Characterizing the *Sphaceloma* Fungus, Causal Agent of Superelongation Disease in Cassava

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ABSTRACT

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The fungus *Sphaceloma manihoticola* causes superelongation disease in cassava, a starchy root crop grown widely in the tropics. Isolates were collected from infected plants grown in six localities of Colombia. Morphological analyses of the fungus showed that colony growth and color are not stable characteristics over time. Pathogenicity studies, using the susceptible cassava variety M Col 22 and the resistant M Ven 77, showed that M Col 22 was tolerant of 29% of pathogen isolates studied and had an intermediate reaction to 71%. Variety M Ven 77 also showed tolerance of 16.2% of the isolates, had an intermediate reaction to 80.6%, and was susceptible to 3.2%. Significant cultivar $\times$ isolate interactions indicated pathogenic specialization. This study is the first to describe this pathogen's molecular characteristics. A homogeneous and reproducible band of about 545 bp was obtained with polymerase chain reaction which, when digested by restriction enzymes, showed an equal pattern of bands for all isolates. The isolates thus belonged to one species. Random amplified polymorphic DNA analysis revealed intraspecific genetic diversity. By better understanding the pathogen, we can apply more appropriate disease management strategies, such as selection of germ plasm tolerant of superelongation disease.

For thousands of years, cassava (*Manihot esculenta* Crantz) has been a basic agricultural crop in the tropics. Archaeological evidence indicates that it was cultivated in Peru about 4,000 years ago and was one of the first crops to be domesticated in America (6). Cassava is cultivated mainly for human consumption, but it is also important as animal feed and has significant industrial use (5). The world production of cassava rose from 70 million tons in 1960 (4) to 159 million tons in 1998, the total harvested area being 16 million hectares. Countries with the highest cassava production are Nigeria, Brazil, Zaire, Thailand, and Indonesia (7).

In Colombia, cassava is cultivated in almost all departments, from sea level to 2,000 meters. About 38% of commercial production is found in the seven departments of northern Colombia that together make up the "North Coast," as the region is locally known. Other significant produc-

tion is found in the hilly Andean region (15). The fungus *Sphaceloma manihoticola*, the causal agent of superelongation disease in cassava, was described for the first time in 1950 by Bitancourt and Jenkins (2), who emphasized host symptomatology and pathogen morphology. In Colombia, the disease was reported for the first time during outbreaks in 1972 (3). In 1976, Krausz (9) confirmed that *S. manihoticola* causes superelongation. Typical symptoms of the disease are an exaggerated lengthening of internodes in young stalks and petioles, caused by the hormone gibberellin A₄ (18); a deformation or involution of young leaves; and the presence of cankers in stalks, petioles, and leaf blades (3). Superelongation is considered a major disease of the crop because it affects stake (i.e., planting material) quality and, when severe, drastically reduces yields. The causal organism is easily dispersed by wind and water and disseminated through the use of infected stakes as seed. Such easy dispersion and the possible existence of physiological races or biotypes of the fungus are two aspects that hinder management of the disease (16). Previous studies have demonstrated variability of morphology and pathogenicity in the fungus (17), but its genetics are still unknown.

The objectives of the study were to collect isolates of the fungus from plants affected by superelongation disease, determine the variability of morphology and pathogenicity of these isolates, and to conduct a preliminary, molecular characteri-

zation of the fungus to determine its genetic diversity.

MATERIALS AND METHODS

Collecting isolates of *Sphaceloma manihoticola*. *Collecting samples.* Samples were collected from plants affected by superelongation disease and showing symptoms on leaves, petioles, and stems. The isolates came from six localities in Colombia, according to three edaphoclimatic zones: Zone I = Carmen de Bolívar (in the Department of Bolívar); Zone II = Carimagua, Puerto López, Granada, and Villavicencio (in the Department of Meta); and a transitional zone = Santander de Quilichao (in the Department of Cauca), which corresponds to an area between Zone II and another zone (V) (Table 1).

Isolating the pathogen. The pathogen was isolated from cankers that were removed from the plant with a sterile scalpel and disinfested with undiluted household bleach (5% sodium hypochlorite) for 60 to 90 s. They were then rinsed with sterile distilled water for 30 s and dried with sterile absorbent tissue. The disinfested samples were placed on fresh potato dextrose agar (PDA) medium, prepared as follows: 200 g of fresh potatoes were washed, diced, and cooked for 10 min in 300 ml of distilled water. The potatoes and liquid were pureed and mixed with an additional 700 ml of distilled water, 17 g of dextrose and 18 g of agar. The mixture was autoclaved for 30 min and supplemented with 1 ml of 100-ppm streptomycin sulfate and 1 ml 25% lactic acid. The plates were incubated for about 15 days at 26°C in the dark. To decontaminate the isolates, a disk of the fungus was put into a solution containing sterile distilled water, 2 drops of 25% lactic acid, and 0.1 ml of 100-ppm streptomycin sulfate. Subsequently, they were decanted, rinsed three times with sterile distilled water, and dried with sterile absorbent tissue. They were then placed on PDA and incubated at 26°C in the dark.

Acquiring monoonidial isolates. For each isolate, a suspension of conidia was prepared to a concentration of 5×10^1 conidia per ml. The suspensions were placed on 2% water agar and incubated at 26°C for 24 to 36 h in the dark. Subsequently, with the help of a microscopic stereoscope, individual germinated conidia were collected with a fine inoculating needle, placed on PDA, and incubated again at 26°C in the dark for about 25 days. Isolates

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were transferred to additional plates containing PDA.

Conserving the isolates. The isolates were maintained in petri dishes on PDA and transferred to new media approximately every 2 weeks. To store the isolates for long periods, we placed them in test tubes on PDA medium and covered them with sterile mineral oil.

Morphological characterization. Monoclonal isolates that were 15 to 20 days old were used. A 5-mm-diameter disk of each fungus was obtained with a hollow punch and placed on PDA. Two replicates per isolate were made, and observations were taken 7, 14, and 21 days after placing on the medium. The morphological characteristics evaluated were: (i) colony growth (length and width), (ii) presence or absence of aerial mycelium, (iii) colony color, and (iv) conidial size (mean of 50 measurements taken per isolate). The morphological characteristics were analyzed by cluster analysis, using Ward's technique, based on minimum variance.

Pathogenicity analysis. The fungus was inoculated onto plants in a growth chamber under controlled conditions. Of the collection, 31 isolates were evaluated in two cassava varieties, the resistant M Ven 77 and the susceptible M Col 22. A randomized complete block design was used, with 10 replicates per isolate per variety and a control inoculated with sterile distilled

water and Tween 20. Pathogenicity was rated according to a scale of 1 to 5, where 1 = plants without visible symptoms, 2 = some spots or cankers on leaves or petioles, 3 = cankers on petioles and stems, severe leaf deformation, 4 = elongation, cankers on leaves, petioles, and stems, and severe leaf deformation with scald, and 5 = dieback after severe lengthening.

In the greenhouse, 10-cm-long stakes were planted in pots; the stakes had been cleaned of fungi by immersion for 5 min in a solution of 0.2% Sistemin (dimethoate), 0.6% Captan, and 0.6% Benlate (benomyl). They were left to dry, then planted, and the aerial extreme of each stake was covered with paraffin. The stakes were planted in a mixture of soil and sand (2:1) and sterilized by steam. Six days after planting, to ensure cleanliness, the stakes received a further application of a solution of 0.2% Sistemin and 0.3% Coljap (N = 30%, P = 3%, and K = 5%). To prepare the inoculum, each of 10 samples of each isolate was placed in a petri dish containing PDA medium and incubated for 21 to 28 days at 26°C in the dark. The inoculum was obtained by suspending the fungus in sterile distilled water with 5 drops of Tween 20 and adjusting the concentration to 3×10^6 conidia per ml. Each stalk to be inoculated was cleaned with a cotton-tipped applicator and dampened with sterile water to remove the waxy layer for easier fungal penetra-

tion. The entire stalk was then inoculated by spraying with a hand atomizer and placed in a growth chamber with temperatures ranging from 26 to 28°C. The relative humidity (RH) was kept between 98 and 100% for 48 h, and afterwards between 50 and 80%. The plants were evaluated 15 days after inoculation.

Molecular characterization of *Sphaceloma manihoticola*. Tests for molecular characterization were done according to the protocols of Lee and Taylor (11). The sizes of bands were obtained according to the ONE Discan 1.0 program (Dimensional Gel Analysis) 1994-1995.

Extracting DNA. The isolates were placed in liquid medium obtained by filtering V8 juice and incubated, under constant agitation, for 15 days at temperatures between 26 and 28°C in the dark. The mycelium was collected and filtered through filter paper Whatman no. 1 under vacuum, was lyophilized (80°C in vacuum) for about 2 h, then macerated with liquid nitrogen to obtain a fine and dry powder. A sample of 0.1 to 0.3 g of macerated mycelium was taken, to which was added 400 µl of extraction buffer (50 mM Tris-HCl, pH 7.2; 50 mM EDTA; sodium dodecyl sulfate at 3%; and 2-mercaptoethanol at 1%). The solution was mixed with vortex action until a homogeneous consistency was obtained. Subsequently, the suspension was incubated at 65°C for 1 h. A solution (400 µl) comprising phenol, chloroform, and isoamyl alcohol was added at a ratio of 2:2:1, and the mixture was gently inverted until a homogeneous consistency was obtained. The mixture was then centrifuged for 15 min at 10,000 rpm, and the supernatant was recovered, taking care not to take any of the interphase liquid. Ten µl of 3M sodium acetate and 0.54 volumes of isopropanol were then added, mixed by inverting, and left to precipitate for 12 to 14 h at -20°C. The mixture was centrifuged again for 5 min at 10,000 rpm, and the supernatant was decanted.

The resulting pellet was washed with 70% ethanol, centrifuged for 5 min at 10,000 rpm, and the supernatant discarded. The tubes were then left inverted over sterile paper for 15 min and dried in a vacuum bell for 1 h. The DNA obtained was resuspended in 100 µl of TE buffer (0.1 mM Tris-HCl, 10 mM EDTA), 1 µl of RNase was added, and the mixture was incubated for 30 min at 37°C. The concentration of DNA was determined with a fluorometer (Hoefer DyNA Quant 200; Fluorometer: Hoefer Scientific Instruments, San Francisco, CA), diluted in filtered (0.2 µm) sterile, bi-distillate water to a concentration of 5 ng/ml, and stored at -20°C.

Amplifying rDNA through PCR. The following reagents were used: a buffer of 10× enzyme (50 mM KCl; 10 mM Tris-HCl, pH 8.3; triton 0.1%), 0.2 mM dNTPS (dATP, dCTP, dGTP, dTTP), 0.5 mM each

Table 1. Origin of *Sphaceloma manihoticola* isolates used in the study. The fungus is the causal agent of superelongation disease in cassava (*Manihot esculenta* Crantz)

Isolate	Cassava variety	Plant part	Origin ^a
0001	M Pse 009	Stem	Carimagua
0010	CM 523-7	Petiole	Carimagua
0011	CM 523-7	Leaf	Carimagua
0016	CM 6633-6	Petiole	Puerto López
0017	CM 6633-6	Leaf	Puerto López
0018	M Bra 97	Petiole	Puerto López
0020	M Bra 97	Petiole	Granada
0021	M Bra 97	Leaf	Granada
0022	CM 7274-1	Petiole	Granada
0024	M Pse 002	Stem	Carimagua
0026	M Pse 002	Petiole	Carimagua
0033	M Bra 237	Stem	Carimagua
0035	M Bra 237	Leaf	Carimagua
0037	M Col 1795	Petiole	Carimagua
0040	M Pse 004	Leaf	Carimagua
0041	M Pse 004	Petiole	Carimagua
0043	CM 1355-2	Petiole	Carimagua
0046	M Bra 458	Leaf	Carimagua
0047	M Bra 458	Petiole	Carimagua
0050	M Bra 590	Petiole	Carimagua
0051	M Ind 33	Stem	Carimagua
0053	M Ind 33	Petiole	Villavicencio
0079	M Col 3306-19	Petiole	Carmen de Bolívar, Bolívar
0082	M Col 1505	Petiole	Santander de Quilichao, Cauca
0088	CM 4365-3	Leaf	Santander de Quilichao, Cauca
0090	M Col 1505	Petiole	Santander de Quilichao, Cauca
0101	Unknown	Stem	Villavicencio
0102	Unknown	Petiole	Villavicencio
0114	M Nga 1	Petiole	Villavicencio
0136	M Ph 14	Leaf	Villavicencio
0168	CG 402-11	Petiole	Villavicencio
1123CP	M Pse 003	Leaf	Carimagua
983CP	M Pse 010	Petiole	Carimagua

^a In the Department of Meta, except where indicated.

primer (ITS4 and ITS5), 3 mM magnesium chloride, and 2 µl of DNA for each sample. Just before mixing the DNA with the cocktail, 0.625 units of *Taq* polymerase enzyme was added and the volume completed with filtered (0.2 µm) sterile, bi-distillate water to a final volume of 25 µl. As negative control, we used the mixture of reagents, replacing the DNA with filtered (0.2 µm) sterile, bi-distillate water. The samples were covered with 72 µl of sterile mineral oil, and the plate was covered with Vinilpel paper. The plate in which the reaction was to be carried out was placed in a thermocycler PTC-100 (MJ Research, Inc., Watertown, MA), and the following program was set:

1. 95°C for 3 min
2. 57°C for 30 s
3. 72°C for 2 min

4. 95°C for 30 s
5. 24 cycles of steps 2 to 4
6. 50°C for 30 s
7. 72°C for 10 min
8. 4°C

The amplified segments were analyzed by electrophoresis in a gel comprising 1.5% agar, a buffer of TBE 1× (trizma-base, boric acid, EDTA, pH 8.0), and ethidium bromide at 10 mg/ml. The electrophoresis chamber was maintained at a constant 58 V for 2 h and 30 min. A marker of 100 bp was included in each gel.

Restriction enzymes. For the analysis with restriction enzymes, a sample of 15 µl of the product from the PCR reaction was taken, and 2 µl of a buffer of 10× enzyme and 1 µl of restriction enzyme was added. The suspension was incubated for 16 h at an average temperature of 37°C, after

which 3 µl of running buffer was added. A gel comprising 1.4% agar, a buffer of TBE 1×, and ethidium bromide at 10 mg/ml was then placed in an electrophoresis chamber for 4 h at a constant 70 V. In each gel, a marker of known molecular weight (1 kb of ladder) was included.

Polymorphism of random amplified DNA (RAPD). Each sample consisted of 2.5 µl of a buffer of the enzyme, 2.5 µl of dNTPs of 0.2 mM (dATP, dCTP, dGTP, dTTP), 1.2 µl of the primer at a concentration of 0.5 mM, 2.5 µl of magnesium chloride at 2.5 mM, and 4 µl of rDNA. Just before mixing the DNA with the cocktail, 0.625 units of *Taq* polymerase was added and raised to a final volume of 25 µl with filtered (0.2 µm) sterile, bi-distillate water. As negative control, we used the mixture of reagents, and the rDNA was replaced by filtered (0.2 µm) bi-distilled water. The samples were covered with 72 µl of sterile mineral oil, and the plate was covered with Vinilpel paper. The plate was then placed in a thermocycler and the following program set:

1. 95°C for 3 min
2. 57°C for 30 s
3. 72°C for 2 min
4. 95°C for 30 s
5. 44 cycles of steps 2 to 4
6. 50°C for 30 s
7. 72°C for 10 min
8. 4°C

The amplified segments were observed in a gel comprising a 1.4% agar, a buffer of TBE 1×, and ethidium bromide at 10 mg/ml. The gel was placed in an electrophoresis chamber at a constant 70 V for 4 h. In each gel, a marker of known molecular weight (1 kb of ladder) was included. The analysis was performed using NTSYS-pc 1.8 (F. J. Rohlf, Exeter Software, New York) statistical package.

RESULTS

Isolation and characterization of *Sphaceloma manihoticola*. Thirty-three isolates were obtained from cassava plants affected by superelongation disease (Table 1). Colonies of all isolates grew slowly (Table 2). A new colony began as a gelatinous or mucous mass that established itself

Table 2. Linear growth, morphology, and pathogenicity of 33 isolates of *Sphaceloma manihoticola* to two varieties of cassava (*Manihot esculenta*)

Isolate	Growth (cm) ^a		Morphology		Pathogenicity ^d	
	Length	Width	Colony color ^b	Aerial mycelium ^c	M Col 22	M Ven 77
0001	1.4	1.2	Y-TBr-DR	+	2.5	2.0
0010	2.4	2.0	Y-TBr	+	3.0	3.0
0011	2.3	2.0	Y-TG	–	2.5	2.5
0016	2.8	1.4	Y-Br-G	+	2.0	2.5
0017	1.3	1.3	Y-TBr	–	1.5	3.0
0018	2.0	1.9	Y-Br-TR	+	3.0	2.5
0020	1.3	1.1	Y	–	2.5	2.0
0021	1.6	1.4	Y-TRo-DR	+	3.0	3.0
0022	2.7	2.2	Y-TBr	+	3.0	3.0
0024	2.4	2.0	Y-TG	+	3.0	3.0
0026	2.8	2.2	Y-BS	+	3.0	3.0
0033	2.8	1.8	BY-BS	–	2.0	3.0
0035	2.0	2.0	Y	+	2.5	2.5
0037	2.6	2.3	Y-Ro-Tro	+	3.0	3.5
0040	2.4	2.0	Y-DR	+	2.5	3.0
0041	3.0	2.5	Y-Wmy	+	1.5	2.0
0043	1.7	1.6	Y-TBr-Br-DR	+	2.5	2.0
0046	2.1	2.0	Y	+	2.5	2.5
0047	2.8	2.6	Y-GM	+	3.0	2.5
0050	2.0	1.7	Y-G-TBr	+	2.5	2.5
0051	2.5	1.8	Y-DR-Br	+	2.0	3.0
0053	1.0	0.8	Tbe	–	2.0	2.0
0079	1.9	1.9	B	+	2.0	2.5
0082	2.4	2.0	Y-GM	–	2.5	3.0
0088	1.7	1.4	Y	+	ND ^e	ND
0090	0.8	0.8	Y	+	2.0	3.0
0101	2.1	1.8	Y-DR-Br	+	2.5	3.0
0102	2.6	2.4	TY	–	3.0	3.0
0114	1.9	1.6	TBr	+	2.5	2.5
0136	2.1	1.9	Y-TBr	–	ND	ND
0168	1.6	1.5	BY	+	2.0	3.0
1123CP	1.6	1.4	Y	+	3.0	3.5
983CP	3.3	2.2	Y-G	–	2.5	4.0
Control	ND	ND	ND	ND	1.0	1.0
DMS 5%	ND	ND	ND	ND	0.621	0.621

^a 21 days after placing fungus on medium.

^b Color codes for fungal colonies: B = black, Br = brown, BS = black spots, BY = bright yellow, DR = dark red, TBr = different tones of brown, TG = different tones of green, TBe = different tones of beige, TR = different tones of red, TRo = different tones of rose, TY = different tones of yellow, DY = tan, G = green, GM = green (margins only), WMy = white (mycelium only), Ro = rose-colored, Y = yellow.

^c + = presence of aerial mycelium; – = absence of mycelium.

^d Average score for damage, rated on a scale where 1 = plants without visible symptoms, 2 = some spots or cankers on leaves or petioles, 3 = cankers on petioles and stems, severe leaf deformation, 4 = elongation, cankers on leaves, petioles, and stems, severe leaf deformation with scald, and 5 = dieback after severe lengthening.

^e Not determined.

Table 3. Analysis of variance for pathogenicity evaluated with a scale of damage between 1 and 9 for 31 isolates of *Sphaceloma manihoticola* obtained from Colombia^a

Source	df	Pathogenicity (mean square) ^b
Replicate	9	0.88 NS
Variety	1	4.08**
Isolates	31	11.76**
Variety × isolate	31	1.34**
CV (%)	31.33	

^a Treatments were arranged in a randomized complete block design with 10 replicates.

^b ** = $P \leq 0.01$; NS = no significant difference.

on the medium and then developed as an extension of the mother colony. A colony's growth, both lengthwise and in width, was irregular and highly variable, whether among replicates of a single isolate or among different isolates. Aerial mycelium appeared in a colony between 21 and 28 days after placement on the medium (Table 2). Isolates 0011, 0033, and 983CP of Carimagua (Meta); 0017 of Puerto López (Meta); 0020 of Granada (Meta); 0082 of Santander de Quilichao (Cauca); and 0053, 0102, and 0136 of Villavicencio (Meta) were late in exhibiting aerial mycelium (at about 28 days). Sometimes the mycelium appeared uniform across the entire colony, and sometimes it appeared only in isolated parts and in different colors: black, brown, yellow, red, green, beige, rose, tan, or white (Table 2). A colony often had several colors. The usual color was yellow, except in colonies of isolates 0079 of Carmen de Bolívar (Bolívar) and 0053 and 0114 of Villavicencio, which were black, beige, and brown, respectively. Areas of new growth changed color as a colony matured. The conidia of all isolates were elliptical and refractive, embedded in a gelatinous matrix (1), with mean dimensions of $5.7 \times 3.2 \mu\text{m}$.

The high variability did not permit differentiation of isolates into morphological groups. No significant differences were observed among isolates. Isolates 0022 from Granada; 0024, 0040, 983CP, and 0050 from Carimagua; and 0168 from Villavicencio lost viability over time and could not be recovered.

Pathogenicity. Symptoms include necrotic leaf spots; cankers on leaf veins, petioles, and stems; leaf and stem distortion; and internode elongation of severely affected plants. The reaction of 31 isolates to the susceptible cultivar M Col 22 ranged from 1.5 to 3.0, and to the resistant cultivar M Ven 77 from 2.0 to 4.0 (Table 2). Cultivar M Col 22 was also tolerant of 29% of the pathogen population studied and

showed an intermediate reaction to the remaining 71% of that population. Cultivar M Ven 77 showed a tolerant reaction (score of 2) to 16.2% of isolates, an intermediate reaction (score of 3) to 80.6%, and a susceptible reaction (score >3) to 3.2%.

For the variable "pathogenicity," the interactions between isolates, variety, and their origin was analyzed by analysis of variance (Tables 3 and 4). Significant differences in pathogenicity were found among the isolates from Carimagua, Granada, Villavicencio, and Puerto López (Meta). In contrast, the isolates from Santander de Quilichao (Cauca) did not show differences. These results indicate host cultivar-pathogen specificity.

Molecular characterization of *Sphaceloma manihoticola*. Amplification and restriction of rDNA. The internal transcribed region of rDNA was amplified with primers ITS4 and ITS5. For all the isolates, a homogeneous band of about 545 bp (Fig.

1) was observed. The product generated by the PCR technique was digested with the endonucleases *CfoI*, *MspI*, *HinfI*, and *MseI* and presented a pattern of similar bands for all isolates and for each enzyme (Fig. 2, Table 5).

Polymorphisms of random amplified DNA. Twenty-five primers were evaluated, of which OPN-18, OPA-03, and OPH-13 were selected because they showed reproducible bands in most of the isolates (Fig. 3). The analysis showed two genetic lineages: one is homogeneous, comprising mostly isolates from Meta (Fig. 4). Within this group, regional differences were found between Carimagua, Puerto López, Granada, and Villavicencio. The other lineage is heterogeneous and is formed by isolates from several departments. The isolates 0102 of Villavicencio (Meta) and 0090 of Santander de Quilichao (Cauca) were separated from these two lineages, possibly indicating other lineages. The results

Table 4. Analysis of variance for pathogenicity evaluated with a scale of damage between 1 and 9 for 31 isolates of *Sphaceloma manihoticola* obtained from different departments in Colombia^a

Source	df	Pathogenicity (mean square) ^b
Replicate	9	0.88 NS
Variety	1	4.08**
Geographic origin of isolates	6	47.06**
Carimagua, Meta	16	3.80**
Puerto López, Meta	2	2.12*
Granada, Meta	2	3.76**
Villavicencio, Meta	4	2.13**
Carmen de Bolívar, Bolívar	0	...
Santander de Quilichao, Cauca	1	1.40 NS
Control isolates	0	...
Variety × origin of isolates	6	0.84 NS
Variety × Carimagua, Meta	16	1.46**
Variety × Puerto López, Meta	2	4.36**
Variety × Granada, Meta	2	0.42 NS
Variety × Villavicencio, Meta	4	0.54 NS
Variety × Carmen de Bolívar, Bolívar	0	...
Variety × Santander de Quilichao, Cauca	1	1.056 NS
Variety × control isolates	0	...
CV (%)	31.34	

^a Treatments were arranged in a randomized complete block design with 10 replicates.

^b ** = $P \leq 0.01$; * = $P \leq 0.05$; NS = no significant difference.

Fig. 1. Amplification of the ITS4 and ITS5 regions of the ribosomal DNA of the fungus *Sphaceloma manihoticola*. M = marker 100 bp. Lanes 1 to 11 = isolates from Carimagua, Puerto López, Granada, Villavicencio (Meta), Carmen de Bolívar (Bolívar), and Santander de Quilichao (Cauca).

Fig. 2. Electrophoresis of fragments of ITS taken from ribosomal DNA of the fungus *Sphaceloma manihoticola* and digested by the endonuclease *HinfI*. Isolate origin: lanes 1 to 13 = isolates from Carimagua (Meta), lanes 14 and 15 = isolates from Puerto López, lanes 16 and 17 = isolates from Granada (Meta), lanes 18 and 19 = isolates from Villavicencio (Meta), M = marker (1 kb).

showed that polymorphism exists among the isolates, revealing genetic diversity in the pathogen (13). Isolates with equal indexes of similarity came from different localities, suggesting dissemination of planting materials among the localities and therefore migration of the pathogen and an exchange of genes (12). No correlation was

found between morphology and pathogenicity of the fungus, nor between interaction and genetic diversity.

DISCUSSION

The characterization of a set of isolates that formed a population of the fungus *S. manihoticola*, the causal agent of supere-

longation disease in cassava, showed the fungus's morphological, pathogenic, and genetic variability. The morphological characteristics of the isolates evaluated were not sufficiently distinctive to differentiate groups. One reason was that those characteristics were too variable and unstable. The second reason was that conidium size was quite uniform among isolates. These findings corroborate those of other authors, who suggest that any changes found may have been induced by environmental factors (9,10,19). Pathogenicity analyses demonstrated a wide range of host-pathogen interaction, a common phenomenon in heterozygotic crops such as cassava that have polygenic resistance to disease (8,19). It should be emphasized that the two cassava varieties studied responded with an intermediate reaction to most isolates. Such response may be a result of the crop's asexual reproduction, whereby clones are generated with a spectrum of mild reaction (2). Therefore, the genetically variable hosts probably could maintain a genetically variable population of the pathogen (8).

This study is the first to describe this pathogen's molecular characteristics. Thirty-one isolates were identified, all belonging to one species and demonstrating wide genetic diversity. It is possible that this variability was induced by selection of the host. The pathogen has had to continually adapt to the changes in the environment in which it lives, such as the introduction of resistant varieties, fertilizers, crop rotation, and fungicides (2). The latter is realized by conidia being trans-

Table 5. Sizes of fragments amplified with primers ITS4 and ITS5 and cut with restriction enzymes

Isolate	Restriction enzyme			
	<i>CfoI</i>	<i>MspI</i>	<i>MseI</i>	<i>HinfI</i>
0001	270, 195 ^a	212, 140, 125	185, 105	351, 296
0010	269, 195	212, 141, 125	185, 109	355, 295
0011	269, 194	212, 141, 125	186, 110	354, 294
0026	269, 194	212, 142, 126	186, 111	353, 298
0033	268, 193	212, 142, 127	186, 109	347, 292
0035	268, 193	212, 143, 128	185, 110	350, 295
0037	268, 192	213, 143, 128	185, 111	354, 294
0041	267, 192	213, 144, 129	185, 112	357, 298
0043	267, 192	213, 143, 127	185, 110	352, 296
0046	268, 193	212, 142, 127	184, 109	346, 291
0047	275, 196	214, 145, 130	185, 111	353, 298
0051	269, 195	214, 144, 129	183, 112	357, 296
1123cp	270, 194	214, 144, 129	184, 110	354, 294
0016	270, 193	214, 143, 128	182, 111	348, 293
0017	270, 193	214, 144, 129	185, 111	357, 296
0018	269, 195	213, 145, 130	183, 112	355, 295
0020	273, 195	214, 145, 130	185, 113	354, 298
0021	272, 194	213, 144, 130	184, 109	357, 296
0053	274, 195	214, 144, 130	185, 114	352, 296
0101	271, 196	214, 145, 131	185, 114	350, 295
0102	272, 194	213, 143, 127	185, 114	353, 298
0114	272, 193	214, 145, 130	185, 114	355, 295
0136	274, 195	213, 144, 129	186, 115	354, 298
0079	274, 195	214, 144, 129	185, 114	355, 295
0082	275, 196	214, 144, 129	185, 114	353, 298
0088	271, 195	213, 144, 129	186, 115	357, 296
0090	274, 195	213, 144, 127	186, 115	356, 198

^a Sizes in bp, according to Program ONE Discan 1.0.

Fig. 3. Band patterns of DNA extracted from the fungus *Sphaceloma manihoticola* and evaluated with primer OPA-03. Lanes 1 to 13 = isolates from Carimagua (Meta), lanes 14 and 15 = isolates from Puerto López, lanes 16 and 17 = isolates from Granada (Meta), lanes 18 to 22 = isolates from Villavicencio (Meta), lane 23 = isolates from Carmen de Bolívar (Bolívar), lanes 24 to 26 = isolates from Santander de Quilichao (Cauca), C = control, M = marker (1 kb).

Fig. 4. Phenetic tree of 27 *Sphaceloma manihoticola* isolates used in this study based on random amplified polymorphic DNA (RAPD) markers. The tree was generated using unweighted pair group method with arithmetic means clustering of a simple genetic distance matrix. The scale represents genetic similarity distance.

ported by rain and wind, thus causing migrations of pathogen populations and, hence, the movement of genes. The result is a movement of new genes and their incorporation into the pathogen's populations, creating a more homogeneous though diverse genotype (14).

This effect could explain why some localities showed more genetic homogeneity, even though they were the sources of the largest number of isolates (e.g., Carimagua and Villavicencio, both in Meta). Indeed, both of these areas contain high levels of potential inoculum throughout the year and are sites for research and exchange of planting material, activities which increase pathogen variability both in these localities and in those farming areas around them (8). These areas show both genetic diversity and genetic similarity among residing isolates of the pathogen. These results help

to better understand the pathogen, its mechanism of action, and its coevolution with its host (14). Such knowledge enables directed management strategies to increase productivity through resistance by developing plants to resist targeted populations of the pathogen rather than to fulfill a specific objective, thereby avoiding the potential economic imbalances that so often negatively affect the poorest farmers, who usually produce most of the cassava in a given cassava-producing region.

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