

# Genetic diversity of *Fusarium oxysporum* isolates from common bean and distribution of mating type alleles

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## Abstract

Fusarium wilt caused by *Fusarium oxysporum* Schltdl. f.sp. *phaseoli* (Fop), is one of the important diseases of the common bean in Iran and many of the bean growing countries. Incidence of the disease has been reported in tropical and semi-tropical regions of the world. This study was carried out to characterize genetic diversity of *F. oxysporum* isolates from the common bean by vegetative compatibility test and random amplified polymorphic DNA (RAPD) analysis, as well as determining the frequency of mating type alleles (idiomorphs) in *F. oxysporum* population. In the present study, a total of 82 *Fusarium* sp. isolates collected from the major common bean growing areas of Tehran province were purified and identified. Among the isolates, 20 (24.4%), which were identified as *F. oxysporum*, were selected for the study. Nitrate non-utilizing (*Nit*) mutants were used to identify vegetative compatibility groups (VCGs). Ten VCGs were identified based on heterokaryon formation, 6 of which were single isolate VCGs. In order to study genetic diversity by RAPD, 6 random primers were used. Amplicons were analyzed and a dendrogram was created. The 20 isolates were grouped into 10 fingerprint groups. The results of VCGs and RAPD analyses suggest high genetic diversity among the 20 *F. oxysporum* isolates obtained from the bean samples. To determine the frequency of mating type alleles (MAT1-1 and MAT1-2) in *F. oxysporum* isolates, PCR was carried out using two pairs of specific primers. Among the tested isolates, two isolates were identified as MAT1-1 and 15 isolates as MAT1-2. There may be a direct relationship between the existence of both mating types

idiomorphs and the large genetic diversity among the isolates.

**Keywords:** *Fusarium oxysporum*; Genetic diversity; Vegetative compatibility groups; RAPD; Mating type idiomorphs

## INTRODUCTION

Bean production is significantly affected by soil-borne pathogens world-wide. One of the widespread and important diseases of the common bean is Fusarium wilt caused by *Fusarium oxysporum* f.sp. *phaseoli* (Fop). The disease has been reported in many bean producing regions including U.S.A., Brazil, Colombia, Spain, Italy, Netherlands, Greece and Central Africa (Hirano and Arie, 2009; Ramos *et al.*, 2007; Cramer *et al.*, 2003; Buruchara and Camacho, 2000; Alves-Santos *et al.*, 1999; Diaz-Minguez *et al.*, 1996; Pastor-Corrales and Abawi, 1987; Ribeiro and Hagedorn, 1979 a,b; Kendrick and Snyder, 1942). There are some reports of *F. oxysporum* as being the causal agent of Fusarium wilt and root rot in different provinces of Iran (Namvar Hamzanlouyi *et al.*, 2006; Faraji *et al.*, 2004; Heidarian and Ershad, 2002).

Awareness of genetic diversity among the isolates in the populations of plant pathogens can lead researchers to understand their genetic structure and dynamics. The latter issue plays a significant role in deducing the evolution of the pathogens and their correlation with plant hosts, which helps us take any preventive measure against them.

Determination of vegetative compatibility groups (VCGs) is a way to study genetic variability in fungal

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populations and was employed for the first time by Puhalla (1985) to distinguish and classify strains of *F. oxysporum*. Characterization of Fop isolates by VCGs has since been employed by various researchers (Namvar Hamzanlouyi *et al.*, 2006; Alves-Santos *et al.*, 1999; Woo *et al.*, 1996).

Molecular markers including random amplified polymorphic DNA (RAPD) can overcome limitations of other techniques, which are time-consuming and labor-intensive. These molecular biology techniques are highly sensitive in differentiating various strains of *F. oxysporum* (Assigbetse *et al.*, 1994; Kelly *et al.*, 1994; Grajal-Martin *et al.*, 1993). Thus, RAPD marker is effective in discriminating between Fop isolates (Namvar Hamzanlouyi *et al.*, 2006; Zanotti *et al.*, 2006; Alves-Santos *et al.*, 2002; Woo *et al.*, 1996).

Although no sexual stage has been known for *F. oxysporum*, strains of this fungus carry mating type idiomorphs, called MAT1-1 and MAT1-2 (Hirano and Arie, 2009; Abo *et al.*, 2005; Arie *et al.*, 2000; Yun *et al.*, 2000). Furthermore, some isolates can produce sterile stromatic tissue, pale or deep violet resembling a *Gibberella*-like perithecium (Booth, 1971). Awareness of the status of mating type idiomorphs in the population of *F. oxysporum*, which has been known as not having sexual form, is very useful to infer the status of the fungus population. It can lead us to a better interpretation of its level of genetic diversity.

The present study aimed to characterize genetic diversity among *F. oxysporum* isolates from the common bean by vegetative compatibility and RAPD analyses and study their potential efficiency to characterize genetic diversity. A further objective of this investigation was to determine the frequency of mating type idiomorphs in the *F. oxysporum* population in Tehran province, which has not been studied so far.

## MATERIALS AND METHODS

**Sampling strategy and collection of isolates:** The major bean cultivation regions in Tehran province including Karaj, Hashtgerd, Pishva and Varamin were visited during 2004-2005. Regarding the cultivated areas and frequency of diseased plants, 5-10 random symptomatic plants per field expressing chlorosis, wilt, leaf drop and/or stunt were carefully extracted from the soil. Then, the crown was cut off vertically up to stem to observe discoloration in the vascular tissue. After that, the samples were placed in plastic bags and transferred to the laboratory as soon as possible.

The approximate 0.5 cm cross-sections of plant stems were placed in sodium hypochlorite solution (containing 1% (v/v) active NaClO<sub>4</sub>) for 2-3 min after washing under tap water. Then, they were transferred to Petri dishes containing a selective agar media containing peptone, pentachloronitrobenzene (PCNB) and streptomycin (Nash and Snyder, 1962), and were placed in the dark at 25°C. After 4-5 days, fungal isolates were purified, stored in 1.5 ml microtubes containing sterile riverbed sand and *Fusarium oxysporum* isolates were subsequently identified on carnation leaf agar (CLA) (Fisher *et al.*, 1982).

**Pathogenicity assay:** Pathogenicity assay was carried out using the method of Haware and Nen (1982) with some modifications. Inoculum was produced by seeding 250-ml flasks containing 100 g of sand-maize meal mixture with small agar plugs cut from the margin of 7-day-old colonies (incubated in the dark at 25°C), at a proportion of 95:5. The seeded flasks were then incubated in the dark at 25°C for 5 to 14 days. The inoculum (100 g) of each flask was completely mixed with 2 kg of sterile soil containing a 1:1 mixture of riverbed sand and pot soil, and was placed in the one-third lower part of a 9-cm plastic pot. The upper two-thirds contained sterile soil. The pots were irrigated daily for 4 days before sowing seeds of two of the most cultivated cultivars in the region (Sun Ray and Dehghan). Four days after inoculation, the disinfected sprouted seeds were placed in pots (2 seeds of each cultivar per pot). Ten pots were considered for each isolate, from which there were 5 pots of Sun Ray and 5 pots of Dehghan. Five pots containing the mixture of sand-maize meal and sterile soil were considered as control. Plants were grown in a greenhouse (22-35°C) with 40-80% relative humidity. Four to 6 weeks later, symptomatic plants expressing wilt, chlorosis, leaf drop, stunt and/or discoloration in the stem vascular tissue were selected and the pathogen was reisolated and grown as a pure culture. Subsequently, the pathogenicity assay was performed twice.

**Isolation and characterization of *nit* mutants:** *Nit* mutants were recovered on minimal medium with chlorate (MMC) containing 3.5% (w/v) KClO<sub>3</sub>. MMC was made by adding NaNO<sub>3</sub>, L-asparagine and KClO<sub>3</sub> to a basal medium (Correll *et al.*, 1987). The amount of KClO<sub>3</sub> can be enhanced up to 6% (Leslie and Summerell, 2006). In this study, for 2 isolates (Fo 9 and Fo 12), chlorate concentration was enhanced by up to 5%, because *nit* mutants could not be obtained on

the medium containing 3.5% (w/v)  $KClO_3$ . To recover *nit* mutants, mycelial plugs from 2-4-day-old cultures grown on PDA were transferred to Petri dishes containing MMC. Then, they were placed in the dark at 25°C. After 5 to 7 days, *nit* mutants were recovered from the tip of the auxotrophic hyphae. After that, the mutants were characterized as *nit1*, *nit3*, or NitM based on their ability to utilize nitrate, nitrite, hypoxanthine, ammonium and/or uric acid (Correll *et al.*, 1987). To characterize *nit* mutants, 5 selective media were made. These media are as follows: nitrate medium (by adding  $NaNO_3$  to the basal medium), nitrite medium (by adding  $NaNO_2$  to the basal medium), hypoxanthine medium (by adding  $C_3H_4N_4O$  to the basal medium), ammonium medium (by adding ammonium tartarate  $[(NH_4)_2C_4H_4O_6]$  to the basal medium) and uric acid medium (by adding  $C_5H_4N_4O_3$  to the basal medium).

**Characterization of vegetative compatibility groups:** One *nit1* and 1 NitM were randomly selected for each isolate and pairings were conducted between these mutants on minimal medium (MM) in order to determine heterokaryon self-incompatible (HSI) isolates. If no NitM had been recovered for an isolate, the pairings were conducted between *nit1* and *nit3*. When heterokaryon formation occurred at the interface of *nit1* and NitM mutants, the isolate was considered to be self-compatible. Recovered mutants from these isolates were paired on MM in all possible combinations. Plates were incubated in the dark at 25°C for 14 to 21 days. Isolates were considered to be vegetatively compatible if prototrophic growth was observed at the zone of anastomosis. Pairings were performed twice at different times.

**DNA extraction:** Genomic DNA was extracted using a modified method of Leslie and Summerell (2006). Mycelial plugs from 2-4-day old cultures grown on potato dextrose agar (PDA) were transferred to 50 ml of potato dextrose broth (PDB) and were incubated in the dark at 28-33°C without shaking, for 4-5 days. The mycelia were collected from liquid medium, washed with sterile water and freeze-dried. Approximately 20 mg of the dried mycelia was thoroughly ground and subjected to 750  $\mu$ l of an extraction buffer containing 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 20 g/l of cetyl trimethyl ammonium bromide (CTAB) and 10 ml/l of 2-mercaptoethanol. Then it was incubated at 65°C for 30 min, subjected to a half volume of phenol/chloroform (25:24) mixture, vortexed for 2-3 s,

and centrifuged at 1000  $\times g$  for 5 min. An equal volume of chloroform was then added to the supernatant and after a 5-6 s vortex, was centrifuged again. DNA was precipitated with isopropanol. After centrifugation at 1000  $\times g$  for 2 min, it was washed in 70% (v/v) ethanol, centrifuged again, air-dried, and suspended in 30-50  $\mu$ l of deionized water.

#### **Random amplified polymorphic DNA (RAPD)**

**analysis:** Six 10-mer random primers CS 16: 5'-CGT TGG ATG C -3', CS 24: 5'-GCG GCA TTG T-3', CS 29: 5'-CCA GAC AAG C-3', Fus 01: 5'-CCG CAT CTA C-3', UBC 6: 5'-CCT GGG CCT A-3' and UBC 188: 5'-GCT GGA CAT C -3', as suggested by Cramer *et al.*, (2003), and synthesized by MWG-Biotech. (Germany), were used for DNA amplification. Polymerase chain reactions were performed in 20  $\mu$ l reactions containing approximately 5-6 ng of genomic DNA and a 16  $\mu$ l master mix consisting of 1 $\times$ PCR buffer (CinnaGen Co., Iran), 2.75 mM  $MgCl_2$ , 0.6 mM dNTPs, 7.5  $\mu$ M of primer and 2.5 unit of *Smar Taq* DNA polymerase (CinnaGen Co., Iran). Reactions were amplified by a CG1-96 thermocycler (Corbett Research, Australia) in three cycles of: 2 min denaturation at 94°C, 1 min dwell at 30°C, 3 min extension at 70°C followed by 45 cycles of 1 min denaturation at 94°C, 1 min dwell at 33°C, 2 min extension at 72°C and a final extension of 10 min at 72°C. Amplified DNA was separated on 1% (w/v) agarose gels in 1 $\times$ TBE (3 h at 85 V) and stained with ethidium bromide. PCR reactions were repeated at least twice.

**Analysis of genetic similarity:** The binary matrix of RAPD data was subjected to the SIMQUAL module in the numerical taxonomy package NTSYS pc-2.02e using Dice's coefficient of similarity, to generate a genetic distance matrix. The matrix was subjected to the SAHN module to create a dendrogram based on unweighted-paired group method with arithmetic averages (UPGMA).

#### **Determination of frequency of mating type idiomorphs by polymerase chain reaction (PCR):**

Two pairs of specific primers suggested by Ker enyi *et al.*, (2004), and synthesized by MWG-Biotech. (Germany) were used to amplify the highly conserved alpha box and HMG box domains found in MAT1-1 and MAT1-2, respectively. The primers were as follows:

fus ALPHA forward: 5' -CGC CCT CT(GT) AA(CT) G(GC)C TTC ATG-3'

*fus* ALPHA reverse: 5' -GGA (AG)TA (AG)AC (CT)TT AGC AAT (CT)AG GGC-3'

*fus* HMG forward: 5'-CGA CCT CCC AA(CT) GC(CT) TAC AT-3'

*fus* HMG reverse: 5' -TGG GCG GTA CTG GTA (AG)TC (AG)GG-3'.

Each PCR reaction mixture (final volume: 20  $\mu$ l) contained approximately 4.2 ng of genomic DNA, 1 $\times$ PCR buffer, 2.5 mM MgCl<sub>2</sub>, 1 mM dNTPs, 3  $\mu$ M of each pair of the primers and 2.5 unit of *Smar Taq* DNA polymerase (CinnaGen Co., Iran). Reactions were then amplified for an initial denaturation at 94°C for 45 s followed by 32 cycles of 45 s at 94°C, 45 s at 55°C, 2.5 min extension at 72°C and a final extension of 10 min at 72°C. Reactions were repeated a minimum of two times.

## RESULTS

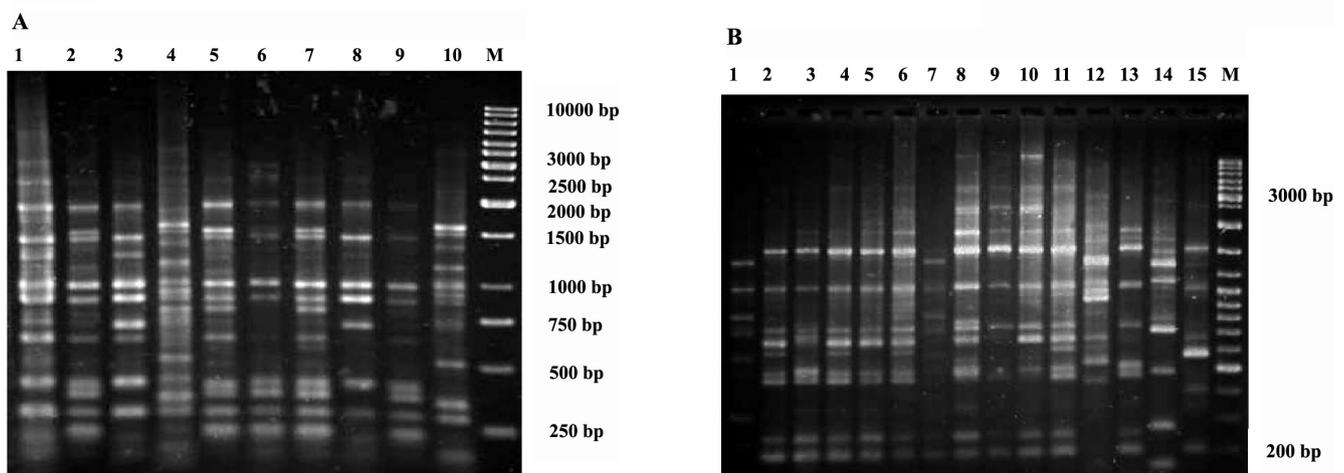
**Pathogenicity assay:** Of the 20 *Fusarium oxysporum* isolates, 2 isolates (Fop 41 and Fop 42-1) were *F. oxysporum* f.sp. *phaseoli* (Fop). They caused chlorosis, wilt and brown discoloration in the stem vascular tissue. The other isolates were not as pathogenic as the above-mentioned isolates, as they caused less visible disease symptoms on the common bean cultivars. Similar results for the pathogenicity assay were achieved in the two independent experiments.

**Characterization of vegetative compatibility groups:** Of the 20 *F. oxysporum* isolates examined, *nit*

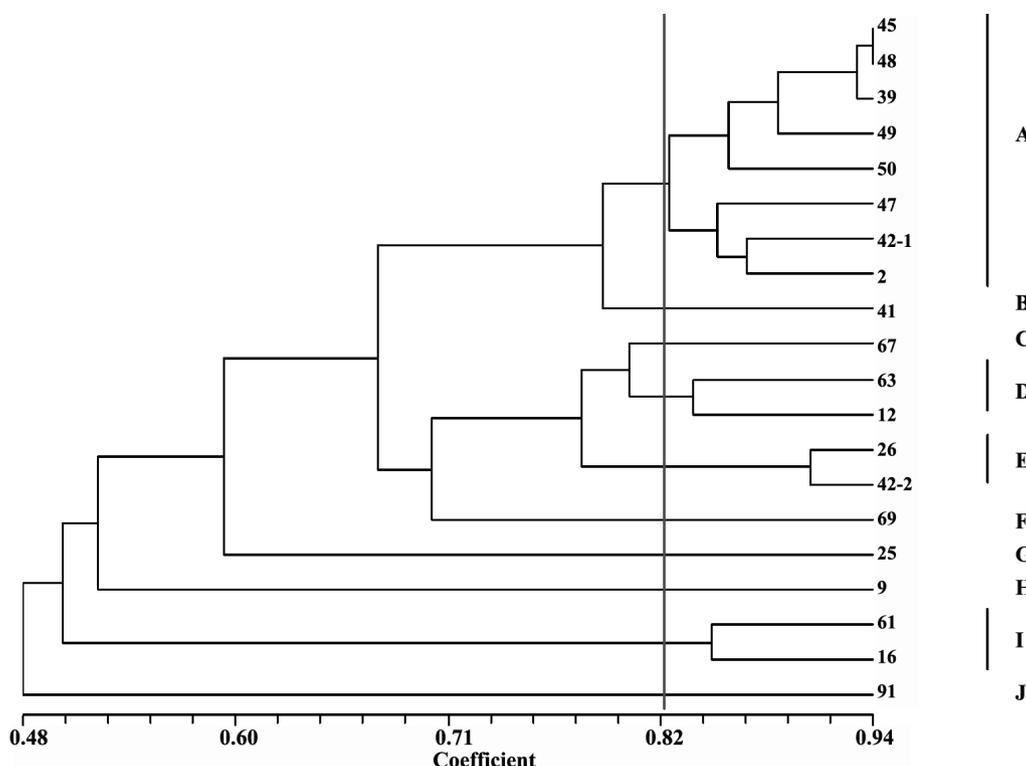
mutants from 18 isolates were recovered on MMC containing 3.5% (w/v) KClO<sub>3</sub>. For 2 isolates (Fo 9 and Fo 12), *nit* mutants could not be obtained after enhancing chlorate concentration to 4%, 4.5% and even 5%. The frequency of mutants was 54.84% for *nit1*, 32.26% for *nit3* and 12.9% for NitM.

One isolate (Fo 39) was identified as heterokaryon self-incompatible (HSI) because of auxotrophic growth at the interface of mutants related to the same isolate. A total of 31 *nit* mutants (containing *nit1*, *nit3* and NitM) recovered from the other 17 isolates, were paired in all possible combinations. Ten VCGs were found, among which one group (VCG<sub>1</sub>) consisted of 5 isolates and 3 groups (VCG<sub>2</sub>, VCG<sub>3</sub> and VCG<sub>4</sub>) contained 2 isolates. The other 6 VCGs (VCG<sub>5</sub> to VCG<sub>10</sub>) were single isolate VCGs. The 2 Fop isolates (Fop 41 and Fop 42-1) which both originated from Karaj, were the only isolates from which all three phenotypic types of *nit* mutants (*nit1*, *nit3* and NitM) could be recovered and were placed in the same VCG (VCG<sub>3</sub>). Similar results were achieved in the two independent repetitions.

**RAPD and analysis of genetic similarity:** A total of 6 RAPD primers generated 103 polymorphic RAPD markers varying from 200 bp to 2900 bp (Fig.1 A and B). Cluster analysis of RAPD banding pattern revealed a high degree of genetic diversity. The 20 *F. oxysporum* isolates were placed in 10 fingerprint groups named A to J (Fig. 2), 6 of which consisted of one isolate. The repetition of the experiment rendered similar results.



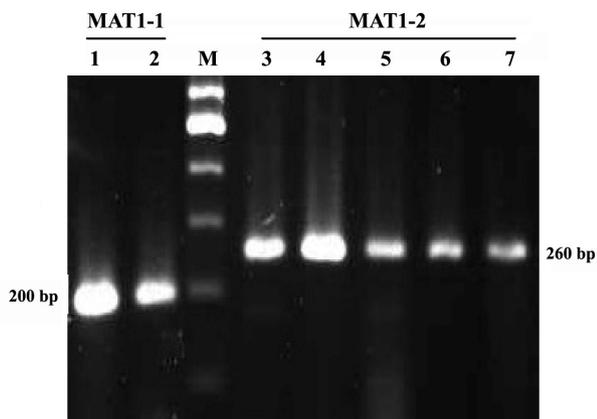
**Figure 1.** 1% (w/v) Agarose gels showing random amplified polymorphic DNA (RAPD) banding patterns in *Fusarium oxysporum* isolates using 10-mer random primers. A: Primer CS 29: Lane 1 = Fo 69, Lane 2 = Fop 42-1, Lane 3 = Fo 63, Lane 4 = Fo 61, Lane 5 = Fo 48, Lane 6 = Fo 47, Lane 7 = Fo 39, Lane 8 = Fo 67, Lane 9 = Fo 45, Lane 10 = Fo 16, and M = GeneRuler™ 1 kb DNA Ladder (MBI Fermentas, Germany). B: Primer UBC 6: Lane 1 = Fo 16, Lane 2 = Fo 45, Lane 3 = Fo 67, Lane 4 = Fo 39, Lane 5 = Fo 47, Lane 6 = Fo 48, Lane 7 = Fo 61, Lane 8 = Fo 63, Lane 9 = Fo 12, Lane 10 = Fop 42-1, Lane 11 = Fo 49, Lane 12 = Fo 91, Lane 13 = Fo 26, Lane 14 = Fo 9, Lane 15 = Fo 25, and M = GeneRuler™ DNA Ladder Mix.



**Figure 2.** Genetic relationship among *Fusarium oxysporum* isolates collected from the major bean cultivation regions in Tehran province. The dendrogram is an unweighted-paired group method with arithmetic averages (UPGMA) tree based on analysis of 6 random amplified polymorphic DNA (RAPD) primers which generated 103 polymorphic RAPD markers. The fingerprint groups (A to J) were separated from one another using Dice's coefficient of similarity (82%).

**Mating type characterization:** Screening the MAT1 idiomorphs revealed that 3 isolates did not show a clear amplification pattern. Among the other 17 isolates, 15 were MAT1-2 and 2 were MAT1-1 (Table 1).

The size of the amplicons from MAT1-1 and MAT1-2 idiomorphs were 200 bp and 260, respectively (Fig. 3). The results of the second experiment confirmed those of the first one.



**Figure 3.** Amplification pattern of the mating type idiomorphs in *Fusarium oxysporum* using specific primers. Lane1 = Fo 16, Lane 2 = Fo 26, Lane 3 = Fop 42-1, Lane 4 = Fo 63, Lane 5 = Fo 50, Lane 6 = Fop 41, Lane 7 = Fo 91, M = GeneRuler™ DNA Ladder Mix. Electrophoresis was carried out on 1.5 (w/v) agarose gel.

## DISCUSSION

Pathogenicity assay revealed that 2 of the 20 *Fusarium oxysporum* isolates (Fop 41 and Fop 42-1), which both originated from Karaj and were placed in the same vegetative compatibility group (VCG<sub>3</sub>), showed higher levels of pathogenicity on the common bean cultivars. They caused wilt, chlorosis and brown discoloration in the stem vascular tissue. They were also grouped in fingerprint groups B and A, respectively, nearly next to each other. The other isolates were not as pathogenic as the above-mentioned ones, since they caused less visible disease symptoms on the common bean cultivars. This result confirmed the report of Cramer *et al.*, (2003), who found that although all 166 *F. oxysporum* isolates had been collected from symp-

**Table 1.** Distribution of fingerprint groups, vegetative compatibility groups and mating type idiomorphs of *Fusarium oxysporum* isolates in Tehran province.

Isolate number	Isolate's binomial name	Geographical origin <sup>a</sup>	Fingerprint group	VCGs <sup>b</sup>	Mating type
Fo 45	<i>F. oxysporum</i> 45	Karaj	A	5	MAT1-2
Fo 48	<i>F. oxysporum</i> 48	Karaj	A	2	MAT1-2
Fo 39	<i>F. oxysporum</i> 39	Karaj	A	HSI	MAT1-2
Fo 49	<i>F. oxysporum</i> 49	Karaj	A	1	MAT1-2
Fo 50	<i>F. oxysporum</i> 50	Karaj	A	7	MAT1-2
Fo 47	<i>F. oxysporum</i> 47	Karaj	A	6	MAT1-2
Fop 42-1	<i>F. oxysporum</i> f.sp. phaseoli 42-1	Karaj	A	3	MAT1-2
Fo 2	<i>F. oxysporum</i> 2	Karaj	A	2	MAT1-2
Fop 41	<i>F. oxysporum</i> f.sp. phaseoli 41	Karaj	B	3	MAT1-2
Fo 67	<i>F. oxysporum</i> 67	Hashtgerd	C	4	MAT1-2
Fo 63	<i>F. oxysporum</i> 63	Hashtgerd	D	8	MAT1-2
Fo 12	<i>F. oxysporum</i> 12	Pishva	D	-	Unknown
Fo 26	<i>F. oxysporum</i> 26	Karaj	E	1	MAT1-1
Fo 42-2	<i>F. oxysporum</i> 42-2	Karaj	E	4	MAT1-2
Fo 69	<i>F. oxysporum</i> 69	Karaj	F	9	Unknown
Fo 25	<i>F. oxysporum</i> 25	Karaj	G	1	Unknown
Fo 9	<i>F. oxysporum</i> 9	Pishva	H	-	MAT1-2
Fo 61	<i>F. oxysporum</i> 61	Hashtgerd	I	1	MAT1-2
Fo 16	<i>F. oxysporum</i> 16	Varamin	I	1	MAT1-1
Fo 91	<i>F. oxysporum</i> 91	Pishva	J	10	MAT1-2

<sup>a</sup>Different cities in Tehran province where the main common bean growing areas are located. <sup>b</sup>Numbers refer to the vegetative compatibility group that each isolate belongs to. - is used for the isolates from which no *nit* mutants could be recovered on MMC. HSI refers to the heterokaryon self-incompatible isolate. All the samples were isolated during 2004-2005.

tomatic bean plants, 4 isolates from the same geographical origin (the Central High Plains) were pathogenic towards the common bean and were placed in the same fingerprint group by random amplified polymorphic DNA (RAPD) analysis.

Among the 20 *F. oxysporum* isolates examined in this study, *nit* mutants from 18 isolates were obtained on MMC containing 3.5% (w/v) KClO<sub>3</sub>. For 2 isolates (Fo 9 and Fo 12), *nit* mutants could not be recovered after enhancing chlorate concentration to 4%, 4.5% and even 5%. One isolate (Fo 39) was identified as HSI. Recovery of HSI isolates have been previously reported by other researchers including Jacobson and Gordon (1988) and Woo *et al.*, (1996). The other 17 *F. oxysporum* isolates were grouped in 10 VCGs. One VCG contained 5 isolates and 3VCGs consisted of 2 isolates. The remaining 6 isolates formed single isolate VCGs. These results suggested a large amount of genetic variability and were in agreement with the findings of Woo *et al.*, (1996), who found 9 VCGs containing 4 single isolate VCGs among 20 *F. oxysporum*

isolates obtained from the common bean; Alves-Santos *et al.*, (1999), who found 96 VCGs among 128 *F. oxysporum* isolates from the common bean, most of which were single isolate VCGs, and indicated that high levels of VCGs diversity among the isolates could be due to sexual exchange; and Namvar Hamzanlouyi *et al.*, (2006), who found 7 VCGs containing 3 single isolate VCGs among 22 *Fusarium oxysporum* f.sp. *phaseoli* (Fop) isolates.

A high genetic diversity was further supported by the variable banding pattern observed in RAPD analysis. A total of 103 polymorphic RAPD markers varying from 250 bp to 2900 bp were generated using 6 random primers, and the 20 *F. oxysporum* isolates formed 10 fingerprint groups, 6 of which contained one isolate. Of these six fingerprint groups, isolates Fo 69 and Fo 91, the members of groups F and J formed single isolate VCG<sub>9</sub> and VCG<sub>10</sub>, respectively. Also, isolates Fo 16 and Fo 61 in VCG<sub>1</sub> formed the two-member fingerprint group I and isolates Fo 2 and Fo 48, the members of VCG<sub>2</sub> were placed in fingerprint group A. In

other cases, no clear correlation was observed between VCGs and RAPD grouping. The obtained results apparently showed high genetic diversity. This is consistent with the results of the study by Woo *et al.*, (1996), who found 13 fingerprint groups among 20 *F. oxysporum* isolates from the common bean using 4 random primers; Cramer *et al.*, (2003), who reported 9 fingerprint groups in 19 *F. oxysporum* isolates from the common bean by using 12 random primers; Namvar Hamzanlouyi *et al.*, (2006), who found 6 fingerprint groups among 22 Fop isolates using 3 random primers; and Zanotti *et al.*, (2006), who found 7 fingerprint groups amongst 20 *F. oxysporum* isolates from the common bean by using 16 random primers.

Given the fact that sexual reproduction in *F. oxysporum* has not been reported either in nature or laboratory conditions so far, the large amount of genetic diversity observed in many studies, demands more attention. Some known mechanisms in *F. oxysporum* such as parasexual recombination (Molnar *et al.*, 1990) and mutations especially in the pathogenicity genes, resulting in the evolution of pathogenic races, can clarify the issue to some extent.

Mating type screening revealed that 3 isolates did not show a clear amplification pattern. Of the other 17 isolates, 15 isolates were MAT1-2 (260 bp) and 2 ones were MAT1-1(200 bp). The size of MAT1-1 and MAT1-2 amplicons confirmed the results of the study by Kerényi *et al.*, (2004). Mating type idiomorphs in *F. oxysporum* populations have been reported by other researchers (Hirano and Arie, 2009; Abo *et al.*, 2005; Arie *et al.*, 2000; Yun *et al.*, 2000). Presence of both MAT1 idiomorphs along with the fairly high genetic diversity found in the population in the present study suggests the probability of sexual recombination.

Comparing the findings of the present study, it can be concluded that: I) Both RAPD analysis and VCGs assays revealed a large degree of genetic diversity. This result confirmed the report of Woo *et al.*, (1996) and Namvar Hamzanlouyi *et al.*, (2006). It appears that the RAPD marker could partially recognize the genetic relationship among the isolates in different VCGs. II) There was a partial correspondence between the results of the mating type assay and VCGs testing. Both isolates with MAT1-1 idiomorphs were grouped in VCG<sub>1</sub> along with 3 MAT1-2 isolates. III) The identified fingerprint groups could partially differentiate among the isolates of different geographical regions. All the 8 members of fingerprint group A originated from Karaj. Also, the 2 isolates in group I were related to Karaj. However, all the isolates with the same origin

could not be placed in the same fingerprint groups. Also, the known VCGs differentiated among the isolates of different geographical regions partially. Possibly, vicinity of the regions has provided an opportunity for distribution of common genetic materials (isolates in the same VCG) to different geographical regions.

There may be a direct relationship between the existence of both mating type idiomorphs and the large amount of genetic diversity among the isolates. In order to help clarify the dynamics of the fungus in the country, further investigations on the presence of MAT idiomorphs in *F. oxysporum* populations in various regions of the country are recommended.

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