Genetic Diversity of *Fusarium proliferatum* Populations from Maize, Onion, Rice and Sugarcane in Iran Based on Vegetative Compatibility Grouping

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Fusarium proliferatum is the causal agent of stalk and root rot disease of maize, foot rot disease of rice, basal and root rot disease of onion and knife cut disease of sugarcane in Iran. In recent years, incidence and severity of these diseases have been increased in Iran. Fifty seven *F. proliferatum* single-spore isolates collected from diseased maize, rice, onion and sugarcane plants at different areas were used to study genetic diversity by determination of vegetative compatibility groups (VCGs). Chlorate-resistant nitrate non-utilizing (*nit*) mutants were recovered from selected isolates of *F. proliferatum* and used in complementation tests. All isolates in which both *nit1* and *NitM* (or *nit3*) mutants were recovered, demonstrated self-compatibility. Vegetative compatibility tests by pairing *nit* mutants identified 30 VCGs among 57 isolates. Twenty-three isolates belonged to single-member VCGs and the remaining 34 isolates, belonged to other seven multimember VCGs. Segregation of *F. proliferatum* isolates obtained from various area and host plants into different VCGs in Iran is reported for the first time. In this study, none of isolates obtained from rice complemented with any other isolates from onion and sugarcane and, non complementation occurred between onion and sugarcane isolates. Also, only one complementation occurred between one isolate of maize and one isolate of sugarcane and rice. Thus, a correlation between VCGs grouping and host preferences was founded. It is concluded that natural populations of *F. proliferatum* in Iran are probably genetically divergent and include isolates representing a potential risk for disease development.

Keywords : *Fusarium proliferatum*, genetic diversity, *Nit* mutant, vegetative compatibility grouping (VCG)

Fusarium proliferatum (Matsushima) Nirenberg [*Gibberella fujikuroi* (Kuhlman) Samuels, Nirenberg & Seifert, mating population D = *Gibberella intermedia*], is a prevalent pathogen of agriculturally important crops with wide host range. This pathogen can parasitize cultivated plants, and/or produce mycotoxins that pose serious hazards to human and animal health (Leslie & Summerell, 2006).

F. proliferatum is the pathogen of some economically importance plants including rice, maize, citrus fruit, banana, orchids, sorghum, asparagus, pine, palm (Leslie & Summerell, 2006), onion (Galvan et al., 2008) and sugarcane (Taherkhani, 1995). Particularly in Iran, it has been known as the causal agent of stalk and root rot of maize (Bujari et al., 1993, Ghiasian et al., 2004), foot rot disease of rice (Abbaszadeh et al., 2007), basal and root rot disease of onion (Rabiee motlagh, 2009) and knife cut disease of sugarcane (Taherkhani, 1995). More importantly, in recent years, the incidence and severity of these diseases have increased drastically in Iran. However, to the best of our knowledge, no comprehensive study on the Iranian *F. proliferatum* populations has been conducted so far.

Most recent assessments of fungal pathogens have used multilocus markers to detect populations (Milgroom, 1996). The ability of strains and/or species from geographically separated locations to recombine, poses the danger of introducing virulence and/ or toxigenic genes into local pathogen populations. One approach that has been used successfully in many fungi to examine population structure, clonality and the potential for gene flow is the study of vegetative incompatibility (Leslie, 1993). Fungal isolates that anastomose and form heterokaryons with each other are vegetatively compatible and are assigned to a single vegetative compatibility group (VCG) (Leslie, 1993). Members of the same VCG can form a stable heterokaryon, and share an identical set of alleles at about 10 *vic* loci (Leslie, 1993). Vegetative compatibility or anastomosis has been widely documented among several fungi and has proved to be a powerful tool in determining fungal genetic

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diversity (Correll et al., 1987, 1988; Nitzan et al., 2002; Puhalla, 1985).

F. proliferatum has not often been studied in depth from a population genetics perspective (Leslie & Summerell 2006). Understanding the genetic structure of a population reflects its evolutionary history and its potential to evolve. However, in order to devise effective strategies to control pathogen growth, and to reduce the impact of disease, it is necessary to build knowledge on the genetic structure of pathogen population to be able to assess the level of pathogen variability and obtain additional insight into their population structure (McDonald, 2004; McDonald and McDermott, 1993). Therefore, knowledge of the genetic structure of the *F. proliferatum* populations might be useful in order to establish effective strategies for controlling the disease (McDonald, 2004). To date, most population genetics studies of *Fusarium* species have been conducted using the vegetative compatibility group as a marker for genotyping fungal isolates (Chulze et al., 2000; Danielsen et al., 1998).

In the previous study, presence of both mating type idiomorphs with favorable frequency and high effective population number among isolates recovered from different hosts of *F. proliferatum*, shows that there is potential for sexual reproduction within these populations and thus, an expansion of the genetic diversity by recombination (Alizadeh et al., 2009). Also, diversity for VCGs was very high in *F. proliferatum* isolates from rice recovered from different regions of Mazandaran province in Iran, where 26 different VCGs were found among 29 isolates (Alian et al., 2005).

The hypotheses of the current study were that there is VCG diversity within *F. proliferatum* populations and genetic exchange through sexual recombination frequently occurs in *G. intermedia* populations. However, weather different isolates of the fungus that infecting different hosts in same or different areas can exchange genetic materials through the anastomosis or they are different populations with distinct features that have own biological behavior. According to the results of the study we can clarify some aspects of their biology.

Our objective was to use VCGs identification to determine the genetic diversity among *F. proliferatum* isolates originating from different hosts in Iran.

Materials and Methods

Isolates. Isolates of *Fusarium* spp. were recovered from the stalks and seeds of 80 maize fields in 15 locations of 11 provinces of Iran (Ardabil, Azarbaijan, Fars, Gorgan, Hamedan, Isfahan, Kerman, Kermanshah, Mazandaran, Qazvin and Tehran) during the 2004-2005 growing seasons.

Other isolates include; 23 isolates from commercial rice field in Guilan and Mazandaran provinces at north of Iran (approximately more than 75% of rice growing areas are located in north of Iran), 20 isolates from onion in Khorasan province and 20 isolates from sugarcane from commercial sugarcane field in Khuzestan province. These isolates were identified using the morphological characteristics described by Leslie and Summerell (2006) and Nelson et al. (1983). PCR assay using species-specific primers confirmed morphological identification of *G. intermedia* isolates of maize and onion (Rahjoo et al., 2008; Rabiee motlagh, 2009). Isolates were obtained through the single spore subcultures and then were stored in filter paper at -20°C . These pure cultures were used as the straining inoculums for all subsequent tests. Fifty-seven isolates including; 20 isolates from maize, 20 isolates from rice, 12 isolates from sugarcane and five isolates from onion were selected for VCG determination, which was based on complementation reaction between NitM (or *nit3*) and *nit1* mutants on minimal medium.

Media. In an initial test, chlorate-resistant mutants were generated on minimal agar medium with chlorate (MMC), Potato dextrose agar medium with chlorate (PDC), Czapek-Dox agar medium with chlorate and water agar medium amended by chlorate (WAC). Minimal medium (MM) was prepared by adding 2 g of NaNO_3 to 1 L basal medium (Correll et al., 1987). MMC was prepared by adding 1-6 g L-asparagine, 2 g NaNO_3 , and 30 g KClO_3 to 1 L of basal medium. PDC was prepared by adding 39 g PDA, and 15 g KClO_3 to 1 L distilled water. CZC was prepared by adding 39 g Czapek-Dox and 30 g KClO_3 to 1 L distilled water and WAC was prepared by adding 20 g agar and 30 g KClO_3 to 1 L distilled water. Subsequently chlorate-resistant mutants were generated on MMC containing 30 g KClO_3 .

Recovery of *nit* mutants. Recovery of *nit* mutants was done according to the methods described by Klittich and Leslie (1988a). Mycelial discs ($1\text{-}2\text{ mm}^3$) from each isolate were placed on minimal medium plus 3% KClO_3 for mutant selection. Plates were incubated at $24\text{-}25^{\circ}\text{C}$ in dark and were examined weekly for the appearance of fast growing sectors. The sectors grow at a visibly faster rate than do the parental culture. This difference may result since wild-type growth may be limited due to the reduction of chlorate to toxic chlorite by nitrate reductase (Solomonson & Vennesland, 1972). Approximately 20-40 chlorate-resistant sectors from each fungal isolates were selected on MMC and transferred to MM. Transfers that grew as expansive but thin mycelial colonies were considered to be *nit* mutants.

Nit mutant phenotypes. The physiological phenotypes of *nit* mutants were investigated by growing them on media containing one of five different nitrogen sources, following the Correll et al. (1987) method.

Complementation test. VCG grouping was based on complementation reaction between NitM (or *nit3*) and *nit1* mutants on minimal medium. Pairings to test vegetative compatibility were made in plates (10 cm in diameter) as previously described by Klittich and Leslie (1988b). Isolates were paired in all possible pairwise combinations and with a positive control. Pairs of isolates that exhibited vigorous aerial growth at the contact zone of the two *nit* mutant, indicating the formation of a heterokaryon, were determined as vegetative compatible and were assigned to the same VCG. Otherwise, isolates were defined as belonging to different VCGs.

Results

Nit mutants isolation. All 57 isolates of *F. proliferatum* spontaneously produced chlorate resistant sectors on MMC. All of them grew on MM as thin mycelium with no aerial mycelia (*nit* mutants) because of their inability to utilize nitrate as a sole nitrogen source. The majority of recovered chlorate resistant sectors were unable to utilize nitrate as a sole nitrogen source and consequently grew as thin expansive colonies with no aerial mycelia in MM. There was a marked difference in the rate at which sectoring occurred and in the recovery of *nit* mutants on MMC. A few sectors that were recovered from each strain were chlorate resistant, but able to utilize nitrate.

Nit mutant phenotype identification. *Nit* mutant phenotype was identified after growth of isolates on media that contained one of five different nitrogen sources. The *nit* mutants could be divided into three phenotypic classes. The *nit* mutants with wild-type growth on both nitrite and hypoxanthine-containing media were classified as *nit1*; those with wild-type growth on medium with hypoxanthine or nitrite as the sole N source were identified as *nit3* and NitM, respectively. The majority of *nit* mutants recovered on all medium were *nit1* mutants. (Table 1). However, the frequency of recovered *nit1* mutants was higher on PDC than on other mediums. Consequently, the relative frequency of nitM mutants recovered was considerably higher on MMC than on other media. Also, the highest relative frequency of *nit3* was observed on MMC. Among the *nit* mutants, the *nit1* class composed 79% of the total number of mutants, while nitM and *nit3* mutants composed 8% and 13% of the total number of mutants, respectively (Table 1).

Table 1. Frequency and phenotype of ninrate nonutilizing (*nit*) mutants recovered from four media

Medium ^a	<i>nit</i> ^b (%)	<i>nit</i> mutants classes ^c		
		<i>nit1</i> (%)	nitM (%)	<i>nit3</i> (%)
PDC	92	90	5	5
MMC	89	64	15	21
CZC	93	78	8	14
WAC	78	86	2	12
Total	88	79	8	13

^a PDC=potato-dextrose agar medium plus chlorate, MMC=minimal agar medium plus chlorate, CZC=Czapek-Dox agar chlorate plus chlorate, WAC=water agar medium plus chlorate.

^b Percent of chlorate-resistant sectors that grew as thin expansive colonies on minimal medium.

^c *Nit* mutant phenotypes determined according to growth on basal medium amended with different nitrogen sources.

Complementation test. All isolates in which were recovered both *nit1* and NitM (or *nit3*) mutants demonstrated self-compatibility. Based on their positive complementation reactions, thirty VCGs were identified among 57 isolates, of these 23 groups had only one member, and the remaining 34 isolates belonged to seven multimember VCGs (Table 2), accounting for a genetic diversity (number of VCGs/ number of isolates) of 0.54. VCG1 was the largest and the most frequent group in Iran and consisted of 20 isolates, VCG2 included four isolates whereas the other five multimember VCGs each had two members.

Discussion

Genetic characterization of prevailing *F. proliferatum* populations is important for effective management of the given diseases. Examination of selected isolates from Iran for vegetative compatibility and sexual fertility (Alizadeh et al., 2009) further confirm the status of the *F. proliferatum* isolates that originating from various hosts in Iran. According to the previous study, based on morphological characteristics and sexual fertility crosses, the 142 isolates were assigned to "D" mating population of *G. fujikuroi*. Furthermore, morphological identification of these isolates as *G. intermedia* was confirmed by sexual crosses. The usability of the diagnostic PCR method for mating type identification was tested on 142 *F. proliferatum* isolates obtained from maize, rice, onion and sugarcane plants from various areas of Iran. Both *MAT-1* and *MAT-2* individuals were identified among these all isolates (Alizadeh et al., 2009). These results showed that there is potential for sexual reproduction within these populations and thus, an expansion of the genetic diversity by recombination.

Distribution of *F. proliferatum* isolates obtained from various area and host plants into different VCGs in Iran is

Table 2. Characterization of *nit* mutants from *F. proliferatum* isolated from different host plants in different locations of Iran.

Isolate (no.) ^a	Location ^b	Host/Infected tissue	Year	Mating type ^c	Phenotype of <i>nit</i> mutants			VCG Group ^d
					A	B	C	
1	Ardabil	Maize- ear	2005	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	1
2	Ardabil	Maize- ear	2005	MAT-1	<i>nit1</i>	–	NitM	3
3	Ardabil	Maize- ear	2005	MAT-1	<i>nit1</i>	–	–	8
4	Ardabil	Maize-ear	2005	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	1
5	Ardabil	Maize-ear	2005	MAT-2	<i>nit1</i>	<i>nit3</i>	–	1
6	East-Azerbaijan	Maize-stalk	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	–	9
7	Fars	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	–	10
8	Fars	Maize-ear	2005	MAT-1	<i>nit1</i>	–	NitM	3
9	Fars	Maize-ear	2005	MAT-1	<i>nit1</i>	–	NitM	1
10	Gorgan	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	1
11	Hamedan	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	1
12	Hamedan	Maize- ear	2005	MAT-2	<i>nit1</i>	–	NitM	1
13	Isfahan	Maize- ear	2005	MAT-2	<i>nit1</i>	<i>nit3</i>	–	2
14	Isfahan	Maize- ear	2005	MAT-2	<i>nit1</i>	<i>nit3</i>	–	4
15	Kerman	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	1
16	Kermanshah	Maize- ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	–	4
17	Mzandaran	Maize-ear	2005	MAT-2	<i>nit1</i>	–	–	11
18	Mzandaran	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	–	12
19	Qazvin	Maize-ear	2005	MAT-1	<i>nit1</i>	–	NitM	1
20	Tehran	Maize-ear	2005	MAT-1	<i>nit1</i>	<i>nit3</i>	–	13
21	Guilan	Rice-foot	2004	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	2
22	Guilan	Rice-foot	2004	MAT-1	<i>nit1</i>	–	NitM	2
23	Guilan	Rice-foot	2004	MAT-1	<i>nit1</i>	–	NitM	14
24	Guilan	Rice-foot	2004	MAT-1	<i>nit1</i>	<i>nit3</i>	–	15
25	Guilan	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	16
26	Guilan	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	17
27	Mazandaran	Rice-foot	2004	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	2
28	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	5
29	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	5
30	Mazandaran	Rice-foot	2004	MAT-1	<i>nit1</i>	–	NitM	6
31	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	6
32	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	7
33	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	–	NitM	7
34	Mazandaran	Rice-foot	2004	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	18
35	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	19
36	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	–	NitM	20
37	Mazandaran	Rice-foot	2004	MAT-1	<i>nit1</i>	<i>nit3</i>	–	21
38	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	22
39	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	23
40	Mazandaran	Rice-foot	2004	MAT-2	<i>nit1</i>	<i>nit3</i>	–	24
41	Khuzestan	Sugarcane	2003	MAT-1	<i>nit1</i>	<i>nit3</i>	–	25
42	Khuzestan	Sugarcane-stem	2003	MAT-2	<i>nit1</i>	–	NitM	1
43	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	–	NitM	1
44	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	<i>nit3</i>	–	26
45	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	–	NitM	1
46	Khuzestan	Sugarcane-stem	2003	MAT-2	<i>nit1</i>	<i>nit3</i>	–	1
47	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	–	NitM	1
48	Khuzestan	Sugarcane-stem	2003	MAT-2	<i>nit1</i>	<i>nit3</i>	NitM	1

Table 2. Characterization of *nit* mutants from *F. proliferatum* isolated from different host plants in different locations of Iran.

Isolate (no.) ^a	Location ^b	Host/Infected tissue	Year	Mating type ^c	Phenotype of <i>nit</i> mutants			VCG ^d
					A	B	C	
49	Khuzestan	Sugarcane-stem	2003	MAT-2	<i>nit1</i>	<i>nit3</i>	–	27
50	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	–	NitM	28
51	Khuzestan	Sugarcane-stem	2003	MAT-2	<i>nit1</i>	<i>nit3</i>	–	29
52	Khuzestan	Sugarcane-stem	2003	MAT-1	<i>nit1</i>	–	–	30
53	North Khorasan	Onion-root	2007	MAT-1	<i>nit1</i>	–	NitM	1
54	North Khorasan	Onion-scale	2007	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	1
55	North Khorasan	Onion-root	2007	MAT-1	<i>nit1</i>	–	NitM	1
56	North Khorasan	Onion-root	2007	MAT-1	<i>nit1</i>	–	NitM	1
57	Rzavi Khorasan	Onion-basal	2007	MAT-1	<i>nit1</i>	<i>nit3</i>	NitM	1

^a Isolate number.^b Locations are different provinces of Iran.^c Mating type data for examined isolates obtained from the study conducted by Alizadeh et al. (2009).^d VCG=vegetative compatibility groups (Total 57 isolates grouped in 30 VCGs based on vegetative compatibility tests by pairing *nit* mutants).

reported for the first time. We identified 30 VCGs among 57 tested isolates. Isolates belonging to VCG1 were collected from eight provinces, indicating that genetic variation across geographic locations occurs in Iran, confirming results obtained in previous studies (Desjardins, 2003; Barga et al., 2009). VCG1 included isolates from maize, onion and sugarcane host plants and VCG2 included isolates from maize and rice. Each of VCG 3, VCG 4 and VCG 5 included two isolates from maize and each of VCG 6 and VCG 7 included two isolates from rice. Beside those, 23 isolates grouped in 23 VCGs that each of them had only one member. This result was similar to those of Alian et al. (2005) that 26 VCG groups identified among 29 isolates. Of those, 23 VCGs were represented each by a single isolates and 6 isolates belonged to three multimember groups, each containing two isolates. In this study, none of isolates from rice complemented with any other isolates from onion and sugarcane. Also, non complementation occurred between onion and sugarcane isolates. In VCG1 only one complementation occurred between one isolate from maize and sugarcane isolates. Also, in VCG2 only one isolate from maize complemented with rice isolates. Therefore except some cases, host preferences observed in complementation tests and assignment of isolates to VCG groups. Missing relationships between DNA fingerprint groups and origin of isolates or other characteristic features are often described for different *Fusarium* species, which causes various plant diseases. For instance, Abd-El Salam et al. (2003) were able to correlate diverse fingerprint patterns of *F. semitectum* isolates with morphological characteristics of fungal cultures, but found no correlation with host genotype or geographic origin. Patino et al. (2001) investigated thirty-three isolates of *F. verticillioides* isolated from

diverse origins and hosts. Analysis of the IGS region by PCR-RFLP discriminated two groups of isolates based on fumonisin production and host preferences. Moretti et al., (2004) reported that, isolates of *F. verticillioides* from banana showed different traits such as pathogenicity, toxin profile, fertility and AFLP fingerprint than members of the same isolates from maize. In the present study, a correlation between VCGs grouping and host preferences was founded. Also, individuals with both mating types, MAT-1 and MAT-2, were found in the multimember VCGs groups indicating that those isolates in same VCGs would not be clones of one another but different individuals.

Isolates from maize belonging to VCG 1 were collected from eight provinces, also indicating that genetic variation across geographic locations occurs in Iran. Since clonal spread by rain splash or wind over the large distances is unlikely, the same VCG genotype was probably introduced into different sites as seed-borne inoculums. Infected maize seed must be a significant vehicle for the dissemination of pathogen genotypes throughout Iran. In addition, we think that fungal populations including VCG1 may be adapted to a maize genotype which was commonly cultivated in 2004-2005.

Molecular phylogenetic analyses of strains from diverse hosts and geographic areas initiated by Desjardins (2003) exhibited high diversity within *F. proliferatum* (*G. intermedia*) isolates, which were recovered from asparagus spears from different locations in Austria and Germany, confirmed considerable genetic variability (Barga et al., 2009). Alian et al. (2005) investigated twenty-nine isolates of *F. proliferatum* recovered from rice from Mazandaran province in Iran and 26 VCG groups identified among 29 isolates. Mohammadian (2008) reported high genetic diver-

sity of *F. proliferatum* isolates obtained from maize and rice in Iran using RAPD molecular marker. In addition, AFLP analysis of the *F. proliferatum* populations obtained from various hosts and geographical areas showed that natural populations of *F. proliferatum* in Iran are genetically divergent and include isolates representing a potential risk for disease development (Alizadeh, 2010).

Present study showed the high genetic diversity within Iranian populations of *F. proliferatum* from different plant hosts in Iran. These results are in agreement with results from previous studies on *F. proliferatum*, demonstrating that the populations of this fungus are genotypically highly diverse in Iran (Alian et al., 2005; Mohammadian, 2008).

Preliminary results on sexual fertility revealed that occurrence of sexual recombination and genetic variations are possible in *G. intermedia* populations in Iran (Alizadeh et al., 2009). Abbaszadeh et al. (2007) recorded that isolates of *F. proliferatum* have the ability to reproduce both sexually and asexually in Iran. Extensive sexual recombination should, however, increase the level of diversity in the *G. intermedia* populations by recombining the alleles present into new combination. Meiotic recombination can generate and maintain genetic variation and results in the reassortment of genes that govern traits such as virulence or toxin production (Cumagun et al., 2002).

Results obtained from other studies (Mohammadian, 2008; Alian et al., 2005; Abbaszadeh et al., 2007; Alizadeh et al., 2009; Alizadeh, 2010) and high degree of VCGs polymorphism in this study supports the assumption that the genotypic diversity of *F. proliferatum* is primarily caused by recombination during the sexual reproduction. And also, the worldwide distribution of *F. proliferatum* and the wide range of hosts from which it has been isolated can support the existence of variability within this species.

In order to improve understanding of the structure of populations, additional isolates from all hosts growing regions should be tested and neutral DNA markers such as AFLP would generate further information on the genetic structure of *F. proliferatum* populations in Iran.

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