European Journal of Plant Pathology **109:** 807–815, 2003.
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Genetic variability and population structure of the wheat foot rot fungus, *Fusarium culmorum*, in Tunisia

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Accepted 14 March 2003

Key words: cereal disease, genetic diversity, RAPD

Abstract

The random amplified polymorphic DNA (RAPD) method was used to investigate the genetic variability and population structure of *Fusarium culmorum* isolated from wheat stem bases. A total of 108 isolates, representing seven geographically distinct populations, was collected from five climatic regions in Tunisia. Pseudo-allelic frequencies were estimated at each of the 25 putative RAPD loci analyzed by scoring for the presence or absence of amplified fragments; 92 haplotypes were found among the 108 strains. The analysis of the population structure did not reveal any trend with regard to geographic origin. Total gene diversity ($H_T^* = 0.318$) was mostly attributable to diversity within populations ($H_S^* = 0.308$). Analysis of molecular variance confirmed that most of the genetic variability was within populations. Genetic differentiation among populations was low to moderate (G_{ST}^* ranged from 0 to 0.190 and averaged 0.041 over all loci). Cluster analysis with UPGMA using genetic distances did not reveal any spatial clustering of the isolates collected from the different geographic regions. Based on these results, we conclude that the *F. culmorum* isolates recovered from different regions in Tunisia might be part of a single population pool.

Introduction

Fusarium foot rot is an economically important disease of cereals in many grain-producing regions of the world (Cook, 1980; Wiese, 1987), especially in areas with low to intermediate rainfall such as North Africa. In Tunisia, a 44% yield loss was reported in 1974 (Ghodbane et al., 1974). The causal agents rot roots, crown, and basal stem tissues resulting in reduced yield and lower grain quality (Wiese, 1987). Disease etiology is often complex and varies regionally (Smiley and Patterson, 1996). A recent survey conducted in Tunisia showed that *Fusarium culmorum* was the principal causal agent (Gargouri et al., 2001). At present, no fungicides are available to control *Fusarium* diseases efficiently, therefore, breeding for resistance is the best disease-management method (Miedaner, 1997). For applying efficient strategies in the breeding process, knowledge about the genetic diversity and structure of naturally occurring pathogen populations is crucial. Molecular markers are useful tools in the analysis of genetic variation in populations of phytopathogenic fungi. The development of random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990) has provided a powerful method for investigating intraspecific genetic variation in many phytopathogenic fungi. RAPD analyses have been used to study genetic variability of *Fusarium* species associated with wheat diseases including *F. graminearum* (Ouellet and Seifert, 1993; Schilling et al., 1996a,b; 1997; Dusabenyagasani et al., 1999), *F. avenaceum* (Yli-Mattila et al., 1996; Nijs et al., 1997) and *F. culmorum* (Nicholson et al., 1993; Schilling et al., 1996a,b; Nijs et al., 1997). To our

knowledge, however, there has been no report on the genetic variability and organization of populations of *F. culmorum* in Tunisia or in neighboring countries. The objective of this study was to analyze genetic variability and population structure of *F. culmorum* in Tunisia.

Materials and methods

Collection of isolates of F. culmorum

A total of 1215 isolates was collected from stem bases of durum wheat (*Triticum durum*) showing typical symptoms of foot rot disease as described by Wiese (1987). Sampling was done during April and May 2000 from 174 fields in five climatic regions in Tunisia, namely humid, sub-humid, superior semi-arid, medium semi-arid and inferior semi-arid (Bortoli et al., 1969). The fields were chosen randomly and were separated by at least 10 km. More than 70% of fungi isolated were identified as *F. culmorum*, based on the Burgess identification key (Burgess et al., 1994). Morphological identification was confirmed by PCR using *F. culmorum*-specific primers (Schilling et al., 1996a). To study genetic variability and population structure of this species in Tunisia, a subset of 108 single-spore isolates representing seven geographically distinct populations from the five climatic regions (Figure 1) was subjected to RAPD analysis.

DNA extraction

Flasks containing 50 ml of PDB (Potato Dextrose Broth) (DIFCO Laboratories, Detroit, MI, USA) were inoculated with three disks from 3–4 days old cultures grown on PDA. Liquid cultures were placed on a rotary shaker (110 rpm) and grown in the dark at 25 °C for 4–5 days. Mycelium was washed with distilled water and collected by centrifugation (5500 rpm), frozen at −80 °C for 2 h and then lyophilized. Freeze-dried mycelium was ground in liquid nitrogen. 150 µl of ground mycelium were incubated in 500 µl of TES buffer (100 mM Tris–HCl, pH 8.0, 10 mM EDTA, 2% SDS) and 50 µg proteinase K for 60 min at 55 °C, then 150 µl of NaCl (5 M) and 65 µl of CTAB (10%) were added and the mixture was incubated for 10 min at 65 °C. DNA was extracted in chloroform : isoamyl alcohol (24 : 1), precipitated with ethanol, and washed twice with 70% ethanol (Möller et al., 1992). Extracted DNA was resuspended in TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA pH 8.0) and stored at −20 °C. DNA was quantified by comparing the band intensity of samples on 0.8% agarose gels to a known amount of lambda DNA standard.

DNA amplification and primer screening

DNA was amplified by the RAPD technique (Williams et al., 1990). Reactions were carried out in a volume of 12.5 µl containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 µM of each dNTP (Pharmacia Biotechnology Inc, Uppsala, Sweden), 0.2 µM of 10-mer RAPD primer from Kits A, C and E (Operon Technologies, Alameda, CA, USA), or 2 µM of primer from 10-mer kits B and W, 1 U of *Taq* DNA polymerase (Boehringer, Mannheim, Germany) and ca. 5 ng DNA. Reactions were conducted in a Perkin-Elmer Cetus Thermal cycler 9600 (Perkin-Elmer Cetus, Norwalk, CT, USA) following

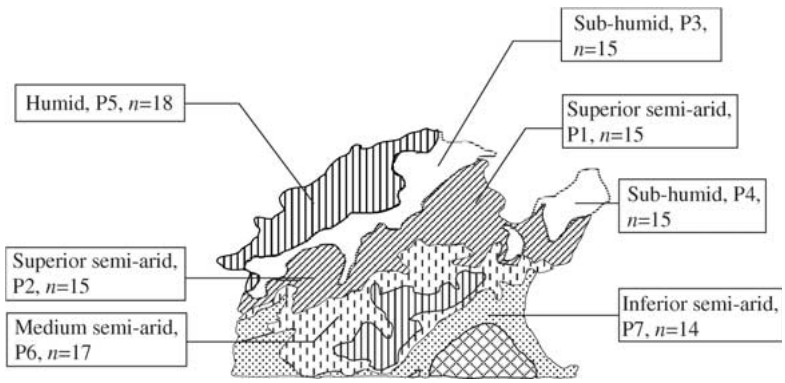

Figure 1. Distribution of the 108 strains in the different climatic regions of Tunisia as described by Bertoli et al. (1969), P: population, *n*: number of isolates.

Table 1. Oligonucleotide primers retained for population analysis and their corresponding sequences

Primer	Sequence
OPA08	GTGACGTAGG
OPA09	CGGTAACGCC
OPA10	GTGATCGCAG
OPA18	AGGTGACCGT
OPA19	CAAACGTCGG
OPB09	TGGGGGACTC
OPB16	TTTGCCCGGA
OPC13	AAGCCTCGTC
OPC14	TGCGTGCTTG
OPC15	GACGGATCAG
OPC16	CACACTCCAG
OPC20	ACTTCGCCAC
OPE19	ACGGCGTATG
OPW03	GTCCGGAGTG

the program of Schilling et al. (1994): 94 °C for 3 min, 35 °C for 1 min, 72 °C for 2 min, one cycle; 94 °C for 1 min, 35 °C for 1 min, 72 °C for 2 min, 43 cycles; 72 °C for 5 min, final extension cycle. The PCR products were separated by electrophoresis on 1% agarose gel in 0.5X TPE (45 mM Tris–Phosphate, 1 mM EDTA, pH 8.0) buffer plus 0.5% Synergel (Diversified Biotech, Boston, MA, USA) and were visualized by fluorescence following ethidium bromide staining. Seventy primers were initially screened with a sample of seven isolates from the seven populations. Fourteen primers that produced polymorphic and reproducible markers were retained for this study (Table 1). Amplifications from each DNA sample were repeated at least once.

Data analysis

Only bright bands that could be clearly distinguished as present or absent for all isolates were scored. RAPD fragments were identified by the name of the primer and the size of band amplified. Fragments were scored as putative loci with two alleles (presence and absence). Data were compiled as a binomial matrix with 108 individual and 25 polymorphic loci. The marker frequency was calculated for each population. For each locus, heterogeneity of the marker frequency between the seven populations was calculated by the likelihood G-test (Sokal and Rohlf, 1981) using SAS for Windows software (release 6.12; SAS institute, Cary, NC, USA). Allelic diversities were estimated using Nei's diversity parameters. The occurrence of any population structure among the bioclimatic regions was studied by dividing the total genetic diversity (H_T) into the genetic diversity of individuals relative to their sub-population (H_S) and the genetic diversity between populations (D_{ST}). The genetic differentiation relative to the total population was calculated as $G_{ST} = D_{ST}/H_T$ (Nei, 1973). All genetic parameters were corrected for small, unequal sample sizes according to Nei and Chesser (1983). An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed using a matrix of Euclidean distances between all pairs of haplotypes (Huff et al., 1994). It consists in partitioning the variance into its hierarchical components. A measure of genetic differentiation was obtained by F-statistics analogs (Φ statistics). The variance components and Φ statistics were tested statistically by non-parametric randomization tests using 1023 repetitions. All analyses were conducted with the Arlequin version 2000 (a software for population genetics data analysis) program (Schneider et al., 2000). A cluster analysis was carried out using a distance matrix based on the genetic distance of Nei and Li (1979) and the unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973). The analysis was performed with the TREECON (software package for the construction and drawing of evolutionary trees, version 1.3b by Yves Van de Peer) program (Van de Peer and De Wachter, 1994) using 500 bootstrap samples.

Results

Sixty-six fragments were amplified with the 14 primers. Twenty-five fragments were polymorphic. Fragment sizes ranged from 0.61 to 3.6 Kb. Polymorphic DNA fragments amplified with primers OPA10 and OPW03 are shown in Figure 2. Ninety-two haplotypes were generated among the 108 individuals. The frequency of nine loci varied significantly ($P < 0.05$) among the seven populations. The analysis of population structure revealed that most of the genetic diversity was within populations, while genetic diversity between populations represented a small proportion of the total. Adjusted values of genetic diversity within populations (H_S^*) ranged from 0.010 to 0.520 and averaged 0.308, accounting for 97% of the total genetic diversity ($H_T^* = 0.318$). The proportion of the total genetic diversity attributable to the population differentiation (G_{ST}^*) ranged from 0 to 0.190 and averaged 0.041 over all loci (Table 2).

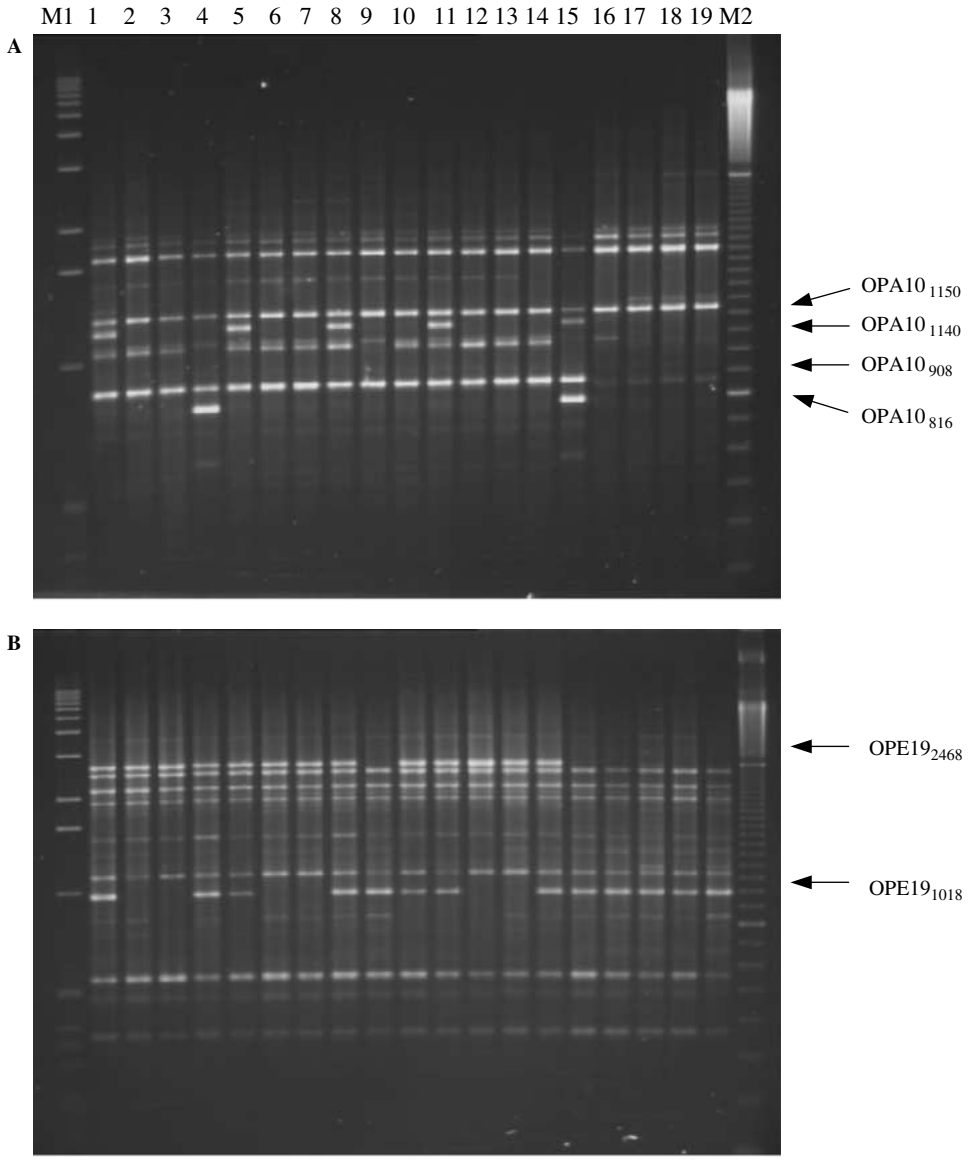

Figure 2. RAPD products from 19 strains of *F. culmorum* amplified with primers OPA10 (A) and OPE19 (B). Lanes M1 and M2 = 1 Kb and 100 bp markers, respectively (GIBCO-BRL). Markers labeled with arrows are polymorphic.

The AMOVA analysis for the 108 individuals revealed that most of the molecular variability was attributable to differences within populations (96.2% of the total variance; $P = 0$). A small yet significant proportion of the variability was explained by differences between populations (3.8%, $P < 0.001$) (Table 3). The cluster analysis with the UPGMA using genetic distances failed to identify any spatial clustering among the different geographic regions (Figure 3).

Discussion

The objective of this study was to estimate the extent of genetic diversity of *F. culmorum* in Tunisia and to determine if any population structure occurred with regard to geographical origin by means of RAPD markers. As was pointed out by Wong et al. (2001), the use of dominant PCR markers would likely underestimate the gene diversity and among

Table 2. Frequency of putative RAPD loci and genetic diversity of seven Tunisian populations of *F. culmorum*

Locus	Frequency of the plus allele in each population							*Pr*	Genetic diversity[a]		
	P1 $n = 14$	P2 $n = 15$	P3 $n = 15$	P4 $n = 15$	P5 $n = 17$	P6 $n = 18$	P7 $n = 14$		H_S	H_T	G_{ST}
$OPA08_{2323}$	0.142	0.066	0.400	0.133	0.000	0.055	0.071	*	0.201	0.219	0.082
$OPA09_{2036}$	0.214	0.266	0.600	0.400	0.411	0.388	0.357	ns	0.474	0.473	0.000
$OPA10_{1150}$	0.500	0.400	0.466	0.400	0.647	0.500	0.428	ns	0.520	0.503	0.000
$OPA10_{1140}$	0.285	0.133	0.266	0.066	0.058	0.166	0.214	ns	0.287	0.285	0.000
$OPA10_{908}$	0.857	1.000	0.933	0.800	0.941	0.944	0.857	ns	0.175	0.173	0.000
$OPA10_{816}$	0.071	0.000	0.333	0.066	0.000	0.055	0.071	*	0.143	0.157	0.089
$OPA18_{933}$	0.142	0.133	0.333	0.133	0.000	0.055	0.071	ns	0.212	0.219	0.030
$OPA19_{2036}$	0.000	0.130	0.330	0.200	0.120	0.280	0.000	*	0.245	0.250	0.020
$OPA19_{612}$	0.357	0.266	0.400	0.000	0.176	0.277	0.571	**	0.383	0.417	0.080
$OPC13_{3050}$	0.714	0.333	0.533	0.600	0.176	0.666	0.928	**	0.412	0.495	0.167
$OPC13_{2036}$	0.785	0.533	0733	0.733	0.705	0.666	0.571	ns	0.453	0.442	0.000
$OPC14_{2036}$	0.214	0.000	0.130	0.066	0.176	0.111	0.142	ns	0.217	0.214	0.000
$OPC15_{1010}$	0.857	0.866	0.733	0.866	0.941	0.888	0.857	ns	0.252	0.244	0.000
$OPC16_{3636}$	0.642	0.666	0.600	0.200	0.470	0.555	0.571	ns	0.486	0.502	0.030
$OPC16_{834}$	0.642	0.733	0.866	0.866	0.820	0.777	0.928	ns	0.317	0.315	0.000
$OPC20_{1760}$	0.500	0.466	0.466	0.666	0.294	0.388	0.500	ns	0.508	0.502	0.000
$OPC20_{1236}$	0.428	0.733	0.600	0.266	0.647	0.388	0.285	ns	0.472	0.503	0.060
$OPE19_{2460}$	0.928	0.866	1.000	0.866	0.823	0.944	0.714	ns	0.213	0.216	0.013
$OPE19_{1010}$	0.642	0.666	0.666	0.600	0.705	0.611	0.857	ns	0.452	0.440	0.000
$OPB09_{1892}$	0.928	0.800	1.000	0.533	0.647	0.833	0.642	**	0.327	0.357	0.080
$OPB09_{1757}$	0.071	0.066	0.000	0.200	0.352	0.055	0.285	*	0.236	0.253	0.060
$OPB09_{852}$	1.000	0.666	0.933	0.933	0.941	0.777	0.857	ns	0.213	0.223	0.040
$OPB16_{2710}$	1.000	1.000	0.933	0.933	0.882	1.000	0.785	ns	0.121	0.125	0.030
$OPW03_{1360}$	1.000	1.000	1.000	0.600	0.941	1.000	0.857	**	0.127	0.158	0.060
$OPW03_{807}$	0.857	0.866	0.933	0.600	0.882	1.000	0.714	*	0.259	0.276	0.190
Average									0.308	0.318	0.041

[a] H_S, genetic diversity of individuals relative to their population; H_T, total genetic diversity; G_{ST}, genetic differentiation of populations; all diversity values were corrected to account for small sample size (Nei and Chesser, 1983); *Pr*, *G*-test heterogeneity of allele frequencies among the seven Tunisian populations; *allele frequencies differed at $P < 0.05$; **allele frequencies differed at $P < 0.01$.

Table 3. Analysis of molecular variance of RAPD haplotypes for 108 isolates of *F. culmorum* from seven Tunisian populations

Source	df	SSD	Φ statistics	Proportion of variance components (%)	*P*
Among populations	6	37.17	0.038	3.8%	0.001
Within populations	101	388.42		96.2%	0.000

df: degrees of freedom; SSD: sums of squared deviations; *P*: Probability of obtaining equal or larger value determined by 1023 randomizations of the treatments.

population genetic variance. The following discussion of the results takes into account the possibility of this slight bias. For this study, seven populations from five bioclimatic zones were analyzed. RAPD analysis revealed a high level of genetic variability in populations of this fungus, as 92 genotypes were identified among the 108 strains studied. Nine out of 25 markers showed significant differences in allele frequencies, while no evidence of population structure was revealed. Indeed, both Nei's diversity indices and AMOVA led to the same conclusion: genetic variability was for the most part accounted for by diversity within populations ($G^*_{ST} = 0.041$; $\Phi = 0.038$).

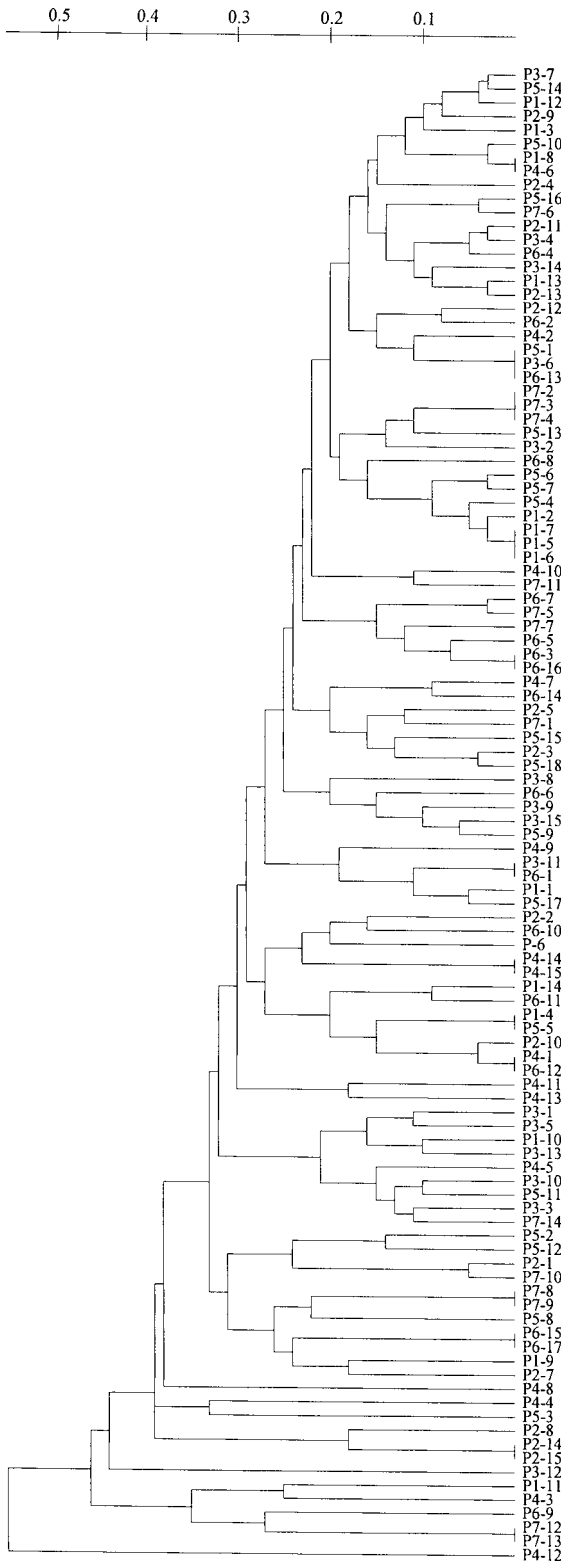

Figure 3. UPGMA dendrogram from RAPD data for 108 strains of *F. culmorum* amplified by 14 primers. UPGMA cluster analysis was based on Nei and Li's distance (1979).

Other authors have reported high levels of genetic diversity in local populations of *F. culmorum* and of related species. Thus, a high level of genotypic variation was detected after RFLP analysis of *F. culmorum* and *F. avenaceum* strains from a single field in England (Nicholson et al., 1993). Similarly, Miedaner and Schilling (1996) found a high degree of genetic variability for aggressiveness within single field populations of *F. culmorum* and *F. graminearum*. On the other hand, Nijs et al. (1997) observed little variation among 17 isolates of *F. culmorum* based on RAPD pattern analysis. However, in addition to the low number of isolates tested, only three primers were used for RAPD amplifications. Interestingly, all isolates showed a different secondary metabolite profile.

No sexual stage has been identified for *F. culmorum*. The observation of high levels of genetic variation in asexually reproducing fungi is not atypical, however. Significant variation for a wide range of markers (pathogenicity, isozymes, ribosomal DNA and colony color) has been reported for populations of the imperfect fungus *Rhyncosporium secalis* (McDermott et al., 1989; Goodwin et al., 1993). High levels of genetic variability have also been observed in *F. oxysporum* (Gordon and Martyn, 1997), in *Alternaria alternata* (Morris et al., 2000) and in asexual populations of *Uromyces appendiculatus* (Groth et al., 1995).

Genetic changes may result from a single gene mutation, which may occur spontaneously or may be mediated by insertion of transposable elements, or by loss of chromosomes or chromosome segments (Kistler and Miao, 1992). For example, a great diversity of electrophoretic karyotypes has been described for *F. oxysporum* (Migheli et al., 1995). Several species of fungi that reproduce asexually can sporulate profusely and give rise to large populations. Even though mutations are rare events, large populations are likely to display some level of genetic diversity over time, as a result of the occurrence and accumulation of nonlethal mutations that have arisen independently from each other. Several lines of evidence suggest that the potential for the buildup of large, genetically diversified populations exists in *F. culmorum*. Under laboratory conditions, *F. culmorum*, unlike *F. graminearum*, sporulates abundantly on malt agar and synthetic media (Champion, 1997). In the field, asexual sporulation

of *F. culmorum* is especially profuse on parasitized stems of oats (Cook, 1968). According to Cook (1980), macroconidia of *F. culmorum* formed on diseased culms or colonized debris can result in population increases of several fold in a single season. In addition, *F. culmorum* exhibits a strong saprophytic ability that may allow isolates with low aggressiveness to propagate even if they cannot invade living host tissue (Burgess and Griffin, 1967). This characteristic of the species would thus allow the coexistence of genotypes with different levels of aggressiveness. Finally, mechanisms favoring genetic exchange are also known to occur in asexually reproducing populations. Thus, somatic recombination through parasexuality has been demonstrated in *F. oxysporum* (Molnar et al., 1990). Whether parasexuality occurs in natural populations of *F. culmorum* remains unknown, however.

The low level of genetic differentiation among Tunisian populations of *F. culmorum* is usually observed in outcrossing fungal species rather than in predominantly asexually propagated populations. Thus, low levels of genetic differentiation among geographic regions have been reported for the sexually reproducing wheat pathogens *Mycosphaerella graminicola* (McDonald et al., 1995), *F. avenaceum* (Yli-Mattila et al., 1996) and *F. graminearum* (Dusabenyagasani et al., 1999). In contrast, in the imperfect fungus *Peridermium harknessii*, 51% of the total variation was attributed to differences among locations: this clustering appeared to be influenced by geographic distribution and stand type (Tuskan et al., 1990).

No clear trends in the distribution of the genetic variability were apparent with regard to the geographic origin within Tunisia. Our work is in concordance with the results of Schilling et al. (1996b) who did not observe any relationship between the groupings among isolates of *F. culmorum* found by means of the RAPD technique, and the host or geographic origin. Similarly, Miedaner et al. (1996) could not match marker-based associations among *F. culmorum* isolates with the degree of aggressiveness as determined in a field experiment. Thus, no structuring has so far been observed at a small or a large geographic scale in this fungal species. This result can be explained by the low level of pathogenic host specialization of *F. culmorum* populations (Miedaner and Schilling, 1996). The lack of a geographic structure in Tunisian populations of *F. culmorum* suggests, in addition, that spore dispersal probably occurs over a wide geographic area.

This research is a study of the genetic diversity and population structure of *F. culmorum* associated with wheat foot rot in Tunisia. For successful *Fusarium* foot rot management and resistance breeding, further studies such as the accurate determination of the aggressiveness of strains are needed to confirm whether this single *F. culmorum* population pool, as determined by RAPD markers, constitutes a single epidemiological unit. Analysis of populations of the wheat pathogen *F. culmorum* at a larger scale would be interesting to better understand the source of the epidemics similarly to the studies done for the cereal pathogens *Rhynchosporium secalis* (Goodwin et al., 1993) and *Setosphaeria turcica* (Borchardt et al., 1998).

Acknowledgements

This work was supported in part by the SERST (Secrétariat d'Etat à la Recherche Scientifique de Tunisie). S. Gargouri was a recipient of a student travel grant from the Tunisia–Canada Cooperation program (ACDI). L. Bernier acknowledges financial support from Fonds FCAR (Québec). The authors are indebted to A. Guermech and A. M'sakni for their help in collecting the isolates, and M. Dusabenyagasani for his help with the molecular analyses.

References

Borchardt DS, Welz HG and Geiger HH (1998) Genetic structure of *Setosphaeria turcica* populations in tropical and temperate climates. Phytopathology 88: 322–329

Bortoli L, Gounot M and Jacquinet JC (1969) Climatologie et Bioclimatologie de la Tunisie septentrionale. Annales de l'INRA Tunisie 42: 1–23

Burgess LW and Griffin DM (1967) Competitive saprophytic colonization of wheat straw. Annals of Applied Biology 60: 137–142

Burgess LW, Summerell BA, Bullock S, Gott KP and Backhouse D (1994) Laboratory Manual for *Fusarium* Research. 3rd edn, University of Sydney, Australia

Champion R (1997) Identifier les champignons transmis par les semences, INRA, Paris

Cook RJ (1968) Influence of oats on soil-borne populations of *Fusarium roseum* f. sp. *cerealis* 'Culmorum'. Phytopathology 58: 957–960

Cook RJ (1980) Fusarium foot rot and its control in the Pacific northwest. Plant Disease 64: 1061–1066

Dusabenyagasani M, Dostaler D and Hamelin RC (1999) Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Canadian Journal of Plant Pathology 21: 308–314

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491

Gargouri S, Hajlaoui MR, Guramech A and Marrakchi M (2001) Identification des espèces fongiques associées à la pourriture du pied du blé et leur répartition selon les étages bioclimatiques. EPPO/OEPP Bulletin 31: 499–503

Ghodbane A, Mahjoub M, Djerbi M, Mlaiki A and Sharen AL (1974) Etude des pertes causées par les pathogènes du blé *Septoria tritici* et *Fusarium* spp. Rapport annuel du ministère de l'agriculture, office des céréales, Tunisie

Goodwin SB, Saghai Maroof MA, Allard RW and Webster RK (1993) Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. Mycological Research 97: 49–58

Gordon TR and Martyn RD (1997) The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35: 111–128

Groth JV, McCain JW and Roelfs AP (1995) Virulence and isozyme diversity of sexual *versus* asexual collections of *Uromyces appendiculatus* (bean rust fungus). Heredity 75: 234–242

Huff DR, Bunting TE and Plumley KA (1994) Use of random amplified polymorphic DNA markers for the detection of genetic variation in *Magnaporthe poae*. Phytopathology 84: 1312–1316

Kistler HC and Miao VPW (1992) New modes of genetic changes in filamentous fungi. Annual Review of Phytopathology 30: 131–152

Mc Dermott JM, McDonald BA, Allard RW and Webster RK (1989) Genetic variability from pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *Rhynchosporium secalis*. Genetics 122: 561–565

McDonald BA, Pettway RE, Chen RS, Boeger JM and Martinez JP (1995) The population genetics of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). Canadian Journal of Botany 73: S292–S301

Miedaner T (1997) Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116: 201–220

Miedaner T, Gang G and Geiger HH (1996) Quantitative-genetic basis of aggressiveness of 42 isolates of *Fusarium culmorum* for winter rye head blight. Plant Disease 80: 500–504

Miedaner T and Schilling AG (1996) Genetic variation of aggressiveness in individual field populations of *Fusarium graminearum* and *F. culmorum* tested on young plants of winter rye. European Journal of Plant Pathology 102: 823–830

Migheli Q, Berio T, Gullino ML and Garibaldi A (1995) Electrophoretic karyotype variation among pathotypes of *Fusarium oxysporum* f. sp. *dianthi*. Plant Pathology 44: 308–315

Möller EM, Bahnweg G, Sandermann H and Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit body and infected plant tissue. Nucleic Acids Research 20: 6115–6116

Molnar A, Sulyok L and Hornok L (1990) Parasexual recombination between vegetatively incompatible strains in *Fusarium oxysporum*. Mycological Research 94: 393–398

Morris PF, Connolly MS and St Clair DA (2000) Genetic diversity of *Alternaria alternata* isolated from tomato in California assessed using RAPD. Mycological Research 104: 286–292

Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA 70: 3321–3323

Nei M and Chesser RK (1983) Estimation of fixation indices and gene diversities. Annals of Human Genetics 47: 253–259

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, USA 76: 5269–5273

Nicholson P, Jenkinson P, Rezanoor HN and Parry DW (1993) Restriction fragment length polymorphism analysis of variation in Fusarium species causing ear blight of cereals. Plant Pathology 42: 905–914

Nijs M, Larsen JS, Games W, Rombouts FM, Wernars K, Thrane U and Notermans SHW (1997) Variation in random amplified polymorphic DNA patterns and secondary metabolite profiles within Fusarium species from cereals from various parts of the Netherlands. Food Microbiology 14: 449–457

Ouellet T and Seifert KA (1993) Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathology 83: 1003–1007

Schilling AG, Möller EM and Geiger HH (1994) RAPDs of *Fusarium culmorum* and *F. graminearum*: Application for genotyping and species identification. In: Schots A et al. (eds) Modern Assays for Plant Pathogenic Fungi (pp 47–56) CAB International, Oxford, UK

Schilling AG, Möller EM and Geiger HH (1996a) Polymerase chain reaction-based assays for species specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. Phytopathology 86: 515–522

Schilling AG, Möller EM and Geiger HH (1996b) Molecular differentiation and diagnosis of the cereal pathogens *Fusarium culmorum* and *F. graminearum*. Sydowia 48: 71–82

Schilling AG, Miedaner T and Geiger HH (1997) Molecular variation and genetic structure in field populations of *Fusarium* species causing head blight in wheat. Cereal Research Communications 25: 549–554

Schneider S, Roessli D and Excoffier L (2000) Arlequin ver. 2.000: A Software for Population Genetics Data Analysis, Genetics and Biometry Laboratory, University of Geneva, Switzerland

Smiley RW and Patterson LM (1996) Pathogenic fungi associated with Fusarium foot rot of winter wheat in the semiarid Pacific Northwest. Plant Disease 80: 944–949

Sneath PHA and Sokal RR (1973) Numerical Taxonomy, W. H. Freeman, San Fransisco

Sokal RR and Rohlf FJ (1981) Biometry, 2nd edn, W.H. Freeman, San Fransisco

Tuskan GA, Walla JA and Lundquist JE (1990) Genetic–geographic variation in *Peridermium harknessii* in the North-central United States. Phytopathology 80: 857–861

Van de Peer Y and De Wachter R (1994) Treecon for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Computer Applications in the Biosciences 10: 569–570

Welsh J and McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213–7218

Wiese MV (1987) Compendium of Wheat Diseases. 2nd edn, American Phytopathological Society, St. Paul, MN

Williams JGK, Kubelik AR, Rafalski JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535

Wong A, Forbes MR and Smith ML (2001) Characterization of AFLP markers in damselflies: prevalence of codominant markers and implications for population genetic applications. Genome 44: 677–684

Yli-Mattila T, Paavanen S, Hannukkala A, Parikka P, Tahvonen R and Karjalainen R (1996) Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathology 45: 126–134