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Morphological characterization and genetic diversity of chickpea wilt fungus *Fusarium oxysporum* f. sp. *ciceri*, using SRAP and RAPD markers

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Abstract
Chickpea (*Cicer arietinum* L.) is the third most important food legume crop of the developing countries after dry bean and pea and *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt is one of the major constraint of chickpea production. A total of thirty Sequence Related Amplified Polymorphism (SRAP) and Fourteen Random Amplified Polymorphic DNA (RAPD) primers were used to determine the genetic variations among the four races (Foc1, Foc2, Foc3 and Foc4) of *Fusarium oxysporum* f. sp. *ciceri*. Among all primers, Me1-Em5, Me1-Em13, Me3-Em11, Me3-Em7, Me4-Em14, Me4-Em18, Me5-Em5, K4, K7, P2 and P21 showed 100% polymorphism between the races. Out of thirty SRAP primer combinations, 25 produced a total of 437 scorable and reproducible amplicons with an average of 18.2 amplicon per primer and all the fourteen RAPD primers together produced a total of 238 scorable amplicons and showed 94.5% polymorphism.

Keywords: Chickpea, chickpea wilt, *Fusarium oxysporum* f. sp. *ciceri*, RAPD, SRAP

Introduction
Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop after bean and pea. Among severe biotic stresses of chickpea, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the limiting factors in successful cultivation of chickpea and is responsible for a significant reduction in yield. *F. oxysporum* f. sp. *ciceri* wilt is the most devastating disease that affects chickpeas, with reports of cases coming from every major growing region each year (Pande *et al*., 2007) [13]. The kind of soil, agroclimatic conditions, and level of host plant resistance affect the frequency of disease occurrance. Wilting can be induced by *F. oxysporum* f. sp. *ciceri* at any stage of growth, from seedling to pod development. Complete yield losses of affected plants are caused by the disease during the seedling and blooming phases. The disease grew more severe due to widespread water stress and high temperatures, which made the crop more vulnerable and resulting in losses in economic yield (Pawar *et al*., 1992; Halila and Strange, 1996; Navas-Cortes *et al*., 2000) [14, 4, 11]. Butler reported the first cases of chickpea wilt in India as early as 1918 [1]. However, chickpea wilt has become more significant since the 1950s, and only in the last few decades has there been a significant advancement in the identification of novel races of this fungus. Haware and Nene (1982) [5] did the first attempt to identify a pathogenic racial pattern in India. They found evidence of race-1 in the Hyderabad region, race-2 in Kanpur, race-3 in Gurdaspur, and race-4 in Hisar and Jabalpur.
In India, chickpeas are mostly farmed with limited resources after the kharif crop is harvested. Farmers in such critical circumstances require low-cost, efficient production technology, which is made possible by the availability of genotypes resistant to wilt. For the purpose of managing this disease economically, extensive research on host plant resistance had previously started (Haware, 1990; Jimenez Diaz *et al*., 1991; Nene and Reddy, 1987) [6, 7, 12]. However, because of the high level of pathogenic variability within the population of *F. oxysporum* f. sp. *ciceri* (Dubey *et al*., 2012) [2] and the undesirable agronomical character associated with the wild donor parent of chickpea (Jimenez-Gasco *et al*., 2004) [8], the deployment of resistant varieties was not extensive. In order to maximise the crop output, farmers and the scientific community face

challenges due to these conditions. According to Sivaramakrishnan *et al*. (2002) [18], the pathogenic diversity among *F. oxysporum* f. sp. *ciceri* isolates leads to the emergence of novel races that trouble stable chickpeas and cause them to wilt.

Hence, the present study was aimed to understand the variability of different races of *Fusarium oxysporum* f. sp. *ciceri*, which would help to develop new resistant varieties and proper management of Fusarium wilt in chickpea using SRAP and RAPD markers.

Materials and Methods

Four races (Foc1, Foc2, Foc3 and Foc4) of *F. oxysporum* f. sp. *ciceri* representing four states (Akola-Maharastra, Kanpur-Uttar Pradesh, Gurudaspur-Haryana and Jabalpur-Madhya Pradesh) of India were selected for the present study. These races maintained in Division of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India were taken for the present study.

DNA extraction from four races of *F. oxysporum* f. sp. *ciceri* was carried out by CTAB (cetyltrimethyl ammonium bromide; 5M NaCl; 0.5M EDTA, 1M TrisHCl, and 2% CTAB) method (Murray and Thompson, 1980). The mycelial mat of fungus were dried and crushed into powder with liquid nitrogen (-196 °C) in sterilized mortar and pestle. The powder was homogenised using pre-warmed (65 °C) extraction buffer and poured into eppendorf tubes (2 ml). The tubes were incubated in hot water bath (65 °C) for one hour and centrifuge them at room temperature for 15 minutes at 12000 rpm. Upper aqueous layer was transferred followed by adding equal volume of chloroform: isoamyl alcohol (24:1) into eppendorf tubes and centrifuged at 12000 rpm for 15 minutes. Transfer the aqueous phase into a fresh eppendorf tube and equal volume of ice chilled isopropanol was added. The tubes were allowed to precipitate overnight at 4 °C and centrifuge the tubes at 10,000 rpm for 8 minutes the following day. The pellet was collected and centrifuged twice at 10,000 rpm for 8 minutes with ethanol (70%) and air dried pellet was dissolved in 20–50 µl TE (Tris-EDTA) buffer depending on the size of the pellet obtained. DNA purity and concentration was estimated by spectrophotometer at 260 nm as well as on 0.8% agarose gel electrophoresis.

Sequence Related Amplified Polymorphism (SRAP)

Genetic diversity analysis of four races of *F. oxysporum* f. sp. *ciceri* were studied using randomly selected thirty different combinations of Sequence Related Amplified Polymorphism (SRAP) primers. The amplification reaction was carried out with total volume of 20 µl reaction mixture for each sample. The mixture contained reagents including template DNA 1 µl, Primer (Forward+Reverse) 2 µl, dNTP 0.4 µl, Taq Polymerase 0.2 µl, 10X PCR Buffer with MgCl2 3.5 µl and 12.9 µl ddH_2O. The PCR reaction was performed in total volume of 20 µl following the PCR set: initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, annealing at 58 °C (depending on the primer given in Table 1) for 1 min, elongation at 72 °C for 1 min, with a final elongation at 72 °C for 10 min. Amplified products were resolved in polyacrylamide gel and the AgNO3 stained gel was photographed using the Gel Doc system.

Table 1: List of Sequence Related Amplified Polymorphism (SRAP) primers used with their nucleotide sequence and annealing temperature

Sr. no.	Primer	Nucleotide sequence (5' to 3')	Annealing temperature (T_m) °C
1	Me1	TGAGTCCAAACCGGATA	45
2	Me2	TGAGTCCAAACCGGAGC	49
3	Me3	TGAGTCCAAACCGGAAT	45
4	Me4	TGAGTCCAAACCGGACC	49
5	Me5	TGAGTCCAAACCGGAAG	47
6	Me6	TGAGTCCAAACCGGACA	47
7	Me9	TGAGTCCAAACCGGAGG	49
8	Me14	GTAGCACAAGCCGGAGC	52
9	Em1	GACTGCGTACGAATTAAT	43
10	Em3	GACTGCGTACGAATTGAC	48
11	Em2	GACTGCGTACGAATTTGC	48
12	Em7	GACTGCGTACGAATTCAA	46
13	Em5	GACTGCGTACGAATTAAC	46
14	Em11	GACTGCGTACGAATTCTA	46
15	Em12	GACTGCGTACGAATTCTC	49
16	Em13	GACTGCGTACGAATTCTG	48
17	Em14	GACTGCGTACGAATTCTT	46
19	Em16	GACTGCGTACGAATTGTC	48
20	Em18	CGAATCTTAGCCGGCAC	49

Random Amplified Polymorphic DNA

Fourteen Random Amplified Polymorphic DNA (RAPD) primers were selected to study the genetic diversity analysis of four races of *F. oxysporum* f. sp. *ciceri*. PCR reaction of 20 µl was prepared for each primer using 1.0 µl of template DNA, 1.0 µl of primer, 0.6 µl of dNTPs, 0.3 µl Taq DNA polymerase, 3.0 µl PCR buffer and 14.1 µl ddH2O. The RAPD amplification protocol is as follows: initial denaturation 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C (depending on the marker as given in Table 2) for 2 min, extension at 72 °C for 1 min and with a final extension at 72 °C for 8 min. The RAPD fragments were separated on a 8% polyacrylamide gel. The gel was stained with an $AgNO_3$ solution and then was photographed using the Gel Doc system.

Table 2: List of Random Amplified Polymorphic DNA (RAPD) primers used with their nucleotide sequence and annealing temperature

Sr. no.	Primer	Nucleotide sequence (5' to 3')	Annealing temperature (T_m) °C
1	K1	TGCGTGCTTG	32
2	K2	ACTTCGCCAC	32
3	K3	GGCTCATGTG	32
4	K4	CAAACGTGGG	32
5	K5	CGAGGTCGACG	38
6	K6	CACCGCCCCAA	38
7	K7	GTCCTCAGTCCC	40
8	OPX 10	CCCTAGACTG	32
9	OPX 13	ACGGGAGCAA	32
10	P2	TACGGCTGGC	34
11	P8	CAGGCCCTTC	34
12	P17	TACGGCTGGC	34
13	P19	GCGGCATTGT	32
14	P21	CCAGACAAGC	32

Data analysis

Diversity and relatedness among four races of *F. oxysporum* f. sp. *ciceri* were estimated as means of scorable DNA bands by using the Jaccard's similarity coefficient. Binary (0/1) matrixes were prepared by using the DNA banding pattern amplifed from diferent SRAP and RAPD markers. Each band was assumed as a character and was scored as 1 and 0 representing the presence and absence of amplification, respectively. Reliable, reproducible bands were scored in very identical positions every time. The similarity coefcient was calculated from a subprogram of numerical taxonomy system version 2.2 (NTSYS-pc version 2.2; Rohlf 1998) [16]. The dendrogram was constructed by an unweighted pair group method with arithmetic averages (UPGMA) within the sequential agglomerative hierarchical nested (SAHN) analysis module of NTSYS-pc.

Results and Discussion

Molecular identification as *F. oxysporum* f. sp. *ciceri*

The ITS1/ITS4 primer pair amplified a single DNA fragment of approximately 550 bp in all races which confirmed the *F. oxysporum* f. sp. *ciceri* fungus. Similar findings were made by Kaur *et al.* (2015) who employed ITS marker to identify isolates of *F. oxysporum* f. sp. *ciceri* by amplifying bands at 550 bp. Gurjar *et al.* (2009) also used ITS primers to identify *F. oxysporum* f. sp. *ciceri* by amplification of its DNA to about 550 bp.

Analysis of SRAP markers efficiency and genetic variability

Thirty SRAP primer combinations were screened to evaluate the genetic variability among four races of *F. oxysporum* f. sp. *ciceri*. Out of thirty combinations, 25 produced a total of 437 scorable and reproducible amplicons with an average of 18.2 amplicon per primer. The amplified product size ranged between 1000 bp to 100 bp. The percent polymorphism generated by SRAP primers were 90.3 percent among all the races of *F. oxysporum* f. sp. *ciceri* (Table 3).

Table 3: Characteristics of Sequence Related Amplified Polymorphism (SRAP) primers used to assess the genetic diversity of *F. oxysporum* f. sp. *ciceri* races

Sr. no.	Primer combinations	Total no. of amplicons	Polymorphic amplicons	Monomorphic amplicons	% polymorphism
1	Me1-Em2	22	20	2	90.9%
2	Me1-Em3	14	12	2	85.7%
3	Me1-Em7	21	17	4	80.9%
4	Me1-Em5	17	17	0	100%
5	Me1-Em11	20	18	2	90%
6	Me1-Em12	18	15	0	83.3%
7	Me1-Em13	17	17	0	100%
8	Me1-Em16	--	--	--	--
9	Me2-Em1	16	12	3	75%
10	Me2-Em3	15	12	3	80%
11	Me3-Em5	16	14	2	87.5%
12	Me3-Em11	23	23	0	100%
13	Me3-Em7	10	10	0	100%
14	Me2-Em12	19	17	2	89.4%
15	Me2-Em5	--	--	--	--
16	Me3-Em16	22	21	1	95.4%
17	Me4-Em1	17	15	2	88.2%
18	Me4-Em2	19	17	2	89.4%
19	Me4-Em14	14	14	0	100%
20	Me4-Em16	22	20	2	90.9%
21	Me4-Em18	21	21	0	100%
22	Me4-Em11	--	--	--	--
23	Me5-Em5	15	15	0	100%
24	Me5-Em3	18	17	1	94.4%
25	Me6-Em5	18	13	5	72.2%

26	Me9-Em13	19	16	3	84.2%
27	Me14-Em5	19	18	1	94.7%
28	Me14-Em11	15	14	1	93.3%
29	Me14-Em3	--	--	--	--
30	Me3-Em6	--	--	--	--
Total bands		437	395	38	---
Average		18.2	16.4	1.6	90.3%

All thirty SRAP primer combinatons together produced a total of 437 scorable amplicons and showed 90.3% polymorphism. Maximum amplicons were generated by Me3-Em11, whereas less amplicons were produced by Me3-Em7 primer combination. Me1-Em16, Me2-Em5, Me4-Em11, Me3-Em6 and Me14-Em3 produced no amplicons in PCR reaction. Me1-Em2 produced a total of 22 amplicons in range 900 to 100 bp, out of which two were monomorphic. The primer showed 90.9% polymorphism. Me1-Em7 produced 80.9% polymorphism with 21 amplicons, whose band size range from 820 to 100 bp. Total 17 amplicons were generated by Me1-Em5 with 100% polymorphism ranging from 900 to 100 bp. The size of 20 amplified products by Me1-Em11 ranged from 900 to 100 bp out of which 90% were polymorphic. While Me1-Em12 amplified races into 18 amplicons with 83.3% polymorphism ranging from 900 to 100 bp.

Me1-Em13 produced a total of 17 amplicons in range 800 to 120 bp, out of which none were monomorphic. The primer showed 100% polymorphism. Me4-Em1 produced 88.2% polymorphism with 17 amplicons, whose band size range from 900 to 120 bp. Total 19 amplicons were generated by Me4-Em2 with 89.4% polymorphism ranging from 900 to 120 bp. The size of 22 amplified products by Me4-Em16 ranged from 800 to 100 bp out of which 90.9% were polymorphic. While Me4-Em18 amplified races into 21 amplicons with 100% polymorphism ranging from 900 to 100 bp. The size of 14 amplified products by Me4-Em14 ranged from 800 to 100 bp out of which 100% were polymorphic. Me3-Em7 produced 100% polymorphism with 10 amplicons, whose band size range from 800 to 100 bp. Me9-Em13 produced a total of 19 amplicons in range 800 to 100 bp, out of which three were monomorphic. The primer showed 84.2% polymorphism. The size of 15 amplified products by Me2-Em3 ranged from 800 to 170 bp out of which 80% were polymorphic. While Me14-Em5 amplified races into 19 amplicons with 94.7% polymorphism ranging from 800 to 100 bp. Me14-Em11 produced 93.3% polymorphism with 15 amplicons, whose band size range from 700 to 120 bp.

Total 19 amplicons were generated by Me2-Em12 with 89.4% polymorphism ranging from 900 to 100 bp. The size of 16 amplified products by Me3-Em5 ranged from 900 to 100 bp out of which 87.5% were polymorphic. While Me3-Em11 amplified races into 23 amplicons with 100% polymorphism ranging from 1000 to 180 bp. Me1-Em3 produced 85.7% polymorphism with 14 amplicons, whose band size range from 600 to 160 bp. Total 18 amplicons were generated by Me6-Em5 with 72.2% polymorphism ranging from 700 to 130 bp. Total 22 amplicons were generated by Me3-Em16 with 95.4% polymorphism ranging from 1000 to 110 bp. The size of 15 amplified products by Me5-Em5 ranged from 600 to 120 bp out of which 100% were polymorphic. While Me5-Em3 amplified races into 18 amplicons with 94.4% polymorphism ranging from 800 to 110 bp. Me2-Em1 produced 75% polymorphism with 16 amplicons, whose band size range from 800 to 160 bp.

Binary similarity matrix and dendrogram from SRAP analysis

The similarity matrix was drawn by using software Ntsys 2.0 on the basis of scoring of bands of all the SRAP primers. The genetic similarity coefficient value based on SRAP markers ranged from 0.45 to 0.52 (Table 4). Lowest coefficient of similarity (0.45) was found between Foc1 (Akola) and Foc2 (Kanpur) and highest (0.52) was between Foc2 (Kanpur) and Foc4 (Jabalpur). The UPGMA based cluster analysis using dice similarity coefficient grouped four races into two major clusters. Cluster A distinct from cluster B with 47 percent similarity. Cluster A generated from UPGMA analysis grouped Foc1 (Akola) alone. Cluster B is further grouped in to two subclusters B_1 and B_2. The Subcluster B_1 consists of Foc2 (Kanpur) and Foc4 (Jabalpur) with 51 percent similarity. Subcluster B_2 grouped Foc3 (Gurudaspur) alone. Subcluster B_1 distinct from B_2 with 49.6 percent similarity (Figure 1).

Table 4: Similarity matrix of Sequence Related Amplified Polymorphism (SRAP) analysis against four races of *F. oxysporum* f. sp. *ciceri*

	Foc1	**Foc2**	**Foc3**	**Foc4**
Foc1	1.00			
Foc2	0.45	1.00		
Foc3	0.48	0.49	1.00	
Foc4	0.46	0.52	0.51	1.00

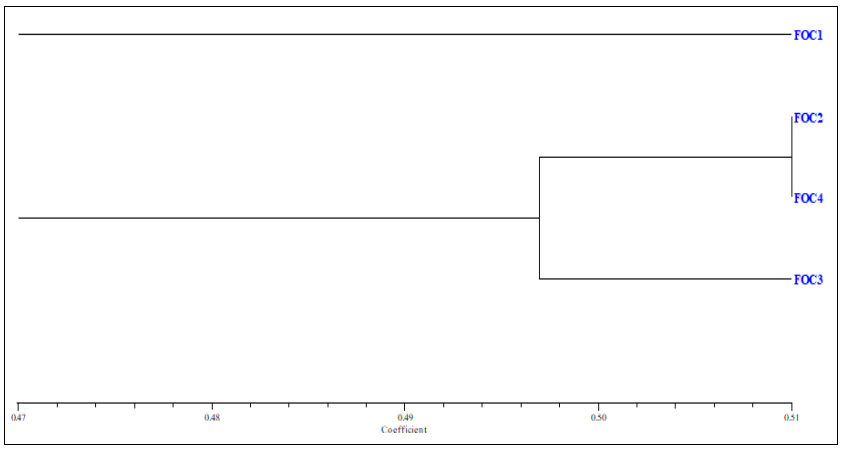

Fig 1: Dendrogram showing genetic similarity between races of *F. oxysporum* f. sp. *ciceri* based on RAPD marker analysis

Machenahalli *et al*. (2021) [9] studied cross infection and molecular characterization of *Colletotrichum* spp. infecting coffee and black pepper using 20 SRAP markers. The UPGMA dendrogram and principal coordinate analysis divided the *Colletotrichum* species into two clusters and placed them in two different coordinates. Based on the Jacquard coefficient index, the similarity between the two strains was 43.0% using SRAP markers. The study showed that the two *Colletotrichum* strains infecting coffee and black pepper are genetically distinct. Dubey *et al*. (2022) [3] studied molecular diversity analysis of 35 isolates of *F. oxysporum* f. sp. *lentis* causing lentil wilt using four different markers and reported that 100% polymorphism was exhibited by SRAP markers. UPGMA analysis clustered the isolates into seven clusters with genetic similarities ranging from 21 to 80%.

Rajput *et al*. (2020) [15] studied diversity assessment of *Diaporthe* species associated with various crops SRAP markers. Random amplifcation of total genomic DNA of 12 isolates of *Diaporthe* spp. with 10 pairs of SRAP primers generated a total of 293 bands ranging from 0.08 to 3.4 kb. The maximum number of the bands (40) was obtained from SRAP primer combination Me2-Em12 followed by Me1- Em2, whereas the maximum PIC value (0.33) was shown by Me1-Em10. Mahmoud *et al*. (2020) [10] studied the applicability of ISSR, SRAP, and SSR as genetic markers to characterize the Fusarium wilt genetic diversity of faba bean and found that, SRAP markers showed higher percentage of polymorphic bands (82.53%) than the ISSR and SSR markers (62.24% and 76.85%, respectively).

Analysis of RAPD markers efficiency and genetic variability

Fourteen RAPD primers were screened to evaluate the genetic variability among four races of *F. oxysporum* f. sp. *ciceri*. The size of amplicons generated from the PCR reaction was compared with 100bp ladder. Fourteen RAPD primers produced a total of 238 scorable and reproducible amplicons with an average of 17 amplicon per primer. The amplified product size ranged between 1000 bp to 100 bp. The percent polymorphism generated by RAPD primers were 94.5 percent among all the races of *F. oxysporum* f. sp. *ciceri* (Table 1).

Table 5: Characteristics of Random Amplified Polymorphic DNA (RAPD) primers used to assess the genetic diversity of *F. oxysporum* f. sp. *ciceri* races

Sr. no.	Primer	Total no. of amplicons	Polymorphic amplicons	Monomorphic amplicons	% polymorphism
1	K1	17	16	1	94.1%
2	K2	13	12	1	92.3%
3	K3	14	11	3	78.5%
4	K4	16	16	0	100%
5	K5	15	14	1	93.3%
6	K6	14	14	0	100%
7	K7	18	17	1	94.4%
8	OPX 10	14	14	0	100%
9	OPX 13	20	18	2	90%
10	P2	19	19	0	100%
11	P8	21	19	2	90.4%
12	P17	19	18	1	94.7%
13	P19	19	18	1	94.7%
14	P21	19	19	0	100%
Total bands		238	225	13	
Average		17	16.07	0.9	94.5%

All fourteen RAPD primers together produced a total of 238 scorable amplicons and showed 94.5% polymorphism. Maximum amplicons were generated by P8, whereas less amplicons were produced by K2. K5 produced a total of 15 amplicons in range 600 to 180 bp, out of which one was monomorphic. The primer showed 93.3% polymorphism. K4 produced 100% polymorphism with 16 amplicons, whose band size range from 600 to 220 bp. Total 17 amplicons were generated by K1 with 94.1% polymorphism ranging from 900 to 160 bp. The size of 13 amplified products by K2 ranged from 900 to 270 bp out of which 92.3% were polymorphic. While K3 amplified races into 14 amplicons with 78.5% polymorphism ranging from 900 to 250 bp.

K6 produced a total of 14 amplicons in range 1000 to 230 bp with 100% polymorphism. OPX 13 produced 90% polymorphism with 20 amplicons, whose band size range from 1000 to 150 bp. Total 18 amplicons were generated by K7 with 94.4% polymorphism ranging from 800 to 160 bp. The size of 19 amplified products by P2 ranged from 800 to 200 bp with 100% polymorphic amplicons. While P8 amplified races into 21 amplicons with 90.4% polymorphism ranging from 900 to 140 bp. The size of 19 amplified products by P17 ranged from 800 to 140 bp out of which 94.7% were polymorphic. P19 produced a total of 19 amplicons in range 700 to 100 bp with 94.7% polymorphism. P21 produced 100% polymorphism with 19 amplicons, whose band size range from 840 to 160 bp. Total 14 amplicons were generated by OPX 10 with 100% polymorphism ranging from 900 to 180 bp.

Binary similarity matrix and dendrogram from RAPD analysis

The similarity matrix was drawn by using software Ntsys 2.0 on the basis of scoring of bands of all the RAPD primers. The genetic similarity coefficient value based on RAPD primers ranged from 0.38 to 0.61 (Table 6). Lowest coefficient of similarity (0.38) was found between Foc2 (Kanpur) and Foc4 (Jabalpur) and highest (0.61) was between Foc1 (Akola) and Foc2 (Kanpur). The UPGMA based cluster analysis using dice similarity coefficient grouped four races into two major clusters. Cluster A distinct from cluster B with 43 percent similarity. Cluster A grouped Foc1 (Akola) and Foc2 (Kanpur) with 61 percent similarity. Cluster B grouped Foc3 (Gurudaspur) and Foc4 (Jabalpur) with 51 percent similarity (Figure 2).

Table 6: Similarity matrix of Random Amplified Polymorphic DNA (RAPD) analysis against four races of *F. oxysporum* f. sp. *ciceri*

	Foc1	Foc2	Foc3	Foc4
Foc1	1.00			
Foc2	0.61	1.00		
Foc3	0.48	0.39	1.00	
Foc4	0.44	0.38	0.51	1.00

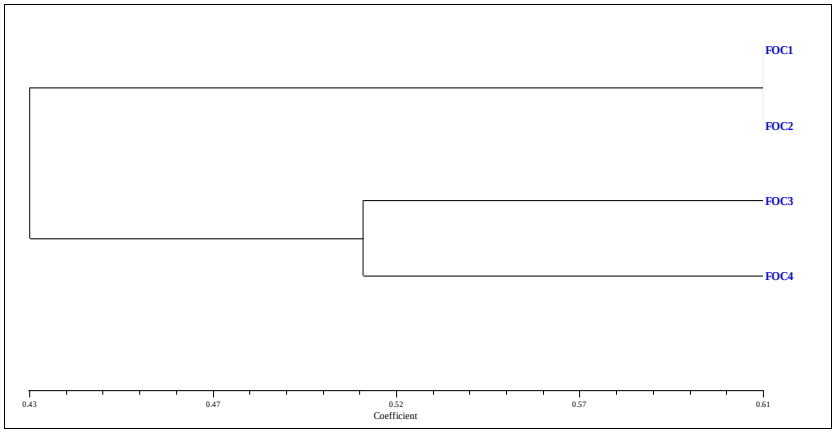

Fig 2: Dendrogram showing genetic similarity between races of *F. oxysporum* f. sp. *ciceri* based on RAPD marker analysis

Singha *et al*. (2016) [17] characterized fusarium wilt of tomato using 40 RAPD primers and out of these, 15 primers that gave reproducible results were selected to observe the relatedness among these isolates. The phylogenetic tree was constructed from the RAPD images consisting of 3 clusters. Jaccard's similarity coefficient was calculated using the RAPD data and it showed the interrelatedness among Fusarium isolates. Thaware *et al*. (2017) [19] also analysed molecular variability of *F. oxysporum* f. sp. *ciceri* isolates by RAPD method. Genetic diversity was analyzed based on data obtained by 10 RAPD primers. The primers have shown polymorphism in the range of 91.66% to 100%. The primers OPA-1, OPA-3, OPA-4, OPA-5, OPA-8 and OPA-9 were showed maximum percent polymorphism (100%). rest of primers were showed percent polymorphism in the range upto 95%. All primers had amplified total number of 144 bands among which 140 were found polymorphic and 4 were found to be monomorphic.

Venkataramanamma *et al*. (2019) [20] worked on thirty two isolates of *F. oxysporum* f. sp. *ciceri* and all the isolates exhibited genetic variation in RAPD banding pattern and three major clusters (I, II and III) were found in the dendrogram. Out of twenty primers, sixteen amplified and gave distinct banding pattern in RAPD analysis. Number of bands obtained was specific to each primer and ranged from 1 to 17. All the 16 primers gave a total of 1687 bands, among which 1458 (86.42%) were polymorphic.

Comparative analysis of SRAP and RAPD profile

Table 7: Similarity matrix of combined Sequence Related Amplified Polymorphism (SRAP) and Random Amplified Polymorphic DNA (RAPD) analysis against four races of *F. oxysporum* f. sp. *ciceri*

	Foc1	Foc2	Foc3	Foc4
Foc1	1.00			
Foc2	0.65	1.00		
Foc3	0.49	0.43	1.00	
Foc4	0.46	0.46	0.59	1.00

The similarity matrix was drawn by using software NTSYS 2.0 on the basis of scoring of bands of all the SRAP and RAPD primers. The genetic similarity coefficient value based on primers ranged from 0.43 to 0.65 (Table 7). Lowest coefficient of similarity (0.43) was found between Foc2 (Kanpur) and Foc3 (Gurudaspur) and highest (0.65) was between Foc1 (Akola) and Foc2 (Kanpur). The UPGMA based cluster analysis using dice similarity coefficient grouped four races into two major clusters. Cluster A distinct from cluster B with 45 percent similarity. Cluster A grouped Foc1 (Akola) and Foc2 (Kanpur) with 65 percent similarity. Cluster B grouped Foc3 (Gurudaspur) and Foc4 (Jabalpur) with 59 percent similarity (Figure 3).

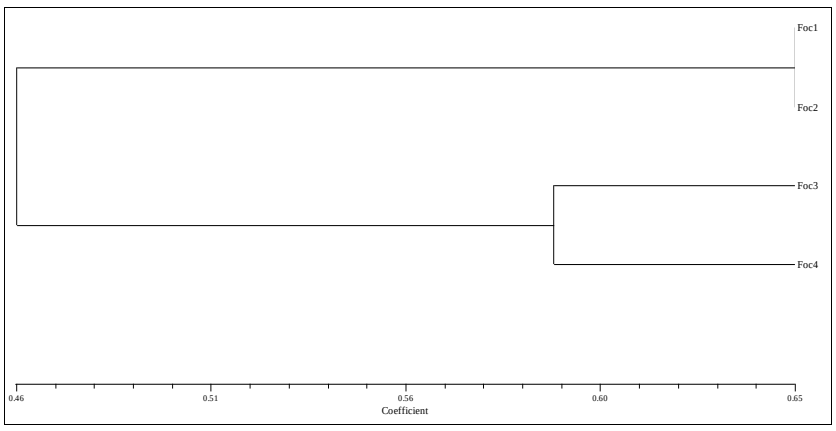

Fig 3: Dendrogram showing genetic similarity between races of *F. oxysporum* f. sp. *ciceri* based on combined SRAP and RAPD marker analysis

Variability of races was clearly distinguished by SRAP and RAPD analysis. All the races expressed a remarkable level of polymorphism between each other. In SRAP analysis Me1-Em5, Me1-Em13, Me3-Em11, Me3-Em7, Me4-Em14, Me4-Em18 and Me5-Em5 differentiated all the races with 100% polymorphism. Among RAPD primers, K4, K7, P2 and P21 showed 100% polymorphism between the races. Comparative analysis of SRAP and SCoT revealed 65 percent similarity among Foc1 (Akola) and Foc2 (Kanpur) and 59 percent similarity among Foc3 (Gurudaspur) and Foc4 (Jabalpur).

Declarations

Availability of data and materials: Not applicable

Competing interests: The authors declare that there is no competing interests

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