Mycologia Iranica 1(2): 75 – 85, 2014 Original Article

Vegetative compatibility and rep-PCR DNA fingerprinting groups of *Fusarium solani* isolates obtained from different hosts and their pathogenicity

M. Fallahi
M. Javan-Nikkhah✉
Kh. B. Fotouhifar
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

M. Moradzadeh Eskandari
Plant Protection ResearchDepartment, Agricultural and Natural Resources Research Center of Khorasan-Razavi province, Mashhad, Iran

Abstract: *Fusarium solani* is the most important pathogen of a variety of host plants worldwide, especially potato, which causes tuber rot in storage and root rot of potato plants in the field. Fifty four isolates obtained from potato, common bean, chickpea and cucurbits (melon, watermelon and cucumber) were subjected to analysis of vegetative compatibility groups (VCGs) and rep-PCR DNA fingerprinting. *Nit* mutants were used to force heterokaryon formation to determine VCGs. Twenty three groups were determined, which were designated as VCG A to VCG W. VCG A was the largest group with 18 isolates and VCG B, VCG C and VCG D were composed of 8, 6 and 3 isolates, respectively. Other groups were identified as two or single-member VCGs. Presence of a high degree of single-member VCGs indicates the large amount of genetic diversity among isolates. Furthermore, the isolates of each host are classified in different VCGs. Dendrogram generated using data of rep-PCR, suggests a high level of genetic diversity among the isolates. No correlation was found between the DNA fingerprinting groups and host or geographical origin of the isolates. Pathogenicity of twenty three *F. solani* isolates as VCGs representatives originated from different hosts was examined on plants and tubers of Agria cultivar of potato. Except for four and two isolates, other isolates were pathogenic on potato plants and tubers, respectively. Pathogenicity tests demonstrated that *F. solani* isolates do not have a host specific behavior and isolates obtained from the non-potato hosts are able to cause disease on potato plants and tubers.

Key words: genetic diversity, *Nit* mutants, host specificity, molecular marker

INTRODUCTION

Fusarium solani (Mart.) Sacc. is one of the most frequently isolated fungi from soil and plant debris (Zhang et al. 2006). This species comprises phytopathogenic and saprophytic strains. Phytopathogenic strains are grouped in *formae speciales* based on their host specificity (Snyder & Hansen 1941, Sakurai & Matuo 1961, Roy 1997). The mentioned pathogen is one of the major causes of potato disease in Iran, but unfortunately, the specific association between the isolates of *F. solani* and potato plants has not been approved and no special host has been established for them (Moradzadeh Eskandari 2010). Specific forms of this fungus are not distinguishable based on the morphological characteristics (Suga et al. 2002). The separation and identification of specific forms and assessment of races diversity within a specific form, solely based on the pathogenicity tests is not accurate. These tests are often influenced by environmental variables, such as temperature, host age and inoculation methods (Correll 1991).

Due to the soil born nature and long-term survival of chlamydospores of this fungus in the soil (Hooker 1981), crop rotation strategy to control the disease cannot be effective. One of the primary goals of plant pathology has been to formulate strategies for disease control. These methods usually have been based on genetic resistance in the host plant or the application of chemical fungicides. Understanding of genetic diversity of fungi is very important to select the best Strategies (Leslie 1993).

Access to the information about the pathogenic variability and genetic transmission potential can be beneficial for researchers to understand the relationships between fungal species and resistance phenotype in breeding programs (McDonald & Linde 2002).

Hence, researchers have used genetic markers for more precise identification and characterization. Determination of vegetative compatibility groups can be a useful method for the identification of specific forms and the establishment of effective strategies for controlling the disease (McDonald & Linde 2002). Fungal isolates that anastomose and form heterok-

Submitted 15 February 2014, accepted for Publication 11 Oct. 2014
✉ Corresponding Author: E-mail: jnikkhah@ut.ac.ir

http://mi.iranjournals.ir

aryons with one another are considered to be vegetative compatible and assigned to a single vegetative compatible group (VCG). Conversely, isolates that are incapable of anastomosing and therefore fail to establish heterokaryons are referred to as vegetative incompatible isolates (Anagnostakis 1982).

Correlations between VCGs and other characters such as pathogenicity could lead to effectual diagnostics (Leslie 1993). On the other hand, due to the existence of self-incompatible isolates in the fungus populations and inability of vegetative compatibility groups in the expression of genetic similarity between these isolates, using more powerful tools is essential to overcome the existing limitations. Thus, the use of molecular markers based on PCR has been considered for this purpose. Repetitive-sequence based polymerase chain reaction (rep-PCR) is based on PCR-mediated amplification of DNA sequences located between specific interspersed repeated sequences in prokaryotic genomes. These repeated elements are termed as BOX, REP and ERIC elements. The objectives of this study were determination of vegetative compatibility groups to clarify the genetic diversity among *F. solani* isolates from different hosts and comparison of the pathogenicity of vegetative compatibility groups' representatives on potato plants in glasshouse and on potato tubers in laboratory to examine the host specificity of the isolates, as well as comparison of vegetative compatibility groups with rep-PCR.

MATERIALS AND METHODS

Fungal isolates

Fifty four isolates of *F. solani* including twenty nine isolates from potato (*Solanum tuberosum*), nine isolates from chickpea (*Cicera rietinum*), nine isolates from common bean (*Phaseolus vulgaris*), two isolates from cucumber (*Cucumis sativus*), three isolates from watermelon (*Citrullus lanatus*), two isolates from melon (*Cucumis melo* var. *inodorus*) collected from different regions of Khorasan-Razavi province in east northern Iran were selected for VCG determination, rep-PCR DNA and pathogenicity tests.

Media

Minimal medium (MM) was prepared by adding 2 g of $NaNO_3$ in l L basal medium (Correll et al. 1987). In an initial test, chlorate-resistant mutants were generated on the medium amended with chlorate ($KClO_3$). In this study, we used three chlorate-containing media, including MMC (MM medium with Chlorate), PDC (Potato Dextrose Agar with Chlorate) and CDAC (Czapek medium with Chlorate). To determine the suitable amount of $KClO_3$, 15, 20, 30, 50 and 70 g/l $KClO_3$ in PDA, MM and CDA was tested. Extremely, 50-70 g/l $KClO_3$ was selected so that MMC was prepared by adding 50-70 g/l $KClO_3$ to 1 L MM medium, PDC was made by adding 39 g PDA, 5 g Davis agar and 50-70 g/l $KClO_3$ to 1 L distilled water and CDAC was prepared by adding 39 g CDA and 50-70 g/l $KClO_3$ in 1 L distilled water.

Generation and characterization of *Nit* mutants

For each isolate, mycelium was transferred from PDA cultures to the medium containing $KClO_3$. Growth of wild-type strains of *F. solani* was restricted by chlorate on MMC according to Correll et al. (1986). The plates were incubated at 24 ± 2°C in the dark for 10-14 days. Then, the rapidly expanding sectors growing away from the restricted growth zone were transferred to MM, and those that grew as the thin expansive colonies with no aerial mycelium on MM were considered as *nit* mutants.

The physiological phenotypes of *nit* mutants were established by growing them on media containing one of the four different nitrogen sources (nitrate, nitrite, hypoxanthine and ammonium tartrate) following Correll et al. (1987) (Table 1).

Complementation tests

Complementation tests were conducted on MM. Three 2 mm^2 agar blocks containing a *Nit* M mutant grown on MM were placed equidistantly apart across the center of the Petri dish, and agar blocks of *nit* 1 or *nit* 3 mutants grown on MM were placed at the three matching positions, opposing the *Nit* M blocks in two rows and 1.5 mm on either side of the *Nit* M blocks. This arrangement provided for complementation between different *nit* 1 or *nit* 3 mutants and a single *Nit* M mutant. Complementation indicating heterokaryon formation was recognized as a line of dense aerial mycelial growth where two *nit* mutants met together on MM (Correll et al. 1987).

Table 1. Identification of *nit* mutants of *Fusarium solani* isolates by growing them on different nitrogen sources (Correl et al. 1987).

Mutant designation	Medium supplement				
	Nitrate	Nitrite	Ammonium	Hypoxanthine	Chlorate
Wild type	+	+	+	+	-
nit 1	-	+	+	+	+
nit 3	-	-	+	+	+
Nit M	-	+	+	-	+
*Crn**	+	+	+	+	+

+: Typical wild-type growing, -: Thin growing with no aerial mycelium, *Crn**: chlorate resistant utilizing nitrate

DNA extraction

Three to four mycelia plugs (each 4 mm in diameter) from PDA cultures were transferred to flasks containing 60 ml of potato dextrose broth, and incubated at room temperature for 6–8 days. Mycelial mass was filtered through a filter paper, washed three times with sterile water, air-dried and then frozen at -80°C and lyophilized prior to use. Lyophilized mycelia were ground in liquid nitrogen into a fine powder with a mortar and pestle. Fungal genomic DNA was extracted using Core-one ^{tm} Plant Genomic DNA isolation Kit (Core Bio, Korea) following the manufacturers' instructions.

Rep-PCR analysis

Rep-PCR reaction was performed using BOX primer (1A-1R 5'-CTACGGCAAGGCGACGCTG ACG-3') (McDonald et al. 2000). PCR was carried out using PCR Master Mix kit (CinnaGen PCR Master Kit). Each reaction consisted of the following components: 10 μL of PCR Master Mix (containing 1 U of *Taq* DNA polymerase, 2 μL PCR buffer, 3 mM $MgCl_2$ and 0.25 mM of each dNTPs), 10 pM primer, 3 μL (30 ng/μl) of fungal genomic DNA and 5.5 μL distilled water in a final reaction volume of 20 μL. PCR was performed in a palm Thermal Cycler (CG1-96, Corbett Research, Australia) with the following PCR program. An initial denaturation step of 5 minutes at 95°C followed by 35 cycles of denaturation at 94°C for 3s and 92°C for 30s, 1 min of annealing at 49°C, extension for 7 min at 72°C and a final extension for 10 min at 72°C.

Data analysis

To determine the genetic relationships among the isolates, the presence or absence of DNA bands was converted into binary data (1 for presence and 0 for absence of each band). Similarity matrix was calculated with Dice's coefficient and the SIMQUAL program of NTSYSpc, ver. 2.1. Cluster analysis was carried out within the SAHN program by using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean).

Virulence of VCGs representatives on potato plants in greenhouse

For greenhouse pathogenicity tests, spore suspensions were created following Romberg & Davis (2007). Macroconidia and microconidia were harvested from 2-week-old cultures grown on PDA at 25°C by adding three ml of sterile water to the plates and scraping the surface of the agar plate with a sterile glass slide. The resulting conidial or mycelial suspension was filtered through eight layers of cheesecloth to remove the mycelial fragments. Conidial concentrations were calculated using a hem cytometer and diluted in water to a concentration of 10^6 macro- and microconidia/ml for pathogenicity tests. The pathogenicity of twenty three *F. solani* isolates obtained from potato**,** common bean**,** chickpea, cucumber, watermelon and melon as VCGs representatives was determined on healthy 8-10 cm-tall potato seedlings of Agria cv., the most frequently potato cultivar in all potato growing regions of Khorasan-Razavi province, Iran. After trimming the roots, the entire root system was immersed in a 10^6 macro- and microconidia/ml suspension of each isolate. Each seedling was transplanted into three separate 15-cm-diameter pots containing sterile soil (5:3:2:1 mixture of farm soil, sand, animal manure and compost). Each treatment was replicated three times. The inoculated plants were maintained in greenhouse at 25°C to 28°C for 45- 50 days, and then removed from the pots. Then, they were scored for disease severity based on a 0–3 scale (0 = No disease, 1 = mild root rot, 2 = vascular discoloration of the root and crown, 3 = vascular discoloration of the root, crown and stem) (Moradzadeh Eskandari 2010).

Virulence of VCGs representatives on potato tubers under lab conditions

Tubers of Agria cv. were used to determine the virulence of the same VCGs representatives as those used in the pathogenicity tests on plants. Initially, the tubers appearing healthy and uniform in size (100-120 g) were selected and washed to remove excess soil, surface sterilized in 0.5% sodium hypochlorite solution for 10 min, rinsed with three changes of sterile distilled water and then air dried. Afterwards, the tubers were wounded with a four mm-diameter cork borer to a depth of four mm (Theron & Holz 1989) and inoculated with all of the fresh *F. solani* mycelia (from 1-week-fresh cultures grown on PDA at 25°C) by putting 7 mm PDA blocks. All the wounded potato tubers were wrapped in paper bags (Manici & Cerato 1994) and incubated at 20°C under dark conditions for three weeks. Blocks of PDA medium were used as control. Each treatment was replicated three times. At the end of incubation, tubers were cut through the inoculation points, and the degree of rot was estimated based on a zero to five (or A-F) scale, basically according to Theron and Holz (1989): 0. (A) - Limited discoloration, no extended dry rot in inoculated areas; 1. (B) - Limited discoloration with the development of dry rot in inoculated areas; 2. (C) - Extensive discoloration with increased dry rot in inoculated areas; 3. (D) - Extensive discoloration with extensive dry rot in inoculated areas; 4. (E) - discoloration and very extensive dry rot, tubers not disappearing completely; 5. (F) - discoloration and very extensive dry rot, tubers disappearing completely.

RESULTS

Determination of VCGs

Production of chlorate-resistant sectors was very low on MMC, PDC and CDAC containing 15 g/l $KC10_3$, but after increasing the concentration of

$KClO_3$ in the three media to 50-70 g/l, most of the isolates readily formed chlorate-resistant sectors (Fig. 1a). Totally, fifty two isolates produced chlorate-resistant sectors. These sectors were then transferred to MM containing $NaNO_3$ as the sole nitrogen source. The sectors with thin expansive growth on MM were considered as *nit* mutants. A few sectors resistant to chlorate were recovered, but they had wild type colony morphology on MM. Such mutants are known as Chlorate resistant isolates utilizing nitrate (Crn) (Bowden & Leslie 1992).

Four different nitrogen sources were used in order to identify phenotypic classes. Three classes of *nit* mutants were recovered representing mutations at a nitrate reductase structural locus (*nit* l, unable to utilize nitrate), a nitrate-assimilation pathway-specific regulatory locus (*nit* 3, unable to utilize nitrate or nitrite) and loci affecting the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (*Nit* M, unable to utilize nitrate, hypoxanthine or uric acid). The frequency of *nit* 1, *nit* 3 and *Nit* M phenotype was 44.74%, 36.84% and 18.42%, respectively. *Nit* l mutants were recovered at a higher frequency than *nit* 3 and *Nit* M mutants.

Complementation between *nit* mutants was indicated by the development of a dense aerial growth where the mycelia of the colonies grew together and anastomosed. When *Nit* M mutants were paired-or were involved in the pairing, complementation occurred more rapidly than those of other *nit* mutant pairs. When *nit* 1 and *nit* 3 mutants were paired, weak vegetative compatibility reactions were obtained. Those isolates that had two mutants available for complementation tests, and had positive intra-strain complementation tests were classified as heterokaryon self-compatible (HSC) and those with negative intra strain complementation tests were considered as heterokaryon self-incompatible (HSI). One of the 52 isolates was HSI. Six multi-strain VCGs were identified among the HSC strains (Fig. 1b). These groups were designated as VCG A to VCG W. VCG A as the largest group had 18 members and VCGs B, C and D comprised of 8, 6 and 3 members, respectively. Each of the VCGs E and F consisted of two members. Seventeen isolates out of 51 HSC isolates were incompatible with all of the other isolates and therefore were considered as single-strain VCGs with designation as VCG G to VCG W. Generally, isolates of each of the VCGs originated from different hosts and usually from different regions (Table 2). VCG A included fourteen isolates of potato, three isolates of chickpea and one isolate of common bean, which belonged to several regions (Chenaran, Sabzevar, Fariman, Torbat-e Heydarieh, Quchan, Nishapur, Mashhad and Birjand). VCG B consisted of seven isolates of potato and one isolate of common bean, obtained from four regions (Chenaran, Fariman, Torbat-e Heydarieh and Mashhad). Three isolates of common bean, one isolate of potato, one isolate of chickpea and one isolate of melon were grouped in VCG C. The members of this VCG were obtained from six regions (Chenaran, Torbat-e Heydarieh, Mashhad, Faruj, Kashmar and Ardabil). VCG D contained 3 isolates of three host plants (potato, common bean and chickpea) and three different regions (Kashmar, Qaen and Torbat-e Heydarieh). Two isolates of chickpea from two different regions (Faruj and Mashhad) were grouped in VCG E. VCG F contained 2 isolate of potato, obtained from Birjand. Therefore, there was no correlation between VCGs and hosts and geographical origin of the isolates (Fig. 1c).

Rep-PCR DNA fingerprinting

Totally, 37 fragments were amplified using BOX primer, ranging in size from 2000 to 3500 bp, all of which were polymorphic (Fig. 2). Cluster analysis revealed that all of the isolates examined were placed in two major fingerprinting groups (designated as Fo_1 and Fo_2) with at least 30% genetic similarity (Fig. 3). Table 3 exhibits diversity of VCGs according to rep-PCR analysis using BOX primer.

Pathogenicity test of VCG representatives on potato tubers revealed that among 23 VCGs representatives tested, 21 isolates showed various virulence and only isolates CH-25 (VCG E) and FW-52 (VCG T) caused no symptoms on tubers of Agria cv. Isolates Fpo-5 (VCG B), Fpo-70 (VCG I) and Fpo-69 (VCG A) from potato possessed the highest virulence (Fig. 5). Details of these tests and isolates grouping are given in table 4.

Fig.1. **(a)** Production of chlorate-resistant sectors on PDA containing 70 g/l $KClO_3$ in the isolate FCV-21 of *Fusarium solani* at 25°C, under dark conditions after 15 days. **(b)** Heterokaryon formation on minimal medium between *nit* 1 and *Nit* M mutants in the isolate C-96 of *F. solani* at 25°C, under dark conditions after 10 days. **(c)** Complementation between *nit* 3 and *Nit* M mutants of *F. solani* isolates on minimal medium, and heterokaryon formation.

Table 2. Characteristics of *nit* mutants of *Fusarium solani* isolates obtained from different host plants in different regions of Khorasan-Razavi province, Iran.

Isolates	Location[a]	Year	Host	Phenotype of nit mutants			VCG [b]
				A	B	C	
2	Quchan	2004	Potato	*nit* 1	*nit* 3	-	A
3	Fariman	2004	Potato	*nit* 1	*nit* 3	*Nit* M	J
10	Fariman	2004	Potato	*nit* 1	*nit* 3	*Nit* M	B
12	Nishapur	2004	Potato	*nit* 1	*nit* 3	-	A
13	Torbat-e Heydarieh	2004	Potato	*nit* 1	*nit* 3	*Nit* M	A
14	Quchan	2004	Potato	*nit* 1	*nit* 3	*Nit* M	A
16	Quchan	2004	Potato	*nit* 1	-	*Nit* M	K
19	Mashhad	2004	Potato	*nit* 1	*nit* 3	*Nit* M	B
20	Torbat-e Heydarieh	2004	Potato	*nit* 1	*nit* 3	*Nit* M	L
F-132	Ardabil	2004	Potato	*nit* 1	*nit* 3	-	A
Fpo-1	Chenaran	2007	Potato	*nit* 1	*nit* 3	-	C
Fpo-9	Birjand	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-19	Birjand	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-8	Birjand	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-16	Birjand	2007	Potato	*nit* 1	*nit* 3	*Nit* M	F
Fpo-45	Sabzevar	2007	Potato	*nit* 1	-	*Nit* M	A
Fpo-44	Chenaran	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-7	Birjand	2007	Potato	*nit* 1	*nit* 3	*Nit* M	B
Fpo-67	Bojnord	2007	Potato	*nit* 1	*nit* 3	-	F
Fpo-62	Fariman	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-60	Quchan	2007	Potato	*nit* 1	*nit* 3	-	G
Fpo-54	Chenaran	2007	Potato	*nit* 1	*nit* 3	-	A
Fpo-69	Esfarayen	2007	Potato	*nit* 1	*nit* 3	-	B,A
Fpo-70	Quchan	2007	Potato	*nit* 1	-	*Nit* M	A
Fpo-74	Torbat-e Jam	2007	Potato	*nit* 1	*nit* 3	-	I
Fpo-76	Fariman	2007	Potato	*nit* 1	*nit* 3	-	H
Fpo-87	Fariman	2007	Potato	*nit* 1	*nit* 3	-	B
Fpo-22	Qaen	2007	Potato	*nit* 1	-	-	B,A
Fpo-5	Chenaran	2007	Potato	*nit* 1	-	*Nit* M	D
LM-WM	Kashmar	2006	Common Bean	*nit* 1	*nit* 3	-	B
LM-13b	Mashhad	2006	Common Bean	*nit* 1	-	*Nit* M	C
LM-23	Chenaran	2006	Common Bean	*nit* 1	-	*Nit* M	C
LM-25	Torbat-e Jam	2006	Common Bean	*nit* 1	*nit* 3	-	B,C
LM-26	Bojnord	2006	Common Bean	*nit* 1	*nit* 3	-	V
LM-40	Bojnord	2006	Common Bean	*nit* 1	*nit* 3	-	Q
LM-6	Fariman	2006	Common Bean	*nit* 1	*nit* 3	*Nit* M	S
LM-11	Mashhad	2006	Common Bean	*nit* 1	*nit* 3	-	R
LM-28	Bojnord	2006	Common Bean	*nit* 1	*nit* 3	-	-
CH-2	Torbat-e Jam	2005	Chickpea	*nit* 1	-	*Nit* M	D
CH-5	Mashhad	2005	Chickpea	*nit* 1	-	-	E
CH-7	Fariman	2005	Chickpea	*nit* 1	*nit* 3	*Nit* M	F
CH-14	Mashhad	2005	Chickpea	*nit* 1	*nit* 3	-	A
CH-25	Faruj	2005	Chickpea	*nit* 1	-	*Nit* M	E
CH-4	Chenaran	2005	Chickpea	*nit* 1	-	*Nit* M	A
CH-KH	Khvaf	2005	Chickpea	*nit* 1	*nit* 3	*Nit* M	O
CH-31	Rashtkhvar	2005	Chickpea	-	-	-	-
FCV-21	Khorasan-Razavi	2006	Chickpea	*nit* 1	*nit* 3	-	A
C-96	Jajrom	2006	Cucumber	*nit* 1	-	*Nit* M	U
C-89	Esfarayen	2006	Cucumber	*nit* 1	-	-	M
C-102	Kashmar	2006	Melon	*nit* 1	*nit* 3	-	C
C-120	Mashhad	2006	Melon	*nit* 1	*nit* 3	-	W
FW-52	Fariman	2002	Watermelon	*nit* 1	*nit* 3	-	T
FW-54	Nishapur	2002	Watermelon	*nit* 1	*nit* 3	-	P
C-67	Jovin	2002	Watermelon	-	*nit* 3	-	-

[a] Location = different regions of Khorasan-Razavi province, Iran
[b] VCG = vegetative compatibility groups (totally, 54 isolates were grouped in 23 VCGs based on vegetative compatibility tests by pairing *nit* mutants)

Rep-PCR DNA fingerprinting

Totally, 37 fragments were amplified using BOX primer, ranging in size from 2000 to 3500 bp, all of which were polymorphic (Fig. 2). Cluster analysis revealed that all of the isolates examined were placed in two major fingerprinting groups (designated as Fo_1 and Fo_2) with at least 30% genetic similarity (Fig. 3). Table 3 exhibits diversity of VCGs according to rep-PCR analysis using BOX primer.

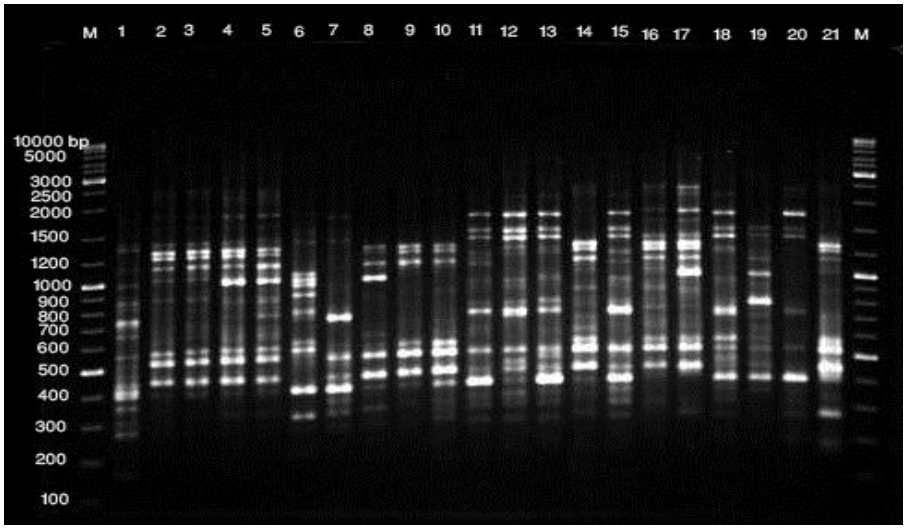

Fig. 2. Electrophoretic patterns on 1.4 % agarose gel of amplified fragments generated from the 21 isolates of *Fusarium solani* by rep-PCR, using BOX-primer. Lane M is the 1 kb DNA ladder; Lanes1-20; *F. solani* isolates (16, Fpo-5, Fpo-69, Fpo-9, Fpo-74, CH-25, LM-40, 12, Fpo-67, Fpo-87, C-102, 14, FW-52, Fpo-76, LM-23, Fpo-44, Fpo-16, LM-25, C-67, CH-2 and Fpo-8).

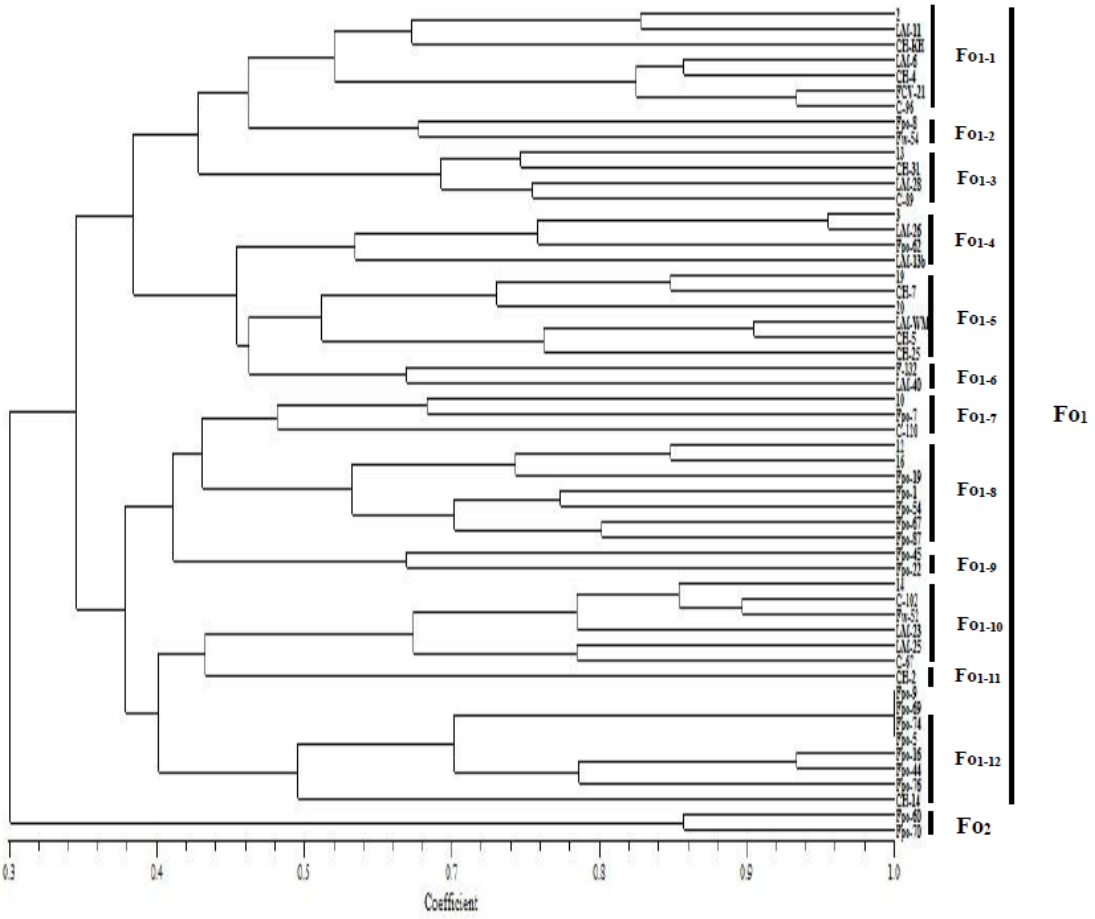

Fig. 3. Dendrogram constructed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on the data of rep-PCR using BOX primer, indicating relationships among *Fusarium solani* isolates obtained from different host plants. Similarity matrix was produced by Dice`s coefficient. Defined main groups, designated as Fo_1 and Fo_2 and subgroups of Fo_1, designated as $Fo_{1\text{-}1}$ to $Fo_{1\text{-}12}$ are indicated on the right.

Table 3. Diversity of *Fusarium solani* VCG groups according to rep-PCR DNA fingerprinting analysis, using BOX primer.

Fingerprinting group	Fingerprinting subgroup	No. of isolates	VCGs	Hosts	Location[a]
Fo_1	Fo_{1-1}	7	VCG A, VCG R, VCG O, VCG S, VCG U	Potato, Common Bean, Chickpea, Cucumbers, Watermelon	Mashhad, Khaf, Fariman, Chenaran and Quchan
	Fo_{1-2}	2	VCG F, VCG P	Potato, Watermelon	Birjandand Nishapur
	Fo_{1-3}	7	VCG A, VCG M	Potato, Common Bean, Chickpea, Cucumbers	Nishapur, Torbat-e Jam, Mashhad, Jajrom, Esfarayen
	Fo_{1-4}	4	VCG J, VCG V, VCG G, VCG C	Potato, Common Bean	Mashhad, Fariman, Bojnord
	Fo_{1-5}	6	VCG B, VCG N, VCG L, VCG D, VCG E	Potato, Common Bean, Chickpea	Mashhad, Fariman, Kashmar, Torbat-e Jam
	Fo_{1-6}	2	VCG C, VCG Q	Potato, Common Bean	Bojnord, Ardabil
	Fo_{1-7}	3	VCG B, VCG F, VCG W	Potato, Watermelon	Mashhad, Fariman, Mashhad
	Fo_{1-8}	7	VCG A, VCGB, VCG K	Potato	Nishapur, Bojnord, Birjand, Chenaran, Fariman, Sabzevar, Qaen
	Fo_{1-9}	2	VCG A, VCG D	Potato	Sabzevar, Qaen
	Fo_{1-10}	6	VCG A, VCG B, VCG C, VCG T	Potato, Common Bean, Melons, Watermelon	Kashmar, Fariman, Chenaran, Torbat-e Heydarieh, Bojnord
	Fo_{1-11}	1	VCG D	Chickpea	Torbat-e Heydarieh
	Fo_{1-12}	8	VCG A, VCG B, VCG H	Chickpea, Potato	Chenaran, Esfarayen, Torbat-e Jam, Birjand
Fo_2	-	2	VCG A and VCG I	Potato	Qochan

a. Sampling regions in Khorasan-Razavi province, Iran. Each region is a town.

Fig. 4. Pathogenicity of *Fusarium solani* isolates on potato plants in greenhouse at 25°C. (**a**) Isolate Fpo-74 obtained from potato (**b**) Isolate CH-25 obtained from chickpea. In each Fig., the infected plant is on the right side and the healthy plant is on the left side. Photographs were taken 30 days after inoculation.

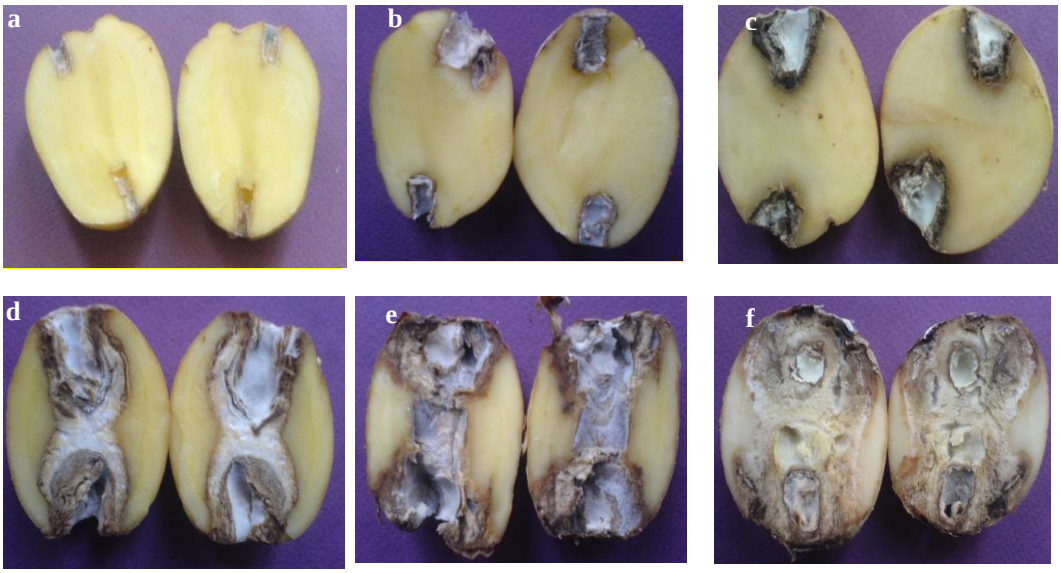

Fig. 5. Tuber rot caused by *Fusarium solani* isolates three weeks after inoculation at 20˚C under dark conditions in the lab. (**a**) No dry rot on potato by the isolate CH-25 obtained from chickpea, with zero disease scale, **(b-f)** Dry rot on potato by the isolates LM-6, CH-7, Fpo-8, Fpo-69 and Fpo-5 with disease scales of 1, 2, 3, 4 and 5, respectively.

Table 4. Pathogenicity of *Fusarium solani* isolates (VCG representatives) on potato plants in greenhouse and on potato tubers under lab conditions.

Isolates	Host	VCG	Disease index on potato plants[a]	Disease severity average on potato tubers [b]
3	Potato	J	0	1
16	Potato	K	1	0.7
20	Potato	L	0	0.7
Fpo-8	Potato	F	2	2.7
Fpo-62	Potato	G	1	2
Fpo-74	Potato	H	3	2
Fpo-70	Potato	I	3	4.3
Fpo-22	Potato	D	1	2.7
Fpo-69	Potato	A	2	4
Fpo-5	Potato	B	3	4.7
LM-13b	Common Bean	C	0	0.4
LM-40	Common Bean	Q	1	0.7
LM-11	Common Bean	R	0	2.5
LM-6	Common Bean	S	1	0.7
LM-26	Common Bean	V	2	0.7
CH-25	Chickpea	E	1	0
CH-7	Chickpea	N	1	2.5
CH-KH	Chickpea	O	1	2.7
C-89	Cucumbers	M	1	4
C-96	Cucumbers	U	1	2.5
C-120	Melons	W	1	2.5
FW-52	Watermelon	T	1	0
FW-54	Watermelon	P	2	2.5

[a] Disease index value on potato plants obtained from three replicates with 3 plants 45 days post-inoculation, 0 = no disease; 1= mild infection; 2 = severe infection; 3 = 100% infection.

[b] Disease severity average on potato tubers based on Theron & Holz (1987) index (b_x) (x = replicate number) obtained from three replicates with 3 tubers 3 week post- inoculation.

$b = (b_1 + b_2 + b_3)/3$

DISCUSSION

Vegetative compatibility groups (VCGs) have been used to examine diversity and population structure in many fungi. Initially, Puhalla (1985) found that there was a correlation between VCG and *forma specialis*. Isolates in the same VCGs belonged to the same *forma specialis* and strains in different *formae speciales* were grouped in different VCGs (Correll 1991).

Vegetative compatibility has been tested in many fungi using nitrate non-utilizing (nit) mutants to demonstrate heterokaryosis (Leslie 1993). Various factors such as temperature, nutrition and the kind of fungus influence generation of $KClO_3$ resistant sectors in the fungi (Klittich & Leslie 1988). *Fusarium solani* strains are more tolerant to chlorate than most strains of *F. oxysporum* and *F. moniliform* (Correll et al. 1987, Klittich & Leslie 1988). In the present study, most *nit* mutants were recovered on PDA containing 50-70 g/l $KClO_3$.

Identification of phenotypes of *nit* mutants showed that *nit* 1 and *Nit* M have highest and lowest frequencies, respectively. Frequency of *nit* mutations in fungi can be influenced by the nitrogen source in medium containing potassium chlorate. Hawthorne et al. (1996) showed that by adding thereonine to the medium containing chlorate, *Nit* M of the species *F. solani* increases.

In this study, *Nit* mutants were obtained for 52 out of 54 isolates of *F. solani*. One out of 52 isolates was heterokaryon self- incompatible (HIS). Hawthorne et al. (1996) reported that there are many self-incompatible isolates among different isolates of *F. solani*, but the importance of self-incompatibility in nature is uncertain. Correll et al. (1989) concluded that HSI in *Gibberella fujikuroi* (*F. monilifome*) was under control of a single gene, and Jacobson and Gordon (1990) suggested that it could be a mutant artifact that arises in fungi maintaining for long periods in an artificial culture.

Fifty one heterokaryon self-compatible (HSC) isolates were grouped in 23 VCGs. Seventeen of 51 HSC isolates were incompatible with all the other isolates, and therefore were regarded as single-member VCGs. Presence of high single-member VCGs indicates the high level of genetic diversity among the isolates. Distribution of *F. solani* isolates obtained from various areas and host plants into different VCGs was observed in the present study. For example, the isolates belonging to VCG A were obtained from potato, common bean and chickpea, and collected from eight regions. VCG D included the isolates from potato, common bean, cucumber and melon, collected from three regions. Besides, only VCG E and VCG F contained the isolates from the same host. VCG E consisted of two isolates from chickpea, and VCG F contained two isolates from potato. Comparison between the isolates from different VCGs and their host origins showed that the isolates from the same host were classified in different VCGs, and had a considerable amount of genetic diversity. Therefore, except for some cases, VCGs grouping in the present study could not separate isolates, especially the potato isolates that had the most frequency in this study based on the host and geographical origin. But in some other studies, VCG grouping could separate isolates of *F. solani*

based on the host origin. For instance, Hawthorn et al. (1996) placed 57 *F. solani* isolates in 35 VCGs, and reported a direct relationship between VCG and host origin of the isolates. Also, there was no correlation between VCG grouping and geographical origins of the isolates in the present study, except for VCG F with two members that were collected from the same region. On the other hand, the isolates of different geographical areas were placed in the same VCG. This suggests the possibility of distribution of the fungus through infected tubers and agricultural equipment, wind and rain in different regions. According to Elmer (1991), existence of the isolates from different regions in the same VCG, or in the other words, existence of a specific VCG in several areas indicates the selective survival of vegetative compatibility groups. This result was similar to the results of Raouffi et al. (2004) and Mohammadi & Banihashemi (2005). On the other hand, some studies have demonstrated the correlation between vegetative compatibility groups and geographical origins. Rahkhodaei (2000) reported that isolates of *F. solani* from potato in each VCG were collected from the same region.

Dendrogram generated using data of BOX primers showed that 54 isolates collected from different regions of Khorasan-Razavi province and from different hosts showed >30% similarity. This result suggests a high level of genetic diversity among the isolates. There was no correlation between the identified fingerprinting groups and host and geographical origins of the fungal isolates. These results are in agreement with results from the previous studies on *F. solani* (Moradzadeh Eskandari 2010, Romberg & Davis 2007, Baghai Raveri et al. 2007). Missing relationships between DNA fingerprinting groups and VCGs observed in this study showed that it could be impossible to separate the isolates based on VCGs using BOX primer. Using VCG grouping and rep-PCR in the present study showed the genetic diversity within *F. solani* isolates, but both methods were unable to classify the isolates based on the host origin. Therefore, using other methods such as investigation of mating populations will generate further information about the host population of *F. solani*. Pathogenicity test of twenty three *F. solani* isolates obtained from different hosts as VCGs representatives on potato plants showed that there was no relation between the host origin and virulence of the isolates, so that two out of four isolates in pathogenicity zero group belonged to potato host. In contrast, non-potato isolates in some cases had more virulence than potato isolates, and were comparable with potato isolates in severity of symptoms. Also, the pathogenicity of twenty three isolates as VCGs representatives on potato tubers showed that except VCG T and VCG E representtatives (from non-potato hosts), the rest of VCGs representatives were able to cause dry rot on potato tubers with diverse virulence. This result confirmed the results of their pathogenicity on potato plants. In a way, the isolates tested in both pathogenicity tests, regardless of the host origin were grouped in different pathogenicity groups. These results indicated that the pathogenicity of *F. solani* isolates was not host specific and the isolates obtained from non-potato hosts were able to cause disease on potato plants and tubers. On the other hand, the isolates obtained from potato showed no symptom on potato plants. This result confirmed the results of previous studies (Moradzadeh Eskandari 2010, Romberg & Davis 2007).

ACKNOWLEDGMENTS

We gratefully acknowledge the High Council for Research of University of Tehran for financially support and providing materials under grant number 7110024/6/24.

REFERENCES

Anagnostakis SL. 1982. Genetic analysis of *Endothia parasitica*, linkage data for four single genes and three vegetative compatibility types. Genetics 102: 25-28.

Baghai Raveri S, Falahati Rastegar M, Jafarpor B, Shokohifar F, Moradzadeh Eskandari M. 2007. DNA fingerprinting of *Fusarium solani* isolates causing wilt and dry rot of potato in Razavi and Northern Khorasan provinces using molecular markers based on PCR. Iranian Journal of Plant Pathology 42: 417-437.

Bowden RL, Leslie JF. 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Experimental Mycology 16: 308–315.

Correll JC. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology 81: 1061-1064.

Correll JC, Klittich CJ, Leslie JF. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycological Research 93: 21-27.

Correll JC, Klittich CJ, Leslie JF. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility test. Phytopathology 77: 1640-1646.

Correll JC, Puhalla JE, Schneider RW. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence and vegetative compatibility. Phytopathology 76: 396-400.

Elmer WH. 1991. Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates and colonization of asparagus residues among groups. Phytopathology 81: 852-857.

Hawthorne BT, Ball RD, Rees-George J. 1996. Use of nitrate non-utilizing mutants to study vegetative incompatibility in *Fusarium solani* (*Nectria*

haematococca) especially members of mating populations I, V and VI. Mycological Research 100: 1075-1081.

Hooker WJ. 1981. Compendium of potato diseases. The American Phytopathological Society Press 125 pp.

Jacobson DJ, Gordon TR. 1990. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. Mycological Research 94: 734-744.

Klittich CJ, Leslie JF. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118: 417-423.

Leslie JF. 1993. Fungal vegetative compatibility. Annual Review of Phytopathology 31: 12–151.

Manici LM, Cerato C. 1994. Pathogenecity of *Fusarium oxysporum* f. sp. *tuberosi* isolates from tubers and potato plants. Potato Research 37: 129-134.

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

McDonald JG, Wong E, White GP. 2000. Differentiation of *Tilletia* species by rep-PCR genomic fingerprinting. Plant Disease 84: 1121–1125.

Mohammadi H, Banihashemi Z. 2005. Distribution, pathogenicity and survival of *Fusarium* spp. the causal agents of chickpea wilt and root rot in Fars province. Iranian Journal of Plant Pathology. 41: 687-706.

Moradzadeh Eskandari M. 2010. Study on the population structure of *Fusarium solani* isolated from potato in Khorasan provinces, determination of phylogenetic relationship among some of its formae speciales. Ph.D thesis, University of Tehran, Iran 150 pp.

Puhalla JE. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Canadian Journal of Botany 63: 179-183.

Rahkhodaei E. 2000. Vegetative compatibility groups and pathogenicity of *Fusarium solani* and *Fusarium oxysporum* from potato in Fars and Khuzestan Provinces. M. Sc. Thesis, University of Shahid Chamran, Ahvaz, Iran 124 pp.

Raouffi M, Farrokhi-Nejad R, Mahmoudi SB. 2004. Population genetic diversity of *Fusarium solani* the causal agent of sugar beet root rot, using vegetative compatibility groups (VCGs) and its relationship to virulence of isolates. Sugar Beet 201: 39-53.

Romberg MK, Davis RM. 2007. Host range and phylogeny of *Fusarium solani* f. sp. *eumartii* from potato and tomato in California. Plant Disease 91: 585-592.

Roy KW. 1997. *Fusarium solani* on soybean roots, nomenclature of the causal agent of sudden death syndrome and identity and relevance of *F. solani* form B. Plant Disease 81: 259–266.

Sakurai Y, Matuo T. 1961. Taxonomy of the causal fungus of trunk-blight of *Xanthoxylum piperitum* and heterothallism in this fungus. Annals of the Phytopathological Society of Japan 26: 112–117.

Snyder WC, Hansen HN. 1941. The species concept in *Fusarium* with reference to Section *Martiella*. American Journal of Botany 28: 738-742.

Theron DJ, Holz G. 1989. *Fusarium* species associated with dry and stem-end rot of potatoes in South Africa. Phytophylactica 21: 175-181.

Suga H, Ikeda S, Taga M, Kageyama K, Hyakumachi M. 2002. Electrophoretic karyotyping and gene mapping of seven formae speciales in *Fusarium solani*. Current Genetic 41: 254-260.

Zhang N, O'Donnell K, Sutton DA, Nalim FA, Summerbell RC, Padhye AA, Geiser DM. 2006. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. Journal of Clinical Microbiology 44: 2186–2190.

گروه های سازگاری رویشی و انگشت نگاری DNA بر اساس rep-PCR در جدایه های *Fusarium solani* بدست آمده از میزبان های مختلف و بیماریزایی آنها

مریم فلاحی[1]، محمد جوان نیکخواه[1]، خلیل بردی فتوحی فر[1] و مجتبی مرادزاده اسکندری[2]

۱- گروه گیاهپزشکی، پردیس کشاورزی و منابع طبیعی دانشگاه تهران، کرج

۲- گروه گیاهپزشکی، مرکز تحقیقات کشاورزی و منابع طبیعی استان خراسان رضوی، مشهد

چکیده: قارچ *Fusarium solani* از مهمترین بیمارگرهای گیاهی می باشد که روی دامنه ی وسیعی از گیاهان از جمله سیب زمینی در دنیا ایجاد خسارت می نماید. این گونه به عنوان یکی از عوامل اصلی پوسیدگی ریشه و غده ی سیب زمینی در انبار و مزرعه محسوب می شود. به دلیل اهمیت این بیماری و شدت زیاد خسارت وارده، ۵۴ جدایه گونه *F. solani* از سیب زمینی، نخود، لوبیا، هندوانه، خربزه و خیار انتخاب شدند تا بر اساس شناسایی گروه های سازگاری رویشی و انگشت نگاری DNA بر اساس نشانگر rep-PCR مورد بررسی قرار گیرند. در این راستا شناسایی گروه های سازگاری رویشی بر اساس جداسازی جهش یافتگان *nit* و بررسی تشکیل هتروکاریون پایدار بین آنها انجام شد. در نهایت ۲۳ گروه سازگاری رویشی (VCG) بین جدایه های مورد بررسی مشخص شد. این گروه ها با اسامی VCG A تا VCG W نام گذاری شدند. گروه VCG A با ۱۸ عضو به عنوان بزرگترین گروه و VCG B، VCG C ، VCG D به ترتیب با هشت، شش و سه عضو مشخص شدند، همچنین دو گروه دو عضوی و ۱۷ گروه به عنوان گروه های تک عضوی شناسایی شدند. وجود تعداد زیاد گروه های تک عضوی نشان دهنده تنوع ژنتیکی نسبتاً زیاد در بین جدایه های مورد مطالعه بود. از طرفی بین منشا میزبانی جدایه ها با گروه های سازگار رویشی مشخص شده، ارتباطی مشاهده نشد و جدایه های مربوط به هر میزبان در گروه های مختلف VCG قرار گرفتند. بررسی دندروگرام رسم شده بر اساس نشانگر rep-PCR نیز نشان دهنده ی تنوع ژنتیکی بالا در جدایه های مورد بررسی در این مطالعه بود. و با توجه به نتایج در این بخش، همبستگی مشخصی بین منشاء جغرافیایی و میزبانی جدایه ها با گروه های مشخص شده با نشانگر rep-PCR مشاهده نشد. همچنین آزمون بیماریزایی ۲۳ جدایه منتخب گروه های سازگار رویشی روی بوته و غده ی سیب زمینی رقم آگریا نشان داد که به ترتیب به جز چهار و دو جدایه که علایمی روی بوته و غده ی سیب زمینی نشان ندادند و در گروه صفر قرار گرفتند، بقیه نمایندگان گروه های VCG حداقل توانایی ایجاد پوسیدگی خفیف ریشه و کاهش رشد در بوته و پوسیدگی خشک در غده سیب زمینی را دارا بودند. این نتایج نشان دهنده ی عدم اختصاصیت میزبانی در جدایه های مورد آزمون بود. چرا که در بین این جدایه ها عدم بیماریزایی جدایه هایی از میزبان سیب زمینی روی گیاه سیب زمینی وجود داشت، از طرف دیگر، جدایه های از میزبان غیر سیب زمینی قادر بودند شدت زیادی از بیماری را روی گیاه و غده سیب زمینی ایجاد نمایند.

کلمات کلیدی: تنوع ژنتیکی، جهش یافتگان *Nit*، نشانگر مولکولی، اختصاصیت میزبانی

مکاتبه کننده: محمد جوان نیکخواه Email: jnikkhah@ut.ac.ir