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Variability in *Fusarium oxysporum* f.sp. *ciceris* causing wilt in chickpea

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Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Matuo and K. Sato is a major constraint in successful cultivation of chickpea. Therefore, in the present study, 24 isolates of *F. oxysporum* f.sp. *ciceris* collected from different chickpea growing areas of Punjab and adjoining states were assessed for morphological variations. Among 24 isolates, the maximum (8.78 mm/days) and minimum (5.00 mm/day) growth rate were exhibited by Foc-21 and Foc-15, respectively. The isolates showed growth pattern from appressed, fluffy to less fluffy and mycelial colour varied from different shades of white to purplish white. A significant variation with respect to size of micro (8.9-16.9 x 3.1-6.3 µm) and macro (21.7-64.9 x 2.7-10.0 µm) conidia was also observed. At pathogenic level, twenty isolates were studied where Foc-3, Foc-7 and Foc-22 showed virulence pattern similar to existing races 1, 2, 3 and 4 on three standard differentials *viz*. JG 62, WR 315 and L 550, whereas the rest of the isolates did not match with any of the existing race reaction. Further, the six selected genotypes could differentiate the isolates into four pathotypes based on their aggressiveness and Foc-8 was found more aggressive (98.48% wilt incidence) whereas Foc-24 was found to be least aggressive (7.22% wilt incidence). At molecular level, the sequences of internal transcribed spacers (ITS) genomic regions of isolates were studied and they showed 99% similarity with Foc sequences by basic local alignment search tool (BLAST) analysis.

Key words: *Fusarium oxysporum* f.sp. *ciceris*, wilt, chickpea, variability, differentials, pathotypes.

INTRODUCTION

Chickpea cultivation is often subjected to several biotic stresses of which diseases like *Ascochyta* blight, *Botrytis* grey mould, *Verticillium* wilt, *Sclerotinia* stem rot, dry root rot and *Fusarium* wilt are important. Among them, *Fusarium* wilt, caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Matuo and K. Sato has assumed serious proportions in the recent years. Throughout the world, annual chickpea yield losses due to this disease vary from 10 to 15% (Trapero-Casas and Jimenez-Diaz, 1985), but can reach even 100% under favourable

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Table 1. Location and source of host variety of different isolates of *F. oxysporum* f.sp. *ciceris*.

Isolate	Location of sample	Host variety
Foc-1	Dhaulakuan, HP	Channa- II
Foc-2	Shivpur, Paonta Sahib, HP	Local variety
Foc-3	Burma papri, Nahan Block, HP	Local variety
Foc-4	Hanumangarh , Rajasthan	P-12
Foc-5	Mehruwala (Dehradun), UK	Local variety
Foc-6	Ludhiana, Punjab	JG-62
Foc-7	Jaipur, Rajasthan	J-7
Foc-8	Nagaur, Rajasthan	Local variety
Foc-9	Sriganganagar, Rajasthan	Local variety
Foc-10	Dhaulakuan, HP	GPF2
Foc-11	Dhaulakuan, HP	GPF2
Foc-12	Phillaur, Punjab	Local variety
Foc-13	Phillaur, Punjab	Local variety
Foc-14	Samba , Jammu	Local variety
Foc-15	Nangal, Punjab	Local variety
Foc-16	Ludhiana, Punjab	Local variety
Foc-17	Sabhawala (Dehradun), UK	Local variety
Foc-18	Bharapur (Sirmour), HP	GPF2
Foc-19	Gurdaspur, Punjab	Local variety
Foc -20	Bikaner, Rajasthan	Local variety
Foc -21	Churu , Rajasthan	N-11
Foc -22	Dhaulakuan, HP	DKG-986
Foc -23	Sahari, Punjab	Local variety
Foc -24	RS, Gurdaspur	Local variety

Table 2. Sequence of ITS primer.

Primer	Sequence
ITS-Fu-f	5`-CAACTCCCAAACCCCTGTGA-3`
ITS-Fu-r	5`-GCGACGATTACCAGTAACGA-3`

conditions (Navas-Cortes et al., 2000). In India, yield losses estimates range from 10 – 90% every year in different regions in different cultivars (Singh and Dahiya, 1973; Jalali and Chand, 1992).

The fungus is both seed- and soil- borne and may survive in soil for up to six years even in the absence of the host (Haware et al., 1996). Considering the nature of damage and survival ability of the fungus, management of the disease is difficult either through crop rotation or application of fungicides. The most practical and cost-efficient method for management of chickpea wilt is the use of resistant varieties (Nene and Haware, 1980; Nene and Reddy, 1987; Bakhsh et al., 2007). However, evolution of new races poses a serious threat to deployment of wilt resistance in chickpea.

Therefore, regular monitoring of variation in new isolates collected from different cultivars and geographically distinct regions over the years is critical for successful resistance breeding programme. The conventional approaches to assess variation among the fungal isolates are morphological and virulence analysis. However, with the advent of DNA based molecular techniques it is now possible to assess genetic variation among the isolates. Genotyping of *F. oxysporum* f.sp. *ciceris* isolates along with virulence analysis may yield some information relevant to breeding. Keeping this in view, the present study was carried out with the objective of assessment of variability in isolates of *F. oxysporum* f.sp. *ciceris* (Foc) collected from chickpea growing areas of Punjab and adjoining states at morphological, pathogenic and molecular level.

MATERIALS AND METHODS

Collection, isolation and maintenance of isolates

A large number of isolations were made from stem and root portions of diseased chickpea plants collected from different chickpea cultivars grown in different locations in Punjab and adjoining states. The infected portion was cut into small bits, was surface sterilized in 0.1% mercuric chloride for 40-60 s followed by rinsing twice in sterilized distilled water. Later, these bits were transferred on PDA in Petri plates under complete and aseptic conditions. Plates were incubated at 25°C in BOD incubator to obtainfungal growth. Finally, a total of 24 isolates were purified and maintained on PDA slants at 4°C for further studies and designated as Foc-1, Foc-2 and so on (Table 1).

Cultural and morphological characterization

In cultural characterization, the fresh cultures were grown from 7 days old culture of each isolate separately and incubated at 25 ± 2°C. Each isolate was replicated thrice. The observations on colony diameter, colony colour, rate and pattern of growth were recorded up to 9 days at regular intervals. For morphological characterization, cultures obtained on PDA slants were examined under compound microscope (*Leica* DM 3000) using image analyzer software at 40x. The size and shape as well as septation of micro and macro conidia of each isolate were recorded.

Pathogenic characterization

For pathogenic characterization, only twenty isolates of *F. oxysporum* f.sp. *ciceris* were included in the study since the remaining four isolates seems to be morphologically similar to one or other isolates. All the isolates were subjected to the patho-genicity tests on susceptible and resistant chickpea germplasm lines (JG-62, WR-315, L-550, L-552, GL-26054 and GLK-24092). Each isolate was cultured on potato dextrose broth (PDB) for 15 days at 25 ± 2°C. Ten surface sterilized seeds of 6 differential varieties were sown in three replications in 4 x 9" poly bags containing autoclaved soil and inoculum of respective isolate. In the control, plants were sown in autoclaved soil only without inoculum. Data on disease incidence (% infected plants) were recorded at regular intervals.

Molecular characterization

All the isolates were characterized using internal transcribed spacers (ITS) primers for *Fusarium* genus and *xylanase 3* gene specific markers (Table 2).

Table 3. Cultural characteristics of different isolates of *F. oxysporum* f. sp. *Ciceris.*

Isolate	Colony diameter (mm) after inoculation		Average growth rate / day (mm)	Mycelial colour	Mycelial texture
	3rd day	9th day			
Foc 1	24.5	82.5	8.29	White	Appressed
Foc 2	31.0	78.0	6.71	Purplish	Fluffy
Foc 3	35.0	85.0	7.14	Dull white	Appressed
Foc 4	30.5	85.0	7.79	Bright white	Appressed
Foc 5	27.0	74.5	6.79	Dull white	Appressed
Foc 6	27.5	71.0	6.21	Creamish white	Less Fluffy
Foc 7	21.0	75.0	7.71	Creamish white	Appressed
Foc 8	26.0	72.0	6.57	White	Fluffy
Foc 9	25.5	71.0	6.50	Creamish white	Fluffy
Foc 10	35.0	85.0	7.14	White	Fluffy
Foc 11	29.0	80.0	7.28	White	Fluffy
Foc 12	25.5	82.5	8.14	White	Fluffy
Foc 13	25.0	72.5	6.78	White	Less Fluffy
Foc 14	22.5	64.5	6.00	Creamish white	Appressed
Foc 15	41.5	76.5	5.00	White	Appressed
Foc 16	20.0	80.0	8.57	Creamish white	Less Fluffy
Foc 17	21.0	74.5	7.64	White	Less Fluffy
Foc 18	25.5	75.5	7.14	Bright white	Appressed
Foc 19	23.5	72.5	7.00	Bright white	Appressed
Foc 20	23.5	69.0	6.50	Creamish white	Fluffy
Foc 21	23.5	85.0	8.78	Bright white	Fluffy
Foc 22	26.5	68.5	6.00	Creamish white	Less Fluffy
Foc 23	38.5	85.0	6.64	Bright white	Appressed
Foc 24	28.5	80.0	7.35	Bright white	Less Fluffy

Fungal DNA extraction from Foc isolates

The cultures from 24 Foc isolates were grown on 100 mL potato dextrose broth in 250 mL Borosil flasks and incubated at 25± 2°C in BOD incubator for fifteen days. DNA from 15 days old broth cultures of each isolate was extracted using modified CTAB extraction method (Murray and Thompson, 1980). DNA was quantified using Thermo Scientific NanoDrop™ 1000 Spectrophotometer and working dilutions were made with DNA concentration of 60 ng/ µl in each isolate.

Amplification of fungal DNA through PCR

Fungal DNA from each isolate was subjected to PCR amplification with ITS primers (ITS- Fu-f and ITS –Fu-r) specific for *Fusarium* genus (Abd- Elsalam et al., 2003). Each PCR reaction mixture of 25 µl contained 2 µl genomic DNA (60 ng/ µl), 5.0 µl 10X PCR buffer, 0.5 µl 2mM dNTPs, 1.5 µl 25 mM $MgCl_2$, 1.0 µl forward primer (20 pmol/µl), 1.0 µl reverse primer (20 pmol/ml), 0.3 µl Taq DNA polymerase (3 U/µl) and 13.7 µl nuclease free water. The PCR amplification was carried out in Eppendorf Mastercycler® pro with initial denaturation at 92°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 0.50 s and extension at 72°C for 1 min. Final extension was carried out at 72°C for 10 min. The amplified PCR product was separated on 2% agarose gel in TBE buffer stained with good view dye (9 µl/ 150 ml buffer) and visualized in gel document system (ImagerTM1200, Alpha Innotech Corp., CA, USA).

The amplified ITS genomic region from each isolate was further sequenced by outsourcing from Xcelris Labs Ltd., Ahmedabad India and compared with other Foc sequences submitted at National Center for Boitechnology Information (NCBI).

RESULTS AND DISCUSSION

Cultural and morphological characterization

All the isolates produced well developed colonies on PDA medium with colony diameter ranging from 64.5 to 85.0 mm after 9 days of incubation. Maximum colony diameter, that is, 85.0 mm was observed in isolates Foc-3, Foc-4, Foc-10, Foc-21 and Foc-23, whereas minimum colony diameter, that is, 64.5 mm was observed in Foc-15. However, when growth rate was calculated, Foc-21 exhibited maximum growth rate of 8.78 mm/day and Foc-15 exhibited minimum growth rate of 5.0 mm/day (Table 3).

The isolates also differed in the growth pattern from appressed (Foc-1, Foc-3, Foc-4, Foc-5, Foc-7, Foc-14, Foc-15, Foc-18, Foc-19 and Foc-23), fluffy (Foc-2, Foc-8, Foc-9, Foc-10, Foc-11, Foc-12, Foc-20 and Foc-21) to less fluffy (Foc-6, Foc-13, Foc-16, Foc-17, Foc-22

Table 4. Morphological characterization of different isolates of *F. oxysporum* f.sp. *ciceris*.

Isolate	Microconidia							Macroconidia						
	Length (µm)			Width (µm)			No. of septa	Length (µm)			Width (µm)			No. of septa
	Max	Min	Mean	Max	Min	Mean		Max	Min	Mean	Max	Min	Mean	
Foc -1	21.0	12.6	16.9	8.2	4.5	6.3	0-1	-	-	-	-	-	-	-
Foc -2	16.2	10.8	13.5	6.5	3.7	5.1	0-1	29.8	18.3	24.0	7.6	4.4	6.0	1-3
Foc -3	17.1	9.9	13.5	8.1	3.0	5.5	0-1	31.7	18.9	25.3	8.3	5.0	6.6	1-4
Foc -4	13.3	9.2	11.2	5.9	4.5	5.2	0	37.4	19.8	28.6	7.0	3.1	5.0	1-3
Foc -5	13.8	7.3	10.5	6.5	3.0	4.8	0-1	77.3	52.6	64.9	8.3	4.2	6.2	1-5
Foc-6	14.6	7.4	11.0	7.1	4.7	5.9	0	70.1	42.6	56.2	13.4	6.6	10.0	1-3
Foc -7	12.1	6.1	9.1	5.0	3.4	4.2	0-1	72.3	50.1	61.2	7.1	3.1	5.1	1-5
Foc-8	17.1	11.0	14.1	7.0	2.7	4.8	0-1	-	-	-	-	-	-	-
Foc-9	15.5	10.2	12.8	5.2	3.8	4.5	0-1	-	-	-	-	-	-	-
Foc-10	12.2	5.7	8.9	4.2	2.1	3.1	0	-	-	-	-	-	-	-
Foc -11	-	-	-	-	-	-	-	61.4	29.1	45.2	8.3	4.0	6.1	1-5
Foc-12	15.8	9.8	12.8	6.9	5.2	6.0	0-1	-	-	-	-	-	-	-
Foc-13	-	-	-	-	-	-	0	60.6	40.7	50.6	5.3	3.2	4.2	1-2
Foc-14	16.6	10.7	13.6	5.1	3.2	4.1	0-1	30.9	17.9	24.4	4.2	2.1	3.1	1-2
Foc-15	16.1	7.8	11.9	4.8	2.0	3.4	0	29.0	17.9	23.4	4.3	2.7	3.5	1-3
Foc-16	13.2	10.2	11.7	6.9	5.0	5.9	0-1	39.0	22.5	30.7	4.3	3.3	3.8	1-3
Foc-17	-	-	-	-	-	-	-	78.7	21.1	49.9	3.1	2.3	2.7	1-3
Foc-18	10.7	8.6	9.6	4.4	3.0	3.7	0	47.1	20.4	33.7	6.2	4.5	5.3	1-3
Foc-19	16.7	12.1	14.4	6.9	2.7	4.8	0	-	-	-	-	-	-	-
Foc-20	-	-	-	-	-	-	-	27.0	16.5	21.7	3.6	2.6	3.1	1-3
Foc-21	15.3	12.2	13.7	4.9	2.9	3.9	0-1	-	-	-	-	-	-	-
Foc-22	17.5	10.8	14.1	6.0	2.4	4.2	0	35.2	20.3	27.7	5.1	3.5	4.3	1-3
Foc-23	13.0	9.7	11.3	5.7	3.4	4.5	0	-	-	-	-	-	-	-
Foc-24	18.0	10.7	14.3	6.1	4.1	5.1	0-1	60.0	21.8	40.9	4.5	3.0	3.7	1-3
CD at 5 %	0.62	0.55		0.40	0.36			2.36	1.28		0.44	0.47		

(-) Not formed; Max- maximum; Min- minimum.

and Foc-24). The cultures of different isolates also exhibited variation in mycelial colour which ranged from different shades of white to purplish. Isolates Foc-4, Foc-18, Foc-19, Foc-21, Foc-23 and Foc-24 showed bright white mycelial colour, whereas, Foc-6, Foc-7, Foc-9, Foc-14, Foc-16, Foc-20 and Foc-22 showed creamish white mycelial colour. White mycelial colour was produced by isolates Foc-1, Foc-8, Foc-10, Foc-11, Foc-12, Foc-13, Foc-15 and Foc-17. Two isolates Foc-3 and Foc-5 produced dull white mycelial colour, whereas one isolate Foc-2 produced purplish mycelial colour. Flat to fluffy, white to pinkish mycelial growth has been previously observed by other workers (Patil et al., 2005; Barhate et al., 2006). Singh et al. (2010) also observed dull white to pinkish white, thin and flat hairy to fluffy growth with irregular margins. The isolates showed considerable variation with respect to size of micro and macro conidia (Table 4). Size of microconidia varied from 5.7-21.0 x 2.00 – 8.2 µm with 0-1 septa, whereas size of macroconidia varied from 16.5-78.7 x 2.1- 13.4 µm with 1-5 septa. Similarly, average length and width of microconidia showed considerable variation (8.9-16.9 x 3.1-6.3 µm) in different isolates of *F. oxysporum* f.sp. *ciceris*. Foc-1 produced largest microconidia having maximum mean length and width (16.9 x 6.3 µm), whereas Foc-10 produced smallest microconidia having minimum mean length and width (8.9 x 3.1 µm). Likewise, average size of macroconidia also varied considerably (21.7-64.9 x 2.7-10.00 µm). Largest macroconidia were produced by Foc-6 with mean size of 56.2 x 10.00 µm, whereas smallest macroconidia were produced by Foc-20 with mean size of 3.1-21.7 µm.

Pathogenic characterization

On a set of six chickpea genotypes *viz*., GL 26054, JG 62, WR 315, GLK 24092, L 550 and L 552, Foc-8 was found more aggressive, causing maximum average wilt incidence (98.48%), whereas Foc-24 was found least aggressive producing minimum average wilt incidence (7.22%) (Table 5). However, Foc-3, Foc-7 and Foc-22

Table 5. Percent wilt incidence induced by different Foc isolates on selected chickpea genotypes.

Isolate/genotype	Wilt incidence (%)						
	GLK 24092	L 550	JG 62	WR 315	L 552	GL 26054	Average
Foc-1	92.31	70.00	83.33	85.71	0.00	50.00	63.56
Foc-2	100.00	92.31	85.71	75.00	100.00	0.00	75.50
Foc-3	90.00	58.33	53.85	0.00	60.00	66.67	54.81
Foc-4	92.86	84.62	88.89	83.33	100.00	75.00	87.45
Foc-5	53.85	53.33	25.00	62.50	0.00	100.00	58.94
Foc-7	69.23	50.00	41.67	0.00	100.00	0.00	43.48
Foc-8	90.91	100.00	100.00	100.00	100.00	100.00	98.48
Foc-9	86.67	69.23	84.62	66.67	0.00	100.00	81.44
Foc-10	100.00	81.25	100.00	77.78	100.00	100.00	93.17
Foc-11	66.67	85.71	15.38	22.22	0.00	50.00	48.00
Foc-13	77.78	68.75	100.00	100.00	100.00	33.33	79.90
Foc-14	92.31	88.89	66.67	100.00	66.67	75.00	81.59
Foc-15	0.00	25.00	16.67	0.00	0.00	0.00	8.33
Foc-16	75.00	72.73	77.78	66.67	100.00	100.00	82.03
Foc-17	69.23	70.00	0.00	0.00	0.00	50.00	37.85
Foc-18	100.00	81.25	100.00	60.00	100.00	50.00	81.88
Foc-20	33.33	50.00	16.67	0.00	100.00	33.33	38.89
Foc-22	42.86	45.45	37.50	0.00	0.00	10.00	27.16
Foc-23	93.33	70.00	55.56	75.00	100.00	100.00	82.31
Foc-24	0.00	33.33	0.00	10.00	0.00	0.00	7.22

Table 6. Grouping of different isolates as different pathotypes.

Pathotype	Isolate
I	Foc-4, Foc-8, Foc-9, Foc-10, Foc-14, Foc-16, Foc-18, Foc-23.
II	Foc-1, Foc-2, Foc-3, Foc-5, Foc-7, Foc-11, Foc-13
III	Foc-17, Foc-20, Foc-22
IV	Foc-24, Foc-15

Table 7. Grouping based on pathogenic variation.

Group	Sub group	Isolate
1	1a	Foc-1, Foc-9, Foc-5, Foc-11 and Foc-17
	1b	Foc-22, Foc-15, Foc-24, Foc-3, Foc-7 and Foc-20
2	2a	Foc-2, Foc-13 and Foc-18
	2b	Foc-14, Foc-16, Foc-23, Foc-8, Foc-4 and Foc-10

behaved like existing in races 1, 2, 3 and 4 on three genotypes JG 62, WR 315 and L 550. Rest of the isolates differed in their virulence behavior on these three genotypes. Among these isolates, it was observed that Foc-17 and Foc- 24 were avirulent on highly susceptible variety JG 62, whereas Foc- 1, Foc-2, Foc-4, Foc-5, Foc-8, Foc-9, Foc-10, Foc-11, Foc-13, Foc-14, Foc-16, Foc-18, Foc-23 and Foc-24 were virulent on highly resistant variety WR 315.

The six selected genotypes could differentiate the level of aggressiveness of all the isolates, thus giving them the status of pathotypes. Therefore, on the basis of mean aggressiveness, the 20 isolates could be converted into four pathotypes as given in Table 6. The isolates were also differentiated on the basis of their virulence reaction on each chickpea genotype, using DARwin software. The isolates were converted into two groups (Table 7). The first group consisted of two sub groups, that is, 1a (Foc-1,

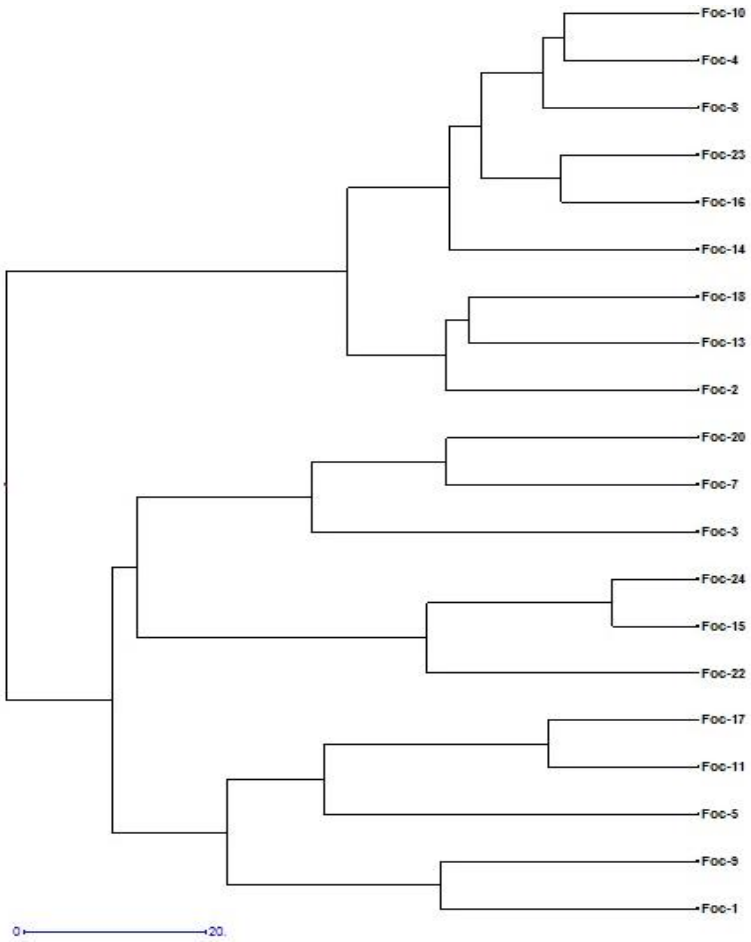

Figure 1. Dendrogram based on pathogenic variation among Foc isolates.

Foc-9, Foc-5, Foc-11 and Foc-17) and 1b (Foc-22, Foc-15, Foc-24, Foc-3, Foc-7 and Foc-20). Similarly, the second group also consisted of two sub groups, that is, 2a (Foc-2, Foc-13 and Foc-18) and 2b (Foc-14, Foc-16, Foc-23, Foc-8, Foc-4 and Foc-10) (Figure 1). The same standard differential was followed by several workers and existence of four races *viz;* race 1, 2, 3 and 4 was reported (Bayraktar and Dolar, 2012; Mandhare et al., 2011; Barhate and Dake, 2006). Recently, a new race (race 6) was reported to occur in India by Sharma et al. (2014).

Molecular characterization

Fusarium genus specific 18 S ribosomal DNA based ITS markers were used for true identification and study of genetic variation among Foc isolates. The ITS primers amplified a region of ~400 bp size from genomic DNA of all isolates (Figures 2 and 3). Further, amplicons were eluted using gel extraction kit, purified and sequenced by outsourcing from Xcelris Labs Ltd., Ahmedabad India. The sequences were aligned using DNA baser software and compared with other Foc sequences from standard database GenBank. Nineteen isolates were further subjected to Basic Local Alignment Search Tool (BLAST) software to compare the sequences of Foc, previously submitted at National Center for Biotechnology Information (NCBI) data base. All sequences resembled 99% similarity with Foc sequences. Dendrogram on the basis of sequence homologies among all the isolates was also generated by using dendroscope and it was found that 20 isolates were further grouped into three major groups (Figure 6). First major group consisted of 17 isolates Foc-13, Foc-14, Foc-3, Foc-2, Foc-4, Foc-15, Foc-17, Foc-23, Foc-11, Foc-8, Foc-20, Foc-5, Foc-22, Foc-1, Foc-24, Foc-18 and Foc-16. The other two groups consisted of Foc-9 and Foc-10. The ITS region sequence of Foc-15 have been deposited at NCBI, with Accession no. KM253762.

In the present study, presence of 700 bp fragment amplified by xylanase 3 gene (Figures 4 and 5) in all the isolates confirmed absence of race 4 in any of the isolates. Foc race 4 was distinguished with xylanase 3 gene by absence of amplification product only in this

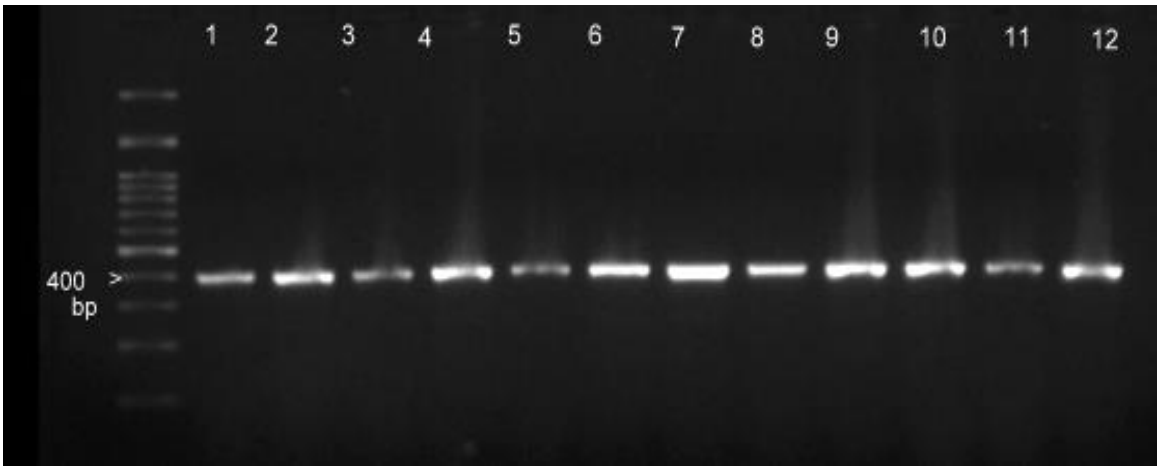

Figure 2. PCR amplification of genomic DNA of Foc isolates (1-12) with ITS markers.

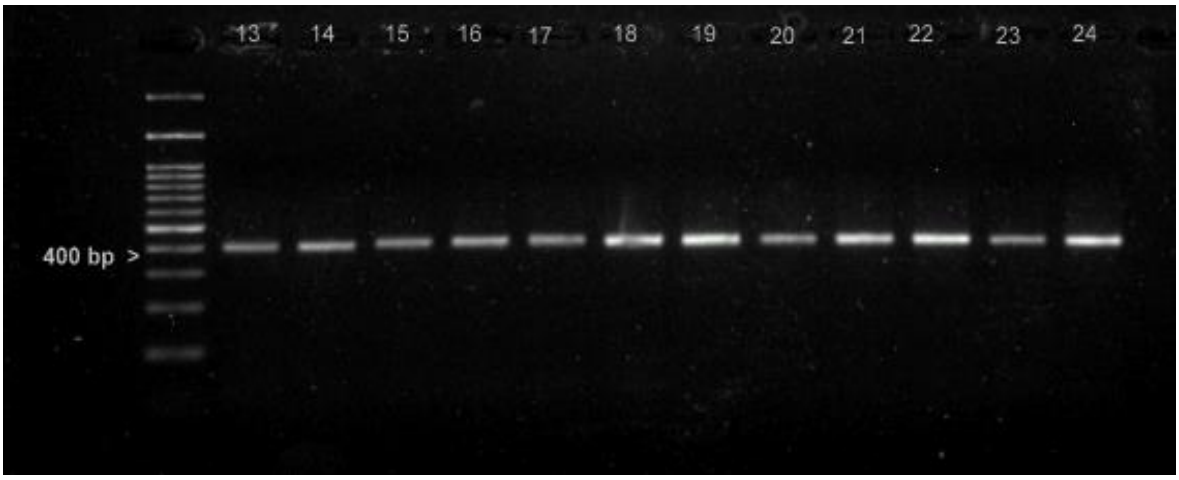

Figure 3. PCR amplification of genomic DNA of Foc isolates (13-24) with ITS markers.

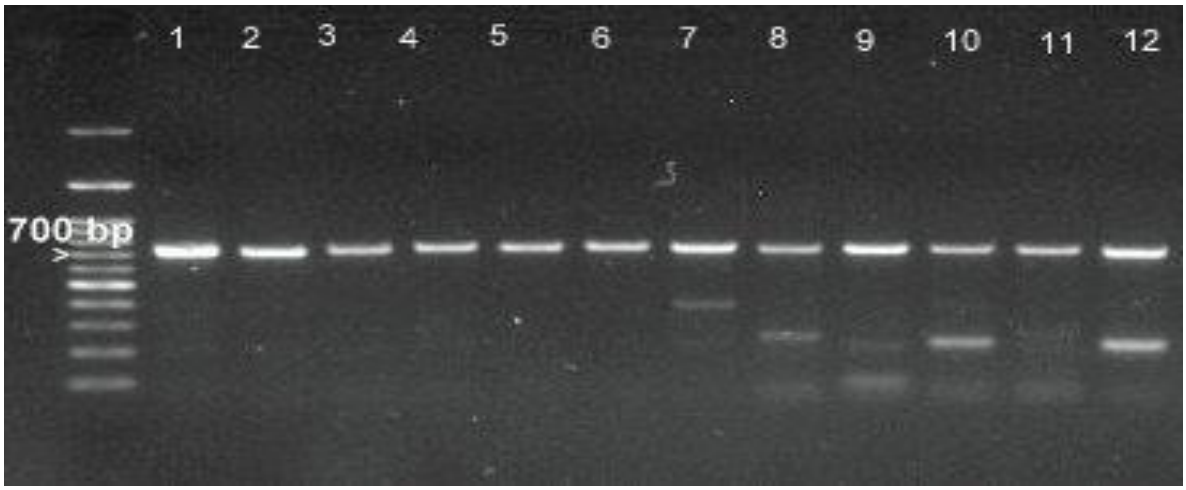

Figure 4. PCR amplification of genomic DNA of Foc isolates (1-12) with *Xylanase 3* gene specific marker

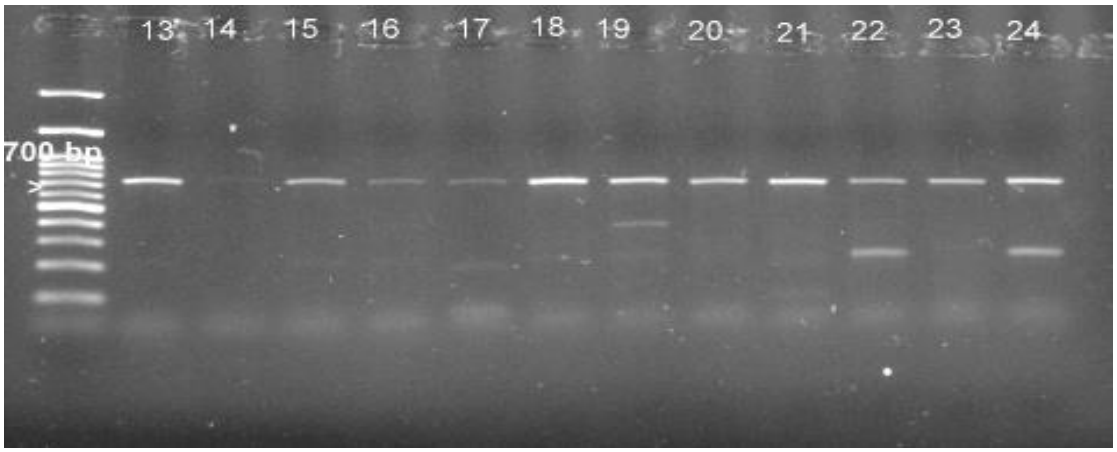

Figure 5. PCR amplification of genomic DNA of Foc isolates (13-24) with *Xylanase 3* gene specific marker.

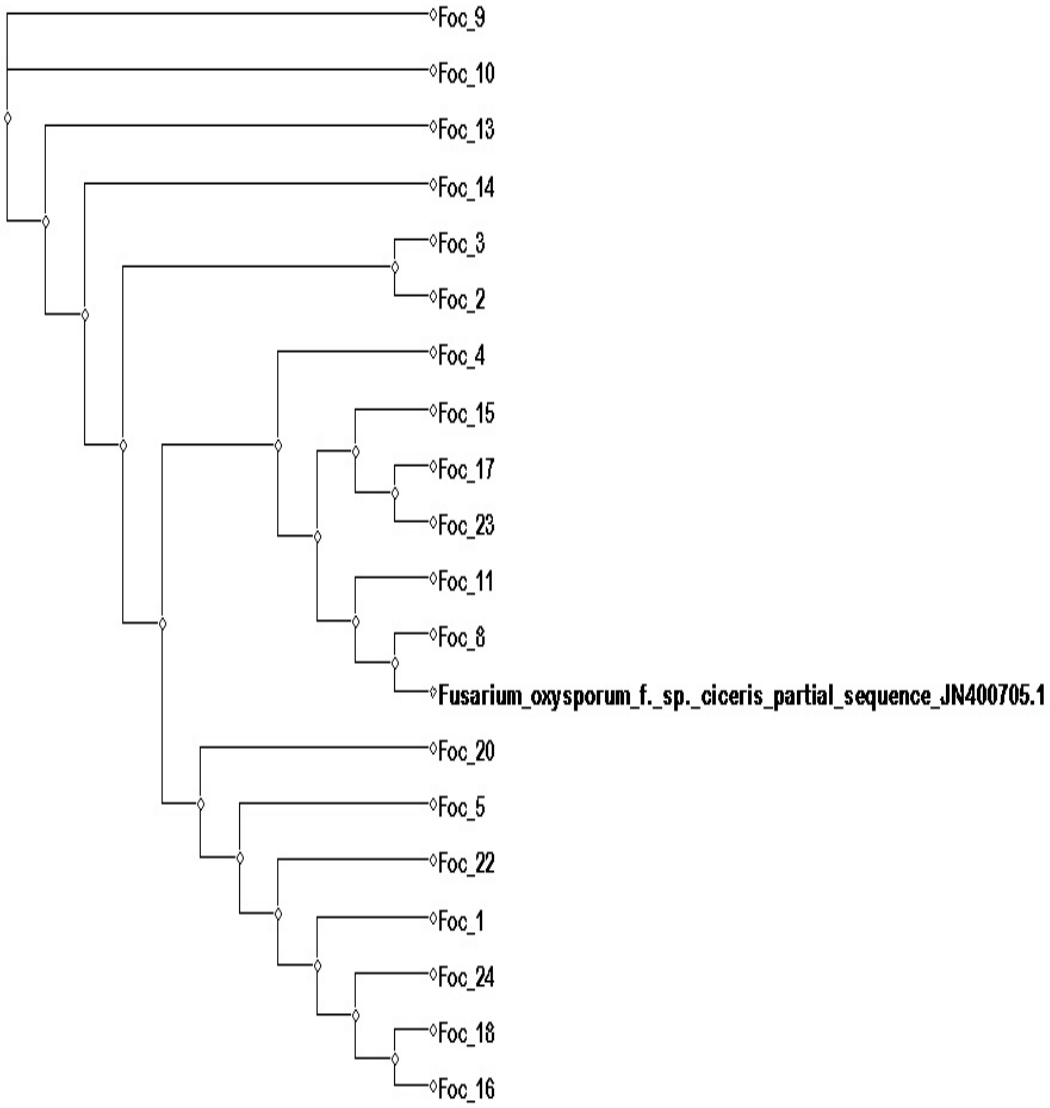

Figure 6. Maximum-likelihood phylogenetic tree based on the alignment of the partial sequences.

race (Gurjar et al., 2009). Any correlation between geographical region and virulence variation with genetic diversity was not observed in the case of both markers (Singh et al., 2006; Sharma et al., 2009; Mandhare et al., 2011; Sivaramakrishnan et al., 2002). In a similar study by Kelly et al. (1994), it was observed that RAPD marker analysis converged the Foc isolates into two groups, yellowing type and wilt syndrome type. RAPDs markers have also been developed into SCAR markers specific to Foc (Durai et al., 2012).

Dubey et al. (2010) observed high level of genetic diversity using ITS-RFLP analysis of Foc isolates, which converged the isolates into six groups. Recently, Sharma et al. (2014) reported occurrence of race 6 in India, which is of Mediterranean and USA region. They also reported application of DArT markers in assessment of genetic diversity in Foc pathogen, with few race specific unique alleles.

Conflict of interests

The authors did not declare any conflict of interest.

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