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Genetic structure of *Fusarium oxysporum* f. sp. *ciceri* populations from chickpea in Ilam province, Iran

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Abstract: Chickpea (*Cicer arietinum* L.) is one of the most important legume crops in Iran. Wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*, is the most important soil-borne disease of chickpea in the world. This disease caused high losses in different regions during recent years. Simple sequence repeat (SSR) were used to estimate genetic diversity in 114 of *F. oxysporum* isolates from six counties in the western of Iran (Asemanabad, Sarableh, Eivan, Badreh, Chardavol and Dareh Shahr). A set of five microsatellite primer pairs revealed 17 alleles in each locus across the populations. A low level of genetic variability was observed among isolates in these regions. Genetic diversity was low (H = 0.269) within populations with corresponding high average gene flow and low genetic distances between populations. The smallest genetic distance was observed between isolates from Chardavol and Darahshahr regions. Effective number of alleles was higher in Asemanabad in comparison to the other populations. The number of (H) and Shannon's Information index (I) were also higher in Badreh (H = 0.430, I = 0.281). The total gene diversity and gene diversities between subpopulation were estimated 0.2004 and 0.188, respectively. Gene diversity attributable to differentiation among populations was 0.0618, while gene flow was 7.589. Cluster analysis based on UPGMA showed the lowest genetic distance between Chardavol and Dareh Shahr, then between Chardavol and Badreh. The dendrogram indicated a clear break between Asemanabad and the remaining populations. These results will be useful in breeding for chickpea root rot resistant cultivars and developing necessary control measures.

Key words: Genetic diversity, gene flow, population differentiation, microsatellite

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops in the world (Barve et al. 2001). This crop is an important component of the cropping systems as it derives more than 70% of its nitrogen from symbiotic nitrogen fixation (Saxena 1990). It is reported to be infected by more than 52 pathogen (Nene et al. 1984). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo & K. Sauto (FOC) is one of the major limiting factors of chickpea production worldwide. The disease is widespread in all chickpea growing areas of the world (Haware & Nene 1982), causing 10 - 90% annual losses (Singh & Dahiya 1973, Jalali & Chand 1992).

General symptoms at the seedling stage include seed rot and sudden drooping more like wilting and damping off (Khare 1980). The disease may cause complete crop loss under favorable conditions, and can be the major limiting factor for cultivation in certain areas (Chaudhary & Amarjit 2002). The disease is difficult to control due to the survival of pathogen in infected soil for years even in the absence of the host plant (Haware et al. 1996). The most effective and practical strategies to control Fusarium wilt of chickpea was reported to be the development of resistant cultivars (Nene & Haware 1980, Haware 1990).

Knowledge of genetic diversity is needed for resistance deployment to be effective and to identify shifts in race or population structure that might occur (McDonald 1997). Identification of diversity by morphological characters is highly variable in *Fusarium* isolates, these characters are influenced by cultural conditions. In recent years, numerous DNA based methods have been increasingly used to study variability in pathogenic *Fusarium* population (Kiprop et al. 2002, Sivaramakrishnan et al. 2002b, Belabid et al. 2004).

However, the effectiveness of resistant cultivars in disease management is restricted by pathogenic variability in FOC. Frequent incidence of virulent forms of FOC has affected chickpea production and beat valuable genetic resistance resources. Therefore,

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accurate and rapid identification of genetic variability is necessary for developing resistant cultivars and effective disease control systems. In the recent years, the genetic variability of the pathogen was characterized by several types of DNA markers such as RAPD (random amplified polymorphic DNA), RFLP (restriction fragment length polymorphism), SSR (simple sequence repeat) and amplified fragment length polymorphism (AFLP) have been increasingly used to study the variability in pathogenic populations of FOC (Sivaramakrishnan et al. 2002, Jimenez-Gasco et al. 2004, Dubey & Singh 2008, Sharma et al. 2009, Jimnez Gasco et al. 2001, Jimnez Gasco & Jimnez-Diaz 2003, Kelley et al. 1994, Nourollahi & Jalali 2017). SSR markers distinguished the four races of *F. oxysporum ciceri* causing varied levels of wilting with differential host cultivars (Barve et al. 2001). Bogale et al. (2005) showed that polymorphism revealed with eight SSR markers should be sufficient for study of genetic diversity in *F. oxysporum* complex.

Knowledge of the amount and distribution of genetic diversity between and within pathogen populations is a prerequisite for the development of resistant cultivars (Nourollahi et al. 2011). Chickpea breeders need to know the genetic structure and population biology of FOC isolates. However, the characterization of resistance is variable regionally, and therefore, knowledge of genetic variability in different chickpea growing areas is required for resistance deployment (Jimenez-Gasco et al. 2001). The objectives of this work were to determine genetic diversity of Iranian FOC isolates in chickpea farms using SSR marker.

MATERIALS AND METHODS

Fungal isolates and pathogenicity test

Chickpea plants wilt symptoms and black lesions on root were randomly collected in 2012-13 from six different regions including Asemanabad, Eivan, Badreh, Chardavol, Darehshahr and Sarableh in the western of Iran. The regions are different in altitude and climate, and separated by substantial mountain ranges from 50-300 kilometer. Isolates of each region was considered as a population (Table 1, Fig 1). Diseased samples were cut into 2- to 5-mm-long pieces, surface sterilized by dipping into domestic bleach solution (5% NaOCl) for 2–3 min, washed three times with sterile distilled water, dried with sterile filter paper and plated on potato dextrose agar (PDA). Samples were incubated for three days in an incubator at 25°C with a 12-h photoperiod to induce production of conidia. The fungus was isolated and purified using the hyphal tip and single spore method (Hawker 1950). Identified isolates were stored for short time on SNA at 4ºC, however for perennial time stored in tube containing sand at 4°C. Detail of the FOC isolates are presented in Table 1.

The pathogenicity of FOC isolates from the different population was tested in the greenhouse condition on wilt susceptible local cultivar artificially inoculated. The FOC isolates mass were multiplied separately on sand maize meal medium. Soil of pots for experiment was autoclaved. The mass multiplied inoculum was mixed with the soil in 1:10 proportion and filled in the pre sterilized pots (Miller 1946). Five seeds of susceptible chickpea cultivar seeds were surface sterilized using 2% sodium hypochlorite for 2 min, rinsed in sterile water three times and were sown in pots for each isolate in three replications. Chickpea seedlings were grown in pots containing sand-maize meal and 10% w/w fungus inoculums. The disease symptoms were recorded in 20-22 days at 25 ± 1C with a relative humidity of 40–50%. The seedlings maintained in sterilized soil without inoculums were served as control. The pathogenicity test was confirmed by proving Koch's Postulate and pathogens were confirmed.

Table 1. Geographical origin of FOC populations from Ilam province, Iran

Isolates	Sampling region	No. of isolates	Population number
FOC_1- FOC_{18}	Asemanabad	18	1
FOC_{19}- FOC_{37}	Eivan	19	2
FOC_{38}- FOC_{54}	Badreh	17	3
FOC_{55}- FOC_{73}	Chardavol	19	4
FOC_{74}- FOC_{92}	Darehshahr	19	5
FOC_{93}- FOC_{114}	Sarableh	22	6

Fig 1. Geographical origins of the six FOC populations from Ilam province, were used in this study.

DNA extraction and SSRs analyses

For extraction of the mycelia mass, liquid cultures were initiated by adding 2–4 mm^2 pieces of fungus plague to 250-mL Erlenmeyer flasks containing 100 mL PDB medium (Potato Dextrose Broth). Flasks were incubated at room temperature approximately 25 °C on a rotary shaker for 6–8 days. Mycelium was collected by filtration through sterile filter paper with a vacuum funnel. Mycelia were harvested, frozen and stored at −20°C. DNA was extracted using a modified

hexadecyl trimethyl-ammonium bromide (CTAB) procedure (Doyle & Doyle 1987). Genomic DNA was isolated from single spore culture of each isolates (Murray & Thompson 1980). Mycelia were ground in liquid nitrogen and suspended in 2% CTAB extraction buffer (1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol). Samples were treated with 5 units RNase at 37°C for 30 min. and then extracted with Chloroform Isoamyl alcohol 24:1 (v/v). DNA in the supernatant was precipitated with isopropanol, rinsed with ethanol, and adjusted to a final concentration of 20 ng.µl^{-1} in TE (pH 7.4). The quality of the extracted DNA was visually checked on 0.8% agarose gels.

A set of five locus-specific primers pairs for SSRs (Table 2) described by Bogale et al. (2005), were selected. Primer aliquots for each marker were prepared by mixing equimolar amounts of appropriate forward and reverse primer in 1× TE (1mM EDTA, 10 mM Tris–HCl, pH 8.0) and used for the amplifi cation of individual microsatellite loci. PCR amplification was performed in a 25 µl reaction volume containing 1.5 mM $MgCl_2$, 0.2 mM of dNTPs mix (100 mM of each dNTPs), 1 µl of each forward and reversed primer, 0.6 U of *Taq* polymerase with 25 ng of template DNA. Amplification was performed using Biometra thermal cycler (USA), PCR conditions for SSR were as Follows, the PCR programmed had one initial denaturation step at 94°C for 3 min Followed by 30 cycles of 94°C for 60 s, annealing for 60 s (appropriate annealing temperature were used for each primers set, Table 2) and 72°C for 2 min. The thermal cycles were terminated by a final extension of 7 min at 72°C. Amplified products were resolved in 2 % agarose gel at 60 V cm-1 using in 1X TBE buffer and stained with DNA Safe Stain at 0.5mg.ml^{-1} and photographed under UV Transillaminator with Gel Doc. Intas. A 1 kbp ladder (Gene Ruler TM, Fermantas,) was used as a molecular size standard.

Table 2. SSR primer sequences used in this study (Bogale et al. 2005).

Locus	Primer sequence (5'→3')	Annealing temperature (Ta)	Repeat motif	Expected size (bp)	PIC	EMR	MI
MB2	F: TGCTGTGTATGGATGGATGG R:CATGGTCGATAGCTTGTCTCAG	57	(GT)11(GA)6	200-500	0.345	4	1.38
MB5	F:ACTTGGAGAAATGGGCTTC R:GGATGGCGTTTAATAAATCTGG	54	(TG)9	900-1000	0.373	4	1.49
MB11	F:GTGGACGAACACCTGCATC R:AGATCCTCCACCTCCACCTC	60	(GGC)7	100-150	0.384	2	0.76
MB13	F:GGAGGATGAGCTCGATGAAG R:CTAAGCCTGCTACACCCTCG	60	(CTTGGAAGT GGTAGCGG)14	150-200	0.346	3	1.03
MB18	F:GGTAGGAAATGACGAAGCTGAC R:TGAGCACTCTAGCACTCCAAAC	61	(CAACA)6	100-300	0.266	4	1.06
Average	-	-	-	-	0.342	3.4	1.14

Molecular analysis of the data

Data analyses of populations were defined according to the geographic locations. The bands generated by SSR primers that were repeatable and clearly visible with a high intensity were scored manually for the presence (1) or absence (0) of bands in each isolate. The pair-wise distance among the isolates was calculated from the binary matrix using the simple mismatch coefficient (Sneath & Sokal 1973) that is recommended for haploid fungi (Kosman & Leonard 2005). Genetic similarity between pairs was estimated using Jaccard`s similarity coefficient. Similarity coefficients were used for the construction of UPGMA (Unweighted Pair Group Method with Arithmetic Average) dendrogram (Rolhf 1990). For each primer pair, the polymorphic information content (PIC), marker index (MI) was calculated. The polymorphic information content (PIC) was calculated using $PIC_i = 2f_i (1-f_i)$, where *i* is the information of marker I, f_i is the frequency of the amplified allele (presence of fragments) and $(1 – f_i)$ is the frequency of the null alleles (Roldan-Ruiz et al. 2000). The genetic variation was measured in terms of genetic diversity and was computed by averaging PIC estimates over all loci (Nei and Li 1979). The marker index (MI) was calculated by MI = PIC x EMR, where EMR is the "effective multiplex relationship" given by the product of the total number of fragments per primer (n) and the fraction of polymorphic fragments (β) (Varshney et al. 2007).

Genotypic diversity (H) among isolates was estimated from allelic frequencies using the equation $H = 1 - \Sigma xi^2$, where, xi is the frequency of the i^{th} allele (Nei 1973). The coefficient of population subdivision (Gst) was computed as (Ht – Hs)/Ht, where, Ht is the total genetic diversity and Hs is the average gene diversity over all subgroups (Nei 1973). the allele frequencies at polymorphic loci, the Nm values (effective migration rate), and the genetic identity among populations for characterize genetic variation, observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (He) and Shannon's information index (I) were calculated in both origin sites and subspecies levels. Mean values of gene diversity in total populations (Ht), gene diversity between populations (Hs), proportion of gene diversity attributable to differentiation among populations (Gst) and estimate of gene flow from Gst (Nm) were obtained across loci (McDermot & MacDonald 1993). Relationships of the populations were estimated from the SSR data using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean), clustering method on the basis of Nei's (1978)

unbiased genetic distance, Based on SSR data, Analysis of molecular variance (AMOVA) tests were performed to assess population variance among and within populations and Principal coordinate analysis (PCA) was performed to evaluate the genetic differences among isolates within populations too, all above calculations were performed using POPGENE ver. 1.31 (Yeh et al. 1999) and Gen Alex ver. 6.5 (Peakall & Smouse, 2006).

RESULTS

Isolates collection and pathogenicity test

A total of 114 isolates of FOC were isolated and purified from infected chickpea plants (Table 1). Based on morphological characteristics, the isolates were identified as FOC. FOC isolates were identified according to their morphological and microscopic characters as described by (Jens et al. 1991, Nelson et al. 1983, Barnett and Hunter 1972, Leslie et al. 2006). Isolates showed significant variations in cultural characteristics, production of microconidia, macro-conidia and chlamydospores. Our morphological identifications were further confirmed by molecular method (PCR) using specific SSR primers used in this study for FOC isolates.

The result of pathogenicity test indicates that FOC isolates were virulent and caused wilting and death of the seedlings in 20-22 days. Characteristic wilt symptoms such as drooping of leaflets and yellowing of the leaves starting from apical part, progressing downward and final wilting of the whole plant were observed. Internal discoloration of the root vascular system was visible in wilted plants.

Allele's frequency and primers information

Five pair SSR primers were used for amplification of SSR loci of 114 FOC isolates. A total of seventeen alleles were produced by SSR primers with average of 3.4 alleles in all loci. All isolates amplified a single band ranging from 100 to 1000 bp. Five microsatellite loci were polymorphic when considered over all isolates. The average of allelic variability per locus (H) was the highest (0.281) in Badreh population and the lowest in Dareh Shahr with 0.242 alleles. The allele's frequency (No. allele) is the highest in Eivan, Badreh and Chardavol with 15 and lowest in Sarableh with 12 alleles (Table 3).

A summary of the genetic diversity data of five microsatellite loci in populations is given in Table 3. Observed allele number (Na = 1.765) was higher in Eivan, Badreh and Chardavol, effective numbers (Ne = 1.533) of alleles was higher in Asemanabad compared to other populations. The amount of genetic diversity (H) and Shannon's Information Index (I) were also higher in Badreh (H = 0.481, I = 0.430) but lower values were estimated for Dareh Shahr (H = 0.242, I = 0.376).

On basis of microsatellite data, the polymorphic information content (PIC) varied from 0.266 (Primer MB18) to 0.384 (Primers MB11), with average of 0.342, which reflects the informative content of the used primers. EMR (effective multiplex relationship ratio) varied from 2 to 4. The marker index (MI), which incorporates the informative content of the marker (PIC), the number of fragments per primer pair and the fraction of polymorphic fragments (EMR), varied from 0.76 (MB11) to 1.49 (MB5) (Table 2).

Genetic variability of populations

The average genetic distance was calculated among the six populations. Nei's pairwise genetic distances between the populations varied from 0.0005 to 0.0233. The lowest genetic distance was found between Chardavol and Dareh Shahr, while the highest genetic distance was revealed between Eivan and Sarableh (Table 4).

Table 3. Genetic diversity estimates in FOC populations based on SSR loci.

Population	No. of alleles	Na	Ne	I	H	P %
Asemanabad	14	1.647	1.471	0.413	0.275	82.35
Eivan	15	1.765	1.405	0.405	0.259	88.24
Badreh	15	1.765	1.457	0.430	0.281	88.24
Chardavol	15	1.765	1.451	0.428	0.279	88.24
Dareh Shahr	14	1.647	1.396	0.376	0.242	82.35
Sarableh	12	1.765	1.459	0.423	0.277	88.24
Average	14	1.725	1.44	0.412	0.269	86.27

I = Shannon's Information Index H = Nei's (1973) gene diversity, Ne = Effective number of alleles, Na = Observed number of alleles, P = Polymorphism %

Table 4. Information about genetic distance between pairs of FOC populations.

Sarableh	Darehshahr	Chardavol	Badreh	Eivan	Asemanabad	Population
					***	Asemanabad
				***	0.0127	Eivan
			***	0.0108	0.0215	Badreh
		***	0.0022	0.0049	0.0165	Chardavol
	***	0.0005	0.0021	0.0043	0.0226	Darehshahr
***	0.0216	0.0091	0.0126	0.0233	0.0181	Sarableh

Cluster analysis (UPGMA) was used to produce a dendrogram showing the genetic relationships between the populations based on the SSR data, the dendrogram showed a distinction between Asemanabad population and the five remaining populations (Fig. 2).

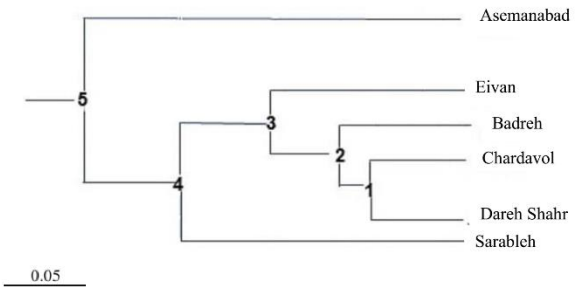

Fig 2. Dendrogram of genetic relationships between each FOC population constructed by UPGMA.

The total gene diversity (Ht) and gene diversities between subpopulations (Hs) were estimated to be 0.2004 and 0.1880, respectively. Gene diversity attributable to differentiation among populations (Gst) was 0.0618, while gene flow (Nm) was 7.589 (data not shown). The genetic similarity varied from 97% to 99%, in isolates with different geographical origins. A dendrogram based on UPGMA analysis showed three major groups containing isolates with different origins.

The AMOVA of genetic variation in FOC populations revealed that 8% of the variance occurred among populations and 92% within populations (Table 5). PCA (Principal Component Analysis) using SSR data showed the genetic differences among isolates within populations and gene flow between different populations (Fig. 3), the first and second principal coordinates account for 25.07% and 19.52% of the variation, respectively. There was no clear separation among individuals from different populations, however isolates in the same populations tended to gather together. This suggests the geographical regions of sampling play important role for the formation of populations. PCA allows for visualizing the patterns of genetic relationship without altering the data itself and finds patterns within a multidimensional data set.

DISCUSSION

The present work was carried out to discover the possible utilization of SSR marker for identifying genetic variation in Iranian FOC isolates. In conclusion, the present study generated significant information about genetic diversity among FOC isolates, collected from different regions. The study also highlighted the fact that genetic analysis using SSR marker is useful tool and have high discriminatory power for studying the diversity in *F. oxysporum* f. sp. *ciceri*.

The advantage of microsatellite markers are their high specificity, high polymorphism, good reproducibility and unambiguous scorability (Tenzer et al. 1999, Sahran & Naef 2008). Alleles vary according to the number of repeat units present but other mutations have also been shown to be responsible for allele length variation in SSR analysis (Burgess et al. 2001, Slippers et al. 2004b).

Table 5. Hierarchical molecular analysis of variation (AMOVA) within and between FOC populations based on SSR markers.

Source of variation	df.	Sum of squares (SS)	Mean of squares (MS)	percentage of variation	*P* value
Among populations	5	31.48	**6.26	8	0.01
Within populations	108	260.41	2.41	92	
Total	113	291.89		100	

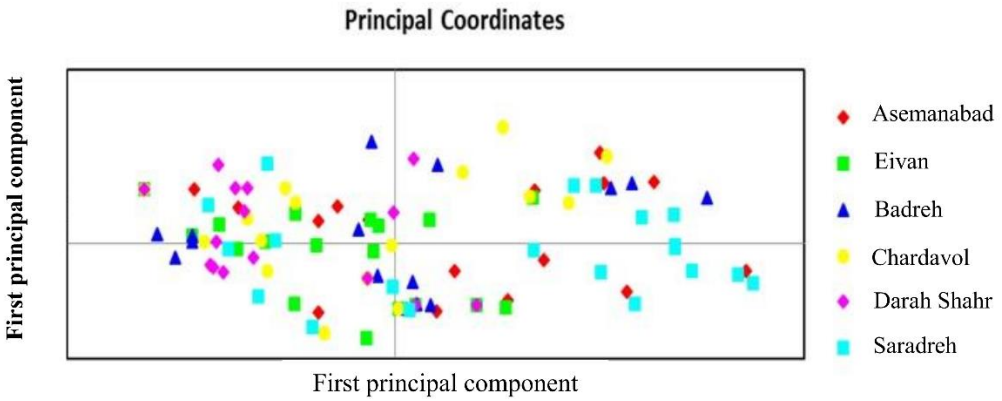

Fig 3. Principal Component Analysis (PCA) based on SSR data for 114 individual FOC isolates belonged to five populations.

SSRs provides a powerful tool for taxonomic and population genetic studies (Britz et al. 2002). In this research, SSR markers divided all the FOC population in two major groups. Earlier, 64 FOC Indian isolates placed into two major categories with ISSR and RAPD markers (Dubey & Singh 2008), similar studies on other plant pathogenic fungi have emphasized the importance of molecular approaches to characterize genetic diversity within and between isolates (Bentley et al. 1995, Nourollahi et al. 2011, Sivaramakrishnan et al. 2002, Belabid et al. 2004). By SSR markers, different allele sizes were recorded in FOC genome, the smallest allele size was detected as 120 bp and the largest one was 1000 bp as reported by Mohammadi et al. (2004). In this study the mean number of PIC value of SSR markers was 0.342, This PIC value indicates that the isolates had a low degree of biodiversity. The variable numbers of alleles per loci is an indication of high level of polymorphism, and Polymorphism has been observed in other fungi as a direct record of genetic evolution (Sanders, 2002, Mwang`Ombe et al. 2007). The polymorphic character of SSRs produces highly discriminating fingerprints that often allow characterization of fungi at a strain level (Migheli et al. 1998). The analysis performed by AMOVA, indicated that most of variation in the populations is accounted for among individual FOC isolates (98%) and low proportion (2%) of the total genetic variations were accounted for differences among populations.

The results showed that there was a low level of genetic variation among the isolates of FOC populations in sampling regions. The Nm (7.589) indices demonstrated high gene flow and low genetic differentiation among populations. This study suggests that frequent gene flow and recombination between populations of FOC are significantly influenced on the evolution and development of this fungus. This subject is in accordance with previous results of Jimenez-Gasco et al. (2002) who stated that *F. oxysporum* f. sp. *ciceri* is a monophyletic group that derived from a small population or a single individual. Molnar et al. (1990) demonstrated that somatic recombination occurred through parasexuality in *F. oxysporum*. High degrees of pathogenic and genetic variation in asexual fungi such as *F. oxysporum* f. sp. *ciceri* are also observed with accumulation of genetic mutations over time (Gordon & Martyn 1997). In this study, FOC isolates showed a different level of diversity within and between populations in comparison with the worldwide isolates in different researches. Similar results were also reported in *F. oxysporum* f. sp. *phaseoli* (Woo et al.1996), *F. oxysporum* f. sp. *ciceri* (Jimenez-Gasco et al. 2001), and *F. oxysporum* f. sp. *lentis* (Nourollahi & Jalali 2017). Similar findings have also been reported in the Ethiopian *F. oxysporum* isolates by AFLP, SSR and ITS sequence analysis (Bogale et al. 2006). In Iranian populations, over 92% of the gene diversity was distributed on a local level within populations. However, there was a high degree of genetic similarity among populations separated by low geographical distances. The low level of gene diversity (Gst = 0.0618) was detected among all populations. The low Gst value indicated little genetic differentiation among the populations and showed little evidence for geographical subdivision among populations (Bayraktar et al. 2010).

The genetic distances were very small, indicates that populations linked by movement of conidia through contaminated seeds, infected plant debris as gene flew because the geographic distances between sampling regions are short with range of 50 to 300 km. Gene flow is one of the evolutionary forces that can have a significant force on the genetic diversity of a population. In the absence of gene flow, genetic drift cause developing different allele frequencies at neutral loci, leading to differentiation in isolate populations (Keller et al. 1997). The high genetic similarity among FOC populations suggests that gene flow occurred across long distances. Infected seed can lead to persistence of genotypes; we consider that infected seed can explain the distribution and diversity of genotypes found at the end of the growing season in natural populations. Genetic drift and selection would limit the number of genotypes present in field populations (Shah et al. 1995). In this study genetic characterization of FOC isolates could be essential for the efficient management of *Fusarium* wilt through use of resistant cultivars in chickpea growing areas. Low FOC genetic diversity in the west of Iran is a warning for breeder to apply the successful use of resistance genes in local disease management; however, understanding of occurrence, distribution, and genetic relatedness of such pathogenic variants is a necessary for developing effective and efficient integrated disease management. Quarantine regulations will need to prevent the introduction of more diverse isolates into these populations and prevent transmission any isolates from this area to other regions of the country. The present investigation will helps to understand the genetic variability among the isolates of FOC in Iran, which will be useful for its manipulation in regional breeding program for developing wilt resistance variety. Understanding the genetic structure of pathogen populations in the present study may provide insights into the epidemiology and evolutionary potential of pathogens and could lead to developing integrated strategies for disease management and breeding programs.

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ساختار ژنتیکی جمعیت های *Fusarium oxysporum* f. sp. *ciceri* جدا شده از نخود در استان ایلام

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چکیده: نخود (*Cicer arietinum* L.) یکی از مهمترین حبوبات در ایران است. بیماری پژمردگی ناشی از *Fusarium oxysporum* f. sp. *ciceri*، یکی از مهم ترین بیماری های خاکزاد نخود در جهان است. این بیماری در سال های اخیر باعث خسارت زیادی در مناطق مختلف شده است. برای بررسی تنوع ژنتیکی در ۱۱۴ جدایه *F. oxysporum* جدا شده از شش منطقه در غرب ایران (آسمان آباد، سرابله، ایوان، بدره، چرداول و دره شهر)، از نشانگر ریزماهواره استفاده شد. از مجموع پنج جفت پرایمر ریزماهواره هفده آلل در هر جایگاه در همه جمعیت ها مشخص گردید. مقدار تنوع ژنتیکی کمی در بین جدایه های این مناطق مشاهده شد. تنوع ژنتیکی در درون جمعیتها (H = 0.269) در اثر میانگین جریان ژنی بالا و فاصله های ژنتیکی کم در درون جمعیت ها پایین بود. کمترین فاصله ژنتیکی بین جدایه های مناطق چرداول و دره شهر مشاهده گردید. تعداد آللهای مؤثر در آسمان آباد در مقایسه با سایر جمعیت ها بیشتر بود. مقدار تنوع ژنتیکی (H) و شاخص اطلاعات شانون (I) در بدره (H = 0.430, I = 0.281) نیز بیشتر بود. تنوع ژنی کل و تنوع ژنی بین زیر جمعیت ها به ترتیب ۰/۲۰۰۴ و ۰/۱۸۸ بود. تنوع ژنی ناشی از تفاوت در میان جمعیت ها ۰/۰۶۱۸ بود در حالیکه جریان ژنی ۷/۵۹۸ بود. تجزیه و تحلیل خوشه ای بر اساسUPGMA، کمترین فاصله ژنتیکی بین چرداول و دره شهر و سپس بین چرداول و بدره را نشان داد. دندروگرام اختلاف آشکاری را در بین آسمان آباد و جمعیتهای باقیمانده نشان داد. این نتایج در برنامه اصلاح ارقام مقاوم پوسیدگی ریشه نخود و گسترش روشهای کنترل مفید خواهد بود.

کلمات کلیدی: تنوع ژنتیکی، جریان ژنی، تمایز جمعیت، ریزماهواره

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