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Genetic structure analysis of *Fusarium oxysporum* f. sp. *phaseoli* populations on common bean by using SSR markers

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Abstract: Determination of the genetic structure of the *Fusarium oxysporum* populations provides different levels of pathogen behavior and environmental compatibility in the management of root rot disease in bean farms. In this study, Short Sequence Repeat (SSR) markers were used to determine the genetic structure and estimate genetic diversity in sixty *F. oxysporum* isolates from five counties in Ilam province located in the west of Iran (Ilam, Ayvan, Malekshahi, Sirvan, Chardavol). A set of five microsatellite primer pairs revealed one allele in each locus across the populations. The average number of alleles (N_a) and the mean number of effective alleles (N_e) observed among populations were 1.484, and 1.438 respectively. The number of Genetic diversity (*H*) and Shannon's coefficient (*I*) were also maximum in Chardavol (H_e = 0.442, *I*=0.333), but the minimum values were estimated in Ilam (H_e = 0.228, *I* = 0.333). The minimum genetic distance was found between Ayvan and Malekshahi (0.046), but the maximum genetic distance (0.151) was revealed between Ilam and Chardavol. The total gene diversity (*Ht*) and genetic variability between the subpopulations (*Hs*) were estimated to be 0.312 and 0.261, respectively. Gene diversity attributable to differentiation among the population (*Gst*) was 0.162, while gene flow (*Nm*) was 0.5717. Cluster analysis based on UPGMA showed the lowest genetic distance between Ayvan and Malekshahi. The dendrogram showed a clear break between the population from Sirvan and Chardavol and other remaining populations. Results from this study could be useful in breeding programs for developing root rot resistant cultivars.

Keywords: Fusarium wilt, population, genetic distance, microsatellite

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an important legume in the world with high nutritional and economic value. Plant pathogens can cause important bean diseases, among which wilt disease caused by *Fusarium oxysporum* f. sp. *phaseoli* (FOP) is one of the most important diseases of common bean (Karimian et al. 2010). Symptoms of Fusarium wilt initially appear as yellowing and wilting and in case of infection at younger ages, plants may also be stunted. *Fusarium oxysporum* isolates exhibit great variability in morphology and aggressiveness (Abbas 1995, Belabid et al. 2004). Pathogenic isolates of *F. oxysporum* often show host specificity and based on the host, may be subdivided into formae speciales (Armstrong et al. 1981). Control of *F. oxysporum* infection in the field is difficult because the pathogen can survive for a long time in the form of mycelium in infected plant debris or in the form of chlamydospores in soil (Haware et al. 1996). Fusarium wilt disease management can be conducted through the use of resistance cultivars (Jalali & Chand 1992).

The genetic diversity of plant pathogens plays a significant role in the understanding of plant pathogens evolution and their correlation with plant hosts, which helps us in applying any preventive measure against them (Karimian et al. 2010). Knowledge of genetic diversity is necessary for resistance development and shifts identification in races or population structure that might occur (McDonald 1997). Identification of diversity by morphological characteristics is highly variable in *Fusarium* isolates and these characteristics are influenced by cultural conditions. Many DNA–based methods have been used to study diversity in pathogenic *Fusarium* populations (Kiprop et al. 2002, Sivaramakrishnan et al. 2002a, Belabid et al. 2004, Nourollahi and Aliaran 2017). Molecular markers have been used to characterize the genetic diversity of different isolates of *Fusarium oxysporum* (Edel et al. 1997, Kistler 2001, Alves–Santos et al. 1999) formae–speciales (Gherbawy 1999, Alves–Santos et al. 2002). RAPD is an effective method for discrim

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inating of FOP isolates (Namvar Hamzanlouyi et al. 2006, Alves–Santos et al. 2002, Woo et al. 1996).

Short sequence repeats (SSRs) are powerful tools for taxonomic and population genetic studies (Britz et al. 2002). Alleles vary according to the number of present repeat units, although it has been shown that mutations could be responsible for allele length variations in SSR analysis (Burgess et al. 2001, Slippers et al. 2004). Because of the high resolution provided by SSRs (Enjalbert et al. 2005), they have also been used in different fungal species, including *Sclerotinia sclerotiorum* (Sirjusingh & Kohn, 2001), *Rhizoctonia solani* (Mwang Òmbe et al. 2007) and *Ascochyta rabiei* (Nourollahi et al. 2011). Some researchers have already worked on a molecular variation of different *F. oxysporum* form specials (Stewart et al. 2006). Bogale et al. (2005) determined that polymorphism shown with eight SSR markers should be enough for study of genetic variability in *F. oxysporum* complex. Based on the studies, microsatellite markers could differentiate the four races of *F. oxysporum* f. sp. *ciceris* causing varying levels of wilting with differential host cultivars (Barve et al. 2001). The ISSR marker has been used for determining genetic variations between several populations of *F. oxysporum* f. sp. *ciceris* (Bayraktar & Dolar 2008).

Most studies of *F. oxysporum* have focused on plant pathogenic isolates (Mohammadi et al. 2004). It is difficult to control pathogenic fungi with a high level of genetic variation, as they adapt more quickly to any control strategy, such as applying resistant cultivar. Therefore, knowledge of the genetic diversity of FOP has contributed to the development of disease control strategies (Kistler 2001). This study was conducted to analyze the diversity and genetic relationships of *F. oxysporum* isolates collected from common bean fields in Ilam province using SSR markers.

MATERIALS AND METHODS

Sampling and fungal isolation

Roots of bean plants with wilt symptoms and black lesions were randomly collected from five different regions, including Ilam, Ayvan, Malekshahi, Sirvan, and Chardavol from 2015 to 2016, in the west of Iran. Isolates of each region were considered as a population (Table 1, Fig 1). Infected samples were cut into 2– to 5–mm–long pieces, surface sterilized by dipping into 5 % sodium hypochlorite, (5% NaOCl) for 2–3 min, washed three times with sterile distilled water, dried with sterile filter paper, and finally, were placed on potato dextrose agar (PDA) plates. All the samples were incubated for three days at 25 °C with a 12–h photoperiod to induce conidia production. The fungus was isolated and purified using the hyphal tip and single spore methods (Hawker 1950). After the identification, characterized isolates were stored for a short time on SNA culture (Leslie et al. 2006) at 4 ºC, and for perennial time, spores of each isolate were stored at 4 °C in micro tubs containing sand. *F. oxyspourum* isolates were initially identified according to the morphological and microscopic characteristics of the macroconidia, phialides, microconidia, chlamydospores, and colony growth traits described by Jens et al. 1991, Nelson et al. 1983, Barnett & Hunter 1972, Leslie et al. 2006, Maina et al. 2017.

Table 1. Geographical origin of *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province.

Sampling region	Origin site	No.Isolates	Population number
Ilam	Ilam	14	1
Ayvan	Khoran	10	2
Malekshahi	Mehr	12	3
Sirvan	Lomar	9	4
Chardavol	Sarableh	15	5

Fig. 1. Geographical location of sampling sites of *Fusarium oxysporum* f. sp. *phaseoli* populations in Ilam province.

Pathogenicity test

A pathogenicity test was done in the greenhouse at Ilam University and one representative isolate from each population was randomly chosen for a pathogenicity test on local cultivar to evaluate the infective ability of isolates as described by Perveen et al. (2007). The standard root dip inoculation technique was used to analyze the pathogenicity of isolates on common bean plants (Alves–Santos et al. 1999). Briefly, roots of one–week–old seedlings were washed in running tap water; about 2 cm pieces was cut from each tip, and subsequently, the roots were dipped for three min in spore suspension with 1×10^6 spores/ml concentration. Inoculated seedlings were transplanted into plastic pots with sterilized vermiculite. Finally, the plants were incubated for 24 h in a controlled climatic chamber at 23–24 °C with 60–75 % relative humidity. *Fusarium* yellows severity was recorded at one–week intervals after inoculation by using the Centro International de Agricultura Tropical scale (Alves–Santos et al. 1999) score as 1 (no external symptoms) to 9 (dead or severely

infected plants with 100 % of the foliage showing wilting, chlorosis, necrosis, and/or premature defoliation).

DNA extraction

DNA was extracted from 60 FOP isolates. To obtain the mycelia mass, liquid cultures were initiated by adding 2–4 mm^2 pieces of filter papers containing the fungus to 250 mL Erlenmeyer flasks containing 100 mL PDB medium (potato dextrose broth). All the 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 and stored at −20 °C. DNA extraction was done according to the CTAB (Cetyltrimethyl–Ammonium Bromide) method (Doyle & Doyle 1987). Mycelia were ground in liquid nitrogen and suspended in a 2 % CTAB extraction buffer (1.4 M NaCl, 0.1 M Tris–HCl, pH 8.0, 20 mM EDTA, 0.2% β–mercaptoethanol). The samples were treated with five 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 $ng\mu l^{-1}$ in TE (pH 7.4). The quality of the extracted DNA was visually checked on 0.8 % agarose gel.

A set of five locus–specific primer pairs for SSRs (Table 2) described by Bogale et al. (2005) were selected. For each primer pair, primer aliquots for each marker were prepared by mixing equimolar amounts of appropriate forward and reverse primers in 1X TE buffer (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) and used for the amplification of individual microsatellite loci. PCR amplification was perfor–med in a 25 µL volume reaction containing 2.5 µL of 10X PCR Buffer, 0.2 mM of dNTPs mix (100 mM of each dNTPs), 1.5 mM $MgCl_2$, 1 µL of each forward and reverse primer, 0.5 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 4 min Followed by 35 cycles of 94 °C for the 30s, annealing temperature (59 –62°C) for 30s (appropriate annealing temperature were used for each primer set (Table 2)), and 72 °C for 5 min. The thermal cycles were terminated by a final extension of 10 min at 72 °C. The amplified products were resolved in 2.0 % agarose gel at 60 V cm^{-1}, using Tris Boric Acid EDTA (1X TBE) buffer and strained with DNA Safe Stain and photographed under UV Trans Laminator with Gel documentation, Intas.

Molecular data analysis

Data analyses of populations were defined according to the geographic locations. The bands generated by SSR primers that were repeatable and visible with 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). Genetic similarity between pairs was estimated using Jaccard's similarity coefficient. Similarity coefficients were used for the construction of UPGMA dendrogram (Rolhf 2009). For each primer pair, the polymorphic information content (PIC) and marker index (MI) were 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 overall 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 $H = 1 – \Sigma X_i^2$, where X_i 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 overall subgroups (Nei 1973). The allele frequencies at polymorphic loci, the N_m values (effective migration rate), and the genetic identity among populations for the characterrizing genetic variation, observed number of alleles (Na), the effective number of alleles (*Ne*), Nei's gene diversity *He*) and Shannon's information (index (*I*) was calculated in both origin sites and subspecies levels.

Mean values of gene diversity in total populations (*Ht*), gene diversity between populations (*Hs*), the proportion of gene diversity attributable to differentiation among populations (*Gst*), and the estimate of gene flow from *Gst* (N_m) were obtained across loci (McDermot & MacDonald 1993). The relationships among populations were estimated using UPGMA dendrograms based on Nei's genetic distances (1978) and also Analysis of molecular variance (AMOVA), which is the hierarchical distribution of genetic variation among and within populations, was assessed. Principal coordinate analysis (PCA) was performed to evaluate the genetic differences among isolates within populations. All above calculations were performed using POPGENE ver. 1.31 (Yu et al. 1999) and Gen Alex ver. 6.5 (Peakall & Smouse 2006).

RESULTS

Morphological features and pathogenicity test

A total of 60 isolates of FOP were isolated and purified from infected bean plants (Table 1). Based on morphological characteristics, the isolates were identified as FOP. The isolates showed significant

Table 2. Characterization of SSR primers used in this study.

Primer locus	Primer sequence (5′–3′)	Repeat motif	Annealing	EMR	MI	PIC
MB9	F:TGGCTGGGATACTGTGTAATTG R:TTAGCTTCAGAGCCCTTTGG	(CA)9	60	300	109.2	0.367
MB10	F:TATCGAGTCCGGCTTCCAGAAC R:TTGCAATTACCTCCGATACCAC	(AAC)6	61	400	114	0.285
MB14	F:CGTCTCTGAACCACCTTCATC R:TTCCTCCGTCCATCCTGAC	(CCA)5	59	400	158.4	0.396
MB17	F:ACTGATTCACCGATCCTTGG R:GCTGGCCTGACTTGTTATCG	(CA)21	60	400	162	0.405
MB18	F:GGTAGGAAATGACGAAGCTGAC R:TGAGCACTCTAGCACTCCAAAC	(CAACA)6	62	400	145.6	0.364
Average				380	137.8	0.363

PIC (the polymorphic information content), EMR (effective multiplex relationship ratio)
MI (marker index)

variations in cultural characteristics, production of microconidia, macroconidia, and chlamydospores. Our morphological identifications were further confirmed by the molecular method using specific SSR primers used in this study for FOP isolates.

Morphological identification was based on characteristics of the macroconidia, phialides, microconidia, chlamydospores, and colony growth traits (Maina et al. 2017). The colony diameter for the isolates at seven days of growth differed among the isolates incubated at 25–26 °C on PDA. Moreover, the isolates showed variations in their micro and macroconidia in terms of size and number of septa. The macroconidia in all isolates had more than one septum. They were slightly sickle–shaped with slightly foot–shaped basal cells and attenuated apical cells. The aseptate microconidia were produced abundantly from short monophialides. The chlamydospores were present at the terminal or intercalary positions, usually in singles and in pairs. The results of the pathogenicity test indicate that all FOP isolates were virulent and caused wilting and death of the seedlings. In the early stage, symptoms appeared as yellowing of the lower leaves, then symptoms progressed up toward upper leaves, at this stage, wilting plants were observed, Vascular discoloration was typically observed in infected plants. Typical symptoms of wilt disease were observed 20–40 days after inoculation.

Primer information and allelic frequency

Based on the microsatellite data, the polymorphic information content (PIC) varied from 0.258 (Primer MB10) to 0.405 (Primers MB17), which reflects the informative content of the used primers. EMR (effective multiplex relationship ratio) varied from three to four. 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 109.2 (MB9) to 162 (MB17) (Table 2).

To investigate the genetic structure of *F. oxysporum* populations, the isolates were divided into five populations, including Ilam, Ayvan, Malekshahi, Sirvan, and Chardavol. From the microsatellite loci used in this study, a total of 300 DNA bands (200–500 bp) were amplified. A total of 16 different alleles were produced by SSR primers (Table 3, Fig. 2).

The average number of bands per gene locus was one. The highest number of bands was 58 at MB14 and the lowest number at 55 at MB9. The average number of alleles (N_a) observed among populations was 1.484 and the mean number of effective alleles (N_e) among populations (1.438), with the highest number of alleles (1.480) in Malekshahi population then that in Chardavol and Ayvan populations with 1.476 and 1.432, respectively and the lowest number of effective alleles was found in Sirvan population with 1.394 (Table 4).

Fig. 2. Banding pattern of MB17 (A) and MB14 (B) and MB10 (C) primers on *Fusarium oxysporum* f. sp. *phaseoli*. M indicates 1Kbp DNA ladder and F1, F2, …, F24 indicate the isolates code.

Genetic diversity among populations

Analysis of obtained SSR data for polymorphism studies among sixty *F. oxysporum* isolates was performed. The total gene diversity (H_t) and gene diversities between subpopulations (H_s) were estimated to be 0.312 and 0.261, respectively. Gene diversity attributable to differentiation among populations (G_{st}) was 0.162, while gene flow (N_m) was 0.571.

The average genetic distance was calculated among the five populations. Nei's pairwise genetic distances between the populations varied from 0.046 to 0.151. The lowest genetic distance was found

Table 3. Number of alleles at each locus across the five populations of *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province.

SSR primer	Number of alleles per population					Total alleles
	Chardavol	Sirvan	Malekshahi	Ayvan	Ilam	
MB9	3	2	3	3	3	12
MB10	1	2	2	2	4	14
MB14	2	3	4	2	2	15
MB17	3	3	3	3	3	13
MB18	3	4	3	3	4	16
Average	2.4	2.8	3	2.6	3.2	14.2

Table 4. Genetic diversity estimates for *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province based on five microsatellite loci.

Population	N_a	N_e	I	H
Ilam	1.211	1.409	0.333	0.228
Ayvan	1.579	1.432	0.395	0.259
Malekshahi	1.579	1.480	0.431	0.288
Sirvan	1.368	1.394	0.363	0.240
Chardavol	1.684	1.476	0.442	0.292
Mean	1.484	1.438	0.393	0.261

between Ayvan and Malekshahi, while the highest genetic distance was revealed between Ilam and Chardavol (Table 5). Cluster analysis (UPGMA) results revealed the genetic relationships between the populations based on the SSR data; the dendrogram revealed a distinction between the Chardavol population and the four remaining populations (Fig.3). PCA (Principal Component Analysis) using SSR data showed the genetic differences among isolates within populations and gene flow between different populations (Fig. 4); accordingly, the first and second principal coordinates were 27.42 % 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.

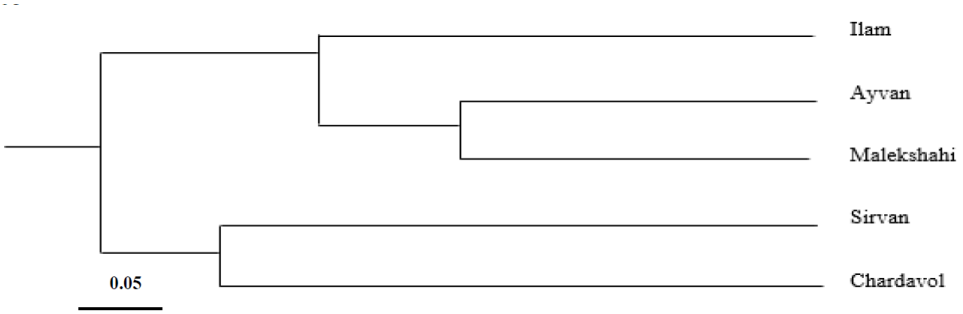

Fig. 3 Dendrogram of genetic relationships between five populations of *Fusarium oxysporum* f. sp. *phaseoli* from Ilam province reconstructed by UPGMA using Nei's genetic distance.

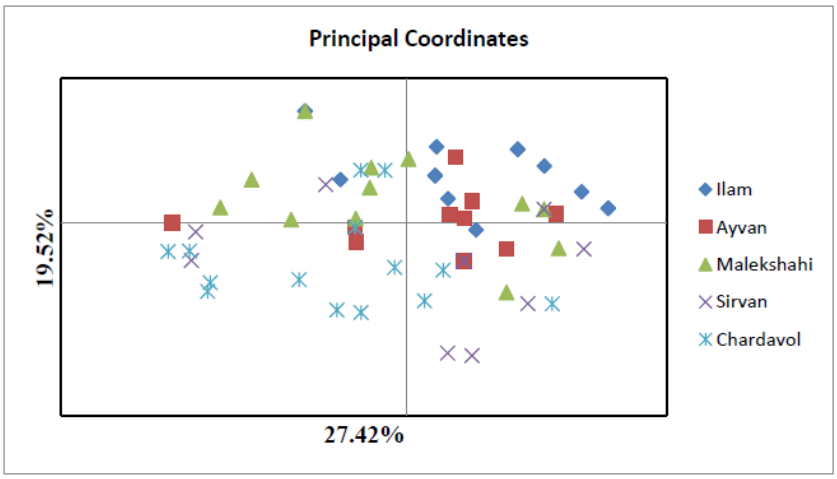

Fig. 4. Principal Component Analysis based on SSR data from *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province.

Table 5. Nei's original measure of genetic distance between pairs of *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province.

Population	Ilam	Ayvan	Malekshahi	Sirvan	Chardavol
Ilam	–				
Ayvan	0.075	–			
Malekshahi	0.054	0.046	–		
Sirvan	0.150	0.074	0.093	–	
Chardavol	0.151	0.085	0.089	0.082	–

Table 6. Analysis of molecular variance (AMOVA) within and between *Fusarium oxysporum* f. sp. *phaseoli* populations from Ilam province.

Source of variation	df.	Sum of squares (SS)	Mean of squares (MS)	Percentage of variation	P value
Among populations	4	29/590	7/398	11	0.126
within populations	55	149/376	2/716	89	
Total	59	178/967		100	

This suggests the geographical regions of sampling play an important role in the formation of populations. The AMOVA of genetic variation in FOP populations revealed that 13 % of the variance occurred among populations and 87 % within populations (Table 6).

DISCUSSION

In this research, the population genetic variability of *F. oxysporum* from five bean–growing regions in the west of Iran was analyzed. Essential information was generated and showed that SSR markers are powerful discriminative tools for studying diversity in *F. oxysporum* f. sp. *phaseoli* populations. The advantage of microsatellite markers is their high specificity, high polymorphism, good reproducibility and unambiguous score ability, which provides a powerful means for taxonomic and population genetic studies (Tenzer et al. 1999, Britz et al. 2002). In microsatellite analysis, alleles modify according to the number of repeated units, and mutation has also been responsible for allelic differences (Burgess et al. 2001, Slippers et al. 2004, Nourollahi & Aliaran 2017). In this study, SSR marker grouped *F. oxysporum* f. sp. *phaseoli* isolates into three major groups based on their geographical regions. In similar studies, 114 *F. oxysporum* f. sp. *ciceris* isolates and 101 *F. oxysporum* f. sp. *lentis* isolates from Iran were placed into two major groups (Nourollahi & Jalali 2016, Nourollahi & Aliaran 2017). In another study, based on ISSR and RAPD markers, 64 *F. oxysporum* f. sp. *ciceris* isolates from India were located into two major classes (Dubey & Singh 2008). In the most of worldwide studies on plant pathogenic fungi using molecular markers have highlighted the importance of molecular methods for illustrating genetic diversity within and between populations (Bentley et al. 1995, Sivaramakrishnan et al. 2002b, Belabid et al. 2004, Nourollahi et al. 2011,). In this study, multiple alleles were recorded in *F. oxysporum* f. sp. *phaseoli* populations, as reported in the similar studies on *Fusarium* species (Mohammadi et al. 2004, Nourollahi & Jalali 2016, Nourollahi & Aliaran 2017). The polymorphic features of SSRs often allow characterization of fungi at strain level (Barres et al. 2006). The variability of alleles per loci showed the level of polymorphism and directly used for showing of genetic evolution (Sanders 2002, Mwang Òmbe et al. 2007, Nourollahi & Jalali 2016). The results showed that there was a low level of genetic variation in the overall isolates of *F. oxysporum* f. sp. *phaseoli* in Ilam province. In these regions, low levels of genetic diversities have been reported among *F. oxysporum* isolates collected from chickpea and lentils farms (Nourollahi & Jalali 2016, Nourollahi & Aliaran 2017). In such studies, the low amount of variation within the populations was perhaps due to exchange of contaminated seeds between sampling regions, infected plant debris. Cultivation of these seeds leads to the distribution of spores of the fungus in the farms (Nourollahi & Jalali 2016).

Analysis of molecular variance (AMOVA) showed that the genetic variance between populations is 13 % in contrast to 87 % for the within population component. Pair–wise genetic distances between the populations were also very similar and they showed a different level of diversity in comparison with the local and international isolates. Similar results were also reported in *F. oxysporum* f. sp. *lentis* (Nourollahi & Jalali 2016), *F. oxysporum* f. sp. *phaseoli* (Woo et al. 1996), *F. oxysporum* f. sp. *ciceris* isolates (Jimenez–Gasco et al. 2001, Nourollahi & Aliaran 2017) and in the Ethiopian *F. oxysporum* isolates using AFLP, SSR, and ITS (Bogale et al. 2006).

The low level of gene diversity (G_{st} = 0.162) was distinguished in these populations. The low G_{st} value directed little genetic variation among the populat–tions and showed little indication for geographical subdivision (Bayraktar et al. 2010). The gene flow (N_m = 7.589) indices revealed high gene flow and low genetic separation in these regions. This study suggests that the gene flow between sampling area played a key role in permanence of the fungus and the similarity of *F. oxysporum* populations. This subject was confirmed by a study that showed *F. oxysporum* f. sp. *ciceris* is a monophyletic group (Jimenez–Gasco et al. 2003).

Gene flow is one of the evolutionary elements that can have a vital role in genetic variability of populations. In the absence of gene flow, genetic drift causes emerging different allele frequencies at neutral loci, leading to variation in populations (Keller et al. 1997). Knowledge of the occurrence severity, distribution, and genetic relatedness of *F. oxysporum* f. sp. *phaseoli* isolates is necessary for developing effective integrated disease management.

In this study, the genetic structure of *F. oxysporum* populations was recognized and the result can provide essential information for plant breeders to introduce tolerant cultivars against *Fusarium* and developing integrated strategies for disease management.

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تجزیه و تحلیل ساختار ژنتیکی جمعیت‌های *Fusarium oxysporum* f. sp. *phaseoli* روی لوبیای معمولی با بکارگیری نشانگرهای ریزماهواره

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چکیده: مطالعه تنوع ژنتیکی جمعیت های *Fusarium oxysporum* سطوح مختلفی از رفتار بیمارگر و سازگاری محیطی برای مدیریت بیماری پوسیدگی ریشه و طوقه در مزارع لوبیا را فراهم می کند. نشانگر توالی تکراری کوتاه برای تعیین ساختار ژنتیکی و تخمین تنوع ژنتیکی در ۶۰ جدایه *F. oxysporum* از پنج منطقه استان ایلام واقع در غرب ایران شامل (ایلام، ایوان، ملکشاهی، سیروان و چرداول) استفاده شد. میانگین الل ها در هر مکان ژنی یک آلل بود. میانگین آلل ها و میانگین آللهای موثر شناسایی شده در جمعیتها به ترتیب ۱/۴۸۴ و ۱/۴۳۸ بود. در هر ژنگاه در جمعیت ملکشاهی بالاترین و در جمعیت سیروان کمترین بود. همچنین شاخص تنوع ژنتیکی (H) و شاخص شانون (I) بیشترین در جمعیت چرداول (H_e =۰/۲۹۲ ، I = ۰/۴۴۲) اما کمترین آن ها در ایلام (I = ۰ /۳۳۳, H_e = ۰ /۲۲۸) بود. کمترین فاصله ژنتیکی بین جمعیت ایوان و ملکشاهی (۰/۰۴۶) و بیشترین فاصله ژنتیکی (۰/۱۵۱) بین ایلام و چرداول مشاهده گردید. همچنین تنوع ژنتیکی کل (Ht) و تنوع ژنی زیر جمعیت ها (H_S) ۰/۳۱۲ و ۰/۲۶۱ به ترتیب تخمین زده شد. تنوع ژنتیکی مؤثر در تمایز بین جمعیت ها (G_{st}) ۰/۱۶۲ بود و میزان جریان ژنی (N_m)**۰/۵۷۱۷** بود. تجزیه کلاستر بر اساس دندروگرام UPGMA نشان داد کمترین فاصله ژنتیکی بین ایوان و ملکشاهی وجود دارد و دندروگرام شکاف واضحی بین جمعیت های سیروان و چرداول با دیگر جمعیت ها را نشان می دهد. نتایج حاصل از این مطالعه می تواند در اصلاح ارقام مقاوم نسبت به پوسیدگی ریشه و توسعه روش های کنترل بیماری مؤثر باشد.

کلمات کلیدی: پژمردگی فوزاریومی، تنوع ژنتیکی، فاصله ژنتیکی، ریزماهواره

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