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Study on Genetic Diversity of *Fusarium oxysporum* f.sp. *dianthi* Based on Vegetative Compatibility Groups and Races in Five Provinces of Iran

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Abstract: *Fusarium* wilt of carnation caused by *Fusarium oxysporum* f.sp. *dianthi* (Fod) W.C.Snyder & H.N. Hensen, is the most important fungal disease in all main carnation growing regions of Iran. In 2020, sampling of infected plants was randomly carried out from carnation greenhouses in five provinces located in the North (Gilan, Golestan, and Mazandaran) and Middle (Tehran and Markazi) of Iran. Eighty-eight fungal isolates were identified as Fod by morphological characters (based on Nelson's key identification), molecular method (PCR amplified of ITS region), and pathogenicity tests (according to the root dipping on sensitive cv. Rendezvous). In order to investigate on biodiversity of Iranian Fod isolates based on Vegetative Compatibility Groups (VCGs) and races, 70 isolates were randomly selected. These isolates were compared with eight Fod isolates from the collection of Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (Di.Va.P.R.A) Patologia vegetale, Italy; which were used as references for races and VCGs. For DNA amplifying, primers R 1.8, R 2.1, and R 4.2 were employed. Amplified fragments of 295 bp and 564 bp were identified to race 1 (or 8; because these primers applied are not able to discriminate between the races), and race 2 (or 5 or 6), respectively. Furthermore, two VCG 0021 and VCG 0022 were identified amongst the Iranian *Fod* isolates. The relationship between the races and VCGs demonstrated that all race 1 (23 isolates) were categorized in VCG 0022, and race 2 (44 isolates) were grouped in VCG 0021. In addition, three isolates did not produce any amplification.
Key words: Carnation, *Fusarium oxysporum* f.sp. *dianthi*, Vegetative Compatibility Groups, Races, Iran

İran'ın Beş İlinde Farklı *Fusarium oxysporum* f.sp. *dianthi* Mantar Türleri Üzerinde Grup ve Irkların Vejetatif Uyumluluğuna Dayalı Bir Araştırma

Öz: *Fusarium oxysporum* f.sp. *dianthi* nin neden olduğu karanfil Fusarium solgunluğu (Fod) W.C.Snyder & H.N. Hensen, İran'ın tüm ana karanfil yetiştirme bölgelerindeki en önemli mantar hastalığıdır. 2019 yılında, İran'ın beş kuzey (Gilan, Golestan ve Mazandaran) ve orta (Tahran ve Orta) vilayetlerindeki karanfil seralarından enfekteli bitki örneklemesi rastgele yapıldı. Morfolojik özelliklere göre 88 mantar izolatı Fod olarak tanımlandı (Nelson'ın anahtar tanımlamasına göre), Moleküler yöntem (ITS bölgesinden amplifiye edilmiş PCR), ve patojenite testleri (hassas Randzo çeşidinde köklerin patojen süspansiyonuna daldırılması yöntemine dayalıdır) İranın gıda izolatlarının biyolojik çeşitliliğini araştırmak için, uyumluluk gruplar (VCG'ler) ve ırklara göre, 70 izolat rastgele seçildi. Bu izolatlar, Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (Di.Va.P.R.A) Patologia vegetale koleksiyonundan sekiz Fod izolatı ile karşılaştırıldı. İtalya; ırklar ve VCG'ler için referans olarak kullanılmıştır. DNA amplifikasyonu için R 1.8, R 2.1 ve R 4.2 primerleri kullanıldı. Sırasıyla ırk 1 (veya 8) için 295 bp ve 564 bp'lik amplifiye fragmanlar tanımlandı. (çünkü uygulanan bu primerler ırkları ayırt edemez) ve sırasıyla ırk 2 veya 5 veya 6. Ayrıca İran gıda izolatları arasında VCG 0021 ve VCG 0022 tespit edilmiştir. Irklar ve VCG'ler

arasındaki ilişki, tüm ırk 1'in (23 izolat) VCG 0022'de sınıflandırıldığını gösterdi ve ırk 2 (44 izolat) VCG 0021'de gruplandırılmıştır. Ayrıca üç izolat herhangi bir amplifikasyon üretmedi.

Anahtar kelimeler: Karanfil, *Fusarium oxysporum* f.sp. *dianthi*, Bitkisel Uyumluluk Grupları, ırklar, İran

Introduction

Dianthus caryophyllus, which is known as the carnation is a perennial ornamental plant that belongs to the *Caryophyllaceae* family. Fusarium vascular wilt, which is one of the most important soil-borne fungal diseases, is caused by *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Fod) (Prill. & Delacr) W.C. Snyder & H.N. Hans. This disease is a major limiting factor in Iran and around the whole world in culturing this ornamental flower (Quirico Migheli, Briatore, & Garibaldi, 1998). Since 1975, when this disease was reported for the first time, many studies have been conducted to identify the races and Vegetative Compatibility Groups (VCGs) of this pathogen in different countries (Baayen, Van Dreven, Krijger, & Waalwijk, 1997); (Cabanás, Valverde-Corredor, & Pérez-Artés, 2012); (Manicom, Bar-Joseph, Kotze, & Becker, 1990); (Basallote-Ureba et al., 2016); (Baayen et al., 1997); (Zandyavari, Zadeh, & Zarrinnia, 2013); (Cer, Benlioglu, & Egerci, 2022). Researches confirm that there are eight different races of this pathogen. Although two other races called, 3 or 7 have been identified for the pathogen; so far they have not been officially accepted and approved (Aloi & Baayen, 1993). In 1979, race 3 (or 7) was identified for *F. oxysporum* var. *redolens* rather than species, but later this race was reported for *F. oxysporum* var *dianthi* (Basallote-Ureba et al., 2016); (Baayen et al., 1997). Therefore, sometimes it is still not completely clear that the species under investigation *Fusarium* belongs to *F. oxysporum* var. *redolens* or *F. oxysporum* var *dianthi* (Baayen et al., 1997). The morphological study conducted by Baayen and Gomes in 1988 showed that both species are identical and all isolates being collected from carnation, should be introduced as *F. oxysporum* f.sp. *dianthi* (Canizares, Gomez-Lama, García-Pedrajas, & Perez-Artes, 2015);. Nevertheless, the studies done in 1995 and 1996 by some researchers on the ITS region of the ribosomal DNA (rDNA) demonstrated that these two species are different and both can be pathogenic for carnation (Jawaharlal, Ganga, Padmadevi, Jegadeeswari, & Karthikeyan, 2009); (Canizares et al., 2015; Jawaharlal et al., 2009).

For the first time in 1989, five VCGs for Fod were identified and now two groups of them are considered for *F. oxysporum* f.sp. *redolens* (Mishra, Mukhopadhyay, & Singh, 2012). However, in 1993, Aloi and Baayen announced that each of the races identified for Fod belongs to a specific Vegetative Compatibility Groups (Aloi & Baayen, 1993). Using total DNA in the RFLP (Restriction Fragment Length Polymorphisms) method with probes developed from Fod displayed that the isolates belonging to the same VCGs are genetically homogeneous (Zandyavari et al., 2013). Similar results in the research conducted with RAPD method and chromosome electrophoresis confirm this issue (Manicom et al., 1990); (García-Núñez et al., 2017); (Q Migheli, Briatore, Andrina, & Garibaldi, 1997).

The present study was undertaken to present genetic diversity in Iranian *F. oxysporum* f.sp. *dianthi* isolates based on identification of VCGs and races in five provinces of Iran.

Material and Methods

Pathogen isolates

All 90 isolates being collected for this study are listed in Table 1. These isolates were collected from five provinces located in the North (Gilan, Golestan, and Mazandaran) and Middle (Tehran and Markazi) of Iran during 2020- 2021.

From each greenhouse 5 - 8 plants with wilt symptoms ranging from chlorosis, and yellows to deformation were pulled out of the soil randomly and separately put in paper bags and transferred to the laboratory in a short time. In the laboratory, the samples were disinfected with sodium hypochlorite solution (containing 1% (V/V), cut into 0.5- 1 cm cross sections, and transferred to Nash & Snyder (Prados-Ligero, Basallote-Ureba, López-Herrera, & Melero-Vara, 2007); (Zandyavari et al., 2013) selective media for a week. In order to purify, the single-spore method was used; then the purified isolates were cultured on Carnation Leaf Agar (CLA) media to produce chlamydospores.

The microconidia, macroconidia, and chlamydospores were considered keys to identifying morphological recognition Fod isolates according to Nelson et al., method (Nelson, Toussoun, & Marasas, 1983).

Table 1. Isolates of Fod from different carnation growing areas in five provinces of Iran Isolates

	PROVINCES	YEAR	VCGS	RACES
01	Tehran	2020	0021	2 (5 or 6)
02	Tehran	2020	0021	2 (5 or 6)
03	Tehran	2020	0021	2 (5 or 6)
04	Tehran	2020	0022	1 (or 8)
05	Tehran	2020	0022	1 (or 8)
06	Tehran	2020	0021	2 (5 or 6)
07	Tehran	2020	0021	2 (5 or 6)
08	Tehran	2020	0021	2 (5 or 6)
09	Tehran	2020	No Fusarium	-
10	Tehran	2020	0022	1 (or 8)
11	Tehran	2020	0021	2 (5 or 6)
12	Tehran	2020	0021	2 (5 or 6)
13	Tehran	2020	0021	2 (5 or 6)
14	Tehran	2020	No identify	No identify
15	Tehran	2020	0022	1 (or 8)
16	Tehran	2020	0022	1 (or 8)
17	Tehran	2020	0022	1 (or 8)
18	Tehran	2020	0021	2 (5 or 6)
19	Tehran	2020	0021	2 (5 or 6)
20	Tehran	2020	0021	2 (5 or 6)
21	Tehran	2020	0022	1 (or 8)
22	Tehran	2020	0021	2 (5 or 6)
23	Tehran	2020	SC*	-
24	Markazi	2020	0021	2 (5 or 6)
25	Markazi	2020	0022	1 (or 8)
26	Markazi	2020	0022	1 (or 8)

27	Markazi	2020	No identify	No identify
28	Markazi	2020	SC	-
29	Markazi	2020	0021	2 (5 or 6)
30	Markazi	2020	NP*	-
31	Markazi	2020	0021	2 (5 or 6)
32	Markazi	2020	0022	1 (or 8)
33	Markazi	2020	0022	1 (or 8)
34	Markazi	2020	SC	-
35	Markazi	2020	0021	2 (5 or 6)
36	Markazi	2020	0021	2 (5 or 6)
37	Markazi	2020	0022	1 (or 8)
38	Markazi	2020	0021	2 (5 or 6)
39	Markazi	2020	0022	1 (or 8)
40	Markazi	2020	0021	2 (5 or 6)
41	Markazi	2020	NP	-
42	Markazi	2020	SC	-
43	Markazi	2020	0021	2 (5 or 6)
44	Markazi	2020	NP	-
45	Markazi	2020	0022	1 (or 8)
46	Gilan	2021	0021	2 (5 or 6)
47	Gilan	2021	0022	1 (or 8)
48	Gilan	2021	0022	1 (or 8)
49	Gilan	2021	0021	2 (5 or 6)
50	Gilan	2021	0021	2 (5 or 6)
51	Gilan	2021	No identify	No identify
52	Gilan	2021	0022	1 (or 8)
53	Gilan	2021	0021	2 (5 or 6)

54	Gilan	2021	NP	-
55	Gilan	2021	0021	2 (5 or 6)
56	Gilan	2021	SC	-
57	Gilan	2021	0021	2 (5 or 6)
58	Gilan	2021	SC	-
59	Gilan	2021	0021	2 (5 or 6)
60	Gilan	2021	0021	2 (5 or 6)
61	Golestan	2021	0021	2 (5 or 6)
62	Golestan	2021	0022	1 (or 8)
63	Golestan	2021	0021	2 (5 or 6)
64	Golestan	2021	No Fusarium	-
65	Golestan	2021	0021	2 (5 or 6)
66	Golestan	2021	0021	2 (5 or 6)
67	Golestan	2021	0022	1 (or 8)
68	Golestan	2021	0021	2 (5 or 6)
69	Golestan	2021	0021	2 (5 or 6)
70	Golestan	2021	0022	1 (or 8)
71	Golestan	2021	0021	2 (5 or 6)
72	Golestan	2021	SC	-
73	Golestan	2021	0021	2 (5 or 6)
74	Golestan	2021	SC	-
75	Golestan	2021	0021	2 (5 or 6)
76	Mazandaran	2021	0021	2 (5 or 6)
77	Mazandaran	2021	0022	1 (or 8)
78	Mazandaran	2021	0021	2 (5 or 6)
79	Mazandaran	2021	0022	1 (or 8)
80	Mazandaran	2021	0021	2 (5 or 6)

81	Mazandaran	2021	SC	-
82	Mazandaran	2021	0021	2 (5 or 6)
83	Mazandaran	2021	NP	-
84	Mazandaran	2021	0021	2 (5 or 6)
85	Mazandaran	2021	SC	-
86	Mazandaran	2021	SC	-
87	Mazandaran	2021	0022	1 (or 8)
88	Mazandaran	2021	NP	-
89	Mazandaran	2021	0021	2 (5 or 6)
90	Mazandaran	2021	SC	-

*SC: self-Compatible *NP: Non- Pathogen

Pathogenicity assay

Cv. Rendezvous cuttings show high susceptibility to Fusarium wilt; so they were selected to do this test according to the method of Cabanás et al., 2012 with some modifications (Cabanás et al., 2012). The purified fungal colonies were planted on PDA (Potato Dextrose Agar) and incubated for five days at 25°C to obtain the inoculum. Each inoculum was diluted in sterile distilled water to give suspensions of 106 CFU.ML-1. The root dipping method was used to incubate carnation cuttings. Inoculated cuttings were cultured in pots containing sterilized soil with a mixture of peat: silt: sand (1:2:2 by volume). Then, plants were maintained at room temperature and 50- 60 % relative humidity. Three replicates were measured for each isolate. Some no inoculated plants were planted as control plants. *Fusarium* wilt symptoms were evaluated every five days for 120 days using the scale: 0 = no symptoms; 1= chlorosis or wilt showed to basal leaves; 2= chlorosis or wilt extending beyond the basal leaves; 3= chlorosis and wilt of one-third to half of the plant; 4= chlorosis and/or wilt symptoms reaching the upper part of the plant, and 5= dead plant (Cabanáset al., 2012).

Identification of Pathogen

Total DNA for pathogen isolates was extracted based on the CTAB method (Turaki et al., 2017). In order to PCR method 17.5µl ddH2O, 2.5µl PCR buffer 10X, 0.75µl MgCl2 (50Mm), 0.5µl dNTPs mix (10Mm), 0.3µl Taq DNA polymerase 5 unit/µl, 2µl DNA, 0.5µl ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 0.5µl ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used in this experiment part (Baayen et al., 1997). PCRs were performed in a Bio-Rad thermal cycler as per the following program: initial denaturation at 94 °C for 3 min, 40 cycles at 94 °C for 1 min, 55 °C for 45 s, and 72 °C for 1 min, and the final extension at 72 °C for 2 min. PCR products were purified by polyethylene glycol and printed on 1.2% agarose gel which demonstrated these isolates vicinity 600bp were *Fusarium* spp. (Baayen et al., 1997).

Vegetative Compatibility Groups (VCGs)

Vegetative compatibility Groups tests were performed for Fod isolates, which were selected randomly for the investigation, according to previously described methods with nit1, nit3, and/or Nit M mutants of the isolates (Puhalla, 1985); (Correll, 1991); (Aloi & Baayen, 1993); (Zandyavari et al., 2013).

A) Obtaining mutants

Potato Dextrose Chlorate (PDC) and Minimal Media Chlorate (MMC) with 20- 40 gr KClO3 were used to obtain nit mutants. Then phenotypic classification of nit mutants was identified according to Correll et al., by using different nitrogen media including Nitrate medium, ammonium medium, nitrite medium, uric acid medium, and hypoxanthine medium. The mutants were classified into three various classes based on their mode of growth on the mentioned media (Correll, 1991).

B) Self- compatibility test

All identified mutants of the isolates were evaluated by self-compatibility test. Almost 1-2 mm^2 block of Nit M from each isolate was placed in the center of a Petri dish containing MM (Minimal Media) and several blocks of nit1 and nit3 cultures from the same isolate were transferred at a distance of 2-4 cm from Nit M. Then the plates were maintained at 25°C for a couple of weeks. The growth of aerial mycelium between Nit M and nit1 and nit3 was considered an indicator of Vegetative Self-Compatibility.

The absence of aerial mycelium, however, was identified as Vegetative Incompatibility, and such isolates were discarded (Mishra et al., 2012); (Puhalla, 1985); (Zandyavari et al., 2013).

C) Determination of vegetative compatibility groups

Nit M of each self-compatibilities isolate was paired with one nit1 or nit3 from other isolates on MM in all possible combinations and was kept at 25°C for two weeks. The growth of a stable heterokaryon and the appearance of wild type in the space area of two nits of isolates a positive result in the compatibility of two contacting isolates and therefore, both isolates belong to one VCG group.

After VCGs determination in Iranian *Fod* isolates; they paired with nits of different VCGs obtained from the culture collection of Dipartimento di Valorizzazione e Protezionedelle Risorse Agroforestali (Di.Va.P.R.A) Patologia vegetale, Italy as tester isolates (Zandyavari et al., 2013).

Determination of races

The isolates were used for further molecular characterization by comparison with eight Fod isolates from the culture collection of Dipartimento di Valorizzazione e Protezionedelle Risorse Agroforestali (Di.Va.P.R.A) Patologiavegetale, Italy which were considered as the reference for races 1, 2, 4 and 8. In order to do this experiment, Chiocchetti et al., 1999 method was applied to identify Fod races by PCR amplification of transposon insertions (Chiocchetti et al., 1999).

PCR reaction

1 to 5 µl template DNA was amplified in a solution containing 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl2, 50 mM KCl, 0.1%Triton X-100, 0.01% (wt/vol) gelatin with the addition of 200 µM each nucleotide, 0.5 µM each primer R2.1 (Race specificity 2) (5’-CTTGTTTCTCGATTTCTGTCTCACG-3’), R4.2 (Race specificity 4) (5’-GGTGATTGGAGGAGGAATACC-3’), and R8.1 (Race specificity 1 or 8) (5’-CGATGAAGTCGGTTTGCGATT-3’), and 0.5 µl of crude recombinant Taq polymerase prepared (Chiocchetti et al., 1999).

PCR reactions were performed according to Chiocchetti et al. method with a thermal cycler (Bio-Rad) programmed as follows: one cycle at 94°C for 5 min, followed by 35 cycles, each consisting of a denaturation step at 94°C for 30 s; an extension stage at 72°C for 60 s; and annealing temperatures decreasing during the first 10 cycles from 60 to 55°C for 30 s according to Chiocchetti et al, (1999). After amplification, the reaction mixture was loaded on a 1.2% agarose gel, separated by electrophoresis, and photographed under UV light with the Gel Doc (Bio-Rad). Amplification experiments were repeated three times (Chiocchetti et al., 1999).

Results

Morphological identification

Macro conidia, which have three or four septate walls, are produced on a specialized structure named sprodochia; microconidia with no or one septate wall are produced on the aerial mycelium, and resistant structures called chlamydospores that formed on the middle or terminal part of the hyphae, are the main factor to identify *Fusarium oxysporum* (Figure 1).

Pathogenicity assay

At 28 days after incubation, the first symptoms of *Fusarium* wilt appeared in some cuttings. The observed differences were not significant in symptom types, while the isolates (various races) varied in the rate and severity of pathogenicity. Since six isolates could not demonstrate the disease on plants after four months, they were discarded from future steps of the study. Eighty- four isolates, which caused the death of carnations, were identified as pathogenic isolates. All these pathogenic isolates were within four months. The control plants, which were uninculated, remained healthy throughout the pathogenicity period (Figure 2).

Identification of pathogen

In this study, the ITS region of rDNA was amplified using genus- specific ITS1 and ITS4 primers. The ITS PCR has assisted to detect polymorphism at the ITS region of rDNA among the *Fusarium* isolates. These results demonstrated that 82 of 84 Iranian *Fusarium* isolates showed a vicinity 600 bp on 1.2% agarose gel and were considered *Fusarium oxysporum* (Fo) (Figure 3). Two isolates belonging to Tehran and Golestan (one isolate from each province) were not *F.oysporum*. Therefore, they were omitted from the investigation.

Eighty- two isolates of *Fusarium oxysporum* f.sp. *dianthi* which was confirmed in molecular tests were cultured on PDC and MMC media. The total number of sectors was 789 and 541 on MMC and PDC media, respectively. Furthermore, 136 and 48 Chlorate Resistant utilizing Nitrate (crn) that were able to grow wild type on MMC and PDC were deleted from the next study’s steps. Most of the mutants were formed on a culture medium with 4% chlorate.

Five different nitrogen resources were used to identify nit mutants’ phenotypic classes. The frequencies of Nit M, nit1, and nit3 phenotypes on PDC were 9.4%, 63.2%, and 9.4%, respectively while those frequencies on MMC were 13.7 %, 54.2 %, and 14.9 % respectively (Table 2). Comparing the number of mutants obtained on both chlorate mediums shows that the highest number of mutants were formed on MMC culture medium.

Twelve pathogenic isolates were self-incompatible, so, were ignored in the determination of VCGs test. Therefore, VCGs test was done for all 70 Iranian *Fod* isolates.

Two groups were obtained by pairing representative nit mutants from seventy Iranian Fod isolates in all combinations. The competition between Iranian isolates and Italian references isolates from the

collection of Di.Va.P.R.A Italy, expressed that Fod isolates from Iran belonged to VCG0021 and VCG 0022. Within the two VCGs, the biggest group (VCG 0021) contained 44 members and VCG 0022 contained 23 members, respectively (Table 1).

Table 2. Frequency and phenotype of nit mutants recovered from PDC and MMC media

Media	No. of Sectors	No. of Nit	Percentage of Nit	Nits' Classes		
				Nit1	Nit3	NitM
MMC	789	653	82.8%	54.2%	14.9%	13.7%
PDC	541	496	91.7%	63.2%	19.1%	9.4%

The results from the present study reveal that there was no wide genetic diversity in mentioned areas in Iran. Moreover, the correlation between provinces and VCGs was not observed because the two mentioned groups were observed in all five provinces (Table 1). Three isolates did not match with any isolates; therefore, they were not grouped in these VCGs (Table 1).

Determination of races

DNA was amplified using primers R4.2, R8.1, and R2.1 as described to the determination of races 4, 2 (5 or 6), and 8 (or 1). Amplified fragments of 295 bp and 564 bp corresponded to races 1 (or 8) and race 2, respectively. Since the primers used do not allow discrimination between these two near- isogenic races, therefore, the isogenic races were reported together. 44 and 23 isolates belonged to race 2 (5 or 6) and race 1 (or 8), respectively. None of the tested isolates be- longed to race 4 (Figure 4). Three isolates, which were collected from Tehran, Markazi, and Gilan (one isolate from each region), did not generate any amplification by using the tested primers and did not group in the mentioned VCGs (Table 1).

As a noticeable point, the pathogenicity symptoms caused by races 2 and 8 were different. Race 2 caused wilting symptoms on carnation cuttings after 28 days and caused complete plant death within 2 months. The symptoms of race 2 isolates mostly started as yellowing and wilting in the basal leaves of the plant and ended with the death of the plant. However, the isolates of race 8 caused the complete death of the plant within 4 months. Their symptoms first appeared on the plant after 40 days. The rate of yellowing and wilting in this race was lower than in race 2.

Furthermore, examining the isolates grouped in VCG and determining races showed that all isolates placed in VCG 0021 were race 2 (5 or 6) and all isolates belonging to VCG 0021 were race 1 (or 8). This result displays that different races have various characteristics that separate their VCG groups.

Discussion

In 2012, Cabanás et al., announced some isolates of *Fod* could show the *Fusarium* wilt on carnation in a pathogenicity test. In the experiment conducted by them, the symptoms caused by races 2, 8, and 4 were different. Although some isolates have been identified as *Fod* in RAPD molecular tests, they did not cause visible symptoms on the cuttings (Cabanás et al., 2012). In addition, in our study, the different races demonstrated different rate and severity of pathogenicity. In some other investigations, the different symptoms of *Fusarium* wilt between various races or isolates (without identification of races) have been reported (Basallote-Ureba et al., 2016); (Zhu et al., 2020); (Liu, Ji, Zhang, Wang, & Liu, 2020).

The result of *Fod* molecular identification in our study was in accordance with some previous studies. (Baayen et al., 1997) studied the identification and genetic diversity in *F.oxysporum* f.sp. *dianthi* and *F. redolens* f.sp. *dianthi* isolates in 1997 (Baayen et al., 1997), and reported *Fod* isolates showed approximately 600 bp on 1.2% agarose gel. In addition, Manicom et al., and Cabanás et al., reported this pathogen isolates displayed 550- 587 bp in molecular tests based on ITS regions (Manicom et al., 1990); (Chiocchetti et al., 1999); (Cabanás et al., 2012).

Some researchers believed that identification and classification of VCGs and races of *Fod* isolates are difficult due to the similarity of its characteristics with *F. redolens* f.sp. *dianthi* (Frd) (R. Baayen et al., 2000); (Bogale, Wingfield, Wingfield, & Steenkamp, 2007); (García-Núñez et al., 2017); (Nelson et al., 1983); (Quirico Migheli et al., 1998). Nevertheless, Waalwijk and Baayen in 1995 and Waalwjik et al., in 1996 showed that these two species were not even sisters and could be distinguished according to sequence differences in the ITS2 region of the rDNA (Waalwijk, de Koning, Baayen, & Gams, 1996). In their research, it was found that using the ITS index, it was found that six VCGs are related to Fod and four VCGs are related to Frd, and no similarity was observed among the members of those groups (Baayen et al., 1997). Therefore, since all the isolates collected for our research (except for three isolates) were grouped in

two relatively large groups in terms of race and VCG, it can be concluded that perhaps the Frd population in these areas was investigated, is very few or do not exist at all. However, according to the three mentioned isolates, it is not possible to declare the absence of Frd certainty.

In this study, we focused on the determination of VCGs and races in five three northern (Gilan, Golestan, and Mazandaran) and two central (Tehran and Markazi) provinces of Iran. In this research, it was found that there are VCGs 0021 and 0021 in Tehran, Gilan, Markazi, Golestan, and Mazandaran provinces. These VCGs are the groups that have been identified in almost every part of the world. In other studies, these groups have been announced in several countries of the world (Quirico Migheli et al., 1998); (R. Baayen et al., 2000); (Zandyavari et al., 2013); (Q Migheli et al., 1997); (Cabanás et al., 2012). However, in some countries and regions, VCG 0020 has also been reported (Quirico Migheli et al., 1998). In a previous study about Iran published in 2013 (Zandyavari et al., 2013), the 0020 group was also reported. Perhaps the absence of VCG 0020 in the recent study is because the investigated areas were more limited or because the genetic diversity of this pathogen has decreased in these areas due to the lack of import of new cuttings from other countries. Nevertheless, VCG 0021 is still the biggest group in Iran.

In these five regions, the largest population of races is related to race 2 (44 isolates) and the lowest population is related to race 8 (23 isolates). Race 2 is a race that has been reported everywhere in the world and always accounts for the largest population. This race can cause disease in different cultivars of cloves such as Pallas (R. Baayen et al., 2000); (Cabanás et al., 2012); (Q Migheli et al., 1997).

Since Europe is the center of *Dianthus caryophyllus* breeding, it is most likely the origin of VCG 0021 and race 2. In addition, because Europe is the exporter of this ornamental flower to the world, this race and VCG have been imported from Europe to other countries that produce this flower. Some VCGs, such as VCG 0028, which includes race 9, and the importance of its pathogenicity and spread are similar to VCG 0021 and race 2; only have been reported from Australia (Zandyavari et al., 2013); (Cabanás et al., 2012); (Bogale et al., 2007); (R. P. Baayen et al., 2000); (García-Núñez et al., 2017). Researchers believe that the origin of this VCG and breed in Australia may be from an unknown source or may have been affected by unknown mutations after being imported from Europe to Australia (García-Núñez et al., 2017); (Aloi & Baayen, 1993).

Since, according to reports, the Italian Flower Riviera has been grown traditionally in the province of Liguria, Italy, and sent to other regions and countries; some researchers believe that the origin of VCG 0022 and races 1 and 8 is from this region (Aloi & Baayen, 1993); (Waalwijk et al., 1996); (Prados-Ligero et al., 2007).

Moreover, although race 4 and VCG 0020 have been reported several times from some countries such as Spain, Italy, and Israel, a group of researchers attributes its origin to carnation-grown areas in America (Aloi & Baayen, 1993); (García-Núñez et al., 2017); (Locke, Marois, & Papavizas, 1985); (Waalwijk et al., 1996); (Cabanás et al., 2012).

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