Output 7
Disease Resistance in Cassava

An important feature of the IP3 project relates to the integration of breeding, entomology, plant pathology and the development and use of tools from biotechnology. In spite of the "divisions" created by the project structure, those four scientific areas have maintained as much a close relationship as possible. In Output 7, the progress related to cassava diseases is summarized.

Activity 7.1. *Characterizing* Xanthomonas axonopodis *pv.* manihotis, *causal agent of bacterial blight in cassava, by AFLP*

Specific objectives

1. To characterize 100 Xam isolates from different regions of Colombia, Venezuela, Brazil, and Cuba to analyze genetic variability in the bacterium.

Materials and methods

Isolates. 85 isolates of *Xam* were used (Table 7.1), collected from different cassava varieties in Colombia, Brazil, Venezuela and Cuba. Most of the isolates were from the CIO collection held by the Biotechnology Unit at CIAT and ORSTOM, while others were obtained from the Cassava Pathology Laboratory at CIAT.

Isolating DNA. *Xam* isolates, conserved in 60% glycerol at –80°C, were cultured onto YDCA medium (5 g yeast extract, 15 g agar, 5 g dextrose, and 10 g sodium carbonate) and left to grow overnight. DNA was later extracted, following the protocol by Boucher et al. (1985). The DNA was then dissolved in 100 µL of TE buffer (Tris-EDTA pH 8.0), and its concentration determined in a spectrophotometer. DNA quality was checked in 0.8% agarose gel.

AFLP. A 1000-ng sample of DNA was digested with two restriction enzymes (*Eco*RI and *Mse*I). The digested fragments were ligated with their respective adapters. Later, 5 µL of the restriction-ligation reaction were amplified, followed by a selective amplification of those fragments that had the nucleotide sequence annexed during ligation. For such amplification, eight combinations of primers were evaluated with five isolates, of which EC/MA was selected.(Gibco-BRL, AFLP Analysis System for Microorganisms).

The amplified products were denatured at 95°C and separated in polyacrylamide gels at 4% (w/v) in 0.5X TBE buffer for electrophoresis (Fig 7.1 and 7.2).

Data analysis. To determine genetic variability among isolates, a phylogenetic tree was constructed, using the SAHN method and the tree option of NTSYS-pc 2.02 (FJ Rohlf, Exeter Software, New York).

Table 7.1. Description of isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of cassava bacterial blight, used in this study.

Isolate no.	Identification	Genotype	Source	Location	Isolate no.	Identification	Genotype	Source	Location
1	CIO 6	*Manihot* sp.		Brazil	44	CIO 353	SRT 1319	Leaf	Brazil
2	CIO 7	*M. esculenta*	Stem	Brazil	45	CIO 356	IAC 576-70	Leaf	Brazil
3	CIO 8	*M. esculenta*	Stem	Brazil	46	CIO 367	SRT 1363	Leaf	Brazil
4	CIO 29	M Pse-004 Sylv	Stem	Meta-Colombia	47	CIO 465	AM 244-17	Leaf	Atlántico-Col.
5	CIO 34	SM 593-8	Stem	Meta-Colombia	48	CIO 469	M Col 2215	Leaf	Atlántico-Col
6	CIO 63	Mona Blanca	Stem	Sincelejo-Col	49	CIO 485	Black -Stick	Leaf	San Andrés-Col
7	CIO 66	M Col 1505	Stem	Sincelejo-Col.	50	CIO 500	Cod 88	Exudate	Meta-Col
8	CIO 68	M Col 2215	Stem	Bolívar-Col.	51	CIO 507	Cod 702	Stem	Meta-Col
9	CIO 71	M Col 2215	Leaf	Bolívar-Col.	52	CIO 725		Leaf	Latipilla-Cuba
10	CIO 148	CM 7274-1	Exudate	Meta-Col.	53	CIO 762	Brava-nativa	Leaf	Vaupés-Col
11	CIO 184	Var. Paraguai	Stem	Brazil	54	CIO 764	Brava-nativa	Leaf	Vaupés-Col
12	CIO 187	M. Joliana	Stem	Brazil	55	CIO 806	M Col 2215	Leaf	Magdalena-Col
13	CIO 191	Cacho Toro	Stem	Venezuela	56	CIO 833	M Ven 25	Leaf	Magdalena-Col
14	CIO 192	Tres Brincos	Stem	Venezuela	57	CIO 841	SM 1791-40	Stem	Córdoba-Col
15	CIO 210	M Ven 77	Stem	Venezuela	58	CIO 849	CM 6119-5	Leaf	Córdoba-Col
16	CIO 212	Lancetilla Negra	Stem	Venezuela	59	CIO 854	SM 1411-5	Leaf	Córdoba-Col
17	CIO 214	SG 104-57	Stem	Venezuela	60	CIO 856	CG 1141-1	Leaf	Atlántico-Col
18	CIO 241	Caribe Medio	Stem	Venezuela	61	CIO 901	M Nga 2	Exudate	Brazil
19	CIO 243	Bonifacio	Stem	Venezuela	62	CIO 905	K45	Exudate	Brazil
20	CIO 249		Stem	Venezuela	63	CIO 909	K140	Exudate	Brazil
21	CIO 259	Paigua Negra		Venezuela	64	CIO 910	K9 P4	Stem	Cauca-Col
22	CIO 276	Tres Brincos		Venezuela	65	CIO 911	K9 P4 Parc 483 Pt 2	Leaf	Cauca-Col
23	CIO 277	Tres Brincos		Venezuela	66	CIO 954	K33	Leaf	Meta-Col
24	CIO 278	Tres Brincos		Venezuela	67	CIO 961		Leaf	Brazil
25	CIO 280	Tres Brincos		Venezuela	68	CIO 964	Yuca dulce	Leaf	Amazonas- Col
26	CIO 281	Paigua Negra		Venezuela	69	CIO 974	MCOL 2215 Parc 25 Pt 4	Leaf	Magdalena-Col
27	CIO 282	Paigua Negra		Venezuela	70	CIO 988	T8	Leaf	Magdalena-Col

28	CIO 283	Paigua Negra		Venezuela	71	CIO 1017		Leaf	Brazil
29	CIO 286	IAC 114-80	Leaf	Brazil	72	CIO 1070	M Bra 383	Stem	Valle-Col
30	CIO 288	IAC 44-82		Brazil	73	CIO 1072	CM 8491	Leaf	Valle-Col
31	CIO 289	IAC 105-88		Brazil	74	CIO 1074	CM 8491	Leaf	Valle-Col
32	CIO 330	Xingu	Leaf	Brazil	75	CIO 1238	SM 1794-2	Leaf	Meta-Col
33	CIO 333	Quro do Vale	Leaf	Brazil	76	CIO 1279	M Bra 383	Leaf	Quindío-Col
34	CIO 335	Mico	Leaf	Brazil	77	XAMJV VII	CM 6740-7	Leaf	Valle-Col
35	CIO 336	Fibra	Leaf	Brazil	78	XAMJV VIII	M Bra 383	Leaf	Valle-Col
36	CIO 337	IAC 44-82	Leaf	Brazil	79	XAMVM 1O	SM 1855-9	Leaf	Meta-Col
37	CIO 338	Fibra	Leaf	Brazil	80	XAMVM 1Y	SM 1642-13	Leaf	Meta-Col
38	CIO 339	IAC 12-829	Leaf	Brazil	81	XAMS-1	SM 1624-2	Leaf	Sincelejo-Col
39	CIO 340	IAC 89-87	Leaf	Brazil	82	XAMS-1B	SM 1624-2	Leaf	Sincelejo-Col
40	CIO 342	IAC 12-829	Leaf	Brazil	83	XAMVM 6	GM 221-38	Leaf	Meta-Col
41	CIO 343	Fibra	Leaf	Brazil	84	XAMVM 7	GM 223-70	Leaf	Meta-Col
42	CIO 346	IAC 144-86	Leaf	Brazil	85	XAMVM 8	GM 220-52	Leaf	Meta-Col
43	CIO 348	Taquari	Leaf	Brazil					

Results

On analyzing the genetic variability of 85 isolates of *Xam* through the AFLP technique, three groups could be distinguished (Figure 7.3). The first group clustered at a similarity level of 0.6, and is formed of isolates from different localities in Colombia. The second group clustered at 0.7, and comprises 81% of the Venezuelan isolates included in this study, and 4 Brazilian isolates. The third group clustered at 0.4, and is formed by most of the Brazilian isolates, 3 isolates from Venezuela, 1 from Cuba, and 3 from Colombia. In this group, clustering below the 0.4 similarity level also occurred, indicating great genetic variability within the Brazilian locations, possibly related to the also high level of genetic diversity observed for the host plant (Roa et al. 1997; Sánchez et al. 1999). When new pathogen strains are introduced into a given area, the genetic diversity already found within the pathogen population is increased, thereby favoring the development of new pathotypes (Restrepo 1999).

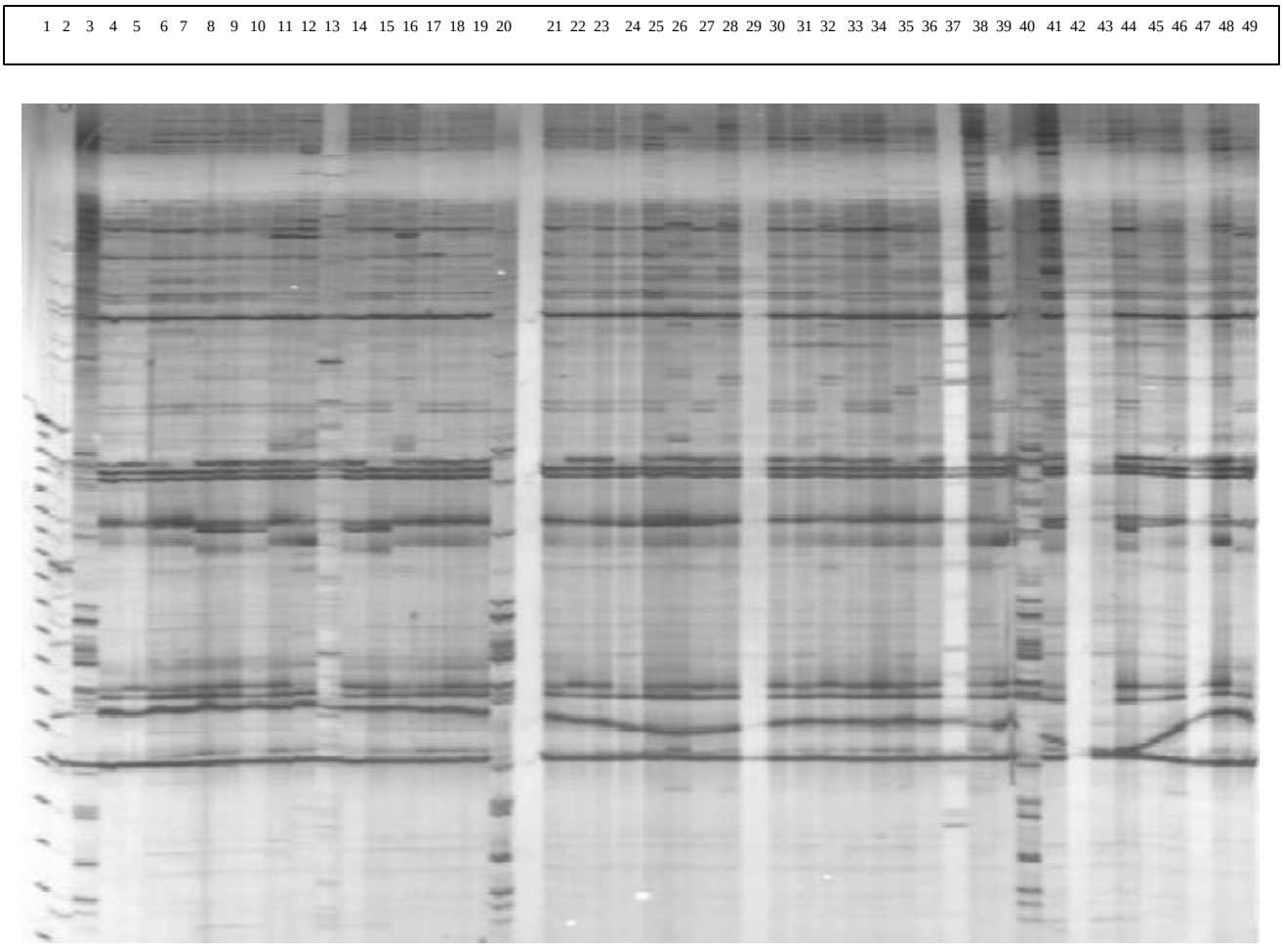

Figure 7.1. Patterns of AFLP for thirty-eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.

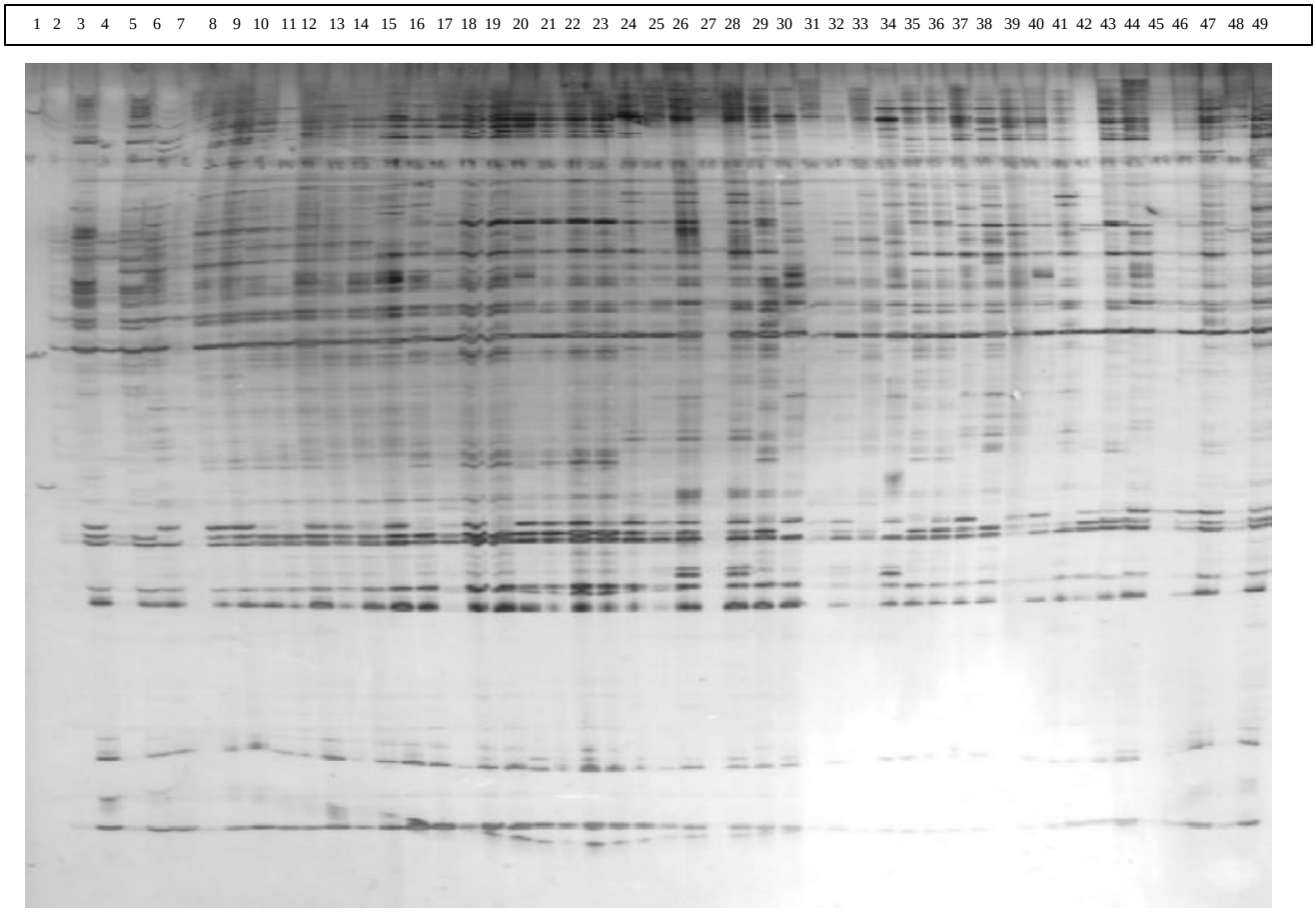

Figure 7.2. Patterns of AFLP for forty-eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.

Cluster analysis led to the formation of three groups of isolates that could be separated by country (i.e., Brazil, Colombia, and Venezuela). Within these large groups, subgroups can be found, based on different areas within the countries. The cluster of these *Xam* populations showed high variability—a significant finding, because a population with high genetic variability can adapt faster to antibiotics and resistant hosts. Several evolutionary factors can affect a population's genetic structure, including size and number of individuals, gene flow, and host selection (Mejía 2002).

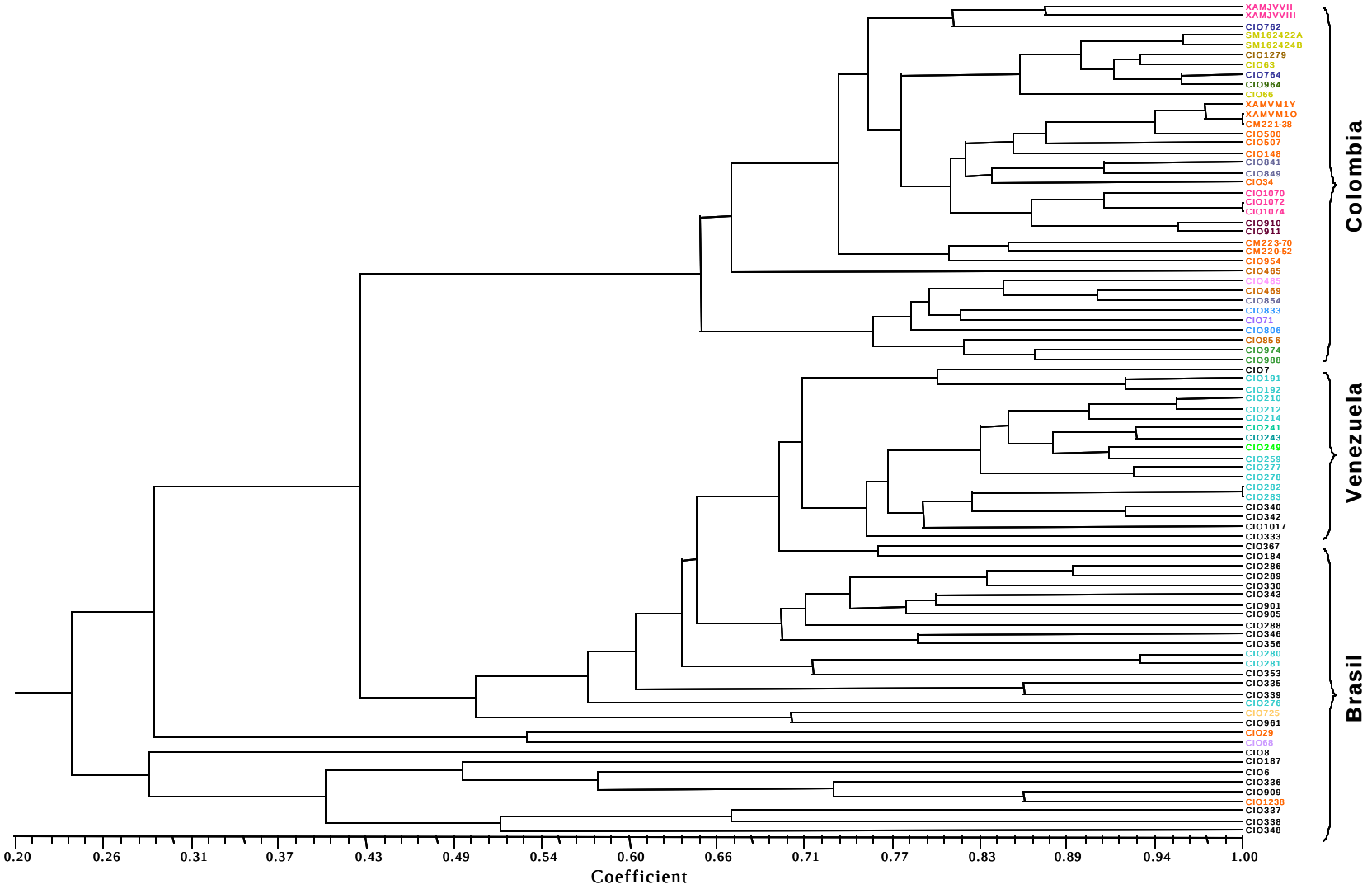

Figure 7.3. Similarity dendrogram of 85 isolates of *Xanthomonas axonopodis* pv. *manihotis*, based on AFLP analysis, using the unweighted pair-group method with arithmetic averaging (UPGMA) program of NTSYS-pc 2.02 (FJ Rohlf, Exeter Software, New York).

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Activity 7.2. Characterizing cassava genotypes for their reaction to cassava bacterial blight under greenhouse conditions, using different bacterial isolates

Specific objectives

1. *To obtain and screen different isolates of Xanthomonas axonopodis pv. manihotis (Xam), causal agent of CBB.*
2. *To analyze the reaction of different cassava genotypes to different isolates of Xam.*
3. *To better understand the host-pathogen interaction for CBB.*

Methodology

Thirty-six genotypes were characterized under greenhouse conditions for their reaction to six isolates of *Xam*, causal agent of CBB. Isolates were obtained from different cassava genotypes in two edaphoclimatic zones in Colombia (Table 7.2). In the greenhouse, the stems of 30-day-old cassava plants of each genotype were injected with a bacterial suspension of the isolates (1×10^6 cfu/mL). Disease severity was recorded at 12, 19, and 26 days after inoculation.

Results

The most aggressive isolates were S1B and VM8, with 94.4% and 91.7% virulence, respectively. Genotype 'Cravela', from Brazil, performed well, showing resistance to two isolates and intermediate resistance to four isolates. Genotypes M Esc Fla 036 and SM 1871-32 presented either intermediate or resistance reactions to 66.7% of the isolates. 'La Reina' (CM 6740-7) was susceptible to one isolate from Villavicencio and to both isolates from Sincelejo, and was also susceptible in the field at Villavicencio. 'Brasilera' (M Col 2737), susceptible in the field at Villavicencio, was also susceptible

in the greenhouse to three isolates from Villavicencio. 'Catumare' (CM 523-7) continued having adequate resistance to CBB in the field, although it was susceptible to three isolates in the greenhouse (Table 7.3).

Table 7.2. Origin in Colombia and cassava genotype source of *Xanthomonas axonopodis* pv. *manihotis* isolates (causal agent of cassava bacterial blight) used to evaluate disease resistance in cassava.

Isolate	Colombian municipality (department)	Genotype source
S1	Sincelejo (Sucre)	SM 1624-2
S1B	Sincelejo	SM 1624-2
VM5	Villavicencio (Meta)	GM 315-01
VM6	Villavicencio	GM 221-8
VM7	Villavicencio	GM 223-0
VM8	Villavicencio	GM 220-52

The correlation coefficient between the field and greenhouse at Villavicencio, based on four control genotypes widely distributed in the field, was 0.76 and 0.98 for isolates VM6 and VM8, respectively, indicating that these isolates could be highly frequent in the field, even though only a few genotypes were analyzed to infer the appropriate correlation.

Table 7.3. Disease reaction [a] of cassava genotypes to six isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of common bacterial blight.

Genotype	Isolate [b]						Total [c]			R + I (%) [d]
	S1	S1B	VM5	VM6	VM7	VM8	R	I	S	
CG 1141-1	5.0	4.5	4.5	4.5	4.5	4.5	0	0	6	0.0
CG 402-11	2.5	4.0	1.0	2.5	4.0	3.5	1	2	3	50.0
CM 2177-2	5.0	5.0	5.0	5.0	5.0	5.0	0	0	6	0.0
CM 3306-4	4.0	5.0	1.5	5.0	4.0	3.5	1	0	5	16.7
CM 3311-4	5.0	5.0	1.0	4.0	4.0	5.0	1	0	5	16.7
CM 523-7 (Catumare)	2.0	5.0	1.0	2.0	4.5	3.5	3	0	3	50.0
CM 6438-14	5.0	5.0	1.0	2.5	5.0	5.0	1	1	4	33.3
CM 6740-7 (La Reina)	3.5	4.0	1.0	2.0	2.0	4.0	3	0	3	50.0
Cravela	3.0	3.0	1.5	1.0	2.5	3.0	2	4	0	100.0
IM 175	5.0	5.0	1.0	4.0	3.5	4.0	1	0	5	16.7
Manipeba Tapioqueira	4.5	4.5	4.0	5.0	4.5	4.5	0	0	6	0.0
M Bra 1045	4.0	4.0	1.5	4.0	4.5	3.5	1	0	5	16.7
M Col 1468	4.0	4.5	3.5	3.5	4.5	4.5	0	0	6	0.0
M Col 1505	4.0	4.5	1.0	4.0	4.0	5.0	1	0	5	16.7
M Col 1522	5.0	5.0	5.0	5.0	4.5	4.5	0	0	6	0.0
M Col 2215	4.5	4.5	5.0	3.0	4.5	4.5	0	1	5	16.7
M Col 2737 (Brasilera)	4.0	5.0	1.0	4.5	4.5	4.5	1	0	5	16.7
M Esc Fla 036	2.5	4.0	1.0	2.5	3.5	3.0	1	3	2	66.7
M Esc Fla 048	3.5	4.5	1.0	1.5	4.0	3.0	2	1	3	50.0

Table 7.3 (cont.)

M Nga 2	5.0	4.5	4.5	4.5	4.5	5.0	0	0	6	0.0
M Nga 19	4.0	4.5	3.5	2.0	4.5	4.5	1	0	5	16.7
M Per 183	4.5	4.5	5.0	4.5	5.0	4.5	0	0	6	0.0
M Pse 004	3.5	4.0	1.5	2.5	3.5	4.5	1	1	4	33.3
M Pse 005	4.0	4.0	1.5	2.5	3.0	5.0	1	2	3	50.0
M Pse 007	3.5	4.5	1.0	2.5	3.5	3.5	1	1	4	33.3
SM 1144-4	5.0	4.5	5.0	4.5	5.0	4.5	0	0	6	0.0
SM 1210-4	3.0	5.0	2.0	3.0	4.0	5.0	1	2	3	50.0
SM 1225-12	4.5	4.5	4.5	4.5	5.0	5.0	0	0	6	0.0
SM 1345-10	4.5	4.5	3.0	4.0	4.5	4.5	0	1	5	16.7
SM 1411-5	5.0	4.5	3.0	5.0	5.0	5.0	0	1	5	16.7
SM 1460-1	4.5	4.5	3.5	3.5	4.5	4.5	0	0	6	0.0
SM 1479-8	4.5	4.5	4.5	3.5	4.5	5.0	0	0	6	0.0
SM 1555-17	4.5	4.5	3.5	4.5	4.5	4.5	0	0	6	0.0
SM 1871-32	4.0	1.5	1.0	2.5	2.5	4.0	2	2	2	66.7
SM 929-5	5.0	5.0	1.0	1.0	4.0	4.0	2	0	4	33.3
SM 985-9	4.5	4.5	4.0	5.0	4.5	5.0	0	0	6	0.0
Genotypes that were:										
Resistant	1	1	19	6	1	0				
Intermediate	4	1	2	9	3	3				
Susceptible	31	34	15	21	32	33				
Virulence (%)[e]	86.1	94.4	41.7	58.3	88.9	91.7				
Correlation[f]	-	-	0.19	0.76	-0.19	0.98				

a. Disease reaction on a scale of 1 to 5: R (resistant) = from 1.0 to 2.0; I (intermediate) = from 2.5 to 3.0; S (susceptible) = from 3.5 to 5.0.
b. For origin and source of isolates, see Table 7.2.
c. Total of isolates to which each genotype is either resistant (R), intermediate (I), or susceptible (S).
d. Percentage of isolates to which each genotype shows both resistance (R) and intermediate resistance (I).
e. Percentage of genotypes susceptible to each isolate.
f. Correlation between disease reaction of isolates from Villavicencio (VM5 to VM8) in the field and greenhouse. Correlation was carried out with control genotypes evaluated in the field at Villavicencio.

Activity 7.3. Characterizing cassava genotypes for their reaction to superelongation disease under greenhouse conditions, using different fungal isolates

Specific objectives

1. *To obtain and screen different isolates of the fungus Sphaceloma manihoticola, causal agent of superelongation disease (SED).*
2. *To analyze the reaction of different cassava genotypes to different isolates of S. manihoticola.*
3. *To better understand the host-pathogen interaction for SED.*

Methodology

Fifteen genotypes were characterized under greenhouse conditions for their reaction to five isolates of *S. manihoticola* that had been collected from different genotypes in Villavicencio and Brazil (Table 7.4). Plants, 35-40 days old, were inoculated by first cleaning petioles and stems with wet cotton wool to remove wax and facilitate fungal

penetration. They were then inoculated with a suspension of the fungus at 3×10^6 spores/mL. Inoculated plants were incubated for 10 days in a growth room at 30°C and 98% humidity, and evaluated at 9, 14, and 21 days after inoculation, on a 1-to-5 severity scale, where 1 indicated no symptoms and 5 death.

Table 7.4. Origin in Colombia and cassava genotype source of *Sphaceloma manihoticola* isolates (causal agent of superelongation disease) used to evaluate disease resistance in cassava.

Isolate	Colombian municipality (department)	Genotype source
SVM-1	Villavicencio (Meta)	CM 9901-4
SVM-2	Villavicencio	CM 8035-37
SVM-3	Villavicencio	CM 6438-14
SVM-4	Villavicencio	GM 227-68
B5	Brazil	M Bra 5

Results

Table 7.5. Disease reaction[a] of 15 cassava genotypes to five isolates of *Sphaceloma manihoticola*, causal agent of superelongation disease, under greenhouse conditions.

Genotype	Isolate[b]					Total[c]			R + I (%)[d]
	SVM-1	SVM-2	SVM-3	SVM-4	B 5	R	I	S	
CG 1141-1	3.0	3.0	3.0	3.5	2.5	0	4	1	80
CM 2177-2 (Cebucán)	3.0	2.5	1.0	2.0	2.5	2	3	0	100
CM 3306-4	3.0	2.0	3.5	3.5	3.0	1	2	2	60
CM 4919-1	3.0	2.0	2.0	3.0	2.5	2	3	0	100
CM 523-7 (Catumare)	3.0	1.5	3.0	3.5	3.0	1	3	1	80
CM 6055-3	2.5	3.5	3.0	3.0	2.5	0	4	1	80
CM 6438-14	3.5	3.0	3.5	3.5	2.5	0	2	3	40
CM 6740-7 (La Reina)	3.5	2.0	3.5	3.5	2.5	1	1	3	40
M Bra 1044	2.5	2.0	2.0	3.5	1.5	3	1	1	80
M Col 1505	2.5	2.0	2.0	2.5	1.5	3	2	0	100
M Nga 2	3.5	2.5	2.0	2.0	2.5	2	2	1	80
M Per 183	3.5	3.0	3.5	3.0	2.5	0	3	2	60
M Tai 8	3.0	2.5	3.5	2.5	3.0	0	4	1	80
M Ven 25	3.0	3.5	2.5	2.5	2.0	1	3	1	80
SM 1411-5	2.5	2.5	1.5	2.0	2.5	2	3	0	100
Genotypes that were:									
Resistant	0	6	6	3	3				
Intermediate	11	7	4	6	12				
Susceptible	4	2	5	6	0				
Virulence (%)[e]	26.7	13.3	33.3	40.0	0.0				

a. Disease reaction on a scale of 1 to 5: R (resistant) = from 1.0 to 2.0; I (intermediate) = from 2.5 to 3.0; S (susceptible) = from 3.5 to 5.0.
b. For origin and source of isolates, see Table 7.4.
c. Total of isolates to which each genotype is either resistant (R), intermediate (I), or susceptible (S).
d. Percentage of isolates to which each genotype shows both resistance (R) and intermediate resistance (I).
e. Percentage of genotypes susceptible to each isolate.

The most aggressive isolate was SVM-4, from Villavicencio, with 40% virulence, whereas the Brazilian isolate B5 was the least virulent. Eleven genotypes were either resistant or intermediate to 80% or more of the isolates. M Col 1505 was the most

resistant genotype, followed by CM 4919-1 and CM 2177-2 (resistant in the field). 'La Reina' (CM 6740-7, and susceptible in Villavicencio) was susceptible to three isolates in the greenhouse, and M Tai 8, susceptible in the field, was also susceptible to isolate SVM-3 in the greenhouse (Table 7.5).

Activity 7.4. Evaluating cassava genotypes for resistance to cassava bacterial blight, superelongation disease, and Phytophthora root rot in the Villavicencio, North Coast, and Quindío regions of Colombia

Specific objective

1. To evaluate the reaction of groups of genotypes to the three diseases.

Methodology: Villavicencio

We evaluated 244 cassava genotypes from a preliminary yield trial for their reactions to CBB and SED under natural disease pressure in Villavicencio, where several pathotypes of the causal agents are present. The experimental design comprised three replicates in a randomized block design, with plots of 12 plants each.

Results: Villavicencio

Eight genotypes presented partial resistance to both diseases, with scores under 2.5 in a severity scale from 1 to 5: CM 9460-34, CM 9460-38, CM 9461-10, CM 9463-2, CM 9463-10, CM 9464-26, SM 2632-17, and SM 2636-44 (Table 7.6). Two of the genotypes most planted by farmers in the zone, 'Brasilera' and 'La Reina', were susceptible to both diseases.

Table 7.6. Disease reaction[a] of cassava genotypes from a preliminary yield trial to cassava bacterial blight (CBB) and superelongation disease (SED), Villavicencio, Department of Meta, Colombia.

Genotype	CBB	SED	Genotype	CBB	SED	Genotype	CBB	SED
Brasilera	4.5	4.0	CM 9472-4	4.0	2.5	SM 2641-2	4.0	4.0
Catumare	3.0	3.0	CM 9472-7	3.0	3.5	SM 2641-7	3.0	2.5
HMC-1	4.5	4.0	CM 9483-4	3.0	2.5	SM 2641-9	3.5	3.0
La Reina	4.0	4.0	SM 1812-92	3.5	3.0	SM 2641-11	3.5	3.0
CM 8746-1	3.5	4.0	SM 2220-18	4.0	3.0	SM 2642-3	4.0	2.5
CM 8747-5	3.0	4.0	SM 2220-19	4.0	2.5	SM 2642-17	3.5	2.0
CM 9449-2	2.5	4.0	SM 2220-20	3.0	2.5	SM 2642-24	3.0	3.0
CM 9449-6	3.0	4.0	SM 2366-44	4.5	2.5	SM 2642-27	3.0	3.5
CM 9449-8	3.0	4.0	SM 2366-45	3.5	3.5	SM 2644-1	4.0	4.0
CM 9450-5	4.0	4.0	SM 2366-46	3.5	4.0	SM 2644-3	3.5	3.0
CM 9451-1	3.5	2.5	SM 2366-49	4.0	4.0	SM 2644-4	4.5	4.0
CM 9452-6	3.5	4.0	SM 2366-50	4.5	2.5	SM 2644-5	4.0	4.0
CM 9452-11	2.5	3.5	SM 2366-57	4.0	2.5	SM 2645-1	4.0	4.0
CM 9452-13	4.0	4.0	SM 2452-13	3.5	4.0	SM 2646-2	3.0	4.0
CM 9452-15	2.5	4.0	SM 2561-32	3.5	4.0	SM 2724-9	3.5	4.0

Table 7.6 (cont.)

CM 9456-26	3.5	3.5	SM 2592-14	4.0	3.0	SM 2724-15	4.0	4.0
CM 9456-40	3.5	3.0	SM 2593-21	4.0	2.5	SM 2724-18	4.0	3.0
CM 9459-1	4.5	2.5	SM 2594-16	4.0	4.0	SM 2726-4	4.0	3.0
CM 9459-2	3.0	3.5	SM 2599-25	3.5	4.0	SM 2726-17	3.5	2.5
CM 9459-6	3.5	3.0	SM 2599-41	4.0	3.0	SM 2727-1	4.0	3.0
CM 9459-10	3.5	3.0	SM 2599-49	3.5	3.5	SM 2727-9	3.5	2.5
CM 9459-11	3.5	2.5	SM 2601-22	4.0	3.5	SM 2727-12	4.0	2.5
CM 9459-12	3.0	3.0	SM 2601-23	4.0	3.5	SM 2727-20	4.0	4.0
CM 9459-13	3.5	2.5	SM 2601-27	4.0	4.0	SM 2727-23	4.0	4.0
CM 9459-15	3.5	3.0	SM 2601-30	3.5	3.0	SM 2727-26	4.0	2.5
CM 9459-18	3.0	2.5	SM 2601-31	4.0	3.0	SM 2727-27	3.5	2.5
CM 9459-21	3.0	2.0	SM 2601-39	4.0	4.0	SM 2727-31	3.5	3.0
CM 9459-22	4.0	2.0	SM 2601-44	3.5	2.5	SM 2727-36	4.0	3.0
CM 9459-24	3.5	3.0	SM 2601-55	4.0	4.0	SM 2727-42	4.0	4.0
CM 9460-1	2.5	3.5	SM 2601-56	4.0	3.5	SM 2727-43	4.0	4.0
CM 9460-3	3.0	4.0	SM 2603-23	4.0	4.0	SM 2728-9	4.0	3.5
CM 9460-9	3.0	3.5	SM 2606-25	4.0	4.0	SM 2730-1	4.0	2.5
CM 9460-12	3.0	3.5	SM 2606-27	3.5	4.0	SM 2730-8	3.0	4.0
CM 9460-13	3.5	4.0	SM 2608-27	3.0	4.0	SM 2730-12	3.5	4.0
CM 9460-15	3.0	3.5	SM 2609-54	4.0	2.5	SM 2730-26	3.0	4.0
CM 9460-16	3.5	2.5	SM 2612-29	4.0	2.5	SM 2730-42	4.0	3.0
CM 9460-17	3.5	2.5	SM 2632-2	3.5	2.5	SM 2730-43	4.0	4.0
CM 9460-25	3.0	2.0	SM 2632-4	3.0	2.5	SM 2738-1	3.5	4.0
CM 9460-34	2.5	2.5	SM 2632-5	3.0	4.0	SM 2739-1	4.0	3.0
CM 9460-35	3.0	3.0	SM 2632-15	3.5	3.5	SM 2739-4	4.0	3.0
CM 9460-37	4.0	3.5	SM 2632-17	2.5	2.5	SM 2786-1	3.0	2.5
CM 9460-38	2.5	2.0	SM 2632-22	4.0	3.0	SM 2786-5	4.0	2.5
CM 9460-39	3.0	4.0	SM 2633-3	3.0	3.0	SM 2786-7	3.5	2.5
CM 9460-40	3.5	3.0	SM 2633-10	3.0	3.0	SM 2786-9	3.5	4.0
CM 9460-41	2.5	3.5	SM 2634-4	3.5	3.0	SM 2786-10	3.0	2.0
CM 9460-42	4.5	3.0	SM 2634-7	4.0	3.0	SM 2786-15	3.5	2.5
CM 9461-1	4.0	2.5	SM 2634-8	3.5	2.5	SM 2786-18	3.5	4.0
CM 9461-2	4.0	2.0	SM 2634-9	4.0	3.0	SM 2787-1	3.0	2.5
CM 9461-3	3.5	3.0	SM 2634-13	3.5	2.5	SM 2787-4	3.5	2.5
CM 9461-5	3.5	2.5	SM 2635-4	3.5	4.0	SM 2787-5	4.5	3.0
CM 9461-6	3.0	3.0	SM 2635-6	3.5	3.0	SM 2787-13	3.5	3.5
CM 9461-7	3.0	3.5	SM 2635-12	3.5	4.0	SM 2790-2	4.5	3.0
CM 9461-8	3.0	3.0	SM 2636-4	4.0	4.0	SM 2790-17	4.0	2.5
CM 9461-10	2.5	2.5	SM 2636-5	3.5	3.0	SM 2790-18	4.0	3.0
CM 9461-11	4.0	3.0	SM 2636-6	3.0	2.5	SM 2790-27	4.0	3.0
CM 9461-12	3.0	3.0	SM 2636-10	3.0	2.5	SM 2790-28	4.0	2.5
CM 9461-13	4.0	2.5	SM 2636-14	3.5	2.5	SM 2790-32	4.0	2.5
CM 9461-14	4.0	2.5	SM 2636-18	3.0	3.0	SM 2791-2	4.0	2.5
CM 9461-15	3.5	3.0	SM 2636-19	4.0	4.0	SM 2791-5	4.0	2.0
CM 9461-17	3.0	3.0	SM 2636-20	4.0	3.0	SM 2791-12	3.5	4.0
CM 9461-18	2.5	3.0	SM 2636-26	3.0	2.0	SM 2791-16	4.0	3.5
CM 9461-21	4.0	2.5	SM 2636-29	4.0	3.5	SM 2791-17	3.5	4.0
CM 9461-32	5.0	3.0	SM 2636-30	4.0	4.0	SM 2792-3	3.5	2.5
CM 9461-35	3.0	2.5	SM 2636-42	3.0	4.0	SM 2792-6	4.0	3.5
CM 9461-36	4.0	3.0	SM 2636-44	2.5	2.5	SM 2792-11	3.0	2.5
CM 9461-51	2.5	3.5	SM 2638-6	3.0	4.0	SM 2792-12	4.0	3.5
CM 9461-53	2.5	3.0	SM 2638-10	4.0	2.5	SM 2792-14	5.0	4.0
CM 9461-56	3.0	3.0	SM 2638-11	3.5	3.0	SM 2792-16	3.5	3.5

Table 7.6 (cont.)

CM 9462-17	3.0	3.0	SM 2638-12	3.0	2.5	SM 2792-28	4.0	4.0
CM 9463-2	2.5	2.5	SM 2638-13	3.0	3.0	SM 2792-31	3.0	3.0
CM 9463-10	2.5	2.5	SM 2638-17	3.5	3.5	SM 2792-32	4.0	4.0
CM 9463-15	3.5	3.0	SM 2638-20	3.5	2.5	SM 2792-36	4.0	2.5
CM 9463-19	4.0	2.5	SM 2638-23	3.0	3.0	SM 2792-37	4.0	2.5
CM 9464-1	3.5	2.5	SM 2638-27	4.0	3.5	SM 2792-38	3.5	4.0
CM 9464-3	3.5	3.0	SM 2638-40	3.5	2.0	SM 2792-42	4.0	3.0
CM 9464-19	2.5	4.0	SM 2638-44	3.0	3.0	SM 2792-43	4.0	2.5
CM 9464-26	2.5	2.5	SM 2640-1	3.5	3.0	SM 2792-50	3.5	3.0
CM 9464-27	3.0	2.5	SM 2640-6	4.0	4.0	SM 2792-52	4.0	3.0
CM 9464-29	2.5	3.0	SM 2640-7	3.0	2.5	SM 2793-7	3.0	2.5
CM 9464-30	3.0	3.0	SM 2640-8	4.0	2.0	SM 2794-2	3.0	3.5
CM 9464-33	2.5	3.5	SM 2640-9	3.0	3.0	SM 2794-18	3.5	3.0
CM 9464-36	3.0	4.0						

a. Disease reaction measured on a scale of 1 to 5, where: For CBB, 1.0 to 2.0 = resistant; 2.5 to 3.0 = intermediate; 3.5 to 5.0 = susceptible. For SED, 1.0 to 2.0 = resistant; 2.5 to 3.5 = intermediate; 4.0 to 5.0 = susceptible.

Methodology: North Coast

A cassava regional assay, conformed by 40 elite genotypes, was conducted in Sincelejo (Department of Sucre, Colombia), for resistance to CBB and SED. Genotypes were planted in a randomized block design with 3 replicates.

Results: North Coast

CBB pressure was low, whereas SED pressure was high. Genotypes SM 1565-17 and SM 1624-2 were the most resistant to SED (Table 7.7).

Table 7.7. Disease reaction[a] of cassava genotypes to cassava bacterial blight (CBB) and superelongation disease (SED) in Sincelejo, Department of Sucre, Colombia.

Genotype	CBB	SED	Genotype	CBB	SED	Genotype	CBB	SED
CG 1141-1	1.5	3.0	M Col 1505	2.0	3.0	SM 1650-7	2.0	4.0
CM 3306-19	2.5	4.0	M Col 2215	2.0	4.5	SM 1657-14	2.5	4.0
CM 3306-4	1.5	3.0	M Per 183	2.0	4.0	SM 1665-2	2.0	3.0
CM 4843-1	2.5	3.0	M Tai 8	2.0	3.5	SM 1669-5	1.5	3.0
CM 4919-1	1.5	4.0	M Ven 25	2.0	3.0	SM 1669-7	2.5	4.0
CM 523-7	1.5	4.0	SBO 216-9	2.5	4.0	SM 1759-29	2.0	3.0
CM 6119-5	1.5	3.0	SGB 765-2	2.0	3.0	SM 1778-45	2.0	4.0
CM 6740-7	3.0	4.0	SGB 765-4	2.0	4.5	SM 1778-53	3.0	4.5
CM 6754-8	2.5	3.0	SM 1411-5	1.5	4.0	SM 1973-23	2.0	4.0
CM 6758-1	1.5	3.0	SM 1438-2	2.5	3.0	SM 1973-25	2.0	3.0
CM 7514-8	2.0	4.0	SM 1511-6	3.0	4.0	SM 643-17	2.0	3.0
CM 8027-3	1.5	4.0	SM 1565-17	2.5	2.0	SM 805-15	3.0	4.0
CM 8475-4	2.0	3.0	SM 1624-2	1.5	2.0			
M Bra 384	2.0	4.0	SM 1627-16	2.0	3.0			

a. Disease reaction measured on a scale of 1 to 5, where: For CBB, 1.0 to 2.0 = resistant; 2.5 to 3.0 = intermediate; 3.5 to 5.0 = susceptible. For SED, 1.0 to 2.0 = resistant; 2.5 to 3.5 = intermediate; 4.0 to 5.0 = susceptible.

Methodology: Cauca

To evaluate the effect of five control practices on *Phytophthora* fungi, which induce root rot in cassava, experimental plots were established on a farm in the Municipality of Pescador (Cabuyal Village District), Department of Cauca, Colombia, in April 2002. The farmer is indigenous and belongs to the Interinstitutional Consortium for Sustainable Agriculture in Hillsides (CIPASLA, its Spanish acronym).

Treatments

Planting stakes were grouped for use in five treatments, which were then evaluated for their effect on the incidence and severity of root rots in the harvested roots of each group. The types of control were:

1. *Biological control*: Strain 14PDA-4 of the fungus *Trichoderma* sp., which attacks root rot fungi (*Phytophthora* spp.), was used to make a suspension of 1×10^6 conidia/mL. Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil near the base of each plant. The effectiveness of the *Trichoderma* strain in controlling *Phytophthora tropicalis* was also evaluated *in vitro* tests and the greenhouse.

2. *No treatment:* Traditional farmer's practice.

3. *Selection of quality planting materials*: Stakes were selected for their health and from middle parts of stems.

4. *Chemical control*: Planting stakes were immersed for 5 min in Ridomil® (metalaxyl) at 3 g/L of water.

5. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

For all treatments, stakes of the regional cassava variety Algodona (M Col 1522) were used, and chicken manure was incorporated into the soil at 2.5 t/ha.

The experimental design was randomized complete blocks, with two replicates and 44 plants per treatment.

As checks, other cassava genotypes that had previously given high yields in field experiments were planted in the same plot, at 38 plants per genotype. These genotypes were CM 7438-14, M Bra 383, SM 1053-23, SM 1058-13, and SM 1937-1. All plots were planted in association with beans.

Results: Cauca

In the trial, the heat treatment did not affect germination (Table 7.8).

Table 7.8 Effect of different practices of root-rot management on germination, Pescador, Department of Cauca, Colombia.

Treatment	Germination (%)[a]
Algodona (M Col 1522)	
Stake selection	96.6
Thermotherapy	93.3
Traditional farmer's practice	95.3
Trichoderma strain 14PDA-4	88.6
Chemical control	98.9
Check varieties	
CM 7438-14	100.0
M Bra 383	100.0
SM 1053-23	94.6
SM 1058-13	100.0
SM 1937-1	97.4
Average	96.5

a. 30 days after planting.

Methodology: Quindío

Different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in two field trials at "La Elena" Farm, Municipality of Montenegro.

Treatments for the first trial

The first trial was planted in June 2001 with the local variety Manzana. Treatments were as follows:

1. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

2. *Chemical control*: Planting stakes were immersed for 5 min in a mixture of Orthocide® (captan) at 4 g/L and Ridomil® at 3 g/L of water.

3. *Biological control*: Strain 14PDA-4 of *Trichoderma* sp. was used to make a suspension of 1×10^4 conidia/mL Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil around the stake, using 100 mL/plant.

4. *No treatment*: Traditional farmer's practice.

All the plots received fertilizers 45 days after planting, that is, 500 kg/ha of the fertilizer mix Nitrax®, DAP, and KCl was applied at a rate of 1:2:2.

The experimental design was randomized complete blocks, with three replicates and 55-60 plants per treatment.

Treatments for the second trial

The second trial was planted in August 2001:

1. *Thermotherapy*: Planting stakes were immersed for 49 min in water heated to 49°C over a wood fire.

2. *Chemical control*: Planting stakes were immersed for 5 min in a mixture of Orthocide® at 4 g/L and Ridomil® at 3 g/L of water.

3. *Biological control*: Strain 14PDA-4 of *Trichoderma* sp. was used to make a suspension of 1×10^4 conidia/mL Planting stakes were then inoculated by immersing them in the suspension for 10 min. The suspension was also applied to the soil around the stake, using 100 mL/plant.

All the plots received fertilizers 45 days after planting, that is, 500 kg/ha of the fertilizer mix Nitrax, DAP, and KCl was applied at a rate of 1:2:2. Four months after planting, 1% of each of Kelatex-Mn® and Kelatex-Zn® were applied to the foliage and 55 kg/ha of the same products to the soil.

The experimental design was the same as for the first experiment.

Results: Quindío

The applied biological control agent *Trichoderma* strain 14PDA-4 reduced root rot strongly in two experiments conducted at Quindío (Table 2 and 3). Traditional farmer's practice resulted in consistently higher levels of root rot. However, the resulting yields were not good, and germination and plant development following treatment were generally low. Thermotherapy showed similar results. It can be concluded that *Trichoderma* or heat affected germination and crop development of stem cuttings obtained from immature cassava plants.

For the cassava variety Chiroza, the biological (*Trichoderma*) and chemical (Orthocide® and Ridomil®) treatments reduced the severity of root rot caused by *Phytophthora* sp. (Table 3), but did not affect disease incidence. The manganese and zinc applications, in contrast, did reduce incidence (data not presented). Yields were fairly high, considering the field had been cropped for 5 cycles and the pressure of Phytophthora root rot was high. Thermotherapy and the zinc and manganese applications gave the highest yields, whereas the chemical and *Trichoderma* treatments had the lowest.

To take advantage of the fairly good control by *Trichoderma*, it is suggested to apply several control practices and improve crop fertilization in future experiments.

Clone HMC-1 showed acceptable levels of root rot infection; incidence was much lower than other varieties or treatments. The variety Reina (Cm 6740-7) will be included in future trials at Quindío.

Table 7.9. Effect of stake treatments—thermotherapy, biological control agent, and chemical control—and fertilizers on the development of cassava variety Manzana and root rot disease, Department of Quindío, Colombia.

Parameter	Control practice				
	Thermo-therapy[a]	Tricho-derma[b]	Chemical	Traditional farmer's	Average
	5 months after planting				
Germination (%)	66.7	41.7	94.0	88.1	72.6
Height (cm)	62.3	56.3	72.3	55.0	61.5
	12 months after planting				
No. stakes/plant	10.6	13.5	16.8	10.1	12.7
Harvested plants (%)	71.7	46.7	87.2	75.0	70.1
Yield (t/ha)					
Commercial roots	1.9	1.3	5.9	4.0	3.3
Noncommercial roots	2.0	2.6	3.8	2.7	2.8
Disease incidence (%)					
Plants with root rot	75.6	90.0	70.0	73.2	77.2
Severity (%)					
Rot (by no. of roots)	44.8	41.3	39.3	40.6	41.5
Rot (by weight)	65.9	44.4	51.0	49.8	52.8
Root rot (t/ha)					
Commercial roots	3.0	2.1	5.9	3.4	3.6
Noncommercial roots	4.1	1.8	2.9	3.0	3.0

a. Roots immersed for 49 min in hot water (47ºC-49ºC) in an oil drum on a wood fire.
b. Strain 14PDA-4.

Table 7.10. Effect of stake treatments—thermotherapy, biological control agent (*Trichoderma* sp.), and chemical control—and fertilizers (Mn + Zn) on the development of the cassava variety Chiroza and Phytophthora root rot, Department of Quindío, Colombia.

Treatment	Root yield (t/ha)		DM[a] content (%)	Infected roots			
				Disease severity (%)		(t/ha)	
	Commer.	Non-commer.		(no.)	(weight)	Commer.	Non-commer.
Three replicates per treatment							
Mn + Zn	19.3	5.3	34.4	55.1	40.4	8.1	1.8
Thermotherapy[b]	19.1	6.9	35.8	34.5	24.8	5.4	1.0
Traditional farmer's practice	18.5	5.4	40.7	35.9	29.5	6.2	0.8
Chemical control	17.9	7.0	35.0	27.5	18.3	3.5	1.0
Trichoderma strain 14PDA-4	16.7	5.5	35.5	31.5	26.0	4.6	1.2
One replicate per treatment							
Catumare	3.5	3.9	41.6	46.2	54.2	3.3	0.8
HMC-1	9.6	8.2	38.2	13.4	21.4	3.5	0.3
Chiroza	3.0	2.3	24.3	61.8	70.3	2.6	1.1
La Reina (Cm 6740-7)	17.2	7.0	38.1	16.0	16.9	4.0	0.1

a. DM = dry matter.
b. Roots immersed for 49 min in hot water (47ºC-49ºC) in an oil drum on a wood fire.

Activity 7.5 Characterizin progeny of four backcross families (M Nga 19 crossed with each of CM 9208-13, CM 9208-26, CM 9208-31, and CM 9208-73) for resistance to cassava bacterial blight in Villavicencio

Specific objectives

1. *To evaluate CBB resistance in F1 of four BC1 families under field conditions at Villavicencio.*
2. *To select the family with the widest segregation among the four families, based on disease frequency distribution, for further molecular marker analysis.*

Methodology

The progeny of four cassava BC1 families were characterized for their reaction to CBB under natural disease pressure in Villavicencio. The four families were:

Family	Cross	Individuals (no.)
GM 315	M Nga 19 × CM 9208-13	357
GM 316	M Nga 19 × CM 9208-26	399
GM 317	M Nga 19 × CM 9208-31	348
GM 318	M Nga 19 × CM 9208-73	238

All four families have a common recurrent male parent, which is resistant to many strains of *Xam*.

The families' reactions to CBB were analyzed, using a frequency distribution graph for each family, based on an average of 6 plants per plot, and a disease severity scale of 1.0 to 5.0, where 1.0 indicates plants with no symptoms and 5.0 death. Resistant plants scored between 1.0 and 2.0 on the scale, intermediate between 2.5 and 3.0, and susceptible more than 3.5.

Results

According to the frequency distribution graphs (Figure 7.1) for CBB resistance, at least 83% of individuals in each of the four families had values of less than 3.0 on the disease severity scale. The families GM 316, GM 317, and GM 318 had more than 69% of individuals with a score between 2.1 and 2.5. The GM 315 family was the most segregated and had the highest standard deviation (0.697) for individuals in each class: 46% of individuals of this family scored between 2.1 and 2.5, thus showing intermediate resistance, whereas 6% scored less than 1.5 (i.e., resistant); and 2% scored more than 4.5 (i.e., susceptible).

Because of its high segregation rate and standard deviation, the GM 315 family was chosen for identifying SSR markers linked to CBB resistance in cassava by segregant analysis of bulks from resistant and susceptible individuals.

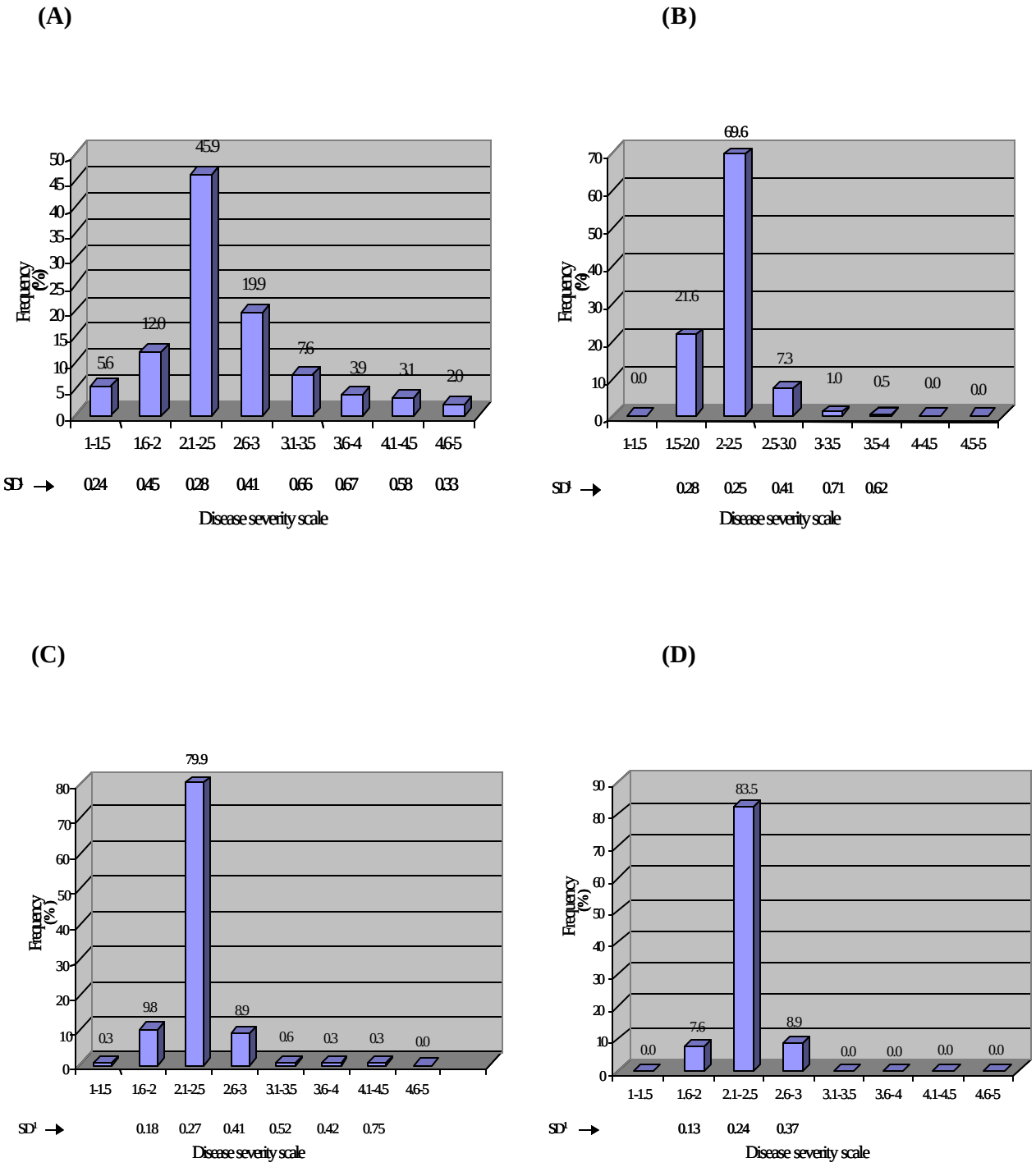

Figure 7.4. Breakdown of four cassava backcrossed families according to resistance level to *Xanthomonas axonopodis* pv. *manihotis*. **(A)** Family GM 315. **(B)** Family GM 316. **(C)** Family GM 317. **(D)** Family GM 318. SD = average of standard deviation inside plot.