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Virulence analysis of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*

Kajal Jankar, SS Mane, ST Ingle, PV Jadhav and MW Marawar

Abstract
Chickpea (*Cicer arietinum* L.), one of the key pulse crop, grown extensively in India as a rainfed and irrigated crop. Wilt is a prominent issue in chickpea production among several biotic restraints. Host differential analysis of 40 isolates of *Fusarium oxysporum* f. sp. *ciceri* (Foc) causing chickpea wilt collected from major chickpea growing states of India, was tested on a 10 different cultivars of chickpea, determined that each isolate from different state varied greatly in terms of virulence. Multiple races were discovered to be prominent in every state based on responses to global differentials. The Foc isolates demonstrated substantial heterogeneity on set of 10 host differential cultivars of chickpea namely JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annegiri, Chafa, L-550, and K-850. One of the most practical and affordable solution to wilt issue, is the establish new sources of resistant cultivars. To test several germplasm lines for wilt, the current experiment was carried out in rabi 2021–2022.

Keywords: Chickpea, race, *Fusarium* wilt, *Fusarium oxysporum* f. sp. *ciceri*, soil inoculation method, virulence

Introduction
Chickpea (*Cicer arietinum* L.) is One of the significant pulse crops grown in Maharashtra and throughout India. It is grown on 9.88 million ha of land in India, with a yield of 10.73 million tonnes and a productivity of 1086 kg/ha in 2020–21. Chickpea yield losses were caused by a multiple reasons, according to reports, there are 52 different pathogens that attack the crop. *Fusarium* wilt, which is caused by *Fusarium oxysporum* f. sp. *ciceri* is main limiting factors for productivity of chickpea. *Fusarium* wilt causes significant economic losses that range from 10 to 40 percent globally. It result in yield losses between 10 to 100% under favorable climatic conditions (Jendoubi *et al*., 2017) [12]. *F. oxysporum* f. sp. *ciceri* is seed and soil borne pathogen. Eight races of the pathogen (races 0, 1A, 1B/C, 2, 3, 4, and 6) were found based on how they responded to a variety of different chickpea cultivars (Haware and Nene 1982; Jimenez-Diaz *et al*. 1993) [10, 11].
Among eight races, four races i.e., race 1A, 2, 3 and 4 are reported only from India. A serious threat in Foc is the introduction of new races, which reduces exploitation of wilt resistance in the crop in a particular region. The spread of new races is a severe danger to Foc because they limit the crop ability to exploit wilt resistance in particular region. The efficacy of the *Fusarium* wilt resistance breeding programme depends on the constant monitoring of variation in new isolates obtained from various varieties or genotypes and geographical areas. In order to ascertain the prevalence of distinct races, the current study aimed to assess the virulence of Foc isolates representing various Agro-ecological zones of India on a set of chickpea differentials.

Materials and Methods
Purification and identification
The isolated fungus was maintained on PDA slants in pure culture using the single spore isolation method. On the basis of morphological characteristics described and reported by Booth (1971 and 1977), the pure culture thus produced was recognised as *Fusarium oxysporum* f. sp. *ciceri*. According to Murray and Thompson (1980) [14], DNA was isolated from Foc isolates using the cetyl trimethyl ammonium bromide (CTAB) technique. 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) [20]. Races of *F. oxysporum* f. sp. *ciceri* are differentiated using a race-specific primer i.e FDP primer.

Race identification of isolates of *Fusarium oxysporum* f. sp. *ciceri* by Race specific primers

Table 1: List and sequence of race specific primer

Sr. No.	List of primers	Sequence
1	FDP-2 F:	CTTGGTGTTGGGATCTGTGTGCAA
	FDP-2 R:	AAT TACAACTCGGGCCCGAGA
2	FDP-9 F:	CCAGACTTCCACTGCGTGTC
	FDP-9 R:	CACGCCAGGACTGCCTCGT
3	FDP-11 F:	GGAGAGCAGGACAGCAAAGACTA
	FDP-11 R:	GGAGAGCAGCTACCCTAGATACACC
4	FDP-25 F:	ATGGGTAAGGA(A/G)GACAAGAC
	FDP-25 R:	GGA(G/A)GTACCAGT(G/C)ATCATGTT

Host differential analysis

A set of 10 distinct chickpea cultivars, including JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annegiri, Chafa, L-550, and K-850, were used to investigate the virulence of 40 representative isolates of Foc. Soil inoculation method were used to study the pathogenic variability of isolates of *F. oxysporum* f. sp. *ciceri* (Nene *et al*., 1981) [16]. Mass multiplication of the inoculum of Foc, Maize: sand meal medium (1:3% w/w) was utilised. In a 1000 ml conical flask, it was prepared by combining 50 g of corn and 150 g of dry sand with 20 ml of distilled water. The conical flask containing Maize: Sand meal medium was autoclaved at 1.05 kg/cm^2 for 30 minutes for three consecutive days, then inoculated with pure culture of each isolate of *F. oxysporum* f. sp. *ciceri* separately under aseptic condition. For two weeks, the inoculated flasks were incubated at room temperature 27±2 °C. In pot experiments, the inoculum was utilised to inoculate soil at a rate of 25 g kg-1. Five seeds sown in each small pot. Chickpea seeds were sown in pot containing un-inoculated soil serve as a control. The plants were checked periodically up to 60 days after sowing (DAS) for initiation of wilt symptoms and percent disease incidence.

Disease assessment and data analysis

Inoculated seedlings were monitored for the onset of disease symptoms and the incidence of wilt percent every after second day. Wilt incidence was determined using formula,

$$\text{Percent wilt incidence} = \frac{\text{No. of plants showing wilt symptoms}}{\text{Total number of plants}} \text{ X } 100$$

Wilt reactions were classified as R-Resistant (0-20%); MS-Moderately susceptible (21-50%); Susceptible (>50%) Haware and Nene 1982 [10]; Dubey *et al*. 2012 [4]. The pathogenicity test was verified by demonstrating Koch's Postulate, and the pathogens was identified as *F. oxysporum* f. sp *ciceri* (Padwick) Snyder and Hansen.

Table 2: List of isolates of *Fusarium oxysporum* f. sp. *ciceri* and their locations selected from different agro-ecological region for host differenetial studies

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 ghat 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	Anantpur	Foc19	14°42'04.1"N 77°35'59.1"E	Deccan plateau (Telangana) and eastern Ghats, hot semi arid ecoregion (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 75°43'26.0″E	Deccan plateau, hot semi-arid eco-region (AER 6.4)
22	Chhattisgarh	IGKV, Raipur	Foc22	21°13'57.54 N 81°43'07.37"E	Chattisgarh/ 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 Kathiwar Penisula Ecoregion (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 Kathiwar Penisula, 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 Kathiwar Penisula, 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 Kathiwar Penisula, 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 Kathiwar Penisula, 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, gujatar Plains and Kathiwar Penisula 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)

Results and Discussion

Identification of *Fusarium oxysporum* f. sp. *ciceri* by Internal Transcript Spacer region (ITS)

All isolates of *F. oxysporum* f. sp. *ciceri* amplified a single DNA fragment at 550 bp using the ITS forward (ITS-1) and reverse (ITS-4) oligonucleotide pair. Similar findings were made by Gayatri Gurjar *et al.* (2009) [7] discovered that *F. oxysporum* f. sp. *ciceri* DNA was amplified to about 550 bp using ITS primers and (Figure 1). Dubey *et al.* (2010) [3], Durai *et al.* (2012) [6], and Datta and Lal (2012) [2] suggested use of the ITS marker at the genomic level for identification.

Race identification of *Fusarium oxysporum* f. sp. *ciceri* isolates by Race specific primers

Race 1 (FDP-25)

The Fibrinogen Degradation Products (FDP-25) primer, shown polymorphic banding patterns at 700bp. Isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from various agro-ecological zones, race-1 is identified using the FDP-25 primer. Race 1 shown 700bp-720bp belongs to the region *viz*. Maharashtra, Andhra Pradesh and Karnataka respectively (Figure 2).

Race 2 (FDP-11)

FDP-11 primer used to distinguish race 2 among all isolates of Foc. In Uttar Pradesh, the isolates of *F. oxysporum* f. sp. *ciceri* from Kanpur, Allahabad, Varanasi, and Harro represent race-2 on 900–1000bp (Figure 3).

Race 3 (FDP-02)

ITS region of the ribosomal gene is the target site of FDP-02. Race 3 distinguished from all isolates of Foc on 300 bp using FDP-02 primer. Race 3 is represented by the Gurdaspur from Punjab (figure 4).

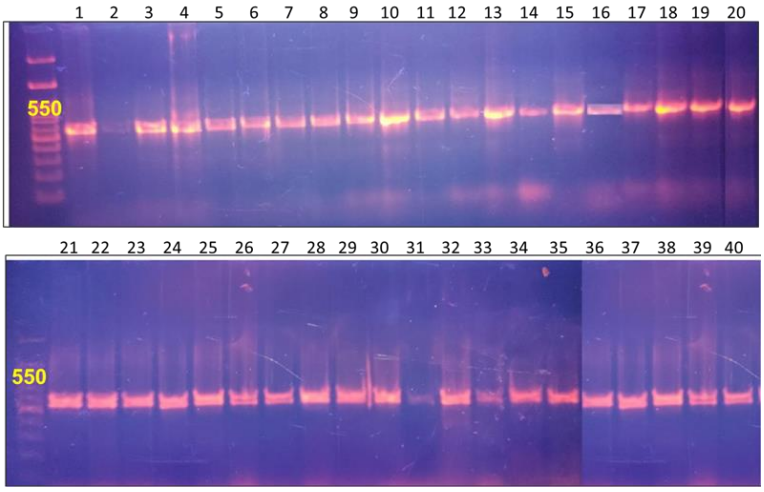

Fig 1: Identification of *F. oxysporum* f. sp. *ciceri* by using ITS primer

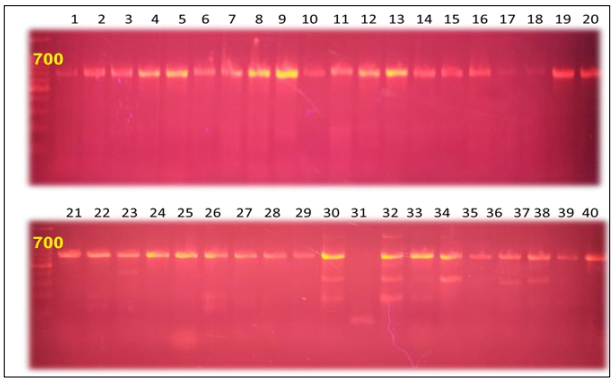

Fig 2: Molecular confirmation of race 1 from isolates of *F. oxysporum* f. sp. *ciceri* by using FDP-25

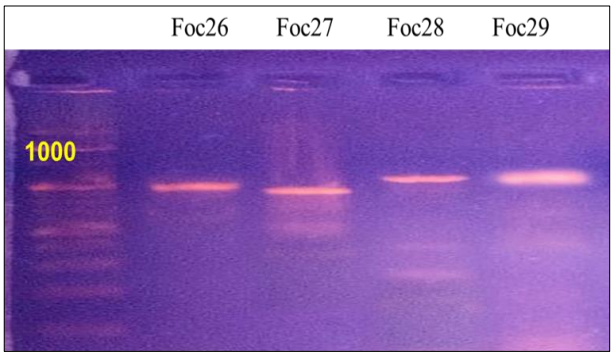

Fig 3: Molecular confirmation of race 2 from isolates of *F. oxysporum* f. sp. *ciceri* by using FDP-11

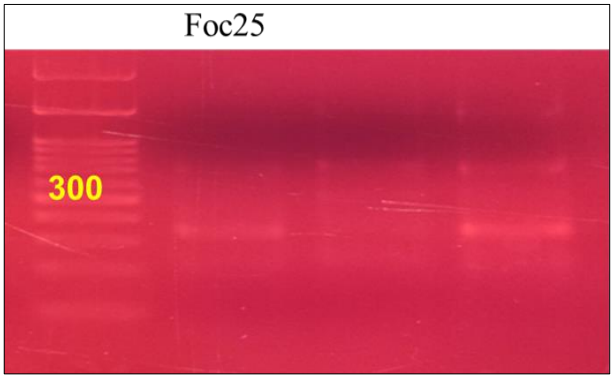

Fig 4: Molecular confirmation of race 3 from isolates of *F. oxysporum* f. sp. *ciceri* by using FDP-02

Race 4 (FDP-09)

Race 4 is identified by using FDP-09 a distinct band of 500 bp/2000bp. Race 4 is represented by Delhi (Haryana) and Jabalpur (MP) (figure 5). Similar result found by Poornima *et al*. (2017) [21] used FDP primer for racial identification of *F. oxysporum* f. sp. *ciceri.*

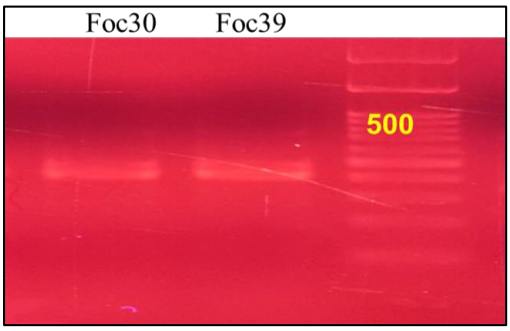

Fig 5: Molecular confirmation of race-4 from isolates of *F. oxysporum* f. sp. *ciceri* by using FDP-09

Host differential analysis

Isolates of *F. oxysporum* f. sp. *ciceri* collected from chickpea-growing regions of Indian were used in an experiment to study the host differential analysis. Pathogenic diversity of these isolates was variable in their disease reaction to chickpea. Cultivar JG-62 shows susceptible against all isolates except Foc 25 Gurdaspur (Race 3). Cultivar C-104 resistant to the Foc 25 Gurdaspur (Race 3) isolate but susceptible to the other isolates. Cultivar JG-74 resistant to all isolates except Race 2 except Race 2 isolates Foc 26 (Kanpur), Foc 29 (Mirzapur) were susceptible and Foc 27 (Allahabad), Foc 28 (Varanasi) were moderately susceptible to JG-74. CPS-1 resistant to Race 1 isolates and susceptible to Race 2 and Race 3 isolates. From Race 4, Foc 30 (Hisar) susceptible and Foc 39 (Jabalpur) moderately susceptible to CPS-1. Cultivar BG-212 resistant to Race 1 isolates except Foc-26, Foc-27, Foc-28, Foc-29 (Race 2) were susceptible. Foc-25 (Race-3) and Foc-30 and Foc-39 from Race-4 were moderately susceptible. WR-315 resistant to race 1 isolates except Foc-40 (Rewa) also shows against Race 2 and Race 4. Race 3 shows susceptible to WR-315 cultivar. Annegiri shows susceptible reaction against all isolates. Chafa shows susceptible against all isolates except Race 3 shows moderately susceptible.

Table 3: Host differential Analysis of forty isolates of *F. oxysporum* f. sp. *ciceri* on ten chickpea cultivar

Host Differentials	Isolates																																							
	Race1																																Race2				Race3	Race4	
	Foc1	Foc2	Foc3	Foc4	Foc5	Foc6	Foc7	Foc8	Foc9	Foc10	Foc11	Foc12	Foc13	Foc14	Foc15	Foc16	Foc17	Foc18	Foc19	Foc20	Foc21	Foc22	Foc23	Foc24	Foc31	Foc32	Foc33	Foc34	Foc35	Foc36	Foc37	Foc38	Foc40	Foc26	Foc27	Foc28	Foc29	Foc25	Foc30	Foc39
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	S	S	S	S	S	S	S	S	S	S	S	S	S	M	S	S
C-104	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
JG-74	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	M	M	S	R	R	R
CPS-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	M
BG-212	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	M	M	M
WR-315	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	M	R	R	R	R	S	R	R
Annegiri	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Chafa	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	S	S
L-550	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	S	S
K-850	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	M	M	M	M	M	M

Cultivar L-550 and K-850 shows susceptible reaction against all isolates except Race 3 shows moderately susceptible to L-550 and Race 2, Race 3 and Race 4 moderately susceptible to K-850.

Dubey *et al*. (2012) [4] seventy isolates of *Fusarium oxysporum* f. sp. *ciceris* (Foc) causing chickpea wilt representing 13 states and four crop cultivation zones of India were analysed for their virulence and genetic diversity on differential responses and the isolates were characterized into eight races of the pathogen on the basis of host differential as race 1 differentiate by resistance of C104 and GPF2 these two cultivar, race 2 by JG 74 and GPF 2, race 3 by JG 74 and C 104, race 4 by BG 212 and KWR 108, race 5 by WR 315 and GPF 2, race 6 by C 104 and KWR 108, race 7 by BG 212 and GPF 2 and race 8 by GPF 2 and DCP 92-3.

Conclusion

DNA-based PCR marker used for racial identification. The isolates were collected from different regions of India were classified into races. The virulence assay reveals no relationship between the virulence patterns and geographic origin of the isolate. Races identification helps to to develop race specific resistant cultivars in different region and also design effective management strategies for *Fusarium* wilt.

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