J. Agric. Res. Technol., 51 (2) : 246-252 (2026) https://doi.org/10.56228/JART.2026.51214

Characterization of Races of Chickpea Wilt in Maharashtra

R. Kolhe[1], S. Latke[2] and P. Kulwal[1],
State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722 (India)
Corresponding author Email : pawankulwal@gmail.com
(Received : 12.01.2026 Accepted : 17.04.2026)

Abstract

The presence of distinct pathogenic races in Fusarium oxysporum f. sp. ciceris is a major concern in chickpea wilt management. Globally, eight races (0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been identified, of which four races (1, 2, 3 and 4) have been reported from India. This study investigated the genetic variability and race composition of Fusarium oxysporum f. sp. ciceri, the causal agent of chickpea wilt in Maharashtra, during 2023–2025. Ten isolates collected from major chickpea growing regions were characterized using cultural, morphological and molecular approaches. Species specific PCR confirmed all isolates, while SSR analysis using twenty primers identified ten polymorphic markers generating 26 alleles with moderate to high PIC values (0.18–0.59). Cluster analysis grouped isolates into two major clusters showing clear geographical differentiation. Race specific primers detected the presence of Race 1 and Race 2, indicating coexistence of multiple pathogenic races in the region. The observed genetic diversity highlights the need for broad spectrum and regionally targeted resistance breeding to improve wilt management in Maharashtra.

Key words : Chickpea wilt, *Fusarium*, SSR markers, Races characterization.

Chickpea (*Cicer arietinum* L.) is a major grain legume originating from the Fertile Crescent and remains central to global food security, with India contributing over 70% of world production (FAOSTAT, 2024; https://www.fao.org/faostat/en/#home). Despite its importance, chickpea yields are severely constrained by *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. ciceris (FOC), first reported in India by Butler in 1918. The pathogen persists in soil for several years through chlamydospores (Smith and Snyder, 1975; Gordon and Martyn, 1997) and causes 10-100% yield losses depending on environmental conditions and pathogen virulence (Haware and Nene, 1992).

*Fusarium oxysporu*m f. sp. *ciceris* produces microconidia (2.5-4.5 x 5-11 µm), macroconidia (3.5-4.5 x 25-65 µm; 3-5 septa) and thick-walled chlamydospores formed singly, in pairs or chains. The fungus has hyaline, septate, branched hyphae and grows across a wide temperature (7-35°C) and pH range (4.0-9.4) with optimal mycelial growth at 25-27°C and pH 5.1-5.9. Infection begins through the root epidermis, progressing to the xylem where micro and macroconidia enable systemic colonization (Leslie and Summerell, 2006). Chlamydospores act as long-term survival structures, persisting in soil for over six years and serving as the primary inoculum for subsequent crops (Smith and Snyder, 1975).

Several races of *Fusarium* have been reported in the literature which are specific to the geographic locations (Venkataramanamma et al., 2025). In Maharashtra, race 1 is known to be predominant. However, there is still ambiguity regarding presence of other races. To address this, the present study employed race specific and microsatellite (SSR) markers for race characterization and to discriminate the different isolates of *F. oxysporum* f. sp. *ciceris* by cultural, morphological characterization among FOC isolates collected from major chickpea growing districts of Maharashtra.

Materials and Methods

Sample collection, isolation and purification : Chickpea plants, naturally infected and showing clear symptoms of wilt and vascular discoloration were collected from ten different districts of Maharashtra which includes Akola, Pune, Dhule, Buldhana, Jalgaon, Latur, Ahilyanagar and Jalna (Table 1). Root and stem sections from these symptomatic plants were used to ensure representative pathogen identification and molecular characterization across varying disease severities. Isolation of the fungus was made by root culture. The resulting fungal cultures were purified by hyphal tip method. Purified cultures were maintained on potato dextrose agar (PDA) by storing it under refrigeration at 4°C. The fungus was isolated, purified and sub cultured in aseptic condition under a laminar flow. The isolates of the pathogen were primarily identified based on colony characters and spores morphology (Booth, 1971). Photomicrographs of the *F. oxysporum* f. sp. *ciceri* isolates were taken by using imaging microscope to describe spore morphology.

Cultural and morphological studies : All ten isolates of *F. oxysporum* f. sp. *ciceri* were separately grown on PDA in petriplates and incubated at 28 ± 2°C for seven days. Observations on cultural characters viz., colony colour and type, growth and pigmentation were recorded a week after inoculation. Morphological characters of spores of different isolates were studied by observing in blue stained slides under imaging microscope. Measurements of macro and microconidia and chlamydospores were made with the help of imaging microscope which shows size of conidia and diameter of chlamydospores.

Table 1. List of *F. oxysporum* isolates collected from various locations of Maharashtra

Location	District	Isolate name
Akola	Akola	Isolate-1
Vadgaon Maval	Pune	Isolate-2
Dhule	Dhule	Isolate-3
Buldhana	Buldhana	Isolate-4
Badnapur	Jalna	Isolate-5
Rahuri	Ahilyanagar	Isolate-6
Latur	Latur	Isolate-7
Junnar	Pune	Isolate-8
Jalgaon	Jalgaon	Isolate 9
Niphad	Nashik	Isolate-10

Molecular characterization

Fungal DNA isolation and race identification : The fungal genomic DNA was extracted from mycelia grown in 250 ml of PDB at 28 °C for 5 days. The mycelia were harvested from broth for further process. For genetic diversity analysis, the genomic DNA of 10 *Fusarium oxysporum* isolates were extracted using modified sodium dodecyl sulphate (SDS) procedure (Asran-Amal, 2012). Genomic DNA was isolated from fungal mycelia using a detergent based (SDS) lysis approach as given in Sakhare et al., (2024). Fresh mycelium was mixed with a lysis buffer to break open cells and RNase was added to remove RNA. Proteins and other contaminants were removed using organic extraction (phenol: chloroform: isoamyl alcohol) and DNA was precipitated from the cleared lysate with isopropanol. The resulting DNA pellet was washed with ethanol to remove salts, dried and finally dissolved in TE buffer for long-term storage. The molecular confirmation of all isolates was done with species specific primer FOC-Fs4 (5'ATCGGCCACGTCGACTCT3'-F, 5'GGCGTCTGTTGATTGTTAGC3'-R).

The *Fusarium oxysporum* f. sp. *Ciceri* specific primers were used for DNA amplification. The amplification was performed in 20 µl reaction volume with 0.1 mM of each dNTP and 100pmol of both forward and reverse primer. PCR was programmed for initial denaturation at 94°C for 5 min and 40 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for

1 min. The amplification was completed with a final extension at 72°C for 5 min. Race identification was carried out using race-specific primers and DNA amplification was performed using a total of twenty primers.

Data Scoring and Analysis : Amplified bands were scored as presence (1) or absence (0). Polymorphism Information Content (PIC) was calculated as described in Borse et al. (2017) using:

$$PIC = 1 - \Sigma(pi^2)$$

A dissimilarity matrix was generated and cluster analysis was performed using the unweighted neighbor-joining method in DARwin 6.0.021 to infer genetic relationships and race grouping among isolates.

Result and Discussion

Cultural characterization : Ten isolates of *Fusarium oxysporum* f. sp. *ciceris* were individually cultured on PDA plates and incubated at 28 ± 2°C for one week. Cultural traits including colony morphology, mean mycelial growth, growth habit, pigmentation and sporulation were recorded after two weeks (Table 2, Fig. 1). Isolates were classified as slow (10 mm day^{-1}), medium (10-12 mm day^{-1}), or fast (>12 mm day^{-1}) growers following the criteria of Dubey et al. (2010).

Morphological characterization : All samples produced both microconidia and macroconidia when observed under the microscope. Microconidia were primarily 1–2 celled and varied from sparsely to abundantly

Table 2. Morphological and cultural characteristics of *Fusarium* isolates studied

Isolate	Colony morphology	Mean mycelial growth mm 7-1 DAI	Growth habit	No. of days taken to cover the plate	Pigmentation	Sporulation
Isolate 1	Circular, compact, aerial mycelia	80.00	Medium	8	Pale pinkish	1-2 celled sparsely dispersed microconidia
Isolate 2	Smooth, circular, sparsely dense mycelia	77.00	Fast	7	Deep pinkish	Sparsely dispersed microconidia with minimum curvature
Isolate 3	Circular, profuse compact aerial mycelia	74.00	Fast	7	Pale yellowish	Sparsely dispersed microconidia
Isolate 4	Circular, compact aerial mycelia	90.00	Medium	9	Pale pinkish	Abundantly dispersed micro and 3-5 septate macroconidia
Isolate 5	Circular, smooth, compact aerial mycelia	77.00	Medium	9	Pale pinkish with centered white	Sparsely dispersed microconidia and macroconidia
Isolate 6	Circular, sparsely dense aerial mycelia	78.67	Medium	9	Pale yellowish	1-2 celled sparsely dispersed microconidia
Isolate 7	Circular, sparsely flattened mycelia	80.67	Medium	8	Yellowish with centered white	1-2 Abundantly dispersed microconidia
Isolate 8	Smooth, circular, compact mycelium	71.33	Medium	10	Deep pinkish	Abundantly dispersed 3-5 celled macroconidia
Isolate 9	Circular, smooth, compact mycelium	70.33	Medium	10	Pale yellowish	Abundantly dispersed 1-2 celled microconidia
Isolate 10	Circular, compact, aerial mycelim	86.00	Fast	7	Pale pinkish	Abundantly dispersed 3- celled macroconidia

Fig. 1. Various isolates of Fusarium obtained after pure culture

dispersed, with some showing slight curvature. Macroconidia were consistently present, typically 3–5 celled and their abundance also varied among isolates. Overall, the isolates showed clear differences in the dispersion and septation of both spore types (Table 2, Fig. 2-3).

Molecular characterization

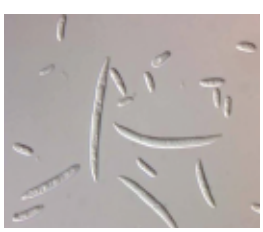

Fig. 2. Macroconidia and microconidia

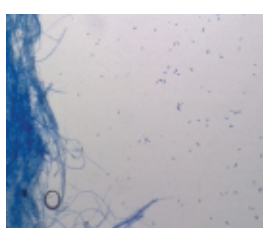

Fig. 3. Microconidia seen when dyed with methylene blue

Race identification : Race identification of *Fusarium oxysporum* f. sp. *ciceris* (FOC) was successfully performed using race specific primer. The primer FOC FS4 produced clear amplification, confirming the molecular identity of the pathogen. It successfully amplified a single allelic band of 1400 bp (Fig. 4). Among the race specific primers, FDP 3 and TEF FU3 (specific to Race 1) showed positive amplification in all the isolates, thereby confirming the presence of Race 1 in these isolates (Fig. 5). Similarly, FDP 11 and FDP 14, which are specific to Race 2 also produced amplification, indicating the presence of Race 2 in the samples analysed (Fig. 6). Among the primers tested, FDP-03 amplified three alleles with fragment sizes between 550–650 bp, while FDP-11 produced three amplicons in the range of 1400–1600 bp. Primer TEF Fu3 generated two alleles of 1250–1300 bp and FDP-14 amplified three alleles within the 200–500 bp range (Table 3). In contrast, no amplification was obtained with the primers specific for other races. The primers FOF 1 and FDP 2 (Race 3), FDP 9 and FDP 7 (Race 4), FDP 17 and FDP 19 (Race 5) and FDP 22 (Race 6) did not show any amplification, suggesting the absence of these races in the samples collected from the study area.

The SSR markers amplified 2-3 alleles with a PIC value of 0.18 to 0.59 suggesting moderate to high variability (Table 3). Markers with PIC values above 0.50 can be considered as informative in discriminating isolates from each other.

Binary similarity matrix and dendogram : A binary similarity matrix was constructed using the combined amplification profiles of 10 primers across 10 isolates of *Fusarium oxysporum* f. sp. *ciceri*. For each primer, the presence (scored as '1') or absence (scored as '0') of DNA bands was recorded for each isolate, with bands displaying identical electrophoretic mobility considered to represent the same genetic locus. This binary data set facilitated the estimation of pairwise genetic similarity between isolates, providing the foundation for subsequent cluster analysis and interpretation of molecular diversity. The dendrogram analysis revealed that the ten

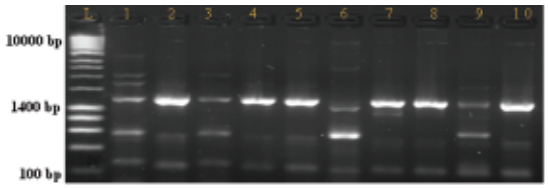

Fig. 4. Molecular identification of Fusarium wilt isolates using FOC FS4 marker

(L- 1kb DNA ladder, Lane 1 containing 1-10 fungal isolates where 1- Isolate-1, 2- Isolate-2, 3- Isolate-3, 4- Isolate -4, 5- Isolate -5, 6- Isolate -6, 7- Isolate -7, 8- Isolate -8, 9- Isolate -9, 10- Isolate -10)

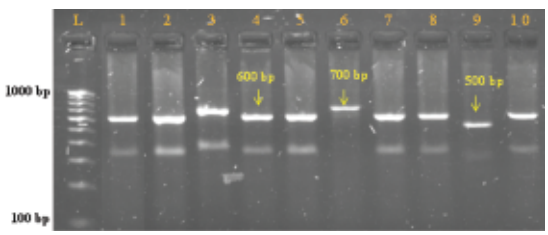

Fig. 5. PCR amplification profile showing banding pattern using race 1 specific FDP-03 marker

(L-100bp DNA ladder, Lane 1 containing 1-10 fungal isolates where 1- Isolate-1,2- Isolate-2, 3- Isolate-3, 4- Isolate -4, 5- Isolate -5, 6- Isolate -6, 7- Isolate -7, 8-Isolate -8, 9- Isolate -9, 10- Isolate -10)

Fusarium oxysporum isolates were separated into two major clusters, designated as Cluster I and Cluster II (Fig. 7).

Cluster analysis revealed a hierarchical separation of the ten *Fusarium oxysporum* f. sp. ciceris isolates into two major clusters. Cluster I showed extensive sub-structuring, ultimately separating isolates from Buldhana, Badnapur, Nashik, Pune, Latur, Junnar and Akola into distinct divisions and subgroups, indicating high genetic variability (62.4%). Subcluster IB contained only the isolate from

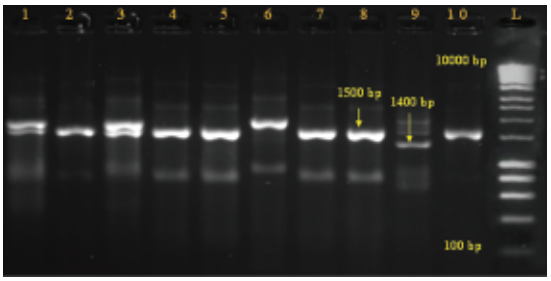

Fig. 6. PCR amplification profile showing banding pattern using race 2 specific marker FDP-11

L-100bp DNA ladder, Lane 1 containing 1-10 fungal isolates where 1- Isolate1, 2- Isolate-2, 3- Isolate-3, 4- Isolate -4, 5- Isolate -5, 6- Isolate -6, 7- Isolate -7, 8-Isolate -8, 9- Isolate -9, 10- Isolate -10)

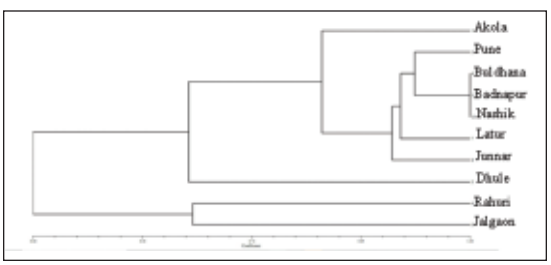

Fig. 7. Consensus tree showing genetic similarities between different isolates of wilt in chickpea

Dhule district. Cluster II was comparatively uniform, consisting of two sub-clusters represented by isolates from Rahuri and Jalgaon and exhibited lower genetic variability (54.7%). Overall, the complex matrix structure suggests that, despite higher diversity in Cluster I, the comparatively stable Cluster II may offer a more reliable basis for interpreting genetic evolutionary relationships.

The results confirm the coexistence of Race 1 and Race 2 of *Fusarium oxysporum* f. sp. ciceris (FOC) in Maharashtra, with no detection of races 3, 4, 5 and 6. The amplification with primers specific to Race 1 (FDP 3, TEF FU3)

Table 3. Marker amplification and product size of primers used

Primer	No. of alleles amplified	Amplified product range	PIC
FOC-Fs4	1	1400	-
FDP-03	3	500-700	0.46
TEF-Fu-3	2	1250-1300	0.18
FDP-11	3	1400-1600	0.34
FDP-14	3	200-500	0.46
MB-2	3	400-550	0.59
MB-5	1	400	-
MB-9	3	300-400	0.56
MB-10	3	400-500	0.54
MB-11	2	150-180	0.23
MB-14	2	180-400	0.18
MB-17	2	200-250	0.32
MB-18	1	300	-

and Race 2 (FDP 11, FDP 14) confirms their presence, reflecting the pathogenic diversity and higher risk of disease outbreaks in the region. This situation aligns with earlier studies in India that identified Race 1 and Race 2 as the dominant races in chickpea-growing areas (Sruthy et al., 2024). The presence of these races underscores the need for breeding broad-spectrum resistant chickpea cultivars to manage the disease effectively, as resistance to a single race may be insufficient under field conditions (Datta and Lal, 2013).

Earlier reports by Sruthy et al., (2024) specifically on Maharashtra and surrounding regions confirm molecular identification and the prevalence of Race 1 and Race 2, while absence of other races indicates their current non-prevalence but suggests continuous monitoring is necessary to detect any new emergences or introductions.

Research on race characterization of fungal isolates causing *Fusarium* wilt in chickpea is valuable for identifying specific fungal races and understanding resistance or tolerance in different cultivars. To achieve better accuracy in genetic characterization, it is highly recommended to combine molecular marker analysis with traditional morphological evaluations, as this approach enhances precision and provides supportive information.

Conclusion

The study confirmed the presence of *Fusarium oxysporum* in all collected isolates using species specific primers and demonstrated substantial genetic variability through SSR marker analysis. Combined molecular and morphological analyses revealed considerable diversity among *F. oxysporum* populations in Maharashtra. Importantly, the investigation successfully identified Race 1 and Race 2 of *F. oxysporum* f. sp. *ciceris* in key chickpea-growing regions, providing essential baseline information for future resistance breeding and disease management programmes.

References

Asran-Amal, A., 2012. Molecular identification of Macrophomina phaseolina by microsatellite-based fingerprint. Plant Path Quarant. 2, 143-151.

Booth, C., 1971. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, pp 237.

Borse, V. V., Kulwal, P. L., Mhase, L. B., Jadhav A. S., (2017) Validation of seed weight-associated ssr markers and their usefulness in distinguishing chickpea genotypes according to seed size. Agric Res. 6, 130-138.

Datta, J., Lal, N., 2013. Genetic diversity of Fusarium wilt races of pigeonpea in major regions of India. African Crop Sci J. 21(3), 201-212.

Dubey, S. C., Singh, S. R., Singh, B., 2010. Morphological and pathogenic variability of Indian isolates of Fusarium oxysporum f. sp. ciceris causing chickpea wilt. Archives Phytopath Plant Prot. 43(2), 174-190.

Gordon, T. R., Martyn, R. D., 1997. The evolutionary biology of Fusarium oxysporum. Ann Rev Phytopath. 35(1), 111-128.

Haware, M. P., Nene, Y. L., Pundir, R. P. S., Rao, J. N., 1992. Screening of world chickpea germplasm for

resistance to *fusarium* wilt. Field Crops Res. 30(1-2), 147-154.

Haware, M. P and Nene, Y. L., 1982. Races of Fusarium oxysporum f.sp. ciceri. Plant Dis. 66: 809-810.

Leslie, J. F., Summerell, B. A., 2006. Fusarium laboratory workshops-A recent history. Mycotoxin Res. 22(2), 73-74.

Sakhare, V. V., Kulwal, P. L., Surnar, S., Raghuwanshi, K. R., 2024. Development of SSR markers and characterization of different isolates of Macrophomina phaseolina causing dry root rot of chickpea (*Cicer arietinum* L.). J Agri Res Tech. 49(3), 525-533.

Smith, S. N., Snyder, W. C., 1975. Persistence of *Fusarium oxysporum* f. sp. vasinfectum. Phytopath. 65, 190-196.

Sruthy, M., Mane, S. S., Ingle, S. T., Jadhav, P. V., Jankar, K., 2024. Morphological characterization and genetic diversity of chickpea wilt fungus *Fusarium oxysporum* f. sp. *ciceri*, using SRAP and RAPD markers. International Journal of Research in Agronomy, 7(7), 29-35.

Venkataramanamma, K., Bhaskara Reddy, B. V., Sarada Jayalakshmi, R., 2025. "A Review on *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* F. Sp. *Ciceris* and its biology". J Expt Agri Intern. 47 (9), 149-162.