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Genetic diversity of *Fusarium moniliforme* detected by vegetative compatibility groups and random amplified polymorphic DNA markers

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Genetic diversity among *Fusarium moniliforme* isolates was analysed using vegetative compatibility group (VCG) and random amplified polymorphic DNA (RAPD) techniques. In the first experiment, RAPD was used to analyse a set of 43 isolates collected from different corn growing areas in Israel and the US. The isolates were assigned to 27 different VCGs. Thirty-two RAPD haplotypes were also detected by analysing 48 polymorphic bands. RAPD could differentiate all the VCGs, except in two cases where two VCGs were assigned a single RAPD haplotype. In six cases, however, molecular variation was detected among isolates belonging to the same VCG. Cluster analysis of the RAPD data showed a very good agreement with the VCG grouping, e.g. isolates of the same VCG were always closely clustered by the molecular data. In a second experiment, 63 isolates of *Fusarium moniliforme* were collected from six corn lines growing in a single corn field. Extensive genetic variation was observed among the isolates: 42 different VCGs and 37 RAPD haplotypes were identified. Once again, RAPD patterns could differentiate nearly all the VCGs. However, in four cases, two different VCGs were grouped into a single RAPD haplotype, while in another three cases, isolates of the same VCG could be differentiated by distinct molecular haplotypes. The variation data was used to gain insight on the population structure and the patterns of genetic variation among geographical locations and within a single field. Hierarchical gene diversity analysis of the RAPD data indicated that most of the genetic variability (81%) was distributed within corn lines in the same field, suggesting that RAPD haplotype, or VCG frequencies, were not significantly affected by the plant genotypes grown in this experiment. Most of the RAPD band combinations did not display significant gametic phase disequilibrium, suggesting that active recombination might be occurring in the field. Our results indicate that by using a small number of primers, similar resolution was obtained by RAPD and VCG analysis, respectively. RAPD analysis is however, simpler to perform and its sensitivity in genotyping individuals within *Fusarium moniliforme* can be increased by analysing more primers, enabling a more detailed population genetic analysis of this important pathogen.

INTRODUCTION

Fusarium moniliforme is the major species that causes ear, stalk and root rot on corn in Israel. In 1994 and 1995, the disease was so severe that in some fields the entire crop had to be discarded. Soil and seeds are apparently the main primary inoculum sources of *F. moniliforme* on corn (Christensen & Wilcoxson, 1966; Headrick & Pataky, 1990; Leslie et al., 1990; Lipps & Deep, 1991). Corn plant residues in the soil are known to be the major overwintering sites of *F. moniliforme* (Christensen & Wilcoxson, 1966; Damicone et al., 1988; Lipps & Deep, 1991). During the growing season, conidia might also be carried by wind (Damicone et al., 1988) and insects (Smelzer, 1958; Farrar & Davis, 1991). The mode of entry of *F. moniliforme* into the kernels is still unclear. Kernel infection might result from either internal growth of the fungus in the plant stalk (Foley, 1962; Christensen & Wilcoxson, 1966) or through wounds in the kernel (Smelzer, 1958; Sutton, 1982; Farrar & Davis, 1991). *F. moniliforme* can be isolated from all plant tissues, including those that do not exhibit disease symptoms. Koehler (1942) found *F. moniliforme* in a decreasing order of prevalence in the silks,

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kernels, pedicels, vascular cylinder and butt of cob, and claimed that this distribution indicates the path of infection.

Knowledge of the genetic structure of pathogen populations has direct agricultural applications. For instance, the genetic variation maintained within a population indicates the speed at which a pathogen evolves (McDonald & McDermott, 1993). This information might eventually be used to predict the efficacy of control measures, e.g. use of resistant cultivars or fungicide application.

To date, most population genetic studies of *F. moniliforme* have been conducted using the vegetative compatibility group (VCG) as a marker for genotyping fungal isolates (Farrokhi-Nejad & Leslie, 1990; Campbell et al., 1992; Kedera et al., 1994). Strains that are vegetatively compatible, i.e. belong to the same VCG, can form a stable heterokaryon, and share an identical set of alleles at about 10 vic loci (Leslie, 1993). The VCG technique is particularly suitable for population genetic studies of *F. moniliforme*, because field isolates of this fungus belong to many VCGs (Leslie et al., 1992). Isolates of *F. moniliforme* belonging to the same VCG are presumed to be clones, and VCG analysis might therefore be used for strain identification (Kedera et al., 1994). The VCG technique has, however, some limitations. First, generation of *nit* mutants (Correll et al., 1987), necessary for VCG assignment, is laborious and time consuming. For some isolates, generation of *nitM* or *nit3* mutants is very difficult, if not impossible. Secondly, self-incompatibility (Jacobson & Gordon, 1988) can make the VCG assignment of an isolate impossible. Finally, from the viewpoint of population biology, VCG analysis assays only one marker, while additional markers are needed for detailed population studies, e.g. determining the extent of genetic variation and its distribution in a pathogen population. Molecular analysis might provide the additional, useful information required for a more profound understanding of this pathogen.

Several molecular techniques are available for investigating genetic variability within plant pathogenic fungi populations. These include RFLP (restriction fragment length polymorphism) analysis of both nuclear and mitochondrial DNA (McDonald & Martinez, 1990; Kim et al., 1992), DNA fingerprinting (Levy et al., 1991), and isozyme markers (Welz & Leonard, 1993). In recent years, PCR (polymerase chain reaction)-based markers, especially RAPD (random amplified polymorphic DNA) have become popular because of their technical simplicity, and potential for rapid screening of large numbers of individuals using minimal amounts of DNA. RAPD markers are especially suitable for haploid plant pathogenic fungi, in which the dominance of the amplified fragments does not affect genetic analysis (Williams et al., 1991). This technique has been successfully used to assess genetic variability within many plant pathogenic fungi (Goodwin & Annis, 1991; Jones & Dunkle, 1993; Schilling et al., 1993; Huff et al., 1994; Kelly et al., 1994), including *Fusarium* section *Liseola* (Amoah et al., 1995, 1996; Voigt et al., 1995; MacDonald & Chapman, 1997). In these studies isolates from different countries were surveyed, and RAPDs could successfully distinguish between mating populations of *Fusarium* section *Liseola*. The researchers also tried to correlate RAPD variation with the host, and the tissue, from which the isolates originated (Amoah et al., 1995, 1996), and to the level of fusaric acid (MacDonald & Chapman, 1997) and gibberellic acid (Voigt et al., 1995) produced by the different isolates. In this study we used both the VCG and RAPD techniques to analyse two sets of *F. moniliforme* isolates. In the first experiment, we analysed the genetic variability in a collection of *F. moniliforme* isolates from different geographical locations, to assess the ability of RAPDs, as compared to the VCG technique, in genotyping isolates of *F. moniliforme*. In the second experiment, we analysed a set of isolates from a single corn field, to demonstrate further the advantage of RAPD in assessing the fine scale genetic structure of *F. moniliforme* within a small area.

MATERIALS AND METHODS

Isolate collection

Set 1

To compare the RAPD technique with VCG, 43 single-conidial isolates of *F. moniliforme* were obtained from either corn plants or seeds from different locations (Table 1). These isolates were collected over a period of two years (1994–95), and were VCG typed separately from Set 2. Ten VCGs are represented by at least two isolates, enabling the examination of genetic variation within VCGs.

Set 2

Sixty-three isolates of *F. moniliforme* were collected in a corn field with different corn breeding lines at the Newe Yaar research station in Northern Israel. A single row (about 90 m) in the middle of

Table 1 Isolates of *Fusarium moniliforme* from different locations used in this study, their VCG assignment and RAPD haplotypes

Isolate	Location	VCG	RAPD haplotype
Z1	Hazor/Galilee	L1	1
Z2	Hazor/Galilee	L1	2
G1	Golan Heights	L2	3
G2	Golan Heights	L2	3
G3	Golan Heights	L3	4
G4	Golan Heights	L3	5
G5	Golan Heights	L3	6
G6	Golan Heights	L13	7[a]
G7	Golan Heights	L4	8
G8	Golan Heights	L4	8
G9	Golan Heights	L4	9
G10	Golan Heights	L26	10
G11	Golan Heights	L5	11
G12	Golan Heights	L5	11
G13	Golan Heights	L5	11
H1	Hula Valley	L6	7
H2	Hula Valley	L6	12
H3	Hula Valley	L6	12
H5	Hula Valley	L7	13
H6	Hula Valley	L7	13
H7	Hula Valley	L7	14
H8	Hula Valley	L8	15
H9	Hula Valley	L14	16
H10	Hula Valley	L9	17
H11	Hula Valley	L9	18
H12	Hula Valley	L15	19
N1	Newe Yaar	L10	20
N2	Newe Yaar	L10	20
N3	Newe Yaar	L11	21
N4	Newe Yaar	L11	21
N5	Newe Yaar	L11	21
N7	Newe Yaar	L12	22
N8	Newe Yaar	L27	23
A1	Ashdod	L16	24
A2	Ashdod	L17	25
A3	Ashdod	L18	26
A4	Ashdod	L19	27
A5	Ashdod	L20	28
A6	Ashdod	L21	29
U1	USA [b]	L23	30
U4	USA [b]	L24	30
Ht2	USA [c]	L22	31
Ht4	USA [c]	L25	32

[a]Haplotypes containing more than two VCGs are underlined. [b]Isolate recovered from seed, cv. 'Jubilee', imported from Rogers and Brothers, Idaho [c]Isolate recovered from seed of A619-isoline with *Ht1* resistance genes against *Exerohilium turcicum*, originated from USA.

the field was selected. The row was divided into 6 segments of equal length (about 14 m). In each row segment a different corn breeding line was grown. Shortly before harvest, three plants were sampled from each row segment, with approximately 4-m spacing between samples. The ear, the crown and the third node above the crown were cut off, put in separate plastic bags and labelled. Laboratory isolation was performed immediately after returning from the field. Tissue pieces from each plant part were surface sterilized by immersion in a solution of 1% NaOCl and 0.05% Tween 20 for 30 s, rinsed twice in sterilized distilled water and transferred to Nash-Snyder medium (15 g Difco peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 20 g agar, 1 g pentachlor-nitrobenzene (PCNB) per L, supplemented with 0.3 g L^{-1} streptomycin; Nelson et al. 1983). After incubation for about a week at 25°C, a small piece of agar with mycelium tips from the growing edge of the fungal colony was transferred to potato dextrose agar (PDA). Species identification was performed according to Nelson et al. (1983). All isolates were cultured from single spores before they were subjected to VCG and RAPD analyses.

Generation and characterization of *nit* mutants

Generation and characterization of *nit* mutants was performed according to the method described by Correll et al. (1987) and Kedera et al. (1994). Mycelial discs from each isolate were placed on medium containing 3% $NaClO_3$ for mutant selection. Hyphal tips from fast-growing sections were transferred to minimal medium for classification and further study. These mutants were assigned to *nit1*, *nit3* and *nitM* phenotypic classes based on differential growth on media containing $NaNO_3$, $NaNO_2$ and hypoxanthine, respectively, as sole nitrogen sources.

VCG assignment

VCG assignment was based on a complementation reaction between *nitM* (or *nit3*) and *nit1* mutants on minimal medium (Correll et al., 1987). Pairs of isolates that exhibited vigorous aerial growth at the contact site of the two *nit* mutant-mycelia, indicating the formation of an heterokaryon, were determined as vegetatively compatible and were assigned to the same VCG. Otherwise, isolates were defined as different VCGs. Pairing tests were conducted in 5-cm Petri dishes. The set-up of the test was as follows: in each Petri dish, four mycelial

discs were placed at the corners of a 1.5×1.5 cm square. At the ends of one diagonal were the *nitM* (or *nit3*, if *nitM* was not available) mutants of the two isolates to be paired, and on the ends of the other diagonal were the *nit1* mutants of the two isolates, respectively. All possible pairs were tested for all isolates for which *nitM* or *nit3* mutants were available.

Fungal culture and DNA preparation for RAPD analysis

Three 0.5 mm mycelial discs from the growing edge of single conidial colonies grown on PDA were used for inoculating flasks containing 50 mL of the medium (17.5 g Czapek-Dox broth and 5 g yeast extract per litre). Inoculated flasks were incubated for 5 days at 25°C without shaking. Mycelia were harvested, frozen at -20°C and lyophilized. Genomic DNA was extracted using the method described by Müller et al. (1992).

PCR-RAPD analysis

Amplification was performed by the method of Schilling et al. (1993) with some modifications. Each amplification mixture (25 μL) contained 25 ng template DNA, 0.1 mM of each dNTP, 2.5 mM MgCl, 10 mM Tris HCl, 50 mM KCl, 0.1% Triton X100, 0.2 mg mL^{-1}gelatin, 5 picomoles of the decamer primer, 1 unit Taq DNA polymerase (Appligene), pH 9.0. An Appligene thermocycler (Crocodyle II model) was programmed for an initial denaturation step at 94°C followed by 40 cycles. Each cycle consisted of 94°C for 1 min, a primer annealing step at 36°C for 1 min and primer extension at 72°C for 2 min Primer extension step of the final cycle was extended by 3 min The reaction products were subjected to electrophoresis on 1.5% agarose gels in $1 \times$TBE, stained with ethidium bromide and visualized by UV light (320 nm).Ten decamer primers (Operon Technologies, Alameda, CA, USA) were initially screened on five isolates, belonging to five different VCGs. Six of the these primers (Table 2), that amplified reproducible and polymorphic DNA fragments, were used for analysing all 43 isolates of Set 1. Only OPR11, OPA3 and OPA4 were used to analyse the isolates from the single field experiment (Set 2; Table 2). At least two separate amplifications were conducted for each isolate/primer combination. RAPD bands were recorded as either being present ('1'), or absent ('0') for each isolate.

Table 2 Primers used for RAPD analysis of 43 Isolates of *Fusarium moniliforme* from different locations (Set 1), and from the single field population (Set 2). The number of polymorphic bands produced by each primer among the isolates in each set is shown

Primer	Nucleotide sequence 5′ to 3′	Polymorphic markers
Set 1		
OPR11	GTAGCCGTCT	16
OPR14	CAGGATTCCC	10
OPR15	GGACAACGAG	5
OPR16	CTCTGCGCGT	10
OPR19	CCTCCTCATC	4
OPR20	ACGGCAAGGA	3
Total		48
Set 2		
OPR11	GTAGCCGTCT	8
OPA 3	AGTCAGCCAC	3
OPA 4	AGTCAGCCAC	2
Total		13

Data analysis

The RAPD data were subjected to parsimony analysis using the PAUP software (Swofford, 1993) to describe the possible genetic relationships among isolates.

Differences in RAPD band frequencies between corn lines were tested by the Fisher's exact test for association, using the PROC FREQ procedure of the SAS package (SAS Institute, 1982). Hierarchical gene diversity analysis (Beckwitt & Chakraborty, 1980) was used to partition genetic variation into components among and within corn lines in the field. For the calculation of gametic phase disequilibrium in our population, we interpreted the presence or absence of a RAPD band as two alleles of a putative locus in the calculation of gene diversity. Given two loci with two alleles each, A and a, and B and b, and letting PAB be the frequency of genotype AB, etc., the gametic phase disequilibrium (D) can be estimated, where

$$D = PAB \times Pab - PAb.$$

Deviations from zero gametic phase disequilibrium ($D = 0$, i.e. equilibrium) were tested statistically by the 2×2 contingency table G-test or by the χ^2 test, wherever the G-test was not possible because of the presence of zero values in any cell of the 2×2 contingency table (Sokal & Rohlf, 1981).

RESULTS

RAPD and VCG analysis of Fusarium isolates from different geographical locations

Out of 43 *Fusarium moniliforme* isolates, 39 were obtained from plant samples collected from different geographical locations in Israel, and the rest were isolated in the laboratory from seeds obtained from the USA. VCG analysis was performed on these 43 isolates (Set 1). For this purpose, *nit* mutants were prepared from single-spore cultures, and these were resolved into 27 different VCGs (designated L1 to L27, Table 1). In most geographical locations, isolates belonged to several VCGs, while isolates from different locations were never assigned the same VCG.

Six RAPD primers (Table 2) produced a total of 48 polymorphic bands among the 43 isolates. According to the patterns of all six primers, the isolates were classified into 32 RAPD 'haplotypes'. Thirty haplotypes contained isolates belonging to a single VCG, and only two, namely haplotypes 7 and 30, contained isolates from two distinct VCGs (Table 1). Thus, RAPDs could differentiate almost every VCG tested. Moreover, molecular variation was also detected within six of the 10 VCGs that contained multiple isolates. Thus, a single RAPD band amplified by the primer OPR 19 (the 1.1 kbp-band, Fig. 1) differentiated between isolates of VCG L1 (Z1 absent, Z2 present), L3 (G3 absent, G4 and G5 present), L7 (H7 absent, H5 and H6 present) and L9 (H10 absent, H11 present – Fig. 1a, b, c). The isolates within L3 could also be distinguished by a RAPD band of 1.23 kbp generated by primer OPR11, present in G3 and G4 and absent from G5 (Fig. 1d). This demonstrates that the RAPD technique might provide in many cases increased resolution for isolate genotyping, as compared to VCG assignment.

The relatedness of the different RAPD haplotypes was further analysed by constructing most-parsimonious trees of the isolates according to their RAPD patterns. In Fig. 2a, a shortest (104-steps long) tree, resulting from a Heuristic search, is shown. A Strict-Consensus tree was computed from over 100 shortest-trees found by such a search (Fig. 2b). Several of the nodes of such tree appeared in the majority of the trees obtained by 'Bootstrap' random-resampling of the data, and are indicated by asterisks. Most interestingly, Parsimony analysis has consistently grouped together isolates of the same VCG, showing that both VCG and RAPD can be related to each other, probably reflecting true genetic distances between isolates. Thus, isolates of the same VCG have never been grouped on distant

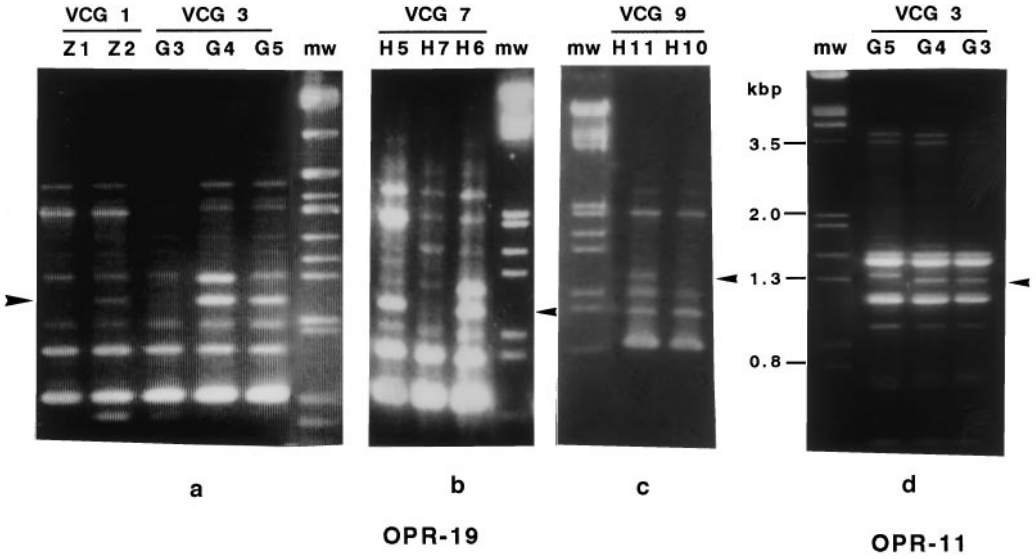

Fig. 1 Random amplified polymorphic DNA markers produced with primers OPR19 and OPR11, detecting genetic variation among isolates within vegetative compatibility groups of *Fusarium moniliforme*. VCG 1: isolates Z1 and Z2; VCG 3: isolates G3, G4 and G5; VCG 7: isolates H5, H7, H6; VCG 9: isolates H11 and H10. Arrows indicate the two differential bands; mw – molecular weight markers.

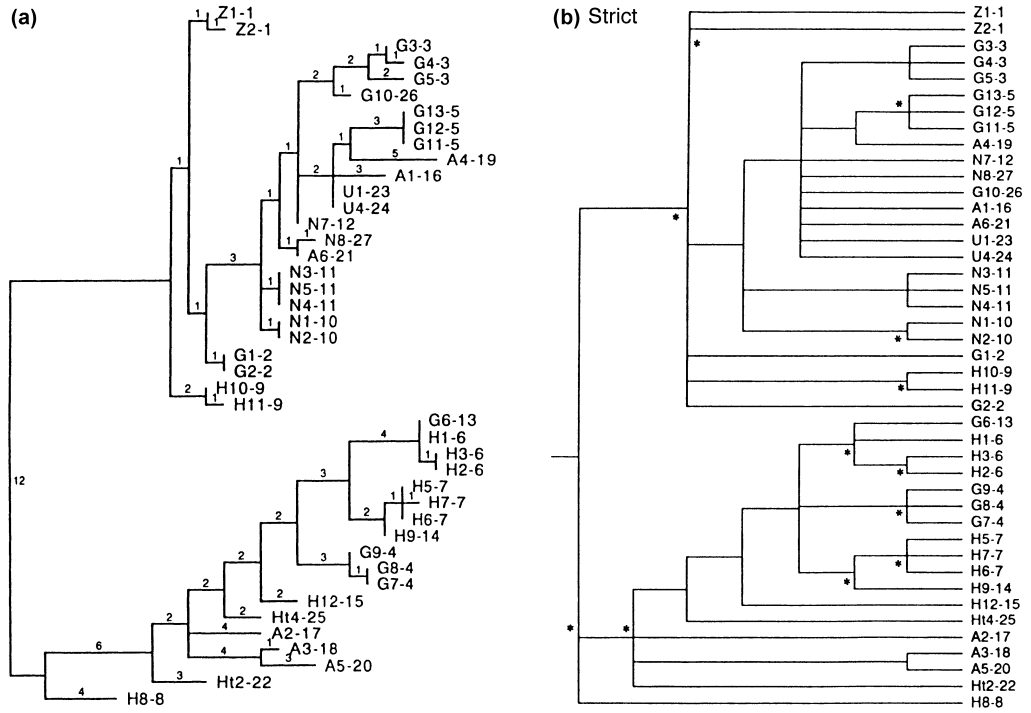

Fig. 2 Cluster analysis of the RAPD data on 48 polymorphic bands among 43 *Fusarium moniliforme* isolates. A. A most parsimonious tree (104 steps long) recovered by Heuristic search using different algorithm options of the PAUP program. Number of steps (i.e. branch length) is indicated above each branch. The first letter of the isolate code indicates geographical location. The VCG assignment of each isolate (Table 1) is aso shown, e.g. Z1–1 – isolate Z1, VCG L1. Note how branches of zero length lead to isolates that share the same haplotype, e.g. isolates U1 and U4. B. A 'Strict Consensus' tree was computed from more than 100 shortest-trees (104 steps long). The tree shows only the nodes that were common to all the shortest trees. The nodes that appeared in the majority (> 50%) of the shortest trees recovered by a 50-replicate random-resampling of the data (the 'Bootstrap test' run by PAUP), are indicated by asterisks.

branches. RAPD, being a more detailed and quantitative method of typing, can also show the relationships between different VCGs, e.g. that isolates belonging to VCGs L6 and L14 are closely related, as are L5 and L19. The isolates can be divided into two major clusters (Fig. 2), but the biological basis for such division remains unclear. The two clusters do not represent geographical separation, because isolates from most locations are distributed between the two clusters. We also could not correlate the clusters with the tissue from which the isolate was derived.

Genetic diversity within a single corn field

We then asked whether significant genetic variation could be detected between *Fusarium moniliforme* isolates from the same corn field. Sixty-three isolates of *Fusarium moniliforme* (Set 2) were collected from one field at Newe Yaar. In most cases, *F. moniliforme* could be isolated from every plant part (crown, stem or seeds), whether the plant displayed disease symptoms or was symptomless. Assignment to VCG turned out to be rather difficult for some isolates: *nit1* mutants were easily generated from all isolates, but *nitM* or *nit3* mutants could be generated only from 53 of the 63 isolates. Our results suggest that the generation of *nit* or *nit3* mutants is isolate-dependent. While in some isolates we could identify one *nitM* or *nit3* mutant from as few as four *nit* mutations, with other isolates we tested as many as 65 independent *nit* mutants and failed to isolate the desired mutation. In spite of this difficulty, we could assign 61 of the 63 isolates to 42 VCGs, designated F1 – F42 (Table 3). Of the 42 identified VCGs, 31 were represented by only a single isolate, and 11 VCGs were represented by 2–4 isolates. It was not possible to

Table 3 Isolates of *Fusarium moniliforme* from a single corn field. Data are classified according to three observed relationships between VCG and RAPD assignments

I. Direct VCG:RAPD correlation			II. Same VCG/different RAPDs			III. Different VCGs/same RAPDs		
Isolate[a]	VCG	RAPD haplotype	Isolate	VCG	RAPD haplotype	Isolate	VCG	RAPD haplotype
01r1	F1	28	01s2	F2	1	03e2	F4	14
01r2	F1	28	07e	F2	20	03s	F4	14
01s1	F1	28	07s	F2	20	14s2	F21	14
01e	F31	– -b	09e	F5	24	06e	F36	6
02e1	F32	19	09r	F5	24	10s1	F28	6
02r1	F32	19	12r	F5	29	12s	F18	15
02e2	F3	22	11s	F7	37	14e	F19	15
02r2	F3	22	11e	F7	37	18r	F26	3
02e3	F10	– -	15s1	F7	35	18s	F25	3
03e1	F11	33	15s2	F7	35			
03r1	F27	7						
03r2	F27	7						
04e1	F33	32						
04e2	F12	12						
05e1	F34	13						
05e2	F13	– -						
05r	F35	10						
05s	F14	11						
06r	F16	16						
06s	F15	27						
07r	F42	36						
08e1	F37	18						
08e2	F37	18						
08r	F17	– -						
09s	F38	31						
10e	F6	17						
10r	F6	17						
10s2	F29	30						
11r	F39	– -						
12e	F40	– -						
13e	F8	9						
13s1	F8	9						
13r	F9	25						
13 s2	?	– -						
14r	F9	25						
14s1	F20	21						
15e1	F6	17						
15e2	F6	17						
16e	F22	5						
16r	F24	2						
16s	F23	4						
17s1	?	26						
17s2	F30	23						
18e	F41	34						

[a] The first two digits in isolate designation represent the plant number; the next letter designates the tissue (e, ear; s, stem; r, crown) from which the isolate was derived. When more than one isolate was collected from the same tissue, an additional digit was added. For example, isolate 08s2 is the 2nd isolate collected from stem ot plant 8. Plants 1–3, 4–6, 7–9, 10–12, 13–15, and 16–18 belong to the six corn lines grown in the row, respectively. [b] Isolates that were not available for RAPD analysis.

assign a VCG to the remaining two isolates, because no *nitM* or *nit3* mutant could be generated from them, and no complementation reaction occurred between their *nit1* mutants and any of the *nit3* and *nitM* mutants available. Field isolates were observed to be very diverse genetically, as a minimum of 2 and a maximum of 4 VCGs were detected in each of the 18 plants sampled (Table 3).

Because a few isolates were lost upon storage, only 56 were available for DNA extraction. To these isolates, 3 primers (OPR11, OPA3 and OPA4), amplifying a total of 13 polymorphic bands, were applied (Table 2). These were sufficient to divide the 56 isolates into 37 RAPD haplotypes (Table 3), and to differentiate nearly all the VCGs. In four cases (RAPD haplotypes 3, 6, 14 and 15), isolates belonging to two VCGs displayed the same haplotype. In most cases, isolates belonging to the same VCG came from the same plant, except in five situations (F2, F5, F6, F7 and F9), where VCGs included isolates from different plants. However, RAPD analysis demonstrated that isolates of F2, F5 or F7, coming from different plants, had different RAPD haplotypes, whilst those from F6 (plants 10, 15, Table 3), and F9 (plants 13, 14), had identical RAPD haplotypes. In conclusion, RAPDs could differentiate isolates from one VCG into different haplotypes, and might provide at least the same resolution as VCG assignment for strain genotyping.

The fungal isolates of this set were derived from corn plants of six genotypes (breeding lines). Frequencies of the different RAPD markers among the isolates derived from each corn line in the field are summarized in Table 4. A Fisher's exact test for heterogeneity showed that most RAPD frequencies did not differ significantly among corn lines. Only in three of the 13 RAPD frequencies were there differences among corn lines, at a 0.05 significance level. Along the same line, hierarchical gene diversity analysis was performed (Table 4), indicating that most (>81%) of the variability in frequency of the 13 polymorphic RAPD bands was distributed within, rather than among, corn lines.

We then asked whether alternative alleles at the 13 putative RAPD loci appear to recombine in the *Fusarium* population under study. For this purpose we calculated, based on the frequency of each RAPD band in the population (Table 4, total frequency values), the apparent gametic phase disequilibrium values among all possible combinations of the 13 RAPD bands $(13\times12)/2=78$ combinations; see Methods). Our results indicated

Table 4 RAPD band frequencies of *Fusarium moniliforme* in six corn lines from a single corn field, and gene diversity calculated for RAPD putative loci

	RAPD band frequency in corn lines								Hier. gene diversity		
RAPD[a]	Line 1 n = 13[b]	Line 2 n = 8	Line 3 n = 8	Line 4 n = 8	Line 5 n = 11	Line 6 n = 8	Total n = 56	Prob.[c]	HT[d]	Gs[e]	GL[f]
OPR11–1.60	0.54	0.38	0.38	0.75	0.64	0.38	0.52	0.533	0.50	0.07	0.93
OPR11–1.50	0.38	0.63	0.50	0.25	0.55	0.50	0.46	0.729	0.50	0.05	0.95
OPR11–1.37	0.38	0.50	0.38	0.00	0.36	0.38	0.34	0.327	0.45	0.09	0.91
OPR11–1.20	0.77	0.88	0.75	0.63	0.82	1.00	0.80	0.582	0.32	0.10	0.90
OPR11–1.10	0.92	0.50	1.00	0.88	0.82	0.88	0.84	0.136	0.27	0.17	0.83
OPR11–1.12	0.31	0.25	0.13	0.00	0.00	0.50	0.20	0.043	0.32	0.19	0.81
OPR11–1.15	0.31	0.13	0.50	0.00	0.45	0.00	0.25	0.037	0.38	0.19	0.81
OPR11–1.18	0.38	0.13	0.00	0.63	0.18	0.25	0.27	0.073	0.39	0.18	0.82
OPA3–0.58	0.77	0.63	1.00	0.63	0.55	0.50	0.68	0.215	0.44	0.12	0.88
OPA3–1.18	0.62	0.88	0.25	0.63	0.45	0.38	0.54	0.165	0.50	0.14	0.86
OPA3–1.95	0.15	0.63	0.38	0.25	0.64	0.63	0.43	0.089	0.49	0.17	0.83
OPA4–0.91	0.15	0.00	0.25	0.25	0.09	0.13	0.14	0.725	0.24	0.06	0.94
OPA4–1.17	0.62	0.38	1.00	0.88	0.73	0.38	0.66	0.032	0.45	0.17	0.83

[a]RAPD bands are indicated by the primer and the band size in kbp. [b]n is the number of isolate tested. [c]Probabilty (2-tail) that the RAPD frequencies among all corn lines are the same (Fisher's exact test); values smaller than 0.05 are underlined. [d]HT is the total genetic diversity, calculated as in Beckwitt & Chakraborty (1980). [e]GS is the fraction of diversity among corn lines out of total diversity. [f]GL is the fraction of diversity within corn lines out of the total diversity.

that 66 out of 78 combinations (86%) did not display significant gametic phase disequilibrium at the 0.05 level (not shown).

DISCUSSION

The two goals of this study were to assess the ability of RAPDs to genotype isolates of *F. moniliforme*, as compared to the VCG method, and to analyse the distribution of genetic variation among isolates of this pathogen. Our results show that RAPD fingerprinting can provide, in most situations, the same resolution for genotyping as VCG. In our data set, the two methods provided, in fact, similar resolution. In a few cases, RAPD divided a VCG into different haplotypes, while in other cases a RAPD haplotype contained more than one VCG. It is, however, likely that we could have obtained greater resolution with RAPDs by increasing the number of bands scored. In conclusion, in spite of the high sensitivity it did provide, we would not recommend the VCG technique alone for strain or clone identification. While VCG assays only one marker, RAPD analyses a number of markers, that can be increased to meet specific needs, simply by using more primers: as a result, more detailed population genetic analyses can be conducted. In addition, the RAPD technique is more time- and labour-efficient than VCG typing.

The distribution of genetic variation among geographical locations in Israel was examined, and two major clusters of isolates were detected with parsimony analysis using the PAUP program (Fig. 2). Isolates from most locations were present on both clusters. This might be the result of gene flow between locations in Israel – or from a common gene pool of *Fusarium* isolates that became established in the different locations. All the sweet corn in Israel was grown from seeds (mainly cv. 'Jubilee') imported from the USA, and seed lots imported before 1994 were found to be infected with *F. moniliforme* (R. Huang, unpublished data). Use of imported seeds might support a continuous gene-flow to various locations, and the fact that the four isolates from US-imported seeds did not cluster separately from the Israeli isolates supports this hypothesis. It has been reported that there are two mating populations, A and F, within *F. moniliforme* (Leslie, 1991; Leslie et al., 1992), and that most isolates collected from corn in the USA belong to mating population A (Campbell et al., 1992). Because we did not determine the mating populations to which the isolates of the present study belonged, we are unable to say whether the two clusters of Israeli isolates represent two mating populations or two subpopulations within the mating population A. In previous studies, the major factor subdividing *Fusarium* section *Liseola* into subgroups that correlated with RAPD patterns was the assignment to mating populations (Amoah et al., 1995, 1996; Voigt et al., 1995; MacDonald & Chapman, 1997). Amoah et al. (1995, 1996) suggested the division of *F. moniliforme* isolates collected in Ghana, from corn plants, into tissue-related subgroups (seeds vs. stalks). MacDonald & Chapman (1997) similarly divided isolates from Kenya, belonging to mating population A, into two subgroups based on RAPDs, with some correlation to the tissue-origin of the isolates. We could not find any correlation between the two clusters we observed and the tissue-origin of our isolates. Most isolates in set 1 were from cobs or seeds, and the rest were from stalks.The fact that some VCGs/haplotypes (in set 2) were found both in the seeds and the stalks, and the systemic nature of *F. moniliforme* infection (Smelzer, 1958; Damicone et al., 1988; Farrar & Davis, 1991) do not lend surpport to the the tissue-related subgrouping hypothesis.

Extensive variation was detected among isolates of *F. moniliforme* collected from a single corn field. Among 63 isolates, 42 VCGs and 37 RAPD haplotypes were identified. If the two ungrouped isolates are taken as two additional VCGs, there would be 0.70 VCGs and 0.66 haplotypes per isolate, which is similar to the diversity of the *F. moniliforme* population in the USA (Farrokhi-Nejad & Leslie, 1990; Campbell et al., 1992). Such diversity in both the USA and Israel might indicate that sexual reproduction occurs, and is probably very important in the life cycle of *F. moniliforme*. It is well known that the genetic diversity of a population tends to decrease during asexual reproduction because of selection and genetic drift, while sexual recombination can maintain high levels of diversity. The fact that the majority (86%) of the RAPD combinations are in gametic phase equilibrium also suggests significant genetic recombination in *F. moniliforme*, because gametic phase equilibrium is typical of a randomly mating population.

To our knowledge, there is no evidence that *F. moniliforme* can be differentially selected by different corn genotypes. Hierarchical gene-diversity analysis shows that most of the diversity in the experimental field was found within corn lines, and RAPD frequencies were not significantly different between lines. Thus, the genetic diversity of *F. moniliforme* is distributed on a very fine scale, while the corn lines in this experiment had a limited

effect, if any, on this distribution. Seed borne inoculum might be important for long distance gene flow, and might cause plant genotype-specific effects on the distribution of fungal genotypes, but compared to the large fungal population in the soil, its effect on the population diversity is probably small. The similarity of RAPD frequencies among corn plants that were spatially separated across the field also indicates that there was significant genetic exchange within the field. This is not unexpected, because conidia can be easily disseminated by wind and insects (Smelzer, 1958; Damicone et al., 1988; Farrar & Davis, 1991).

When isolates of the same VCG were found in two different plants, further RAPD analysis showed that in most cases they could be divided into different RAPD haplotypes, each appearing in a different plant (Table 3). More RAPD information might be required to determine whether isolates sharing the same haplotype and the same VCG belong to the same clone. For a fungus like *F. moniliforme*, that might be using an open mating system, isolates with the same RAPD pattern or with the same VCG assignment are not necessarily clones. Nevertheless, in eight out of 16 plants, isolates belonging to the same VCG and haplotype were found in different tissues. Although we cannot demonstrate their clonal origin, it is likely that the frequent presence of such isolates in different parts of the same plant was caused by systemic infection. This is based on the observation that only in two cases were isolates of the same VCG and haplotype found in different plants, as compared to their common occurence within plants. Systemic infection of corn plants by *F. moniliforme* has been well documented (Foley, 1962; Bacon & Hinton, 1996).

All plants sampled had multiple infections. As many as four VCGs or RAPD haplotypes were found in each plant, even when only 2–5 isolates per plant were collected. It is probable that with more extensive sampling, more VCGs or haplotypes would have been found. Multiple infection might have an important impact on the evolution of the fungus, because it is one of the prerequisites for sexual or parasexual recombination. As a result, many plants are likely to be infected by fungal individuals of opposite mating type, and sexual recombination might occur in the field. In addition, infection by different individuals of the same VCG might result in heterokaryon formation and parasexual recombination in vivo.

DEDICATION

We wish to dedicate this article to the memory of Prof. Yehouda Levy, an enthusiastic and capable phytopathologist and a beloved teacher and friend. Prof. Levy passed away at the age of 47 on July 18 1996.

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