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GENETIC DIVERSITY IN POPULATION OF *FUSARIUM SOLANI* FROM CUMIN IN IRAN

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Abstract: Nineteen isolates of *Fusarium solani* were recovered from crown and root rotted parts of cumin plants collected from the major cumin producing area in Iran during 1999–2000 using Nash-Snyder media. One hundred and fifty eight *nit* mutants of *F. solani* were generated using PDA amended with 3% and 5% potassium chlorate. Of the *nit* mutants generated, 47/7%, 26/6% and 25/9% were *nit1*, *nit3* and *nitM*, respectively. *Nit* mutants were used to force heterokaryon formation to determine VCGs and their relation to pathogenecity and geographic origin. Fifteen VCGs were determined for *F. solani* isolates, that 12 were single members VCGs. There was no specific relation between VCGs and geographic origin in *F. solani* isolates. This is the first research on the genetic diversity of *F. solani* from cumin.

Key words: Cumin, *Fusarium solani*, Vegetative compatibility groups, *nit* mutant

INTRODUCTION

Cumin (*Cuminum cyminum* L.) belongs to the family *Apiaceae* and is believed to native of the Mediterranean and Near Eastern regions. Cumin is grown for production of the dry ripe fruits. It was known to the ancient Egyptians as a spice and medicinal plant. In addition to its common use as spice in our daily life, recent studies have indicated its pharmaceutical and medicinal importance (Aruna and Sivaramakrishnan 1996). It is mainly cultivated in India, Egypt, Libya, Iran, Pakistan and Mexico (Peter and Nybe 2002). Cumin is cultivated on 30960 hectares and production of 15 000 tones in Iran, mainly in the provinces of Khorasan and Kerman (Nooras Mofrad *et al*. 2005). Fusarium species are the most important pathogens that affect cumin fields in Iran. These pathogens are distributed via seeds and soil. *F. oxysporum* and *F. solani* were isolated from infected plants and soil of Khorasan fields (Nooras Mofrad *et al.* 2005).

The objectives of this study were determination of genetic diversity among Iranian isolates of *F. solani*, the agent of cumin wilt, based on VCGs and identification of the most effective chlorate media for generation of *nit* mutants of *F. solani* for VCG complementation pairings.

MATERIALS AND METHODS

Sample collection, culture isolation and identification

Infected plants were collected during 1999–2000. Isolates of *F. solani* were recovered from the top and lateral roots of cumin plants showing typical root rot symptoms. Top root and side root samples were trimmed, washed in running tap water to remove soil, blotted dry and cut into 10-mm segments. Root segments were sterilized with 0.5% NaOCl for 1 min. Isolations from symptomatic roots were made on Nash-Snyder media. Each longitudinal section was placed into a separate Petri dish containing PDA (Burgess *et al*. 1994). Fusarium colonies were observed microscopically. Those colonies identified as *F. solani* were transferred to carnation-leaf agar (CLA) and PDA. Single spore isolation made from each colony. Isolates were identified morphologically to species based on characteristics of macroconidia, phialides, microconidia, chlamydospores and colony growth traits (Leslie and Summerell 2006).

Pathogenecity tests

Ten seeds of cumin were planted in each of 30 4-cm-diameter plastic pots containing a soil mixture. After seed germination, a plug of *F. solani* culture was put on the base of a seedling. Negative controls were treated same way but lacked fungi. The pots were placed in a greenhouse at 25±2°C. Symptoms were evaluated after 3–6 days of incubation (Dang 1995).

Vegetative compatibility

VCGs were determined using nitrate non utilizing (*nit*) mutants as a visual indicator of heterokaryon formation (Klittich and Leslie 1988). *Nit* mutants were generated from each of *F. solani* isolates on potato dextrose agar containing 3% KClO3. The concentration of KClO3 was increased to 5% for isolates that were not restricted by 3% KClO3 (Kistler *et al*. 1998). The fast growing, chlorate-

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resistant sectors originating from the initially restricted colony, which grew sparsely but expansively on Puhalla's minimal medium (MM), were considered *nit* mutants (Puhalla 1985). *Nit* mutants were phenotypically classified by their growth on basal medium (MM without NaNO3) amended with one of several *nit*rogen sources (Correll *et al*. 1987). Several *nit1* and *nitM* mutants from all isolates were stored in sterile distilled water at 4°C. Before complementation tests between the isolates, vegetative self-incompatibility of each isolate was examined (Jacobson and Gordon 1988). *Nit1* and *nitM* mutants of all isolates of *F. solani* were then paired in all possible combinations on minimal medium and the plates were incubated at 25°C in the dark. Vegetatively compatible isolates were recognized by the robust growth at the interface of the two colonies after 10 days (Klittich and Leslie 1988).

RESULTS AND DISCUSSION

Nineteen isolates of *F. solani* were recovered from crown and root rotted parts of cumin plants collected from the major producing areas (Mashhad, Sarakhs, Ayask, Khavaf, Boshroye and Bostagh) in Iran during 1999–2000 (Table 1).

Table 1. Vegetative compatibility groups of *F. solani* used in the study

Isolate	Origin	VCG group
Fs1	Mashhad	A
Fs2	Mashhad	A
Fs3	Khavaf	B
Fs4	Sarakhs	B
Fs5	Ayask	B
Fs6	Ayask	C
Fs7	Ayask	C
Fs8	Mashhad	D
Fs9	Roshtkhar	E
Fs10	Nasrabad	F
Fs11	Sarakhs	G
Fs12	Bushroyeh	H
Fs13	Torogh	I
Fs14	Sarakhs	J
Fs15	Sarakhs	K
Fs16	Ayask	L
Fs17	Ayask	M
Fs18	Bostagh	N
Fs19	Ayask	O

Colonies of isolates were woolly to cottony with cream to white aerial mycelium and a cream reverse. Sporodochia were blue-green or blue, conidiophores had simple or branched monophialides, macroconidia were moderately curved, thick-walled, usually 3–5 septate, microconidia were one to three-celled, chlamydoconidia occurred both singly and in pairs (Fig. 1).

Isolates of *F. solani* were examined for their pathogenecity on seedlings of cumin. Results of pathogenecity tests revealed that all isolates were pathogenic to cumin and produce crown and root rot.

F. solani isolates produced chlorate-resistant sectors on media complemented with chlorate. One hundred and fifty eight *nit* mutants were generated from 19 isolates of *F. solani* using PDA amended with 3% and 5% potassium chlorate. The number of sectors were observed at 3.0% (w/v) of chlorate concentration. The *nit* mutants were divided into three classes; *nit1* (a mutation of nitrate reductase structural locus), *nit3* (a mutation of nitrate-assimilation pathway specific locus) and *nitM* (mutations that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity). The most frequent phenotype was *nit1* (47/7%), followed by *nit3* (26/6%) and *nitM* (25/9%) among total 158 mutants (Table 2). According to the literature data the frequency of mutant *nit1* is higher than the frequency of other types of *nit* mutants (Klittich and Leslie 1988).

Nit mutants were used to force heterokaryon to determine distribution of VCGs and their relation to pathogenecity and geographic origin. Several *nit1* and *nitM* mutants from each isolate were selected for complementation tests. No self-incompatibility was observed between complementary *nit* mutants recovered from the same isolate. Rare occurrence of self-incompatibility was also observed in the various formae speciales of *F. oxysporum* (Katan *et al*. 1994). On the other hand, 16 isolates out of 28 were self-incompatible in *F. graminearum* (Moon *et al.* 1999). This difference might be due to the existence or absence of gene(s) or mutations controlling heterokaryon self-incompatibility (Correll *et al.* 1987; Klittich and Leslie 1988).

Based on pairing complementary *nit* mutants of all isolates, mainly with *nit1* and n*itM* mutants, the 19 isolates were grouped into fifteen VCGs that 12 VCGs have single member. VCGs A, B and C had 2, 3 and 2 isolate members respectively (Table 1).

Fusarium species are the most important plant pathogens in the world (Booth 1971). Seventeen years ago, Puhalla developed a method by which isolates within different formae specials of *F. oxysporum* could be classified in VCGs (Puhalla 1985). Vegetative compatibility provides means for characterizing variation based on genetics of the fungus rather than on the host–pathogen interaction

Table 2. Frequency and phenotypes of *nit* mutants recovered from *F. solani* on cumin

Phenotype of *Nit* mutants	Growth on nitrogen sources				Total *Nit* mutants
	Nitrate	Nitrite	Hypoxanthine	Amonium Tartarate	
Nit1	–	+	+	+	75(47/7%)
Nit3	–	–	+	+	42(26/6%)
NitM	–	+	–	+	41(25/9%)

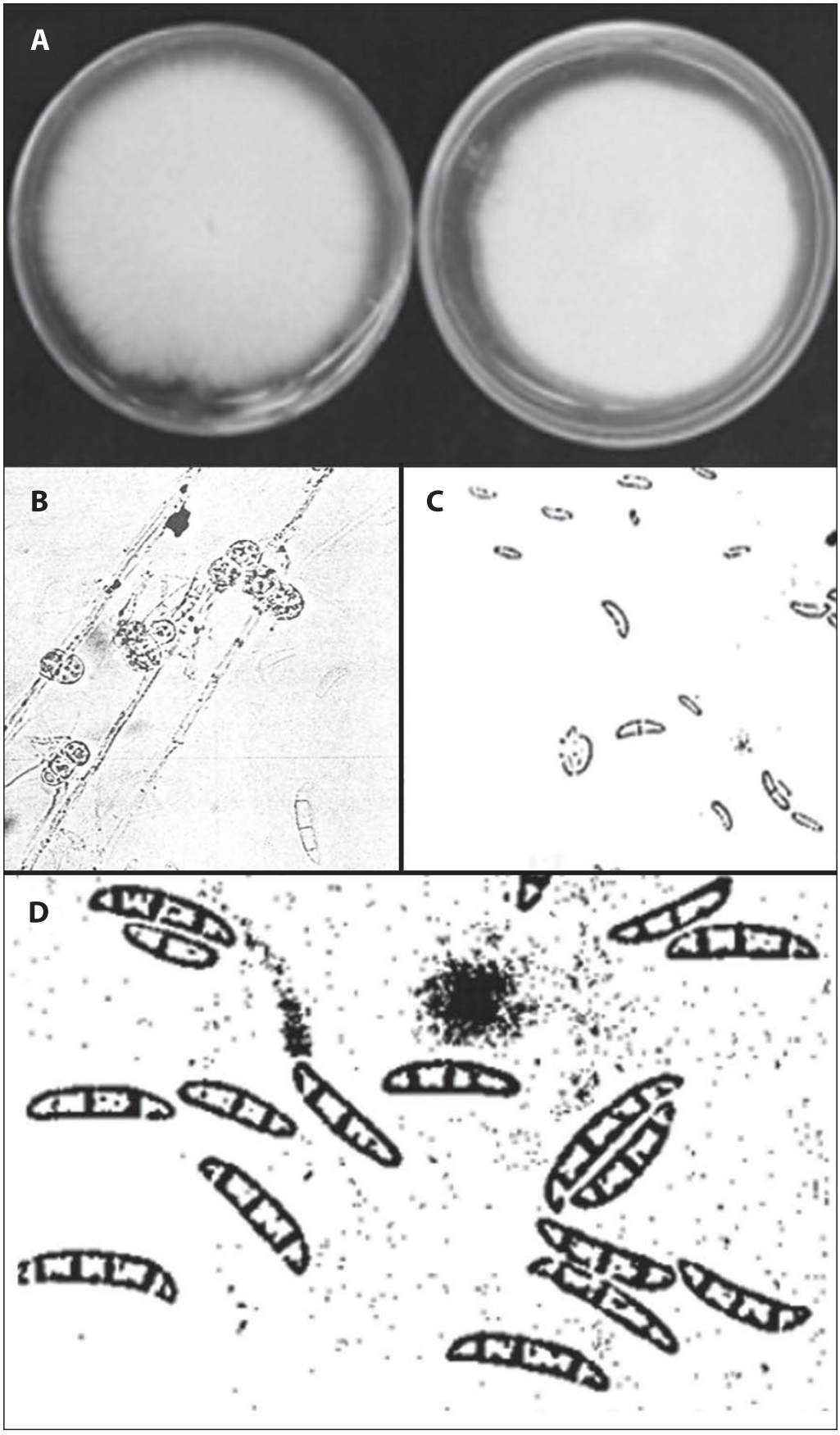

Fig. 1. Morphological characteristics of *F. solani* from cumin colony on PDA (A), Chlamydospores (B), Microconidia (C), Macroconodia (D)

(Leslie 1993). Isolates in a VCG often share pathological and physiological attributes as well as geographic origin. Consequently, vegetative compatibility has been used to study the origins of and relatedness among plant pathogenic fusaria (Katan 1999). In general, strains within a VCG tend to be more genetically similar than strains in different (Leslie 1993).

This study demonstrated a high degree of VCGs heterogeneity among Iranian isolates of *F. solani*. The 19 *F. solani* isolates, from diseased cumin plants sampled from Iran belonged to fifteen VCGs. Sharifi and his group (Sharifi *et al*. 2008) showed high genetic diversity in Iranian isolates of *F. solani* that were isolated from potato. Hawthorne and Rees-George have accommodated 22 isolates of *F. solani* in nine different VCGs and demonstrated high genetic variability with *F. solani* (Hawthorne and Rees-George 1996). There was no correlation between VCG and geographical origin of isolates.

The high genetic diversity observed in *F. solani* isolates from cumin in Iran might be due to the occurrence of a sexual state of the fungus and the genetic recombination that occurs as a result of sexual reproduction. Another possibility is that there are several species to be described within the *F. solani* species complex. It has been discovered that the classification of *F. solani* needs to be revised. Morphological, molecular and VCG techniques are required for study and classification of *F. solani* populations.

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POLISH SUMMARY

GENETYCZNA RÓŻNORODNOŚĆ POPULACJI *FUSARIUM SOLANI* Z KMINU RZYMSKIEGO W IRANIE

W latach 1999–2000 z głównych areałów Iranu produkujących kmin rzymski uzyskano, ze zgniłych części nadziemnych i korzeni kminu, dziewiętnaście izolatów *Fusarium solani*, wykorzystując pożywki Nash-Snyder. Przy użyciu pożywki PDA wzbogaconej 3% i 5% chloranem potasu otrzymano 158 mutantów *nit F. solani*. Z uzyskanych mutantów *nit* 47/7%, 26/6% i 25/9% było odpowiednio mutantami *nit*1, *nit*3 i *nit*M. Mutanty *nit* użyto do otrzymania heterokarionów w celu określenia grup VCG i ich odniesienia do patogeniczności oraz pochodzenia geograficznego. Dla izolatów *F. solani* określono 15 grup VCG, z których 12 było pojedynczymi częściami składowymi grup wegetatywnej kompatybilności (VCG). Nie było specyficznego związku pomiędzy grupami VCG i geograficznym pochodzenia izolatorów *F. solani.* Jest to pierwsze doniesienie dotyczące genetycznej różnorodności *F. solani* z kminu rzymskiego *Fusarium*.