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Molecular diversity in Indian isolates of *Fusarium oxysporum* f.sp. *ciceri* by issr analysis

PN Rakhonde, SS Mane, AD Gawande, RM Wadskar, GF Vyavhare and AD Harne

Abstract
Eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* causing wilt of chickpea representing nine states and eight Agro climatic region of India were analysed for their virulence and genetic diversity. 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 (51-70%) and seven moderately pathogenic (21-50%) to cv. JG-62. The isolates of the pathogen showed high variability in causing wilt incidence on set of twelve host differential cultivars of chickpea namely JG-62, BG-212, L-550, JG-74, CPS-1, WR-315, DCP-92-3, KWR-108, JG-12, Annegiri, IPC-2004-52 and K-850. On the basis of host differential reaction, eighteen isolates could be classified into five races. The same set of isolates was used for molecular characterization with inter simple sequence repeats (ISSR) - polymerase chain reaction (PCR). Unweighted paired group method with arithmetic average grouped the isolates into five categories at a genetic similarities ranging from 50 to 94 per cent.

Keywords: chickpea, *Fusarium oxysporum* f. sp. *ciceri*, ISSR, molecular variability, virulence, wilt, etc.

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 its susceptibility to several fungal, bacterial and viral diseases (Dubey and Singh 2008) [6]. Among the diseases, the wilt caused by *Fusarium oxysporum* f.sp *ciceri* (Padwick) Snyder and Hansen is an important disease for major productivity loss in chickpea worldwide (Haware and Nene 1982) [10]. The losses caused by early wilting range from 77 to 94 Per cent, while the losses caused by late wilting range from 24 to 65Per cent (Haware and Nene 1980) [9]. The disease has been reported from all the chickpea-growing states of India. Its incidence varies from 14.1 to 32.0Per cent (Dubey *et al*. 2010) [11]. The cultivation of resistant varieties 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) [14] because of their high pathogenic variability that limits the effectiveness of their resistance (Jimenez-Diaz *et al*. 1993) [12]. 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 differential chickpea cultivars (Haware and Nene 1982; Jimenez-Diaz *et al*. 1993) [12, 21]. All these races have distinct geographic distribution. In India races 1, 2, 3 and 4 were reported, while races 0, 1B/C, 5 and 6 were reported from the Mediterranean region and the USA, thus showing area-specific distribution patterns.
The aim of the present study was to analyse the virulence of FOC isolates representing various Agro ecological regions of India on a set of chickpea differentials to determine the prevalence of various races and their diversity by using ISSR markers. The knowledge generated in this study could be utilized in resistance breeding programme.

Materials and Methods

S. No	Location	State
A	Akola	Maharashtra
B	Nagpur	Maharashtra
C	Parbhani	Maharashtra
D	Badnapur	Maharashtra
E	Rahuri	Maharashtra
F	Jabalpur	Madhya Pradesh
G	Dharansara (Rajnandgaon)	Chhattisgarh
H	Raipur	Chhattisgarh
I	Kanpur	Uttar Pradesh
J	Allahabad	Uttar Pradesh
K	Varanasi	Uttar Pradesh
L	Delhi	Delhi
M	Gurdaspur	Punjab
N	Nimboda	Rajasthan
O	Udaipur	Rajasthan
P	Bikaner	Rajasthan
Q	ICRISAT	Andhra Pradesh
R	Dharwad	Karnataka

Fig 1: Collection of isolates of *Fusarium oxysporum* f.sp. *ciceri* representing nine states in India

Eighteen isolates of FOC representing nine states (Fig. 1) and 8 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, were selected for the present study (Table 1).

Table 1: List of different isolates of *Fusarium oxysporum* f.sp. *ciceri* from India

S. No	Isolates	Location	State of India	GPS Location	Agro climatic region	Sub-Agro climatic zones
1	FOC-1	Akola	Maharashtra	N- 20^{0}42'18.65" E- 077^{0}03'16.19"	IX. Western Plateau and Hill region	Central Plateau
2	FOC-2	Nagpur	Maharashtra	N- 21^{0}07'51.56" E- 079^{0}04'15.28"	IX. Western Plateau and Hill region	Central Vidarbha
3	FOC-3	Parbhani	Maharashtra	N- 19^{0}15'02.21" E- 076^{0}47'45.33"	IX. Western Plateau and Hill region	Central Plateau
4	FOC-4	Badnapur	Maharashtra	N- 19^{0}52'06.47" E- 075^{0}42'24.79"	IX. Western Plateau and Hill region	Central Plateau
5	FOC-5	Rahuri	Maharashtra	N- 19^{0}22'12.53" E- 074^{0}38'58.07"	IX. Western Plateau and Hill region	Scarcity region
6	FOC-6	Jabalpur	Madhya Pradesh	N- 23^{0}12'50.67" E- 079^{0}57'52.90"	VIII. Central Plateau and Hills region	Kymore Plateau and Satpura Hills
7	FOC-7	Dharansara (Rajnandgaon)	Chhattisgarh	N- 21^{0}06'27.56" E- 081^{0}05'21.52"	VII. Eastern Plateau and Hills region	Wainganga
8	FOC-8	Raipur	Chhattisgarh	N- 21^{0}14'04.91" E- 081^{0}41'49.99"	VII. Eastern Plateau and Hills region	Wainganga
9	FOC-9	Kanpur	Uttar Pradesh	N- 26^{0}29'34.93" E- 080^{0}16'20.20"	V. Upper Gangetic Plains region	Central Plains
10	FOC-10	Allahabad	Uttar Pradesh	N- 25^{0}24'96.47" E- 081051'00.99"	V. Upper Gangetic Plains region	Central Plains
11	FOC-11	Varanasi	Uttar Pradesh	N- 25^{0}15'25.72" E- 082^{0}59'21.63"	IV. Middle Gangetic Plains region	Eastern Plain Zone of Uttar Pradesh
12	FOC-12	Delhi	Delhi	N- 28^{0}38'23.77" E- 077^{0}09'27.41"	VI. Trans-Gangetic Plains region	Plains
13	FOC-13	Gurdaspur	Punjab	N- 32^{0}02'36.29" E- 075^{0}23'12.36"	VI. Trans-Gangetic Plains region	Foot hill of Shivalik
14	FOC-14	Nimboda	Rajasthan	N- 24^{0}09'40.54" E- 073^{0}48'00.57"	VIII. Central Plateau and hills region	Southern plain of Rajasthan
15	FOC-15	Udaipur	Rajasthan	N- 24^{0}34'52.81" E- 078^{0}42'12.55"	VIII. Central Plateau and Hills region	Southern Plain of Rajasthan
16	FOC-16	Bikaner	Rajasthan	N- 28^{0}05'36.83" E- 073^{0}21'06.26"	XIV. Western dry region	Western dry region
17	FOC-17	ICRISAT (Hyderabad)	Andhra Pradesh	N- 17^{0}30'15.87" E- 078^{0}16'15.93"	X. Southern Plateau and Hills region	South Telangana
18	FOC-18	Dharwad	Karnataka	N- 15^{0}29'51.91" E- 074^{0}59'09.67"	X. Southern Plateau and Hills region	Northern dry region of Karnataka

The pure cultures of FOC were obtained using 'single spore technique' (Haware and Nene 1982) [10]. Microscopically *Fusarium oxysporum* f.sp. *ciceri* identified on the basis of morphological characters by Booth (1977) [3] and molecularly

identified by using SCAR primer Foc0-12f (5'GGCGTTTCGCAGCCTTACAATGAAG3') and Foc0-12r (5'GACTCCTTTTTCCCGAGGTAGGTCAGAT3') Jimenez-Gasco and Jimenez-Diaz (2003) [13]. The virulence of eighteen representative isolates of the pathogen was tested on a set of 12 host differential cultivars of chickpea JG-62, BG-212, L-550, JG-74, CPS-1, WR-315, DCP-92-3, KWR-108, JG-12, Annegiri, IPC-2004-52 and K-850 according to the pot culture inoculation method and wilt reactions were graded as R-Resistant (0-20 %); MS-Moderately susceptible (<20-50 %); S-Susceptible (>50 %) Haware and Nene 1982; Dubey *et al*. 2012 [7, 10]. On the basis of the resistant reactions, the cultivars were identified to differentiate the races of the pathogen.

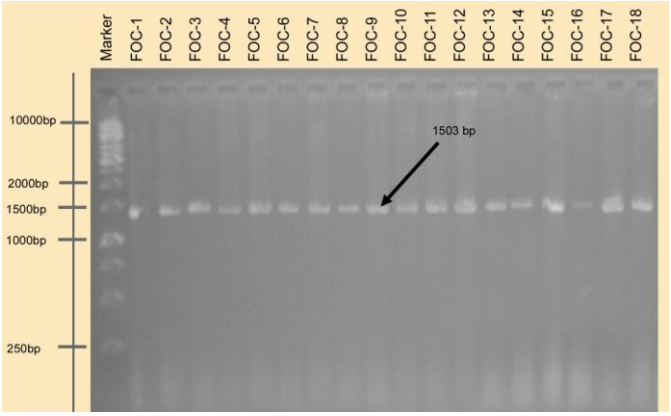
DNA was extracted from FOC isolates using cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). ISSR-PCR was performed by using protocol Yuan Lin *et al*. (2012) [16]. PCR reactions was performed in a total volume of 12.5 µl of the reaction containing 10 mM Tris-HCl with KCl (pH 8.0), 25 mM $MgCl_2$, 0.24 µM primer, 10 mM dNTPs, and 2 U Taq polymerase (Invitrogen). Amplification was performed as follow: initial denaturation (94ºC, 10 min) followed by 40 cycles of denaturing (94ºC, 1 min), annealing temperature were adjusted depending on G+C content of ISSR primers (Table 4) 45s, extension (72ºC, 2 min) and a final extension step (72ºC, 10 min). The products of PCR amplification were separated by horizontal gel electrophoresis in 1.8Per cent 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. A 1 kb ladder (Gene Ruler TM, Fermantas, France) was used as a molecular size standard ruler. All PCR assays were repeated at least twice. In all eleven primers were selected from IDT (Integrated DNA Technologies) were used.

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) [22] 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

Detection on the Basis of SCAR Marker

All the isolates yielded the 1503 bp band with the SCAR marker, hence confirms the culture of *Fusarium oxysporum* f.sp. *ciceri* as per the result of Jimenez Gasco and Jimenez-Diaz (2003) [13].



Detection of *Fusarium oxysporum* f.sp. *ciceri* by SCAR marker

Pathogenicity Test

Table 2: Pathogenicity test of *Fusarium oxysporum* f.sp. *ciceri* against susceptible variety JG-62

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

Category	Per cent wilt
1. Non-pathogenic (NPI)	0%
2. Weakly pathogenic (WPI)	1-20%
3. Moderately pathogenic (MPI)	21-50%
4. Moderately Susceptible (SPI)	51-70%
5. Highly pathogenic (HPI)	>70%

The virulence spectrum of the fungal pathogens obtained from diseased chickpea plants from 9 Indian states was determined

on a susceptible chickpea cultivar (JG-62). All the eighteen isolates proved to be pathogenic of which, 3 isolates were highly pathogenic, 8 strongly pathogenic and 7 were moderately pathogenic. (Table 2) The pathogenicity test was confirmed by proving Koch's Postulate and pathogens were confirmed as *Fusarium oxysporum* f.sp *ciceri* (Padwick) Snyder and Hansen.

Pathogenic Variability

Pathogenic variability of these isolates was variable in their disease reaction to chickpea. The isolates were grouped into five categories based on disease reactions on the twelve differential cultivars. The reactions of isolates on differentials FOC-1, FOC-2, FOC-3, FOC-4, FOC-5, FOC-17 and FOC-18 came under one group as race-1. These isolates were distinguished from the others using JG 74 along with CPS 1, BG 212 and WR315 as resistant reaction. Race-2 differentiates resistant reaction of cultivar WR-315, KWR-108 and K 850 showed in the isolates FOC-9, FOC-10 and FOC-11. Whereas FOC-13 resistance against JG 74, DCP 92-3, JG 12 and IPC 2004-52 and showed susceptible against WR 315 came under one group as race-3 whereas race-4 include FOC-6, FOC-7, FOC-8 and FOC-12 These isolates were distinguished from the others using JG 74 along with WR 315 resistant, whereas FOC-14, FOC-15 and FOC-16 shows different host differential reaction in which WR-315 and DCP-92-3 shows resistant reaction there is possibility of race-4A (Table 3).

Table 3: Reaction of host differential to *Fusarium oxysporum* f.sp. *ciceri* under sick soil method

Host differential	Host Differential Reaction																	
	Isolates																	
	FOC-1	FOC-2	FOC-3	FOC-4	FOC-5	FOC-6	FOC-7	FOC-8	FOC-9	FOC-10	FOC-11	FOC-12	FOC-13	FOC-14	FOC-15	FOC-16	FOC-17	FOC-18
JG-62	S	S	S	S	S	S	MS	S	S	S	MS	S	S	S	MS	S	S	S
BG-212	R	R	R	R	R	MS	MS	S	S	S	MS	MS	MS	S	S	S	R	R
L-550	S	S	S	S	S	S	S	S	S	S	MS	MS	MS	MS	S	MS	MS	S
JG-74	R	R	R	R	R	R	R	R	MS	MS	MS	R	R	MS	MS	MS	R	R
CPS-1	R	R	MS	MS	R	MS	MS	MS	MS	S	S	MS	MS	S	MS	S	MS	R
WR-315	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
DCP-92-3	MS	MS	MS	S	S	MS	S	S	S	S	MR	S	R	MS	R	R	MS	MS
KWR-108	MS	MS	S	S	R	S	S	MS	R	R	R	MS	MS	S	MS	MS	MS	MS
JG-12	MS	MS	MS	MS	MS	S	R	S	S	S	R	MS	R	MS	MS	MS	S	MS
Annegiri	S	S	S	S	S	S	S	S	MS	S	MS	S	MS	S	MS	MS	S	S
IPC-2004-52	S	S	S	MS	S	S	MS	MS	R	S	MS	MS	R	MS	MS	MS	S	S
K-850	S	S	MS	MS	MS	MS	MS	MS	R	R	R	S	MS	MS	MS	MS	S	S

R- Resistant (0-20 %); MS-Moderately susceptible (<20-50 %); S-Susceptible (>50 %)

Pathogenic variability of this pathogen has been well demonstrated since 1982 and the existence of four races in India has been reported earlier by Haware and Nene (1982) [10] and stated that the line reported resistant at one location may not be resistant to another location. They recorded susceptible reaction with Kanpur isolates on JG 74. Isolates of Gurdaspur and Ludhiana were distinguished using cultivars C104 and JG 74 (resistant to pathogen) and called as race 3. Isolates of Hisar and Jabalpur were also designated earlier as race 4 using cultivar CPS-1, as moderately susceptible to the pathogen. Pathogenic variability in *F. oxysporum* f. sp. ciceri causing wilt of chickpea has also been reported by other workers Desai *et al*. (1992) [4]; Kapoor *et al*. (1992) [15]; Rahman *et al*. (1998) [21]; Sharma *et al*. (2004); Srivastava *et al*. (2004) [24]; Dubey and Singh (2008) [6] in India.

Barhate *et al*. (2006) [1] also observed that race 1 was wide spread in all Tehsils of Ahmednagar district. While, Honnareddy and Dubey (2006) [11] grouped the 25 isolates as seven different races. Race 1 isolates were distinguished from the others using K-850 along with C-104, JG-74, CPS-1, BG-212 and WR-315 as resistant reactions. Race 2 isolates were distinguished by WR-315 and JG-74 cultivars as resistant. Race 3 was distinguished using cultivars C-104 and JG-74 as resistant reaction. While isolates which showed resistance reaction against WR-315 and BG-212 were distinguished as race 4. Race 5 isolates were distinguished using cultivar L-550 along with K-850, BG-212, JG-74 and C-104 as resistant reactions. Whereas, the isolates which showed resistant reaction against Annegiri along with WR-315, CPS-1 and C-104 were grouped as race 6. And the one which were distinguished by Chaffa and showed resistant reaction along with WR-315, CPS-1 and C-104 were called as race 7.

Dubey *et al*. (2012) [7] seventy isolates of *Fusarium oxysporum* f.sp. *ciceri* (Foc) causing chickpea wilt representing 13 states and four crop cultivation zones of India were analysed for their virulence and genetic diversity on differential responses and the isolates were characterized into eight races of the pathogen on the basis of host differential as race 1 differentiate by resistance of C104 and GPF2 these two cultivar, race 2 by JG74 and GPF2, race 3 by JG74 and C104, race 4 by BG212 and KWR108, race 5 by WR315 and GPF2, race 6 by C104 and KWR108, race 7 by BG212 and GPF2 and race 8 by GPF2 and DCP92-3.

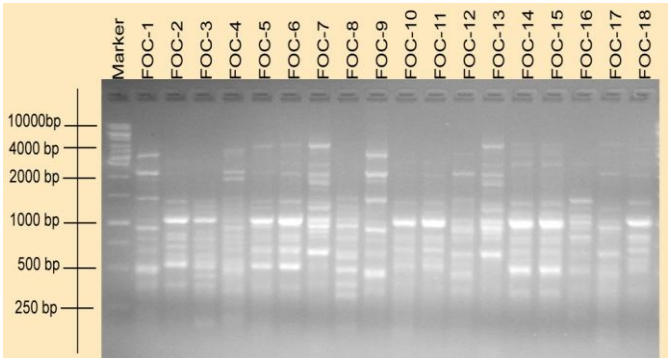
ISSR Analysis

Genetic variation was detected among eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri* using ISSR-PCR marker. Out of the eleven ISSR primers screened for amplification of DNA all primer produced reproducible and scorable bands with high degree of polymorphism. ISSR primers were amplified a total of 113 bands out of which 98 found polymorphic. Maximum of 15 bands were amplified by two primer $(AG)_8T$ and $(AG)_8YT$ followed by 14 bands from $(AC)_8YT$ and the least bands 3 were amplified by $(GA)_8YT$ primer (Table 4).

Table 4: G+C content (%) and annealing temperatures of the primers and per cent polymorphism observed in ISSR primers

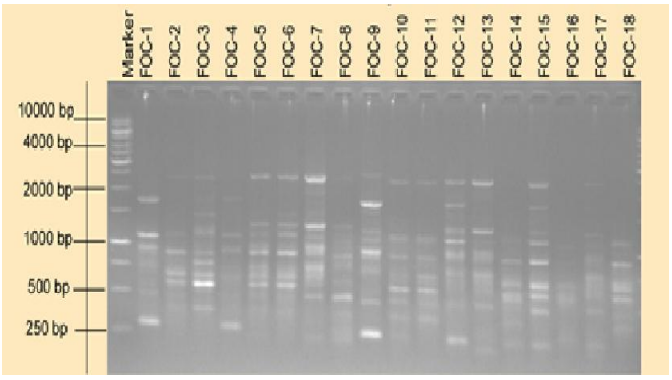
S. No	Primer	G+C Content (%)	Annealing Temp (ºC)	Bands	Polymorphic Bands	% polymorphism
1	$(AC)_8YT$	47.2	52	14	09	64.28
2	$(AG)_8G$	52.9	52	13	12	92.30
3	$(AG)_8T$	47.1	50	15	13	86.66
4	$(AG)_8YT$	47.2	52	15	12	80.00
5	$(ATG)_6$	33.3	50	13	11	84.61
6	$(CA)_8RT$	47.2	52	10	10	100
7	$(GA)_8T$	47.1	50	09	08	88.88
8	$(GA)_8YT$	47.2	53	03	02	66.66
9	$(TG)_8RT$	47.2	52	07	07	100
10	$(GA)_9RY$	50.0	54	07	07	100
11	$(AC)_8T$	47.1	50	07	07	100
	Total			113	98	86.72

A binary similarity matrix of combined data of ISSR primers for the eighteen isolates of FOC was prepared by scoring bands. Similarity coefficient value ranged from 0.5044-0.9380 across eighteen isolates of FOC indicating high degree of variation in respect to genetic similarity. This ultimately means high range of genetic diversity among the isolates studied. The maximum genetic similarity (93.80%) is found in between two isolates from north-eastern region of India, FOC-10 and FOC-11. The ISSR primers clustered all 18 isolates into five main clusters (Fig.2).



ISSR pattern of *Fusarium oxysporum* f.sp. *ciceri* isolates with primer $(AC)_8YT$

In the first group, seven isolates FOC-1, FOC-4, FOC-2, FOC-3 and FOC-5 from western plateau and hill region with FOC-17 and FOC-18 from southern plateau and hills region separated in one cluster as a race-1 with 70.50 per cent genetic similarity. The second group consisted of virulent isolates FOC-6 from central plateau and hills region with FOC-7 and FOC-8 from eastern plateau and hills region and FOC-12 from trans-gangetic plains region with sub group plains with 75 per cent genetic similarity as a race-4. The third group consisted of virulent isolates, FOC-9 and FOC-10 from upper gangetic plains region and FOC-11 from middle gangetic plains region with 73 per cent genetic similarity as a race-2. The fourth group consisted FOC-14 and FOC-15 from central plateau and hills region with sub group southern plain of Rajasthan and FOC16 from western dry region variable from all other isolates with 82.80 per cent genetic similarity as a race-4A, whereas FOC-13 from trans-gangetic plains region with sub group foot hill of shivalik shows 50.44 per cent genetic similarity with all other isolates as a race-3 (Table 5).



ISSR pattern of *Fusarium oxysporum* f.sp. *ciceri* isolates with primer $(AG)_8T$

Table 5: A binary similarity coefficient of ISSR analysis against eighteen isolates of *Fusarium oxysporum* f.sp. *ciceri*.

FOC -1	FOC -2	FOC -3	FOC -4	FOC -5	FOC -6	FOC -7	FOC -8	FOC -9	FOC -10	FOC -11	FOC -12	FOC -13	FOC -14	FOC -15	FOC -16	FOC -17	FOC -18
1.0000																	
0.7876	1.0000																
0.7345	0.8938	1.0000															
0.8938	0.8230	0.7876	1.0000														
0.7433	0.8141	0.8141	0.7610	1.0000													
0.5663	0.6548	0.6371	0.5663	0.7168	1.0000												
0.5398	0.6283	0.6106	0.5929	0.6902	0.8672	1.0000											
0.5929	0.6283	0.6283	0.6106	0.7079	0.7610	0.7876	1.0000										

0.6017	0.5840	0.6017	0.6371	0.6283	0.6460	0.6548	0.6371	1.0000									
0.6017	0.6548	0.6725	0.6194	0.7168	0.6814	0.6902	0.6902	0.7345	1.0000								
0.6106	0.6460	0.6637	0.6106	0.7256	0.6725	0.6991	0.6814	0.7256	0.9380	1.0000							
0.5221	0.5752	0.5929	0.5575	0.6371	0.7610	0.7876	0.6991	0.6902	0.6371	0.6637	1.0000						
0.5044	0.5044	0.5044	0.5044	0.6194	0.6725	0.6991	0.5929	0.6017	0.6371	0.6283	0.6460	1.0000					
0.6017	0.6902	0.6725	0.6371	0.7522	0.6460	0.6017	0.6371	0.6460	0.7168	0.7079	0.6371	0.6017	1.0000				
0.6283	0.6283	0.6106	0.6106	0.6902	0.6194	0.5752	0.6106	0.6017	0.6725	0.6460	0.6637	0.5398	0.8672	1.0000			
0.6194	0.6902	0.6548	0.5840	0.7168	0.6460	0.6194	0.6725	0.5752	0.7345	0.7256	0.6371	0.5486	0.8407	0.8141	1.0000		
0.6814	0.6637	0.6637	0.6991	0.7433	0.6548	0.6814	0.6106	0.6017	0.6194	0.6106	0.7168	0.5752	0.6548	0.7168	0.5840	1.0000	
0.7168	0.7522	0.7345	0.7168	0.7610	0.6902	0.6991	0.6283	0.6017	0.6902	0.7345	0.6814	0.5398	0.7079	0.6637	0.7256	0.7876	1.0000

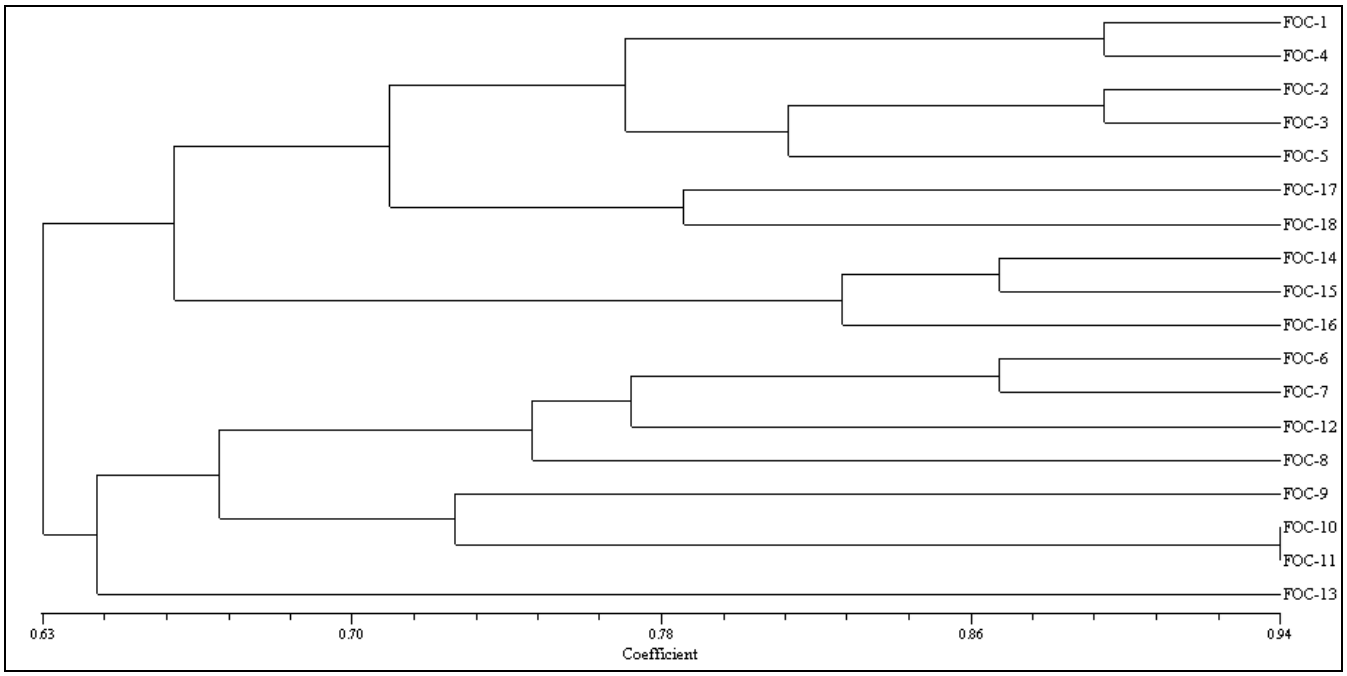


Fig 2: UPGMA dendrogram obtained by ISSR analysis of *Fusarium oxysporum* f. sp. *ciceri* isolates based on Jaccard's Similarity Coefficient

Earlier, Bayraktar and Dolar (2009) [2] analysed the genetic variation among the isolates of *Fusarium oxysporum* f.sp. *ciceri* using molecular markers Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) polymorphism. 74 isolates were assessed using 30 arbitrary decamer primers and 20 ISSR primers. Average cluster analysis of RAPD, ISSR and RAPD + ISSR database divided the isolates into three major groups. Groups 1, 2 and 3 consisted of 41, 18 and 15 isolates resp. These methods revealed a considerable genetic variation among Turkish isolates. And also Dubey and Singh (2008) [6] observed high level of variability in the pathogen by ISSR markers. Out of 13 ISSR primers screened, only seven primers amplified all the isolates of the pathogen. All 48 bands were polymorphic with maximum polymorphism in ISSR 12. Banding pattern obtained for 64 isolates of the pathogen with all primers was analysed and the dendrogram obtained after cluster analysis showed that at 25Per cent genetic similarity all isolates could be grouped into two categories. The first group of 22 isolates consisted of 8 isolates from Rajasthan and 14 from Punjab while the remaining 42 isolates were clustered in the second category. Whereas, Yuan Lin *et al*. (2012) [16] thirty isolated strains from Hebei were tested by ISSR analysis to analyse the genetic diversity between of *Fusarium oxysporum* Schl. The isolated 30 strains were clustered into 2 genetic lineages at 0.86 genetic similarities. The genetic lineages of 30 strains showed no obvious relation with their geographical originals. Similarly, Mohd. Arif *et al*. 2008, Gayatri Gurjar *et al*. 2009, Mohammadi *et al*. 2011, Dubey *et al*. 2012 [7, 8, 19] and Madhuri Katkar 2012 [17] have been used ISSR marker in variability study successfully.

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