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Genetic diversity and population structure of Iranian isolates of *Fusarium oxysporum* f. sp. *ciceris*, the causal agent of chickpea wilt, using ISSR and DAMD-PCR markers

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Abstract

Fusarium oxysporum f. sp. *ciceris* (FOC), is one of the most important soil-borne diseases of chickpea in the world. In order to study the genetic diversity of Iranian FOC isolates, sixty five isolates of the pathogen were isolated from wilted chickpea plants from different chickpea growing areas of West of Iran. Phylogenetic analysis using ISSR and DAMD-PCR markers grouped FOC isolates into six and five distinct groups, respectively. Both ISSR and DAMD-PCR markers showed a high level of polymorphism and were found to be effective in determining genetic diversity in FOC isolates. The genetic structure of 65 FOC isolates showed the highest peak at $K = 5$ indicating the that the collected FOC isolates should be divided into five populations. The results of FOC grouping by ISSR and DAMD-PCR markers showed relatively low correlation with geographic origins. Overall, our results showed a high genetic diversity level in Iranian FOC isolates, which might be mediated by gene mutation or chromosomal segment loss and may suggest a longer evolutionary period for FOC isolates from the chickpea growing area in the west of Iran. The results of the presented study will be useful to chickpea breeders for effective selection of durable resistance sources.

Key words: chickpea, DAMD-PCR, *Fusarium oxysporum* f. sp. *ciceris*, genetic diversity, ISSR.
Abbreviations: DAMD, direct amplified of minisatellite DNA; FOC, *Fusarium oxysporum* f. sp. *ciceris*; ISSR, inter-simple sequence repeat.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops as a cheap protein source in many countries in Asia and Africa (Jannatabadi et al. 2014; Hajibarat et al. 2015). The countries located in the Middle-East such as India, Turkey, Pakistan and Iran, are the major chickpea producers in Asia (Mohammadi, Talebi 2015). In Iran, chickpea production is low, which may be attributed to several abiotic and biotic stresses such as drought, cold and fungal diseases (Patil et al. 2005; Jendoubi et al. 2017). Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceris* (FOC), is one of the major fungal diseases in chickpea production worldwide (Jalali, Chand 1992; Cachinero et al. 2002). This disease has been reported in most chickpea production areas in the world, however, the chickpea producing countries that are more threatened by this disease are Iran, India, Pakistan, Spain and Tunisia (Chand, Khirbad 2009). Depending upon the environmental conditions, this disease can be devastating to chickpea yield 10 to 15% each year in regular conditions, and can reach up to 100% in some improved chickpea cultivars in unfavorable agroclimatic conditions (Mohamed et al. 2015).

FOC symptoms can develop at any stage of plant growth with higher incidence during the flowering and pod filling stages (Jiménez-Díaz et al. 2015; Jendoubi et al. 2017). This pathogen is soil and seed-born and in the absence of the chickpea host, it can survive on plant debris in soil for more than six years (Haware et al. 1978). Based on disease symptoms, pathotypes can be divided into two groups as distinct yellow or wilting syndromes (Jiménez-Gasco et al. 2004; Jiménez-Díaz et al. 2015). The yellowing pathotype causes foliar yellowing, vascular discoloration and late plant death, while the wilting pathotype induces very fast chlorosis, vascular discoloration and early plant death (Jiménez-Fernández et al. 2013; Jendoubi et al. 2017). Based on disease severity and virulence of FOC isolated on a set of chickpea differential lines, eight distinct races of FOC (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been identified (Jiménez-Gasco et al. 2004; Sharma, Muehlbauer 2007).

Considering the nature and survival ability of FOC fungi, management of wilt disease through fungicide and agronomical practices are difficult and are not environment friendly methods. Thus, the most effective methods for management of chickpea wilt are the use of resistance sources in breeding programs (Bakhsh et al. 2007). However, evolution of new races poses a serious threat to deployment of wilt resistance in chickpea. Analysis of plant

pathogen populations is useful for improving cultivars that are resistant to the pathogen populations (Aghamiri et al. 2015). This information helps breeders to develop new strategies to achieve durable disease resistance (Talebi et al. 2010).

In the recent years, molecular genetic markers have been widely used for assessing the genetic variation and origin identification in many species (Hajibarat et al. 2015). FOC, the causal agent of chickpea wilt has a high diversity in nature and assessment of variability in the population of FOC has been made by several workers at morphological, pathogenic and molecular levels. Genetic diversity in *F. oxysporium* causing diseases as well as in FOC isolates has been explored using different types of molecular markers such as RAPD (Sivaramakrishnan et al. 2002; Mohamed et al. 2015); SCAR (Jimenez-Gasco, Jimenez-Dıaz 2003); SSR (Mohamed et al. 2015); AFLP (Jimenez-Gasco et al. 2001; Madhuri, Mane 2012); ITS-RFLP (Gurjar et al. 2009); and BOX-and rep-PCR (Muiru et al. 2010; Kashyap et al. 2016). The aim of this study was to investigate the genetic variability and population structure analysis of 65 FOC isolates originated from north-west Iran (Kurdistan and Kermanshah provinces) using inter-simple sequence repeat (ISSR) and direct amplified of minisatellite DNA (DAMD) molecular markers.

Materials and methods

Isolation of Fusarium oxysporum *f. sp.* ciceri *and DNA extraction*

Sixty five isolates of *Fusarium oxysporum* f. sp. *ciceri* were collected from eight chickpea cultivation zones from two provinces (Kordestan and Kermanshah) located in north-west Iran (Table 1). All FOC isolated were obtained from the infected stems of chickpea plants showing vascular discoloration symptoms. Infected plant parts were washed and cut into small pieces. All samples surface disinfected by 1% hypochlorite sodium for 2 min and then washed twice with sterilized distilled water and plated on potato dextrose agar. Plates were incubated at room temperature (25 °C) for six days with a 12 h photoperiod (Mohamed et al. 2015). FOC cultures were purified from single-spore culture and incubated on potato dextrose broth (potato 200 g, dextrose 20 g, agar 18 g and 1 L water) for eight days. Fungal mycelium was harvested from cultures and used for DNA extraction using CTAB method as described by Kumar et al. (2013). DNA concentrations were estimated by both spectrophotometry (260/280) and gel electrophoresis (0.8% agarose gel) and used for PCR analysis in final concentration of 30 ng μL^{-1}.

Pathogenicity test

Pathogenicity tests of FOC isolates were carried out in controlled greenhouse conditions using a root dip inoculation method (Pande et al. 2007). Isolated FOC were grown in potato dextrose broth media and incubated for seven days at room temperature. The inoculum suspensions were filtered and adjusted to 5×10^6 conidia mL^{-1} concentration. Seedlings of the wilt-susceptible chickpea cv. Bivanij were grown in perlite for 14 days. Seedlings were carefully uprooted and roots of seedlings were dipped in inoculum of each isolate for 2 to 3 min. Root-inoculated plants were transferred to sterile soil plus sand and pots kept in controlled greenhouse at 24 °C. Five pots with three plants in each pot were used for each isolate. Disease symptoms were observed 20 to 25 days after inoculation. Disease severity of each isolates was evaluated at 28 days after inoculation based on percentage of plants death for each isolates and graded as avirulent (0% wilt), less virulent (1 – 20% wilt), moderately virulent (21 – 50% wilt) and highly virulent (> 51% wilt) (Kashyap et al. 2016).

Table 1. Description of the isolates of *Fusarium oxysporum* f. sp. *ciceris*, collection site, morphological features and pathogenicity test on cv. Bivanij

No.	Isolate	Origin (province/collection site)	Colony color	Clamydospore position	Disease severity (% of wilt incidence)
1	Foc1	Kermanshah/Sarab-Nilofar	Yellowish	Intercalary	35.1
2	Foc2	Kermanshah/Sarab-Nilofar	White	Intercalary	91.2
3	Foc3	Kermanshah/Sarab-Nilofar	Yellowish	Intercalary	92
4	Foc4	Kermanshah/Sarab-Nilofar	Yellowish	Both	39.8
5	Foc5	Kermanshah/Sarab-Nilofar	Pinkish white	Intercalary	42.8
6	Foc6	Kermanshah/Sarab-Nilofar	White	Absent	44.6
7	Foc7	Kermanshah/Sarab-Nilofar	Pinkish white	Intercalary	38.5
8	Foc8	Kurdistan/Kamyaran	Yellowish	Intercalary	34.2
9	Foc9	Kurdistan/Kamyaran	Yellowish	Intercalary	12.7
10	Foc10	Kurdistan/Kamyaran	Yellowish	Both	26.4
11	Foc11	Kurdistan/Kamyaran	Pinkish white	Absent	29.4
12	Foc12	Kurdistan/Kamyaran	White	Intercalary	21.7
13	Foc13	Kurdistan/Kamyaran	Pinkish white	Intercalary	24.2

continued

Table 1. *continued*

No.	Isolate	Origin (province/collection site)	Colony color	Clamydospore position	Disease severity (% of wilt incidence)
14	Foc14	Kurdistan/Kamyaran	Yellowish	Intercalary	16.7
15	Foc15	Kurdistan/Kamyaran	Yellowish	Intercalary	27.2
16	Foc16	Kurdistan/Kamyaran	White	Absent	29.5
17	Foc17	Kermanshah/Sararood	Yellowish	Intercalary	44.7
18	Foc18	Kermanshah/Sararood	Yellowish	Intercalary	91.7
19	Foc19	Kermanshah/Sararood	Pinkish white	Intercalary	42.8
20	Foc20	Kermanshah/Sararood	Pinkish white	Intercalary	90.7
21	Foc21	Kermanshah/Sararood	Yellowish	Both	46.5
22	Foc22	Kermanshah/Sararood	White	Absent	44.5
23	Foc23	Kermanshah/Sararood	Pinkish white	Intercalary	42.8
24	Foc24	Kermanshah/Sararood	Yellowish	Intercalary	46.1
25	Foc25	Kermanshah/Sararood	Pinkish white	Intercalary	45.9
26	Foc26	Kermanshah/Sararood	White	Intercalary	42.8
27	Foc27	Kermanshah/Dorood Faraman	Yellowish	Absent	49.7
28	Foc28	Kermanshah/Dorood Faraman	Yellowish	Intercalary	46.5
29	Foc29	Kermanshah/Dorood Faraman	White	Intercalary	49.6
30	Foc30	Kermanshah/Dorood Faraman	Yellowish	Both	48.2
31	Foc31	Kermanshah/Dorood Faraman	White	Intercalary	49.4
32	Foc32	Kermanshah/Dorood Faraman	Pinkish white	Intercalary	47.5
33	Foc33	Kermanshah/Dorood Faraman	Pinkish white	Intercalary	91.5
34	Foc34	Kurdistan/Kani-Charmoo	Yellowish	Intercalary	18.2
35	Foc35	Kurdistan/Kani-Charmoo	White	Intercalary	25.8
36	Foc36	Kurdistan/Kani-Charmoo	Pinkish white	Intercalary	19.1
37	Foc37	Kurdistan/Kani-Charmoo	Yellowish	Both	29.6
38	Foc38	Kurdistan/Kani-Charmoo	Yellowish	Intercalary	31.5
39	Foc39	Kurdistan/Kani-Charmoo	Pinkish white	Absent	30.8
40	Foc40	Kurdistan/Kani-Charmoo	Pinkish white	Intercalary	34.2
41	Foc41	Kurdistan/Kani-Charmoo	Yellowish	Absent	31.2
42	Foc42	Kurdistan/Kani-Charmoo	Yellowish	Intercalary	28.8
43	Foc43	Kurdistan/Kani-Charmoo	Yellowish	Both	29.6
44	Foc44	Kurdistan/Kani-Charmoo	Yellowish	Absent	29.1
45	Foc45	Kurdistan/Kani-Charmoo	White	Intercalary	30.4
46	Foc46	Kermanshah/Gomateh	Pinkish white	Intercalary	44.2
47	Foc47	Kermanshah/Gomateh	yellowish	Intercalary	46.2
48	Foc48	Kermanshah/Gomateh	Pinkish white	Intercalary	39.1
49	Foc49	Kermanshah/Gomateh	Yellowish	Absent	35.8
50	Foc50	Kermanshah/Gomateh	white	Intercalary	45.2
51	Foc51	Kermanshah/Gomateh	Yellowish	Intercalary	44
52	Foc52	Kermanshah/Gomateh	Yellowish	Intercalary	46
53	Foc53	Kermanshah/Korani	Pinkish white	Intercalary	37.1
54	Foc54	Kermanshah/Korani	Yellowish	Both	35.5
55	Foc55	Kermanshah/Korani	White	Absent	33.4
56	Foc56	Kermanshah/Korani	Yellowish	Intercalary	39.1
57	Foc57	Kermanshah/Korani	Yellowish	Intercalary	32
58	Foc58	Kermanshah/Korani	Pinkish white	Absent	42
59	Foc59	Kermanshah/Korani	White	Intercalary	41
60	Foc60	Kermanshah/Korani	Yellowish	Absent	42.1
61	Foc61	Kermanshah/Mahidasht	Pinkish white	Intercalary	46.8
62	Foc62	Kermanshah/Mahidasht	Yellowish	Intercalary	49.1
63	Foc63	Kermanshah/Mahidasht	white	Absent	45.6
64	Foc64	Kermanshah/Mahidasht	Pinkish white	Intercalary	47.6
65	Foc65	Kermanshah/Mahidasht	Pinkish white	Both	43.8

Table 2. Description of ISSR and DAMD-PCR markers used for genetic diversity analysis in *Fusarium oxysporum* f. sp. *ciceris* isolates

Type	Marker	Sequence (5/ to 3/)	GC (%)	Tm (°C)
ISSR	UBC807	AGAGAGAGAGAGAGAGT	47	50
	UBC812	GAGAGAGAGAGAGAGAA	47	45
	UBC815	CTCTCTCTCTCTCTCTG	52	44
	UBC816	CACACACACACACACAT	47	48
	UBC818	CACACACACACACACAG	52	52
	UBC828	TGTGTGTGTGTGTGTGA	47	52
	UBC864	ATGATGATGATGATGATG	33	52
	UBC868	GAAGAAGAAGAAGAAGAA	33	48
	UBC878	GGATGGATGGATGGAT	50	54
	UBC880	GGAGA GAGAGGAGA	60	50
DAMD-PCR	URP1F	ATCCAAGGTCCGAGACAACC	55	50
	URP2F	GTGTGCGATCAGTTGCTGGG	60	50
	URP6R	GGCAAGCTGGTGGGAGGTAC	65	50
	URP4R	AGGACTCGATAACAGGCTCC	55	50
	URP9F	ATGTGTGCGATCAGTTGCTG	50	50
	URP13R	TACATCGCAAGTGACACAGG	50	50
	URP17R	AATGTGGGCAAGCTGGTGGT	55	50
	URP25F	GATGTGTTCTTGGAGCCTGT	50	50
	URP30F	GGACAAGAAGAGGATGTGGA	50	50
	URP38F	AAGAGGCATTCTACCACCAC	50	50

Genetic diversity analysis by ISSR and DAMD-PCR molecular markers

For ISSR analysis, a set of 10 primers (UBC set, University of British Columbia, Canada) were used to assessing genetic diversity among FOC isolates (Table 2). Ten DAMD-PCR markers (originally derived from the repeat elements of weed rice) (Kang et al. 2002) were used in this study (Table 2). PCR amplification was performed in 20 µL reaction containing 1× PCR buffer, 30 ng sample DNA, 2.5 µM primer, 200 µM of each dNTP, 1.5 – 2.5 mM MgCl2 and 1.5 units of Taq DNA polymerase (Cinnagene, Iran). All amplification were carried out in a Eppendorf thermocyclers as follows: 94 °C for 3 min, followed by 35 cycles of denaturation at 93 °C for 45 s, annealing at optimum Ta for 45 s, and extension at 72 °C for 90 s. A final extension cycle at 72 °C for 10 min followed. PCR products were separated on 1.4% agarose gels, stained with ethidium bromide and scored for the presence or absence of bands.

Data analysis

PCR products of ISSR and URP (Universal Rice Primer) primers were scored visually. For each marker, bands were scored visually for the presence (1) and absence (0) of bands for all the 65 FOC isolates. Cluster analysis using un-weighted pair-group method with arithmetic averages (UPGMA) using the similarity matrixes were performed by DARwin program package (Perrier and Jacquemoud-Collet, 2006). For both ISSR and URP-based clusters the fit of dendrograms obtained were checked by bootstrapping using 1000 replications, and only bootstrap values higher than 50% are presented. Polymorphic information content (PIC) values were calculated for each ISSR and URP primers according to the formula: $PIC = 1 - \Sigma(Pij)^2$, where Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers. Marker index (MI) was obtained by multiplying the average PIC with the Effective multiplex ratio. Effective multiplex ratio (EMR) is the product of the number of polymorphic loci per primer (n) and the fraction of polymorphic fragments (Perrier et al. 2003). For the analysis of population structure, a Bayesian model-based analysis was performed using STRUCTURE 2.1 software (Pritchard et al. 2000). A Monte Carlo Markov chain method was used to estimate allele frequencies in each of the K populations and the degree of admixture for each individual plant. The number of clusters was inferred using 10 independent simultaneous runs with 10000 replications using the admixture model and correlated allele frequencies with the K value ranging from 1 to 10.

Results

Pathogenicity test of the FOC isolates

Disease severity and vascular discoloration caused by FOC isolates on cv. Bivanij showed variability between isolates. In general, among all the isolates, FOC isolates collected from the Kermanshah province showed more aggressiveness and caused the maximum wilt incidence (>80%) on the susceptible cv. Bivanij. Isolates Foc2, Foc3, Foc18, Foc20 and Foc33 showed maximum wilt incidence (~92%) and isolates from Kurdistan showed less aggressiveness. Isolates Foc9, Foc14, Foc34, Foc36 showed least wilt incidence

(>20%) in comparison to other FOC isolates. The other isolates differed in their virulence behavior and showed moderately virulent (21 – 50% wilt damage).

R and DAMD-PCR diversity pattern

ISSR and DAMD-PCR analysis revealed clear and scorable bands per primer. Ten ISSR markers amplified 155 bands, of which 87 bands (56.1%) were polymorphic (Table 3) with an average of 8.7 polymorphic bands per primer. The maximum and minimum number of polymorphic bands were obtained using UBC816 (12 bands) and UBC880 (6 bands), respectively. The PIC values for the 10 ISSR primers ranged from 0.22 (UBC880) to 0.43 (UBC816) with an average of 0.36 per primer. The marker index (MI) of the primers ranged from 0.99 (UBC880) to 4.11 (UBC816) (Table 3). Ten DAMD-PCR markers produced 88 bands across 65 FOC isolates, of which 55 were polymorphic (62.5%) with an average of 5.5 polymorphic bands per primer (Table 3). Most of the DAMD-PCR markers generated six polymorphic bands and the least number of polymorphic bands was observed for URP9F (4 bands). The PIC values for the DMAD-PCR primers ranged from 0.19 (URP9F) to 0.32 (URP2F) with an average of 0.25 per primer. The marker index (MI) of the primers ranged from 0.69 (URP9F) to 2.59 (URP2F) (Table 3). Un-weighted pair-group (UPGMA) clustering using ISSR and DAMD-PCR molecular dataset grouped the FOC isolates in six and five distinct groups, respectively (Fig. 1 and Fig. 2). Similarity between genotype clustering in ISSR and DAMD analyses based on the Mantel coefficient test showed relatively high, but non-significant correlation ($r = 0.41$).

Table 3. Description of the polymorphism, PIC and marker index (MI) values of ISSR and DAMD-PCR markers used in the genetic diversity analysis of FOC isolates

Primer	Number of amplified bands	Number of polymorphic bands	PIC	MI
ISSR	14	10	0.41	3.87
UBC807	12	9	0.36	2.39
UBC812	16	11	0.42	3.98
UBC815	16	10	0.40	3.21
UBC816	18	12	0.43	4.11
UBC818	14	8	0.34	2.19
UBC828	19	12	0.39	2.77
UBC864	14	10	0.38	3.01
UBC868	16	10	0.35	2.67
UBC878	17	11	0.34	2.46
UBC880	13	6	0.22	0.99
DAMD-PCR	10	7	0.35	2.78
URP1F	9	6	0.31	2.54
URP2F	12	6	0.32	2.59
URP6R	9	5	0.26	1.28
URP4R	10	6	0.29	1.41
URP9F	9	4	0.19	0.69
URP13R	10	5	0.24	0.89
URP17R	11	6	0.23	0.82
URP25F	9	6	0.25	0.91
URP30F	9	5	0.24	0.84
URP38F	10	6	0.21	0.77

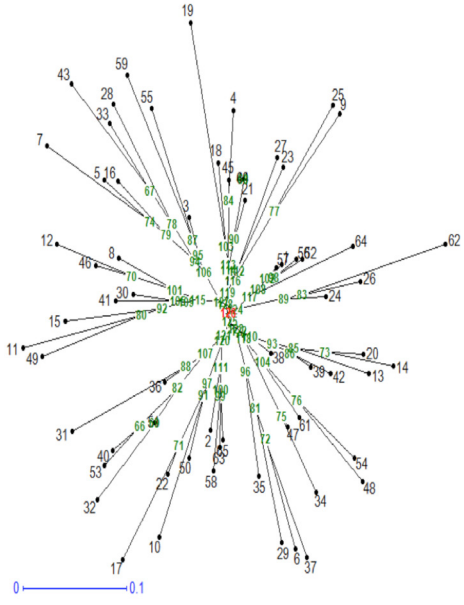

Fig. 1. Cluster analysis of 65 of *Fusarium oxysporum* f. sp. *ciceris* isolates obtained by ISSR markers.

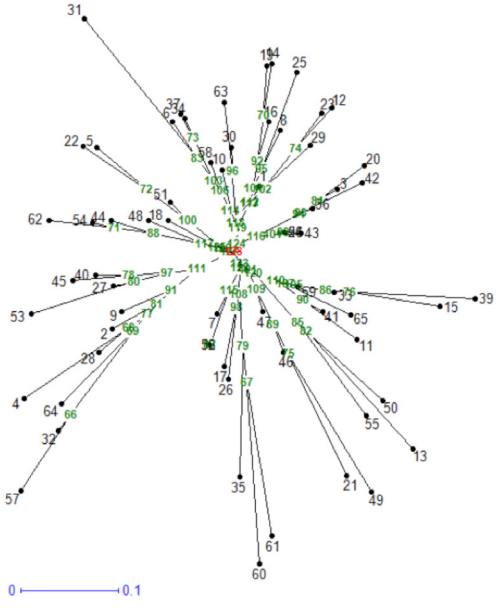

Fig. 2. Cluster analysis of 65 of *Fusarium oxysporum* f. sp. *ciceris* isolates obtained by DAMD-PCR markers.

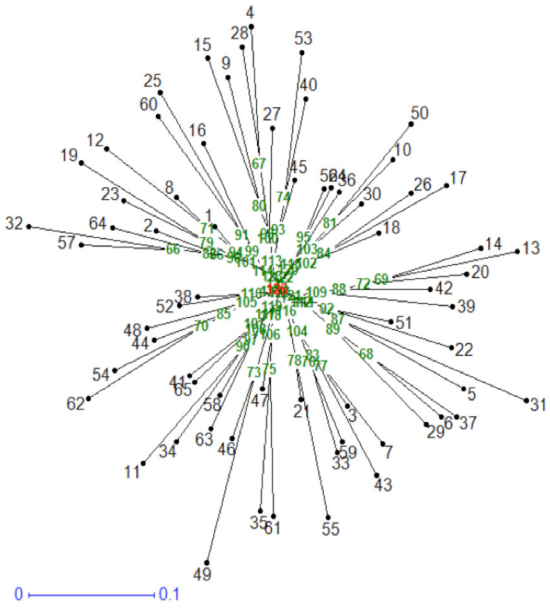

Fig. 3. Cluster analysis of 65 of *Fusarium oxysporum* f. sp. *ciceris* isolates obtained by combined data (ISSR + DAMD-PCR markers).

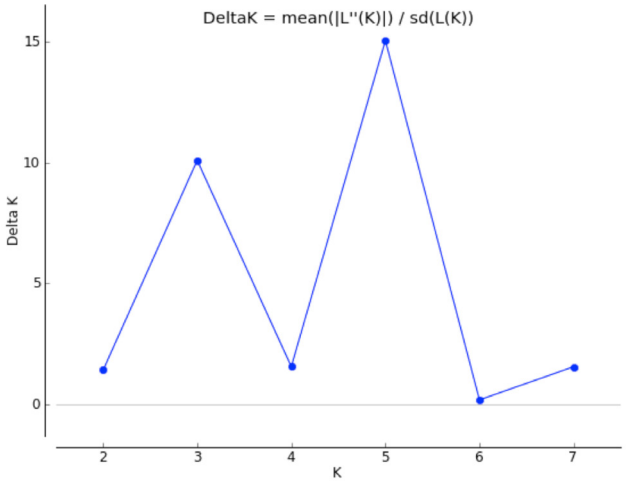

Fig. 4. Determination of the optimal value of K, based on three independent runs and K ranging from 1 to 10 based on 65 of *Fusarium oxysporum* f. sp. *ciceris* isolates using ISSR + DAMD-PCR markers.

Diversity and population structure of FOC isolates by combined data

The general dendrogram (Fig. 3) that was constructed using the combined data of all molecular markers used in this study (ISSR and DAMD-PCR) grouped the FOC isolates into five major clusters. Cluster I comprised 12 FOC isolates, mostly from the Kermanshah province, except two isolates from Kordestan. Cluster II and II contained 8 and 9 FOC isolates, respectively, which most of them collected from the Kermanshah province. Cluster IV and V comprised 30 and 6 FOC isolates. All isolated FOC in cluster V originated from the Kermanshah province. The general dendrogram was generally consistent with the dendrograms generated by ISSR and DAMD-PCR markers. The cophenetic coefficient for different molecular marker types (0.76 for ISSR and 0.72 for DAMD-PCR) indicated good fit for clustering. The genetic structure of 65 FOC isolates was further explored using the Bayesian clustering model implemented in the STRUCTURE software. The results showed the highest peak at $K = 5$ indicating the presence of five major clusters (Fig. 4). These results mean that the collected FOC isolates should be divided into five populations (Fig. 5).

Discussion

The study of genetic diversity within in any pathogenic fungal species is critical for disease management and mainly attributed to important factors such as sexual habitat, gene flow and migration within populations and selection pressure (McDonald 1997; Aghamiri et al. 2015). *Fusarium oxysporum* f. sp. *ciceris* (FOC), a causal agent of chickpea wilt, is highly variable in morphology, virulence ability that consists of different races and pathotypes (Jendoubi et al. 2017). Thus, study of the natural genetic diversity pattern is very fundamental for disease management. The present study showed the genetic diversity level in 65 FOC isolates collected from west of Iran using two comparative

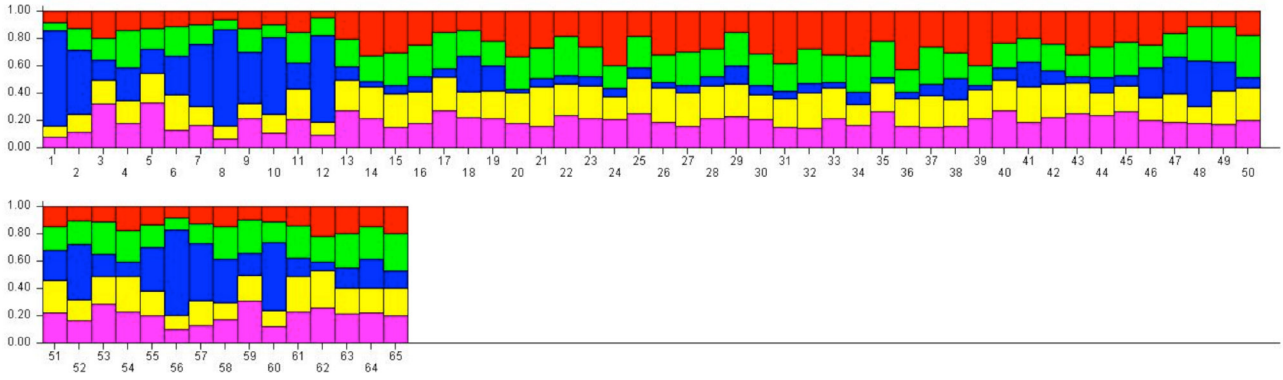

Fig. 5. Population structure of 65 of *Fusarium oxysporum* f. sp. *ciceris* isolates based on ISSR and DAMD-PCR markers.

molecular markers techniques, ISSR and DAMD-PCR. In our study, two different types of markers showed high level of polymorphism and were found to be effective in determining genetic diversity in FOC isolates. Their efficiency was evident from the high number of polymorphic bands, PIC and the marker index. A high level of diversity in FOC isolates using SSR (Mohamed et al. 2015; Kashyap et al. 2016), RAPD (Sivaramakrishnan et al. 2002), ERIC and BOX (Kashyap et al. 2016) and AFLP (Sivaramakrishnan et al. 2002) markers had been reported earlier. In our study, two different markers showedthe relatively same efficiency with regard to average polymorphism and PIC value. Although both ISSRs and DAMD-PCR markers showed relatively similar grouping of FOC isolates, ISSR efficiency was evident from high number of polymorphic bands, PIC and the marker index. To our knowledge, the present study is the first analysis of the genetic diversity of FOC isolates using DAMD-PCR markers.

The results of the present study showed that FOC isolates from Iran varied in morphology, virulence and genetic structure. Cultural and morphological characteristics such as colony color, clamydospore position and diseases severity have been used to discriminate or characterize the isolates of FOC (Kashyap et al. 2016). FOC isolates showed different categories on the basis of colony color, clamydospore position and virulence severity. However, there was no strong correlation noticed between morphology, virulence and geographical origin of the isolates.

According to isolate virulence, FOC isolates were graded in four groups as avirulent (0% wilt), less virulent (1 – 20% wilt), moderately virulent (21 – 50% wilt) and highly virulent (> 51% wilt). The results showed that about 20% of isolates were highly virulent (> 50% wilt damage) and the remaining isolates were moderately or less virulent. Cluster analysis using ISSR and DAMD-PCR markers grouped the FOC isolates in five distinct clusters. The results obtained from the STRUCTURE analysis are in good agreement with those obtained from Un-weighted pair-group (UPGMA) clustering generated by ISSR and DAMD-PCR markers. It is also clear from the results that both molecular markers revealed highly genetic variation between FOC isolates from different geographical regions.

A high rate of genetic diversity occurred in FOC isolates, which might be mediated by gene mutation, chromosomal segment loss and transposable elements or recombination processes in fungal genome (Mehrabi et al. 20111; Soren et al. 2016). Kashyap et al (2016) showed that the genetic similarity among the Indian FOC isolates was not correlated with virulence, race compositions or geographic origin of the isolates. These finding are in agreements with previous studies FOC isolates from India and Sudan (Soren et al. 2016; Mohamed et al. 2015). the pathogenicity test for race identification of FOC isolates was not used in this study. Therefore, complementary pathogenicity studies and specific molecular markers analysis for characterization of FOC races are very useful and may be helpful to reveal correlation between FOC virulence and geographical origin (Mohamed et al. 2015).

The high genetic variability observed between FOC isolates is very important to pathogens to adapt to environmental changes such as overcoming a new resistance gene in a crop or sensitivity towards crop protection agents (Mehrabi et al. 2011). Molecular diagnostic tools and the availability of many new whole genome sequences of fungi will enable to answer this question. The results form cluster analysis generated by ISSR and DAMD-PCR markers indicated that the genetic similarity among the Iranian FOC isolates could be partly explained by geographic origin. It can be inferred from our results that chickpea FOC isolates have a heterothallic origin. Previous studies on genetic diversity in FOC are in agreement with the hypothesis that Fusarium spp. fungi exhibit a sporadic and cryptic sexual cycle (Dubey et al. 2010) and it also suggested that all fungi with no known sexual stage are originally heterothallic, and most of them should display a sexual reproduction (Turgeon 1998; Taylor et al. 1999; Kashyap et al. 2016). In conclusion, the genetic variation identified in this study can provide useful information for disease management and identification of effective resistance sources to FOC in chickpea wilt resistance breeding programs.

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