MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION OF *Fusarium oxysporum ciceri* ISOLATES FROM DIFFERENT CHICKPEA AREAS IN IKR, IRAQ

Emad M. Al-Maaroof **Kazhal H. Rahim**
Professor **Researcher**
College of Agricultural Engineering Sciences, University of Sulaimani, IKR, Iraq
Email: emad.ghalib@univsul.edu.iq

ABSTRACT

Thirty-seven different *F. oxysporum ciceri* isolates were isolated from 55 chickpea fields at Sulaimani and Halabja governorates during 2021. Fourteen Foc isolates were pathogenic and displayed various morphological traits. The ideal temperature for Foc ranged 25-30°C. PDA was the best media for the fungal growth. The isolates showed a variety of growth patterns, including appressed, flat/velvet, fluffy to partial fluffy, and cottony. However, Foc-28 displays nerve type mycelium growth on PDA at 25ºC. Microconidia (6.1-8.7x2.9-4.98µm), macroconidia (10.0-20.1x2.7-5.2µm), chlamydospores (7.2-15.0x6.8-10.7µm), and colony diameter on PDA were all significantly differed among the isolates. Significant differences in virulence of 14 Foc isolates were detected on 10 chickpea wilt differentials. The isolates were classified in to two groups and ten physiological races accordingly. Each isolate's genomic DNA was amplified by the ITS primers to a maximum size of 400bp, producing a single band for each accession. The basic local alignment search tool (BLAST) analysis supported the morphological identification, whereby the closest match 99-100% in the NCBI GenBank database. The accession numbers for the sequences were registered at the NCBI gen bank under different codes. The phylogenetic tree proposed two major clades with stains distributed across the dendrogram irrespective of their geographic status.

Key words: *Cicer arietinum*, Fusarium wilt, Physiological races, Virulence analysis.

التوصيف الجزيئي والفسيولوجي لعزلات الفطر *Fusarium oxysporum ciceri* من مناطق انتاج الحمص المختلفة في كردستان العراق

عماد محمود المعروف **كزال حسن رحيم**
أستاذ **باحث**
كليه علوم الهندسة الزراعيه، جامعه السليمانيه، سليمانيه، اقليم كردستان، العراق.

المستخلص

تم عزل 37 عزلة مختلفة من الفطر *F. oxysporum ciceri* من 55 حقلاً من حقول الحمص في محافظتي السليمانية وحلبجة خلال عام2021. اظهرت أربعة عشر عزلة من الفطر Foc إمراضيه عالية على بادرات الحمص والماش وأسفرت عن صفات مظهرية متباينة. تراوح معدل درجة الحرارة المثلى لنمو الفطر بين 25– 30 س° وكان الوسط الغذائي PDA من أفضل الاوساط لنمو الفطر. أبدت عزلات الفطر الممرض أنماطا متنوعة من النمو منها، مضغوط، مسطح/مخملي، زغبي إلى شبه زغبي، وقطني. الا ان العزلة Foc–28 تميزت بمظهر نمو يشبه الأعصاب على الوسط PDA عند درجة حرارة 25 س°. لوحظ وجود فروقات معنوية بين عزلات الفطر في ابعاد المايكروكونيديا (6.1–8.7x2.9–4.98µm)، الماكروكونيديا (10.0– 20.1x2.7–5.2µm)، السبورات الكلاميدية (7.2–15.0x6.8–10.7µm) واقطار مستعمرات الفطر على الوسط الغذائي PDA. تم الكشف عن وجود اختلافات معنوية في فوعة أربعة عشر عزلة من الفطر Foc على عشرة أصناف تفريقيه لذبول الحمص الفيوزارمي. صنفت العزلات على أساسها الى مجموعتين وعشر سلالات فسيولوجية. تم تضخيم الحامض النووي الجينومي لكل سلالة بواسطة بادئات ال ITS الى حجم اقصى مقداره ٤٠٠ زوج قاعدي، مما أسفر عن انتاج حزمة مفردة لكل سلسلة. دعم تحليل BLAST لبيانات تسلسل ITS–rDNA نتائج التشخيص المورفولوجي لعزلات الفطر مع نسبة تشابه ٩٩–١٠٠٪ مع الفطر Foc في بيانات بنك الجينات NCBI . تم تسجيل تسلسل جينوم عزلات الفطر Foc في بنك الجينات العام NCBIتحت أرقام انضمام مختلفة. اقترحت شجرة النشؤ والتطور مجموعتين رئيسيتين مع عزلات الفطر موزعة عبر مخطط الشجرة بغض النظر عن حالتها الجغرافية.

الكلمات مفتاحية: *Cicer arietinum*، الذبول الفيوزارمي، سلالات فسيولوجية، تحليل الفوعة.

Received:12/11/2022, Accepted:5/2/2023

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a significant pulse crops that is typically grown in the semi-arid regions with poor soils (27). Biotic and abiotic stresses, significantly decrease the average yields to 0.9-1.8t/ha across a variety of cultivation locations, which are below the theoretical potential (22). *Fusarium* wilt incited by *F. oxysporum* f. sp. *ciceri* (Foc) is one of the most important fungal disease that can severely damage chickpea crops worldwide (6). The disease might spread, through infected plant debris. Disease symptoms displays 25 days after sowing, including wilting, and plant decline. However, according to other studies, disease symptoms start to show up at podding stage. Typically, around the start of flowering, disease symptoms are more obvious (25). In general, disease symptoms can be seen at any plant development stage, but they are most noticeable during flowering and podding stage (late wilt) (13). Vascular tissues discoloration, development of cavities between xylem and phloem, and growth of mycelium, microconidia, macroconidia, and chlamydospores are other signs of the disease (13). Pathogenic variability has been found among Foc isolates based on the symptoms they inflect on the host. Wilting pathotypes and the yellowing pathotypes of Foc have been identified. The former causes quick and severe chlorosis flaccidity, vascular discoloration, and early plant death, while the latter results in progressive foliar yellowing, vascular discoloration and late plant death (36). Foc affects a wide range of hosts at all stages. The pathogen causes systemic infection and can survive in the soil for at least six years without a host (14). Disease management is challenging, whether it is done by crop rotation or the chemical application, due to the nature of the damage and the fungus' ability to survive (8). Resistant cultivars is one of the most practical and cost-effective strategies for controlling *Fusarium*, even if these cultivars don't perform well everywhere because to the limited efficacy of their resistance and the high pathogenic variability (35). Chaudhry et al. (9) evaluated 196 accessions of chickpeas and found that none of them were resistant. Although resistance to Foc in chickpea is race-specific, no evidence of resistance being overcome has been found to date, suggesting that there may not have been much or any selection for races that can overcome resistance in this patho-system. Understanding of the pathogenic diversity and the pathogen races that are present in a target area is essential to start breeding program for disease resistance. Numerous conventional and molecular breeding programs have been carried out globally to develop resistant chickpea cultivars. Morphological and pathogenicity assessments are conventional techniques for assessing differences among the fungal isolates (3). However, a DNA- based molecular approach is currently helping to assess genetic variation among the pathogen isolates (4, 20). The current study was carried out to identify the virulent Foc isolates using the available standard taxonomic keys based on colony morphology and conidial characteristics of each isolate and the confirmation of the identity was achieved with the help of PCR.

MATERIALS AND METHODS

Isolation, purification and identification of *F. oxysporum ciceri* isolates

The pathogens were isolated using stem and root samples collected from infected chickpea plants from diverse locations in Sulaimani and Halabja. The samples surface were sterilized by 1% NaOCl for 2–3 min, then rinsed with sterilized distilled water (SDW) and dried on filter papers. Three pieces of 3-5mm were placed in 90mm petri plates containing Potato Dextrose Agar (PDA) supplemented with 0.01% streptomycin. The plates were kept at 25 ± 2ºC to recover the pathogen for five days. Using the hypha-tip isolation method, the fungal colonies were recovered, purified and stored at 4ºC on PDA slants before use. The pathogens were identified based on their morphological and cultural traits (26).

Pathogenicity of *F. oxysporum ciceri* isolates on chickpea and mung bean seedlings

Chickpea and mung bean seeds were sterilized by 1% NaOCl for 2-3min, rinsed twice in SDW, air-dried on filter papers then planted in the sterilized plastic containers. Two seedlings with two leaves were carefully removed from the containers and placed in flasks filled with SDW.

Cotton plug used to secure the roots placement lower the water level in the flask. The spores were taken off from the surface 37 Foc isolate 7-days cultures and put into 10ml SDW. Spore suspension of each pathogen was adjusted to 10^6spores/ml. and placed in 250ml water in flasks and mixed thoroughly. Three replicates were used for each treatment. The flasks were incubated at 25°C, and the leaves were observed for infection symptoms. Disease scoring was recorded within 24-96 hr. using the following scale (2). +++= High pathogenic(HP), disease symptoms appeared on the seedlings after 48hr., ++= Pathogenic (P), disease symptoms appeared after 72hr., += Weak pathogenic (WP), disease symptoms appeared after 96hr., - = Non Pathogenic (NP), No symptoms appeared on the seedlings after 96hr. The infected seedlings were examined for any fungal development in the plant tissue after symptoms appearance. The fungi were re- isolated from the infected seedlings to prove Koch's hypothesis (21). **Cultural and morphological traits of Foc** Fourteen Foc isolates generated from the pathogenicity test were assessed for cultural and morphological characteristics such as texture, pigmentation, margin, mycelium shape, microconidia and macroconidia shapes, and chlamydospore type after one week. Spores dimensions were measured using six randomized spores from each isolate using compound microscope at 400x magnification. A cork-borer was used to remove 5mm discs from 7-days-old Foc isolates and deposited in the centers of PDA plates and incubated at 15°C, 20°C, 25°C, and 30°C for 15days. Three plates were used for each isolate. The average radial mycelium growth in millimeters was calculated every 24h. The experiment was run in complete randomized design (CRD) with three replicates. ANOVA was used to analyze the data, and LSD was used to compare the means of treatments at P≤ 0.05. 5mm inoculums of 7-day-old Foc isolates were placed in the center of petri plates containing PDA, Sabouraud Dextrose Agar (SDA), and Malt Extract Agar (MEA) to select the ideal medium for Foc growth. Three replicates were used for each isolate. The plates were incubated at 25°C in a CRD with three replicates. Growth diameter and colony color were measured for 15days.

Phenotyping of *F. oxysporum ciceri* isolates.

The experiment was conducted at the plastic house of the Sulaimani Directorate of Agriculture Research using 14 Foc pure culture isolates and 10 chickpea differentials during 2021/22. Two discs of 50mm diameter were taken from a 7-day-old culture of each isolate and placed in plastic bag with 100g of sand: maize meal (9:1) and incubated for 14 days at 25°C (15, 31). The inoculums were thoroughly combined with 1kg of sterilized farm soil, sand, and cow dung manure mixture at a ratio of 1:1:1 (w/w/w) in 20cm plastic pots. PDA only was added to the control treatment. Five seeds from each differential were surface-sterilized by 3% NaOCl for 2-3min, rinsed twice in SDW, air-dried on filter papers, and then planted in each infested soil mixture pots. The experiment was laid out in factorial RCBD with three replicates. The pots were watered as they needed and daily monitored for 6 weeks. Disease scoring was periodically conducted with ten days' intervals using a five-point severity scale (32). Vascular discoloration was tested for Foc presence. The percentage of diseases severity was calculated using the following formula (24).

DS(%)=

$$\frac{\sum(No.of\ seed.of\ deg.0\times 0)+-\mp(No.of\ seed.of\ degr.5\times 5)}{The\ total\ number\ of\ tested\ seedlings\times 5}\ x100$$

The isolates were divided into the following pathotypes. Av=A virulent (1-20 % severity), MV= Moderately Virulent (20.1-30%severity), V=Virulent (31.1-50% Severity), HV= highly virulent (50.1-100%).

Molecular identification of *F. oxysporum ciceri* isolates by PCR

DNA was extracted from eight Foc isolates using molecular method. A loop of 7-day mycelium of each isolate was transferred to 1.5ml Eppendorf tubes using Add Prep genomic DNA isolation kit. DNA was then quantified using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer and working dilutions were created at a DNA concentration of 50ng/µl for each isolate. The 1x TBE buffer prepared by dissolving 100g of fine package in 500ml of SDW and then shaken it in Thersequencemo-shaker until it melted. 1% agarose gel was prepared for electrophoresis as described in (30). The ITS-Fu primer:

(f:5`CAACTCCCAAACCCCTGTGA-3`, r:5`-GCGACGATTACCAGTAACGA-3`) was used to identify Fusarium isolates (1). Each 20µl PCR reaction mixture contains 10µl Master mix, 1.0µl forward primer, 1.0µl reverse, 2µl nuclease free water, and 6µl DNA template (50ng/µl). PCR amplification conditions included an initial denaturation at 92°C for 1min., cycling conditions were 94 °C for 1 min, 52 °C for 50 s, 72 °C for 1 min (30 cycles), followed by a final extension at 72 °C for 10 min. The amplified PCR product was separated on 1% agarose gel in TBE buffer (0.5g gel, and 50ml TBE buffer) stained with 5µl safe dye and visualized in gel document system (Sygene, UK). The bioinformatics software GENEIOUS prime (22) was used to view the DNA Sanger sequencing chromatograms that were produced, Sequences in both directions were aligned, modified, and cut before being preserved for later analysis. The GENEIOUS alignment technique was used to align several sequences pairwise and multiple times for each sequence. Each alignment was manually tweaked and improved before using .

Phylogenetic reconstruction

Phylogenetic inference in this study was based on Maximum Likelihood (ML) using the bootstrap method of 1000 replications. The ML analysis was performed using MEGA version 11 (34). The Juke-Cantor (JC) model of consistent rates across sites was the most effective nucleotide substation model. Phylogenetic and molecular evolutionary studies were performed. *Ascochyta rabiei* used as an out-group fungus.

RESULTS AND DISCUSSION

Isolation, purification and identification of *F. oxysporum ciceri* isolates

The infected chickpea plants were identified in the fields based on the key symptoms like wilting, withering, and yellowing of plants as well as improper branching, drooping petioles and rachises, browning of vascular bundles, and drying and yellowing of leaves from the base to the top. When the plant uprooted, they exhibit stem shrinkage above and below the collar area. The area that has shrunk may be 2.5cm long or longer. Seedlings that are affected don't decay on the stem or root surface. The internal stem tissues, however, exhibit dark brown to black discoloration when cut transversely or split open vertically from the collar downward. Thirty seven isolates were isolated from 50 different infected chickpeas fields in nine districts within Sulaimani and Halabja governorates in IKR (Table 1). The isolated organisms were identified as *F. oxysporum ciceri* (Pass.) Labor based on the morphology of the cultures, growth rate, descriptions, and dimensions of microconidia, macroconidia, and chlamydospore using the taxonomic characteristics of the pathogen (26).

Pathogenicity test of Foc isolates:

Quick pathogenicity test of 37 Foc isolates obtained from chickpea fields of Sulaimani and Halabja on chickpea and mung bean seedlings revealed from four interaction categories between each *Fusarium* isolate and both chickpea and mung bean seedlings (Table 1). High pathogenic group; exhibit disease symptoms on chickpea and mung bean seedlings after 48hr of inoculation. The high pathogenic isolates include Foc-4, Foc-16, Foc-17, Foc-18, Foc-20, Foc-24, Foc-28, Foc-30 and Foc-32 that explored high pathogenicity on both chickpea and mung bean seedlings. Pathogenic group; start to exhibit disease symptoms on chickpea and mung bean seedlings after 72hr. The pathogenic isolates include Foc-2a, Foc-2b, Foc-3b, and Foc-26 that showed pathogenicity on both seedling types; Isolates Foc-14, Foc-23, Foc-29 and Foc-100 that showed high pathogenicity on chickpea seedlings and pathogenicity on mung bean seedlings, and Isolates Foc-22, Foc-27, and Foc-31 that showed pathogenic and high pathogenic reaction on chickpea and mung bean seedlings respectively.

The weak pathogenic group; include isolates Foc-1, Foc-6, Foc-7, Foc-9, Foc-19, and Foc-25 that showed pathogenicity on both seedling types; Isolates Foc-3a, and Foc-13 that explored pathogenic reaction on chickpea and weak pathogenicity on mung bean and isolates Foc-12, and Foc-21 that explored weak pathogenicity and pathogenicity on chickpea and mung bean seedlings respectively.

The non-pathogenic group; including isolates Foc-15, and Foc-81 that not produce any symptoms on the seedlings within 96hr.,

and isolate Foc-11a, and Foc-11b that showed weak pathogenicity on chickpea and nonpathogenic on mung bean as specified in Table 1. As a result of pathogenicity of the isolates on the seedlings, only 14 Foc isolates were selected among the 37 tested isolates for further studies. Significant differences were detected between *F. oxysporium* isolates with the control treatment.

Table 1. Pathogenicity of different *F. oxysporum ciceri* isolates collected from various Chickpea growing areas in Sulaimani and Halabja governorates during the growing season 2020/21

Foc isolate	Chickpea	Mung bean	Mean	Foc isolate	Chickpea	Mung bean	Mean
Foc-1	*+	+	+	**Foc-17**	+++	+++	+++
Foc-2a	++	++	++	**Foc-18**	+++	+++	+++
Foc-2b	++	++	++	**Foc-19**	+	+	+
Foc-3a	++	+	++	**Foc-20**	+++	+++	+++
Foc-3b	++	++	++	**Foc-21**	+	++	+++
Foc-4	+++	+++	+++	**Foc-22**	++	+++	+++
Foc-5	++	+++	+++	**Foc-23**	+++	++	+++
Foc-6	+	+	+	**Foc-24**	+++	+++	+++
Foc-7	+	+	+	**Foc-25**	+	+	+
Foc-8	+	++	+++	**Foc-26**	++	++	++
Foc-9	+	+	+	**Foc-27**	++	+++	+++
Foc-10	+	++	+++	**Foc-28**	+++	+++	+++
Foc-11a	+	-	+	**Foc-29**	+++	++	+++
Foc-11b	+	-	+	**Foc-30**	+++	+++	+++
Foc-12	+	++	+++	**Foc-31**	++	+++	+++
Foc-13	++	+	+++	**Foc-32**	+++	+++	+++
Foc-14	+++	++	+++	**Foc-81**	-	-	-
Foc-15	-	-	-	**Foc-100**	+++	++	+++
Foc-16	+++	+++	+++	**Mean**	++	++	++

- +++ = Highly pathogenic (HP), disease symptoms appeared on Chickpea and Mung bean seedlings after 48hr., ++ = Pathogenic (P), disease symptoms appeared on Chickpea and Mung bean seedlings after 72hr., + = Weak pathogenic (WP) disease symptoms appeared on Chickpea and Mung bean seedlings after 96hr., - = Non Pathogenic (NP) No any symptom appearance on Chickpea and Mung bean seedlings after 96hr

Cultural and Morphological characteristics of *F. oxysporum ciceri* isolates: *F. oxysporum* isolates were identified based on the cultures morphology, growth rate, descriptions and dimensions of microconidia, macroconidia, and chlamydospore using the taxonomic characteristics of the pathogen (26). Pure culture of Foc isolates were obtained by multiplying single conidia of each isolate on PDA at 25±2^{o}C. Variations were detected among Foc isolates in terms of cultural traits, growth patterns, pigmentation, and sporulation as well as the number and dimensions of conidia and chlamydospores. The fungus grew flat/velvet, fluffy to partial fluffy, and cottony, however Foc-28 was morphologically distinct from the others due to developing mycelium growth in the form of nerves on PDA at 25±2^{o}C, becoming felted and wrinkled in older cultures (Table 2).

Table 2. Morphological characterization of different *Fusarium oxysporum ciceri* isolates collected from various chickpea growing areas in Sulaimani and Halabja during 2020/21 season

Foc Isolate	Morphotype or texture	Pigmentation in light growth	Pigmentation in dark growth	margin	Shape of Myceliu m	Shape of Microconidia	Shape of Macroconidia	Type of Chlamydospore
Foc-4	Velvet	White	Pale Creamy	Entire	Regular	Elliptical	Slightly curved	Intercalary formation
Foc-5	Flat/velvet	Creamy white	Pale orange	Wavy	Irregular	Oval	Slightly curved	Intercalary formation
Foc-14	Flat/velvet	White	Creamy	Entire	Regular	Oval	Slightly curved	Terminal formation
Foc-16	Cottony	Pink	Whitish pink	Entire	Regular without sector	Oval	Slightly curved	Intercalary formation
Foc-17	Cottony	Pale pink	Pink	Wavy entire	Regular without sector	Oval	Straight	Terminal formation
Foc-18	Fluffy	Whitish	Whitish pink	wavy	Irregular without sector	Oval	Slightly curved	Intercalary formation
Foc-20	Fluffy	Dark pink	Pink	Entire	Irregular	Elliptical	Straight	Chain formation
Foc-23	Fluffy	Purplish white	Whitish pink	Wavy entire	Regular without sector	Elliptical	Straight	Intercalary formation
Foc-24	Less fluffy	Creamy pink	Dark Creamy	Wavy entire	Irregular	Oval	Straight	Terminal formation
Foc-28	Nerve shape	White	White	Irregular	Regular with sector	Oval	Straight	Intercalary formation
Foc-29	Cottony	Creamy white	White	Wavy entire	Irregular	Elliptical	Slightly curved	Terminal formation
Foc-30	Fluffy	White	Pale pink	Wavy entire	Regular without sector	Elliptical	Straight	Intercalary formation
Foc-32	Fluffy	Pale pink	Pale pink	Wavy entire	Regular with sector	Elliptical	Slightly curved	Intercalary formation
Foc-100	Less fluffy	Pale pink	Whitish pink	Entire	Regular	Oval	Slightly curved	Terminal formation

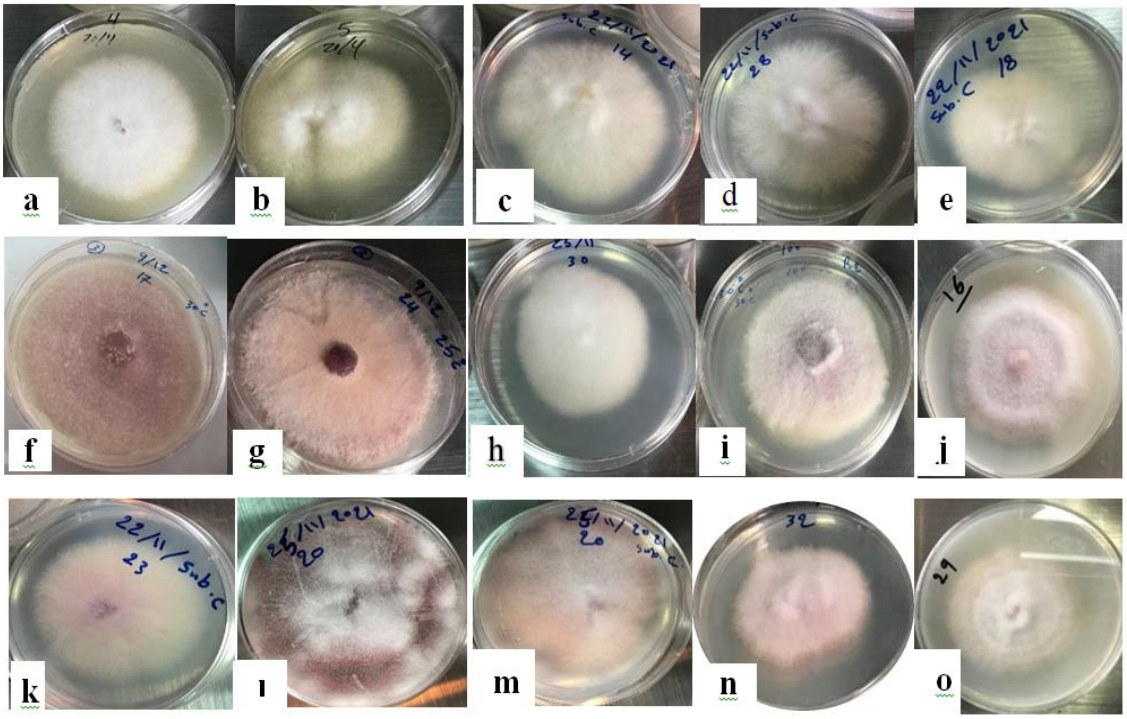

Figure 1. Morphology of different *F. oxysporum* f. sp. *ciceri* isolates (a-o) on PDA at 25°C after 7days

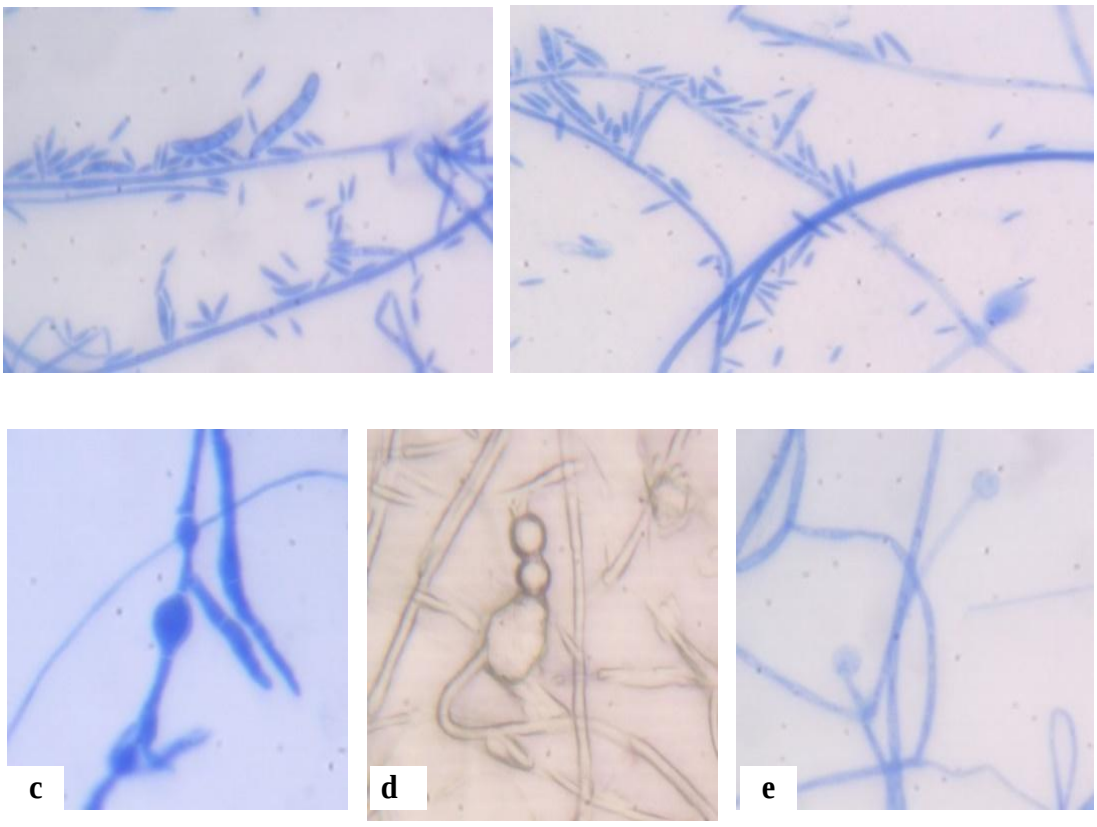

Figure 2. *F. oxysporum ciceri* spore types (a): Macroconidia, (b): Microconidia, (c - e): Chlamydospores formation types in vitro: (c) Intercalary, (d) Chain, (e) terminal formation (400X).

The isolates develop a range of pigmentations in light growth in PDA after 96hr. creamy white, white and pale pink. When comparing Foc-16 pink, and Foc-20 we can observe that they start out as pale pink and then change to dark pink, if we describe the precise color of the isolate mycelium, we can see that it is initially white before changing to a light pink color, as in the case of Foc-100, and that initially is white before changing to a purplish white color over time, as in the case of Foc-23. This change start from the middle to the end of Fusarium growth, as shown in Figure1. The isolates showed four different mycelium margins types: entire, wavy entire, wavy, and Irregular. Five different mycelium morphologies, including regular, regular with sector, regular without sector, irregular and irregular without sector. Microconidia morphologies ranged from oval in 57% of the isolates to elliptical in 43% of the isolates, while the macroconidia morphologies ranged from straight in 43% of the isolates to slightly curve in 57%. Three different types of chlamydospores were produced by the fungal isolates, Intercalary formation in 57% of the isolates, terminal formation in 35% and chain formation only in Foc-20 (Table 2, Figure 2). The dimensions of microconidia, macroconidia, and chlamydospores were significantly different between Foc isolates (Table 3). Foc-4 has the largest average macroconidia dimensions (20.09*4.02µm) and significantly surpassed all other isolates except Foc-14 and Foc-30, while the smallest macroconidia dimensions was detected in Foc-16. The largest macroconidia dimension was detected in Foc-30 (8.69*4.94µm), which was significantly surpassed all other isolates, and the lowest dimension was detected in Foc-24. The chlamydospore area's varied from 49.1µm in Foc-14 to 160.4µm in Foc-16. All the isolates microconidia, macroconidia, and chlamydospores falls within the original stock culture of Foc (29). Microconidia dimensions ranged from 5.1-12.8*2.5-5.0µm according to Dubey et al. (10), in contrast, macroconidia had 1 to 5 septa and measured between 16.5-7.9*4.0–5.9µm. Gupta et al. (12) found the macroconidia size ranged from 16.7-6.6*3.3-6.7µm whereas microconidia was 3.9-10.0*1.7-5.0µm.

Table 3. Conidia and chlamydospore dimensions of different *F. oxysporum ciceri* isolates from various chickpea growing areas in Sulaimani and Halabja grown on PDA at 25±2ºC.

Foc Isolate	Macroconidia		Microconidia		Chlamydospore	
	Dimension (µm)	Area (µm^2)	Dimension (µm)	Area (µm^2)	Dimension (µm)	Area (µm^2)
Foc-4	20.09 ^{a} * 4.02 ^{bc}	80.76 ^{a}	7.44 $^{a-e}$ * 3.07 ^{ef}	22.84 ^{de}	12.09 $^{b-d}$ * 9.74 ^{ab}	117.76 ^{bc}
Foc-5	15.32 $^{b-d}$ * 2.92 ^{ef}	44.73 $^{c-e}$	8.38 ^{ab} * 4.01 ^{bc}	33.60 ^{bc}	13.55 ^{ab} * 10.49 ^{ab}	142.14 ^{ab}
Foc-14	18.52 $^{a-c}$ * 4.14 ^{bc}	76.67 ^{ab}	8.13 ^{ab} * 4.22 ^{b}	34.31 ^{b}	7.24 ^{f} * 6.77 ^{d}	49.01 ^{e}
Foc-16	10.02 ^{e} * 3.21 $^{d-f}$	32.16 ^{e}	6.11 ^{e} * 4.98 $^{d-f}$	30.43 ^{de}	15.03 ^{a} * 10.67 ^{a}	160.37 ^{a}
Foc-17	15.01 $^{b-d}$ * 2.90 ^{ef}	43.53 $^{c-e}$	6.74 $^{c-e}$ * 2.89 ^{f}	19.48 ^{e}	12.40 $^{a-d}$ * 9.02 $^{a-c}$	111.85 ^{bc}
Foc-18	14.02 ^{de} * 4.44 ^{ab}	62.25 $^{a-c}$	7.51 $^{a-d}$ * 3.53 $^{c-e}$	26.51 ^{d}	13.08 $^{a-c}$ * 8.45 $^{b-d}$	110.53 ^{bc}
Foc-20	13.72 ^{de} * 2.90 ^{ef}	39.79 ^{de}	8.02 $^{a-c}$ * 3.17 $^{d-f}$	25.42 ^{de}	10.23 $^{c-e}$ * 9.09 $^{a-c}$	92.99 ^{cd}
Foc-23	13.20 ^{de} * 3.65 ^{cd}	48.18 $^{c-e}$	7.30 $^{b-e}$ * 3.64 ^{cd}	26.57 ^{d}	8.71 ^{ef} * 7.35 ^{cd}	64.02 ^{de}
Foc-24	19.21 ^{ab} * 2.70 ^{f}	51.87 $^{c-e}$	6.49 ^{de} * 2.93 ^{f}	19.02 ^{e}	13.73 ^{ab} * 10.53 ^{ab}	144.58 ^{ab}
Foc-28	15.22 $^{b-d}$ * 3.79 $^{b-d}$	57.68 $^{b-d}$	7.43 $^{a-e}$ * 3.59 ^{cd}	26.67 ^{d}	14.44 ^{ab} * 10.47 ^{ab}	151.19 ^{ab}
Foc-29	13.61 ^{de} * 3.24 $^{d-f}$	44.10 $^{c-e}$	7.08 $^{b-e}$ * 3.64 ^{cd}	25.77 ^{de}	10.52 $^{c-e}$ * 8.37 $^{b-d}$	88.05 $^{c-e}$
Foc-30	14.78 ^{cd} * 5.16 ^{a}	76.26 ^{ab}	8.69 ^{a} * 4.94 ^{a}	42.93 ^{a}	10.39 $^{c-e}$ * 9.11 $^{a-c}$	94.65 ^{cd}
Foc-32	13.27 ^{de} * 4.02 ^{bc}	53.35 ^{cd}	7.49 $^{a-d}$ * 3.63 ^{cd}	27.19 ^{cd}	11.80 $^{b-d}$ * 9.19 $^{a-c}$	108.44 $^{b-d}$
Foc-100	16.63 $^{a-d}$ * 2.92 ^{ef}	48.56 $^{c-e}$	7.28 $^{b-e}$ * 3.05 ^{ef}	22.20 ^{de}	12.41 $^{a-d}$ * 7.37 ^{cd}	91.46 $^{c-e}$
Mean	15.19 $^{b-d}$ * 3.57 $^{c-e}$	54.23 ^{cd}	7.43 $^{a-e}$ * 3.66 ^{cd}	27.19 ^{cd}	11.83 $^{d-f}$ * 9.04 $^{a-c}$	106.94 $^{b-d}$

*** Each no. is a mean of three replicates.**

**** Means with different letters within each column are significantly different at p≤0.05 based on Revised LSD Test.**

Physiological characteristics of Foc isolates: Foc isolates underwent divergent mycelial growth at different temperature regimes of 15, 20, 25, and 30°C on PDA. The growth of Foc isolates varied significantly ($P \geq 0.05$) at different temperatures. After 96hr of incubation at 15-30°C, the mean fungal diameter varied from 50- 63.3mm in Foc-24 and Foc-23, respectively (Table 4). Foc--23 significantly surpassed all other isolates except Foc-18 and Foc-30. The maximum mean growth of Foc isolates observed at 25°C (77.3mm), significantly exceeding all other temperatures, while the minimum temperature observed at 15°C was 33.0mm. Above and below 25°C, the colony growth was hindered. The optimum temperature range was between 25°C to 30°C. Each isolate exhibited exceptional development at 25°C. Foc-23 exceeded isolates Foc-5, Foc-14, Foc-17, Foc-24, and Foc-29 in term of maximum and minimum growth of 79.0mm and 64.3mm respectively at 25°C. The isolates thrived at 30 and 20°C, with maximum and minimum growth of 70mm and 69mm for Foc-23 and 55.3mm and 47.0mm for Foc-24 and Foc-28 respectively. Low growth was observed at 15°C, with maximum and minimum growth of 40mm and 31mm for Foc-18 and Foc-24 respectively. Similar studies have also been presented by other researchers **(**19, 25). Sharma et al**.** (31) demonstrate that the ideal development of the disease occurred at 25°C. The largest colony diameter reported by Mina and Dubey **(**23) was 85mm at 28°C. PDA, SDA, and MDA had significant impact on the mycelia growth of various Foc isolates, significant differences were detected between isolates (Table 5).The highest mycelial growth of Foc (74.9mm) was observed on PDA, significantly exceeding all other media, followed by SDA (47.2mm), while the lowest growth was observed on MEA. Out of the three tested media, PDA provides the best growth and sporulation conditions for Foc isolates, with a maximum growth of 79mm for isolate Foc-23 that significantly surpassed on 30% of the tested isolates and a minimum growth of 64.3mm for isolate Foc-29. On SDA, Foc-14 reported a maximum growth of 56.0mm, which significantly surpassed morethan 71% of the tested isolates, whereas Foc-24 recorded a minimum growth of 31.3mm. Foc-18 grew to a maximum growth of 54.0mm on MDA, which was significantly more than 86% of the tested isolates, while Foc-20 grew to a minimum of 38.3mm (Table 5). Some Foc isolates have different colors on different media like Foc-24. Similar outcomes were observed when working with *M. phaseolina* (18). Several synthetic and non-synthetic culture media have a significant impact on morphological and cultural traits of the fungi. Distinct fungi have different nutritional requirements for growth (19). However, the mannitol and dextrose promote the best fungal development requirements (28).

Phenotyping of *F. oxysporum ciceri* isolates

Phenotyping of fourteen Foc isolates collected from various chickpea production area in Sulaimani and Halabja, showed significant variation in the pathogenicity and virulence of the isolates on ten chickpea differentials (Tables 6). The isolates were classified in to two groups and ten physiological races based on their pathogenicity and virulence on a set of ten chickpea differential genotypes.

Table 4. Effect of temperature on mycelial growth of different *F. oxysporum ciceri* isolates grown on PDA at control conditions after 96hr.

Foc isolate	Colony diameter (mm)				Mean growth
	15°C	20°C	25°C	30°C	
Foc-4	31.00 ^{vw}	53.00 ^{p}	78.67 ^{a}	68.00 $^{g-i}$	57.67 ^{ef}
Foc-5	35.00 $^{s-u}$	61.00 $^{k-m}$	70.33 ^{fg}	58.00 $^{m-o}$	56.08 ^{fg}
Foc-14	38.00 ^{rs}	62.67 $^{j-l}$	74.00 $^{c-e}$	70.67 $^{e-g}$	61.34 ^{bc}
Foc-16	32.67 ^{uv}	48.33 ^{q}	77.67 ^{ab}	69.67 ^{fg}	57.09 ^{f}
Foc-17	37.00 $^{r-t}$	53.33 ^{p}	75.00 $^{b-d}$	74.00 $^{c-e}$	59.83 ^{cd}
Foc-18	40.00 ^{r}	64.00 ^{jk}	77.00 $^{a-c}$	69.00 $^{f-h}$	62.50 ^{ab}
Foc-20	35.00 $^{s-u}$	49.00 ^{q}	77.67 ^{ab}	64.00 ^{jk}	56.42 ^{fg}
Foc-23	35.00 $^{s-u}$	69.00 $^{f-h}$	79.00 ^{a}	70.00 ^{fg}	63.25 ^{a}
Foc-24	31.00 ^{vw}	48.00 ^{q}	66.00 $^{h-j}$	55.33 ^{op}	50.08 ^{i}
Foc-28	34.00 $^{t-v}$	47.00 ^{q}	78.00 ^{ab}	61.00 $^{k-m}$	55.00 ^{g}
Foc-29	31.00 ^{vw}	57.00 ^{no}	64.33 ^{jk}	58.67 $^{m-o}$	52.75 ^{h}
Foc-30	37.00 $^{r-t}$	61.00 $^{k-m}$	78.00 ^{ab}	72.00 $^{d-f}$	62.00 ^{ab}
Foc-32	33.00 ^{uv}	59.00 $^{l-n}$	77.33^{a-c}	75.00 $^{b-d}$	61.08 ^{bc}
Foc-100	28.67 ^{w}	66.00 $^{h-j}$	76.00 $^{a-c}$	65.00 ^{ij}	58.92 ^{de}
Mean	34.17 $^{v-x}$	57.02 ^{pq}	74.93 $^{b-d}$	66.45 $^{h-k}$	58.14 $^{o-q}$

*** Each no. is a mean of three replicates.**

**** Means with different letters within each column are significantly different at p ≤ 0.05 based on Revised LSD Test**

Table 5. Effect of various culture media on the mycelia growth of *Fusarium oxysporum ciceri* isolates under control conditions at 25°C after 96hr.

Foc isolate	Colony diameter (mm)*			Mean
	PDA	SDA	MDA	
Foc-4	78.67 ^{a}	42.67 $^{m-q}$	53.00 $^{e-h}$	58.11 $^{g-j}$
Foc-5	70.33 ^{c}	49.33 $^{h-k}$	44.67 $^{l-o}$	54.78 $^{j-n}$
Foc-14	74.00 ^{bc}	56.00 ^{e}	49.33 $^{h-k}$	59.78 $^{g-i}$
Foc-16	77.67 ^{ab}	51.33 $^{f-i}$	40.33 $^{p-r}$	56.44 $^{h-l}$
Foc-17	75.00 ^{ab}	55.33 ^{ef}	49.67 $^{g-j}$	60.00 ^{gh}
Foc-18	77.00 ^{ab}	53.67 $^{e-g}$	54.00 ^{ef}	61.56 ^{fg}
Foc-20	77.67 ^{ab}	46.33 $^{j-m}$	38.33 ^{r}	54.11 $^{k-n}$
Foc-23	79.00 ^{a}	52.33 $^{e-h}$	47.67 $^{i-l}$	59.67 $^{g-i}$
Foc-24	66.00 ^{d}	31.33 ^{s}	41.00 $^{o-r}$	46.11 $^{r-t}$
Foc-28	78.00 ^{ab}	40.33 $^{p-r}$	41.00 $^{o-r}$	53.11 $^{l-p}$
Foc-29	64.33 ^{d}	37.67 ^{r}	41.00 $^{o-r}$	47.67 $^{q-s}$
Foc-30	78.00 ^{ab}	54.00^{ef}	41.67 $^{n-r}$	57.89 $^{g-k}$
Foc-32	77.33 ^{ab}	46.67 $^{j-m}$	45.33 $^{k-n}$	56.44 $^{h-l}$
Foc-100	76.00 ^{ab}	44.33 $^{l-p}$	39.33 ^{qr}	53.22 $^{l-o}$
Mean	74.93 ^{ab}	47.24 $^{j-m}$	44.74 $^{l-o}$	55.63 $^{j-m}$

***average of three replicates**

**** Means with different letters within each column are significantly different at p ≤ 0.05 based on Revised Least Significant Difference Test.**

Table 6 Phenotyping of fourteen *F. oxysporum ciceris* isolates collected from major chickpea growing area across Sulaimani and Halabja on ten chickpea differentials under artificial inoculation conditions.

isolate	Disease severity percentage										Average
	Flip09-99c	Flip09-100c	Flip09-128c	Flip09-163c	Flip09-182c	Flip09-206c	Flip09-209c	Flip09-420c	Flip09-424c	ILC482	
Foc-4	HV 52.0^{p-t}	V 40.0^{d1-f1}	MV 28.0^{j1}	AV 16.0^{m}	HV 74.3^{cd}	HV 68.0^{gh}	HV 60.0^{j}	V 40.0^{d1-f1}	HV 68.0^{gh}	HV 64.0^{i}	HV 51.0^{r-u}
Foc-5	V 40.0^{d1-f1}	HV 55.1^{k-o}	HV 52.0^{p-t}	V 40.0^{d1-f1}	V 48.0^{v-y}	HV 56.0^{k-m}	HV 65.7^{hi}	AV 20.0^{l1}	HV 68.6^{f-h}	HV 68.0^{gh}	HV 51.3^{r-u}
Foc-14	HV 54.3^{k-q}	HV 59.8^{j}	HV 64.0^{i}	HV 68.6^{f-h}	V 34.3^{hli1}	HV 68.0^{gh}	HV 68.0^{gh}	AV 12.0^{n1}	HV 50.5^{s-v}	V 44.0^{a1-c1}	HV 52.4^{o-t}
Foc-16	HV 76.0^{bc}	HV 51.4^{q-u}	HV 76.0^{bc}	AV 16.0^{m1}	V 44.0^{a1-c1}	HV 84.0^{a}	HV 52.0^{p-t}	AV 16.0^{m1}	HV 68.6^{f-h}	HV 52.0^{p-t}	HV 53.6^{l-r}
Foc-17	MV 24.0^{k1}	AV 20.0^{l1}	V 40.0^{d1-f1}	HV 72.0^{de}	HV 56.0^{k-m}	V 48.9^{u-x}	V 36.0^{g1h1}	HV 64.4^{i}	HV 56.0^{k-m}	HV 52.0^{p-t}	V 46.9^{w-z}
Foc-18	MV 28.0^{j1}	HV 71.4^{d-f}	HV 65.7^{hi}	V 34.3^{hli1}	AV 16.0^{m1}	HV 52.0^{p-t}	AV 20.0^{l1}	HV 65.7^{hi}	HV 65.7^{hi}	V 36.00^{g1h1}	V 45.5^{y-a1}
Foc-20	HV 64.4^{i}	V 36.0^{g1h1}	HV 57.1^{jk}	HV 52.0 $^{p-t}$	V 36.0^{g1h1}	HV 60.0^{j}	HV 64.0^{i}	V 32.0^{i1}	MV 24.0^{k1}	HV 62.9^{i}	V 48.9^{u-x}
Foc-23	HV 68.0^{gh}	HV 68.0^{gh}	HV 72.0^{de}	V 34.3^{hli1}	HV 84.0^{a}	HV 68.2^{gh}	V 32.0^{i1}	V 36.0^{g1h1}	V 32.0^{i1}	HV 56.3^{kl}	HV 55.1^{k-o}
Foc-24	V 32.0^{i1}	HV 51.4^{q-u}	MV 28.0^{j1}	HV 72.4^{de}	V 40.0^{d1-f1}	MV 28.0^{j1}	MV 28.0^{j1}	MV 28.0^{j1}	V 48.0^{v-y}	HV 65.7^{hi}	V 42.2^{b1-d1}
Foc-28	AV 12.0^{n1}	HV 68.0^{gh}	AV 20.0^{l1}	HV 54.3^{k-q}	HV 76.0^{bc}	HV 71.4^{d-f}	HV 60.0^{j}	MV 24.0^{k1}	HV 68.6^{f-h}	HV 51.1^{r-u}	HV 50.5^{s-v}
Foc-29	HV 76.0^{bc}	AV 20.0^{l1}	HV 52.0^{p-t}	AV 12.0^{n1}	HV 52.0^{p-t}	AV 16.0^{m1}	V 40.0^{d1-f1}	V 32.0^{i1}	V 37.1^{f1-h1}	HV 76.0^{bc}	V 41.3^{c1-e1}
Foc-30	V 32.0^{i1}	AV 20.0^{l1}	HV 56.0^{k-m}	V 44.4^{z-b1}	AV 16.0^{m1}	HV 68.0^{gh}	HV 60.0^{j}	HV 68.6^{f-h}	HV 52.0^{p-t}	V 32.0^{i1}	V 44.9^{z-b1}
Foc-32	HV 70.0^{e-g}	HV 77.8^{b}	HV 82.2^{a}	V 35.6^{h1}	HV 82.2^{a}	HV 84.0^{a}	HV 71.4^{d-f}	HV 54.3^{k-q}	HV 52.0^{p-t}	HV 64.4^{i}	HV 67.4^{gh}
Foc-100	MV 24.0^{k1}	HV 54.3^{k-q}	V 48.0^{v-y}	HV 64.0^{i}	MV 24.0^{k1}	AV 20.0^{l1}	HV 76.0^{bc}	HV 50.8^{r-v}	HV 54.3^{k-q}	HV 54.3^{k-p}	V 47.0^{w-z}
Cont.	AV 2.2^{p1}	AV 0.89^{p}	AV 1.67^{p}	AV 1.6^{p}	AV 0.9^{p}	AV 1.9^{p1}	AV 1.0^{p1}	AV 3.3^{p1}	AV 6.7^{o1}	AV 1.7^{p1}	AV 2.2^{p1}
Mean	S 46.62^{x-a1}	S 49.5^{t-w}	HS 52.9^{n-s}	S 44.0^{a1-c1}	S 48.8^{u-x}	HS 56.6^{k}	HS 52.4^{o-t}	S 38.8^{e1-g1}	HS 53.2^{m-s}	HS 55.6^{k-n}	S 49.9^{t-v}

*** Av=A virulent (1-20 % severity), MV= Virulent (20.1-30% severity), V= Virulent (31.1-50% Severity), HV= highly virulent (50.1-100%).**

**** S= Susceptible (30.1-50% Severity), HS= Highly Susceptible (51% or more of plants wilted).**

***** Means with different letters within each column are significantly different at p≤0.05 based on Revised Least Significant Difference Test.**

G1 represent the high pathogenic isolates including, isolates Foc-4, Foc-5, Foc-14, Foc-16, Foc-23, Foc-28, and Foc-32; G2 involve the pathogenic isolates like Foc-17, Foc-18, Foc-20, Foc-24, Foc-29, Foc-30, and Foc-100. Foc-32 incited the highest mean disease severity (67.39%) on the differentials and significantly surpassed all other isolates, whereas Foc-29 produced the lowest mean disease severity (41.3%). Race 1 characterized by its high aggressiveness and showed virulence's against all the tested differentials genotypes. It was represented by four isolates such as Foc-20, Foc-23, Foc-24, and Foc-32. Races 2, 3, 4 and 5 were a virulent on one genotype only, race 2 represented by Foc-4, that was a virulent on Flip09-163c; race 3 represented by two isolates Foc-5, and Foc-14, that were a virulent on Flip09-420c; race 4 represented by Foc-17, that was a virulent on Flip09-100c; and race 5, represented by Foc-100, that was a virulent on Flip09-206c. Races 6, 7, 8, and 9 were a virulent on two genotypes, represented by Foc-16, Foc-18, Foc-28, and Foc-30, that were a virulent on (Flip09-163c and Flip09-420c), (Flip09-182c and Flip09-209c), (Flip09-99c and Flip09-128c), and (Flip09-100cand Flip09-182c) respectively; Foc-29 representing the tenth race, that was a virulent on three genotypes (Flip09-100c, Flip09-163c and Flip09-206c). All the differential genotypes exhibited susceptible to high susceptible reaction to Foc despite the isolates. The highest disease severity (55.6%) was recorded for the genotype ILC482, which was significantly surpassed all other genotypes and was highly susceptible to all Foc isolates, followed by Flip09-424c. The lowest disease severity was recorded on the differential genotype Flip09-420c which was tolerant to three Foc isolates (Foc-5, Foc-14, and Foc-16). As a result of virulence variation, Foc isolates divided into two groups and ten physiological distinct races. The severity of various Foc races can be used to distinguish between them on 10 chickpea differentials. Foc-32 was one of the most aggressive and economically damaging race, showing gradual plant yellowing and wilting within 45 days of inoculation. Other studies have found the previously described yellowing or wilting pathotypes based on the disease symptoms shown in pathogenicity tests (27). The yellowing pathotypes results in slow foliar yellowing with vascular discoloration and late plant mortality, in contrast to the wilting pathotypes that cause rapid and severe chlorosis, flaccidity, vascular discoloration, and early plant death (36). Plants infected with the wilting cause races continue to grow without any obvious leaf yellowing for three to four weeks after inoculations. Sharma et al. (31) described a distinct disease symptom known as slow wilting characterized by longer latent period and low disease incidence.

Molecular characterization of *F. oxysporum ciceri* isolates: The ITS region was successfully amplified from the DNA of all *F. oxysporum* isolates in the study by the fungal-specific universal primer pairs ITS-Fu-f and ITS-Fu-r. Amplification of nuclear ITS sequences gave a single band for each accession. The lengths of the sequences as determined by gel electrophoresis were ~400 bp in size (Figures 3). The Basic Local Alignment Search Tool (BLAST) analysis of the ITS rDNA sequence data, supported the morphological identification, whereby the closest match 99–100% similarity with *F. oxysporum* in the NCBI GenBank database. The accession numbers for the sequences were registered at the NCBI Gene registery under the codes OP824784,OP824785, OP824786, OP824787, OP824788, OP824789, OP824790, and OP824791 (Table 7). A dendrogram was constructed based on the ITS sequences using GENEIOUS Prime 2022 and Mega 11 software by Maximum Likelihood (ML) methods. The phylogenetic tree proposed two major clades with isolates distributed across the dendrogram irrespective of their geographic status. These sequences showed poor resolution, and the bootstrap values were low. This is because the polymorphism resulting from the alignment of the sequences is a single nucleotide polymorphism. The outgroup species *Aschochyta rabiei* were well resolved

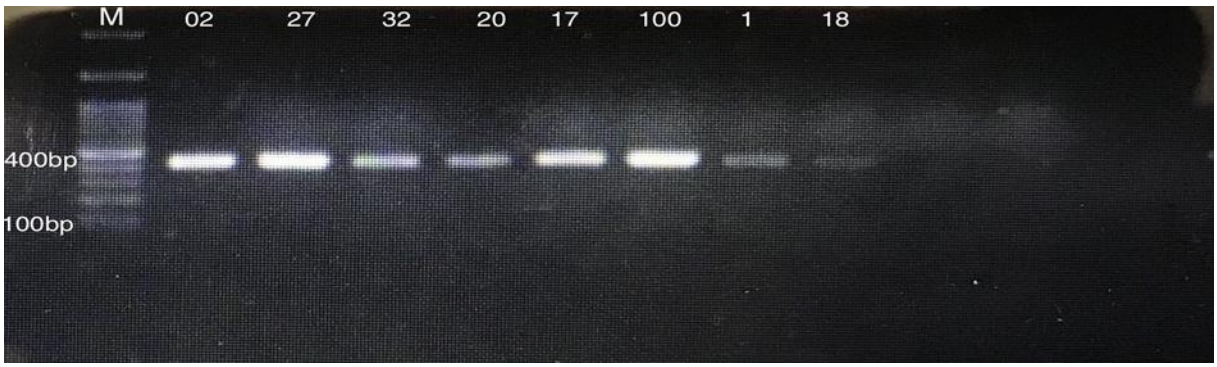

Figure 3. PCR products amplification with specific primer of *Fusarium oxysporum* on the 1% agarose gel, location and sample, m= (3000 bp DNA ladder), (1-8) DNA of the Fungal isolates 1= Foc-02, 2= Foc-27, 3= Foc-32, 4= Foc-20, 5 = Foc-17, 6= Foc-100, 7= Foc-1, 8= Foc-18

Table 7. Identification results of ITS Sequence for various Foc isolates from chickpea fields, Sulaimani, Iraq

Sample	Fungal identified	Accession Numbers	Location	Query Cover %	Identic Number %	Accession Number of BLAST Identification	Country Identification
1	*Fusarium oxysporum* f.sp. *ciceris* (Foc-1)	OP824784	Bakrajo	100	100		
2	*Fusarium oxysporum* f.sp. *ciceris* (Foc-02)	OP824785	Bakrajo	100	100		
3	*Fusarium oxysporum* f.sp. *ciceris* (Foc-17)	OP824786	bakrajo	100	100		
4	*Fusarium oxysporum* f.sp. *ciceris* (Foc-18)	OP824787	Grdy byran/ Barznja	100	97.2	KP992931 KU671029 KM253762	India
5	*Fusarium oxysporum* f.sp. *ciceris* (Foc-20)	OP824788	Taxta raq/ Barznja	100	95.1		
6	*Fusarium oxysporum* f.sp. *ciceris* (Foc-27)	OP824789	Kupay hajy/ Barznja	100	96.3		
7	*Fusarium oxysporum* f.sp. *ciceris* (Foc-32)	OP824790	Kanypanka/arbat	100	99.4		
8	*Fusarium oxysporum* f.sp. *ciceris* (Foc-100)	OP824791	Bakrajo	100	96.5		

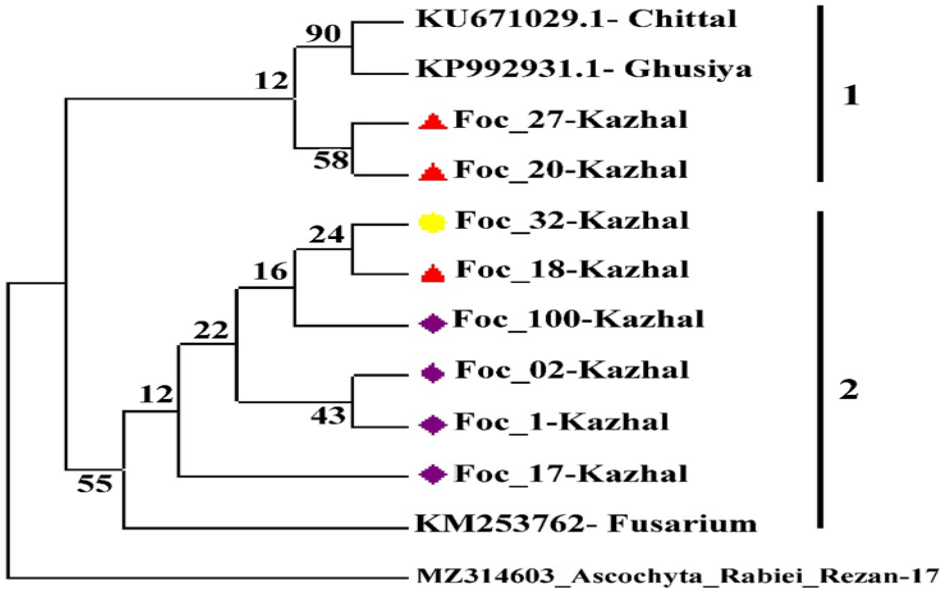

Figure 4. Maximum likelihood tree inferred from the ITS sequence data set of 11 *F. oxysporum ciceri* isolates.

The bootstrap support values (BP) are given at nodes. The tree is rooted to *Aschochyta rabiei* (MZ314603). MEGA 11 was used to analyze evolutionary processes

from the target sequences *F. oxysporum*. Bootstrapping indicated that each branch corresponding to the particular clade which was poorly supported (mostly bellow 60%). Clade I was found to be heterogeneous with Foc-20, Foc-27, KU671029 and KP992931 (11), clade II contained the other Foc isolates with only the Indian Foc isolate KM253762 (17) which was clustered out of the local Foc isolates (Fig 4). All the pathogenic Foc isolates in the current study were typically distributed throughout the whole phylogenetic tree and mixed with the Indian Foc isolate in the clades. Interestingly, two single membered clades uniquely consisted of Foc-27 and Foc-20 seem to be identical and represent the same isolate with relatedly high bootstrap values, besides that they are collected from the same location (Barznja). This isolate is closely related to the Indian Foc isolates KU671029.1 and KP992931.1. Further, isolate Foc-1 and Foc-2 which are seem to be identical too and represent another, they are also collected from Bakrajo location. However, although Foc-32 (Kanypanka), Foc-18 (Barznja), and Foc-100 (Bakrajo) collected from different location, they were clustered in a smaller clade and may be represented two close different isolates. Isolate Foc-17 (Bakrajo) however is nested in a same clade II but it is different from them and all of the clade I isolates. In addition of clustering Foc KM253762 with the rest of current study isolates. The capacity to recognize the pathogen races variation in a particular area of chickpea production is important for breeding programs and the efficient use of the available resistance resources. New procedures must be developed in order to identify the pathogen and its physiological races quickly, reliably, and repeatedly. The ITS PCR assays differentiate the Foc by the variation of genetic diversity between the eight isolates that were collected from different geographic locations and show variations in their pathogenicity on chickpea differentials in the greenhouse, the pathotype, and geographic location of the eight Foc races isolates vary as well. Foc races 1A through 6 are associated with wilting pathotype, while races 0 and 1B/C are associated to the yellowing pathotype. Races 0, 1A, 1B/C, 5, and 6 have been identified in California and the Mediterranean regions (15, 16), while other reports refer to the presence of races 1A, 2, 3, and 4 in India (21). Races 2, 3, and 4 were recently discovered in Turkey (7), and Ethiopia (33). Races 0 and 1B/C, 4 and 5, were also reported previously in Iraq **(**5). There was no correlation between geographic location, virulence variation, and genetic diversity in the case of ITS markers. According to the current study, Foc is a serious disease that affect numerous chickpea fields in Sulaimani and Halabja governorates in Iraqi Kurdistan. Significant differences were found in the macroscopic and microscopic traits among Foc isolates. Foc isolates were divided into two groups and ten distinct physiological races by phenotyping the isolates on 10 differentials. Each isolate's genomic DNA was amplified by the ITS primers to a maximum size of 400bp, producing a single band for each accession. All the isolates' ITS sequences were registered at the NCBI gen bank under various accession numbers. This is the first report in Iraq on the full identification of Foc isolates using morphological and molecular methods.

REFERENCES

1. Abd-Elsalam, K.A., I.N. Aly, M.A. Abdel-Satar, M.S. Khalil, and J.A. Verreet. 2003. PCR identification of Fusarium genus based on nuclear ribosomal-DNA sequence data. Af. J. of Biot. 2(4): 82-85. https://doi.org/10.5897/AJB2003.000-1016

2. Al-Maaroof, E.M. and N.M. Saber. 2019. Occurrence and biological control of cucumber damping off disease under protected cultivation in Sulaimani, Iraq. Int. J. of Annov. Appr. In Agr. 3(2): 229-246. https://doi.org/10.29329/ijiaar.2019.194.9

3. Al-Maaroof, E.M and P.H. Saed. 2023. Resistance of Iraqi wheat cultivars to common bunt disease and molecular diagnosis of the available *Bt genes* in each cultivar. Iraqi Journal of Agricultural Sciences, 54(6): 1760-1772. https://doi.org /10.36103/ijas.v54i6.1875

4. Al-Maaroof, E.M and R.M. Saleh, 2022. Physiological and molecular characterization of *Ascochyta rabiei* isolates from various chickpea areas across IKR, Iraq. The Iraqi J. of Agri. Sci. 53(2): 297-314. https://doi.org/10.36103/ijas.v53i2.1537

5. Al-Taae, A.K., H.A. Hadwan, and S.E. Al-Jobory. 2013. Pathogenic variability in isolates of *Fusarium oxysporum* f. sp. *ciceris* in Iraq. Sci. J. Univ. Zakho 1(A): 108-114. https://sjuoz.uoz.edu.krd/index.php/sjuoz/article/view/52

6. Amine, E., A. Douira, M. Ilyass and S. Ahmed. 2022. Integrating sowing date with Chickpea genotypes in managing Fusarium wilt in Morocco. Agriculture. 12(6): 773. https://doi.org/10.3390/agriculture12060773

7. Bayraktar, H., and F. S. Dolar. 2012. Pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates from chickpea in Turkey. Pak. J. Bot. 44: 821-823. http://142.54.178.187:9060/xmlui/handle/123456789/15403

8. Cha, J.Y., S. Han, H.J. Hong, H. Cho, D. Kim, Y. Kwon, and J.F. Kim. 2016. Microbial and biochemical basis of a Fusarium wilt-suppressive soil. The ISME J. 10(1): 119-129. https://doi.org/10.1038/ismej.2015.95

9. Chaudhry, M., M. Ilyas, F. Muhammad, and M. Ghazanfar. 2007. Sources of resistance in chickpea germplasm against fusarium wilt. Mycopath. 5(1): 17-21. http://111.68.103.26/journals/index.php/mycopath/article/viewFile/194/99

10. Dubey, S., S.R. Singh, and B. Singh. 2010. Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. Arch. of Phytopath., and Pl. Prot. 43(2): 174-190. https://doi.org/10.1080/03235400802021108

11. Golakiya, B., M. Bhimani, and L. Akbari. 2018. Characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Int.* J. Curr. Microbiol. App. Sci. 7(3): 1152-1162. https://doi.org/10.20546/ijcmas.2018.703.137

12. Gupta, O., M. Khare, and S. Kotasthane. 1986. Variability among six isolates of *Fusarium oxysporum* f. sp. *ciceri* causing vascular wilt of chickpea. Ind. Phytopathol. 39: 279-281

13. Hashem, A., B. Tabassum, and E.F. Abdallah. 2020. Omics approaches in chickpea Fusarium wilt disease management. Management of Fungal Pathogens in Pulses. Springer. 57-72. https://doi.org/10.1007/978-3-030-35947-8_4

14. Haware, M., Y. Nene, and M. Natarajan. 1996. The survival of *Fusarium oxysporum* f. sp. *ciceri* in the soil in the absence of chickpea. Phytopath. Med. 35(1): 9-12. https://www.jstor.org/stable/42685234

15. Jiménez-Gasco, M., E. Pérez-Artés, and R.M. Jiménez-Diaz. 2001. Identification of pathogenic races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f. sp. *ciceri* with random amplified polymorphic DNA (RAPD). Eu. J. of Plant Path. 107(2): 237-248. https://doi.org/10.1023/A:1011294204630

16. Jimenez-Gasco, M.M., and R.M. Jimenez-Díaz. 2003. Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f. sp. *ciceri* and its pathogenic races 0, 1A, 5, and 6. Phytopath. 93: 200-209. https://doi.org/10.1094/PHYTO.2003.93.2.200

17. Kaur, A., V. K. Sharma, A. Sirari, J. Kaur, G. Singh, and P. Kumar. 2015. Variability in *Fusarium oxysporum* f. sp. *ciceris* causing wilt in chickpea. African Journal of Microbiology Research. 9(15): 1089-1097. https://doi.org/10.5897/AJMR2014.7267

18. Khanzada, S.A., S.M. Iqbal, and A.M. Haqqani. 2003. Physiological studies on *Macrophomina phaseolina*. Mycopath.1:31-34. http://111.68.103.26/journals/index.php/mycopath/article/viewFile/5573/2608

19. Landa, B.B., J.A. Navas-Cortés, M. del Mar Jimenez-Gasco, B. Retig, and R.M. Jiménez-Díaz. 2006. Temperature response of chickpea cultivars to races of *Fusarium oxysporum* f. sp. *ciceri*, causal agent of Fusarium wilt. Pl. Dis. 90(3): 365-374. https://doi.org/10.1094/PD-90-0365

20. Lara B.Y., and B.S. Almaliky. 2024. Morphology, molecular identification, pathogenicity and first report of *Fusarium equiseti* associated with root rot and wilt disease of Catharanthus roseus in Baghdad, Iraq. Iraqi Journal of Agricultural Sciences, 55(6): 2153-2163. https://doi.org/10.36103/tjptrn68

21. Maitlo, S., R. Syed, M. Rustamani, R. Khuhro, and A. Lodhi. 2016. Influence of inoculation methods and inoculum levels on the aggressiveness of *Fusarium oxysporum* f. sp. *ciceri* on chickpea plant growth. Int. J. of Agri. and Biol. 18: 31-36. https://doi.org/10.5555/20163061056

22. Manjunatha, L., A. Puyam, G. Prema, M. Bandi, R. Kumar and T. Kavitha. 2022.

Chickpea biotic stresses, genomic designing for biotic stress, resistant pulse crops. Springer. 117-159. https://doi.org/10.1007/978-3-030-91043-3_2

23. Mina, U., and S.C. Dubey. 2010. Effect of environmental variables on development of Fusarium wilt in chickpea (*Cicer arietinum*) cultivars. Ind. J. Agric. Sci. 80(3): 231-234. https://epubs.icar.org.in/index.php/IJAgS/article/view/245

24. Nath, N., A. Ahmed, and F. Aminuzaman. 2017. Morphological and physiological variation of *Fusarium oxysporum* f. sp. *ciceri* isolates causing wilt disease in chickpea. Int. J. of Env. Agri. and Biot. 2(1): 202-212. http://dx.doi.org/10.22161/ijeab/2.1.25

25. Navas-Cortés, J.A., B.B. Landa, M.A. Méndez-Rodríguez, and R.M. Jiménez-Díaz. 2007. Quantitative modeling of the effects of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceri* races 0 and 5 on development of fusarium wilt in chickpea cultivars. Phytopathol. 97(5): 564–573. https://doi.org/10.1094/PHYTO-97-5-0564

26. Nelson, P.E., M.C. Dignani, and E.J. Anaissie. 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. Clinical microbiology reviews. 7(4): 479-504. https://doi.org/10.1128/cmr.7.4.479.

27. Pampana, S., A. Masoni, M. Mariotti, L. Ercoli, and I. Arduini. 2018. Nitrogen fixation of grain legumes differs in response to nitrogen fertilisation. Experimental Agriculture. 54(1): 66-82. https://doi.org/10.1017/S0014479716000685

28. Paulkar, P.K., B.T. Raut, and K.B. Kale. 2001. Nutritional studies on four isolates of *F . oxysporum* f. sp. *ciceri*. New Agri. 12: 89-91. https://doi.org/10.15740/HAS/IJPS/11.2/213-217

29. Reddy, S.A., C. Kushwaha, and B.D Prasad. 2022. Analyzing influence of thermal and osmotic stress on native isolates of *Fusarium oxysporum* f. sp. *cubense*. Ind. Phytopathol.75: 863-868. https://doi.org/10.1007/s42360-022-00514-8

30. Sanghvi, G., K. Bhimani, D. Vaishnav, T. Oza, G. Dave, and N. Sheth. 2016. Mitigation of acrylamide by l-asparaginase from Bacillus subtilis KDPS1 and analysis of degradation products by HPLC and HPTLC. Springer Plus. 5(1): 1-11. https://doi.org/10.1186/s40064-016-2159-8

31. Sharma, K.D., W. Chen, and F.J. Muehlbauer. 2005. Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. Pl. Dis. 89:385-390. https://doi.org/10.1094/PD-89-0385

32. Shawket, S., S. Bashir, M.N. Mughal, R.u.R. Mir, F. Bhatt, and T. Shah. 2018. Identification of sources of resistance against wilt (*Fusarium oxysporum* f.sp. *ciceri*) in Chickpea genotypes under temperate agro-climatic conditions of kashmir. Int. J. Cur. Mic. App. Sci. 7(9):195-199. https://doi.org/10.20546/ijcmas.2018.709.025

33. Shehabu, M., S. Ahmed, K. Sakhuja. 2008. Pathogenic variability in Ethiopian isolates of *Fusarium oxysporum* f. sp. *ciceri* and reaction of chickpea improved varieties to the isolates. Int. J. of Pest Man. 54(2): 143-149. https://dio.org/10.1094/PD-89-0385

34. Tamura, K., G. Stecher, D. Peterson, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. and Evol. 30(12): 2725-2729. https://dio.org/10.1093/molbev/mst197

35. Upasani M.L., B.M. Limaye, G.S. Gurjar, S.M. Kasibhatla, and V.S. Gupta. 2017. Chickpea-*Fusarium oxysporum* interaction transcriptome reveals differential modulation of plant defense strategies. Sci Rep.7: 1-12. https://dio.org/10.1038/s41598-017-07114-x

36. Younesi, H., M. Darvishnia, E. Bazgir and K. Chehri. 2021. Morphological, molecular and pathogenic characterization of *Fusarium spp.* associated with chickpea wilt in western Iran. J. of Pl. Prot. 61(4): 402–413. https://dio.org/10.24425/jppr.2021.139250