International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(6): 383-390
www.biochemjournal.com
Received: 21-04-2024
Accepted: 29-05-2024

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Genetic diversity assessment of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt as revealed by the URP and SRAP marker

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DOI: https://doi.org/10.33545/26174693.2024.v8.i6e.1340

Abstract
Chickpea (*Cicer arietinum* L.), one of the key pulse crop, grown extensively in India as a rainfed and irrigated crop. Wilt is a devastating disease in chickpea production among several biotic constraints. A investigation of the geographic distribution and racial complexity of isolates of *Fusarium oxysporum* f. sp. *ciceri* (Foc) representing various agro-climatic zones of India was carried out at molecular level. The potential of these molecular markers to assess the genetic variability of *Fusarium oxysporum* f. sp. *ciceri* was examined using DNA-based Universal Rice Primers (URP) and Sequence Related Amplified Polymorphism (SRAP) markers. The present study provided new information on these pathogens based on their races and their dominant existence in this region that has not been reported before. The isolates were categorised into six categories using the Unweighted paired group method with arithmetic average analysis (UPGMA). The genetic similarities coefficient values from the combined data of the URP primer and SRAP primer ranged from 0.4944 to 0.8782 across 40 isolates of *F. oxysporum* f. sp. *ciceri*. There is heterogeneity in the majority of Indian populations representing several races of the pathogen, and these isolates are divided into different clusters individually. The present racial profiling of the Indian population of pathogen and its distribution pattern is completely novel. The present race profiling for the Indian population of the pathogen and its distribution pattern is entirely new. The knowledge generated in this study could be utilized in resistance breeding programme.

Keywords: Chickpea, Race, *Fusarium* wilt, *Fusarium oxysporum* f. sp. *ciceri*, genetic diversity, URP primer, SRAP primer

Introduction
Chickpea (*Cicer arietinum* L.) is one of the significant pulse crops grown in Maharashtra and throughout India. Low yield of chickpea is ascribed to its vulnerability to several fungal, bacterial and viral diseases (Dubey and Singh 2008) [6], according to reports, there are 52 different pathogens that attack the crop. Among the diseases, the wilt is an important reason for major significant reduction in chickpea productivity. Different pathogens can cause chickpea wilt, but the most prevalent one is *Fusarium oxysporum* f. sp. *ciceri*. Other pathogens that can cause the disease include *Rhizoctonia bataticola*, *Fusarium solani*, *Botrytis cinerea*, etc. Pathogen attack the plant at two main stages i.e. first at seedling stage 20 -25 days after sowing (Sept-Oct) and second at reproductive stage (Feb-Mar). Dry and warm climate is flourish for the spreading of wilt (Pande *et al*., 2005) [18]. Pathogen attack the roots of plant and penetrate in the vascular tissues, and blocking the vascular tissues, leading to reduction or interruption of water flow to the aerial parts of the plant. As the result of wilt, yellowing and drooping of leaves, observed in the infected plant as the disease progress, ultimately plant dies after showing these symptoms. Globally, Fusarium wilt causes large economic losses that can vary from 10 to 40 percent (Nene *et al*., 1987) [17]. In favourable climatic conditions, it results in yield losses ranging from 10 to 100 percent (Jendoubi *et al*., 2017) [13]. *F. oxysporum* f. sp. *ciceri* is seed and soil borne pathogen. Eight races of the pathogen were identified based on their responses to various chickpea cultivars: races 0, 1A, 1B/C, 2, 3, 4, and 6 (Jimenez-Diaz *et al*., 1993) [12].
Out of the eight races, four-races 1A, 2, 3, and 4 have been reported from India. The emergence of new races of *F. oxysporum* f. sp. *ciceri* poses a significant concern since it diminishes the ability of crop to exploit wilt resistance in a given area.

The continuous monitoring of variation in new isolates obtained from different varieties or genotypes and geographical areas is essential to the effectiveness of the Fusarium wilt resistance breeding programme.

Analysis have been made to the detection and genetic characterisation of phytopathogenic fungus, including race differentiation in formae speciales of *F. oxysporum*, using Universal Rice Primer (URP) and Sequence Related Amplified Polymorphism (SRAP) Primer. However, 40 isolates of *F. oxysporum* f. sp. *ciceri* representing races were taken for the current investigation from almost all the major chickpea-growing states of India. The disease is extremely difficult to manage by using fungicides and crop rotation because of its survival ability and mode of damage. The best and most effective strategy to mitigate disease-related losses is to cultivate resistant cultivars. (Nene and Reddy, 1987; Bakhsh *et al*., 2007) [1, 17].

There are various mean for identifying the pathogen strains. However, molecular based approach is the most accurate and easiest way to identify and characterize the genetic variation within the pathogens. These approaches include PCR based URP and SRAP Primer.

In order to ascertain the prevalence of distinct races, examine genotyping of the isolates using these approaches is helpful in identification of microbial species and isolates as well as for generating information that is useful for the disease-resistance breeding programme. In this context, the present study was conducted to assess the genetic variability in the pathogens of wilt complex that were collected from chickpea growing areas of India.

Materials and Methods

Collection and isolation of wilted samples

Wilted samples were gathered from different geographical locations of India. Fungus was isolated on two percent agar media. Potato Dextrose Agar (PDA) Media was used to purify the fungus. The isolated fungus was maintained on PDA slants in pure culture using the single spore isolation method. This pure culture was identified as *Fusarium oxysporum* f. sp. *ciceri* based on morphological features that described and reported by Booth (1977) [13]. For the extraction of DNA, fungal isolates were grow on Potato Dextrose Broth (PDB) to get mycelium (Farooq *et al*. 2005) [18]. Using the ITS-1 and ITS-4 primers, isolates of *F. oxysporum* f. sp. *ciceri* were identified at the molecular level (White *et al*. 1990) [25].

Table 1: List of isolates of *Fusarium oxysporum* f. sp. *ciceri* and their locations selected from different agro-ecological region

Sr. No.	State	Location	Isolates	GPS Location	Agro ecological region
1	Maharashtra	Akola	Foc1	$20^0$43’07.83’’N $77^0$09’25.80’’E	Deccan plateau, hot moist semi-arid eco-region (AER 6.3)
2		Amravati	Foc2	$21^0$12’08.4’’N $77^0$27’19.9’’E	Deccan plateau, hot semi-arid eco-region (AER 6.3)
3		Nagpur	Foc3	$21^0$10’39.6’’N $79^0$02’07.5’’E	Central highlands, hot sub humid eco-region (AER 10.2)
4		Washim	Foc4	19°58'54.4"N 76°47'15.9″E	Eastern Maharashtra plateau, hot moist semi-arid eco-region (AER 6.3)
5		Hingoli	Foc5	19°44'18.0°N 77°08'33.4"E	Deccan plateau, hot semi-arid eco-region (AER 6.2)
6		Parbhani	Foc6	19°15'02.6″N 76°47'41.9″E	Deccan plateau, hot semi-arid eco-region (AER 6.1)
7		Nashik	Foc7	20°01'35.0″N 73°49'57,3"E	Deccan plateau, for semi-arid eco-region (AER 6.1)
8		Rahuri	Foc8	19°05'42.8°N 74°45'07.7"E	Deccan plateau, hot semi-arid eco-region (AER 6.1)
9		Satara	Foc9	17°40'42.5"N 74°00'19.4"E	Deccan plateau, for semi-arid eco-region (AER 6.1)
10		Patri	Foc10	20°01'11.0″N 74°57'56.3″E	Deccan plateau, for semi-arid eco-region (AER 6.1)
11		Koda	Foc11	20°26'19.0"N 75°47'58.0″E	Deccan plateau, hot semi-arid eco-region (AER 6.2)
12		Badnapur	Foc12	19°52'27.5"N 75°43'20.4"E	Deccan plateau, hot semi-arid eco-region (AER 6.2)
13		Killa (KVK)	Foc13	18°25'19.7"N 73°10'36.1"E	Western gnat and coastal plain, hot humid per humid eco-region (AER 19.1)
14		Nanded	Foc14	19°08'18.6"N 77°19'26.7"E	Deccan plateau, hot semi-arid eco-region (AER 6.1)
15		Latur	Foc15	18°24'48.3"N 76°33'09.8"E	Deccan plateau, hot semi-arid eco-region (AER 6.2)
16		Osmanabad	Foc16	18°11'08.7"N 76°02'10.8"E	Deccan plateau, hot semi-arid eco-region (AER 6.2)
17	Telangana	Hyderabad	Foc17	17°18'25.9"N 78°24'29.6″E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
18		ICRISAT	Foc18	17°30'39.6″N 78°16'31.5" E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
19	Andhra Pradesh	Anantapur	Foc19	14°42'04.1"N 77°35'59.1"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
20	Tamil Nadu	Rameshwar	Foc20	17°33'23.3″N 78°16'48.7"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
21	Karnataka	Bijapur	Foc21	16°49'31.0"N	Deccan plateau, hot semi-arid eco-region (AER 6.4)

				75°43'26.0″E	
22	Chhattisgarh	IGKV, Raipur	Foc22	21°13'57.54 N 81°43'07.37"E	Chhattisgarh/ Mahanadi basin Agro-eco-region (AER 11.0)
23	Gujarat	NAU, Navsari	Foc23	20°56'52.1"N 72°57'07.6"E	Central (Malwa) highlands, Gujarat Plains and Kathiawar Peninsula Eco region (AER 5.2)
24	Rajasthan	MPUAT, Udaipur	Foc24	24°34'57.05N 73°42'12.73 E	Northern Plain (and central highland) including Aravallis, hot semi-arid ecoregion (AER 4.2)
25	Haryana	Gurdaspur	Foc25	32°02'54.4"N 75°25'53.7"E	Western Plain, Kachchh and part of Kathiawar Peninsula, hot arid ecoregion (AER 2.3)
26	Uttar Pradesh	IIPR, Kanpur	Foc26	26°29'38.4"N 80°16'19.5″E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
27		Allahabad	Foc27	25°17'25.2"N 81°48'37.5″E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
28		Varanasi	Foc28	25°16'26.0″N 82°59'31.6″E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
29		Mirzapur	Foc29	25°07'57.6″N 82°33'53.9"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
30	Haryana	CCS HAU, Hisar	Foc30	29°08‘22.19″N 75°42'53.88"E	Western Plain, Kachchh and part of Kathiawar Peninsula, hot arid ecoregion (AER 2.3)
31	Uttar Pradesh	Abohar7676	Foc31	30°08'06.3″N 74°12'43.6″E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
32	Haryana	Sikohpur7677	Foc32	28°23'42.6"N 76°59'21.1"E	Western Plain, Kachchh and part of Kathiawar Peninsula, hot arid ecoregion (AER 2.3)
33		Ludhiana7679	Foc33	30°54'00.64”N 75°48'00.57"E	Western Plain, Kachchh and part of Kathiawar Peninsula, hot arid ecoregion (AER 2.3)
34	Rajasthan	Jaitsar 7683	Foc34	26°34'08.5″N 72°01'06.7″E	Northern Plain (and central highland) including Aravallis, hot semi-arid ecoregion (AER 4.2)
35	Gujarat	Junagarh7686	Foc35	21°29'59.2"N 70°27'01.1"E	Central (Malwa) highlands, Gujarat Plains and Kathiawar Peninsula Ecoregion (AER 5.2)
36	Uttar Pradesh	Dholi7687	Foc36	25°32'59.6"N 82°01'00.5″E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
37	Andhra Pradesh	Guntur7689	Foc37	16°18'24.8"N 80°26'09.0"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
38	Karnataka	Raichur7690	Foc38	16°11'55.4"N 77°19'48.1"E	Deccan plateau, hot semi-arid eco-region (AER 6.4)
39	Madhya Pradesh	Jabalpur7692	Foc39	23°12′27.6″N 79°57'26.0"E	Central Highlands, Hot subhumid (dry) ecoregion (AER 10.1)
40		Rewa7693	Foc40	24°32'11.8"N 81°18'14.6″E	Central Highlands, Hot subhumid (dry) ecoregion (AER 10.1)

Extraction of genomic DNA

According to Murray and Thompson (1980), DNA was isolated from *F. oxysporum* f. sp. *ciceri* isolates using the cetyl trimethyl ammonium bromide (CTAB) technique. Seven days old mycelial mat of 40 isolates of *F. oxysporum* f. sp. *ciceri* was air dried on blotter paper to remove moisture. DNA isolation was done using this dried mycelium mat. Approximately, one gram of air-dried fungal mat was frozen in liquid nitrogen (-196 °C) and crushed into powder form with the help of sterilized mortar and pestle. The powder was transferred to 2 ml Eppendorf tube and extraction buffer that had been heated (65 °C) was quickly added to homogenise it. The contents of the tube were shaken for one min and the tubes were incubated for one hour at 65 °C in a water bath, with gentle shaking performed every 15 min. The tubes were taken out of the hot water bath and centrifuged at 12,000 at room temperature rpm for 20 min.

The upper aqueous phase was poured into a new 2 ml Eppendorf tube, and to denature the proteins, add equal volume of chloroform: isoamyl alcohol (24:1) and gently stirred for 5 minutes. The content of the tubes were gently mix for five min and centrifuged for 15 min at 10000 rpm. After centrifugation, the aqueous phase was transferred to a new tube, and equal volume of ice chilled isopropanol was added. After centrifuged for 10 min at 12000 rpm and supernatant was decanted. The pellet was washed with 70 percent ethanol twice and suspended in TE buffer. The DNA solution was treated with RNase at 37 ºC for one hr. The concentration and purity of DNA were measured using spectrophotometry at 260 nm and on 0.8% agarose gel electrophoresis.

Genetic diversity analysis by Universal Rice Primers (URP)

Table 2: List of universal rice primers (URP) with their sequences

Sr.	List of primers	Sequence	Annealing temperature (°C)
1	URP 1F	ATCCAAGGTCCGAGACAACC	54
2	URP 2F	GTGTGCGATCAGTTGCTGGG	56
3	URP 2R	CCCAGCAACTGATCGCACAC	56
4	URP 4R	GGCAAGCTGGTGGGAGGTAC	54
5	URP 6R	GGCAAGCTGGTGGGAGGTAC	58
6	URP 9F	ATGTGTGCGATCAGTTGCTG	52
7	URP 13R	TACATCGCAAGTGACACAGG	52
8	URP 17R	AATGTGGGCAAGCTGGTGGT	54
9	URP 25R	GATGTGTTCTTGGAGCCTGT	52
10	URP 30F	GGACAAGAAGAGGATGTGGA	52
11	URP 32F	TACACGTCTCGATCTACAGG	52
12	URP 38F	AAGAGGCATTCTACCACCAC	50

*Annealing temperature varied from primer to primer

Analysis of genetic diversity among 40 isolates of *F. oxysporum* f. sp. *ciceri*, 12 URP primers were selected. The 20 µl of PCR mixture was used for URP primers. It included 50 ng template DNA 1 µl, 3 µl 10X PCR buffer with $MgCl_2$ 25 mM, 0.5 µl 10 mM dNTP, 1 µl 10pM primer, Taq polymerase (5U/µl) 0.3 µl) nuclease free water 14.2 µl.

Table 3: Steps used for PCR-URP Primer

Name of step		Temperature	Time
Initial Denaturation		94 °C	5 min.
35 cycles	- Denaturation	94 °C	30 sec.
	- Annealing	55 °C*	45 sec.
	- Extension	72 °C	1 min.
Final Extension		72 °C	10 min.

Resolved PCR product on 10 percent Polyacrylamide Gel Electrophoresis (PAGE). Differentiate PCR product and the size of the bands was estimated using a 100 bp DNA ladder. A dendrogram of the Unweighted Pair Group Technique of Arithmetic Mean (UPGMA) was produced based on the banding patterns of URP Primers.

Genetic diversity analysis by Sequence Related Amplified Polymorphism (SRAP)

Fourteen SRAP primer combinations were selected to study molecular variability among *F. oxysporum* f. sp. *ciceri* isolates.

Table 4: List of Sequence Related Amplified Polymorphism (SRAP) primers with their sequences

Sr. No.	List of primers	Sequence	Annealing temperature (°C)
1	ME 1	TGAGTCCAAACCGGATA	45
2	ME 2	TGAGTCCAAACCGGAGC	49
3	ME 3	TGAGTCCAAACCGGAAT	45
4	ME 5	TGAGTCCAAACCGGAAG	47
5	EM 1	GACTGCGTACGAATTAAT	43
6	EM 2	GACTGCGTACGAATTTGC	48
7	EM 3	GACTGCGTACGAATTGAC	48
8	EM 4	GACTGCGTACGAATTTGA	54
9	EM 6	GACTGCGTACGAATTAAC	54
10	EM 7	GACTGCGTACGAATTCAA	46
11	EM 16	GACTGCGTACGAATTGTC	48

*Annealing temperature varied from primer to primer

Polymerase chain reactions (PCRs) were performed using 50 ng of DNA 1 µl, 3.5 µl 10X PCR buffer with $MgCl_2$ 25 mM, 0.4 µl 10 mM dNTP, 2 µl (forward+reverse) primer 10 pM, *Taq* polymerase (5U/µl) 0.2 µl and nuclease free water 12.9 µl.

The PCR product was resolved on 10 percent Polyacrylamide Gel Electrophoresis (PAGE).

Table 5: Steps used for PCR-SRAP Primer

Name of step		Temperature	Time
Initial Denaturation		94 °C	3 min.
5 cycles	- Denaturation	94 °C	30 sec.
	- Annealing	35 °C	45 sec.
	- Extension	72 °C	1 min.
35 cycles	- Denaturation	94 °C	30 sec.
	- Annealing	50 °C*	45 sec.
	- Extension	72 °C	1 min.
Final Extension		72 °C	5 min.

Results and Discussion

The cultural and morphological variation is the key factor for classification of the fungus by conventional taxonomic method. Still, it might be challenging to classify isolates according to important metrics such as mycelial growth rate and colony pattern. Consequently, allele-based classification, which is currently a possibility for modern genetic classification and is mostly based on the genetic composition of the isolates, is a legitimate and significant option.

Genetic diversity analysis by Universal Rice Primers (URP)

A total of twelve distinct URP primers were used to assess the genetic diversity of the isolates of *F. oxysporum* f. sp. *ciceri*. Out of that, seven URP primers were amplified a total of 114 scorable and reproducible amplicons, off which 104 found polymorphic amplicons with an average 16 amplicon per primer. The URP primers generated 91.22 percent polymorphism among the 40 isolates of *F. oxysporum* f. sp. *ciceri*.

Maximum 21 amplicons were amplified by URP 6R primer in the range of 110 to 1300bp and least 15 amplicons were amplified by URP 2F, URP 17R, URP 30F primer in the range of 100-950bp, 110 to 1000bp, 160 to 1080bp respectively.

Table 6: Percent polymorphism observed in Universal Rice Primers (URP)

Sr.	Primer	Total amplicons	Polymorphic amplicons	Amplicon size (bp)	% Polymorphism
1	URP 2F	15	14	100-950	93.33
2	URP 4R	16	14	110-870	87.50
3	URP 6R	21	21	110-1300	100.00
4	URP 9F	16	13	120-1150	81.25
5	URP 13R	16	16	120-1100	100.00
6	URP 17R	15	13	110-1000	86.66
7	URP 30F	15	13	160-1080	86.66
Total		114	104		91.22
Average		16.28	14.85		

Montakhabi *et al*. (2018) [15] used 10 URP primers which exihibits variability of 65 Iranian isolates of *F. oxysporum* f. sp. *ciceri* which produced 88 bands, among that 55 were polymorphic and 62.50 percent polymorphism were reported.

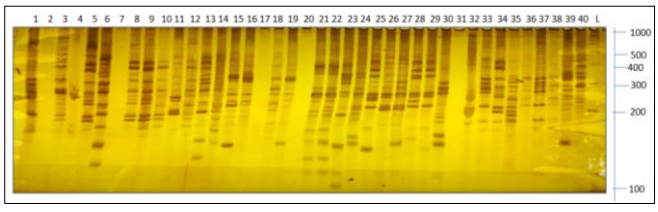

Fig 1: Banding profile of *F. oxysporum* f. sp. *ciceri* generated by URP 6R Primer

Genetic diversity analysis by Sequence Related Amplified Polymorphism (SRAP)

Initially all possible primer combinations were screened, among that 14 SRAP primer combinations were employed to evaluate the genetic diversity among 40 selected isolates of *F. oxysporum* f. sp. *ciceri*. The Polymerase Chain Reaction (PCR) amplified products of each primer were

resolved on 10 percent Polyacrylamide Gel Electrophoresis (PAGE). DNA ladder (100bp) used to compare the size of amplicons generated from the PCR reaction.

The 14 SRAP primers produced reproducible and scorable amplicons with high degree of polymorphism. The number of amplicons generated were primer and isolate dependent and ranged from 11 to 16. A total of 170 amplicons generated, out of which 157 amplicons were polymorphic with average of 12.14 amplicon per primer. The level of polymorphism was 92.35 percent. The primer ME1/EM16 and ME2/EM3 amplified maximum 16 amplicons and primer ME3/EM7, ME5/EM1, ME5/EM2, ME5/EM16, ME2/EM7, ME2/EM16 and ME2/EM2 amplified the least 11 amplicons.

Table 7: Percent polymorphism observed in Sequence Related Amplified Polymorphism (SRAP) primers

Sr.	Primer combination	Total amplicons	Polymorphic amplicons	Amplicon size (bp)	% Polymorphism
1	ME1/EM2	12	12	170-700	100.00
2	ME1/EM16	16	15	180-1000	93.75
3	ME2/EM3	16	14	70-1100	87.50
4	ME3/EM3	12	12	130-950	100.00
5	ME3/EM7	11	10	160-1100	90.90
6	ME5/EM1	11	09	110-1250	81.81
7	ME5/EM2	11	11	80-1000	100.00
8	ME5/EM16	11	09	150-780	81.81
9	ME2/EM7	11	10	110-1300	90.90
10	ME1/EM3	12	10	90-900	83.33
11	ME2/EM16	11	10	120-950	90.90
12	ME2/EM2	11	11	100-900	100.00
13	ME3/EM2	12	12	140-1400	100.00
14	ME3/EM16	13	12	90-1200	92.30
Total		170	157		92..35
Average		12.14	11.21		

The SRAP markers developed by Soren *et al*. (2016) were employed against Indian isolates of *F. oxysporum* f. sp. *ciceri*, concluded that SRAP fingerprinting of 15 primers produce on an average of 154 reproducible bands ranging from 100-2100bp, of which 147 bands were polymorphic and reported 97 percent polymorphism.

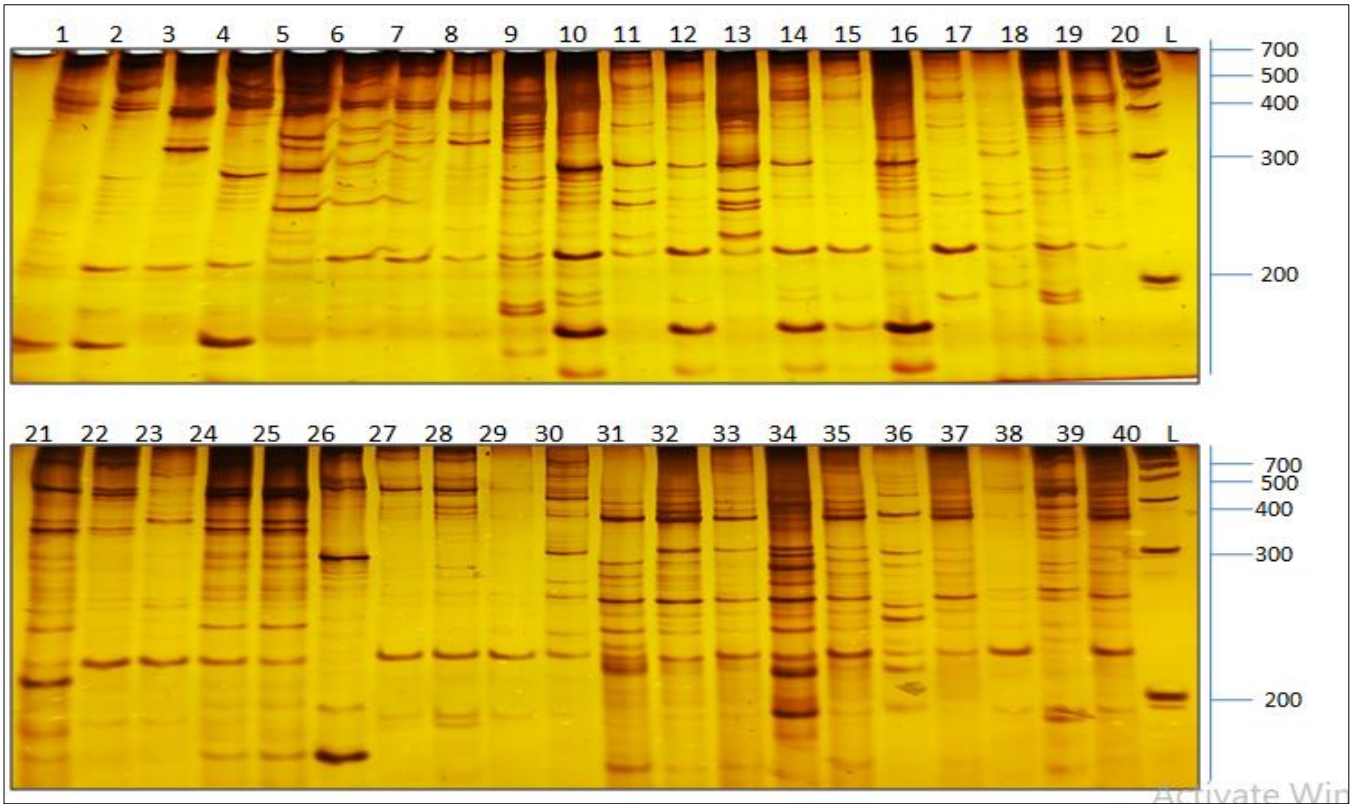

Fig 2: Banding profile of *F. oxysporum* f. sp. *ciceri* generated by SRAP ME5/EM16 Primer

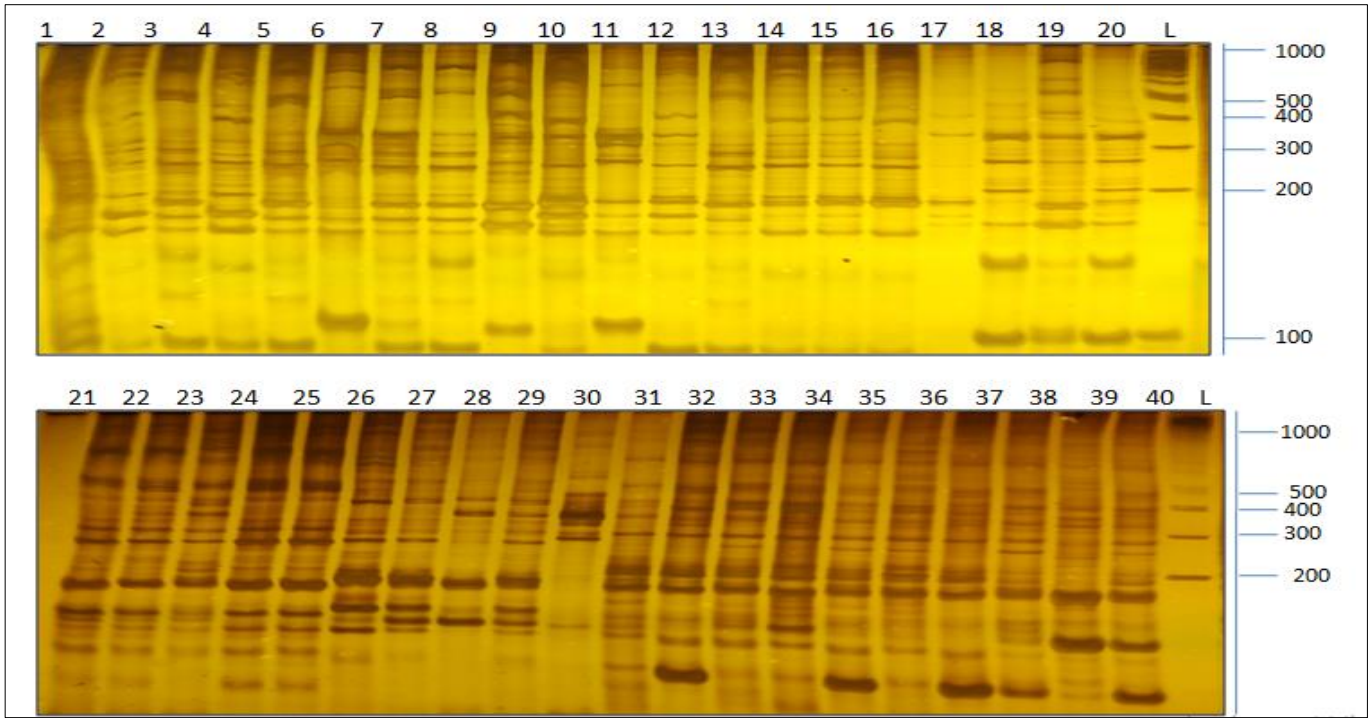

Fig 3: Banding profile of *F. oxysporum* f. sp. *ciceri* generated by SRAP ME2/EM7 Primer

Binary similarity matrix and Dendrogram

A binary similarity matrix of combined data from 21 URP and SRAP primers for the 40 *F. oxysporum* f. sp. *ciceri* isolates was prepared by scoring bands for presence or absence. DNA bands of same mobility (molecular weight) were assumed to be identical.

On the basis of calculated similarity matrix the similarity between two genotypes can be predicted. The pathotype showing similarity index of "1" are presumed to be 100 percent similar while that of "0" are 100 percent genetically dissimilar. The present study found that the similarity coefficient values across 40 isolates of *F. oxysporum* f. sp. *ciceri* ranged from 0.4944 to 0.8782, indicating high degree of variation in respect to genetic similarity, which ultimately means high range of genetic diversity among the isolates studied.

Genetic similarity estimate (Jaccard's coefficient) based on URP and SRAP banding pattern were used for cluster analysis to present genetic relationship in the form of dendrogram. The isolate Foc-10 (Aurangabad) was found to had higher value of similarity coefficient (0.8782) to Foc-12 (Badnapur) this was indicative of similarity between these two isolates, had higher similarity index as compared to other isolates. Whereas, isolate Foc-2 (Amravati) had the lower value of similarity coefficient 0.4944 to Foc-32 (Sikohpur) which indicate least similarity between them that indicate the higher diversity amongst the isolates of *F. oxysporum* f. sp. *ciceri*.

The cluster analysis of similarity index from URP and SRAP data showed highest molecular variability and distributed the 40 isolates of F. oxysporum f. sp. ciceri in six major clusters A, B, C, D, E and F, that represented in the dendrogram (Figure 4). The cluster A included 24 isolates Foc-1 (Akola), Foc-11 (Jalna, Koda), Foc-13 (Raigad), Foc-10 (Aurangabad), Foc-12 (Jalna, Badnapur), Foc-14 (Nanded), Foc-16 (Osmanabad), Foc-15 (Latur), Foc-9 (Satara), Foc-19 (Anantapur), Foc-21 (Bijapur), Foc-22 (Raipur), Foc-24 (Udaipur), Foc-23 (Navsari), Foc-25 (Gurdaspur), Foc-26 (Kanpur), Foc-39 (Jabalpur), Foc-40 (Rewa), Foc-32 (Sikohpur), Foc-37 (Guntur), Foc-33 (Ludhiana), Foc-34 (Jaitsar), Foc-35 (Junagarh) and Foc-36 (Dholi), these isolates came under race 1 except Foc-25 from Gurdaspur (race 3) and Foc-26 from Kanpur (race 2). Whereas, cluster B included five isolates Foc-3 (Nagpur), Foc-5 (Hingoli), Foc-7 (Nashik), Foc-8 (Ahmadnagar) and Foc-6 (Parbhani). In cluster C included Foc-27 (Allahabad), Foc-28 (Varanasi), Foc-29 (Mirzapur), Foc-30 (Hisar) and Foc-38 (Raichur) five isolates, among that Foc-27, Foc-28, Foc-29 were race 2 isolates came under same cluster except Foc-30 (race 4). Cluster D includes Foc-18 (ICRISAT) isolate only. Cluster E includes Foc-2 (Amravati) and Foc-4 (Washim) from Maharashtra (race 1). Cluster F includes Foc-17(Hyderabad), Foc-20 (Rameshwar) and Foc-31 (Abohar). The dendrogram is represented in Figure 4.

Dubey *et al*. (2022) [8] found genetic diversity analysis of 32 isolates of *F. oxysporum* f. sp. lentis through RAPD, URP, ISSR and SRAP markers. The URP, ISSR and SRAP markers gave 100 percent polymorphism except RAPD gave 98.9 percent polymorphism. The isolates were grouped into seven clusters with genetic similarities ranging from 21 to 80% using unweighted paired group method with arithmetic average (UPGMA) analysis. The isolates were grouped into two major clades with 28 isolates into one clade and 4 remaining isolates in another clade.

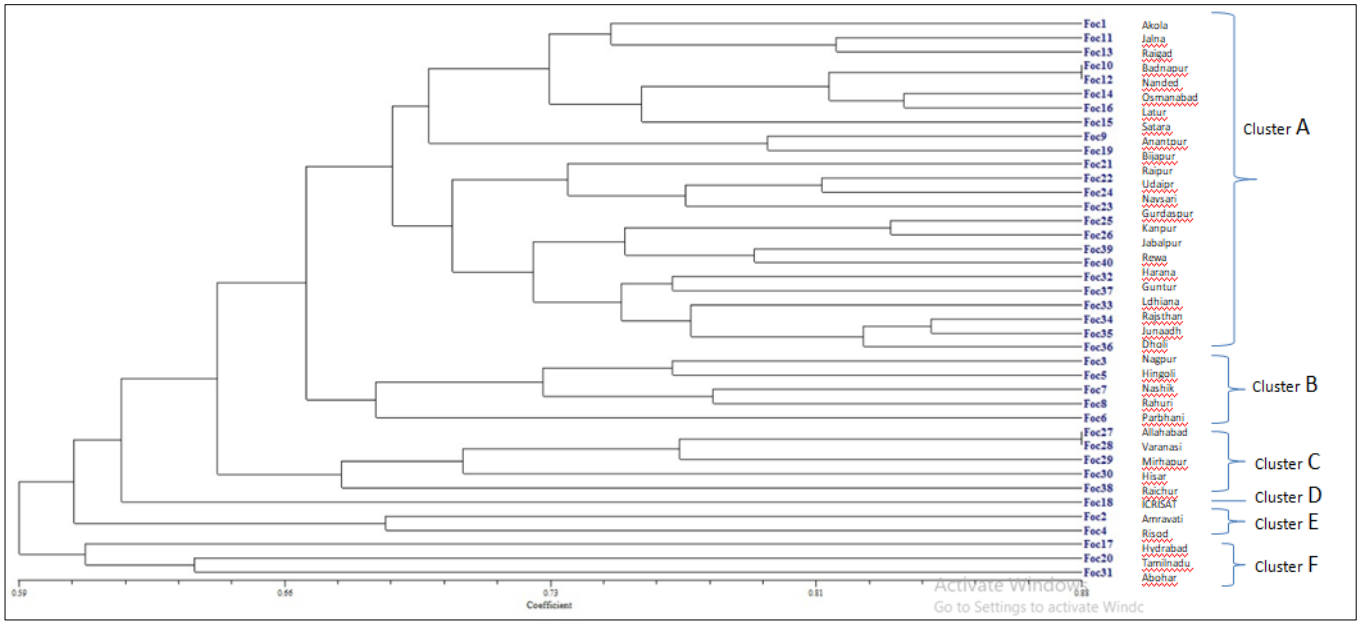

Fig 4: UPGMA dendrogram of *Fusarium oxysporum* f. sp. *ciceri* based on Universal Rice Primers (URP) and Sequence Related Amplified Polymorphism (SRAP) primers

Conclusion

The study reveals the existence of genetic variation in pathogen at species level establishing the evidence about the gradual genetic evolution of novel species alongside the existing one. High rate of this genetic change occurred in the isolates might be mediated by single gene mutation, insertion of transposable elements, or by loss of chromosomal segments and cropping system might favor evolution of large population of fungus. The reason could also be due to man-made dispersal of different genotypes during farmers to farmers seeds exchange programme.

Acknowledgments

Funding: University Grand Commission, New Delhi.

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