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Genetic Diversity Among *Colletotrichum falcatum* Isolates Causing Red Rot of Sugarcane in Subtropical Region of India

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Abstract

Silver Genetic diversity of *Colletotrichum falcatum* causing red rot of sugarcane was assessed based on morphological, pathological and molecular characteristics especially from sub-tropical Indian conditions. Sixteen isolates of this pathogen were collected based on the extensive survey on prominent varieties grown in the region along with some elite selections. Morphological observations (colony colour, mycelium pattern and sporulation) grouped the isolates into two distinct types (C1: light type and C2: dark type). However, quantitative data on colony diameter showed five clusters for these isolates. Pathogenic characterization of these isolates on fourteen standard differentials formed six groups, ingroup 1: (CF-Pb-1) isolates Cf-157, Cf-249 and Cf-248 were the most virulent while group 6 (CF-Pb-6) isolates Cf-60 and Cf-247 were the least one. The genetic relatedness among the isolates using Random Amplified Polymorphic DNA (RAPD) analysis revealed sufficient molecular polymorphism, which in turn confirmed the variation in virulence of different isolates. The data categorized different isolates into two major clusters and five independent lineages. Polymorphic information content (PIC) ranged from 0.701 to 0.929. Isolate Cf-223 was found to be genetically most diverse among all the isolates. Present study inferred that morphological grouping of most of the isolates showed positive correlation with the pathogenic variability while molecular diversity did not showed such associations.

Keywords: *Colletotrichum falcatum*, sugarcane, variability, diversity, molecular marker

Introduction

Sugarcane (*Saccharum spp.*) is one of the most important agro-industrial crops of India next to textile industry. It is is grown in both tropical and sub-tropical belts of the country, later has a major concern with respect to the total pool of sugar production in the country. Of the various biotic stresses of sugarcane, red rot (caused by *Colletotrichum falcatum*) is a devastating fungal disease posing a serious threat to sugarcane cultivation in India (Freeman, 1997; Alexander and Viswanathan, 1996). This disease causes losses in both cane yield and sugar recovery to the tune of 29.07% and 30.8%, respectively (Hussnain and Afghan, 2006).

It has been reported that this disease became highly destructive in the northwestern part of the country due to favourable environmental conditions of high humidity and ideal temperature during crop season in this area (Tiwari *et al.*, 2010). The development and use of resistant cultivars is the most reliable, long lasting, economical and integral mean for the control of this disease (Sengar *et al.*, 2009). However, the major bottleneck in assessing durable resistance among different varieties/ genotypes to red rot is the highly variable nature of pathogen in the form of different strains, which results in frequent breakdown of host resistance under field conditions. *Colletotrichum* spp. infecting diverse hosts have a high degree of pathogenic variability (Sharma *et al.*, 2005). Several epiphytotics of red rot have been reported in the past, resulted in the complete failure of many elite cultivars like Co 1148, Co 7717 and CoJ 64. The pathogen undergoes adaptive changes in relation to the host varieties cultivated, which subsequently leads to alterations in the virulence pattern of the fungus (Satyavir, 2003; Duttamajumdar, 2008).

The typical morphological and cultural features of *C. falcatum* include acervulli with setae, presence or absence of teleomorph, colony colour, sporulation and growth rate are used used for its genetic characterization (Viswanathan *et al.*, 2003; Malathi *et al.*, 2010). The earlier workers have designated races/ isolates of *C. falcatum* with emphasis on cultural and morphological characters which is time consuming and expertise specific. Thereafter, the use of differential hosts was found as a viable option for evaluation

of pathogenic variability (Beniwal *et al*., 1989; Sharma *et al*., 1999). Since, the virulence assays are subject to environmental variations and sometimes leading to disease escape. This variability and high adaptability of the pathogen endangers the cultivation of different elite cultivars. In the recent past, various DNA- based characterization methods have been used successfully in identification of different *Colletotrichum* spp. infecting different hosts (Madan *et al*., 2000; Latha *et al*., 2003; Kumar *et al*., 2010). Therefore, present study was undertaken to apply different approaches morphological, pathological and molecular to investigate the existing variability of *C. falcatum* isolates in northern India.

Materials and methods

Collection, isolation and maintenance of isolates

Sixteen isolates of *C. falcatum* were isolated from red rot infected stalks of 15 sub-tropical sugarcane cultivars from different sugar mill areas of Punjab, Haryana and Uttar Pradesh (Tab. 1).

Tab. 1. Isolates of *Colletotrichum falcatum* used in this study and their place of collection

S. No.	Isolate	Variety	Source of collection
1	Cf-60	CoJ-64	Budhewal (Punjab)
2	Cf-65	Co-1148	Dasuya (Punjab)
3	Cf-157	Co-84	Budhewal (Punjab)
4	Cf-204	CoJ-83	Buttar Sevian (Punjab)
5	Cf-223	CoS-88230	Mukerian (Punjab)
6	Cf-241	Co-7717	Fazilka (Punjab)
7	Cf-245	Co-89003	Nawanshahr (Punjab)
8	Cf-248	CoC-90063	Gurdaspur (Punjab)
9	Cf-249	CoJ-86	Budhewal (Punjab)
10	Cf-250	Sel 32-00	Gurdaspur (Punjab)
11	Cf-251	Sel 64-00	Ladhowal (Punjab)
12	Cf-252	Sel 69-00	Gurdaspur (Punjab)
13	Cf-253	Sel 21-00	Ladhowal (Punjab)
14	Cf-254	CoJ-85	Gurdaspur (Punjab)
15	Cf-255	CoS-767	Kaithal (Haryana)
16	Cf-256	CoS-8436	Shahjahanpur (U.P)

These isolates were purified by single spore isolation and maintained on oat-meal agar medium with periodic sub-culturing and stored at 4±1 °C for subsequent use.

Morphological and cultural variability

The oat meal agar Petri plates were inoculated in the centre with actively growing 5mm diameter mycelial disc having spores obtained from 7 days old culture and each isolate was replicated thrice. The cultures were incubated at 25±1 °C in a BOD incubator. The morphological and cultural characteristics viz., colony diameter (mm), growth pattern, sporulation were recorded after 7 days of incubation. Sporulation intensity was calculated with the help of haemocytometer while conidial size (length and breadth) of each isolate was measured with the ocular micrometer.

Pathogenicity assay

Fourteen sugarcane differentials namely Baragua (*S. officinarum*), Khakai (*S. sinense*), SES 594 (*S. spontaneum*), CoS 767, Co 975, BO 91, CoC 671, Co 7717, Co 997, CoJ 64, Co 1148, Co 419, Co 62399 and Co 8436 were used to study pathogenic variability of *C. falcatum* under field conditions. These differentials were inoculated by plug method (Srinivasan and Bhatt, 1961) and disease data was recorded after 60 days of inoculation by splitting the canes longitudinally. Observations were recorded on 0-9 scale (Srinivasan and Bhatt, 1961) on the number of internodes transgressed by the pathogen, lesion width and white spots. The score was rated as Resistant (0-4.0), Intermediate (4.1-6.0) and Susceptible (> 6.1).

Molecular assay

Monoconidial cultures of all the isolates were grown on oat meal broth at 25±1 °C for 7 days. The mycelium was dried and ground to a fine powder in liquid nitrogen using pre-cooled pestle and mortar. Total genomic DNA of individual isolate was extracted using the CTAB (Cetyl trimethyl ammonium bromide) method as modified by Saghai Maroof *et al* (1984). Isolated DNA was purified by using 1 μL RNase A (50 μg/mL) and resolved on 0.8% agarose gel. A working DNA solution was made by diluting the DNA stock to 25 ng/μL with distilled water for polymerase chain reaction (PCR) amplification.

PCR amplification

A set of 80 RAPD primers from Operon Technologies USA were first validated on single isolates. Among these 22 primers that produced consistent amplicons were further used on 16 isolates. For RAPD analysis, a 20-μL PCR volume containing 2.0 μL 10X buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl and 1.5 mM $MgCl_2$), 4.0 μL dNTP mix (10 mM; Bangalore Genei), 6 μL primers (50 ng/μL), 0.3 μL Taq polymerase (Bangalore Genei; 5 U/μL), 2 μL DNA template (25 ng/μL) and 5.7 μL sterilized distilled water was used. The thermal cycling profiles for RAPD were, denaturation of genomic DNA at 94 °C for 5 min, primer annealing at 37 °C for 1 min and extension of primer at 72 °C for 2 min. Forty additional PCR cycles were carried out at 94 °C for 1 min, 37 °C for 1 min and 72 °C for 2 min. An additional cycle at 72 °C for 5 min was run at the end of these cycles. The PCR products were resolved by electrophoresis on 1.5% agarose gel in 0.5X TBE buffer at 50 V for 3 h. The gels were visualized under UV light and photographed using gel documentation system (UVP Transillumination).

Data analysis

The RAPD allele sizes were determined depending on the position of bands relative to the ladder (1 Kb). Total numbers of alleles were recorded for each RAPD marker in all the 16 isolates by giving allele number as 1, 2, 3, 4 and so on. The amplified bands were recorded as 1 (band present) or 0 (band absent) in a binary matrix. The PIC values described by Botstein *et al.* (1980) are used to refer the relative value of each marker with respect to the amount of polymorphism exhibited. PIC values for each of the 22 markers were estimated as per Nei (1987). Statistical analysis for RAPD data were conducted with software

NTSYSpc version 2.02e (Rohlf, 1998) by using Jaccard's coefficient. Similarity was computed with SIMQUAL function for RAPD primers (binary data) and SIMINT function (interval data) for morphological/ cultural parameters using NTSYSpc version 2.02e. SHAN module of NTSYS was used for cluster analysis of both molecular (RAPD) and pathogenic data. Dendrograms were generated using Unweighted Pair Group Method based on Arithmetic average (UPGMA).

Results and discussion

Morphological and cultural variability

Visual observations showed that all the isolates used in the study possessed specific morphological features of *C. falcatum* when cultured on oat meal agar medium in Petri dishes. Based on morphological characteristics (colony colour, growth pattern, sporulation and conidial shape and size), all the 16 isolates were placed in two groups (C1- light type and C2 dark type). Isolates of group C1 had whitish grey mostly fluffy colonies produced medium or high sporulation. Twelve isolates fell in this group. Isolates of group C2 had grey, less fluffy colonies with low sporulation and 4 isolates viz., Cf-65, Cf-251, Cf-252 and Cf-256 were included in this group. Abott (1935, 1938) also characterized the different isolates of red rot into two distinct dark and light races on the basis of colour and texture and revealed that dark type sporulated sparingly and light type sporulated abundantly. In this study average length and width of conidia varied between 27.0-45.0 and 5.6-10.0 μm, respectively. None of the isolates resembled with each other for all the morphological characters studied. However, within a single group, different isolates showed similarity in colony growth, colour and sporulation. Further, these isolates were categorized into five groups based on colony diameter. Two isolates in group I namely Cf-157 and Cf-249 were relatively fast growing with colony diameter ranging from 85.0- 86.5 mm while isolate Cf-255 in group V exhibited slow colony growth with diameter of 73.0 mm (Tab. 2). Such morphological and cultural variation in *C. falcatum* had been studied by earlier workers in different sugarcane varieties (Prakasam and Venkatareddy, 1961; Sharma, 1970). The findings of Jothi (1989) support the present results who divided 30 isolates of *C. falcatum* into 5 groups based on colour, texture and sporulation. The mycelial growth variation among different strains have also been documented by Viswanathan *et al* (2000) who worked on nine major *C. falcatum* pathotypes.

Pathogenic variability

Pathogenic variability of the sixteen isolates on a set of fourteen differential lines revealed that all the isolates exhibited susceptible to intermediate reaction on cvs. Khakai, CoC-671, CoJ-64 and Co-997 whereas Cvs SES-594, BO-91, Co-8436 and Baragua were either resistant or intermediate to all the isolates. Six differential hosts *viz*., CoS-767, Co- 7717, Co-1148, Co- 419, Co- 62399 and Co-975 showed a differential behavior to red rot reaction on all the isolates (Tab. 3). Based on the disease severity (Tab. 4), isolates Cf-157, Cf-245, Cf-248 and Cf-249 were found the most virulent (71.43% virulence frequency) followed by Cf-254 (64.28%) whereas isolate Cf-60 was the least virulent (28.58%). Pathogenic variability in *C. falcatum* based on virulent pattern has also been reported by earlier workers who used different sets of differentials using plug and nodal methods of inoculation (Khirbat *et al* 1980; Beniwal *et al* 1989). Our results are in complete agreement with the findings of Prakasam *et al* (2000) who reported identification of five races of the pathogen based on differential host studies *viz*., Cf-1148, Cf-7717, Cf-671, Cf-90063 and Cf-92061.

Molecular variability

DNA fingerprinting of all the sixteen *C. falcatum* isolates was done with 10 oligonucleotide primers. Of 80

Tab. 2. Colony characters of different isolates of *Colletotrichum falcatum*

Isolate No.	Colony colour	Mycelium pattern	Sporulation	Length*1 (μm)	Breadth*1 (μm)	Isolate No.
Cf-60	+WG (L)	More fluffy	Medium**	45.00	7.5	Cf-60
Cf-65	Grey (D)	Less fluffy	Medium	28.75	7.5	Cf-65
Cf-157	++GW (L)	Fluffy	Low*	31.00	7.5	Cf-157
Cf-204	WG (L)	Less fluffy	Low	27.50	9.0	Cf-204
Cf-223	WG (L)	Less fluffy	Low	29.50	10.0	Cf-223
Cf-241	WG (L)	Less fluffy	Medium	27.75	6.5	Cf-241
Cf-245	GW (L)	Fluffy	Medium	38.00	10.0	Cf-245
Cf-248	GW (L)	More fluffy	Medium	29.00	6.5	Cf-248
Cf-249	WG (L)	Fluffy	Medium	29.00	7.5	Cf-249
Cf-250	GW (L)	Fluffy	High***	40.00	9.0	Cf-250
Cf-251	Grey (D)	Less fluffy	Medium	38.50	9.0	Cf-251
Cf-252	Grey (D)	Less fluffy	Medium	34.00	8.5	Cf-252
Cf-253	WG (L)	Less fluffy	Low	30.50	10.0	Cf-253
Cf-254	GW (L)	Fluffy	High	27.00	5.6	Cf-254
Cf-255	WG (L)	More fluffy	Medium	32.50	10.0	Cf-255
Cf-256	Grey (D)	Fluffy	Medium	30.00	6.5	Cf-256

*Low = 2.08 to 16.67 X 10^5/9mm disc
**Medium = 16.67 to 29.17 X 10^5/9mm disc
*** High = 29.17 to 41.67 X 10^5/9mm disc
+WG = Whitish grey; ++GW = Greyish white L= Light; D = Dark
*1 Average of 50 observations

Tab. 3. Expression of red rot isolates on sugarcane differentials by plug method of inoculation (after 60 days)

S. No	Isolate No	Host variety	Differential hosts													
			Bara-gua	Kha kai	SE S 594	CoS 767	BO 91	CoC 671	Co 7717	Co 997	CoJ 64	Co 1148	Co 419	Co 62399	Co 975	Co 8436
1	Cf-60	CoJ 64	R	S	R	R	R	S	R	S	S	X	R	R	X	R
2	Cf-65	Co 1148	R	S	R	R	R	S	R	S	S	S	X	S	X	R
3	Cf-157	CoJ 84	R	S	R	S	R	S	S	S	S	S	S	S	S	R
4	Cf-204	CoJ 83	R	S	R	R	R	S	S	S	S	X	S	S	S	R
5	Cf-241	Co 7717	R	S	R	R	R	S	S	S	S	R	X	R	R	R
6	Cf-245	Co 89003	R	S	R	S	R	S	S	S	S	S	S	S	S	R
7	Cf-223	CoS 88230	R	S	R	R	R	S	S	S	S	X	X	S	X	R
8	Cf-248	CoC 90063	R	S	R	S	R	S	S	S	S	S	S	S	S	R
9	Cf-249	CoJ 86	R	S	R	S	R	S	S	S	S	S	S	S	S	R
10	Cf-250	Sel 32-00	X	S	R	R	R	S	R	S	S	S	R	S	R	R
11	Cf-251	Sel 64-00	R	S	R	R	R	S	R	X	S	S	X	S	R	R
12	Cf-252	Sel 69-00	X	S	R	R	R	S	R	S	S	S	S	S	R	R
13	Cf-253	Sel 21-00	R	S	R	R	R	S	R	X	S	S	X	S	R	R
14	Cf-254	CoJ 85	X	S	R	X	R	S	S	S	S	S	S	S	X	R
15	Cf-255	CoS 767	R	S	R	S	R	S	R	S	S	S	R	R	R	R
16	Cf-256	CoS 8436	X	S	R	R	R	S	X	S	S	X	R	S	S	R

Score 0-4 = Resistant (R)
4.1-6 = Intermediate (X)
>6.1 = Susceptible (S)

Tab. 4. Virulence frequency of isolates of *Colletotrichum falcatum*

S. No.	Isolate	Variety	Number of differentials infected	Virulence frequency (%)
1	Cf-60	CoJ-64	4	28.58
2	Cf-65	Co-1148	6	42.86
3	Cf-157	Co-84	10	71.43
4	Cf-204	CoJ-83	8	57.14
5	Cf-223	CoS-88230	6	42.86
6	Cf-241	Co-7717	5	35.71
7	Cf-245	Co-89003	10	71.43
8	Cf-248	CoC-90063	10	71.43
9	Cf-249	CoJ-86	10	71.43
10	Cf-250	Sel 32-00	6	42.86
11	Cf-251	Sel 64-00	5	35.71
12	Cf-252	Sel 69-00	5	35.71

primers initially used for amplification, 22 were selected which were most polymorphic and consistently produced 10-18 amplicons (Tab. 5) of 0.3-2.7 kb size. Among these 22 primers, 10 primers were able to give unique bands in various isolates. Primer S103 amplified maximum bands (Fig. 1) while primer S110 and S114 amplified the least

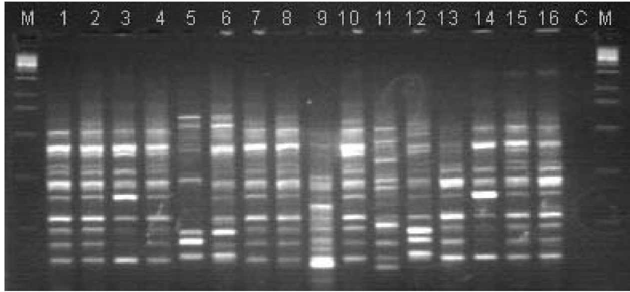

Fig. 1. PCR amplification products obtained with primer S 103, Lane M indicates standard DNA ladder of 100-bp marker and 1-16 indicates Cf-65, Cf-60, Cf-254, Cf-254m, Cf-223, Cf-250, Cf-249, Cf-255, Cf-248, Cf-253, Cf-256, Cf-252, Cf-204, Cf-241, Cf-251 and Cf-245 respectively, C indicates control

bands. Information generated from these banding patterns was used to study the genetic distance between the test isolates. Primer S119 generated specific amplification pattern in isolates Cf-204, Cf-249 and Cf-254 with single unique band while 2 unique bands were found in isolate Cf-248. S119 RAPD primer was more prominent and could be used to identify 13 to 16 isolates as it showed highest efficiency of 81.25%. Similarity coefficient based on DNA amplification using RAPD primers was estimated using Jaccard's coefficient of similarity (Sneath and Sokal, 1973). The similarity coefficient ranged from 0.31 to 0.83 (Tab. 6). Molecular data revealed that isolate Cf-223 showed least similarity coefficient 0.31) with Cf-253 isolate indicating their diverse nature at molecular level while isolate Cf-204 showed high similarity (0.83) with Cf-245 indicating their close relationship. The polymorphic information content (PIC) values ranged from 0.701 (primer S1118) to 0.929 (primer S103) with an average of 0.867 for all 22 primers. Cluster analysis was conducted to analyze the genetic similarities among different isolates as revealed by RAPD marker system (Fig. 2). At 60 per cent coefficient of similarity one major cluster was identified with eleven isolates while rest five isolates (Cf-223, Cf-248, Cf-250, Cf-252 and Cf-256) showed independent lineages. This cluster was found very heterogeneous as it comprised of isolates from 7 different districts of Punjab state and one from Haryana. All the isolates were associated at 40% similarity level. Isolate Cf-254 was the most diverse at molecular level as compared to other isolates. However, at 70 per cent coefficient of similarity two major clusters were identified which comprised of 11 isolates. Remaining 5 isolates showed independent lineages. Lineage I was formed by 6 isolates viz. Cf-65, Cf-60, Cf-254, Cf-254m, Cf-249 and Cf-255. Lineage II comprised of 5 isolates viz. Cf-253, Cf-251, Cf-204, Cf-245 and Cf-241. One isolate Cf-256 from UP formed independent lineage V along with 4 isolates Cf-250, Cf-252, Cf-248 and Cf-223 from Punjab which formed lineage III, IV, VI and VII. Isolates from Gurdaspur formed individual lineage, indicating that *C. falcatum* isolates from Gurdaspur were genetically distinct and these are well differentiated from isolates of other regions of Punjab.

Tab. 5. Primers used in RAPD analysis of isolates of *Colletotrichum falcatum*

S. No.	Primer Code	Primer sequence	Polymorphic Information Content (PIC) values	No. of amplified fragments
1	S 103	5'-AGACGTCCAC-3'	0.929	18
2	S 104	5'-GGAAGTCGCC-3'	0.850	13
3	S 108	5'-GAAACACCCC-3'	0.882	12
4	S 110	5'-CCTACGTCAG-3'	0.895	10
5	S 111	5'-CTTTCCGCAGT-3'	0.867	15
6	S 114	5'-ACCAGGTTGG-3'	0.747	10
7	S 115	5'-AATGGCGCAG-3'	0.922	14
8	S 116	5'-TCTCAGCTGG-3'	0.926	15
9	S 118	5'-GAATCGGCCA-3'	0.799	11
10	S119	5'-CTGACCAGCC-3'	0.791	17
11	S 1101	5'-TCACGTACGG-3'	0.926	15
12	S 1103	5'-CTTCCCTGTGT-3'	0.900	17
13	S 1104	5'-GAGGGACCTC-3'	0.903	16
14	S 1105	5'-GGGCTATGCC-3'	0.893	13
15	S 1106	5'-CTCGGGATGT-3'	0.907	17
16	S 1108	5'-ACCACGAGTG-3'	0.850	16
17	S 1110	5'-CAGACCGACC-3'	0.890	11
18	S 1111	5'-AGATGCGCGG-3'	0.927	16
19	S 1113	5'-CACGGCACAA-3'	0.901	16
20	S 1114	5'-TGGTTGCGGA-3'	0.788	14
21	S1118	5'-ACGGGACTCT-3'	0.701	17
22	S 1120	5'-ACCAACCAGG-3'	0.877	13

The clustering in RAPD dendrogram was not associated with the geographic locations from which the isolates were obtained. Different pathogens have been reported to possess a high degree of molecular variability when evaluated by RAPD markers like *Colletotrichum accutatum* (Guerber *et al.*, 2003; Whitelaw *et al.*, 2007), *Colletotrichum gloeosporiods* (Telhinhas *et al.*, 2005). However, Thottappilly *et al.* (1999)

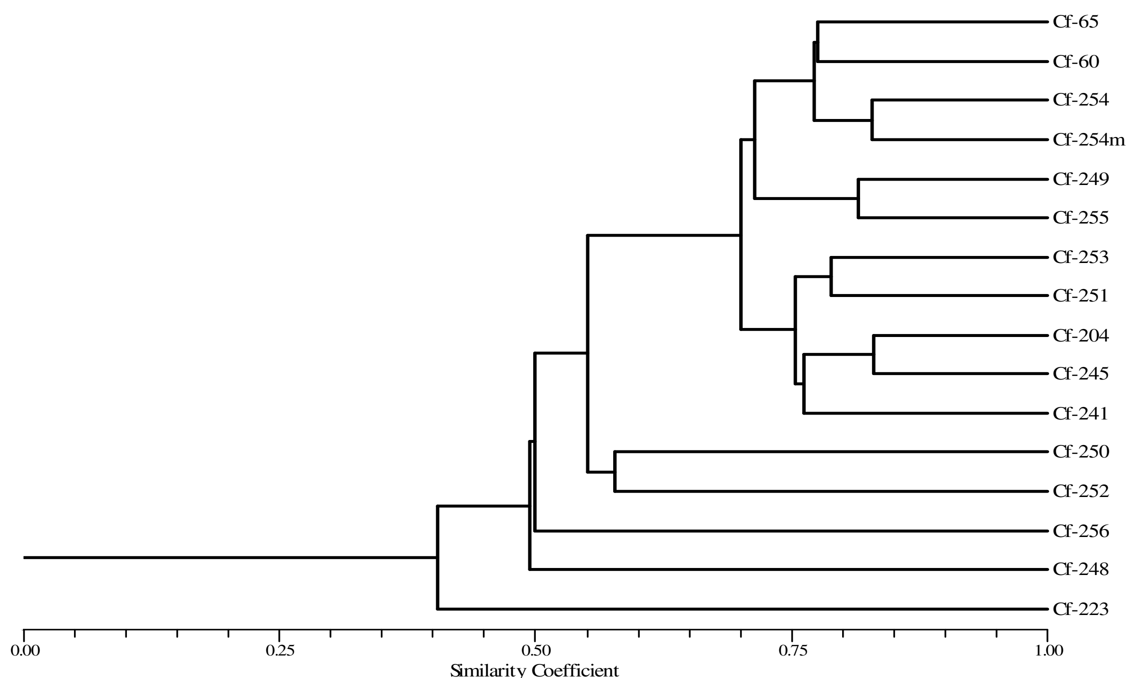

Fig. 2. Dendrogram obtained by unweighted paired group method showing molecular diversity among different isolates of *Colletotrichum falcatum*

Tab. 6. Similarity matrix generated by Jaccard's coefficient using molecular data

No.	Isolate No.	Cf65	Cf60	Cf254	Cf254m	Cf223	Cf250	Cf249	Cf255	Cf248	Cf253	Cf256	Cf252	Cf204	Cf241	Cf251	Cf245
1	Cf65	1.00															
2	Cf60	0.62	1.00														
3	Cf254	0.66	0.66	1.00													
4	Cf254m	0.67	0.64	0.74	1.00												
5	Cf223	0.00	0.00	0.00	0.00	1.00											
6	Cf250	0.36	0.35	0.34	0.37	0.00	1.00										
7	Cf249	0.52	0.51	0.53	0.54	0.00	0.36	1.00									
8	Cf255	0.48	0.52	0.52	0.54	0.00	0.37	0.69	1.00								
9	Cf248	0.34	0.30	0.28	0.32	0.00	0.27	0.32	0.33	1.00							
10	Cf253	0.48	0.53	0.49	0.50	0.00	0.31	0.51	0.56	0.31	1.00						
11	Cf256	0.34	0.34	0.32	0.29	0.00	0.30	0.28	0.28	0.26	0.32	1.00					
12	Cf252	0.33	0.34	0.30	0.33	0.00	0.32	0.30	0.32	0.22	0.37	0.27	1.00				
13.	Cf204	0.53	0.46	0.50	0.59	0.00	0.35	0.53	0.50	0.35	0.50	0.30	0.29	1.00			
14.	Cf241	0.57	0.51	0.55	0.57	0.00	0.36	0.55	0.54	0.30	0.52	0.31	0.30	0.60	1.00		
15.	Cf251	0.54	0.48	0.51	0.54	0.00	0.36	0.49	0.52	0.31	0.65	0.30	0.33	0.55	0.62	1.00	
16	Cf245	0.53	0.54	0.51	0.55	0.00	0.34	0.49	0.54	0.33	0.59	0.34	0.32	0.76	0.63	0.60	1.00

categorized 51 isolates of *C. gloeosporioides* into four groups using RAPD analysis, which were previously categorized on the basis of morphology and virulence. They did not find any correlation between classification of different isolates by RAPD and rate of growth of isolates in culture or their geographic origin. Padder *et al.* (2007) studied five *C. lindemuthianum* populations causing anthracnose in common bean to determine genetic diversity, migration and the probable rate of spread of races capable of overcoming resistance present in elite cultivars on the basis of allele frequencies of 12 RAPD markers. They suggested that naturally occurring gene flow is likely to be increased by the human activity for comprehensive cultivation of beans in the near future.

It is evident from the morphological, pathological and molecular analysis used in this study that *C. falcatum* causing red rot of sugarcane in the northern region of India possesses high degree of variability. However, it also inferred that morphological grouping of most of the isolates possess positive correlation with pathogenic variability whereas molecular diversity did not showed such correlation. The molecular analysis needs to be further validated with advanced markers like SNPs to get a précised and comprehensive results on existing variability of this pathogen for their successful exploitation in future breeding programme.

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