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Pathogenic variability among specific isolates of *Fusarium oxysporum* f. *sp. ciceri* causing chickpea wilt

Sujata Dandale, SS Mane, ST Ingle, PV Jadhav, AN Patil, RS Nandanwal, KB Jankar, RR Tatte and PN Kalane

Abstract
The chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is seed and soil borne disease at is considered to be as one of the major factors of low productivity. The present study was conducted to determine pathogenic variability of twenty five isolates collected from different agro ecological regions of India. In the reference to isolates corresponding to four races of chickpea pathogen. The highly wilt susceptible genotype JG-62 by pot culture (sick soil) method. The five isolates were found moderately resistant to wilt, fifteen were found highly virulent and five were strongly virulent to wilt disease in soil inoculation method. None of the isolates found non-pathogenic/avirulent in the experiment. This is an alarming situation for chickpea grower. The virulence assay does not show correlation between virulence patterns with geographical origin of isolate, mycelium growth rate, pigmentation.

Keywords: Fusarium wilt, agro-ecological regions, soil inoculation method, virulence, pathogenicity

Introduction
Chickpea (*Cicer arietinum* L.) is an important pulse crop, commonly known as Gram or *Bengal gram* belongs to family *leguminoceae* (Dhar and Gurha, 1998) [5]. The Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is seed and soil borne disease of economic important. India has diverse agro-ecosystem and varied climatic conditions. The *F. oxysporum* f. sp. *ciceri* caused significant losses in yield and are primarily responsible for huge losses in yield. The pathogen survives in soil in the form of chlamydospores upto 6 years in the absence of the host plants (Haware *et al*., 1986) [14]. Chemical control of wilt is not feasible and economical because of the soil as well as seed-borne nature of the pathogen. The most practical and cost-effective method for management of *Fusarium* wilt of chickpea is the use of resistant genotypes.

India is the largest producer of chickpea (65.49 %) in the world. In India, chickpea is grown on 10.57 million ha area with production of 11.16 million tones and productivity of 1056 kg ha-1(Anonymous 2020) [1]. The Fusarium wilt is one of the major limiting factors chickpea production (Jalali and Chand 1992) [17]. The disease is widespread in chickpea growing areas of the world and is reported from at least 33 countries (Nene *et al*. 1996) [21]. The pathogen penetrates the roots and provokes either root rots or tracheomycosis when they invade the vascular system, causing the wilt. 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) [20]. 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) [20], thereby confirming the diagnosis of fusarium wilt. The disease can be observed in a susceptible cultivar JG-62 within 25 days after sowing in fusarium wilt sick soil and this is known as 'early wilt' (Haware and Nene 1980) [12]. 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 stage 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, out of which 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) [15]. 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) [17]. However, the high pathogenic variability in the FOC may limit the effectiveness of resistance (Haware and Nene 1982) [13]. Moreover; development of resistant varieties has been hampered because of their undesirable agronomic characteristics (Honnareddy and Dubey 2006) [16].

Material and Method

Survey and sample collection

A rapid roving survey was conducted during *Rabi* seasons of 2016-17, 2017-18 at diverse geographical locations and major chickpea growing region of India (Table 2). At each location on farmer fields the plants were collected, showing the symptoms of wilting, paler, grayish green colored leaves, In both seedling and adult plant infections, inspection of roots showed a brown to black discoloration of internal vascular tissue (pith and xylem). Such wilted samples were collected and brought to laboratory for isolation.

The 90 chickpea plant showing symptoms were collected from diverse agro ecological region. The 25 isolates representing 4 races were selected for the pathogenicity study of chickpea wilt.

Purification and Identification

The isolated fungus brought in pure culture by single spore isolation technique and was maintained on PDA slants for further studies. The pure culture thus obtained was identified as *Fusarium oxysporum* f. sp. *ciceri* on the basis of morphological characters published and reported by Booth (1971 and 1977) [2, 3].

90 isolates of *Fusarium oxysporum* f. sp. *ciceri* were identified on molecular level by using ITS-1 and ITS-4 primers (White *et al* 1990) [30]. The ITS forward (ITS-1) and reverse (ITS-4)oligonucleotides pair amplified a single DNA fragment of approximately 550 bp in all *F. oxysporum* f. sp. *ciceri* isolates representative of all races. Gayatri Gurjar *et al*. (2009) [10] also found the similar results that ITS primers amplified *F. oxysporum* f. sp. *ciceri* DNA approximately to 550bp and used for identification.

Similarly earlier worker Amandeep Kaur *et al*. (2015) [18] also used ITS marker for identification of *F. oxysporum* f. sp. *ciceri* isolates showed 99% similarity with *F. oxysporum* f. sp. *ciceri* sequence by basic local alignment search tool (BLAST) analysis. Use of ITS marker at genetic level was earlier implicated by Dubey *et al*. (2010) [7], Durai *et al*. (2012) [8], Datta and Lal (2012) [4].

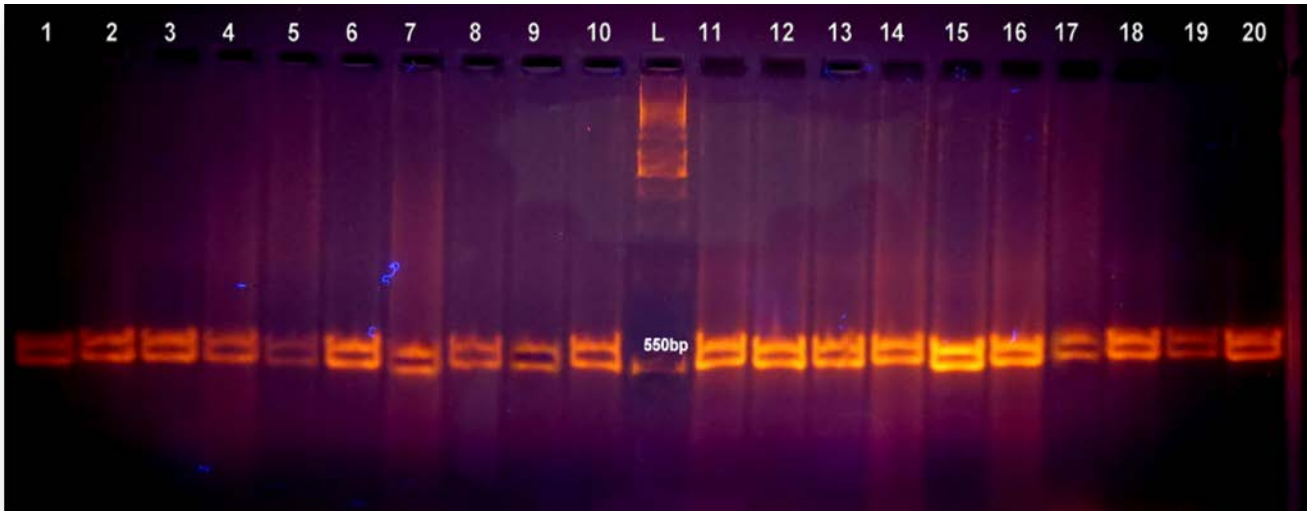


Fig 1: Identification of F.oxysporum f.sp.ciceri by using ITS primer

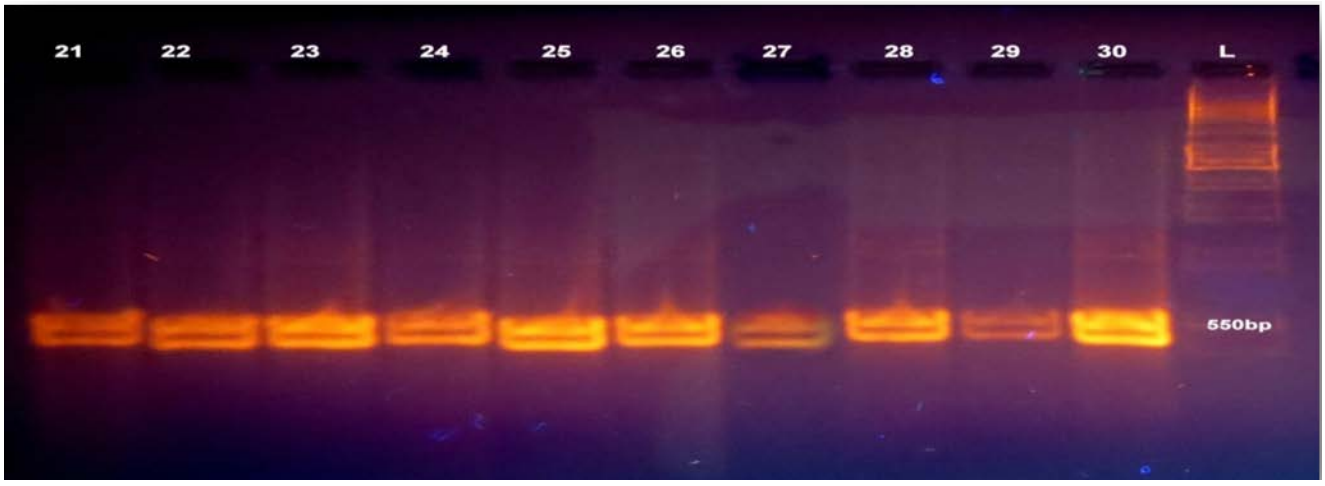


Fig 2: Identification of F.oxysporum f.sp.ciceri by using ITS primer

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

With the gaining importance of *Fusarium* wilt disease of chickpea and use of DNA markers for pathogen identification, the present study envisages to differentiate the four races of FOC mostly found in India. Race 1 had a yellowish tinge; race 2 was found to be creamish, pinkish tinge in race 3 and 4.

Race 1 (FDP-03)

The Fibrinogen Degradation Products (FDP 03) targets the mitochondrial rRNA gene designed basically for *Verticillium sp.* (Li *et al*. 1994) [19]. The FDP-03 has shown polymorphic banding pattern around 700bp among the different races. Fibrinogen Degradation Products (FDP-03) primer is used for identification of race-1 from the isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from different agro-ecological regions. The 700bp-720 bp indicates the race 1 belongs to the region *viz*. Maharashtra, Karnataka and Andhra Pradesh respectively (Figure 3, 4, 5).

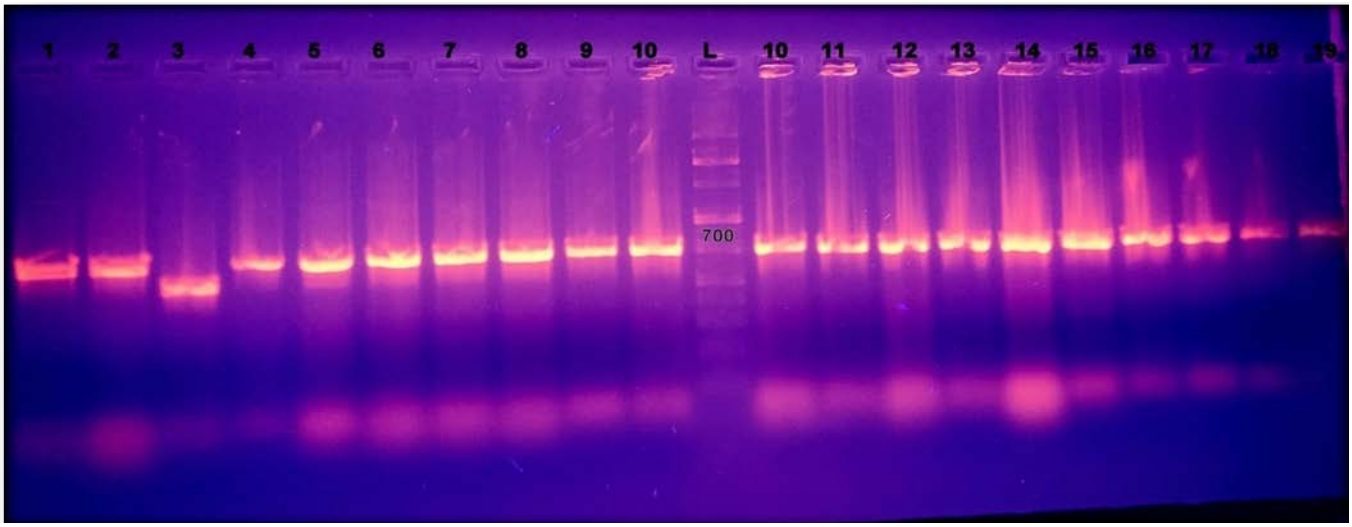


Fig 3: Molecular confirmation of race-1 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-03)

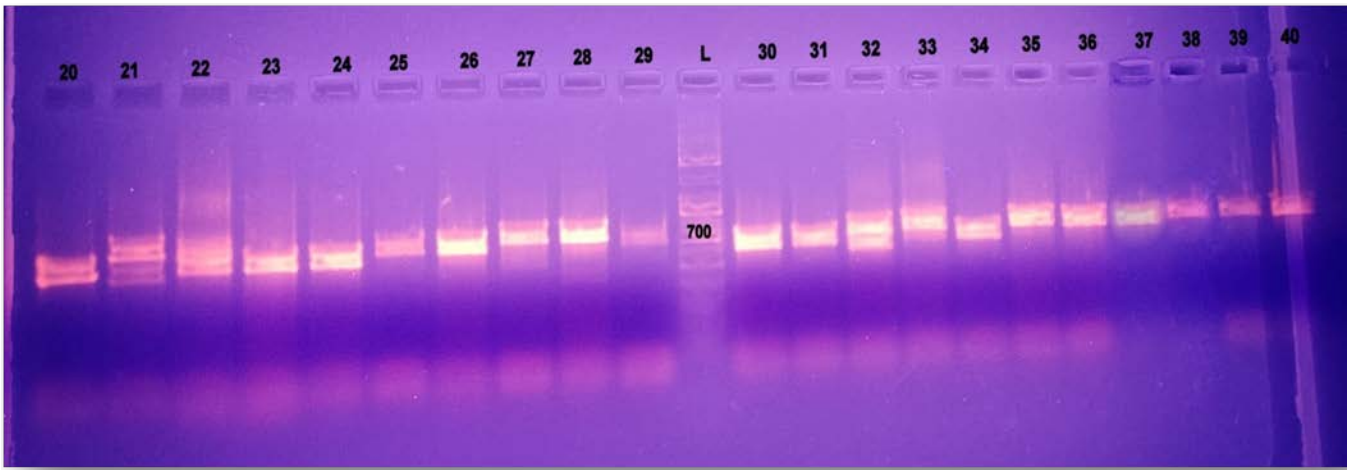


Fig 4: Molecular confirmation of race-1 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-03)

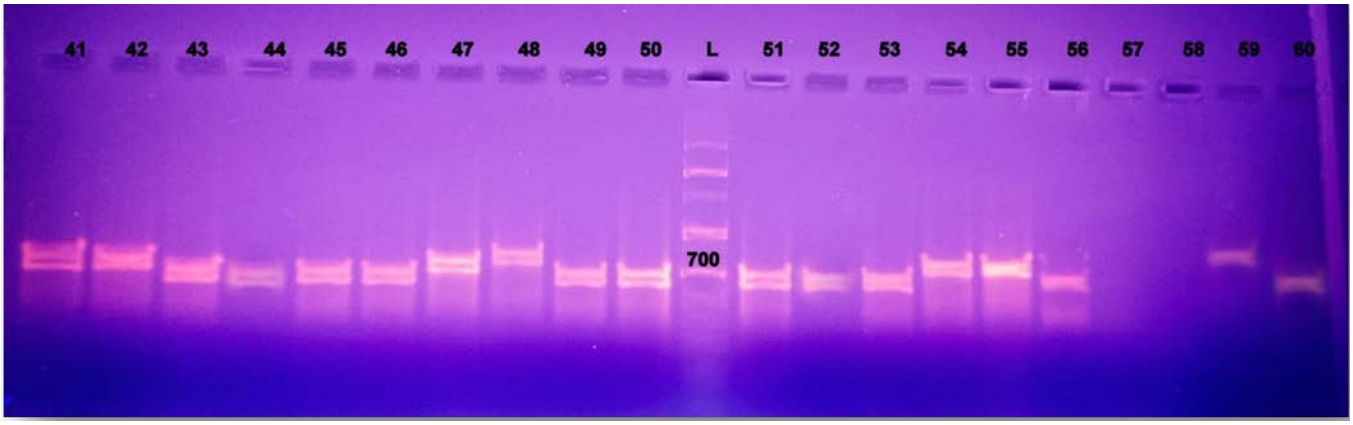


Fig 5: Molecular confirmation of race-1 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-03)

Race 2 (FDP-14)

FDP14 specifically for race 2, whereas Ortiz *et al*. (2011) [23] reported 1kb band for race 6 in their study.

The isolates of *F. oxysporum* f. sp. *ciceri* belonged from Uttar Pradesh viz. Kanpur, Allahabad, Varanasi and Harro represents race-2 on 900-1000bp (Figure 6)

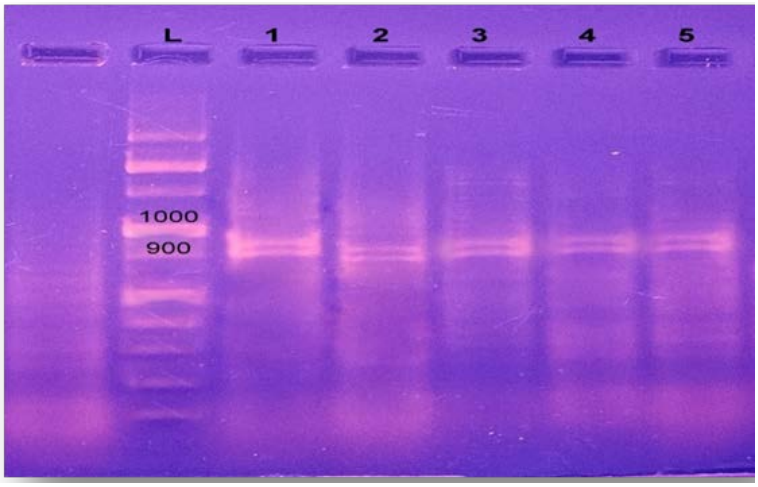


Fig 6: Molecular confirmation of race-2 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-14)

Race 3 (FDP-02)

FDP 2 targeted the ITS region of the ribosomal gene. The primer FDP-02 is used to distinguish the isolates of FOC in race-3 on 300bp. The Gurdaspur from Punjab represents race3 (figure 7).

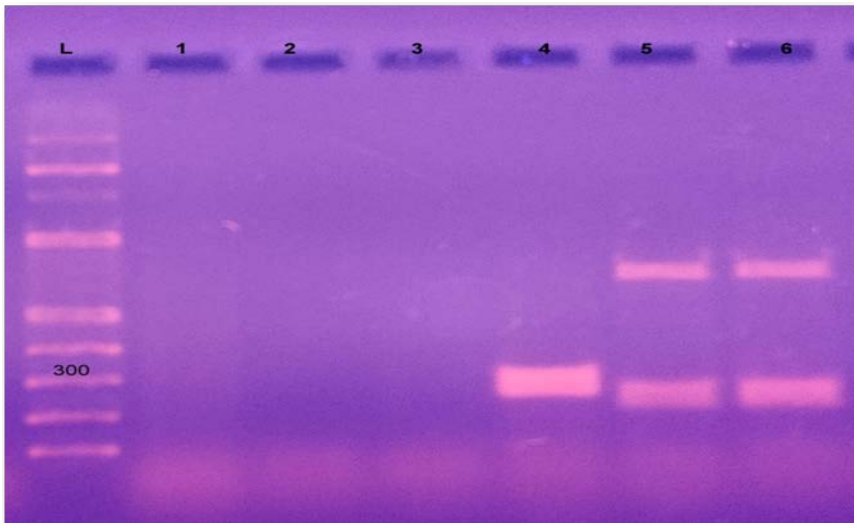


Fig 7: Molecular confirmation of race-3 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-02)

Race 4 (FDP-09)

FDP 9 distinguishes race 4 with a specific band of 500bp/2kb. The primer FDP-09 is used to distinguish the isolates of FOC in race-4 on 500bp/ 2000bp. The Jabalpur (MP) and Delhi (Haryana) represents race 4(figure8).

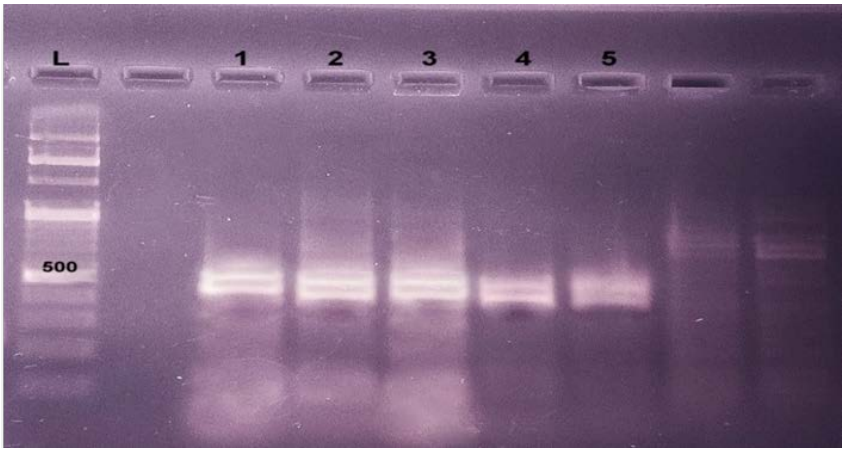


Fig 8: Molecular confirmation of race-4 from isolates of *F.oxysporum* f.sp.*ciceri* by using (FDP-09)

Table 1: List of isolates of *Fusarium oxysporum* f. sp. *ciceri* and their designation selected from different agro-ecological zones for cultural and pathogenic variability studies

Sr. No.	Foc* Designation	Location(State)	Date of collection	GPS location	Agro ecological region
1	FOC-1B	1 B Akola (MS)	25/12/2016	$20^0$43'07.83''N $77^0$09'25.80''E	Deccan Plateau, hot moist semi-arid with medium land deep clayey Black soils(6.3)
2	FOC-12	Amravati(MS)	30/12/2016	21°12'08.4"N 77°27'19.9"E	Deccan plateau, hot semi-arid eco-region (AER 6.3)
3	FOC-19	Wani Yavtmal (MS)	01/01/2017	20°04'00.7"N 78°57'18.1"E	Deccan Plateau, Hot Semi-Arid Eco-Region(6) Western Maharashtra plateau, hot moist semi-arid eco- sub region (6.3)
4	FOC-24	Lonar (MS)	05/01/2017	19°58'57.2"N 76°31'06.7"E	Western Maharashtra plateau, hot moist semi-arid eco- sub region (6.3)
5	FOC-26	Parbhani (MS)	06/01/2017	19°15'02.6"N 76°47'41.9"E	Deccan Plateau, Hot Semi-Arid Eco-Region (6.2)
6	FOC-33	Jalgao Sap.(MS)	08/01/2017	20°25'04.3"N 75°50'15.6"E	Deccan plateau, hot semi-arid eco-region (AER 6.3)
7	FOC-43	ICRISAT (AP)	07/01/2017	17°30'39.6"N 78°16'31.5"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
8	FOC-7689	Guntur (AP)	10/01/2017	16°18'24.8"N 80°26'09.0"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
9	FOC-46	Rameshwar(TN)	12/01/2017	17°33'23.3"N 78°16'48.7"E	Deccan plateau (Telangana) and eastern Ghats, hot semi-arid Eco region (AER 7.3)
10	FOC-7685	Dharwad (KT)	**	15°29'23.6"N 74°58'50.5"E	Deccan plateau, hot semi-arid eco-region (AER 6.4)
11	FOC-60	Kanpur (UP)	26/03/2017	26°29'38.4"N 80°16'19.5"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
12	S-15	Allahabad (UP)	25/02/2018	25°17'25.2"N 81°48'37.5"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
13	S-16	BHU, Varanasi(UP)	26/03/2017	25°16'26.0"N 82°59'31.6"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
14	FOC-67	Mirzapur(UP)	25/02/2018	25°07'57.6"N 82°33'53.9"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
15	FOC-74	Harro (UP)	24/02/2018	25°07'20.4"N 81°45'13.7"E	Northern plain hot sub humid (dry) eco-region (AER 9.2)
16	FOC-64*	Gurdaspur (PJ)	20/03/2017	32°02'54.4"N 75°25'53.7"E	Northern Plain (and central highland) including Aravallis, hot semi-arid Eco region (AER 14.2)
17	FOC-7692	Jabalpur (MP)	**	23°10'55.4"N 79°59'04.5"E	Central Highlands, Hot subhumid (dry) Eco region (AER 10.1)
18	FOC-7693	Rewa (MP)	**	24°32'11.8"N 81°18'14.6"E	Central Highlands, Hot subhumid (dry) ecoregion (AER 10.1)
19	FOC-51	Bhopal (MP)	21/02/2017	23°14'09.0"N 77°24'31.5"E	Central Highlands, Hot subhumid (dry) ecoregion (AER 10.1)
20	FOC-53	Raipur(CG)	15/02/2017	$21^0$13'57.54''N $81^0$43'07.37''E	Chhattisgarh/ Mahanadi basin Agro-eco-region (AER 11.0)
21	FOC-54	Dharansa (CG)	16/02/2017	23°13'10.4"N 82°11'55.1"E	Chhattisgarh/ Mahanadi basin Agro-eco-region (AER 11.0)
22	FOC-7675	Alwar (RJ)	**	27°34'34.7"N 76°35'17.4"E	Northern Plain (and central highland) including Aravallis, hot semi-arid ecoregion (AER 4.2)
23	FOC-59	New Delhi(HR)	**	28°37'59.4"N 77°09'09.8"E	Western Plain, Kachchh and part of Kathiwar Penisula, hot arid ecoregion (AER 2.3)
24	FOC-58	Hissar(HR)	19/03/2017	29°09'03.5"N 75°42'18.9"E	Western Plain, Kachchh and part of Kathiwar Penisula, hot arid ecoregion (AER 2.3)
25	FOC-7677	Sikohpur (HR)	**	28°23'42.6"N 76°59'21.1"E	Western Plain, Kachchh and part of Kathiwar Penisula, hot arid ecoregion (AER 2.3)

*Foc- *Fusarium oxysporum* f. sp. *ciceri*, ** samples procured from ITCC, New Delhi

Soil inoculation method

The pathogenic variability of isolates *F. oxysporum* f. sp. *ciceri* was tested by soil inoculation methods (Nene *et al*., 1981)[22] on wilt susceptible chickpea cultivar JG 62.

Preparation of mass Inoculum

Maize: Sand meal medium (1:3 % w/w) was used for mass multiplication of inoculum of *F. oxysporum* f. sp. *ciceri*. It was prepared by mixing 50 g maize and 150 g dry sand with 20 ml distilled water in 1000 ml conical flask. The conical flask was autoclaved at 1.05 kg/cm^2 for 30 minutes for three consecutive days. The autoclaved Maize: Sand meal medium were then inoculated with pure culture of each isolate of *Fusarium oxysporum* f.sp. *ciceri* separately under aseptic condition. The inoculated flasks were incubated at room temperature27±2°C for 2 weeks. The mass multiplied inoculum of the test pathogens was mixed to soil in at the rate 25 g kg^{-1} to make the soil sick.

Pot culture method

The inoculum was used for soil inoculation at 25 g kg^{-1} soil in pot experiments. Ten seeds/per big pot and 5 seeds/per small pot of wilt susceptible JG-62 were sown in each pot and with 3 replications. In case of control, chickpea seeds were sown in

pots containing un-inoculated soil. The plants were observed periodically up to 60 days after sowing (DAS) for days of initiation of wilt symptoms and per cent disease incidence.

Disease assessment and data analysis

After every second day, inoculated seedlings were observed for initiation of disease symptoms and per cent wilt incidence. Wilt incidence were calculated by using formula,

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

The isolates of *Fusarium oxysporum* f. sp. *ciceri* were tentatively divided into five groups on the basis of virulence as follows.

Category	Per cent wilt
i) Non-pathogenic isolates (NPI)	0%
ii) Weakly pathogenic isolates (WPI)	1-20%
iii) Moderately pathogenic isolates (MPI)	21-50%
iv) Strong pathogenic isolates (SPI)	51-70%
v) Highly pathogenic isolates (HPI)	>70%

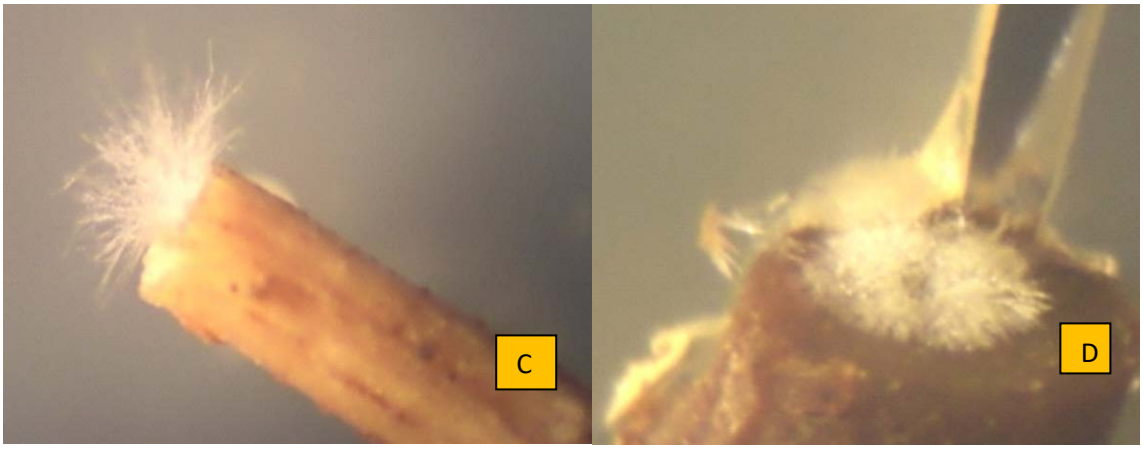
Results

Isolation and Purification

Isolation of *F. oxysporum* f. sp. *ciceri* from infected tissue

The wilted plants collected during survey and brought to the laboratory. The isolations were made from stem and root portions of wilted chickpea plants, collected from different agro ecological zones in India. The infected portion was cut into small bits of 1-2mm, was surface sterilized in 0.1% mercuric chloride for 1min followed by rinsing twice in sterilized distilled water. Later, these bits were transferred on PDA in Petri plates under aseptic conditions. The plates were incubated at 27±2 °C in BOD incubator. The growth thus obtained for each isolate was purified by single spore method. Finally, a total of 90 isolates were purified and maintained on PDA slants at 4°C for further studies and designated as Foc-1, Foc-2 and so on.

Fig 9: A.Wilt at vegetative stage, B. Field detection backening of vascular bundle



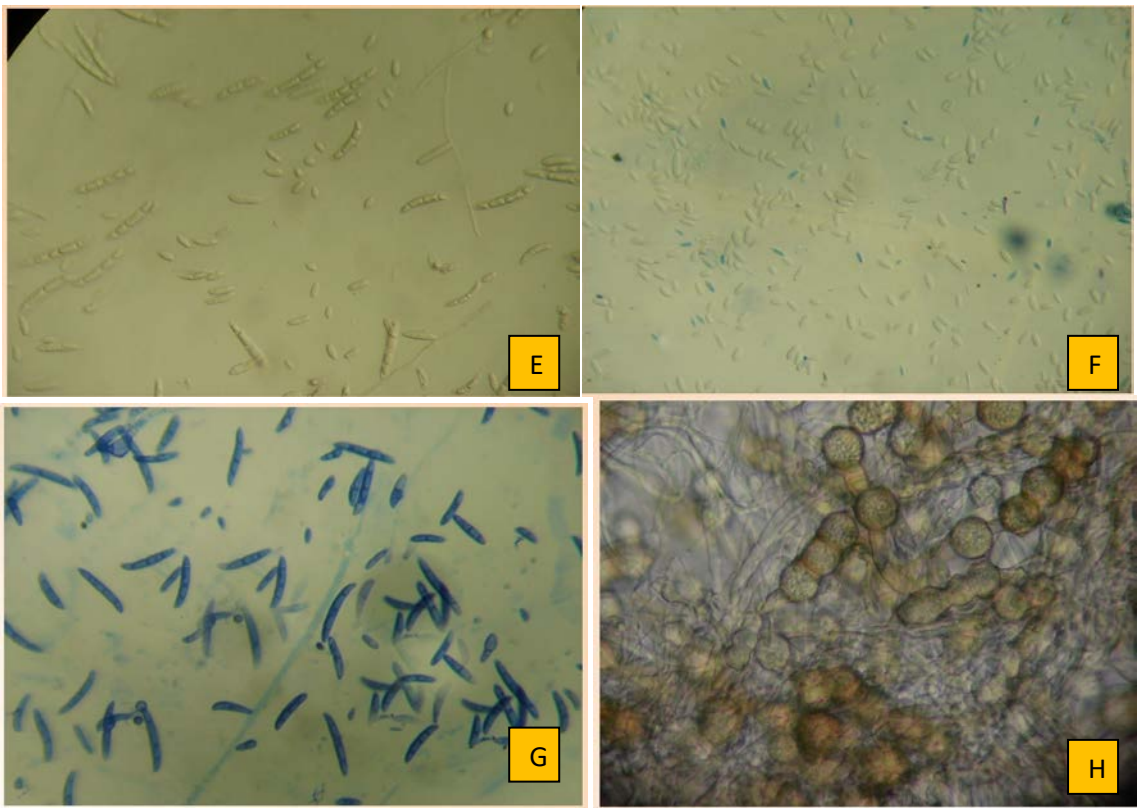


Fig 10: C. and D. The Fusarium isolated from xylem bundle of chickpea, E. & G. Macroconidia, F Microconidia, H. Chlymadospores

Purification *F. oxysporum* f. sp. *ciceri*

The isolated fungus brought in pure culture by single spore isolation technique and was maintained on PDA slants for further studies (figure 10). The pure culture thus obtained was identified as *Fusarium oxysporum* f. sp. *ciceri* on the basis of morphological characters published and reported by Booth (1971 and 1977)[2, 3].

Cultural variability on PDA media

The present investigations variations in pigmentation were observed in *Fusarium oxysporum* f. sp. *ciceri* isolates on reverse side of PDA plates. Based on pigmentation, *F. oxysporum* f. sp. *ciceri* isolates were categorized into three groups, Group-I (White - dull white colour), Group-II (Pink - Red - Saffron colour) and Group-III (Yellow-orange colour).Group - I included seven isolates viz., Foc-13, Foc-14, Foc-16, Foc-18, Foc-22, Foc-23, Foc-24. Group-II included 12 isolates viz., Foc-3, Foc-12, Foc-17, Foc-19, Foc-25 and last group-III included 15 isolates viz., Foc-1, Foc-2, Foc-4, Foc-5, Foc-6, Foc-7, Foc-8, Foc-9, Foc-10, Foc-11, Foc-15, Foc-20, Foc-21Similar variations for pigmentations were earlier recorded by Prasad *et al*. (2008) [25] and Saxena and Singh (1987) [26]. Difference on the surface as well as reverse side of fungal colonies was distinct in PDA media (Sharma and Pandey, 2010) [28]. Singh *et al*. (2010) [29] also observed the variation in pigmentation of *F. oxysporum* f. sp. *ciceri* isolates belonging from Kanpur, Varanasi and Allahabad as pale pinkish to white colour on PDA medium.

Pathogenic variability

An experiment was set up to study the pathogenic variability existing in different isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from different Agro-ecological regions of India. Twenty five isolates of *F. oxysporum* f. sp. *ciceri* (Table 1) were screened with highly wilt susceptible genotype JG 62 in sick soil by pot culture method.

Pot culture method

The sterilized soil was inoculated with sand maize meal inoculum of *Fusarium oxysporum* f. sp. *ciceri* isolates. The 15 seeds of JG-62 were sown in each inoculated plastic pot with individual isolates. After germination, ten seedlings per pot were maintained. Three pot per isolates were kept and observations i.e. days to initial wilting and complete wilting were recorded. The seedling maintained in sterilized soil served as control (figure 11).

The disease incidence recorded in JG 62 in response to25 respective *F. oxysporum* f. sp. *ciceri* isolates. The 25 isolates all showed pathogenic in nature. The wide range of variation in wilt incidence was recorded. The data revealed that variation in wilt incidence in the test isolates were 33 to 100 per cent. The Isolates Foc-16, Foc-20 were caused average wilt incidence 96.67 per cent followed by Foc-1, Foc-7, Foc-12 (93.33%). Out of 25 *F. oxysporum* f. sp. *ciceri* isolates eight viz., Foc-8, Foc-10, Foc-13, Foc-18 andFoc-22, were found moderately pathogenic with average 21-50 per cent wilt while isolates of *F. oxysporum* f. sp. *ciceri* viz., Foc-4, Foc-14, Foc-23, Foc-24, Foc-25, were found strongly pathogenic showed 51 to 70 per cent wilt. The fifteen isolates viz., Foc-1, Foc-2, Foc-3, Foc-5, Foc-6, Foc-7, Foc-9, Foc-11, Foc-12, Foc-15, Foc-16, Foc-17, Foc-19, Foc-20, Foc-21 were observed highly pathogenic produced more than 70 per cent wilt incidence (Table 2).No wilt symptoms were observed in control plants. Similarly Amandeep Kaur *et al*. (2015) [18] also found variability in pathogenic nature among twenty *F. oxysporum* f. sp. *ciceri* isolates.

Fig 11: I. Initioation of wilt symptoms, J. Pathogenic variability *F.oxysporum* f.sp.*ciceri* isolates by pot culture method, K .disesed and healthy plant

The expression of wilting in individual isolates was varied from each other. The average expression of wilting was observed from 18 to 42 days after sowing. There was relation between virulence and expression of symptoms. The most of the virulent isolates express the symptoms upto 19-23 days after sowing. The earlier similar observation was observed by Giri (2002) [9] in *Fusarium ud um* isolates causing wilt of pigeonpea, showed variability in incubation period and no relation between virulence and expression of symptoms. Mamta Sharma *et al*. (2009)[27] also found the variation of in days of disease development and disease incidence among 48 isolates of *F. oxysporum* f. sp. *ciceri.*

Table 2: Pathogenic variability among *Fusarium oxysporum* f. sp. *ciceri* by pot culture method on susceptible cultivar JG 62

Sr.	IsolatesCode	Total plants	Wilted plants	Wilt Incidence (%)	Days for initiation of Wilting(DAS)	Disease reaction	Race
1	FOC-1B	30	28	93.33	20	Highly virulent	Race-1
2	FOC-12	30	22	73.33	22	Highly virulent	Race-1
3	FOC-19	30	23	76.67	30	Highly virulent	Race-1
4	FOC-24	30	18	60.00	22	Stronglyvirulent	Race-1
5	FOC-26	30	25	83.33	19	Highly virulent	Race-1
6	FOC-33	30	22	73.33	32	Highly virulent	Race-1
7	FOC-43	30	28	93.33	35	Highly virulent	Race-1
8	FOC-7689	30	11	36.67	28	Moderatelyvirulent	Race-1
9	FOC-46	30	25	83.33	30	Highly virulent	Race-1
10	FOC-7685	30	12	40.00	28	Moderately virulent	Race-1
11	FOC-60	30	26	86.67	28	Highly virulent	Race-2
12	S-15	30	28	93.33	18	Highly virulent	Race-2
13	S-16	30	14	46.67	25	Moderately virulent	Race-2
14	FOC-67	30	19	63.33	18	Strongly virulent	Race-2
15	FOC-74	30	24	80.00	33	Highly virulent	Race-2
16	FOC-64*	30	29	96.67	30	Highly virulent	Race-3
17	FOC-7692	30	26	86.67	30	Highly virulent	Race-4
18	FOC-7693	30	12	40.00	21	Moderately virulent	Race-4
19	FOC-51	30	26	86.67	29	Highly virulent	Race-4
20	FOC-53	30	29	96.67	19	Highly virulent	Race-4
21	FOC-54	30	23	76.67	28	Highly virulent	Race-4
22	FOC-7675	30	15	50.00	30	Moderately virulent	Race-4
23	FOC-59	30	17	56.67	19	Strongly virulent	Race-4
24	FOC-58	30	18	60.00	30	Strongly virulent	Race-4
25	FOC-7677	30	16	53.33	28	Strongly virulent	Race-4

WP: Wilted plants, WI: Wilt incidence

At the end of experiment *F. oxysporum* f. sp. *ciceri* was recovered from wilted plants and its morphological characters showed resemblance with original isolates and Koch's Postulates were confirmed.

Conclusion

The rapid roving survey in the major chickpea growing areas of India revealed the prevalence of *Fusarium* wilt at all the locations. The disease incidence ranged from 1.71 to 57.93 per cent. The Chickpea wilt incidence observed more severe at reproductive stage of crop growth. The racial identification were carried out using DNA based PCR marker. The isolates which are collected from different geographical locations of India were differentiate into races. The virulence assay does not show correlation between virulence patterns with geographical origin of isolate, mycelium growth rate, pigmentation.

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