

Isolation and molecular characterization of *Fusarium solani* as a causal agent of Fusarium wilt on cowpea (*Vigna unguiculata*)

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Abstract

Lahuf, A. A., Abdulmoohsin, R. G. & Hameed, Z. L. (2023). Isolation and molecular characterization of *Fusarium solani* as a causal agent of Fusarium wilt on cowpea (*Vigna unguiculata*). *Bulg. J. Agric. Sci.*, 29(5), 946–950

During season of 2020, cowpea (*Vigna unguiculata*) plants exhibiting sever wilt symptoms were collected from several fields in Kerbala Province of Iraq. A fungus was consistently isolated from the symptomatic tissues and identified as *Fusarium solani*, based on its cultural and morphological features, as well as the sequence analysis of the internal transcribed spacer (ITS), translation elongation factor-1 alpha (TEF-1 α) and actin (actA). The pathogenicity of this fungus was verified on cowpea plants that reproduced the typical wilt symptoms. The fungal pathogen was re-isolated from these diseased plants and reidentified. To best of our knowledge, this is the first record of *F. solani* causing cowpea wilt in Kerbala, Iraq.

Keywords: Cowpea; wilt disease; *Fusarium solani*

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.), belonging to the family of Fabaceae, is native to West Africa, and currently is commonly grown worldwide (Singh, 2020). It is one of the world-leading legume crops with the global production of 7.249.060 t in 2018. However, in Iraq, the production is low accounting for ~ 0.005% of total world production with yield of 0.418 t/ha and production of 396 t (FAOSTAT, 2020). Various biotic and abiotic factors, have contributed in this production reduction involving many fungal pathogens that are commonly and widespread on this crop (Al-Ani et al., 1989; Abdulmoohsin et al., 2019).

Fusarium solani is one of those pathogens, that cause various diseases in many agriculturally essential crops (Jaber & Lahuf, 2020). This species is complex representing a biologically and genetically complex group that adapt numerous formae speciales (Schroers et al., 2016). Newly, several molecular identification approaches, implicating the phylogenetic analysis of various genetic markers, such as

internal transcribed spacer (ITS) regions, RNA polymerase II (RPB1), translation elongation factor-1 α (TEF-1 α) and actin (actA) genes, have been developed for identification of this species and others (O'Donnell et al., 2008; Lahuf et al., 2020).

During a survey conducted for evaluating the occurrence of cowpea crop diseases in 2020, sever wilt symptoms were observed in cowpea fields in the Kerbala Province of Iraq, with approximately 10-20% of disease incidence. The symptoms displayed on the seedlings as leaves withering and turning into color yellow then brown, afterward they dried up and dropped off. The transport tissues were colored to brown or black and in severe infestation the seedlings fell off and died. On the other hands, the symptoms appeared at the lower leaves, when the plants reached the flowering stage, yellowing color started from the edges and turned to brown, followed by wilting, and falling the leaves. After which, the infection extended to the top and the symptoms emerged on other leaves, until the plant had completely wilted. Additionally, the xylem tissues became dark brown in color.

Thus, the objective of current study was to identify the etiology of this disease via morphological and molecular characterizations, as well as the pathogenicity assessment.

Material and Methods

Sampling, isolation, and purification of the pathogen

Approximately 100 samples of cowpea plants displaying wilt symptoms were randomly collected from different fields in three sites (Aljadwal algharbi– 32°30'22.4"N 44°11'34.1"E, Al Alabrahimia–32°39'43.3"N 44°08'23.5"E and Alhusaynia– 32°42'43.3"N 44°14'06.6"E) of Kerbala Province, Iraq, from 4/6 to 25/7 of 2020. The symptomatic samples were washed under tap water and excised into 0.5-1 cm pieces. These pieces were then surface sterilized with 2% of NaOCl for 2 min, rinsed three times in sterilized distilled water, and placed into plates containing Potato Dextrose Agar (PDA) media for 2–3 days at 25±2°C in the darkness (Shehan et al., 2023). Pure cultures were attained by transferring 0.5 cm mycelia plugs from the ledge of the growing fungal colonies to fresh PDA, amended with the antibiotic chloramphenicol (10 mg/L) (Lahuf et al., 2018).

Morphological and molecular identification of the pathogen

After two weeks of incubation at 25+2°C in dark, a total of 50 isolates were obtained. The morphology and color of cultural growth of colonies was examined. As well as, the type, shape, and dimensions of 50 conidia arbitrarily selected were measured by a light microscope at 40 X magnification. Due to all recovered isolates were identical in morphological features, a representative isolate, Cowp-F.so1, was selected for further characterization and assessment.

To identify the representative isolate molecularly, genomic DNA was extracted from one-week old pure culture, using a previous developed method (Lahuf et al., 2019). The phylogenetic analysis of three genetic markers covering the internal transcribed spacer (ITS) region, the translation elongation factor-1 alpha (TEF-1 α) gene and actin (actA) gene were evaluated to confirm the identification of the fungal agent associated with diseased cowpea plants. The selected genetic markers were PCR-amplified employing specific primer sets, ITS1-ITS4 for ITS-rDNA region (White et al., 1990), TEF1/TEF2 for TEF-1 α gene (O'Donnel et al., 1998) and ACT-512F/ACT-783R for actA gene (Carbone & Kohn, 1999).

The PCR reaction was carried out, using the Ready-To-Go PCR Beads kit (G.E. Healthcare, Illinois, USA), in a final volume of 25 μ l containing the reaction mixture ingredients, provided as beads besides 1 μ l of each primer sets (5 pmol),

and 2 μ l of the genomic DNA extracted. The PCR conditions of ITS region amplification included an initial denaturation step at 94°C for 4 min, followed by 35 repeated cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 s and elongation at 72°C for 1 min. The final elongation was at 72°C for 5 min. On the other hand, The PCR conditions of TEF-1 α and actA genes amplification comprised of an initial denaturation at 94°C for 3 min, and 40 repeated cycles of denaturation at 94°C for 30 s, annealing at 52°C (TEF-1 α gene) and 48°C (actA gene) for 30 s and extension at 72°C for 45 s, in addition to the final extension at 72°C for 5 min (Lahuf et al., 2022).

The PCR amplicons were sent to Macrogen, Inc. (Seoul, South Korea) for purification and sequencing. The Generated sequences were edited through BioEdit v7.0.9.0 and A plasmid Editor v2.0.61. Subsequently, they compared using Blastn tool against fungal publicly available sequences deposited at the GenBank database in National Center for Biotechnology Information. The phylogenetic analysis was conducted in MEGA 11 (v.11.0.13), using MUSCLE for sequences alignment and neighbor-joining method, with a bootstrap test of 1000 replications for producing the phylogenetic trees. The genetic markers sequences of the fungus isolated were then submitted to the GenBank to obtain specific accession numbers (Abass & Lahuf, 2023).

Pathogenicity assessments

The pathogenicity of fungus identified was determined by preparing the fungal inoculum at concentration of 1x10⁶ (conidia/ml), using the same method of Lahuf (2019). The conidial suspension was then added (A five μ L/plant) on a two weeks-old healthy cowpea plants, while the control plants were inoculated with sterilized distilled water only. Each treatment was triplicates. After inoculation, the development of the wilt disease was verified in inoculated plants whereas no disease symptoms were observed on plants of control. Furthermore, the fungal pathogen was re-isolated from diseased plants to confirm its identification and in order to fulfill the Koch's postulates (Hameed et al., 2021).

Results and Discussion

The incidence of cowpea wilt disease was estimated to be in the average range of 10 – 20% in all the surveyed fields of the three regions. A total of 50 isolates were obtained from the diseased cowpea samples, and were consistently had similar cultural and morphological characterizations.

The fungal colony were initially white aerial hypha and 7 days later turned to dark brown. They produced also light to dark brown pigment in the medium (Figure 1A and 1B). The

microconidia were transparent in oval, or fusiform shape with average size of 6.6 to 14.5×3.2 – $5.5 \mu\text{m}$ and had 0 to 2 transversal septa. On the contrary, the macroconidia were thick-walled profusely produced in cylindrical curved shape, variable in length (31.3 to 40.2×4.5 – $5.6 \mu\text{m}$), and usually with 3 to 5 transversal septations (Figure 1C). Furthermore, after approximately two weeks the chlamydospores were observed as individual and intercalary with average size of 6.4 – $8.8 \mu\text{m}$. These cultural and morphological attributes were compatible with previous descriptions of *Fusarium solani* (Leslie & Summerell, 2006).

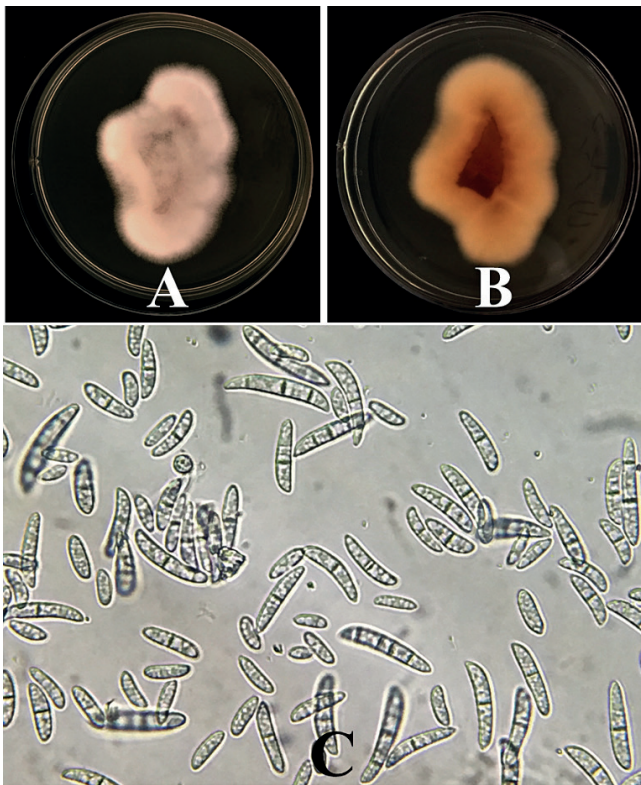


Fig. 1. Morphological characteristics of the *Fusarium solani* isolate Co. Karbala-IQ 1. Colony characters of Co. Karbala-IQ 1 on PDA (A) upper surface, (B) bottom surface, (C) Microscopic observation of the Co. Karbala-IQ 1 conidia

The morphological identification was confirmed molecularly through amplification and sequencing of the region and genes of ITS, TEF-1 α and actA. These sequences were deposited and had GenBank Accession numbers (MW076176.1, for ITS, MW080736.1 for EF-1 α and MW080737.1 for actA). The BLASTn analysis presented strong evidence of 100% similarity of these three mark-

ers' sequences with numerous corresponding sequences of international *F. solani* strains. The phylogenetic analysis demonstrated clustering of the representative isolate, *F. solani* isolate Co. Karbala-IQ 1, identified in this study with various *F. solani* strains depending on multiple alignments of the TEF-1 α sequence (Figure 2). As determined by the cultural and morphological characteristics, as well as the confirmation of molecular identification, the fungus isolated in this study was identified as *F. solani* (Mart.) L. Lombard & Crous.

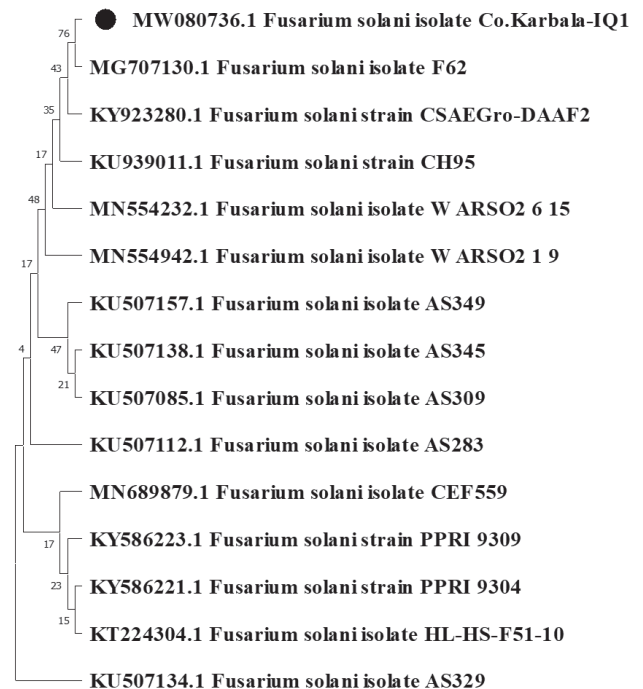


Fig. 2. Phylogenetic analysis of the *Fusarium solani* isolate Co. Karbala-IQ 1 (indicated with a black dot). Phylogenetic tree was concluded from the translation elongation factor 1-alpha gene sequences of numerous *F. solani* strains by means of the neighbour-joining method via MEGA 11. Bootstrap values supporting the branch were indicated at the nodes

The pathogenicity test was proved that the associated fungus was pathogenic through developing the wilt symptoms clearly on the inoculated cowpea young plants, and were comparable to those naturally infected plants. However, the control plants were symptomless (Figure 3). The pathogen, *F. solani*, was re-isolated from the symptomatic tissues, and observed to confirm the Koch's postulates.

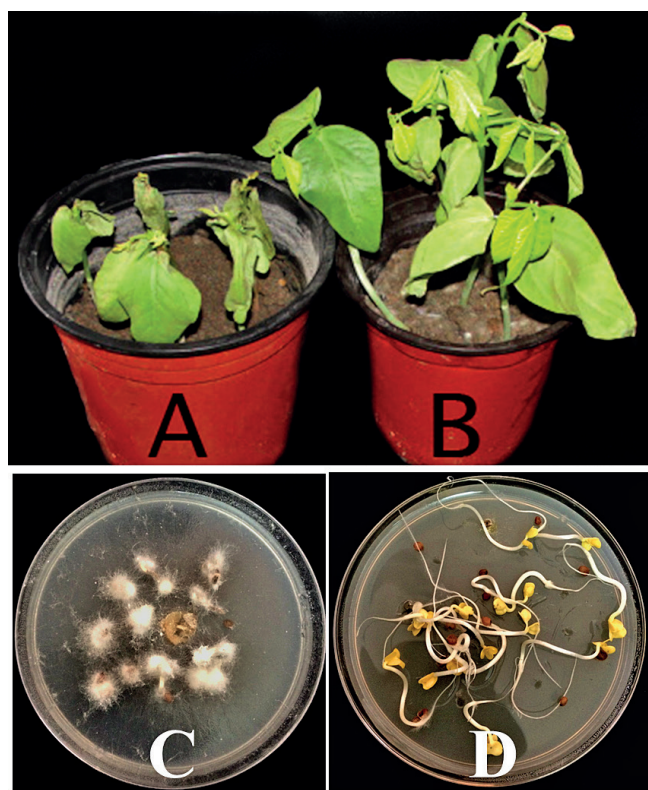


Fig. 3. Pathogenicity test of the fungal isolate *Fusarium solani* Co. Karbala-IQ 1. (A) Typical symptoms of fusarium wilt on young cowpea plant, (B) healthy young cowpea plant

Conclusions

The object of current study was to identify and characterize of the causal agent of cowpea wilt disease in Kerbala Province, Iraq. Worldwide, *F. solani* is previously well-known to cause various diseases on several agricultural important crops, vegetable, and trees (Coleman et al., 2016; Zhu et al., 2019). For instance, it was recorded as a pathogen of root rot on cowpea (*Vigna unguiculata*) and *Zingiber officinale* (Hussein, 2019; Liu et al., 2019). As well as, it was reported causing wilt disease on *Gossypium barbadense* and *Melia dubia* (Zhu et al., 2019; Pandey et al., 2018). However, to best of our knowledge this is the first report of *F. solani* causing wilt of cowpea crop in Kerbala, Iraq.

In view of this study, this causative agent could be a possible threat to cowpea crop production in the Kerbala Province and other Iraqi provinces. Thus, appropriate and effective cultural, biological, and chemical approaches should be evaluated to allocate direct management practices for the control.

Declaration of competing interest

The authors announce that they have no known competing financial interests or personal relationships that could have appeared to affect the results reported in the current study.

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References

- Al-Ani, R. A., Jarjis, M. M. & Jaber, K. S. (1989). Diseases of Field Crops. Higher Education Press, Baghdad, Iraq, p.650.
- Abdulmoohsin, R. G., Lahuf, A. A., Husain, Y. N. & Hameed, Z. L. (2019). Bioefficiency of some indigenous biocontrol agents against *Rhizoctonia solani* causing cowpea seed rot and preemergence damping-off. *IOP Conf. Ser.: Earth Environ. Sci.*, 388, 012011. doi:10.1088/1755-1315/388/1/012011.
- Abass, M.O. & Lahuf, A.A. (2023). High-Throughput Sequencing and Bioinformatic Analysis Reveal Presence of the Endogenous Pararetrovirus Tobacco vein clearing virus Genome in the tomato (*Solanum lycopersicum*) host genome. *Arab. J. Plant Prot.*, 41(1), 77-84. https://doi.org/10.22268/AJPP-41.1.077084
- Carbone, I. & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, 91, 553-556.
- Coleman, J. J. (2016). The *Fusarium solani* species complex: ubiquitous pathogens of agricultural importance. *Mol. Plant Pathol.*, 17(2), 146-158.
- Food and Agriculture Organization of the United Nations (FAO). Database Results, Geneva (2020). Available: <http://www.fao.org/faostat/en/#data/QC>.
- Hameed, Z.L., Lahuf, A.A., Jasim, M.T., Mohsen, H.M., Kadim, B.J., Saleh, S.A. & Mohamed, A.F. (2021). First Report of *Alternaria alternata* causing brown leaf spot on apricot (*Prunus Armeniaca*) in Kerbala Province of Iraq. *IOP Conf. Ser.: Earth Environ. Sci.*, 910, 012080.
- Hussein, S. N. (2019). Evaluate some of chemical and biological agents in controlling root rot disease of cowpea *Vigna unguiculata* (L.) Walp. *Iraqi J. Agric. Sci.*, 24(1), 125-135.
- Jaber, M.H. & Lahuf, A. A. (2020). Survey, pathogenicity and molecular identification of novel *Fusarium* species causing seed decay and damping-off disease of wheat crop in Kerbala Province, Iraq. *Plant Cell Biotechnol. Mol. Biol.*, 21, 1-14.
- Lahuf, A. A. (2019). First report of *Fusarium proliferatum* causing stem and root rot on lucky bamboo (*Dracaena braunii*) in Iraq. *Hell. Plant Prot. J.*, 12, 1-5.
- Lahuf, A. A., Kareem, A. A., Mohammadali, M. T., Mohsen, H. M. & Li, J. (2020). First report of *Alternaria alternata* causing leaf spot on quince (*Cydonia oblonga*) in Iraq. *Plant Dis.*, 104(5), 1538-1538.
- Lahuf, A. A., Jaafar, O. H. & Hameed, Z. L. (2019). A simple,

- rapid, safe and low-cost method to extract DNA from phytopathogenic fungi. *Asian J. Agric. & Biol.*, 7(2), 197-203.
- Lahuf, A. A., Jaafar, O. H., Al-mosoy, M. & Hameed, Z. L.** (2018). First record of the crown rot fungus *Fusarium equiseti* affecting *Triticum aestivum* L. and *Aptenia cordifolia* in Iraq. *Asian J. Agric. Biol.*, 6(4), 543-548.
- Lahuf, A. A., Kadhim, A. Q., Hameed, Z.L. & Almulla, A. M. N.** (2022). First report of Paecilomyces rot on rose (*Rosa* sp.) in Iraq. *J. Plant Pathol.* 104, 1195-1195.
- Leslie, J. F., Summerell, B. A. & Bullock, S.** (2006). The Fusarium Laboratory Manual. 2(10). *Blackwell Pub.*, Ames, IA, USA.
- Liu, X., Cao, A., Wang, Q., Yan, D., Ouyang, C. & Fang, W.** (2019). First Report of Small Yellow Ginger Root Rot Caused by *Fusarium solani* in Jiangxi, China. *Plant Dis.*, 103(6), 1643.
- O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., Zhang, N. & Geiser, D. M.** (2008). Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. *J. Clin. Microbiol.*, 46(8), 2477-2490.
- Pandey, A., Juwantha, R., Chandra, S., Kumar, A., Kannoja, P., Khanna, D. & Pandey, S.** (2018). First report of *Fusarium solani* causing wilt of *Melia dubia*. *For. Pathol.*, 48, e12398.
- Schroers, H. J., Samuels, G. J., Zhang, N., Short, D. P., Juba, J. & Geiser, D. M.** (2016). Epitypification of *Fusarium solani* and its assignment to a common phylogenetic species in the *Fusarium solani* species complex. *Mycologia*, 108, 806-819.
- Shehan, S.J., Abdalmoohsin, R.G. & Lahuf, A.A.** (2022). First report of *Ectophoma multirostrata* causing root rot of *Celosia argentea* in Iraq. *New Dis. Rep.*, 46, e12126. <https://doi.org/10.1002/ndr.12126>
- Singh, B. B.** (2020). Cowpea: The Food Legume of the 21st Century. *Willy Publications*, Christchurch, New Zealand.
- White, T. J., Bruns, T., Lee, S. & Taylor, J.** (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Applications*. *Academic Press*, San Diego, CA, 315-322.
- Zhu, Y., Abdelraheem, A., Sanogo, S., Wedegaertner, T., Nichols, R. & Zhang, J. F.** (2019). First Report of *Fusarium solani* Causing Fusarium Wilt in Pima Cotton (*Gossypium barbadense*) in New Mexico, USA. *Plant Dis.*, 103(12), 3279.

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