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Isolation and molecular identification of Fusarium oxysporum from Tur crop in Marathwadha region

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Abstract
The study investigated Fusarium isolates associated with wilt-affected pigeonpea plants collected from eight districts of the Marathwada region of Maharashtra. Eight isolates, designated FO-PP-01 to FO-PP-08, were obtained and characterized based on cultural and morphological features. The selected isolate FO-PP-01 was further identified using ITS1–5.8S rRNA–ITS2 region sequencing. ITS amplification produced an approximately 500–600 bp fragment, and sequence comparison with GenBank reference sequences supported its affinity with Fusarium oxysporum. Neighbor-Joining analysis showed that FO-PP-01 clustered closely with selected F. oxysporum reference sequences. The study demonstrates the usefulness of combining morphological and molecular approaches for characterization of pigeonpea wilt-associated Fusarium isolates. Further multilocus and pathogenicity studies are recommended for definitive species and pathogenicity confirmation.

Keywords: Pigeonpea wilt, Fusarium oxysporum, ITS sequencing, Molecular identification, Phylogenetic analysis.

1. Introduction

Pigeonpea (Cajanus cajan (L.) Millsp.) is an important leguminous crop extensively cultivated in tropical and subtropical regions, significantly contributing to India's rainfed farming systems. The agricultural commodity is valued for its exceptional dietary protein and is vital for food and nutritional security. Pigeonpea promotes sustainable agricultural methods by enabling biological nitrogen fixation and exhibiting resistance in semi-arid environments. India is the leading worldwide producer of pigeonpea, with Maharashtra serving as a crucial state for its cultivation, mostly in rainfed conditions (Saxena et al., 2010; Varshney et al., 2012).

Despite its importance, the yield of pigeonpea is constrained by numerous biotic and abiotic influences. Fusarium wilt is a major soil-transmitted disease that poses a biotic constraint on pigeonpea farming. The disease is characterised by chlorosis, drooping, and withering of leaves, vascular discolouration, and finally the death of affected plants, leading to considerable yield losses even in ideal environmental conditions. Fusarium udum Butler is recognised as the principal pathogen causing Fusarium wilt in pigeonpea throughout India. Marked variations in pathogenicity and genetic diversity have been recorded among F. udum isolates sourced from different pigeonpea farming regions (Reddy et al., 1993; Srivastava

et al., 2022). Moreover, Fusarium oxysporum has been recorded in association with wilt symptoms in pigeonpea, highlighting the imperative for accurate identification of Fusarium isolates related to the crop (Kale et al., 2024).

The categorisation of Fusarium species has traditionally depended on cultural and morphological characteristics, such as colony colour, growth patterns, pigmentation, and the morphology of macroconidia, microconidia, and chlamydospores. However, considerable morphological variation can occur across isolates, and closely related Fusarium species may exhibit shared characteristics, hindering species-level classification (Leslie and Summerell, 2006). Molecular techniques have consequently become crucial for the accurate identification and characterisation of fungal infections. The internal transcribed spacer (ITS) region of ribosomal DNA is widely employed for fungal identification, while protein-coding genes such as translation elongation factor 1-alpha (TEF-1α) provide superior differentiation among closely related Fusarium species (O'Donnell et al., 1998; Geiser et al., 2004).

PCR-based amplification followed by DNA sequencing provides a reliable approach for confirming the identity of fungal isolates obtained from affected plants. A comparative examination of sequences using databases such as GenBank facilitates the categorisation of isolates into species based on their molecular similarity to established reference sequences. The utilisation of multilocus molecular methodologies has improved the accuracy and reliability of Fusarium identification, particularly within species complexes where ITS sequences may not provide sufficient distinction (O'Donnell et al., 2015; Torres-Cruz et al., 2020).

Considering the importance of pigeonpea cultivation in Maharashtra and the potential impact of soil-borne Fusarium pathogens on productivity, an in-depth analysis of Fusarium isolates associated with pigeonpea fields is essential. As a result, this study aimed to isolate, identify, and molecularly characterise Fusarium oxysporum associated with pigeonpea (tur) cultivation in the Marathwada area of Maharashtra. The study involved isolating the pathogen from infected plant samples, characterising it using cultural and physical characteristics, amplifying it via PCR, and conducting DNA sequencing for molecular confirmation. The findings will provide significant understanding of the occurrence and molecular traits of F. oxysporum associated with pigeonpea in Marathwada and may lay the groundwork for future studies on pathogenicity, genetic diversity, and disease control strategies.

2. Material and methodology

2.1. Experimental site

Laboratory (plate and pot culture) and field experiments were conducted at the Department of Botany, Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra, respectively.

2.2. Disease samples

Stems, leaves and pods of the respective crops exhibiting typical symptoms of the diseases were collected in paper bags from the fields surveyed during the Kharif season of 2023–2024. The diseased samples were brought to the laboratory and subjected aseptically to tissue isolation on Potato Dextrose Agar (PDA) medium for the isolation and identification of the respective fungal pathogens, namely Fusarium oxysporum, Samples were collected from different location of Marathwadha region.

2.3. Culture media

Potato Dextrose Agar (PDA) was used as the basal culture medium for the isolation, purification, and maintenance of pure cultures of Fusarium oxysporum, Macrophomina phaseolina, Colletotrichum lindemuthianum, and Cercospora canescens. For mass multiplication of the respective soil- and seed-borne pathogens, suitable solid substrates such as sand-maize meal medium were used as per the requirement of the pathogen. For studying the cultural and morphological characteristics of the fungal pathogens, synthetic ready-made media (HiMedia, India) and non-synthetic media prepared in the laboratory were used. The required culture media and ingredients were obtained from the Department of Botany, Mukta Nand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra.

Erysiphe polygoni, being an obligate biotrophic fungus, was maintained on naturally infected and susceptible mungbean plants rather than on artificial culture media. Disease development and pathogen characteristics were studied using infected host plant material under suitable environmental conditions.

2.4. Variety

Seeds of selected varieties/genotypes of tur (Cajanus cajan), mungbean (Vigna radiata), and blackgram (Vigna mungo) representing resistant and susceptible reactions to the respective diseases were obtained from available sources including Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, agricultural universities/research institutes in India, and were used for laboratory, pot culture and field experiments. The details of the varieties/genotypes used in the present investigations are presented in Table 1.

Table 1 Selected resistant and susceptible variety of pulses Ref. (https://www.icar-iipr.org.in/varity)

Crop	Variety/ genotype	Disease studied	Reaction	Important characteristics
Tur (Cajanus cajan)	**Maruti (ICP 8863)**	Fusarium wilt (Fusarium oxysporum), Macrophomina phaseolina	Resistant	Medium-duration, semi-spreading and indeterminate type; good adaptation to peninsular India; highly resistant to Fusarium wilt. Macrophomina phaseolina.
	ICPB 2049	Fusarium wilt (Fusarium oxysporum),Macrophomina phaseolina	Susceptible	Pigeonpea genotype reported as susceptible to Fusarium wilt; suitable as a susceptible parent/check for wilt studies.

2.5. Chemicals and glassware

Standard chemicals, reagents, fungicides, culture media, and other materials required for the experimentation were obtained from the Department of Botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra. The common glassware (Borosil, J-Sil and Corning make), viz., Petri dishes, test tubes, conical flasks, volumetric flasks, measuring cylinders, glass rods, beakers, funnels, pipettes, etc., were obtained from the Department of Botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra.

2.6. Equipment

The laboratory equipments viz., Autoclave, Hot air oven, Laminar airflow Cabinet, BOD Incubator, Refrigerator, Binocular Research Microscope, Electronic balance, pH meter, Refrigerator etc. available at the Department of botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra.

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2.7. Survey

In order to determine the occurrence of stem rot and pod rot disease, a roving survey was carried out in the groundnut-growing regions of the eight districts in the Marathwada region during the Kharif and Rabi seasons of 202–2024. The records kept at the Sub-Divisional Agriculture Officer's office in the areas to be surveyed were used to identify groundnut growing fields and pockets. A sample area of 2.0 m^2 was chosen from each groundnut field inspected in order to record observations on the occurrence of stem rot and pod rot. In this area, the total number of groundnut plants and pods exhibiting characteristic symptoms of stem rot and pod rot were tallied. Using the following formula, the data on stem rot and pod rot incidence was used to estimate their % incidence.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants showing disease symptoms}}{\text{Total number of plants observed}} \times 100$$

2.8. Symptomatology

Visual observations were made for manifestation of the groundnut stem rot and pod rot typical symptoms in the groundnut fields surveyed and diseased samples (stem / pod rot) were collected in paper bags and brought to the laboratory for further studies.

2.9. Isolation

Naturally infected plant parts showing typical symptoms of the respective diseases were collected during the Kharif season of 2023–2024 from the surveyed fields of the study area and brought to the laboratory for isolation of the associated fungal pathogens (Table 3.5). The collected samples were washed thoroughly with sterile distilled water, blot dried and cut into small pieces of approximately 5 mm using a sharp sterilized blade. The cut pieces were surface sterilized with 0.1 per cent aqueous mercuric chloride ($HgCl_2$) solution for two minutes and subsequently washed three times with sterile distilled water to remove traces of the sterilizing agent. The sterilized pieces were blot dried and aseptically transferred onto autoclaved and cooled Potato Dextrose Agar (PDA) medium in sterilized Petri plates under aseptic conditions in a laminar-air-flow cabinet. The inoculated plates were incubated in a BOD incubator at 25 ± 2°C. The developing fungal colonies were observed periodically

for their growth and morphological characteristics. The growing fungal cultures were purified by hyphal-tip isolation and repeated sub-culturing on PDA medium. The purified cultures of Fusarium oxysporum, were maintained on PDA slants in test tubes and stored under refrigerated conditions for further studies.

2.10 Fungal culture collection and selection of isolates

Fungal samples associated with wilt-affected pigeonpea (Cajanus cajan L.) plants were collected from different pigeonpea-growing regions of Maharashtra, covering districts such as Chhatrapati Sambhajinagar, Jalna, Beed, Latur, Nanded, Parbhani, Dharashiv and Hingoli. Diseased plant samples showing typical wilt symptoms were collected from different fields and brought to the laboratory for isolation of associated fungal pathogens. The samples were processed using standard isolation techniques, and the resulting fungal cultures were purified and maintained on suitable culture media. Based on colony characteristics, microscopic observations and preliminary identification, several Fusarium isolates were obtained from the collected samples. Among these, representative isolates showing typical cultural and morphological characteristics of Fusarium oxysporum were selected for further molecular characterization. The isolate obtained from Chhatrapati Sambhajinagar was designated as FO-PP-01, while isolates from other locations were assigned unique codes such as FO-PP-02, FO-PP-03, FO-PP-04 and so on. The selected isolates were subsequently subjected to PCR amplification and DNA sequencing for molecular confirmation of their identity (Table 2).

Table 2. Collection and identification of Fusarium oxysporum isolates from wilt-affected pigeonpea fields in different regions of Maharashtra.

Sr. No.	District / Place of collection	Crop	Disease	Fungal pathogen	Isolate code
1	Chhatrapati Sambhajinagar	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-01
2	Jalna	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-02
3	Beed	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-03
4	Latur	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-04
5	Nanded	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-05
6	Parbhani	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-06

7	Dharashiv	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-07
8	Hingoli	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-08

2.11 Mass multiplication of fungal isolate

The Fusarium oxysporum isolate associated with stem canker of pigeonpea was mass multiplied on a sterilized sand–maize medium. The medium was prepared using partially broken maize grains and sand, moistened with distilled water, and sterilized by autoclaving at 121°C (15 psi) for 45–60 minutes. After cooling, the medium was inoculated aseptically with actively growing mycelial discs of Fusarium oxysporum and incubated at 25–28°C for 14–15 days. The thoroughly colonized substrate was used as the inoculum source for artificial inoculation in the field experiment.

2.12Molecular Identification of Fungal Isolates

2.12.1 Genomic DNA extraction

Genomic DNA was extracted from purified fungal isolates using the Plant Mini Kit (QIAGEN, Germany; Cat. No. 69104) following the manufacturer's instructions. Fresh actively growing mycelium was collected, homogenized and subjected to lysis, followed by purification through spin columns. The purified DNA was eluted in elution buffer and stored at −20°C until further analysis. DNA concentration and purity were checked using a microvolume spectrophotometer and its integrity was confirmed by agarose gel electrophoresis.

2.12.2 PCR amplification

The fungal internal transcribed spacer (ITS) region was amplified using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as described by White et al. (1990). PCR was carried out in a 25 µL reaction mixture containing template DNA, PCR buffer, $MgCl_2$, dNTPs, ITS1 and ITS4 primers, Taq DNA polymerase and nuclease-free water. Amplification was performed using an Applied Biosystems Veriti™ 96-Well Thermal Cycler (USA). The PCR programme consisted of initial denaturation, 35 amplification cycles of denaturation, annealing and extension, followed by a final extension, based on the established ITS amplification protocol.

2.12.3 Agarose gel electrophoresis and PCR product purification

The amplified PCR products were separated on agarose gel using a Bio-Rad Mini-Sub® Cell GT Horizontal Electrophoresis System (Bio-Rad Laboratories, USA). The amplified bands were visualized and documented using a Bio-Rad Gel Doc™ system. The desired bands were excised carefully and purified using the QIAquick Gel Extraction Kit (QIAGEN) according to the manufacturer's instructions.

2.12.4 Sanger sequencing and phylogenetic analysis

The purified ITS-PCR products were submitted to Eurofins Scientific Ltd., Pune, Maharashtra, for Sanger sequencing using the corresponding ITS primers. The obtained sequences were quality checked, edited and assembled to generate consensus sequences. The sequences were compared with reference sequences

in the NCBI GenBank database using BLASTn for molecular identification. Phylogenetic analysis was performed using MEGA2 software, and the evolutionary relationships among the fungal isolates and reference sequences were determined (Kumar et al., 2001).

3. Results

3.1 District wise fungal sample collection

Table 3. Collection and identification of Fusarium oxysporum isolates from wilt-affected pigeonpea fields in different regions of Maharashtra.

Sr. No.	District / Place of collection	Crop	Disease	Fungal pathogen	Isolate code
1	Chhatrapati Sambhajinagar	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-01
2	Jalna	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-02
3	Beed	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-03
4	Latur	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-04
5	Nanded	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-05
6	Parbhani	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-06
7	Dharashiv	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-07
8	Hingoli	Pigeonpea (Cajanus cajan)	Wilt	Fusarium oxysporum	FO-PP-08

The data presented in Table 3.5 indicate that fungal cultures associated with wilt-affected pigeonpea plants were collected from eight different locations representing major pigeonpea-growing areas of the Marathwada region of Maharashtra (Table 3). The sampling locations included Chhatrapati Sambhajinagar, Jalna, Beed, Latur, Nanded, Parbhani, Dharashiv and Hingoli. Diseased pigeonpea plants exhibiting typical wilt symptoms were collected from these locations and processed for fungal isolation. Based on cultural and morphological characteristics, the recovered isolates were identified as Fusarium oxysporum. To facilitate further laboratory investigation, the isolates were assigned unique identification codes ranging from FO-PP-01 to FO-PP-08, corresponding to their respective collection locations. The collection of isolates from geographically diverse areas provides an opportunity to assess the occurrence and distribution of F. oxysporum associated with pigeonpea wilt in the region.

The occurrence of Fusarium species in pigeonpea-growing areas is of particular importance because Fusarium wilt is one of the major soil-borne diseases affecting pigeonpea production. Although Fusarium udum is widely recognized as the principal causal pathogen of pigeonpea wilt, the association of other Fusarium taxa with wilt-affected pigeonpea plants emphasizes the need for accurate species-level identification. Srivastava et al. (2022) reported considerable pathogenic and genetic diversity among

Fusarium isolates associated with pigeonpea wilt, highlighting the importance of characterizing isolates from different geographical regions. Similarly, Leslie and Summerell (2006) emphasized that cultural and morphological characteristics are useful for preliminary identification of Fusarium isolates, but molecular approaches are often required for reliable species-level confirmation.

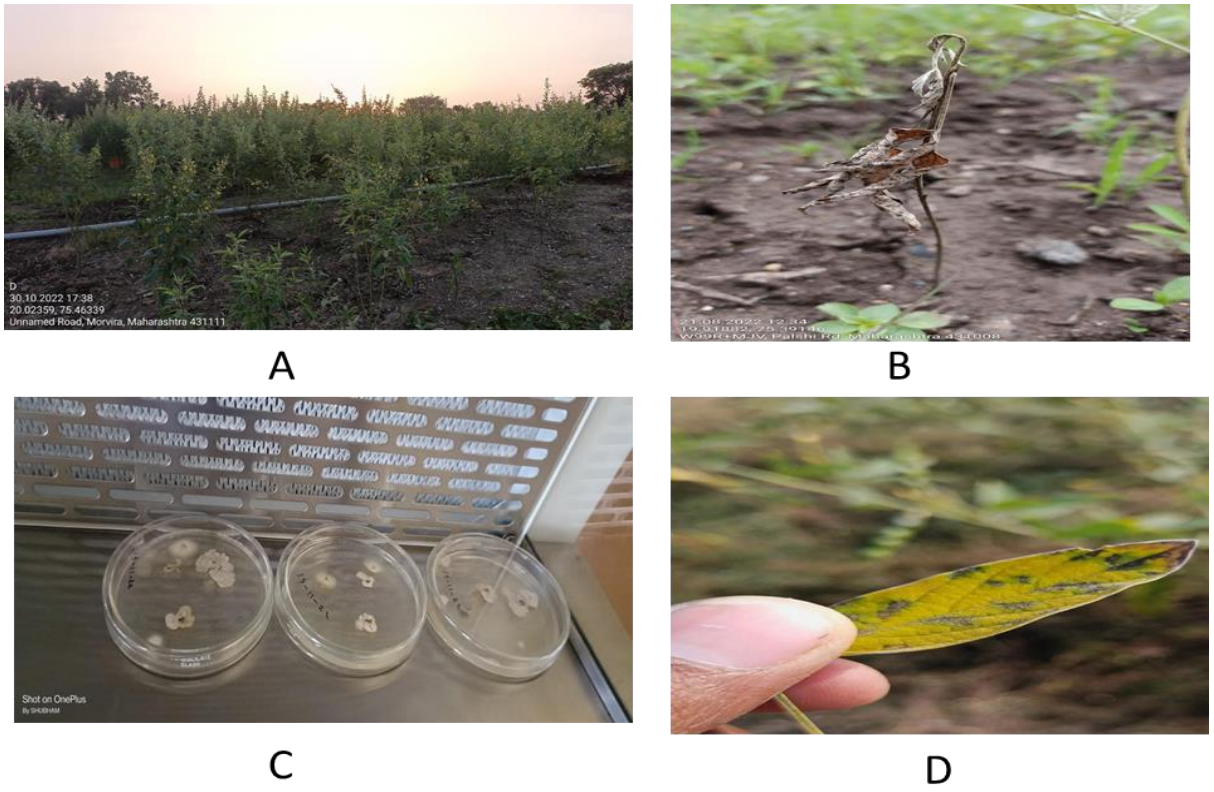
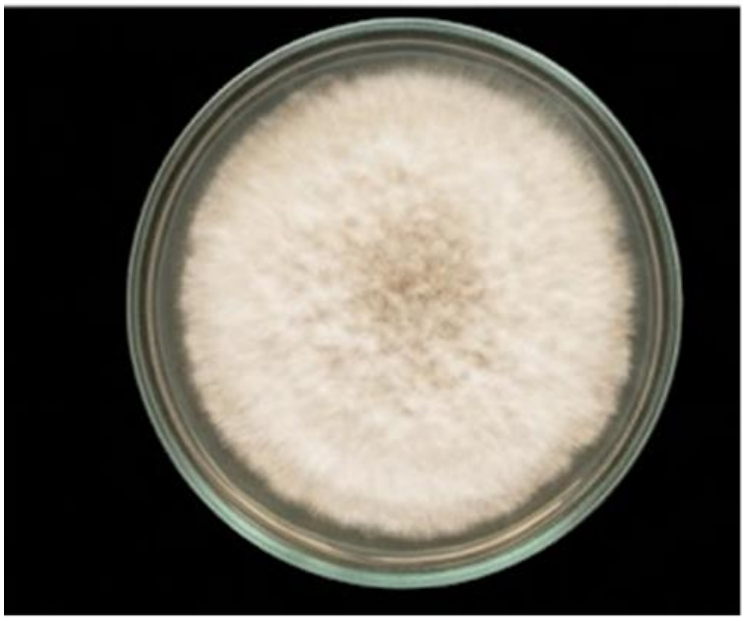


Figure 1. Field symptoms, sample collection and laboratory isolation of Fusarium oxysporum associated with wilt-affected pigeonpea: A) pigeonpea field from which diseased samples were collected; B) wilt-affected pigeonpea plant showing drying and wilting symptoms; C) laboratory isolation of the fungal pathogen from infected plant tissues; and D) diseased pigeonpea leaf showing yellowing, chlorosis and necrosis symptoms.

Figure 1 illustrates the field collection, disease symptoms, and laboratory isolation of the fungal pathogen associated with wilt-affected pigeonpea plants. Panel A shows the pigeonpea field from which diseased plant samples were collected for isolation and identification of the associated fungal pathogen. Panel B shows a wilt-affected pigeonpea plant exhibiting characteristic symptoms such as drying, wilting and progressive plant decline, which are commonly associated with Fusarium wilt. Panel D shows a symptomatic pigeonpea leaf displaying yellowing, chlorosis and necrosis, indicating the progression of disease infection. Panel C represents the laboratory isolation work, where infected plant tissues were placed on culture medium under aseptic conditions for the recovery of the associated fungal pathogen. The development of fungal growth from the infected tissue provided the primary culture for subsequent purification, cultural and morphological identification, and molecular characterization of the Fusarium isolate. Overall, the figure documents the process from field-level disease observation and sample collection to laboratory isolation of the suspected Fusarium pathogen.



A

Figure 2. Different fumgal culture isolates A : Fusarium oxysporum (Cajanus cajan)

Table 4. Sequencing result of isolate fungal strain. FO-PP-01(Cajanus cajan) , Fusarium oxysporum
>FO-PP-01_Fusarium oxysporum partial sequence TCTCTAGGGGGAAATCTCTGAGCAAGCAAAACCAAGGAGACAACTCCCAAACCCCCGGG AACATACCAATTGTTGCCTCGGCGGATCAACTACTCCGCTCCCGGTAACACGGGACGGCC CGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAA TCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGC GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC CCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGG TTTGGTGTTGGGGATCGGCGAGCCCTATTTCAAGCCGGCCCCGAAATCTAGTGGCGGTCT CGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGTACGCGGCGCGGCCAAA CCCGTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCGAAACT TAAGCATATCATTAAGCCGGAGAGA

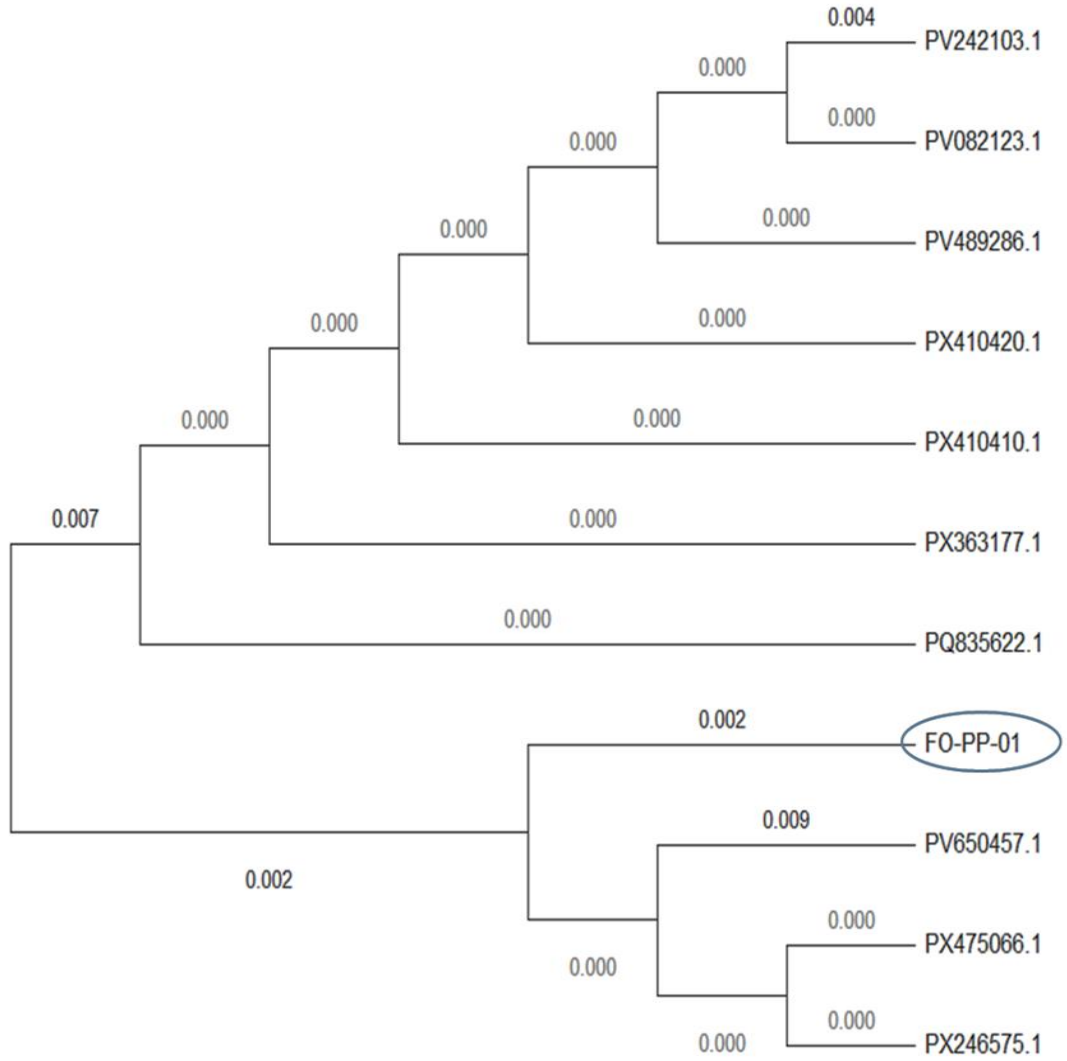


Figure 3. Phylogenetic analysis of FO-PP-01(Cajanus cajan) , Fusarium oxysporum

The selected fungal isolates were further subjected to molecular characterization using ITS1–5.8S rRNA–ITS2 region-based primers. The amplified sequences obtained from these isolates are also presented in Table 4. Fungal cultures associated with wilt-affected pigeonpea plants were collected from different pigeonpea-growing regions of Maharashtra, particularly from the Marathwada region. A total of eight representative isolates were obtained from Chhatrapati Sambhajinagar, Jalna, Beed, Latur, Nanded, Parbhani, Dharashiv and Hingoli districts. The collected samples exhibited typical symptoms of pigeonpea wilt and were processed for isolation of the associated fungal pathogen. Based on cultural and morphological characteristics, the recovered isolates were identified as Fusarium oxysporum and designated with unique isolate codes from FO-PP-01 to FO-PP-08 according to their respective collection locations. The occurrence of Fusarium isolates across geographically separated pigeonpea-growing areas indicates their widespread association with wilt-affected plants in the region. Fusarium wilt is an important soil-borne disease of pigeonpea, and variation among isolates collected from different geographical regions has previously been reported (Leslie and Summerell, 2006; Srivastava et al., 2022). The selected isolates were therefore maintained for further molecular characterization to confirm their identity and assess their genetic relatedness.

Figure 3 Shows the Neighbor-Joining phylogenetic analysis of FO-PP-01, a Fusarium oxysporum isolates obtained from Cajanus cajan. The phylogenetic tree was constructed using ITS rDNA sequences to determine the molecular relationship of the selected isolate with closely related reference sequences. FO-PP-01 was grouped in a cluster with reference sequences PV650457.1, PX475066.1 and PX246575.1, indicating a close genetic relationship with these sequences. The short branch lengths observed within this cluster suggest low genetic divergence among the sequences. In comparison, PV242103.1, PV082123.1, PV489286.1, PX410420.1, PX410410.1, PX363177.1 and PQ835622.1 formed separate lineages, indicating comparatively greater genetic divergence from FO-PP-01. Thus, the ITS-based phylogenetic analysis supported the molecular identification of FO-PP-01 as Fusarium oxysporum .

4. Discussion

The current study uncovered the presence of Fusarium isolates linked to wilt-affected pigeonpea plants gathered from eight districts in the Marathwada region of Maharashtra, specifically Chhatrapati Sambhajinagar, Jalna, Beed, Latur, Nanded, Parbhani, Dharashiv, and Hingoli. The impacted flora displayed typical wilting signs, such as yellowing, chlorosis, desiccation, drooping, and gradual deterioration. Eight fungal isolates were obtained and labelled as FO-PP-01 through FO-PP-08 based on their specific collecting sites. The extraction of isolates from pigeonpea cultivation regions that are geographically distinct suggests the prevalent presence of Fusarium linked to wilt-affected pigeonpea in the examined area. Pande et al. (2013) indicated that Fusarium wilt is a critical soil-borne affliction affecting pigeonpea and highlighted its importance in pigeonpea cultivation.

The retrieved isolates were classified as Fusarium oxysporum based on their cultural and physical traits. The noted colony traits and standard morphological attributes corroborated their initial identification. Leslie and Summerell (2006) highlighted the significance of cultural and physical traits for the initial identification of Fusarium isolates. Nevertheless, physical resemblance among closely allied Fusarium taxa can complicate precise species-level identification. Lombard et al. (2019) emphasised the significant taxonomic intricacy present in the F. oxysporum species complex and advocated for the application of molecular techniques for accurate identification. The current collection of Fusarium isolates from various geographical regions offers valuable resources for additional research on pathogen diversity and disease control.

The chosen isolate underwent molecular characterisation across the ITS1–5.8S rRNA–ITS2 region. PCR amplification yielded a distinct fragment measuring roughly 500–600 bp, thereby validating the successful amplification of the designated fungal ITS region. The sequence derived for FO-PP-01 was analysed against reference sequences found in the NCBI GenBank database utilising BLASTn. ITS-based identification corroborated the association of FO-PP-01 with Fusarium oxysporum. White et al. (1990) pioneered the application of the internal transcribed spacer area for the amplification and sequencing of fungal ribosomal DNA, which has since been extensively utilised for fungal identification.

The Neighbor-Joining phylogenetic assessment additionally corroborated the molecular identification of FO-PP-01. The isolate exhibited a close clustering with F. oxysporum reference sequences PV650457.1, PX475066.1, and PX246575.1, characterised by short branch lengths that suggest little sequence divergence. Alternative reference sequences, such as PV242103.1, PV082123.1, PV489286.1,

PX410420.1, PX410410.1, PX363177.1, and PQ835622.1, established distinct lineages and exhibited relatively higher genetic divergence from FO-PP-01. Comparable molecular investigations have revealed genetic diversity within Fusarium populations and highlighted the efficacy of molecular markers in evaluating their interrelations (Dubey, 2016; Laurence et al., 2014).

The current results hold significance considering the recognised diversity of pathogens linked to pigeonpea wilt. Fusarium udum is acknowledged as the primary pathogen responsible for pigeonpea wilt; nonetheless, significant pathogenic and genetic diversity has been seen among Fusarium isolates linked to pigeonpea (Ravikumara et al., 2022). Reddy and Raju (1996) also illustrated differences in infection and colonisation patterns of Fusarium across pigeonpea genotypes. Consequently, recognising FO-PP-01 as strongly associated with F. oxysporum underscores the necessity for precise characterisation of Fusarium isolates linked to wilt symptoms in various pigeonpea cultivation areas.

In summary, the amalgamation of field symptoms, cultural and morphological traits, ITS amplification, sequence comparison, and phylogenetic analysis corroborated the initial identification of FO-PP-01 as Fusarium oxysporum. Nevertheless, ITS by itself may lack adequate resolution within the F. oxysporum species complex; hence, supplementary loci like TEF1-α and pathogenicity investigations might yield more robust validation (Laurence et al., 2014; Lombard et al., 2019). The isolates gathered in this study may provide significant resources for additional research on pathogenic variability, genetic diversity, and comprehensive management of pigeonpea wilt.

5. Conclusion

The study established the occurrence of Fusarium isolates associated with wilt-affected pigeonpea across eight districts of Marathwada. Cultural, morphological and ITS-based molecular characterization supported the identification of the selected FO-PP-01 isolate as closely related to Fusarium oxysporum. The phylogenetic clustering demonstrated its genetic relationship with reference F. oxysporum sequences, providing a basis for further pathogenicity and disease-management studies.

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