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Existence of two genetically distinct populations of *Colletotrichum gloeosporioides* Penz in mango from India

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ABSTRACT: Seventy nine isolates of *Colletotrichum gloeosporioides* representing all mango growing areas in India were collected and characterized based on morphological, species- specific PCR and sequence analysis of the internal transcribed spacer (ITS) region of r DNA. Based on cultural and morphological criteria of *Colletotrichum gloeosporioides*, 79 isolates were categorized into two groups designated respectively as slow growing grey (SGG) and fast growing grey type (FGG). Genetic variation, revealed by sequence analysis of ITS region and ITS RFLP patterns, were found between two morphological groups. Phylogenetic analysis based on the sequences of ITS region clearly separated into two distinct clusters. Pathogenicity assays on detached fruits of five varieties of mango confirmed the apparent difference between the groups of isolates. It is therefore concluded that the pathogen responsible for mango fruit rot anthracnose in India, belongs to two sub populations of *C. gloeosporioides*. The implications of these findings for *C. gloeosporioides* induced anthracnose control strategies and resistance breeding are discussed.

Keywords : Anthracnose, *Colletotrichum*, ITS- RFLP, mango, PCR

INTRODUCTION

Mango (*Mangifera indica* L.) is native to India and South East Asia. It is grown throughout tropics and subtropics worldwide and is being promoted as cash crop for small holders (Arauz, 2000). India is the leading producer of mango, accounting 51 per cent of world production in an area of 11,36,668 ha (FAO, 1999; Arauz, 2000). Anthracnose of mango, caused by *Colletotrichum* Species, is the most serious and wide spread disease throughout production areas, leading to substantial pre and post harvest losses (Fitzell *et al.*, 1984; Jeffries *et al.*, 1990; Prusky, 1996; Arauz, 2000). The disease is severe on young leaves, inflorescences, fruits and even causes tree dieback (Jeffries *et al.*,1990; Arauz, 2000).The post harvest phase on fruits is the most economically significant throughout the world (Arauz,, 2000). The markets demand very high quality fruit and even low levels of anthracnose on fruits make it unsaleable (Prusky,1996). *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (teleomorph *Glomerella cingulata* Stonem.(Spauld and Schrenk) is the most important pathogen on mango (Fitzell *et al.*,1984; Jeffries *et al.*,1990; Prusky,1996) although *C. gloeosporioides* Penz var *minor* Simmonds (Fitzell and Peak, 1984) and *C. acutatum* Simmonds (Fitzell, 1979) are also recorded.

C. gloeosporioides is considered cumulative species composed of diverse subpopulations (Peres *et al.*, 2002; Sutton, 1992; Free man *et al.*,1998; Vinnere *et al.*, 2002). Different levels of genetic variation according to host have been widely reported for *C. gloeosporioides* (Hodson *et al.*,1993; Sreeenivasaprasad *et al.*, 1993; Hayden *et al.*, 1994; Freeman *et al.*, 1998). Two distinct populations were evident among the isolates of *C. gloeosporioides* on tropical pasture legume (Braithwaithe *et al.*, 1990 a,b ; Munaut *et al.*,1988; Masel *et al.*,1996) and citrus (Liyanange *et al.*,1993). Polymorphisms have also been recorded when representative isolates of *C. gloeosporioides* from almond, apple, avocado and mango were compared based on mt DNA-RFLP (Freeman and Shabi, 1996; Freeman *et al.*, 1996,2000). For effective disease control strategies and development and deployment host of resistance, information on the distribution of pathotypes/ races in an area is prerequisite. Due to lack of reliable morphological criteria and differential host sets for such studies in *C. gloeosporioides*, DNA based molecular markers are being used currently to characterize and analyze the taxonomic complexities in *Colletotrichum* species and to measure the variability among the individual species (Sreenvasaprasad *et al.*, 1996; Lardener *et al.*, 1999; Freeman *et al.*, 2001; Talhinhas *et al.*, 2005; Schiller *et al.*, 2006).Combined application of molecular diagnostic tools and along with host reactions could be an appropriate and reliable approach for measuring variability in *C. gloeosporioides* isolates.

The objective of the present study was to determine whether diversity exits within species of *C. gloeosporioides* infecting mango in India using morphological criteria, species-specific PCR, restriction endonuclease digest analysis of PCR- amplified ITS region of r DNA, ITS sequencing and pathogenicity assays.

MATERIALS AND METHODS

Collection, isolation and maintenance of isolates

A total of 79 isolates of *Colletotrichum* were recovered from the mango fruit samples showing typical symptoms of anthracnose during May and June of 2003, 2004 and 2005 from different mango production areas in Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra and Uttar Pradesh States of India (Table 1). For isolation, 10-15 lesion bits (2 x 2mm) were cut from each sample and washed with sterile distilled water ,then surface sterilized with 0.1per cent mercuric chloride solution for 30 s to 1 min and again rinsed with sterile distilled water and these surface sterilized bits were aseptically transferred onto potato dextrose agar plates (PDA) containing 100 ppm streptocycline and incubated at 25 ± 1°C in dark 7 days. Monoconidial cultures were obtained through single spore isolation technique. The single spored cultures were maintained in PDA slants at 25 ± 1°C in dark under the paraffin oil.

Table 1. Geographical origin of *Colletotrichum* isolates

Isolate	Year of Isolation	Source	Location	Genetic Group
CM 1	2004	Fruit	Hessaraghatta, Bangalore, Karnataka	I
CM 2	2004	Fruit	Hirehalli, Tumkur, Karnataka	I
CM 4	2004	Fruit	Nagadasanahalli, Bangalore, Karnataka	I
CM 6	2004	Fruit	Ramanagar, Krishnagiri Tamilnadu	I
CM 10	2004	Fruit	Ramanagar, Krishnagiri, Tamilnadu	I
CM 11	2004	Fruit	Hyderabad, Andhra Pradesh	I
C M12	2004	Fruit	Hyderabad, Andhra Pradesh	I
CM 13	2004	Fruit	Krishnagiri, Tamilnadu	I
CM 14	2004	Fruit	Nagadasanhalli, Bangalore, Karnataka	I
CM 17	2004	Fruit	Hosur, Dharmapuri,Tamilnadu	I
CM 18	2004	Fruit	Krishnagiri, Tamilnadu	I
CM 19	2004	Fruit	Hosur, Tamilnadu	I
CM 20	2004	Fruit	Kaveripatnam, Tamilnadu	I
CM 21	2004	Fruit	Kaveripatnam, Tamilnadu	I
CM 22	2004	Fruit	Hosur, Tamilnadu	I
CM 23	2004	Fruit	Krishnagiri, Tamilnadu	I
CM 24	2004	Fruit	Ramandoddi, Tamilnadu	I
CM 25	2004	Fruit	Hosur, Tamilnadu	I
CM 27	2004	Fruit	Krishnagiri, Tamilnadu	I
CM 28	2004	Fruit	Phianur, Tamilnadu	I
CM 32	2004	Fruit	—	I
CM 33	2004	Fruit	Hosur, Tamilnadu	I
CM 34	2004	Fruit	Hebbur, Tumkur, Bangalore, Karnataka	I
CM 36	2004	Fruit	Jadere,(Srinivasapur),Kolar, Karnataka	I
CM 37	2004	Fruit	Mulbagal, Kolar, Karnataka	I
CM 38	2004	Fruit	Jamanahalli(Mulgagal) , Kolar, Karnataka	I
CM 39	2004	Fruit	Doddamaladoddi(Srinivasapur), Kolar, Karnataka	I

CM 40	2004	Fruit	Hobepalli(Srinivasapur), Kolar, Karnataka	I
CM 41	2004	Fruit	Sundarapalya (Srinivasapur), Kolar, Karnataka	I
CM 42	2004	Fruit	Tamanahalli(Mulgagal), Kolar, Karnataka	I
CM 43	2004	Fruit	Hebata (Srinivasapur), Kolar,Karnataka	I
CM 44	2004	Fruit	Lakshmisagara, Bangalore, Karnataka	II
CM 45	2004	Fruit	Bandapalli (Srinivasapur), Kolar,Karnataka	I
CM 46	2004	Fruit	Immarakunta (Srinivasapur) Kolar, Karnataka	I
CM 47	2004	Fruit	Hebata (Srinivasapur), Kolar,Karnataka	I
CM 48	2004	Fruit	Dhimbla (Srinivasapur), Kolar, Karnataka	I
CM 49	2004	Fruit	Ghattapalli(Srinivasapur), Kolar,Karnataka	I
CM 50	2004	Fruit	Nimakayapalli(Chintamani), Kolar,Karnataka	I
CM 51	2004	Fruit	Ronur(Srinivasapur), Kolar,Karnataka	I
CM 52	2004	Fruit	Yeldur(Srinivasapur) Kolar, Karnataka	I
CM 53	2004	Fruit	Ariyakunta(Srinivasapur), Kolar, Karnataka	I
CM 54	2004	Fruit	Hebata (Srinivasapur), Kolar, Karnataka	I
CM 55	2004	Fruit	Gaddur (Mulgagal), Karnataka	I
CM 56	2004	Fruit	Srinivasapur, Karnataka	I
CM 57	2004	Fruit	Bandapalli(Srinivasapur), Kolar, Karnataka	I
CM 58	2004	Fruit	Kalluru(Srinivasapur), Kolar, Karnataka	I
CM 59	2004	Fruit	Muthakapalli(Srinivasapur), Kolar, Karnataka	I
CM 60	2004	Fruit	Imarakunta(Srinivasapur), Kolar, Karnataka	II
CM 62	2004	Fruit	Misganahalli(Srinivasapur), Kolar, Karnataka	I
CM 63	2004	Fruit	Chowdanahalli(Srinivasapur), Kolar, Karnataka	I
CM 64	2004	Fruit	Bandapalli(Srinivasapur), Kolar, Karnataka	I
CM 65	2004	Fruit	Thadagolgarma (Srinivasapur), Kolar, Karnataka	I
CM 69	2004	Fruit	Doddaballapur, Bangalore, Karnataka	II
CM 70	2004	Fruit	Devanahalli, Bangalore, Karnataka	I
CM 71	2004	Fruit	Lucknow, Uttar Pradesh	I
CM 72	2004	Leaf	Rehmankhera, Uttar Pradesh	I
CM 73	2004	Leaf	Lemoor, Ranga reddy, Andhra Pradesh	I
CM 74	2004	Leaf	Edualpally, Mehaboobnagar, Andhra Pradesh	I
CM 75	2004	Fruit	Nagulapalli, Mehaboobnagar, Andhra Pradesh	I
CM 76	2004	Fruit	Mekhaguda, Mehaboobnagar, Andhra Pradesh	I
CM 77	2004	Fruit	Guttapalli, Ranga reddy, Andhra Pradesh	I
CM 78	2005	Fruit	Tumkur, Karnataka.	I
CM 79	2005	Fruit	Irala, Andhra Pradesh	I
CM 81	2005	Fruit	Mallagunta, Andhra Pradesh	I
CM 82	2005	Fruit	Gudipala, Andhra Pradesh	I
CM 83	2005	Fruit	Penumur, Andhra Pradesh	I
CM 84	2005	Fruit	Gudiatum, Tamilnadu	I
CM 85	2005	Fruit	Bangarupalyam, Andhra Pradesh	I
CM 86	2005	Fruit	Tavanampalli, Andhra Pradesh	I
CM 87	2005	Fruit	Madanapalle, Andhra Pradesh	I

CM 88	2005	Fruit	Pileru, Andhra Pradesh	I
CM 89	2005	Fruit	Bangarupalyam, Andhra Pradesh	I
CM 93	2005	Fruit	Bangarupalyam, Andhra Pradesh	I
CM 94	2005	Fruit	Ramakuppam, Andhra Pradesh	I
CM 96	2005	Fruit	Rajanakunte, Bangalore	II
CM 97	2006	Leaf	Dasanapura, Bangalore	II
CM 98	2006	Leaf	Nuzuvid, Andhra Pradesh	I
CM 100	2006	Leaf	Kondapura, Andhra Pradesh	I
CM 104	2006	Twig	Vijayawada, Andhra Pradesh	I

Morphological examination

For morphological examination, petri plates with PDA were inoculated with mycelial disks, 5mm diam, taken from margin of 4-5 day old cultures grown in dark at 25 ± 1°C. Colony diameter (three replicates and four measurements) and morphological characteristics (texture, density, zonation, margin, presence of conidial masses and colour on both sides) were recorded from the cultures grown on PDA under the same conditions mentioned above. The length and breadth of 100 conidia for each isolate were measured at x 400 magnification under Zeiss bright field microscope using Axio Imager A1 soft ware. The length / breadth ratio was determined and the shape was recorded.

Species- specific PCR

Total fungal DNA was extracted from mycelia obtained from 100 ml of potato dextrose broth for 4- 6 days at 25 ±1°C. Mycelium was harvested from liquid cultures by filtration through whatman no.3 filter paper and damp dried and subsequently ground into a fine powder in liquid nitrogen. DNA was extracted from the frozen mycelial powder employing a slightly modified method of Raedor and Broda (1985) by incubating at 37°C for 10 min after the phenol: chloroform: isoamyl alcohol(25:24:1) precipitation. This was followed by precipitation with 0.54 volumes of isopropyl alcohol and centrifugation at 10,000 g for 2 min. The DNA pellet was washed with 70% cold ethanol, dried at room temperature overnight (16hr) and then pellet was re-suspended in 30 µl 10mM TE buffer (pH.8).DNA was stored at -20°C.

Diagnostic PCR with species-specific primers

ITS region of r DNA of fungal isolates were amplified by PCR with universal primer pair ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC (White *et al.*,1990) and with specific primer to *C.gloeosporioides* CgInt, (GGCCTCCCGCCTCCGGGCGG) (Mills *et al.*, 1992) together with ITS4. PCR reactions (50µl) were performed with primer pair ITS1/ITS4 and species- specific primer/ ITS4 in Eppendorf master cycler by 34 cycles of denaturation at 94°C for 60s, annealing at 55°C for 60s, and extension at 72°C for 1.5 min with an initial denaturation of 5 min at 94°C before cycling and final extension of 5 min at 72°C after cycling. Amplified PCR products were separated in 2 % agarose gels in Tris-Borate- EDTA(TBE) buffer and visualized under UV after staining with ethidium bromide (0.5µg/ml).

Restriction enzyme digestion of amplified r DNA

Amplified PCR product was digested with different restriction enzymes namely *Alu I, Msp I, Rsa I, Pvu II, Hinf I, Taq I* and *Tru II.* Each 20 µl reaction mixture contained 10µl PCR product, 0.6 units enzyme, 2µl 10x enzyme buffer and remaining sterile distilled water was added. The digestion was carried out overnight at 37°C (except *Taq* I, *Tru* II at 65°C) in a water bath. Restricted products were analyzed with 2.5% Agarose gel and were observed with UV Transluminator with ethidium bromide staining. The size of the restriction fragments was estimated by comparison with known DNA marker (50 bp molecular DNA ladder).

Sequencing of the ribosomal ITS region

Amplified ITS region of r DNA products (560bp) with primer pair (ITS 1 and ITS 4) were purified using PCR product purification kit KT 72 (Genei, Bangalore). The purified product (10-12 ng) was used for PCR cycle sequencing using Big Dye Terminator ready reaction Mixture kit (Applied Bio systems, USA) and analyzed with ABI 3100 analyzer capillary machines. The sequences from *C. gloeosporioides* on mango; Group I

CM17(JF796311), CM41(JF796329), CM88(JF796357) and CM89(JF796358) and Group II CM44(JF796332), CM60(JF796344), CM69(JF796352) and CM96 (JF796362) were deposited in the Genbank (www.ncbi.nlm.nih.gov).

Phylogenetic analysis

A multiple-sequence alignment was performed with similar reference sequences of other *Colletotrichum* isolates available in the Gene Bank database using CLUSTAL X (Thompson *et al.,* 1994) and a BLAST similarity test was also performed. The regions of sequence ambiguity and positions that was not available for all of the sequences compared were omitted before undertaking the phylogenetic analysis. Phylogenetic trees were constructed from the aligend sequences with original data set and 100 bootstrap data sets generated by the Clustal X. The trees generated for ITS region of r DNA sequences using *Alternaria alternata* as the out group sequences to allow the trees to be rooted. The final trees were displayed using TREEVIEW (Page, 1996) and NJ PLOT program.

Pathogenicity assays

Pathogenicity tests were performed with both morpho groups using detached and ripe fruits of five mango cultivars (Banganapalli, Raspuri, Rumani, Arka Anmol, and Totapuri). Inoculum was obtained from fungal colonies grown on PDA for 7 days at 25 ± 1°C in the darkness. Conidia were harvested and dispersed in sterile distilled water and concentration was adjusted to 5 x 10^5 conidia/ml. Fruits are washed with sterilized distilled water and surface disinfected by immersion for 30 s in (1%) sodium hypochlorite solution, followed by rinsing in sterile distilled water and inoculation by deposition of a 20-µl droplet of conidial suspension containing (1%) gelatin on the gently pin pricked fruit surface. The inoculated fruits with appropriate controls were placed in the sterilized tray containing moistened blotting paper and cotton plugs and covered with polythene sheet to maintain 100 percent relative humidity and kept at 25 ± 1°C and symptoms were recorded after 7 days of inoculation. The lesion area (mm^2) was calculated using π x l x w, where l and w are half length and width of the lesion respectively (Fagan, 1988).

RESULTS AND DISCUSSION

Morphological and cultural variability

All the isolates exhibited typical morphological criteria of *C. gloeosporioides*. Based on the differences on colony characteristics such as colony colour, growth, colony diameter and conidial shape and size, 79 isolates were categorized into two morphological groups. Type I cultures produced relatively slow growing (7.70 - 9.91 ± 2.28 mm/day) and light grey colonies (SGG) with appressed mycelia and contained majority of the isolates (Fig.1). Type II cultures grew faster (10.16 -16.70 ±2.49 mm/day) and produced typical gray and fluffy colonies(FGG) and consists of only five isolates(CM 44,60,69,96 and 97). The conidia of type I isolates were mostly rounded at both ends while conidia of type II isolates had mostly obtuse at the apex, tapered towards the truncate base although some conidia were rounded at both ends. The size of conidia of type I isolates were slightly lower (10.93 -13.21 ±1.49 µm) than that of type II isolates(12.61-15.12 ± 0.53 µm).

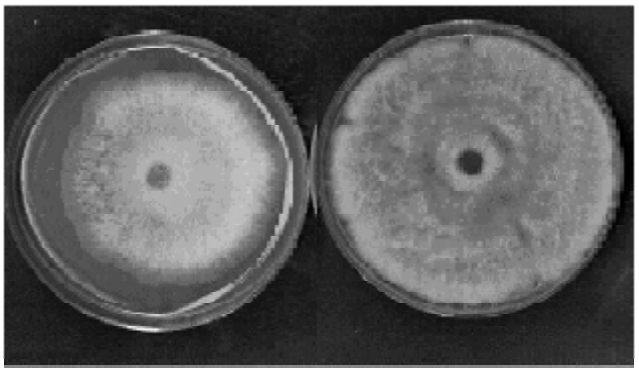

Fig. 1. Colony morphology of groups of *Colletotrichum gloeosporioides*

Molecular identification with species- specific PCR

All 79 isolates of *C. gloeosporioides* tested positive in PCR amplification of the ITS region of r DNA using *C. gloeosporioides* –species-specific primer Cglnt and the

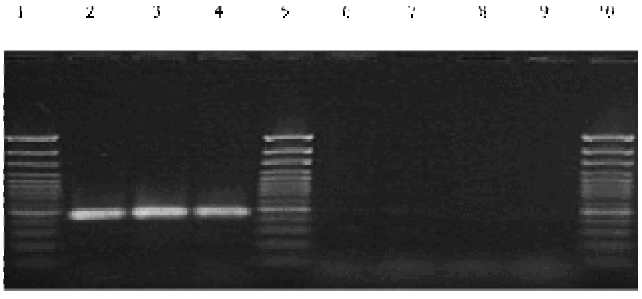

Fig. 2. PCR amplification of ITS region of ribosomal DNA of *Colletotrichum gloeosporioides* and *Gloeosporium* with primer pair Cglnt and ITS 4 Lane 1,5 & 10, marker (100 bp ladder); lane 2-4 *Colletotrichum gloeosporioides* CM 8,CM 50, CM 67 respectively ; lane 6, *Gloeosporium sp* , Lane 7, *Phomopsis sp* Lane 8, *Lasiodiplodia theobromae*, Lane 9, *Alternaria alternata*

ITS 4 primer pair and PCR products contained a single band of 450bp (Fig. 2). CgInt did not amplify a product from the DNA of *Gloeosporium, Phompsis, Lasiodiplodia theobromae* and *Alternaria alternata* isolates, commonly found on mango.

Polymorphism of r DNA ITS 1-2 restriction digests

Restriction endonuclease digest patterns of PCR – amplified r DNA ITS 1-2 regions were compared for the both groups of *C. gloeosporioides* that were identified by morphology. All the seven restriction enzymes such as *Alu* I, *Msp* I, *Rsa* I, *Pvu* II, *Hinf* I, *Taq* I, *Tru* II distinguished between both groups of *C. gloeosporioides* by recognizing unique restriction sites (Fig.3).

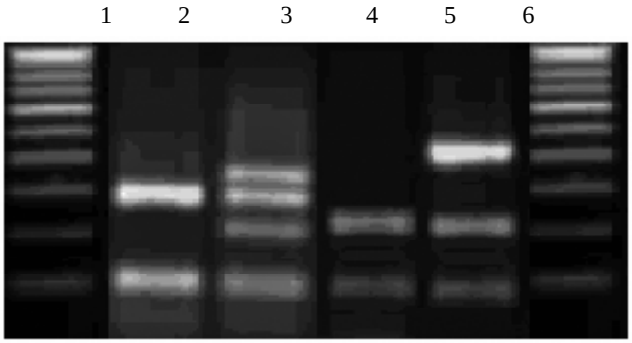

Fig. 3. Restriction digestion patterns of two subgroups (CM 8 and CM 60) based on ITS region of ribosomal DNA. Lane 2-3 *Msp I* and Lane 4-5 *Tru* II, Lane 1 and 6, marker (100 bp ladder); lane 2 and 4 CM 8, lane 3 and 5 CM 60.

Nucleotide sequencing of the ribosomal ITS 1 and ITS2 region

The BLAST similarity search confirmed the results obtained by the species- specific PCR analysis as the ITS sequences obtained from the *C. gloeosporioides* isolates shared sequence identity with published ITS sequences of *C. gloeosporioides*. Based on phylogentic trees generated using sequences of ITS 1and ITS 2 regions (Fig.4), two clusters were evident that corresponded to the two subgroups previously identified by morphological and restriction digestion patterns of ITS region. Sub group I contained 74 isolates and sub group II contained remaining five isolates (CM 44,60,69,96 and 97).

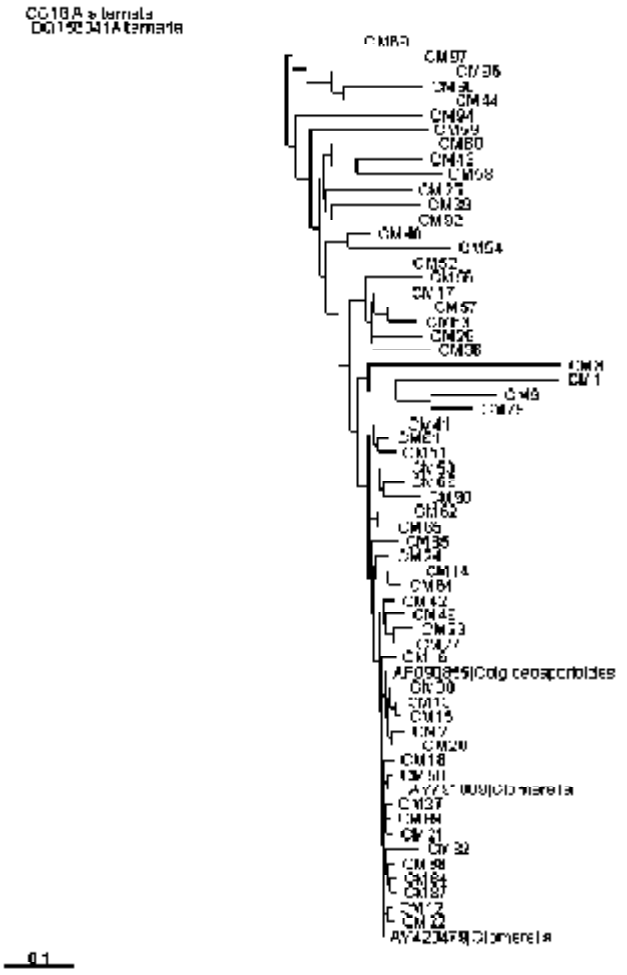

Fig. 4. Internal transcribed spacer 1 and 2 based phylogentic trees of *C. gloeosporioides* isolates and published sequences.

Pathogenicity assays

The isolates of *C. gloeosporioides* representing the molecular and phenotypic diversity observed above were

Table 2. Pathogenicity of *Colletotrichum* isolates on detached mango fruits

	Lesion area (mm^2)				
Isolate group	**Raspuri**	**Anmol**	**Rumani**	**Banganapalli**	**Totapuri**
CM 8	204.1 ± 6.20*	349.3 ± 8.30	23.55 ± 1.86	78.50 ± 2.56	101.96±3.80
CM 60	11.30 ± 3.00	21.58 ± 2.80	07.33 ± 1.32	06.90 ± 1.01	14.60±1.20

* Mean of 15 fruits ± SD, Three replications and five fruits for each replication.

tested for their pathogenicity on mango fruits. The slow growing and light grey isolates (SGG) were more virulent on irrespective of the mango variety tested compared to fast growing grey types (FGG) (Table.2). The SGG types produced dark brown/black sunken necrotic lesions with orange masses of conidia on abundant aerial white/grey mycelia while, FGG types incited pale brown with little aerial mycelium and conidial masses.

Our aim was to investigate the genetic structure and pathogenic diversity of Indian isolates of *C. gloeosporioides* from mango collected from different agroclimatic conditions. The overlapping morphology between isolates of *C. gloeosporioides* makes reliable distinction on the basis of morphology difficult. In this study, a combination of morphological characterization, molecular techniques and pathogenicity tests, allowed identification of groups within *C. gloeosporioides* that are responsible for mango anthracnose. Cultural characteristics such as colony colour and growth rate and conidial morphology revealed into two distinct types of isolates corresponding to *C. gloeosporioides* as has been observed with previous investigations of various hosts (Braithwaite *et al.,* 1990 a,b; Liyanage *et al.,* 1993; Thottappilly *et al.,* 1999; Talhinhas *et al.,* 2005). Further, application of species- specific PCR using primers based on ITS region of rDNA (Mills *et al.,* 1992) provided rapid and reliable diagnosis of isolates belonging to *C. gloeosporioides* from mango. Species- specific PCR using ITS region of r DNA has been widely advocated for rapid identification of *Colletotrichum* species (Free man *et al.,* 2001;Talhinhas *et al.,* 2005; Schiller *et al.,* 2006).

The results of the present study show that, from a large samples of *C. gloeosporioides* from mango and different geographical locations, there is considerable variation in restriction digestion patterns and sequencing of ITS region of r DNA and phylogenetic analysis sharply divided the *C. gloeosporioides* populations into two molecular groups, which mostly correlated with morphological groups based on different colony and conidia characteristics. This is the first report of on the existence of diverse molecular groups among *C. gloeosporioides* populations associated with mango anthracnose. Repeated sampling of *C. gloeosporioides* from mango in Sri Lanka also revealed greater amount of diversity in r DNA and mt DNA restriction fragment banding patterns (Alahakoon *et al.,* 1994a,b). In contrast, *C. gloeosporioides* from mango from around the world found the isolates relatively homogenous based on r DNA restriction fragment banding patterns and distinct from other fruit species (Hodson *et al.,*1993). Different species of *Colletorichum* have been reported to posses a high degree of variability based on DNA based markers. Sequencing of ITS region of r DNA, ITS RFLP and RAPD patterns have been used for intraspecific characterisation of *Colletotrichum* spp (Freeman *et al.,* 2001; Talhinhas *et al.,* 2005; Schiller *et al.,* 2006). This approach has been found useful for proper identification of races /pathotypes in *C. gloeosporioides* infecting *Stylosanthes* as it yielded pathogenically distinct r DNA restriction digestion patterns (Braithwaite *et al.,* 1990a,b). Madan *et al*. (2000) identified two distinct clusters among the isolates of *C. falcatum* causing anthracnose on sugarcane based on DNA polymorphism. Thottappilly *et al.* (1999) grouped 51 isolates of *C. gloeosporioides* into four groups based on RAPD analysis, which were correlated with morphological and virulence. Race specific DNA profiles were obtained among the isolates of *C. lindemuthianum* (Mesquita *et al.,* 1998). Sharma *et al.* (2005) classified 37 isolates of *C. capsici* inciting anthracnose on chillies into five groups using DNA profiles, which were correlated with virulence. Moreover, two genetic and phenotypic groups within *C. gloeosporioides*, identified in this study, also showed differences in their virulence on detached mango fruits, with SGG isolates dominant group tending to be more virulent than that of FGG isolates. Due to its high aggressiveness, the SGG populations widely spread and prevalent in all geographic locations, where highly fluctuating weather conditions prevails while FGG populations restricted to only Bangalore and Kolar districts of Karnataka, where moderate climatic conditions prevails. The differences in pathogen population between these regions probably reflect the differences in germplasm used and the agricultural practices employed. In fact, farmers in Bangalore and Kolar regions have planted different varieties of mango in addition to application of intensive agricultural practices including wide usage of chemical fertilizers and plant protection measures compared to other mango growing areas of India. *C. gloeosporioides* represents a complex group of many related forms. Many researchers have identified sub groups within population of *C. gloeosporioides* (Agostini *et al.*,1992; Liyange *et al.*,1993;Elmer *et al.*,2001; Abang *et al.,* 2005, 2006). Most traits used to delineate these groups are linked with pathogenicity tests on particular host. In fact, molecular, morphological and physiologically tests usually revealed high degree of homogeneity among isolates of *C. gloeosporioides* from one host (Freeman *et al.*,1998)

but there are numerous examples have been demonstrated in which a high degree of heterogeneity has been observed (Freeman *et al.,* 1998). The emergence of two distinct populations on one host is not uncommon and has been demonstrated on citrus (Liyange *et al.,* 1993), *Stylosanthes* spp. (Chakraborty *et al.,* 1999; He *et al.,* 1995), lupins (Elmer *et al.,* 2001) and yam (Abang *et al.,* 2006). Mango is indigenous to North East India and North Burma in the foot hills of the Himalayas and is said to have originated in the Indo – Burma region (Mukherjee, 1951) and nearly 1000 varieties of mango cultivars exist in India (Iyer and Mukanda 1998). Thus, it is presumed to be centre of diversity of its pathogen, *C. gloeosporioides*. This can influence pathogen variability because diversity of *C. gloeosporioides* has been shown to be extensive at site where native or naturalized host populations occur compared to sites where the host has been introduced recently(Weeds *et al.*,2003). Genotypic markers may serve as pathogenicity markers, if pathogenicity patterns can be correlated with specific genotypic makers. Such markers can be used for identification of races / pathotypes in perennial crops like mango. Combining molecular markers with pathogenicity assays has also been found to be highly useful method of investgating spatial and host differentiation in *C. gloeosporioides* (Freeman *et al.,* 1996).

This study has clearly demonstrated that diverse sub groups in *C. gloeosporioides* populations associated with anthracnose on mango in India. This work has also shown the variation in the virulence of the mango anthracnose pathogen isolates, which has implications for both disease control and the host adaptability of pathogen populations. Further, studies are very much essential to understand the spread and severity of the disease in relation to environmental factors and any interchange of the pathogens among different hosts such as grape, citrus, avocado, passion fruit, chillies, which are normally growing in the vicinity of mango in India.

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