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Genetic Diversity in the Fungus *Fusarium solani* f.sp. *cucurbitae* Race 1, the Casual Agent of Root and Crown Rot of Cucurbits in Iran, using Molecular Markers

[1]M.R. Alymanesh, [2]M. Falahatirastegar, [2]B. Jafarpour and [2]E. Mahdikhanimoghadam
[1]College of Agriculture, Ferdowsi University of Mashhad, Iran
[2]Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

Abstract: *Fusarium solani* f.sp. *cucurbitae* race 1 is a pathogen on cucurbit plants. In this study genetic diversity among 26 isolates of *Fusarium solani* f.sp. *cucurbitae* race 1 was studied using Restriction Fragment Length Polymorphism (RFLP) of ITS (Interal Transcribed Spacer) regions and Random Amplified Polymorphic DNAs (RAPD) markers. Outcome of digestion with six restriction enzymes including *Eco*R I, *Rsa* I, *Bme* 18I, *Msp* I, *Hae* III and *Hin*d III, together with the patterns of restriction fragment length polymorphism of ITS regions divided the isolates into two groups. Deoxy Ribonuckin Acid DNA pattern was obtained for the isolates using 12 random primers and genetic distance between them was calculated and relationships (by cluster analysis) determined. Among the primers used, seven primers showed polymorphism. Genetic distance between isolate pairs ranged from 0.03 to 0.48. Genetic diversity was high (e.g., the isolates were distributed into 10 genetic groups at a similarity percentage of 75). The lowest distance was observed between isolates 50 and 73 and the highest distance observed between isolates 50 and 73 with isolate 102. Restriction fragment length polymorphism results show diversity in ITS regions, without any correlation to geographic origin and RAPD. However, this genomic regions usually have high constancy in species, but in this study diversity was shown in ITS regions even for race 1. The data suggest that taxonomical situation of *Foc* race 1 probably needs revision.

Key words: RFLP, RAPD, *Fusarium solani* f.sp. *cucurbitae*, ITS, ITS1 and ITS4 primers, restriction enzymes, genetic diversity

INTRODUCTION

Fusarium solani f.sp.*cucrubita*. Fungus race 1 is a pathogen of cucurbits which is pathogenic for main products such as watermelon, melon, cantaloupe, cucumber and gourd, therefore it causes root, crown and fruit rots. This pathogen has two races with race determining being based on the tissue specificity, so that race 1 is pathogenic on root, crown and fruit but race 2 is pathogenic only on fruit. Race 2 of this pathogen is reported only in limited areas in the world and has less significance (Armengol *et al*., 2000; Boughalleb, 2005). Among the methods which researchers have used to analyze the phylogenetics of *Fusarium solani* species are the fallowing: rDNA-IGS regions, rDNA-ITS regions, large submit RNA gene (Lee *et al*., 2000) and translation elongation factor-alpha (*tef*). Internal Trans Cribed (ITS) regions are probably the most widely sequenced regions of DNA in fungi. rDNA-IGS and rDNA-ITS regions show a higher degree of diversity than other regions such as SSU and LSU (Depriest and Been, 1992). Different primers are used to study for fungus such as universal primers ITS1 and ITS4 (Hibbett and Vilgalys, 1991). Two Enzymes, *Eco*RI and *Hae*III has been used to study the diversity in ITS of the fungus *F. solani* (Brasileiro *et al*., 2004). Also, four restriction enzymes has been used to compare formae specialis of *F. solani* (Suga *et al*., 2000). Direct analysis of DNA polymorphism provides us with information about intra- and inter-specific diversity. To survey this kind of diversity is possible by many molecular methods (Jana *et al*., 2003). The other molecular methods require primary information about the sequence of DNA; but the RAPD (Random Amplified Polymorphic DNA) molecular markers is a simple method with no need to data about the sequence of DNA. Random Amplified Polymorphic DNAs (RAPD) is a valuable technique implemented to survey fungal genomes because it is possible to compare different isolates quickly. For example, it can quickly identity pathogens and prevalence of specific races. Informing about the genetic diversity of plant pathogenic fungi is a primary task for providing resistant cultivars (Benthy *et al*., 1998; Naghavi *et al*., 2005). There is no

Corresponding Author: M.R. Alymanesh, College of Agriculture, Ferdowsi University of Mashhad, Iran

accurate data about the genetic diversity status of this fungus in Iran. This fungus has an extensive host range and very high rate of diversity in pathogenicity and morphology. Also, the condition of determining its race is different from other formae specials of the *Fusarium* genus. Thus, in this research we tried to use RAPD and investigate rDNA ITS region to study the genetic diversity of race 1 of this fungus.

MATERIALS AND METHODS

Isolates: The name of isolates is mentioned in Table 1. Samples from infected plants were taken during 2004-2005 from 101 cucurbit plants, in townships of three provinces (khorasan Razavi, Northern khorasan and Fars). Isolates of *Fusarium solani* were isolated from different growth stages of watermelon, melon and cucumber. Pathogenicity test was conducted by the Root-dipping method on seedlings of watermelon, melon, cucumber and squash and pathogenic isolates were obtained. Formae specialis detection test was also performed on seedlings of tomato, pea and bean (non-host plants). In conclusion, some isolates showed pathogenicity only on cucurbit plants and were confirmed as *Fusarium solani* f.sp *cucurbitae*. The isolates that showed pathogenicity on fruit in addition to root and crown was detected race 1. Race determination were conducted by molecular methods using the FSC1 primer which has been designed based on *tef 1*α gene (Mehl and Epstein, 2007). Primer sequence are as fallows:

FSC1-EF1 (forward): 5' GCTAA CAATCATCTACAGAC 3'
FSC1-EF2 (reverse): 5' GACGGATGAGAGAGCAAC 3'

DNA extraction: For DNA extraction the fungus was cultured on PDA medium for 7 days. A bulk of fungus was then removed and placed in liquid PDB medium. Mycelium was grown in 100 mL of PDB on a rotary shaker at 180 rpm for three days at 25-28°C. Then glass containers were placed under the light for 2 weeks. At this time the fungus was ready for DNA extraction, which was accomplished based on CTAB (Cetyl Tremetyl Ammonium Bromide) Method. DNA quality and quantity

Table 1: List of 26 isolates of *Fusarium solani* f.sp. *cucurbitae* race 1 studied in three provinces of Iran

No.	Code	Locale	City	Province	Host
1	30	Gorde	Estahban	Fars	Watermelon
2	41		Maharloo	Fars	Cantaloupe
3	50	Chahardah	Khaf	Khorasan Razavi	Watermelon
4	52	Chalindar	Khaf	Khorasan Razavi	Watermelon
5	58	Vicinity	Khaf	Khorasan Razavi	Watermelon
6	67	Joveyn	Sabzevar	Khorasan Razavi	Watermelon
7	70	Abravan	Mashhad	Khorasan Razavi	Watermelon
8	71	Jade gomrok	Serakhs	Khorasan Razavi	Watermelon
9	73	Vicinity	Mashhad	Khorasan Razavi	Watermelon
10	76	Nobonyad	Serakhs	Khorasan Razavi	Watermelon
11	86	Vicinity	Esfarayen	Khorasan Shomali	Cucumber
12	89		Sankhast	Khorasan Shomali	Watermelon
13	90		Chenaran	Khorasan Shomali	Cucumber
14	93	Vicinity	Bojnord	Khorasan Shomali	Cucumber
15	96	Ghoshan	Jajarm	Khorasan Shomali	Cucumber
16	98	Ghale ghasab	Serakhs	Khorasan Razavi	Melon
17	99	Ghosh Alijan	Serakhs	Khorasan Razavi	Melon
18	118	Fark	Kashmar	Khorasan Razavi	Cantaloupe
19	120	Tape salam	Mashhad	Khorasan Razavi	Melon
20	122		Mashhad	Khorasan Razavi	Melon
21	102	Vicinity	Khaf	Khorasan Razavi	Melon
22	83	Vicinity	Serakhs	Khorasan Razavi	Melon
23	42	Vicinity	Shiraz	Fars	Cantaloupe
24	38	Vicinity	Estahban	Fars	Watermelon
25	584		Torbat Haidarie	Khorasan Razavi	Watermelon
26	81	Vicinity	Kashmar	Khorasan Razavi	Watermelon

ITS1 : TCCGTAGGTGAACCTGCGG- 3'
ITS2 : TCCTCCGCTTATTGATATGC-3'

Amplification reactions were performed in 1.5 µL $MgCl_2$ (50 Mm), 0.5 µL PCR buffer (10X), 0.5 µL d NTPs (10 Mm), 0.5 µL Taq DNA polymerase (5 Unit μL^{-1}), 2.5 µL PrimerITs1 (10 µm), 2.5 µL PrimerITs4 (10 µm), 2 µL Genomic DNA (50 ng mL^{-1}) and 35.5 µL dd H_2O in a final volume of 50 µL. PCR conditions consisted of an initial denaturation of 4 min at 95°C followed by 35 cycles of 1 min at 92°C, 1 min at 58°C and 2 min at 72°C, with to final extension of 5 min at 72°C. A reaction without adding DNA was used as control. Size markers of 50 or 100 bp were used.

Amplified fragment length polymorphism was finally confirmed by running the PCR-product on 8% polyacrilamide gel. 10X TBE buffer was used to prepare the polyacrilamide gels. Six restriction enzymes were used to digest PCR products in order to compare probable polymorphism of products between different isolates of race1 *Fusarium solani* f.sp. *cucurbitae*.

OPK 19	5' - CTCCTGCCAA - 3'
VBC 53	5' - CTCCCTGAGC - 3'
RCO 9	5' - GATAACGCAC - 3'
VBC 300	5' - GGCTAGGGCG - 3'
OPK 15	5' - CTCCTGCCAA - 3'
VBC 83	5' - GGGCTCGTGG - 3'
VBC 222	5' - AAGCCTCCCC - 3'
VBC 228	5' - GCTGGGCCGA - 3'
VDC 6	5' - CCTGGGCCTA - 3'

and sequences of primers is mentioned in Table 2. Amplification reactions were performed in 1.5 µL $MgCl_2$ (50 Mm), 0.5 µL PCR buffer (10X), 0.5 µL d NTPs (10 Mm), 0.5 µL Taq DNA polymerase (5 Unit μL^{-1}), 2.5 µL PrimerITs1 (10 µm), 2.5 µL PrimerITs4 (10 µm), 2 µL Genomic DNA(50 ng mL^{-1}) and 35.5 µL dd H_2O in a final volume of 50 µL. When all of PCR reaction parameters were optimized. A reaction without using DNA was used as negative control. Polymerase Chain Reaction (PCR) conditions included a pre-denaturation for 3 min at 94°C, 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C and extension for 2 min at 72°C, with to final extension of 5 min at 72°C. Desirable results were obtained following different improvements in RAPD-PCR, such as adding PCR enhancers like DMSO at 5% (v/v of total reaction volume). Polymerase Chain Reaction (PCR) products were run on 1.5% agarose gel. After staining the gel with ethidium bormide for 15 min and decolouring with distilled water for 5 min, the bands were observed. Pictures from electrophoresis gels were provided and each band considered as a locus (presence of the band was scored as 1 and its absence as 0). All bands were studied except weak and incomplete ones. The variable binary similarity matrix was prepared using Nei coefficient by the NTSYS (Numerical Taxonomy System of multivariate) program version pc2.02e Dendrograms were prepared by UPGMA (Unweighted Pair Group Method with Arithmetical Averages) analysis (Nei, 1972, 1987).

RESULTS

Race identification: Thirty seven isolates of *Fusarium solani* were isolated. Thirty three pathogenic isolates were obtained. Twenty six isolates out of 33 showed pathogenicity only on cucurbit plants and were confirmed as *Fusarium solani* f.sp cucurbitae. The 26 isolates

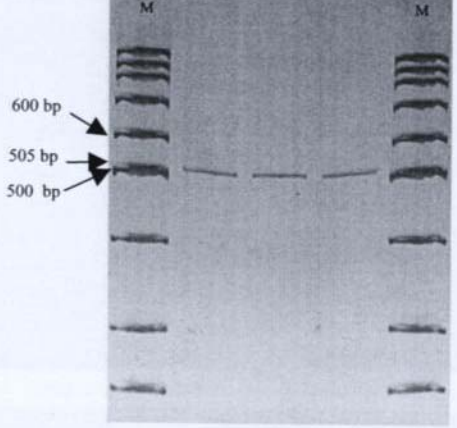

Fig. 1: Specific primer for race 1 produced specific band on polyacrylamid gel 8% (M: Size marker)

Specific primer was used to determine the race 1. fungus *Fusarium solani* f.sp. cucurbit generated the certain band (505 bp) in polyacrylamid Gel 8% (Fig. 1).

Studying rDNA ITS regions

Amplification with primers ITS1 and ITS4 resulted in fragments of approximately 580 and 625 bp which were specified as code 1 and 2 (Table 3, Fig. 2A). Digestion of the ITS amplification products with *Eco*RI resulted in two banding patterns producing two fragments of 310 and 270 bp for the group1 and 2 fragments of 310 and 300 bp for the group 2 (and little fragments which probably could not be observed in the 8% acrylamid gel). The minor differences in the size of the bands on the acrylamid gel is clearly observable in Fig. 2B. Digestion with *Hae*III did not show a difference between groups 1 and 2 or within each group and in all of the isolates fragments of 80, 95, 130 and 245 bp were observed (and little fragments which probably could not be observed in the 8% acrylamid gel) (Fig. 2E). Digestion of the ITS amplification products with Bme18I showed one banding pattern with two cutting sites in all of the isolates and the restriction sites gave rise to three fragments of 300, 150 and 130 bp (Fig. 2D). Digestion with *Msp*I resulted in two banding patterns producing two fragments of 365 and 215 bp for the group 1 and two fragments of 410 and 215 bp for the group 2 (Fig. 2C). Restriction enzymes *Rsa*I and *Hind*III were lacking cleavage sites in all of the isolates. *Eco*RI and *Msp*I could show polymorphism in isolates which in

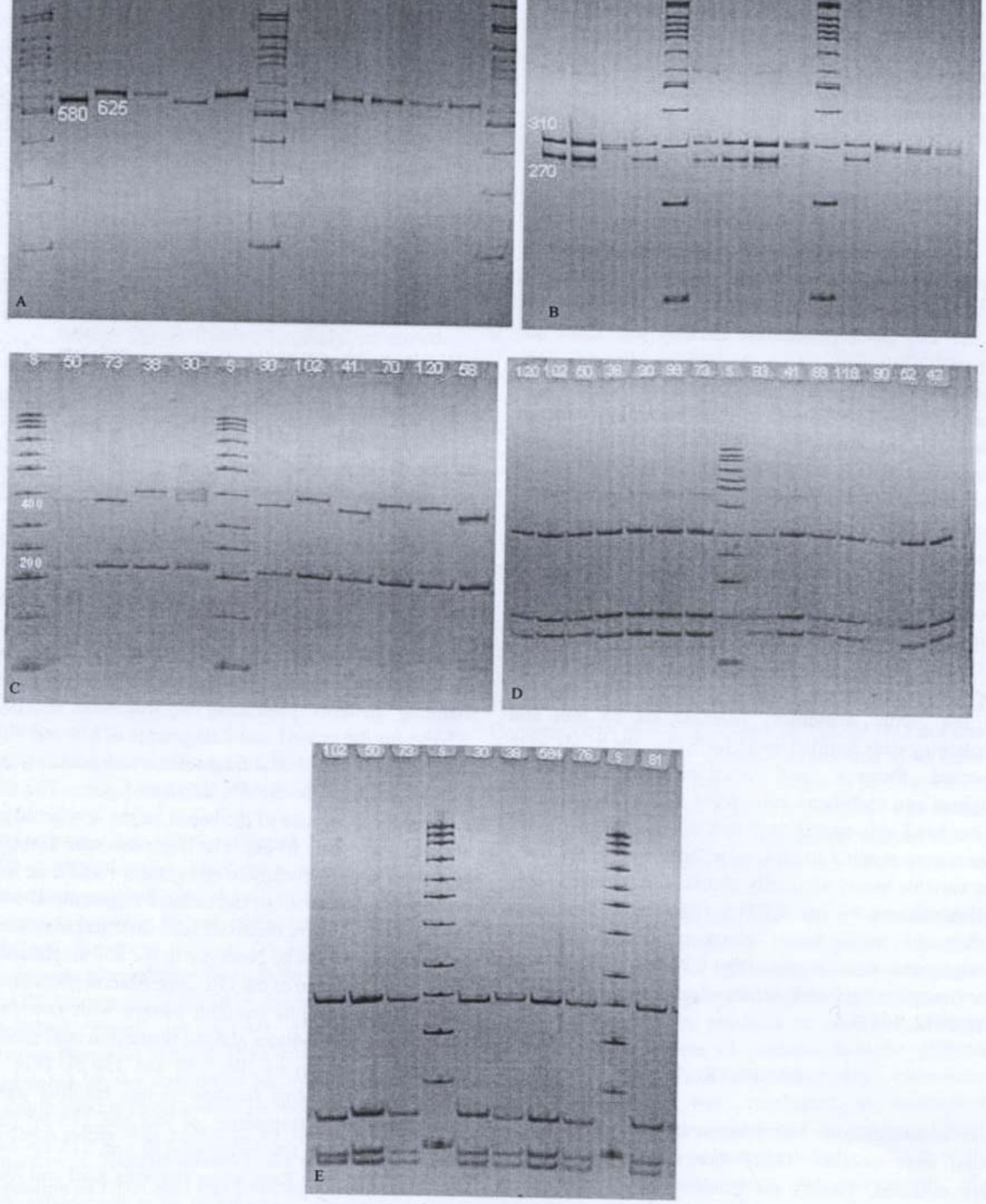

Fig. 2: (A) Restriction Fragment Length Polymorphism of ITS1 and ITS4 primers; separated into 2 groups, (B)

118	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
122	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
71	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
98	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
81	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
41	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
30	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
86	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
73	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
50	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
52	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
42	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
67	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
83	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
58	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
584	1	580	310, 270	80, 95, 130, 245	580	580	130, 150, 300	215, 365
76	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
70	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
120	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
96	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
99	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
93	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
38	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
89	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
102	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410
90	2	625	310, 300	80, 95, 130, 245	625	625	130, 150, 300	215, 410

Table 4: The RAPD primers that revealed diversity between isolates

No.	Name	Primer sequence	No. amplify locus	No. polymorphic bands	No. genotype
1	RCO 8	5' - GGATGTCGAA - 3'	12	10	12
2	OPK 19	5' - CTCCTGCCAA - 3'	6	5	5
3	VBC 199	5' - GCTCCCCCAC - 3'	5	4	5
4	RCO 9	5' - GATAACGCAC - 3'	21	18	23
5	VDC 82	5' - GGGCCCGAG - 3'	15	14	20
6	VBC 83	5' - GGGCTCGTGG - 3'	22	18	22
7	VBC 222	5' - AAGCCTCCCC - 3'	12	12	18
Total	-		93	81	

RAPD marker study: Seven out of 12 primers used showed polymorphism (Table 4). Opk 15 after many repeats (even in low annealing temperature) wasn't able to amplify genomic DNA. In negative control reactions also no band was observed. These primer causes 93 loci to amplify with sharp bands which could be numbered. Total number of polymorphic bands was 81. Each primer for each isolate generated 1-12 bands. Polymrase Chain Reaction (PCR) products ranged from 500 to 3500 kbp. VBC83 and Rco9 primers generated the most polymorphic bands and Rco9 primer identified the highest diversity by identifying 23 genotype (Fig. 3, 4). Genetic distance matrix and calculated genetic similarity are shown in Table 5 and dendrogram is depicted in Fig. 5. The results of cluster analysis and dendrogram drawing showed that there is not any logical relationship between genetic groups

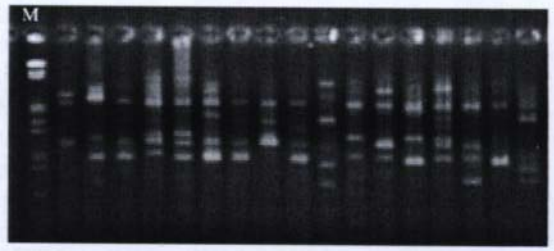

Fig. 3: Pattern of bands amplified with VBC83 primer (M: Size marker)

clusters and the isolates gathered from some different regions were placed in one cluster. Genetic distance between pairs of isolates is variable from 0.03 to 0.48. The least distance was between isolates 50 and 73 (from

the isolates were distributed into 10 genetic groups at a similarity percentage of 75 (Fig. 5). Three isolates from Fars province were placed in one group but isolate 30 from this province was placed in a different group. Isolates of Khorasan Razavi and Khorasan Shomali (e.g., isolates of Khaf and Serakhs) were not placed in one group.

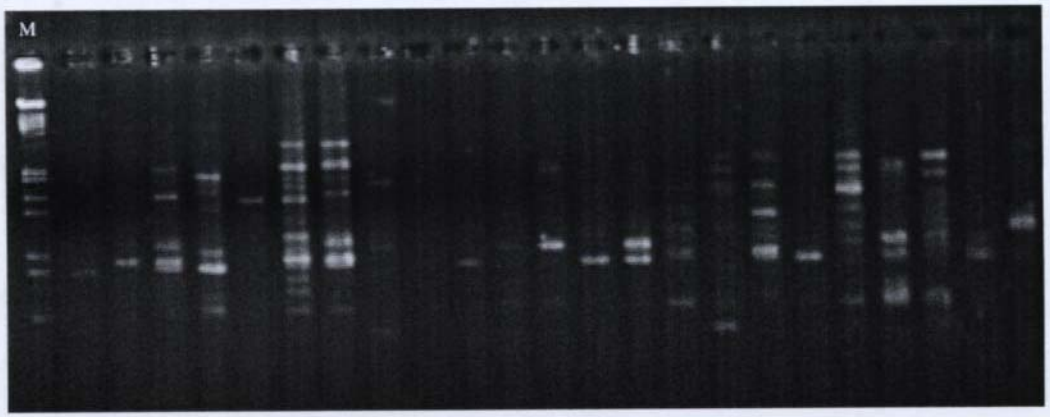

Fig. 4: Pattern of bands amplified with RCO9 primer (M: Size marker). {Some isolates did not produce any bands with some primers like RCO9 even after several repeats. (That is usual in a microorganism with small genome such as fungi) or weak and incomplete bands which was ignored in RAPD, produced (just bands which are repeatable and clear after several reactions are used in calculations). In these figures which two primers reveal high diversity purpose is exposure of the topic in figures (that with these primers some isolates produced many bands on the other hand some isolates did not produce any bands with the same primers or produced weak and unrepeatable bands). Because the subjects above said are clear so they usually are not explained in the text of RAPD articles. By the way when we have many isolates and comparison of all of them are difficult. Comparison is accomplished on several small gels and is utilized standard ladder}

Table 5: Genetic distance matrix (nether numbers) and genetic similarity (upper numbers) among 26 isolates

Isolates	38	41	42	30	86	89	93	96	102	83	52	584	58
38	****	0.8025	0.7407	0.716	0.7531	0.8395	0.8519	0.7901	0.5926	0.8272	0.8272	0.7778	0.8025
41	0.2201	****	0.7901	0.7901	0.7531	0.8395	0.8272	0.7901	0.642	0.8765	0.8765	0.8272	0.8519
42	0.3001	0.2356	****	0.7284	0.716	0.7778	0.7407	0.7531	0.4815	0.7901	0.8148	0.8148	0.7407
30	0.334	0.2356	0.3169	****	0.716	0.7778	0.7654	0.7284	0.5802	0.7654	0.7654	0.7407	0.6914
86	0.2836	0.2836	0.334	0.334	****	0.7654	0.7778	0.7407	0.6173	0.7531	0.7778	0.7037	0.7531
89	0.1749	0.1749	0.2513	0.2513	0.2673	****	0.8395	0.9012	0.6296	0.8642	0.8642	0.8148	0.8642
93	0.1603	0.1898	0.3001	0.2673	0.2513	0.1749	****	0.8148	0.6667	0.8765	0.8519	0.8272	0.8519
96	0.2356	0.2356	0.2836	0.3169	0.3001	0.104	0.2048	****	0.6296	0.8148	0.8395	0.7654	0.8148
102	0.5232	0.4432	0.7309	0.5443	0.4824	0.4626	0.4055	0.4626	****	0.6667	0.6173	0.5432	0.5926
83	0.1898	0.1306	0.2356	0.2673	0.2836	0.146	0.1318	0.2048	0.4055	****	0.8765	0.8272	0.8272
52	0.1898	0.1318	0.2048	0.2673	0.2513	0.146	0.1603	0.1749	0.4824	0.1318	****	0.8272	0.8272
584	0.2513	0.1898	0.2048	0.3001	0.3514	0.2048	0.1898	0.2673	0.6103	0.1898	0.1898	****	0.8272
58	0.2201	0.1603	0.3001	0.3691	0.2836	0.146	0.1603	0.2048	0.5232	0.1898	0.1898	0.1898	****
71	0.5443	0.3871	0.3691	0.4824	0.4626	0.4055	0.4242	0.4432	0.7056	0.4242	0.4242	0.3169	0.4242
73	0.104	0.1318	0.2356	0.2673	0.1898	0.1178	0.104	0.1749	0.4432	0.104	0.104	0.1898	0.1603
76	0.146	0.1749	0.3169	0.3169	0.2048	0.1318	0.146	0.1603	0.3871	0.1749	0.1749	0.2048	0.1749
98	0.1898	0.1318	0.2673	0.2356	0.3169	0.1178	0.1603	0.146	0.4055	0.1603	0.1318	0.2201	0.1898
50	0.077	0.1318	0.2048	0.2673	0.1898	0.1178	0.104	0.1749	0.4824	0.104	0.104	0.1898	0.1603
90	0.2513	0.2513	0.2356	0.3691	0.3169	0.2673	0.1603	0.3001	0.4824	0.1898	0.2201	0.2513	0.2513
81	0.334	0.2673	0.2836	0.3514	0.4432	0.2836	0.2048	0.3169	0.5878	0.2356	0.3001	0.2356	0.2673
67	0.334	0.3001	0.3169	0.3871	0.4824	0.3514	0.2673	0.4242	0.5926	0.334	0.2673	0.2356	0.3001

42	0.6914	0.7901	0.7284	0.7654	0.8148	0.7901	0.7531	0.7284	0.7037	0.7407	0.7778	0.6914	0.6708
30	0.6173	0.7654	0.7284	0.7901	0.7654	0.6914	0.7037	0.679	0.7284	0.6914	0.7037	0.716	0.646
86	0.6296	0.8272	0.8148	0.7284	0.8272	0.7284	0.642	0.6173	0.7654	0.7037	0.6914	0.6543	0.7329
89	0.6667	0.8889	0.8765	0.8889	0.8889	0.7654	0.7531	0.7037	0.8272	0.7654	0.7284	0.8148	0.7329
93	0.6543	0.9012	0.8642	0.8519	0.9012	0.8519	0.8148	0.7654	0.8642	0.7037	0.8148	0.8272	0.7826
96	0.642	0.8395	0.8519	0.8642	0.8395	0.7407	0.7284	0.6543	0.8025	0.716	0.7037	0.716	0.6708
102	0.4938	0.642	0.679	0.6667	0.6173	0.6173	0.5556	0.6049	0.6296	0.5926	0.5062	0.6173	0.6211
83	0.6543	0.9012	0.8395	0.8519	0.9012	0.8272	0.7901	0.716	0.8395	0.679	0.7407	0.7778	0.7329
52	0.6543	0.9012	0.8395	0.8765	0.9012	0.8025	0.7407	0.7654	0.8148	0.7284	0.7407	0.7531	0.7081
584	0.7284	0.8272	0.8148	0.8025	0.8272	0.7778	0.7901	0.7901	0.7407	0.7037	0.7407	0.8025	0.7329
58	0.6543	0.8519	0.8395	0.8272	0.8519	0.7778	0.7654	0.7407	0.8395	0.7037	0.7407	0.8272	0.7702
71	****	0.6543	0.6667	0.6049	0.6543	0.7037	0.6667	0.6667	0.5679	0.6049	0.716	0.6049	0.5839
73	0.4242	****	0.8889	0.8765	0.9753	0.8272	0.7654	0.7654	0.8889	0.7037	0.7654	0.7778	0.7826
76	0.4055	0.1178	****	0.8642	0.8889	0.7654	0.7284	0.7037	0.8272	0.6914	0.7284	0.7654	0.795
98	0.5026	0.1318	0.146	****	0.8765	0.7531	0.7407	0.7407	0.7901	0.679	0.716	0.7778	0.7454
50	0.4242	0.025	0.1178	0.1318	****	0.8519	0.7654	0.7654	0.8889	0.7037	0.7654	0.7778	0.8075
90	0.3514	0.1898	0.2673	0.2836	0.1603	****	0.7654	0.7654	0.7901	0.679	0.8148	0.7531	0.7329
81	0.4055	0.2673	0.3169	0.3001	0.2673	0.2673	****	0.8272	0.7284	0.6914	0.7531	0.7654	0.6957
67	0.4055	0.2673	0.3514	0.3001	0.2673	0.2673	0.1898	****	0.679	0.642	0.7037	0.716	0.6957
70	0.5658	0.1178	0.1898	0.2356	0.1178	0.2356	0.3169	0.3871	****	0.6914	0.7531	0.7654	0.7454
120	0.5026	0.3514	0.3691	0.3871	0.3514	0.3871	0.3691	0.4432	0.3691	****	0.716	0.7037	0.6832
122	0.334	0.2673	0.3169	0.334	0.2673	0.2048	0.2836	0.3514	0.2836	0.334	****	0.7654	0.6708
118	0.5026	0.2513	0.2673	0.2513	0.2513	0.2836	0.2673	0.334	0.2673	0.3514	0.2673	****	0.7702
99	0.5381	0.2451	0.2294	0.2939	0.2139	0.3107	0.3629	0.3629	0.2939	0.3809	0.3993	0.2611	*****

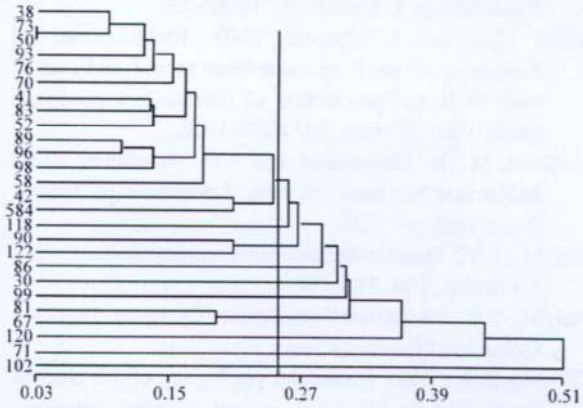

Fig. 5: RAPD analysis dendrogram: for example isolates are set into 10 genetic groups at a 75% similarity level

DISCUSSION

Generally *F. solani* has an extensive host range and very high rate of diversity in pathogenicity and morphology (Brasileiro *et al.*, 2004). Among 9 formae specials were described for *F. solani* which were all resolved as phylogenetically distinct species by the molecular phylogeny. Of these, *F. solani* f. sp. *cucurbitae* races 1 and 2 appear to represent reproductively isolated (based on the tissue specificity) (Boughalleb, 2005), So, these indicate special position of this formae speciale and its races among *Fusarium* species. The abundant diversity between different isolates of different geographical areas in this fungus has different causes. One of the reasons can be sexual reproduction in *F. solani* f.sp. *cucurbitae*; although teleomorph is very rare in nature and ascospore does not play an important role in survival of the fungus (Armengol *et al.*, 2000) and there is not report of presence teleomorph in Iran. Cropping resistant species is one of the diversity causes in a lot of pathogens, because by cropping resistant species only limited genotypes of pathogens will have the ability of pathogenicity. The geographic factor do not have a logical relation with ITS and RAPD diversity and thus some isolates which were collected from different provinces has more similarity as revealed by the genetic distance in RAPD analysis than some isolates from different areas of a township with little distance. Isolates 102 and 50, both from two neighboring areas in khaf had the greatest genetic distance from each other; while isolates 38 and 50 which were from two different provinces with long geographic distance (Fars and Khorasan Razavi, respectively) had very low genetic distance from each other and isolates 30 and 38 from two near, neighboring geographic area were placed in two separate ITS groups. Although, the geographic distance

in placing genetic results, isolates of a geographic area in two independent groups also placing two isolates from two different area in a group show this fact. There was remarkable differences even in different farms of an area. Also, the factors of ITS and RAPD did not have a logical relationship with each other and some isolates with low genetic distance in RAPD (such as isolates 38 and 73) were placed in two different groups 1 and 2 in ITS. In this survey all isolates are race 1, but according to PCR-RFLP results which shows diversity in ITS regions and to the fact that usually this genomic regions have high constancy in species. The *tef 1α* gene commonly is used for DNA sequence-based identification in *Fusarium* sp. because there frequently is insufficient variation in the Internal Transcribed Spacer (ITS) region (Mehl and Epstein, 2007). But in this study diversity was shown in ITS regions even for race 1. Also, the results of RAPD showed high genetic diversity in race 1 of this fungus. The data (two ITS groups and high genetic diversity computed by RAPD) suggest that taxonomical situation of *Foc* race 1 probably needs revision.

Understanding the relation among fungal isolates variability may help driving the progress of breeding programs or the use of recombinant DNA technology towards producing resistance cultivars (Naghavi *et al.*, 2005). Also, integrated taxonomic studies using molecular methods, studying other regions of genome and analyzing all of them, studying isolates from other geographical regions in Iran and other parts of the world and comparing their population in order to more accurately specify the condition of this race and the rate of its genetic diversity appears to be necessary.

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