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Research Article

Population genetic structure of *Fusarium verticillioides* the causal agent of corn crown and root rot in Ilam province using Microsatellite markers

Ahmad Seydi nejad and Khoshnoud Nourollahi*

Department of Plant Protection, Faculty of Agriculture, Ilam University, Ilam, Iran.

Abstract: Analysis of genetic diversity of *Fusarium verticillioides* populations concludes different levels of information in management of crown and root rot disease in corn farms. Simple sequence repeat (SSR) markers were used to determine genetic structure and estimate genetic diversity in 77 *F. verticillioides* isolates from major producing areas in Ilam province, Iran such as: Dehloran, Mosiyan, Dasht Abas, Mehran, Eivan, Holeylan and Darreh Shahr during 2016 - 2017. Nine microsatellite (SSR) primer pairs revealed that the average number of alleles in populations were 34, the number of alleles in populations varied from 27 alleles in Dehloran and Mosiyan as the lowest to 40 alleles in Darreh shahr as the highest. Observed alleles (N_a) number and effective number of alleles (N_e) were higher in Mehran (N_a = 1.860; N_e = 1.463) compared to other populations. The genetic diversity (H_e) was higher in Holeylan (H_e = 0.284) and Shannon's information index (I) was also higher in Mehran (I = 0.436) but lower values were estimated for Mosiyan (H_e = 0.195; I = 0.303). The lowest genetic distance was found between Dehloran and Mosiyan (0.013) while the highest genetic distance was revealed between Dehloran and Darehshahr (0.139). Total gene diversity (H_t) and gene diversities between subpopulations (H_s) were estimated at 0.292 and 0.249 respectively. Gene diversity attributable to differentiation among populations (G_{st}) was 0.147, while gene flow (N_m) was 2.890. Cluster analysis based on UPGMA showed the lowest genetic distance between Dehloran and Mosiyan and then Dasht abas. The dendrogram indicated a high genetic distance between Darehshahr and the six remaining populations. Results from this study will be useful in breeding program of crown and root rot resistant cultivars and developing control methods for this disease.

Keywords: corn, genetic distance, root rot, SSR

Introduction

Corn *Zae mays* L. is the highest yielding produce among cereals and regarding cultivation area is next to wheat and rice, but stands first as forage. *Fusarium verticillioides* (Saccardo) Nirenberg [*Gibberella fujikuroi* (Sawada) Wollenw] is a prevalent pathogen of agriculturally important crops (Leslie, 1991, 1995). *Fusarium verticillioides* has been associated with human and animal toxicities since it was first described in 1881 (Saccardo, 1881). This species and other anamorphs of *G. fujikuroi* are the most common fungi associated with corn production in North America and other temperate regions in the world

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* **Corresponding authors,** e-mail: k.nourollahi@ilam.ac.ir
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(White, 1999). *Fusarium* species are capable of causing seedling disease, root rots, stalk rots and ear rots of corn as well as damaging stored grain (Mohammadi and Mofrad, 2011). Although yield usually is not much affected, kernel infection by *G. fujikuroi* is of concern because of the loss of grain and seed quality and the potential occurrence of fumonisins and other mycotoxins (Munkvold and Desjardins, 1997). Members of *F. verticillioides* and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*) belong to the Liseola section of *Fusarium* (Nelson *et al*., 1983), and are well-known pathogens of corn, causing stalk and ear rot (Nelson *et al*., 1981; Leslie *et al*., 1990) and also rice root rot and stunting of crown (Hsieh *et al*., 1977). Crown and root rot is primarily a seed borne disease. Soil temperature of 35 ºC is most favorable for infection (Nyvall, 1999). Spores of this pathogen are distributed by wind between corn fields (Ooka and Kommedahl, 1977). Long distance distribution by infected seed (Mohammadi and Mofrad, 2011). Asexual sporulation is clearly the most successful and impressive reproductive mechanism because of the large number of conidia that is produced by a single colony (Kendrick, 2003). Symptoms of the disease are a white to pink or salmon-colored mold on the ear and brown to black lesions on the crown and root. Infection of developing corn kernels may occur through the silks. Through holes and fissures in the pericarp or at points where the pericarp is torn by the emerging seedling and as a result of systemic infection of the corn plant by *F. verticillioides* (Glenn *et al*., 2003). *Fusarium verticillioides* produces abundant, mostly single-celled microconidia in long chains (Glenn *et al*., 2004). For more than 20 years, assessments of fungal pathogens have used multilocus markers to detect population genetics (Milgroom, 1996). DNA-based techniques have increasingly become a tool for understanding the genetic diversity and also phylogenetic relationships of *Fusarium* spp. Researchers have already worked on molecular variation in *Fusarium* spp. (O'Donnell, 2000; Datta *et al.,* 2011). Ren *et al*. (2012) showed high polymorphism and high genetic diversity among *F. verticillioides* isolated from corn using SSR markers. Plant pathologists should study the population genetic of plant pathogenic fungi, because pathogens evolve (Momeni and Nazari, 2016). Pathogens population must constantly adapt to changes in their environment to survive (McDonald, 1997). Defining the genetic structure of populations is a logical first step in studies of fungal population genetics because the genetic structure of a population reflects its evolutionary history and its potential to evolve (McDonald, 1997). Knowledge derived from genetic structure of pathogen populations has direct agricultural applications. For instance, the genetic variation maintained within a population indicates the speed at which a pathogen evolves (McDonald and McDermott, 1993). This information may eventually be used to predict how long control measures such as fungicides and resistant cultivars are likely to be effective (McDonald and Linde, 2002). Several studies have recorded the genetic distinction of the mating populations of the *G. fujikuroi* complex species, including vegetative compatibility (Leslie, 1993), isozyme analysis (Huss *et al.*, 1996) and electrophoretic karyotyping (Xu and Leslie, 1996). Knowledge of genetic structure of *F. verticillioides* populations is useful for development of effective strategies in controlling the disease (McDonald, 2004). Several molecular techniques are available for investigating genetic variability among populations of fungal plant pathogens. Microsatellites also known as simple sequence repeats (SSRs), which are randomly distributed throughout the genome of fungi and other eukaryotes (Li *et al.,* 2002; Wostemeyer and Kreibich, 2002; Sahran and Naef, 2008), provide a powerful tool for taxonomic and population genetic studies (Britz *et al.,* 2002). 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.,* 2004). The objective of present study was to collect corn samples with crown and root rot symptoms from corn farms in Ilam province and to determine population genetic structure of *F. verticillioides* using SSR markers.

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Materials and Methods

Samples and fungal isolates

Corn plants in panicle stages with symptoms of brown to black lesions on crown and root were randomly sampled from 35 corn fields (each field ≥ 1 ha) in seven different regions as populations in the west of Iran, Ilam province, during 2016 - 2017 growing season (Fig. 1., Table 1). A total of five symptomatic plants were collected per field.

Each sample were cut into 3 - 5 mm long pieces, were surface-sterilized with 0.5% sodium hypochlorite for 1 - 2 min and 70% ethanol for 30 seconds rinsed three times with sterile distilled water and air dried with sterile filter paper. The sterilized samples were placed on a general medium, potato dextrose agar (PDA), and Nash and Snyder medium (Taherkhani *et al.,* 1998; Jo *et al.,* 2008). Samples were incubated 3 - 5 days at 25 °C with a 12 - h photoperiod for production of conidia. Nash and Snyder is a selective medium for *Fusarium* species and facilitates the formation of large, easily recognizable colonies (Chen *et al.,* 2007). The fungal isolates were isolated and purified using the hyphal tip and single spore method (Hawker, 1950). *Fusarium* colonies were observed microscopically, those colonies identified as *F. verticillioides*, were transferred to carnation-leaf agar (CLA), potassium chloride agar (KCLA) and potato dextrose agar (PDA) (Merck Company). Isolates were identified morphologically to species based on characteristics of the macroconidia, microconidia, phialides, chlamydospores and colony growth traits (Leslie and Summerell, 2006). Obtaining 98 isolates of *Fusarium* section Lesiola, among which *F. verticillioides* was the most frequent (78.5% of the population), followed by *F. proliferatum* (21.5%). Identified isolates were stored for short time on SNA at 4 °C, but for long time stored in tube containing glycerol at 4 °C.

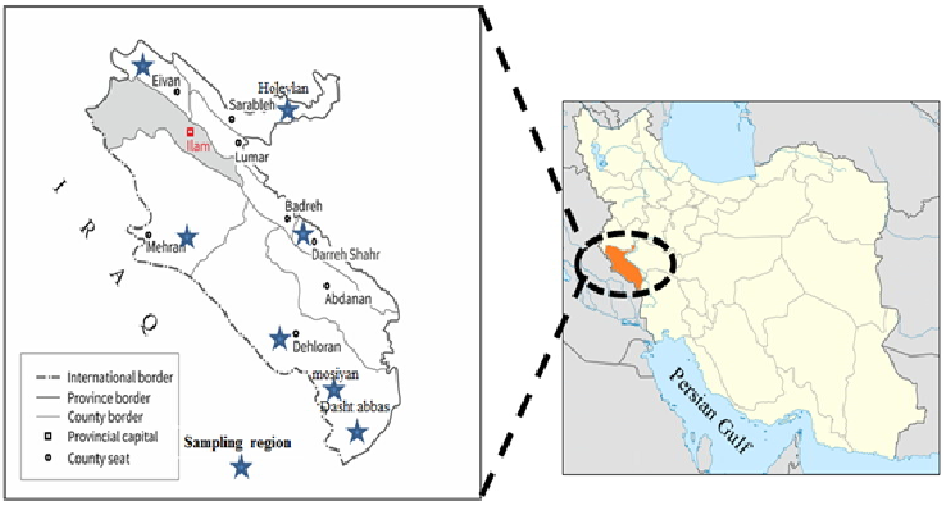

Figure 1 Geographical origins of the seven *Fusarium verticillioides* populations used in this study.

Table 1 Location and number of isolates in each *Fusarium verticillioides* population.

Populations	No. of isolates in population	Code of isolates	Location	Province
1	12	FvC1-FvC12	Dehloran	Ilam
2	10	FvC13-FvC22	Mosiyan	Ilam
3	9	FvC23-FvC31	Dasht abbas	Ilam
4	12	FvC32-FvC43	Darreh shahr	Ilam
5	12	FvC44-FvC55	Mehran	Ilam
6	11	FvC56-FvC66	Holeylan	Ilam
7	11	FvC67-FvC77	Eivan	Ilam

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Pathogenicity test

For inoculation, 100 g of corn seeds soaked for 24 hours in distilled water sterilized at 121 °C for 20 minutes, then inoculated with 1 × 2 cm mycelial block of 7 days-old cultures on PDA plates. The flasks containing the inoculated seeds were shaken daily to prevent clumping of inoculated seeds. Pathogenicity test was carried out on the susceptible local cultivars under greenhouse conditions. Germinated seeds of corn plants were planted in pots containing sterilized soil. The corn seedling was inoculated by adding propagules to the soils around plant roots, covered with white plastic bags to keep high humidity for 24 hours. Control treatments were corn plants that were grown in sterilized soil without inoculation. Twenty-five days after inoculation, plants were observed for the development of disease symptoms and *F. verticillioides* was reisolated from inoculated plants.

DNA extraction

Liquid cultures were initiated by adding 2-4 mm^2 pieces of filter papers containing of *F. verticilioides* to 250 ml Erlenmeyer flasks containing 100 ml PDB medium (potato dextrose broth plus 2 g yeast extract per liter). Flasks were incubated at room temperature approximately 25 °C and shaken on a rotary shaker for 7 - 14 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 Cetyl Trimethyl Ammonium Bromide (CTAB) procedure (Doyle and Doyle, 1990). 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 isoamylalcohol 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 in TE (pH 7.4) and stored at -20 °C (Weising *et al.,* 1991; Bayraktar, 2010).

SSR amplification and analysis

Nine SSR primer pairs (Table 2) were selected based on their high PIC as described by Ren *et al.* (2012). Primer aliquots for each marker were prepared by mixing equimolar amounts 20 pmoles of appropriate forward and reverse primer in 1 × TE (1 mM EDTA, 10 mM Tris–HCl, pH 8.0). PCR amplification was performed in a 25 µl reaction volume containing 2.5 µl of 10X PCR buffer, 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 Mnotach 60 thermal cycler., PCR conditions for SSR were as follows; one initial denaturation step at 95 °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 primer set, between 52 - 59 °C) and 72 °C for 60 s. The thermal cycles were terminated by a final extension of 5 min at 72 °C. Amplified products were resolved in 3% agarose gel at 60 V using Tris Boric Acid EDTA (1X TBE) buffer and stained with DNA Safe Stain at 0.5mg/ ml and photographed under UV Trans laminator with Gel Doc. Intas. A 1 kbp ladder was used as a molecular size standard.

Statistical analysis

Populations were defined as different geographic regions. The clear and intense amplified DNA bands were scored as binary digit code of "0" (for absence) and "1" (for presence), respectively. 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). Genetic distance of the populations was estimated from the SSR data using the UPGMA, clustering method on the basis of Nei's (1978) unbiased genetic distance. All data analyses were performed using POPGENE ver. 1.31 (Yeh *et al.,* 1999), Gen Alex ver. 6.501 (Peakall and Smouse, 2012) and NTYsys ver. 1.07c (Rolhf, 1990).

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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 (Rohlf, 1990). For each primer pair, polymorphic information content (PIC), marker index (MI) was calculated. The polymorphic information content (PIC) was calculated using PICi = 2fi (1 – fi), where i is the information of marker$_i$, fi is the frequency of the amplified allele (presence of fragments) and (1 – fi) 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 *EMR, where EMR is the "effective multiplex relationship" given by the product of the total number of fragments per primer and the fraction of polymorphic fragments (Varshney *et al.,* 2007). Genotypic diversity (H_t) among isolates was estimated from allelic frequencies using the equation H = 1 - Σ xi2, where xi is the frequency of the i^{th} allele of a particular locus (Nei, 1973). The coefficient of population subdivision (G_{st}) was computed as $(H_t - H_s)/H_t$, where, H_t is the total genetic diversity and H_s is the average gene diversity over all subgroups (Nei, 1973). The allele frequencies at polymorphic loci, the N_m values (effective migration rate), and the genetic identity among populations for characterizing genetic variation, observed number of alleles (N_a), effective number of alleles (N_e), Nei's gene diversity (H_e) and Shannon's information index (I) were calculated for each locus and population. Mean values of gene diversity in total populations (H_t), gene diversity between populations (H_s), proportion of gene diversity attributable to differentiation among populations (G_{st}) and estimate of gene flow (N_m) from G_{st} were estimated across loci (McDonald and McDermot, 1993).

Table 2 SSR primers of *Fusarium verticillioides* used in this study (Ren *et al*., 2012).

Primer locus	Repeat of cloned allele	Primer Sequences	Expected Size (bp)	Tm (°C)
3H02	$(ATG)_{11}$	F:ATCACCAAAACAGAGCAAAG R:GTGGCTGAGAAAGACAAGAA	181	52
4H18	$(TTTC)_6$	F:TGATGCGGTCAAAGAATGG R:ACTGGAGCAGATGAAGAGC	152	56
5H08	$(CTTT)_6$	F:ACCAACTAACATCCCGAATC R:CGTAAACTCAAACGCAAGG	410	58
5H12	$(GAAA)_7$	F:GGCACCAACATTCCTGACG R:AACCGCCTACAAGCACCA	404	59
6H02	$(GTTC)_{14}$	F:AACGGTATAGCGAGAAACG R:GGACGGAGTCGAATGAAGT	222	55
7H21	$(GA)_{25}$	F:TTATGTCTGCCCAATCGC R:CCAGTTTCGCATCTGTCG	442	57
8H01	$(AAG)_9$	F:TGGTATGATGGCTGGTTCG R:ACTAGAGGCTGGGTTGGTGT	485	55
9H05	$(ATGG)_{11}$	F:AGGCACGAGTGGATAAGGC R:TTGGAAGCAAGCGAAGGA	221	56
9H09	$(GT)_{18}$	F:ATCGGTGGTTTCTTGCTGC R:GCTCCCAACTGCCTACCTACA	263	59

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Results and Discussion

Isolation

Ninety-eight isolates of *Fusarium* in the section Liseola were isolated from 350 corn plants with symptom of crown and root rot disease. Among them 77 isolates were *F. verticillioides*. Previous studies also showed that *F. verticillioides* were predominant in corn fields.

Pathogenicity test

Results proved that all *F. verticillioides* isolates were pathogenic on corn. In the pathogenicity test, inoculated roots showed brown to black lesions and all isolates caused discoloration of the corn crowns and roots while no such symptoms were observed in control treatments.

Distribution of alleles at polymorphic SSR loci

All nine microsatellite loci were polymorphic with a total of 43 alleles detected. Among which3H02, 4H18, 5H08, 5H12, 6H02**,** 7H21, 8H01, 9H05 and 9H09 showed a total of 5, 6, 5, 5, 4, 4, 5, 4 and 5 alleles per locus, respectively (Table 3, Fig. 2). Moreover, the average number of alleles per locus in populations was the highest (6 alleles) in Mehran and Holeylan populations and the lowest (2 alleles) in Dehloran and Mosiyan population (Table 4).

Table 3 Number of alleles at each locus across the five populations of *Fusarium verticillioides* from Ilam province.

SSR primer	Number of alleles						
	Dehloran	Mosiyan	Dasht abas	Darreh shahr	Mehran	Holeylan	Eivan
3H02	5	4	4	5	4	4	4
4H18	3	3	3	4	6	6	4
5H08	3	3	3	4	5	4	5
5H12	3	3	3	4	4	4	4
6H02	3	2	3	4	4	3	4
7H21	3	3	3	4	4	4	4
8H01	2	3	3	5	4	5	5
9H05	3	3	3	4	4	4	4
9H09	2	3	4	4	5	4	5

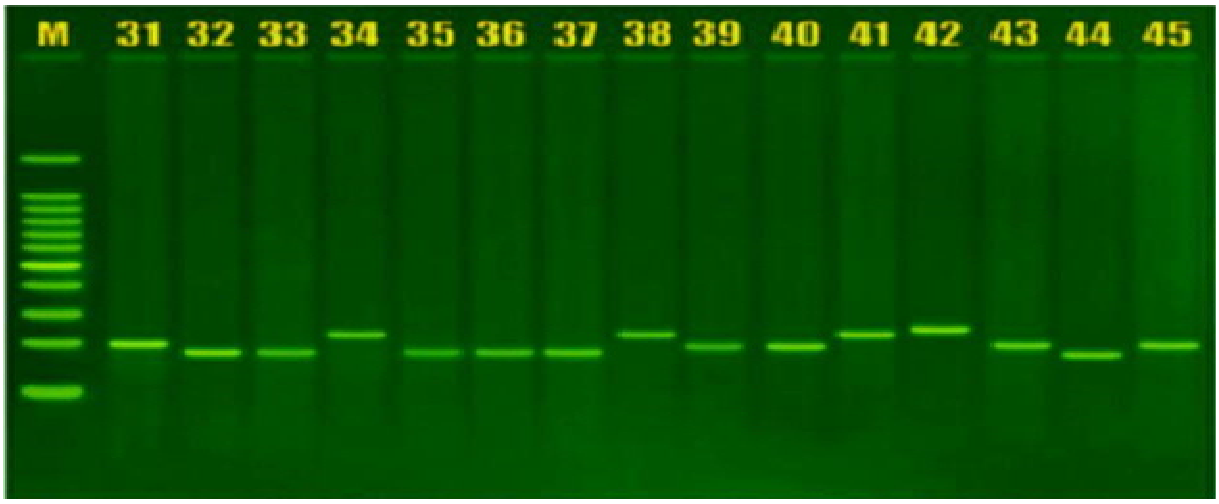

Figure 2 Amplification profile of *Fusarium verticillioides* isolates using 6H02 SSR primer pairs. Numbers indicate the isolates (31 - 45), M indicates size marker (Ladder 1 kb).

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Table 4 Number of alleles, percentage of polymorphism and polymorphic information content obtained from SSR primers among *Fusarium verticillioides* populations.

SSR primer	Number of alleles	Number of Polymorphic alleles	Percentage of polymorphism	Polymorphic information Content (PIC)
3H02	5	5	100	0.228
4H18	6	6	100	0.281
5H08	5	5	100	0.288
5H12	5	5	100	0.294
6H02	4	4	100	0.325
7H21	4	4	100	0.335
8H01	5	5	100	0.289
9H05	4	4	100	0.313
9H09	5	5	100	0.277

Primers characteristics

A summary of characteristics of nine microsatellite loci is given in Table 4. Number of alleles, percentage of polymorphism and Polymorphic Information Content (PIC) of SSR primers were evaluated. The PIC value varied from 0.33 (primer 7H21) to 0.228 (primers 3H02), which reflects the informative content of the primers. The total number of alleles, percentage of polymorphic alleles of all SSR markers were 43 and 100%, respectively. A total of 43 amplified alleles were polymorphic, and average number of polymorphic alleles per primer was 4.77. Percentages of polymorphic alleles were 100%. Total allelic number in populations varied from 27 (Dehloran and Mosiyan) to 40 (Mehran). Allele diversity of populations ranged from 0.195 in Mosiyan to 0.284 in Holeylan (Table 5).

Genetic variability of populations

Various population genetic parameters in nine microsatellite loci for all populations are given in Table 6. Observed allele number (N_a) and effective numbers (N_e) of alleles were higher in Mehran compared to other populations. The value of gene diversity (H_e) were higher in Holeylan (H = 0.284) and Shannon's Information index (I) were also higher in Mehran (I = 0.436) but lower values were estimated for Mosiyan (H_e = 0.195; I = 0.303).

Based on the SSR data, average genetic distance was calculated between seven populations. The lowest genetic distance was found between Dehloran and Mosiyan populations (0.013), while the highest was revealed between Darreh shahr and Dehloran populations (0.139), (Table 7).

Genetic relationships among populations

Nei's genetic distances were estimated between populations. Cluster analysis based on UPGMA dendrogram to show the genetic relationships among populations (Fig. 3). The lowest genetic dissimilarity was found between Dehloran and Mosiyan and then Dasht abas. Moreover, the dendrogram showed the highest dissimilarity between Darreh shahr population and the six remaining populations. In this study, genetic diversity within and among seven populations of *F. verticillioides* sampled from Ilam was evaluated using SSR markers. The advantage of microsatellite markers over random amplified polymorphic DNA and polymerase chain reaction restriction fragment length polymorphism markers are their high specificity, high polymorphism, good reproducibility and unambiguous scorability (Tenzer *et al.,* 1999; Sahran and Naef, 2008). These results are in accordance with results from previous studies on *F. verticillioides*, demonstrating that the population of this fungus is genotypically highly diverse (Danielsen *et al*., 1998; Chulze *et al*., 2000). The results showed that allele frequencies were different among nine SSRs loci. The allele frequencies showed that *F. verticillioides* may spread as

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asexual stage in all populations. The sample sizes per population and SSRs primers were too small in this experiment for a statistically powerful test of gametic equilibrium. In this study the genetic similarity detected between isolates within the seven populations was probably due to exchange of corn seeds between sampled regions and geographical closeness as well. SSR results indicated that seven populations of *F. verticillioides* had low degrees of gene diversity, but Mehran maintained higher genetic diversity than the other regions (Table 6). Different research has been carried out on international populations of *F. verticillioides*, and a different level of genetic diversity was detected within and between populations (Amoah *et al.,* 1995). High genetic variability among *F. verticillioides* isolates from different hosts has been reported by RFLPs of ribosomal DNA and RAPDs analysis in Ghana (Amoah *et al.,* 1995). Mitter *et al.* (2002) reported high genetic variation among *F. moniliforme* isolates from different geographical regions in India and different hosts by RAPD markers. In Iranian populations, over 91% of the gene diversity was distributed on a local level within populations (Fig. 4).

Table 5 Number of alleles and mean number of allelic diversity in *Fusarium verticillioides* populations.

Population	Number of alleles	Mean number of Allelic diversity
Dehloran	27	0.200
Mosiyan	27	0.195
Dasht abas	29	0.232
Darreh shahr	38	0.273
Mehran	40	0.282
Holeylan	38	0.284
Eivan	39	0.279

Table 6 Genetic diversity of *Fusarium verticillioides* populations based on microsatellite loci.

Population	N	R%	N_a	N_e	H_e	I
Dehloran	12	62.79	1.256	1.330	0.200	0.305
Mosiyan	10	62.79	1.256	1.310	0.195	0.303
Dasht abas	9	67.44	1.369	1.387	0.232	0.351
Dareh shahr	12	88.37	1.767	1.442	0.273	0.421
Mehran	12	93.02	1.860	1.463	0.282	0.436
Holeylan	11	88.37	1.767	1.463	0.284	0.435
Eivan	11	90.70	1.814	1.452	0.279	0.432

Na = Observed number of alleles
Ne = Effective number of alleles
He = Nei's (1973) gene diversity
I = Shannon's information index
R= the percentage of polymorphic loci

Table 7 Genetic distance between *Fusarium verticillioides* populations.

Population	Dehloran	Mosiyan	Dasht - abas	Darreh - shahr	Mehran	Holeylan	Eivan
Dehloran	0						
Mosiyan	0.013	0					
Dasht abas	0.020	0.018	0				
Darreh - shahr	0.139	0.136	0.122	0			
Mehran	0.044	0.046	0.037	0.071	0		
Holeylan	0.097	0.091	0.060	0.066	0.048	0	
Eivan	0.109	0.104	0.079	0.052	0.056	0.042	0

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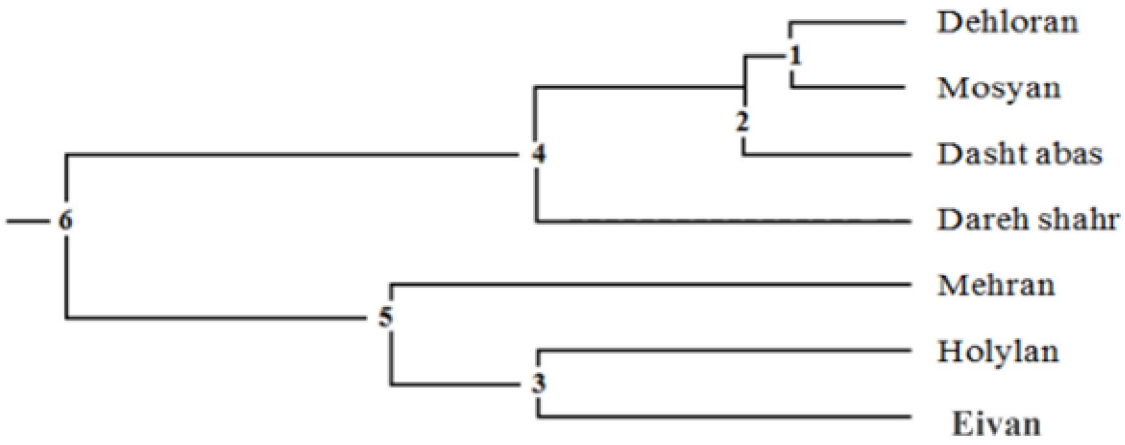

Figure 3 Dendrogram constructed with UPGMA based on Jaccard`s similarity coefficient among seven populations of *Fusarium verticillioides* originated from Ilam province.

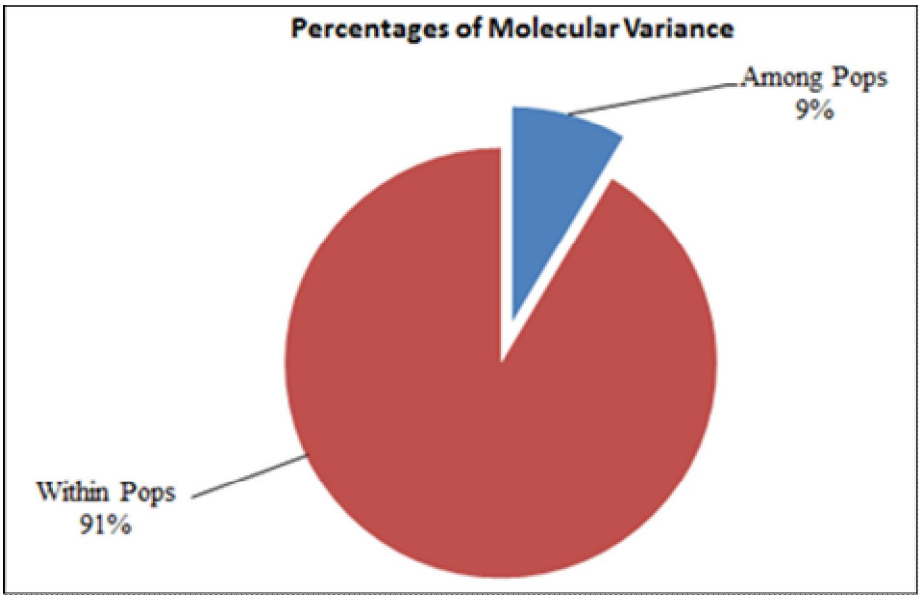

Figure 4 Analysis of molecular variance (AMOVA) in *Fusarium verticillioides* populations from Ilam province.

However, there was a high degree of genetic similarity among populations separated by short geographical distances like Dehloran and Mosiyan. The low level of gene diversity (G_{st}) was detected among all seven populations. Low G_{st} value (0.147) indicated low genetic differentiation among the seven populations and showed evidence for geographical subdivision among populations (Bayraktar, 2010). The genetic distances were very small and the geographic distances between populations ranged from 20 to 375 km, this indicates that populations linked by movement of conidia within populations. Gene flow may have occurred among these populations with infected plant debris, infected seeds, agricultural vehicles and fungal spores. Gene flow has a significant role on the genetic diversity of populations. In the absence of gene flow, genetic drifts cause developing different allele frequencies at neutral loci, leading to differentiation in

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isolate populations (Keller *et al.,* 1997). The high genetic similarity among populations of *F. verticillioides* suggests that gene flow has occurred across long distances. N_m averaged 2.890 in all loci and populations, suggesting a high level of gene flow needed to prevent populations from diverging by genetic drift (Keller *et al.,* 1997). Another factor that has an important role in the genetic diversity of *F. verticillioides* populations is the mating system. With this assumption if the sexual reproduction of *F. verticillioides* occurs in nature, the sexual spores (ascospores) may play a major role in population biology. Moreover, 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. If the main source of primary inoculums was asexual spores from seeds, it is expected to find some clones that were distributed among different locations in a field. Genetic drift and selection would limit the number of genotypes present in field populations, unless sexual reproduction generated new genotypes and wind dispersal distributed ascospores among populations every year (Shah *et al.,* 1995). Depending on environmental factors, genetic diversity of *F. verticillioides* populations may possess potentially a risk of incidence of severe disease and high loss in corn farms in these regions. These data can help breeders in screening for resistant cultivars based on genetic diversity of *F. verticillioides* populations for local disease management. Understanding population genetic structure of *F. verticillioides* in the present study may provide insights into the epidemiology and evolutionary potential of the pathogen and could lead to improved strategies for managing the disease. Moreover, neutral DNA markers such as RAPD and AFLP (McDonald and McDermott, 1993) would generate further results on the genetic structure of *F. verticillioides* populations in corn.

Reference

Amoah, B. K., Rezanoor, H. N., Nicholson, P. and Macdonald, M. V. 1995. Variation in the *Fusarium* section Liseola. Pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different host in Ghana. Plant Pathology, 44: 563-572.

Bayraktar, H. 2010. Genetic diversity and population structure of *Fusarium oxysporum* f. sp. *cepae*, the causal agent of *Fusarium* basal plate rot on Onion, using RAPD Markers. Agricultural Sciences, 16: 139-159.

Britz, H. C., Coutinho, T. A., Wingfield, B. D. and Wingfield, M. J. 2002. Sequence characterized amplified polymorphic markers for the pitch canker pathogen, *Fusarium circinatum*. Molecular Ecology, 3: 577.

Burgess, T., Wing, M. and Wing, B. 2001. Simple sequence repeat markers distinguish among morphotypes of *Sphaeropsis sapinea*. Applied and Environmental Microbiology, 67: 354-362.

Chen, Y., Wang, J. X., Zhou, M. G., Chen, C. J. and Yuan, S. K. 2007. Vegetative compatibility of *Fusarium graminearum* isolates and genetic study on their carbendazim resistance recombination in China. Phytopathology, 97: 1584-1589.

Chulze, S. N., Ramirez, M. L., Torres, A. and Leslie, J. F. 2000. Genetic variation in *Fusarium* section Liseola from no-till maize in Argentina. Applied and Environmental Microbiology, 66: 5312-5315.

Danielsen, S., Meyer, U. M. and FunckJensen, D. 1998. Genetic characteristics of *Fusarium verticillioides* isolates from maize in Costa Rica. Plant Pathology, 44: 615-622.

Datta, S., Choudhary, R. G., Shamim, M. D., Singh, R. K. and Dhar, V. 2011. Molecular diversity in Indian isolates of *Fusarium oxysporum* f. sp. *lentis* inciting wilt disease in lentil (*Lens culinaris* Medik). African Journal of Biotechnology, 10: 7314-7323.

Doyle, J. J. and Doyle, J. L. 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12: 13.

[DOR: 20.1001.1.22519041.2020.9.1.5.2]

Glenn, A. E, Richardson, E. A. and Bacon, C. W. 2004. Genetic and morphological characterization of a *Fusarium verticillioides* conidiation mutant. Mycologia, 96: 968-980.

Glenn, A. E., Meredith, F. I., Morrison, W. H. and Bacon, C. W. 2003. Identification of intermediate and branch metabolites resulting from biotransformation of 2-benzoxazolinone by *Fusarium verticillioides*. Applied and Environmental Microbiology, 69: 3165-3169.

Hawker, L. E. 1950. Physiology of fungi. University of London Press Ltd, London.

Hsieh, H. W., Smith, S. N. and Snyder, W. C. 1977. Mating groups in *Fusarium moniliform*. Phytopathology, 67: 1041-1043.

Huss, M. J., Campbell, C. L., Jennings, D. B. and Leslie, J. F. 1996. Isozyme variation among biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section Liseola). Applied and Environmental Microbiology, 62: 3750-3756.

Jo, Y. K., Chang, S. W., Rees, J. and Jung, G. 2008. Reassessment of vegetative compatibility of *Sclerotinia homoeocarpa* using nitrate-nonutilizing mutants. Phytopathology, 98: 108-114.

Keller, S. M., Mcdermott, J. M., Pettway, R. E., Wolfe, M. S. and Mcdonald, B. A. 1997. Gene flow and sexual reproduction in the wheat glum blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). Phytopathology, 87: 353-358.

Kendrick, B. 2003. Analysis of morphogenesis in hyphomycetes: new characters derived from considering some conidiophores and conidia as condensed hyphal systems. Canadian Journal of Botany, 81: 75-100.

Kosman, E. and Leonard, J. 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Molecular Ecology, 14: 415-424.

Leslie, J. F. 1991. Mating populations in *Gibberella fujikuroi* (Fusarium section Liseola). Phytopathology, 81: 1058-1060.

Leslie, J. F. 1993. Fungal vegetative compatibility. Annual Review of Phytopathology, 31: 127-151.

Leslie, J. F. 1995. *Gibberella fujikuroi*: available populations and variable traits. Canadian Journal of Botany, 7: 282-291.

Leslie, J. F. and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell. 388 p.

Leslie, J. F., Pearson, C. A., Nelson, P. A. and Toussoun, T. A. 1990. *Fusarium* spp. From maize, sorghum and soybean fields in the central and eastern United States. Phytopathology, 86: 343-350.

Li, Y. C., Korol, A. B., Fahima, T., Beiles, A. and Nevo, E. 2002. Microsatellites: genomic distribution, putative functions and mutational a review mechanisms. Molecular Ecology, 11: 2453-2465.

McDonald, B. A. 1997. The population genetics of fungi: tools and techniques. Phytopathology, 87: 448-453.

McDonald, B. A. and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology, 40: 349-379.

McDonald, B. A. and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi. Bioscience, 43: 311-319.

Milgroom M. G., 1996. Recombination and the multilocus structure of fungal populations. Annual Review of Phytopathology, 34: 457-477.

Mitter, N., Srivastava, A. C., Ahamad, S., Sarbhoy, A. K. and Agarwal, D. K. 2002. Characterization of gibberellin producing strains of *Fusarium moniliforme* based on DNA polymorphism. Mycopathologia, 153 (4): 187-193.

Mohammadi, A. and Mofrad, N. N. 2011. Investigation on genetic diversity of *Fusarium verticillioides* isolated from corn using vegetative compatibility groups and relation of VCGs to the pathogenicity. Agricultural Technology, 7 (1): 143-148.

Momeni, H., and Nazari, F. 2016. Population Genetic Structure among Iranian Isolates of

[DOR: 20.1001.1.22519041.2020.9.1.5.2]

Fusarium verticillioides. Plant Pathology Microbiology, 7: 6.

Munkvold, G. P. and Desjardins, A. E. 1997. Fumonisins in maize, can we reduce their occurrence? Plant Diseases, 81: 556-565.

Nei, M. 1973. Analysis of the genetic diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA, 70: 3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 76: 379.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceeding of the national academy of science USA, 76: 5269-5273.

Nelson, P. E., Toussoun, T. A. and Cook, R. J. 1981. *Fusarium*, diseases, biology, taxonomy. The Pennsylvania State University Press. University Park, PA, USA.

Nelson, P. E., Toussoun, T. A. and Marasas, W. 1983. *Fusarium* species: An illustrated manual for identification. The Pennsylvania State University Press. University Park, PA, USA.

Nirenberg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Canadian Journal of Botany, 59: 1599-1609.

Nyvall, R. F. 1999. Field crop diseases. Iowa State University Press, USA.

Odonnell, K., Cigenlink, E. and Nirenberg, H. I. 2000. Molecular systematic and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia, 90: 467-493.

Ooka, J. J. and Kommedahl, T. 1977. Wind and rain dispersal of *Fusarium moniliforme* in corn fields. Phytopathology, 67: 1023-1026.

Peakall, R. and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28: 2537.

Ren, X., Zhu, Z. D., Li, H. J., Duan, C. X. and Wang, X. M. 2012. SSR marker development and analysis of genetic diversity of *Fusarium verticillioides* isolated from maize in China. Scientia Agriculture Sinica, 45: 52-66.

Roldan-Ruiz, I., Dendauw, J. E., Van Bockstaele, E., Depicker, A. and Loose, M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding, 6: 125-126.

Rolhf, F. J. 1990. NTSYSPc, Numerical taxonomy and multivariant analysis system. Version 2.02. Applied Biostatistics. New York.

Saharan, M. S. and Naef, A. 2008. Detection of genetic variation among Indian wheat head scab pathogens (*Fusarium* spp. isolates) with microsatellite markers. Crop Protection, 27: 1148-1154.

Shah, D., Bergstrom, G. C. and Ueng, P. P. 1995. Initiation of *Septoria nodorum* blotch epidemics in winter wheat by seed born *Stagonospora nodorum*. Phytopathology, 85: 452-457.

Slippers, B., Burgess, T., Wingfield, B. D., Crous, P. W., Coutinho, T. A. and Wingfield, M. J. 2004. Development of simple sequence repeat markers for *Botryosphaeria* spp. with *Fusicoccum* anamorphs. Molecular Ecology Notes, 4: 675-677.

Sneath, P. H. A. and Sokal, P. R. 1973. Numerical taxonomy. San Fransisco: W. H. Freeman and Company.

Taherkhani, K., Alizadeh, A., Farokhinejad, R. and Sharifitehrani, A. 1998. Identification of causal agents of sugarcane *Fusarium* diseases in Khuzestan Province. In: Proceedings 13th Iranian Plant Protection Congress, 23-27 Aug., Karaj, Iran, P. 120.

Tenzer, I., Ivanissevich, S. D., Morgante, M. and Gessler, C. 1999. Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. Phytopathology, 89: 748-753.

Varshney, R. K., Chabane, K., Hendre, P. S., Aggarwal, R. K. and Graner, A. 2007. Comparative assessment of EST-SSR.; EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild cultivated and elite barleys. Plant Science, 173: 638-649.

Weising, K., Kaemmer, D., Epplen, J. T., Weigand, F., Saxena, M. and Kahl, G. 1991. DNA fingerprinting of *Ascochyta rabiei*

[DOR: 20.1001.1.22519041.2020.9.1.5.2]

with synthetic oligodeoxynucleotides. Current Genetics, 19: 483-489.

White, D. G. 1999. Compendium of Corn Diseases. 3rd Ed. The American Psychopathological Association, 78 pp.

Wostemeyer, J. and Kreibich, A. 2002. Repetitive DNA elements in fungi (Mycota): impact on genomic architecture and evolution. Current Genetics, 41: 189-198.

Xu, J. R. and Leslie, J. F. 1996. A genetic map of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*). Genetics, 143: 175-189.

Yeh, F. C., Yang, R. C. and Boyle, T. 1999. Microsoft Window-based freeware for population genetic analysis (POPGENE, ver. 1.31), ftp://ftp.microsoft.com /softlib/ mslfiles/hpgl.exe.

[DOR: 20.1001.1.22519041.2020.9.1.5.2]

ساختار ژنتیکی جمعیت‌های *Fusarium verticillioides* عامل پوسیدگی ریشه و طوقه ذرت در استان ایلام با استفاده از نشانگرهای ریزماهواره

احمد صیدی‌نژاد و خشنود نوراللهی*

گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه ایلام، ایلام، ایران.
پست الکترونیکی نویسندگان مسئول مکاتبه: k.nourollahi@ilam.ac.ir
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چکیده: بررسی تنوع ژنتیکی جمعیت‌های قارچ *Fusarium verticillioides* سطوح مختلفی از اطلاعات در مورد مدیریت بیماری پوسیدگی ریشه و طوقه در مزارع ذرت را فراهم می‌کند. از نشانگرهای SSR برای تعیین ساختار ژنتیکی و تخمین تنوع ژنتیکی در ۷۷ جدایه *F. verticillioides* از مناطق کشت عمده ذرت در استان ایلام از جمله: دهلران، موسیان، دشت عباس، مهران، ایوان، هلیلان و دره‌شهر در سال زراعی ۱۳۹۵-۱۳۹۴ استفاده شد. با به‌کارگیری نه جفت پرایمر SSR، میانگین تعداد آلل‌ها در جمعیت‌ها ۳۴ آلل بود که فراوانی آلل‌ها در جمعیت دهلران و موسیان با ۲۷ آلل، کم‌ترین و در جمعیت دره‌شهر با ۴۰ آلل، بیش‌ترین میزان فراوانی را داشتند. تعداد آلل‌های مشاهده شده (N_a) و تعداد آلل-های مؤثر (N_e) در مهران (N_a = ۱/۸۶۰, N_e = ۱/۴۶۳) نسبت به دیگر جمعیت‌ها بیش‌تر بود. بیش‌ترین تنوع ژنتیکی (H_e) در هلیلان (H_e = ۰/۲۸۴) و بیش‌ترین شاخص شانون (I) در مهران (I = ۰/۴۳۶) بود اما کم‌ترین میزان برای موسیان (H_e = ۰/۱۹۵, I = ۰/۳۰۳) برآورد شد. کم‌ترین فاصله ژنتیکی بین دهلران و موسیان (۰/۰۱۳) بود درحالی‌که بیش‌ترین فاصله ژنتیکی بین دهلران و دره‌شهر (۰/۱۳۹) وجود داشت. تنوع ژنتیکی کل (H_t) و تنوع ژنی بین زیر جمعیت‌ها (H_s) به‌ترتیب ۰/۲۹۲ و ۰/۲۴۹ تخمین زده شد. میزان تنوع ژنتیکی مؤثر در تمایز بین جمعیت‌ها (G_{st}) ۰/۱۴۷ و میزان جریان ژنی (N_m) ۲/۸۹۰ بود. تجزیه کلاستر براساس UPGMA نشان داد که کم‌ترین فاصله ژنتیکی بین دهلران، موسیان و سپس دشت‌عباس وجود دارد. این دندروگرام بیش‌ترین فاصله ژنتیکی را بین دره‌شهر و شش جمعیت دیگر نشان می‌دهد. نتایج حاصل از این مطالعه در راستای تولید ارقام مقاوم به بیماری پوسیدگی ریشه و طوقه و همچنین توسعه روش‌های کنترل بیماری می‌تواند مورد استفاده قرار گیرد.

واژگان کلیدی: ذرت، فاصله ژنتیکی، پوسیدگی ریشه، SSR

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