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Full Length Research Paper

Analysis of Iranian isolates of *Fusarium solani* using morphological, pathogenicity and microsatellite DNA marker characterization

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Many economically important phytopathogenic species are nested within *Fusarium solani* species complex. Since most of them lack Latin binomials, morphological and molecular phylogenetic analyses were conducted on strains selected to represent the known pathogenic and genetic diversity of this pathogen. To evaluate methods used in classifying strains of this pathogen, 33 *F. solani* isolates collected from different geographical regions of Iran, were cultured and characterized using morphology, pathogenicity and microsatellite DNA. All the isolates showed high variability in aerial mycelial growth, mycelia texture, pigmentation (mycelia colour) when cultured on potato dextrose agar medium, and conidial measurements on Spezieller Nahrstoffarmer agar medium. Colonies were grouped into fluffy and fibrous based on mycelial texture; buff, umber, loteous, pale loteous, ochreous and dark brown based on mycelia colour; and long, medium and short macroconidial length. Of the 33 isolates, 26 were pathogenic on *Solanum tuberosum*, *Hordeum vulgare* and *Cicer arietinum*. DNA analysis showed that the isolates showed a high genetic diversity. Ten clusters were reconstructed using microsatellite variation. However, the microsatellite groupings were independent colony characteristics and virulence of the isolates.

Key words: Genetic diversity, simple sequence repeat (SSR) marker, phylogenetic analyses, virulence, *Fusarium solani*.

INTRODUCTION

Members of the genus *Fusarium* are among the most important plant pathogens in the world. *Fusarium* species are a widespread cosmopolitan group of fungi that commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Fungi in this genus cause a huge range of diseases on a wide range of host plants. The fungus can be soil-, air-, and water-borne or carried in or on plant residue or seeds, and can be recovered from any part of a plant from roots, shoots, flowers and or cones, as well as seeds (Summerell et al., 2003). *Fusarium* taxonomy has been plagued by changing species concepts, with as few as nine, to over 1000 species recognized by various taxonomists during the past century (Summerell et al., 2003). Differing opinions on species identification was largely stabilized since the 1980s following the publications by Gerlach and Nirenberg (1982) and Nelson et al. (1983) that defined widely accepted morphological species. Since then, however, application of biological Leslie (2001) and phylogenetic Nirenberg and O'Donnell (1998) species concepts to new, as well as existing strain collections has resulted in further splitting of many of the previously described species.

The genus *Fusarium* has been divided into infrageneric groupings called sections based on conidium and colony

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morphology. Section *Martiella* as circumscribed by Wollenweber and Reinking (1935), consisted of five species, ten varieties and four forms, including *Fusarium solani*. *F. solani* (Mart.) Appel and Wollenw. (teleomorph Haematonectria haematococca Berk. and Br.), as recognized by Snyder and Hansen (1941) and Nelson et al. (1983), represented the only species within section *Martiella*. The other four species recognized by Wollenweber and Reinking (1935) were treated as synonyms of *F. solani* Snyder and Hansen (1941). However, four and six species, respectively were accepted within this section in taxonomic treatments of this genus by Booth (1971) and Gerlach and Nirenberg (1982). The *Martiella* fusaria are usually reported as the polytypic species *F. solani* f. sp. or mating populations (MP) of *Nectria haematococca* even though the seven MP originally identified by Sakurai and Matuo (1959, 1960, 1961a, b), Matuo and Sakurai (1965) and Matuo and Snyder (1973) represent reproductively isolated biological species. *F. solani* is sub-classified into *formae specialis (phaseoli, pisi, cucurbitae, batatas, radicicola, robiniae, mori, piperis, eumartii, xanthoxyli, hibisci, lycopersici and phaseoli*) based on host specificity (Suga et al., 2000a, b). Variations in the degree of virulence in *formae specialis*, as well as genetic diversity in isolates of different origins revealed that the *N. haematococca*. *F. solani* complex is composed by several phylogenetic species responsible for biologically distinct phytopathologies (O'Donnell, 2000). Therefore, the knowledge of the genetic diversity within this pathogen should help to understand the causes of different disease manifestations.

F. solani is widely found in soil and constitutes one of the most important phytopathogens in agriculture. It infects cultivars like soybean (*Glycine max*), bean (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), potato (*Solanum tuberosum*), among others (Olivieri et al., 2002; Poltronieri et al., 2002), causing root and fruit rot, as well as wilting of the plant upper parts. As an opportunist pathogen, it can cause superficial mycoses in humans and animals (Dijk et al., 1980; Stover, 1981). Recent molecular phylogenetic analyses of DNA sequences, however, indicate the *F. solani* species complex (also known as section *Martiella*) comprises at least 26 phylogenetically distinct species (O'Donnell, 2000), including many undescribed species considered as *F. solani* f. sp. *glycines* and *F. solani* f. sp. *phaseoli* (Burkh.) W.C. Snyder and H.N. Hansen. Molecular and morphological analyses of North American isolates of the sudden death syndrome (SDS) pathogen indicate that they are genetically homogeneous and closely related to a root-rot pathogen of *Phaseolus vulgaris* L., *F. solani* f. sp. *phaseoli* (Achenbach et al., 1996; O'Donnell, 2000; Li et al., 2000; Rupe et al., 2001). More recently, Aoki et al. (2003) studied North American and Argentinean isolates of the soybean SDS pathogen, using comparative morphology and genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al., 2000).

MATERIALS AND METHODS

Fungal isolates and identification

The *F. solani* isolates used in the study are listed in Table 1. Single-conidium cultures of all isolates were morphologically characterized both on potato-dextrose agar (PDA, Merck, Darmstadt, Germany) and on synthetic nutrient-poor agar (SNA, Nirenberg, 1976). Isolates were identified according to Gerlach and Irenberg (1982).

Colony characteristics

A small portion of each isolate was transferred onto a PDA plate and incubated at room temperature for 8 days. The cultures from these plates were used to determine the nature of aerial mycelial growth, mycelial texture and pigments (colour) produced on the mycelia. The nature of aerial mycelia growth and mycelial texture were determined by visual observation while pigmentation on the mycelium was determined with the help of a mycological colour chart (Rayner, 1970).

To study the conidial morphology, *F. solani* isolates were sub-cultured onto Spezieller Nahrstoffarmer agar (SNA) medium. The plates were incubated at room temperature in a 12 h light/dark cycle for ten days. A block of 1 cm^2 of agar from SNA was cut and mounted directly on a slide with a drop of water and a cover slip. Morphology of macroconidia and microconidia was described and measurements made in micrometers (mm). 50 conidia per isolate were measured to determine the length, width and the number of septa, using a compound microscope with a micrometer. Determination of the width was by measuring the widest section of the conidia.

Pathogenicity test

Pathogenicity tests for all isolates of *F. solani* were done on *S. tuberosum*, *Hordeum vulgare* and *Cicer arietinum*. Two-month old, washed and dried susceptible potato (*S. tuberosum* L.) tubers variety Agria were used to estimate pathogenicity and virulence of *F. solani* isolates because it is a relatively accurate and convenient assessing disease development on these organs. Using a steel rod (2 mm in diameter), two holes (5 mm deep) were formed in potato tubers, one at the apical and one at the stem end. Each isolate was inoculated on three tubers at the two holes on each tuber (Theron and Holz, 1989). For the inoculum preparations, fungal isolates were grown for two weeks on carnation-leaf agar (CLA) at 25°C. Conidia were washed by distilled water, counted using a haemocytometer, the suspensions diluted to 1 × 104 conidia ml^{-1} and 200 µL of suspension laid in each inoculation point. After the inoculation, tubers were placed in paper bags and incubated in a germinator at 10°C and 90% relative humidity, for two weeks.

At the end of incubation, tubers were cut through the inoculation points, and the degree of rot was estimated on a zero to five scale, basically according to Theron and Holz (1989) as estimated by the LEICA QWin 550 CW software (Germany). For pathogenicity test on *H. vulgare* and *C. arietinum*, the inocula were obtained from cultures grown on PDA for one week. 5 ml of sterile distilled water was added to each PDA plate and scraped gently with the edge of a glass slide to obtain a spore suspension. The suspension was then sieved in four layers of cheesecloth to remove mycelial fragments. The spore concentration was determined with the help of haemocytometer. The planting media was composed of soil, manure (cow dung) and sand in the ratio 2:1:1 by volume (Isanda, 1995). The planting media was steam sterilized at 80°C overnight, allowed to cool and kept in 20 cm diameter polythene sleeves and kept in a glasshouse with a prevailing temperature of 23 to 30°C with a 12 h photoperiod. A soil inoculation method of Abawi and

Table 1. Host, location, colony characteristics and pathogenicity of 33 *Fusarium solani* isolates.

Isolate	Host	Location	Macro-conidia (µM)	Phialide (µM)	Micro-conidia (µM)	Pathogenicity	Mycelia colour
F 1	*Cucumis melo (root)*	Varamin	30.3 × 4.6	81.5 × 3.8	10.7 × 3.7	3	Buff
F 2	*Cucumis melo (root)*	Varamin	29.6 × 4.6	81.6 × 3.3	10.4 × 3.6	0	Loteous
F 3	*Cucumis melo (root)*	Varamin	30.0 × 6.5	99.9 × 4.1	10.3 × 4.0	0	Dark brown
F 4	*Cucumis melo (crown)*	Varamin	32.6 × 5.1	77.2 × 3.4	13.5 × 3.6	0	Buff
F 5	*Beta vulgaris*	Shahrekord	21.6 × 6.3	99.1 × 4.1	11.5 × 5.0	5	Dark brown
F 6	*Beta vulgaris*	Kerman	29.5 × 4.9	74.7 × 3.9	10.5 × 4.0	2	Umber
F 7	*Beta vulgaris*	Dezful	30.1 × 5.1	83.6 × 4.6	14.2 × 4.0	3	Ochreous
F 8	*Pyrus malus (root)*	Semirom	34.8 × 5.1	83.6 × 4	13.8 × 4.4	2	Dark brown
F 9	*Solanum lycopersicum*	Bojnurd	28.6 × 5.2	78.4 × 4	16.4 × 4.0	4	Pale loteous
F 10	*S. lycopersicum*	Bojnurd	34.7 × 5.4	73.6 × 4.1	14.5 × 4.5	4	Buff
F 11	*S. lycopersicum*	Bojnurd	48.2 × 4.1	73.5 × 3.8	12.9 × 4.7	1	Ochreous
F 12	*Pyrus malus (root)*	Samirom	29.3 × 4.9	74.4 × 3.9	12.8 × 4.6	4	Buff
F 13	*Pyrus malus (root)*	Samirom	29.1 × 5.3	81.2 × 4.4	13.4 × 4.4	2	Buff
F 14	*Triticum aestivum (root)*	Kuhdasht	29.5 × 5.5	86.1 × 4.4	15.2 × 4.3	0	Ochreous
F 15	*Oryza sativa (crown)*	Kolale	34.0 × 5.3	81.2 × 5.6	15.8 × 5.1	3	Dark brown
F 16	*Cynodon dactylon (crown)*	Kolale	34.5 × 5.6	81.3 × 5.4	16.7 × 4.6	2	Loteous
F 17	*Avena sativa (root)*	Marand	28.2 × 5.1	63.2 × 3.6	11.7 × 4.1	4	Buff
F 18	*Avena sativa (root)*	Marand	27.1 × 5.2	76.4 × 4.3	16.1 × 5.6	2	Loteous
F 19	*Glycine max (root)*	Chaghalvandi	30.2 × 5.1	61.6 × 4.1	14.5 × 4.4	2	Pale loteous
F 20	*Glycine max (root)*	Aleshtar	31.7 × 4.9	51.4 × 4.5	14.9 × 4.7	3	Loteous
F 21	*Glycine max (root)*	Aleshtar	30.7 × 4.3	84.3 × 4.3	14.1 × 4.7	1	Buff
F 22	*Glycine max (root)*	Kuhdasht	28.1 × 5.3	66.8 × 3.8	11.4 × 4.0	0	Buff
F 23	*Hordeum vulgare*	Khorasan	33.7 × 4.7	85.1 × 4.8	13.1 × 4.8	2	Loteous
F 24	*H. vulgare*	Bafgh	30.8 × 4.8	80.3 × 3.5	12.1 × 3.9	0	Buff
F 25	*H. vulgare*	Kuhdasht	31.3 × 4.9	67.7 × 4.1	14.2 × 3.8	2	Buff
F 26	*H. vulgare*	Kuhdasht	28.7 × 4.5	85.1 × 3.9	13.3 × 4.0	2	Umber
F 27	*Solanum tuberosum*	Ardebil	29.4 × 4.6	80.1 × 3.7	9.3 × 4.2	3	Umber
F 28	*S. tuberosum*	Damavand	32.6 × 4.1	90.6 × 3.8	12.5 × 3.8	4	Buff
F 29	*S. tuberosum*	Firuzkuh	34.8 × 4.4	112.5 × 3.3	11.3 × 3.6	2	Buff
F 30	*Helianthus* sp.	Mashhad	31.8 × 4.9	79.5 × 4.3	11.8 × 4.1	4	Buff
F 31	*Solanum melongena*	Golestan	34.1 × 4.8	94.3 × 3.7	14.1 × 4.6	2	Buff
F 32	*Euzophera bigella*	Mazandaran	37.0 × 4.6	101.4 × 3.9	9.4 × 3.4	4	Buff
F 33	*S. tuberosum*	Shahrud	30.7 × 4.8	52.5 × 3.4	9.2 × 2.8	0	Buff

Table 2. Microsatellites sequence, size ranges, mean number of alleles and polymorphic information content (PIC) obtained from genotyping 33 *Fusarium solani* isolates.

Marker	Primer sequence (F + R)	Tm (°C)	Size range (bp)	Number of allele	PIC
RB23	CAG CCG TCT TTC TCT CTC C GCC TTG AAT CACTACCTCCA	58.8 57.3	167 - 190	2	0.33
RE14	TAC CCA TTG CCT TGT TTC C ACT CCG CGT TCT GCT AGA G	54.5 58.8	260 - 268	2	0.06
RE102	GGA CTT GTC AGC GTC AAG TCA ACC ATC TCA AGG TAT GTC	56.0 55.9	175 - 250	4	0.40
BC5	CGT TTT CCA GCA TTT CAA GT CAT CTC ATA TTC GTT CCT CA	51.7 47.0	164 - 182	3	0.23
AY117125	GCT TCT ACC ATA GTG AC ACC ACG AGT GCT TCT ACC ATA CTC	45.7 47.5	225 - 285	6	0.78
AF513014	GGCATATTGAGTATGGTATGGAT GCTC CCG AGA TCT TGT TCA	54.0 47.3	182 - 287	6	0.70
AY212027	GAG CTG TGC GCG AGT CTG TG ACT GCT CCT TCGA GTC GTC A	63.5 59.4	168 - 474	4	0.69
				Mean = 3.85	

Pastor-Corrales (1990) was adopted. A spore suspension of each isolate was mixed with steam-sterilized soil at a rate of 3×10^4 conidia per g of soil. Four bean seeds were planted in pots filled with steam sterilized soil mixture and covered with approximately 3 cm of infected soil and watered daily. Non inoculated soils were planted with barley and pee seeds were used as controls. Data on percentage plants showing symptoms was taken five weeks after sowing. The experiment was laid in a complete randomized design with three replicates.

DNA extraction

DNA was extracted from 33 single spore isolates of *F. solani*. Erlenmeyer flasks (500 ml) containing 200 ml of glucose-yeast medium (1 g NH_4 H_2PO_4, 0.2 g KCl, 0.2 g $MgSO_4.7H_2O$, 5 g yeast extract (Difco), 1 ml $ZnSO_4.7$ H_2O (1%), 1 ml $CuSO_4.5H_2O$ (1%), 10 g glucose) were inoculated with three 5 mm^2 agar blocks excised from margins of seven day old cultures on PDA medium, placed on a rotary shaker at 120 rpm and incubated at 27 to 28°C under a dark condition for seven days. The mycelia were harvested by filtration through two layers of cheesecloth; freeze dried using liquid nitrogen and ground to a fine powder using a mortar and pestle. DNA isolated following Raeder and Broda (1985).

DNA microsatellite analysis

Seven SSR marker (microsatellite) pairs adopted from other fungal taxa are indicated in Table 2 (Mwang'ombe et al., 2008). PCRs were performed on Eppendrof thermocycler. The medium temperature PCR protocol comprised 30 s at 94°C, 30 s annealing step at (3°C lower of the melting temperature of primers), 30 s at 72°C, with a reduction in the annealing temperature by 0.5°C per cycle (10 cycles), this was followed by 30 cycles for 30 s at 94°C, 30 s at annealing temperature, 30 s at 72°C followed by a 10 min extension at 72°C, and a final 5 min at 10°C. PCR reactions were performed in a final volume of 25 µL and contained 0.4 U *Taq* polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP, 0.5 µM of primers and 40 ng of template DNA. Only clearly different bands were accepted, obtained images were scored manually and independently by three persons.

The PCR fragments were fractionated and sized under denaturing conditions on a 3.5% agarose gel (Metaphor). The gels were scored for the presence or absence of amplified fragments. Observed (Ho) and expected (He) heterozygosities as well as genetic parameters such as the size range and number of alleles per locus were estimated for each microsatellite locus using Popgene version 1.31 software. The percentage of observed heterozygosity was calculated as the ratio between heterozygote individual genotypes and the total number of analyzed genotypes for each locus. Expected heterozygosity was estimated based on the probability that two individuals taken at random from a given sample would have different alleles at a locus (Nei, 1987), an estimated Shannon's (1949) information index as a measure of gene diversity and fixation index estimates (FIS) as a measure of heterozygote deficiency or excess (Wright, 1978). In addition, the polymorphism information content (PIC) was also estimated as the

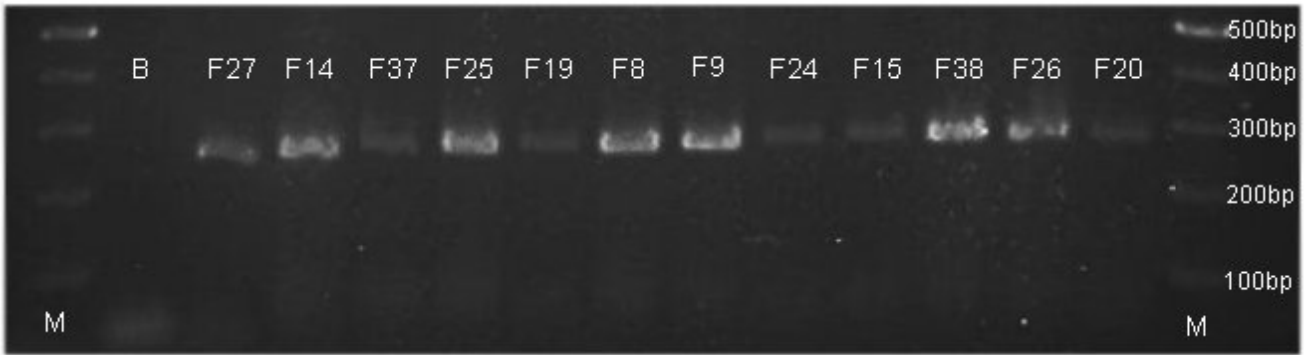

Figure 1. Microsatellite analysis of *Fusarium solani* isolates amplified with the RE14 primer. PCR amplicons were separated on a 3.5% agarose gel in 1X TBE. Bands were stained with ethidium bromide and visualized on UV-Transeluminator. (Marker: #SM0323, Fermentas).

probability that an individual is informative with respect to the segregation of its inherited alleles (Botstein et al., 1980). Cluster analysis and two-dimension were performed with NTSYS software (Version 2.0.1.5).

RESULTS AND DISCUSSION

Colony characteristics

The *F. solani* isolates exhibited high variability in colony characteristics on PDA and SNA media (Table 1). Mycelial texture was either fluffy (61.6% isolates) or fibrous (38.4%). The mycelial colors observed were buff (45% isolates), loteous (0.15% isolates), dark brown (0.12% isolates), umber (0.09% isolates), ochreous (0.09% isolates), and pale loteous (0.06% isolates). The hyphae of the cultured isolates were highly branched, slender, septate and produced conidia and chlamydospores. The microconidia observed on the isolates were 0 to 1 septate, and their length ranged from 9.3 to 16.7 µM, while the width ranged from 2.8 to 5.6 µM (Table 1). The most commonly observed macroconidia from all the isolates ranged from 3 to 5 septate and their length ranged from 21.6 to 37 µM, while the width ranged from 4.1 to 6.5 µM (Table 1). The monophialides were simple to branched, with 0 to 4 septate and their length ranged from 51.4 to 112.5 µM, while the width ranged from 3.3 to 5.6 µM (Table 1).

Pathogenicity

The results show that *F. solani* isolates are non host-specific on *H. vulgare*, *S. tuberosum* and *C. arietinum*, and therefore uses potato as a host to scoring pathogenicity. *F. solani* isolates F2, F3, F4, F14, F22, F24 and F33, obtained from the roots and crowns of *Cucumis melo*, *Triticum aestivum*, *G. max*, *H. vulgare* and *S. tuberosum* did not cause any symptoms when inoculated in potato tubers. These isolates were therefore considered non-pathogenic. Isolate F32 from the insect *Euzophera bigella* produced disease symptoms in the pathogenicity test on potato tubers; in this isolate disease severity was high. Of the four isolates obtained from *C. melo* (F1, F2, F3, F4), three were non-pathogenic, while all three isolates from *S. lycopersicum* (F9, F10, F11), the three from *P. malus* (F8,F12, F13), seven out of nine from gramineous host (F14, F15, F16, F17, F18, F23, F24, F25, F26), three from *B. vulgaris* (F5, F6, F7) and three out of four isolates from *S. tuberosum* (F27, F28, F29, F33) were pathogenic. Isolates F5 from *B. vulgaris* had the highest virulence. This isolate was collected from Shahrekord which is one of the coldest Iranian regions, characterized by subzero temperatures and snow cover in winter.

DNA microsatellite results

In order to determine the genetic relationship among of populations of fungal isolates, a separate matrix was used to the data obtained from the 27 polymorphic alleles and the 33 isolates of *F. solani* were clustered (Figures 1 to 4). The microsatellite primers exhibited 27 clear polymorphism alleles from the 33 fungal samples. The number of polymorphic alleles per locus ranged from 2 to 6 (Table 2). The allele sizes and their frequencies are given in Table 4. Genetic diversity at the seven SSRs was high. The observation of a higher diversity may mean a very rapid evolutionary diversification in this group following divergence from their common ancestor. Multiple sources of molecular data suggest to an evolutionary diversification and loss of host specificity within member forms of *F. solani*. Observed heterozygosity (H_O) and the value expected under Hardy-Weinberg (H_E), fixation index and Shannon's index are given in Table 3, and the mean numbers of alleles (MNA) and PIC are given in Table 2. PIC was estimated for the seven markers with values ranging from 0.06 (RE14) to 0.69 (AY212027). H_O was significantly lower than H_E. Fixation

Figure 2. Microsatellite analysis of *Fusarium solani* isolates amplified with the Ay117125 primer. PCR amplicons were separated on a 3.5% agarose gel in 1X TBE. Bands were stained with ethidium bromide and visualized on UV-Transeluminator. (Marker: #SM0323, Fermentas).

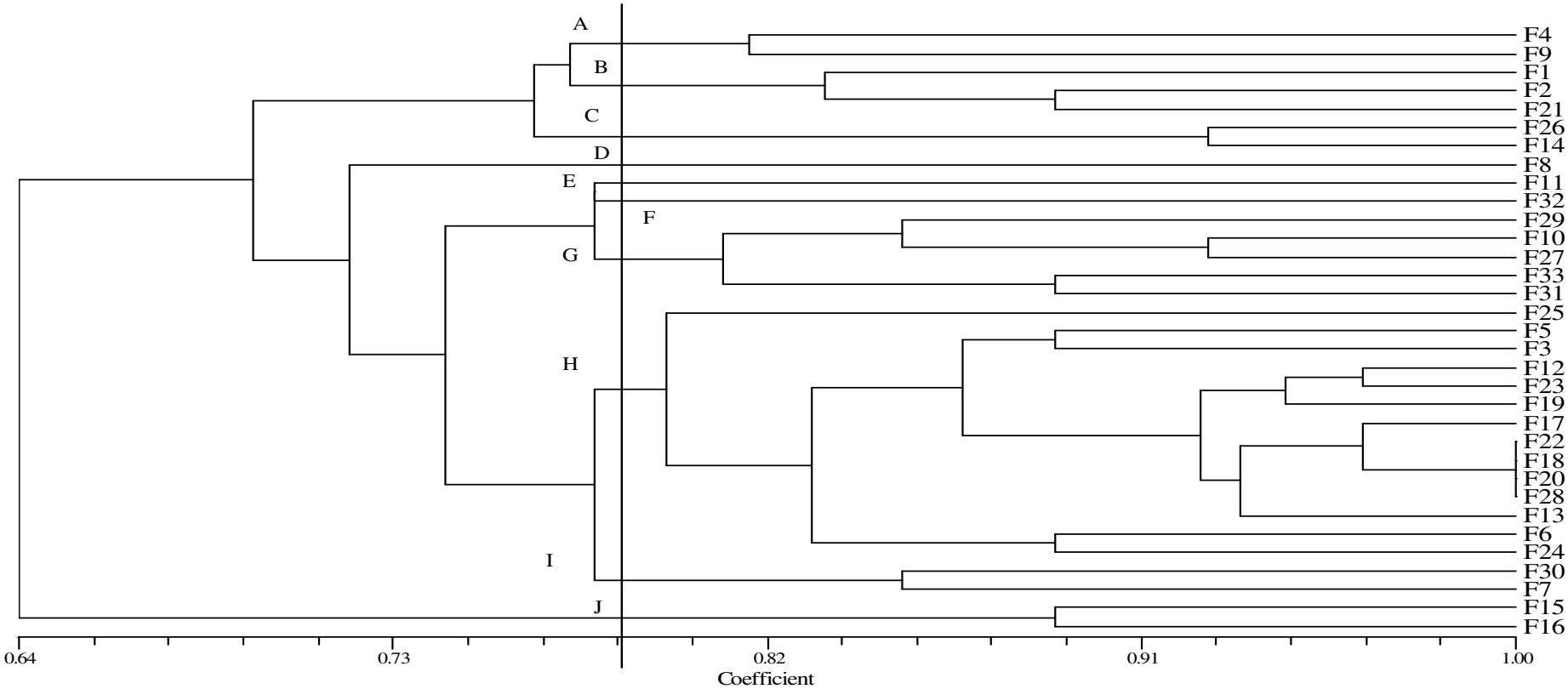

Figure 3. Dendrogram showing the genetic relationships among 33 *Fusarium solani* isolates obtained from Iran based on Jaccard's similarity coefficient at seven microsatellite loci.

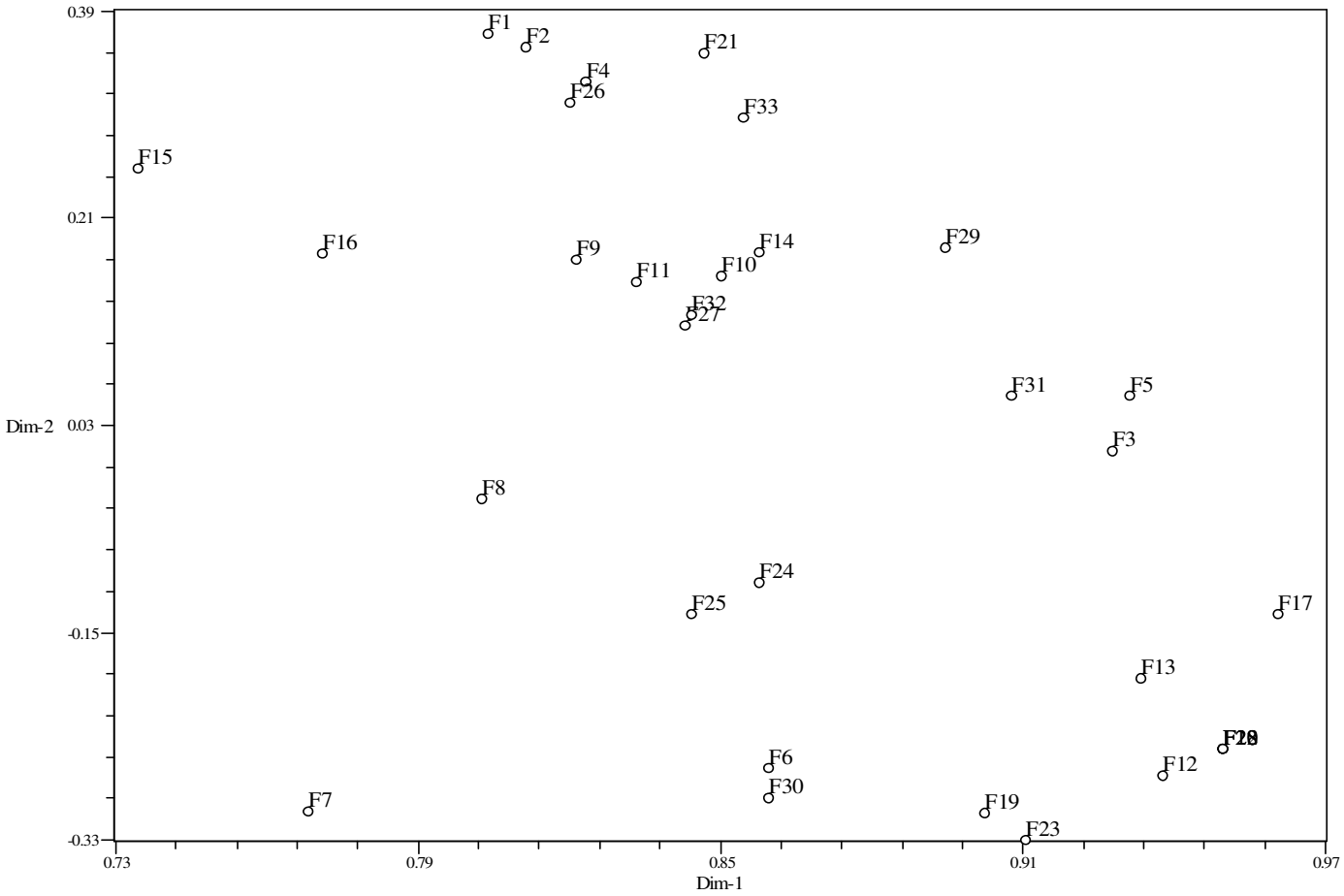

Figure 4. Two-dimension plot by principal component analysis (NTSYS PC) of 33 *Fusarium solani* isolates using genetic diversity data for 27 alleles at seven microsatellite loci.

Table 3. Microsatellites expected (H_E) and observed (H_O) heterozygosity, fixation index, and Shannon's index obtained from genotyping 33 *Fusarium solani* isolates.

Marker	Expected heterozygosity	Observed heterozygosity	Fixation index	Shannon's index
AF 513014	0.6757	0.2692	0.5938	1.4068
Ay 117125	0.7981	0.2273	0.7086	1.62
Ay 212027	0.6996	0.44	0.3582	1.2141
BC 5	0.2396	0	1	0.4702
RB 23	0.3394	0	1	0.5168
RE 14	0.0678	0	1	0.15
RE 102	0.3734	0.0357	0.9026	0.7505
Mean	0.4562	0.1388	0.7947	0.8754

index with values ranging from 0.35 to 1 (mean 0.79), in BC5, RB23 and RE14 primers fixation index was 1 and Shannon's index and PIC were significantly lower than other primers (Table 2). These primers were not suitable for evaluation of genetic diversity in *F. solani* isolates. Moreover, in previous study (Mwang'ombet et al., 2008) the number and size range of bands reported for these primers were low. The highest genetic diversity was observed in AY117125 and AF513014 primers.

Furthermore, in cluster analysis, 33 isolates of *F. solani* were divided into 10 clades in 78% Jaccard's similarity (Figures 3). Clade A had two members (F4 and F9). Of the three isolates that existed in clade B, two belonged to same host and region, while Clade C had two members that

Table 4. Size and frequency of each allele obtained per locus from genotyping 33 *Fusarium solani* isolates.

AF513014 (bp)	F	Ay117125 (bp)	F	Ay212027 (bp)	F	BC 5 (bp)	F	RB23 (bp)	F	RE14 (bp)	F	RE102 (bp)	F
287	0.09	**285**	0.21	**474**	0.36	**182**	0.60	**190**	0.78	**268**	0.84	250	0.09
272	0.12	**266**	0.21	371	0.30	170	0.06	167	0.21	260	0.03	225	0.06
232	0.06	262	0.06	**300**	0.36	164	0.03					**200**	0.66
218	0.09	246	0.03	168	0.06							175	0.06
200	0.15	235	0.18										
182	0.48	225	0.12										

Bold figures are most frequent alleles. F, Frequency.

were isolated from gramineous host at the same region. Clades D to H had one member Isolate, F32, from the insect *E. bigella* which differed from other isolates. In this isolate, the length of phialides and macroconidia were higher than other isolates. Clade G had five members (F10, F27, F29, F31 and F33), with all of these isolates isolated from Solanaceous hosts. Clade I have 13 member including F3, F5, F6, F12, F13, F17, F18, F19, F20, F22, F23, F24 and F28 indicated that four isolates (F18, F20, F22 and F28) have 100% similarity. Clade J had two members (F15 and F16) that were collected from *Oryza sativa* and the weed of *O. sativa* farms (*Cynodon dactylon*) at the same region. However, the microsatellite grouping was partly dependent with host but not dependent colony characteristics and virulence of the isolates.

As a second step, principle component analysis (PCA) was done, and to do this, a correlation matrix of the data was calculated with NTSYS software (Figures 4). The high genetic diversity for these isolates, both in phenotype and genotype suggest that the lineage could be diversifying. These isolates were collected from various host and geographical area in Iran, which could be a reason for this high diversity. Genetically, *F. solani* carries a number of dispensable “B” chromosomes that do not segregat e in a 1:1 manner following meiosis (Miao et al., 1991). These “B” chromosomes may carry one or more copies of some transpo-sable elements (Enkerli et al., 1997; Shiflett et al., 2002) and are implicated in pathogenicity towards pea (Kistler et al., 1996; Wasmann and Van Etten, 1996). Based on CHEF gel karyotype and light microscopic observations, the various mating populations known in *F. solani* have five to 13 chromosomes (Nazareth and Bruschi, 1994; Suga et al., 2002; Taga et al., 1998), a result consistent with the suggestion that these entities should be described as species.

F. solani is known to be very persistent in soil and capable of surviving in infested soils almost limitlessly, and some strains may become patho-genic or non-pathogenic. This study was based on a limited number of microsatellite DNA markers. A more expansive study using more SSR makers to include both pathogenic and nonpathogenic isolates and other related *Fusarium* species is needed to better understand the variation in this fungus. This is, however, the first study on the genetic diversity of *F. solani* in Iran using micro-satellite analysis.

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REFERENCES

Abawi GS, Pastor-Corrales MA (1990). Root rots of beans in Latin America and Africa: Diagnosis, research methodologies and management strategies. CIAT, Cali. Colombia, p. 114.

Achenbach LA, Patrick J (1996). Use of RAPD markers as a diagnostic tool for the identification of *Fusarium solani* isolates that cause soybean sudden-death syndrome. Plant Dis. 80: 1228-1232.

Aoki T, O’Donnell K, Homma Y, Lattanzi AR (2003). Sudden death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex–*F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia, 95: 660-684.

Booth C (1971). The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment lengh polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Dijk CT, Vats TS, Berg WH, Landwehr AJ (1980). *Fusarium solani* infection of a hypertensive leg ulcer in a diabetic. Mykosen, 23: 603-606.

Enkerli J, Bhatt G, Covert SF (1997). *Nht1*, a transposable element cloned from a dispensable chromosome in *Nectria haematococca*. Mol. Plant-Mic. Int. 10: 742-749.

Isanda GO (1995). Assessment of the level of bean (*Phaseolus vulgaris* L.) infection by Colletotrichum lindemuthianum (Sacc. Magn.) Brit. and CAV. It’s implication

on disease incidence and severity. Msc. Thesis. University of Nairobi.
Gerlach W, Nirenberg HI (1982). The genus *Fusarium*-a pictorial atlas. Mitt. Biol. Bundes. Land- Forst. (Berlin–Dahlem) 209: 1-406.
Kistler HC, Meinhardt LW, Benny U (1996). Mutants of *Nectria haematococca* created by a site-directed chromosome breakage are greatly reduced in virulence toward pea. Mol. Plant-Mic. Int. 9: 804-809.
Leslie JF (2001). Population genetics level problems in the *Gibberella fujikuroi* species complex. in: *Fusarium*: Paul E. Nelson Memorial Symposium. pp. 113-121
Li S, Tam YK, Hartman GL (2000). Molecular differentiation of *Fusarium solani* f. sp. *glycines* from other *F. solani* based on mitochondrial small subunit rDNA sequences. Phytopathol, 90: 491-497.
Matuo T, Sakurai Y (1965). *Fusarium solani* f. sp. *robbiniae* one of the casual fusaria of the twig blight of *Robinia psedocacia*. Ann. Phytopathol. Soc. Japan, 30: 562-656.
Matuo T, Snyder WC (1973). Use of morphology and mating population in the identification of formae speciales in *Fusarium solani*. Phytopathol, 63: 562-565.
Miao VP, Covert SF, Van Etten HD (1991). A fungal gene for antibiotic resistance in a dispensable ("B") chromosome. Science, 254: 1773-1776.
Mwang'ombe AW, Kipsumbai PK, Kiprop EK, Olubayo FM, Ochieng JW (2008). Analysis of Kenyan isolates of *Fusarium solani* f. sp. *phaseoli* from common bean using colony characteristics, pathogenicity and microsatellite DNA. Afr. J. Biotechnol. 7: 1662-1671.
Nazareth SW, Bruschi CV (1994). Electrophoretic karyotype of *Fusarium solani*. Microbiology, 140: 1373-1375.
Nei M (1987). Molecular Evolutionary Genetics. New York: Columbia Univ Press.
Nelson PE, Tousoun TA, Marasas WFO (1983). *Fusarium* Species: An illustrated manual for identification. Pennsylvania State University, University Park.
Nirenberg HI (1976). Untersuchungen über die morphologische und iologische Differenzierung in der *Fusarium* Sektion *Liseola*. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft (Berlin–Dahlem) 169: 1–117.
Nirenberg HI, O'Donnell K (1998). New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia, 90: 434-458.
O'Donnell K (2000). Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia, 92: 919-938.
Olivieri F, Zanetti ME, Oliva CR, Covarrubias AA, Casalongue CA (2002). Characterization of an extracellular serine protease of *Fusarium eumartii* and its action on pathogenesis related proteins. Eur. J. Plant Pathol. 108: 63-72.
Poltronieri LS, Trinidad DR, Albuquerque FC, Duarte MLR, Cardoso SS (2002). Incidence of *Fusarium solani* in annulled in the state of Para. Brazil. Fitopatol. Bras. 27: 544.
Raeder V, Broda P (1985). Rapid preparation of DNA from filamentous fungi. Lett. Appl. Microbiol. 1: 17-20.
Rayner RW (1970). A mycological colour chart. Commonwealth Agricultural Bureaux.
Rupe JC, Correll JC, Guerber JC, Becton CM, Gbur EF, Cummings MS, Yount PA (2001). Differentiation of the sudden death syndrome pathogen of soybean, *Fusarium solani* f. sp. *glycines*, from other isolates of *F. solani* based on cultural morphology, pathogenicity, and mitochondrial DNA restriction fragment length polymorphisms. Can. J. Bot. 79: 829-835.
Sakurai Y, Matuo T (1959). On the form name and race of *Hypomyces solani* (Rke. et Berth.) Snyd. et Hans. which is pathogenic to the mulberry trees. Ann. Phytopathol. Soc. Japan, 24: 219-223.
Sakurai Y. Matuo, T (1960). Studies on the intraspecific groups in *Fusarium solani* (1). Res Rep Fac Textile Sericulture, Shinshu Univ. 10: 21-32.
Sakurai Y, Matuo T (1961a). Taxonomy of the casual fungus of trunk-blight of *Xanthoxylum piperitum* and heterothallism in this fungus. Ann. Phytopathol. Soc. Japan, 26: 112-117.
Sakurai Y, Matuo T (1961b). Studies on the intraspecific groups in *Fusarium solani* (2). On the differentiation in utilization of carbon sources among the intraspecific groups. Ann. Phytopathol. Soc. Japan, 26: 118-124.
Shiflett AM, Enkerli J, Covert SF (2002). *Nht2*, a *copia* LTR retrotransposon from a conditionally dispensable chromosome in *Nectria haematococca*. Curr. Genet. 41: 99-106.
Snyder WC, Hansen HN (1941). The species concept in *Fusarium* with reference to section *Martiella*. Am. J. Bot. 28: 738-742.
Stover RH (1981). *Fusarium solani* in the tropics. In: Nelson PE, Tousson TA Cook RJ (1981). *Fusarium:* Disease, biology and taxonomy. Pennsylvania: The Pennsylvania State University Press, pp. 114-120.
Suga H, Hasegawa T, Mitsui H, Kageyama K, Hyakumachi M (2000a). Phylogenetic analysis of the phytopathogenic fungus *Fusarium solani* based on the rDNA-ITS region. Mycol. Res. 104: 1175-1183.
Suga H, Oyabu K, Ito M, Kageyama K, Hyakumachi M (2000b). Detection of intron-like sequences in the small subunit rDNA 3′ region of *Fusarium solani*. Mycol. Res. 104: 782-787.
Suga H, Ikeda S, Taga M, Kageyama K, Hyakumachi M (2002). Electrophoretic karyotyping and gene mapping of seven *formae speciales* in *Fusarium solani*. Curr. Genet. 41: 254-260.
Summerell BA, Salleh B, Leslie JF (2003). A utilitarian approach to Fusarium identification. Plant Dis. 87: 2.
Taga M, Murata M, Saito H (1998). Comparison of different karyotyping methods in filamentous ascomycetes: A case study of Nectria haematococca. Mycol. Res. 102: 1355-1364.
Taylor JW, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000). Phylogenetic species recognition and species concepts in Fungi. Fungal Genet. Biol. 31: 21-31.
Theron DJ, Holz G (1989). Fusarium species associated with dry and stem-end rot of potatoes in South Africa. Phytophylactica 21: 175-181.
Wasmann CC, Van Etten HD (1996). Transformation-mediated chromosome loss and disruption of a gene for pisatin demethylase decrease the virulence of Nectria haematococca on pea. Mol. Plant-Mic. Int. 9: 793-803.
Wollenweber HW, Reinking OA (1935). Die Fusarium ihre Beschreibung, Schadwirkung und Bekampfung. Berlin: Paul Parey, p. 355.
Wright S (1978). Variability within and among natural populations. Vol. 4. The Univ. Of Chicago Press, Chicago.