Output 11

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 biotechnology tools. Despite the "divisions" created by the project structure, these four scientific areas have maintained as close a relationship as possible. In Output 7, progress related to cassava diseases is summarized.

Activity 11.1 Characterizing cassava genotypes for their reaction to super-elongation disease (SED) under greenhouse conditions, using different isolates

Specific objective

To evaluate the resistance to superelongation disease (SED) of promising cassava genotypes for the Colombian Eastern Plains (agroecological zone 2)

Methodology

We evaluated 84 promising cassava genotypes under greenhouse conditions for their resistance to SED, caused by the fungus *Sphaceloma manihoticola*. We inoculated 38 genotypes and the control (M Tai 8) with two isolates (SQ-1 and SQ-2) obtained from Santander de Quilichao (Department of Cauca). The other 45 were inoculated with isolate SV-9, obtained from Villavicencio (Meta). Inoculation was carried out by spraying leaves with a suspension of spores at 1.2×10^6 per milliliter. The plants were planted into plastic bags that were distributed in a randomized complete block design with four replications. The inoculated plants were incubated for 5 days at a relative humidity (RH) of 98% and 30°C. Reaction to the disease was evaluated at days 7, 14, and 21 after inoculation, according to a scale of severity of 1.0 to 5.0, where 1.0 corresponds to no symptoms and 5.0 to plant death.

Results

Tables 7.1 and 7.2 show the genotypes' reaction to *S. manihoticola*. Of the 39 genotypes (including the control) evaluated with isolates SQ-1 and SQ-2, 10% were resistant and 57.5% were intermediately resistant to SQ-1, and 17.6% resistant and 44.1% intermediately resistant to SQ-2. Of the 46 genotypes (including the control) inoculated with isolate SV-9, 23.9% were resistant and 65.2% were intermediately resistant.

Table 11.1 Reaction, under greenhouse conditions, of 38 promising cassava genotypes and susceptible check to two isolates of the fungus *Sphaceloma manihoticola* obtained from Santander de Quilichao, Department of Cauca, Colombia.[a]

	Isolate			Isolate	
Genotype	SQ-1	SQ-2	Genotype	SQ-1	SQ-2
CG 1141-1	2.0	4.0	M Per 183	3.0	3.0
CM 3306-19	3.5	2.5	SM 1144-4	3.0	3.5
CM 3306-4	3.0	3.5	SM 1438-2	2.5	2.5
CM 4919-1	4.0	4.0	SM 1460-1	4.5	4.5
CM 6055-3	4.0	4.5	SM 1479-8	2.5	4.0
CM 6119-5	2.5	2.0	SM 1557-17	3.5	4.0
CM 6754-8	3.5	3.0	SM 1665-2	3.0	3.0
CM 6758-1	2.5	3.0	SM 1821- 7	4.0	4.0
CM 7514-7	2.5	3.0	SM 1871-32	4.0	4.0
CM 9459-13	2.5	2.5	SM 2219-9	3.5	4.5
CM 9460-12	3.0		SM 2330-1	1.0	
CM 9460-15	2.5	3.0	SM 2632-4	4.0	1.5
CM 9460-2	3.0		SM 2730-1	2.5	1.0
CM 9460-40	4.0		SM 2786-10	1.0	2.5
CM 9461-5	3.5		SM 2792-31	4.0	3.0
CM 9463-15	3.0	2.0	SM 2792-32	4.0	1.5
CM 9464-26	4.5	3.0	SM 653-14	3.5	4.0
CM 9464-36	4.0		SM 985-9	3.5	3.5
M Bra 703	3.5	4.5	M Tai 8 (check)	4.0	4.0
M Col 1505	4.0	4.0			
	Resistant (%)		10.0	17.6	
	Intermediate (%)		57.5	44.2	
	Susceptible (%)		32.5	38.2	

a. Resistance is measured according to a scale of 1.0 to 5.0, where scores of 1.0–2.0 indicate resistant; 2.5–3.5, intermediately resistant; and 4.0–5.0, susceptible plants.

Table 11.2. Reaction, under greenhouse conditions, of 45 promising cassava genotypes for the Colombian Eastern Plains and susceptible check to isolate SV-9 of the fungus *Sphaceloma manihoticola* obtained from Villavicencio, Department of Meta, Colombia.[a]

Genotype	SV-9	Genotype	SV-9	Genotype	SV-9
Brasilera	2.5	CM 9463-10	2.5	SM 2640-6	3.0
CM 4574-7	3.0	CM 9463-19	2.5	SM 2640-7	2.5
CM 6438-14	2.5	CM 9464-19	2.5	SM 2640-9	2.0
CM 6740-7	3.0	CM 9464-29	2.0	SM 2644-3	2.0
CM 9459-2	2.0	CM 9464-30	2.5	SM 2726-17	2.5
CM 9459-10	2.0	CM 9464-33	2.5	SM 2727-12	2.0
CM 9460-9	2.0	SM 2452-13	2.5	SM 2727-31	4.0
CM 9460-13	3.0	SM 2601-44	4.0	SM 2739-4	3.0
CM 9460-41	2.5	SM 2634-8	2.0	SM 2786-1	2.5
CM 9461-1	3.0	SM 2636-10	2.5	SM 2786-7	2.5
CM 9461-3	2.5	SM 2636-18	2.0	SM 2787-1	2.5
CM 9461-10	3.5	SM 2636-44	3.0	SM 2787-4	4.0
CM 9461-15	3.0	SM 2638-13	2.0	SM 2791-16	2.5
CM 9461-51	2.5	SM 2638-20	4.0	SM 2792-42	2.5
CM 9461-56	3.0	SM 2638-44	2.5	M Tai 8 (check)	4.0
CM 9462-17	2.0				
		Resistant (%)	23.9		
		Intermediate (%)	65.2		
		Susceptible (%)	10.9		

a. Resistance is measured according to a scale of 1.0 to 5.0, where scores of 1.0–2.0 indicate resistant; 2.5–3.5, intermediately resistant; and 4.0–5.0, susceptible plants.

Activity 11.2. Characterizing cassava genotypes for their reaction to cassava bacterial blight (CBB) under greenhouse conditions, using different isolates

Specific objective

To evaluate the resistance to cassava bacterial blight (CBB) of promising cassava genotypes for the Colombian Eastern Plains (agroecological zone 2)

Methodology

We evaluated, under greenhouse conditions, 65 promising cassava genotypes for agroecological zone 2 (Colombian Eastern Plains) for their resistance to CBB, caused by the bacterium *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). We inoculated 17 genotypes with isolate VM9 and another 48, with isolates VM10, VM11, and VM12, all three obtained from Villavicencio (Meta). Inoculation was by injection of a suspension of the bacterium at a 0.5 nm absorbance, equivalent to 1×10^6 cfu/mL. The plants were arranged in plots with four replications, with the isolate as the principal plot and the genotype as subplot. The susceptible controls were M Col 1505 and M Col 1522. The inoculated plants were incubated for 5 days at an RH of 98% and 30°C. Reaction to the disease was evaluated at days 7, 14, and 21 after inoculation, according to a scale of severity of 1.0 to 5.0, where 1.0 corresponds to no symptoms and 5.0 to plant death.

Results

Tables 7.3 and 7.4 list the reactions of the evaluated genotypes to *Xam*. Genotypes CM 9459-13, CM 9460-41, and CM 9463-15 scored as high as 2.0 to isolate VM9. Genotypes CM 9460-13 and SM 2640-6 showed resistance to isolate VM11, while CM 9460-9 was resistant to isolate VM10. Genotype SM 2638-44 was intermediately resistant (scoring 3.0) to the three isolates. Commercial variety La Reina (CM 6740-7) showed an intermediate reaction to isolates VM10 and VM11 and was highly susceptible to VM12. The commercial variety Brasilera was susceptible to all three isolates. These results confirm field evaluations carried out in previous years (2002 and 2003 annual reports of Project IP-3). Both elite genotypes CM 4574-7 and CM 6438-14 were susceptible to isolate VM10, and CM 6438-14 also to VM12.

Table 11.3 Reaction, under greenhouse conditions, of 17 promising cassava genotypes for the Colombian Eastern Plains and susceptible check to isolate VM9 of the bacterium *Xanthomonas axonopodis* pv. *manihotis*, obtained from Villavicencio, Department of Meta, Colombia.[a]

Genotype	VM9	Genotype	VM9
CM 9459-13	2.0	CM 9464-29	3.0
CM 9460-12	4.0	CM 9464-36	2.5
CM 9460-15	4.0	SM 2632-4	4.0
CM 9460-40	5.0	SM 2638-13	3.5
CM 9460-41	2.0	SM 2730-1	3.0
CM 9461-5	3.5	SM 2786-10	4.0
CM 9462-26	2.5	SM 2792-31	4.0
CM 9463-15	1.0	SM 2792-32	4.0
CM 9464-26	4.0	M Col 1505 (check)	4.0
	Resistant (%)	17.6	
	Intermediate (%)	35.3	
	Susceptible (%)	47.1	

a. Resistance is measured according to a scale of 1.0 to 5.0, where scores of 1.0–2.0 indicate resistant; 2.5–3.5, intermediately resistant; and 4.0–5.0, susceptible plants.

Table 11.4 Reaction, under greenhouse conditions, of 48 promising cassava clones for the Eastern Plains and a susceptible check to three isolates of *X. axonopodis* pv. *manihotis*, from Villavicencio, Meta Department.

Genotype	Isolate			Genotype	Isolate		
	VM10	VM11	VM12		VM10	VM11	VM12
Brasilera	4.0	4.0	4.0	SM 2601-44	3.5	4.0	4.0
CM 4574-7	4.0	3.5	3.0	SM 2634-8	3.0	4.0	
CM 6438-14	4.0	3.0	4.0	SM 2636-10	3.0	3.5	3.5
CM 6740-7	3.0	3.0	4.5	SM 2636-18	4.5	5.0	3.0
CM 9459-10	4.0	3.0		SM 2636-26	4.5	5.0	
CM 9459-2	3.0	2.5		SM 2636-42	5.0	4.0	4.0
CM 9460-13	3.5	2.0	3.5	SM 2636-44	3.0	3.0	3.5
CM 9460-41	3.0	4.0	3.0	SM 2636-6	4.0	4.0	4.0
CM 9460-9	2.0	4.5	3.5	SM 2638-13	3.5	4.0	4.0
CM 9461-1	4.5	4.0	3.5	SM 2638-20	3.5	3.5	3.0
CM 9461-10	4.0	3.0		SM 2638-44	3.0	3.0	3.0
CM 9461-15	4.0	2.5	3.0	SM 2640-6	3.0	2.0	4.0
CM 9461-3	3.0	2.5	3.5	SM 2640-7	3.0		
CM 9461-51	3.0	4.5		SM 2726-17	3.5	3.0	3.5
CM 9461-56	5.0	3.5	4.5	SM 2727-12	4.5	4.5	5.0
CM 9462-17	2.5	4.0		SM 2727-31	4.5	3.5	4.0
CM 9463-10	3.0	2.5	3.5	SM 2739-4	4.0	3.0	4.0
CM 9463-19	2.0	4.5	3.0	SM 2786-1	3.0	3.5	3.0
CM 9464-19	2.5	3.0	3.0	SM 2786-7	4.0	4.0	4.0
CM 9464-29	3.0	4.0	4.5	SM 2787-1	3.5	4.0	3.0
CM 9464-30	3.0	4.0	4.0	SM 2787-4	4.0	3.5	3.0
CM 9464-33	2.0	1.5	2.5	SM 2790-18	5.0	4.5	
M Col 1505 (check)	4.0	4.0	4.0	SM 2791-16	3.0	3.5	4.0
M Col 1522 (check)	4.0	4.0	4.0	SM 2792-42	3.5	3.0	4.0
SM 2452-13	3.0	3.0	3.0	SM 2792-43	4.0	4.5	
		Resistant (%)		5.9	6.0	0.0	
		Intermediate (%)		51.0	46.0	53.7	
		Susceptible (%)		43.1	48.0	46.3	

a. Resistance is measured according to a scale of 1.0 to 5.0, where scores of 1.0–2.0 indicate resistant; 2.5–3.5, intermediately resistant; and 4.0–5.0, susceptible plants

Activity 11.3 Evaluating cassava genotypes for their resistance to super-elongation disease (SED) in Santander de Quilichao, Department of Cauca, Colombia

Specific objective
To evaluate cassava crosses for resistance to SED

Methodology
We evaluated 219 cassava individuals from eight different families for resistance to SED in Santander de Quilichao, under natural disease pressure. The individuals were evaluated in furrows of six plants each. Resistance was determined according to a scale of 1 to 5, where 1 corresponded to no symptoms and 5 to plant death. Plants were considered resistant if they scored 1.0–2.0.

Results
The families with the highest percentages of resistant individuals were GM 308, GM 312, and GM 313. Although disease pressure in 2004 was less than in the previous year, for the second year consecutively, the following genotypes showed resistance: GM 310-26 (M Ecu 72 × SM 1278-2), GM 312-6, GM 312-23 (M Ecu 72 × SM 1673-10), and GM 313-19 (M Ecu 72 × SM 1741-1). Table 11.5 summarizes the reactions of each family.

Table 11.5 Resistance of eight cassava families to superelongation disease under the conditions of Santander de Quilichao, Colombia. The female parent for all eight families was M Ecu 72.

Family	Male parent	Number of individuals	No. of resistant individuals[a]	Resistance (%)
GM 306	M Per 183	29	4	13.8
GM 308	CM 6740-7	28	16	57.1
GM 309	SM 1219-9	26	7	26.9
GM 310	SM 1278-2	28	11	39.3
GM 311	SM 1636-24	26	2	7.7
GM 312	SM 1673-10	28	18	64.3
GM 313	SM 1741-1	27	14	51.9
GM 314	HMC-1	27	4	14.8
	Total	219	76	

a. Resistance was measured according to a scale of 1.0 to 5.0, where scores of 1.0–2.0 indicate resistant; 2.5–3.5, intermediately resistant; and 4.0–5.0, susceptible plants.

Activity 11.4 Evaluating cassava genotypes growing in Pescador, Department of Cauca, Colombia, for their resistance to Phytophthora root rots (PRRs)

Specific objective
To evaluate elite cassava genotypes for resistance to PRRs in the field, with farmer participation

Methodology
On a farm in Pescador, located at 1500 m above sea level, five promising cassava genotypes for industrial use were evaluated for starch production, adaptation, farmers' preferences, and resistance to PRRs. We established plots with 20 plants each, arranged in a randomized complete block design with three replications. A group of farmers from the region evaluated the genotypes for their adaptation, height, and yield, and ranked the materials according to their preferences.

Results
Genotype SM 1053-23 had the highest yield (1.8 kg/plant). It was also the most preferred by the farmers, not only for its yield but also for its similarity with the local variety Algodona. This latter is well accepted on the market for the quality and quantity of its starch. The farmers rejected the other four genotypes for their low yields or short plant stature (Table 11.6).

Table 11.6 Characteristics of six elite cassava genotypes, destined for industrial use, and local check that were evaluated by a group of farmers from Pescador, Department of Cauca, Colombia.

Variety	Yield (kg/plant)	Stem height (cm)	Preference ranking	Farmers' observations
CM 7438-14	0.58	50	–	Very low heigth; low yields
M Bra 383	0.57	100	–	The only one with a purple root peel; low yields
SM 1053-23	1.80	150	1	Most preferred because of its high yields and similar to 'Algodona', although root peel is slightly paler
SM 1058-13	0.80	50	–	Yields are too low
SM 1937-1	0.78	70	–	Disliked
Algodona (check)	1.30	80	2	Yields are good

Activity 11.5. Evaluating of cassava genotypes growing in the departments of Sucre and Córdoba, for their resistance to FSD and SED.

We evaluated a total of 145 cassava clones for their resistance to frogskin disease (FSD) and superelongation disease (SED). At Montería, Córdoba, 81 clones from an Advanced Yield Trial were planted and, at La Unión, Sucre, 64 clones from a Preliminary Yield Trial. The experimental design was random complete blocks with three replications. Plant germination was evaluated at day 30 after planting, and showed rates between 3% and 100%. No symptoms of the two diseases were observed. For SED, further evaluations will be made when the plants are 6, 9, and 12 months old and, for FSD, at harvest.

Activities 11.6 and 11.7 Identifying the association between foliar resistance and root resistance to Phytophthora tropicalis. Determining resistance in roots and leaves to P. tropicalis during its penetration and post-penetration phases.

Objective
To identify different types of resistance of 22 cassava clones to *Phytophthora tropicalis*, a causal agent of root rots.

Introduction
A methodology was developed to evaluate resistance of cassava roots and leaves to *P. tropicalis* during its penetration and post-penetration phases. The methodology was then validated in 22 cassava clones. Finally, the relationship between root resistance and leaf resistance was established in terms of the pathogen's two infection phases.

Methodology
Plant materials. In Palmira (Department of Valle del Cauca, Colombia), roots were selected from 10-month-old plants of 26 cassava clones. The roots of clones CM 523-7, CM 7951-5, HMC-1, M Per 183, SM 1855-15, and SM 2160-2 were evaluated, with two replications over time, because they showed contrasting reactions to infection by *P. tropicalis* when both leaves and roots were inoculated.

Roots were harvested and washed with potable water, disinfested with 1% sodium hypochlorite for 5 min, followed by 50% ethanol for 5 min, and then washed again in sterilized deionized water.

Leaves nos. 3, 4, and 5, taken from the apical part of the stem of 22 clones, were harvested in Palmira and Jamundí (Valle del Cauca, Colombia), disinfested in 10% ethanol, and washed twice in deionized water. In addition to the two evaluations of leaves from the field, leaves were also taken from clones CM 523-7, CM 7463-2, HMC-1, M Col 2760, SM 1219-9, SM 1642-22, and SM 1660-4 grown for 1 month in a screen house located at CIAT, Palmira. These clones had contrasting reactions to *P. tropicalis* during the first two evaluations.

Phytophthora tropicalis.
For the experiments, we used isolate no. 44 from the collection held at CIAT and identified as *P. tropicalis*.

Inoculum for leaf lobes.
Zoospores on disks of cassava leaves inoculated with isolate no. 44 were obtained as follows: disks, with 8-mm diameters, of cassava leaves were taken from 1-month-old plants growing in a screen house at Palmira. They were then disinfected by immersion for 1 min in 10% ethanol and then washed with deionized water.

Eight disks, placed on two slides, were added to each of one petri dish per clon, holding moist paper toweling. The slides kept the leaf disks away from the towel. The disks were then inoculated with fragments of mycelium in suspension, grown in nutritive broth. The petri dishes were incubated in the laboratory at temperatures between 25°C and 28°C and in alternating 12 h light and 12 h dark. The presence of sporangia was checked daily.

Sporangia were harvested with a needle and then suspended in water containing 0.01% of Tween® 80. To liberate the zoospores, the suspension was then incubated for 37 min at about 4°C. To inoculate the leaf lobes, a concentration of 1×10^4 zoospores/mL of inoculum was used. This concentration was selected as optimal through a test of serial dilutions that had previously been carried out. The concentration of inoculum was ascertained by counting the zoospores in a hemacytometer under a light microscope.

Inoculum for roots.
The pathogenicity of the isolate was recovered by a re-isolation of inoculated roots. The roots were inoculated by placing a fragment of culture, 5 mm in diameter, in a perforation made with a punch (López and Lozano 1992). Re-isolation was obtained 5 days after inoculation by planting infected tissue in V8A medium modified with antibiotics and fungicides (Sánchez 1998). The root fragments were disinfested in 50% ethanol for 30 s or 60 s. The inoculum (hyphae) was obtained from the margins of colonies growing in V8 agar culture medium containing penicillin.

***Genetic resistance to* P. tropicalis.**
Resistance to the penetration phase was determined according to the percentage of lesions with diameters greater than 5 mm, obtained at several inoculation sites in leaves and roots. The area covered by the lesion was used to indicate the degree of resistance during the post-penetration phase of infection in roots and leaves.

Determining resistance to* P. tropicalis *in cassava roots.
Cassava roots were inoculated without wounding by placing 3 to 9 disks of mycelial growth of *P. tropicalis* on each root's surface at several sites. The disks measured 5 mm in diameter and 1 mm thick. Each disk was then covered with masking tape. Each root was placed on a sterilized, moistened paper towel in a plastic bag. We evaluated four roots per cassava clone and incubated them at temperatures between 20°C and 25°C in the dark. As checks, one root per cassava clone was inoculated with a disk of medium culture with no mycelial growth. The 22 clones were organized according to a randomized complete block design.

At days 6, 9, and 12 after inoculation, transverse cuts were made to one (days 6 and 9) or two (day 12) roots per clone at the site where the inoculum was placed. The number of lesions was determined, and the infected area estimated (%). To determine the area infected by the pathogen, we took into account the area showing fluorescence under ultraviolet light at 365 nm (Spectroline®, Lonlife™ Filter, Ultraviolet Fluorescence Analysis Cabinet).

To evaluate resistance to post-penetration, roots were inoculated by perforating the peel. Evaluation was carried out 6, 9, and 12 days later by making a transverse cut through each root section and determining the percentage of area infected, according to the symptoms described above.

Determining resistance to* P. tropicalis *in leaves.
From each clone, two leaves (four lobes), 5 cm long, were taken. Their extremes were covered in paraffin and the lobes placed on two slides in a petri dish containing moistened paper toweling.

Evaluating resistance to* P. tropicalis *during infection of leaf epidermis.
To determine resistance to penetration by the pathogen, 3 drops of 30 µL each of the suspension of zoospores (1×10^4 zoospores/mL) were deposited on the lower surface of the leaf lobe without wounding, using a 200-µL micropipette. Each drop was covered with a sterilized disk of filter paper (Whatman® No. 1), measuring 6 mm in diameter.

Evaluating resistance to* P. tropicalis *in the post-penetration phase of leaf infection.
To determine the resistance of leaf tissue, the midrib and blade of each leaf lobe was perforated with a punch with a 1-mm diameter. On the lower side of the lobe, the perforation was covered with a piece of sticky tape with a 6-mm diameter to prevent loss of the inoculum. Each lobe was inoculated with 30 µL of a suspension of *P. tropicalis* at a concentration of about 1×10^4 zoospores/mL placed within the perforation, using a 200-µL micropipette. As negative checks, two leaf lobes of each clone were inoculated with sterilized distilled water.

All the lobes were incubated in the laboratory at temperatures between 20°C and 25°C. At 72, 96, 120, and 144 h of incubation, the lobes were evaluated in terms of the number of lesions formed and severity, using a semi-quantitative scale.

Experimental design.
Roots. We evaluated 26 cassava clones inoculated with *P. tropicalis*, using four roots per clone and organized according to a randomized complete block design. Each root was inoculated between three and nine sites, which were each evaluated separately. The experimental unit was one root. The roots—from clones CM 523-7, CM 7951-5, HMC-1, M Per 183, SM 1855-15, and SM 2160-2—were evaluated twice (both times harvested from the same place) because they showed contrasting reactions to infection by *P. tropicalis* through leaf inoculation. For the first evaluation, six roots per clone were used and the roots organized according to the randomized complete block design. For the second inoculation, four roots per clone were evaluated, using the same design.

Lobes. Leaf lobes of the clones (in all, 21 clones were evaluated) were organized according to the randomized complete block design. For each clone, four lobes (cut from two plants) were inoculated on their lower side at three inoculation sites, each of which was evaluated separately. The upper surface of the lobe, which had the perforation, had only one inoculation site. The 21 clones were evaluated twice: leaves from Cali (one method of inoculation—perforation of lobe) and then those from Jamundí (two methods of inoculation—with and without perforation of lobe). In addition to the two evaluations of leaves from the field, we also evaluated leaves from clones CM 523-7, CM 7463-2, HMC-1, M Col 2760, SM 1219-9, SM 1642-22, and SM 1660-4, cultivated in a screen house at CIAT, Palmira. These clones contrasted in their reaction to *P. tropicalis* during the first two evaluations.

Statistical analysis.

The roots were evaluated on 3 separate days (days 6, 9, and 12 after inoculation). Observations made on the infected area of root parenchyma were standardized to generate an average per clone. It should be pointed out that each evaluation was destructive, preventing repeated evaluations of a root.

To determine the significance of differences found between the reactions of clones in resisting the different phases of infection by *P. tropicalis* in roots and leaves, we carried out an analysis of variance and a LSD or Tukey's test, using the analytical package STATISTIX 8.0 (1985–2003). The relationship between reaction at penetration and at post-penetration was proven by regression analysis.

Results

Evaluating resistance to* P. tropicalis *during infection of root peel and leaf epidermis.

Without wounding the roots, we obtained infection in all 26 clones inoculated with *P. tropicalis* (Figures 7.1 and 7.2). Differences were significant ($P < 0.05$) among the clones evaluated for root-peel resistance in the penetration phase, according to the percentage of observed lesions. By clone, lesions per root averaged between 18.8% (M Bra 383), and 97.3% (SM 1642-22). Table 11.7. The percentage of lesions was relatively high in commercial varieties such as CM 523-7 (ICA Catumare, 66.2%), SM 2160-2 (59.8%), and M Per 183 (42.2%). Few lesions were recorded for M Col 2737 (26.8%), SM 1855-15 (31.9%), SM 1219-9 (23.8%), and CM 7951-5 (57.0%). These clones formed a cluster at the level of intermediate resistance (Tukey's test, $\alpha = 0.05$).

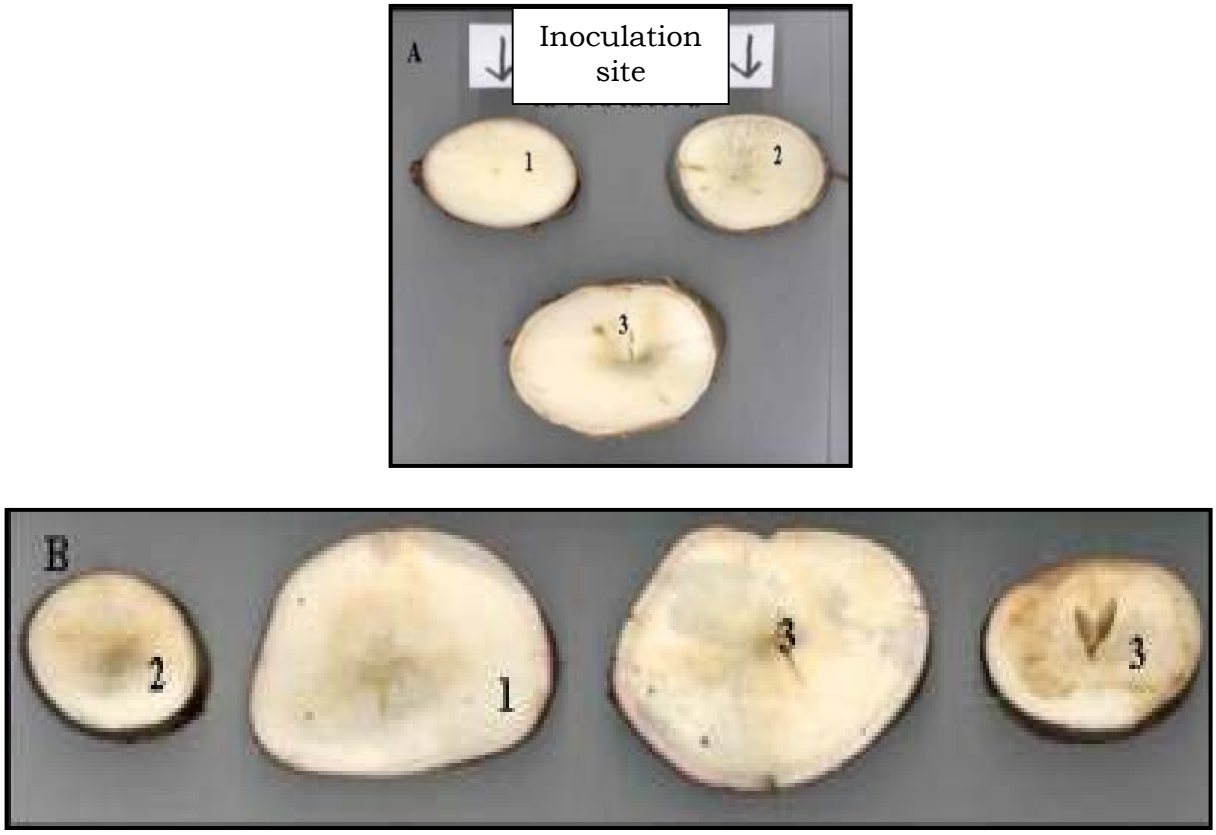

Figure 11.1 Symptoms obtained by inoculating roots with *Phytophthora tropicalis*, a causal agent of root rots. **(A)** No lesions. **(B)** With lesions: (1) presence of scopoletin caused by infection of root by the pathogen; (2) progress towards the root medulla; and (3) maceration of tissue.

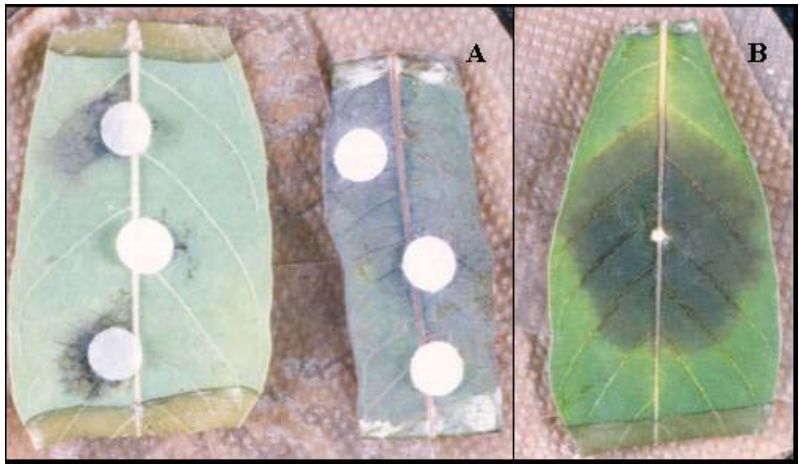

Figure 11.2. Symptoms obtained through inoculating leaf lobes with *Phytophthora tropicalis*, a causal agent of root rots. **(A)** No lesions. **(B)** With lesion.

Table 11.7 Resistance to penetration and tissue resistance in roots and leaves of 26 cassava clones after inoculation with *Phytophthora tropicalis*, a causal agent of root rots. Averages are based on one experiment, otherwise values in parentheses indicate the number of experiments carried out.

		Root resistance			Leaf lobe resistance	
	Clone	To penetration[a]	Of tissue[b]		To penetration[c]	Of tissue[d]
1	M Bra 383	18.8	54.4		–[e]	2.4 (2)
2	SM 1219-9	23.8	21.0		64.6	2.7 (2)
3	M Col 2737	26.8	6.4		–[e]	3.0 (2)
4	M Col 2760	28.8	40.8		66.0	3.1 (2)
5	SM 1855-15	31.9 (2)	19.4		81.3	3.6 (2)
6	(Manihoica P-13)[f]	38.2 (2)	–[e]		36.8 (2)	3.1 (3)
7	SM 2211-3	36.4	32.7		69.4 (2)	4.0 (3)
8	SM 2073-1	36.9	76.8		56.3	2.7 (2)
9	M Per 183 (Peruana)[g]	42.2 (2)	46.8		72.9	3.0 (2)
10	CM 8370-11	47.3	60.1		–[e]	3.2 (2)
11	SM 2085-7	47.5	70.9		72.9	2.4 (2)
12	CM 7951-5	57.0 (2)	25.6		84.1	2.5 (2)
13	SM 2198-4	55.6	56.4		72.9	2.9 (2)
14	SM 2160-2	59.8 (2)	64.3		72.9	2.7 (2)
15	SM 1871-33	59.8	54.0		100.0	3.2 (2)
16	SM 1520-16	59.8	41.8		–[e]	3.5 (2)
17	M Tai 8 (variety Taí)	59.8	41.3		86.8	3.2 (2)
18	SM 2058-2	61.7	43.1		64.6	3.5 (2)
19	SM 1965-1	64.0	46.0		64.6	3.3 (2)
20	ICA Catumare	66.2 (2)	58.5		81.3 (2)	3.4 (3)
21	CM 8370-10	65.2	50.6		66.9	3.2 (2)
22	CM 6660-21	67.3 (2)	33.4		99.6	3.6 (2)
23	SM 1779-7	68.5	50.4		46.3	3.6 (2)
24	SM 1660-4	72.3	42.7		92.4 (2)	3.3 (3)
25	CM 7463-2	79.8	69.0		86.5 (2)	2.4 (3)
26	SM 1642-22	97.3	46.2		77.9 (2)	2.8 (3)
	Minimum	18.8	6.4		36.8	2.4
	Maximum	97.3	76.8		100.0	4.0
	Average	52.8	46.1		73.5	3.1
	St. Deviation	18.9	16.8		15.6	0.4

a. No perforation of the inoculated tissue; resistance measured as of lesions per inoculated root at day 10 after inoculation
b. Inoculated tissue perforated; resistance measured as percentage of lesion area per inoculated root at day 10 after inoculation.
c. No perforation of the inoculated tissue; resistance measured as percentage of lesions per inoculated leaf lobe at day 3 after inoculation.
d. Inoculated tissue perforated; resistance is measured in terms of percentage of lesion area per inoculated lobe at day 5 after inoculation; scale of 1 to 5, where 1 = healthy tissue; 2 = mild symptoms of disease; 3 = intermediate symptoms; 4 = severe symptoms; 5 = whole leaf lobe is infected.
e. Not determined.
f. Check clone with a relatively high level of resistance to root rots, according to cassava farmers in Colombia.
g. Check clone with a high level of resistance to postharvest physiological deterioration of roots (Teresa Sánchez, 2004, CIAT, unpublished data), but low levels of resistance to *P. tropicalis*.
h. Separation from the mean, according to Tukey's test at 5%.

The percentage of lesions on leaf lobes, 3 days after inoculation, fluctuated between 36.8% and 100.0% (Table 11.7), with clones HMC-1 (36.8%) and SM 1779-7 (46.3%) being the most resistant, and SM 1871-33 (100.0%), CM 6660-21 (99.6%), and SM 1660-4 (92.4%) the most susceptible (LSD, $\alpha = 0.05$).

Evaluating resistance to* P. tropicalis *in the post-penetration phase of root and leaf.
Lesion size in roots varied significantly ($P < 0.05$) among the clones. SM 1660-4 was the most susceptible, with 64.3% of area infected (across two experiments; LSD, $\alpha = 0.05$). Detecting significant differences in percentage of lesions between clones, evaluating the lower side of leaf lobes was difficult. A combination ANOVA of experiments 1 and 2 did not help detect significant differences among the clones. However, HMC-1 (scale score of 3.1) and M Col 2760 (3.1) showed adequate levels of resistance in both experiments.

We did not observe resistance in leaf tissue, as all the clones showed high degrees of susceptibility. However, we did see significant differences ($P < 0.05$) among the clones for leaf susceptibility to pathogen invasion, according to lesion size. Lesion sizes, averaging across three experiments, 5 days after inoculation (LSD, $P < 0.05$), for CM 7463-2 (scale score of 2.5), SM 1642-22 (2.8), and HMC-1 (3.0) were moderate (scoring 3.1 or less), whereas the relatively large lesions (scoring equal to or more than 3.0) were produced in SM 2211-3 (3.8), CM 523-7 (3.5), and SM 1660-4 (3.4).

Check clones: HMC-1 and M Per 183. Clone HMC-1 has a relatively high level of resistance to root rot in the field, according to evaluations carried out by cassava farmers in Colombia. Table 11.6 shows that this clone shares the best position with respect to resistance to penetration in root peel. Its reaction for leaf resistance (lesion size) to the pathogen is significantly smaller than that of the most susceptible clones. We need to include in trials validating the methodology, a larger number of clones with known reaction in the field to root rots. Clone M Per 183 has a high level of resistance to postharvest physiological deterioration of roots but with low level of resistance to *P. tropicalis*.

CM 523-7 and its progeny.
Table 11.6 shows that the root peel and parenchyma of clone CM 523-7 (ICA Catumare) is susceptible to *P. tropicalis*. However, clone SM 1855-15 (female parent is CM 523-7) has considerable levels of resistance in both root tissues (Tukey's test, $\alpha = 0.05$).

Relationships during different infection phases between root resistance and leaf resistance.
A correlation of +0.31 (25 clones) was observed for root resistance during (in root peel) and after penetration (in root parenchyma), indicating that the two phases are moderately associated.

We did not obtain correlation (leaves of 22 clones, -0.01) between the reactions caused by the penetration and post-penetration phases of the pathogen in leaves. These two forms of resistance within each tissue (root or lobe) are apparently independent, suggesting that resistance to penetration cannot be predicted by tissue resistance.

Relationship between root resistance and leaf resistance.
The analysis of Pearson's correlation carried out for the reactions during penetration phase of the pathogen in the leaves and roots of 22 clones showed a moderately positive relationship between the two organs (r = +0.37, r^2 = 0.14; Figure 7.3). The moderately positive correlation obtained among the leaves and roots suggests that leaves can be used to predict resistance of roots in cassava populations. To corroborate the validity of using leaf reaction as indicator of resistance to *P. tropicalis*, a representative population of cassava should be evaluated.

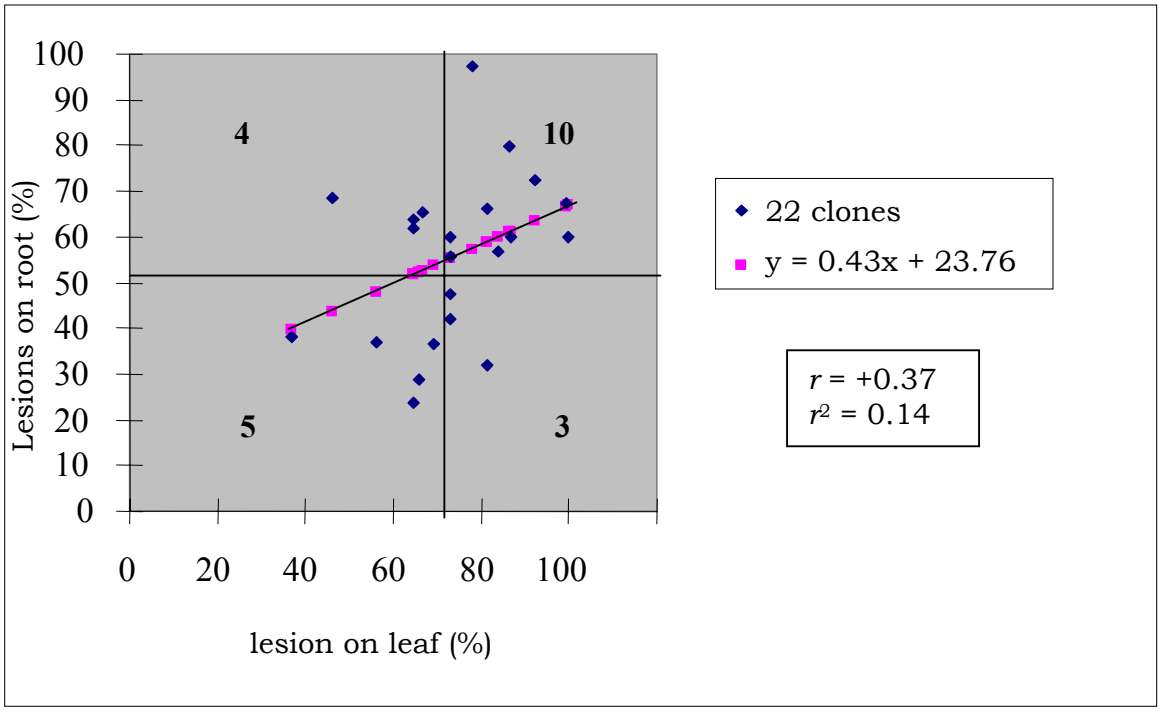

Figure 11.3 Relationship between percentage of lesions (at day 10 after inoculation) in roots and percentage of lesions (day 3) in leaf lobes of 22 clones inoculated with *Phytophthora tropicalis,* a causal agent of root rots. The number in each quadrant indicates the number of clones. The line represents a linear relationship between the two parameters.

Analysis of the 22 clones indicates that most of the clones (15 of 22 clones, or 68%) have similar levels of resistance to penetration in the leaf and root peel (Figure 7.3). Four clones showed a level of leaf resistance that was higher than average, but their level of root-peel resistance to penetration showed they were susceptible. Assuming there is no interest in selecting susceptible clones, then the reactions of 44% of clones (i.e., 4 of 9 clones) selected for their leaf reaction were false because they did not have higher levels of resistance in the root peel.

For the 22 clones, the correlation between resistance in root parenchyma (size of infected area on day 10 after inoculation) and resistance in leaf tissue (size of infected area on day 5) is -0.43, with r^2 = 0.19.

Discussion and conclusions

Agrios (1988) specified that resistance can be effective at the site of the pathogen's entry (i.e., penetration) or during its development within the host's tissue (i.e., post-penetration). In our study, the different cassava clones analyzed demonstrated that different levels of susceptibility exist for both phases of penetration and post-penetration during infection of leaves and roots by *P. tropicalis*. Such results occur according to inoculation method, suggesting that mechanisms of resistance operating in the penetration and post-penetration phases of the pathogen are different (Iwaro et al. 1997).

We observed high clonal variation in the resistance of root peel and parenchyma of cassava. No previous research has demonstrated that unlesioned cassava roots can serve as sites of infection for *Phytophthora*. This is the first report to determine resistance to *Phytophthora* in root peel.

Although the responsible factors for *P. tropicalis* infection of root and leaf should be characterized completely, results suggest that two levels of resistance possibly exist in the roots. This implies that the selection of clones for resistance to root rot caused by *Phytophthora* should be carried out independently for penetration and for post-penetration, so that their combination may be used for breeding, thus increasing existing levels of resistance in cassava to *P. tropicalis*.

The low correlation obtained between resistance of root peel to penetration and resistance of root parenchyma to post-penetration indicates that the mechanism conferring resistance is not systemic within the root.

In several clones, we observed reductions in the size and number of root lesions, which may indicate quantitative resistance to *P. tropicalis*. Quantitative resistance is basically characterized by reduced growth rate, size, and number of lesions, latent period, and capacity to sporulate. These are controlled by several quantitative heredity genes (Parlevliet 2003).

We found a negative correlation of -0.43 (r^2 = 0.19) between resistance of leaf tissue and resistance of root parenchyma. A biological reason may be that the translocation of photosynthetic products, which varies according to the physiological phases of the cassava plant, probably interferes with the level of resistance to *P. tropicalis* in leaves and roots.

This study indicates that resistance in root peel may be predicted by calculating the percentage of lesions found in leaf lobes inoculated in the laboratory or obtained from field plants. Thus, large populations of progenies can be evaluated during the plants' first phase in the field, hence, saving time and costs.

The methodology developed for pre-selecting clones resistant to root rots by evaluating leaf lobes inoculated with *P. tropicalis* results in greater efficiency of cassava genetic-improvement programs. Adult roots, which may take a year to develop, are not required. Another advantage is the possibility of evaluating clones in the greenhouse (e.g., for transgenic plants) or clones that are not adapted to the agroecological zones where germplasm banks are located. Obtaining leaves is easier than obtaining adult roots, especially in the greenhouse.

The lack of a relationship between leaf reactions and root reactions in the post-penetration phase of the pathogen suggests that selection for this component of resistance is in the roots' enlargement phase. In Activity 11.8 we discuss the close relationship between fluorescence of uninoculated roots and resistance to root rot caused by *P. tropicalis*, enabling evaluation to be more efficient.

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STATISTIX 8.0. 1985–2003. Analytical software.

Activity 11.*8 Determining the biochemical markers and agronomic traits associated with* resistance to root rot caused by *Phytophthora tropicalis*

11.8.1. Palmira.

Objective

To optimize the selection of cassava clones resistant to root rot caused by *P. tropicalis* through the use of biochemical and morphological markers

Methodology

Plant materials.

In Palmira (Department of Valle del Cauca, Colombia), roots of 10-month-old plants were selected from 26 cassava clones.

Biochemical markers.

The cassava roots were washed with running water to eliminate soil residues, peeled with a

steel knife, and the peel then dried for 2 days at 40°C in an incubator. A mill was used to pulverize the peel. For each clone, iron and manganese contents were determined by atomic absorption spectrophotometry.

Fluorescence (scopoletin) in root parenchyma.
Once harvested, six roots of each clone were washed with running water, then disinfected with 1% sodium hypochlorite for 1 min and 50% ethanol for 5 min, and given a final wash with sterilized deionized water. Each root received three 3-cm-long incisions to a depth of 1 cm. Three centimeters were cut off each end of the root and the new ends covered with cellophane. Each root was wrapped in moist, sterilized, paper toweling, placed in a plastic bag, and incubated at 20°C to 25°C in the dark. To determine the area presenting fluorescence (scopoletin), the percentage of the fluorescent area was evaluated 10 days after harvest, based on seven transverse cuts made on each root on the day of evaluation. To measure the fluorescence in the roots, we used a dark booth with ultraviolet light at 365 nm (Spectroline®, Longlife™ Filter, Ultraviolet Fluorescence Analysis Cabinet).

***Resistance to root rot caused by* P. tropicalis.**
Two types of resistance were evaluated: (1) resistance of peel to penetration, based on the frequency of lesions in the parenchyma with diameters greater than 1 mm, and obtained at several points of inoculation on the root; and (2) the size of lesion area was used to indicate resistance after the pathogen penetrated the parenchyma.

Statistical analysis.
We carried out analyses of variance, using STATISTIX 8.0 (1985–2003), to determine significant differences among the clones for area of fluorescence (scopoletin). Correlations were calculated between resistance and the following parameters: iron, manganese, iron-to-manganese ratio, and fluorescence (scopoletin). The correlations were then evaluated according to Pearson's coefficient and r^2.

Results

***Relationship between iron and manganese contents in root cortex and resistance to* P. tropicalis.**
Iron content in the root peel of the 16 clones is highly variable, fluctuating between 78.5 and 413.6 ppm (Table 11.8). A coefficient of correlation was estimated as being -0.28 between Fe content and resistance (% of lesions in roots) to *P. tropicalis*.

Although Mn is found in relatively low quantities—between 3.6 and 26.0 ppm (Table 11.8)—in the root peel and parenchyma of the 16 clones, positive correlation was found with resistance to *P. tropicalis* (r = +0.21). This indicated a slight tendency for the percentage of lesions to increase with higher Mn content. A correlation of -0.53 (r^2 = 0.28) was found between resistance to penetration (% lesions on day 10) and Fe and Mn contents (in ppm) in the peel of 16 of the clones (Figure 7.4). The correlation between Fe and Mn contents is +0.42. Iron and manganese contents in the peel are not associated with resistance to *P. tropicalis* in the parenchyma.

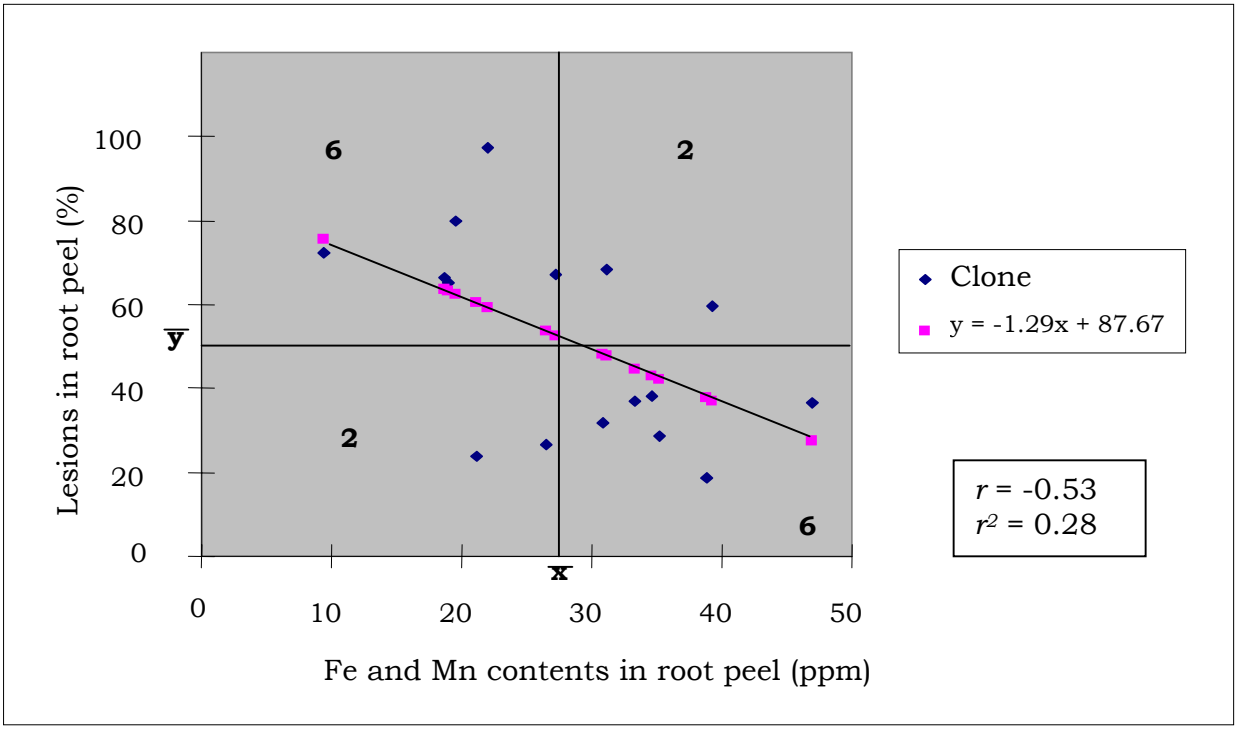

Figure 11.4. Relationship between resistance to *Phytophthora tropicalis* in roots (percentage of lesions, 10 days after inoculation) and the ratio of Fe to Mn contents in the root peel of 16 cassava clones. The bold number in each quadrant indicates the number of clones. The diagonal line represents a linear relationship between the two parameters.

Relationship between resistance to* P. tropicalis *and fluorescence (scopoletin). Significant differences ($P < 0.05$) among the 26 clones were found with regard to area of fluorescence (scopoletin) (Table 11.8). Figure 7.5 presents the simple linear regression between fluorescence and the area of root parenchyma affected by *P. tropicalis*, based on averages of 25 of the clones. The coefficient of correlation for this association is +0.52, with $r^2 = 0.28$. Only clones M Bra 383 and SM 1779-7 showed low presence of fluorescence and high susceptibility to the pathogen (higher than the average for the other 25 clones). A correlation of -0.06 was found between the area of fluorescent parenchyma and resistance to *P. tropicalis* in the peel, thus indicating that no association exists between these evaluation parameters.

Table 11.8 Biochemical characteristics of the roots of 26 cassava clones and their resistance to *Phytophthora tropicalis*, causal agent of root rot.

	Peel				Resistance	
	Fe (mg/kg)	Mn (mg/kg)	Fe/Mn (mg/kg)[a]	Fluorescence in parenchyma	Peel	Parenchyma
CM 523-7	119.1	6.4	18.7	47.8	47.8	58.5
CM 6660-21	255.6	9.4	27.2	17.6	17.6	33.4
CM 7463-2	184.7	9.4	19.6	60.8	60.8	69.0
CM 7951-5	–[c]	–	–	40.5	40.5	25.6
CM 8370-10	138.9	7.3	18.9	43.8	43.8	50.6
CM 8370-11	–	–	–	54.2	54.2	60.1
HMC 1	413.6	11.9	34.6	43.4	43.4	–
M Bra 383	278.9	7.2	38.8	33.2	33.2	54.4
M Col 2737	152.7	5.8	26.5	56.9	56.9	6.4
M Col 2760	239.4	6.8	35.3	34.3	34.3	40.8
M Per 183	–	–	–	41.7	41.7	46.8
M Tai 8	–	–	–	21.2	21.2	41.3
SM 1219-9	135.8	6.4	21.2	38.3	38.3	21.0
SM 1520-16	–	–	–	25.5	25.5	41.8
SM 1642-22	78.5	3.6	22.1	35.8	35.8	46.2
SM 1660-4	244.3	26.0	9.4	31.0	31.0	42.7
SM 1779-7	145.4	4.7	31.1	31.6	31.6	50.4
SM 1855-15	126.6	4.1	30.8	22.0	22.0	19.4
SM 1871-33	–	–	–	51.8	51.8	54.0
SM 1965-1	–	–	–	46.5	46.5	46.0
SM 2058-2	–	–	–	38.7	38.7	43.1
SM 2073-1	130.4	3.9	33.4	60.5	60.5	76.8
SM 2085-7	–	–	–	75.5	75.5	70.9
SM 2160-2	296.9	7.6	39.3	50.5	50.5	64.3
SM 2198-4	–	–	–	57.5	57.5	56.4
SM 2211-3	306.2	6.5	46.9	41.4	41.4	32.7
Minimum	78.5	3.6	9.4	17.6	17.6	6.4
Maximum	413.6	26.0	46.9	75.5	75.5	76.8
Average	202.9	7.9	28.4	42.4	42.4	46.1
St. Deviation	90.9	5.3	9.7	13.8	13.8	16.8

a. Ratio Fe/Mn in dry matter of root peel
b. Area (%) presenting fluorescence (scopoletin) evaluated 10 days after harvest
c. Not determined

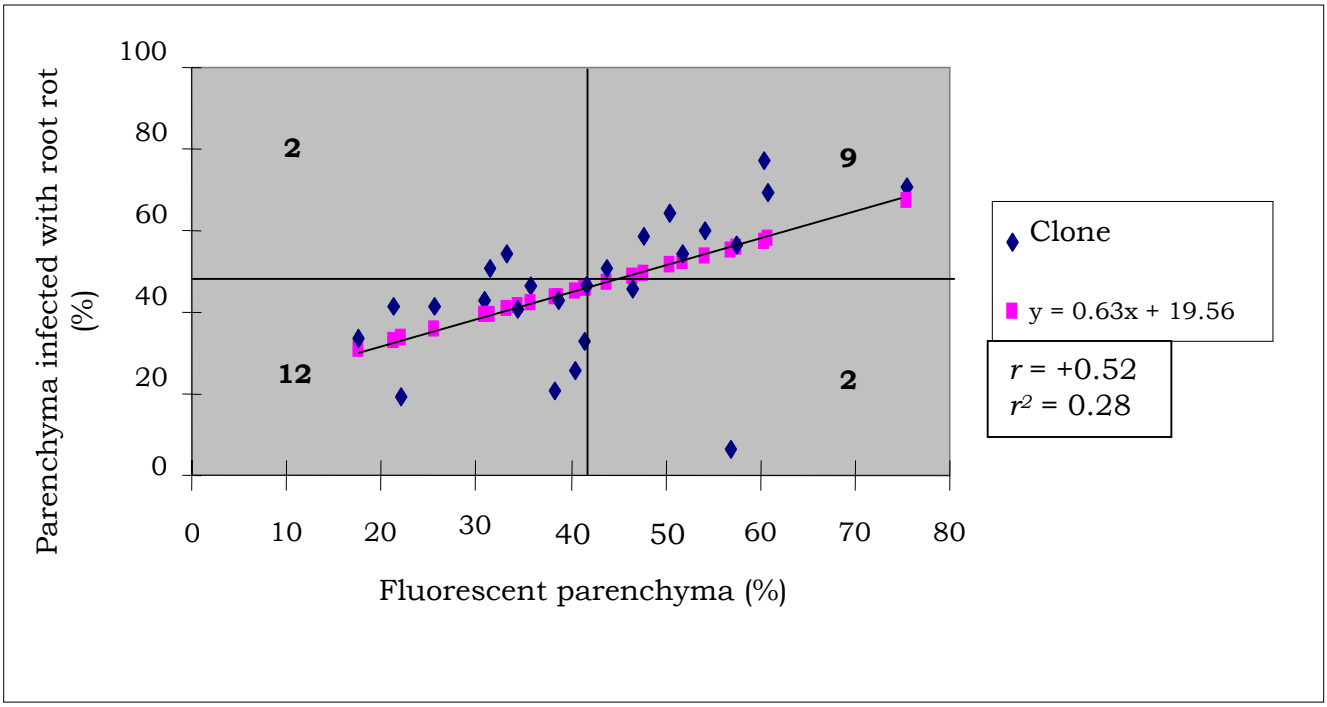

Figure 11.5. Relationship between the presence of scopoletin as observed by long-wave ultraviolet light and resistance of root tissue to *Phytophthora tropicalis* (causal agent of root rot) in 25 cassava clones. Both parameters are expressed as percentage of the area of a transverse cut of a root. The bold number in each quadrant indicates the number of clones. The diagonal line represents a linear relationship between the two parameters.

Discussion and conclusions

We demonstrated that Fe and Mn contents in the root peel of 16 selected cassava clones and the ratio of these two microelements explain 28% of the phenotypic variation in resistance found in the clones. García Mata and co-workers (2001) reported the effect of iron scarcity on *Phytophthora infestans*, showing that infection in cut potato leaves by *P. infestans* was drastically reduced when deferoxamine—an exogenous iron chelator—was applied. More analysis is needed to interpret these results. In tubers, messenger RNA of ferritin increased after treatment with an elicitor. These results suggest that iron has a function in the interaction between potato and *P. infestans*. This is corroborated by findings that several soybean lines, resistant to different races of *Phytophthora sojae*, are tolerant of iron deficiency, which causes chlorosis of the plant (Helms et al. 2002; Orf and Denny 2000). However, Kaitany et al. (2000) report that plants of 12 soybean cultivars suffering high nutritional deficiency, particularly of iron, are more susceptible to *P. sojae*. A similar situation may occur in cassava.

In our study, a close relationship was found between the absence of scopoletin—a coumarin found in very low concentrations in fresh roots but which increases considerably after harvest (Rickard 1982)—and resistance to *P. tropicalis* in cassava roots. This discovery will make preselection of clones simpler and faster because inoculating root parenchyma with *P. tropicalis* will not be necessary. The use of this evaluation parameter in relation to resistance to *Phytophthora* is unknown for plants. However, the relationship between fluorescence of an area and deterioration is reported by Rickard (1982). Agrios (1988) mentions scopoletin in relation to resistance.

We recommend integrating genetic improvement of cassava for biofortification, resistance to root rot caused by *P. tropicalis*, and postharvest deterioration. The magnitude of the genotype-by-environment interaction for iron and manganese contents and area of root parenchyma with scopoletin is currently under study.

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STATISTIX 8.0 1985–2003. Analytical Software.

11.8.2 Jamundí and Rozo.

Objective

To validate the relationship between percentage of scopoletin and morphological traits of roots with two types of resistance to root rots caused by *Phytophthora tropicalis* and with resistance to physiological deterioration in 60 cassava clones

Methodology

Plant materials.
Roots were harvested from 34 cassava clones from a 14-month-old crop growing in the village district of Rozo, Municipality of Palmira, Department of Valle del Cauca, Colombia. Roots were also harvested from 26 cassava clones from a 12-month-old crop growing in Jamundí, Valle del Cauca.

Root morphology.
For the study, a detailed analysis was carried out of the root. Three sections of the root were evaluated separately: proximal (the part closest to the stem), central part, and distal part (the part corresponding to the root's growing point). For each of these sections, evaluations of the variables described below were carried out.

Inoculum.
Isolate no. 44 from the CIAT collection, identified as *Phytophthora tropicalis*, was used. It had grown for 5 days in a V8 culture medium that was modified with antibiotics and fungicides. The medium without pathogen growth was also used as a negative control.

***Resistance to* Phytophthora tropicalis.**
Based on previous field evaluations (Loke 2004), four categories of resistance were defined according to the damage observed: resistant, with less than 10% of root area infected; moderately resistant, with 10% to 30% infected; susceptible, with 30% to 60%; and highly susceptible, with more than 60%. These categories were used to differentiate the clones according to their resistance to the pathogen.

Two types of resistance to *P. tropicalis* were evaluated, according to Iwaro et al. (1997a). The first is resistance to the pathogen's penetration phase, and the second is resistance to the pathogen's post-penetration phase. The methodology is described in detail in Activity 11.8.1. Roots were organized in an experimental design of random complete blocks, with 10 replications and two roots as negative control. The experimental unit consisted of one root with three inoculation points corresponding to the root's proximal, central, and distal parts.

The roots evaluated for resistance to penetration were inoculated without perforating the peel. Ten days later, the presence or absence of rot in the parenchyma was recorded, expressed as (1) percentage of lesions with respect to the total number of inoculation points for each root section; and (2) percentage of area infected in each root section, making a transverse cut and evaluating the percentage of damage as determined by tissue maceration, yellowing, and a smell of fermentation, which are typical symptoms of *P. tropicalis* infection (Loke 2004).
To evaluate resistance to post-penetration, roots were inoculated by perforating the peel. Evaluation was carried out 5 days later by making a transverse cut through each root section and determining the percentage of area infected, according to the symptoms described above.

Resistance to postharvest physiological deterioration.
Ten roots per clone were disinfected by immersing for 5 min in a solution of 1% sodium hypochlorite and then again for 5 min in 50% ethanol. About 2 cm were cut off from each end of each root and each distal extreme was covered with a film of PVC that was secured with a rubber band. Deterioration thus began in the proximal part of the root, permitting evaluation according to a scale for physiological deterioration (Wheatley et al. 1985). The disinfected roots were placed in sterilized plastic bags containing moist paper towelling. The bags were arranged according to a random complete block design with 10 replications. The experimental unit was one root.

Deterioration was evaluated at 10 days, measuring the advance of deterioration through the parenchyma by making five transverse cuts through the root and using Wheatley's scale (1985) of 11 values, as follows:

Scale		Percentage
0	to	0 (no damage)
1	to	10
2	to	20
3	to	30
4	to	40
5	to	50

and so forth until 10 corresponds to 100%. This last value indicates a total change of color in the parenchymatous tissues and xylem bundles from white to bluish maroon in the form of vascular streaks.

Scopoletin. We evaluated the percentage of fluorescence appearing in the transverse cuts of the roots seen in a dark box under ultraviolet light at 365 nm. The roots were inoculated with *P. tropicalis*, using the non-perforation method. Five transverse cuts were made to roots exposed to physiological deterioration.

An adjustment was made to also determine the presence of scopoletin in the parenchyma, that is, the sum of the percentage of macerated tissue caused by inoculation (no perforation) of the peel and the percentage of fluorescent tissue determined under ultraviolet light. This adjustment was applied because where maceration of the parenchyma occurred because of *P. tropicalis*, scopoletin also appeared.

To analyze results, we used a random complete blocks design with 10 replications and two negative controls. The experimental unit was one root.

Peel thickness.
Thickness was determined, using five roots per clone and making two measurements in each section (proximal, central, and distal) of the root with a precision calibrator on fresh roots

from the field. The experimental design was random complete blocks, with five replications; and the experimental unit was one root.

Root hardness.
Hardness was determined in five roots per clone, using a penetrometer that measured resistance in kilograms per square centimeters. Two evaluations were made for each root, in each of the proximal, central, and distal sections of each root. An experimental design of random complete blocks was used, with five replications. The experimental unit was one root.

Moisture content.
To determine the percentage of moisture in the roots, the peel (i.e., outer peel and bark) and parenchyma were removed from three roots per clone. The tissues were then broken up into tiny pieces and dried at 60°C for 2 days. To calculate the percentage of moisture, the samples were weighed immediately after being fragmented, and again immediately after having been removed from the oven, and the weights compared.

Colors of outer peel, bark, and parenchyma.
Five roots per clone were evaluated for the colors of these tissues according to Fukuda and Guevara (1998):

External color of root:	1 = white or cream; 2 = yellow; 3 = pale maroon; 4 = dark maroon
Bark color:	1 = white or cream; 2 = yellow; 3 = pink; 4 = red
Color of root parenchyma:	1 = white; 2 = cream; 3 = yellow; 4 = pink

Data analysis.
Results were processed through the statistical analysis program. The following analyses were carried out: analysis of variance; separation of means, using Tukey's comparison test; and correlations between variables at 5% significance.
Values of resistance to the pathogen, and resistance to physiological deterioration, were transformed by standardizing the normal curve because inoculations had to be done over different seasons, as the number of clones did not permit execution at one date.

Results

Resistance to P. tropicalis *during the pathogen's penetration phase.* Tables 7.9a, 7.9b, 7.10a, 7.10b indicate that results for the non-perforation method were as follows: the most resistant clones in the crop from Jamundí were SM 1871-33 (0% of area was infected), SM 2211-3 (1.33%), SM 1855-15 (3%), and SM 2141-1 (7%), among which no significant differences were observed, according to Tukey's means test (α = 5%).

Table 11.9a. Resistance of the roots of 26 cassava clones to *Phytophthora tropicalis* in terms of rots, lesions, and area with scopoletin, Jamundí, Colombia.

	% rots, perforation method				% rots, non-perforation method				% lesions in parenchyma, non-perforation method				% area with scopoletin, non-perforation method			
Clones	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]
CM 6660-21	34.5	40.5	42.5	39.2	21.2	41.5	55.0	39.2	100.0	100.0	100.0	100.0	65.7	85.5	96.5	82.6
CM 7463-2	43.5	50.0	60.5	51.3	0.0	40.0	56.5	32.2	0.0	100.0	100.0	66.7	47.5	89.0	95.5	77.3
CM 7951-5	50.5	54.0	65.0	56.5	37.5	61.0	53.0	50.5	100.0	100.0	100.0	100.0	85.0	93.5	100.0	92.8
CM 8370-10	42.5	48.0	54.0	48.2	30.5	43.0	26.5	33.3	100.0	100.0	100.0	100.0	65.5	69.0	69.5	68.0
CM 8370-11	53.5	61.0	71.0	61.8	18.0	23.0	32.5	24.5	100.0	100.0	100.0	100.0	71.0	71.1	82.9	75.0
M Col 2759	61.0	71.5	64.0	65.5	46.0	68.5	45.0	53.2	100.0	100.0	100.0	100.0	97.0	99.5	97.0	97.8
M Per 183	55.0	59.5	68.5	61.0	16.5	17.5	27.0	20.3	100.0	100.0	100.0	100.0	39.0	41.5	57.5	46.0
M Tai 8	34.2	37.2	37.7	36.4	0.0	21.5	27.5	16.3	0.0	100.0	100.0	66.7	16.0	51.5	57.5	41.7
SM 1520-16	43.0	51.0	59.5	51.2	0.0	0.0	32.5	10.8	0.0	0.0	100.0	33.3	69.0	100.0	100.0	89.7
SM 1520-18	60.5	70.5	74.0	68.3	0.0	0.0	31.0	10.3	0.0	0.0	100.0	33.3	15.0	37.0	53.5	35.2
SM 1642-22	35.5	49.5	59.5	48.2	0.0	20.5	24.5	15.0	0.0	90.0	90.0	60.0	21.0	52.0	57.0	43.3
SM 1660-4	58.5	59.5	76.5	64.8	41.5	47.0	69.5	52.7	100.0	100.0	100.0	100.0	87.0	95.0	99.0	93.7
SM 1779-7	36.5	63.8	69.0	56.4	40.0	51.0	71.5	54.2	100.0	100.0	100.0	100.0	98.0	94.5	96.0	96.2
SM 1855-15	51.0	50.0	69.0	56.7	2.5	3.5	3.0	3.0	20.0	20.0	20.0	20.0	28.0	30.5	28.5	29.0
SM 1871-33	69.5	75.5	86.5	77.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.5	19.0	19.0	17.2
SM 1959-1	36.5	40.5	43.0	40.0	1.0	5.5	41.0	15.8	10.0	30.0	100.0	46.7	88.5	93.0	99.5	93.7
SM 1965-1	57.8	67.0	71.0	65.3	23.0	31.5	4.0	19.5	100.0	100.0	40.0	80.0	49.0	63.0	25.5	45.8
SM 2052-4	64.0	67.5	79.0	70.2	1.5	54.5	65.0	40.3	20.0	100.0	100.0	73.3	98.5	100.0	97.0	98.5
SM 2058-2	53.5	61.5	69.0	61.3	0.5	26.5	60.0	29.0	10.0	100.0	100.0	70.0	94.0	100.0	100.0	98.0
SM 2073-1	44.0	57.5	60.5	54.0	27.5	21.0	2.0	16.8	100.0	100.0	20.0	73.3	44.0	45.0	21.0	36.7
SM 2085-7	27.0	36.0	53.0	38.7	78.0	9.5	68.0	51.8	100.0	30.0	100.0	76.7	100.0	100.0	100.0	100.0
SM 2141-1	36.4	45.0	55.0	45.5	10.5	9.0	1.5	7.0	100.0	100.0	30.0	76.7	24.0	22.5	11.5	19.3

Continued

Cont. Table 11.9a

Clones	% rots, perforation method				% rots, non-perforation method				% lesions in parenchyma, non-perforation method				% area with scopoletin, non-perforation method			
	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]
SM 2160-2	53.0	56.5	80.5	63.3	68.5	69.5	76.0	71.3	100.0	100.0	100.0	100.0	100.0	99.5	99.5	99.7
SM 2198-4	52.0	57.0	63.0	57.3	27.0	31.0	38.0	32.0	90.0	90.0	90.0	90.0	58.0	70.5	85.5	71.3
SM 2211-3	39.0	43.0	56.5	46.2	1.0	1.5	1.5	1.3	20.0	20.0	20.0	20.0	11.5	12.0	20.0	14.5
SM 653-14	42.5	59.2	60.0	53.9	35.0	33.5	50.5	39.7	100.0	100.0	100.0	100.0	64.5	68.0	85.0	72.5
Maximum	69.5	75.5	86.5	77.2	78.0	69.5	76.0	71.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Minimum	27.0	36.0	37.7	36.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.5	12.0	11.5	14.5
Average	47.5	55.1	63.4	55.3	20.3	28.1	37.0	28.5	60.4	76.2	81.2	72.6	59.6	69.3	71.3	66.7
Standard deviation	16.0	17.6	16.4	13.4	9.4	12.6	12.5	8.8	17.9	19.8	21.6	16.1	12.5	12.6	17.7	10.2
Variation coefficient (%)	33.7	31.9	25.8	24.3	46.2	44.8	33.9	30.8	29.6	26.0	26.7	22.2	21.0	18.2	17.7	15.3
Tukey α=5% non-Transformed [b]	26.3	28.9	26.9	22.1	15.4	20.7	20.6	14.5	70.0	60.0	41.0	26.7	20.7	21.0	21.1	17.0

a. Average of the three sections of root, that is, proximal, central, and distal.
b. Values are not standardized.

Table 11.9b. Resistance of the roots of 26 cassava clones to *Phytophthora tropicalis* in terms of morphological characteristics, and resistance to physiological deterioration, Jamundí, Colombia.

	Physiological deterioration		Hardness of root				Thickness of peel				Color [c]			Humidity %	
Clones	%	% area with scopoletin	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Outer peel	Bark	Paren-chyma	Bark	Paren-chyma
CM 6660-21	24.0	49.2	44.4	40.2	48.2	44.3	2.2	2.4	2.0	2.2	1	1	1	78.24	66.12
CM 7463-2	15.2	36.5	50.2	38.8	46.8	45.3	2.6	3.4	2.2	2.7	2	2	1	74.36	62.63
CM 7951-5	36.4	60.0	50.7	41.6	62.8	51.7	2.2	2.3	1.9	2.1	3	3	1	74.76	60.61
CM 8370-10	22.4	64.6	48.1	40.7	48.6	45.8	2.8	4.7	1.9	3.1	3	3	1	73.48	62.01
CM 8370-11	22.4	46.1	67.3	36.5	32.1	45.3	2.7	3.3	2.5	2.8	4	2	2	75.54	63.26
M Col 2759	36.4	46.5	57.9	39.3	35.1	44.1	2.5	2.5	1.9	2.3	3	3	2	82.35	61.19
M Per 183	38.0	52.8	61.6	47.3	37.7	48.9	2.9	3.2	2.3	2.8	3	4	1	76.89	67.38
M Tai 8	4.2	21.4	56.4	47.2	37.4	47.0	3.3	2.8	2.0	2.7	1	2	2	73.72	61.24
SM 1520-16	20.4	28.1	74.9	52.7	38.5	55.4	2.5	2.7	2.0	2.4	3	1	2	71.30	60.00
SM 1520-18	19.4	57.5	55.9	48.1	54.5	52.8	2.4	2.4	2.0	2.3	2	2	1	67.63	60.85
SM 1642-22	12.2	31.6	48.8	52.2	49.3	50.1	2.7	2.6	1.8	2.4	3	1	1	68.51	60.35
SM 1660-4	20.4	58.6	47.2	37.3	40.5	41.6	2.1	2.2	1.7	2.0	2	2	1	79.32	66.43
SM 1779-7	39.6	57.8	52.8	40.7	46.2	46.6	3.5	3.1	2.4	3.0	3	2	2	73.57	59.96
SM 1855-15	55.2	68.5	64.6	69.6	60.4	64.9	2.9	3.2	2.1	2.7	3	2	1	68.71	59.95
SM 1871-33	28.0	48.8	66.6	65.8	57.9	63.4	2.6	2.9	2.4	2.6	4	4	1	69.20	56.43
SM 1959-1	23.4	54.9	59.6	45.0	40.3	48.3	2.3	2.3	1.7	2.1	3	2	1	76.50	69.97
SM 1965-1	50.0	61.4	56.7	57.1	57.7	57.2	2.8	3.0	1.5	2.4	3	2	1	71.18	63.35
SM 2052-4	21.0	36.9	38.9	35.6	32.6	35.7	2.5	2.4	2.0	2.3	3	1	1	77.62	65.50
SM 2058-2	26.0	55.2	55.0	50.8	54.9	53.6	2.0	2.3	1.8	2.0	3	2	1	72.26	61.22
SM 2073-1	13.4	50.8	55.9	50.8	43.9	50.2	2.8	2.6	2.0	2.4	3	1	1	70.86	58.15
SM 2085-7	36.6	60.1	36.5	32.8	28.1	32.5	2.3	2.1	1.7	2.0	3	1	1	76.85	62.08
SM 2141-1	25.6	33.1	72.3	54.4	46.1	57.6	2.8	3.1	2.4	2.8	3	2	1	70.50	58.24
SM 2160-2	21.2	38.7	53.8	53.6	51.0	52.8	2.3	2.4	1.9	2.2	3	1	1	72.00	60.00

Continued

Cont. Table 11.9b

Clones	% Physiological deterioration		Hardness of root				Thickness of peel				Color [c]			Humidity %	
	%	area with scopoletin	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Outer peel	Bark	Paren-chyma	Bark	Paren-chyma
SM 2198-4	24.4	52.2	60.1	47.9	73.6	60.5	2.1	2.0	2.1	2.0	2	2	1	69.85	57.41
SM 2211-3	20.4	33.2	52.5	54.6	39.2	48.8	2.5	2.8	2.0	2.4	3	2	1	73.37	66.38
SM 653-14	20.6	33.5	63.8	63.0	118.5	81.7	2.4	2.3	1.7	2.1	3	3	1	71.03	55.62
Maximum	55.2	68.5	74.9	69.6	118.5	81.7	3.5	4.7	2.5	3.1	4	4	2	82.35	69.97
Minimum	4.2	21.4	36.5	32.8	28.1	32.5	2.0	2.0	1.5	2.0	1	1	1	67.63	55.62
Average	26.0	47.6	55.9	47.8	49.3	51.0	2.6	2.7	2.0	2.4	-[d]	-	-	73.45	61.78
Standard deviation	28.5	28.8	10.9	6.9	16.8	7.1	0.3	0.9	0.9	0.6	-	-	-	-	-
Variation coefficient (%)	109.4	60.5	19.5	14.4	34.1	14.0	9.7	33.0	14.7	14.5	-	-	-	-	-
Tukey α=5% non-transformed[b]	46.8	47.3	26.1	16.5	40.1	17.0	0.6	2.1	0.7	0.8	-	-	-	-	-

a. Average of the three sections of root, that is, proximal, central, and distal.
b. Values are not standardized.
c. See text: *Colors of outer peel, bark, and parenchyma*
d. Values from one replication.

Table 11.10a. Resistance of the roots of 34 cassava clones to *Phytophthora tropicalis* in terms of rots, lesions, and area with scopoletin, Rozo, Palmira, Colombia.

Clones	% rots, perforation method				% rots, non-perforation method				% lesions in parenchyma, non-perforation method				% area with scopoletin, non-perforation method			
	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]
CM 523-7	42.50	25.50	37.50	35.17	11.0	42.0	67.5	40.2	70.0	70.0	100.0	80.0	96.5	95.5	99.5	97.2
CM 6438-14	46.00	41.00	34.50	40.50	0.0	6.0	16.5	7.5	0.0	0.0	0.0	0.0	9.5	22.5	36.0	22.7
CM 9582-14	41.00	44.00	64.00	49.67	80.5	80.5	66.5	75.8	20.0	40.0	30.0	30.0	100.0	100.0	97.5	99.2
CM 9582-16	36.50	43.00	47.50	42.33	60.0	65.5	72.0	65.8	0.0	0.0	0.0	0.0	95.0	94.5	95.0	94.8
CM 9582-17	42.00	42.00	67.00	50.33	30.5	42.5	49.0	40.7	50.0	50.0	50.0	50.0	55.5	81.5	85.0	74.0
CM 9582-18	36.00	37.00	44.50	39.17	59.0	59.0	75.0	64.3	20.0	20.0	20.0	20.0	90.0	89.0	100.0	93.0
CM 9582-20	39.00	30.00	33.50	34.17	49.0	60.0	61.0	56.7	20.0	20.0	20.0	20.0	87.5	92.5	99.5	93.2
CM 9582-28	58.00	52.50	52.50	54.33	4.5	8.5	16.0	9.7	30.0	10.0	10.0	16.7	9.0	17.0	26.5	17.5
CM 9582-29	33.50	36.00	47.00	38.83	3.0	44.5	44.5	30.7	90.0	90.0	90.0	90.0	49.0	79.5	82.5	70.3
CM 9582-30	49.00	47.00	46.50	47.50	5.0	11.5	29.5	15.3	0.0	40.0	70.0	36.7	14.0	25.0	48.0	29.0
CM 9582-32	35.50	22.00	36.50	31.33	26.0	21.5	69.0	38.8	20.0	0.0	80.0	33.3	54.0	69.5	98.5	74.0
CM 9582-55	19.50	16.50	9.50	15.17	0.5	1.0	19.0	6.8	20.0	50.0	20.0	30.0	15.5	22.0	46.0	27.8
CM 9582-62	31.50	40.00	31.50	34.33	7.0	2.5	42.5	17.3	40.0	40.0	40.0	40.0	26.0	32.0	73.5	43.8
CM 9582-63	44.50	30.00	49.50	41.33	10.0	0.0	0.0	3.3	30.0	30.0	30.0	30.0	22.5	18.0	27.0	22.5
CM 9582-64	37.00	39.50	63.00	46.50	0.0	0.0	0.0	0.0	10.0	40.0	20.0	23.3	14.0	18.5	16.0	16.2
CM 9582-9	28.00	44.50	43.00	38.50	30.0	30.0	25.0	28.3	40.0	40.0	40.0	40.0	30.0	30.0	30.0	30.0
CMC 40	62.00	66.50	69.00	65.83	2.5	14.5	7.5	8.2	100.0	40.0	0.0	46.7	14.5	29.5	20.0	21.3
HMC-1	11.50	17.00	36.00	21.50	20.0	21.5	22.0	21.2	0.0	40.0	50.0	30.0	20.5	24.0	24.0	22.8
M Bra 1044	34.50	67.00	90.50	64.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M Bra 1045	28.50	20.50	20.00	23.00	40.0	40.0	40.0	40.0	90.0	90.0	80.0	86.7	41.0	40.5	41.5	41.0
M Bra 12	48.50	17.50	13.50	26.50	2.0	7.0	5.0	4.7	10.0	0.0	0.0	3.3	11.2	19.5	16.5	15.7
M Bra 532	22.00	25.50	42.00	29.83	20.0	20.0	20.0	20.0	100.0	100.0	100.0	100.0	20.0	20.0	20.0	20.0
M Col 1505	24.00	27.50	26.00	25.83	50.0	50.0	50.0	50.0	100.0	100.0	100.0	100.0	50.0	50.0	50.0	50.0
M Col 2066	6.50	9.50	40.50	18.83	40.0	40.0	40.0	40.0	100.0	100.0	100.0	100.0	44.0	45.2	45.0	44.7
M Cr 81	38.00	31.50	44.00	37.83	20.0	20.0	20.0	20.0	90.0	90.0	90.0	90.0	29.5	30.5	34.5	31.5

Continued

Cont. Table 11.10a

Clones	% rots, perforation method				% rots, non-perforation method				% lesions in parenchyma, non-perforation method				% area with scopoletin, non-perforation method			
	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]
M Nga 2	14.50	14.50	12.50	13.83	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	2.0	1.5	2.0	1.8
M Per 183	72.00	41.50	16.00	43.17	26.5	24.5	31.5	27.5	30.0	50.0	70.0	50.0	99.0	97.5	99.5	98.7
M Tai 8	17.00	14.00	0.00	10.33	100.0	40.0	0.0	46.7	20.0	80.0	100.0	66.7	100.0	100.0	100.0	100.0
SM 1210-4	19.50	40.50	35.50	31.83	30.0	10.0	10.0	16.7	70.0	50.0	90.0	70.0	52.0	50.5	59.0	53.8
SM 1411-5	27.00	41.00	49.50	39.17	0.0	10.0	16.5	8.8	30.0	70.0	100.0	66.7	29.0	39.0	45.0	37.7
SM 1460-1	40.00	48.00	68.00	52.00	20.0	0.0	80.0	33.3	10.0	10.0	50.0	23.3	88.0	96.0	100.0	94.7
SM 1479-8	31.50	26.50	47.50	35.17	23.5	22.5	27.5	24.5	80.0	90.0	100.0	90.0	66.5	65.5	69.5	67.2
SM 1555-17	42.00	59.00	62.00	54.33	29.5	30.5	36.5	32.2	90.0	90.0	100.0	93.3	68.5	69.0	84.0	73.8
SM 1741-1	40.50	57.00	67.00	54.83	24.0	22.0	28.0	24.7	90.0	80.0	100.0	90.0	59.5	58.5	69.0	62.3
Maximum	72.00	67.00	90.50	65.83	100.0	80.5	80.0	75.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Minimum	6.50	9.50	0.00	10.33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average	35.26	35.74	42.56	37.85	24.2	24.9	32.0	27.0	46.2	50.6	57.4	51.4	46.0	50.7	57.1	51.2
Standard deviation	16.51	17.09	17.41	11.56	27.6	27.9	28.2	24.5	35.6	46.5	33.2	29.6	26.6	26.5	26.6	25.1
Variation coefficient (%)	46.83	47.84	40.91	30.55	113.9	111.8	88.2	90.4	77.3	91.8	57.9	57.6	57.8	52.3	46.6	49.1
Tukey α=5% non-transformed[b]	28.13	29.11	29.65	19.70	47.0	47.5	48.0	41.6	60.8	79.1	56.5	50.41	45.3	45.2	45.3	42.8

a. Average of the three sections of root, that is, proximal, central, and distal.
b. Values are not standardized.

Table 11.10b. Resistance of the roots of 34 cassava clones to *Phytophthora tropicalis* in terms of morphological characteristics, and resistance to physiological deterioration, Rozo, Palmira, Colombia.

	Physiological deterioration		Hardness of root				Thickness of peel				Color [c]			Humidity %	
Clones	%	% area with scopoletin	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Outer peel	Bark	Paren-chyma	Bark	Paren-chyma
CM 523-7	44.8	49.9	91.5	62.0	64.7	72.7	2.0	2.6	2.0	2.2	3	3	1	61.8	56.2
CM 6438-14	49.2	65.8	64.4	50.7	48.3	54.5	2.4	2.6	1.3	2.1	3	1	1	67.5	61.3
CM 9582-14	62.4	58.0	58.1	44.7	43.9	48.9	2.7	2.2	1.6	2.2	3	1	2	71.5	60.2
CM 9582-16	41.4	47.2	56.7	60.7	39.5	52.3	2.3	2.8	1.8	2.3	3	1	2	67.8	58.5
CM 9582-17	42.0	45.5	78.4	55.9	46.5	60.3	3.0	2.5	1.9	2.4	3	1	2	67.8	56.3
CM 9582-18	33.8	32.5	57.4	52.3	46.5	52.1	2.2	2.9	2.4	2.5	3	1	2	68.2	58.2
CM 9582-20	50.0	62.7	45.5	44.5	50.1	46.7	3.0	3.8	2.3	3.0	3	1	2	67.0	58.5
CM 9582-28	43.4	41.2	57.7	51.1	37.8	48.8	3.5	3.6	3.3	3.5	3	1	2	70.9	61.0
CM 9582-29	15.2	31.4	40.2	36.3	32.1	36.2	2.2	2.5	1.6	2.1	3	1	2	65.7	59.6
CM 9582-30	44.8	49.6	64.1	46.1	53.4	54.6	2.6	2.7	1.8	2.4	3	1	2	70.8	61.3
CM 9582-32	29.6	31.9	59.0	52.7	40.5	50.7	2.6	2.8	2.2	2.5	3	1	3	71.1	67.7
CM 9582-55	35.6	54.1	35.8	33.6	37.4	35.6	2.6	2.7	2.0	2.4	3	1	2	69.9	65.5
CM 9582-62	28.8	41.4	58.5	60.5	49.9	56.3	2.6	3.5	2.9	3.0	3	1	2	69.6	55.8
CM 9582-63	46.2	42.4	39.7	39.7	31.2	36.9	1.9	1.9	1.4	1.7	3	1	2	74.3	65.2
CM 9582-64	22.8	16.6	66.7	43.0	39.4	49.7	2.2	2.5	2.1	2.3	3	1	3	71.5	66.1
CM 9582-9	37.0	37.4	86.0	72.6	48.9	69.2	3.6	3.8	2.4	3.3	3	1	2	69.4	59.7
CMC 40	31.4	75.9	54.4	52.8	39.2	48.8	1.9	2.3	1.2	1.8	3	3	1	72.2	61.8
HMC-1	18.0	26.8	71.8	68.0	43.9	61.2	2.2	2.6	2.1	2.3	4	4	1	74.4	67.7
M Bra 1044	10.2	10.8	52.1	43.1	54.9	50.0	2.3	2.5	2.0	2.3	3	2	2	68.4	65.1
M Bra 1045	47.0	38.2	88.8	81.4	80.0	83.4	2.3	3.1	2.2	2.5	3	1	2	61.8	57.7
M Bra 12	39.8	44.9	65.5	67.5	69.9	67.6	2.5	3.0	2.2	2.6	2	3	1	72.5	64.9
M Bra 532	30.0	54.9	58.3	53.3	42.8	51.4	2.5	3.3	2.8	2.9	4	1	1	71.5	59.4

Continued

Cont. Table 11.10b

Clones	Physiological deterioration		Hardness of root				Thickness of peel				Color [c]			Humidity %	
	%	% area with scopoletin	Prox.	Cent.	Distal	Avge[a]	Prox.	Cent.	Distal	Avge[a]	Outer peel	Bark	Paren-chyma	Bark	Paren-chyma
M Col 1505	43.4	39.8	75.8	60.0	57.3	64.4	5.0	2.4	1.7	3.0	3	3	1	69.1	55.7
M Col 2066	39.8	49.6	61.2	58.9	34.0	51.4	1.7	2.0	1.4	1.7	4	3	1	68.5	64.2
M Cr81	29.2	29.0	51.5	38.7	44.0	44.7	3.2	3.4	2.4	3.0	4	2	1	70.5	56.6
M Nga 2	57.0	55.4	100.4	77.0	49.0	75.5	3.4	3.2	2.2	2.9	3	2	1	66.4	60.6
M Per 183	34.4	37.6	49.3	40.2	35.7	41.7	2.4	2.4	1.9	2.2	4	3	1	74.2	67.1
M Tai 8	33.8	40.1	66.0	71.1	44.8	60.6	3.0	3.5	2.3	3.0	1	2	2	67.6	57.1
SM 1210-4	16.2	20.9	56.0	36.6	39.1	43.9	2.0	2.2	1.8	2.0	3	2	1	76.5	65.8
SM 1411-5	16.8	29.2	56.5	46.6	65.3	56.1	2.2	3.0	2.5	2.6	2	1	1	68.9	61.8
SM 1460-1	48.6	65.9	48.3	38.5	32.0	39.6	2.5	2.7	1.7	2.3	3	1	1	68.2	63.3
SM 1479-8	10.0	24.6	50.5	39.5	34.7	41.6	1.7	2.2	1.4	1.8	3	1	1	77.2	72.2
SM 1555-17	9.0	5.3	45.2	42.6	40.4	42.7	1.8	2.0	1.7	1.8	4	3	1	73.0	56.3
SM 1741-1	27.2	26.2	48.4	59.2	58.9	55.5	2.1	4.4	1.7	2.7	3	1	1	70.2	60.4
Maximum	62.4	75.9	100.4	81.4	80	83.4	5	4.4	3.3	3.5	4	4	3	77.2	72.2
Minimum	9	5.3	35.8	33.6	31.2	35.6	1.7	1.9	1.2	1.7	1	1	1	61.8	55.7
Average	34.4	40.7	60.6	52.4	46.3	53.1	2.5	2.8	2	2.4	- [d]	-	-	69.9	61.4
Standard deviation	29.2	28.7	13.3	10.8	8.5	6.4	1.3	0.9	0.3	0.6	-	-	-	-	-
Variation coefficient (%)	84.8	70.5	22	20.6	18.3	12.1	50.1	31.5	17.3	23.4	-	-	-	-	-
Tukey α=5% non-transformed[b]	49.7	48.8	31.9	25.9	20.5	15.4	3.1	2.1	0.8	1.4	-	-	-	-	-

a. Average of the three sections of root, that is, proximal, central, and distal.
b. Values are not standardized.
c. See text: *Colors of outer peel, bark, and parenchyma*
d. Values from one replication

For the clones established at Rozo, the most resistant to the disease were M Bra 1044 (0%), CM 9582-16 (0%), CM 6438-14 (0%), M Bra 532 (3.3%), CM 9582-55 (4.66%), SM 1460-1 (6.83%), HMC-1 (7.5%), CM 9582-64 (8.16%), CM 9582-30 (8.83%), and M Per 183 (9.66%). No significant differences were observed (Tukey's, α = 5%). These results confirmed observations made by CIAT (1999) on the resistance in the field of M Bra 1044, M Bra 532, and HMC-1. Moreover, the parents and various individuals of the family CM 9582 (M CR 81 × M Bra 1045) were reported as resistant by Llano (2003). Clone CM 6438-14 ('Vergara') is an elite line for agroecological zone 2 (Eastern Plains). The other clones of Jamundí showed variation in infected area between 10.33% and 71.33%, whereas the clones established in Rozo showed a range between 15.33% and 75.83% of the area affected by the pathogen.

Tables 11.9 and11.10 show the percentages of lesions in the parenchyma for each root section (proximal, central, distal, and the mean of the three). The most resistant of the Jamundí clones for the penetration phase showed the lowest average of lesions (less than 20% of inoculation points). The exception was SM 2141-1, which showed 76.7% of lesions in the parenchyma, indicating that the mechanism for resistance to penetration in the peel of this clone was different to that of the others.

The Rozo clones with resistance to the pathogen's penetration phase—M Bra 1044, CM 9582-16, CM 6438-14, and M Bra 532—also had low average percentages for lesions in the parenchyma (less than 3.33%), indicating that they would also have resistance to the post-penetration phase. The other clones, resistant to the penetration phase, had percentages of lesions between 23.33% and 50%, indicating that variation existed between clones in terms of mechanisms of resistance in the peel.

Resistance to* P. tropicalis *during the pathogen's post-penetration phase.
Evaluations of resistance to *P. tropicalis* in its post-penetration phase (with the perforation method) showed that none of the genotypes established at Jamundí were resistant. In Rozo, clones M Tai 8, M Nga 2, CM 9582-55, M Col 2066, HMC-1, and M Bra 532, among others, were moderately resistant, having a percentage of damage that was less than 20% Tables 11.9 and 11.10. The results obtained with the last two clones confirmed that the peel is an important factor in cassava's resistance to *P. tropicalis*.

Resistance to postharvest physiological deterioration.
The clones established in Jamundí showed 4.2% to 55.2% physiological deterioration, with clone M Tai 8 being the least affected. No significant differences were observed, however, with clones showing 50% deterioration. Similar results were observed for the Rozo clones, where SM 1555-17 showed 9% deterioration and M Nga 2 showed 57%, a not-significant difference according to Tukey's (α = 5%).

Root morphology.
No significant correlations were observed between evaluations for resistance to physiological deterioration and rot caused by *P. tropicalis*, and the morphological traits studied. These findings corroborated those reported by Iwaro et al. (1997b) for cacao. The lack of association of morphological traits with resistance to *P. tropicalis* corroborates the studies conducted by Alvarez et al. (2003), which indicated that resistance to *P. tropicalis* is controlled by minor genes, with considerable influence from the environment.

Scopoletin. Tables 11.9 and 11.10 show the percentages of root area with scopoletin in the 60 clones evaluated in Jamundí and Rozo. The correlation of presence of scopoletin with damage caused by *P. tropicalis* was found to be +0.6 between the area of parenchyma affected and the area with scopoletin. Likewise, the correlation of percentage of root area with scopoletin with postharvest physiological deterioration was +0.7.

The correlations observed for scopoletin with *P. tropicalis* inoculated by the non-perforation method with physiological deterioration show that scopoletin is an expression of root deterioration, whether microbial or physiological (Agrios 1978; Wheatley et al. 1985).

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Activity 11.9 Determining the QTLs that most contribute to phenotypic variance of resistance to Phytophthora root rots, and identifying the linkage group(s) where they are located

Objective
To locate the QTLs associated with resistance to root rots caused by *Phytophthora tropicalis* in the parental maps of cassava family K

Introduction
Deciphering the complexity of genetic resistance is a key element of plant breeding, particularly for diseases such as cassava root rot. We evaluated individuals of cassava family K for their reaction to root rots to better understand the genetics of resistance to *P. tropicalis*. Genetic improvement for resistance to the disease can be more quickly and effectively achieved by using molecular markers.

Methodology

Plant materials.
In 2000 and 2001, we inoculated and evaluated the roots of 92 cassava clones belonging to family K (M Nga 2 × CM 2177-2) from Santander de Quilichao, Department of Cauca, Colombia.

Pathogen.
We used isolate 44 as inoculum. It was identified as *P. tropicalis* by sequencing the ITS region of its ribosomal DNA.

Inoculation.
We inoculated 10 to 12-month-old cassava roots, and extracted a cylinder of root, using a punch with an 8-mm diameter. A disk of mycelial growth, measuring 5 mm in diameter, was deposited in the orifice, which was then covered with the fragment of root just extracted. Each inoculated root was placed in a plastic bag and on a plastic tray, and incubated at 22°C in the dark for 7 days.

Evaluation.
Each inoculated root was cut transversely and the following measurements made: lengths and widths of lesion and cut, root length, and depth of inoculum in the root. The data were then processed through the Excel program.

Data analysis. For analysis, the experimental unit was a root.

Analyzing for QTLs.

The roots of 92 clones of cassava family K were harvested at CIAT. To analyze for QTLs, we used parental maps based on the segregation of alleles of the maternal (M Nga 2) and paternal clones (CM 2177-2) according to different classes of markers (172 in maternal and 192 in paternal). These markers were restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), isoenzymes, microsatellites, expressed sequence tags (ESTs), and known genes.

The analysis and mapping were carried out with the QGene program, version 36, on McIntosh equipment, using simple regression or single-marker analysis. The dependent variable was the reaction to the pathogen and the independent variable was the number of alleles in the marker's locus, depending on the segregation of the individual. To minimize the detection of false positives, a significant association between the DNA marker and resistance to *Phytophthora* was determined if the probability of no QTL being present was less than 0 05. The degree of phenotypic variation explained by each marker was obtained with the coefficient of regression (r^2).

Results

Resistance of family K to *P. tropicalis* was based on results obtained in 2003.

Table 11.11 shows the results of the regression analysis for the simple marker as the percentage of infected area of roots inoculated in the laboratory. The markers identified eight QTLs located in the linkage groups C, H, J, N, Q, and V of the maternal map. The QTLs explained between 1.3% and 9% of phenotypic variance. NS911 (microsatellite) was the most significant QTL, located in linkage group V. The markers identified six QTLs located in linkage groups A, D, I, M, and N of the paternal map. In this group, the most significant QTL was rGY32 (RFLP), which explained 11% of phenotypic variance and was found in linkage group A.

Discussion and conclusions

The variability in the expression of resistance from year 2000 to 2003 indicates that cassava family K presents polygenes (Llano 2003). The QTLs associated with resistance to *P. tropicalis* in this study are different from the ones reported by Llano (2003). Nor were the linkage groups the same. Environment usually influences phenotypic expression, generating variation. Even so, certain clones of family K that had expressed intermediate resistance in 2000 continued expressing it in 2001.

Both parents of family K are susceptible to *P. tropicalis*. However, a group of clones in this family presented intermediate resistance. This indicates that the parents were heterozygotes and that both have resistance genes. Fregene et al. (1997) demonstrated that family K is heterozygous.

This work is the first to report on an analysis of QTLs for resistance to root rot caused by *P. tropicalis* in a cassava population generating a map for each parent.

The presence of individuals that are more resistant than the two parents and the detection of QTLs associated with the molecular markers of the map derived from the maternal parent of family K show that alleles for resistance that come from both parents contribute to resistance

in the progenies (transgressive segregation). Such characteristics are well known in heterozygotic species and are useful for combining genetic factors of resistance in the same cultivar (Jorge et al. 2001).

Table 11.11. QTLs that most explain phenotypic variance for resistance of cassava to root rot caused by *Phytophthora tropicalis*, as described by the percentage of infected area of the root.

Linkage group [a]	Markers[b]	*F*[c]	V[d] (%)	*P*[e]
Maternal map				
C (3)	rGY172	0 29	5.4	<0 500
H (8)	SSRY178	0.315	1.3	<0 500
J (10)	CDY76	0.163	4	<0 500
	K2a	0 40	8.6	<0 500
N (14)	SSRY13	0 78	4.2	<0 500
Q (17)	SSRY911	0 47	5.7	<0 500
V (22)	NS911	0 07	9	<0 070
	GY153	0 49	4.5	<0 500
Paternal map				
A (1)	rGY32	0 29	11	<0 100
D (4)	SSRY313	0.315	3.4	<0 500
I (9)	GY88	0.163	3.3	<0 500
	SSRY51	0 40	5.7	<0 500
M (13)	SSRY299	0 78	3.4	<0 500
N (14)	SSRY105	0 47	4.8	<0 500

a. Numbers in parentheses refer to linkage group number; b. Markers; c. F value; d. Phenotypic variance explained; e. Probability for no QTL associated

The markers rGY32, NS911, and K2a, which together explain 28.6% of phenotypic variation, will be evaluated in selection trials of cassava genotypes to identify individuals resistant to root rot caused by *P. tropicalis*.

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Activity 11.10 Detecting phytoplasmas in cassava affected by frogskin disease (FSD), using nested PCR.

Specific objective
To detect phytoplasmas in cassava plants affected by frogskin disease (FSD)

Methodology

Plant tissues.
Asexual planting materials (stakes) from 40 plants of the commercial cassava varieties Catumare and Manzana were obtained. Twenty of the plants came from Rozo and Palmira, Department of Valle del Cauca, Colombia, and were either moderately infected (Catumare) or severely affected (Manzana) by FSD. The other 20 plants were disease-free and came from Montenegro, Department of Quindío, Colombia.

The stakes, 20 cm long, were planted in plastic bags containing pasteurized soil that was free of FSD. The bags were placed on plates to prevent contamination during watering.

All the plants were fertilized periodically and left in anti-aphid cages. The plants and cages were fumigated periodically, rotating the following products: Vertimec® 1.8% CE (abamectin at 0.5 cc/L of the commercial product), Malathion® (malathion at 1 cc/L), and Sistemin® (dimethoate at 3 cc/L).

As control we used healthy 'Secundina' from in vitro plants, placing them in the cages with the varieties being evaluated. They also functioned as monitors for the presence of insect vectors.

The trials were established in a greenhouse and screen house under different conditions of relative humidity and temperature. The greenhouse had an RH of 31% to 98%, and temperatures varied from 19°C to 28°C. Four replications of 10 plants were used per variety in each of the greenhouse and screen house, and placed in the same cages of their respective varieties.

Healthy plants from Armenia, Quindío, were also established under equal conditions in the same greenhouse and screen house but in separate cages.

Insects.

In a separate experiment, Homopterans (*Scaphytopius marginelineatus*) were collected from cassava crops infected with FSD, and breeding was established in cages containing diseased plants. After a couple of generations, adult insects were transferred to healthy plants to test for transmission of disease (CIAT Cassava Entomology Section, personal communication, 2004).

DNA extraction. Total DNA was extracted as described by Gilbertson et al. (1983).

Nested-PCR analysis.
We amplified 50 ng of genomic DNA, using nested PCR with the universal primers R16F2/R16R2 and the primers specific to the 16SrIII group (X-disease), R16(III)F2/R16(III)R1. The cocktail was prepared with 2 mM dNTPs, 1X Taq buffer, 2.5 mM $MgCl_2$, 1 U Taq polymerase, and 10 µM of each primer. The conditions for amplification were 94°C for the initial denaturation for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 3 min, and a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel.

RFLP analyses.
All amplified PCR products were digested with the restriction endonucleases *Alu*I, *Rsa*I, and *Taq*I to confirm the presence of a single group of phytoplasmas associated with the disease. The restriction products were analyzed by electrophoresis on 5% polyacrylamide gel. These enzymes had been used previously to classify FSD phytoplasmas.

DNA sequencing.
The amplified PCR products were cleaned, using a purification kit (QIAGEN) and then sequenced by automated dideoxy sequencing (ABI PRISM® 377-96 DNA Sequencer, Applied Biosystems). The sequences obtained were homologized with the sequences reported in GenBank to identify the organism detected in the evaluated samples.

Results

DNA extraction.
A total of 320 DNA samples were obtained from infected plant tissues —160 from roots and 160 from leaf midribs and petioles—and another 80 from healthy tissues. All samples were from the varieties Catumare and Manzana **(**Table 11.12). In addition, 17 samples were extracted from tissues at different developmental stages of the insect *S. marginelineatus*, processing 1 to 2 individuals per sample (Table 11.13).

Nested-PCR analysis.
Of the 320 infected-plant-tissue samples evaluated, 262 were detected as having a phytoplasma (82%); of the 17 samples from insects fed on diseased plants, 50% showed amplification; and of the 80 healthy plant tissues, no amplifications were obtained.

Table 11.12 Tissues evaluated, using nested PCR and sequencing, to determine the incidence of phytoplasmas in cassava plants infected with frogskin disease.

		Samples processed[a]		No. of positive	% nested	PCR[b] sequencing	
Tissue	Variety	Roots	P and MR	samples	PCR	Roots	P and MR
Infected[c]	Catumare[d]	80	80	124	77	8	10
Infected	Manzana[e]	80	80	138	86	10	12
Healthy[f]	Catumare	20	20	0	0	-	-
Healthy	Manzana	20	20	0	0	-	-

a. Total number of samples processed in the greenhouse and screen house; P = leaf petioles; MR = leaf midribs.
b. The same number of samples was taken for both greenhouse and screen house. P = leaf petioles; MR = leaf midribs.
c. Seed came from plots infected with frogskin disease in Rozo and Palmira, Valle del Cauca.
d. Moderately infected.
e. Severely infected.
f. Seed came from plots free of frogskin disease in Montenegro, Quindío.

Table 11.13 Identifying phytoplasmas in Homopterans (*Scaphytopius marginelineatus*) as evaluated by nested PCR with primers R16F2/R16R2 and R16(III)F2/R16(III)R1.

Sample	Genotype[a]	Stage	Nested PCR[b]
1[a]	M Col 2063 (I)	Adult	+(S)
1B	M Col 2063 (I)	Nymph	+
1C	M Col 2063 (I)	Nymph	-
2[a]	M Col 2063 (I)	Adult	+
2B	M Col 2063 (I)	Nymph	-
2C	M Col 2063 (I)	Nymph	+
3B	M Col 2063 (I)	Nymph	-
3C	M Col 2063 (I)	Nymph	+
4[a]	M Col 2063 (H?)	Adult	-
4B	M Col 2063 (H?)	Nymph	-
4C	M Col 2063 (H?)	Nymph	-
SE1	M Col 2063 (I)	Adult	-
Ss1	M Col 2063 (H?)	Adult	-
F1	Bean(H)	Adult	-
383 (1)	M Bra 383(I)	Male nymph	-
383 (2)	M Bra 383(I)	Female nymph	-
383 (3)	M Bra 383(I)	Adult	+(S)

a. Cassava germplasm materials facilitated and qualified as healthy or diseased by the CIAT Virology Unit. (I) = infected; (H) = healthy.
b. (S) Samples sequencing.

The presence of a phytoplasma was shown by visualization in agarose gels. Bands of about 800 bp—typical of the 16SrIII group—appeared when the primer pair R16(III)F2/R16(III)R1 was used. The rates of detecting the presence of phytoplasmas in plants (82%) (Figure 7.6A) and insects (50%) (Figure 7.6B) are high, considering that a rate of no detection of phytoplasmas is possible in plants presenting symptoms typically associated with them. Lack of detection could be attributed to substances in plant-tissue extracts inhibiting amplification, irregular distribution of phytoplasmas in the plant, or low concentrations of the microorganism in either plant or insect tissues (Chen and Liao 1975; Lee et al. 1994; Bianchini 2001).

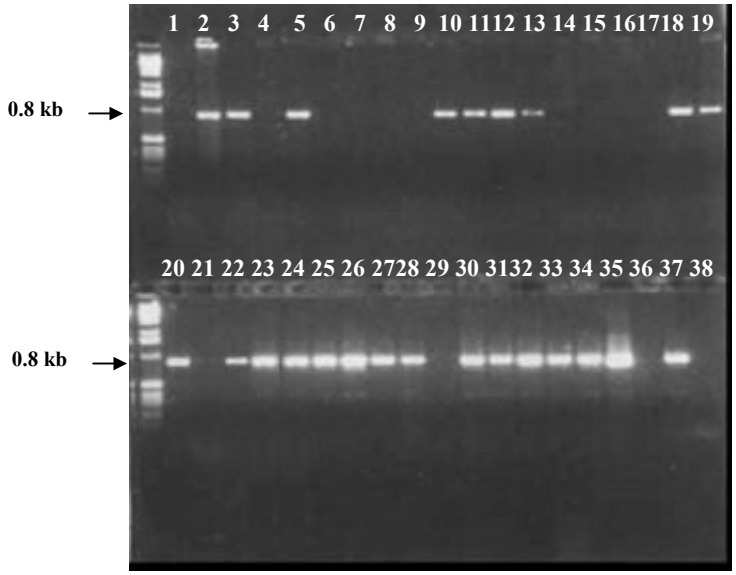

Figure 11.6A. DNA from infected tissues amplified with primers R16F2/R16R2 nested with R16(III)F2/R16(III)R1. Lanes 1–19 = Screenhouse tissues; lanes 20–38 = Glasshouse tissues.

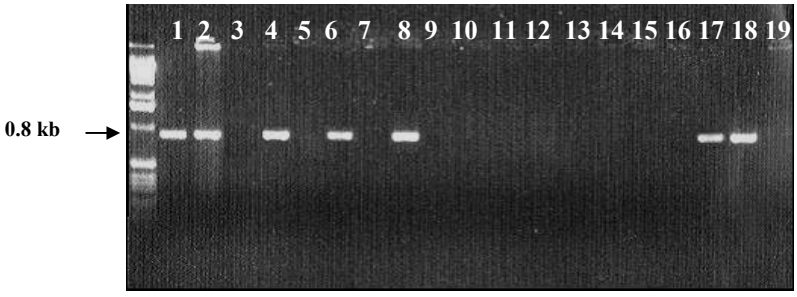

Figure 11.6B. Presence of phytoplasm typical of the group 16Sr III for *S. marginelineatus*, fed on infected plants, line 1,2,4,6,8 and 17; line 18 positive control and line 19 negative control, 1kb: Marker of molecular weight.

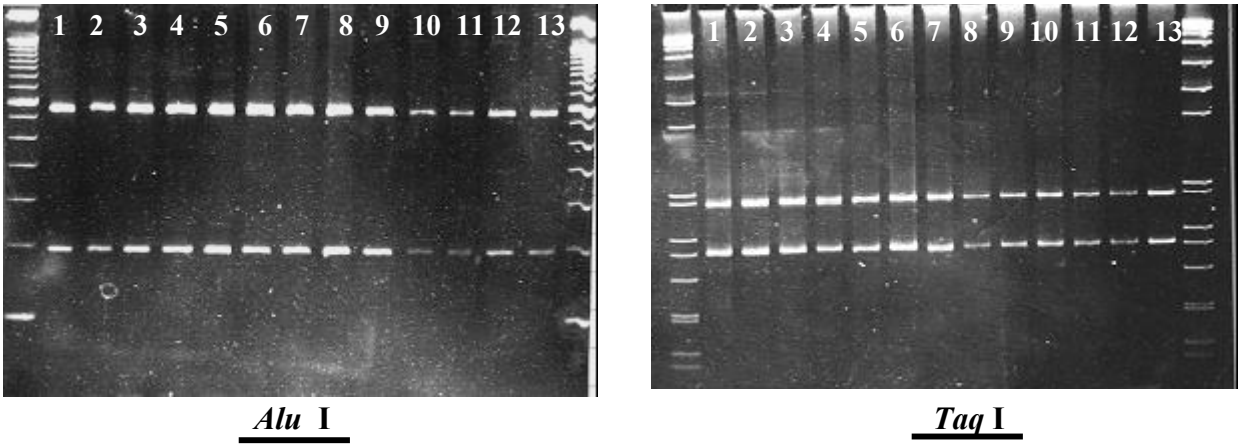

Figure 11.7. Cuts of the amplified product with primers R16(III)F2/R16(III)R1 by restriction enzymes *Alu*I and *Taq*I. Lines 1-6 (Glasshouse) and 7-13 (Screenhouse).

RFLP analyses.
The band pattern obtained for the 262 samples amplified with the three evaluated enzymes enabled us to confirm that the products belonged to the 16SrIII group (X-disease) (Figure 7.7).

DNA sequencing.
For the DNA amplifications, we took representative tissue samples from the roots and leaves (midrib and petiole) of infected cassava varieties in the field and in the greenhouse and screen house where the trials took place. The 40 fragments of plant DNA and 2 of insect DNA (Table 11.12 and 7.13) were then directly sequenced, purifying the PCR products.

The sequence analysis of the 42 fragments revealed that the cassava phytoplasma was similar to Cirsium white-leaf phytoplasma (GenBank accession no. AF373106, 16SrIII or X-disease group), with a sequence homology of 100% in fragments measuring 800 bp. These findings thus confirmed that the amplified products belong to a phytoplasma associated with FSD in cassava (CIAT 2003).

A homology of 90% was found among the sequenced fragments from insect tissue and from tissues of the varieties Manzana and Catumare. Given these homology results, being based on the nested-PCR technique, new transmission trials are being evaluated by Cassava Entomology Section at CIAT. The Section will first evaluate plants regarded as healthy or diseased and then evaluate plants on which those homopterans insects identified as possible vectors have fed.

This study shows evidence of an association between FSD and phytoplasmas. By applying molecular tools, a phytoplasma was successfully detected in FSD-infected cassava roots and leaf midribs.

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Activity 11.11 Identifying phytoplasmas by sequencing PCR products

Specific objective
To identify, through DNA sequencing, the phytoplasma associated with frogskin disease (FSD) of cassava.

Methodology
Plant tissues. Roots, petioles, and leaf midribs of both FSD-infected and healthy cassava plants, grown in the field and greenhouse, were processed. We evaluated 41 samples from cassava genotypes and varieties of three areas of Colombia—Atlantic Coast, Valle del Cauca, and Cauca—where FSD has high incidence. The goal was to confirm the presence of phytoplasmas in plants showing symptoms of FSD (Table 11.14).

Table 11.14. List of DNA fragments obtained from samples of tissues of 41 cassava varieties infected with frogskin disease. The samples were amplified by nested and direct PCR, using universal primers and primers specific for phytoplasmas.

Variety	Tissue	Site[a]	PCR	Primers[b]
CM 6740-7	Leaf midrib	Agrovélez	+	A
CM 6740-7	Root	Agrovélez	+	A
CM 6740-7	Leaf midrib[c]	CIAT-greenhouse	+	A
CM 6740-7	Root	CIAT-greenhouse	+	A
CM 6740-7	Leaf midrib[c]	Santa Elena-field	+	C
CM 6740-7	Root	Santa Elena-field	-	C
Parrita	Shoot	Agrovélez	+	B
Parrita	Leaf midrib	Agrovélez	+	B
Parrita	Stem	Agrovélez	+	B
Parrita	Petiole	Agrovélez	+	B
Parrita	Root	Agrovélez	+	B
Parrita	Leaf midrib	CIAT-greenhouse	-	B
Parrita	Petiole	CIAT-greenhouse	-	B
Parrita	Stem	CIAT-greenhouse	-	B
Parrita	Rootlet	CIAT-greenhouse	-	B
Catumare	Leaf midrib	Montenegro	-	B
Catumare	Root	Montenegro	-	B
Catumare	Leaf midrib[c]	Rozo-field	+	B
Catumare	Root	Rozo-field	+	B
Catumare	Leaf midrib	CIAT-screen house	+	C
Manzana	Leaf midrib	Montenegro	-	B
Manzana	Root	Montenegro	-	B
Manzana	Leaf midrib	Rozo	+	B
Manzana	Root	Rozo	+	B
M Bra 383	Root	Quilichao	+	B
M Bra 383	Root	Quilichao	+	B
M Bra 383	Root	CIAT-field	+	B
M Bra 383	Root	CIAT-field	+	B
CM 849-1	Leaf midrib	Agrovélez	+	B
CM 849-1	Petiole	Agrovélez	+	B
CM 849-1	Stem	Agrovélez	+	B
CM 849-1	Rootlet	Agrovélez	+	B
SM 1219-9	Leaf midrib	CIAT-field	+	B
SM 1219-9	Root	CIAT-field	+	B
CM 2177-2	Leaf midrib	CIAT-field	+	B
CM 2177-2	Root	CIAT-field	+	B
CM 4919-1	Leaf midrib	CIAT-field	+	B
CM 4919-1	Root	CIAT-field	+	B
M Col 2063	Leaf midrib[c]	CIAT-greenhouse	+	B
M Col 2063	Root	CIAT-greenhouse	+	B
M Col 2063	Leaf midrib	CIAT-screen house	+	B
M Col 2063	Root	CIAT-screen house	+	B
M Bra 383	Leaf midrib[c]	CIAT-greenhouse	+	B
M Bra 383	Rootlet	CIAT-greenhouse	+	B
Venezolana	Root	Sincelejo-field	+	A-B
Venezolana	Root	Sincelejo-field	+	A-B
CM 3306-9	Leaf midrib[c]	CIAT-greenhouse	+	B

Continued

Continued

Variety	Tissue	Site[a]	PCR	Primers[b]
CM 3306-9	Petiole	CIAT-greenhouse	+	B
CM 3306-19	Petiole	CIAT-greenhouse	+	B
M Bra 856-54	Leaf midrib[c]	CIAT-greenhouse	+	B
M Bra 856-54	Petiole	CIAT-greenhouse	+	B
M Col 634	Leaf midrib	Quilichao-field	+	C
M Col 634	Root	Quilichao-field	+	C
M Bra 829	Leaf midrib	Quilichao-field	+	C
M Bra 829	Root	Quilichao-field	-	C
M Per 16	Leaf midrib	Quilichao-field	+	C
M Per 16	Root	Quilichao-field	+	C
M Bra 856	Leaf midrib	Quilichao-field	+	C
M Bra 856	Root	Quilichao-field	+	C
M Bra 856	Leaf midrib	Quilichao-field	+	C
M Bra 856	Root	Quilichao-field	+	C
M Chn 2	Leaf midrib	Quilichao-field	-	C
M Chn 2	Root	Quilichao-field	-	C
HMC 1	Leaf midrib	Quilichao-field	+	C
HMC 1	Root	Quilichao-field	+	C
M Arg 2	Leaf midrib	Quilichao-field	-	C
M Arg 2	Root	Quilichao-field	-	C
M Bra 325	Leaf midrib	Quilichao-field	+	C
M Bra 325	Root	Quilichao-field	+	C
M Bra 839	Leaf midrib	Quilichao-field	+	C
M Bra 839	Root	Quilichao-field	+	C
M Col 1178	Leaf midrib	Quilichao-field	+	C
M Col 1178	Root	Quilichao-field	+	C
M Col 1468	Leaf midrib	Quilichao-field	+	C
M Col 1468	Root	Quilichao-field	+	C
M Cub 74	Leaf midrib	Quilichao-field	-	C
M Cub 74	Root	Quilichao-field	+	C
M Bra 886	Leaf midrib[c]	Quilichao-field	+	C
M Bra 886	Root	Quilichao-field	+	C
M Bra 882	Leaf midrib	Quilichao-field	-	C
M Bra 882	Root	Quilichao-field	-	C
CM 5460-10	Leaf midrib[c]	CIAT-screen house	+	C
CM 5460-10	Petiole	CIAT-screen house	+	C
SM 909-25	Leaf midrib[c]	CIAT-screen house	+	C
SM 909-25	Petiole	CIAT-screen house	+	C
CG 6119-5	Leaf midrib[c]	Santa Elena-field	+	C
CG 6119-5	Root	Santa Elena-field	+	C
M Per 335	Root	Santa Elena-field	+	C
ICA Nataima	Leaf midrib	Santa Elena-field	-	C
ICA Nataima	Root	Santa Elena-field	+	C
SM 1201-5	Leaf midrib	Santa Elena-field	-	C
GM 228-14	Leaf midrib	Santa Elena-field	-	C
CM 9582-64	Leaf midrib	Rozo-field	+	A-B-C
CM 9582-64	Root	Rozo-field	+	A-B-C
CM 9582-65	Leaf midrib	Rozo-field	+	A-B-C
CM 9582-65	Root	Rozo-field	+	A-B-C
CM 9582-24	Leaf midrib	Rozo-field	+	A-B-C
CM 9582-24	Root	Rozo-field	+	A-B-C
M CR 81	Leaf midrib	Rozo-field	+	A-B-C
M CR 81	Root	Rozo-field	+	A-B-C

Continuation of Table 11.14

a. Agrovélez S.A., CIAT, Rozo, and Santa Elena are found in the Department of Valle del Cauca; Quilichao in Cauca; Sincelejo in Atlántico; and Montenegro in Quindío.
b. Primers used for amplification were (A) P1/P7–R16F2N/R16R2, (B) R16mF2/R16mR1–R16F2N/R16R2, and (C) R16F2/R16R2–R16(III)F2/R16(III)R1.
c. Also showing foliar symptoms of mosaic and deformation of leaf blade.

Sequencing the 16S rRNA region.

DNA obtained from plants with symptoms of FSD was used to amplify fragments of the 16S region of ribosomal DNA, using polymerase chain reaction (PCR) and two pairs of universal primers P1/P7 and R16mF2/R16mR1. The products were re-amplified, using nested PCR and primers R16F2N/R16FR2, to detect and confirm that the phytoplasma is associated with the disease. The products of the nested PCR (1.2 kb) were digested with the enzymes *Alu*I, *Mse*I, *Rsa*I, and *Taq*I. The band patterns obtained with the restriction fragment length polymorphism (RFLP) technique made it possible to locate the group to which the phytoplasma belongs.

These results were confirmed by re-amplifying the products R16F2/R16R2 with primers R16(III)F2/R16(III)R1 (0.8 kb) specific to the 16SrIII group. The fragments of 1.2 kb and 0.8 kb were cloned and sequenced. Purified PCR products were ligated in pGEM®-T Easy Vector, which was introduced into the *Escherichia coli* strain DH5-α by electroporation at 2.4 kV/cm^2.

Transformants were selected on a blue-white screen by plating on an LB/ampicillin/IPTG/Xgal medium. Positive inserts were observed with plasmid restriction with *Eco*RI and electrophoresis in 1.5% agarose gel. Different-sized fragments were selected and sequenced by automated dideoxy sequencing (ABI PRISM® 377-96 DNA Sequencer, Applied Biosystems).

Results

A phytoplasma was successfully detected, using nested PCR, in all FSD-infected tissues. Of the methods used in this study, PCR was the most sensitive for detecting, identifying, and classifying phytoplasmas.

A sequence from a cloned fragment, obtained from an infected cassava plant, showed a 99% homology with the Chinaberry yellows phytoplasma and 100% with that of Cirsium white leaf. These results allow us to infer that a phytoplasma possibly plays a role in this disease.

As criteria, we took the number of correctly read bases of the amplified fragment, amplifications of the characteristic symptoms of the disease, and differences of genotype, and obtained two complete sequences, measuring 1260 and 1298 bp of 16Sr DNA gene region of two different cassava varieties, M Col 2063 (Y17) (leaf midrib and petiole) and SM 1219-9 (Y29) (external phloem from roots), which were classified and reported in GenBank with the accession numbers AY737646 (1260 bp) and AY737647 (1298 bp).

Acknowledgments
We thank the following people for their support: Tom Harrington, Joe Steimel, and Gary Polking, Iowa State University, for DNA sequencing analysis; the CIAT Virology Unit and Cassava Entomology for facilitating some cassava genotypes; and Agrovélez S.A., Jamundí, Valle del Cauca.

Activity 11.12/Designing specific primers for high-specificity detection of a phytoplasma associated with frogskin disease (FSD) of cassava

Specific objective
To obtain high specificity in the technique and improve it for detecting phytoplasmas in cassava plants with symptoms of FSD, weeds, and potential insect vectors

Methodology

Sequencing and analyzing phytoplasma rDNA.
We previously described obtaining complete sequences of DNA fragments through PCR from samples of two cassava varieties. They were reported to GenBank, which gave them accession numbers AY737646 and AY737647 (CIAT 2004). We conducted analyses of homology with these sequences against 24 sequences of the 16SrIII group and accessions of phytoplasmas representing at least 14 primary phytoplasma groups, using multiple alignments among the sequences (DNAMAN, version 5.2.2, Lynnon BioSoft). Specific differences in nucleotides were sought, seeking a series of bases that would be specific to the cassava phytoplasma. The homology of the sequences was calculated (in %) by taking the identical number of bases over the difference of aligned sequences and total size of gaps (in %). "Gap (%)" is the number of gaps of all sequences over the size of aligned sequences.

We used the option "Quick Alignment" to perform pairwise alignment with all sequences, using the method developed by Wilbur and Lipman (1983). With this method, DNAMAN aligns each pair of sequences, constructs a homology tree from the results of pairwise alignment, and finally builds up alignment based on the homology tree with the previously established alignment. This tree is set up with the distance matrix, using the UPGMA method (Sneath and Sokal 1973). The matrix can be built up only with Observed Divergence (this method uses directly unmatched residues divided by compared length between two sequences. No correction is applied to distances). After the tree is constructed, dynamic programming is finally used to optimize group alignment (Feng and Doolittle 1987; Thompson et al. 1994).

The phylogenetic analysis was constructed with the distance matrix, using the neighbor-joining method (Saitou and Nei 1987). Bootstrapping statistically shows typical variations (Felsenstein 1985). It involves creating a new data set by sampling randomly with replacements, so that the resulting data set has the same size as the original, but some characters have been left out and others are duplicated. The method assumes that the characters evolve independently. Phylogenetic analysis of the 16Sr RNA sequences was

resolved, using the PAUP Software Program, version 3.1.

Results

Designing primers.

The results of the phylogenetic and homology analyses show that the FSD phytoplasma clustered closely with other known X-disease (16SrIII) group strains, thus supporting its assignment to this group. We found multiple differences among the sequences of the FSD phytoplasma and the group 16SrIII phytoplasmas (Figure 7.8), generating sufficient information to design primers. The primers for the specific amplification of the phytoplasma associated with FSD were designed with the assistance of the program PRIMER 3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi [2004]), taking into account certain criteria such as the contents of G + C and A + T, close to 50%, a minimum of nitrogenous bases, absence of extensive palindrome sequences within the primers, and that mating among their pairs was minimum. The primers obtained were synthesized by Integrated DNA Technologies, Inc.

wwbp	5’	AGGATAACAATTGGAAATAG	3’	wwbp	5’	TAAAAGATCTTCTTTGAAGG	3’
Slfp	5’	AGGATAACAATTGGAAATAG	3’	Slfp	5’	TAAAAGATCTTCTTTGAAGG	3’
Wxp	5’	AGGATAACAATTGGAAATAG	3’	Wxp	5’	TAAAAGATCTTCTTTGAAGG	3’
Y17	5’	AGGATAACGATTGGAAATAG	3’	Y17	5’	TAAAAGACCTTTTTTGAAGG	3’
Y29	5’	AGGATAACGATTGGAAATAG	3’	Y29	5’	TAAAAGACCTTTTTTGAAGG	3’
wwbp	5’	ACTAGAGTGAGATAGAGGCA	3’	wwbp	5’	CTTGCTGGGTCTTTACTGAC	3’
Slfp	5’	ACTAGAGTGAGATAGAGGCA	3’	Slfp	5’	CTTGCTGGGTCTTTACTGAC	3’
Wxp	5’	ACTAGAGTGAGATAGAGGCA	3’	Wxp	5’	CTTGCTGGGTCTTTACTGAC	3’
Y17	5’	ACTAGAGTGAGTTAGAGGCA	3’	Y17	5’	CTTGCTGGGACTTTACTGAC	3’
Y29	5’	ACTAGAGTGAGTTAGAGGCA	3’	Y29	5’	CTTGCTGGGACTTTACTGAC	3’
wwbp	5’	CTGGTAGTCCACGCCGTAAA	3’	wwbp	5’	CCAATCTCAAAAAATCAATC	3’
Slfp	5’	CTGGTAGTCCACGCCGTAAA	3’	Slfp	5’	CCAATCTCAAAAAATCAATC	3’
Wxp	5’	CTGGTAGTCCACGCCGTAAA	3’	Wxp	5’	CCAATCTCAAAAAATCAATC	3’
Y17	5’	CTGGTAGTCCACACCGTAAA	3’	Y17	5’	CCAATCTCACAAAATCAATC	3’
Y29	5’	CTGGTAGTCCACACCGTAAA	3’	Y29	5’	CCAATCTCACAAAATCAATC	3’

Figure 11.8. Some differences found in region 16Sr DNA between phytoplasmas of the 16SrIII groupand the cassava frogskin disease phytoplasma. Wwbp (Walnut witches'-broom phytoplasma**),** Slfp **(**Strawberry leafy fruit phytoplasma), Wxp **(**Western X phytoplasma)**,** and Y17 indicate the cassava frogskin disease phytoplasma AY737646 (1260 bp). Y29 indicates the cassavafrogskin disease phytoplasma AY737647 (1298 bp).

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Activity 11.13 Detecting phytoplasmas by electron microscopy

Specific objective
To detect, through electron microscopy, phytoplasma structures in tissues infected with FSD that was positive to nested PCR

Methodology

Tissues.
Portions of roots exhibiting typical FSD symptoms were chosen from four different cassava genotypes. Typical symptoms are small longitudinal fissures distributed all over the root. On healing, these fissures develop "lips". The root portions for each variety comprised different cuts directed mainly at the phloem. Healthy plant samples were also processed to act as control (Table 11.15).

We also processed Homopteran insects (*S. marginelineatus*) collected from cassava crops infected with FSD and bred them in cages with different susceptible cassava genotypes that would show severe symptoms of the disease. Three individuals per developmental stage of the insect were taken as samples (Table 11.15) (CIAT Cassava Entomology, personal

communication, 2004). The tissue fragments were cut into 1 × 2 mm pieces to be prefixed in 2%–3% glutaraldehyde (0.1 M phosphate buffer, pH 7.3). Complete insects were also fixed in the same buffer.

Table 11.15. Processed samples of insects (*Scaphytopius marginelineatus*) and cassava plant tissues for detecting phytoplasmas by electron microscopy.

Tissue sample	Insect's developmental stage	Cassava genotype	Electron microscopy
Insect SE1	Adult	M Col 2063	In process
Insect Ss1[a]	Adult	M Col 2063	In process
Insect 383 (1)	Male nymph	M Bra 383	In process
Insect 383 (2)	Female nymph	M Bra 383	In process
Insect 383 (3)	Adult	M Bra 383	In process
Roots		CM 9582-64	+
Roots		CM 9582-65	+
Roots		CM 9582-24	+
Roots		M CR 81	+

a. Tissues in this sample were healthy, whereas tissues in the other samples were infected.

Electron microscopy.
The samples for electron microscopy were prepared by making ultra-thin (60–90 nm) sections with a Reichert Ultracut S ultramicrotome (North Central Instruments, Plymouth, MN). After post-fixation and precontrasting in uranyl acetate, they were dehydrated in an acetone series 50, 70, 90 (15 min each) and 100% (15 min, three times), and were embedded in Spurr's resin. A previous 18-h infiltration with acetone-Spurr (1:1) was done to facilitate the entry of resin into the tissues. The ultra-thin sections were mounted on copper grills, and images taken, using a Megaview III digital camera system with SIS software (Soft Imaging System Corp., Lakewood, CO) on a JEOL 1200EX woburn, MA scanning/transmission electron microscope (Japan Electron Optics Laboratory, Peabody, MA).

Results

In the previous studies, diverse tissues (stem, leaf midrib, petioles, and roots) were evaluated for numerous cassava plants, but only some could be compared with the results obtained for nested PCR. In this study, guided by the results of the nested PCR, root tissues of four cassava genotypes susceptible to FSD were first examined. These showed severe symptoms of the disease. Cells characteristic of phytoplasmas were detected in root phloem. The phytoplasma structures observed were pleomorphic, comprising round, elongate, dumbbell, and ring-shaped elements, mostly 150 to 250 nm wide and 1000 nm long (Figure 7.9). The phytoplasma structures were limited only to phloem tubes and were never seen in large quantities (Andersen *et al.* 2001). The insect-tissue samples are still being processed.

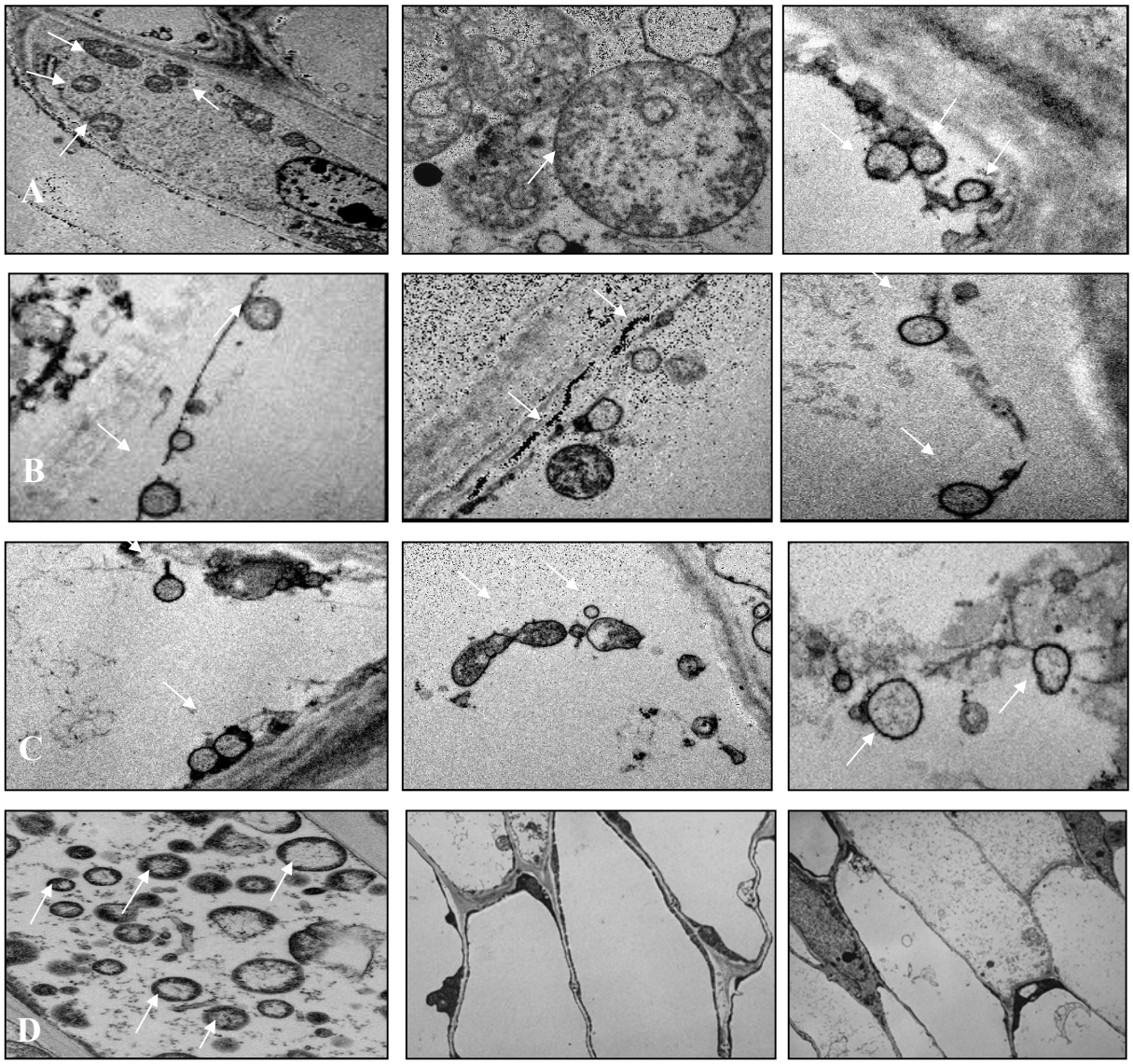

Figure 11.9. Micrographs, taken by cell transmission microscopy, of phytoplasmas FSD. (A) and (B) Infected cassava petiols. (C) Infected cassava roots and (D) Positive control (Periwinkle) and healthy cassava petiols. Photos (Alvarez, 2004).

Reference

Andersen, M.T.; Beever, R.E.; Sutherland, P.W.; Forster, R.L.S. 2001. Association of "*Candidatus* Phytoplasma australiense" with Sudden Decline of Cabbage Tree in New Zealand. *Plant Disease 85*: 462-469.

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Activity 11.14 To develop and validate sustainable methods to prevent and control FSD and SED.

To determine the effect of sustainable methods of preventing and controlling FSD and SED, experiments on thermotherapy and fertilization are recently started or ongoing.

Objective
To examine the effect of heat treatment on stakes of cassava plants affected by superelongation disease (SED), their germination, and yield

Methodology
We used thermotherapy to treat six stakes from each of 168 cassava genotypes taken from a field affected by SED. The stakes were immersed in hot water at 49°C for 49 min and then planted at Santander de Quilichao. The percentage of germination and yield were estimated, and the data analyzed by T test.

Results
The group of genotypes treated with thermotherapy had a germination rate of 90.2%, and yield was 22.2 t/ha, whereas the untreated group had a germination rate of 98.5%, and yield was 22.9 t/ha. The T test for germination gave a value of 1.79 and a probability of 0 77, whereas for yield the value was 0.41 and the probability of 0.66. The results indicated that there were no significant differences between the two treatments.

Acknowledgments
Bernardo Arias, Cassava Entomology, CIAT.

Activity 11.15 Multiplying cassava genotypes to ensure sufficient cuttings for disease-resistance evaluations

We propagated 220 promising cassava genotypes on a farm located in Rozo, Palmira (Department of Valle del Cauca, Colombia) for use in greenhouse experiments on varietal resistance, genetic studies, and disease management. The group included two populations for studies on resistance to *Phytophthora* spp., causal agents of PRRs.

Activity 11.16 DNA sequence analysis of specific regions of cassava resistance genes analogs (RGAs).

Objective
Report in GenBank sequences of resistence gene analogs isolated from cassava.

Table 11.16. Sequences of resistance gene analogs from cassava, submitted to the GenBank database.

Accession GenBank	Name	Size (bp)	Organism	Isolate/ Clone	Host/ source genotype	Location in Colombia
AY730038	*Manihot esculenta* resistance gene analog clone N37 NBS-LRR	325	*M. esculenta*	N37	M Bra 1045	Palmira
AY730040	*Manihot esculenta* resistance gene analog clone N38 NBS-LRR	474	*M. esculenta*	N38	M Bra 532	Palmira
AY730041	*Manihot esculenta* resistance gene analog clone K1 NBS-LRR	496	*M. esculenta*	K1	M Bra 532	Palmira
AY737490	*Manihot esculenta* resistance gene analog clone N33 NBS-LRR	342	*M. esculenta*	N33	M Bra 1045	Palmira
AY745762	*Manihot esculenta* resistance gene analog clone N31 NBS-LRR	210	*M. esculenta*	N31	CM6438-14	Palmira
AY745763	*Manihot esculenta* resistance gene analog clone P32 kinase	449	*M. esculenta*	P32	CM 3311-4	Palmira
AY745764	*Manihot esculenta* resistance gene analog clone W5 kinase	487	*M. esculenta*	W5	CM 7772-13	Palmira
AY745765	*Manihot esculenta* resistance gene analog clone X1 kinase	441	*M. esculenta*	X1	CM 3311-4	Palmira
AY745766	*Manihot esculenta* resistance gene analog clone X5 kinase	535	*M. esculenta*	X5	CM 7772-13	Palmira
AY745767	*Manihot esculenta* resistance gene analog clone X9 kinase	336	*M. esculenta*	X9	CM 6438-14	Palmira
AY745768	*Manihot esculenta* resistance gene analog clone W6 kinase	117	*M. esculenta*	W6	CM 6438-14	Palmira
AY745769	*Manihot esculenta* resistance gene analog clone W10 kinase	442	*M. esculenta*	W10	CBB resistant bulk	Villavicencio
AY745770	*Manihot esculenta* resistance gene analog clone P36 kinase	336	*M. esculenta*	P36	CM 3311-4	Palmira
AY745771	*Manihot esculenta* resistance gene analog clone P41 kinase	599	*M. esculenta*	P41	M Nga 19	Palmira

Methodology
Conserved regions of DNA corresponding to resistance genes analogs from cassava resistant varieties to *Phytophthora* sp. .— MBra 532, MBra 1045, and MCr 81— and *Xanthomonas axonopodis* pv *manihotis*— CM 3311-4, CM 7772-13, CM 6438-14, CM 7772-13, M Bra 1045, and M Nga 19— were amplified using degenerated primers amplifying of DNA, using PCR with degenerated NBS (nucleotide-binding sites) and Pto kinase primers. Amplified DNA was purified and then was ligated in pGEM-T Easy vector, which was introduced into the *Escherichia coli* strain DH5-a by electroporation at 2.4 kV/cm^2. Transformants were selected on blue/white color screening by plating on LB/ampicillin/IPTG/X-gal media. Positive inserts were observed by plasmid restriction with *Eco*RI and electrophoresis in 1.5% agarose gel. Different-sized fragments were selected for sequencing by automated dideoxy sequencing (ABI Prism 377-96 DNA Sequencer), using a DNA-sequencing kit from Applied Biosystems. Using the DNAMAN software with the option Assambly, different fragments of each microorganism or gen were aligned to obtain complete sequences. In order to report the sequences in the GenBank the read bases and their taxonomic classification at morphologic and molecular level were analyzed of each species.

Clones sequenced were homologated with known resistance genes, using the Blastx tool, in the NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) database. With this tool, we identify that clones as resistance gene analogs, wich were submitted in that database (Table 11.16).

Activity 11.17 Training researchers from Latin America, the Caribbean, and Africa on managing cassava diseases and research technology

2003

23 October. Philipp Aerni, Senior Researcher, Center for Comparative and International Studies (CIS) of the Swiss Federal Institute of Technology (ETH) at Zürich
Disease diagnosis, molecular characterization of pathogens, molecular markers associated with resistance to cassava diseases, and management of cassava diseases

2004

10 February. José Ventura and Ernesto Espinoza, INIVIT, Cuba
Disease detection and diagnosis in cassava

15 February. Natali Cortés, Student, Tver State University, Moscow, Russia
Applied biotechnology to detect and control phytopathogenic agents

15 March. Okechukwu Eke-Okoro (Nigeria), Titus Alicai (Uganda), Christopher Omongo (Uganda), William Sserubombwe (Uganda), Mayanne Apok (Uganda), Steven Tumwesigye (Uganda)
Disease diagnosis, molecular characterization of pathogens, molecular markers associated with resistance to cassava diseases, management of cassava diseases

20 May. Colombo–Japanese Association (15 participants)
Integrated management of diseases for cassava, plantain, palm, and flowers

31 May to 12 June. CIAT (30 participants)

International course on modern systems of cassava production, processing, and use
Organized by CLAYUCA

9 September. Reinaldo Tovar. Universidad Nacional Experimental de Guayana.
Puerto Ordaz, Venezuela
Thermotherapy for *in vitro* production of cassava plantlets

30 September. Manuel Valdivié Instituto de Ciencia Animal (ICA), Cuba
Management of cassava diseases

1 October. Gustavo Córdova. Instituto Nicaragüense de Tecnología
Agropecuaria. Managua, Nicaragua.
Biological control of pathogens

Activity 11.18 Train students, farmers, technicians, and researchers through field days and meetings on modern, sustainable, cassava production systems in different regions of Colombia to manage major cassava diseases, emphasizing selection of stem cuttings

2003

4 November. Five people from Chemonics International and farmers of Putumayo. Disease diagnosis and management for cassava and plantain

2004

February. Lorena Escobar. Universidad Nacional de Colombia. Isolation of *Phytophthora* species from chili pepper

March. Liliana Cadavid and Susana Mejía, Biology Students, Universidad del
Valle, Cali, Colombia. Isolation, detection, and pathogenicity tests of pathogens

29 February. 18 participants, including farmers and students from Pereira, Department of Risaralda
Disease diagnosis and management for cassava

12 March. 12 participants, including Chemonics International, Fundación Futuro Ambiental, and Fundación Catatumbo, and farmers. Disease diagnosis and management for cassava, rubber, cacao, and vanilla.

24 March. Meeting with Nicolás Cock Duque in Ecoflora
Integrated management of diseases through plant extracts

16 May. 30 participants, including farmers, functionaries from
national bodies, and NGOs in Orito, Department of Putumayo
Diagnosis and management of frogskin and other cassava diseases

18 May. Dr Octavio Vargas, Mitsui & Co., Ltd. Natural products in disease management

4 June. 37 Students, University of Caldas
Cassava and plantain diseases

5 June. 350 participants, including farmers, functionaries from national bodies, and private enterprises in Montería, Department of Córdoba, at the release of new cassava varieties
Management of cassava diseases, with a presentation on the diagnosis and management of frogskin disease

1 July. 6 farmers, Pescador, Department of Cauca
Diagnosis and management of cassava diseases

22 July. Visitors from the Colombian Association of Banana Growers (AUGURA) and CENIBANANO, including Luis Fernando Patiño, León Toné Gaviria, and Ramiro Jaramillo Sosa of the Board of Directors of CENIBANANO
Integrated management of diseases for plantain, cassava, and oil palm

26 July to 6 August. Hernán Zapata, Agrobiológicos SAFER (Natural Control)
Isolation and conservation of pathogens and biocontrollers, inoculum preparation, and pathogenicity tests

Attendance at meetings in 2004

8–14 March. Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia

31 July to 4 August. Annual Meeting of the American Phytopathological Society (APS), Anaheim, CA

13–15 August. XXV National Congress of Phytopathology, Cali, Colombia (held by ASCOLFI)

Activity 11.19. Publications in 2004

Articles

Calle F; Pérez JC; Gaitán W; Morante N; Ceballos H; Llano GA; Alvarez E. Genetics of relevant traits in cassava (*Manihot esculenta* Crantz) adapted to acid-soil savannas. Euphytica. (In press.)

Hurtado PX; Alvarez E. Búsqueda de genes análogos de resistencia asociados con la resistencia al añublo bacterial de la yuca. Fitopatol Colomb 27(2):59–64.

Hurtado PX; Alvarez E; Fregene M; Llano GA. Detección de marcadores microsatélites asociados con la resistencia a *Xanthomonas axonopodis* pv. *manihotis* en una familia de yuca (bc1). Rev Fitopatol Colomb. (In press.)

Llano GA; Alvarez E; Muñoz JE; Fregene M. Identificación de genes análogos de resistencia a enfermedades en yuca (*Manihot esculenta* Crantz), y su relación con la resistencia a tres especies de *Phytophthora*. Acta Agron 53(1/2). (In press.)

Loke JB; Alvarez E; Vallejo FA; Marín J; Fregene M; Rivera S; Llano GA. Análisis de QTLs de la resistencia a pudrición de raíz causada por *Phytophthora tropicalis* en una población segregante de yuca (*Manihot esculenta* Crantz). Acta Agron. (In press.)

Presentations at meetings in 2004

8–14 March. Llano GA; Alvarez E; Fregene M; Muñoz JE. Identification of resistance-gene analogs in cassava (*Manihot esculenta*), and their relationship to three *Phytophthora* species. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page 121.

8-14 March. Loke JB; Alvarez E; Corredor JA; Folgueras M; Jaramillo G; Ceballos H. Preliminary evidence between foliar and root resistance to root rot caused by *Phytophthora tropicalis* in cassava. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page.79.

8–14 March. Loke JB; Alvarez E; Fregene M; Marín J; Rivera S; Llano GA; Mejía JF. QTL mapping for resistance to root rot caused by *Phytophthora tropicalis* in cassava. Poster presented at the Sixth International Scientific Meeting of the Cassava Biotechnology Network, Cali, Colombia. Page 158.

5 June. Loke JB; Pérez JC; Alvarez E; Cuervo M; Mejía JF; Llano G.; Pineda B. Cuero de Sapo: *Una Enfermedad de la Yuca* -Once Preguntas Muy Interesantes de Agricultores-. Poster presented during the release of new cassava varieties. Montería, Córdoba.

11–13 August. Alvarez E; Mejía JF; Llano GA; Loke JB. Detección de un fitoplasma asociado a cuero de sapo de yuca (*Manihot esculenta* Crantz) en Colombia. Paper presented at the XXV ASCOLFI Congress, Cali.

Activity 11.20 Two postgraduate theses in cassava for the Universidad Nacional de Colombia (Palmira) and the Universidad de los Andes (Bogotá, Colombia)

Paula X. Hurtado. Evaluación de marcadores microsatélites y genes análogos, asociados a la resistencia de yuca a *Xanthomonas axonopodis* pv. *manihotis*. Universidad de los Andes—Bogotá. For a Master's in Biology, emphasizing Plant Molecular Biology.

John B. Loke. Análisis genético de la resistencia de yuca (*Manihot esculenta* Crantz) a *Phytophthora tropicalis*, causante de pudrición radical. Universidad Nacional de Colombia—Palmira. For a Master's in Plant Breeding.

Activity 11.21 Two undergraduate theses currently being undertaken in cassava for the Universidad de Caldas, Manizales, Colombia

Alejandro Corredor. Evaluación de la asociación de Marcadores bioquímicos y morfológicos con la resistencia a pudrición de raíz (*Phytophthora tropicalis*) y el deterioro fisiológico en yuca (*Manihot esculenta* Crantz). Universidad de Caldas, Manizales. For a degree in Agronomy.

Activity 11.22 Concept notes and projects developed.

Identification of insect vectors and alternative hosts of phytoplasmas causing cassava frogskin disease. Presented to USAID. Funds requested: US$ 12 00 for 1 year. Approved.

Desarrollo de estrategias de manejo de cuero de sapo y superalargamiento en yuca, mediante investigación participativa. Presented to Ministerio de Agricultura y Desarrollo de Colombia. Funds requested: US$ 14.546 for 1 year. Approved.

Combating Hidden Hunger in Latin America: Biofortified crops with improved Vitamina A, Essential Minerals and Quality Protein (English). Presented to CIDA. Funds requested: US$ 122.880 for 6 years. Approved.

Manejo Integrado de Enfermedades del Cultivo de Yuca. Presented to Ministerio de Agricultura y Desarrollo de Colombia and IICA. Funds requested: US$ 77.700. Submitted.

Pest and disease resistance, drought tolerance and increased shelf life genes from wild relatives of cassava and the development of low-cost technologies to pyramid them into elite progenitors. Presented to The generation challenge programme. Funds requested: US$ 289.200 per year, for 3 years. Submitted.

Personnel

Staff

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Juan Fernando Mejía	Paula Hurtado	

Students and Technicians

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3 months
Técnico Agroforestal**,** Mitú, Vaupés:
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2 months
Colegio Bolívar, Cali:
Cristina Londoño
3 months
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Mariluz Folgueiras
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UMATAs (Mitú, La Tebaida, and Montenegro)
Universidad Nacional de Colombia—Palmira (Valle del Cauca, Colombia)

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Corporación para el Desarrollo Sostenible del Norte y Oriente Amazónico (CDA, Vaupés, Dr E. Polo and R. Peña)
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