana* fruits exhibiting anthracnose symptoms were collected from a fruit stall. Diseased leaves of *Ficus religiosa* and *Hevea brasiliensis* with characteristic necrotic spots were field collected.

The fungus was isolated from symptomatic tissues of each host via cut separation of small square pieces of diseased tissues. These were surface sterilized in 0.5% chlorox (NaOCl) solution for 3- 5 minutes followed by rinsing with sterile distilled water. The tissues were aseptically transferred onto PDA medium.

Pure cultures were maintained by sub-culturing at 14 day intervals. Conidia suspensions of each isolate were prepared by flooding 14 day old sporulating cultures with sterile distilled water and the mycelium was gently rubbed with a sterile glass spreader in order to dislodge conidia. The resulting suspension was filtered through glass wool. A loopful of conidia suspension was streaked across a thin plate of tap water agar and the plates were incubated at room temperature for about 18 hours. Monoconidial isolates were obtained from germinating spores on water agar and each isolate was given an acronym (*C. frutescens* – Cf; *C. papaya* –Cp; *M. indica* – Mi; *P. americana* – Pa; *F. religiosa* – Fr and *H. brasiliensis* – Hb).

Morphological studies: Seven day old starter cultures were examined under light and stereomicroscopes in order to identify suitable characters with variations in character status, for the phenetic analysis of *C. gloeosporioides.* This was repeated three times in order to overcome any deviations.

Colony characteristics: The increase in colony diameter was assessed by first demarcating the margins of the colonies each day and then measuring the diameter along two perpendicular axes. Colony colour was described using the degree of pigmentation of the colonies. In addition, nature of the colony margin, elevation of the colonies, presence /absence of sectoring and nature of the fungal mycelium were also recorded.

Conidial characters: Suspensions of conidia of each isolate were prepared and the concentration was adjusted to $1x10^7$ conidia/ml using a haemocytometer. The conidia were examined under light microscope and the length and width of 100 conidia per isolate were measured using an eye piece graticule at x100 magnification. In addition, the shape of conidia and presence or absence of visible conidial masses were also recorded.

Characters of appressoria: Suspensions of conidia (1×10^5 conidia/ml) were prepared and 10 µl of the suspensions were placed on separate sterile glass slides and the slides were incubated in a moisture chamber for 10 -12 hours. Growth of appressoria was monitored and at the end of the incubation period a drop of cotton blue lacto phenol was added to arrest further development of the germinating conidia and stain the fungal structures. Finally the appressoria were examined and the length and width of 75 appressoria per slide were measured as described above and average lengths and widths were determined. In addition, shape and colour of appressoria were also recorded.

Cross inoculation studies: Diseased fruits of *P. americana, M. indica* and *Colletotrichum* infected leaves of *H. brasiliensis* of clone RRIC 100 were obtained. Pathogens were isolated on PDA as described previously. Conidia suspensions were prepared from 5 day old

cultures. Drops (15 µl) of conidia suspensions from each of three isolates were applied on to ripe fruits of *P. americana, M. indica, C. papaya* and copper brown young *H. brasiliensis* leaves of clone RRIC 100 (Table 1). Controls were treated with 15µl drops of sterile distilled water and four replicates were used for each treatment. All the treated and control fruits were incubated at 100% RH and at room temperature (28^{o}C) until the characteristic anthracnose lesions developed. After six days of incubation, lesion diameter was measured.

The phenetic analysis: The characters with variation and non-overlapping character states were identified and coded into a data matrix. A cluster analysis and a Principal Component Analysis (PCA) was carried out using PC-ORD software.

RESULTS AND DISCUSSION

A total of 40 *C. gloeosporioides* isolates were studied for morphological and physiological data. The different characters and character states identified and coded for the phenetic analysis are given in Table 2.

Morphological studies:

Colony characteristics: The colony colour ranged from intense white to highly intense blackish ash. A distinct sequence of colour changes especially at the central region, was evident in *C. gloeosporioides* isolates obtained from *C. papaya* (Fig.1a) and *F. religiosa* (Fig. 1c) compared to the rest of the isolates. The lower surface of the cultures did not undergo a significant variation in colour with time.

Several different types of mycelia were found within the *C. gloeosporioides* isolates as dense aerial, even felted mat and compact aerial mycelium in tufts. Most of the colonies were grouped under dense aerial (Fig. 1c) and sparse aerial mycelium in tufts (Fig.1a) type while the even felted mat type was observed only in isolates obtained from *C. frutescens* (Fig.1b).

The elevation of the colonies also differed among the isolates. The highest elevation (>0.5 cm) was observed in the isolates obtained from *C. papaya, M. indica and F. religiosa* whereas the *P. americana* colonies showed the least elevation (<0.3 cm). The highest average growth rate ranged between 0.79 – 1.1 cm/ day was shown by isolates obtained from *C. papaya* whereas the least value ranged between 0.56 – 0.7 mm/day was shown by *M. indica* isolates.

Conidial morphology: The conidia shape was basically cylindrical with obtuse ends (Fig. 2a), except in isolates obtained from *H. brasiliensis* that consisted of few cylindrical conidia with obtuse ends and narrowed centre (Fig. 2b). The conidia lengths varied from 11.92 – 15.67µm among isolates whereas the conidia widths were more or less similar in most isolates. The highest mean conidia length (15.67 ± 0.8 µm) was in the isolates obtained from *F. religiosa* where *G. cingulata*, the teleomorphic state being the causal agent of the leaf spot disease. The least mean conidial length (11.92 ± 0.5µm) was recorded by isolates obtained from *H. brasiliensis.*

Characters of appressoria: During the present study all the isolates produced appressoria at the end of the germ-tube. The intensity of brown colour of the appressoria of some isolates increased drastically with time due to melonization. The colour of appressoria at the end of the incubation period was evaluated since under laboratory conditions *C. gloeosporioides* has proved to form three types of appressoria as hyaline, slightly pigmented and melanized (Estrada *et al.*, 2000). In this study the most intense brown colour was shown by *M. indica* isolates whereas the faintest brown colour was shown by the *P. americana* isolate. However, non of the isolates produced hyaline appressoria.

Table 1. Combinations of cross inoculation of isolates of *P. americana, H. brasiliensis* and *M. indica* onto the commodities *P. americana, H. brasiliensis, M. indica* and *C. papaya.*

Isolate/Commodity	*P. americana* (A)	*H. brasiliensis* (R)	*M. indica* (M)	*C. papaya* (P)
CgA[a]	CgAA	CgAR	CgAM	CgAP
CgR[b]	CgRA	CgRR	CgRM	CgRP
CgM[c]	CgMA	CgMR	CgMM	CgMP

[a] *C. gloeosporioides* of *P. americana,* [b] *C. gloeosporioides* of *H. brasiliensis,*[c] *C. gloeosporioides* of *M. indica*

Table 2. **List of characters used for the phenetic analysis of the phytopathogen *C. gloeosporioides.***

Character	Character states
Colony characters	
1. Rate of colony growth per day	0.5 – 0.69 mm/day; 0.7- 0.99mm/day; > 1.0 mm/day
2. Colony colour at the upper surface	Intense white; White; Off white; Moderate blackish ash; Slightly intense blackish ash; Highly intense blackish ash
3. Sequence of colony colour change on the upper surface	Absent; Present
4. Colony colour at the lower surface	Intense white; White; Off white; Moderate blackish ash; Slightly intense blackish ash; Highly intense blackish ash
5. Sequence of colour change on the lower surface	Absent; Present
6. Nature of the colony margin	Entire; Other
7. Elevation of the colony	< 0.3 cm; 0.31 – 0.5 cm; >0.51 cm
8. Sectoring	Absent; Present
9. Nature of the mycelium	Dense aerial; Even felted mat; sparse aerial mycelium in tufts
Conidial characters	
10. Length of conidia (μm)	<12.00 μm; 12.01 – 13.99 μm; >14.00 μm
11.Width of conidia (μm)	1-3.5 μm; >3.6 μm
12. Production of conidial masses	Absent; Present
13. Shape of conidia	Cylindrical with obtuse ends; Other
Appressorium characters	
14. Length of appressoria (μm)	7.9 – 8.99 μm; 9.0 – 11.2 μm
15.Width of appressoria (μm)	5.0 - 6.0μ m; >6.1 μm
16. Shape of appressoria	Lobed; Ovate; Round
17. Colour of appressoria	Ash; Blackish ash; Brown; Pale blackish ash

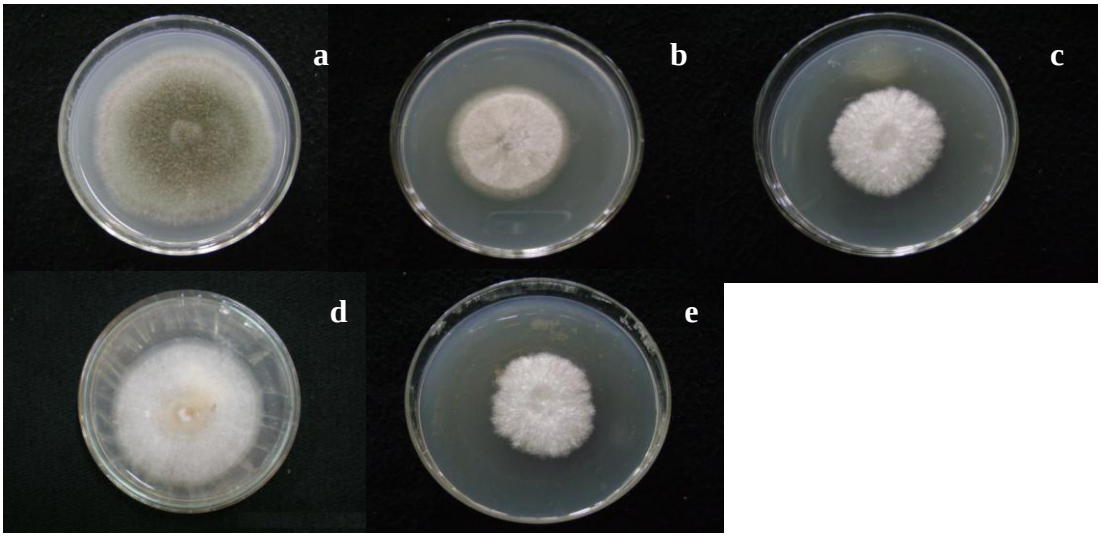

Figure 1. Monoconidial isolates of *C. gloeosporioides* obtained from (a) *C. papaya*, (b) *C. frutescens*, (c) *F. religiosa*, (d) *H. brasiliensis* and (e) *M. indica.*

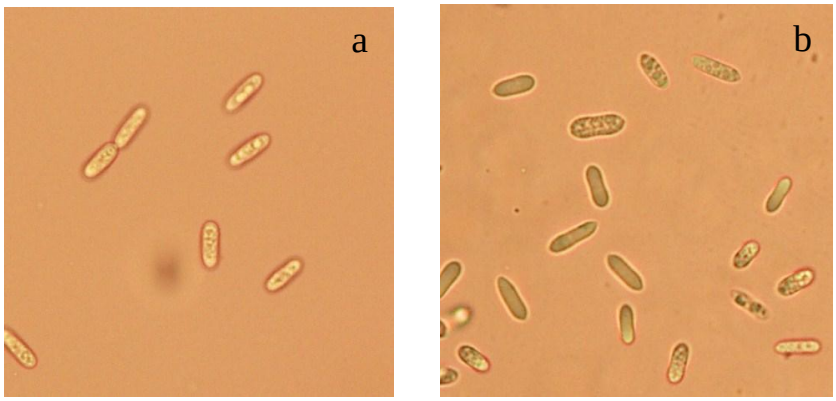

Figure 2. Different conidia types observed among *C. gloeosporioides* isolates obtained from *H. brasiliensis*: (a) cylindrical conidia with obtuse ends and (b) cylindrical conidia narrowing at the centre (x 400).

Several differences were observed in isolates with respect to the morphology of appressoria. Most of the isolates consisted of lobed or round appressoria (Figs.3a and b). Ovate type of appressoria were produced only with *C. frutescens* isolate (Fig. 3c). The appressoria dimensions ranged from 5.0-6.8 µm in width and 7.37 - 11.05 µm in lengths respectively, which is consistent with those of Du *et al*. (2005).

Cross inoculation studies: *C. gloeosporioides* isolates when inoculated developed the largest lesions (8.5-7.4 mm diameter) on *H. brasiliensis* leaves, whereas the smallest lesions (4.1 mm) were observed in fruits of *P. americana* (Fig. 4). Isolates obtained from infected *P. americana*, *M. indica* and *H. brasiliensis* produced lesions on all three hosts, *C. papaya*, *M. indica* and *H. brasiliensis.* In contrast, only the isolate obtained from *P. americana* could produce the characteristic anthracnose lesions on its original host, *P. americana*.

Phenetic analysis: Phenetic analysis includes numerical evaluation of the affinity/similarity between taxonomic units and ordering of these units into taxa on the basis of their affinities (Mayr, 1965).

Cluster analysis: The phenogram obtained from the phenetic analysis of morphological and physiological data is shown in Fig. 5.

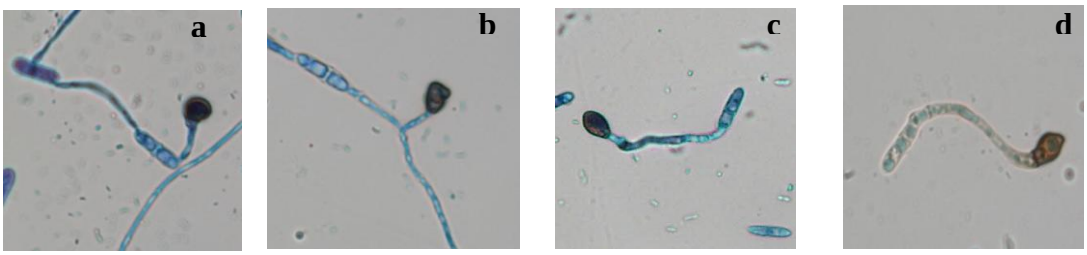

Figure 3. Appressoria of *C. gloeosporioides* isolates from (a) *M. indica*, (b) *C. papaya*, (c) *H. brasiliensis* and (d) *G. cingulata* isolate from *F. religiosa* (10 x10 x 40).

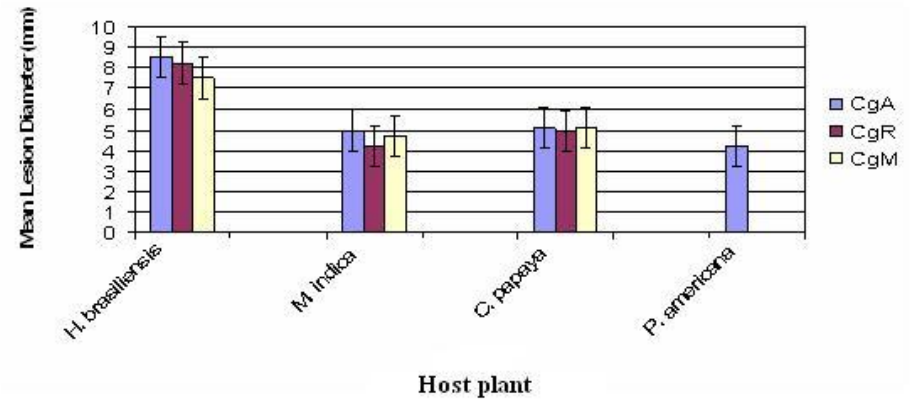

Figure 4. Mean lesion diameter on different crops following cross inoculation with *C. gloeosporioides* isolates.

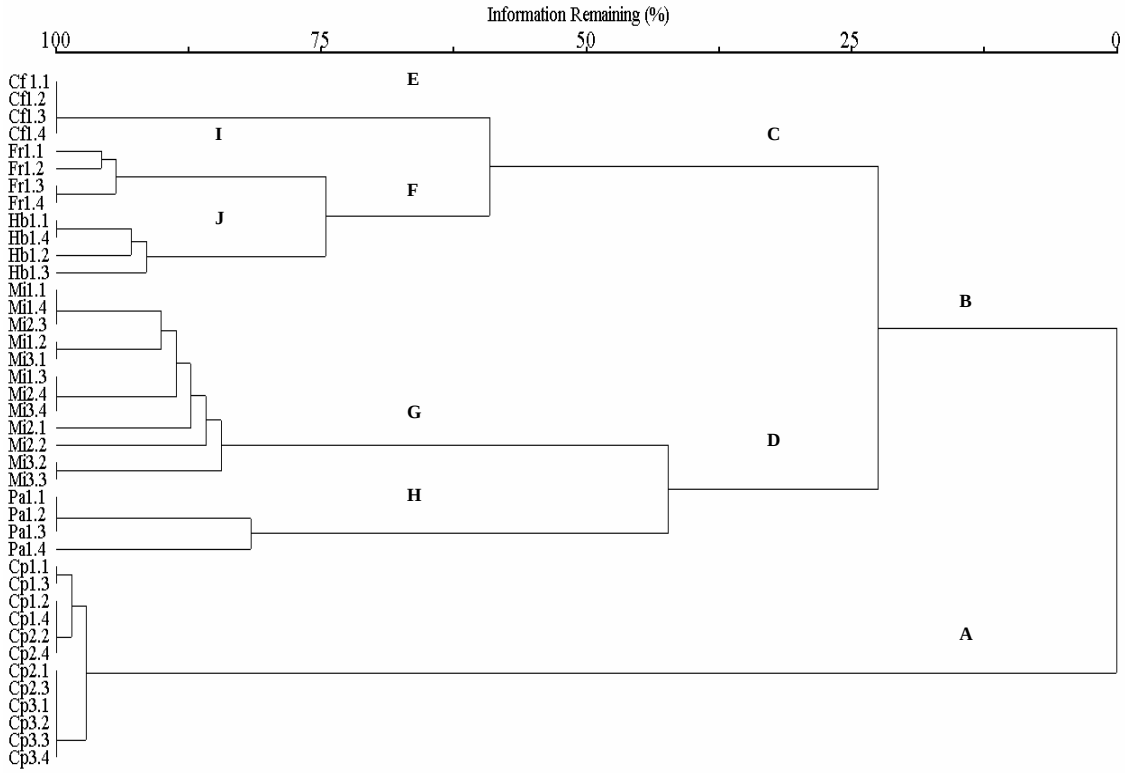

Figure 5. Dendrogram obtained following phenetic analysis of different isolates of *C. gloeosporioides*.

The 40 isolates separated into two distinct groups at the initial stage, separating *C. papaya* isolates (cluster A) from the rest of the isolates (cluster B). The formation of such a distinct groups may have resulted from the considerable morphological diversity that these isolates exhibited compared to other isolates. For example, wooly nature of the mycelium and intense blackish ash colour at the lower surface were observed only in these isolates. Further, conidia length of more than 13.99 μm was recorded only from *C. papaya* isolates. A previous study conducted with molecular data has also obtained similar results supporting the separation of *Colletotrichum* isolates from different hosts (Mills *et al*., 1992b).

Considering the 25% similarity level, two major phenetic groups can be identified where cluster C includes isolates from *C. frutescens, F. religiosa* and *H. brasiliensis* while the other (cluster D) includes isolates from *M. indica* and *P. americana*. The unity of the isolates of *C. frutescens, F. religiosa* and *H. brasiliensis* indicates the presence of a high degree of similarity among them. These isolates share similar growth rates (0.7 - 0.99 mm/day) and similar colony colour (off white) at the lower surface.

On the other hand, the isolates from *M. indica* and *P. americana* were grouped together to form cluster D, suggesting a close morphological similarity between these two sets of isolates. This may be due to the similarity among these isolates in colony colour at the upper surface which was of intense white colour, absence of sequence of colony colour change, conidia length (>13.99 μm) and width (<3.5 μm).

Cluster C has further separated into two groups at around 60% similarity level separating *C. frutescens* isolates (cluster E) from those of *F. religiosa* (cluster F) and *H. brasiliensis. C. frutescens* isolates consisted of white coloured, even felted mat of mycelium while the other two isolates consisted of moderate blackish ash and dense aerial mycelium. Further, in C. *frutescens* isolates the sequence of colour change at the upper surface was not observed unlike the other two sets of isolates. These features may have led to the separation of *C. frutescens* isolates, forming a distinct cluster.

Similarly, cluster D has separated into two groups at 40% similarity level, separating isolates of *P. americana* (cluster H) and *M. indica* (cluster G). *P. americana* isolates had moderate blackish ash colour lower colony surface whereas in *M. indica* the lower surface colony colour was off white. In addition, there were differences in the colonial elevation, colour of appressoria etc. between these two sets of isolates.

However, it is well established that the *C. gloeosporioides* isolates from *M. indica* show low levels of genetic variability in contrast to *C. papaya* and *P. americana* isolates. Both RFLP analysis of rDNA and Polymerase Chain Reaction (PCR) amplification of variable intergenic spacer region of the rDNA cluster have indicated that *C. gloeosporioides* isolates from *M. indica* do not exhibit geographically related groupings (Mills *et al*., 1992a, 1992b). This may be either due to the introduction of the pathogen from a single source at wide scale or due to the selection pressure towards a single pathogen type.

Cluster F has two groups around 75% similarity level separating isolates of *F. religiosa* (cluster I) and *H. brasiliensis* (cluster J) from each other. The elevation of *F. religiosa* colonies was more than 0.5 mm while the elevation of *H. brasiliensis* isolates was 0.31-0.5mm. Further, the appressoria colour of *F. religiosa* isolates was brown whereas in *H. brasiliensis* it was blackish ash. The origin of clusters I and J may have been due to these features.

Colletotrichum gloeosporioides isolates from different hosts have formed distinct groups within the phenogram. This may be due to the unique morphological and physiological character combinations which the different isolates possessed. This is in agreement with the findings of an earlier study of ribosomal DNA (rDNA) of *C. gloeosporioides* isolated from diverse hosts including *P. americana, M. indica, C. papaya* and *Hevea brasiliensis* covering different regions of the world, using RFLP (Mills *et al*., 1992b). The resulting rDNA repeat unit sizes were more or less similar for one particular host species. Such grouping is believed to be due to the separate evolution of *C. gloeosporioides* populations due to either ecological barriers or the rare sexual reproduction of this fungus. Both these situations are expected to limit the mixing of genes of distinct populations (Mills *et al*., 1992b). Identification of this type of grouping is

important not only for the development of disease management practices but also for the investigation of resistant cultivars via plant breeding programmes (Manners *et al*., 1992). Further, the host specificity can be used for the benefit of field of agriculture. For example, *C. gloeosporioides* f.sp. *malvae* which is host specific has been used for biological control of *Malva pussila*, which is a common farmyard and green house weed (Mortensen, 1988). However, it has also been proved that the isolates obtained from the same host are not identical but there are variations among them with respect to biochemical and molecular characteristics (Maymon *et al.*, 2006).

Despite forming distinct host specific groupings, *C. gloeosporioides* has the ability to cause cross infections (Freeman and Shabi, 1996). The cross infection potential of *C. gloeosporioides* has been studied under laboratory conditions (Alahakoon *et al*., 1994; Freeman and Shabi, 1996) where *C. gloeosporioides* isolates were found more virulent on the host which they were originally isolated from. The success of cross infection is proved to be dependant on the genetic adaptability of the fungus once in contact with a new host as well as the cultivar variety of the host specie. Crops such as avocado, mango, papaya etc. are often grown in mixed plantations. Therefore, it is important to have a sound knowledge of the host range and the cross inoculation capability of the fungus under natural conditions (Freeman *et al.*, 1998).

Principal component analysis:

Principal component analysis was carried out using the data matrix resulting from the phenetic analysis. Different combinations of the first six dominant eigenvectors were examined. The first three axes together explain 77.74% of the total variance (Table 3). Upon consideration of the eigenvector values, the characters such as colony colour both at upper and lower surfaces, nature of the mycelium, colour of appressoria and average growth rate per day appear to contribute substantially towards the resolution among different isolates.

The best dispersion is given by the first two axes, which together explain 64.75% of the total variation (Fig. 6). The horizontal axis supports the separation of isolates obtained from *C. papaya* and *M. indica* from each other whereas, the vertical axis exhibits separation of isolates of *H. brasiliensis* from those of *F. religiosa* further strengthening the data obtained from the cluster analysis.

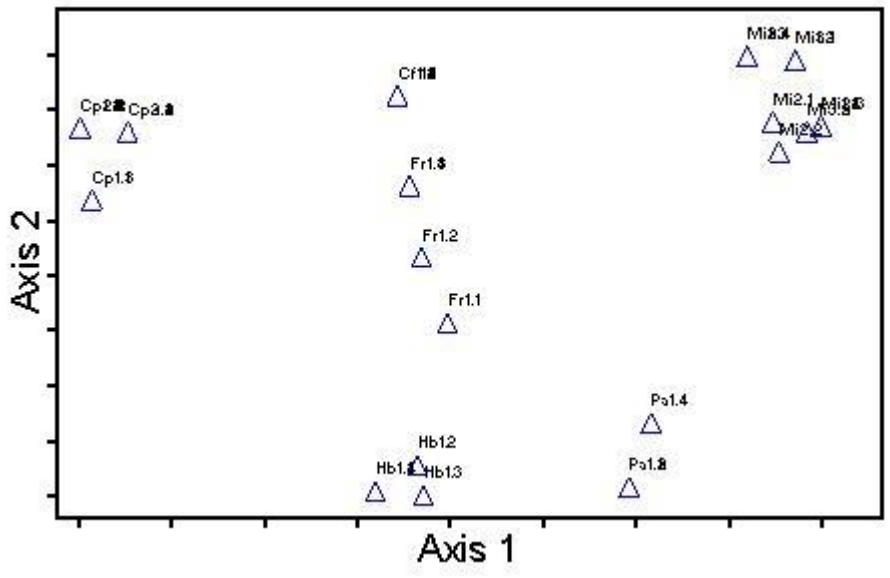

Figure 6. Scatter plot resulting from data for phenetic analysis of the pathogen *C. gloeosporioides* (Cp = *C. papaya,* Cf = *C. frutescens*, Fr = *F. religiosa,* Hb = *H. brasiliensis*, Mi = *M. indica* and Pa =*Persea americana*).

Table 3. Loadings of first three principal components from principal component analysis of data for phenetic analysis of *C. gloeosporioides.*

Principal component (PC)	PC1	PC2	PC3
Eigenvalue	7.023	2.057	1.804
Cumulative % variance	50.163	64.855	77.744
Character 1	-0.2881	0.0197	0.0666
Character 2	-0.3338	-0.1059	-0.0304
Character 3	-0.3126	-0.1339	0.0894
Character 4	-0.3041	0.0941	0.356
Character 5	-0.315	0.1843	0.2986
Character 7	-0.047	-0.5777	0.3266
Character 9	-0.3294	0.2427	0.1068
Character 10	0.2478	0.1564	0.2828
Character 11	0.3349	0.0355	0.2711
Character 13	-0.3032	-0.3154	-0.1433
Character 14	-0.128	0.3193	0.4895
Character 15	0.0539	-0.3025	0.3238
Character 16	-0.2476	0.2511	-0.3641
Character 17	0.2435	0.3915	0.0401

CONCLUSION

The results of the phenetic analysis suggest a high degree of similarity among *C. gloeosporioides* isolates obtained from different hosts and the presence of host specific populations. Isolates of *C. papaya* form a distinct population well separated from the other isolates. Isolates from *Capsicum frutescens*, *H. brasiliensis* and *F. religiosa* exhibit similar morphological characters, while isolates of *P. americana* and *M. indica* share similar morphological features. The study reveals the cross infection potential of *C. gloeosporioides* isolates obtained from different host species, *P. americana. M. indica, C. papaya* and *H. brasiliensis*.

REFERENCES

Alahakoon, P.W., Brown, A.E. and Sreenivaprasad, S. (1994). Cross infection potential of genetic groups of *Colletotrichum gloeosporioides* on tropical fruits. *Physiological and Molecular Plant Pathology* **44:**93-103.

Bailey, J. A., O'Connell, R. J., Pring, R. J., and Nash, C. (1992). Infection strategies of *Colletotrichum* species. In: *Colletotrichum*: Biology, Pathology and Control. Bailey, J. A. and. Jeger, M. J., eds. CAB International, UK 88-120.

Du, M., Scardl, C.L., Nuckles, E.M. and Vaillancourts, L.J. (2005). Using mating-type gene sequences for improved phylogenetic resolution of *Collectotrichum* species complexes. *Mycologia* **97:**641-658.

Estrada, A.B., Dodd, J.C. and Jeffries, P. (2000). Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides. Plant Pathology* **49:**608-618.

Freeman, S., Talma, K. and Shabi, E. (1995). Characterization of *Colletotrichum gloeosporioides* isolates from avocado and almond fruits with molecular and pathogenicity Tests. *Applied and Environmental Microbiology* **62:**1014-1020.

Freeman, S. and Shabi, E. (1996). Cross-infection of subtropical and temperate fruits by *Colletotrichum* species from various hosts. *Physiological and Molecular Plant Pathology* **49:** 395-404.

Freeman, S., Talma, K. and Shabi, E. (1998). Characterization of *Colletotrichum* species responsible for anthracnose disease in various fruits. *Plant Disease* **82:** 596- 605.

Gunnell, S.P and Gubler, D.W. (1992). Taxonomy and Morphology of *Colletotrichum* species pathogenic to strawberry. *Mycologia* **84:**157-165**.**

Hong, S.K., Kim W.G., Yun, H.K. and Choi, K.J. (2008). Morphological variation, genetic diversity and pathognicity of *Colletotrichum* species causing grape ripe rot in Korea. *Plant Pathology* **24:** 269-278.

Manners, J.M., Masel, A., Braithwaite, K.S. and Irwin, J.A. (1992). Molecular analysis of *Colletotrichum gloeosporioides* pathogenic on the tropical pasture legume *Stylosanthes.* In: *Colletotrichum* Biology, pathology and control. Bailey, J.A. and Jeger M.J., eds. CAB International, UK. 250-264.

Maymon, M., Zveibil, A., Pivonia, S., Minz, D., and and Freeman, S. (2006). Identification and characterization of benomyl-resistant and –sensitive populations of *Colletotrichum gloeosporioides* from statice (*Limonium* spp.). *Phytopathology* **96:**542-548

Mayr, E. (1965). Numerical Phenetics and Taxonomic Theory. *Systematic Zoology* **14:**73-97.

Mills, P.R., Sreenivaprasad, S. and Brown, A.E. (1992a). Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. *FEMS Microbiology Letters* **98:** 137-143.

Mills, P.R., Hodson, A. and Brown, A.E. (1992b). Molecular differenciation of *Colletotrichum gloeosporioides* isolates infecting tropical fruits. In: *Colletotrichum* Biology, pathology and control. Bailey, J.A. and Jeger M.J., eds. CAB International, UK. 269-287.

Mortensen, K. (1988). The Potential of an Endemic Fungus, *Colletotrichum gloeosporioides*, for Biological Control of Round-Leaved Mallow (*Malva pusilla*) and velvet leaf (*Abutilon theophrasti*). *Weed Science* **36:**473-478.

Sutton, B.C. (1992). The Genus *Glomerella* and its Anamorph *Colletotrichum.* In: *Colletotrichum* Biology, pathology and control. Bailey, J.A. and Jeger M.J., eds. CAB International, UK. 1-11.

Von Arx, J. A. (1957). Die Arten der Gattung *Colletotrichum* Cda. *Phytopathologische Zeistchrift .Z.* **29:**413- 468.