Genetic variability among isolates of *Colletotrichum falcatum* Went causing red rot of sugarcane in Tamil Nadu, India

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Molecular variability in *C. falcatum*

- Red rot is the major constraint to sugarcane cultivation in India
- Red rot causes significant loss in cane yield and reduction in sugar recovery
- The fungus is notorious for its variability and break down of red rot resistance in sugarcane varieties by forming new pathotypes/ races
- Using 13 sugarcane differential hosts, 11 pathotypes of *C. falcatum* have been reported in different zones of India (Satyavir, 2006)

C. falcatum races in India

- **North West Zone of India** - Cf 01 (Co 1148), Cf 02 (Co 7717), Cf 03 (CoJ 64), Cf 07 (CoJ 64), Cf 08 (CoJ 64/Co J84), Cf 09 (CoS 767)
- **East coast zone of India-** Cf 04 (Co 419), Cf 05 (Co 997), Cf 06 (CoC 671) and Cf 10 (35A 261) (Satyavir et al. 2001)
- Singh (2005) reported the existence of a new race of *C. falcatum* in North Western zone of India on the basis of infection.

Current status of physiological races of *C. falcatum* in India

Sr. No	RACE No	Source	LOCATION / ZONE	Co 997	Co 1148	KHAKAI	CoJ 64	Co 62399	Co 419	Co 975	Co 7717	CoC 671	BARAGUA	CoS 767	BO 91	SES 594
1	CF 01	Co 1148	NORTH WEST	S	S	S	S	S	R	S	R	S	R	R	R	R
2	CF 02	Co 7717	NORTH WEST	S	R	S	I	I	I	R	S	S	R	R	R	R
3	CF 03	CoJ 64	NORTH WEST	S	R	S	S	R	R	R	R	I	R	R	R	R
4	CF 04	CO 419	EAST COAST	I	S	S	S	R	S	S	S	S	R	R	R	R
5	CF 05	Co 997	EAST COAST	S	I	I	I	R	R	R	R	S	R	R	R	R
6	CF 06	CoC 671	EAST COAST	S	I	I	S	R	R	I	R	S	S	R	R	R
7	CF 07	CoJ 64	NORTH WEST	S	S	S	S	R	I	R	R	R	R	R	I	R
8	CF 08	CoJ 64 CoJ 84	NORTH WEST	S	S	S	S	S	I	S	S	S	R	I	R	R
9	CF 09	CoS 767	NORTH WEST	S	S	S	S	R	I	I	R	I	R	S	R	R
10	CF 10	85A261	EAST COAST	S	R	R	--	--	S	S	S	S	S	R	--	R

S = Susceptible R = Resistant I = Intermediate

Colletotrichum spp. toxins

- Several species of *Colletotrichum* produce phytotoxins (Abang et al. 2003; Amusa, 2006; Alleyne & O,Garro 2008)
- Toxins are involved in pathogenesis in several species
- Eg. Colletotrichins produced by *C. nicotianae* on *Nicotiana tabacum* (Gohbara et al. 1976)
- Acetyl colletotrichium and colletodiol produced by *C. capsici* (Grove et al. 1966)
- Glycoprotein toxins produced by *C. kahkwae* on coffee, *C. gloeosporioides* on yam (Alleyne & O,Garro 2008), citrus and mango (Garcia-Pajon & Collado 2003; Amusa 2006)

C. falcatum toxin

- ***C. falcatum* toxin is anthroquinone compound** (Olufolaji & Bamgboye 1986)
- Partially purified toxin from the culture filtrate of *C. falcatum* induced electrolytes from sugarcane leaves (Mohanraj et al. 2003) . The susceptible cultivar Co 87044 recorded a significant increase in loss of electrolytes as compared to that of a resistant cultivar BO 91.
- *C. falcatum* toxin can be used as a molecular marker in the search for resistant genotypes of sugarcane (Ramesh Sundar et al.,1999)
- Naik & Vedamurthy (1997) used toxin of *C. falcatum* in the selection of red rot resistant genotypes of sugarcane.

Objectives

Analysis of different isolates of *C. falcatum* from Tamil Nadu, India for their ability to produce toxin and genetic diversity using RAPD analysis

Places in Tamil Nadu, India, where red rot infected sugarcane samples were collected

Location	District	Cultivar	Isolate description
Kallakurichi	Villupuram	Co 86032	Cf 86032
Nellikuppam	Cuddalore	CoC 90063	Cf 90063a
Nellikuppam	Cuddalore	93v297	Cf 93v297a
Melpattampakkam	Cuddalore	Si 95020	Cf 95020
Padamathur	Sivaganga	Coc 671	Cf 671a
Devakottai	Sivaganga	CoC 671	Cf 671b
Ariyakudi	Sivaganga	CoC 671	Cf 671c
Viswanathapuram	Cuddalore	Co 8368	Cf 8368
Melpattampakkam	Cuddalore	Si 95045	Cf 95045
Arasamangalam	Cuddalore	CoC 98061	Cf 98061
Nattham East	Cuddalore	93v297	Cf 93 v 297b
Nantheiswaramangalam	Cuddalore	Co 93009	Cf 90063b
Sethiathope	Cuddalore	Co 93009	Cf 90063c
Kandarkottai	Cuddalore	Si 94025	Cf 94025
Thalavanur	Villupuram	Co 87012	Cf 87012a
Thirumanikuli	Villupuram	Co 87012	Cf 87012b
Pahour		Si 94045	Cf 94045
Semmandalam	Cuddalore	Co 8371	Cf 8371
Vampathur	Villupuram	Co 91017	Cf 91017
Thiyagavalli	Cuddalore	Co 6304	Cf 6304

Production of toxin by different isolates of *C. falcatum* as measured by induction of electrolyte leakage from sugarcane leaf tissue

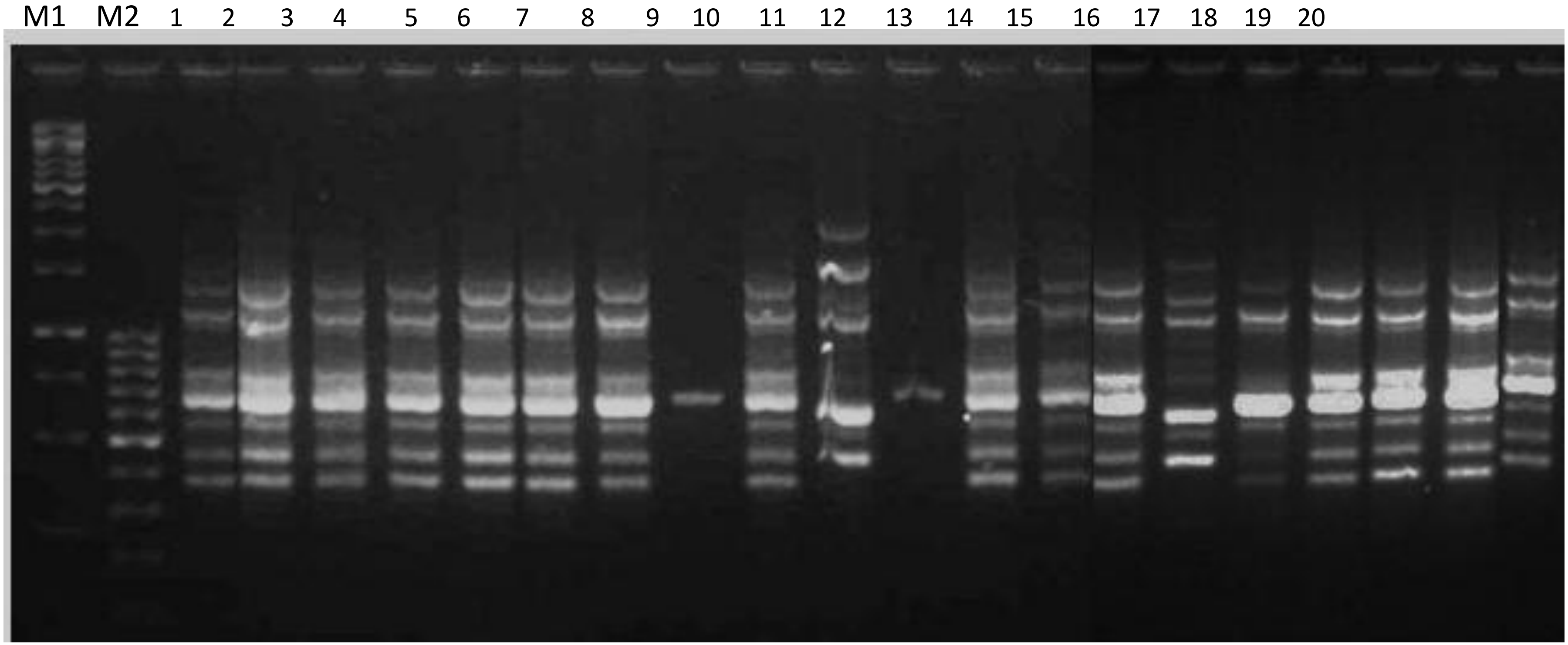
C. falcatum isolates	Electrolyte leakage (µS)*
Cf 86032	180
Cf 90063a	220
Cf 93v297a	180
Cf 95020	210
Cf 671a	300
Cf 671b	205
Cf 671c	225
Cf 8368	100
Cf 95045	200
Cf 98061	235
Cf 93v297b	295
Cf 90063b	190
Cf 90063c	260
Cf 94025	230
Cf 87012a	270
Cf 87012b	200
Cf 94045	170
Cf 8371	170
Cf 91017	155
Cf 6304	255
Control	40
CD(P=0.05)	17.52

RAPD analysis of isolates of *C. falcatum*

Sequences of RAPD primers used to study the genetic variability among isolates of *C. falcatum*

Primers	Nucleotide sequence 5'- 3'
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-03	CATCCCCCTG
OPB-06	TGCTCTGCCC
OPB-15	GGAGGGTGTT
OPC-02	GTGAGGCGTC
OPC-07	GTCCCGACGA
OPC-08	TGGACCGGTG
OPD-01	GGACCCAACC
OPD-07	TTGGCACGGG
OPE-01	CCCAAGGTCC
OPE-02	GGACTGCAGA
OPE-09	CTTCACCCGA
OPL-05	ACGCAGGCAC
OPL -07	AGGCGGGAAC
OPL-08	AGCAGGTGGA
OPL-12	GGGCGGTACT

RAPD analysis of isolates of *C. falcatum* using primer OPL-05



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Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
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RAPD analysis of isolates of *C. falcatum* using primer OPL-07

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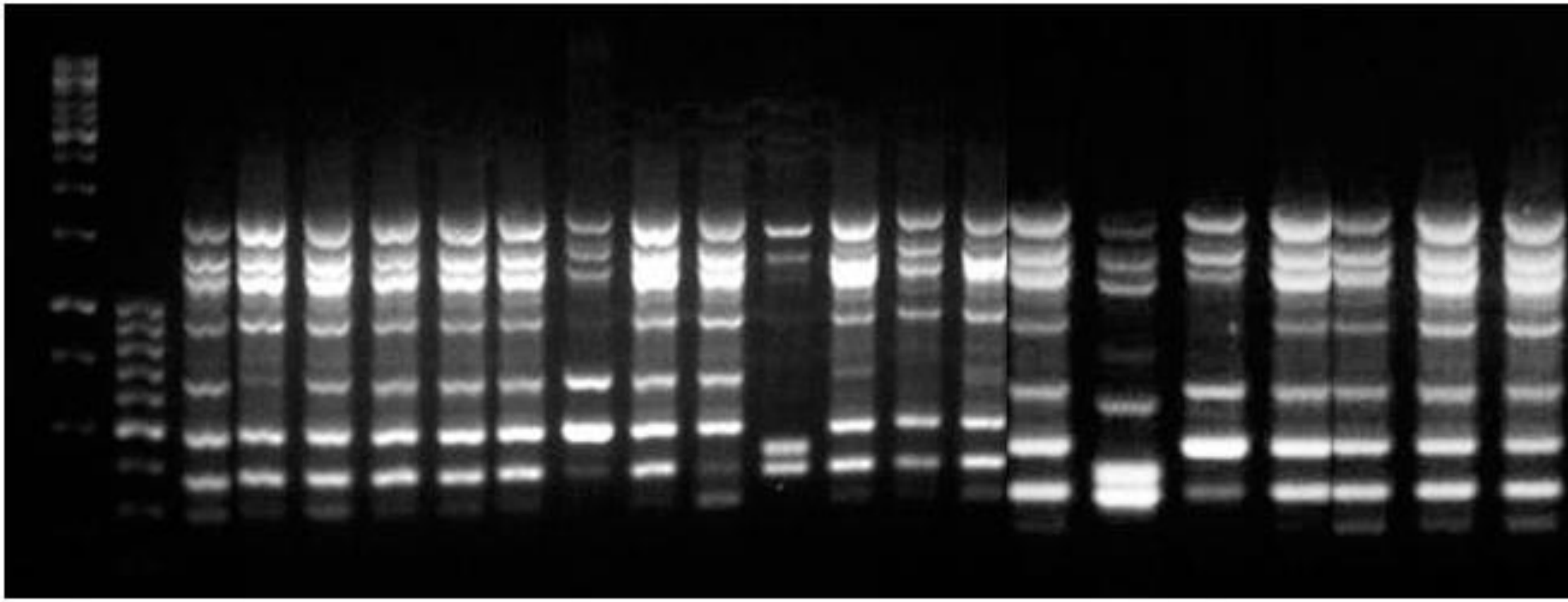

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Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
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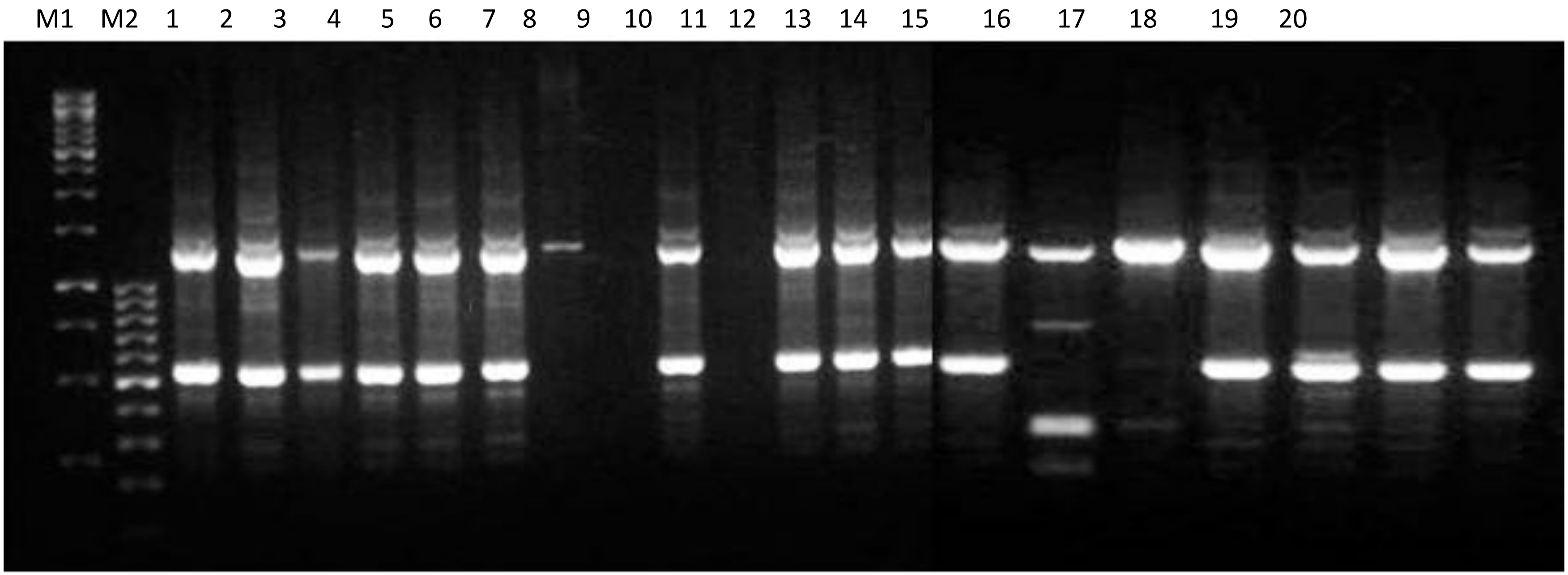
RAPD analysis of isolates of *C. falcatum* using primer OPL-08



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Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13

Lane 14:Cf 14
Lane 15: Cf 15
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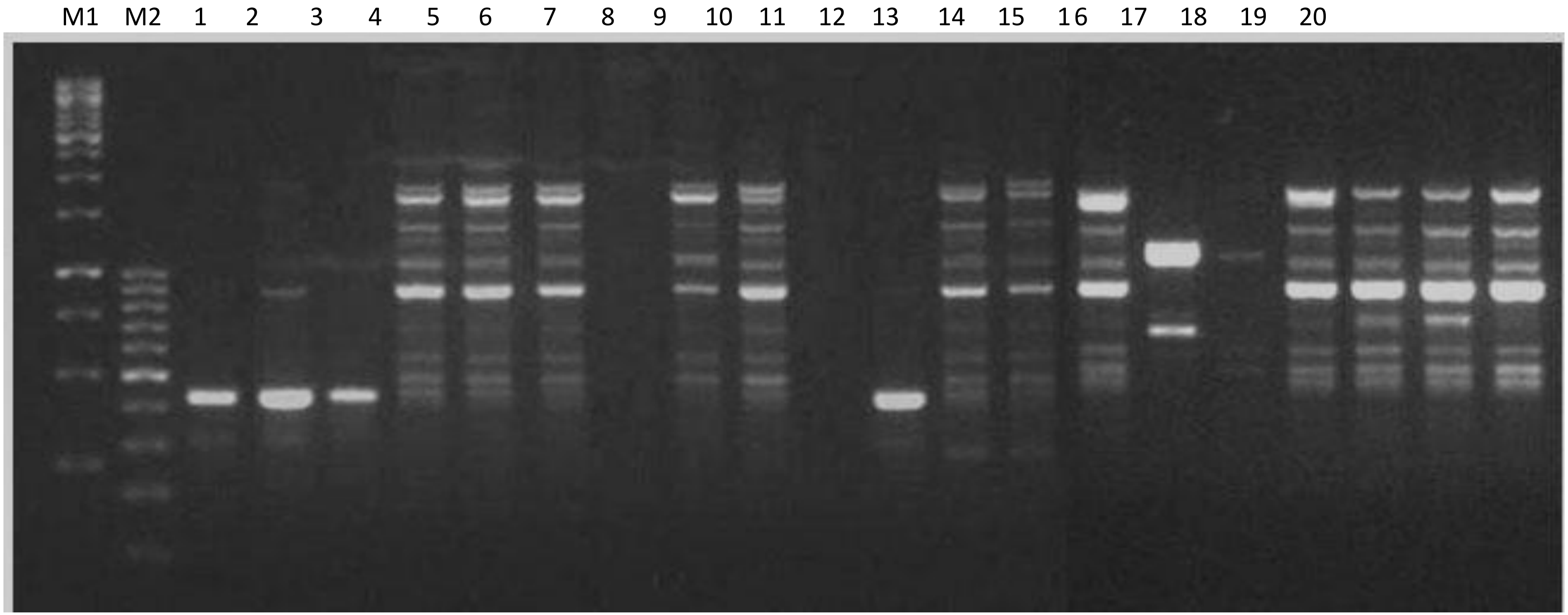
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPB- 15



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Lane 8: Cf 8
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Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13

Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPE-01

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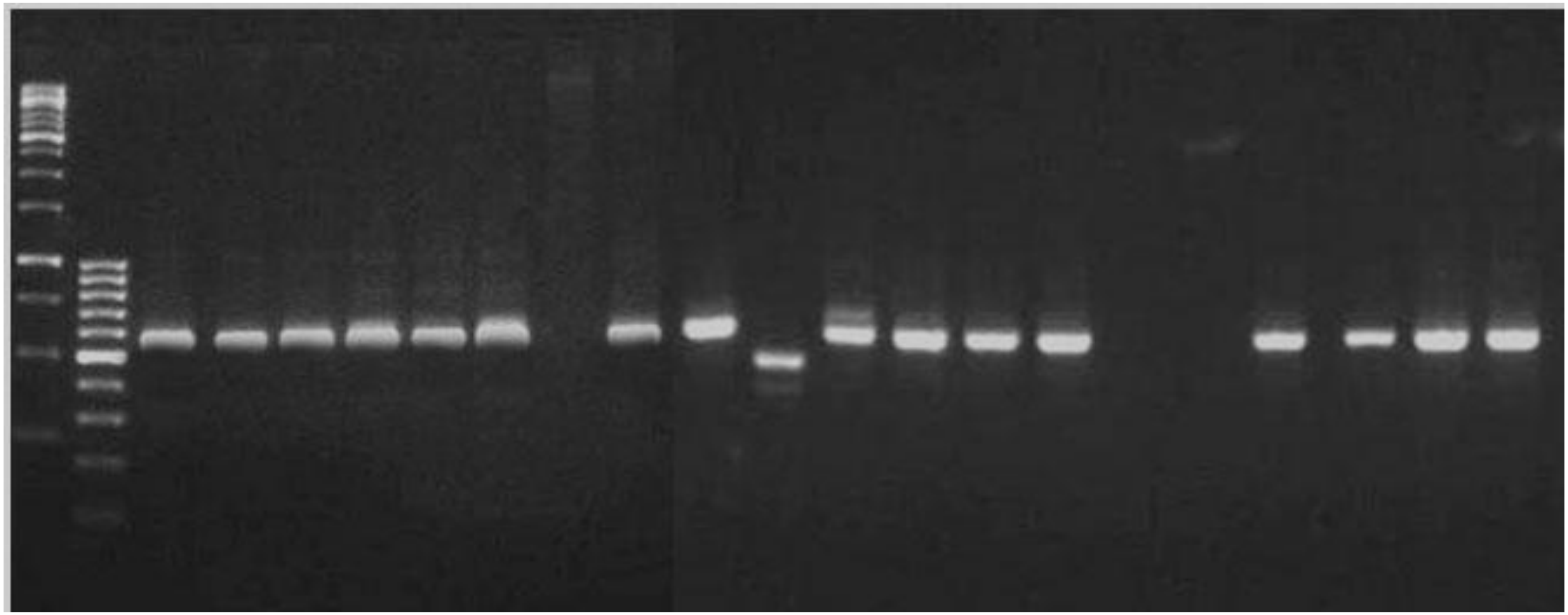

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Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPL-12

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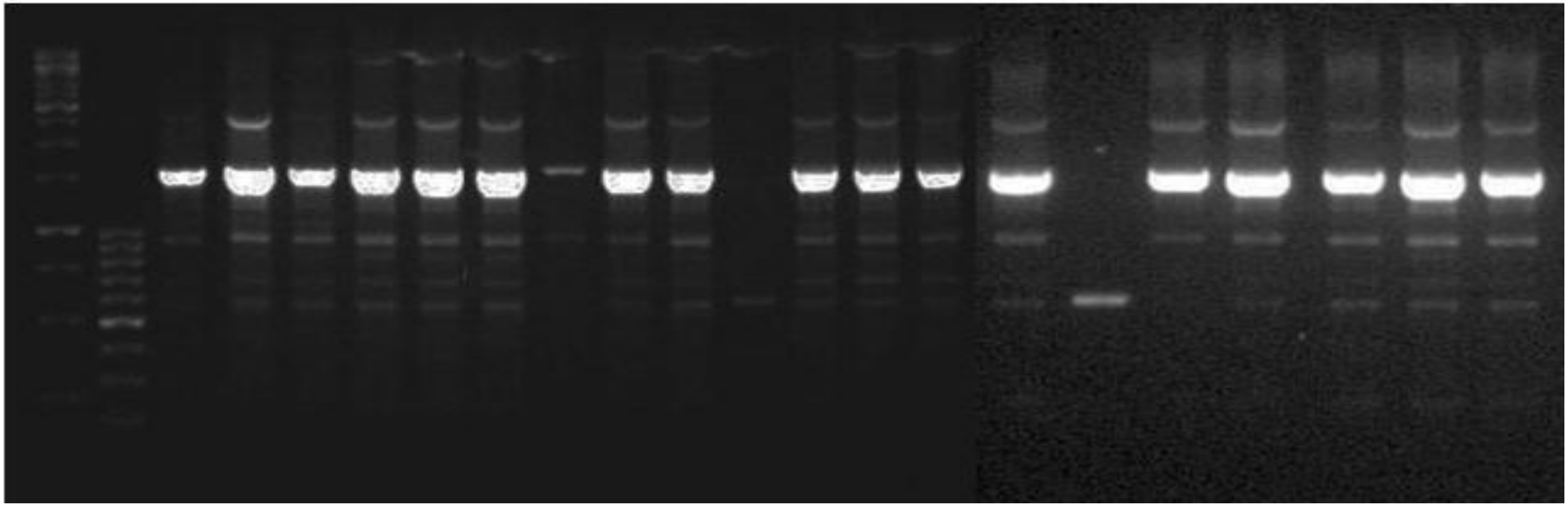

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Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
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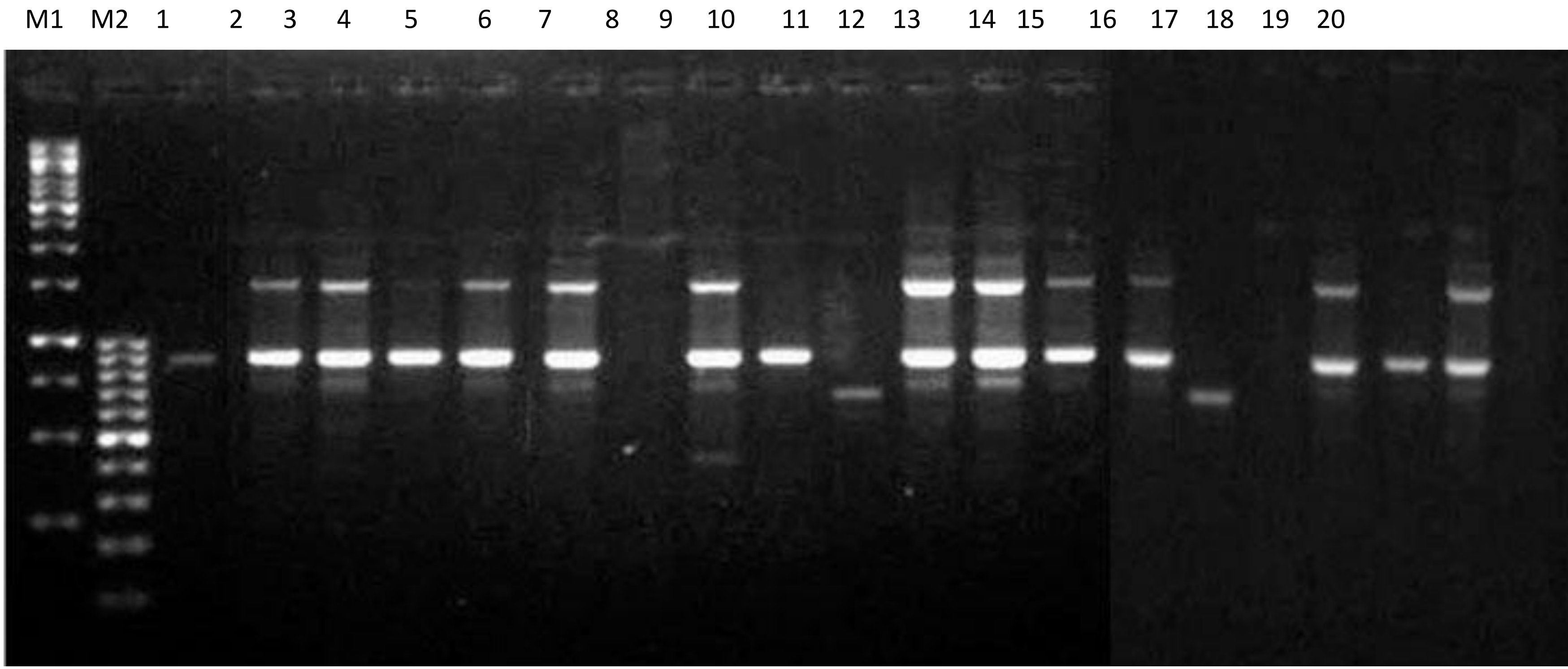
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPA 1



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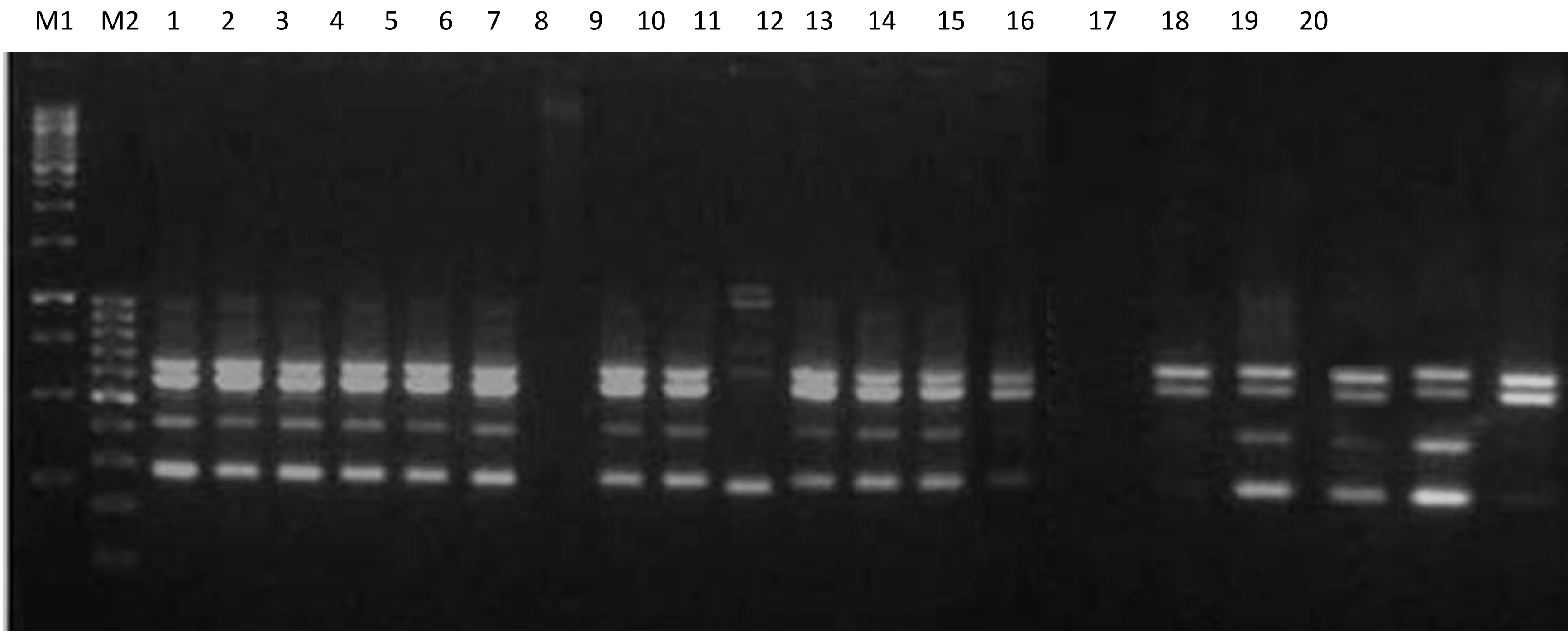
Lane 8: Cf 8
Lane 9: Cf 9
Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13

Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPA 2



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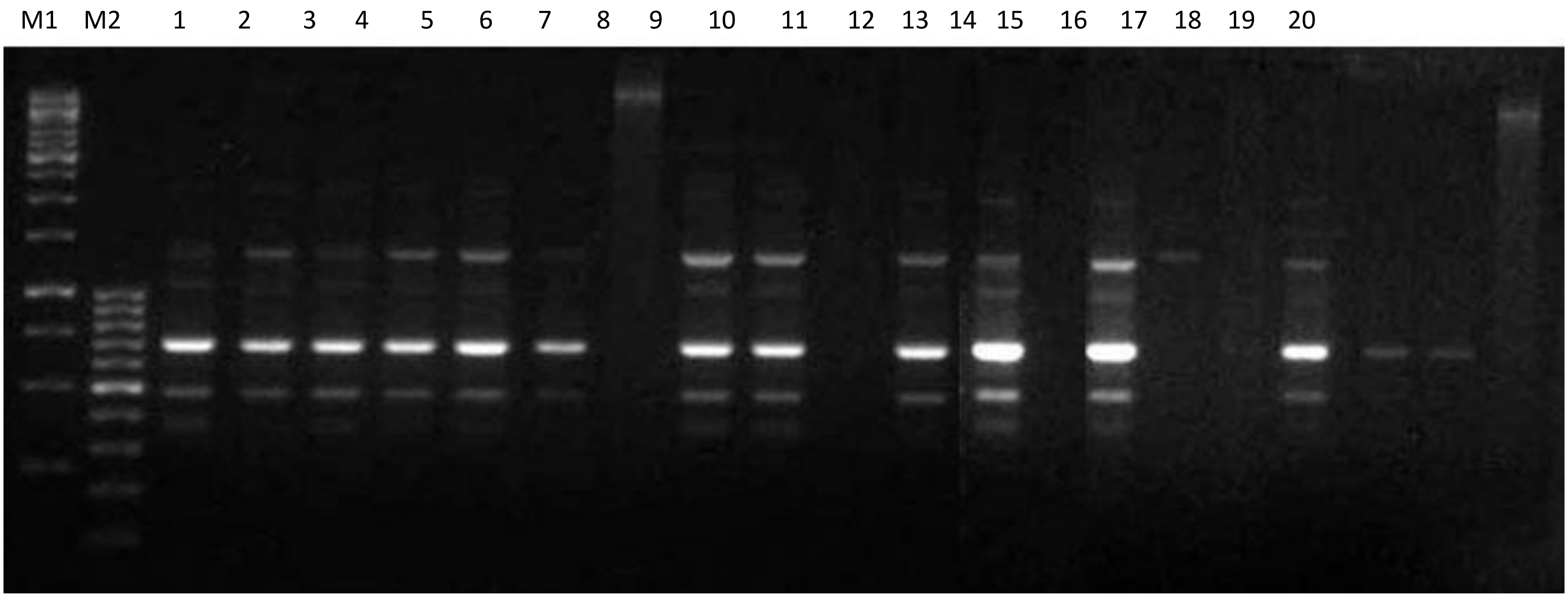
Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
Lane 20: Cf 20

RAPD analysis of isolates of *C. falcatum* using primer OPA 5



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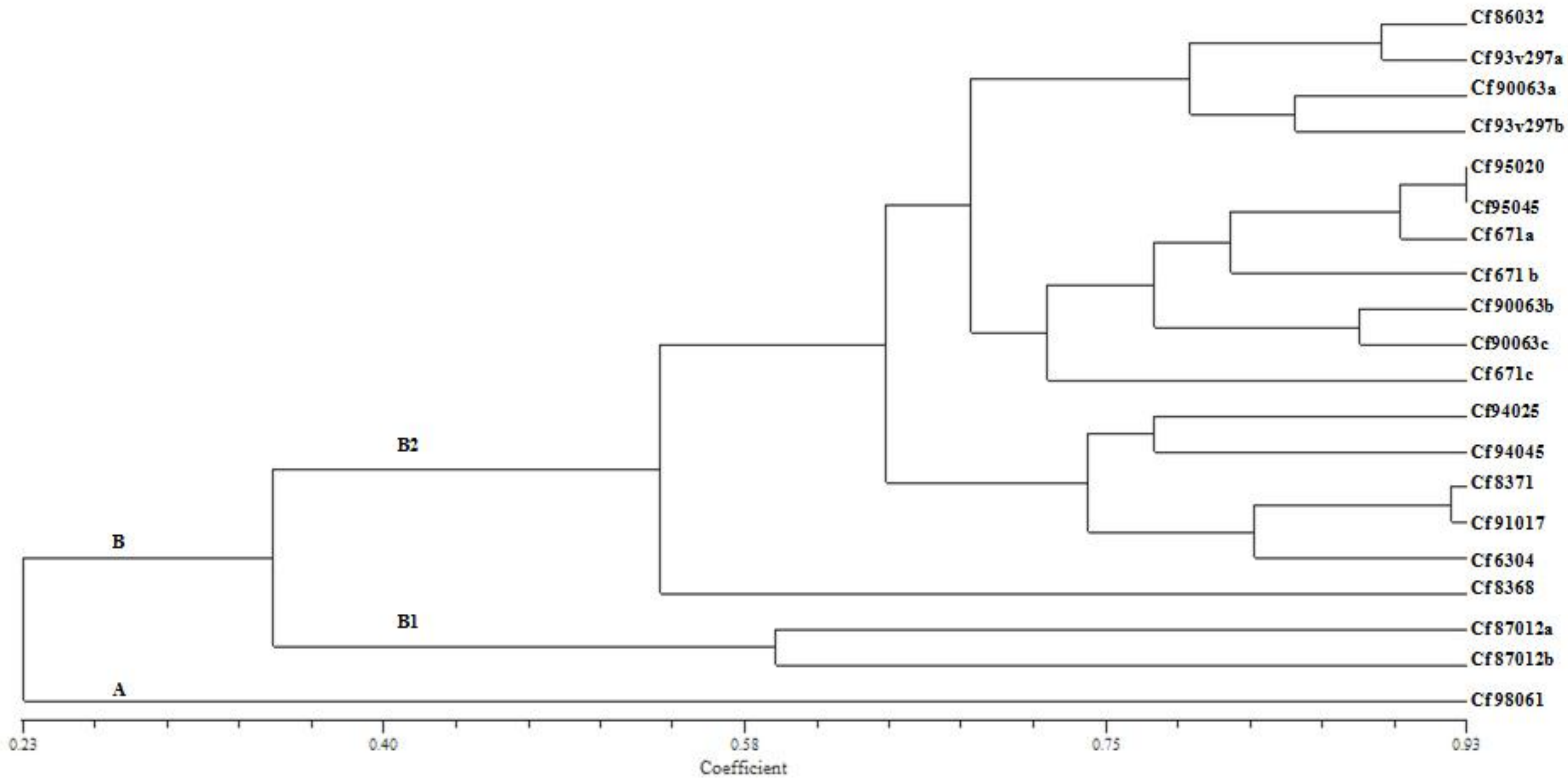
Lane 10: Cf 10
Lane 11: Cf 11
Lane 12: Cf 12
Lane 13: Cf 13
Lane 14:Cf 14
Lane 15: Cf 15
Lane 16: Cf 16
Lane 17: Cf 17
Lane 18: Cf 18
Lane 19: Cf 19
Lane 20: Cf 20

Genetic similarity coefficient matrix for *C. falcatum* isolates based on RAPD profile

C.falcatum Isolates	Cf 86032	Cf 90063a	Cf 93v297a	Cf 95020	Cf 671a	Cf 671b	Cf 671c	Cf 8368	Cf 95045	Cf 98061	Cf 93v297b	Cf 90063b	Cf 90063c	Cf 94025	Cf 87012a	Cf 87012b	Cf 94045	Cf 8371	Cf 91017	Cf 6304
Cf 86032	1.000																			
Cf 90063a	0.750	1.000																		
Cf 93v297a	0.861	0.825	1.000																	
Cf 95020	0.681	0.777	0.711	1.000																
Cf 671a	0.652	0.822	0.717	0.954	1.000															
Cf 671b	0.565	0.733	0.630	0.863	0.909	1.000														
Cf 671c	0.388	0.400	0.432	0.395	0.377	0.414	1.000													
Cf 8368	0.476	0.545	0.547	0.600	0.608	0.521	0.230	1.000												
Cf 95045	0.697	0.717	0.727	0.930	0 .888	0.800	0.404	0.651	1.000											
Cf 98061	0.195	0.222	0.238	0.229	0.220	0.239	0.296	0.146	0.208	1.000										
Cf 93v297b	0.648	0.717	0.684	0.613	0.622	0.533	0.270	0.685	0.627	0.150	1.000									
Cf 90063b	0.595	0.723	0.659	0.847	0.851	0.765	0.355	0.622	0.866	0.224	0.600	1.000								
Cf 90063c	0.511	0.608	0.577	0.772	0.777	0.853	0.384	0.534	0.790	0.232	0.547	0.795	1.000							
Cf 94025	0.651	0.711	0.720	0.760	0.765	0.680	0.390	0.568	0.818	0.166	0.581	0.744	0.666	1.000						
Cf 87012a	0.209	0.234	0.222	0.240	0.230	0.224	0.266	0.136	0.220	0.321	0.166	0.211	0.191	0.229	1.000					
Cf 87012b	0.388	0.400	0.358	0.395	0.377	0.414	0.440	0.263	0.372	0.206	0.305	0.326	0.384	0.390	0.357	1.000				
Cf 94045	0.622	0.680	0.652	0.729	0.734	0.653	0.311	0.543	0.744	0.234	0.521	0.750	0.638	0.777	0.297	0.404	1.000			
Cf 8371	0.568	0.630	0.565	0.717	0.687	0.711	0.375	0.425	0.695	0.227	0.466	0.632	0.659	0.727	0.266	0.447	0.772	1.000		
Cf 91017	0.555	0.617	0.586	0.666	0.673	0.695	0.365	0.446	0.680	0.250	0.488	0.620	0.681	0.750	0.260	0.400	0.795	0.923	1.000	
Cf 6304	0.500	0.533	0.500	0.622	0.595	0.651	0.441	0.355	0.636	0.230	0.395	0.541	0.634	0.750	0.275	0.484	0.674	0.837	0.815	1.000

Unweighted pair group method arithmetic average dendrogram constructed from RAPD data indicating the relationship among the isolates of *C. falcatum*



Salient findings

- Genetic relatedness of 24 isolates of *C. falcatum* differing in toxin production potential was investigated by RAPD analysis using 18 decamer primers
- Analysis of the genetic coefficient matrix derived from the scores of RAPD profiles showed that minimum and maximum percent similarities among the tested *C. falcatum* isolates were in the range of 19 to 95% respectively
- Phylogenetic analysis by the UPGMA identified two main clusters.
- Both highly toxigenic and moderately toxigenic isolates were interspersed in the two groups, indicating that no association exists between RAPD profile and the production of toxin in *C. falcatum*.


Thank you