

## 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, these 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. Characterization of 50 genotypes under greenhouse conditions regarding their reaction to 12 different isolates of common bacterial blight (CBB).**

##### **Specific objectives**

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

Thirty genotypes were characterized under greenhouse conditions regarding their reaction to nine isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent CBB. Eight of the isolates were collected from different genotypes and edaphoclimatic zones in Colombia and one in Brazil (Table 7.1). Under greenhouse conditions, 30-day-old cassava plants of each genotype were inoculated with the isolates, injecting the stem with a bacterial suspension of  $1 \times 10^6$  cfu/ml. Disease severity was recorded at 10, 17, and 24 days after inoculation.

Table 7.1. Origin and source of isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of common bacterial blight (CBB), obtained from cassava to evaluate disease resistance.

<b>Isolate</b>	<b>Origin (department/country)</b>	<b>Genotype source</b>
JV 7A	Jamundí (Valle, Colombia)	La Reina
VM 2	Villavicencio (Meta, Colombia)	M Bra 489
VM 7	Villavicencio (Meta, Colombia)	SM 2069-1
Cio 63	Sincelejo (Sucre, Colombia)	Mona Blanca
Cio 71	Segovia (Sucre, Colombia)	M Col 2215
Cio 148	Granada (Meta, Colombia)	CM 7274-1
Cio 367	Campinas (Brazil)	SRT 1363 Abacate
Cio 465	INYUCAL (Atlántico, Colombia)	AM 244-17
Cio 466	INYUCAL (Atlántico, Colombia)	AM 244-17 (exudate)

The most aggressive isolates were Cio 148, with 44.8% virulence, and Cio 367, with 63.3% virulence. Fourteen varieties, equivalent to 47%, presented either intermediate or resistance reactions to 88.9%-100% of the isolates. Genotypes M Bra 383, SM1779-8, and SM1862-25 were resistant to 77.8%- 87.5% of the isolates (Table 7.2).

Table 7.2. Disease reaction<sup>a</sup> of cassava genotypes to nine isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of common bacterial blight (CBB).

Genotypes	Isolates <sup>b</sup>									Total <sup>c</sup>			R + I (%) <sup>d</sup>
	VM2	VM 7	JV7A	Cio71	Cio63	Cio148	Cio367	Cio465	Cio466	R	I	S	
Brasilera	-	4.0	3.0	4.0	4.5	3.5	3.5	4.5	4.0	0	1	7	12.5
CM 4574-7	-	3.5	2.5	3.5	3.0	3.5	4.0	3.5	3.0	0	3	5	37.5
CM 6921-3	2.5	2.0	1.0	2.5	4.0	4.0	3.0	4.0	3.5	2	3	4	55.6
CM 7514-7	1.5	3.0	2.0	1.5	2.5	3.5	3.5	2.5	2.5	3	4	2	77.8
CM 8370-11	1.5	2.5	1.0	1.0	2.5	2.5	3.0	2.0	2.5	4	5	0	100.0
CM 8370-14	1.5	1.5	2.0	1.5	2.5	3.0	3.5	3.0	2.0	5	3	1	88.9
La Reina	2.0	2.0	1.5	2.0	3.0	2.0	3.5	3.0	2.5	5	3	1	88.9
M Bra 383	2.0	1.5	1.5	1.0	3.0	2.5	2.0	2.0	2.0	7	2	0	100.0
M Bra 466	-	3.5	3.5	4.0	4.0	4.0	4.5	4.0	4.0	0	0	8	0.0
M Bra 489	1.5	2.5	1.0	2.0	3.0	4.0	3.5	3.5	3.5	3	2	4	55.6
M Col 1505	-	3.5	3.0	4.0	3.5	3.5	4.5	3.5	5.0	0	1	7	12.5
M Col 2307	2.0	2.5	1.5	2.5	3.0	3.0	4.0	2.5	3.5	2	5	2	77.8
M Per 183	2.0	1.5	1.5	2.0	2.5	3.0	2.5	2.0	2.5	5	4	0	100.0
M Tai 8	1.0	2.0	2.0	2.5	2.5	2.0	1.0	3.0	2.5	5	4	0	100.0
M Cr 32	2.0	2.0	2.0	2.0	2.0	3.5	2.0	2.5	3.0	6	2	1	88.9
SM 985-9	-	4.0	5.0	5.0	4.0	4.0	5.0	5.0	4.5	0	0	8	0.0
SM 1219-9	1.5	3.0	1.5	1.0	3.0	2.5	3.5	3.0	4.0	3	4	2	77.8
SM 1460-1	1.5	3.0	2.0	1.5	2.0	4.0	3.0	2.5	2.0	5	3	1	88.9
SM 1545-25	1.5	1.5	1.0	1.5	2.0	3.0	4.0	2.5	2.5	5	3	1	88.9
SM 1565-15	1.5	2.0	1.5	2.0	4.0	2.0	4.0	2.5	3.0	5	2	2	77.8
SM 1673-11	1.5	2.0	1.5	1.5	3.0	3.0	3.5	2.5	3.5	4	3	2	77.8
SM 1741-1	1.5	2.0	1.0	2.5	2.5	3.0	2.5	2.5	2.5	3	6	0	100.0
SM 1779-8	2.0	2.0	1.0	1.0	3.0	-	2.0	2.0	2.0	7	1	0	100.0
SM 1820-8	1.0	2.0	1.5	1.5	2.5	2.5	2.5	2.0	2.0	6	3	0	100.0
SM 1828-11	-	4.0	4.0	4.0	3.5	4.5	5.0	4.0	4.5	0	0	8	0.0
SM 1859-26	-	3.5	3.5	3.5	4.0	3.0	4.0	3.5	4.0	0	1	7	12.5
SM 1822-12	2.0	1.5	1.5	2.5	3.0	3.0	4.0	2.5	3.0	3	5	1	88.9
SM 1862-25	2.0	1.5	1.5	2.0	2.0	2.5	2.0	3.0	2.0	7	2	0	100.0
SM 1928-11	1.5	2.5	1.0	2.0	4.0	3.5	4.0	2.5	2.5	3	3	3	66.7
SM 2069-57	1.0	2.0	1.0	2.0	3.5	4.0	3.5	3.5	2.5	4	1	4	55.6
Total													
Resistant	22	16	23	18	4	3	5	5	6				
Intermediate	1	7	3	5	16	13	6	15	13				
Susceptible	0	7	4	7	10	13	19	10	11				
Virulence (%) <sup>e</sup>	0.0	23.3	13.3	23.3	33.3	44.8	63.3	33.3	36.7				
Correlation <sup>f</sup>	0.3	0.5	-	-	-	-	-	-	-				

<sup>a</sup> Disease reaction: resistant (R) = from 1.0 to 2.0; intermediate (I) = from 2.5 to 3.0; susceptible (S) = from 3.5 to 5.0.

<sup>b</sup> Isolates: See Table 7.1.1.

<sup>c</sup> Total of isolates to which each genotype is either resistant (R), intermediate (I), or susceptible (S).

<sup>d</sup> Percentage of isolates to which each genotype shows both resistance (R) and intermediate resistance (I).

<sup>e</sup> Percentage of genotypes susceptible to each isolate.

<sup>f</sup> Correlation between disease reaction of isolates from Villavicencio (VM2 and VM7) in the field and greenhouse. Correlation was carried out with genotypes evaluated in the field in each zone.

**Activity 7.2. Evaluation of 50 cassava genotypes for their resistance to common bacterial blight (CBB) and superelongation disease (SED) in Villavicencio and Matazul.**

**Specific objectives**

- 1) To evaluate the reaction of 50 genotypes to two different diseases and measure their root yield potential.
- 2) To correlate disease reactions in the field and in the greenhouse.

A total of 104 cassava genotypes were characterized for their reactions to several pathotypes of CBB and SED under natural disease pressure in Villavicencio (Meta); 37 genotypes were evaluated in Matazul (Puerto López, Meta).

Table 7.3. Disease reaction<sup>a</sup> of cassava genotypes to common bacterial blight (CBB) and super-elongation disease (SED) in Villavicencio and Matazul (Meta, Colombia).

Genotype	CBB	SED	Genotype	CBB	SED
Villavicencio					
Brasilera	3.5	3.0	SM 1555-17	2.5	2.5
CM 2600-2	2.5	2.0	SM 1673-11	3.0	2.0
CM 2772-3	3.0	1.5	SM 1855-9	3.5	2.5
CM 8746-1	2.5	3.0	SM 1871-39	3.5	2.0
CM 8747-5	2.5	4.0	SM 2069-1	3.5	2.5
HMC-1	4.0	3.0	SM 2069-2	3.5	2.0
Ica Catumare	3.0	2.0	SM 2220-18	4.0	2.0
Ica Cebucán	3.0	2.0	SM 2220-19	4.0	2.5
K 3	3.5	2.5	SM 2366-44	3.5	2.0
K 7	3.5	2.0	SM 2366-45	3.5	2.5
K 9	3.0	2.5	SM 2366-46	3.0	4.0
K 12	3.5	1.5	SM 2366-49	4.0	2.0
K 14	3.0	2.5	SM 2366-50	4.5	2.0
K 17	3.5	2.0	SM 2366-57	4.0	2.5
K 22	3.5	2.5	SM 2452-13	2.5	3.0
K 26	3.0	2.5	SM 2561-32	3.5	2.5
K 31	2.5	2.0	SM 2632-22	3.5	3.0
K 36	2.5	1.5	SM 2632-4	3.0	2.0
K 43	3.0	2.0	SM 2632-15	3.5	2.0
K 49	3.0	1.5	SM 2633-10	3.0	3.0
K 50	3.5	2.0	SM 2634-13	3.5	2.5
K 58	3.5	4.0	SM 2634-4	3.5	2.0
K 69	3.0	2.0	SM 2635-12	3.5	4.0
K 80	2.5	1.5	SM 2636-19	4.0	4.0
K 81	3.0	2.5	SM 2636-20	4.0	2.0
K 93	3.0	1.0	SM 2636-29	4.0	2.0
K 94	3.0	1.0	SM 2636-30	4.0	3.5
K 105	2.5	2.5	SM 2636-4	3.5	3.5
K 111	3.0	1.5	SM 2636-42	2.5	2.5
K 113	1.5	1.0	SM 2636-44	2.5	2.0
K 115	2.5	2.0	SM 2638-11	3.5	3.0
K 122	3.0	1.5	SM 2638-12	3.0	2.5

K 127	2.5	2.5	SM 2638-13	2.5	3.5
K 128	3.5	1.5	SM 2638-17	3.5	2.0
K 129	3.5	2.0	SM 2638-20	3.5	2.0
K 130	2.5	2.5	SM 2638-23	2.5	2.0
K 140	2.5	2.0	SM 2638-27	3.5	3.0
K 144	3.5	2.0	SM 2638-6	3.0	4.0
K 150	3.0	2.0	SM 2640-1	3.5	2.5
La Reina	4.0	4.0	SM 2640-6	4.0	2.0
M Bra 466	3.5	2.0	SM 2640-9	3.0	2.0
M Bra 489	3.0	2.0	SM 2641-2	4.0	2.0
M Bra 881	3.0	1.5	SM 2642-3	3.5	2.0
M Bra 902	3.5	1.0	SM 2644-1	3.5	3.0
M Col 1505	3.5	2.0	SM 2644-4	4.0	4.0
M Col 2307	3.0	2.5	SM 2644-5	4.0	4.0
M Col 2329	3.0	2.5	SM 2645-1	4.0	4.0
M Col 2409	2.5	2.0	SM 2646-2	3.5	3.5
M Cub 74	4.0	1.0	SM 2786-1	2.5	2.5
SG 104-74	3.0	2.0	SM 2786-18	3.5	3.0
SM 1225-12	3.0	2.0	SM 2786-5	3.5	2.0
SM 1468-9	3.0	2.5	SM 2786-7	3.0	2.5
Matazol					
Brasilera	3.0	2.5	SM 1460-1	1.5	2.5
CM 6055-3	1.0	3.0	SM 1468-9	4.0	2.0
CM 6697-2	2.0	2.5	SM 1545-19	1.0	2.0
CM 6975-14	3.5	2.0	SM 1555-17	1.5	2.0
Ica Catumare	1.5	2.0	SM 1588-1	1.5	2.0
Ica Cebucán	1.0	3.5	SM 1673-10	1.5	2.0
La Reina	1.0	2.0	SM 1682-2	2.0	2.0
M Col 2387	1.0	2.5	SM 1828-11	1.5	2.0
M Cr 32	2.0	2.0	SM 1855-9	3.0	2.0
M Esc Fla 39	1.0	2.0	SM 1871-32	1.5	2.5
M Ven 77	1.0	4.0	SM 1871-38	1.5	2.0
SG 104-74	2.0	2.5	SM 1871-42	2.0	2.5
SM 1143-22	3.5	2.0	SM 2061-1	2.0	2.0
SM 1215-1	3.5	2.0	SM 2069-1	1.5	2.0
SM 1223-20	2.0	2.5	SM 2069-4	2.0	2.0
SM 1345-10	1.5	2.0	SM 2069-55	1.5	2.0
SM 1361-8	1.5	2.0	SM 2069-57	1.5	3.0
SM 1363-3	1.0	1.5	SM 2219-9	2.0	2.0
SM 1411-5	1.5	2.0			

<sup>a</sup> CBB = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.0; and susceptible, from 3.5 to 5.0.

SED = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.5; and susceptible, from 4.0 to 5.0.

In Villavicencio, one genotype was resistant to CBB, 49 showed intermediate resistance, and 54 were susceptible, while 55 genotypes were resistant to SED, 39 showed intermediate resistance, and 10 were susceptible. Genotype K 113 showed high resistance to both diseases (Table 7.3). La Reina (CM 6740-7), the new commercial variety for the Eastern Plains, was susceptible to both CBB and SED. Brasilera, a genotype asked for by farmers in the department of Meta, is also susceptible to both diseases, as shown in Tables 7.3 and 7.4.

CBB pressure was lower in Matazol than in Villavicencio, and 31 genotypes showed resistance to the disease, 2

showed intermediate resistance, and 4 were susceptible, whereas 23 genotypes were resistant to SED, 13 showed intermediate resistance, and 1 was susceptible. Genotypes Brasileira, Ica Catumare, Ica Cebucán, La Reina, SG 104-74, SM 1468-9, SM 1555-17, SM 1855-9, and SM 2069-1 were evaluated at both sites.

An early disease assessment trial was conducted at La Libertad, Villavicencio, with 244 cassava genotypes. No CBB-resistant genotypes were found, 129 genotypes presented intermediate resistance, and 115 were susceptible to the disease. In the case of SED, 47 genotypes were resistant, 164 showed intermediate resistance, and 33 were susceptible. Disease severity will increase depending on environmental conditions. Trial results are being evaluated (Table 7.4).

A correlation coefficient of 0.3 and 0.5 between field and greenhouse results for isolates VM2 and VM7, from Villavicencio was obtained. This correlation indicates that isolate VM7 shows higher prevalence in the field.

Table 7.4. Disease reaction<sup>a</sup> of 5-month-old cassava genotypes to common bacterial blight (CBB) and super-elongation disease (SED) in Villavicencio (Meta, Colombia).

Genotype	CBB	SED	Genotype	CBB	SED	Genotype	CBB	SED
Brasileira	4.5	4.0	CM 9472-4	4.0	2.5	SM 2641-2	4.0	2.0
Catumare	3.0	3.0	CM 9472-7	3.0	2.5	SM 2641-7	2.5	2.0
HMC-1	4.5	4.0	CM 9483-4	2.5	3.5	SM 2641-9	2.5	3.0
La Reina	4.0	2.5	SM 1812-92	2.5	2.0	SM 2641-11	2.5	2.5
CM 8746-1	2.5	3.0	SM 2220-18	4.0	2.0	SM 2642-3	3.5	2.5
CM 8747-5	2.5	4.0	SM 2220-19	4.0	2.5	SM 2642-17	2.5	2.0
CM 9449-2	2.5	3.0	SM 2220-20	2.5	2.0	SM 2642-24	2.5	3.0
CM 9449-6	2.5	2.5	SM 2366-44	3.5	2.0	SM 2642-27	2.5	2.5
CM 9449-8	2.5	3.5	SM 2366-45	3.5	2.5	SM 2644-1	3.5	3.0
CM 9450-5	4.0	4.0	SM 2366-46	2.5	4.0	SM 2644-3	2.5	2.5
CM 9451-1	2.5	2.0	SM 2366-49	4.0	2.0	SM 2644-4	4.0	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	2.5	SM 2366-57	4.0	2.5	SM 2645-1	4.0	4.0
CM 9452-13	4.0	4.0	SM 2452-13	2.5	3.0	SM 2646-2	3.0	3.5
CM 9452-15	2.5	3.0	SM 2561-32	3.5	2.5	SM 2724-9	3.5	4.0
CM 9456-26	2.5	2.5	SM 2592-14	4.0	3.0	SM 2724-15	3.5	4.0
CM 9456-40	3.5	2.5	SM 2593-21	4.0	2.5	SM 2724-18	3.0	2.5
CM 9459-1	4.5	2.5	SM 2594-16	4.0	3.5	SM 2726-4	4.0	3.0
CM 9459-2	2.5	3.0	SM 2599-25	3.0	4.0	SM 2726-17	3.5	2.5
CM 9459-6	3.0	3.0	SM 2599-41	4.0	3.0	SM 2727-1	4.0	3.0
CM 9459-10	2.5	3.0	SM 2599-49	3.5	3.5	SM 2727-9	3.0	2.5
CM 9459-11	3.0	2.5	SM 2601-22	4.0	3.5	SM 2727-12	4.0	2.5
CM 9459-12	2.5	2.0	SM 2601-23	4.0	3.0	SM 2727-20	4.0	4.0
CM 9459-13	2.5	2.0	SM 2601-27	4.0	4.0	SM 2727-23	4.0	4.0
CM 9459-15	3.0	2.0	SM 2601-30	3.5	3.0	SM 2727-26	4.0	2.5
CM 9459-18	2.5	3.0	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	3.0	SM 2727-31	3.5	3.0
CM 9459-22	4.0	2.0	SM 2601-44	2.5	2.5	SM 2727-36	4.0	3.0
CM 9459-24	3.5	2.0	SM 2601-55	4.0	2.5	SM 2727-42	2.5	4.0
CM 9460-1	2.5	2.5	SM 2601-56	4.0	3.0	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	3.0
CM 9460-9	2.5	3.0	SM 2606-25	4.0	4.0	SM 2730-1	4.0	2.5
CM 9460-12	3.0	2.0	SM 2606-27	2.5	3.0	SM 2730-8	2.5	3.0
CM 9460-13	2.5	3.0	SM 2608-27	3.0	3.0	SM 2730-12	3.0	2.5
CM 9460-15	2.5	3.0	SM 2609-54	4.0	2.5	SM 2730-26	3.0	4.0
CM 9460-16	2.5	2.5	SM 2612-29	4.0	2.5	SM 2730-42	4.0	3.0

Continuation Table 7.4.

CM 9460-17	3.0	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	2.0	SM 2738-1	2.5	3.5
CM 9460-34	2.5	2.5	SM 2632-5	2.5	3.5	SM 2739-1	4.0	3.0
CM 9460-35	3.0	2.5	SM 2632-15	3.0	2.5	SM 2739-4	3.5	3.0
CM 9460-37	3.5	3.0	SM 2632-17	2.5	2.5	SM 2786-1	2.5	2.5
CM 9460-38	2.5	2.0	SM 2632-22	3.5	3.0	SM 2786-5	3.5	2.0
CM 9460-39	2.5	4.0	SM 2633-3	2.5	3.0	SM 2786-7	3.0	2.5
CM 9460-40	3.5	3.0	SM 2633-10	3.0	3.0	SM 2786-9	2.5	2.0
CM 9460-41	2.5	2.5	SM 2634-4	3.5	2.0	SM 2786-10	2.5	2.0
CM 9460-42	4.5	3.0	SM 2634-7	2.5	2.0	SM 2786-15	2.5	2.5
CM 9461-1	4.0	2.5	SM 2634-8	3.0	2.0	SM 2786-18	3.5	3.0
CM 9461-2	4.0	2.0	SM 2634-9	3.5	2.5	SM 2787-1	3.0	2.5
CM 9461-3	2.5	3.0	SM 2634-13	3.5	2.0	SM 2787-4	3.0	2.5
CM 9461-5	2.5	2.0	SM 2635-4	2.5	2.0	SM 2787-5	4.5	2.5
CM 9461-6	3.0	3.0	SM 2635-6	3.5	3.0	SM 2787-13	3.5	3.5
CM 9461-7	2.5	2.0	SM 2635-12	3.5	4.0	SM 2790-2	4.5	2.0
CM 9461-8	2.5	3.0	SM 2636-4	3.5	3.5	SM 2790-17	4.0	2.5
CM 9461-10	2.5	2.5	SM 2636-5	2.5	2.5	SM 2790-18	3.0	3.0
CM 9461-11	2.5	3.0	SM 2636-6	2.5	2.0	SM 2790-27	4.0	2.5
CM 9461-12	2.5	2.5	SM 2636-10	2.5	2.5	SM 2790-28	4.0	2.5
CM 9461-13	4.0	2.5	SM 2636-14	2.5	2.0	SM 2790-32	4.0	2.5
CM 9461-14	4.0	2.5	SM 2636-18	2.5	2.0	SM 2791-2	4.0	2.5
CM 9461-15	3.5	3.0	SM 2636-19	4.0	4.0	SM 2791-5	3.5	2.0
CM 9461-17	3.0	2.5	SM 2636-20	4.0	2.0	SM 2791-12	3.0	3.0
CM 9461-18	2.5	3.0	SM 2636-26	2.5	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	3.0	4.0
CM 9461-32	5.0	3.0	SM 2636-30	4.0	3.5	SM 2792-3	3.0	2.0
CM 9461-35	3.0	2.5	SM 2636-42	2.5	2.0	SM 2792-6	4.0	3.5
CM 9461-36	4.0	3.0	SM 2636-44	2.5	2.5	SM 2792-11	2.5	2.5
CM 9461-51	2.5	2.5	SM 2638-6	3.0	4.0	SM 2792-12	4.0	2.5
CM 9461-53	2.5	3.0	SM 2638-10	2.5	2.0	SM 2792-14	5.0	4.0
CM 9461-56	3.0	2.5	SM 2638-11	3.5	3.0	SM 2792-16	3.0	2.5
CM 9462-17	3.0	3.0	SM 2638-12	3.0	2.5	SM 2792-28	4.0	3.0
CM 9463-2	2.5	2.5	SM 2638-13	2.5	3.0	SM 2792-31	3.0	3.0
CM 9463-10	2.5	2.5	SM 2638-17	3.5	2.0	SM 2792-32	2.5	4.0
CM 9463-15	3.5	3.0	SM 2638-20	3.5	2.0	SM 2792-36	3.0	2.5
CM 9463-19	4.0	2.5	SM 2638-23	2.5	2.5	SM 2792-37	4.0	2.5
CM 9464-1	2.5	2.5	SM 2638-27	3.5	3.0	SM 2792-38	3.0	4.0
CM 9464-3	3.5	3.0	SM 2638-40	2.5	2.0	SM 2792-42	4.0	3.0
CM 9464-19	2.5	4.0	SM 2638-44	2.5	2.0	SM 2792-43	4.0	2.0
CM 9464-26	2.5	2.5	SM 2640-1	3.5	2.5	SM 2792-50	3.5	2.5
CM 9464-27	2.5	2.5	SM 2640-6	4.0	2.5	SM 2792-52	4.0	3.0
CM 9464-29	2.5	3.0	SM 2640-7	3.0	2.5	SM 2793-7	2.5	2.5
CM 9464-30	2.5	2.5	SM 2640-8	2.5	2.0	SM 2794-2	2.5	2.5
CM 9464-33	2.5	3.5	SM 2640-9	3.0	2.0	SM 2794-18	3.5	3.0
CM 9464-36	2.5	4.0						

<sup>a</sup> CBB = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.0; susceptible, from 3.5 to 5.0.  
 SED = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.5; susceptible, from 4.0 to 5.0.

**Activity 7.3. Evaluation of 7 cassava varieties for their resistance to *Phytophthora* spp. in on-farm trials established in two departments of Colombia (Cauca and Quindío), where root rots are endemic.**

**Specific objectives**

1) To evaluate the reaction of 7 different cassava genotypes to root rots under field conditions.

Trials were conducted with the active participation of farmers and UMATA technicians in the Departments of Cauca and Quindío.

**Department of Cauca**

In the Department of Cauca, two trials were established in the village districts of San Jerónimo and Mondomito, Municipality of Santander of Quilichao, to evaluate the control of some practices over *Phytophthora* spp., fungi which induce root rot. The treatments include evaluation of varieties for resistance to root rots.

The following treatments were evaluated for their effect on the incidence and severity of root rots:

**Treatment**

- 1 2.5 t/ha chicken manure + 300 kg/ha of the chemical fertilizer Agropremix® (15% N, 10% P<sub>2</sub>O<sub>5</sub>, 12% Zn, 2% B, 0.75% Cu, 3% S, and 0.01% of Mo)
- 2 2.5 t/ha chicken manure + potassium sulfate (180 kg/ha K<sub>2</sub>O)
- 3 2.5 t/ha chicken manure + potassium chloride (180 kg/ha K<sub>2</sub>O)
- 4 2.5 t/ha chicken manure + thermotherapy (stakes immersed in water heated over a wood fire to 49°C for 49 min)
- 5 *Trichoderma* strain 14PDA-4 (1 × 10<sup>4</sup> conidia/mL)
- 6 *Trichoderma* strain 19TSM-3A (1 × 10<sup>4</sup> conidia/mL)
- 7 Cassava variety La Reina (CM 6740-7)
- 8 Stake selection
- 9 2.5 t/ha chicken manure (traditional farmer's practice)

For all treatments, chicken manure was incorporated at 2.5 t/ha. The cassava regional variety Verdecita (M Col 1505) was planted with vegetative seed obtained from a farm located in San Jerónimo, where the disease was present. The two best strains of the *Trichoderma* fungus were selected to control *Phytophthora* spp. in *in vitro* tests and in the greenhouse. Cassava stakes were inoculated with *Trichoderma* by immersion for 10 min in a suspension with a concentration of 1 × 10<sup>4</sup> conidia/mL. We then applied 100 mL of the suspension at the base of each plant, and again every 45 to 60 days throughout the crop's cycle. Stakes were selected for their health and from the middle parts of stems.

The experimental design used for these plantings was a randomized complete block design with three replicates and 20 plants per treatment. Treatment 6 was applied only in San Jerónimo.

Following farmers' customs, for the San Jerónimo trial, dolomitic lime was applied at 500 kg/ha and fertilizers were applied 35 days after planting. In contrast, in Mondomito, fertilizers were applied at planting and no lime was applied. The performance of the elite genotype CM 6740-7 ('La Reina') was evaluated.

Plant height and stake production per plant were greatest when the trial was fertilized with Agropremix. Table 7.5 shows the effect of the treatments on yield and incidence of rotten roots. All treatments surpassed the control in stake production per plant. Yield under all treatments in San Jerónimo was very low because of low-fertility soil and the plot's history of six cassava crops previous to the trial. Chemical fertilization did not increase yield,

whereas treatments with *Trichoderma* 14 PDA-4 and selection of stakes improved yields by 33.6% and 25.8%, respectively, although root-rot incidence was higher than for the control. In contrast, *Trichoderma* 19 TSM-3A helped reduce root rots. Potassium sources also helped reduce rots. The variety La Reina showed no root rots.

The Mondomito trial could not be harvested because of public order problems.

Table 7.5. Effect of root-rot management on yield and incidence of rotten roots, Farm “Villa Fernanda”, San Jerónimo Village District, Santander de Quilichao, Cauca.

Treatment	Plant height (m)	Stake production per plant	Yield (T/ha)	Root rot disease		
				Incidence (% affected plants)	Severity (Kg affected roots/ha)	Percentage of affected roots
Agropremix	2.1	10.2	3.63	14	183	4.8
K <sub>2</sub> SO <sub>4</sub>	1.9	8.4	3.2	5	50	1.5
KCl	2	8.5	3.6	5	67	1.8
Stake selection	2	9.4	4.38	4	150	3.3
Thermotherapy	2	8.2	3.95	23	150	3.7
Control, traditional farmer's practice	1.9	7.9	3.48	16	100	2.8
Trichoderma strain 14PDA-4	2	8.6	4.65	17	175	3.6
Trichoderma strain 19TSM-3A	2	9.1	3.15	5	33	1.0
Cassava variety Reina (CM 6740-7)	2.8	8.4	5.15	19	0	0.0

### Department of Quindío

The different control practices for *Phytophthora* spp. were evaluated for disease incidence and severity, and for yield in four field trials in the Municipalities of Montenegro and La Tebaida. Two experiments were established on the Farms “El Jardín” (La Tebaida) and “Guayaquil” (Montenegro) to evaluate the effect of some management practices for controlling *Phytophthora* spp. Variety HMC-1 was used, and the treatments were as follows:

#### Treatment

- 1 Fertilization with KCl (180 kg/ha K<sub>2</sub>O).
- 2 Fertilization with K<sub>2</sub>SO<sub>4</sub> (180 kg/ha K<sub>2</sub>O).
- 3 Farmer fertilization: Farm “El Jardín” applied 350 kg/ha of a mixture of ammonium sulfate and borax at a rate of 50:1.5; Farm “Guayaquil” applied 500 kg/ha of a mixture of Nitrax-DAP-KCl at a rate of 1:2:2. Fertilizers were applied 45 days after planting.
- 4 Stakes given thermotherapy (49°C for 49 min).
- 5 Stakes immersed for 5 min in Orthocide® (captan, 4 g/L of the commercial product) and Ridomil® (metalaxyl, 3 g/L of the commercial product).
- 6 Stakes immersed in Lonlife® (ascorbic acid) at 4%.
- 7 Biological control: stakes immersed for 10 min in a suspension of *Trichoderma* (1 × 10<sup>4</sup> conidia/mL), strains 19TSM-3A and 41 PDA-3A. The area around the stake was treated with 100 mL/plant of the fungal suspension.
- 8 Varietal resistance, using genotypes ‘HMC-1’, ‘ICA Catumare’, ‘M Per 183’ (‘Peruana’), and the local variety ‘Chiroza’ (M Col 2066).

The experimental design was a randomized complete block design, with three replicates and 20 plants per treatment. The treatments with thermotherapy and *Trichoderma* were as described for the trials in Cauca (Treatments 4, 5, and 6).

The highest yields were obtained with the crop management practices suggested by CIAT: stake immersion and periodic applications of a suspension of the biological agent *Trichoderma* strain 14 PDA-4. Compared with local practices, applications of potassium sulfate and potassium chloride improved yield. The incidence of drying was only 13% (scale of 2 or 3), a low level for evaluating the effects of treatments. In general, germination and plant development were good. The application of Micobiol® increased plant height considerably (Table 7.6).

Table 7.6. Effect of stake treatments, including hot water, biocontrol, chemical control, fertilizers, and varietal resistance, on cassava development, root rot disease, and cassava bacterial blight in a trial established in the Department of Quindío, Colombia.<sup>a</sup>

Control practices	Plant height (m) <sup>b</sup>	Root yield (t/ha)	Stakes per plant	Bacterial blight		Root Rot Disease		
				Incidence (% affected plants)	Severity (%)	Incidence (% affected plants)	Severity (T affected roots/ha)	% affected roots
Variety HMC-1								
Thermotherapy <sup>c</sup>	1.73	62 a	36 a	21 a	89	2 a	3.7 a	5.6
Biocontrol with <i>Trichoderma</i> spp. <sup>d</sup>	1.89	63 a	36 a	16 a	89	2 a	1.8 a	2.8
Micobiol® <sup>e</sup>	2.31	60 a	37 a	12 a	56	1.3 a	0.2 a	0.3
Ridomil (metalaxyl)	1.91	70 a	39 a	16 a	89	1.7 a	1.0 a	1.4
Potassium chloride (KCl)	1.90	70 a	37 a	18 a	100	2 a	0.3 a	0.4
Potassium sulfate (K <sub>2</sub> SO <sub>4</sub> )	1.90	80 a	38 a	24 a	100	2 a	1.1 a	1.4
Local varieties								
Manzana	1.93	41 a	36 a	21 a	100	2 a	7.1 a	14.8
HMC-1	1.86	51 a	37 a	22 a	100	1.8 a	6.1 a	10.7

a. Duncan's multiple range test, alpha ≤ 0.05.

b. At 7 months after planting.

c. Oil drum on wood fire, with the water's temperature at 49°C for 49 min.

d. Strain 14 PDA-4.

e. Contains *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thuringiensis*.

f. Duncan's multiple range test, alpha ≤ 0.05.

g. At 7 months after planting.

h. Oil drum on wood fire, with the water's temperature at 49°C for 49 min.

i. Strain 14 PDA-4.

j. Contains *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thuringiensis*.

At Farm "El Jardín", the highest cassava yield was obtained with 'ICA Catumare', which surpassed by more than 20 t/ha the varieties HMC-1, Chiroza, and M Per 183, whose yields ranged between 32.0 and 38.7 t/ha. At Farm "Guayaquil", 'ICA Catumare' and 'HMC-1' surpassed 'Chiroza' (Table 7.7).

Table 7.7. Effect of management practices for root rots on plant growth in cassava, Farm “El Jardin”, La Tebaida, Quindío, and Farm “Guayaquil”, Montenegro, Quindío.

Treatment	El Jardín		Guayaquil		Average	
	Plant height (m)	No. of stakes/plant	Plant height (m)	No. of stakes/plant	Plant height (m)	No. of stakes/plant
Fertilization						
KCl (180 Kg/ha K <sub>2</sub> O)	1.81	8.4	2.14	9.9	1.98	9.2
K <sub>2</sub> SO <sub>4</sub> (180 Kg/ha K <sub>2</sub> O)	1.92	10.3	1.92	7.1	1.92	8.7
Control farmer <sup>a</sup>	1.88	11.8	1.86	8.9	1.87	10.4
Control without fertilization	1.89	10.1	1.89	9.2	1.89	9.7
Stake treatment						
Thermotherapy (49°C during 49 min)	1.92	9.7	1.86	6.5	1.89	8.1
Orthocide® (4 g/L) + Ridomil® (3 g/L) <sup>b</sup>	1.74	9.0	1.85	8.7	1.80	8.9
Lonlife® 4%	-	-	2.14	8.5	2.14	8.5
Biological control						
Trichoderma strain 41PDA-3A	1.75	8.2	2.07	7.7	1.91	8.0
Trichoderma strain 19TSM3A	1.88	10.7	1.92	6.9	1.90	8.8
Varietal resistance						
Chiroza	2.59	18.3	2.34	17.2	2.47	17.8
HMC-1	1.81	10.7	2.30	10.5	2.06	10.6
Ica Catumare	2.03	11.0	2.80	13.9	2.42	12.5
M Per 183	1.82	10.2	-	-	1.82	10.2

Farmers’ fertilization management, which involved high doses, led to the highest yields, but also to the highest incidence of root rots. Although Farm “Guayaquil” obtained the higher yield (28.9 t/ha) with the *Trichoderma* strain 41 PDA-3A, it was not consistent with what happened on Farm “El Jardin”, where yield (28.3 t/ha) was much lower than the control without fertilizer (47.9 t/ha; Table 7.8).

Table 7.8. Effect of root-rot management practices on yield and on incidence of rotten roots at the Farms “El Jardín” (La Tebaida, Quindío) and “Guayaquil” (Montenegro, Quindío).

Treatment	El Jardín			Guayaquil			Average		
	Root yield (t/ha)	Roots affected by Root Rot (kg/ha)	Root Rot (%)	Root yield (T/ha)	Roots affected by Root Rot (kg/ha)	Root Rot (%)	Root yield (t/ha)	Roots affected by Root Rot (kg/ha)	Root Rot (%)
Fertilization									
KCl (180 Kg/ha K <sub>2</sub> O)	42.6	0	0.0	23.4	439	1.8	33	220	0.7
K <sub>2</sub> SO <sub>4</sub> (180 Kg/ha K <sub>2</sub> O)	29.9	0	0.0	22.3	0	0.0	26.1	0	0.0
Farmer’s control <sup>a</sup>	50.5	0	0.0	23	1869	7.5	36.8	935	2.5
No fertilization	47.9	0	0.0	19.2	575	2.9	33.6	288	0.8
Stake treatment									
Thermotherapy (49°C/ 49 min)	35.1	123	0.3	20.8	1768	7.8	28	946	3.3
Orthocide® (4 g/L) + Ridomil® (3 g/L) <sup>b</sup>	37.3	0	0.0	27.9	514	1.8	32.6	257	0.8
Lonlife® 4% + (ascorbic acid)	-	-	-	23.4	114	0.5	23.4	114	0.5
Biocontrol with Trichoderma									
Strain 41PDA-3A	28.3	0	0.0	28.9	247	0.8	28.6	124	0.4
Strain 19TSM 3A	32.4	0	0.0	14.7	41	0.3	23.6	21	0.1
Varietal resistance									
Chiroza	38.6	0	0.0	15.5	3086	16.6	27.1	1543	5.4
HMC-1	38.7	0	0.0	25.2	24	0.1	32	12	0.0
ICA Catumare	59.5	597	1.0	28.9	1028	3.4	44.2	813	1.8
M Per 183	32	3009	8.6	-	-	-	32	3009	8.6

<sup>a</sup> Farm “El Jardín”: ammonium sulfate + borax (50:1.5) at 300 kg/ha. Farm “Guayaquil”: Nitrox-DAP-KCl (1:2:2) at 500 kg/ha.

<sup>b</sup> At Farm “Guayaquil”, Orthocide® was replaced by copper oxychloride.

When potassium sulfate was used, root rots were not present. Stake treatment with Lonlife® led to the greatest reductions of root rots. The varieties most affected by root rots were Chiroza and M Per 183, whereas variety HMC-1 had the least root rots. The *Trichoderma* strain 19 TSM-3A helped perceptibly to reduce root rots, although the resulting yields were not good (Table 7.8).

At Farm “El Jardín”, 65-day-old plants were affected by the bacterium *Xanthomonas axonopodis* pv. *manihotis* in some treatments. The bacterium was not present in treatments with K<sub>2</sub>SO<sub>4</sub>, thermotherapy, nor in the genotypes ‘ICA Catumare’ and ‘Chiroza’, which have shown acceptable resistance to the disease, whereas ‘HMC-1’ and ‘M Per 183’ are susceptible. As the crop aged, incidence of the bacterium became insignificant.

At Farms “Las Mercedes” and “El Jardín”, where incidence of cassava bacterial blight is high, some 35-day-old plants were evaluated as being affected by *Xanthomonas axonopodis* pv. *manihotis* in treatments with KCl, farmers’ control, *Trichoderma* spp., chemical control, and in genotypes ‘M Per 183’ and ‘HMC-1’.

### Comparing Departments

Table 7.9 compares selected trials carried out during the project. Thermotherapy of cassava stakes before planting and the use of *Trichoderma* are practices that have a good effect on yield. The use of KCl is recommended for Quindío. The variety La Reina (CM 6740-7) is a very good option for farmers in Cauca. The Chiroza, the variety traditionally planted in the Eje Cafetero, produced much less than did ‘ICA Catumare’ or ‘HMC-1’.

Table 7.9. Cassava yield under management for root rots. Averages across five trials established in the Quindío and Cauca Departments of Colombia.

Treatment	Root yield (T/ha)						
	Quindío				Cauca		Average
Montenegro (Cantores)	Montenegro (Guayaquil)	La Tebaida (El Jardín)	Average	Santander de Quilichao (San Jerónimo)	Quilichao (El Turco)		
Thermotherapy	62	21	35	39.3	4	15	11.5
Trichoderma	63	22	30	33.5	3.9	-	3.9
KCl	70	23	43	45.3	4	-	4
K <sub>2</sub> SO <sub>4</sub>	-	22	30	26	-	9	9
Manzana	41	-	-	41	-	-	-
Chiroza	-	15	39	27	-	-	-
La Reina (CM 6740-7)	-	-	-	-	5	-	5
Ica Catumare	-	29	59	44	-	-	-
HMC-1	-	25	39	32	-	-	-
M Per 183	-	-	-	-	-	-	-
Farmer <sup>a</sup>	51	23	51	41.7	4	15	9.5

<sup>a</sup> Montenegro and La Tebaida: HMC-1; Santander de Quilichao: Verdecita

### Activity 7.4. Characterization of F<sub>1</sub> progeny and parental material of families K (M Nga2 x CM 2177-2) and CM 9582 (M Bra 1045 x M Cr 81) regarding their resistance to *Phytophthora* root rot.

#### Specific objectives

- 1) To evaluate individuals from families K and CM 9582 for their reaction to root rot.
- 2) To understand the genetic of resistance to *Phytophthora* spp.

Cassava roots from 38 individuals of family CM 9582 (M Bra 1045 x M Cr 81) and its parents were inoculated with fungal discs of *Phytophthora* isolates 44 (*P. tropicalis*), P12 (*P. melonis*), P4 (*P. palmivora*), and 69 (*Pythium* sp.). Root damage was determined by measuring width and length of lesions at 5 days after inoculation. Variety M Bra 12 was used as control.

Four groups of varieties were formed by Ward’s minimum variance cluster analysis, with 94.5% reliability, based on disease resistance. Root lesions in these groups ranged from 1.8 cm<sup>2</sup> to 9.17 cm<sup>2</sup> for the resistant group, from 9.18 cm<sup>2</sup> to 13.88 cm<sup>2</sup> for the moderately resistant group, from 13.89 cm<sup>2</sup> to 20.13 cm<sup>2</sup> for the intermediate group, and from 20.14 cm<sup>2</sup> to 25.46 cm<sup>2</sup> for the susceptible group (Table 7.10).

Table 7.10. Cassava genotypes from family CM 9582 (M Bra 1045 x M Cr 81) evaluated under laboratory conditions for their resistance to different *Phytophthora* isolates.

Genotype	Isolate <sup>a</sup>				Average lesion size (cm <sup>2</sup> )
	44	P4	P12	69	
CM 9582-1	16.3	10.6	6.9	-	10.48
CM 9582-2	16.9	7.8	4.0	-	7.65
CM 9582-3	24.4	29.8	-	16.8	19.18
CM 9582-4	15.5	19.5	1.8	-	10.03
CM 9582-5	28.8	11.6	-	7.1	14.56
CM 9582-6	20.2	-	6.2	-	9.61
CM 9582-7	16.4	4.1	4.7	-	7.60
CM 9582-8	6.4	13.9	3.2	-	5.87
CM 9582-9	23.1	23.0	-	20.8	18.28
CM 9582-10	31.5	22.5	3.8	-	17.06
CM 9582-11	34.3	26.3	32.8	30.0	21.33
CM 9582-12	22.3	17.2	4.6	-	13.19
CM 9582-13	15.9	22.4	-	31.7	21.07
CM 9582-14	19.0	9.4	3.2	-	7.91
CM 9582-15	21.0	16.4	-	20.8	17.90
CM 9582-16	19.0	16.8	4.1	-	9.35
CM 9582-17	18.0	10.4	-	8.4	9.83
CM 9582-18	20.6	9.8	-	16.4	11.70
CM 9582-20	17.9	13.6	12.3	17.0	10.56
CM 9582-21	15.5	16.4	3.8	-	9.75
CM 9582-22	24.3	25.6	16.6	33.0	16.93
CM 9582-23	24.7	17.9	27.2	22.2	17.30
CM 9582-24	32.9	31.8	-	19.6	25.46
CM 9582-25	15.2	12.9	-	30.0	17.93
CM 9582-26	25.6	13.1	28.6	24.3	17.41
CM 9582-27	15.3	12.6	6.3	-	10.42
CM 9582-28	22.0	-	-	-	11.00
CM 9582-29	16.6	-	6.2	-	8.99
CM 9582-30	10.4	8.8	3.0	-	6.53
CM 9582-31	16.3	18.5	2.3	-	10.65
CM 9582-32	19.6	15.0	5.2	-	7.67
CM 9582-33	39.7	20.0	7.6	-	23.72
CM 9582-34	24.6	22.9	9.8	30.7	17.13
CM 9582-35	15.9	22.2	4.9	-	9.95
CM 9582-36	17.6	13.2	2.5	-	8.33
CM 9582-37	17.2	13.7	4.1	-	9.54
CM 9582-38	17.8	16.1	4.9	-	11.63
CM 9582-40	10.3	11.1	7.2	-	7.85
M Bra 1045	22.7	15.6	-	24.6	15.64
M Cr 81	8.5	16.9	4.3	-	7.82
M Bra 12	16.8	20.6	10.2	29.3	12.12
Average	20.8	16.9	9.14	22.5	
Duncan 5%	6.94	6.94	6.94	6.94	

<sup>a</sup> Origin of isolates: 44, Quindio, Colombia; P4, Colombia; P12, Brazil; 69, Colombia.

Results indicated that 34.2% of the individuals were resistant, 28.9% moderately resistant, 15.8% intermediately resistant, 15.8% susceptible and 5.3% highly susceptible. M Bra 1045 proved susceptible to isolates 69 and 44, and intermediately resistant to P4, while M Cr 81 was resistant to isolates 44 and P12 and intermediately resistant to P4. The control variety M Bra 12 was susceptible to isolates 69 and P4, but showed intermediate or moderate resistance to isolates 44 and P12. Isolate 69 was considered the most aggressive, followed by 44.

Roots from 74 other individuals of the family CM 9582 and 115 individuals of the family K (M Nga 2 x CM 2177-2) were inoculated with *P. tropicalis*. Figures 7.1. and 7.2. show the distribution of total individuals per group according to degree of resistance to this pathogen. In the case of CM 9582, 73.8% of the population was resistant and moderately resistant, with root rot ranging from 9.1% to 23.2%. For family K, 13% of the individuals was moderately resistant, with root rot ranging from 25% to 40%. No resistant materials were found in the family.

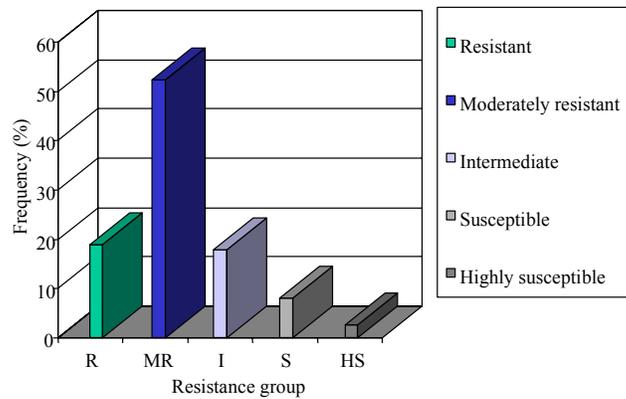


Figure 7.1. Breakdown of family CM9582 (M Bra 1045 x M Cr 81) according to degree of resistance to *Phytophthora tropicalis* inoculated on roots.

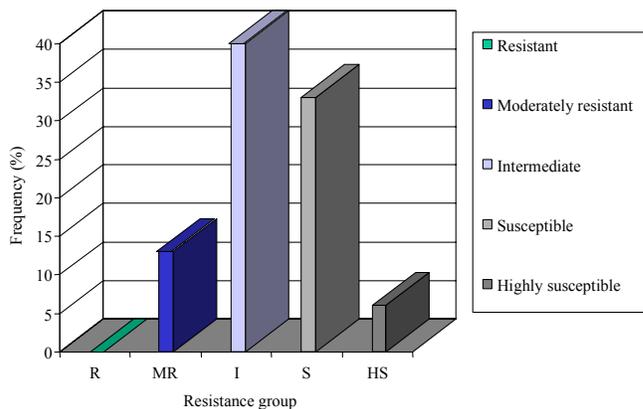


Figure 7.2. Breakdown of family K (M Nga 2 x CM 2177-2), inoculated with *Phytophthora tropicalis* on roots, according to degree of resistance.

**Activity 7.5. Evaluation of probes of resistance gene analogs in individuals of the K family regarding resistance to Phytophthora root rot.**

**Specific objectives**

- 1) To develop molecular markers associated with genes involved in resistance to root rots.

**Materials and methods**

DNA was extracted from leaf tissues of two cassava parental genotypes, M Nga 2 and CM 2177-2, using the Gilboston-Dellaporta protocol. M Nga 2 is intermediately resistant to *Phytophthora tropicalis* and susceptible to *Phytophthora* isolate MTR6, while CM 2177-2 is susceptible to *P. tropicalis* and resistant to isolate MTR6. Genomic restriction with the enzymes *Eco* RI, *Eco* RV, *Hae* III, *Hind* III, *Dra* I, and *Taq* I was done after gel depurination and denaturation. The digested DNA was transferred overnight to a Hybond N+ membrane, using 10 x SSC (NaCl and trisodic citric acid) as transferring solution. The DNA was fixed on the membrane by ultraviolet light in a Stratalinker.

*Escherichia coli* DH5- $\alpha$  cells were transformed by electroporation, introducing pGEM-T Plasmid Vector System (Promega), containing 10 disease resistance gene analogs isolated from maize and rice. Transformed cells were kept at -80 °C in glycerol 30%. Minipreps were prepared with Concert Rapid Plasmid Purification Systems (Gibco-BRL) from transformed cells. A PCR, using primers T7/SP6, M 13F/M13R, T3/T7, was done to amplify inserts, which were then used as probes by marking with <sup>32</sup>P[dATP] to hybridize the with restricted cassava genome of the parents described above.

**Results**

Ten RGAs were successfully multiplied in *Escherichia coli* DH5- $\alpha$  by Cell-Porator<sup>®</sup> Voltage Booster from Gibco BRL, at 2.4 Kv/cm<sup>2</sup>. The transformants were selected on blue/white color screening, using LB/ampicillin/IPTG/X-Gal plates. The complete digestion of genomic DNA was observed using the six enzymes indicated above. Southern analysis for each enzyme and variety was performed. Afterwards, all filters were hybridized with seven different probes from rice and maize, labeled with <sup>32</sup>P. The probe Pic 15, a NBS gene from maize, showed bands hybridized to both parents, at different molecular weight with *Eco* RV (1500 bp for CM 2177-2 and 1600 bp for M Nga 2), *Hind* III (1600 bp for CM 2177-2 and 1500 bp for M Nga 2), *Dra* I (1400 bp for CM 2177-2 and 1500 bp for M Nga 2) (Figure 7.3). This probe will be evaluated first on several resistant and susceptible individuals from crosses between these parents, using the established methodology, and then with the entire population conformed by 144 individuals to determine whether polymorphisms relate to disease reaction.

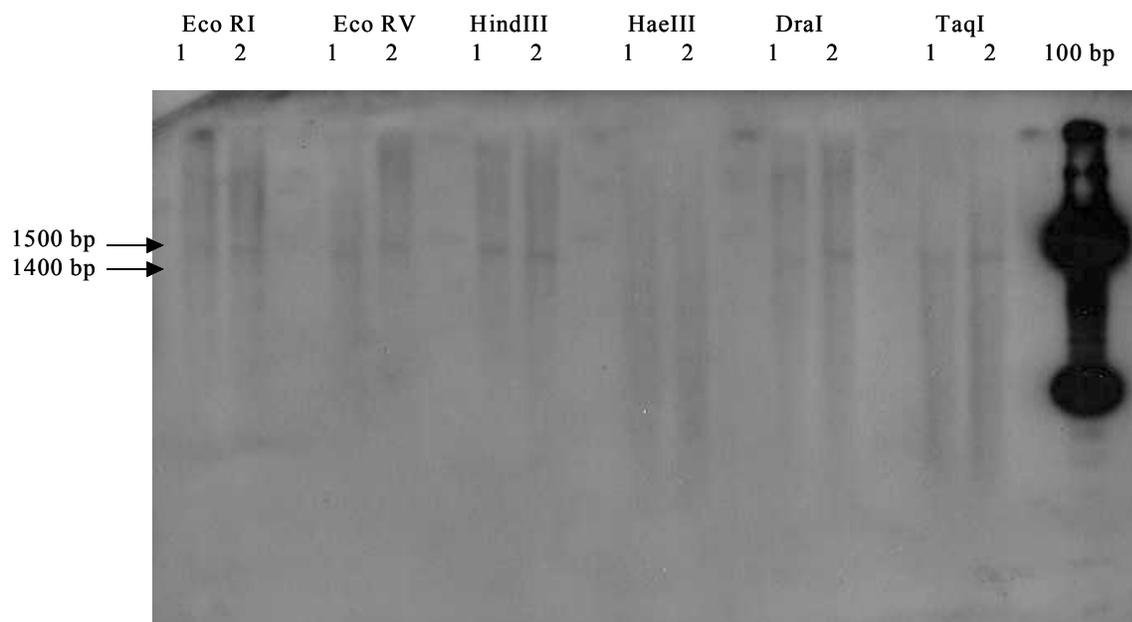


Figure 7.3. Hybridization of probe Pic 15 from maize to DNA digested with six enzymes from M 2177-2 (1) and M Nga 2 (2), parents of the K family of cassava.

**Activity 7.6.** *Use of PCR with degenerated primers as well as low-annealing-temperature PCR to detect polymorphisms between cassava genotypes resistant and susceptible to *Phytophthora* spp.*

#### **Specific objectives**

- 1) *To develop molecular markers associated with resistance to root rots.*

Although the overall sequence homology among disease resistance genes is low and insufficient to be detected by cross hybridization using RFLP, the conserved domains in resistance genes offer opportunities for PCR-amplification and isolation of similar sequences in other plant species.

Five set of primers used in rice by Chen et al. (1998), corresponding to conserved domains in disease resistance genes, were used to amplify similar sequences in cassava DNA from genotypes both resistant and susceptible to *Phytophthora* spp. Each PCR reaction was performed in 25- $\mu$ l volume consisting of 0.2 mM dATP, dCTP, dGTP and dTTP each; 2.5 mM MgCl<sub>2</sub>; 0.25 X Q solution (Qia Gen kit for PCRs); 1.5 U of *Taq* polymerase, 1  $\mu$ M primer; 2.5  $\mu$ l 10X *Taq* polymerase buffer; and 100 ng template DNA. For control reactions, template DNA was substituted by sterile distilled H<sub>2</sub>O. Amplification was carried out in an MSJ-Research PTC-200 thermal cycler programmed for 5 min at 94 °C, 1 min at 45 °C, and 2 min at 72 °C. A 2.5-min ramp time was used between the 94 °C denaturation and the 45 °C annealing steps.

The primer NBS is a sequence from conserved motifs of the nucleotide-binding site in tobacco N and *Arabidopsis* RPS2 gene (Yu et al., 1998). XLRR is a sequence based on the leucine-rich repeat region of the RPS2 and Xa 21

from rice (Chen et al., 1998). Pto is a sequence for potato kinase (Leister et al., 1996). Pox amplifies an intron region of a peroxidase gene in tomato. WIPK amplifies the conserved region of MAK kinase from parsley (Y12875), tobacco (D61377), *Arabidopsis* (MPK3), and *Medicago sativa* (MMK4) (Ligterink et al., 1997).

Primers used are:

XLRR f: 5'- CCGTTGGACAGGAAGGAG- 3'

XLRR r: 5'- CCCATAGACCGGACTGTT-3'

WIPK 1: 5'- GGTCGTGGTGCTTATGGAAT-3'

WIPK 2: 5'-CCATGAAGATGCAACCGAC-3'

NBS f1: 5'- GGAATGGGNGGNGTNGGNAARAC-3'

NBS r1: 5'- YCTAGTTGTRAYDATDAYYYTRC-3'

Pto 1: 5'- ATGGGAAGCAAGTATTCAAGGC-3'

Pto 2: 5'- TTGGCACAAAATTCTCATCAAGC-3'

Pox 1f: 5'- GGAGCTTCTCTCATTCGTCT-3'

Pox 1r: 5'-TAGCAGAATACCTCCATCAC-3'

DNA (100 ng) from three cassava varieties resistant to *Phytophthora* spp. (M Bra 1045, M CR 81, and K 64) and three susceptible (M Nga 2, M Cr 54, and K 142) was amplified. Rice DNA was used as control.

The PCR product was electrophorized in 2% agarose gel in 0.5X TBE buffer. A 100-bp DNA ladder was used to estimate the size of each amplified DNA fragment (Figures 7.4 and 7.5). In addition, 4% polyacrylamide gel electrophoresis was run using a 330-bp DNA ladder (Figures 7.6 and 7.7).

Fifteen clones were obtained with NBS primer and two clones from Pto primer in PCR, obtained from DNA of resistant genotype M Bra 1045. These were ligated in PGEM-T Easy vector. The transformant *E. coli* DH 5- $\alpha$  was obtained by electroporation and conserved in glycerol, at -80 °C. The clones will be sequenced to search for homologies with disease resistance genes reported in Gene Bank (ncbi). With sequences matching resistance genes, primers will be designed to amplify DNA from a segregant population.

Different size bands were observed with electrophoresis in agarose gel (Figures 7.4. and 7.5). Polymorphisms were observed among cassava varieties when polyacrylamide gel was used, but resistant varieties could not be distinguished from susceptible ones (Figures 7.6., 7.7., and 7.8).



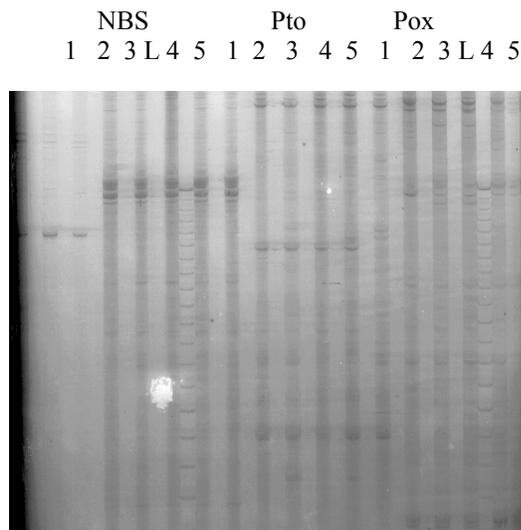


Figure 7.6. DNA amplification of resistant and susceptible cassava varieties with primers NBS, Pto and Pox, corresponding to conserved domains related with disease resistance. Cassava varieties used were M Bra 1045 (1), M Cr 81 (2), K 64 (3), M Col 2066 (4), and K 142 (5). L stands for the 330-bp ladder.

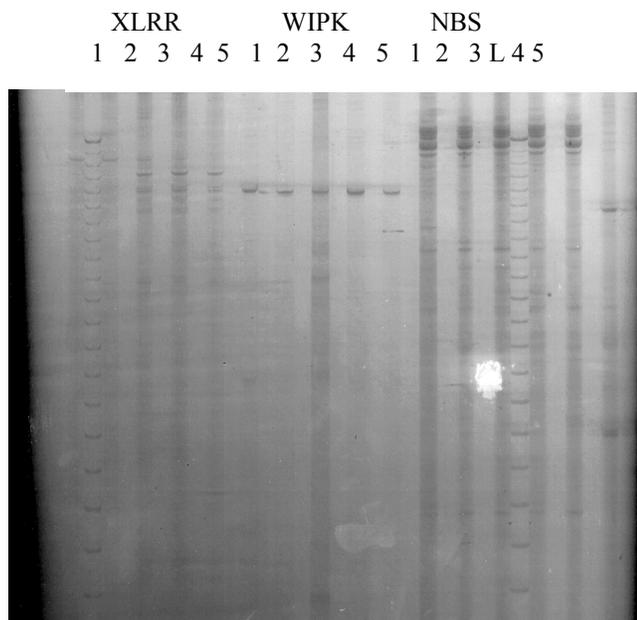
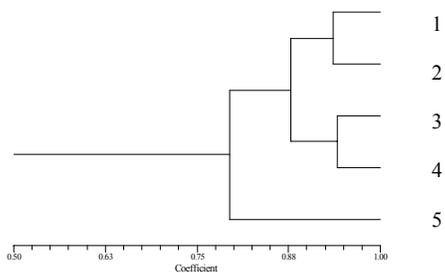
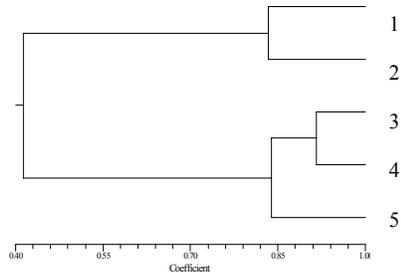


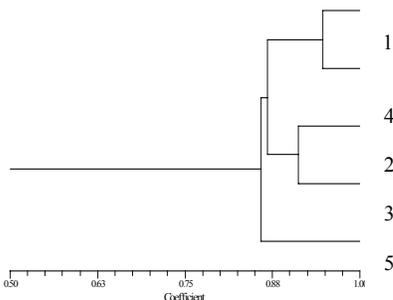
Figure 7.7. DNA amplification of resistant and susceptible cassava varieties with primers XLRR, WIPK, and NBS, corresponding to conserved domains related with disease resistance. Cassava varieties used were M Bra 1045 (1), M Cr 81 (2), K 64 (3), M Col 2066 (4), and K 142 (5). L stands for the 330-bp ladder.



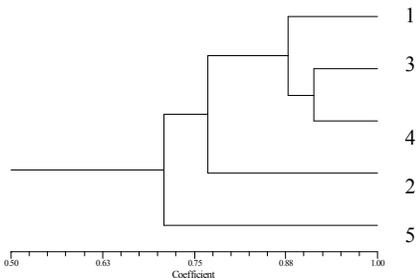
NBS



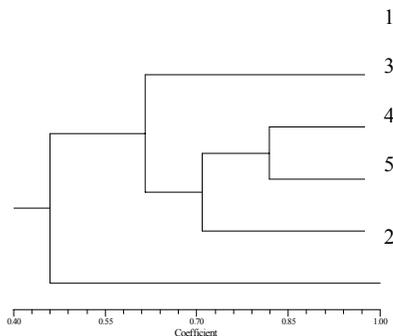
XLRR



Pox



Pto



WIPK

Figure 7.8. Dendrograms of DNA amplified with primers NBS, XLRR, Pox, Pto, and WIPK, used to amplify sequences related to disease resistance and electrophorized on polyacrylamide gel. DNA was used from three cassava genotypes resistant to *Phytophthora tropicalis*—M Bra 1045 (1), M Cr 81 (2), and K 64 (3)—and two genotypes—M Col 2066 (4) and K 142 (5)—susceptible to the pathogen.

**Activity 7.7. Evaluation of cassava bacterial blight and super-elongation disease in cassava varieties from the North Coast of Colombia.**

A total of 381 plants of nine cassava genotypes from Colombia's North Coast were evaluated in the greenhouse at CIAT-Palmira to propagate clean material at CIAT HQ. Plants were kept for 30 days in a chamber with 100% RH to induce symptom expression.

Table 7.11. Evaluation of common bacterial blight (CBB) and super-elongation disease (SED) in in natural infected cuttings of cassava varieties from North Coast of Colombia.

Genotype	Plants						
	No. planted	No. evaluated	Without symptoms (%)	Dead (%)	Affected by CBB (%)	Affected by SED (%)	Affected by other diseases (%)
CG 1141-1	50	33	82	-	6	-	12
CM 3306-4	60	55	36	7	5	36	16
CM 4919-1	80	72	30	4	1	61	4
CM 6119-5	40	25	48	4	-	40	8
CM 6754-8	120	114	44	10	-	40	6
CM 7514-8	40	36	19	14	-	64	3
SM 643-17	40	3	67	33	-	-	-
SM 805-15	40	27	30	7	4	37	22
SM 1201-5	40	16	88	6	-	6	-

Of the plants evaluated, 42% showed no visible disease symptoms, 1.8% were affected by CBB, 40.2% by SED, and 8.4% by other diseases or physiological problems (Table 7.11). A total of 7.4% of the plants died during disease incubation.

**Activity 7.8. Multiplication of promising cassava genotypes to ensure sufficient planting material for both greenhouse and field experiments.**

A total of 286 promising cassava genotypes are being propagated at CORPOICA-Palmira and at a farm located in Ginebra (Valle del Cauca, Colombia) for greenhouse experiments on varietal resistance, genetic studies, and disease management.

**Activity 7.9. Evaluation of frog skin disease (FSD) in cassava varieties in Palmira.**

In a field at Corpoica-Palmira with 95% incidence of frog skin disease (FSD), M Bra 1045 only presented 10% incidence, which was attributed to the low presence of whiteflies observed on this material.

Cassava varieties affected by FSD were grafted on M Bra 1045 to evaluate this genotype resistance. Disease symptoms on roots will be recorded.

**Activity 7.10. Use of meristem culture to clean cassava cuttings of frog skin disease (FSD).**

Table 7.12. lists the 52 cassava genotypes in cleaning process to produce FSD-free material that is used by CIAT's Cassava Pathology Program for experiments on varietal resistance, genetic studies, and disease management.

Table 7.12. Genotypes in cleaning process to produce material free of frog skin disease.

Genotype	Project <sup>a</sup>	Genotype	Project
Cedinha	Brazil – <i>Phytophthora</i> resistance	CM 9582-20	<i>Phytophthora</i> resistance
CM 6975- 8	CBB	CM 9582-22	<i>Phytophthora</i> resistance
CM 6975-14	CBB	CM 9582-23	<i>Phytophthora</i> resistance
CM 7052-2	CBB	CM 9582-25	<i>Phytophthora</i> resistance
CM 7661-12	CBB	CM 9582-26	<i>Phytophthora</i> resistance
CM 7661-15	CBB	CM 9582-27	<i>Phytophthora</i> resistance
CM 7666-10	CBB	CM 9582-28	<i>Phytophthora</i> resistance
CM 7666-25	CBB	CM 9582-29	<i>Phytophthora</i> resistance
CM 7666-31	CBB	CM 9582-30	<i>Phytophthora</i> resistance
CM 7670-4	CBB	CM 9582-31	<i>Phytophthora</i> resistance
CM 7772-2	CBB	CM 9582-34	<i>Phytophthora</i> resistance
CM 7772-11	CBB	CM 9600-2	<i>Phytophthora</i> resistance
CM 7772-15	CBB	CM 9600-5	<i>Phytophthora</i> resistance
CM 7803-1	CBB	CM 9600-6	<i>Phytophthora</i> resistance
CM 7811-9	CBB	CM 9600-17	<i>Phytophthora</i> resistance
CM 7811-15	CBB	CM 9600-20	<i>Phytophthora</i> resistance
CM 8370-14	CBB	CM 9600-21	<i>Phytophthora</i> resistance
CM 9582-2	<i>Phytophthora</i> resistance	CM 9600-24	<i>Phytophthora</i> resistance
CM 9582-5	<i>Phytophthora</i> resistance	CM 9600-25	<i>Phytophthora</i> resistance
CM 9582-9	<i>Phytophthora</i> resistance	CM 9600-31	<i>Phytophthora</i> resistance
CM 9582-10	<i>Phytophthora</i> resistance	CM 9600-39	<i>Phytophthora</i> resistance
CM 9582-11	<i>Phytophthora</i> resistance	IM 175	<i>Phytophthora</i> resistance
CM 9582-14	<i>Phytophthora</i> resistance	Lapa Blanca	Native from Vaupés
CM 9582-16	<i>Phytophthora</i> resistance	M BRA 71	<i>Phytophthora</i> resistance
CM 9582-17	<i>Phytophthora</i> resistance	M BRA 703	CBB and SED
CM 9582-18	<i>Phytophthora</i> resistance	M COL 2737	CBB

<sup>a</sup> CBB = common bacterial blight; SED = superelongation disease; *Phytophthora* resistance in F<sub>1</sub> of CM 9582 (M Bra 1045 x M Cr 81); CM 9600 (M Cr 81 x M Cr 54).

### Activity 7.11. Adoption of CIAT varieties by indigenous groups in Mitú (Vaupés, Colombia).

CIAT varieties CM 2772-3, Ica Catumare, and M Bra 97 were adopted by women of the Tukano indigenous group in Mitú (Vaupés, Colombia). These varieties were planted together with native varieties in several *chagras* (small rural properties) of five indigenous communities, following the traditional planting arrangements that consist of 3 to 30 or more varieties associated with other crops. CIAT variety M Bra 1045 is also being grown in *chagras* of two communities outside the project's area of influence. So far its performance has been acceptable and its good quality has made it appropriate for preparing different foods.

**Activity 7.12. Training of farmers, technicians, and extension agents in participatory research, cassava management, oil palm cultivation, and disease control strategies.**

**Seminars**

Field day on participatory research, incorporation of ash and organic matter (dead leaves and branches from forest surfaces) to improve soil quality, and varietal selection (18 October 2000). Among the 115 participants were technicians from SENA, CDA, JER School, Secretaría de Desarrollo del Vaupés, NGOs, and Seima Central (Mitú).

*Phytophthora* in palms: diagnosis, isolation, and disease management. Asociación de Micología de Colombia, Bogotá (February 2001).

Advances in project management of powdery mildew in rose. Asocolflores, CIAT-Palmira (February 2001).

Seminar on integrated management of cassava diseases and pests, held at CIAT-Palmira (13 July 2001). Among the 19 participants were farmers, technicians from Umatas (Northern Cauca), and students and professors from the Universidad Nacional de Colombia-Palmira.

Field day on integrated management of root rots, held at La Elena Farm, Municipality of Montenegro (Quindío, Colombia) (8 August 2001). The 12 participants included farmers and technicians.

**Training**

Four oil palm technicians trained in bud rot control strategy in Villanueva (Casanare) and Paratebueno (Cundinamarca) (January, March, and April 2001).

Ten professors and students of the Universidad Nacional de Colombia (Palmira) trained in molecular techniques (4-6 April 2001).

Members of 10 indigenous communities in Mitú trained in participatory research, incorporation of ash and organic matter (dead leaves and branches on forest surface) to improve soil quality, and varietal selection (20 April 2001). The 77 participants included technicians from SENA, CDA, JER, Secretaría de Desarrollo, and NGOs.

36 individuals trained in integrated disease management in cassava, presented at “*Curso intensivo sobre el manejo agronómico y poscosecha del cultivo de la yuca con fines industriales*”, Corpoica, Villavicencio (24-26 April 2001).

31 farmers and technicians trained in integrated disease management in cassava, held in El Tambo (Cauca, Colombia) (29 June 2001).

Case study on participatory research to control cassava root rots, presented at the course “*Methods and techniques of farmer participation in research*”, held at CIAT-Palmira (29 June 2001). The 24 participants included CIAT research assistants, professionals from the Ministries of Agriculture of Cuba and Costa Rica, professionals from INIA (Chile).

1-day training offered to Carlos Yepes from Congelagro in research advances in major cassava diseases. CIAT-Palmira (5 July 2001).

18 students and technicians from Sena-Buga (Valle del Cauca, Colombia) trained in integrated disease management in cassava, held at CIAT-Palmira (17 August 2001).

6-month training offered to Mariana Valencia, microbiologist from Levapan S.A., in RAPD and AFLP (February-August 2001).

1-day training offered to Ramón Arbona (Dominican Republic) in research and management of common bacterial blight and superelongation disease in cassava (23 August 2001).

### **Publications**

- ✓ Handbook on “Investigación participativa para el control de pudriciones de yuca con comunidades indígenas de Mitú”. Print run: 500.
- ✓ “Evaluación de la adaptación de variedades de yuca con resistencia a *Phytophthora* spp., mediante investigación participativa en comunidades indígenas de Mitú (Vaupés, Colombia)”. Submitted to *Acta Agronómica*, a journal of Universidad Nacional de Colombia-Palmira (in press).
- ✓ Alvarez, E. and J. F. Mejía. 2001. Assessing virulence and genetic variability of *Sphaceloma manihoticola*, causal agent of superelongation in cassava, in Brazil and Colombia, using RAMS and AFLP. Salt Lake 2001. APS, SON and MSA Joint Meeting August 25-29. Phytopathology 91:S101. Publication no. P-2001-0004-MSA.
- ✓ Alvarez, E. and J. F. Mejía, T. L. 2001. Molecular and pathogenicity characterization of *Sphaceloma manihoticola* isolates from Central-South Brazil. Valle. Plant Disease. In preparation.
- ✓ CIAT in Perspective 2000-2001. People power in the Amazon. p 28.
- ✓ “Evaluación de la adaptación de variedades de yuca con resistencia a *Phytophthora* spp., mediante investigación participativa en comunidades indígenas de Mitú (Vaupés, Colombia)”. *Acta Agronómica*, journal from Universidad Nacional de Colombia, Sede Palmira. In Press.
- ✓ La yuca en el tercer milenio. Integrated Disease Management. Chapter in Handbook for Cassava Crop. 2001. CIAT.
- ✓ “Manual para la identificación de plagas y enfermedades” (Pocket Handbook for Disease Diagnostic.). CIAT, 2001.

### **Ongoing thesis work**

Loke, J.B. Identifying and isolating major genes conferring resistance to causal agents of the root rots *Phytophthora drechsleri*, *P. nicotianae*, and *P. cryptogea* in a segregating population of cassava (*Manihot esculenta* Crantz). Universidad Nacional de Colombia-Palmira, Colombia.

Llano, G.A. Evaluación de la homología de sondas heterólogas en el genoma de yuca y su asociación con la resistencia a *Phytophthora* spp. Thesis work for MS in Agricultural Sciences with emphasis on Plant Breeding. Universidad Nacional de Colombia-Palmira, Colombia.

Celis, A. Determinación del agente causal de la enfermedad “marchitamiento letal” en palma de aceite. Project initiation: 1 September 2001.

Trujillo, O.F. Producción sostenible de yuca en un sistema agroforestal indígena de Mitú (Vaupés), con participación comunitaria. Project initiation: 1 September 2001.

### **Linkages with Other CIAT Projects and with CIAT's Partner Institutions**

BIOTEC (based at CIAT, Colombia)  
CLAYUCA (based at CIAT, Colombia)  
Instituto Agronómico de Campinas (IAC), Brazil

Instituto de Investigaciones de Viandas Tropicales (INIVIT), Cuba  
IPRA (based at CIAT, Colombia)  
Secretaría de Agricultura del Vaupés (Mitú, Colombia)  
UMATAs from Mitú, Santander de Quilichao, Buenos Aires, Caicedonia, La Tebaida, and Montenegro (Colombia)  
Universidad Nacional de Colombia-Palmira, Colombia

***Donors***

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Palmar del Oriente  
Universidad Nacional de Colombia-Palmira (DINAIN)  
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