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MORPHOLOGICAL CHARACTERIZATION AND MOLECULAR PHYLOGENY OF *COLLETOTRICHUM CAPSICI* CAUSING LEAF SPOT DISEASE OF TURMERIC

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ABSTRACT
Leaf spot disease is one of the major economic constraint which hampers turmeric production. Twenty isolates of *Colletotrichum capsici* causing leaf spot of turmeric were evaluated for their morphological, pathogenic, virulence and genetic characterization using random amplified polymorphic DNA (RAPD-PCR). The isolates were categorized into seven groups, based on the morphological characteristics, produced cottony colonies with zigzag to ring or circular pattern of growth. However, differences were obtained in colony colour, shape and size of conidia. Isolates were classified into four groups designated as highly resistant, moderately resistant, moderately sensitive and highly sensitive group based on the effect of propiconzole. The 5.8 S rDNA of ITS region was amplified which confirmed the specific amplicon size of 590 bp. The molecular polymorphism among isolates were analysed by means of RAPD-PCR and the genetic coefficient matrix derived from the scores of RAPD profile showed that minimum and maximum per cent similarities among isolates were in the range of 70 to 96 percent respectively. The cluster analysis by unweighted pair-group method with arithmetic average (UPGMA), separated the isolates into four clusters which confirming the genetic diversity among isolates. However, morphological, virulence and RAPD grouping of isolates suggested no correlation among the test isolates.

INTRODUCTION

Leaf spot disease caused by *Colletotrichum capsici* is the most important economic constraint which hamper turmeric (*Curcuma longa* L) production in major turmeric growing regions of the India, and often results in high yield losses (Uma Devi, 2008). It is main problem at the active vegetative growth and rhizome formation stage of turmeric. Most of the turmeric cultivars available today are equally susceptible to leaf spot disease, causing extensive yield losses to the turmeric production. The species of *C. capsici* (Butler and Bisby), *C. gloeosporioides* (Penz.) have been reported as causal agents of turmeric leaf spot in India (Chawda *et al*., 2012). *C. capsici* has been reported to have a wide putative host range associated with symptoms of foliar blight, leaf spot diseases (Shenoy *et al*., 2007).

The occurrence of different virulent strains of *C. capsici* has been well documented in India (Sharma *et al*., 2005). Numerous cases have been reported in which several *Colletotrichum* species or biotypes are associated with a single host (Peres *et al*., 2002) making their identification by morphological and physiological methods more difficult. The use of molecular marker techniques has improved the accuracy and speed of identification of *Colletotrichum* spp. (Cai *et al*., 2009). Among these molecular techniques, DNA fragment analysis RAPD (PCR), has been extensively used to investigate relationships among isolates of *Colletotrichum* spp. (Madhavan *et al*., 2010, Sangdee *et al*., 2011). Similarly, nucleotide sequence information for the 5.8S rDNA gene and the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) has been used to design *Colletotrichum* species specific primers for diagnostic purposes and for phylogenetic analysis (Thalhinhas *et al*., 2002). Analysis of virulence and genetic diversity is one step towards understanding the pathogen population. The present study is planned to investigate the diversity of *C. capsici* isolates causing leaf spot disease of turmeric using the morphological, pathological, virulence and RAPD analysis.

MATERIALS AND METHODS

Collection and isolation of pathogen

Samples of typical leaf spot symptoms on turmeric leaves were collected from different turmeric growing states of southern India during 2011-2012. The infected portion were cut into small pieces and surface sterilized by dipping in 0.1% $HgCl_2$ for 1 min, and rinsed three times with sterile distilled water and transferred onto the surface of water agar. The mycelium growing out of the plant tissue was sub-cultured to potato dextrose agar (PDA) and incubated at 28 ± 2^{o}C for 7 to 10 days. The pure cultures of the *Colletotrichum* were obtained by single spore isolation method using the procedure described by Choi *et al*. (1999) with modifications. The isolates were identified based on morphological and cultural charactertics of pathogens (Than *et al*., 2008a). After confirming *C. capsici* by microscope examination, one

Table 1: Sequences of RAPD primers used to study the genetic diversity among isolates of *Colletotrichum capsici*

Primer	Sequence
OPA1	5′- CAGGCCCTTC - 3′
OPA2	5′- TGCCGAGCTG - 3′
OPA3	5′- AGTCAGCCAC - 3′
OPA4	5′- AATCGGGCTG - 3′
OPA5	5′- AGGGGTCTTG - 3′
OPA6	5′- GGTCCCTG AC - 3′
OPA7	5′- GAAACGGGTG - 3′
OPA8	5′- GTGACGTAGG - 3′
OPA9	5′- GGGTAACGCC - 3′
OPA10	5′- GTG ATCGCAG - 3′
OPA11	5′- CAATCGCCGT - 3′
OPA12	5′- TCGGCGATAG - 3′
OPA13	5′- CAGCACCCAC - 3′
OPA14	5′- TCTGTGCTGG - 3′
OPA15	5′- TTCCGAACCC - 3′
OPF01	5′- GGGAATTCGG - 3′
OPF07	5′- CCGATATCCC - 3′
OPF10	5′- GGAAGCTTGG - 3′

monoconidial culture from each isolate was prepared and used in this study (Table 2).

Examination of cultural and morphological characteristics:

The isolates were cultured on PDA at 28 ± 2°C for 7 days, after which mycelial disks were transferred to the center of a new PDA medium. The colony morphology and colony colour of each isolate on PDA medium were examined daily from 5-10 days. For sporulation the culture were maintained in 12 hour light and dark alternatively, then conidia were harvested from each isolate and mounted in water. The size and shape of twenty five conidia were measured under a image analyzer (LABOMED iVu5100, Labo America Inc, USA. Scope image 9.0 exe, software 9.1v for spore measurement) light microscope (Sangdee *et al*., 2011).

Pathogenicity test under in *vitro* and glasshouse condition:

Pure cultures of each isolate are grown on PDA for 7–14 days at 28 ± 2°C under alternating 12 hour fluorescent light and12 hour dark cycle to induce sporulation (Than *et al*., 2008b). The conidial suspension was harvested, filtered and centrifuged at 5000rpm. The mass of spore sedimentation was collected, resuspended with sterilized distilled water and spore density was adjusted to a concentration of 1×10^6 spore/ml using a haemocytometer. Freshly collected immature and untreated leaves are washed under running tap water for 60 seconds followed by surface sterilization by immersing the leaves in 70% ethanol for 3 minutes, 1% sodium hypochlorite solution for 3 minutes and then rinsing three times in sterilised distilled water for 2 minutes each time and drying with sterile tissue paper and then air drying (Sanders and Korsten, 2003; Montri *et al*., 2009). The surface sterilized turmeric leaves were pinpricked with sterile needle then placed in the petridish which is equipped with moist cotton. The drop of 6μl of 10^6 spores per ml was placed on the pinpricked or wounded spots and incubated in moist chamber at 26°C and 95% relative humidity. The sterile water was used instead of spore suspension served as a control under *in -vitro* condition. In

Table 2: Cultural and morphological characterization of different isolates of *C. capsici* causing turmeric leaf spot

Isolate	Location	Colony morphology	Colony colour	Conidia shape	Morphology group	Growth reduction(%)	Virulence group	RAPD group
Cc1TNAU	Erode-TN	Zigzag cottony colonies, Smooth	Grey	Fusiform, Medium	CC-I	32.55	CCV-I	IV
Cc2TNAU	Coimbatore -TN	Circular cottony colonies	Grey	Fusiform, Medium	CC-II	100.00	CCV-IV	IV
Cg1TNAU	Salem -TN	Ring like zonation, Smooth	White	Fusiform, Large	CC-III	100.00	CCV-II	II
Cc3TNAU	Dharmapuri -TN	Zigzag cottony colonies, Smooth	Grey	Fusiform,Medium	CC-I	42.77	CCV-II	IV
Cc4TNAU	Karur -TN	Circular cottony colonies, Rough	White	Fusiform, Large	CC-II	100.00	CCV-III	III
Cc5TNAU	Namakkal -TN	Ring like zonation,Rough	Grey	Fusiform, large	CC-III	100.00	CCV-IV	III
Cc6TNAU	Ksishanagiri -TN	Zigzag cottony colonies, Smooth	Grey	Fusiform, Medium	CC-I	81.97	CCV-II	IV
Cg2TNAU	Perumbalur -TN	Round cottony colonies	White	Fusiform, Medium	CC-III	29.36	CCV-II	I
Cc7TNAU	Villupuram -TN	Zigzag colonies	Grey	Fusiform, Large	CC-IV	6.87	CCV-I	IV
Cg3TNAU	Trichy -TN	Circular, Smooth	White	Fusiform, Large	CC-V	9.52	CCV-I	I
Cc8TNAU	Nizamabad – A.P	Ring like growth,	Grey	Fusiform, Large	CC-III	18.36	CCV-I	II
Cc9TNAU	Guntur – A.P	Ring like growth, Rough	Black	Fusiform, Medium	CC-VI	56.89	CCV-III	IV
Cc10TNAU	Warangal – A.P	Zigzag colonies, Smooth	Grey	Fusiform, Medium	CC-IV	13.68	CCV-I	IV
Cc11TNAU	Kozhikode - KL	Zigzag zonation, Rough	White	Fusiform, Medium	CC-VII	50.16	CCV-II	IV
Cc12TNAU	Palakkadu- KL	Cicular Zonations, Rough	White	Fusiform, Large	CC-II	56.45	CCV-III	IV
Cc13TNAU	Wayanad - KL	Zigzag cottony colonies, smooth	Light brown	Fusiform, medium	CC-I	24.60	CCV-II	IV
Cc14TNAU	Belgaum - KA	Zigzag colonies, smooth	Grey	Fusiform, Large	CC-IV	16.84	CCV-I	III
Cc15TNAU	Mysore - KA	Ring like zonation, Rough	Grey	Fusiform, Medium	CC-III	100.00	CCV-IV	IV
Cc16TNAU	Chamarajnagar - KA	Circular colonies, Rough	White	Fusiform, Medium	CC-II	100.00	CCV-III	IV
Cc17TNAU	Gulbarga - KA	Zigzag cottony colonies, Smooth	White	Fusiform, Large	C-III	100.00	CCV-III	IV

another experiment, the conidial spore suspension @ 1 × 10^6 spore/ml was prepared and sprayed at 3-4 leaf stage on turmeric plants under glass house condition. The inoculated plants were covered with polythene sheets for incubation and maintenance of temperature and relative humidity. The appearance of symptoms was observed four days after inoculation (Than *et al*., 2008a).

Effect of propiconazole on mycelial growth

The mycelial discs of all the isolates were transferred in to the center of the PDA medium containing 500μg mL^{-1} of the active ingredient of propiconazole and incubated at 28 ± 2°C for 12 days and mycelial growth rate was calculated. The percent reduction over control in the mycelial growth of *C. capsici* on PDA medium containing propiconazole was calculated using the procedure described by Sangdee *et al*., 2011. All tests consisted of three replicates.

Isolation of genomic DNA

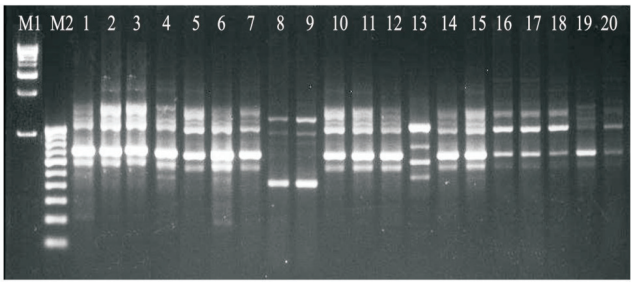
For DNA extraction, pure culture of each isolate was grown in potato dextrose broth, for 10 days at room temperature (28 ± 2°C). The mycelia were harvested by filtration and frozen in liquid nitrogen. Freeze-dried mycelium (1g) was ground to a fine powder using liquid nitrogen, and DNA was extracted, according to standard protocols (Murray and Thompson 1980). The genomic DNA was checked by agarose gel electrophoresis and stored at –20°C for further use.

Molecular detection of *C. capsici* using 5.8 ITS rDNA region

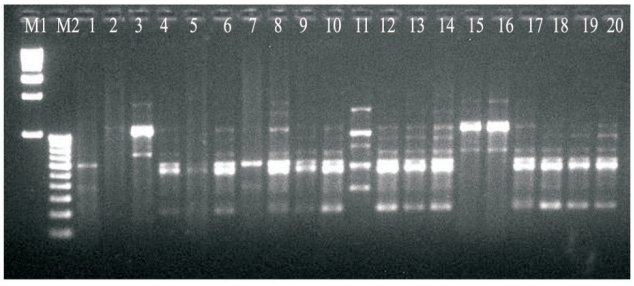
Amplifications of ITS region of the isolates were carried out using the general PCR with conserved primers ITS-1F (5'-GT CCTAACAAGGTTTCCGTA-3'; J297952) and ITS-4R (5'-TTCTCCGCTTATTGATATGC-3'; AJ297953). PCR was executed with a 20 µL reaction volume, 2.0 units of Taq polymerase (Bangalore Genei Pvt Ltd, Banglore, India), 2μL of 10X buffer, 1.5μL of 2.5 mM $MgCl_2$, 1μL of 2.5 mM dNTP, 2μL of 10μM primer, 4μL of genomic DNA and sterile distilled water. PCR amplifications were performed in a thermal cycler (Eppendorf Master Cycler nexus gradient, German) and denaturation was executed at 94°C for 5 min before PCR cycling. The reaction cycle consisted of 45 sec at 94°C for denaturation, 45 sec at 46 °C for annealing, and 1min at 72 °C for extension. A total of 35 cycles was performed with final extension at 72°C for 10 min (Shenoy *et al*., 2007). Products of the polymerase chain reaction were analyzed by electrophoresis in 1.5 per cent agarose gels in electric fields of potential gradient 2 V cm^{-1}. The gel was viewed in an UV transilluminator and the banding pattern was photographed and analyzed. The sizes of the PCR products were determined by comparison with standard 100bp ladder (Bangalore Genei Pvt. Ltd., Bangalore, India).

Molecular diversity using RAPD analysis

In total, eighteen 10-mer primers were used for RAPD analysis (Table 1). All the RAPD primers were purchased from Operon (Operon Biotechnologies, Cologne, Germany) and used as single primers. PCR amplification was performed using a Eppendorf nexus gradient master cycler and a 20μL total volume containing 2.0 units of Taq polymerase (Bangalore Genei Pvt Ltd, Banglore, India), 2μL of 10X buffer, 1.5μL of 2.5 mM $MgCl_2$, 1μL of 2.5 mM dNTP, 2μL of 10μM primer, 4μL of genomic DNA and sterile distilled water. The PCR was performed, using Eppendorf – Master Cycler nexus gradient S (Eppendorf, A G, Hamburg, Germany), with an initial denaturation step for 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C, with a final extension for 10 min at 72°C. Following amplification, 20¼l of each PCR product was separated by electrophoresis in 2%



OPA-02



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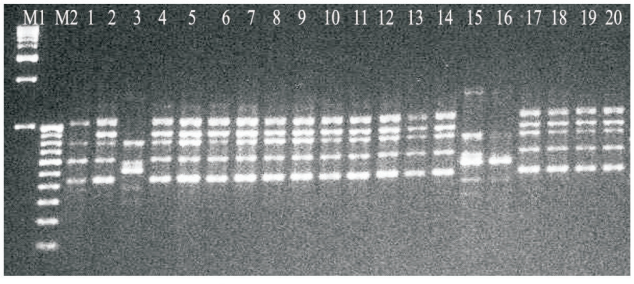
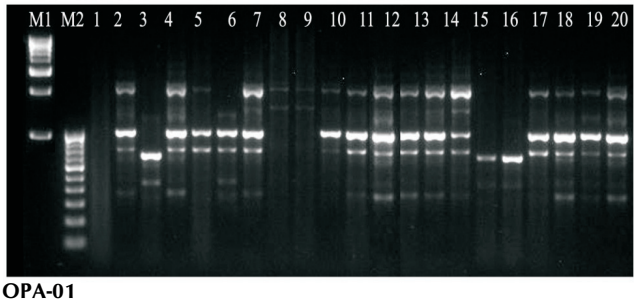


OPA-13



OPA-01

Figure 1: RAPD profiles of *Colletotrichum capsici* isolates using random primer OPA-02, OPA-09, OPA-13 and OPF-01. M1 = 1kb DNA ladder and M2 = 100bp DNA ladder. (1)Cc1TNAU, (2)Cc2TNAU, (3)Cg1TNAU, (4)Cc3TNAU, (5)Cc4TNAU, (6)Cc5TNAU, (7)Cc6TNAU, (8)Cg2TNAU, (9)Cc7TNAU, (10)Cg3TNAU, (11)Cc8TNAU, (12).Cc9TNAU, (13)Cc10TNAU, (14)Cc11TNAU, (15)Cc12TNAU (16)Cc13TNAU, (17)Cc14TNAU, (18)Cc15TNAU, (19)Cc16TNAU and (20)Cc17TNAU

(w/v) agarose gel in Tris-acetate-EDTA (TAE) buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0). A 100- base pair (bp) ladder (Bangalore Genei Pvt Ltd, Banglore, India) was used as a size standard. To visualise DNA, gels were stained with ethidium bromide (0.1mg L^{-1}) and then photographed under transmitted ultraviolet light, using an AlphaImager 2000 (Alpha Innotech, San Leandro, CA, USA). All RAPD analyses were repeated at least three times for each isolate only the RAPD bands which appeared consistently were evaluated for polymorphism (Madhavan *et al.*, 2010).

RESULTS

Examination of cultural and morphological characteristics

The isolates of *Colletotrichum* spp. were identified based on size and shape of the conidia and confirmed as a *C. capsici*. Twenty isolates were assigned to seven morphological groups (CC-I to CC-VII) based on the differences in morphological characteristics (colony color, colony diameter and conidial shape and size). Various isolates produced zigzag cottony, ring or circular like with zigzag zonation colonies on PDA with a color of greyish-white to dark grey or light brown on the ventral surface whereas the reverse of the colonies was mainly black. The colony diameter of different groups ranged from 67 to 87 mm after 12 days incubation. The colonies of group CC-I produced zigzag cottony, smooth surface with grey to grey colonies, CC-II produced circular cottony colonies with white to grey colour, whereas the isolate in group CC-III and CC-VII possessed ring like or circular growth with zigzag zonation of colonies. The conidia shape of the different groups was fusiform with both their ends are curved and pointed. Average length and width of conidia varied between 19.36 to 28.53 µm and 3.25 to 4.65 µm, respectively. Twenty isolates were grouped into two groups large (24.25 to 28.53 µm × 4.00 to 4.65 µm) and medium (19.36 to 24.25 µm × 3.25 to 4.00 µm) based on the length and width of the conidia respectively (Table 2).

Effect of propiconazole on mycelial growth:

The isolates were classified into four groups based on their reaction to propiconazole fungicide. The first group were highly resistant (<25% inhibition) and consisted of isolates, Cc4TNAU, Cc8TNAU, Cc10TNAU, Cc13TNAU and Cg3TNAU; second group, moderately resistant (<50% inhibition – Cc1TNAU, Cc3TNAU, Cg2TNAU); third group, moderately sensitive (<75% inhibition- Cc9TNAU, Cc11TNAU), whereas the fourth group were highly sensitive (>75 to 100% inhibition) and consisted of the isolates Cc2TNAU, Cc4TNAU, Cc5TNAU, Cc6TNAU, Cg1TNAU, CcCc15TNAU, Cc16TNAU and Cc17TNAU (Table 2).

Pathogenicity test

The variable pathogenicity was observed upon inoculation of *C. capsici* on the leaves of turmeric. All the isolates were pathogenic and produced leaf spot symptoms e.g. brown spots on the upper surface of the young leaves, spots are irregular in shape and white or grey in the centre. Later, two or more spots may coalesce and formed an irregular patch covering almost the whole leaf upon turmeric leaves after inoculation. The sporulation and acervuli formed 12 days after inoculation. Based on the development of acervuli on inoculated leaves the isolates were designated into four groups (CCV-I, CCV-II, CCV-III and CCV-IV). The first group, CCF-I was designed a mildly virulent strain consisting of 6 isolates, Cc1TNAU, Cc7TNAU, Cc8TNAU, Cc10TNAU, CcTNAU14 and Cg3TNAU. Four isolates, Cg1TNAU, Cg2TNAU, Cc3TNAU, Cc6TNAU, Cc11TNAU and Cc13TNAU, were assigned to

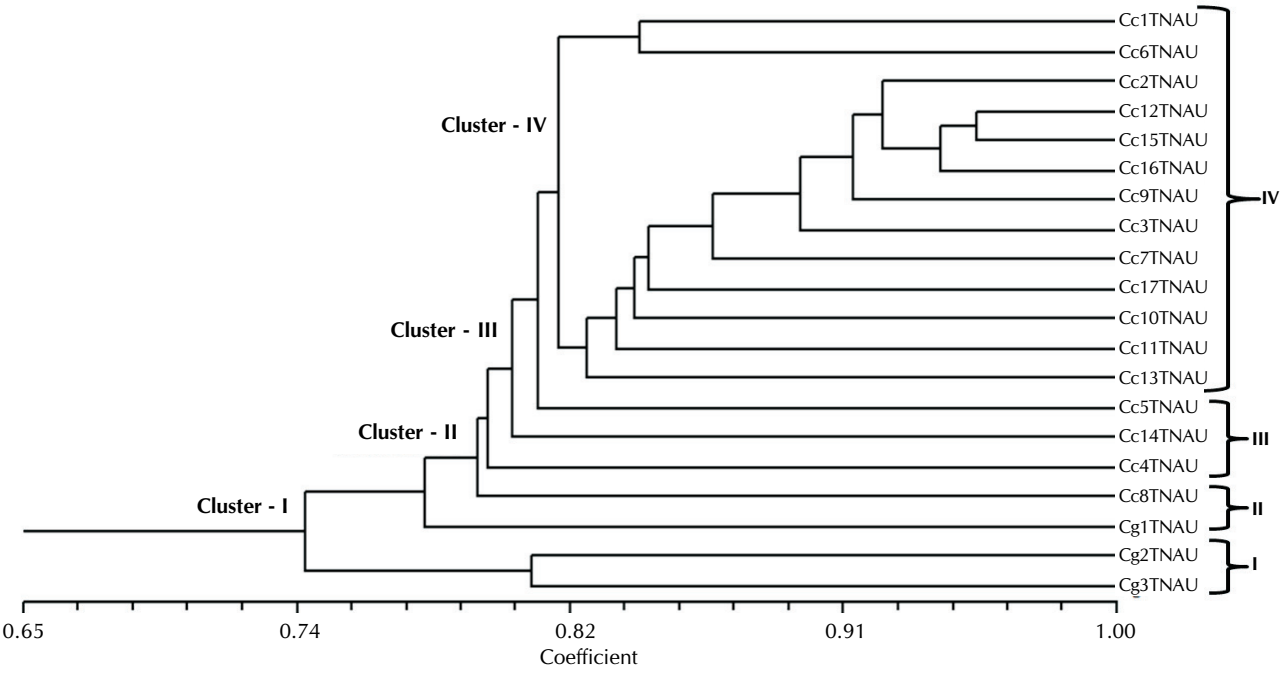


Figure 2: Dendrogram showing the relationship between the twenty *C. capsici* isolates. This was derived from cluster analysis of the RAPD allelic patterns

Table 3: Genetic similarity coefficient matrix for *Colletotrichum capsici* isolates from turmeric based on RAPD profile

Isolates	Cc1 TNAU	Cc2 TNAU	Cg1 TNAU	Cc3 TNAU	Cc4 TNAU	Cc5 TNAU	Cc6 TNAU	Cg2 TNAU	Cc7 TNAU	Cg3 TNAU	Cc8 TNAU	Cc9 TNAU	Cc10 TNAU	Cc11 TNAU	Cc12 TNAU	Cc13 TNAU	Cc14 TNAU	Cc15 TNAU	Cc16 TNAU	Cc17 TNAU
Cc1TNAU	1																			
Cc2TNAU	0.82	1																		
Cg1TNAU	0.72	0.79	1																	
Cc3TNAU	0.81	0.91	0.79	1																
Cc4TNAU	0.77	0.82	0.77	0.80	1															
Cc5TNAU	0.80	0.85	0.77	0.83	0.76	1														
Cc6TNAU	0.84	0.85	0.78	0.83	0.78	0.81	1													
Cg2TNAU	0.74	0.77	0.68	0.77	0.70	0.69	0.77	1												
Cc7TNAU	0.77	0.88	0.78	0.84	0.79	0.81	0.80	0.75	1											
Cg3TNAU	0.69	0.76	0.68	0.76	0.69	0.70	0.77	0.81	0.74	1										
Cc8TNAU	0.77	0.82	0.75	0.81	0.75	0.78	0.82	0.70	0.77	0.73	1									
Cc9TNAU	0.85	0.92	0.80	0.87	0.82	0.84	0.84	0.76	0.85	0.75	0.82	1								
Cc10TNAU	0.79	0.86	0.75	0.82	0.79	0.76	0.80	0.73	0.84	0.71	0.75	0.83	1							
Cc11TNAU	0.77	0.86	0.75	0.82	0.79	0.78	0.78	0.74	0.85	0.71	0.77	0.84	0.81	1						
Cc12TNAU	0.83	0.92	0.83	0.89	0.83	0.86	0.86	0.83	0.87	0.81	0.83	0.91	0.85	0.85	1					
Cc13TNAU	0.78	0.85	0.74	0.80	0.82	0.75	0.79	0.73	0.80	0.70	0.76	0.84	0.80	0.78	0.86	1				
Cc14TNAU	0.79	0.82	0.77	0.79	0.73	0.78	0.78	0.70	0.77	0.67	0.77	0.82	0.77	0.77	0.83	0.76	1			
Cc15TNAU	0.85	0.94	0.83	0.92	0.85	0.86	0.88	0.81	0.89	0.79	0.85	0.93	0.87	0.87	0.95	0.88	0.87	1		
Cc16TNAU	0.82	0.91	0.82	0.88	0.82	0.83	0.85	0.79	0.86	0.78	0.82	0.89	0.86	0.84	0.94	0.83	0.86	0.94	1	
Cc17TNAU	0.83	0.86	0.77	0.82	0.77	0.78	0.78	0.71	0.79	0.71	0.79	0.87	0.81	0.81	0.85	0.82	0.84	0.89	0.84	1

group CCF-II (moderately virulence), with the remaining isolates assigned to group CCF-III and CCF-IV (Cc2TNAU, Cc4TNAU, Cc5TNAU, Cc9TNAU, Cc12TNAU, Cc15TNAU, Cc16TNAU, Cc17TNAU- severely virulent isolates) (Table 2).

Molecular detection of *C.capsici* using 5.8 ITS rDNA region

The DNAs of all the 20 isolates of *C. capsici* were used in PCR with the general primers ITS1 and ITS4 for the amplification of the rDNA region comprising the two noncoding internal transcribed spacers ITS1 and ITS2 and the 5.8S rRNA gene. All isolates amplified a PCR product of approximately 590 bp of 5.8S rDNA region which depicts molecular based confirmation of *C. capsici*.

Random amplified polymorphic DNA (RAPD) analysis:

A total of 20 isolates of *C. capsici* were tested for their genetic variability by RAPD analysis, using 18 random primers. Of these, 10 random primers *viz*., OPA-01, OPA-02, OPA-03, OPA-05, OPA-09, OPA-13, OPA-15, OPF-01, OPF-07 and OPF-10 produce easily scorable and consistent banding patterns, which were used for RAPD analysis of test isolates. The generated fingerprints were evaluated for overall clearness of the banding pattern. The primers showed polymorphism and consistently produced 5 to 9 bands of 0.3-2.4 kb, although majority was below 1.2 kb. The RAPD profiles produced with the primers OPA-02, OPA-09, OPA-13 and OPF-01 are shown in Fig. 1.

The RAPD scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) described by Rohlf (1993). A dendrogram was constructed based on Jaccard's similarity coefficient using the marker data from *Colletotrichum* isolates with UPGMA. Analysis of the genetic coefficient matrix (Table 3), derived from the scores of RAPD profile, showed that minimum and maximum % similarities among the *C. capsici* isolates were in the range of 70 to 96%, respectively. Cluster analysis, using UPGMA, clearly separated the isolates into 4 clusters (I, II, III and IV) confirming some level of genetic diversity among the isolates of *C. capsici* from turmeric (Fig. 2). Cluster I consisted of only two isolates (Cg2TNAU and Cg3TNAU) with similarity coefficient of 0.69 and cluster II consisted of 2 isolates (Cg1TNAU and Cc8TNAU) with the similarity coefficient of 0.78; Cluster III consists of Cc5TNAU and Cc14TNAU with the similarity coefficient of 0.84. All the remaining isolates belonged to cluster IV, with similarity coefficient ranges from 0.87 to 0.96. However, 30.1% polymorphism was found, indicating that all isolates used in this study have approximately similar genetic identity. In the present study RAPD data failed to reveal a relationship between clustering in the dendrogram and in pathogenicity but all the isolates from Kerala and Karnataka were clustered into cluster IV group shows close genetic identity. However other isolates were genetically varied with respect geographical distribution.

DISCUSSION

Colletotrichum capsici causing leaf spot disease of turmeric is responsible for major economic losses in turmeric production in India. In this study, the pathogenicity test confirmed that the species *C. capsici* was responsible for leaf spot disease of turmeric in India. The expression of disease symptoms was

homogeneous among the isolates of *C. capsici* in the pathogenicity test. However, the degree of disease severity, virulence and aggressiveness varied among the isolates which were measured quantitatively. Among seven groups studied for morphological characterization of *C. capsici* based on cultural morphology, spore shape and size showed an overlap in colony color and conidial shape and size. This result was in agreement with a previous study by Sandgee *et al*. (2011) who found a morphometric overlap of conidial size within *Colletotrichum* species. Moreover, Cai *et al*. (2009) observed differences in colony colour of *Colletotrichum* populations. Seven, morphological groups and pathological groups didnot showed any clear cut relationship among isolates of *Colletotrichum*. The combination of these two characteristics has been successfully used to categorize *Colletotrichum* species (Thind and Jhooty, 1990; Than *et al*., 2008a). All the twenty isolates showed hyaline and short conidiophores bearing hyaline fusiform conidia. The conidia measured varied between 19.36 to 28.53 µm and 3.25 to 4.65 µm length and width respectively with a centrally placed oil globule. These characters agreed with the original descriptions given by Hyde *et al*., 2009. The average size of the spores however, did not vary among the isolates and it was further reported that the conidial size was 12.0- 17.0 x 3.5-6.0μm *C. gloeosporioides* isolates obtained from apple, peach, pecan and other hosts varied greatly in their growth, virulence and conidial size (Bernstein *et al*., 1995). Prema *et al*. (2011) reported that sixteen isolates of *C. musae* causing anthracnose of banana showed hyaline and short conidiophores bearing single hyaline cylindrical conidia. The conidia measured 14.7μm x 7.1 µm with a centrally placed oil globule.

Sharma *et al*. (2005) reported considerable pathogenic variability proposed that 15 pathotypes of *C. capsici* existed among 37 isolates from different regions of chilli growing areas in India. However pathotype differences were based on quantitative differences in host reaction, i.e., level of virulence and aggressiveness in chilli. Propiconazole showed the highest level of spore germination inhibition at 0.1μgml-1 concentration. Propiconazole was showing strong inhibition of both, mycelial growth and colony development. In general, concentrations beyond 5μg mL^{-1} completely arrested growth, biomass increase, spore germination and sporulation (Gopinath *et al*., 2006). Similar results were obtained by De los Santos and Romero (2002) when strawberry-crown rot fungus (*C. acutatum*) was tested in *vitro* against various fungicides. Our results are agreement with Sandgee *et al*. (2011) reported the resistance to carbendazim for spore germination, mycelial growth and biomass increase for virulence characterization.

PCR amplification of the 5.8S-ITS region of DNA, subsequent sequence analysis and PCR-RAPD analysis of the rDNA product revealed unequivocally the existence of *C. capsici* causing leaf spot disease of turmeric. For the detection of *Colletotrichum* spp. our results were found agreement with the results of Tapia-Tussell *et al*., 2008 and Shenoy *et al*., 2007. The ITS region is the most widely sequenced region but there are some concerns as to whether ITS sequence data can provide adequate resolution to determine and differentiate *Colletotrichum* species. Crouch *et al*. (2009) have revealed a high error rate and frequency of misidentification (86%) based on ITS sequence similarity comparison within the *C. graminicola* species complex. The ITS sequences named *C. gloeosporioides* found that >86% had considerable evolutionary divergence from the type specimen of *C. gloeosporioides* (Cannon *et al*., 2008), and most likely represent other *Colletotrichum* species.

The RAPD allelic patterns were divided the isolates of *C. capsici* into four clusters in the phylogentic tree dendrogram. These did not correlate with the data from cultural morphology and virulence patterns. Ratanacherdchai *et al*. (2010) analysed the genetic diversity among isolates of *C. gloeosporioides* and *C. capsici* from Thailand by Inter simple sequence repeat (ISSR) analysis and reported that there were two distinct groups of *C. gloeosporioides* and *C. capsici*. Furthermore, genetic diversity was correlated with geographic distribution, while there was no clearrelationship between genetic diversity and pathogenic variability among isolates of *C. gloeosporioides* and *C. capsici*. Our results were agreement with previous studies in which RAPD analysis was shown not to correlate with growth rates in culture and geographic region of different *Colletotrichum* sp. isolates (Sharma *et al*., 2005; Madhavan *et al*., 2010 and Sadgee *et al*., 2011). However, the RAPD approach has been useful for proper identification and categorization of *Colletotrichum* sp. isolates (Lee *et al*., 2007; Cai *et al*., 2009). We conclude that *C. capsici* in southern states of TamilNadu consists of variable populations based on cultural morphology, reaction to propiconazole, virulence pattern and RAPD analysis. Molecular phylogenetic grouping obtained by RAPD analysis did not correlate with morphological characteristics and virulence pattern. In the present study RAPD data failed to reveal a relationship between clustering in the dendrogram and in pathogenicity but all the isolates from Kerala and Karnataka were clustered into cluster-IV group shows close genetic identity. However other isolates were genetically varied with respect to geographical distribution. However, RAPD analysis can be used to classify *C. capsici* more rapidly than these other methods (Lee *et al*., 2007; Talhinhas *et al*., 2005 and Thottappilly *et al*., 1999). Therefore, molecular phylogenetic grouping based on RAPD analysis represents a powerful tool for studying genetic diversity in *C. capsici*.

Pathogen diversity plays a major role in disease dynamics and consequently, in the success of disease management strategies, including the development of cultivars resistant to diseases. The results of the present study demonstrate that there is a certain level of genetic diversity among isolates of *C. capsici* causing leafspot disease of turmeric in Tamil Nadu. Pathogenicity tests revealed that these isolates expressed different levels of virulence. The genetic variability among the isolates of *C. capsici* should be taken in to account when *C. capsici* isolates are used for screening of turmeric genotypes for leaf spot resistance.

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REFERENCES

Bernstein, B., Zehe, E.I., Dean R.A. and Shabi, E. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan and other hosts. *Plant Disease*. **79:** 478-482.

Cai, L., Hyde, K. D., Taylor, P.W. J., Weir, B. S., Waller, J. M., Abang, M. M., Zhang, J. Z., Yang, Y. L., Phoulivong, S., Liu, Z. Y., Prihastuti, H., Shivas, R. G., McKenzie, E. H. C. and Johnston, P.R. 2009. A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity*. **39:**183-204.

Cannon, P.F., Buddie, A.G. and Bridge, P.D. 2008. The typification of *Colletotrichum gloeosporioides*. *Mycotaxon*. **104:** 189-204.

Chawda, S. K., Sabalpara, A. N. and Pandya, J. R. 2012. Epidemiology of turmeric (*Curcuma longa*) leaf spot caused by *Colletotrichum gloeosporioides* (Penz and Sacc). *The Bioscan*. **7(2):** 441-443.

Choi, Y. W., Hyde, K. D. and Ho, W. H. 1999. Single spore isolation of fungi. *Fungal Diversity*. **3:** 29-38.

Crouch, J.A., Clarke, B.B. and Hillman, B.I. 2009. What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis. A case study using the falcate-spored graminicolous *Colletotrichum* group. *Mycologia*. **101:** 648-656.

De los Santos, G. and Romero, M. F. 2002. Effect of different fungicides in the control of *Colletotrichum acutatum*, causal agent of anthracnose crown rot in strawberry plants. *Crop Protection*. **21:** 17-23.

Gopinath, K., Radhakrishnana, N.V. and Jayaraja, J. 2006. Effect of propiconazole and difenoconazole on the control of anthracnose of chilli fruits caused by *Colletotrichum capsici*. *Crop Protection*. **25:** 1024-1031.

Hyde, K.D., Cai, L., Cannon, P.F., Crouch, J.A., Crous, P.W., Damm, U., Goodwin, P.H., Chen, H., Johnston, P.R., Jones, E.B.G., Liu, Z.Y., McKenzie, E.H.C., Moriwaki, J., Noireung, P., Pennycook, S.R., Pfenning, L.H., Prihastuti, H., Sato, T., Shivas, R.G., Tan, Y.P., Taylor, P.W.J., Weir, B.S., Yang, Y.L. and Zhang, J.Z. 2009. Colletotrichum - names in current use. *Fungal Diversity*. **39:** 147-182.

Lee, D. H., Kim, D. H., Jeon, Y., Uhm, J. Y. and Hong. S. B. 2007. Molecular and cultural characterization of *Colletotrichum* spp. causing bitter rot of apples in Korea. *Plant Pathology Journal*. **23(2):** 37-44.

Madhavan, S., Paranidharan, V. and Velazhahan, R. 2010. RAPD and virulence analyses of *Colletotrichum capsici* isolates from chilli (*Capsicum annuum*). *Plant Diseases and Protection*. **117(6):** 253-257.

Montri, P., Taylor, P. W. J. and Mongkolporn, O. 2009. Pathotypes of *Colletotrichum capsici* the causal agent of chili anthracnose, in Thailand. *Plant Disease*. **93:** 17-20.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*. **8:** 4321.

Peres, N. R., Kuramae, E. E., Dias, C. M. and De'souza, N. 2002. Identification and characterization of *Colletotrichum* spp. affecting fruit after harvest in Brazil. *J. Phytopathology*. **150(3):** 128-134.

Prema, R. T., Prabakar, K., Faisal, M. P., Kathikeyan, G. and Raguchander. T. 2011. Morphological and Physiological Characterization of *Colletotrichum musa*e the Causal Organism of Banana Anthracnose. *World J. Agri. Sciences*. **7(6):** 743-754.

Ratanacherdchai, K., Wang, H. K., Lin, F. C. and Soytong, K. 2010. ISSR for comparison of cross-inoculation potential of *Colletotrichum capsici* causing chilli anthracnose. *African J.Microbiol.Res*. **4:** 76-83.

Rohlf, F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system, v. 2.0. Exeter Software. Setauket, New York.

Sanders, G. M. and Korsten, L. 2003. Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Microbiol.Research*. **128:** 143-150.

Sangdee, A., Sachan, S. and Khankhum, S. 2011. Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chilli in the North-east of Thailand. *African J.Microbiol. Research*. **5(25):** 4368-4372.

Sharma, P. N., Kaur, M., Sharma, O. P., Sharma, P. and Pathania, A. 2005. Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western. *Indian J. Phytopathology*. **153:** 232-237.

Shenoy, B. D., Jeewon, R., Lam, W. H., Bhat, D. J., Than, P. P., Taylor, P. W. J. and Hyde, K. D. 2007. Morpho-molecular characterisation and epitypification of *Colletotrichum capsici* (*Glomerallaceae, Sordariomycetes*) the causative agent of anthracnose in chilli. *Fungal Diversity*. **27:** 197-211.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. and Oliveira, H. 2005. Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Microbiology*. **71:** 2987-2998.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. and Oliveira, H. 2002. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology*. **92:** 986-996.

Tapia-Tussell, R., Quijano-Ramayo, A., Cortes-Velazquez, A., Lappe, P., Larque-Saavedra, A. and Perez-Brito. D. 2008. PCR-based detection and characterization of the fungal pathogens *Colletotrichum gloeosporioides* and *Colletotrichum capsici* causing anthracnose in papaya (*Carica papaya* L.) in the Yucatan Peninsula. *Molecular Biotechnology*. **40:** 293–298.

Than, P. P., Jeewon, R., Hyde, K. D., Pongsupasamit, S., Mongkolporn, O. and Taylor, P. W. J. 2008a. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*. **57:** 562-572.

Than, P. P., Prihastuti, H., Phoulivong, S., Taylor, P. W. J. and Hyde, D. 2008b. Chilli anthracnose disease caused by *Colletotrichum* species. *J. Zhejiang Univ Sci.* **9:** 764-778.

Thind, T. S. and Jhooty, J. S. 1990. Studies on variability in two *Colletotrichum* species causing anthracnose and fruit rot of chillies in Punjab. *Indian Phytopathology*. **43:** 53-58.

Thottappilly, G., Mignouna, H. D., Onasanya, A., Abang, M., Oyelakin, O. and Singh, N. K. 1999. Identification and differentiation of isolates of *Colletotrichum gloeosporoides* from yam by Random amplified polymorphic DNA marker. *African Crop Science Journal*. **7:** 195-205.

Uma Devi, G. 2008. Efficacy of fungicides against *Colletotrichum* leaf spot of turmeric. *Indian J.Plant Protection*. **36(1):** 112-113.

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