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MORPHOLOGICAL, PATHOGENIC AND MOLECULAR CHARACTERIZATION OF *Fusarium oxysporum* f.sp. *ciceri* ISOLATES FROM MAHARASHTRA, INDIA

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ABSTRACT

Vascular wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (FOC) is considered as one of the major factors of low productivity in chickpea. The present study was conducted to determine the morphological, pathogenic and random amplified polymorphic DNA (RAPD) variability of twenty isolates of FOC collected from the Maharashtra State of India, along with four reference isolates corresponding to four known FOC races. Pathogenicity of each isolate was confirmed using the wilt susceptible chickpea genotype JG-62. The mycelia of all the isolates were septate, hyaline and profusely branched. All the FOC isolates produced micro- and macro-conidia in pure culture within seven days after inoculation. Based on the abilities of the isolates to cause disease on an international set of chickpea differentials and genetic variability estimated by the RAPD technique, these 24 isolates were grouped into two pathotypes, i.e. pathotype I and pathotype II.

[***Keywords***: *Fusarium oxysporum*, fungal morphology, chickpea, pathogenicity, RAPD, India]

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops grown in the Asian subcontinent. India is the largest producer (25%), importer (20%) as well as consumer in the world. In India, chickpea is grown on 8.25 million ha area with production of 7.5 mt and productivity of 855 kg ha^{-1} (Anonymous 2009). In Maharashtra State of India, the crop is grown on 1.32 million ha area with a total production of 1.21 mt (Anonymous 2008). The national average of chickpea productivity in India is stagnant over recent decades and is lower (855 kg ha^{-1}) than that in Maharashtra State alone (917 kg^{-1}) (Anonymous 2008, 2009). One of the major factors limiting chickpea production is *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (FOC) (Jalali and Chand 1992). The disease is widespread in chickpea growing areas of the world and is reported from at least 33 countries (Nene *et al*. 1996). In severe epidemic, the disease can cause 100% annual losses (Navas-Cortes *et al*. 2000).

The pathogen penetrates the roots and provokes either root rots or tracheomycosis when they invade the vascular system, causing the wilt disease. The pathogen typically invades only living root tissues, kills the plant and then proliferates on the dead tissue. The plants, when uprooted, may show uneven shrinkage at the collar (Nene *et al.* 1978). There is no external rotting of roots and pith, however, when the roots are split vertically, internal discoloration may be seen extending to the stem, due to infection of the xylem tissues of the root and stem. Transverse sections of the infected roots examined under the microscope show the presence of hyphae and spores of the fungus in the xylem (Nene *et al.* 1978), thereby confirming the diagnosis of vascular wilt.

The disease can be observed in a susceptible cultivar within 25 days after sowing in infected soil and this is known as 'early wilt' (Haware and Nene 1980). Isolates of FOC may induce either fast wilting or a progressive yellowing syndrome, which develops 15-40 days after inoculation depending on the cultivar. Wilting may also occur during reproductive growth and is known as 'late wilt'. Plants grown from infected seeds wilt faster than the plants grown from clean seeds.

F. oxysporum f.sp. *ciceri* is a highly variable pathogen. Eight races of this pathogen have been reported, of which six (1A, 2, 3, 4, 5 and 6) cause wilting symptoms, whereas the races 0 and 1B/C cause yellowing syndrome (Gowda *et al*. 2009). The wilt caused by the pathogen can kill the susceptible genotypes within 3-4 weeks of sowing. Four FOC races (1A, 2, 3 and 4) are prevalent in India, of these the race 1A is most virulent.

Management of the disease is difficult either through crop rotation or application of fungicides

because of its soil borne nature. The pathogen can survive in soil for up to six years even in the absence of the host (Haware *et al*. 1996). Instead, the use of wilt resistant chickpea cultivars is potentially the most effective and eco-friendly method of managing the disease (Jalali and Chand 1992). However, the high pathogenic variability in the FOC may limit the effectiveness of resistance (Haware and Nene 1982). Moreover, development of resistant varieties has been hampered because of their undesirable agronomic characteristics (Honnareddy and Dubey 2006).

Morphological identification of FOC into races is difficult. The classical method of race identification involves inoculation of differential chickpea cultivars with a particular FOC isolate and determining its pathogenicity. This is a time consuming procedure requiring at least 40 days for the analysis and reactions can be influenced by various environmental parameters such as temperature and humidity (Haware and Nene 1982). Also, there are several sets of differential cultivars available and some of the differentiation is based on intermediate reactions (Sharma *et al*. 2005). To overcome these problems, several other approaches have also been attempted. Previously, serological and electrophoretic variability of proteins isolated from Indian FOC races has been studied by Desai *et al.* (1992a). Based on antigens, they identified close relationships among races 1, 2 and 3, while race 4 was different. Biochemical analysis of the four FOC races has revealed variation in total sugar and amino acid content for race 3 as compared to races 1, 2 and 4 (Desai *et al*. 1992b).

DNA-based molecular markers have also been used for studying the variability in FOC races. Races 0, 1B, 1C, 5 and 6 were distinguished by random amplified polymorphic DNA (RAPD) finger-printing (Jiménez-Gasco *et al*. 2001) and sequence characterized amplified regions (SCAR) markers have been developed for races 0 and 6, while a race 5 specific identification assay has been developed using touchdown PCR (Jiménez-Gasco and Jiménez-Diaz 2003). The *Eco*RI restriction patterns of nuclear ribosomal DNA of Indian FOC races have suggested that races 1 and 4 are more similar to each other than races 2 and 3 (Chakrabarti *et al*. 2001). In recent years, variation in phytotoxicity of representative isolates of FOC has been examined (Gopalakrishnan and Strange 2005). Although DNA marker based variability for Indian FOC races has been assessed to a great extent, molecular markers have not been developed as yet for these races.

In such circumstances, the use of molecular markers for characterization of genetic variability of FOC appears to be a better proposition. McDormett *et al*. (1994) reported that a RAPD technique is useful in characterizing genetic variation in a wide range of organisms. The objective of this study was to analyze the genetic variability of FOC isolates collected from different regions of Maharashtra along with the reference isolates of four known races in India, using the RAPD technique.

MATERIALS AND METHODS

Fungal Isolates

Twenty FOC isolates obtained from chickpea wilted plants collected from 20 different chickpea growing areas of Maharashtra, India (Table 1; Fig. 1) and four isolates of known races in India (races 1, 2, 3 and 4) were used in this study for comparison. These 20 isolates, designated as isolates I_1 to I_{20}, were confirmed for its species based on morphology and Koch's postulate on universal susceptible cultivar JG-62. The four reference isolates were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad.

Table 1. Location of collected *Fusarium* infected chickpea plants in Maharashtra, India.

Isolate	Place	District
Western Maharashtra		
I_1	Mohol	Solapur
I_2	Pandharpur	Solapur
I_3	Sangli	Sangli
I_4	MPKV, Rahuri	Ahmednagar
I_5	Sinnar	Nashik
I_6	Gadhinglaj	Kolhapur
I_7	Vathar	Satara
I_8	Malegaon	Nashik
I_9	NARP, Kolhapur	Kolhapur
I_{10}	Nira	Pune
I_{11}	Agril. College, Dhule	Dhule
Vidharbha		
I_{12}	Devulagaon	Akola
I_{13}	Dr. PDKV, Akola	Akola
I_{14}	Shegaon	Akola
I_{15}	Agril. College, Nagpur	Nagpur
I_{16}	Amravati	Amravati
Marathwada		
I_{17}	Parbhani	Parbhani
I_{18}	Latur	Latur
I_{19}	ARS, Badnapur	Jalna
I_{20}	Nanded	Nanded

Fig. 1. The map of areas where *Fusarium oxysporum* f.sp. *ciceri* isolates were collected from chickpea growing areas of Maharashtra State, India.

Morphological Variation

All the isolates were grown on potato dextrose agar (PDA) at room temperature (26 ± 2°C) in triplicate. After seven days, the width of mycelia, the length and width of microconidia and macroconidia, and the diameter of chlamydospores were measured using filar micrometer under a light microscope at magnification of 40 x. The diameter of chlamydospores was recorded at 30 days after incubation. The results were compiled from each experiment and analyzed statistically following the procedure described by Panse and Sukhatme (1967).

Differential Reaction

Each isolate was cultured in 250-ml flasks containing 100 ml potato dextrose broth (PDB) for 15 days at room temperature (26 ± 2°C). Surface sterilized seeds of 10 differential varieties (JG-62, JG-74, Chafa, Annigeri, K-850, L-550, BG-212, C-104, CPS-1 and WR-315) obtained from the project coordinator, Indian Institute of Pulses Research, Kanpur, India were sown in pro-trays containing autoclaved sand-farm yard manure in 1:1 proportion. Fifty cups of the growing medium were used for each variety with five seeds in each cup. Fifteen days old seedlings of each variety were uprooted carefully and the roots were subsequently washed under running tap water and then with sterile water. Roots were injured with a scissors and 10 seedlings of each variety were transplanted to pro-trays after dipping in the culture of each isolate for 5 minutes. Number of seedlings wilted were recorded up to 20 days. The percentage of wilted plants was calculated and the varieties were categorized as resistant (<10% plant mortality), moderately resistant (>10-20% plant mortality), and susceptible (>20% plant mortality) (Nene *et al.* 1981).

DNA Isolation

Total genomic DNA was extracted from 0.5 mg mycelia obtained from seven days old FOC cultured on PDA using the lysis buffer CTAB and polysaccharide precipitation with phenol and chloroform: isoamyalcohol (24:1) according to the procedure described by Du Teau and Leslie (1988). DNA pellet was dissolved in 100 µl of TE buffer. The DNA purity was checked spectrophotometrically using Nanodrop, ND-1000 UV visible spectrophotometer (USA) at 260 nm. The 260/280 ratio was about 1.7, indicating high purity of the DNA. The DNA integrity was checked by electrophoresis (Bio-Rad subcell model 96 USA) on 0.8% (w/v) agarose gel containing 0.5 µg ml^{-1} of ethidium bromide and run in TBE buffer at 6 v cm^{-1}. The band intensity was measured on gel documentation unit (Fluor Chem™ Alpha Innotech, USA) and compared to that of calf thymus standard DNA.

RAPD Analysis

Six decamer primers obtained from Operon Technologies, Inc., USA, were used to amplify the DNA. DNA amplification was carried out in a thermal cycler (Eppendorf, Germany) with the conditions as described by Kelly *et al*. (1994) as follows: one cycle of initial denaturation at 95°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 1 minute, annealing at 34°C for 1 minute and primer extension at 72°C for 2 minutes. A final extension at 72°C for 15 minutes was carried out at the end of the cycles. Amplifications were performed in 25-µl reaction volumes containing 100 ng of DNA, 1x PCR buffer, 2mM of $MgCl_2$, 0.2 mM of each dNTP, 0.4 mM of primer and 1 U of *Tag* DNA polymerase. About 8 µl of mineral oil was overlaid on the top of each reaction mixture.

Separation of Amplified PCR Products on Agarose Gel Electrophoresis

Amplified products were separated by electrophoresis in 1.5 % (w/v) agarose gel containing 0.5 µg ml^{-1} ethidium bromide in 1 TBE buffer and run at constant 80 V for 2 hours and 30 minutes. The PCR products were visualized under UV transilluminator (Fluor Chem TM Alpha Innotech, USA) and photographed. RAPD reproducibility was confirmed by repeating the experiment twice. The amplified DNA products appeared in the gel were scored as '1' (presence) or '0' (absence). A binary matrix of combined data from the six primers for the 24 isolates was prepared. The software package NTSYS-pc version 2.02 (Rohlf 1990) was used to cluster the isolates by an unweighted paired group method with arithmetic averages (UPGMA), based on Dice similarity coefficient (Sokal and Sneath 1963). A dendrogram was generated using the SAHN method of NTSYS-pc.

RESULTS AND DISCUSSION

Morphological Variation

The mycelia of all isolates were septate, hyaline, profusely branched and highly varied in width (Table 2). The mycelium width of isolates I_5 and I_8 were only 0.92 and 1.15 µm, respectively, which were significantly shorter than the rest of the isolates. A high degree of variability in chlamydospores diameter was observed. Isolates I_5 and I_8 showed significantly shortest diameter of chlamydospores compared with that of the remaining isolates.

All FOC isolates produced microconidia in pure culture within 7 days after inoculation. A significant variation in microconidia size was also observed (Table 2), with the average length and width ranged from 2.3 to 13.8 µm and 0.69 to 4.6 µm, respectively. The microconidia of isolates I_5 and I_8 were again significantly shorter than the average microconidia length and width of the remaining isolates. Macroconidia were generally 2 to 4 septate, curved and pointed at both ends. The mean length and width of macroconidia were highly varied, ranged from 6.4 to 34.5 µm and 0.92 to 4.6 µm, respectively. The great variability in relative size of macro- and microconidia, width of mycelium and diameter of chlamydospores as was observed in FOC isolates from Maharashtra State was not an isolated case. Previously, Chattopadhyay and Gupta (1967) and Booth (1977) also reported high morphological size variation in FOC isolates collected worldwide. This morphological variation among the isolates might be due to genetic differences among them, as all the isolates were cultured under identical laboratory conditions.

Molecular Characterization

The six RAPD primers amplified a total of 79 bands, of which 68 bands (86%) were polymorphic (Table 3 and 4). Each primer thus produced an average of 13 bands with the size ranged from 220 to 3,700 bp. Maximum number of bands (18) were produced by the primer

Table 2. Morphological variation of *Fusarium oxysporum* f.sp. *ciceri* isolates from Maharashtra, India.

Isolate	Mycelium width (µm)	Macroconidia						Microconidia						Chlamydospore diameter (µm)
		Length (µm)			Width (µm)			Length (µm)			Width (µm)			
		Max	Min	Mean[1)]	Max	Min	Mean[1)]	Max	Min	Mean[1)]	Max	Min	Mean[1)]	
I_1	1.84	9.2	8.05	8.62	1.15	1.15	1.15	6.9	4.6	5.75	1.15	0.69	0.92	5.70
I_2	1.40	13.8	6.90	10.35	1.61	1.15	1.38	6.9	5.8	6.35	2.30	1.15	1.72	6.90
I_3	2.99	13.8	11.5	12.65	3.22	3.22	3.22	4.6	2.3	3.45	1.15	0.69	0.92	9.20
I_4	1.84	20.7	13.8	17.25	4.60	3.45	4.02	6.9	5.8	6.35	0.92	0.92	0.92	5.70
I_5	0.92	9.5	6.40	7.95	0.92	0.92	0.92	3.5	2.3	2.90	0.69	0.69	0.69	3.00
I_6	1.61	18.4	11.50	14.95	1.15	1.15	1.15	5.8	2.3	4.05	1.15	1.15	1.15	5.70
I_7	4.60	11.5	9.20	10.35	1.84	1.15	1.49	4.6	2.3	3.45	1.15	0.69	0.92	10.30
I_8	1.15	9.1	6.50	7.80	0.98	0.97	0.97	3.0	2.3	2.65	0.71	0.71	0.71	3.20
I_9	1.84	11.5	9.20	10.35	2.76	2.30	2.53	8.0	5.8	6.90	0.97	0.99	0.98	4.60
I_{10}	1.84	29.9	16.70	23.30	2.99	1.61	2.30	13.8	6.9	10.35	2.76	1.15	1.95	5.70
I_{11}	1.45	34.5	18.40	26.45	4.60	2.30	3.45	11.5	6.9	9.20	2.30	1.15	1.72	5.20
I_{12}	1.38	11.5	6.90	9.20	1.15	0.92	1.03	6.9	2.3	4.60	1.38	0.69	1.03	5.70
I_{13}	3.45	16.1	11.50	13.80	3.45	2.30	2.87	13.8	6.9	10.35	2.30	1.61	1.95	5.70
I_{14}	2.30	20.7	16.10	18.40	3.45	2.30	2.87	6.9	4.6	5.75	2.30	1.15	1.72	6.30
I_{15}	2.76	11.5	10.30	10.90	1.84	1.61	1.72	5.0	3.2	4.10	1.15	1.15	1.15	6.20
I_{16}	1.85	11.5	6.90	9.20	1.15	0.92	1.03	6.9	2.3	4.60	2.76	1.15	1.95	5.70
I_{17}	2.30	16.1	11.50	13.80	2.30	1.81	2.05	9.2	6.9	8.05	4.60	1.61	2.25	5.20
I_{18}	4.60	16.1	11.50	13.80	2.30	1.61	1.95	11.2	4.6	7.90	3.00	1.15	2.07	5.70
I_{19}	2.30	16.1	11.50	13.80	2.30	1.61	1.95	5.7	3.9	4.80	1.61	1.15	1.38	5.70
I_{20}	2.76	11.5	9.20	10.35	1.84	1.15	1.49	4.6	3.5	4.02	2.30	1.15	1.72	6.80
Race 1	2.30	9.2	8.90	9.05	1.84	1.15	1.49	3.3	3.8	3.55	1.15	0.92	1.03	4.90
Race 2	1.54	11.5	9.20	10.35	1.38	1.15	1.26	4.6	2.3	3.45	1.15	1.15	1.15	4.60
Race 3	1.57	9.2	8.05	8.62	1.95	1.97	1.96	4.6	2.8	3.70	1.15	0.92	1.03	8.00
Race 4	1.56	13.8	8.05	10.92	2.30	1.38	1.84	6.9	2.3	4.60	1.15	1.15	1.15	5.00
CD at 5%	0.16			0.70			0.13			0.33			0.09	0.55
CV (%)	2.54			1.80			2.43			2.09			2.28	3.15

[1)]Mean of three independent observations

CD = critical difference, CV = coefficient of variability.

Table 3. Unique DNA polymorphism bands of *Fusarium oxysporum* f.sp. *ciceri* isolates from Maharashtra, India, based on the six RAPD primers.

Primer	Size of unique bands (bp)	Isolate/race with unique bands
OPB-3	1,620	I_2
OPB-4	330	I_2
	2,250	I_4
OPB-5	550	I_2
	3,540	I_{12}
OPB-6	600	I_5
OPB-7	970	(Race 3)
	1,460	I_8
OPB-8	260	I_5
	1,610	(Race 3)
	2,650	I_{18}

OPB-5 and the least (10) by OPB-4. OPB-6 produced the highest number of polymorphic bands (92%), whereas OPB-8 the lowest (77%). All the primers produced 11 unique bands, which might or might not be race- or isolate-specific. The diversity observed in the 20 isolates is mainly being attributed to genetic dissimilarity revealed by the unique banding patterns in RAPD profile exhibited by each isolate.

Two unique bands generated by the primers OPB-7 and OPB-8 were present only in race 3. Similarly, the 1,620 bp fragment amplified by the primer OPB-3 and the 600 bp produced by the primer OPB-6 were identified only in isolates I_2 and I_5, respectively. These specific bands can be converted into SCAR markers which may be useful for identification of race- or region-specific isolates. These SCAR markers would facilitate the development of region-wise *Fusarium* wilt tolerance.

The high level of polymorphism observed in the present investigation indicates high diversity among the 20 isolates collected from different regions of Maharashtra. Some of these isolates appeared similar in respect of morphological characters and virulence patterns; however, the RAPD technique differentiated them. This indicates that molecular markers have high discrimination power than morphological traits and

pathogenic reactions. The diversity observed in this study could mainly be attributed to genetic dissimilarity revealed by the unique banding patterns in RAPD profile exhibited by each isolate.

A dendrogram was constructed based on the RAPD data (Fig. 2 and 3), in which the isolates were grouped in two major clusters at a coefficient index 0.28. The cluster A comprised most of the isolates of different locations including the four reference race-specific isolates. The cluster A was further partitioned in two clusters, A_1 which comprised only isolate I_1, collected from Mohol, a Western Maharashtra region, and A_2 which comprised the rest of the isolates. The cluster A_2 was further partitioned into six small sub-clusters, of which two (A2I and A2V) had only one isolate each. Two isolates I_5 and I_8, which were collected from Sinnar (District Nashik) and Malegaon in Western Maharashtra, respectively formed an independent cluster (B). These two isolates were clearly distinct from the remaining isolates with respect to their morphological characters, by having shorter mycelium width, shorter chlamydospore diameter, and smaller macro- and microconidia (Table 3).

It was interesting to see that all the four standard races clustered together in the dendrogram. This indicates that the RAPD primers used to discriminate the isolates were not effective to distinguish the standard races. This might be because of the known fact that RAPD are random markers and hence, these might have not been able to target the genetic regions that are responsible for race specificity of the four standard races. On the contrary, the RAPD clustering correlated more-or-less with the geographic origin of the isolates. It is expected that the genetic variation among the isolates derived from different geographic regions is distributed randomly in the genome, and this might have been captured by the RAPD markers, which are also randomly distributed in the genome.

Reaction of Chickpea Differentials to FOC Isolates

The 20 FOC isolates collected from Maharashtra were proven to be pathogenic on susceptible chickpea cultivar JG-62 under artificial inoculation. Characteristics of wilt symptoms such as drooping of leaves

Table 4. Random amplified polymorphic DNA (RAPD) primers used to amplify informative DNA polymorphisms in *Fusarium oxysporum* f.sp. *ciceri* isolates from Maharashtra, India.

Primer	Sequence (5' to 3')	Number of generated bands	Number of polymorphic bands (%)	Number of unique bands (%)	Fragment size (kb)
OPB-3	CATCCCCCTG	11	10 (91)	1 (9)	0.22-2.02
OPB-4	GGACTGGAGT	10	8 (80)	2 (20)	0.33-2.29
OPB-5	TGCGCCCTTC	18	16 (89)	2 (11)	0.39-3.51
OPB-6	TGCTCTGCCC	13	12 (92)	1 (8)	0.41-2.51
OPB-7	GGTGACGCAG	14	12 (86)	2 (14)	0.45-1.79
OPB-8	GTCCACACGG	13	10 (77)	3 (23)	0.26-3.70

Numbers in parentheses are percentages to the number of generated bands.

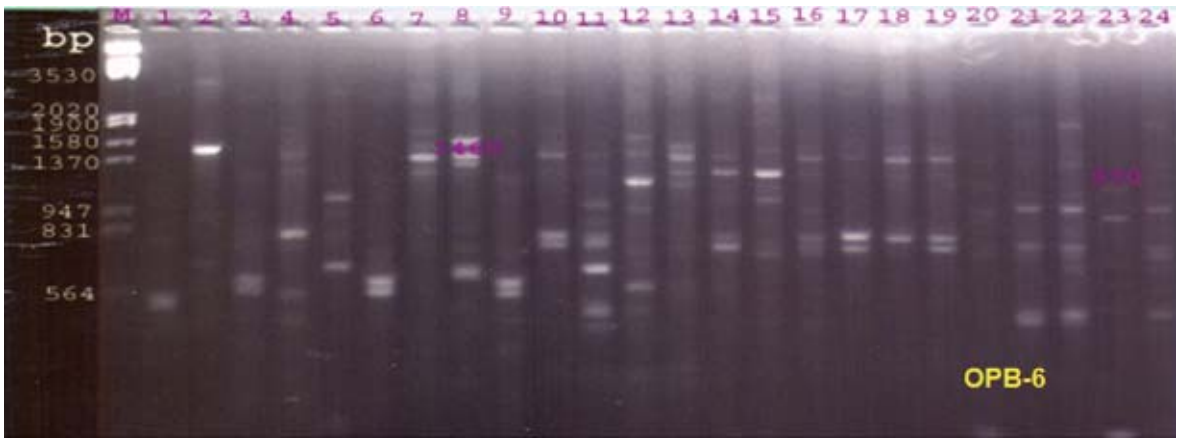

Fig. 2. Random amplified polymorphic DNA profile of *Fusarium oxysporum* f.sp. *ciceri* (FOC) isolates generated by primer OPB-6. Lane M is the 4 kb DNA ladder. Lane 1-20: FOC isolate I_1 to I_{20}, Lane 21-24: FOC race 1 to 4.

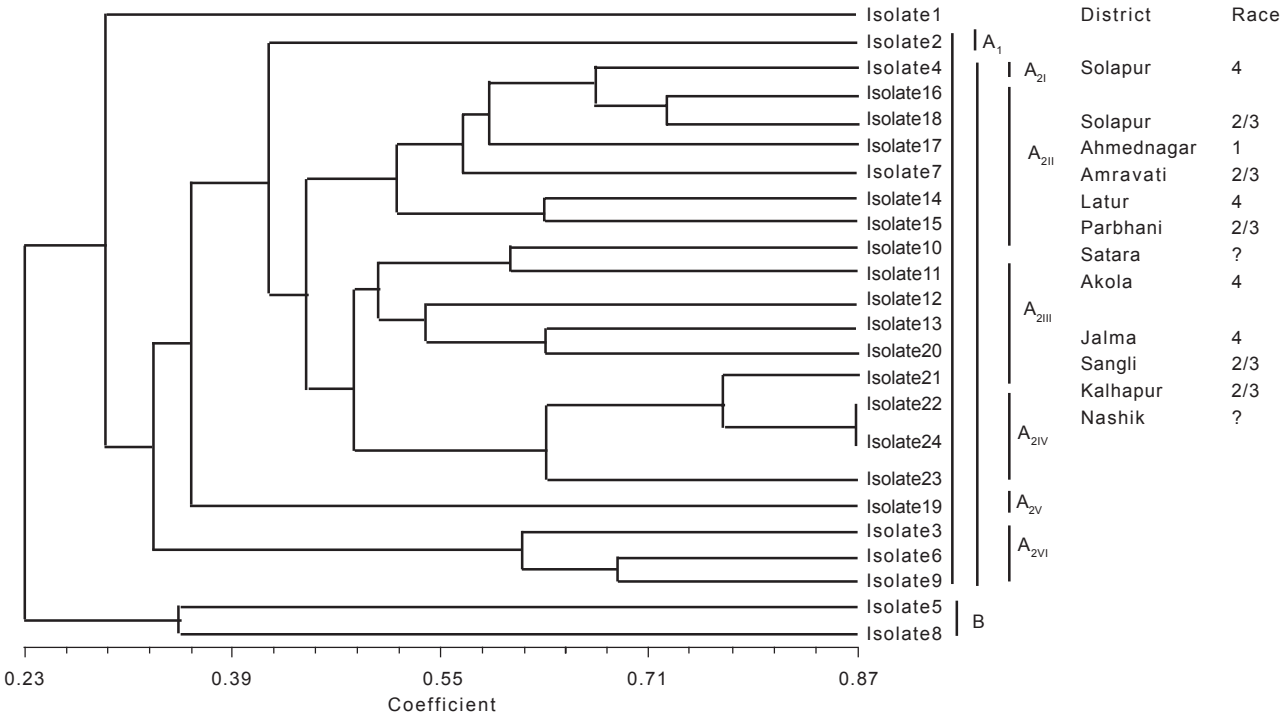

Fig. 3. Unweighted pair group method (UPGMA) cluster analysis on RAPD markers of *Fusarium oxysporum* f.sp. *ciceri* isolates from Maharashtra State, India, based on Dice similarity coefficient.

and internal discoloration of root tissues were conspicuous on infected plants (Frisullo *et al.* 1989). Pathogenicity tests of these isolates along with its four races on ten chickpea differentials revealed the existence of pathogenicity variability among these isolates (Table 5). The cultivars JG-62, CPS-1, Annigeri and Chafa exhibited susceptible reaction to all isolates, whereas L-550 and C-104 were resistant to isolates I_5 and I_8, but susceptible to the rest of the isolates. The cultivars JG-74 and WR-315 showed resistant or moderately resistant reaction against all the FOC isolates. On the basis of wilt differential reactions, the isolates could be grouped into two pathotypes. Isolates I_5 and I_8, which were the least virulent on L-550 and C-104, belonged to pathotype I while the remaining isolates were grouped in pathotype II.

The existence of pathogenic variability in FOC was also reported by Gupta *et al.* (1986), Rahman *et al.* (1998), and Paul *et al.* (2001). Based on a number of FOC isolates collected from various regions in India, Haware and Nene (1982) could identify isolates from Maharashtra and Hyderabad as race 1, isolates from Kanpur as race 2, isolates from Budaspur as race 3, and isolates from Hissar and Jabalpur as race 4. The present study was based on limited number of isolates and therefore could not provide clear picture on the race composition in India.

Honnareddy and Dubey (2006) further conducted a study to determine pathogenic and genetic variability of FOC isolates collected from different parts of India. Pathogenic virulence study on 25 isolates of the pathogen on international set of differential cultivars was accomplished for characterization of new isolates in the known four races of the pathogen. Genetic variability within 24 isolates representing seven races of FOC was assessed by RAPD. At the same time, Singh *et al.* (2006) analyzed 30 isolates of FOC obtained from rhizosphere soil of chickpea from different locations in Northern India. The amount of genetic variation was evaluated by RAPD and IGS analysis. Genetic similarity between each of the isolates was calculated and results indicated that there was little genetic variability among the isolates collected from the different locations.

Similarly, genetic variability among 43 isolates of FOC collected from nine states of India, including the four well-characterized races of the pathogen were assessed using the molecular markers, RAPD and amplified fragment length polymorphism (AFLP). These molecular markers established the distinctness of race 1 and race 2 pathogenic isolates and the close similarity of pathogenic isolates of race 3 with that of race 4. The high levels of DNA polymorphism observed with the molecular markers suggested the rapid evolution of new recombinants of the pathogen in

Table 5. Reaction of international set of differential chickpea cultivars to twenty isolates and four races of *Fusarium oxysporum* f.sp. *ciceri.*

Cultivar	Isolate																				Race			
	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13}	I_{14}	I_{15}	I_{16}	I_{17}	I_{18}	I_{19}	I_{20}	1	2	3	4
JG-62	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CPS-1	S	S	S	S	HS	S	S	HS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
L-550	S	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
BG-212	MR	MR	MR	MR	R	MR	MR	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
K-850	MR	MR	MR	MR	R	MR	MR	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
Annigeri	HS	S	S	HS	HS	S	S	HS	S	HS	S	S	HS	S	S	S	HS	HS	HS	HS	HS	S	S	HS
Chafa	S	S	S	S	HS	S	S	HS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
JG-74	R	R	R	R	MR	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
WR-315	R	R	R	R	MR	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C-104	S	S	S	HS	R	S	S	R	HS	S	HS	S	HS	S	HS	HS	S	S	S	HS	HS	S	S	S

R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

the chickpea growing fields (Sivaramakrishnan *et al.* 2002). In a very recent study, 48 isolates of FOC collected from different chickpea growing regions in India were evaluated for genetic variations using AFLP. Out of 48 isolates, 41 were found pathogenic and seven were non-pathogenic. Pathogenic isolates differed in their virulence, however, there was no apparent correlation between geographical origin and virulence of the isolates. UPGMA cluster analysis and principle coordinate analysis distinctly classified 48 isolates into two major groups, pathogenic and non-pathogenic. The pathogenic isolates could be further clustered into six major groups at 0.77 genetic similarities. Region specific grouping was observed within few isolates (Sharma *et al.* 2009).

The isolates I_4, I_{13} and I_{20} had pathogenicity reaction identical to that of race 1, when tested on the set of differentials and hence these isolates might belong to race 1. Similarly, the isolates I_2, I_3, I_6, I_7 and I_{12} might belong either to races 2 or 3, as they had pathogenicity reaction identical to that of races 2 or 3. Likewise, the isolates I_1, I_{10}, I_{17}, I_{18} and I_{19} were similar to race 4 in the pathogenicity reaction and hence might belong to race 4. However, the isolates I_5, I_8, I_9, I_{11}, I_{15} and I_{16} demonstrated different pathogenicity reactions than any of these four races. Of these, the isolates I_5 and I_8 had identical morphology and pathogenicity reaction and they were clustered together in cluster B in the UPGMA dendrogram. This indicates that these isolates might belong to different races than races 1, 2, 3 or 4. The isolates I_9, I_{11}, I_{15} and I_{16} showed identical reaction to each other, which was different from that of races 1, 2, 3 or 4. However, they were clustered in the cluster A along with the races 1, 2, 3 and 4. This is quite intriguing and it is possible that these isolates either belong to a new race, or need to be differentiated using a bigger set of chickpea differential lines.

CONCLUSION

The 20 Indian FOC isolates displayed high morphological variability as well as RAPD polymorphisms, indicating high genetic diversity among the isolates. On the basis of wilt differential reactions, the 20 isolates were grouped into two pathotypes, pathotype I and pathotype II. In the UPGMA dendrogram, the isolates were grouped in two major clusters; cluster A comprising most of the isolates including the four reference race-specific isolates, whereas two isolates, I_5 and I_8 formed another cluster B. These two isolates were also clearly distinct from the isolates with respect to their morphological characters as well as pathogenicity on a set of chickpea differentials and belonged to pathotype I. The isolates I_5, I_8, I_9, I_{11}, I_{15} and I_{16} demonstrated pathogenicity reaction different than any of the four standard races, which indicate that these isolates either belong to a new race, or need to be differentiated using a bigger set of chickpea differential lines.

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