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MOLECULAR AND PATHOGENIC VARIABILITY AMONG INDIAN ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* CAUSING WILT IN CHICKPEA

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KEYWORDS

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ABSTRACT

Chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is the major constraint in chickpea production. During present investigations eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* representing nine states and eight Agro climatic region of India were analysed for their genetic and pathogenic variability study. All the isolates proved to be pathogenic to susceptible cv. JG-62. From which, three isolates were found highly pathogenic (71-100%), eight were strongly pathogenic and seven moderately pathogenic to cv. JG-62. Genetic diversity was studied among the eighteen virulent isolate using RAPD. Fifty randomly selected primers were screened against the eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri*, out of these fifty 10-mer primers, 49 primers produced polymorphic results. A total 622 amplicons were amplified with the 49 primers with an average 12.69 bands per primer. Out of 622 bands, 601 bands were polymorphic and the level of polymorphism was 96.62 per cent. Primer OPA-19 amplified maximum (18) bands while OPA-6 amplified minimum (7) bands. The range of similarity coefficient value ranged from 0.5048 (Parbhani and Rajnandgaon) to 0.9260 (Jabalpur and Rajnandgaon) across eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* indicating high degree of variation in respect to genetic similarity.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops cultivated in tropical and temperate regions. Low yield of chickpea is attributed to various biotic and abiotic stresses. Amongst the biotic stress, the wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Matuo and K. Sato is major constraint in productivity of chickpea worldwide (Haware and Nene, 1982). It is reported from all the chickpea-growing states of India and its incidence varies from 14.1 to 32.0% (Dubey *et al*. 2010) and causes an annual loss of 10% (Singh and Dahiya, 1973). The cultivation of resistant cultivar is one of the most prudent and cost-effective practices available for the management of Fusarium wilt, but these varieties do not perform satisfactory in different locations (Jimenez-Gasco *et al*. 2004) because of their high pathogenic variability that limits the effectiveness of their resistance (Jimenez-Diaz *et al*. 1993). Eight races of the pathogen (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) were identified by their reaction on a set of host differential chickpea cultivars (Haware and Nene 1982; Jimenez-Diaz *et al*. 1993). All these races have distinct geographic distribution. Races 1, 2, 3 and 4 were reported only from India, while races 0, 1B/C, 5 and 6 were reported from the Mediterranean region and the USA, thus showing area-specific distribution patterns.

From the combined analysis of RAPD and AFLP markers it appeared that the most predominant race of the pathogen prevalent in the chickpea growing states of India is race-1 with 17 isolates followed by race-2 with 15 isolates. Race-3 and race-4 appear to be rare as only six pathogen isolates belong to these two together Sivaramakrishnan *et al*. (2002). The molecular markers such as Random Amplified Polymorphic DNA (RAPD) is an important tool used successfully for variability study by Kelly *et al*. (1994), Jimenez–Gasco *et al*. (2001), Honnareddy and Dubey (2006), Singh *et al*. (2006), Dubey and Singh (2008), Bayraktar and Dolar (2009), Mandhare *et al*. (2011), Dubey *et al*. (2012), Sanjana Ingle *et al*. (2012) and Madhuri Katkar and Mane (2012). The aim of the present study was to analyse the virulence and molecular diversity of Foc isolates representing various states/ agro ecological regions of India.

MATERIALS AND METHODS

Collection, isolation and maintenance of isolates

Chickpea wilt samples were collected from eighteen locations of nine states representing eight Agro climatic region of India namely Western Plateau and Hill region, Central Plateau and Hills region, Eastern Plateau and Hills region, Upper Gangetic Plains region, Middle Gangetic Plains region, Trans-Gangetic Plains region, Western dry region and Southern Plateau and Hills region of India (Table 1).

The roots of wilted plant were cut into small pieces and surface sterilized by using $HgCl_2$ (0.1%) followed by rinsing twice with sterilized distilled water. These small bits were transferred to PDA plates under asceptic condition and incubated at 27 $\pm$ 1ºC in BOD incubator. Finally 18 isolates of *Fusarium oxysporum* f. sp. *ciceri* were obtained.

Purification and identification of fungi

The pure cultures of *Fusarium oxysporum* f. sp. *ciceri* were purified by using 'Single Spore Culture Technique' and identified microscopically as *Fusarium oxysporum* f.sp. *ciceri* on the basis of morphological characters by Booth 1977 and molecularly identified as *F. oxysporum* f.sp. *ciceri* by using SCAR primer Foc0-12f (5'GGCGTTTCGCA GCCTTACA ATGAAG3') and Foc0-12r (5'GACTCCTTTTT CCCGAGGTA GGTCAGAT3') Jimenez-Gasco and Jimenez-Diaz (2003). Eighteen purified cultures of *F. oxysporum* f.sp. *ciceri* were maintained at 4ºC on PDA slants for further studies.

Pathogenic variability

The eighteen isolates of *F. oxysporum* f.sp. *ciceri* were purified by single spore method and mass multiplied separately on sorghum grain medium. The mass multiplied inoculum was mixed with the sterilized soil in 1:3 proportion and filled in the pre sterilized pots. Five seeds of susceptible chickpea cultivar JG-62 were sown per pots for each isolate in 3 replications. The per cent emergence and post-emergence mortality were recorded upto 30 days after sowing (DAS). The seedlings maintained in sterilized soil without inoculums were served as control.

Molecular variability

Fungal DNA extraction

Fungal DNA was extracted from pure cultures of *F. oxysporum* f.sp. *ciceri* isolates multiplied in potato dextrose broth at 25 ± 1ºC on a shaking incubator (120 rpm) for 7 days by using cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications. The DNA was dissolved in TE (10 mM Tris–hydrochloric acid and 1 mM sodium EDTA, pH 8) and stored at -20°C. The quality and quantity of DNA were estimated by spectrophotometer.

Amplification of Fungal DNA

Fungal DNA of each isolates was subjected to amplication by using RAPD marker. 50 RAPD primers (19 OPA series, 9 OPB series, 1 OPC series, 1 OPE series and 20 OPF series) were selected to evaluate molecular variability within *Fusarium oxysporum* f.sp. *ciceri* isolates (Table 2). Each reaction mixture contained total 12.5 ìl reaction containing 10 mM Tris-HCl with KCl (pH 8.0), 25 mM $MgCl_2$, 0.24 ìM primer, 10 mM dNTPs, and 2 U Taq polymerase (Invitrogen). Amplification was performed as follow, Initial denaturation (94ºC, 10 minutes) followed by 35 cycles of denaturing (94ºC, 60 sec.), annealing (37ºC, 60 sec.) for different primers (Table 3), extension (72ºC, 2 minutes) and a final extension step (72ºC, 10 minutes) in Eppendorf gradient (Germany) thermocycler.

The PCR amplified products were separated by horizontal gel electrophoresis in 1.2% agarose gel prepared with 1x TBE buffer and run with the electric potential difference of 80 V for 135 min and subsequently stained with ethidium bromide solution 0.5 mg.ml^{-} and photographed with Gel Doc under UV translaminator. Lourmat T-5 × 20-2A. A 1kb ladder (Gene RulerTM, Fermantas, France) was used as a molecular size standard ruler.

Observations and data analysis

Comparison of each primer's profile was made on the basis of the presence or absence of PCR fragments at positions. Using the NTSYS-pc v.2.0 (Exeter Biological Software, Setauket, NY, USA) numerical taxonomy package program (Rohlf, 1998), a genetic similarity matrix was created with Dice's coefficient of similarity. The genetic similarity matrix was subjected to cluster analysis with an unweighted pair-grouped method with arithmetic average (UPGMA) to generate a dendrogram. In addition to comparison of the dendrogram formed using these marker systems, cophenetic value matrices were calculated, which were later compared by the Mantel test.

RESULTS AND DISCUSSION

Pathogenicity and pathogenic variability

The pathogenicity test indicates that all the 18 isolates of *Fusarium oxysporum* f. sp. *ciceri,* proved to be pathogenic to susceptible cv. JG-62. The isolates of *F. oxysporum* f. sp. *ciceri* were tentatively divided in five groups *viz.,* Non pathogenic (0% wilting), weakly pathogenic (1-20% wilting), moderately pathogenic (21-50% wilting), strongly pathogenic (51-70% wilting) and highly pathogenic (> 70% wilting) based on their pathogenic ability on cv. JG-62. Out of which three isolates (viz., FOC-4, FOC-7, FOC-11, FOC-12, FOC-13, FOC-14, FOC-

Table1: List of different isolates of *Fusarium oxysporum* f.sp. *ciceri* from India

Sr. No.	Isolates	Location	State	GPS Location	Agro climatic region	Sub-Agro climatic zones
1	FOC-1	Akola	Maharashtra	N-20º42'18.65''E-077º03'16.19''	IX. Western Plateau and Hill region	Central Plateau
2	FOC-2	Nagpur	Maharashtra	N-21º07'51.56''E-079º04'15.28''	IX. Western Plateau and Hill region	Central Vidarbha
3	FOC-3	Parbhani	Maharashtra	N-19º15'02.21''E-076º47'45.33''	IX. Western Plateau and Hill region	Central Plateau
4	FOC-4	Badnapur	Maharashtra	N-19º52'06.47''E-075º42'24.79''	IX. Western Plateau and Hill region	Central Plateau
5	FOC-5	Rahuri	Maharashtra	N-19º22'12.53''E-074º38'58.07''	IX. Western Plateau and Hill region	Scarcity region
6	FOC-6	Jabalpur	Madhya Pradesh	N-23º12'50.67''E-079º57'52.90''	VIII. Central Plateau and Hills region	Kymore Plateau and Satpura Hills
7	FOC-7	Rajnandgaon	Chhattisgarh	N-21º06'27.56''E-081º05'21.52''	VII. Eastern Plateau and Hills region	Wainganga
8	FOC-8	Raipur	Chhattisgarh	N-21º14'04.91''E-081º41'49.99''	VII. Eastern Plateau and Hills region	Wainganga
9	FOC-9	Kanpur	Uttar Pradesh	N-26º29'34.93''E-080º16'20.20''	V. Upper Gangetic Plains region	Central Plains
10	FOC-10	Allahabad	Uttar Pradesh	N-25º24'96.47''E-081051'00.99''	V. Upper Gangetic Plains region	Central Plains
11	FOC-11	Varanasi	Uttar Pradesh	N-25º15'25.72''E-082º59'21.63''	IV. Middle Gangetic Plains region	Eastern Plain Zone of Uttar Pradesh
12	FOC-12	Delhi	Delhi	N-28º38'23.77''E-077º09'27.41''	VI. Trans-Gangetic Plains region	Plains
13	FOC-13	Gurdaspur	Punjab	N-32º02'36.29''E-075º23'12.36''	VI. Trans-Gangetic Plains region	Foot hill of Shivalik
14	FOC-14	Nimboda	Rajasthan	N-24º09'40.54''E-073º48'00.57''	VIII. Central Plateau and hills region	Southern plain of Rajasthan
15	FOC-15	Udaipur	Rajasthan	N-24º34'52.81''E-078º42'12.55''	VIII. Central Plateau and Hills region	Southern Plain of Rajasthan
16	FOC-16	Bikaner	Rajasthan	N-28º05'36.83''E-073º21'06.26''	XIV. Western dry region	Western dry region
17	FOC-17	ICRISAT (Hyderabad)	Andhra Pradesh	N-17º30'15.87''E-078º16'15.93''	X. Southern Plateau and Hills region	South Telangana
18	FOC-18	Dharwad	Karnataka	N-15º29'51.91''E-074º59'09.67''	X. Southern Plateau and Hills region	Northern dry region of Karnataka

Table 2: List of RAPD primers screened against the *Fusarium oxysporum* f. sp. *ciceri* isolates

Sr. No.	Primer Screened	Sequence	Annealing Temp. (°C)
1	OPA-1	CAGGCCCTTA	32
2	OPA-2	TGCCGAGCTG	34
3	OPA-3	AGTCAGCCAC	32
4	OPA-4	AATCGGGCTG	32
5	OPA-5	AGGGGTCTTG	32
6	OPA-6	GGTCCCTGAC	34
7	OPA-7	GAAACGGGTG	32
8	OPA-8	GTGACGTAGG	32
9	OPA-9	GGGTAACGCC	34
10	OPA10	GTGATCGCAG	32
11	OPA-12	TCGGCGATAG	32
12	OPA-13	CAGCACCCAC	34
13	OPA-14	TCTGTGCTGG	32
14	OPA-15	TTCCGAACCC	32
15	OPA-16	AGCCAGCGAA	32
16	OPA-17	GACCGCTTGT	32
17	OPA-18	AGGTGACCGT	32
18	OPA-19	CAAACGTCGG	32
19	OPA-20	GTTGCGATCC	32
20	OPB-3	CATCCCCCTG	34
21	OPB-5	TGCGCCCTTC	34
22	OPB-6	TGCTCTGCCC	34
23	OPB-7	GGTGACGCAG	34
24	OPB-10	CTGCTGGGAC	34
25	OPB-11	GTAGACCCGT	32
26	OPB-14	TCCGCTCTGG	34
27	OPB-15	GGAGGGTGTT	32
28	OPB-17	AGGGAACGAG	32
29	OPC-20	ACTTCGCCAC	32
30	OPE-11	GAGTCTCAGG	32
31	OPF-1	ACGGATCCTG	32
32	OPF-2	GAGGATCCCT	32
33	OPF-3	CCTGATCACC	32
34	OPF-4	GGTGATCAGG	32
35	OPF-5	CCGAATTCCC	32
36	OPF-6	GGGAATTCGG	32
37	OPF-7	CCGATATCCC	32
38	OPF-8	GGGATATCGG	32
39	OPF-9	CCAAGCTTCC	32
40	OPF-10	GGAAGCTTGG	32
41	OPF-11	TTGGTACCCC	32
42	OPF-12	ACGGTACCAG	32
43	OPF-13	GGCTGCAGAA	32
44	OPF-14	TGCTGCAGGT	32
45	OPF-15	CCAGTACTCC	32
46	OPF-16	GGAGTACTGG	32
47	OPF-17	AACCCGGGAA	32
48	OPF-18	TTCCCGGGTT	32
49	OPF-19	CCTCTAGACC	32
50	OPF-20	GGTCTAGAGG	32

15) were found highly pathogenic, eight strongly pathogenic (viz., FOC-1, FOC-3, FOC-5, FOC-6, FOC-8, FOC-9, FOC16, FOC-18) and seven moderately pathogenic (viz., FOC-4, FOC-7, FOC-11, FOC-12, FOC-13, FOC-14, FOC-15) (Table 3 and 4). Paulkar *et al*. (2002) observed the pathogenic variability among four isolates of *F. oxysporum* f.sp. *ciceri* under laboratory condition. Differentiation among 86 isolates of *F. oxysporum* f.sp. *ciceri* collected from 84 locations of Madhya Pradesh were also reported by Om Gupta *et al*. (1986). Pathogenic variability of *F. oxysporum* f.sp. *ciceri* isolates based on wilting in cv. JG-62 was reported earlier by Prasad and Padwick (1939), Haware and Nene (1979), Kewate (1986), Shubha Trivedi and Gurha (2005), Barhate *et al*. (2006) and Srivastava and Agrawal (2006). The pathogenicity test was confirmed by proving Koch's Postulate and pathogens were confirmed as *Fusarium oxysporum* f. sp *ciceri* (Padwick) Snyder and Hansen.

RAPD analysis

Genetic variation was detected among eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* using RAPD marker. Out of the 50 randomly selected primers screened for amplification

Table 3: Pathogenicity test of *Fusarium oxysporum* f.sp. *ciceri* against susceptible variety JG-62

Place	Isolates	Total plants	Germina-tion	% Germination	Wilted plants	% Wilting	Wilting (DAI)
Akola	FOC-1	15	14	93.33	8	57.14	22
Nagpur	FOC-2	15	13	86.67	10	76.92	19
Parbhani	FOC-3	15	13	86.67	8	61.54	21
Badnapur	FOC-4	15	14	93.33	6	42.86	22
Rahuri	FOC-5	15	14	93.33	8	57.14	32
Jabalpur	FOC-6	15	15	100.00	8	53.33	31
Dharansara	FOC-7	15	14	93.33	7	50.00	24
Raipur	FOC-8	15	14	93.33	8	57.14	24
Kanpur	FOC-9	15	12	80.00	8	66.67	30
Allahabad	FOC-10	15	15	100.00	13	86.67	30
Varanasi	FOC-11	15	15	100.00	5	33.33	20
Delhi	FOC-12	15	14	93.33	5	35.71	31
Gurudaspur	FOC-13	15	15	100.00	6	40.00	17
Nimboda	FOC-14	15	14	93.33	4	28.57	21
Udaipur	FOC-15	15	14	93.33	5	35.71	22
Bikaner	FOC-16	15	13	86.67	8	61.54	23
ICRISAT	FOC-17	15	12	80.00	12	100.00	26
Dharwad	FOC-18	15	12	80.00	8	66.67	24
	Control	15	13	86.67	00	00	00

Table 4: Grouping of *F. oxysporum* f. sp. *ciceri* isolates on the basis of pathogenicity

Sr. No	Group (% Mortality)	*F. oxysporum* f. sp. *ciceri*
1	Non-Pathogenic(0 %)	(0)
2	Weakly Pathogenic(1-20 %)	(0)
3	Moderately Pathogenic(21-50 %)	FOC-4, FOC-7, FOC-11, FOC-12, FOC-13, FOC-14, FOC-15 (7)
4	Strongly Pathogenic(51-70 %)	FOC-1, FOC-3, FOC-5, FOC-6, FOC-8, FOC-9, FOC16, FOC-18 (8)
5	Highly Pathogenic(>70 %)	FOC-2, FOC-10, FOC-17. (3)

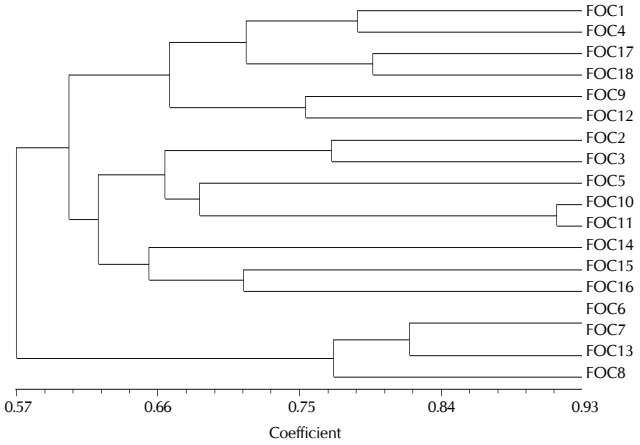

Figure 1: UPGMA dendrogram obtained by RAPD analysis of *Fusarium oxysporum* f. sp. *ciceri* isolates based on Jaccard's similarity coefficient

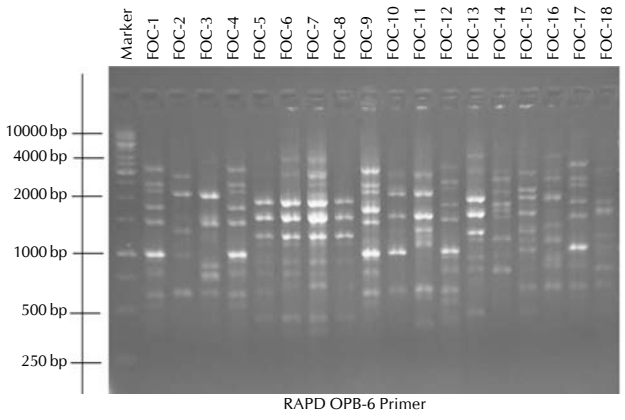

Figure 2: Banding pattern generated by RAPD OPB-6 primer

of DNA of eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri*, 49 produced reproducible and scorable bands with high degree of polymorphism while primer OPB-3 observed no banding pattern. A total 622 amplicons were obtained with the 49 primers with average fragment of 12.69 bands per primer. Out of 622 bands 601 were found to be polymorphic and the level of polymorphism was 96.62 per cent. Primer OPA-19 amplified maximum fragments (18) and primer OPA-6 amplified the least fragment (7) (Table 5).

Similarity coefficient value ranged from 0.5048 to 0.9260 across eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* indicating high degree of variation in respect to genetic similarity (Table 6). Isolates FOC-6 (Jabalpur) was found to have higher value of similarity coefficient (0.9260) to FOC-7 (Dharansara) followed by FOC-10 (Allahabad) and FOC-11 (Varanasi) (0.9099) whereas isolate FOC-4 (Badnapur) had the lower value of similarity coefficient (0.5048) to FOC-7 which indicate least similarity between them.

The cluster analysis of similarity index from RAPD data shows highest molecular variability among the isolates and distributed the eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* in four major clusters A, B, C and D that is represented in the

Table 5: Per cent polymorphism observed in RAPD primers

Sr. No.	Primer	Bands	Polymorphic bands	% Polymorphism
1	OPA-1	11	10	90.90
2	OPA-2	10	10	100
3	OPA-3	10	09	90.00
4	OPA-4	10	08	80.00
5	OPA-5	14	13	92.85
6	OPA-6	07	07	100
7	OPA-7	12	12	100
8	OPA-8	12	12	100
9	OPA-9	14	14	100
10	OPA10	15	15	100
11	OPA-12	13	13	100
12	OPA-13	15	12	80.00
13	OPA-14	15	15	100
14	OPA-15	10	10	100
15	OPA-16	12	12	100
16	OPA-17	16	15	93.75
17	OPA-18	13	12	92.30
18	OPA-19	18	17	94.44
19	OPA-20	13	13	100
20	OPB-3	NA	NA	NA
21	OPB-5	12	12	100
22	OPB-6	16	16	100
23	OPB-7	12	11	91.66
24	OPB-10	14	14	100
25	OPB-11	13	13	100
26	OPB-14	13	13	100
27	OPB-15	12	11	91.66
28	OPB-17	15	15	100
29	OPC-20	13	12	92.30
30	OPE-11	13	13	100
31	OPF-1	10	09	90.00
32	OPF-2	12	11	91.66
33	OPF-3	08	08	100
34	OPF-4	10	10	100
35	OPF-5	12	12	100
36	OPF-6	10	10	100
37	OPF-7	16	15	93.75
38	OPF-8	15	13	86.66
39	OPF-9	16	16	100
40	OPF-10	14	13	92.85
41	OPF-11	08	08	100
42	OPF-12	18	18	100
43	OPF-13	14	13	92.85
44	OPF-14	13	13	100
45	OPF-15	13	13	100
46	OPF-16	11	11	100
47	OPF-17	10	10	100
48	OPF-18	13	13	100
49	OPF-19	12	12	100
50	OPF-20	14	14	100
	Total	622	601	96.62

NA- Not Amplified

dendrogram. The cluster A included the isolates FOC-1 (Akola), FOC-4 (Badnapur), FOC-17 (ICRISAT), FOC-18 (Dharwad), FOC-9 (Kanpur) and FOC-12 (Delhi) whereas, cluster B included five isolates FOC-2 (Nagpur), FOC-3 (Parbhani), FOC-5 (Rahuri), FOC-10 (Allahabad) and FOC-11 (Varanasi). In cluster C included FOC-14 (Nimboda), FOC-15 (Udaipur) and FOC-16 (Bikaner) and cluster D includes four isolates FOC-6 (Jabalpur), FOC-7 (Dharansara), FOC-13 (Gurdaspur) and FOC-8 (Raipur) (Fig. 1). Similarly Mandhare et al. (2011) estimated

Table 6: A Binary Similarity Coefficient of RAPD analysis against eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri*.

FOC1	FOC2	FOC3	FOC4	FOC5	FOC6	FOC7	FOC8	FOC9	FOC10	FOC11	FOC12	FOC13	FOC14	FOC15	FOC16	FOC17	FOC18
1.0000																	
0.6173	1.0000																
0.5900	0.7700	1.0000															
0.7861	0.6125	0.5884	1.0000														
0.6720	0.6752	0.6414	0.6350	1.0000													
0.5353	0.5257	0.5305	0.5144	0.6736	1.0000												
0.5225	0.5032	0.5112	0.5048	0.6672	0.9260	1.0000											
0.5819	0.5498	0.5418	0.5385	0.6913	0.7990	0.7893	1.0000										
0.7041	0.5466	0.5418	0.6929	0.6109	0.5643	0.5482	0.5723	1.0000									
0.6205	0.6784	0.6575	0.5482	0.6816	0.5450	0.5128	0.5980	0.6430	1.0000								
0.6173	0.6784	0.6672	0.5610	0.6945	0.5418	0.5160	0.5852	0.6045	0.9099	1.0000							
0.6800	0.5546	0.5627	0.6913	0.5900	0.6141	0.5916	0.6028	0.7540	0.5546	0.5578	1.0000						
0.5771	0.5482	0.5627	0.5562	0.6864	0.8167	0.8199	0.7250	0.5803	0.5385	0.5418	0.5916	1.0000					
0.5948	0.6012	0.6350	0.5771	0.5916	0.5932	0.6028	0.5755	0.5787	0.5819	0.5723	0.6125	0.6221	1.0000				
0.6093	0.6575	0.6366	0.5916	0.6704	0.6527	0.6205	0.6093	0.5996	0.6254	0.6189	0.6334	0.6816	0.6704	1.0000			
0.5980	0.6302	0.6221	0.6093	0.6527	0.5643	0.5739	0.5916	0.5466	0.6398	0.6398	0.6061	0.6157	0.6430	0.7154	1.0000		
0.7491	0.6173	0.6093	0.7218	0.6559	0.5643	0.5418	0.5755	0.6816	0.5819	0.5819	0.6993	0.5803	0.6366	0.6607	0.6109	1.0000	
0.7154	0.6736	0.6527	0.6816	0.6832	0.5498	0.5401	0.5675	0.5996	0.6189	0.6318	0.6077	0.5659	0.6993	0.6205	0.6189	0.7958	1.0000

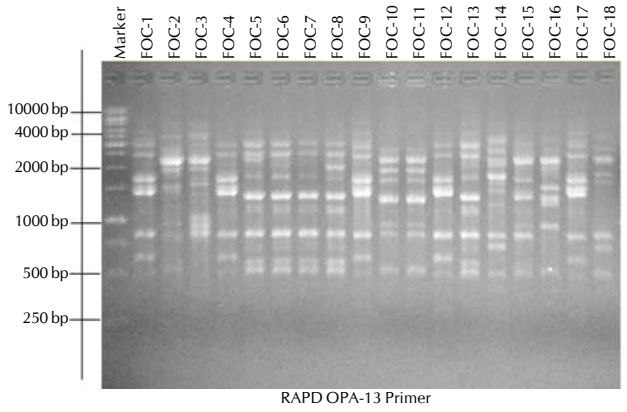

Figure 3: Banding pattern generated by RAPD OPA-13 primer

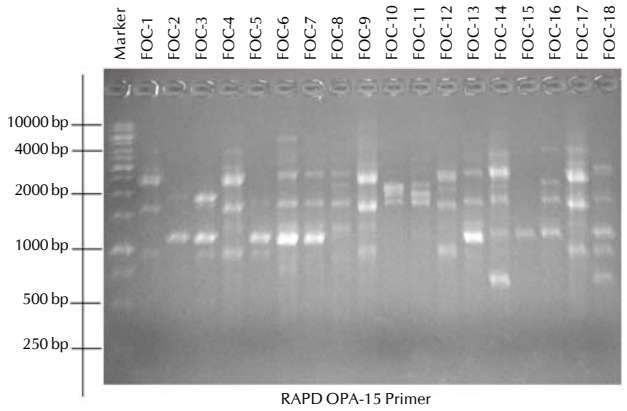

Figure 4: Banding pattern generated by RAPD OPA-15 primer

genetic variability in *Fusarium oxysporum* f.sp. *ciceri* isolates from Maharashtra by the RAPD techinique, these 24 isolates were grouped into two pathotype, i.e. pathotype I and pathotype II. Honnareddy and Dubey (2006) determine the genetic variability among 24 isolates representing seven races of *Fusarium oxysporum* f.sp. *ciceri* by RAPD. With the help of RAPD analysis, Kelly *et al*. (1994) and Jimenez–Gasco *et al*. (2001) separated isolates of *Fusarium oxysporum* f.sp. *ciceri* into two groups that correlated with the pathotypes that cause yellowing and wilting disease syndrome in Chickpea.

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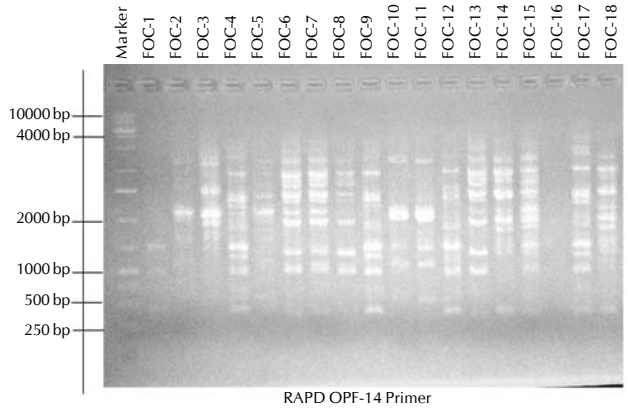

Figure 5: Banding pattern generated by RAPD OPF-14 primer

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