International Journal of Plant & Soil Science

Volume 34, Issue 24, Page 937-948, 2022; Article no.IJPSS.95509
ISSN: 2320-7035

Morpho-molecular Identification and Pathogenicity of *Fusarium oxysporum* Pathogens Associated with Vascular wilt in Cowpea (*Vigna unguiculata* L.)

A. M. Shahiba [a#*], V. G. Jayalekshmy [b†], A. Sajeena [c‡] and Arun Chacko [a#]

[a] Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Trivandrum, Kerala, India.
[b] Department of Seed Science and Technology, College of Agriculture, Vellayani, Trivandrum, Kerala, India.
[c] Department of Plant Pathology, IFSRS, Karamana, Trivandrum, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i242721

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/95509

Original Research Article

Received: 28/10/2022
Accepted: 30/12/2022
Published: 31/12/2022

ABSTRACT

Fusarium oxysporum (Fo), causes vascular wilt in cowpea is a serious pathogen, which leads to severe yield loss and high plant mortality. Variability among the pathogen is very low, due to the lack of sexual reproduction, and low mutation rate. In this study ten different isolates of Fo, collected from various of locations, were evaluated and tested for morphology, molecular biology,

[#] PhD Scholar;
[†] Professor and Head;
[‡] Assistant Professor;
**Corresponding author: E-mail: shahiba6408@gmail.com;*

and pathogenicity as well. Fo consists of asexual spores, macroconidia, microconidia and chlamydospores, with varied dimensions and produced some pigments in culture media. Sand maize media (10%) was used in pathogenicity test and was found the best than other methods for screening Fo in cowpea. The Fo DNA were amplified with Internal Transcribed Spacer (ITS) primer and the obtained sequences were deposited in the gene bank (GenBank) database. The sequences were compared with the available database and showed 99-100% homology with Fo. Whereas, the phylogenetic study revealed that the isolates were not distant from *Fusarium* species. The phylogenetic analysis helps to understand the evolutionary relationship among the species identified.

Keywords: Fusarium; phylogenetics; pathogenicity; molecular primer; database.

1. INTRODUCTION

Fusarium oxysporum (Order: *Hypocreales*, Family*: Nectriaceae*), is a common and active soil saprophyte that attack organic matter and some specific forms are plant pathogens. More than 120 forms (*formea speciales*, ff. spp.) were identified based on host species specificity. However, according to scientific and/ or economic importance Fo is suggested the fifth of the top plant pathogens in quarantine in several countries [1]. The saprophytic ability of the pathogen enables it to survive either as mycelium or spore in the soil plant debris [2]. The pathogen mainly produces three types of asexual spores, one or two celled microconidia, three to five celled macroconidia and, dormant, round, and thick walled chlamydospores [3]. *Fusarium oxysporum* considered as low genetic variable species, due to lack of sexual reproduction, which leads to little genetic flow and low mutation rates [4]. Plants growing in a soil contaminated with the fungus, will become infected, and the pathogen invades the plant either with its sporangial germ tube or mycelium through the roots [5]. The fungal mycelium grows through the root cortex, intercellularly, reaches to xylem vessels, and then advances upwards towards the stem and crown of the plant [6]. The mycelium produces microconidia which spreads through the xylem pits to adjacent cells. Due to the growth of mycelium within the plant's vascular tissue, plant becomes thirsty, closes stomata, wilts the leaves, and eventually dies [7]. Infection of vascular vessels, turns the tissues to brown colour due to the pigments produced by the pathogen, accordingly, the browning is strong evidence of *Fusarium* wilt [2]. In the older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant [8,9].

Legumes are mostly affected by *Fusarium* wilt, mainly chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris* L.), Alfalfa (*Medicago sativa* L.) etc, which cause devastating damages in legume species worldwide [10]. Each crop infected by a host specific *Fusarium* sp., usually characterizes Fo ff. spp., however there are examples that reveal a broader host range, infecting more than one legume species [11]. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum,* in cowpea, produces high plant mortality with severe overall yield loss [12]. As a soil born disease, the management of these pathogen is not under control, even though it requires continuing management practices and soil solarization. Because of the presence of dormant chlamydospores in the soil for several years, occurrence of disease during cropping season is unbeatable [13]. Complete management of pathogen requires studying the morphology, growth pattern, pathogenicity, and the environmental requirements for the Fo establishment. This study focussed on the variability, morphology, and molecular identification of pathogen (Fo) causing *Fusarium* wilt in cowpea.

2. MATERIALS AND METHODS

2.1 *Fusarium* Isolates and Culture Condition

The infected cowpea plants (*Vigna unguiculata* L.) were collected from ten different cowpea fields, stem and roots were washed in a running water to remove adhered soil particles. The infected collar portion and root bits were cut into small pieces of 1-2 mm size and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) followed by three times washes with sterile water. The 4-5 small bits were transferred aseptically into a petridish containing solidified

Potato Dextrose Agar (PDA) amended with streptomycin sulphate and tetracycline hydrochloride to minimize chances of bacterial growth. Plates were incubated at 28ºC and observed periodically. The fungal isolates were grown on PDA to study their growth, variability, and colony characters. A fungus disc of 5 mm was cut from a 3 days old culture plate by sterilized cork and kept at the centre of each fresh Petri dishes with 15ml of sterilized and solidified PDA, incubated for 3 days at 28± 2ºC temperature. The grown fungal isolates transferred into fresh PDA slants for maintenance and stored at 4ºC [14]. Three days after inoculation (DAI), the colony characters, mycelial growth and spore characters were recorded.

2.2 Morphological and Cultural Evaluation of Fungal Strain

A periodic observation for the ten different fungal strains was undergone to study the rate of growth (mm/day), and microscopic characterization of microconidia, macroconidia, and presence of chlamydospore. The external appearance of culture, production of pigments and the pattern of growth in each strain isolated were studied [15].

2.3 Pathogenicity Testing of Fungal Strain

The isolates were assessed for liability to cause diseases in the healthy cowpea plants. Different inoculation methods, *viz.,* Sand Maize Media (SMM), SMM+ Pin Prick, Pin Prick and Control were tested to know the effective way for inoculation. The sand: maize (9:1) media (SMM) was prepared by adding 90g of riverbed sand and 10g of maize meal with 20ml of distilled water in 250ml of Erlenmeyer flask. The medium was autoclaved in the flask at 15 lb for 20 minutes. After cooling the medium, 10 bits of fungal culture along with PDA was aseptically transferred into the medium containing flask and closed air tightly. Allowed the fungus to multiply inside the medium for 2 weeks. SMM was mixed with sterile soil (10% w/w), kept it for one week and healthy cowpea seedlings of two-week-old was transplanted to it. Pin Prick method was done by creating pin holes on the leaves and stem of two-week-old cowpea seedling, and introduced the conidial suspension (1×10^6 cfu) [16] using sterile cottons. To standardise the concentration of SMM was done at different percentages *viz*., 1%, 2%, 5%, 10%, 15%, 20% and 25% SMM, along with control, were inoculated in sterile soil (w/w) for fixing the effective concentration to cause disease in healthy plants, and two-week-old cowpea seedlings were transplanted to it, and monitored regularly. The fungal strain showed the *Fusarium* symptoms in healthy plants were carried to molecular identification with universal primers, to obtain ITS region amplification for comparison with reference database from NCBI.

2.4 Molecular Identification of Fungal Strain

2.4.1 Genomic DNA extraction and ITS amplification

DNA was extracted from 5 pathogenic *Fusarium* spp. isolates using NucleoSpin® Plant II Kit (Macherey-Nagel). The eluted DNA was stored at 4°C. The quality of DNA was checked using 0.8% Agarose Gel Electrophoresis (AGE). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). The ITS region of fungal strain was amplified with universal primers ITS-1F (5'TCCGTAGGTGAACCTGCGG3') and ITS-4R (5'TCCTCCGCTTATTGATATGC3') [17]. The details of amplification mixtures and profile were depicted below,

Amplification mixtures		Amplification profile (40 Cycles)		
2X Phire Master Mix	5Ml	Initial Denaturation	98 °C	30 Sec
D/W	4µL	Denaturation	98 °C	5 Sec
Forward Primer	0.25µL	Annealing	58 °C	10 Sec
Reverse Primer	0.25µL	Extension	72 °C	15 Sec
DNA	1µL	Final extension	72 °C	60 Sec

2.4.2 Sequencing of ITS Region Using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 [18].

2.4.3 Comparison of ITS sequences with reference database deposited in NCBI

The sequences were trimmed, assembled and consensus sequences were obtained using BioEdit software. The obtained nucleotide sequence was searched through Basic Local Alignment Search Tool for nucleotides (BLASTN) at GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) to compare with ITS reference sequence available in the database for identification of the isolate. Multiple sequence alignment of ITS sequence with reference sequence from gene bank was performed using Clustal W available in BioEdit Software. Phylogenetic evolutionary relationship analysis of the fungal ITS sequences was performed using Molecular Evolutionary Genetic Analysis software (MEGA 7). Phylogenetic analysis was carried out using Neighbor-joining method with 100 bootstraps run [19].

3. RESULTS AND DISCUSSION

3.1 Morphological and Cultural Evaluation of Fungal Strain

Ten different cultures were obtained (Table 1), isolated, and the morphological and cultural evaluation were done. Each isolate was distinct in the appearance, some produced pigments in the PDA media, which turned the colour of culture plate. Most of the isolates produced cottony white raised mycelium, pale yellow to dusky red colour pigmentation and moderate to profuse sporulation on PDA medium. Varied colour of *Fusarium* culture plate from normal white to violet, brown, reddish violet, yellowish pink, and dark pink were observed in different isolates of *F. oxysporum* f sp. *Ciceris* [20,21]. Characterized greyish white to pinkish colour pigments production in *F. solani* isolates. *Fusarium* spp. has been recognized as a natural source of coloured polyketidic secondary metabolites; among that red naphthoquione pigments like bikaverin, aurofusarin, fusarubin, bostrycoidin, javanicin and novarubin, as most abundant followed by hydroxyanthraquinoic pigments; but there is no relationship between pathogenicity and pigments produced [22]. The concomitant production of bikaverin along with 2 other novel pigments by phytopathogenic *Fusarium oxysporum* LCP531 strain isolated from soil were reported [23]. Pigmentation was the characteristic evident for the presence of *Fusarium* pathogen. No odor was detected. *Fusarium* pathogen is well known by its three types of asexual spores, macrospores, microspores and chlamydospores depending on the host availability and prevailing environmental conditions but, the sexual or teleomorphic stage is unknown. The shape and septations of these spores make each pathogen species unique, and these are one of the morphological markers for species identification. Macroconidia showed 3-5 septate, curved or straight with pointed or round ends; microconidia with one or no septum, unicellular oval, or kidney shaped, and chlamydospores are singly or in pairs, intercalary or terminal in position with globular or oval shape. The ratio between production of micro conidia and macro conidia depends on the environment and the species produced [24]. Normally the number of microconidia is much more than that of macroconidia, and most abundantly and frequently produced by the fungus under all conditions [2].

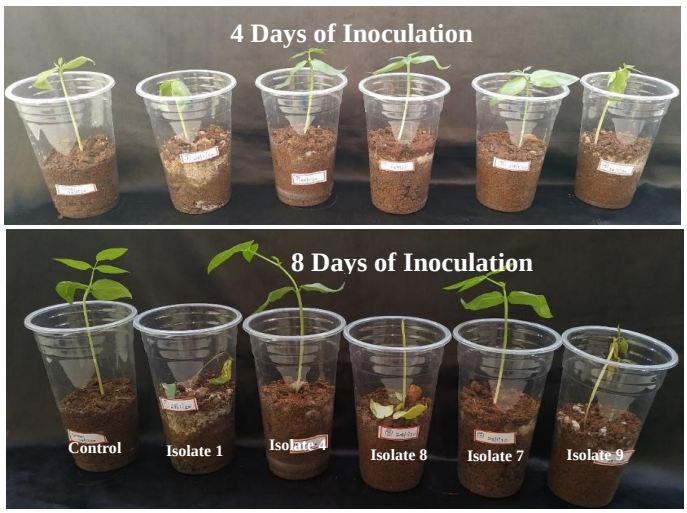
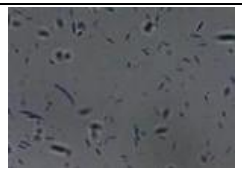
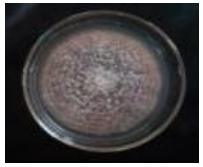
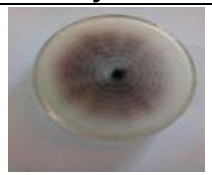
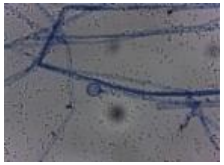
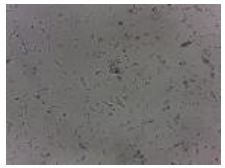
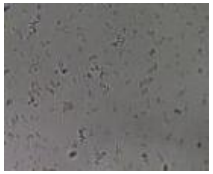
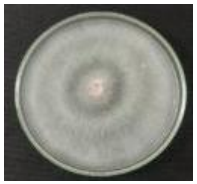
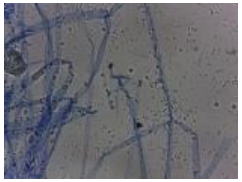
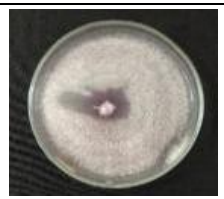
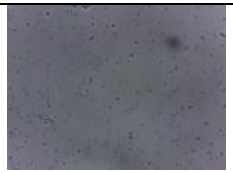
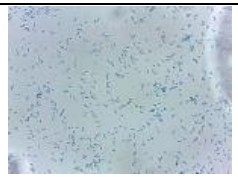
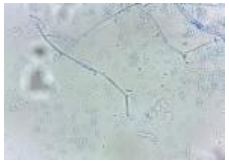
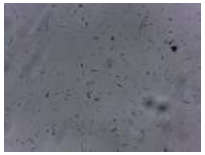
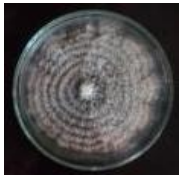
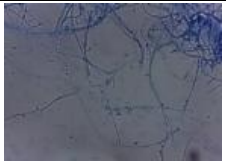
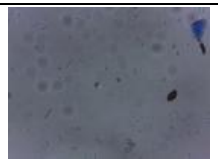
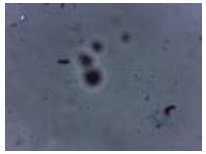
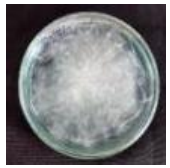
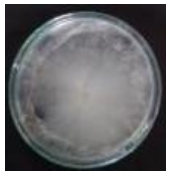
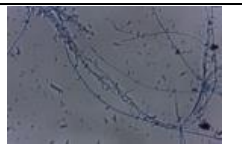
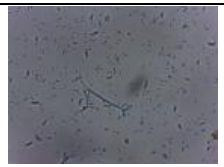
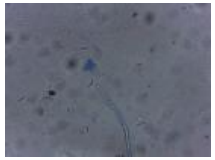
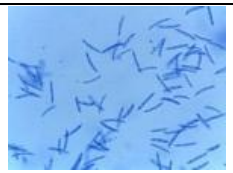


Fig. 1. Pathogenicity analysis of isolated *Fusarium* pathogens

Table 1. Morphological evaluation of ten isolates of *Fusarium* spp.

	Features	Microspores	Megaspores	Surface of colony on PDA	Reverse of colony on PDA	Mycelium
Isolate 1	Growth rate 5mm/day at 24°C; White raised mycelia with reddish brown culture; aseptate microconidia (12.879×3.570 µm); 3-4 septate macroconidia (32.409×5.297 µm), little curved; intercalary and axial chlamydospores present; false head appearance,					
Isolate 2	Growth rate 20mm/day at 24°C; little raised mycelium without pigment production, micro and macro conidia were absent; chlamydospores present					
Isolate 3	Growth rate 20mm/day at 24°C; little raised mycelium with white and light pink colour at centre, micro and macro conidia were absent; chlamydospores present					
Isolate 4	Growth rate 6mm/day at 24°C; White raised mycelia with little pink coloured culture; aseptate microconidia (11.830×3.914 µm); 3 septate macroconidia (30.528×4.762 µm); intercalary and axial chlamydospores;					

	Features	Microspores	Megaspores	Surface of colony on PDA	Reverse of colony on PDA	Mycelium
Isolate 5	Growth rate 3mm/day at 24°C; White raised mycelia without pigmentation; aseptate microconidia (8.419×2.547 µm); 2 septate macroconidia (28.870×3.629µm); intercalary and axial chlamydospores;					
Isolate 6	Growth rate 3mm/day at 24°C; White raised mycelia with little pink coloured culture; aseptate microconidia (9.802×1.974 µm); 2 septate macroconidia (26.692×2.662 µm); no chlamydospores were present;					
Isolate 7	Growth rate 6.5mm/day at 24°C; White raised mycelia with red to dark brown coloured culture; aseptate microconidia (10.548×2.843 µm); 2 septate macroconidia (27.764×3.541 µm); intercalary chlamydospores were present;					
Isolate 8	Growth rate 4mm/day at 24°C; White raised mycelia with white coloured culture; a septate microconidium (7.340×1.973 µm); 2 septate macroconidia (25.591×2.502 µm); intercalary chlamydospores;					

	Features	Microspores	Megaspores	Surface of colony on PDA	Reverse of colony on PDA	Mycelium
Isolate 9	Growth rate 6.5mm/day at 24°C; suppressed mycelia with light yellow coloured culture; aseptate microconidia (8.339×2.159 µm); 2 septate macroconidia (28.659×2.751 µm); axial chlamydospores;					
Isolate 10	Growth rate 6mm/day at 24°C; suppressed mycelia with dark brown coloured culture; aseptate microconidia (12.435×4.914 µm); 5-6 septate macroconidia (37.620×10.772 µm); axial chlamydospores;					

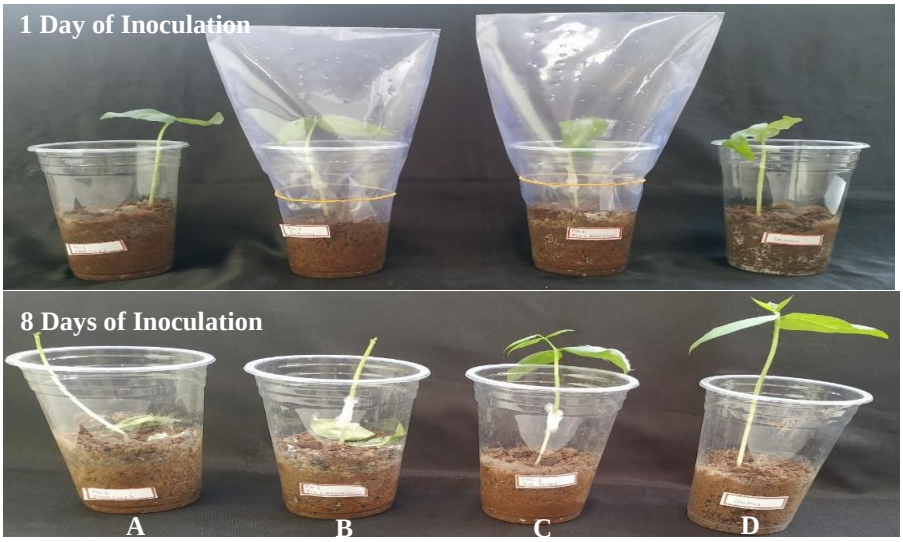


Fig. 2. Inoculation methods for *Fusarium* isolates*;* A) SMM (10%), B) Pin Pricking+ SMM (10%), C) Pin Pricking, D) Control

The growth rate of isolated pathogen in PDA media was varied from 3mm/day to 20mm/day. Microconidia was sized from 7.340 to 12.879 µm by 1.794 to 4.914 µm. microconidia was 9-10 × 1-3 µm in *Fusarium solani* causing dry root rot in citrus [25], and 6.33 ×1.50 µm of *Fusarium oxysporum* f. sp. *Chrysanthemi* associated with vascular wilt of chrysanthemum [26]. While, size of macroconidia of isolates ranged from 25.591 to 37.620 µm by 2.502 to 10.772 µm. Macroconidia size varied from 37.5-50 (42) µm in length by 5-6.3 (5.5) µm in width of *Fusarium oxysporum* associated with *Carya illinoinensis* seedlings [27], 15.11 × 2.88 µm of *Fusarium oxysporum* f. sp. *Chrysanthemi* associated with vascular wilt of chrysanthemum [26]. Chlamydospores are thick-walled dormant spores, which are intercalary or terminal in position. These are the main survival structures of *Fusarium oxysporum* under field condition and severe disease developing than microconidia do in tomato [28]. The dormant spores get germinated under favourable environmental condition as well as by the presence of host plant root exudates. Chlamydospores of *Fusarium oxysporum* germinated efficiently in the root exudates of tomato and reached up to 98% of germination after10 h of incubation period [29].

3.2 Pathogenicity Testing of Fungal Isolates

Among the ten isolates, five isolates were selected according to the cultural and morphological evaluation as *Fusarium* pathogen, and pathogenicity test was carried out (Fig. 1). Inoculation methods provides sufficient inoculum load to the plants for causing the disease. So, the different methods were assessed using the *Fusarium* pathogen, in which inoculation with sand maize base showed sufficient pathogenicity than pin prick method of inoculation (Fig. 2). The rice-grain inoculation to test pathogenicity of *R. solani*, *M. phaseolina*, *F. oxysporum* and *F. solani* on various pepper cultivars were recommended [30]. Great significant variations were observed among the isolates of the pathogens in causing disease. The isolates 1,8, and 9 were showed fusarium wilt symptoms after 8 days of inoculation with leaf necrosis, stem basal discoloration and total wilting of seedlings in the inoculated plants. Isolates 4 and 7, also showed symptoms to some extent, because of the less virulent capacity of these isolates than other three virulent strain. The isolate 1 was more virulent among the isolates, so different inoculation percentage were tried to fix an effective one. Soil inoculation of 10% of pathogen multiplied sand maize (9:1) media was most effective percentage to cause disease. Ten percentage soil inoculation produced *Fusarium* symptoms, and might be used for disease screening using the culture (Fig. 3). Coriander plants showed *Fusarium* symptoms, when 5% sand maize media was used for disease screening [31], 4% soil inoculation for *Fusarium oxysporum* f sp. *ciceri* screening in chickpea [32], and 10% inoculation for *Fusarium oxysporum* f.sp. *lycopersici* in tomato were used [33].

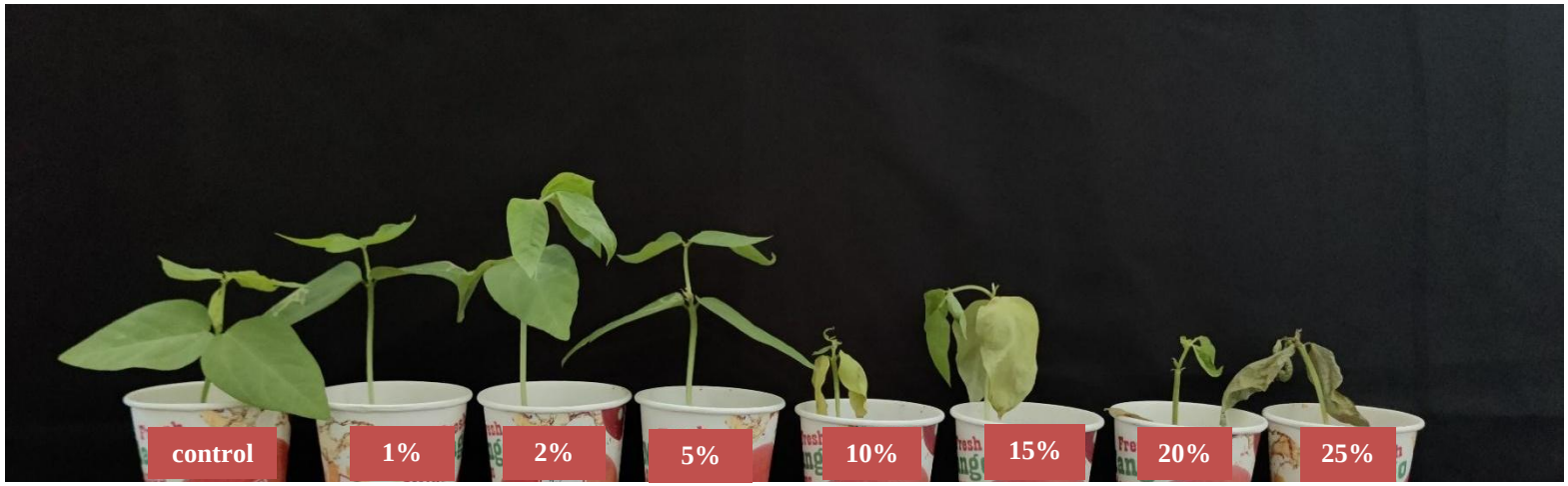


Fig. 3. Different inoculation percentages of *Fusarium* isolates

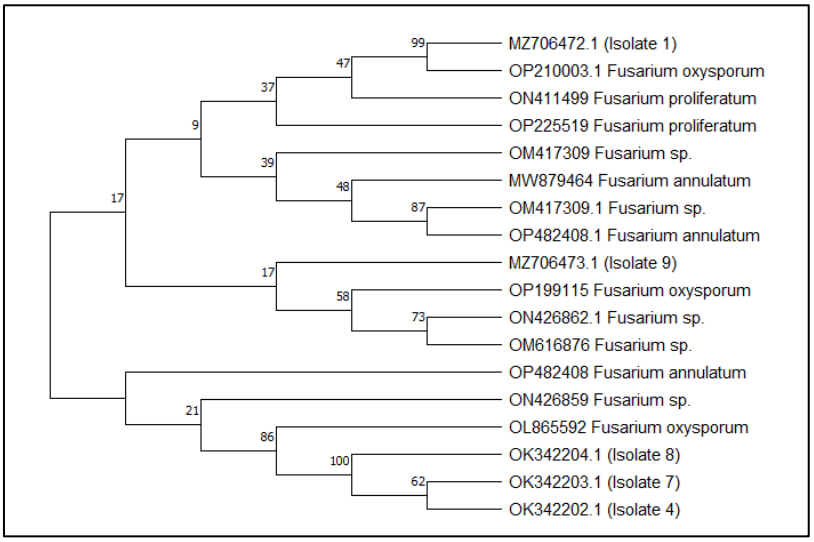


Fig. 4. Phylogenetic analysis of Fusarium isolates with the database sequences

Table 2. Identity percentage and homology sequences of *Fusarium* isolates with GenBank database

Isolates	Accession number	Identity percentage (%)	Homology sequence
Isolate 1	MZ706473.1	100%	*Fusarium* sp. strain (OM417309.1)
Isolate 4	MZ706472.1	100%	*Fusarium oxysporum* isolate (OP684344.1)
Isolate 7	OK342204.1	100%	*Fusarium* sp. strain (MG098654.1)
Isolate 8	OK342203.1	100%	*Fusarium oxysporum* isolate (MK239978.1)
Isolate 9	OK342202.1	100%	*Fusarium oxysporum* isolate (MK968007.1)

3.3 Molecular Identification of Fungal Strain and Phylogenetic Analysis

The accurate identification of pathogen species by visual examination of morphological criteria is very difficult and erroneous. Molecular identification provides unique barcode for determination and identification of different fungal isolates, taxonomy, molecular evolution, population genetics and fungus-plant interaction [34]. Molecular identification was carried out by DNA barcoding using ITS region sequencing. The data were trimmed, assembled and consensus sequences were obtained, deposited, and provided accession number, and it was compared to those in the database using NCBI-BLAST. Five isolates were identified using DNA barcoding with an identity range between 99-100% (Table 2). The ITS rDNA are the excellent candidate genes for phylogenetic analysis because they are universally distributed, functionally constant, sufficiently conserved, and of adequate length to provide a deep view of evolutionary relationship [35]. Phylogenetic analysis showed the isolates to be related to the *Fusarium* database deposited in National Centre for Biotechnology Information **(**NCBI). Isolate 1 was related to *Fusarium oxysporum* (OP210003.1) by 99% bootstrap (Fig. 4).

4. CONCLUSION

The study was done for morpho-molecular identification and pathogenetic analysis of *Fusarium* isolates obtained from diseased cowpea plants from different geographical locations. The *Fusarium* pathogen was consisting of microconidia, macroconidia and chlamydospores, without sexual stage. Each pathogen varied in their conidial characters and pathogenicity in terms of its disease development. Sand maize (9:1) media is the best for pathogen culturing and inoculation method (with 10% inoculum). The ITS region was amplified with universal primers and the sequences were obtained, deposited in the NCBI database. Homology percentage was calculated and 99-100% similarity was observed with the

already deposited database. Phylogenetic analysis helped in understanding the evolutionary relationship among the pathogen isolates. So, the five isolates were identified as *Fusarium oxysporum* using morphological and molecular data obtained.

ACKNOWLEDGEMENT

The authors wish to thank financial contributions from the Department of Science and Technology (DST) as INSPIRE Fellowship and Kerala Agricultural University (KAU). We also thank Integrated Farming Systems Research Station (IFSRS), Karamana for providing laboratory facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sampaio AM, Araújo SDS, Rubiales D, Vaz Patto MC. *Fusarium* wilt management in legume crops. Agronomy. 2020; 10(8):1073.
2. Agrios GN. Plant Pathology, 3rd. ed. Academic Press, Inc.: New York. 2005;803.
3. Roncero MIG, Hera C, Ruiz-Rubio M, Maceira FIG, Madrid MP, Caracuel Z, Calero F, Delgado-Jarana J. Roldán-Rodríguez R. Martínez-Rocha AL, et al. *Fusarium* as a model for studying virulence in soilborne plant pathogens. Physiol. Mol. Plant Pathol. 2003;62:87–98.
4. McDonald BA, Linde C. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 2002;40:349–379.
5. Perez-Nadales E, Di Pietro A. The membrane mucin Msb2 regulates invasive growth and plant infection in *Fusarium* oxysporum. Plant Cell. 2011;23:1171–1185.
6. Perez-Nadales E, Almeida Nogueira MF, Baldin C, Castanheira S. El Ghalid M, Grund E, Lengeler K, Marchegiani E, Mehrotra PV, Moretti M, et al. Fungal model systems and the elucidation of pathogenicity determinants. Fungal Genet. Biol. 2014;70:42–67.
7. Bishop CD, Cooper RM. An ultrastructural study of vascular colonization in three vascular wilt diseases. Colonization of susceptible cultivars. Physiol. Plant Pathol. 1983;23:323–343.
8. Smith IM, Dunez J, Phillips DH, Lelliott RA, Archer SA. European handbook of plant diseases. Blackwell Scientific Publications: Oxford. 1988;583.
9. Michielse CB, Rep M. Pathogen profile update: *Fusarium oxysporum*. Mol. Plant Pathol. 2009; 10:311–324.
10. Williams AH. Sharma M, Thatcher LF, Azam S, Hane JK, Sperschneider J, Kidd BN, Anderson JP, Ghosh R, Garg G, et al. Comparative genomics and prediction of conditionally dispensable sequences in legume-infecting *Fusarium oxysporum* formae speciales facilitates identification of candidate effectors. BMC Genome. 2016;17:191.
11. Edel-Hermann V, Lecomte C. Current status of *Fusarium oxysporum* formae speciales and races. Phytopathol. 2019;109:512–530.
12. Omoigui LO, Danmaigona CC, Kamara AY, Ekefan EJ, Timko MP. Genetic analysis of *Fusarium* wilt resistance in cowpea (*Vigna unguiculata* Walp.). Plant Breeding. 2018; 137(5):773-781.
13. Okiror MA. Genetics of resistance to *Fusarium udum* in pigeon pea [*Cajanus cajan* (L.) Millsp.]. Indian J. Genet. Plant Breed. 2002;62:218–220.
14. Aneja KR. Experiments in microbiology, plant pathology and biotechnology. New Age International. (P) Ltd. Publishers, New Delhi. 2007;60.
15. Lombard L, Sandoval-Denis M, Lamprecht SC, Crous PW. Epitypification of *Fusarium oxysporum* - clearing the taxonomic chaos. Persoonia; 2019.
16. Davydenko K, Nowakowska JA, Kaluski T, Gawlak M, Sadowska K, García JM, Diez JJ, Okorski A, Oszako T. A comparative study of the pathogenicity of *Fusarium circinatum* and other *Fusarium* Species in Polish Provenances of *P. sylvestris* L. Forests. 2018;9(9):560.
17. White TJ, Bruns T, Lee SJWT, Taylor J. Amplification, and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications. 1990;18(1):315-322.
18. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R. Stones-Havas S, Sturrock S, Thierer T, Geneious v5. 1; 2010.

19. Mkumbe BS, Sajidan Pangastuti A, Susilowati A. September. Phylogenetic analysis based on internal transcribed spacer region (ITS1-5.8 S-ITS2) of *Aspergillus niger* producing phytase from Indonesia. In AIP Conference Proceedings. AIP Publishing LLC. 2014;1: 020015:2018,
20. Honnareddy N, Dubey SC. Pathogenic and molecular characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Curr. Sci. 2006;661-666.
21. Chavan SS. Studies on fungal diseases of patchouli with special reference to wilt caused by *Fusarium solani* (Mart.) Sacc. University of Agricultural Sciences, Dharwad. 2007;98.
22. Caro Y, Venkatachalam M, Lebeau J, Fouillaud M, Dufosse L. Pigments, and colorants from filamentous fungi, In *Fungal Metabolites* (Merillon, J.M. and Ramawat K. G. eds,). Cham: Springer International Publishing. 2017;499-568.
23. Lebeau J, Petit T, Clerc P, Dufosse L, Caro Y. Isolation of two novel purple naphthoquinone pigments concomitant with the bioactive red bikaverin and derivates thereof produced by *Fusarium oxysporum*. Biotechnol. Progress. 2019; 35(1):2738.
24. Wyatt TT, Wösten HA, Dijksterhuis, J., Fungal spores for dispersion in space and time. Adv. Appl. Microbiol. 2013; 85:43-91.
25. Chandran MR, Kumar MR. Studies on cultural and morphological variability in isolates of *Fusarium solani* (Mart.) Sacc., incitant of dry root-rot of Citrus. Current Biotica. 2012;6(2):152-162.
26. Anand C, Revathy N, Manonmani K, Balasubramanian P. Morphological and cultural characterization of *Fusarium oxysporum* f. sp. *Chrysanthemi* Associated with Vascular Wilt of Chrysanthemum. Res. J. Agric. Sci. 2018;9(4):856-859.
27. Poletto T, Muniz MFB, Fantinel VS, Harakava R, Rolim JM. Characterization and pathogenicity of *Fusarium oxysporum* associated with *Carya illinoinensis* seedlings. Floresta e Ambiente. 2020;27.
28. De-Cal A, Pascual S, Melgarejo P. Infectivity of chlamydospores vs microconidia of *Fusarium oxysporum* f. sp. *lycopersici* on tomato. J Phytopathol. 1997;145:231–233.
29. Akhter A, Hage-Ahmed K, Soja G, Steinkellner S. Potential of *Fusarium* wilt-inducing chlamydospores, in vitro behaviour in root exudates and physiology of tomato in biochar and compost amended soil. Plant and Soil. 2016; 406(1):425-440.
30. Guney İG, Guldur E. Inoculation techniques for assessing pathogenicity of *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum* and *Fusarium solani* on pepper seedlings. Türkiye Tarımsal Araştırmalar Dergisi. 2018;5(1):1-8.
31. Ashwathi S, Ushamalini C, Parthasarathy S, Nakkeeran S. Morphological and molecular characterization of *Fusarium* spp. associated with vascular wilt of coriander in India. J. Pharmacognosy and Phytochem. 2017;6(5):1055-1059.
32. Kala C, Gangopadhyay S, Godara SL. Eco-friendly management of wilt caused by *Fusarium oxysporum* f. sp. *ciceri* in chickpea. Legume Res. 2016;39(1):129-134.
33. Manikandan R, Harish S, Karthikeyan G, Raguchander T. Comparative proteomic analysis of different isolates of *Fusarium oxysporum* f. sp. *lycopersici* to exploit the differentially expressed proteins responsible for virulence on tomato plants. Frontiers in Microbial. 2018;9: 420.
34. Alsohaili SA, Bani-Hasan BM. Morphological and molecular identification of fungi isolated from different environmental sources in the Northern Eastern desert of Jordan. Jordan J. Biol. Sci. 2018;11(3).
35. Madigan MT, Martinko JM, Stahl DA, Clark DP. Brock Biology of Microorganisms.13th ed. Pearson Prentice Hall, New Jersey. 2012;456.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/95509