# PATHOGENIC VARIABILITY IN ISOLATES OF *FUSARIUM OXYSPORUM F. SP. CICERIS*

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***(Accepted for publication: June 9, 2013)***

## Abstract

**Twenty isolates of *Fusarium oxysporum* f. sp. *ciceris* were isolated from wilted chickpea plants obtained from different districts of north part of Iraq to assess variability in pathogenecity of the populations. Each isolate was tested on 12 differential chickpea varieties . Isolates showed highly significant variation in wilt severity on the differential varieties . Based on the reaction types that induced on differential varieties, isolates were grouped into four groups, First group included isolates FocS1 , FocQ7 , FocQ10 , FocF13 , FocH17 and FocH18 The Second group included isolates FocS2 , FocS3 , FocS4 , FocQ5 , FocQ8 , FocQ9 , FocF11 , FocF12 , FocF14 and FocH19 The Third group included isolates FocF15 , FocH16 , FocH20 .Where the isolate FocQ6 was placed in the Fourth group .Results showed that the percentage of genetic similarity was ranged 42% to 100% and was 42% between the first group and other groups and 72% between the three groups the rest and thus this indicate the presence of four races of the fungus which are zero, 4, 5, and 1B / C this represent the first record of these races in Iraq.**

***Keywords****:chickpea wilt*; *Cicer arietinum*; *Fusarium oxysporum f. sp. ciceris*; *pathogenic variability*

## Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume in Iraq and is grown mainly in the north part of the country for domestic consumption purposes. In the 2010 cropping season, chickpea was cultivated on 10500 ha of land with a productivity of about 700Kg/ha as compared to 3 t/ha in the world(FAO,2010) . This low productivity was due to a variety of a biotic and biotic stresses. Chickpea is attacked by a number of soil-borne and foliar diseases as well as field and storage insect pests. Among the soil-borne diseases affecting chickpea, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the major yield-limiting factor, wherever the crop is grown in north Iraq The wilt can be observed in susceptible genotype within 25 days after sowing in the field. The pathogens attack the roots of plants and cause wilting as a result the whole plant shows drooping of leaves and paler color than healthy plants. The plant finally collapses and dies. Such plants do not show external rotting and look healthy, when cut vertically downward from the collar region, show brown streak of the internal tissues. (Al-Taae,1997;Al-Taae *et.al*,2010).

Throughout the world, annual chickpea yield losses due to Fusarium wilt vary from 10 to 15% (Trapero-Casas and Jimenez-Diaz 1985), but can reach even 100% under certain conditions (Navas-Cortes *et. al*. 2000). In Ethiopia, about 30% yield loss of chickpea due to wilt/root rot has been reported, where *F. oxysporum* f. sp. *ciceris* was isolated from more than 50% of the root samples (Mengistu and Fusarium wilt of chickpea can be managed using resistant cultivars, adjusting sowing dates, and fungicidal seed treatment (Navas-Cortes *et. al*. 1998).

The occurrence of pathogenic races in *F. oxysporum f. sp. ciceris* is known from other parts of the world (Haware and Nene 1982; Cabrera de la Colina *et. al*.1985; Phillips 1988; Jimenez-Diaz *et. al*. 1993; Kelly *et. al*. 1994), however, until now the presence of pathogenic variability in populations of *F. oxysporum f. sp. ciceris* in Iraq has not been studied. Therefore, in order to strengthen the breeding efforts that aims at boosting chickpea productivity and production through the development of wilt resistant chickpea varieties, this study was undertaken with the aims of assessing the pathogenic variability in isolates of *F. oxysporum f. sp. ciceri*s, causing chickpea wilt.

## Materials and methods

Collection of wilted chickpea plant samples :

Wilted chickpea plant samples were collected from four major chickpea-growing districts, which are in the production domain of north Iraq(Ninevah and Erbil provinces). In each district, ten chickpea fields were observed and wilted plants were systematically collected by traversing each field diagonally.

Isolation, purification and identification of Fusarium isolates:

Five pieces of plant tissue (1–2 mm) were taken from the collar region of each wilt-affected plant and the remaining part was split lengthwise and checked for browning of vascular tissue to confirm fusarial wilting (Haq and Jamil 1995). pieces of plant tissue, taken only from plants that showed clear vascular discoloration, were surface disinfected with 1% sodium hypochlorite solution for 2–3 min, rinsed twice in distilled sterilized water, dried on filter paper and were plated on 2%water agar in 9-cm Petri dishes (Haware and Nene 1982). The plates were incubated at 25 C for 5 days. After incubation, a loopful of conidial mass was transferred from actively growing Fusarium colony to 10 mL of distilled and sterilized water to prepare conidial suspension. The conidial suspension was diluted 10 times to obtain well-separated conidia, and from the final dilution one loopful was taken and streaked on solidified 2% water agar medium. After 24 h of incubation at 25C, a single colony was cut from well-separated colonies and transferred to a Petri dish containing solidified potato dextrose agar (PDA) . A total of 20 isolates of Foc were isolated.FocS1-FocS4 from Shakhan ,FocQ5-FosQ10 from Al-Qush,FosF11-FosF15 from Fyda and FosH16-FosH20 from Harer regiens. Fusarium isolates were identified microscopically by their morphological characteristics such as abundance of micro-and fewer macroconidia, short and unbranched monophialides, white to creamy-white colour on PDA medium and production of chlamydospores.

Inoculum preparation and infestation of soil:

Each isolate of Foc was multiplied on petri dishes (9 cm diameter) containing 20 ml of PDA by transferring a small amount of infested sand from the test tube. From 7-day-old culture, two discs (1 cm diameter each) were removed with sterile cork borer and transferred to 100 g of sand: maize meal (9:1, autoclaved twice at 121C for 20 min) in marmalade glass jars and incubated for 14 days at 25C (Jimenez-Gasco*et. al.* 2001).The inoculums raised in each marmalade jar was mixed thoroughly with 2 kg sterilized soil in 15-cm plastic pots. The pots were disinfested with 2.5% sodium hypochlorite solution for 5 min, rinsed in distilled water and air-dried before use. The isolates were allowed to become established in the infested soil/ sand mixture for 1 week before planting of chickpea seeds.

Seeds of deferent chickpea deferential varieties were surface-sterilized by immersion in 2.5% sodium hypochlorite solution for 2–3 min, rinsed twice in distilled sterilized water, air-dried in the laboratory and planted in each pot containing previously sterilized and then artificially infested with pathogen soil–sand mixture.

Pots were arranged in a randomized complete block design with three replications for each isolate-deferential line or isolate-variety combination and maintained on greenhouse benches for 60 days. .

Data on the responses of all deferential varieties and improved varieties to deferent isolates of the pathogen was recorded at weekly intervals. Disease development on deferential varieties and varieties was scored on per plant basis using a five-point severity scale based on percentage of foliar tissue showing wilt symptoms (0 = 0%, 1= 1to 20%, 2= 21 to 50%, 3 = 51 to 100%, and 4= dead plants). Then, on the basis of average disease score, differential varieties and improved varieties were categorised into reaction types such as resistant, moderately resistant and susceptible . Vascular discolouration was checked by splitting the stem and tap roots of two to three plants chosen randomly to confirm that the disease is caused by Foc (Haq and Jamil 1995). Based on similarity/dissimilarity of reactions of differential varieties, isolates were designated into different groups.These values (zero and 1) were entered in the program (NTSYS). A result of the analysis a matrix included transactions genetic similarity between isolates, including the work of the genetic family tree Dendrogram of all isolates that collected in groups based on the extent of similarity or genetic variation among them, and using: (UPGMA) Unweighted Pair Group Method with Arithmetic

## Results

Pathogenic variability in *F. oxysporum* f. sp.*ciceris* :

All the 20 isolates of *F. oxysporum* f. sp.*ciceris* (Foc) collected from farmer's fields ' infected and produced different levels of disease severity on the differential varieties of chickpea. reaction types calculated also differed among isolates.The isolates showed highly significant variation

In a study of genetic variability for twenty isolates of the fungus Foc collected from

different areas of chickpea planted in Ninevah and Erbil provinces .The results presented in Tables (1 and 2) showed that there was difference in the pathogenicity of these isolates in affecting chickpea differential varieties . The isolates were classified in four groups depending on the reaction of differential varieties . The first group, which included isolates FocS1 , FocQ7, FocQ10 , FocF13 , FocH17 and FocH18 .From the results obtained , it was concluded that these isolates belong to the race 0 which is known internationally (Cabrera de la Colina *et al.*1985). The race 0 was non-pathogenic to the JG-62 variety, while this variety was susceptible to other races of the pathogen(Haware and Nene, 1982, Jimenez-Gasco *et al*, 2004 , Sharma *et al*, 2005 , Landa*et al*, 2006 and Jimenez-Fernandez *et al*, 2011) .

The isolates caused severe and progressive yellowing without wilting on L-550 variety, this is fully accomplished with the finding of Correll (1991) and Kelly *et al* (1994) that the 0 race cause yellowing symptoms without wilting. The isolates FocS2 , FocS3 , FocS4 , FocQ5 , FocQ8 , FocQ9 , FocF11 , FocF12, FocF14 and FocH19 which conceive pathogencity placed in the second group, which gave results comparable to race four (Phillips, 1988 ,Honnareddy and Dubey, 2006 , Sharma *et al*, 2005 and Jimenez-Fernandez *et al*, 2011), these isolates were able to affect all varieties except WR-315 and K850, which were resistant to these isolates .These isolates caused severe infection on six varieties (Annigeri , JG-62 , ICC4475 , CHAPP2 , L550 and C-104).The third group, consists of FocF15 , FocH16 and FocH20 which gave different reactions in the infection of chickpea differential varieties and depend on resistance reaction of WR-315 and BJ212 varieties and moderate susceptibility of the varieties K850 , UC27 and CPS-1 .These isolates are considered to be very close to race 5 (Jimenez-Gasco*et al*, 2004 , Sharma *et al*, 2005 and Jimenez-Fernandez *et al*, 2011) and caused severe infection on the chickpea differential varieties (Annigeri, JG-62 , ICC4475 , CHAPP2 , PCH-15 and C-104 ). Where the isolate FocQ6 is placed in the fourth group , which belong to 1B / C race Depending on the reaction of the UC27 verities which was susceptible to the isolate FocQ6 while the two varieties K850 and PCH-15 were with moderate susceptiblity (Jimenez-Gasco *et al*, 2004 and Jimenez-Fernandez *et al*, 2011 ) .

It is clear from the results that 30% of the isolates belonged to the first group which is known as race 0 and 50% of the isolates belongs to the second group which is known as race 4 and 15% of the isolates belonging to the third group which is known as race 5five and 5% of the isolates belonged to the fourth group which is known as race 1B / C .These results are in agreement to those mentioned by Haware and Nene (1982), which revealed that there were four races of the fungus in India . Dolar (1997) reported the existence of the races 0, 2 and 3 in Turkey, while Shehabu *et.al*(2008) by screening 24 isolates of *F.oxysporum* f. sp. *ciceris* which were isolated from wilted chickpea plants obtained from different districts and 'wilt sickplots' of central Ethiopia to assess variability in pathogenecity of the populations, the isolates were grouped into four corresponding races of the fungus and there weree four races of the pathogen in Ethiopia.

Depending on the pathogenicity of these isolates a dendrogram was constructed to show the genetic variation of isolates based on the extent of the genetic variation among them the results of genetic analysis has given four groups (Figure 1) The first group included isolates FocS1 , FocQ7 , FocQ10 , FocF13 , FocH17 and FocH18,the second group included isolates FocS2 , FocS3 , FocS4 , FocQ5 ,FocQ8 , FocQ9 , FocF11 , FocF12 , FocF14 and FocH19,the third group satisfaction to isolates FocF15 , FocH16 and FocH20 and the fourth group included one isolate ( FocQ6). It is clear from this dendrogram that the proportion of genetic similarity among these isolates were from 42% to 100% . It was 42% between the first group and the other groups and 72% between the rest three groups. Results in Table (1) indicate the presence of four races of the fungus, which are given in Table (2) namely (zero) , (4) , (5) and (1B / C) .These results represent the first record of these races in Iraq .It is clear that there are differences in the pathogenicity of the isolates of different regions ,which is due to the difference in the phenomenon of sexual reproduction in fungi accounting for the accumulation of variables and the occurrence of genetic differences and as a result globallythere are eight races which are distributed in the world (Haware and Nene, 1982 and Jimenez-Diaz *et al*, 1993 and Taylor *et al*, b 1999, Jimenez-Gasco *et al*, 2004).

**Table( 1).** Reaction of 12 chickpea differential varieties to the 20 isolates of *Fusarium oxysporum* f.sp.*ciceris*

| Isolate No. | Differential varieties | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| FocS1 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocS2 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocS3 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocS4 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocQ5 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocQ6 | S | S | S | MS | S | S | MS | S | R | MS | R | R |
| FocQ7 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocQ8 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocQ9 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocQ10 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocF11 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocF12 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocF13 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocF14 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocF15 | S | S | S | MS | MS | S | S | S | R | MS | R | R |
| FocH16 | S | S | S | MS | MS | S | S | S | R | MS | R | R |
| FocH17 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocH18 | S | R | S | R | S | S | R | S | MS | S | S | R |
| FocH19 | S | S | S | R | MS | S | MS | S | S | MS | MS | R |
| FocH20 | S | S | S | MS | MS | S | S | S | R | MS | R | R |

1.Annigeri, 2 .JG-62, 3ICC4475 , 4K850 ,5UC27 ,6CHAPP2
7PCH-15,8 C-104 ,9L550 ,10CPS-1 ,11BJ-212 and 12WR-315

Table( 2). Reaction of 12 chickpea differential varieties to four races of *Fusarium oxysporum* f.sp.*ciceris*

| Differential varieties | Races | | | |
|---|---|---|---|---|
| | 0 | 4 | 5 | 1B/C |
| Annigeri | S | S | S | S |
| JG-62 | R | S | S | S |
| ICC4475 | S | S | S | S |
| K850 | R | R | MS | MS |
| UC27 | S | MS | MS | S |
| CHAPP2 | S | S | S | S |
| PCH-15 | R | MS | S | MS |
| C-104 | S | S | S | S |
| L550 | MS | S | R | R |
| CPS-1 | S | MS | MS | MS |
| BJ212 | S | MS | R | R |
| WR-315 | R | R | R | R |

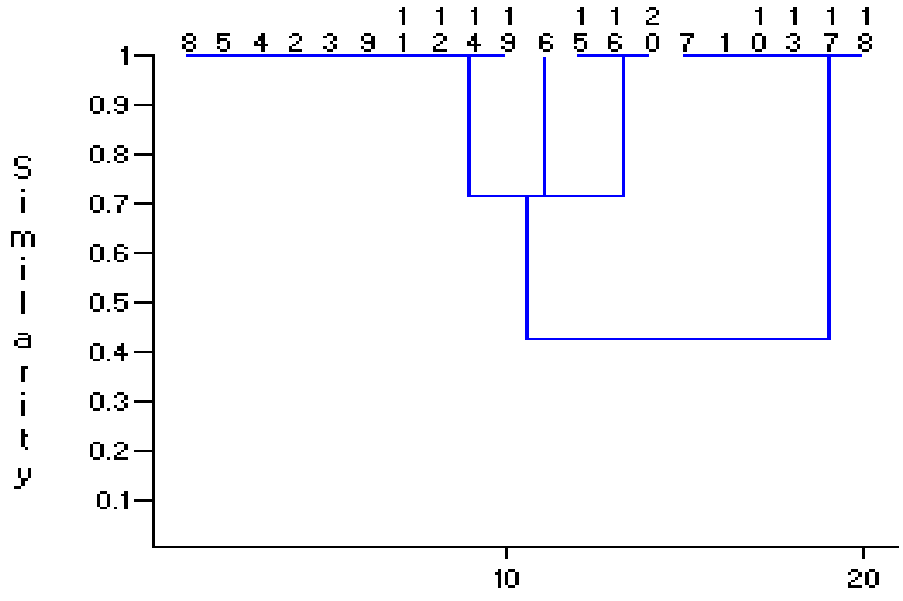


**Fig. (1 ).** Phylogenetic of 20 isolates of the fungus *Fusarium oxysporum f.sp. ciceris* from Ninevah and Erbil provinces using UPEGMA programs showing the similarity between groups.

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## التباين الوراثي لعزلات الفطر *Fusarium oxysporum* f. sp. *ciceris*

**الملخص**

في دراسة التباين الوراثي لعشرين عزلة من الفطر *Fusarium oxysporum* f. sp. *ciceris*(Foc) جمعت من مناطق مختلفة لزراعة الحمص في محافظتي نينوى وأربيل وبالاعتماد على القدرة الإمراضية لهذه العزلات عُملت شجرة التباين الوراثي Dendrogram التي تقوم بتجميع العزلات الى مجاميع بناءً على مدى التباين الوراثي فيما بينها فقد أعطت نتائج التحليل الوراثي أربع مجموعاتالمجموعة الأولى ضمت العزلات FocS1 و FocQ7 و FocQ10 و FocF13 و FocH17 و FocH18 والمجموعة الثانية ضمت العزلات FocS2 و FocS3 و FocS4 و FocQ5 و FocQ8 و FocQ9 و FocF11 و FocF12 و FocF14 و FocH19 أما المجموعة الثالثة فضمت العزلات FocF15 و FocH16 و FocH20 والمجموعة الرابعة ضمت عزلة واحدة وهي FocQ6 ، ومن النتائج نلاحظ أن نسبة التشابه الوراثي بلغت من 42% إلى 100% وكانت 42% مابين المجموعة الأولى وبقية المجموعات و 72% مابين المجموعات الثلاثة البقية وبالتالي هناك أربع سلالات للفطر وهي صفر و 4 و 5 و1B/Cويعد تسجيل هذه السلالات الأول في العراق .

## جیاوازی بۆماوەیی بۆ کەڕووە لەبەرگیراوەکانی *Fusarium oxysporum* f. sp. *ciceris*

**پوختە**

**لە توێژینەوەیەکی بۆماوەیی کە بۆ بیست جۆر کەڕووی لەبەرگیراوە لە جۆری *Fusarium oxysporum* f. sp. *ciceris* (Foc) کە لە ناوجە کشتوکاڵیە جیاوازەکان کۆ کراونەتەوە و لە بەرگیراون وەك حمص لە سوریا وە پارێزگای موصل ، پارێزگای هەولێر لە عێراق . بە پشت بەستن بە توانای نەخۆش کردنی هەر جۆرێك لەو کەڕووانە بۆ ڕووەك داری جیاکەرەوەی بۆماوەیی بۆ هەر 20 بیست جۆر کەڕووی لەبەرگیراوە داڕێژرا Dendrogram ، ئەم جۆرە داڕێژرانەش کۆکردنەوە و دابەشکردنی کەڕووە لەبەرگیراوەکان لەسەر بنەمای جیاوازی یۆماوەیی لەخۆ دەگرێت ، وە لە ئەنجامی شیکردنەوەی بۆماوەیی دەرکەوت کەوا هەر بیست جۆرە کەڕووە لەبەرگیراوەکە دابەش دەکرێت بۆ چوار بەش ، بەشی یەککەم FocQ7 ، FocQ10 ، FocF13 ، FocH17 ، FocH18 لەخۆ دەگرێ ، بەشی دووەم FocS2 ، FocS3، FocS4 ، FocQ5 ، FocQ8 ، FocQ9 ، FocF11 ، FocF12 ، FocF14 لەخۆ دەگرێ ، بەشی سێیەم FocF15 و FocH16 و FocH20 لەخۆ دەگرێ ، وەبەشی چوارەم تەنها یەك کەڕووی لەبەرگرەوە لەخۆ دەگری کە ئەویش FocQ6 . لەئەنجامدا بۆمان دەردەکەوێت کەوا ڕێژەی لەیەکجوونی بۆماوەیی دەگاتە 42٪ تا 100٪ ، وە ئەم 42٪ دەکەوێتە نێوان بەشی یەککەم و بەشەکانی تر ، 72٪ دەکەوێتە نێوان بەشە ماوەکەی تر ، کەواتە چوار جۆر توخمە کەڕوو دیای کران کە ئەوانیش 4 ، 5 ، 1B/C ، ئەمەش بە یەکەم تۆمار دادەنرێت لە سەرتاسەری عێراق .**