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Variation among *Fusarium oxysporum* f. sp. *ciceris* isolates causing chickpea root and crown rot from Kurdistan province

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Abstract: Fusarium wilt, root and crown rot caused by *Fusarium oxysporum* f. sp. *ciceris*, (FOC) is the highly significant soil-borne disease of chickpea in the Kurdistan province of Iran. The distribution of pathogenic races of FOC in Kurdistan province was determined during this research. Infected plant samples were collected from 42 fields in the chickpea production area of the Kurdistan province. The causative microorganism of the disease was isolated and purified from each sample, and then FOC isolates were identified by morphological characters. After the pathogenicity test and evaluation of pathogenic variability on the susceptible cultivar Kaka, the DNA extraction, the molecular identification of species, and races of pathogenic isolates were performed using FOC-specific linked primers. Among the collected isolates, 37 were identified as *Fusarium oxysporum* f. sp. *ciceris*. Molecular identification of races using SCAR-linked markers (1B/C, 0, 2,3,4,5, 6, and 1A) revealed that 28 out of 37 isolates belonged to race 0, and other isolates belonged to race 1B/C. There was no relationship between the prevalence of races and their geographical distribution. Identification of the races is crucial for the evaluation of resistance and the development of new commercial cultivars. The application of resistant cultivars is a fundamental approach for the integrated management of the Fusarium wilt, root, and crown rot for durable chickpea production.

Keywords: *Cicer arietinum*, diversity, pathogenicity, races

INTRODUCTION

The second most cultivated legume is Chickpea (*Cicer arietinum* L.) in the world, after dry bean (*Phaseolus vulgaris* L.) and dry pea (*Pisum sativum* L.). The chickpea crop is infected by several fungal and bacterial diseases. One is Fusarium wilt, root, and crown rot caused by the soil-borne fungus *Fusarium oxysporum* Schelchtend.: Fr. f. sp. *ciceris* (Padwick) Mantuo & K. Sato (FOC), a disease that can cause enormous damage on a global scale (Jalali and Chand, 1992). Although average annual yield losses of 10-15% reported (Trapero-Casas and Jimenez-Diaz, 1985; Jalali and Chand, 1992). If it became prevalent complete crop loss can be made (Haware and Nene, 1982; Halila and Strange, 1996). Using resistant cultivars is a cost-efficient and widely applied method for handling chickpea disease. (Jalali and Chand, 1992). However, pathogen virulence variability in FOC limits the effectiveness of this strategy (Haware and Nene, 1982; Trapero-Casas and Jimenez-Diaz, 1985; Jimenez-Diaz *et al*., 1993). For disease management and efficient use of available resistant cultivars, it is vital to recognize pathogen races of FOC. There are several reports of FOC races and genetically diverse populations over chickpea cultivation in the countries of Ethiopia, India, Spain, Sudan, Syria, Tunisia, Turkey, and Iran (Alloosh *et al*., 2019: Bayraktar *et al*., 2012; Gurjar *et al*., 2009; Haware and Nene, 1982; Jimenez-Diaz *et al*., 1989; Shehaboo *et al*., 2008; Zamani *et al*., 2004). Based on the reaction of chickpea differential lines and molecular markers, researchers have identified two groups and eight races of FOC (0, 1A, 1B/C, 2,3,4,5, and 6). While monitoring the aboveground symptoms of infected chickpea plants, two groups have been described; one group generates yellowing and the other group; that cause wilting (Trapero-Casas and Jimenez-

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Diaz 1985). According to previous research, races 1A, 2,3,4,5, and 6 were described with vascular discoloration and wilting advanced to plant death and placed in the wilting group. The other group has the races 0 and 1B/C distinct with yellowing change in plants (Trapero-Casas and Jimenez-Diaz 1985; Jimenez-Diaz *et al*., 1993). Historically, races 1A, 2,3, and 4 from India were described (Haware and Nene, 1982). Then the incident of races 2, 3, and 4 from Ethiopia and India were reported, but from Turkey, only races 2 and 3 were detected (Haware and Nene, 1982; Jalali and Chand, 1992; Shehabu *et al*., 2008). Later several reports for the detection of races from diverse regions were published, including races 0, 1A, 1B/C, 5, and 6 from California (USA) and Spain; races 0 and 1A, 1B/C from Syria, Tunisia, and Turkey; races 0, 1A, and 6 from Palestine; races 1A and 6 from Morocco; races 0 and 2 in Sudan; races 0, 2, and 3 in Turkey and race 0 from Lebanon (Bayraktar and Dolar 2012; Jimenez-Diaz *et al*., 1993; Halila and Strange 1996; Mohamed *et al*., 2015). Investigation of FOC genetic diversity at the level of races and populations is a primary step for handling the chickpea disease. Thus, this study aimed to estimate the genetic diversity of FOC isolates collected from infected chickpea fields in the Kurdistan province of Iran.

MATERIAL AND METHOD

Infected plant sampling

Infected chickpea samples with yellowing, root rot, and wilting symptoms were collected from 42 chickpea fields in Kurdistan province, Iran in 2019. Latitude and longitude information of each sampling location were recorded. The samples were transferred to the laboratory of the National Plant gene bank of Iran, Seed and Plant Improvement Institute, and stored at 4 ˚C in a refrigerator.

Isolation and purification of the disease agent

Segments of the infected tissues were cut from the root and crown parts of samples with disease symptoms. The samples were first washed with sterilized distilled water and then disinfected with sodium hypochlorite (containing 1% of active substance) for one minute and washed again with sterilized distilled water and dried on sterilized filter paper. Then they were transferred to a PDA culture medium and kept in the incubator for 5-7 days at 25-30 ˚C. Then each fungal isolate was purified separately by the standard single-spore method. To store the isolates, 1.5 cc vials were filled to half with a mixture of 10 parts washed sand, one part perlites, and two parts straw, and 0.5 cc distilled water and sterilized using an autoclave. After the growth of the single spores, a piece of culture medium containing the grown isolate was transferred to the vial for long-term storage and subsequent identification.

Morphological identification of isolates

To evaluate the macroscopic characteristics of isolates, each isolate was cultured on the PDA media and stored at 25 °C and 8/16-hour photoperiod of fluorescent light. After 14 days the phenotypic characteristics such as colony growth rate, colony color, and color diffusion in the agar of the fungal isolates were measured. In addition, microscopic characteristics of each isolate such as shape and size of macroconidia, microconidia, and chlamydospores, the color of, sporodochium, phialide type, and shape of conidia on conidiophore after growth on CLA medium were recorded after three weeks using the Ogawa model microscope with 40X and 100X magnifications. Characteristics of sporodochium and macroconidia; such as presence or absence, color, size, shape and terminal and primary cell type, reproduction method, and the number of transverse bands were recorded. In addition, characteristics of microconidia such as the presence or absence of microconidia, sporulation type, type of arrangement (single, false head and chain), number of transverse bands, phialide type (mono or poly phialide), characteristics of chlamydospores including presence or absence, place of production, wall type, arrangement (single, batch, double and chain), presence or absence of false chlamydospores and presence or absence of swollen cells in filaments were measured. For each sample, the dimensions of 50 spores were measured and their average was calculated. Then based on their morphological characteristics *Fusarium* species were identified using Leslie & Summerell (2006) and Nelson *et al.* (1983).

Pathogenicity tests and pathogenic variability

The pathogenicity tests of *Fusarium* isolates were carried out based on Nene and Haware's (1980) method. Seeds of Wheat, tomato, Parsley, bean, and chickpea were disinfested with 0.25% sodium hypochlorite (NaOCl 0.25%) for five min. Then they were planted in 15 cm pots filled with sterilized soil and kept for two weeks at 25 ± 1°C and 12/12 h photoperiod. When the seedlings became three to four-leafed, they were inoculated with each *Fusarium oxysporum* isolates, First, the topsoil of each pot was removed and then 15 ml of fusarium spores' suspensions with 5×10^6 spores /ml concentration was added to the base of each seedling and the top soil was replaced. Then pathogenic variability of each isolate was measured according to Dubai *et al*., (2010). The percentage of wilting and yellowing was scored at 15 days and 30 days after sowing Kaka cultivar chickpea seeds under artificially inoculated conditions as mentioned earlier. According to the reaction of the seedlings, 30 days past sowing, the isolates were grouped into three categories weakly pathogenic (up to 30% wilt), moderately pathogenic (31 to 50% wilt) and highly pathogenic (above 50% wilt). (Dubai *et al*., 2010). The experiment was performed in a completely randomized design with three replications. PDB culture medium without Fusarium spores was used as a control treatment.

Molecular identification of isolates

Using the technique described by Zhong and Steffenson (2001), total genomic DNA was extracted from Fusarium species. Nearly 100 mg mycelium of each isolate was finely grounded and suspended in 400 µL of extraction buffer (50 mM Tris-HCl (pH 8.0), 150 mM EDTA, 1% sarkosyl, and 300 µg proteinase K

per mL). The suspensions were transferred into a 1.5 mL microtube, mixed by vortex for 10 seconds, and incubated for 30 minutes at 65°C. Then, 400 µL amount of chloroform: isoamyl alcohol (24:1) was inserted, and mixed for 10 min, then centrifuged at 10,000 rpm for 10 min at 4°C. An equal amount of isopropanol was mixed with the clear aqueous phase of suspension and stored overnight for precipitation of DNA at -20°C. Finally, the DNA was recovered by centrifugation at 10,000 rpm for three min and precipitated with 70% ethanol. The DNA pellet was then rinsed twice with one ml of 10 mM Tris-HCl and 0.1 mM EDTA or sterile distilled water buffer. The purity and amount of extracted DNA were determined using NanoDrop 1000 Spectrophotometer (Thermo Scientific, U.S.A).

Polymerase chain reaction

A standard polymerase chain reaction (PCR) assay was conducted utilizing the primer pair FOC-f (5′- GGCG TTTCGCAGCCTTACAATGAAG-3′) and FOC-r (5′- GACTCCTTTTTCCCAGGTAGGTCAGAT-3′) that was used to amplify a DNA fragment of 1500 bp for the detection of FOC isolates by PCR (Arvaio-Ortiz *et al.*, 2011). The final volume of PCR reaction mixture was 25 µL containing 12.5 µl 2X PCR Master Mix, 8.5 µl of distilled water, one µl (10 pmol/ml) specific F primer, one µl (10 pmol/ml) specific R primer, 20 ng of fungal DNA. The following conditions: initial denaturation at 94°C for 10 minutes; 36 cycles of denaturation for one minute at 94 °C, annealing for one minute at 58 °C, initial extension for one minute at 72°C, and final extension of five minutes at 72°C applied. Electrophoresis of 0.8% agarose gels was used for detecting PCR products (Arvaio-Ortiz *et al.*, 2011; Shokri *et al*., 2020). The 1 Kb DNA Ladder (SMOBIO, DM3100) was used, which shows (0.25-10 Kb) fragments bands and isolates compared to them.

Genetic diversity and races analyses

In order to determine the genetic diversity and the FOC races, eight molecular markers were used (Table 1). The PCR amplifications were accomplished similarly to the above description. The thermocycling program for each primer was calculated with their proper annealing temperatures (Table 1). The PCR cycles were carried out as described earlier.

RESULTS

Based on the results obtained from sampling, isolation of fungi, pathogenicity test, and molecular studies on selected samples from 42 infected fields in Kurdistan province, 37 *Fusarium oxysporum* f. sp *ciceris* isolates were identified (Fig. 1).

Morphological characteristics

Different species were identified among *Fusarium* isolates. The highest frequency was two species complexes of *F. oxysporum* and *F. solani*. The *F. oxysporum* is a complex species and has been reported as a cause of vascular wilt disease in many plant species from all over the world. In addition, this species is known as a common soil saprophyte. On the PDA medium the colony morphology is very diverse. The hyphae are low or very high in cotton shape and their color varies from white to purple. In some isolates, large numbers of orange or purple macroconidia form on the filament mass observed in the center of the colony. On CLA medium, macroconidia were usually produced in large numbers on orange color sporodochia and some isolates had no or few sporodochia. Macroconidia were short to medium (4.5 µm×35-50 µm) in length and ranged from crescent to smooth, thin-walled, often with three transverse (septate) in shape. The primary cell was short and, in some isolates, slightly hooked or curved. The basal cell was foot-shaped and sometimes terminates. Macroconidia were produced on extremely small branching conidiophores as sporodochia. The microconidia were often without transverse walls and in elliptical, elongated, and sometimes fragmentary shapes. Microconidia were often short (2.5-3 µm× 5-7 µm) and produced in large numbers in the form of false heads on short monofilaments. Chlamydospores formed in large numbers within hyphae on the surface of the CLA medium. Occasionally, chlamydospores were observed as clusters or short chains within or at the ends of strings. The chlamydospores wall's surface appeared smooth or rough (Fig. 2).

Pathogenicity test and pathogenic variability

The 37 isolates of *Fusarium oxysporum* species caused wilting and yellowing after 15 days post-inoculation only on the chickpea seedling. The other seedlings remain healthy.The pathogenic variability of each isolate was determined according to Dubey *et al*. (2010) separately based on the percentage of yellowing and wilting. The disease aggressiveness of each isolate was significantly different ($P \leq 0.01$) (Tables 1 and 2). Isolates related to the Kamyaran region showed the highest aggressiveness (64.88%), and isolates related to the Saqez region showed the lowest aggressiveness (18.44%). There is a difference in susceptibility between the two stages of maturity and seedling since the disease occurs during the seedling stage until flowering and seed closure. Plants were more susceptible in the seedling stage.

Table 1. Primers used for molecular identification of pathogenic races of *Fusarium oxysporum* f. sp *ciceris*

Primers	Primer sequence (5′ to 3′)	Melting point (°C)	Race	Amplicon size (Kbps)
FOC- 0f	GGAGAGCAGGACAGCAAAGACTA	65	R0	0.9
FOC- 0r	GGAGAGCAGCTACCCTAGATACACC	69		
FOC- B/C1f	GAGAGCAGGGTCAGCGTAGATAG	66	RB/C1	0.5
FOC- B/C1r	GCAGCAGAAGAGGAAGAAAATGTA	62		
FOC- 5f	GGAAGCTTGGCATGACATAC	58	R5	0.9
FOC- 5r	AAGCTTGGGCACCCTCTT	56		
FOC- 6f	GAGCAGTCAATGGCAATGG	57	R6	1.0
FOC- 6r	AGAGCAGGGTCAGCGTAGATA	61		
OPF-12	ACGGTACCA	32	RB/C1-R0-R2	1.1-0.39-0.9
OPF-06	GGGAATTCGG	32	RB/C1- R3	1.9-2
OPF-10	GGAAGCTTGG	32	RB/C1- R4- R5	0.51- 0.95- 0.9
OPL-01	ACCTGGACAC	32	RA1- R0- RB/C1	1- 1.2- 2.7

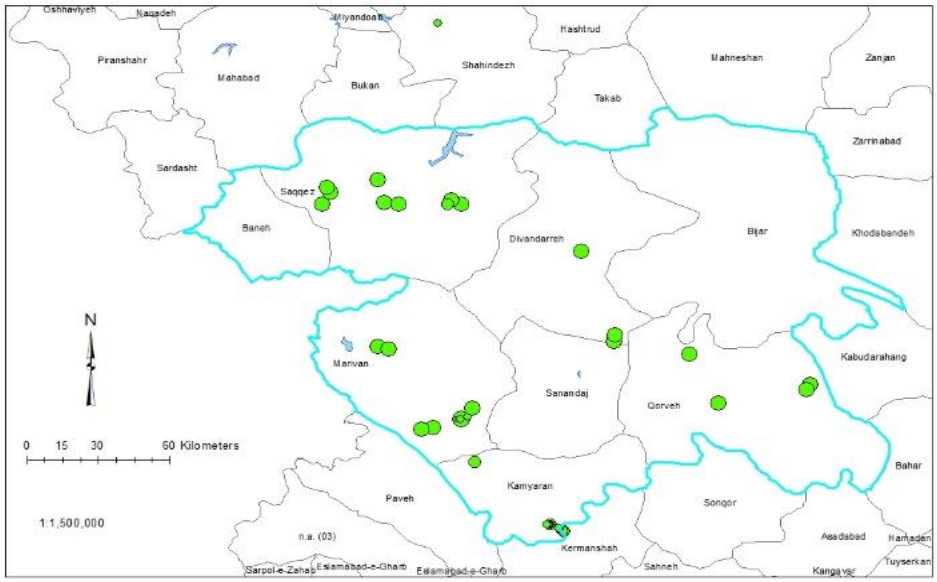

Fig. 1. Distribution map of *Fusarium oxysporum* f. sp *ciceris* isolates sampled from the Kurdistan province of Iran in 2019 indicated by green circles

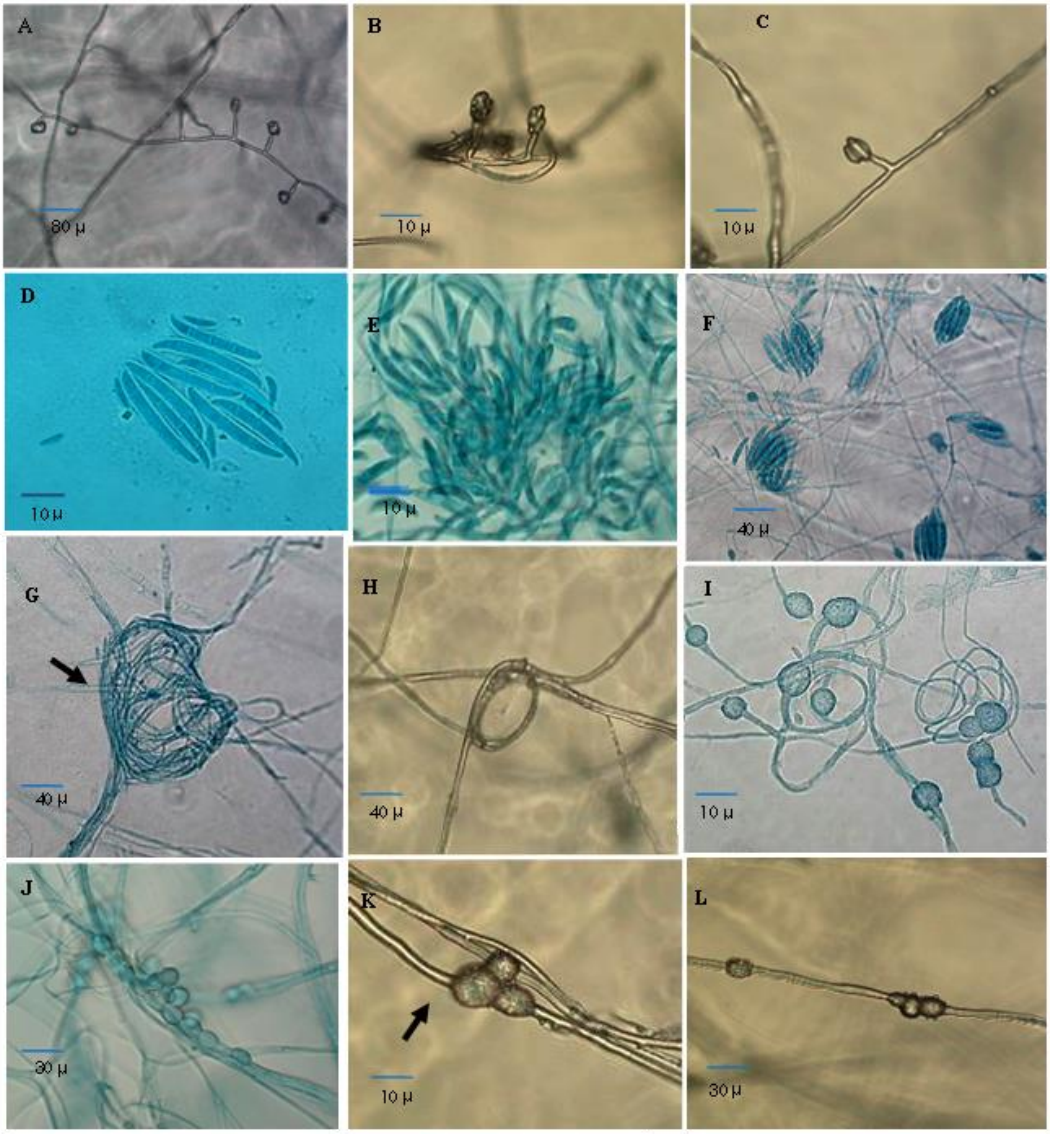

Fig. 2. Microscopic properties of *Fusarium oxysporum* species complex: A, B, C: Microconidia and Phialide, D, E, F: Macroconidia, G, H: Coil hyphae: I, J, K, L: Types of Chlamydospore on CLA medium

Pathogenicity test and pathogenic variability

The 37 isolates of *Fusarium oxysporum* species caused wilting and yellowing after 15 days post-inoculation only on the chickpea seedling. The other seedlings remain healthy. The pathogenic variability of each isolate was determined according to Dubey *et al*. (2010) separately based on the percentage of yellowing and wilting. The disease aggressiveness of each isolate was significantly different (P≤0.01) (Tables 1 and 2). Isolates related to the Kamyaran region showed the highest aggressiveness (64.88%), and isolates related to the Saqez region showed the lowest aggressiveness (18.44%). There is a difference in susceptibility between the two stages of maturity and seedling since the disease occurs during the seedling stage until flowering and seed closure. Plants were more susceptible in the seedling stage.

Molecular identification of *Fusarium oxysporum* f. sp. *ciceris*

All isolates that had the morphological characteristics of *Fusarium oxysporum* and pathogenicity on chickpeas were selected for molecular studies with a pair of FOC scar primers. Those isolates which amplified DNA bands of 1500 bp on agarose gel 0.8% were identified as *Fusarium oxysporum* f. sp. *ciceris* (Figure 3).

Table 2. Results of variance analysis of Disease Severity (DS) based on the wilting, root, and crown rot of chickpea

Pathogenic Virulence		
Highly Pathogenic (>50% wilt incidence)	Moderately Pathogenic (>30 to 50% wilt incidence)	Weakly Pathogenic (Up to 30% wilt incidence)
FOC numbers	FOC numbers	FOC numbers
62.2.1, 63.2.1, 62.1.3, 60.1.3, 40.1.2, 34.2.6, 64.2.4, 51.2.2, 28.2.6, 62.2.1	51.2.7, 55.2.7, 38.2.3, 43.2.3, 52.1.1, 64.2.4, 64.2.2, 58.1.6, 43.2.4, 38.2.2, 35.1.5, 34.1.1, 37.1.2, 33.1.2	34.2.3, 64.1.2, 38.2.5, 63.2.5, 44.2.6, 59.2.3, 61.1, 34.1.7, 63.2.2, 51.2.4, 52.1.3, 63.2.4, 60.1.2, 53.2.6

Kaka cultivar

Table 3. Grouping of isolates into different pathogenic groups based on aggressiveness on chickpea variety Kaka

Source	factor1	Sum of Squares	df	Mean Square
factor1	Linear	122.635	1	122.635
	Quadrati c	43.905	1	43.905
factor1 * T	Linear	11598.532	36	322.181**
	Quadrati c	20850.261	36	579.174**
Error(factor1)	Linear	5568.333	74	75.248
	Quadrati c	4677.667	74	63.212

** Indicates significance at $\propto \leq 0.01$

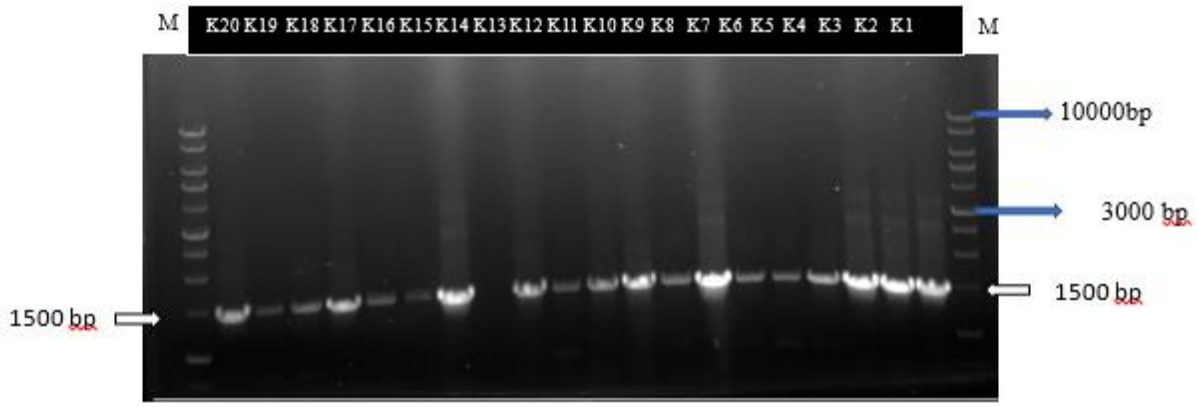

Fig. 3. Identification of *Fusarium oxysporum* f. sp. *ciceris* by DNA bands of 1500 bp in agarose gel 0.8% amplified using FOC-f and FOC-r primer pairs

Identification of FOC races

The FOC isolates races determined by molecular markers (Arvaio-Ortiz *et al.*, 2011; Shokri *et al*., 2020). Two Pathogenic races (0 and 1B/C) were identified among the isolates. Twenty-eight isolates were identified as race 0 and molecularly confirmed by using primer R0, which amplified 900 bp DNA fragment (Figure 4), and nine isolates were identified as race 1B/C, which was confirmed by using the 1B/C primer, produced a band of 500 bp long (Figure 5). Therefore, the prevalent races were race 0 (75.6%) and race 1B/C (24.4%) in Kurdistan province.

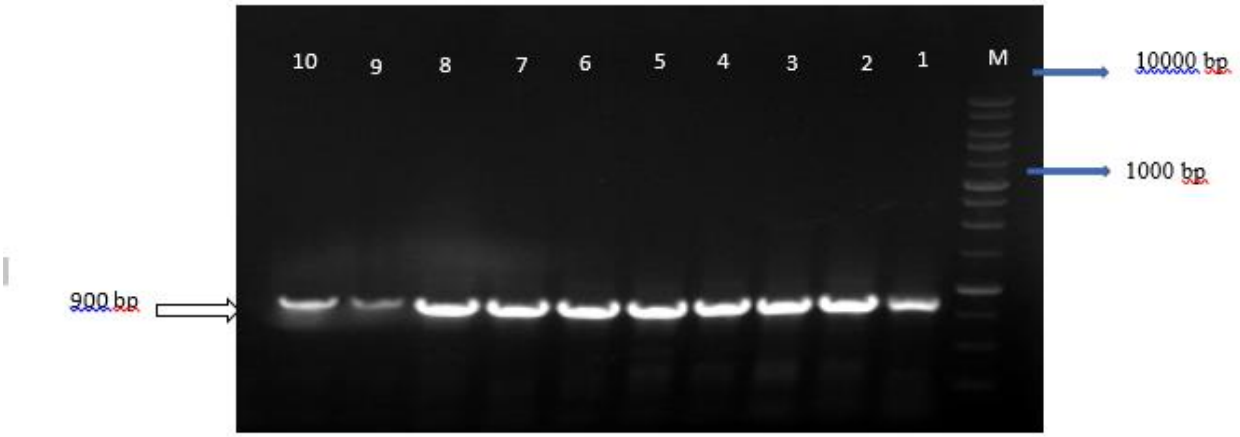

Fig. 4. Molecular confirmation by DNA bands of 900 bp on agarose gel 0.8% for detection of the race 0 of *Fusarium oxysporum* f.sp *ciceris* isolates

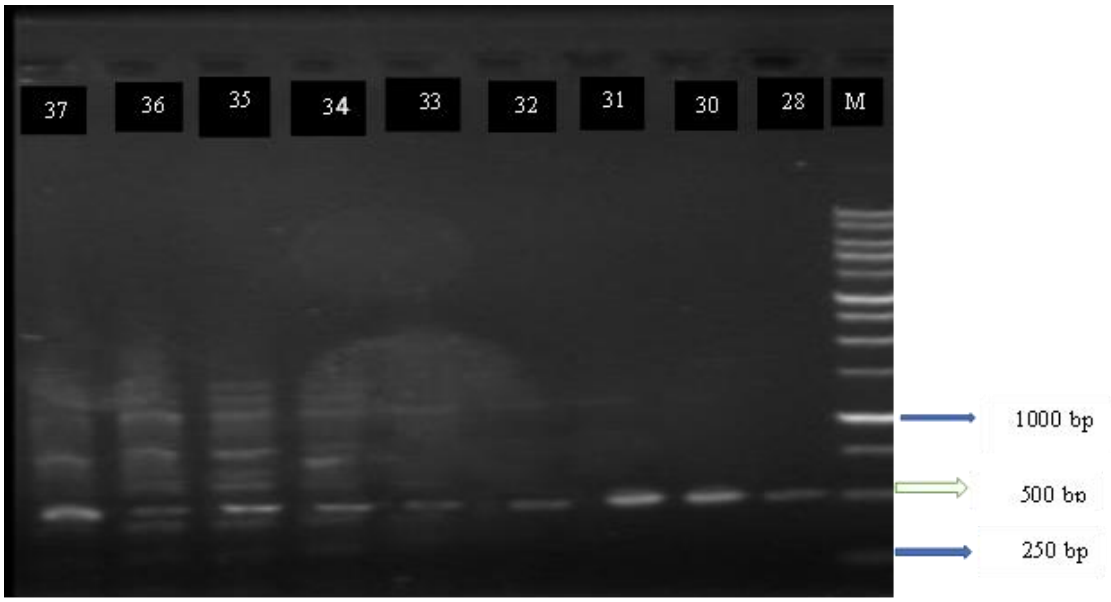

Fig. 5. Molecular confirmation by DNA bands of 500 bp on agarose gel 0.8% for detection of the race 1B/C of *Fusarium oxysporum* f.sp *ciceris* isolates.

Discussion

A high challenge in Iranian Kurdistan's chickpea production is Fusarium wilt. During this research, sampling was done from chickpea-infected fields in

Kurdistan province-Iran to identify the prevalent causative pathogen of chickpea rot. FOC was isolated from plants with vascular wilt disease symptoms. The morphological characteristics of the isolates were consistent with the characteristics mentioned in the fusarium taxonomy literature (Leslie and Summerell 2006; Nelson *et al*. 1983). In this study, the two physiological, races 0 and 1B/C were identified. There was no relationship between the prevalence of races and their geographical distribution over the province. Two types of FOC; one creates yellowing and the other wilting were described (Jimenez-Diaz *et al*., 1989). In addition to two types of FOC, eight pathogenic races (A1, 1B/C, 0, 2, 3, 4, 5, and 6) have been reported that have a wide geographical distribution in the world (Nene, 1988). Races 0, A1, 1B/C, 5, and 6 have been reported from the Mediterranean and California (Halila and Strange 1996; Jimenez-Diaz *et al*. 1993; Jimenez-Gasco *et al*. 2001), races A1, 2, 3, and 4 from the Indian (Haware and Nene 1982), races 2 and 3 from Ethiopia (Shehaboo *et al*. 2008) and Turkey (Bayraktar and Dolar 2012) and race 4 from Ethiopia (Shehaboo *et al*. 2008). Isolates with yellowing-type symptoms corresponded to races 1B/C and 0 (Jimenez-Diaz *et al*., 1989). During our study, the isolates of Kurdistan province with yellowing symptoms on plants were found to be races 0 and 1B/C too. Isolates with wilting-type symptoms i. e., A1, 2, 3, 4, 5, and 6 presenting FOC isolates in another group. They were also reported from six different regions of Sudan. Based on the similarity coefficient, 14 isolates from Lebanon, Sudan, and Syria were investigated using SCAR, SSR, and RAPD markers. The isolates were grouped into two major clusters with seven groups. The study divided races 0 and 2 isolates from Sudan into one cluster, while races 1B/C and 6 isolates from Syria and Lebanon went into another (Mohamed *et al*., 2015). Later race 0 was identified by using FOC race-specific primers in Turkey (Tekeoglu *et al*. 2017). Here we detect race 0 in Kurdistan-Iran with a similar race-specific primer.

The results of the experiments of Montakhabi *et al.* (2018) on 65 *Fusarium* isolates obtained from the infected areas of western Iran showed that phylogenetic analysis could group FOC isolates using ISSR into six and by DAMD-PCR markers into five distinct groups. Overall, their results showed high genetic diversity in Iranian FOC isolates. It could be a sign of the long-time presence of FOC in the chickpea growing areas besides the effectiveness of ISSR and DAMD-PCR markers in determination of genetic diversity of FOC isolates. In another experiment, the pathogenicity, mating type alleles, and genetic diversity of the 65 FOC isolate were compared by genic and EST-SSR molecular markers (Montakhabi *et al*. 2020). The virulence patterns of 86% FOC isolates reported between 21–50% of wilt damage placed then in moderately virulent group. Montakhabi *et al*. (2020) described the presence of the MAT-1 allele in 60% and the MAT-2 allele in 40% of FOC isolates. Their cluster analysis divided FOC isolates into four groups during the comparison of FOC isolates using 10 SSR markers. They found a clear distinction between the isolates from Kurdistan and those collected from Kermanshah province. It could be accurate base on our finding confirming the presence of only two races: 0 and 1B/C, in the Kurdistan province.

In phylogenic and evolutionary studies, mating-type screening is valuable. But it is more effective to identify the type of prevalent race, virulence, and aggressiveness ability that have been done during this study. That information could utilize for the identification of effective resistance sources in the host and practically enable the use of them for the Fusarium wilt management. Races 0, 1B/C, 5, and 6 were determined using SSR primer in Mexico (Arvaio-Ortiz *et al*., 2011 In the western and northwestern provinces of Iran, races 0, 1B/C, 1A, 6, and 5 using SCAR markers were reported by Shokri *et al*. (2020), and in this study, two races 0 and 1B/C using SCAR markers, which confirms the distribution and importance of the presence of these two races in the Kurdistan province of Iran. Furthermore, the aggressiveness and race of each isolate were assessed in this study. According to the results, isolate 62 of race 0 caused the highest leaves yellowing and root and crown rot on the susceptible variety. It demonstrates that race 0 has great potential to cause damage, and the chickpea cultivars of Kurdistan province should be evaluated for resistance to this race before introduction. In similar research carried out in India, 25 *Fusarium oxysporum* f.sp *ciceris* isolates belonging to four races (1A, 2, 3, and 4) were collected, and to evaluate their aggressiveness, the susceptible variety JG2 was utilized. Their tests showed that the aggressiveness of isolates varied between 53.33% to 93.33%, which belonged to isolates of race 4 and 1A, respectively (Dandale *et al*., 2021). The presence of resistant local cultivars in some fields of Kurdistan province compared to elsewhere could be explained by the prevalence of the races 0 and 1B/C there. The quick and appropriate identification of races of FOC is promising. The molecular marker race identification using FOC-specific primers is similar to those of Aloosh and Shokri, indicating that linked molecular markers perform well in identifying races (Alloosh *et al*., 2019; Shokri *et al*., 2020). Resistant cultivars cultivation is one of the best ways to control this disease. Determining the prevalent races 0 (75.6%) and 1B/C (24.4%) in Kurdistan province is very important. Considering the high population of race 0 in Kurdistan, evaluation of the resistance level of cultivars to this race for cultivation is recommended. The prevalence of two races in Kurdistan province indicated that FOC has low genetic diversity in Kurdistan, but the presence of other races with low populations must be considered, and the reaction of resistant cultivars in the field should be carefully monitored. There are similar publications for low genetic variation among the FOC pathogen population from some regions of Turkey, India, Sudan, and Iran. (Bayraktar *et al*., 2012;

Sharma *et al*., 2014; Mohamed *et al*., 2015). There is a report of other races of this fungus in the country (Shokri *et al*., 2020). So, during seed exchange for Kurdistan province quarantine for other races should be considered.

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تنوع بین جدایه های *Fusarium oxysporum* f. sp *ciceris* ایجاد کننده پوسیدگی ریشه و طوقه نخود در استان کردستان

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چکیده: پژمردگی، پوسیدگی ریشه و طوقه فوزاریومی با عامل *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *ciceris*, بیماری بسیار تاثیرگذار خاکزاد نخود در استان کردستان ایران است. طی تحقیق حاضر وضعیت گسترش نژادهای بیماریزای فرم مخصوص نخود این قارچ در استان کردستان تعیین گردید. نمونه‌های گیاهی همراه با علایم بیماری از ۴۲ مزرعه آلوده در مناطق کشت نخود استان کردستان جمع آوری گردید. ابتدا جدا سازی، خالص سازی شناسایی مشخصات ظاهری میکروارگانیسم عامل بیماری مرتبط با هر نمونه اقدام شد. آزمون بیماریزایی و سنجش توان بیماریزایی بروی رقم حساس کاکا انجام شد. سپس استخراج DNA جدایه ها، شناسایی گونه و شناسایی فرم مخصوص با استفاده از نشانگرهای پیوسته برای فرم مخصوص انجام شد. در بین جدایه‌های جمع آوری شده ۳۷ جدایه به عنوان فرم مخصوص *Fusarium oxysporum* f. sp. *ciceris* تعیین شدند. شناسایی نژاد جدایه‌ها با نشانگرهای اسکار پیوسته (۱B/C، ۰، ۲، ۳، ۴، ۵، ۶ و ۱A) نشان داد که ۲۸ جدایه متعلق به نژاد ۰ و بقیه متعلق به نژاد ۱B/C بودند. ارتباطی بین مناطق جغرافیایی گسترش نژادهای بیماری و نژاد رایج مشاهده نشد. شناسایی نژادهای عامل بیماری به منظور ارزیابی منابع مقاومت به بیماری و اصلاح ارقام مقاوم جدید تجاری ضروری است. بکار گیری ارقام مقاوم در مدیریت تلفیقی کنترل این بیماری و تولید پایدار نخود، رویکردی اصولی است.

کلمات کلید: بیماریزایی، تنوع، نژادها ، *Cicer arietinum*

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