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MORPHOLOGICAL AND CULTURAL VARIABILITY OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* ISOLATES OF MARATHWADA REGION (MAHARASHTRA), INDIA

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops in India, but its productivity is severely constrained by *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. The present study was undertaken to assess the morphological and cultural variability of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from eight districts of the Marathwada region, Maharashtra. A total of eight isolates were purified and characterized on PDA medium. Significant variability was observed among isolates in terms of microconidia, macroconidia and chlamydospore dimensions, as well as colony growth rate, pigmentation, growth pattern, margin, and sporulation ability. Microconidia ranged from 2.21–4.52 × 1.40–2.20 µm (0–1 septa), while macroconidia measured 4.91–10.39 × 1.85–2.54 µm (2–4 septa). Chlamydospore size varied between 5.0–9.3 µm with both smooth and rough textures. Colony growth ranged from 46.00–80.33 mm after 7 days, with colours varying from white to pinkish white and pigmentation from brownish yellow to pinkish brown. Sporulation ability varied from good to excellent. The study confirmed considerable morphological and cultural diversity among Foc isolates, which may influence pathogenic variability and disease management strategies.

Key words : Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, Wilt, Morphological variability, Cultural variability, Marathwada.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the world's most important legume crops, valued for its high protein content and its ability to improve soil fertility through biological nitrogen fixation. It plays a crucial role in ensuring food security and supporting the livelihoods of smallholder farmers, especially in semi-arid areas.

In India, chickpea is cultivated on approximately 10.91 million hectares (Mha), producing 13.75 million tonnes (Mt) with an average yield of 1,260 kg/ha (ICAR-IIPR, 2022). Maharashtra accounts for nearly 26% of the country's chickpea production, with about 2.83 Mha under cultivation and an average productivity of around 1,080 kg/ha (Hake and Jadhav, 2021). In the Marathwada region, chickpea is grown on 0.66 Mha, yielding 0.66 Mt, although productivity is lower (918 kg/ha) due to climatic variability (Jadhav *et al*., 2018; Raut and Raut, 2020). However, chickpea production faces significant challenges from Fusarium wilt disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *ciceri* (Padwick), which infects the plant's vascular system, causing wilting and plant death. Under favorable environmental conditions, this disease can cause severe yield losses, sometimes up to 100%, posing a major threat to chickpea cultivation (Gurjar *et al*., 2018).

Fusarium oxysporum f. sp. *Cicero (Foc)* is a seed- and soil-borne, facultative saprophytic fungus belonging to the family Nectriaceae (Kingdom: Fungi, Phylum: Ascomycota, Class: Sordariomycetes, Order: Hypocreales). The pathogen survives in the soil in the form of conidia (microconidia and macroconidia), chlamydospores and mycelia (Jimenez-Diaz *et al*., 2010). Since the disease is soil borne, it is difficult to manage

either through crop rotation or chemical applications. The pathogen has the ability to survive in soil for up to six years even in the absence of host plants (Haware *et al*., 1986). Because of this persistence, utilization of resistant cultivars remains the most feasible option for management.

However, *F. oxysporum* f. sp. *ciceri* is a highly variable pathogen, and its morphological, cultural, and pathogenic variability can reduce the durability of host resistance. Isolates often differ in colony growth rate, pigmentation, sporulation capacity, and conidial dimensions, as well as in virulence on chickpea genotypes. Recent studies have emphasized such variability: Qureshi *et al*. (2021) reported considerable diversity in morphological traits among Indian isolates, Amandeep *et al*. (2022) observed wide variation in cultural and sporulation behavior, while Sharma *et al*. (2023) highlighted significant differences in pathogenic aggressiveness. This diversity complicates the use of resistant cultivars, as genotypes effective against one isolate may succumb to others. Hence, regional characterization of pathogen populations is crucial for developing durable resistance and effective wilt management strategies. Therefore, the present study was undertaken to determine the morphological and cultural variability of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from the Marathwada region of Maharashtra.

Materials and Methods

Chickpea plants exhibiting typical symptoms of chickpea wilt disease were collected from three different agroclimatic zones of Marathwada region, Maharashtra during *Rabi* season of 2020-21. Such symptomatic plants showing visible symptoms such as yellowing, withering, drying of leaves, complete drying of plants, partial wilting and vascular discoloration etc. were collected in the paper bags, labelled with requisite information, brought to the laboratory and subjected to the tissue isolation on potato dextrose agar medium.

Isolation and purification of Fusarium wilt pathogen

Chickpea plants showing typical wilt symptoms were collected from Parbhani, Nanded, Aurangabad, Jalna, Beed, Hingoli, Latur and Osmanabad districts of Marathwada region. Tissue isolations (Tuite, 1969) were made to isolate associated pathogen from wilted plants collected from different locations and purified using hyphal tip technique. The pure cultures of the isolates were maintained on PDA slants and stored at 4 ± 1°C in laboratory for further studies.

Designation of pathogen isolates

The pathogens isolated from different districts (Parbhani, Beed, Osmanabad, Nanded, Jalna, Aurangabad, Latur and Hingoli) of Marathwada region were designated for the ease of use in further studies.

Morphological and cultural studies for identification of pathogen isolates

The morphological and cultural studies of the eight pathogen isolates were made on PDA incubated at 28 ± 2°C for 7-10 days. Observations on cultural characters *viz*., growth rate, colony colour, reverse colony colour, colony growth pattern, elevation of the colony, margin of the colony and sporulation etc. were recorded after a week of incubation. For counting sporulation, sporulating culture of the 10 days old test isolates in Petri plates were6 flooded with 10 ml of distilled water. The surface of culture plate gently scraped with camel hair brush, to obtain spore suspension. Temporary mount on glass slide of the spore suspension was prepared, mounted under research microscope (10X objective lens), counted the spores under five random microscopic fields and averaged. Based on following scale (Kumar and Choudhary, 2006), the test isolates were categorized.

Grouping of isolates based on sporulation

Grade	Sporulation	No. of spores per microscopic field
-	Absent	Nil
+	Poor	1-10
++	Fair	11-30
+++	Good	31-50
++++	Excellent	More than 50

Kumar and Choudhary (2006)

For morphological characters, temporary mounts in Lactophenol cotton blue stain (Appendix II) on glass slides of the sporulating cultures of eight test isolates were prepared separately and covered with glass slide. Observations on length and breadth of microconidia and macroconidia, septation of microconidia and macroconidia of each test isolate were recorded by using J image software, TS view and with the help of the compound microscope at 40X magnification under 10 random microscopic fields. Identification of pathogen was done based on morphological and cultural characters of fungus.

Results and Discussion

Symptomatology

Symptoms observed during survey in the chickpea fields were yellowing and drooping of leaves. At severe stage, partial wilting or complete wilting of plant has been observed. When roots of the plant split open longitudinally,

(Pure cultures of 8 *Fusarium oxysporum* f. sp. *ciceri* isolates)

Fig. 1 : *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region, Maharashtra.

Table 1 : Designation of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region, Maharashtra.

S. no.	Location	Isolate code
1	Hingoli	FOC 1
2	Osmanabad	FOC 2
3	Latur	FOC 3
4	Jalna	FOC 4
5	Nanded	FOC 5
6	Beed	FOC 6
7	Aurangabad	FOC 7
8	Parbhani	FOC 8

it showed internal brown vascular discoloration of the plant (Fig. 1).

Collection, Isolation and designation of Fusarium wilt pathogen isolates

Chickpea plants showing typical wilt symptoms were collected from Parbhani, Nanded, Aurangabad, Jalna, Beed, Hingoli, Latur and Osmanabad districts of Marathwada region. Tissue isolations (Tuite, 1969) were made to isolate associated pathogen from wilted plants collected from different locations. All the eight isolates of pathogen obtained were purified using hyphal tip technique (Tuite, 1969).

The Fusarium isolates collected from eight districts of Marathwada region were identified based on morphological and cultural characteristics. The identified Fusarium isolates were designated, based on location of collected samples and presented in Table 1 and Fig. 1.

Eight pathogen isolates collected from different districts of the Marathwada region (Maharashtra) were examined for their morphological features on PDA medium. The characteristics of microconidia, macroconidia and chlamydospores showed noticeable variability (Table 2 and Fig. 2). The average size of microconidia among the isolates ranged from 2.21–4.52 × 1.40–2.20 µm. The largest microconidia were observed in isolate FOC 8 (4.52 × 2.20 µm), followed by FOC 5 (4.05 × 2.00 µm), whereas the smallest were recorded in FOC 4 (2.21 × 1.40 µm). Microconidia were generally

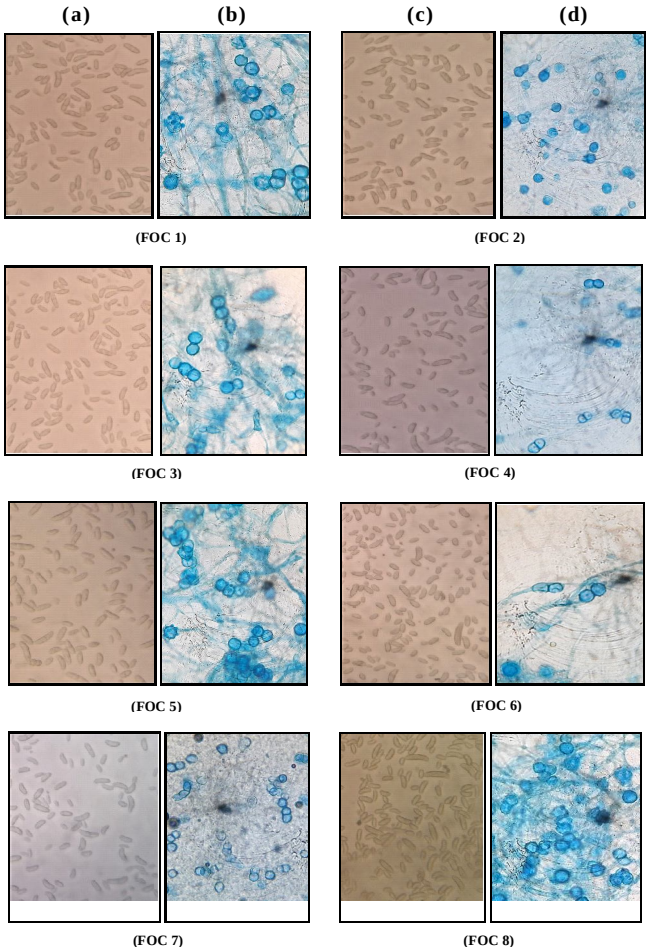


Fig. 2 : Morphological characters of *Fusarium oxysporum* f. sp. *ciceri* isolates (a) macroconidia and microconidia, (b) chlamydospores.

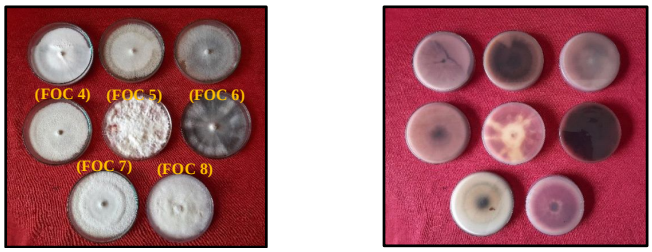
round to oval in shape, with septation ranging from 0–1. Most isolates produced single-septate microconidia, but FOC 4, FOC 6 and FOC 7 were non-septate.

Macroconidia size varied more widely, ranging from 4.91–10.39 × 1.85–2.54 µm. The largest macroconidia were recorded in FOC 8 (10.39 × 2.54 µm), followed by FOC 5 (10.05 × 2.47 µm), while the smallest were in FOC 6 (4.91 × 2.30 µm). All macroconidia were elongated, sickle-shaped with round ends. Septation ranged between 2–4, with the maximum septation (2–4) observed in FOC 8, whereas isolates FOC 2, FOC 6 and FOC 7 produced only two-septate macroconidia.

Chlamydospore size also showed variation, ranging from 5.0–5.3 µm to 9.2–9.3 µm. The largest were noted in FOC 8 (9.2–9.3 µm), followed closely by FOC 5 (9.1–9.2 µm) and FOC 1 (9.0–9.1 µm). The smallest were observed in FOC 7 (5.0–5.3 µm). Chlamydospores were globose to sub-globose in shape, with five isolates (FOC 2, FOC 3, FOC 4, FOC 6 and FOC 7) producing smooth-walled structures, while three isolates (FOC 1, FOC 5 and FOC 8) produced rough-walled ones.

Table 2 : Morphological characters of *Fusarium oxysporum* f. sp. *ciceri* isolates.

S. no.	Isolates	Microconidia		Macroconidia		Chlamydospores	
		Avg. size (µm) Length × Breadth	Septation (No.)	Avg. size (µm) Length × Breadth	Septation (No.)	Size (µm)	Texture
1	FOC 1	3.64 × 1.72	1	6.49 × 2.25	2-3	9.0-9.1	Rough
2	FOC 2	3.71 × 1.59	1	6.15 × 2.14	2	6.0-6.2	Smooth
3	FOC 3	3.55 × 1.41	1	6.04 × 2.05	2-3	8.9-9.0	Smooth
4	FOC 4	2.21 × 1.40	0	5.75 × 1.95	2-3	5.6-5.9	Smooth
5	FOC 5	4.05 × 2.00	1	10.05 × 2.47	2-3	9.1-9.2	Rough
6	FOC 6	2.35 × 1.95	0	4.91 × 2.30	2	7.6-7.8	Smooth
7	FOC 7	2.80 × 1.82	0	8.96 × 1.85	2	5.0-5.3	Smooth
8	FOC 8	4.52 × 2.20	1	10.39 × 2.54	2-4	9.2-9.3	Rough



(Dorsal view) (Ventral view)

Fig. 3 : Cultural characters of *Fusarium oxysporum* f. sp. *ciceri* isolates.

Overall, the morphological variability of the isolates was evident across all structures. Microconidia size ranged from 2.21–4.52 × 1.40–2.20 µm with mostly single septa or no septa. Macroconidia ranged from 4.91–10.39 × 1.85–2.54 µm with 2–4 septa and were characteristically sickle-shaped. Chlamydospores were globose to sub-globose and varied from 5.0–9.3 µm in size, with both smooth and rough textures. These findings are consistent with earlier reports by Qureshi *et al*. (2021), Kadam *et al*. (2012), Mandhare *et al*. (2011) and Mahajan *et al*. (2019), who also documented variability in conidial dimensions, septation and chlamydospore features. Similarly, Joshi *et al*. (2012) and Golakiya *et al*. (2018) observed that chlamydospores could be either rough or smooth-walled, intercalary or terminal, and formed singly, in chains or in pairs.

Cultural variability of *Fusarium oxysporum* f. sp. *ciceri* isolates

The cultural characters of eight isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from Marathwada were studied on PDA medium after seven days of incubation. Considerable variability was observed among the isolates in terms of growth rate, colony colour, pigmentation, colony form, and sporulation ability (Table 3 and Fig. 3). Mycelial growth ranged from 46.00 mm (FOC 4) to 80.33 mm (FOC 8), with FOC 8 and FOC 5 showing the fastest growth. Intermediate growth was recorded in FOC 1, FOC 6, FOC 7, FOC 2, and FOC 3. Colony colour ranged from white (FOC 1, FOC 4, FOC 7), creamy white (FOC 2), hyaline (FOC 3, FOC 6) to pinkish white (FOC 5, FOC 8). Reverse colony pigmentation also varied, with brownish yellow (FOC 1, FOC 3, FOC 4), reddish brown (FOC 2, FOC 6), pinkish brown (FOC 5, FOC 8) and light yellow (FOC 7) being recorded.

Colony growth pattern differed considerably across isolates. FOC 1, FOC 2, FOC 3, FOC 4 and FOC 6 produced subdued and compact colonies, whereas FOC 5, FOC 7 and FOC 8 exhibited aerial strandy and cottony growth. Colony elevation ranged from flat appressed (FOC 1, FOC 2, FOC 3, FOC 4, FOC 6), to convex (FOC 5, FOC 8), and umbonate (FOC 7). Similarly, colony margins were either uniformly round (FOC 2, FOC 3, FOC 6, FOC 7, FOC 8) or wavy (FOC 1, FOC 4, FOC 5). Sporulation ability was generally high, with six isolates (FOC 1, FOC 2, FOC 3, FOC 5, FOC 6, FOC 8) rated as excellent, while two isolates (FOC 4 and FOC 7) showed good sporulation.

Overall, the study demonstrated broad cultural variability among the Foc isolates. Growth rate varied between 46.00–80.33 mm, colony colour from white to pinkish cottony white and pigmentation from brownish yellow to pinkish brown. Colonies exhibited diverse growth patterns ranging from compact to aerial strandy, with variations in elevation and margin type. Sporulation was consistently good to excellent. These observations agree with earlier findings of Amandeep *et al*. (2015), Patil *et al*. (2017), Nath *et al*. (2017) and Younsei *et al*. (2021), who also reported variability in colony morphology, pigmentation, margins and sporulation intensity of Foc isolates. Patra and Biswas (2016) recorded radial growth ranging from 72–87 mm with pigmentation from pinkish to pale yellow, while Qureshi *et al*. (2021) further noted differences in colony elevation (umbonate, crateriform,

Table 3 : Cultural characters of *Fusarium oxysporum* f. sp. *ciceri* isolates.

S. no.	Isolates	*Growth rate after 7 days(mm)	Colony colour	Reverse colony colour/ pigmentation	Colony growth pattern	Elevation of colony	Margin of the colony	**Sporulation
1	FOC 1	70.33	White	Brownish Yellow	Subdued and compact growth	Flat appressed	Wavy	++++
2	FOC 2	60.00	Creamy white	Reddish Brown	Subdued and compact growth	Flat appressed	Uniformly round	++++
3	FOC 3	57.00	Hyaline	Brownish Yellow	Subdued and compact growth	Flat appressed	Uniformly round	++++
4	FOC 4	46.00	White	Brownish Yellow	Subdued and compact growth	Flat appressed	Wavy	+++
5	FOC 5	79.33	Pinkish white	Pinkish Brown	Aerial strandy cottony growth	Convex	Wavy	++++
6	FOC 6	69.33	Hyaline	Reddish Brown	Subdued and compact growth	Flat appressed	Uniformly round	++++
7	FOC 7	65.67	White	Light Yellow	Aerial strandy cottony growth	Umbonate	Uniformly round	+++
8	FOC 8	80.33	Pinkish White	Pinkish Brown	Aerial standy cottony growth	Convex	Uniformly round	++++
S.Em(±)		**0.456**	-	-	-	-	-	-
C.D. (P=0.01)		**1.380**	-	-	-	-	-	-

* Mean of three replications, **Sporulation: (++++) = Excellent, (+++) = Good, (++) = Fair, (+) = Poor, (-) = No spores.

convex, flat) and margin types (entire or undulate). The present results reaffirm the highly diverse cultural nature of *F. oxysporum* f. sp. *ciceri*.

Conclusion

The present study revealed considerable morphological and cultural variability among *Fusarium oxysporum* f. sp. *ciceri* isolates collected from different districts of the Marathwada region. Significant differences were observed in microconidial and macroconidial dimensions, septation, and chlamydospore size and texture, as well as in colony growth rate, pigmentation, growth pattern, elevation, margins, and sporulation ability. Such variability highlights the adaptive potential of the pathogen and poses challenges for breeding durable resistance in chickpea. The findings emphasize the importance of continuous regional monitoring and characterization of pathogen populations to identify stable sources of resistance and to design effective wilt management strategies suited to local conditions.

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