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Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea*, causal agent of powdery mildew of cucurbits

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Abstract

Strains of *Sphaerotheca fuliginea*, one of the causal agents of powdery mildew of cucurbits, were examined for differences in virulence, mating type and DNA polymorphism. The 28 strains were chosen to be diverse according to host and geographic origin. Characterization of virulence phenotypes was based on the expression of symptoms on 4 species of cucurbits and 6 cultivars of melon. Two pathotypes, capable of attacking either cucumber cv. 'Marketer' and melon cv. 'IranH' and squash cv. 'Diamant' or cucumber cv. 'Marketer' and melon cv. 'IranH' were observed. Tests on melon cultivars revealed 3 races. In tests of sexual compatibility with reference strains, heterothallism was observed for all isolates. Frequency of the two mating types differed significantly in the population. DNA polymorphism was determined both by restriction fragment length polymorphism (RFLP) of the ribosomal internal transcribed spacers (ITS) and 5.8S DNA amplified by the polymerase chain reaction and by random amplified polymorphic DNA (RAPD). For any one of the 11 restriction enzymes tested all strains presented an identical pattern of ITS RFLP. RAPD analysis, using 22 primers which provided reproducible patterns, revealed a relatively low degree of polymorphism. Furthermore, cluster analysis based on RAPD data (152 markers) did not separate groups within the species *S. fuliginea*. No association could be found between virulence, mating type, geographical and host origin and RAPD patterns. The lack of association between phenotypic and molecular markers and the close fit to linkage equilibrium for the characters examined suggest that recombination may play a role in populations of *S. fuliginea*.

Introduction

Powdery mildew occurs throughout the world, on cucurbits grown in greenhouses or in the field. It is the principal disease on cucurbits in greenhouse culture (Sitterly, 1978). The disease can be caused by three different species, *Sphaerotheca fuliginea* (Schlecht) Poll., *Erysiphe cichoracearum* D.C. and *Leveillula taurica* (Lev.) Arnaud, but *S. fuliginea* is the species most commonly found in regions with a temperate climate (Bertrand, 1991; Kenigsbuch and Cohen, 1992; Kooistra, 1968; Sowell, 1982; Vakanoulakis et al., 1994).

While the powdery mildew fungi tend to have a narrow host range, often restricted to one species (Yarwood, 1978), strains of *S. fuliginea* have been shown to attack several species in the family *Cucurbitaceae* (Bertrand, 1991). A population of *S. fuliginea* isolated from France was divided into two pathotypes based on pathogenicity tests on four different host species. One group was pathogenic on cucumber (*Cucumis sativus* cultivar 'Marketer'), melon (*Cucumis melo* cv. 'Vedrantais'), and squash (*Cucurbita pepo* cv. 'Diamant'), while the second group was pathogenic only on cucumber cv. 'Marketer' (Bertrand, 1991).

Much effort has been devoted to breeding for resistance to *S. fuliginea* and to elucidating its inheritance. In melon, resistance has been attributed to a single dominant gene in the case of cultivar PMR45 (Kenigsbuch and Cohen, 1992). For other cultivars, however, a

more complex situation was reported, with control by various combinations of several dominant, recessive and modifier genes (Kenigsbuch and Cohen, 1992). Complex gene combinations have also been implicated in the resistance of cucumber to *S. fuliginea* (Zijlstra et al., 1995). For squash, resistant cultivars have been described but specific genes remain to be identified (Cohen et al., 1993). While information is lacking for cucumber and squash, five races of *S. fuliginea* have been described on melon (Bertrand, 1991; McCreight et al., 1987; Molot and Lecoq, 1986; Thomas, 1978). Knowledge on the prevalence and geographic distribution of these races is essential for the choice of appropriate strains to be used in breeding programs and for the implementation of gene deployment strategies (Thomas et al., 1984). The limited data available suggest that the geographic distribution of races may be complex and changing over time (Bertrand, 1991; Mohamed et al., 1995; Sowell, 1982).

In contrast with powdery mildew on other crops such as cereals (Wolfe and McDermott, 1994), information regarding the variability and the ecology of *S. fuliginea* is rare. For instance, the question of the prevalence and epidemiological relevance of the sexual stage of the pathogen remains largely unanswered (Bertrand, 1991; McGrath, 1994) even though cleistothecia have been observed occasionally in the field throughout the world (McGrath et al., 1996) and the presence of the two mating types was established (Bertrand, 1991; McGrath, 1994).

The objective of this work was then to study the genetic diversity among 28 isolates of *S. fuliginea* coming from different host plants and geographic origins, in terms of virulence and DNA polymorphism. To this end, we have used restriction fragment length polymorphism of the ribosomal internal transcript spacers and 5.8S DNA amplified by PCR (ITS-RFLP) and random amplified polymorphic DNA (RAPD). These methods are known to be useful tools for examining genetic diversity in populations of plant pathogenic fungi (Chen et al., 1993; McDermott et al., 1993; Peever and Milgroom, 1994).

Materials and methods

Collection of fungal isolates

Twenty-eight samples of *S. fuliginea* were isolated from mildewed cucurbits, collected in France and in other countries. They were chosen in order to be diverse in geographical and host origin (Table 1). All isolates were single spored and grown on surface disinfested melon cotyledons maintained in Petri dishes on MS agar (mannitol, 20g/l, sucrose, 10g/l, agar, 7g/l, tetracycline hydrochloride 25mg/l), as described by Bertrand (1991). These cultures were transferred to fresh cotyledons every 3 weeks.

Plant material and determination of virulence phenotypes

Cucumber, *Cucumis sativus* cv. 'Marketer', melon, *C. melo* cv. 'IranH', squash, *Cucurbita pepo* cv. 'Diamant' and watermelon, *Citrullus lanatus* cv. 'Sugar Baby' were used for pathotype determination. Six genotypes of melon, 'IranH', 'Védrantais', 'PMR45', 'PMR5', 'PI414723' and 'Edisto 47' were used for race determination according to Mohamed et al. (1995). Seeds were kindly provided by the plant breeding station (INRA, Montfavet, France). Plants were grown in a mildew-free greenhouse compartment and used at the 3–4 leaf stage (about 1 month of culture).

Each isolate was tested in a leaf disk assay as described by Epinat et al. (1993). Symptoms were rated individually for each leaf disk at 10 days after inoculation and were classified into 10 categories from 0 to 9 as described by Mohamed et al. (1995). On a given cultivar, strains with average scores lower than 3.0 were classified as avirulent and those with scores above 3.0 were considered virulent.

Mating type determination

Mating type was determined by pairing a single spore isolate with each of the two reference strains, Scc1 (mating type –) and Sm3 (mating type +). The tests were conducted as described by Bertrand (1991) on surface disinfested *Lagenaria ciceraria* cv. 'Minibottle' cotyledons maintained in Petri dishes on MS agar. Spores of the two strains to be mated were deposited on lines 2–3 mm apart. Cotyledons were examined 3–4 weeks after inoculation and the presence or absence of cleistothecia was recorded. Comparison with *Mat*1-1 and *Mat*1-2 defined by McGrath (1994) was done, using the two reference strains, C2 (*Mat*1-1) and A4 (*Mat*1-2) kindly provided by Dr. M.T. McGrath (Department of Plant Pathology, Cornell University, Riverhead, NY, USA). Hybridization with these strains revealed that *MAT1-1* is equivalent to mating type '+' and *MAT1-2* is equivalent to mating type '–'.

Table 1. Geographic, host origin, mating type, pathotype and race designation of 28 isolates of *Sphaerotheca fuliginea*

Isolate	Region	Host	Mating type	Pathotype[b]	Virulence on differential cultivars[c]	Race
Scc2	France (45)[a]	Cucumber	*Mat*1-1	A	1	0
Slg1	Cyprus	*Lagenaria*	*Mat*1-2	B	1,2	1
Scc3	France (66)	Cucumber	*Mat*1-1	B	1,2	1
Scc4	France (13)	Cucumber	*Mat*1-1	B	1,2	1
Scr3	France (84)	Squash	*Mat*1-1	B	1,2,3	2
Scr7	France (01)	Squash	*Mat*1-1	B	1,2	1
Sm28	France (84)	Melon	*Mat*1-2	B	1,2,3	2
Scr5	France (84)	Squash	*Mat*1-1	B	1,2,3	2
Scr8	France (43)	Squash	*Mat*1-2	B	1,2,3	2
Sm35	France (84)	Melon	*Mat*1-1	B	1,2,3	2
Sm18	France (84)	Melon	*Mat*1-1	B	1,2,3	2
Sm11	France (84)	Melon	*Mat*1-1	B	1,2,3	2
Sm46	U.S.A	Melon	*Mat*1-1	B	1,2,3	2
Scc14	France (45)	Cucumber	*Mat*1-1	B	1,2,3	2
Scr2	Turkey	Squash	*Mat*1-1	B	1,2,3	2
Scr14	France (01)	Squash	*Mat*1-1	B	1,2,3	2
Scr17	Sudan	Squash	*Mat*1-1	B	1,2,3	2
Scr16	Sudan	Squash	*Mat*1-1	B	1,2,3	2
Scc1	France (45)	Cucumber	*Mat*1-2	A	1,2	1
Scc15	France (45)	Cucumber	*Mat*1-1	B	1,2	1
Slg2	France (84)	*Lagenaria*	*Mat*1-1	B	1,2,3	2
Sm3	Tunisia	Melon	*Mat*1-1	B	1,2	1
Sm10	France (84)	Melon	*Mat*1-1	B	1,2,3	2
Sm5	Senegal	Melon	*Mat*1-1	B	1,2,3	2
Sm13	France (84)	Melon	*Mat*1-1	B	1,2,3	2
Scc5	France (13)	Cucumber	*Mat*1-1	B	1,2,3	2
Sm48	Italy	Melon	*Mat*1-1	B	1,2,3	2
Scc6	France (69)	Cucumber	*Mat*1-1	A	1,2	1

[a] Numbers in parentheses indicate district codes, as follows: 01 = Ain, 13 = Bouches du Rhône, 43 = Haute-Loire, 45 = Loiret, 66 = Pyrénées-orientales, 69 = Rhône, 84 = Vaucluse.
[b] Pathotype A: growth on Cucumber cv. 'Marketer' and Melon cv. 'IranH'; Pathotype B: growth on 'Marketer', 'IranH', and Squash cv. 'Diamant'.
[c] Melon differential cultivars: 1: 'IranH', 2: 'Védrantais', 3: 'PMR45', 4: 'PI414723', 5: 'PMR5', 6: 'Edisto47'.

DNA isolation

For each isolate, DNA was extracted from 50–100 mg (fresh weight) of spores collected from 25 melon cotyledons 10 days after inoculation. We used the method of Möller et al. (1992) modified as follows: fresh conidia were ground and suspended in extraction buffer (100 mM Tris-HCl pH7.5; 2 mM EDTA; 2%(w/v) SDS). DNA was extracted twice with one volume of $CHCl_3$/isoamylalcohol (24/1 v/v). Before precipitation, 5 μl of RNAse A (25 mg/ml stock) was added to each sample. After precipitation with one volume of cold isopropanol, the pellets were washed with 70% (v/v) ethanol, air dried and dissolved in 20–50 μl of TE (10 mM Tris-HCl pH8; 0.1 mM EDTA). The samples were left to stand overnight at 4 °C. The DNA was quantified by comparison with known quantities of lambda DNA (Promega, Madison, WI) on 1% (w/v) agarose gels, run in 1X TAE buffer (0.04 M Tris, 0.008 M sodium acetate, 0.001 M EDTA) and stained with ethidium bromide. After quantification, the DNA was stored at –20 °C.

Primers and amplification conditions

To amplify the ITS and the 5.8S regions of ribosomal genes in *S. fuliginea*, we used primers PN23 (5′-CACCGCCCGTCGCTACTACCG-3′) and PN34 (5′-TTGCCGCTTCACTCGCCGTT-3′) (Mouyna and Brygoo, 1993). The synthesized primers were purchased from Eurogentec (Seraing, Belgium).

In preliminary tests, 95 ten-base primers (Operon Technologies Inc., Alameda CA 94501, USA) were taken randomly from kits A to N and were used to determine which primers produced reproducible amplified DNA fragments in two isolates of *S. fuliginea*. Each primer was tested at least twice to verify the consistency of RAPD patterns. Twenty-two of the primers that yielded consistent banding patterns (monomorphic or polymorphic fragments) were selected for the study of the 28 strains (Table 2). For each strain the primers were tested at least twice in independent experiments to identify reproducible fragments in the RAPD patterns.

Amplification reactions were performed in volumes of 25 μl containing 10 to 20 ng of genomic template DNA; 0.28 mM each of dATP, dCTP, dGTP and dTTP (Boehringer Mannheim, Germany); 2.5 mM $MgCl_2$ (Promega, Madison, WI, U.S.A.); 15 pM primer (Operon Technologies Inc., Alameda CA, U.S.A); 2.5 μl of 10X*Taq* polymerase buffer (Promega); 1 unit of *Taq* DNA Polymerase (Promega) in sterile distilled water. Sterile distilled water was used in place of the template DNA as a negative control to test for contaminations. Amplifications were performed with a DNA thermal cycler (PHC-3, Techne, Cambridge, England) programmed as follows for RAPD assays: one first treatment at 93 °C for 5 min, followed by 45 cycles of 1 min at 93 °C, 1 min at 40 °C, and 2 min at 72 °C. A final extension step of 10 min at 72 °C was conducted after the 45 cycles, followed by cooling to 10 °C until recovery of the samples. The amplification products were separated by electrophoresis on 2% (w/v) agarose gels stained with ethidium bromide and photographed under UV light. For PCR amplification of the ITS region, the annealing step of the cycles was run with 1 min at 55 °C instead of 1 min at 40 °C.

Restriction enzyme digestions

The amplified ITS regions were digested with one of 11 restriction enzymes (*Cfo*I, *Dde*I, *Eco*RI, *Hae*III, *Hinf* I, *Hpa*II, *Rsa*I, *Sau*3A, *Sau*96I, *Sma*I, *Taq*I) according to the manufacturer's recommendations (Boehringer Mannheim). Digestion mixtures were prepared as 5 units of enzyme, 6 μl of enzyme buffer (supplied by the manufacturer), 38 μl of sterile distilled water and 15 μl of the PCR product per tube. Digestions were run for one hour at temperatures recommended by the manufacturer for each given enzyme. Restricted DNA was then analyzed on 2% agarose gels.

Analysis of molecular data

RAPD polymorphism among isolates was scored from gel photographs. Fragments comprised between 100 and 2000 bp were scored. For each primer, non reproducible fragments between the repetition tests were disregarded. Comparison of profiles for each primer was done on the basis of the presence *versus* absence of RAPD products presumed to be of the same length (same velocity of migration). RAPD fragments of the same size were scored as identical. The presence or absence of a fragment were considered as alternative alleles at the same locus.

To describe genetic variation among the strains of *S. fuliginea* tested, the diversity index *H* defined by Nei (1987) was determined. This index was first computed for the 152 loci identified for the whole group of 28 strains. It was also computed for the 136 loci identified for the subset of 20 isolates originating from France.

Gametic disequilibrium among each pair of polymorphic RAPD loci was estimated in order to make inferences on the reproductive biology of this pathogen. Gametic disequilibrium is a measure of association between alleles at different loci. The null hypothesis tested (H_0) was the independence of loci; if the loci are linked, then they are not independent. If an organism reproduces asexually, then the entire genome is effectively linked since there is no segregation. Statistical significance of the disequilibrium between two loci was tested using a Fisher exact test with the 20 isolates from France and was realized with the population genetic software package Genepop (version 2.0) (Institut des Sciences de l'Evolution, Montpellier, France; Raymond and Rousset, 1995a and b). Association between two loci was tested from contingency tables (2×2) of the whole allelic combinations observed in the population. Fisher exact test was used instead of a χ^2 test because of the small number of strains analyzed in this study (Zar, 1984). The significance was corrected by the sequential Bonferroni method (Rice, 1989).

To estimate the genetic relationships among the 28 strains, a phylogenetic tree was constructed. The

Table 2. Code and sequence of the 22 primers used in this study; for each primer tested, the total number of RAPD bands, the number of polymorphic DNA fragments and the number of RAPD phenotypes were defined

Primer	Nucleotide sequence	Scored bands	Polymorphic bands	RAPD phenotypes	Primer	Nucleotide sequence	Scored bands	Polymorphic bands	RAPD phenotypes
OPB08	GTCCACACGG	10	9	8	OPC08	TGGACCGGTG	9	7	8
OPC12	TGTCATCCCC	3	3	3	OPD08	GTGTGCCCCA	8	3	4
OPE03	CCAGATGCAC	6	2	3	OPE18	GGACTGCAGA	8	5	5
OPF01	ACGGATCCTG	7	4	6	OPF03	CCTGATCACC	5	4	7
OPF13	GGCTGCAGAA	5	4	8	OPF14	TGCTGCAGGT	6	4	6
OPF19	CCTCTAGACC	1	1	2	OPG02	GGCACTGAGG	9	3	5
OPG03	GAGCCCTCCA	5	0	1	OPG04	AGCGTGTCTG	9	3	5
OPG05	CTGAGACGGA	4	2	3	OPG13	CTCTCCGCCA	7	4	5
OPH02	TCGGACGTGA	8	6	8	OPH07	CTGCATCGTG	9	7	6
OPH11	CTTCCGCAGT	5	3	4	OPH19	CTGACCAGCC	10	6	9
OPI03	CAGAAGCCCA	10	4	7	OPN01	CTCACGTTGG	9	7	6

genetic similarity between two strains was calculated based on Dice's coefficient with the SIMQUAL program of NTSYS-pc version 1.8 (Exeter Software, Setauket NY, USA): *Dice = 2a/(2a+b+c),* where *a* is the number of shared RAPD fragments, *b* the number of fragments present only in isolate 1; and *c* the number of fragments present only in isolate 2. This program was also used to calculate Jaccard's coefficient (*J = a/[a+b+c]*) and the simple matching coefficient (*SM = a+d/[a+b+c+d]*) where *d* is the number of fragments absent in isolates 1 and 2, as a measure of genetic diversity for each pair of isolates. For each coefficient, the similarity matrix was used to construct dendrograms with the help of the unweighted pair grouping by mathematical averaging (UPGMA) (Sokal and Sneath, 1963) methods using the SAHN and TREE programs of NTSYS.

Results

Pathogenicity and mating type tests

Among twenty-eight isolates collected from various geographic regions, two pathotypes and three races could be distinguished on the basis of their pathogenicity towards different host species, and virulence towards differential melon cultivars (Table 1). Three isolates, identified as 'pathotype A' were pathogenic on cucumber 'Marketer' and melon 'IranH'. One of these (Scc2) was characterized as race 0 and the two others (Scc1 and Scc6) as race 1 on melon. All the other strains, identified as 'pathotype B', were pathogenic to cucumber 'Marketer', melon 'IranH', and squash 'Diamant'. In this group, 6 isolates were race 1, and the 19 others were race 2 'Europe' as defined by Mohamed et al. (1995). The race 2 'USA' and the race 3 types were not encountered. No strain was able to grow on watermelon 'Sugar Baby'.

All isolates produced cleistothecia, with one (but not the other) of the two reference strains. Twenty-four clones were mating type *Mat*1-1, and four were mating type *Mat*1-2, as they produced cleistothecia with either strain Scc1 or Sm3, respectively (Table 1).

ITS-RFLP patterns

For all strains of *S. fuliginea*, primers PN23 and PN34 allowed the PCR amplification of a fragment of similar size (about 800 bp) (Figure 1-A). The restriction enzymes *Eco*RI and *Sma*I failed to digest this fragment, but it was digested by all of the 4-base recognition restriction enzymes tested. For a given endonuclease, all the strains showed the same pattern. This is illustrated in Figure 1-B for three strains of *S. fuliginea* with six restriction enzymes.

Polymorphism assessed by RAPD analysis

Among the 95 primers screened with two isolates of *S. fuliginea*, 21 failed to yield amplification products in our conditions and 52 yielded non reproducible amplification patterns. The 22 primers yielding clear, intensive and reproducible patterns were selected for

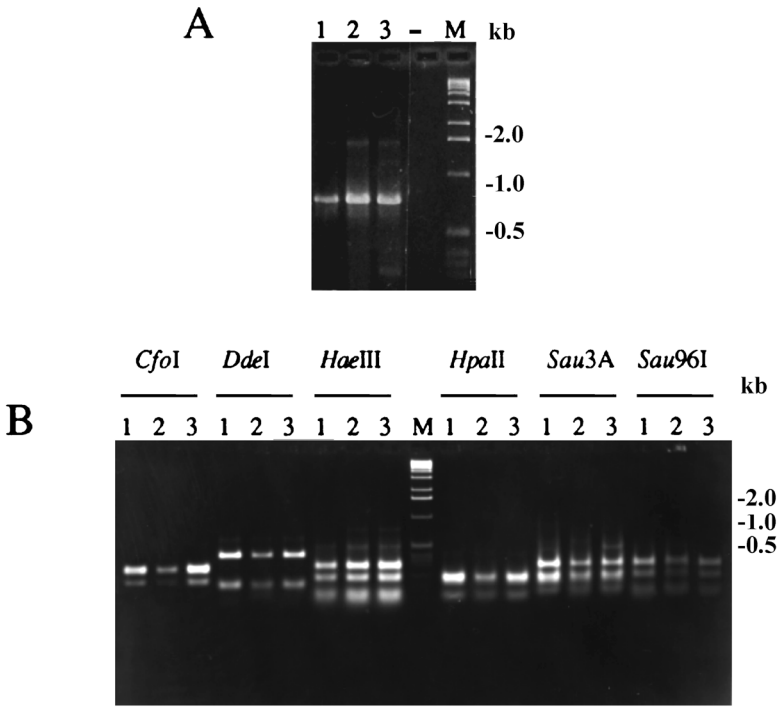

Figure 1. Agarose gels showing: (A), amplification of the ITS region (ITS1, ITS2 and 5.8S); and (B), restriction patterns of PCR-amplified rDNA digested with*Cfo*I, *Dde*II, *Hae*III, *Hpa*II, *Sau*3A and *Sau*96I. Lanes marked 1, 2, and 3 are isolates Scc2, Slg1 and Sm46. Lanes marked M are 1 kb ladder DNA, and lane marked – is control with sterile water.

the analysis of the collection of 28 isolates (Table 2). Profiles of 1 to 5 fragments were observed, showing fragments 100 to 2000 bp in size. The strains examined were identical for a large number of RAPD loci (Table 2) and no single primer differentiated all 28 strains. One to 9 RAPD patterns were obtained with each primer (Table 2). Examples of profiles produced by two of the primers are shown in Figure 2. With primer OPG03, all strains presented the same profile and only one RAPD pattern was obtained. With primer OPN01, polymorphism was observed among the 28 strains: out of nine fragments scored, six were polymorphic and six RAPD patterns were observed. In total, 152 distinct RAPD fragments were recorded for the 28 isolates tested with the 22 primers. Eighty-nine fragments (or 59% of those observed) were polymorphic, but among the 28 isolates tested, only 32 (21%) were present with 10–90% frequency. In the subset of 20 French strains, 54% of the RAPD fragments were polymorphic (73 out of 136) and only 14% (19 out of 136) were not rare among the 20 isolates. Similarity matrixes derived from these data revealed a relatively low level of diversity among the 28 strains tested despite the fact that 27 different RAPD patterns were observed (Figure 3). The two isolates most different based on RAPD markers (isolates Scc5 and Scc6) showed very high levels of similarity: Dice's, Jaccard's and the SM coefficients of similarity were for these two isolates 78%, 64%, and 74%, respectively. For all other 26 isolates, percent similarity was even higher. The overall genetic diversity among isolates was very low based on Nei's index of diversity calculated on the basis of the 152 or 136 RAPD loci: it was 0.09 for the population of 28 strains and 0.10 for the subset of 20 French strains.

Phenograms based on UPGMA clustering of similarity matrixes, derived from Dice's coefficient, Jaccard's coefficient and SM coefficient, were nearly identical in topology. These three trees placed Scc6 as the most distinct isolate. Figure 3 shows the phylogenetic tree obtained with Dice's coefficient. A lack of clustering was observed and races, mating types, hosts or regions of origin could not be distinguished on the basis of the molecular profiles (Figure 3). Two strains, Sm28 and Scr7, had identical patterns although they were collected from different hosts in different regions,

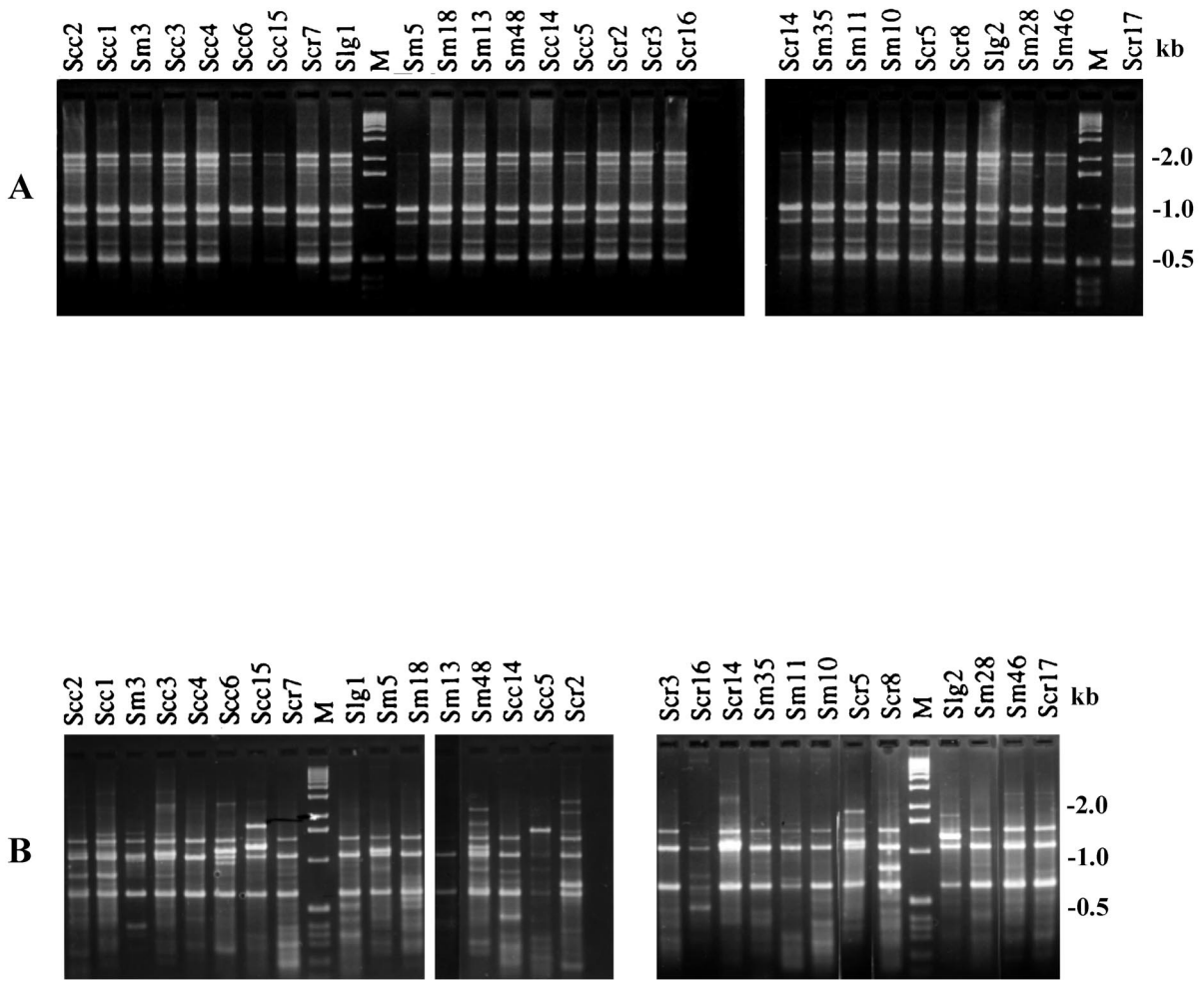

Figure 2. Random amplified polymorphic DNA of 28 strains of *Sphaerotheca fuliginea* with primers OPG03 (A) and OPN01 (B) showing relative homogeneity between isolates. Lanes marked M are 1 kb ladder DNA, and lane marked – is control with sterile water.

and they represented different pathotypes and races. Moreover, strains coming from USA, Turkey, Cyprus, Sudan, Tunisia, Senegal or Italy were classified with the French ones in the phenogram.

The percentage of pairs of loci with gametic disequilibrium was calculated based on the 19 polymorphic RAPD fragments present in the French population. Among 171 combinations of pairs of loci, the null hypothesis of gametic equilibrium was rejected for only 14. This suggests that 8% of the combinations were in disequilibrium in the French population.

Discussion

Molecular methods are a useful tool for examining genetic diversity in populations of plant pathogenic fungi (McDermott et al., 1993; Chen et al., 1993; Peever and Milgroom, 1994). In our study, no polymorphism was found in the ITS regions of *S. fuliginea* and a low level of genetic diversity was revealed by RAPDs even though the 28 isolates in our collection originated from a variety of sampling sites on several continents. Such a situation is not unique among plant pathogenic fungi (Ouellet and Seifert, 1993; Sreenivasaprasad et al., 1993; Van der Vlugt-Bergmans et al., 1993; Délye et al., 1995). Van der Vlugt-Bergmans et al. (1993),

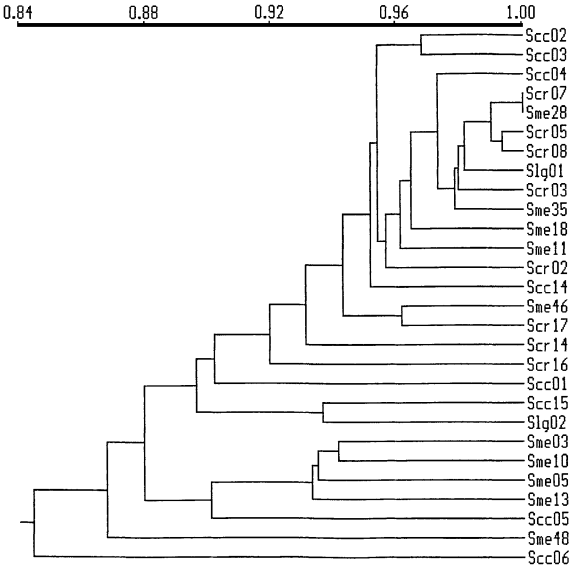

Figure 3. Phenetic tree of the 28 isolates of *S. fuliginea* examined in this study. The tree was produced using UPGMA clustering of a similarity matrix based on Dice coefficient calculated from 152 individual DNA bands produced by RAPD with 22 different primers. A similar phenetic tree was produced using the Jaccard and the simple matching coefficients calculated from the same RAPD data set, but is not shown.

using RAPD markers, showed that similarity between 10 strains of *Botrytis cinerea* always exceeded 82% according to the mathematical model of Nei and Lee (1979) which corresponds to the Dice's coefficient. Using the same model, an even lower rate of polymorphism (almost 95% similarity) was observed by Délye et al. (1995) among 13 isolates of *Uncinula necator*. Very few data are available concerning the valuation of the Nei's diversity index (Nei, 1987) based on RAPD data. The allelic diversity in French population of *S. fuliginea* was estimated to 0.10 (averaged over 136 loci). This result is much lower than those observed with 41 French strains of *E. cichoracearum*, $H = 0.26$ (averaged over 147 loci) (Bardin, Carlier, Nicot and Lemaire, unpublished), and is comparable to those reported by Peever and Milgroom (1994) who examined 10 RAPD loci in populations of *Pyrenophora teres*. They estimated a diversity range from 0.08 in North Dakota to 0.17 in New York. The low level of genetic diversity suggests a close genetic relationship between all these strains even though they came from eight different countries and from different hosts.

DNA polymorphisms were detected among isolates within races of *S. fuliginea* and the dendrogram obtained in this study did not show clustering according to any of the specific ecological traits examined. Strains with different geographic origins, isolated from different hosts or representing diverse pathotypes, races or mating types were not clustered in genetically distinct groups. The lack of association between virulence and RAPD markers suggests that the evolution of virulence may have been independent of the evolution of the RAPD markers tested. Chen et al. (1993), by using 11 primers (50 polymorphic RAPD fragments), also demonstrated that the overall association between virulence and RAPD in *Puccinia striiformis* was low. Among the 26 races included in their study, the authors discriminated only one by RAPD. McDermott et al. (1993) observed that virulence loci and RAPD markers were on the average evolving independently within *Erysiphe graminis* f. sp. *hordei*. This result may provide useful elements for the understanding of the ecology of *S. fuliginea*. This knowledge may have important consequences for disease resistance breeding in cucurbits. One such element concerns the occurrence of the sexual form of *S. fuliginea*. Although the teleomorph has occasionally been observed on cucurbits in a wide range of countries (McGrath et al., 1996), and compatible mating types have been found in North America (McGrath, 1994) and Europe (Bertrand, 1991), the question of its prevalence and epidemiological relevance remains largely unanswered (Bertrand, 1991; McGrath, 1994). Attempts to obtain infection of cucurbits with ascospores were not successful in laboratory conditions with cleistothecia coming from nature or from *in vitro* culture (Bertrand, 1991; McGrath, 1994). Conversely, the lack of clustering, the absence of correlation between phenotypic and molecular markers, the presence of the two mating types in the population and the low level of gametic disequilibrium observed support the hypothesis that recombination could occur in populations of *S. fuliginea*. Indeed, random associations between neutral loci are expected in a random-mating population. This suggests that ascospores may play a role in the spread of this fungus in nature, but further sampling will be necessary to confirm this hypothesis.

Another such element concerns the importance of migration on the population structure of this fungus. No association between molecular markers and geographic origin of strains was detected suggesting that all the strains tested in this study share a nearly similar genetic background wherever their geographical origin. This suggests an absence of differentiation according to geographic distance. Moreover the fact

that similar RAPD patterns were found between strains isolated from different geographical origins suggests that migration could have occurred recently between these locations. But further sampling will be necessary to confirm this hypothesis. However, the capacity of certain powdery mildew fungi to be disseminated by wind has been known for a long time (Hermansen et al., 1978; Yarwood, 1978) but no information is available for *S. fuliginea*. For *Erysiphe graminis*, gene flow may be a major determinant in the distribution of genetic variation across the whole of Europe (Wolfe and McDermott, 1994). In the future, larger samples from different populations could provide more information about long-distance migration of spores of *S. fuliginea*.

In summary, our results demonstrate that molecular markers such as RAPD promise to increase our understanding of the ecology and population genetic of *S. fuliginea*. It could allow us to make inferences regarding the occurrence of sexual reproduction and patterns of dispersal of this species. This knowledge is of great importance to evaluate the factors that lead to emergence of new races and to predict future disease development.

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