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Population Genetic Structure of *Fusarium oxysporum* f. sp. *ciceris*, the Causal Agent of Chickpea Wilt, Using SSR Markers in West and Northwest of Iran

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ABSTRACT

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the major yield-limiting factors in chickpea, particularly in west and northwest of Iran. Simple Sequence Repeats (SSR) were used to determine genetic structure of *Fusarium oxysporum* f. sp. *ciceris* populations from three infected areas located in west and northwest provinces of Iran during 2016-2017. A set of six microsatellite primer pairs revealed 35 alleles in each locus with an average of 5.83 per locus. The PIC value varied from 0.20 (FOAG11) to 0.39 (FODF7) with an average of 0.27 per locus. The average number of effective alleles (N_e), Number of private alleles (N_a), allelic variability per locus (H), Shannon's Information Index (I) and percentage of Polymorphic Loci (%PL) in three populations were 1.33, 1.97, 0.2208, 0.357, and 74.29, respectively. The average genetic distance was calculated among the three populations. Nei's pair-wise genetic distances between the populations varied from 0.0202 to 0.98. The total gene diversity (H_t) and gene diversities between subpopulations (H_s) were estimated to be 0.2208 and 0.2079, respectively. Gene diversity attributable to differentiation among populations (G_{st}) was 0.0585, while gene flow (N_m) was 8.0412. The AMOVA of genetic variation in *Fusarium oxysporum* f. sp. *ciceris* populations revealed that 5% of the variance occurred among populations and 95% within populations. PhiPT value was 0.054. The lowest genetic distance was found between Kurdistan and West Azerbaijan with Lorestan populations, while the highest genetic distance was observed between Lorestan and Kermanshah populations.

Keywords: *Cicer arietinum* L., Gene flow, Genetic diversity, Microsatellite, Nei's genetic distances**.**

NTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown all around the world. The cultivation area of this crop in Iran is 456 thousand hectares with an average yield of 440 kg ha^{-1}, (FAO, 2019). Kermanshah, Lorestan, and Kurdistan provinces have the highest chickpea cultivated area in Iran with 18.7, 16.7, and 12.2%, respectively (Anonymous, 2016).

Chickpea is infected by a few pathogenic diseases to many biotic stresses (Nene and Reddy, 1987), among which the most important diseases are ascochyta blight and fusarium wilt caused by *Fusarium Oxysporum* f. sp. *Ciceris* (FOC)(Padwick) Matuo and K. Sato is one of the major limiting factors of chickpea production worldwide (Haware and Nene, 1982a). The disease is widespread in all chickpea growing areas of the world (Haware and Nene, 1982a), causing 10-90% annual losses (Singh and Dahiya, 1973; Jalali and Chand, 1992). General symptoms at the seedling stage include seed rot and sudden drooping more like wilting and damping off (Khare, 1980).

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First record of the disease in Iran was in 1963 (Behdad, 2010).

Globally, eight races of FOC (Races R0, R1A, R1B/C, R2, R3, R4, R5 and R6) have been identified based on their reactions on a set of chickpea differential lines and using diagnostic molecular markers (Haware and Nene, 1982b; Phillips, 1988; Cabrera *et al*., 1985; Jimenez-Gasco and Jimenez-Diaz, 2003; Gurjar *et al*., 2009).

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 is reported to be the development of resistant cultivars (Haware, 1990). Knowledge of genetic diversity is needed for resistance deployment to be effective and to identify shifts that may occur in race or population structure (McDonald, 1997). Chickpea breeders need to know the genetic structure and population biology of FOC isolates. However, 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).

Identification of diversity by morphological characters is highly variable in *Fusarium* isolates, these characters are influenced by cultural conditions (Nene and Reddy, 1987).

Molecular methods are an easy, fast and low-cost way to determine diversity in pathogenic fungi (Kelly *et al*., 1994; Jimenez-Gasco *et al*., 2001; Jimenez-Gasco and Jimenez-Diaz, 2003; Gurjar *et al*., 2009). In recent years, the genetic variability of the pathogen was characterized by several types of DNA markers such as RAPD, RFLP, SSR and Amplified Fragment Length Polymorphism (AFLP) have been increasingly used to study the variability in pathogenic populations of FOC (Kelly *et al*., 1994; Jimenez-Gasco *et al*., 2001; Sivaramakrishnan *et al*., 2002a; Jimenez-Gasco and Jimenez-Diaz, 2003; Jimenez-Gasco *et al*., 2004; Dubey and Singh, 2008; Sharma *et al*., 2009; Nourollahi and Aliaran, 2017). Genetic variability among 43 isolates of FOC, collected from nine states of India including the four well-characterized races of the pathogen were assessed using RAPD and AFLP markers. Principal coordinate analysis of the similarity index data generated from the molecular marker studies mostly gave three different clusters (Sivaramakrishnan, 2002b). A total of 76 FOC isolates from Sudan and 14 FOC isolates from Syria and Lebanon have been collected to investigate the genetic diversity. Based on the similarity coefficient, the results indicated two major clusters including seven subclusters (Elmahi Mohamed *et al*., 2015). Ninety-nine isolates from different regions of the world have been studied using RAPD markers to identify different FOC isolates (Jimenez-Gasco *et al*., 2001). Genetic diversity of different FOC isolates in Khorasan Province was investigated, but no relationship between the geographical origin of the isolates and the genotypic groups were detected (Zaker Tavallaee, 2003). Fourty-seven isolates of FOC were analyzed using ISSR markers in Ilam Province and the isolates were clustered into 24 groups based on similarity coefficient. This result demonstrates the high genetic diversity among the isolates (Azimi *et al*., 2017). In India, SSR markers distinguished the four races of FOC with differential host cultivars (Barve *et al*., 2001). Fourty-five FOC isolates were studied using five pairs of microsatellite primers in Kermanshah Province (Nourollahi *et al*., 2017).

The main objective of this investigation was to determine genetic structure of FOC populations in chickpea cultivating areas in west and northwest of Iran using SSR marker so that their results can be used in disease management programs and breeding for resistant chickpea cultivars.

MATERIALS AND METHODS

Samples of infected chickpea plants were collected from different locations in Lorestan (Pol-e-Dokhtar, Kuhdasht, Azna, Gerit, Basatabad, Aleshtar and Khorramabad),

Kermanshah (Gilan-e-Garb, Islamabad-e-Garb, Tazehabad, Kerend-e-Gharb, Mahidasht, Kuzaran, Javanrud, Ravansar, Harsin and Kamyaran), and Kurdistan and West Azerbaijan (Saqqez, Zarrineh, Dehgolan, Hezar Kanian, Ghiam-e-Waldian, Sardasht, Baneh, Bukan, Firuraq, Shahin Dezh, Heydarlu and Oshnavieh) Provinces (as three populations) located in west and northwest of Iran, during 2016-2017 (Figure 1). The locations are different in altitude and climate, separated by substantial mountain ranges from 60-550 kilometer. Thirty isolates were selected within each location. Isolates of each location was considered as a population. The samples showed typical wilt symptoms as discoloration of stems.

Roots and basal stems of diseased chickpea plants were washed carefully with running tap water to remove any adhering soil. The samples were cut into small pieces (3–5 mm) and surface sterilized by dipping into 1% aqueous solution of sodium hypochlorite for 1–2 minutes, washed three times with sterile distilled water, blotted on sterilized filter papers and cultured in PDA media. The plates were incubated for three days in an incubator at 25˚C with a 12 hour photoperiod to induce production of conidia. Colonies developing on 1.4 strength PDA were sub-cultured, then transferred to synthetic nutrient agar (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose and 20 g agar L^{-1} distilled water) at the same condition as described above. Plates were examined under stereo microscope. Colonies on PDA were floccose and ranged in color from white to pale violet. Both macroconidia and microconidia were formed and chlamydospores were observed in culture for all isolates. *Fusarium* spp. was identified based on descriptions of Nelson *et al*. (1983), and Leslie and Summerell (2006). After morphologic identification of *Fusarium* isolates, monoconidial culture of each isolate was obtained and used for further analyses. The plates were incubated at 25˚C for 4-5 days.

Fungal mycelium was harvested through a fine mesh screen, frozen and stored at -20˚C. Molecular identification of all isolates was made using the primer pairs FOC-f (5′-GGCGTTTCGCAGCCTTACAATGAAG-3′) and FOC-r (5′-GACTCCTTTTTCCCGAGGT AGGTC AGAT-3′) (Arvaio-Ortiz *et al*., 2011).

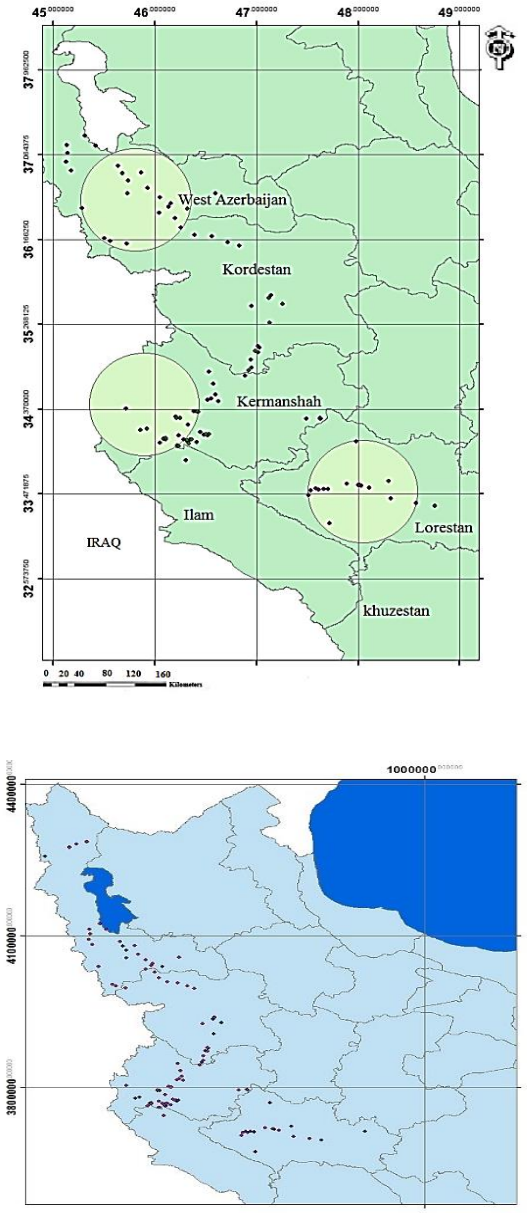

Figure 1. Location of sampling areas in west and northwest of Iran. Each location is shown with a bright green circle on the map.

DNA Extraction and SSR Analysis

Six primers pairs described by Jill *et al*. (2014) (Table 1) were used to study the genetic structure of FOC populations. Primer pairs were manufactured by Metabion international AG, Germany Company.

DNA was extracted using a modified hexadecyl trimethyl-ammonium bromide (CTAB) procedure (Doyle and Doyle, 1987). Genomic DNA was extracted from single spore culture of each isolate (Murray and Thompson, 1980). Mycelia were ground on PDA media, harvested through a fine mesh screen (50-70 mgr) and suspended in 2% CTAB extraction buffer (1.4 M NaCl, 0.1M Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol). Samples were treated with 2 units RNase at 37˚C for 30 minutes 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**.**

Primer aliquots for each marker were prepared by mixing equimolar amounts of appropriate forward and reverse primer in 1x TE (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) and used for the amplification 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 20 ng of template DNA). Amplification was performed using Biored T100 thermal cycler (Sangapur), PCR conditions for SSR were as follows, 94˚C for 4 minutes, followed by 36 cycles of 94˚C for 1 minute, the optimum annealing temperature for 1 minute (appropriate annealing temperature were used for each primers set, Table 1), and 72˚C for 1 minute, followed by 72˚C for 6 minutes. Amplified products were resolved in 1.5% agarose gel at 110V in 1x TBE buffer and stained with DNA Safe Stain at 0.5 mg mL^{-1} and photographed under UVitec (UK Company). Intas. A 1 kb ladder (Thermo Scientific Company, Catalog No. SM0243) was used as a molecular size standard**.**

Table 1. Characteristics of primers (Jill *et al*., 2014**)** used to study genetic structure of FOC based on SSR marker.

SSR locus	Primers	Primer sequence (5′ to 3′)	Expected size (bp)	Tm (ºC)	EMR[a]	MI[b]	PIC[c]
FoAB11	FoAB11F	GGCCGCCCAGAAGAGGTAG	170-210	56	7	1.54	0.22
	FoAB11R	ATTGGAGCGGAAAAGAAACACG					
FoAD1 2	FoAD12F	TCGAGGAAGACACTGCACTAAAAA	150-240	56	7	1.61	0.23
	FoAD12R	CCCCAAGGTCAGACTCACTCAG					
FoAG11	FoAG11F	TGTAAGATGCTCTCTCT	150-700	56	11	2.2	0.20
	FoAG11R	AAAGGTAGTGATGCC					
FoDC5	FoDC5F	AGAAACAAGAACCCCATATCGC	130-200	60	4	1	0.25
	FoDC5R	ACTTAAACAGGAAAGGGACGGA					
FoDD7	FoDD7F	CGATTGACTACCGGGTGAACTTGT	310-350	56	3	0.93	0.31
	FoDD7R	AGGGCGAGGGTGAGGGTGAGA					
FoDF7	FoDF7F	GGCGTTGGGCGTTGCTAA	270-290	54	3	1.17	0.39
	FoDF7R	ATTTGTTGGGATTCCTTCAGAC					

[a] Effective Multiplex Ratio, [b] Marker Index, [c] Polymorphic Information Content.

Data Analysis

For data analysis, each population was defined according to the geographic region (sampling area). The amplified bands were scored co-dominantly for each primer as (1) presence and (0) as absence for each SSR locus. PhiPT, as an indicator for molecular variance, between individuals within a population was calculated by the following formula:

PhiPT= AP/(WP+AP)

Where, AP indicates the diversity of Populations and WP is the level of Population diversity. Two matrices including genetic

distance matrix and geographic distance matrix were obtained from all isolates studied in this study.

The pair-wise distance among the isolates was calculated from the binary matrix using the simple mismatch coefficient (Sneath and Sokal, 1973) that is recommended for haploid fungi (Kosman and Leonard, 2005). The raw binary data was analyzed further to obtain dissimilarity coefficient. Genetic distances between all pairs of populations were calculated and an Un-weighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram was constructed based on Nei's genetic distance using the NTSYS-pc 2.10s software. The population diversity description, Principal Coordinate Analysis (PCoA) and Analysis Of Molecular Variance (AMOVA) were performed using Gen Alex ver. 6.5 (Peakall and Smouse, 2006). For each primer pair, the Polymorphic Information Content (PIC), Marker Index (MI), Effective Multiplex Ratio (EMR), Number of different alleles (N_a), Number of effective alleles (N_e), corrected fixation index [G_{st}' (Nei)], Gene flow (N_m), Nei's gene diversity (H), mean values of gene diversity in total populations (H_t), gene diversity between populations (H_s), and Shannon's Information Index (I) were calculated for each population using software POP GENE ver. 1.31 (Yeh *et al*., 1999).

The Ewens-Watterson (E-W) Test was conducted to determine the effect of natural selection on the distribution of alleles in different locus (Manly *et al*., 1972). In the formula **F**= $\sum_{i=1}^{k} ni^2/n^2$, **F** is the Ewens-Watterson value; ni is the frequency of the allele i; n is the number of locuses, and k is the number of inhabitants in the population. This test was performed for all loci and for different populations. First, the mean value of E-W was calculated and then the upper and lower limits were determined at 95% probability level. Calculations were performed by the POP GENE ver. 1.31 software.

RESULTS

FOC Isolates

Based on morphological characteristics, the isolates were identified as FOC. Isolates showed significant variations in cultural characteristics, production of microconidia, macroconidia and chlamydospores. Our morphological identifications were further confirmed by molecular method (PCR) using specific SSR primers used in this study for FOC isolates. From 255 isolates obtained from plant samples, 235 isolates belonged to Fusarium genus. Among them, 116 isolates, after DNA amplification with specific primers, formed a 1,500 bp band that belong to FOC (Figure 2). Out of 116 isolates identified, 90 isolates (30 isolates per population) belonging to three locations were selected to population genetic structure.

Pathogenicity Test Results

The sterilized soil of each vase containing seeds or seedlings of chickpea sensitive cultivar Beevanij was inoculated by Fusarium isolates in greenhouse conditions. Three weeks after inoculation, the symptoms started as paleness, yellowing, and drying of seedlings. To prove the pathogenicity test, the FOC was re-isolated. Therefore, collected isolates were pathogenic isolates and were chosen for further study.

SSR Polymorphism and Genetic Diversity

Six pair SSR primers were used for amplification of SSR loci of 90 FOC isolates. All isolates amplified a single band ranging from 100 to 1,000 bp. A total of 35 alleles ranging in size from 150 to 700 bp were detected (Tables 1). The number of alleles scored per locus varied from 3 (FODD7 and FODF7) to 11 (FOAG11) with an average of 5.83 across all six loci. The PIC value varied

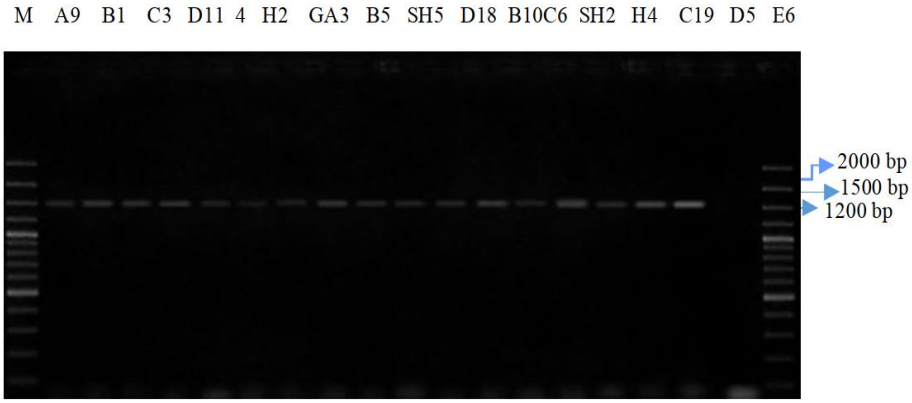

Figure 2. DNA band of 1500 bp in agarose gel 0.8% amplified using FOC-f and FOC-r primer pairs for the specific detection of FOC obtained from Iranian chickpea specimens with symptoms of wilt in the western and north western provinces of Iran. Above shape: Different isolates of the fungus. M: Marker for DNA size.

from 0.20 (FOAG11) to 0.39 (FODF7) with an average of 0.27 per locus (Table 1).

Population Genetic Structure

The genetic diversity indices for FOC populations are summarized in Table 1. The average Number of effective alleles (N_e), Number of private alleles (N_a), the average of allelic variability per locus (H), Shannon's Information Index (I) and percentage of polymorphic loci (%PL) were calculated (Table 2). Observed allele number (N_a= 1.8571) was higher in Kermanshah and lower in Kurdistan and West Azerbaijan. Effective numbers (N_e= 1.3504) of alleles were higher in Lorestan compared to other populations. The amount of genetic diversity (H) was higher in Lorestan, and Shannon's Information Index (I) was higher in Kermanshah (I= 0.3367) but lower values were estimated for Kurdistan and West Azerbaijan (I= 0.3049). The percentage of Polymorphic Loci (%PL) was higher in Kermanshah (%PL= 85.71) compared to other populations.

Genetic Variability of Populations

The pair-wise genetic distance and population matrix of Nei genetic identity are presented in Table 3. The average genetic distance was calculated among the three populations. Nei's pair-wise genetic distances between the populations varied from 0.0202 to 0.98. The lowest genetic distance was found between populations of Kurdistan and West Azerbaijan with Lorestan, while the highest genetic distance

Table 2. Genetic diversity estimates in three FOC populations based on SSR loci.[a]

Population	No of isolates	N_a	N_e	H	I	%PL
Lorestan	30	1.7429	1.3504	0.2149	0.3328	74.29
Kermanshah	30	1.8571	1.3207	0.2090	0.3367	85.71
Kurdistan and West Azerbaijan	30	1.6286	1.3319	0.1997	0.3049	62.86
Average	-	1.9714	1.3370	0.2208	0.3570	74.29

[a] N_a= Observed number of alleles, N_e= Average effective number of alleles, H= Nei's (1973) gene diversity, I= Shannon's Information Index [Lewontin (1972)], %PL= Percentage of Polymorphic Loci.

was between Lorestan with Kermanshah populations (Table 3).

Cluster analysis based on UPGMA was used to produce a dendrogram showing the genetic relationships between the populations based on the SSR data. In a dendrogram based on the genetic distance with the UPGMA algorithm, the populations of three centers were categorized into three groups, although the population of Lorestan and Kurdistan were more closely related to the population of the Kermanshah (Figure 3).

Table 3. Information about genetic distance between pairs of FOC populations.

Population	Lorestan	Kermanshah	Kurdistan and West Azerbaijan
Lorestan	****	0.9751	0.9800
Kermanshah	0.0252	****	0.0716
Kurdistan and West Azerbaijan	0.0202	0.0288	****

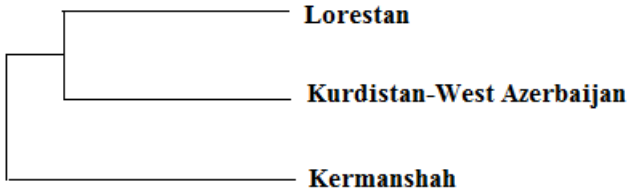

Figure 3. Dendrogram of genetic relationships between each FOC population constructed by UPGMA algorithm.

The total gene diversity (H_t) and gene diversities between subpopulations (H_s) were estimated to be 0.2208 and 0.2079, respectively. Gene diversity attributable to differentiation among populations (G_{st}) was 0.0585, while gene flow (N_m) was 8.0412 (data not shown). A dendrogram based on UPGMA analysis showed three major groups containing isolates with different origins (Figure 3). The AMOVA of genetic variation in FOC populations revealed that 5% of the variance occurred among populations and 95% within populations (Table 4). PhiPT value is 0.054. It is calculated to be an indicator for molecular variance and shows the relation between individuals within a population, and this index is dependent on two factors of diversity between populations and the extent of diversity within populations. The results of the analysis of molecular variance showed a significant difference among the three populations studied from the FOC (Table 4). The frequency of the bands multiplied and the number of specific bands in the populations of Lorestan, Kermanshah, and Kurdistan-West Azerbaijan were (26, 1), (30, 6) and (22, 2) respectively (Figure 4).

PCoA (Principal Component Analysis) using SSR data showed the genetic differences among isolates within populations and gene flow between different populations (Figure 5).

Table 4. 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	2	20.356	10.178**	5	0.01
Within populations	87	327.367	3.763	95	
Total	89	347.722	-	100	

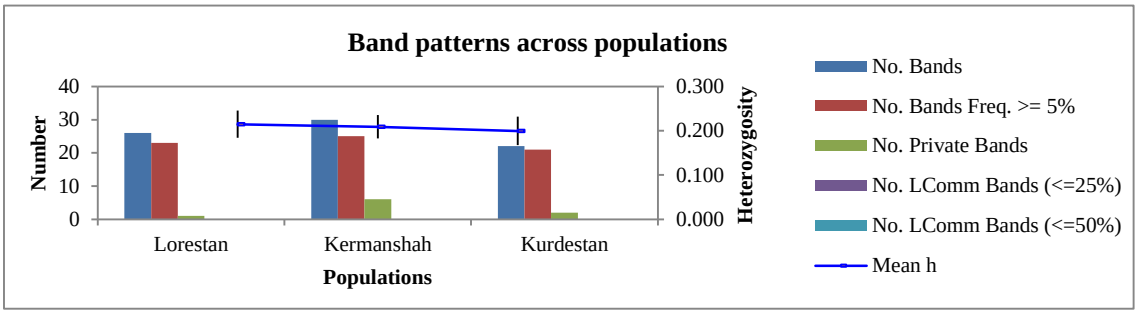

Figure 4. Total band patterns for binary (haploid) data by FOC populations.

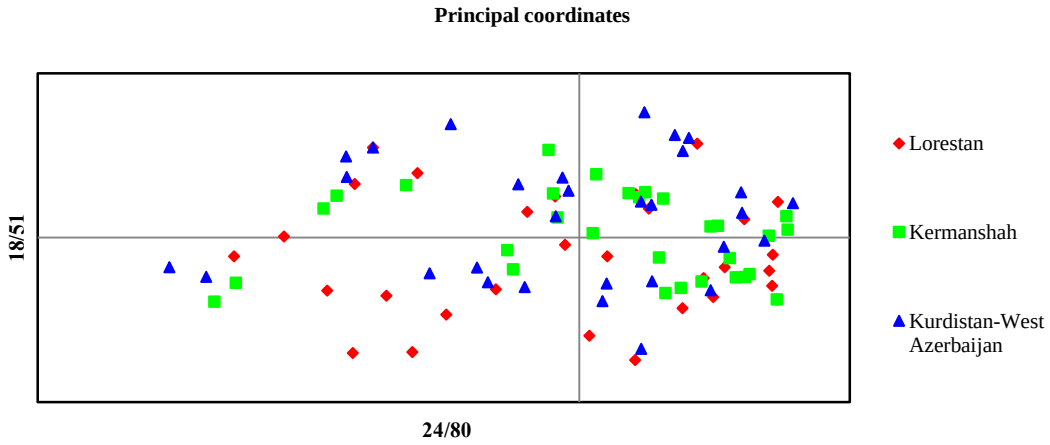

Figure 5. Principal Component Analysis (PCoA) based on SSR data for 90 individual FOC isolates belonging to three populations.

PCoA allows for visualizing the patterns of genetic relationship without altering the data itself and finds patterns within a multidimensional data set. The first and second principal coordinates account for 24.80 and 18.51% of the variation, respectively. There was no clear separation among individuals from different populations, however, isolates in the same populations tended to gather. This suggests the geographical regions of sampling play important role for the formation of populations.

Ewens-Watterson Test

If the F value (sum of square of allelic frequency) of the Ewens-Watterson test for neutrality of microsatellite markers lies between the two lower and upper limits of 95% confidence, then the assumption is correct, which indicates that the natural selection is not effective on the distribution of the alleles in the relevant locus. In this study, the results of the Ewens-Watterson test showed that, for all studied loci, the obtained F value was between the lower and upper limit of 95%, indicating that the natural selection had no significant effect on the distribution of the alleles in the different loci, all loci were neutral and not linked with some selection traits.

DISCUSSION

SSR markers are very useful tools as a genetic marker for a variety of applications in plants (Kalia *et al*., 2011). This study was carried out to discover the possible utilization of SSR markers for identifying genetic variation in FOC isolates from the west and northwest provinces of Iran. The study also highlighted the fact that genetic analysis using SSR markers is a useful tool with high discriminatory power for studying the diversity in FOC (Tenzer *et al*., 1999; Saharan and Naef, 2008). In this study, we investigated the population genetic structure for 90 FOC isolates, belonging to three populations from three sampling areas, by six SSR primer pairs. The six primer pairs primed the amplification of 35 alleles. Alleles were varied 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*., 2004). In this research, SSR markers divided all the FOC populations in three major groups. Previously, 64 FOC Indian isolates were placed in two major categories with ISSR and RAPD markers (Dubey and Singh, 2008). Seventy-six FOC isolates from Sudan and 14 FOC isolates from Syria and Lebanon showed two major clusters as well (Elmahi Mohamed *et al*., 2015). 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; Sivaramakrishnan *et al*., 2002b; Belabid *et al*., 2004; Nourollahi *et al*., 2011).

In this study, the mean number of PIC (Polymorphic Information Content) value of SSR markers was 0.27, which indicated that the isolates had a low degree of diversity. In 64 FO isolates using 71 microsatellites, 71 alleles were amplified, and the polymorphic content of markers (PIC) was reported as 0.25. This polymorphism indicates a low degree of biodiversity (Bogale *et al*, 2005). 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 average number of alleles (5.83) detected in this study was higher than previous report by Nourollahi *et al*. (2017). The polymorphic character of SSR produces highly discriminating fingerprints that often allow characterization of fungi at a strain level (Migheli *et al*., 1998). The AMOVA analysis indicated that most of variation was within the populations (95%) and low proportion (5%) was among the populations. In another study on FOC isolates from Kurdistan and Kermanshah, genetic diversity within and among populations was 93.5 and 6.5%, respectively (Montakhabi *et al*, 2020). The FOC isolates from Turkey showed high genetic diversity due to differences within the population. In their study, the gene flow rate (N_m) was 3.009, which occurs due to the movement of the disease causal agent between infected areas (Bayraktar, 2012). In this study, the N_m (8.0412) index demonstrated high gene flow and low genetic differentiation within populations. This study suggests that frequent gene flow and recombination between populations of FOC might significantly influence the evolution and development of this fungus. This subject is in accordance with the study that indicated a monophyletic group of FOC isolates derived from a small population or a single individual (Jimenez-Gasco *et al*., 2002) and somatic recombination occurred through para sexuality in FO (Molnar *et al*., 1990). High pathogenic and genetic variation in asexual fungi such as FOC were also observed with occurrence of genetic mutations (Gordon and Martyn, 1997). In this study, FOC isolates showed a different level of diversity within and between populations. However, there was a high degree of genetic similarity among populations separated by low geographical distances. High gene flow (N_m= 8.0412) was detected and caused genetic differentiation to be at the lowest value (G_{st}= 0.0585). Low values of G_{st} showed low genetic difference

and low geographical subdivision among populations (Bayraktar *et al*., 2010).

The presence of high gene flow always causes the populations of this fungus to adapt to diverse environmental conditions. The geographic distances between sampling regions were short (60 to 550 km) and variability of the populations was affected by movement of conidia, contaminated seeds, infected plant debris, as gene flew. The absence of large natural barriers such as the sea, ocean or huge mountain ranges, as well as the continuous cultivation of this crop and the presence of sensitive and tolerant cultivars of chickpea have caused the gene flow of this fungus to be high in Iran. Gene flow is one of the evolutionary forces that can have a significant force on the genetic diversity of populations. In the absence of gene flow, genetic drift causes development of 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 short 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 detecting new FOC strains and can be used for introducing resistant cultivars in chickpea growing areas. Although high genetic diversity within FOC populations makes it difficult for breeders to produce a variety of resistant cultivars for each region, low genetic diversity between FOC populations allows them to use these cultivars quickly in other areas as needed. However, understanding of occurrence, distribution, and genetic relatedness of such pathogenic variants is necessary for developing effective and efficient integrated disease management. Knowledge of the genetic diversity of FOC is very important for development of resistant chickpea cultivars and adoption of quarantine measures. Molecular markers have proven to be powerful tools for dissecting genetic diversity and better identification of all races, especially when diagnostic markers are employed (Gurjar *et al*., 2009).

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ساختار ژنتیکی جمعیت قارچ *Fusarium oxysporum* f. sp. ciceris، عامل پژمردگی نخود معمولی، با استفاده از نشانگرهای SSR در غرب و شمال غرب ایران

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چکیده

پژمردگی نخود معمولی توسط قارچ *Fusarium oxysporum* f. sp. ciceris یکی از فاکتورهای مهم در کاهش عملکرد محصول نخود در مزارع غرب و شمال غرب کشور می باشد. در سال های زراعی ۹۶-۱۳۹۵ با استفاده از نشانگر SSR، تعیین ساختار ژنتیکی جمعیت های قارچ Fusarium oxysporum f. sp. ciceris در مزارع غرب و شمال غرب ایران انجام گرفت. با کاربرد شش آغازگر SSR، ۳۵ آلل در جایگاه ژنی و با متوسط ۵/۸۳ آلل در هر جایگاه ژنی تکثیر شد. میزان چند شکلی آغازگرها (PIC) از ۰/۲ (FOAG11) تا ۰/۳۹ (FODF7) با متوسط ۰/۲۷ در هر جایگاه ژنی بدست آمد. متوسط آلل های موثر(Ne)، تعداد آلل های اختصاصی (Na)، تنوع آللی در هر جایگاه ژنی (H)، شاخص شانون (I) و درصد چند شکلی جایگاه ژنی (PL%) در سه جمعیت به ترتیب، ۱/۳۳، ۱/۹۷، ۰/۲۲۰۸، ۰/۳۵۷ و ۷۴/۲۹ بود. میانگین فاصله ژنتیکی بین سه جمعیت محاسبه شد. فاصله ژنتیکی نئی بین بین جمعیت ها از ۰/۰۲۰۲ تا ۰/۹۸ متغیر بود. تنوع ژنی کل (Ht) و تنوع ژنی بین زیرجمعیت ها (Hs) به

به ترتیب، ۰/۲۲۰۸ و ۰/۲۰۹۷ تخمین زده شد. تنوع ژنی ناشی از تمایز بین جمعیت ها (Gst) ۰/۰۵۸۵، در در حالی که جریان ژنی (Nm)، ۸/۰۴۱۲ بود. آنالیز تنوع ژنتیکی (آموا) در جمعیت های قارچ *Fusarium oxysporum* f. sp. ciceris نشان داد که ۵ ٪ از واریانس مربوط به تنوع بین جمعیت ها و ۹۵ ٪ تنوع در درون جمعیت های قارچ رخ داده است. PhiPT، ۰/۰۵۴ محاسبه گردید. کمترین فاصله ژنتیکی بین جمعیت های کردستان و آذربایجان غربی با جمعیت لرستان، در حالی که بیشترین فاصله ژنتیکی بین جمعیت لرستان با جمعیت کرمانشاه مشاهده شد.