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Virulence variation and RAPD polymorphism in African isolates of *Phaeoisariospis griseola* (Sacc.) Ferr., the causal agent of angular leaf spot of common bean

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Abstract

Fifty four isolates of *Phaeoisariopsis griseola*, the agent of common bean angular leaf spot disease from the Great Lakes Region of Africa, were characterised according to their virulence behaviour and their molecular patterns. Virulence properties were revealed through the inoculation of 29 genotypes of *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus polyanthus*. Differences in reaction types revealed high variability among these isolates. Most of them, even when collected within the same location, showed differences in their respective reactions on many plant genotypes. For molecular typing, RAPD amplifications were performed for each isolate using five random primers. Isolates with different patterns were collected within one region. Simultaneously, similar molecular patterns were found in isolates collected at different sites. However, the average of molecular similarity, based on the percentages of shared bands for each isolates pair, was higher among isolates collected within one site. No direct correlation between molecular pattern and pathotype was observed.

Abbreviations: ALS – angular leaf spot; RAPD – randomly amplified polymorphic DNA; RT – reaction type.

Introduction

The angular leaf spot (ALS) disease of common bean (*Phaseolus vulgaris* L.) caused by *Phaeoisariopsis griseola* (Sacc.) Ferr. (*Isariopsis griseola* Sacc.) is found in more than 60 countries world-wide (Guzmán et al., 1995) and results in yield losses up to 80% (Schwartz et al., 1981). Thus, breeding for disease resistance to ALS represents a priority for several national bean breeding programs especially in the African Great Lakes Region where subsistence agriculture prevails. Adaptation of pathogen populations to overcome host resistance represents the main drawback of this strategy. Understanding the genetic structure and the virulence pattern of pathogen populations represents a prerequisite to the development of a rational strategy of resistance gene deployment. This goal can be pursued by two distinct approaches. Comparing the increasing availability of genetic markers, such as isozymes and molecular markers, to virulence data allows the analysis of how pathogenic variability is generated and maintained (Leung et al., 1993; Milgroom, 1997). Variability within *P. griseola* revealed by isoenzymatic analysis (Boshoff et al., 1996) and RAPD patterns (Guzmán et al., 1995; Chacón et al., 1997) has already been reported. Phylogeny inferred from RAPD and isozyme markers divided *P. griseola* isolates into two major groups, the first one (Andean) being generally recovered from Andean gene pool materials whereas the second (Mesoamerican) was recovered from the Mesoamerican gene pool. Forty-one of the 44 isolates originating from Malawi were related to the Andean group (Guzmán et al., 1995).

A traditional pathogen race survey, based on the differential reactions of cultivars to inoculation, generates detailed pictures of the virulence structure and represents the second strategy of analysis. This kind of analysis only reveals pathogen properties related to the host selection effect on the pathogen populations (Kolmer et al., 1995) but remains the ultimate reference of virulence analysis.

Some evidence of pathogenic variability in *P. griseola* has been described based on pathogenicity on differential bean cultivars (Alvarez-Ayala and Schwartz, 1979). Similar variability was also suggested by field trials in Africa (Aggarwal et al., 1996) and India (Srivastava et al., 1995). Moreover pathogenic behaviour of a few randomly chosen *P. griseola* isolates collected by Guzmán et al. (1995) showed association between pathogenicity and molecular phylogeny suggesting coevolution of *P. griseola* with common bean. Similar works have been done with *Colletotrichum lindemuthianum* populations, the agent of common bean anthracnose. In Latin America, molecular and virulence analyses of *C. lindemuthianum* showed the existence of two major groups of strains (González et al., 1998). Data obtained by Sicard et al. (1997) for the same pathogen revealed an adaptation of strains on cultivars of the same geographic origin. This knowledge may be very useful for any program breeding for resistance against bean anthracnose.

The importance of *P. griseola* pathogen diversity prevailing in the African Great Lakes Region remains poorly known. The objective of our study was to analyse *P. griseola* isolates originating from that region in order to understand how ALS disease management by genetic resistance within this specific region may be performed. Pathogen diversity was determined in terms of races or pathotypes as defined by inoculation of different plant genotypes and in terms of molecular patterns using the RAPD technique after which, a comparison between the virulence and molecular diversity was performed. This is the first large-scale analysis of Central African *P. griseola* isolates collected from different areas within a region where cultivars are cultivated in variable mixtures.

Materials and methods

Collection of fungal isolates

A collection of 54 isolates of *P. griseola* was obtained from naturally-infected bean leaves. Forty-four isolates originated from countries of the Great Lakes Region of Africa (Burundi, Rwanda, Zaïre and Kenya), while 10 isolates were collected in Brazil and Colombia (Table 1). Isolates were maintained on V8 juice agar medium (per litre: 200 ml V8, 3 g $CaCO_3$ and 18 g agar) and kept in the dark at $\pm 20\,^{\circ}C$. Colonies descended from a single spore were conserved in a cold room ($4\,^{\circ}C$) for inoculation of different plant genotypes and molecular analyses.

Differential plant genotypes

A set of twenty-nine plant genotypes was used for the inoculation with *P. griseola* (Table 2). The set consists of 17 genotypes of *P. vulgaris* (including 8 differential varieties sent us by CIAT), 6 accessions of *P. coccineus* and 6 accessions of *P. polyanthus* received from the Tropical Crop Husbandry Unit of the Agricultural University of Gembloux. All these genotypes, except two accessions of *P. coccineus* (NI1108 and NI819) are current cultivars.

Inoculation of plant genotypes and scoring of symptoms

Each isolate was multiplied from a conserved sample by culturing on V8 juice agar medium. Conidia were harvested from 12-day-old cultures, suspended in distilled water and adjusted at a concentration of 2×10^4 conidia per ml. The first trifoliate leaves were inoculated by spraying the inoculum to run off under a pressure of $2.8\,kg/cm^2$ until saturation. In order to control the reproducibility of symptom development, a susceptible reference (variety Aroana inoculated by the isolate KGM1) was included in each inoculation series.

Inoculated plants (3 for each isolate $\times$ genotype combination) were incubated for 4 days in a humid chamber (RH of 95%) with a 16 h light photoperiod. Plants were maintained in a greenhouse ($25\,^{\circ}C$) for another 12 days and evaluated for symptoms according to the visual scale defined by Schoonhoven and Pastor-Corrales (1992). Values reflecting the percentage of infected leaf area ranged from 1 (no visible lesion) to 9 (more than 25% of the leaf area covered by lesions). Reaction type categories were determined according to the averages of these symptom scores attributed for each plant pathogen combination.

Table 1. List of *Phaeoisariopsis griseola* isolates

Number	Isolate	Origin Country	Location	Collection date	Sent by
1	KGM1	Burundi	Muhingira	January 1994	ISABU[1]
2	KGM2	Burundi	Muhingira	January 1994	ISABU[1]
3	KGM3	Burundi	Muhingira	January 1994	ISABU[1]
4	KGM4	Burundi	Muhingira	January 1994	ISABU[1]
5	KGM5	Burundi	Muhingira	January 1994	ISABU[1]
6	KGM6	Burundi	Muhingira	January 1994	ISABU[1]
7	KGM7	Burundi	Muhingira	January 1994	ISABU[1]
8	KGM8	Burundi	Muhingira	January 1994	ISABU[1]
9	KGR1	Burundi	Rubagabaga	January 1994	ISABU[1]
10	KGR2	Burundi	Rubagabaga	January 1994	ISABU[1]
11	KGR3	Burundi	Rubagabaga	January 1994	ISABU[1]
12	KGR4	Burundi	Rubagabaga	January 1994	ISABU[1]
13	KGR5	Burundi	Rubagabaga	January 1994	ISABU[1]
14	KGR6	Burundi	Rubagabaga	January 1994	ISABU[1]
15	NMM1	Burundi	Murama	January 1994	ISABU[1]
16	NMM2	Burundi	Murama	January 1994	ISABU[1]
17	NMM3	Burundi	Murama	January 1994	ISABU[1]
18	KF1	Kenya	Unknown	April 1994	University of Nairobi
19	FK4	Kenya	Unknown	April 1994	University of Nairobi
20	KK1	Burundi	Kabuye	July 1994	ISABU[1]
21	KK2	Burundi	Kabuye	July 1994	ISABU[1]
22	KK3	Burundi	Kabuye	July 1994	ISABU[1]
23	KK4	Burundi	Kabuye	July 1994	ISABU[1]
24	KK5	Burundi	Kabuye	July 1994	ISABU[1]
25	KK6	Burundi	Kabuye	July 1994	ISABU[1]
26	RN1	Colombia	Rio Negro	February 1994	CIAT[2]
27	RN2	Colombia	Rio Negro	February 1994	CIAT[2]
28	RN4	Colombia	Rio Negro	February 1994	CIAT[2]
29	RN8	Colombia	Rio Negro	February 1994	CIAT[2]
30	RN10	Colombia	Rio Negro	February 1994	CIAT[2]
31	BR1	Brazil	Goiás	December 1995	EMBRAPA[3]
32	BR2	Brazil	Ceará	December 1995	EMBRAPA[3]
33	BR3	Brazil	Minas Gerais	December 1995	EMBRAPA[3]
34	BR4	Brazil	Espírito Santo	December 1995	EMBRAPA[3]
35	BR5	Brazil	Pernambuco	December 1995	EMBRAPA[3]
36	RDA1	Rwanda	Unknown	April 1996	University of Rwanda
37	RDA2	Rwanda	Unknown	April 1996	University of Rwanda
38	RDA3	Rwanda	Unknown	April 1996	University of Rwanda
39	RDA6	Rwanda	Unknown	April 1996	University of Rwanda
40	RDA7	Rwanda	Unknown	April 1996	University of Rwanda
41	BGA2	Burundi	Gitega	April 1996	University of Burundi
42	BGA4	Burundi	Gitega	April 1996	University of Burundi
43	BGA5	Burundi	Gitega	April 1996	University of Burundi
44	ZA1	Zaïre	Unknown	May 1996	University Kinshasa
45	ZA2	Zaïre	Unknown	May 1996	University Kinshasa
46	ZA3	Zaïre	Unknown	May 1996	University Kinshasa
47	ZA4	Zaïre	Unknown	May 1996	University Kinshasa
48	ZA5	Zaïre	Unknown	May 1996	University Kinshasa
49	ZA6	Zaïre	Unknown	May 1996	University Kinshasa
50	ZA7	Zaïre	Unknown	May 1996	University Kinshasa
51	ZA8	Zaïre	Unknown	May 1996	University Kinshasa
52	ZA9	Zaïre	Unknown	May 1996	University Kinshasa
53	ZA10	Zaïre	Unknown	May 1996	University Kinshasa
54	ZA11	Zaïre	Unknown	May 1996	University Kinshasa

[1]ISABU: Institut des Sciences Agronomiques du Burundi.
[2]CIAT: Centro Internacional de Agricultura Tropical.
[3]EMBRAPA: Empresa Brasileira de Pesquisa Agropecuaria.

Table 2. List of genotypes of *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus polyanthus*

Genotype	Species	Origin
BAT76	*P. vulgaris*	ISABU[1]
CALIMA	*P. vulgaris*	ISABU
AROANA	*P. vulgaris*	ISABU
A340	*P. vulgaris*	ISABU
A345	*P. vulgaris*	ISABU
A285	*P. vulgaris*	ISABU
A410	*P. vulgaris*	ISABU
A140	*P. vulgaris*	ISABU
PRELUDE	*P. vulgaris*	Used in Belgium
BAT1647	*P. vulgaris**	CIAT[2]
SEAFARER	*P. vulgaris**	CIAT
CORNELL49242	*P. vulgaris**	CIAT
MONTCALM	*P. vulgaris**	CIAT
A339	*P. vulgaris**	CIAT
G5686	*P. vulgaris**	CIAT
BAT332	*P. vulgaris**	CIAT
POMPADOUR CHECA	*P. vulgaris**	CIAT
NI15	*P. coccineus*	Rwanda
NI16	*P. coccineus*	Rwanda
NI1108	*P. coccineus*	Mexico[3]
NI819	*P. coccineus*	Mexico[3]
NI229	*P. coccineus*	Zaïre
NI666	*P. coccineus*	Puerto Rico
NI429	*P. polyanthus*	Costa Rica
NI519	*P. polyanthus*	Mexico
NI1208	*P. polyanthus*	Colombia
NI1010	*P. polyanthus*	Colombia
NI1011	*P. polyanthus*	Colombia
NI373	*P. polyanthus*	Venezuela

[1]ISABU: Institut des Sciences Agronomiques du Burundi.
[2]CIAT: Centro Internacional de Agricultura Tropical.
[3]Wild genotypes.
*Varieties sent by CIAT are differentials identified by Correa (1988).

DNA extraction

Erlenmeyer flasks (500 ml) containing 250–300 ml of liquid medium (per liter: 10 g glucose, 5 g yeast extract, 4 g KH_2PO_4, 0.9 g K_2HPO_4, 1 g NH_4Cl, 0.25 g $MgSO_4 \cdot 7H_2O$ and pH about 5.3) (Wöstemeyer, 1985) were inoculated with 10–15 agar disks of 1 cm in diameter. The cultures were placed on a rotary shaker (115 rpm) and incubated at $\pm 20\,^{\circ}C$ with a 16 h light photoperiod for 12 days. Mycelia were harvested by filtration through cheesecloth. Samples were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. DNA extraction was performed according to the CTAB procedure using TE buffer for final dilution of the samples (Doyle and Doyle, 1990).

RAPD analysis

RAPD reactions were carried out with 10-base oligonucleotide primers (OPK7: AGCGAGCAAG, OPK9: CCCTACCGAC, OPK10: GTGCAACGTG, OPL17: AGCCTGAGCC and OPL18: ACCACCCACC) (Operon Technologies, USA). PCR reactions were performed in a 50 µl final volume containing 5 µl of the PCR reaction buffer 10× conc. (Boehringer Mannheim), 0.4 µm oligonucleotide primer, 4 mM $MgCl_2$, 200 µm of each dNTP, 50 ng of genomic DNA from *P. griseola* and 1 unit of Taq polymerase (Boehringer Mannheim). Amplification conditions consisted of an initial step of DNA denaturation of 3 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1 min at 35 °C and 2 min at 72 °C and a final step of 10 min at 72 °C in a thermocycler (Biometra TRIO-Thermoblock). Amplification products were separated by electrophoresis in a 1.5% agarose gel and observed under UV light after gel staining with ethidium bromide.

DATA analysis

Symptom intensity was used to determine the reaction type (RT): resistance (R = symptom score $\leq$3), partial resistance (PR = symptom score >3–6) and susceptibility (S = symptom score >6–9). These RTs were introduced into a matrix and analysed by hierarchical cluster analysis using the average linkage method with the statistical program SYSTAT. Distances between isolates were calculated as the percentage of genotypes on which RTs were not similar for the considered isolates. The results were included in a dendrogram representing distances between isolates. Assuming that all plant genotypes contain different resistance genes, pathogen complexity was evaluated for each isolate by the number of susceptible genotypes. A Simpson diversity index, $H_s = 1 - \sum_i [n_i(n_i-1)/N(N-1)]$ where n_i represents the number of isolates within the ith phenotype and N the sample size (Groth and Roelfs, 1987), was calculated for Burundese isolates for which we precisely know the collection sites. This index takes values between 0 (if all strains are similar) and 1 (if each strain is different from all the others).

Bands observed by RAPD analyses were recorded in relation with their migration within the gel. It was assumed that bands of the same molecular weight in different individuals were identical. For each individual, the presence or absence of each band was determined

and designated by 1 or 0 respectively. The generated distance matrix was used for hierarchical cluster analysis using the average linkage method with the statistical SYSTAT program. Distances between isolates were calculated as the percentage of unshared bands. A dendrogram showing the distances between isolates according to their respective RAPD patterns derived from this analysis.

For the isolates collected in Burundi, molecular similarity percentages, calculated as the percentages of shared bands for each isolates pair, were calculated.

Pearson correlation coefficients between RAPD data (bands) and virulence properties (RTs on plant genotypes) were calculated using the SAS System. In order to determine the significance of the correlation, the proportion of coefficients greater than the significant level at an error of 5% was determined.

Results

Reaction type analysis

The three categories of RT (resistance, partial resistance and susceptibility) were observed with some plant genotypes according to the inoculated isolate. For example, genotype BAT76 appeared resistant to isolate KGR1, partially resistant to KGM1 but was susceptible to isolate KGM2. In the case of compatible reactions (grouping susceptible and partially resistant RT), the symptoms appeared 10 days after *P. griseola* inoculation. In the case of susceptible RTs, symptom intensity reached the highest score (9 on the CIAT scale) 16 days after inoculation.

The different isolates exhibited a high diversity in virulence when inoculated on the 29 plant genotypes. A total of 53 different virulence patterns were observed in the 54 isolates analysed. Figure 1 illustrates the distribution of the complexity of African isolates. The value of that parameter ranged from 0 (for isolates KGM4 and ZA7, which failed to produce compatible reaction on any tested genotype) to 24 (for isolates appearing virulent on a high proportion of genotypes). The more virulent an isolate appeared on a high number of genotypes, the more distant it was from the group of isolates (KGM4, ZA7) on the dendrogram (Figure 2).

The distribution of isolates within the different groups of the dendrogram could not be related to their geographical origin. Isolates collected in the same location showed differences in their patterns of virulence. For example, isolates NMM1, NMM2 and NMM3 originating from Murama in Burundi caused compatible reactions (susceptibility or partial resistance) respectively on 2, 19 and 14 genotypes. A similar result was observed with isolates originating from Gitega (BGA2, BGA4 and BGA5) which established compatible reactions on 13, 24 and 21 genotypes respectively. Table 3 shows the diversity index calculated by the Simpson formula for Burundese isolates. Within most of the considered groups, this value is equal to 1, meaning that each isolate has a virulence pattern different from those of all the other isolates of the same location.

RAPD analysis

Preliminary RAPD amplifications allowed the selection of 5 primers giving rise to repeatable results. The 54 *P. griseola* monospore isolates were analysed with

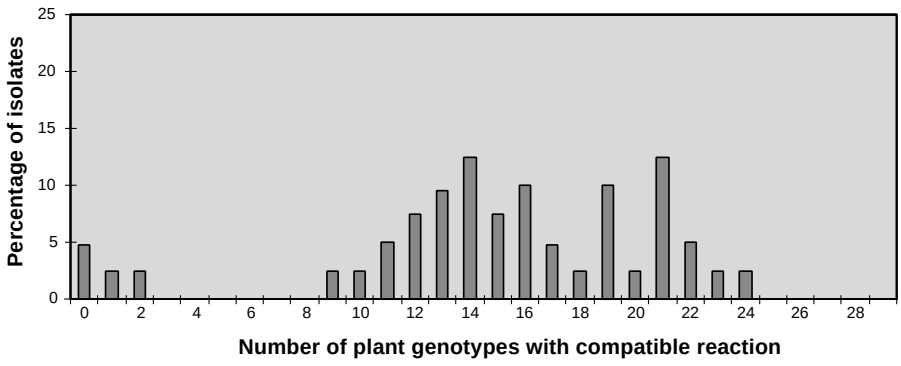

Figure 1. Analysis of the pathogen complexity of *P. griseola* isolates according to reactions caused on the 29 plant genotypes. For each isolate, the complexity evaluated by the number of genotypes with a compatible reaction (susceptible or partially resistant) was established.

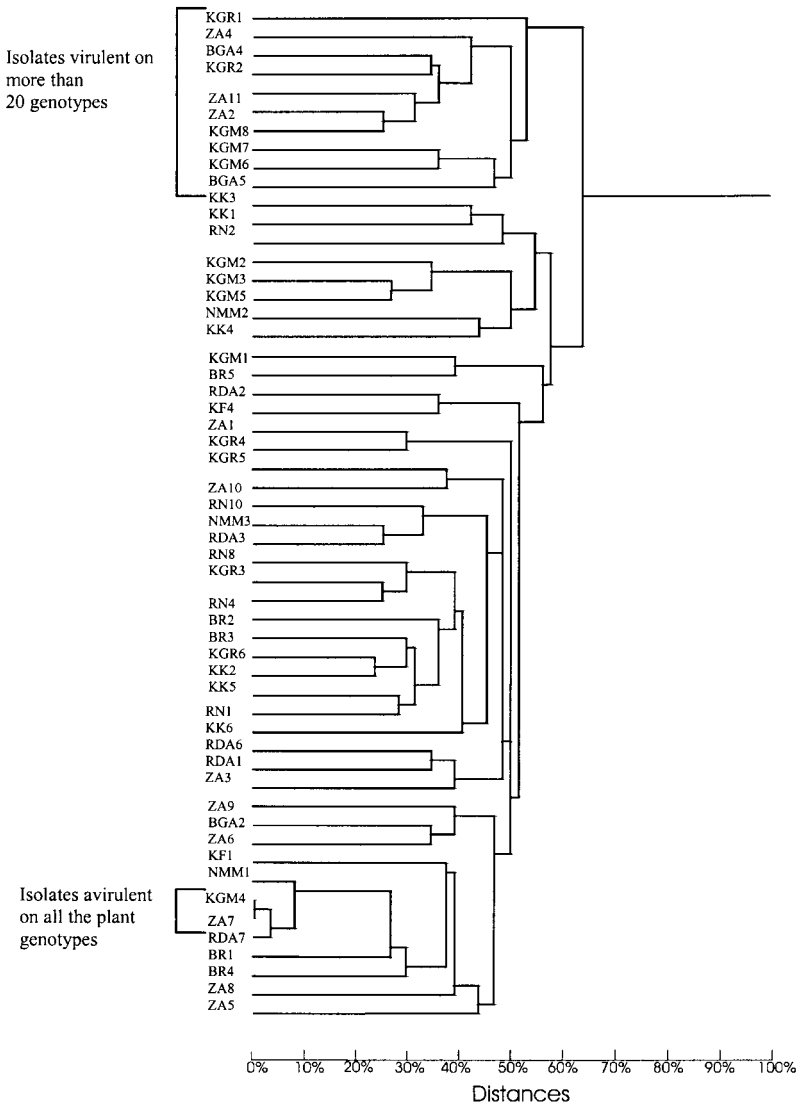

Figure 2. Dendrogram of 54 *P. griseola* isolates based on the RT (resistance, partial resistance and susceptibility) caused by their inoculation on 29 plant genotypes. Distance between 2 isolates indicates the percentage of plant genotypes on which isolates caused different reactions. Distances varied from 0% in case of identical RT to 100% if the RT falled into a different category for each of the inoculated genotypes.

the 5 oligonucleotide primers. An analytical example of the banding patterns obtained with RAPD using OPL17 is shown in Figure 3. Information on banding patterns obtained was used to determine genetic distances between isolates and to construct dendrogram revealing clusters (Figure 4). Isolates ZA7, ZA10 and KGR3 failed to yield amplification products and were not included in this cluster analysis. These 3 isolates showed RAPD amplification with other primers, meaning that the absence of bands with the 5 primers was not caused by inadequate quality of DNA (results not shown). Among the remaining 51 isolates, there was no group of isolates without any difference in RAPD products, where 51 RAPD patterns were recorded. All the 74 amplified bands were polymorphic because not one of them was common to all the isolates.

Table 3. Simpson diversity index on the base of the virulence properties of isolates originating from the same locality in Burundi

All Burundese isolates*	0.94
Isolates of the group KGM	0.93
Isolates of the group KGR	1
Isolates of the group NMM	1
Isolates of the group KK	1
Isolates of the group BGA	1

*Letters abbreviate the isolates origin.
KGM: Kayanza–Gatara–Muhingira.
KGR: Kayanza–Gatara–Rubagabaga.
NMM: Ngozi–Mwumba–Murama.
KK: Kayanza–Kabuye.
BGA: Burundi–Gitega.

A lack of clustering according to the regions of origin was observed. For example, isolate NMM3 was differentiated from NMM1 and NMM2 with a distance of 10% on the dendrogram, whereas all three isolates were collected in the same location. Other particular groups situated at low distances on the dendrogram contain isolates whose geographic origins were different. This was the case for the isolate groups (KGM3, KF1) and (BGA4, RDA6) where distances were of 2.7%. Within the first group, isolate KGM3 was from Burundi while isolate KF1 was from Kenya. For the other group, isolate BGA4 was from Burundi while isolate RDA6 was from Rwanda. However, molecular similarity (percentage of shared bands within all isolates pairs) for Burundese isolates was globally higher for isolates collected within the same area (same hill in this case) than between areas (Table 4).

Pathotype and molecular variation

No direct relationship between molecular pattern and pathotype structure was observed. In fact, correlation between molecular and virulence data was not significant. For example, isolates NMM1 and NMM2, which presented similar molecular patterns, exhibited different virulence properties. Distances between isolates were greater for virulence compared to those of molecular clustering. Indeed, the highest distances were 63% for virulence properties and 32% for RAPD patterns.

Discussion

Our results confirmed the existence of many pathotypes within *P. griseola* but revealed a level of variability not previously described. The majority of our isolates (53 of the 54 isolates analysed) exhibited different virulence profiles even for isolates originating from the same location. We used high numbers of plant genotypes and pathogen isolates, which could explain such a level of virulence polymorphism detected. Indeed, the number of virulence patterns increases with the size of the differential set (Chen et al., 1993). However, our differential genotypes possess unknown resistance factors, which greatly limits our capacity to interpret these data in terms of population structure. Moreover, the conclusions of any pathogen race survey rests on the validity of the sampling strategy. In our case, this one was achieved through the goodwill of researchers and farmers and had no systematic character.

Despite the relative inaccuracy of the data derived from the *P. griseola* race survey, the results highlight the existence of a great diversity of races, some of which were able to overcome the resistance of most of the plant genotypes available. In Central Africa, the existence of that variability could be assigned to selective effects exercised by widely used mixtures of cultivars (Wolfe et al., 1997).

Variability revealed by isozyme and RAPD patterns has already been reported within *P. griseola* populations (Boshoff et al., 1996; Guzmán et al., 1995). In our study, very low polymorphism was found in the ITS regions (Busogoro et al., 1997), while RAPD analysis identified a high polymorphism since no group of isolates with similar patterns was observed among the 51 isolates considered for the cluster analysis. Coexistence in the same area of isolates exhibiting different RAPD patterns was frequently observed.

The dendrogram obtained in this study did not show clustering according to the geographical origins, although molecular similarity was higher for isolates collected within the same site. Our dendrogram based on RAPD patterns did not exhibit 2 major groups of isolates. This is contrary to the results of Guzmán et al. (1995) and Chacón et al. (1997). This might indicate that our isolates were collected from the same common bean gene pool materials. In fact, genotypes of Andean gene pool are predominant within the African Great Lakes Region (Correa, 1988; Gepts and Bliss, 1988; Khairallah et al., 1990). While pathotypic structure is primarily dictated by host selection, phylogeny inferred from neutral molecular markers can reflect the relationship among strains. Based on the suitability of neutral markers for the determination of gene flow (Milgroom and Fry, 1997), RAPD patterns allowed the origin of inoculum for *Crinipellis perniciosa* (Andebrhan and

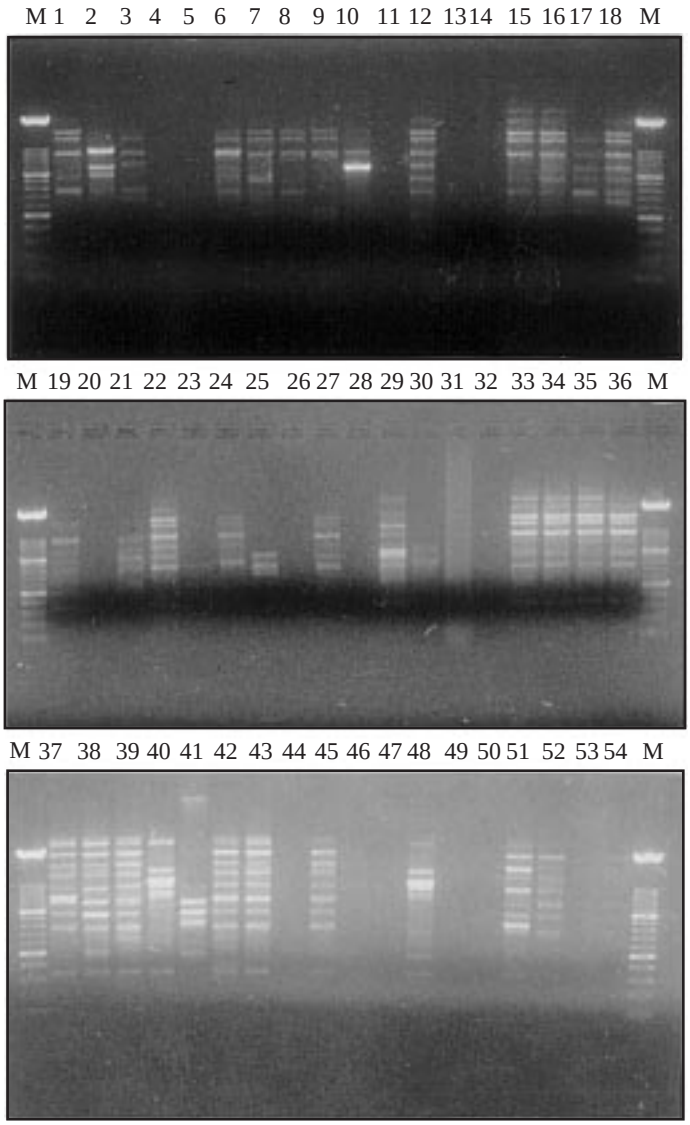

Figure 3. Agarose-gel (1.5%) electrophoresis and staining with ethidium-bromide of the RAPD-amplified fragments from genomic DNA of the 54 *P. griseola* isolates by using the primer OPL17. Lanes 1 to 54 correspond to the numbers assigned to the *P. griseola* isolates (Table 1). Lanes M: Molecular weight size marker (100 bp ladder).

Furtek, 1994) and for *Magnaporthe poae* (Huff et al., 1994) to be determined. By a similar approach, Sicard et al. (1997) were able to track migrations of *Colletotrichum lindemuthianum* in South America. Such an interpretation of our RAPD data is impossible due to the level of variability among the *P. griseola* isolates.

Different mechanisms, including single mutations, migrations and sexual recombination, lead to the appearance of diversity within pathogen populations (Burdon and Roelfs, 1985; Leung et al., 1993; Drenth et al., 1996). Similar RAPD patterns found between isolates collected from different geographical origins suggest that migration could have occurred between these locations, but further sampling would be necessary to confirm this hypothesis.

For a pathogen without known sexual reproduction, as in the case of *P. griseola*, the observed diversity is unlikely to be explained by sexual recombination.

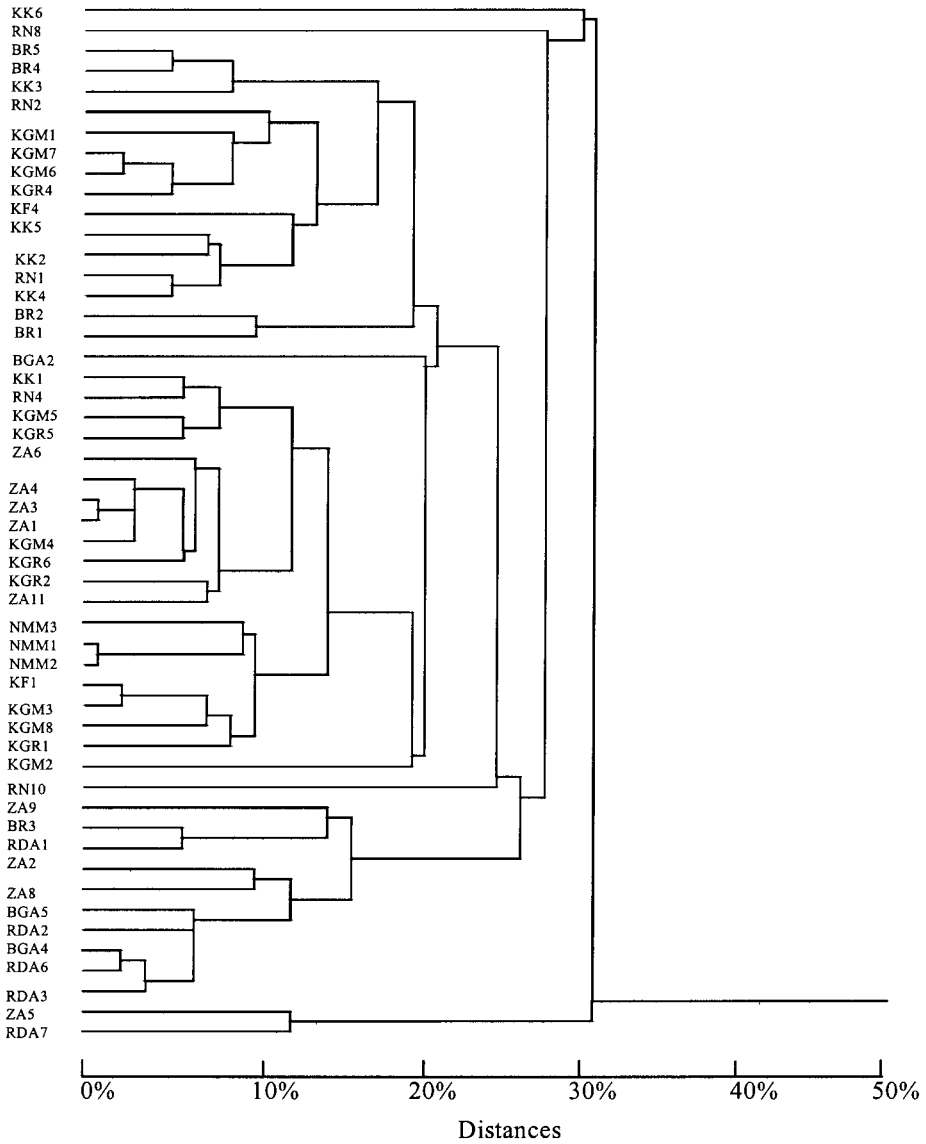

Figure 4. Dendrogram of 54 *P. griseola* single-spore isolates based on their respective patterns generated by RAPD amplifications with 5 primers (OPK7, OPK9, OPK10, OPL17 and OPL18). Distance between 2 isolates indicates the percentage of unshared amplified fragments.

However, single mutations, some chromosomal aberrations like deletions, translations and chromosomal losses (Kistler and Miao, 1992) and the presence of transposons (Kempken and Kück, 1998) may cause increased variability in fungi.

Our results have important implications regarding breeding for ALS resistance. It is not possible to identify dominant virulence structures in the African Great Lakes Region. In the presence of such a pathogenic variation, monocultures of cultivars with a single specific resistance gene would quickly select for corresponding virulence (Tapsoba, 1996). Complementary analysis might allow understanding some epidemiological aspects like the population dynamics which is necessary for defining the resistance management by genes rotation or mixtures of varieties.

Table 4. Average of molecular similarity (%) according to RAPD patterns within and between groups of isolates collected in Burundi

Isolate Groups	KGM	KGR	NMM	KK	BGA
KGM	54				
KGR	31	47			
NMM	31	25	79		
KK	39	26	22	61	
BGA	20	18	24	25	76

*Letters abbreviate the isolates origin.
KGM: Kayanza–Gatara–Muhingira.
KGR: Kayanza–Gatara–Rubagabaga.
NMM: Ngozi–Mwumba–Murama.
KK: Kayanza–Kabuye.
BGA: Burundi–Gitega.

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