Project IP3

Improved cassava for the developing world

Marker Assisted Selection of CMD Resistance

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Annual Report - 2002

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IMPROVED CASSAVA FOR THE DEVELOPING WORLD

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SUMMARY ANNUAL REPORT 2002

IP-3 PROJECT

Title: Improved Cassava for the Developing World

Inputs:

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Cooperators:

Within CIAT:

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José Ignacio Sanz (PE-3)
Francisco Morales (PE-1)
Nathan Russell (Communications Unit)

Budget:

SOURCE	AMOUNT US $	PROPORTION (%)
Unrestricted Core	357,032	18%
Restricted Core	0	0%
Carry over from 2001	5,284	0%
Sub-total	**362,316**	**19%**
Special Projects	1,578,028	81%
Total Project	**1,940,344**	**100%**

Research Highlights in 2002

A Whitefly Resistant Cassava Variety

Whitefly resistance in agricultural crops, in general, is rare and control of whiteflies usually requires the continued use of agrochemicals. Pesticide use reduces farmer income, and is especially detrimental to small farmers who do not have easy access to credit for purchasing costly inputs. Host Plant Resistance (HPR) offers a low cost, efficient and easily employed technology for controlling major pests in cassava, such as whiteflies. Several good sources of resistance to whiteflies have been identified in cassava and high-yielding, whitefly resistant cassava hybrids are being developed. The hybrid CG 489-31 (CIAT Breeding Code) is officially being released by CORPOICA in November 2002, under the varietal name of Nataima-31. This variety was developed over a 15-year period in a CIAT-CORPOICA (MADR) collaborative effort. NATAIMA-31 provides cassava farmers, especially small farmers, with a high quality, high yielding cassava variety that will require little or no pesticide use. NATAIMA-31 also has moderate levels of resistance to thrips and mites. This may be the first food crop variety released for whitefly resistance.

Detection of a Phytoplasma Associated with Frog Skin Disease in Cassava (*Manihot esculenta* Crantz) in Colombia

Frog Skin Disease (FSD), an important cassava disease that affects roots, with unknown causal agent, has been reported with increasing frequency in Colombia, Brazil and Venezuela. Yield losses close to 90% have been reported in commercial fields in Colombia. The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were used in a nested PCR assay to detect and confirm that phytoplasmas were associated with FSD. To characterize and subsequently classify the phytoplasma, two pairs of universal primers (P1/P7 and R16F2n/R2) were used to amplify the 16S ribosomal DNA gene. 1.2 kb fragments were amplified from roots, stems, leaves and flower samples from symptomatic plants. Sequence analysis of the cloned fragment revealed that the cassava phytoplasma was similar to the Chinaberry yellows phytoplasma (GenBank acc. no. AF495657, 16SrXIII Mexican periwinkle virescence group) and Cirsium white leaf phytoplasma (GenBank acc. no. AF373106, 16SrIII X-disease group), both with a sequence homology of 100% and 99% respectively. The presence of phytoplasma was confirmed by DAPI and Dienes staining methods and by grafting. This is the first report of phytoplasma associated to FSD in cassava.

Development of Genetic Markers for Disease Resistance

Five DNA fragments, amplified using a degenerate NBS primer from a cassava genotype, resistant to *Phytophthora* root rot, were cloned in a PGEM-T Easy vector and sequenced. Based on the resulting sequences, five specific NBS primers were designed to amplify resistance regions in the DNA family. From 486 SSR primers evaluated as CBB resistance markers, 13 primers were selected as promising. From this, primer SSRY65 showed differences between CBB resistant and susceptible cassava individuals and can be considered a putative SRR marker for resistance to this disease. QTL analysis showed that resistance of root rot caused by *Phytophthora tropicalis* is polygenic in the K family. In addition eight QTLs were detected. The putative marker NS911 segregates with resistance, explaining 9% of the phenotypic variance.

Marker-Assisted Selection (MAS) for Breeding Resistance to the Cassava Mosaic Disease (CMD) at CIAT.

Molecular marker assisted selection (MAS) for breeding for resistance to the Cassava Mosaic Disease (CMD) has been implemented at CIAT. The SSR marker NS158 tightly associated with *CMD2*, the dominant CMD resistance gene, was able to predict with 95% accuracy resistant genotypes in an experiment with over 2000 genotypes scored for CMD resistance in the field at IITA, Ibadan Nigeria. The marker probably has a higher accuracy of predicting resistant varieties, the 5% recombinants found might be due to uneven disease pressure in the field. A second phase of molecular breeding for CMD resistance breeding at CIAT has also been initiated. CMD resistant progenies bearing *CMD* were crossed to elite parents of CIAT's cassava gene pools, and to high carotene or high protein content genotypes of wild *Manihot* species. Seeds harvested from the crosses were germinated *in vitro* from embryo axes in preparation for MAS and to permit sharing of the CMD resistant genotypes with collaborators in Africa and India. These plants will also be the basis of breeding for CMD resistance in CIAT cassava gene pools.

Resistance Genes for Green Mites from Manihot esculenta sub species flabellifolia

A very high level of resistance to the cassava green mites (CGM) was found in four inter-specific hybrid families. The families had an almost equal number of susceptible and resistant genotypes, which suggests a simple mode of inheritance of resistance. This new source makes rapid deployment of mite resistance via molecular marker assisted selection very attractive. Bulk segregant analysis (BSA), using 500 SSR markers, was therefore used to identify a putative molecular marker, SSRY 330, associated with resistance. At the same time, genetic crosses have been made between some inter-specific hybrids having high CGM resistance and good storage root formation, and elite parents of CIAT gene pools. About a thousand sexual seeds were obtained, they will be established and evaluated for resistance to CGM and also analyzed with the marker SSRY330 to provide a proof of concept for breeding resistance to CGM via MAS.

Improving β-Carotene Content in Cassava.

A very wide segregation for root parenchyma color, from white to pink, was observed in an S_1 family derived from the Colombian land race MCol72. β-carotene was determined for two genotypes pink root color by high performance liquid chromatography. One of the genotypes AM273-23 had the highest beta-carotene content so far recorded in the characterization of β-carotene in CIAT 's cassava germplasm bank. MCol72 has a root color that is normally described as cream colored or 4 on the CIAT scale. The discovery of such wide segregation for root color in an S_1 cross from a cream colored variety provides an ideal population for bulk segregant analysis (BSA) of β carotene content to identify markers associated with β-carotene for marker-assisted breeding for increased β-carotene. The recovery of a genotype with the highest β-carotene so far recorded at CIAT from a much lower content genotype provides insights into improving β-carotene content in cassava. BSA for marker discovery and an experiment to determine the complementation of favorable alleles of genes controlling beta carotene content from diverse sources has been initiated towards a more efficient method of improving beta carotene in cassava.

Identifying sources with high levels of proteins in the roots.

An extensive evaluation of six hundred cassava genotypes for micronutrients in the roots and leaves produced useful information regarding the genetic variation in nutritional properties of different cassava tissues. Very relevant was the finding of several genotypes with protein levels in the roots above 5%. Although results are very preliminary, it was very interesting to note that many of these genotypes originated from Meso-America, particularly Guatemala. This would suggest that an introgression from wild relatives of *Manihot esculenta* growing in that region, may have occurred. As a result, an effort is currently undergoing to recover accessions from this region to confirm this finding.

First systematic study on the inheritance of agronomically-relevant traits.

Three different diallel studies respectively for the sub-humid, the acid-soil savannas and the mid-altitude valleys were harvested during the year. The experiments included nine or ten parents. Each F1 family cross was represented by 30 clones. The field trials were conducted at two different locations within the respective eco-region, with three replications. Genetic variation among and within families was measured, providing a unique quantitative genetic model for analyzing the inheritance of all the traits evaluated. Two Ph.D. student theses will be derived from these analyses. Families providing interesting segregations will be identified and used as populations for more detailed analysis using molecular markers.

Developing a model for incorporating the use of doubled-haploids in cassava breeding.

Cassava breeding is difficult and inefficient. Several alternatives have been evaluated to overcome some of the problems for the genetic improvement of this crop. The use of doubled-haploids through tissue culture protocols represents an excellent example of the potential benefit of biotechnology tools for traditional breeding. A detailed breeding scheme was developed, limitations for its implementation were identified, and alternative strategies for overcoming them proposed. The relevance of this new approach for cassava breeding, its advantages and current bottlenecks have been extensively exposed at different scientific forums. Furthermore, potential donors expressed their interest in financing the implementation of this idea.

Selection of parental genotypes for new crosses using information on their general combining ability.

Cassava breeding is difficult and inefficient. Several alternatives have been evaluated to overcome some of the problems for the genetic improvement of this crop. One of the major problems in cassava breeding has been that no data on the progenies of each parent used in the project was produced in the early stages of selection. It was not possible, therefore, to select parental lines based on objective, tangible information, but rather on the empirical appreciation emerging after many years of using them. A new breeding scheme involved taking data on all the progenies evaluated from the very first evaluation trial (*Clonal Evaluation Trial* stage), regardless they were selected or not. Although the data is not perfectly balanced, data on the mean performance of all the progeny from a given parent has been produced and used to decide if the parental line continues to be in the breeding nurseries or not.

Studies on the effect of different post-harvest handling of cassava roots to prevent post-harvest physiological deterioration.

The availability of a large chamber with controlled temperature and moisture allows more detailed studies on post-harvest deterioration (PPD). The effect of dry matter and carotene contents in the roots on PPD was further established. The effects of temperature and reduced oxygen availability (roots inside plastic bags) was analyzed. Alternative methods for preserving cassava roots (different from freezing or waxing them) for as long as two or three weeks can be developed.

Publication of a new book on cassava production and processing.

CIAT and CLAYUCA have jointly produced a book covering updated technologies for cassava production and processing. The book has 586 pages and comes with a pocket sized guide for identification of symptoms of the most important diseases, pests and nutritional deficiencies in the field.

Project IP-3: Improved Cassava for the Developing World

Project Description

Objective: To develop germplasm, methods, and tools for increased productivity and value of the cassava crop that will result in increased income and development of rural communities involved in cassava production and processing.

Outputs:
1. Genetic base of cassava and related *Manihot* species evaluated and available for cassava improvement.
2. Genetic stocks of improved gene pools developed and transferred to national programs.
3. Collaboration with other institutions in place.
4. Developing a new approach for cassava breeding integrating biotechnology tools.
5. Field germplasm bank maintained.
6. Breeding for resistance to insects and other arthropods, and alternative methods for their control developed.
7. Disease resistance in cassava identified.
8. Development and use of biotechnology tools for cassava improvement.

Gains: The rural populations in Africa, Asia, and Latin America and the Caribbean benefit by increased productivity, enhanced value of the products produced, and flexibility obtained through the availability of different processing alternatives for cassava.

Milestones:

2002 A set of three different diallels evaluated and analyzed to gain knowledge of the inheritance of traits of agronomic relevance. Initiation of shift in the breeding scheme from hybrid production to the design of parental lines.

2003 The first set of S1 lines planted in the field. Better understanding of methods for controlling postharvest physiological deterioration. Better understanding of germplasm and processing procedures for the production of fried cassava chips.

2004 The first *trapiche yuquero* (cassava mill) begins full production of cassava flour. Other alternative uses being scaled up. S2 inbred lines produced. Comparison of different sources of planting materials of the same clone, to determine the rate of decline in their performance due to pathogenic and non-pathogenic organisms in the planting stocks.

2005 The first hybrids from parental lines with some degree of inbreeding produced. First results from the newly developed protocol for the production of double haploids. Molecular marker(s) for trait(s) different than resistance to ACMV identified and used for selection.

2006 Field comparison of hybrids from inbred versus non-inbred parents conducted. Recombination of S2 inbred lines and production of S3 lines through conventional self-pollination.

Users: Immediate beneficiaries are farmers growing cassava for cash crop or subsistence. Other beneficiaries are processing industries related to cassava (for animal feed, processed food, starch, or derived products).

Collaborators: IITA and IFPRI (CGIAR centers), NARS in Asia (particularly, Thailand, Vietnam, China, India, Indonesia) and Latin America (particularly, Brazil, Colombia, Cuba, Haiti, Venezuela, Dominican Republic and Peru), CLAYUCA, and the private sector involved in cassava processing.

CGIAR system linkages: IITA cassava breeding (50%); Crops (10%); Biofortification Initiative (25%); Protecting the Environment (5%); Training (3.4%); Networks (6.6%). Participates in the Global Cassava Strategy.

Project: Improved Cassava for the Developing World
Project Manager: Hernán Ceballos

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To improve the livelihoods of rural populations in Latin America, Africa, and Asia by increasing cassava productivity, while protecting the environment and enhancing the value of products derived from this crop.	Increased productivity of cassava clones. Widened uses for cassava. Increasing the area planted to the crop.	National statistics of different countries where projects have been implemented. Recognition of private sector (processing).	
Purpose To develop methods and tools that will make the genetic improvement of cassava more efficient and to identify valuable germplasm for the breeding project. Eventually, a technology package involving germplasm, cultural practices, and processing alternatives will be made available to rural communities.	By the end of year 2005, the project has consolidated the technology packages for alternative industrial uses of cassava and strengthened the reliability and sustainability of the crop as a source of food security for subsistence farming.	Reports and project documents of our partner institutions. Reports from the processing sector.	Political and institutional support for sustainable rural and agricultural development at the reference sites and targeted countries is maintained. Natural disasters and civil strife do not impede progress toward the project's goal. Absence of drastic changes in the price of maize as a commodity that would greatly affect cassava competitiveness.
Output 1 Genetic base of cassava and related *Manihot* species evaluated and made available for cassava improvement.	Evaluation of genetic diversity for: carotene, proteins and key minerals content in roots and foliage. Starch quality and properties in cassava roots. Evaluation of the effect of carotene content in reducing post-harvest physiological deterioration.	Articles published. Annual reports and project proposals. Clones developed to take advantage of findings from this output.	Natural disasters or civil strife do not impede progress toward achieving the project's goal. Cassava field germplasm bank is maintained.
Output 2 Genetic stocks of improved gene pools developed and transferred to national programs.	Changes in the breeding scheme implemented and consequences measured. Development of a new product in cassava: lines with varying degree of inbreeding. Selection of parental lines based on general combining effects. Number of clones for each	Project home page. Annual reports and working documents. Scientific publications. Shipment of germplasm to collaborators in different countries.	Natural disasters or civil strife do not impede progress toward the project's goal. Adequate funding for research activities.

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Output 2 (cont.)	agroecological region with outstanding performance. Evaluation of diallel sets for sub-humid, acid-soil savannas and mid-altitude valleys to analyze the inheritance of important traits.		
Output 3 Collaboration with other institutions in place.	Continue the consolidation of CLAYUCA. Continue with joint research and collaboration with IITA. Continue the support of cassava breeding projects in Asia. Training of visiting scientists. Close interaction with private sector involved in cassava processing.	Case studies published. Annual reports and working documents. Submission of joint research proposals. Support from private sector.	Natural disasters or civil strife do not impede progress toward the project's goal.
Output 4 Developing a new approach for cassava breeding integrating biotechnology tools.	Development of a protocol for producing doubled haploids in cassava. Promote the idea among cassava breeding projects and actively search for financial resources to implement it.	Annual reports and working documents. Scientific publications. Financial support for the project.	Natural disasters or civil strife do net impede progress toward the project's goal. Adequate funding for research activities.
Output 5 Field germplasm bank maintained.	Continue the collection of data for a complete data set from the germplasm bank. Introduce new germplasm from countries around the world.	Project proposals and reports. Accessions planted and maintained in the field. Introduction of new accessions.	Natural disasters or civil strife do not impede progress toward the project's goal.
Output 6 Breeding for resistance to insects and other arthropods, and alternative methods for their control developed.	Search for molecular marker(s) associated with resistance to whiteflies. Introgression of resistance to whiteflies into breeding stocks. Evaluation of reaction to insects and arthropods in breeding stocks. Development of methods for the biological control of soil insects.	Annual reports and working documents. Scientific publications. Development of commercial products for the biological control of cassava pests.	Natural disasters or civil strife do not impede progress toward the project's goal. Adequate funding for research activities.
Output 7 Disease resistance in cassava identified.	Identification of the causal agent of the frogskin disease and modes of transmission. Implementation of the molecular marker associated with resistance to ACMV.	Annual reports and working documents. Scientific publications. Training manuals. Development of a diagnostic kit for frogskin disease.	Natural disasters or civil strife do not impede progress toward the project's goal. Adequate funding for research activities.

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
	Identification of germplasm and cultural practices for controlling root rots in cassava. Identification of parental material resistant to bacterial blight and superelongation disease.		
Output 8 Development and use of biotechnology tools for cassava improvement.	Identification of populations showing interesting segregations for further analysis, using molecular markers. Testing of the hypothesis that hybrids from inbred lines perform better than those produced from non-inbred parents. Identification of genes of commercial, environmental, or consumer preference value for genetic transformation. Identification of traits of agronomic relevance to be associated with molecular markers for MAS. Collaboration for the development of QTLs of agronomic relevance	Annual reports and working documents. Scientific publications. Training manuals.	Natural disasters or civil strife do not impede progress toward the project's goal. Adequate funding for research activities.

Table of Contents

OUTPUT 8:   Development and use of biotechnology tools for cassava improvement
Activity

OUTPUT 1
Genetic base of cassava and related *Manihot* species evaluated and available for cassava improvement.

The overall objective of this output is to improve the nutritional status of people living in marginal environments of the tropics, by selecting and promoting cassava genotypes with high and good bio-availability of micronutrients and vitamins. Related traits are the need for a better understanding of the biochemical and genetic basis of post-harvest physiological deterioration and starch quality traits.

Activity 1.1. Evaluation of genetic diversity for carotene content in cassava roots. Collaborative project with IFPRI.

Rationale.

This is one of the many collaborative activities between projects **SB2** and **IP3**. Most of the emphasis in relation to cassava breeding has been centered on increasing root production and concentration of starch. Since cassava is a staple in regions where there are severe deficiencies of micro-nutrients; the crop can be used as a vehicle to deliver vitamins and minerals in higher concentrations. Improving the efficiency with which cassava acquires micro-nutrients and accumulates them in the roots and leaves can have an enormous potential not only in terms of human nutrition, but also in terms of crop production.

In many respects, PPD resembles wound responses found in other better studied plant systems but cassava appears to lack the wound healing capacity which is normally associated with the inhibition of wounding responses. An important component of these wound responses are the oxidative processes. Carotene is known to have antioxidant properties. Therefore, PPD was measured in a sample of genotypes to evaluate the potential correlation between this pro-vitamin and PPD. A major problem regarding PPD is obtaining reliable data, because this trait is seriously affected by the environment, and handling of the roots. Repeatability is not as good as desirable, so some efforts have been made to reduce experimental error in the measurements.

In the field of human nutrition (and animal nutrition as well) there is an increasing amount of evidence of a synergistic effect between vitamins and certain minerals. It seems that iron and zinc contents in the diet increase vitamin A absorption and vice versa. Therefore, for the study of micro-nutrients availability from cassava roots and leaves, it is also important to measure mineral contents, because of the putative synergistic effects in their availability in the process of digestion.

Specific Objectives:
a) *To continue the screening of cassava elite clones and landraces from CIAT's germplasm collection for total carotene and minerals content in roots and leaves;*
b) *To correlate vitamin contents with physiological post-harvest deterioration.*
c) *To search for genotypes with higher protein content in the roots.*

Materials and methods

Harvesting and sampling. Root samples from 99 clones from the germplasm or the cassava-breeding project at CIAT were used for this study. Clones with colored roots were favored for the study. This represents a departure from the activities carried out previously, when white and colored roots were analyzed. The emphasis now focuses in identifying clones with the highest carotene content in the roots to be used in breeding and basic studies. Few white rooted clones were also included for the comparison of different methodologies for quantifying carotenes content. Harvest took place at around 10 months of age (normal harvesting time for cassava at CIAT) and commercial size, disease-free roots were taken to represent each clone.

Carotene concentration measurements.

Colorimetric method. The extraction procedure outlined by Safo-Katanga et al. (1984), was modified by extracting root parenchyma with petroleum ether. The extraction protocol for leaves had to be modified due to the presence of tannins and chlorophylls. The modified protocol included several extractions with petroleum ether 35-65 °C and washing steps with methanol in order to minimize the interference from the others pigments. A sample of 5 g was taken out of the root or leaves, randomly selected 10 to 11 months after planting. The quantification was done by ultraviolet spectrophotometry using a Shimadzu UV-VIS 160A recording spectrophotometer. UV detection was done at l = 455nm for root extracts and l = 490 nm for leaf extracts.

HPLC method. Starting from the method used for the colorimetric quantification of total carotenes, aliquots (20 ml) of petroleum extract were completely dried by rota-evaporation. Then the dry extract was dissolved in 1 ml of HPLC mobile phase (methanol: methyl-terbutyl-ether: water, 80:15:4 v/v), centrifuged at 14000 rpm and 10 µl were injected in the HPLC system using a YMC carotenoid 5 um, 250 mm x 4.6mm column. Separation was done by isocratic elution with a mixture of acetonitrile:methylene chloride: methanol, 70:20:10 v/v, as mobile phase at 1 ml min-1 and 30 °C. β-carotene was detected by monitoring absorption at 450 nm. Identification and quantification was done by comparing retention times and uv-vis spectra, with a standard of β-carotene (Sigma C-0126).

Post-Harvest Physiological Deterioration (PPD) measurements. Five commercially sized roots (minimum length 18 cm) were randomly chosen. Roots were analyzed using the method of Wheatley et.al. (1985) with one modification: prepared roots were stored under ambient conditions for 7 days instead of 3 days. The proximal and distal root ends were cut off and the distal end was covered with Clingfilm. After 7 days, seven transversal slices, 2 cm thick were cut along the root, starting from the proximal end. A score of 1-10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1=10%, 2=20%, etc). The mean score of PPD for each root was calculated.

Results

Analysis of total carotene content. Carotene concentration in the roots, based on the colorimetric method, ranged from 0.141 mg/100 g FW to 1.071 with a mean of 0.416 mg/100 g FW and a standard deviation of 0.240 (Table 1.1). These values are similar to those found in the group of more than 1000 accessions evaluated the previous years (see 2000 and 2001 Annual Reports).

Table 1.1 Evaluation of roots from 99 cassava clones for carotene content in the roots (mg/100 g FW) using to quantifying methodologies.

Parameter	Colorimetric method	HPLC method	
	Total carotenes	β-carotene	α-carotene
Average	0.416	0.521	0.039
Standard Deviation	0.240	0.402	0.042
Maximum	1.071	1.688	0.194
Minimum	0.141	0.077	0.000

Activity 1.2. Determination of the actual proportion of different carotenoids included in the total carotene measurement.

Rationale
In the measurement of total carotenes several chemical entities are considered. These entities vary greatly in their vitaminic activity (i.e. β-carotene being much more efficient than α–carotene). Because the ultimate interest is to evaluate the nutritional value of high-carotene cassava roots, it is necessary to get an approximation to the relationship between the "total carotene" variable and "vitaminic activity".

Total carotene is a relatively easy measurement to carry out. On the other hand, the determination of the different carotenoids requires HPLC procedures that are expensive and slow. The methodologies for measuring carotenes have been described above in **Activity 1.1**.

Objectives
a) *To determine the proportion of different carotenoids making up the "total carotenes" measurement obtained in this output.*
b) *To produce preliminary data on how constant (across different genotypes) is the proportion of the different carotenoids making up the total carotene measurement.*

Results
Comparison of the two methods for quantifying carotenes. The results of the two methodologies for measuring carotenes have been presented in Table 1.1. There is a striking difference between the results of the two methodologies. Adding up α- and β-carotenes estimated by the HPLC method yielded estimations about 34% larger than the total carotene quantification based on the colorimetric method (0.560 versus 0.416 mg/100 g FW). Moreover, the HPLC method allowed concluding that about 93% of the carotenes present in the roots is β-carotene. The relationship between total carotenes by colorimetry and β-carotene by the HPLC method is illustrated in Figure 1.1.

The ratio of α- over β-carotene was relatively uniform with an average of 0.07, ranging from non-detectable levels of α-carotene to a maximum of 0.19. One sample was not considered in this range because of its atypical value (a ratio of 0.43).
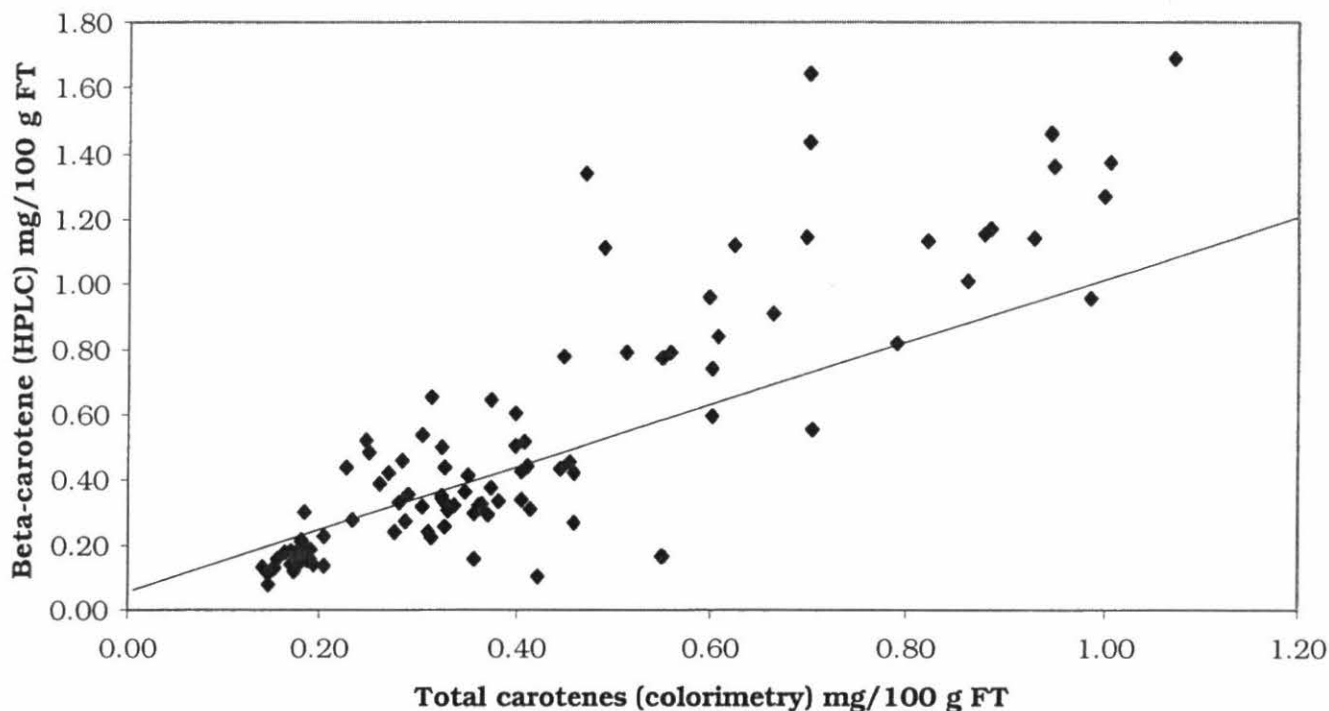
Figure 1.1. Relationship between total carotenes (measured by colorimetry) and β-carotene (measured by HPLC) in root samples from 99 cassava clones.

Activity 1.3. Further refinement of the association between post-harvest physiological deterioration with carotene content and other relevant traits in the root.

Rationale

One of the major constraints upon cassava production as a commercial crop is the rapid deterioration of its roots. Post-harvest physiological deterioration (**PPD**) often begins rapidly within 24 hours (Beeching et al., 1998). It consists of discoloration of the parenchyma as blue-black vascular streaks. Because of PPD, cassava roots need to be consumed shortly after harvesting (van Oirschot, et al., 2000). The short post-harvest storage life of cassava is a characteristic that limits the marketability of the roots. It implies high marketing margins and risks, as well as restricted management flexibility for farmers and processors.

Objectives

a) *To further refine the relationship between PPD and carotene content and other relevant traits.*

b) *To improve the facilities where PPD measurements are taken, to reduce environmental effects.*

Results

Data from previous years were consolidated and analyzed. A total of 1315 clones produced a very solid database. HCN does not seem to have a strong effect on PPD, whereas carotene did reduce and/or delay the onset of PPD (ρ = -0.123). Figure 1.2 illustrates the relationship between PPD and carotene, and suggests the occurrence of a threshold effect with carotene values lower that 50 mg having gradually lesser effect in reducing or delaying PPD. Sugar contents were negatively associated with PPD probably due to their negative correlation with dry matter content. Based on data presented (sample size=1315) the best regression model for PPD was:

$$PPD = -19.227 + 1.275 \text{ (Dry matter content)} - 14.554 \text{ (Carotene)}$$
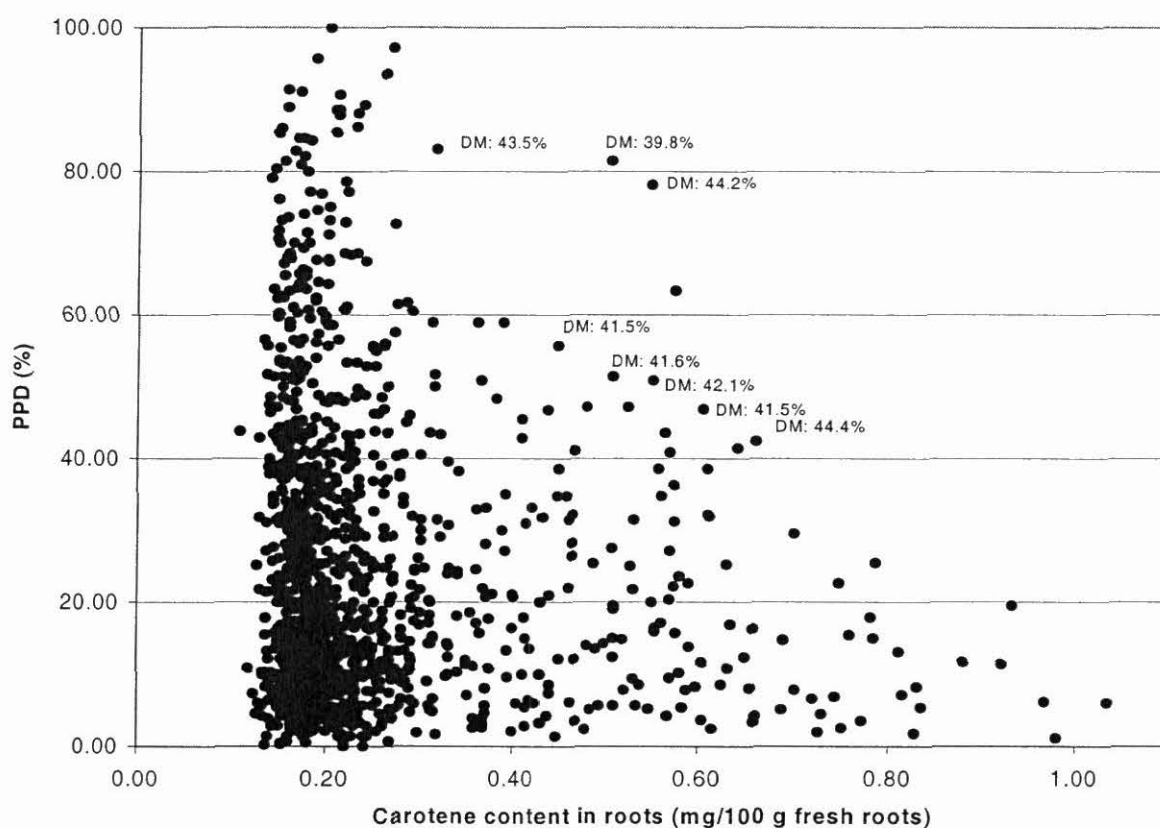
Figure 1.2 Relationship between carotene content (mg/100 g FT) and PPD (%) analyzed in a sample of 1315 cassava roots. Most data points in the upper periphery of the distribution came from root samples with dry matter content (%) considerably higher than the average for the sample analyzed.

In this model, the positive and negative associations of PPD with dry matter and carotene content, respectively, is further reinforced. The adjusted R^2 value for this model, however, was still low (0.132). The large amount of variation that remains unaccounted for is probably due to the large variations for PPD observed at low carotene contents values (Figure 1.2) or around the mean dry matter content. Also, evaluation of PPD is prone to large experimental

errors, because roots are left at room temperature (Wheatley et al., 1985) for seven days. Atmospheric changes in temperature and relative humidity are known to severely affect PPD development (Zapata, 2001). Current measurements on PPD had to be carried out at different harvesting times, because of restriction in the availability of materials from the germplasm bank and limitations in the number of clones that could be processed at any given time. Therefore, PPD estimates were probably affected by variations in the environmental conditions under which they were taken.

A chamber with controlled temperature and, within certain ranges, relative humidity has been constructed with the support of CONGELAGRO-Mc Cain Foods. This chamber (Figure 1.3) is large enough to be able to evaluate the reaction to PPD of thousands of cassava roots simultaneously. Also since measurements are made under controlled conditions, the results will be comparable from batch to batch, in evaluations taken months apart from each other.
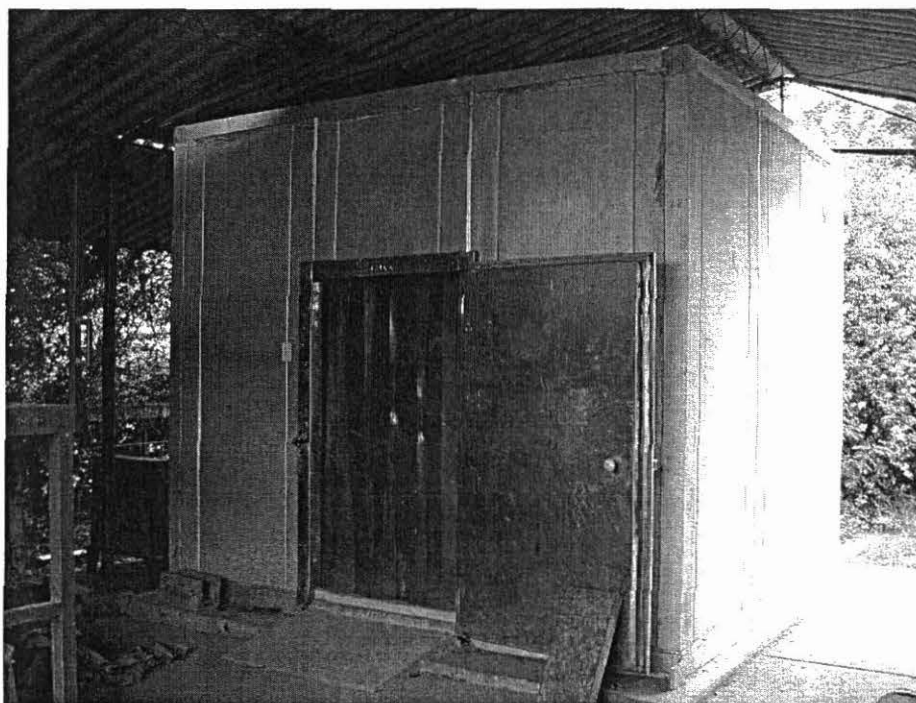
Figure 1.3. Photograph of the chamber with controlled temperature and relative humidity for more reliable and comparable measurements of PPD.

Activity 1.4. Further analyze other nutritional traits in cassava roots and leaves.

Rationale
Considerable amount of information has been produced in the previous analyses of cassava leaves and roots. The consolidated data provided a better base for relevant trends. Crude protein content in the roots was estimated (based on N measurements) in root samples of

600 cassava clones (CIAT, 2001) and further analyzed with other traits, as well as, looking at the geographical distributions (if any) associated with particular traits.

Objective
1) To consolidate and further analyze data obtained in the last three years in search of useful information regarding the nutritional quality of cassava roots and/or leaves.

Results

Regarding protein content in the roots (estimated through N measurements), the mean crude content of 3.06 % agrees with those reported in the literature. The few clones with high protein content (ranging from 5.75 to 8.31%) are remarkable. However, new root samples from the same clones will have to be evaluated again to confirm current expectations, and to have a better estimation of the effect of genotype by environment interaction in the expression of this trait. The weak correlation between nitrogen content and cyanogenic potential would suggest that a fraction of the nitrogen detected originated in the cyanogenic glucosides. This association, however, was low enough to allow for the expectation of developing high protein-low HCN clones.

Table 1.2. List of the best 33 clones regarding crude protein content in the roots from a sample of 600 cassava clones. CM and SM codes identify clones derived from CIAT's cassava breeding project. The remaining clones are from the germplasm bank collection. Highlighted are the clones from Central America.

Clone and protein (%)		Clone and protein (%)		Clone and protein (%)	
CM 5620-3	8.31	MCOL 2436	6.25	MBRA 101	5.94
SM 1406-1	8.13	MBRA 26	6.25	MCOL 219	5.94
MCOL 689B	7.75	*MCR 136*	6.13	*MGUA 33*	5.94
MCOL 1563	7.38	*MGUA 9*	6.13	CM 7310-1	5.88
MGUA 76	6.94	*MGUA 91*	6.06	MCOL 678	5.88
MCR 142	6.63	*MMEX108*	6.06	*MMEX 95*	5.81
CM 696-1	6.44	SM 629-6	6.00	*MGUA 79*	5.81
CM 3199-1	6.44	SM 673-1	6.00	MBRA 300	5.75
SM 734-5	6.44	MCOL 2532	6.00	MCOL 2459	5.75
MCR 38	6.31	*MGUA 19*	6.00	MBRA 1384	5.75
MGUA 86	6.31	CM 3236-3	5.94	MCOL 2694	5.75

A remarkable feature regarding protein content in the roots is that 12 out of the best 30 clones originated in Meso-America: Costa Rica, Guatemala and Mexico (Table 1.2). This

proportion (40 %) is much higher than that of clones representing this region (6.3%) in the total sample of 600 clones. This would suggest that a genetic introgression from meso-american non-cultivated *Manihot* species might have occurred, resulting in a high frequency of cassava clones with increased protein content. About a dozen *Manihot* species grow wild in Middle America (mainly *M. aesculifolia, M. gualanensis, M. isoloba, M pringlei,* and *M. oaxacana*), and can readily cross with *M. esculenta* (Brücher, 1989). Distinctive characteristics of cassava clones from Central America (particularly Guatemala) have been reported using simple sequence repeat markers (CIAT, 2001).

Activity 1.5. *To estimate the relative importance of genotype by environment interactions in the expression of carotene content in cassava roots.*

Rationale
Cassava is characterized by a notable genotype by environment interaction in many traits. Carotene content in the roots and leaf tissues is considered to be a relatively simple and stable trait, poorly affected by environmental factors. However, some preliminary data was required to confirm this assumption.

Objective
1) *To determine the relative importance of genotype by environment interaction in the expression of carotenes in the roots.*
2) *To measure the amount of carotenes remaining after different processing techniques by the HPLC quantifying method.*
3) *To determine the effect of freezing at –80 or –20 °C or upon lyophilization.*

Materials and methods
Four trials were conducted in four different locations in the Acid soil savannas. In the trials three contrasting cassava clones (for carotene content in the roots) were grown. Roots from each clone were harvested and delivered to CIAT-Palmira for carotene content analysis. The methodology used was the traditional colorimetric method described above.

Results

Very little genotype by environment interaction for carotene content in the roots was observed as illustrated in Figure 1.4. No relevant ranking cross-over was observed and the clone with the highest level of carotene content in the roots clearly maintained its superiority through the four environments evaluated. The variations among locations, with Santa Cruz showing the highest levels of carotene content and La Libertad and Cabuyal with the lowest can be explained by variations in dry matter content in the roots.

The results on stability of carotenes after different processing alternatives (boiling, sun drying and oven drying is underway. Preliminary results confirm that boiling the roots is the processing method with lowest loss of carotenes (about 75% of the original levels still present after boiling).

The last studies aim at establishing the stability of carotenes after a period of storage under three different conditions (at –80 or –20 °C or upon lyophilization). The purpose of this experiment is to determine alternatives for storing cassava roots for a certain period of time until their carotene content can be measured. Currently, all measurements are conducted with fresh roots, creating bottlenecks at harvest time, and limiting the number of samples that can be analyzed each growing cycle.
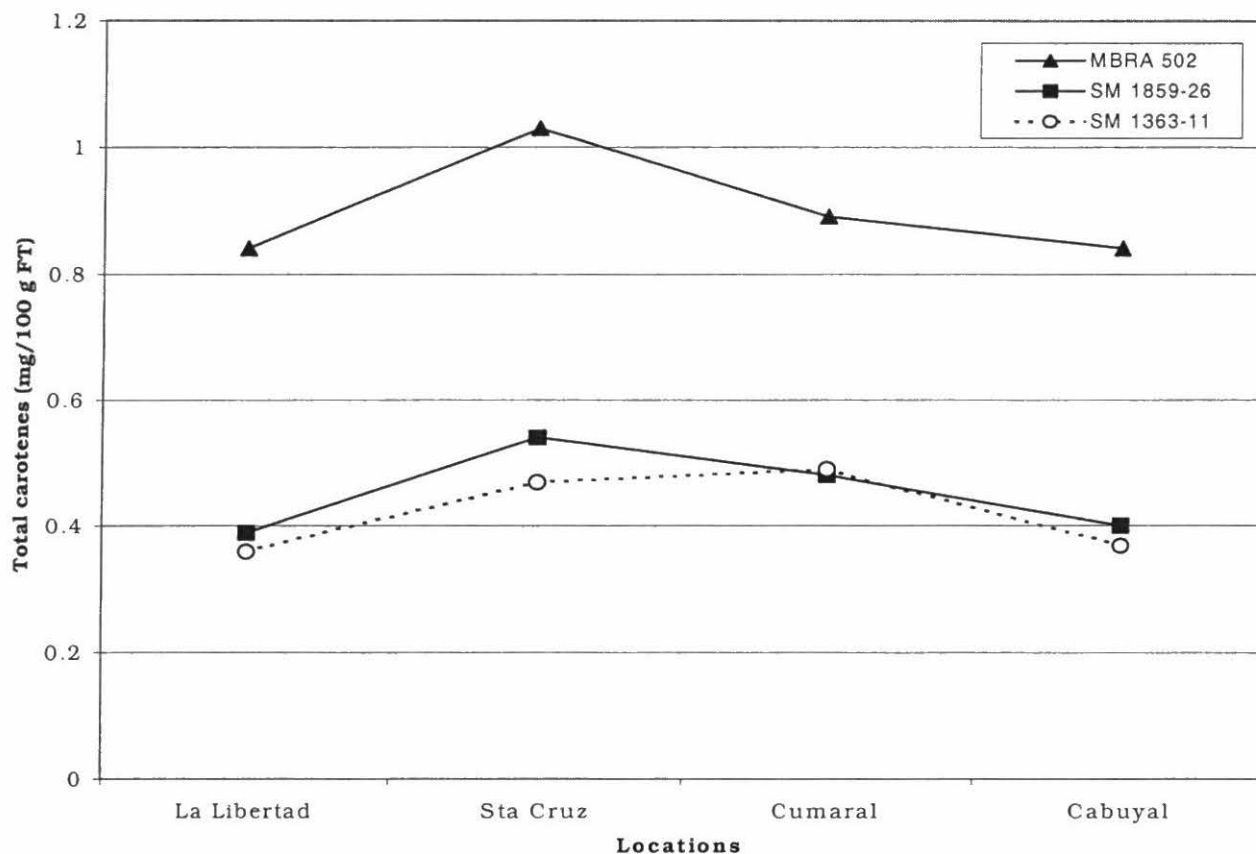
Figure 1.4. Stability of carotene content in the roots from three contrasting clones grown in four different environments in the Acid Soil Savannas of Colombia.

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Genetic stocks and improved gene pools developed and transferred to national programs.

The overall objective of this output is to produce genetically improved cassava germplasm, by recombining selected parental genotypes and then evaluating the segregating progenies under adequate environmental conditions. Recombinant seed and/or vegetative propagules from elite clones are then shipped to our collaborators in Africa, Asia and Latin America. The activities described below may not follow the exact order used to describe them in the respective work plan. This change has been made for more logical and, hopefully, easier to understand description of the research carried out. In addition to germplasm we are also producing information and developing technologies that will make the breeding process more efficient.

Activity 2.1 Selection of progenitors based on previous cycle results and information from other outputs (i.e., resistance/tolerance, root quality traits, etc.).

Rationale
The selection of parents to build populations for future breeding work represents the core of our improvement efforts, since it will determine the genetic progress we will achieve in the future. There are two types of populations developed: open pollinated and controlled crosses. We generally employ open pollination (polycrosses) to develop populations for target ecosystems. We have consistently developed polycrosses for the sub-humid tropics, acid soil savannas, semi-arid tropics, mid-altitude and highland tropics, and sub-tropics. In the case of controlled crosses, we used them to develop progenies for specific traits, special studies or the combination of elite experimental material with local landraces that need to be improved, but they can also be used for adaptation to target ecosystems as well.

Specific Objectives
a) *To identify, a set of elite clones, based on information of evaluation trials at several locations, and new objectives defined for the project. These clones are recombined to start a new cycle of selection.*
b) *To include as progenitor, for each agro-ecological zone, at least one genotype with high-carotene, yellow roots*
c) *To base the selection of parental lines increasingly on information from the performance of their progenies (≈ general combining ability).*

Materials and Methods
Only genotypes that have been selected over 2 consecutive years in *Advanced Yield Trials* are selected to participate as parents for the following generation. Among those genotypes, we select clones with outstanding performance for the most important agronomic traits. After the analysis of variance is conducted with data across two years, those genotypes exceeding at least one standard deviation from the overall mean are considered as parents for the next generation. Sometimes we also include landraces or already released cultivars that can contribute special features to the

progenies generated. Lately, thanks to the modifications introduced to the evaluation process selection of parents is greatly affected by data of the progenies they produce ($\approx$ general combining ability).

The information provided by pathologists, entomologists and quality specialists in relation to sources of resistance or special traits is used to select genotypes for controlled crosses. These controlled crosses are developed upon specific requests from National Programs that want their main landrace, or released varieties, crossed to genotypes with specific traits; or requests from CIAT scientists that want to pyramid genes, or develop segregating progenies for gene tagging.

As will be described below, one of the major changes introduced in the cassava breeding scheme at CIAT has been to take and record data on all progenies starting at the first evaluation stage (*Clonal Evaluation Trials*). The kind of information obtained allows a gross estimation of *general combining ability* (simply defined, it is the capacity of an individual to produce a good progeny) of parental lines employed in generating the clones included in those trials. This information is increasingly influencing the decisions of materials that will continue to be used as parents and those that will not.

Results

The parents selected for the development of gene pools targeted to specific ecosystems is presented in Table 2.1. The agronomic performance of these materials is described further down in this document. Seed will be harvested from July, 2003 through December, 2003. F1 plants will grow until the planting of the trials early in 2004. A major decision to take in the genetic improvement of crops is how to choose materials for use as parents that will produce new varieties with increased production potential and adequate adaptation to the environmental conditions under which they will be cultivated.

The principal criterion for selecting parents to date has been their performance *per se*. Unfortunately, however, good clones do not necessarily give rise to good progeny, hence the need to precisely estimate the traits that the progeny of each individual will produce. Until now, data was recorded starting at the *Preliminary Yield Trials*, which meant that no balanced information was available on **all** progeny produced by a given individual, but only on those that had passed the first stages of selection. The new modality implies taking data for all and each clone evaluated, whether or not it will be eventually selected. This permits the development of a solid database for selecting parents in terms of the progeny they produce (which, from the genetic viewpoint, is what really matters) and not merely based on their innate traits, as was done in the past.

During this year, the 58 genotypes listed in Table 2.1 were selected to produce a new generation of crosses. These materials had stood out for their excellent performance *per se*, and for demonstrating good levels of *general combining ability* in relation to the results observed in the respective *Clonal Evaluation Trials* (see sections *2.4.1, 2.4.2, and 2.4.3* for more detail). The agronomic performance of some of these materials *per se* is also described below. At the bottom of the table parental lines for special purpose crosses have also been listed. The seed produced from the current crossings will be harvested until December 2003.

Table 2.1. Parental lines to be used in crosses for different ecosystems, relevant for cassava production in the world.

#	General purpose crosses		
	Sub-humid tropics	**Inter-regional**	**Acid-soil savannas**
1	CG 1141-1		CM2177-2¶
2	CM 2772-3		CM 2772-3
3	CM 3306-4	CM 4919-1	CM 4574-7
4	CM 3306-19	CM 6754-8¶	CM 6438-14¶
5	CM 4919-1	SSM 1411-5	CM 6740-7
6	CM 6119-5	SM 1438-2	CM 6921-3
7	CM 7514-8	MTAI 8‡	CM 7951-5
8	CM 7514-7		SM 909-25
9	CM 6754-8¶		SM 1219-9
10	CM 4365-3		SM 1363-11
11	SM 1210-5		SM 1460-1
12	SM 1411-5		SM 1565-15
13	SM 1433-4		SM 1741-1
14	SM 1438-2		SM 2219-11
15	SM 1511-6	CM 6438-14¶	HMC-1
16	SM 1565-17	CM 6740-7	MBRA 383
17	SM 1665-2	CM 7951-5	MCOL 2737
18	SM 1669-5	SM 1219-9	MECU 64§
19	SM 1669-7	MBRA 383	MECU72§
20	MTAI 8 (Rayong 60)‡		MPER 335§
21	MTAI 16 (Kasetsart 50)‡		MPER 415§

#	Specific purpose crosses		
	Yellow roots with low HCN levels	**Resistance to white flies**	**High-altitude environments**
1	CM 489-1	MECU 72	CM 7514-7
2	CM 2772-3	CG 489-4	CM 7951-5
3	CM 3750-5	CG 489-23	SM 909-25
4	SM 1433-4	CG 489-31	SM 1219-9
5	MBRA 1A	CG 489-34	SM 1460-1
6	MCOL 1734		SM 1557-17
7	MCOL 2056		SM 1741-1
8	MCOL 2061		MBRA 383
9	MCOL 2206		MECU 72
10	MCOL 2316		
11	MCOL 2564		
12	MMAL 66		
13	MTAI 2 (Rayong 2)		

¶ Forage materials. § Resistance to white flies. ‡ Result of the CIAT-Thailand collaboration.

Planting materials were also selected from these parents to seed the *F1* in July 2002. In addition to crossing these lines they were also self-pollinated to begin an S_2 recurrent selection scheme to improve each of them for tolerance to inbreeding. The justification for this approach is given later when the description of a cassava-breeding

scheme based on the production of doubled-haploids described in Output 4.

Because project activities expanded to areas where CIAT had not previously worked intensely (e.g., Middle Magdalena River and Urabá, in Colombia), hybridizations for these areas will, this year, be conducted as follows: (1) polycrosses and crosses for the two most important cassava-producing regions (Sub-humid and Acid Soil Savannas). Similar needs exist for inter-Andean valleys that can be fulfilled by materials for the Acid Soil Savannas. (2) For new regions, for which the project had not developed specifically adapted materials, production of *interregional* crosses, combining the best five materials of the North Coast with clones adapted to the Acid Soil Savannas and vice versa. These progenies are also expected to produce germplasm with broad adaptation.

For environments affected by white flies sources carrying resistance to whitefly (MECU 64, MECU 72, MPER 335 and MPER 415) have been included. This pest has become the one true constraint to cassava cultivation in that region of Colombia. For the high-altitude tropics, crosses will be carried out within a group of clones recently identified as excellent based on their cooking quality, good acceptability to farmers, and, in some instances resistance to white flies (MECU 72).

Activity 2.2 Establishment of crossing blocks and production of recombinant seed from previously established blocks.

Rationale
Populations developed for specific ecosystems represent the basis for our cooperation with National Programs and **IITA** (International Institute of Tropical Agriculture, Ibadan, Nigeria). The development of genetic stocks is gaining importance through the years. Genetic stocks are produced based on the recombination of a set of genotypes that excel for a particular trait, and we would like to upgrade that trait beyond its natural range of variation (i.e. look for transgressive segregation in broader adaptation). Stocks developed for inheritance studies or to support molecular mapping of specific traits are constructed by the recombination of contrasting genotypes (i.e. resistance to ACMV, African Cassava Mosaic Virus). Often times our aim is to pyramid genes responsible for different sources of resistance (i.e. bacterial blight). As we shift our emphasis from applied breeding to more basic research supporting breeding (i.e. molecular marker assisted selection or MAS) genetic stocks will become even more important.

Parental population development in the future will concentrate more in targeting specific crosses between genotypes selected by NARS and complementary sources of genetic information from our genetic enhancement program or our global germplasm collection.

Specific Objectives
a) *To produce large number of seed by sexual crosses (either polycrosses or controlled) recombining desirable traits from selected parental materials, and deliver them to NARS in Africa, Asia and Latin America.*

Materials and Methods

For polycrosses we use the design developed by Wright 1965 for polycrosses in forage species. For this type of design there is a need to have a number of clones equal to a prime number minus one (i.e. 12, 16, 18, etc.). The design allows for each genotype to have the same probability of being surrounded by any other genotype of the selected group. Knowledge on flowering capacity is important in order to select a group of materials with synchronized flowering. When there are considerable differences we have to implement delayed planting and/or pruning of the earliest flowering genotypes. At harvest the seed from different plants of the same genotype are combined together and named as a half-sib family (**SM**).

For controlled crosses, we plant 10 to 20 plants depending on the flowering capacity of the genotype in question. The fruit developed from each flower has the potential to produce 3 seeds, but in average we obtain no more than 1 seed per pollination. This is due to the sensitivity of the stigma to the manipulation during pollination. Seeds from the same cross are mixed together and name as a full-sib family (**CM**). Because the number of CM families produced in the last few years has reached 10,000, we had began utilizing a new code for full-sib families (**GM**).

Results

A total of 91279 recombinant cassava seeds was produced at CIAT's Experiment Station, Palmira, during June 2001 to October 2002 (Table 2.2).

Table 2.2. Production of recombinant cassava seed at CIAT, Palmira, Valle del Cauca, Colombia, between June 2001 and October2002.

Purpose of cross	Controlled Crosses	Poli-crosses	TOTAL
Wide adaptation	3253		3253
Self pollinations	5085		5085
Yellow roots	5200		5200
Specific adaptation to:			
Sun-humid environment	9775	21434	31209
Acid soil savannas	5236	20591	25827
Mid-altitude valleys	3471	17234	20705
Total	**32020**	**59259**	**91279**

From each cross, a given number of botanical seed was obtained to initiate selection (stage *F1* in Figure 2.1). About 14000 seeds of the total produced, were planted (Table 2.3) to initiate stage *F1*. Of these, about 62% (i.e., 8500) could be transplanted, because either some seed did not germinate or the emerged plantlets were too weak to survive transplanting.

Table 2.3.Cassava seed processed for producing F1 plants for various purposes at CIAT, Palmira Valle del Cauca, Colombia, between June 2000 and August 2001.

Purpose of crosses	Planted seed	Transplanted seedlings
Sub-humid environment (A)	3971	2266
Acid soil savannas (B)	3866	2645
Mid-altitude valleys (C)	3844	2168
Para Tolima-Huila (A+C)	1063	725
Para Magdalena Medio (A+B)	1085	741
Total	13829	8545

Although the recombinant seed was produced at CIAT, the generated seedlings were transplanted to fields outside the Experiment Station and under conditions of isolation from other cassava crops. Thus, the generated *F1* plants grew and were maintained under conditions where possibilities of contamination from frogskin disease were minimized. This strategy, as can be seen in the description of results from different *Clonal Evaluation Trials*, was highly successful in virtually eliminating the incidence of this disease from the nurseries for cassava improvement at CIAT. The production of botanical seed within the CIAT Experiment Station did not represent high risk because this disease, which is probably induced by a virus, viroid or phytoplasm, is not transmitted through botanical seed.

Activity 2.3 Generation and distribution of advanced breeding materials for National Programs.

Rationale
Breeding for Asia has mainly centered on the issue of increased productivity of dry matter per hectare. Yield and root dry matter concentration have been the primary traits for selection, with almost no emphasis given to pests and diseases, or cooking quality. The results obtained in Asia for 15 years, has revealed the possibility to select for broader adaptation of genotypes. We have the case of *Rayong 60* and *Kasetsart 50* with good performance in a range of Asian countries. The production of germplasm for Asia has been moved from Thailand to Colombia due to budget constraints. However, because of the development attained by several NARS in Asia, the provision of recombinant material from Colombia can satisfy their needs. A CIAT soil scientist based in Thailand still coordinates the cassava network for Asia, but covering a broader spectrum of activities.

For Africa, our breeding efforts have been traditionally channeled through our collaboration with the International Institute of Tropical Agriculture (**IITA**) in Nigeria.

As a result extensive germplasm with Latin American "blood" has been introduced to Africa in a long introgression project financed by the International Fund for Agriculture Development (**IFAD**). The purpose of this special project was, among several others, to introgress Latin American cassava germplasm into Africa, in order to increase the genetic base of the crop in that continent, particularly for drought tolerance. This introgression process requires crosses to combine the desirable traits of Latin American germplasm, with resistance to the African Cassava Mosaic Virus (**ACMV**) disease.

Materials and Methods

The same approaches as the ones implemented for other regions of the word (polycrosses and controlled crosses) have been implemented, but a greater proportion of segregating progenies from controlled crosses is usually produced. Elite germplasm identified from the evaluations across the Asian region is periodically sent back to Colombia, to be used as a parental material in new cycles of selection.

Table 2.4. Shipments of recombinant seed produced within the project from September 2001 through September 2002.

Continents	Genotypes in-vitro	Crosses (families)	Plants (in-vitro)	Seeds in the shipment
Latin America				
In-vitro	200		15555	
Hybrid seed		130		8926
Asia				
In-vitro	635		1270	
Hybrid seed		668⁋		34865
Europe + USA				
In-vitro	9		45	
Hybrid seed				
Total				
In-vitro	843		16870	
Hybrid seed		798		43791

⁋ Hybrid seed from four crosses with wild relatives.

Results

A considerable fraction of the seed produced by the project has been transferred to National Programs in different regions of the world. As shown in Table 2.2, close to 91279 recombinant seeds were produced between June 2001 and October 2002 and about 50% of that seed (43,791) has been shipped to our collaborators (Table 2.4). The retirement of our cassava breeder stationed in Thailand, implied that since 1998 an increasing proportion of recombinant seed originated in CIAT-HQ. In the future, we

foresee that the flux of improved germplasm between CIAT-HQ, and the Thai breeding program will continue, and it will be through us that other National Programs will receive progenies involving the latest selections in Thailand. In November 2000, two scientists from Thailand came to CIAT to receive training in tissue culture (for recovering the shipments of *in vitro* plants) and to be exposed to the breeding scheme we are now following. Upon their return to Thailand they had been receiving several shipments of vitroplants containing the core collection of the germplasm bank. The last shipment of vitroplants from the core collection was successfully sent during 2002. Two more scientists from Thailand arrived in September 2002 to undergo further training in biotechnology.

Because of a self-imposed restriction for in-vitro shipments of cassava germplasm CIAT shipped a limited number of vitro-plants in the last two years. This restriction, however, has been gradually eliminated and therefore CIAT will increase the shipment of vitro-plants. To recover the lost time, we have produced about 8000 vitro-plants from a set of the best 31 clones available from our breeding program. Several plants from each clone have been or will be sent before the end of the year to countries in Asia, Latin America and the Caribbean and to IITA. As a result of this comprehensive on-station participatory evaluation and selection with the farmers, and NARS partners of the various countries, promising improved genotypes with desirable characteristics for end users will be identified (as has been the case in the past) under the local environmental conditions in each of the participating countries.

Activity 2.4. Selection of recombinant progenies for broad and specific adaptation within major agro-ecosystems (sub-humid; semi-arid; highland and acid soil savanna).

Rationale
Our strategy for cassava germplasm development is centered on the development of improved gene pools for specific edapho-climatic zones with importance for cassava production, as defined in Table 2.5. The most relevant ecosystems are the semi-arid and sub-humid tropics, for which we devote the majority of our efforts. The main selection activity is conducted in sites selected to represent the conditions of the target ecosystem. For every genotype that was tested in those sites, a copy was maintained at CIAT-HQ. This location is considered to be free of bacterial blight and some important viruses, and to maintain that condition, the introduction of vegetative material from other areas is restricted. In case vegetative material has to be brought to HQ, then it has to pass through quarantine, which usually takes more than a year.

Specific Objectives
a) *To modify the evaluation procedure to make it more efficient and to adapt it to the new breeding objectives.*
b) *To develop and evaluate superior germplasm adapted to particular ecosystems.*
c) *To develop genetic stocks useful for other CIAT projects.*
d) *To evaluate diallel crosses for quantitative genetics analyses.*

Materials and Methods

For each of the zones we conduct a recurrent selection program, with a progressive set of stages as described in Figure 2.1. As the stages progress, we give more emphasis to traits of lower heritability, because we have more planting material for each genotype, and the evaluation can be conducted in bigger plots with replications. Certain selection criteria are of general importance across ecosystem (i.e. yield potential, dry matter content), while others are specific for each ecosystem (i.e. pest and diseases).

Traditionally, the progenies generated from the crossing blocks (**F1**) were planted in screen houses and transplanted to the field after 2 months at CIAT. At 6 months after planting, 2 stakes were harvested from each plant and given a consecutive number according to the plant. One of the stakes was planted at CIAT, the other one, was planted at the main selection site (**F1C1**). Selection was conducted at harvest on individual plants at the main selection site. Planting material taken from the selected genotypes, at CIAT, was used subsequently to establish a non-replicated, 6-plant plot, both at CIAT and at the main selection site (***Clonal Evaluation*** stage). Evaluation was done using the central 3 plants. Selections were transferred to the following stage (***Preliminary Yield Trial***) and planted in non-replicated, 20-plant plots. Evaluation was done in the central 6 plants, and selections were then passed to the ***Advanced Yield Trials*** at 1 or 2 sites, with 3 replications of 25-plant plots. Genotypes selected over 2 consecutive years at the *Advanced Yield Trial* level were considered as "**elite genotypes**" and incorporated in the germplasm collection and the crossing blocks. Since each year a new breeding cycle was initiated, all the stages were simultaneously being conducted in each site.

Some modifications have been already implemented. A major constraint of the traditional evaluation methodology was that the first three stages of selection (*F1C1*, *Clonal Evaluation*, and *Preliminary Yield Trial*) were based on non-replicated plots. In addition large amount of material was maintained at HQ just to have duplicates of the very few materials that would reach the status of "*elite genotype*", in each cycle. Therefore, the changes introduced will speed up the selection process, allow for the evaluation of larger number of progenies and, hopefully, will increase the efficiency of the selection process. The main changes are as follows:

1) The *F1* plants will be grown for 10 months rather than 6. At that age they can produce up to 8-10 stakes. The stakes will be sent to the proper evaluation site for the *Clonal Evaluation*. This implies that the *F1C1* stage is eliminated and that no duplicate of each genotype is necessarily maintained at CIAT-HQ.

2) The *Clonal Evaluation Trials* will be based on up to eight plants, rather that six as before. An important modification for the sub-humid environment is that most measurements at the *Clonal Evaluation Trial* will be carried out in two stages: at the normal harvest time only two plants will be harvested to measure % of dry matter. This trait varies considerably with the time of harvest or age of the plant. Therefore to estimate it correctly, the plants need to be harvested at the proper time. The remaining six plants of each plot will be harvested just prior to normal planting time (one week before). Yield potential will be estimated visually (as had been done traditionally at the *F1C1* and *Clonal Evaluations* stages) based on the volume of roots produced by the six plants or, if possible, by weighing the total production of roots. Few other traits will also be taken using visual scores: plant

architecture, foliar health (for insects and diseases separately), above ground biomass (for an estimate of harvest index), and root aspect. A selection index software will be used to make an efficient and fast selection of the approximately 1000-2000 genotypes evaluated at this stage, for each ecosystem.

Table 2.5. Main ecosystems for cassava production, representative production regions, and main breeding sites.

Description	Representative Countries / Regions	Evaluation Sites
Sub-humid tropics (rainfall: 800- 1500 mm /year, bimodal rainfall distribution)	Colombia (Atlantic Coast & Santanderes); NE. Brazil; NE. Thailand; Dominican Republic, Haiti; N. and W. Venezuela; Mexico (Yucatan Peninsula); subhumid belt of Africa.	Caracolí Santo Tomás Betulia Barrancabermeja
Acid soil savannas (rainfall: 1500 – 3000 mm/year, short dry period, low pH)	Plains of Colombia & Venezuela; Brazil (Cerrado); Mexico (Tabasco); Cuba; W. African savannas; Philippines; Panama (Ocu)	La Libertad Matazul Sder de Quilichao Barrancabermeja
Humid tropical lowlands (rainfall: above 3000 mm/year, no clear dry period)	Amazon basin (Brazil, Colombia, Peru); W. Java & Sumatra; Malaysia; S. Vietnam; Equatorial West Africa	La Libertad Putumayo
Mid-altitude tropics (800-1400 masl)	Andean zone; central Brazilian highlands; mid-altitude areas of Nigeria, Cameroon, East Africa	Palmira Sder de Quilichao Barrancabermeja Tolima-Huila
High-altitude tropics (1400-2000 masl)	Andean zone; Rwanda; Burundi	Popayán Mondomo Armenia
Subtropics (latitudes higher than the tropics)	S Brazil; Argentina; China; N Vietnam; Cuba; Paraguay; S Africa	Sta Catarina (Brazil)
Semiarid (rainfall: below 800 mm/year, unimodal)	NE Brazil; NE Colombia; (Guajira) semiarid belt of West Africa; Tanzania; Mozambique; Ecuador (Coast)	Guajira Santo Tomas NE Brazil Huila

masl; meters above sea level

3) The changes described above allow taking stakes from no less than six plants (except for those cases were stakes did not germinate or plants died), rather than three, as in the past. These six plants will produce more than 30 cuttings, which will be used for the first replicated trial based on three replications and two row plots with ten plants per plot. It is recognized that this evaluation will result in some competition effect among neighboring plots. However, it is hoped that the number of replications will neutralize most of these effects. Also, row spacing between plots can be increased and the plant-to-plant distance within the plot reduced. This will maintain the density unchanged, while favoring competition among plants from the same genotype.

4) A final important modification to the evaluation process is that data will be taken and analyzed for **all** the progenies evaluated. In the past, data was taken only for those families that went beyond the *Clonal Evaluation* stage. Therefore it was difficult to estimate *combining ability* of parental materials, because most of the crosses did not produce data (they had been discarded in the field before any data was taken). The changes introduced will allow us, in the future, to base the selection of the parental materials on its breeding value (*general combining ability*) rather that its performance *per se*, or empirical appreciation of their potential as progenitor.

The main advantages of the new evaluation scheme can be summarized as follows:

&The duplication of materials maintained at CIAT-HQ is avoided until they reach status of "elite genotype".

&The selection of large number of segregating progenies, at the *F1C1* stage, which was based on single plant observations, is avoided.

&The time required to reach the stage of replicated trials is minimized.

&The total length of each cycle of selection is reduced by almost a year.

&Data records will allow for selecting parental material based on *general combining ability*.

&The total cost for each cycle of selection should be reduced.

&Selection will be less subjective by using appropriate software (specifically developed for that purpose).

&For environments with rains concentrated in one season, there is a possibility of selecting clones able to maintain high dry matter upon the arrival of the rains.

Time [¶]	Stage (old system)	Stage (new system)	Time [¶]
0	Crossing of selected parental genotypes ↓	Crossing of selected parental genotypes ↓	0
6	**F1** (3000-5000) *[6 months]* 1 plant / 1 site / 1 rep ↓	**F1** (3000-5000) *[10 months]* 1 plant / 1 site / 1 rep ↓	10
18	**F1C1** (2000-4000) *[1 year]* 1 plant / 1 site / 1 rep ↓	**Clonal evaluation** (1000-1500) *[1 year]* 6-8 plants / 1 site / 1 rep ↓	22
30	**Clonal evaluation** (500-1000) *[1 year]* 6 plants / 1 site / 1 rep ↓	**Preliminary yield trial** (150-300) *[1 year]* 10 plants / 1site / 3 rep ↓	34
42	**Preliminary yield trial** (100-200) *[1 year]* 20 plants / 1-2 sites / 1 rep ↓	**Advanced yield trial** (40-80) *[2 years]* 25 plants / 2-3 sites / 3 reps	58
66	**Advanced yield trial** (30-60) *[2 years]* 25 plants / 2-3 sites / 3 reps		

ELITE GERMPLASM

Germplasm collection	Regional trials	Crossing blocks	Participatory research

[¶] Time in months after germination of botanical seed.

Figure 2.1. Basic cassava breeding schemes applied for each of the priority ecosystems. On the right is the new scheme currently under implementation (shaded area). Later stages of selection are made following the old system (shaded area on left).

Preparing new F1 field

About 12,000 recombinant, botanical seeds were germinated early in 2002, and approximately 8,500 of the resulting plantlets were transplanted at the Colombian Centro de Investigación de la Caña de Azucar (CENICAÑA). This material represents the *F1* stage described in Figure 2.1.

Basic description of the selection index used for ranking the segregating clones in different types of trials.

Below, results for each agroecological area are presented, together with results of the best genotypes according to a **selection index**. This index is a tool for genetically improving crops, and integrates, into a single value, information on various relevant traits. In most cases, the index was estimated according to the following formula:

Selection index = [FRY * 10] + [DMC * 8] - [PT * 3] + [HI * 5]

where,
>   FRY = fresh root yield
>   DMC = dry matter content
>   PT = plant type using a 1(excellent) to 5 (very poor) visual scale
>   HI = harvest index

In this formula, the weighting of each variable is evident. **Fresh root yield** is multiplied by 10 to maximize the influence of this trait on the end-result. **Dry matter content** is multiplied by 8, also to increase its relevance in the selection process. This is important because roots with high dry matter content can be dried more quickly, or else, starch extraction made significantly easier. In both cases, processing costs are reduced.

Plant type integrates several important aspects for cassava: (1) plant health, inasmuch as a plant with a lot of foliage is not likely to have been severely attacked by leaf diseases and pests (at least, not during evaluation); (2) photosynthesis was functioning up to evaluation time; and (3) general plant architecture, as on this depends the quantity of vegetative seed (stakes) produced and the ease with which the farmer can care for the crop. Because a 1 to 5 score is used (where 1=excellent and 5=very poor plant type), the formula uses a negative term for this trait.

Finally, the **harvest index** estimates how much of the plant biomass represents the product with economic value. For now, the index is estimated in terms of the ratio of root production to the plant's total biomass.

A technical clarification: these indexes are severely affected by the unit by which each trait is measured, for example, dry matter content, which fluctuates around 35.0%, would have a much greater effect than does the harvest index, which fluctuates between 0 and 1. To avoid this problem, each variable is converted into what are statistically known as *standardized values*, which obviate the issue of units.

The most relevant results obtained in six major cassava-producing regions of

Colombia during the cycle that finished with harvests during March to May, 2002 are summarized below.

Diallel mating designs were used as part of the Clonal Evaluation stage at each environment.

An important aspect of the hybridization strategies executed during the last two years were the production and planting of recombinant seed following an scheme of diallel crosses. Therefore, not only did we produce a group of progenies with such high potential that some will hopefully surpass the agronomic performance so far reached, but also we could begin a genetic study without precedent for cassava. These diallel trials were harvested during the first semester of 2002 and produced information essential for understanding the mode of inheritance of traits with agronomic relevance. Two Ph.D. students: a woman from Vietnam and a man from Uganda are using this data for their respective dissertations.

The diallel trials for the North Coast (Table 2.6), Acid Soil Savannas (Table 2.7) and inter-Andean valleys (Table 2.8) were recently harvested. As a result, the *Clonal Evaluation* stage for March-May 2001 to March-May 2002 for the three environments has been represented by these relevant studies.

Table 2.6. Parents involved in the diallel experiment for the sub-humid environment of the North Coast of Colombia. The code assigned to each cross is above the diagonal[¶].

Parental Clones	CM 6754-8	CM 8027-3	SM 805-15	SM 1565-17	SM 1411-5	SM 1219-9	SM 1657-12	SM 1665-2
Rayong 60	CM 9106	CM 9148	CM 9178	CM 9966	CM 9958	GM 266	GM 289	GM 291
CM 6754-8		CM 9921	CM 9945	CM 9907	CM 9954	GM 236	GM 237	GM 238
CM 8027-3			CM 9703	CM 9926	CM 9923	GM 246	GM 247	GM 248
SM 805-15				CM 9949	CM 9946	GM 250	GM 251	GM 252
SM1565-17					CM 9957	CM 9952	GM 280	GM 281
SM 1411-5						GM 255	GM 272	GM 273
SM 1219-9							GM 258	GM 259
SM 1657-12								GM 287

[¶] Only 30 plants were used to represent each F1 cross in the diallel study. Remaining plants from each cross were planted in an ordinary *Clonal Evaluation Trial*.

For scientific validity, a singular planting was carried out: from each crossing, 30 of the best F1 plants grown in CENICAÑA were selected to obtain at least eight stakes of excellent quality. Six of those stakes were used to plant the trials and the others were planted in nurseries to serve as seed source. The six stakes for the trials were distributed in three replicates located at two representative sites. Every F1 (with a few exceptions) from the diallel cross experiment were made up of 30 genetically different individuals conforming a full-sib family (both parents known and in common). Each individual was represented in the trials by six plants, as mentioned above. Many of these families can provide the basis for molecular marker studies that will facilitate future work on cassava genetic improvement. It should be pointed out that in addition

of the30 individual clones from each F1 cross, sister clones could also be found in the *Clonal Evaluation Trials*.

Table 2.7. Parents involved in the diallel experiment for the acid-soil savannas in the Eastern plains of Colombia. The code assigned to each cross is above the diagonal [¶].

Parental Clones	MTAI-8 Rayong 60	CM 7033-3	CM 4574-7	CM 6740-7	MPER-183	SM 1219-9	SM 1565-15	SM 2058-2	SM 2219-11
HMC-1	CM 8035	GM 244	GM 224	GM 234	CM 9733	GM 264	GM 277	GM 299	GM 303
MTAI-8		CM 9127	GM 226	GM 235	GM 307	GM 266	GM 279	GM 301	GM 305
CM 7033-3			GM 219	GM 227	GM 245	GM 240	-.-	GM 241	GM 243
CM 4574-7				CM 9460	GM 225	GM 220	GM 221	GM 222	GM 223
CM 6740-7					CM 9642	CM 9901	GM 229	GM 232	GM 233
MPER-183						GM 265	GM 278	GM 300	GM 304
SM 1219-9							GM 256	GM 261	GM 263
SM 1565-15								GM 275	GM 276
SM 2058- 2									GM 298

[¶] Only 30 plants were used to represent each F1 cross in the diallel study. Remaining plants from each cross were planted in an ordinary *Clonal Evaluation Trial*.

Table 2.8. Parents involved in the diallel experiment for the mid-altitude valleys of Colombia. The code assigned to each cross is above the diagonal [¶].

Parental Clones	SM 1219-9	SM 1741-1	SM 1278-2	SM 1636-24	SM 1673-10	HMC-1	MPER-183	MECU-72
CM 6740-7	CM 9901	CM 9903	GM 228	GM 230	GM 231	GM 234	CM 9642	GM 308
SM 1219-9		CM 9953	GM 254	GM 257	GM 260	GM 264	GM 265	GM 309
SM 1741-1			GM 269	GM 284	GM 292	GM 296	GM 297	GM 313
SM 1278-2				GM 267	GM 268	GM 270	GM 271	GM 310
SM 1636-24					GM 283	GM 285	GM 286	GM 311
SM 1673-10						GM 293	GM 294	GM 312
HMC-1							CM 9733	GM 314
MPER-183								GM 306

[¶] Only 30 plants were used to represent each F1 cross in the diallel study. Remaining plants from each cross were planted in an ordinary *Clonal Evaluation Trial*.

2.4.1. Selections for the Sub-Humid Tropical Environment

For logistic reasons, improvement activities developed for several regions of the Northern Coast of Colombia were centralized initially in Barranquilla. Many of the materials evaluated there can then be transferred to the more humid region in the Departments of Córdoba and Sucre, and to the Middle Magdalena (Department of Santander). The results for this eco-region are described in Tables 2.9 to 2.24 Table 2.9 lists all trials, whereas the other tables show results specific to each one.

Table 2.9. Trials conducted in the sub-humid ecosystem (North Coast of Colombia) in the 2000-2001 cycle[q].

Trial	Site	N° of genotypes	N° of reps	Observations
F1	CIAT-Palmira	2267 (1)	1	Plants are left growing in the field for 10 months.
Clonal Evaluation	S. Tomás	1350 (8)	1	See Table 2.11 to 2.13
Diallel Trial	Pitalito	1080 (1)	3	Planted again this cycle
Diallel Trials	S. Tomás	1080 (1)	3	See Table 2.14
	Pitalito	1080 (1)	3	
Preliminary Yield Trial	Pitalito	225 (10)	3	See Tables 2.18 to 2.20
Advanced Yield Trial	Santo Tomás	88 (25)	3	Eliminated by poor germination originated back in a 1999 flood.
Regional Trials [§]	Sub-humid	40 (25)	3 x 4	See Table 2.21 (four locations)
	Humid	40 (25)	3 x 4	See Table 2.22 (four locations)
	Urabá	40 (25)	3 x 3	See Table 2.23 (three locations)

[q] Values in parentheses refer to the number of plants per plot. [§] A total of 11 locations were involved in the Regional Trials

A total of 2267 of seedlings from botanical seeds (targeting this environment) were transplanted at CENICAÑA in an isolated field surrounded by sugar cane. Several more seeds had been put to germinate but many did not germinate or died soon after. The planting of the *F1* stage is isolated to reduce as much as possible infection by diseases that can be found at later stages of the evaluation process. Seedlings from botanical seed are considered to be disease-free and efforts are made to maintain this condition for as long as it can possibly be done. Table 2.10 describes the families for the *Clonal Evaluation Trial* that will be planted in 2003, their progenitors and the number of clones representing each family.

A summary of the results from the *Clonal Evaluation Trial* for the Sub-Humid environment harvested this year is presented in Table 2.11. The effect of harvesting time is apparent from the last two columns of that Table. In the March harvest, conducted during the middle of the dry period, dry matter content averaged 32.40% across the whole experiment. After the rains began in May, cassava showed a typical decrease in dry matter content with an average of 29.57%.

Table 2.10. Families, progenitors and number of genotypes representing each family planted as F1 in CENICAÑA for the Sub-humid environment. The specific male progenitor of SM families is not known but pollen came from a group of selected clones.

	Family	Mother	Father	# clones		Family	Mother	Father	# clones
1	CM 9775	CM 7514-7	MNGA 19	61	26	SM 2828	CM 7389-9	Unknown	55
2	CM 9791	SM 1433-4	MNGA 19	63	27	SM 2829	CM 7395-5	Unknown	43
3	CM 9794	SM 1438-2	MNGA 19	58	28	SM 2832	SM 805-15	Unknown	39
4	CM 9797	SM 1511-6	MNGA 19	41	29	SM 2834	SM 1411-5	Unknown	41
5	CM 9912	CM 7514-8	SM 1433-4	64	30	SM 2835	SM 1431-2	Unknown	35
6	CT 54	R 5	KU 50	32	31	SM 2836	SM 1433-4	Unknown	33
7	CT 57	R 60	KU 50	32	32	SM 2839	SM 1565-17	Unknown	39
8	CT 59	R 90	R 60	76	33	SM 2882	CM 3372-4	Unknown	54
9	GM 281	SM 1665-2	SM 1565-17	48	34	SM 2947	CM 6754-8	Unknown	49
10	GM 288	SM 1657-12	SM 2192-6	23	35	SM 2948	CM 8027-3	Unknown	50
11	SM 2546	SM 890-9	Unknown	76	36	SM 2949	SM 805-15	Unknown	35
12	SM 2547	SM 1068-10	Unknown	70	37	SM 2951	SM 1433-4	Unknown	38
13	SM 2612	SM 1600-4	Unknown	61	38	SM 2952	SM 1438-2	Unknown	52
14	SM 2615	CM 4365-3	Unknown	42	39	SM 2954	SM 1521-10	Unknown	43
15	SM 2618	CM 7389-9	Unknown	51	40	SM 2955	SM 1565-17	Unknown	22
16	SM 2620	CM 7514-8	Unknown	55	41	SM 2956	SM 1619-3	Unknown	29
17	SM 2621	SM 643-17	Unknown	35	42	SM 2957	SM 1657-12	Unknown	33
18	SM 2626	SM 1201-5	Unknown	37	43	SM 2958	SM 1657-14	Unknown	21
19	SM 2629	SM 1422-4	Unknown	59	44	SM 2959	SM 1665-2	Unknown	11
20	SM 2667	CM 6438-14	Unknown	54	45	SM 2960	SM 1754-21	Unknown	29
21	SM 2733	SM 1210-10	Unknown	51	46	SM 2962	SM 2192-6	Unknown	31
22	SM 2773	SM 737-38	Unknown	86	47	SM 2963	MTAI 8	Unknown	40
23	SM 2777	SM 1210-10	Unknown	50	48	SM 2964	MVEN 25	Unknown	53
24	SM 2779	SM 1411-5	Unknown	58	49	SM 2982	CM 2772-3	Unknown	28
25	SM 2783	SM 1511-6	Unknown	47	50	SM 3000	CG 1141-1	Unknown	34

Table 2.11. Results of the selection carried out in the *Clonal Evaluation Trial* at Santo Tomás, Department of Atlántico, from 1967 clones evaluated during May 2001 to May 2002.

Parameter or Genotye	Yield (t/ha)		Harvest Index (0 to 1) [¶]	Plant type (1 to 5) [§]	Dry matter content (%)	
	Fresh roots	Dry matter			March	May
Results from the 1967 clones evaluated						
Maximum	68.58	19.58	0.86	5.00	42.07	38.25
Minimum	0.00	0.00	0.15	1.00	16.39	16.30
Mean	23.89	6.89	0.57	2.77	30.92	27.72
Std. Dev.	9.27	2.74	0.10	0.93	3.39	3.64
Results from the 277 clones selected						
Maximum	68.58	19.58	0.86	4.00	40.24	38.25
Minimum	12.63	3.63	0.40	1.00	21.64	19.24
Mean	35.22	11.02	0.62	2.39	32.40	29.57
Std. Dev.	7.81	2.20	0.08	0.69	2.71	2.79
Best 10 clones selected across the entire Clonal Evaluation Trial						
GM 290-9	52.47	16.72	0.70	3	33.3	29.3
CM 9907-80	68.58	19.58	0.63	2	29.1	27.1
CM 9907-38	50.98	14.82	0.71	2	30.6	26.0
CM 9907-41	39.32	12.34	0.86	1	32.0	29.8
CM 9958-40	38.39	13.04	0.67	2	34.9	31.9
CM 9966-45	37.44	10.81	0.73	1	29.3	28.1
GM 290-54	47.60	14.86	0.56	2	31.8	29.9
CM 9957-75	30.33	10.52	0.85	2	35.3	33.2
GM 259-69	39.87	12.64	0.62	2	30.4	34.1
GM 236-40	39.03	11.75	0.65	2	31.1	28.2

[¶] The harvest index is obtained by dividing the production of commercial roots by total biomass (roots + aerial parts). Preferred harvest indexes are > 0.5.

[§] Plant type integrates under one value, plant architecture, leaves health, and capacity to produce stakes on a scale where 1 = excellent and 5 = very poor is used.

Selection of the 277 clones that passed to the next stage of evaluation was based on the **Selection Index** described above. The superiority of the selected fraction is apparent. Mean fresh root production was 35.22 t/ha in the selected fraction against 23.89 from the entire population. Converted to dry matter productivity these figures were 11.02 and 6.89 t/ha, respectively. Likewise, the average harvest index was considerably higher in the selected fraction (0.62) than in the whole trial (0.57). Finally dry matter content was about 2% higher in the selected fraction than in the total of clones evaluated, regardless of the harvesting time. The contributions of the Ministry of Agriculture and Rural Development of Colombia and of the Fondo Nacional Avícola (FONAV) of the Federación Nacional de Avicultores de Colombia (FENAVI) have been fundamental to the project's significant growth, enabling the project to now identify, with more certainty, outstanding germplasm.

Plant type was evaluated twice, when the two harvestings took place. Table 2.11 presents only the result of the first evaluation date for this trait. There was an excellent phenotypic correlation ($\rho=0.628$) between the two measurements. Also dry

matter content measured in March and in May showed good positive correlation (ρ=0.706). The two measurements of harvest index showed the highest correlation coefficient (ρ=0.740). Finally, fresh root yield showed the lowest correlation (ρ=0.533), although this value is considered to be quite acceptable for a complex trait such as yield.

Worth mentioning is the fact that three out of the ten best clones came from the same family (CM 9907). This situation is adequate to illustrate a weakness of the current scheme. Because of logistical reasons, planting the different clones from the same family together, one after the other, greatly facilitates the planting operations, which is quite cumbersome given the size of the experiment. Proceeding this way, however, creates an undesirable confounding effects because all the clones from a given family are grouped together in the same part of the field. It is possible, therefore, that family CM 9907 was favored by being located in the best part of the field. Since the average of each family's performance is used to get indications of the *general combining ability* of their progenitors, it is convenient to avoid planting all the clones from a given family together but, split in three more or less equal-sized groups. In this way, the *Clonal Evaluation Trial* will be divided into the equivalent of three replications. Since the selection is based on individual clones, this procedure may not help in the selection process. However, the average of each family (as shown below in Table 2.12) will certainly be more precise by dividing the family in the three proposed groups.

Table 2.12 shows the average for each of the 52 families planted in the *Clonal Evaluation Trial* harvested in May 2002. The total number of clones making up the family and the proportion that was selected is also provided. All this information is very valuable for identifying progenitors that tend to produce better (or worse) progenies. Family CM 9923 was made up of 34 clones, and 24 of them were selected (70.6%). About 14% of the clones were selected in the whole trial (277 out of 1967). Therefore, a family that showed such a high degree of success is quite remarkable.

Several families (CM 9703, GM210, GM 237, GM 280, and GM 281) were represented by several clones, of which none was selected. This suggests that for one reason or another, these families were genetically inferior to the rest of the families, and therefore, that the parents that originated them lacked enough positive genetic attributes. On the other hand, in addition to the already mentioned CM 9923, families GM 290 and CM 9946, showed excellent performances. Moreover, the information presented in Table 2.12 allows making some inference about the reasons for the general performance of a given family. For instance, CM 9954 had the best average for plant type (1.57) and CM 9945 the worst (3.46). Family GM 237 had an even worse average for plant type (4.75) but it was represented only by two clones. Harvest indexes in some families were adequate (GM291, GM 248 and CM 9926), while in others was undesirably low (GM237, GM 216, and GM 212).

Dry matter content is a variable of great economic relevance in this region. Families CM 9921 and GM 212 presented an average of 31.8%, followed by GM 218 and CM 9923 with 31.2%. In contrast, GM 237, GM 281, and GM 280 had a average of low dry matter content in the roots. The most important trait (root productivity) showed sharp contrasts. Families CM 9923, CM 9926 and GM 290 produced more that 30 t/ha of fresh roots. On the other hand, families GM 237, GM 280, and GM 290 produced less than 15 t/ha.

Table 2.12. Results from the *Clonal Evaluation Trial* (Santo Tomás, Atlántico), of 1967 genotypes. The averages of each of the 52 families, number of clones representing them and proportion of selected clones is presented.

Family	# clones	% select. Clones	% Fol. Ret.	Yield Fresh (t/ha)	Yield DM (t/ha)	% DM	Plant Type (1-5)	HI (0-1) ¶	SI §
CM 8209	70	11.0	8.6	22.2	6.59	29.8	2.69	0.51	-3.0
CM 9106	5	20.0	40.0	21.4	6.14	28.0	2.90	0.65	-2.2
CM 9148	31	3.0	9.7	20.8	6.11	28.9	3.24	0.53	-9.0
CM 9178	40	4.9	27.5	20.8	5.97	28.9	3.01	0.57	-5.6
CM 9703	18	0.0	11.1	21.4	6.13	29.6	3.03	0.56	-4.2
CM 9907	50	28.0	18.0	28.4	8.05	28.9	2.58	0.64	9.1
CM 9921	15	40.0	33.3	26.5	8.28	31.8	3.03	0.65	9.76
CM 9923	34	70.6	17.3	32.2	9.93	31.2	2.03	0.59	19.2
CM 9926	21	19.0	19.1	30.9	8.61	28.5	3.00	0.67	9.2
CM 9945	25	4.0	28.0	19.7	5.74	29.5	3.46	0.64	-5.4
CM 9946	8	42.9	37.5	23.1	7.24	30.8	2.31	0.54	4.5
CM 9949	35	11.0	28.6	22.6	6.38	28.4	2.71	0.54	-4.1
CM 9952	41	17.0	7.3	29.7	7.80	26.3	2.88	0.64	3.7
CM 9954	15	6.7	26.7	25.5	7.15	28.7	1.57	0.51	6.66
CM 9957	47	23.4	6.4	26.3	7.95	30.8	2.59	0.58	10.0
CM 9958	59	35.6	22.0	27.0	8.09	30.6	2.28	0.58	9.95
CM 9966	44	16.0	22.7	26.1	7.42	28.6	2.69	0.59	2.3
GM 210	15	0.0	20.0	18.2	5.51	30.1	3.13	0.51	-9.67
GM 211	71	11.3	18.3	20.7	6.42	31.0	2.87	0.52	-3.1
GM 212	56	1.8	16.1	20.4	6.39	31.8	3.05	0.48	-4.7
GM 213	79	7.6	1.3	20.7	5.89	28.4	2.51	0.51	-5.7
GM 214	67	12.0	0.0	21.3	6.51	31.1	2.63	0.51	-0.71
GM 215	37	5.4	10.8	20.6	5.81	28.6	2.82	0.54	-6.62
GM 216	29	3.4	27.6	17.0	5.09	26.1	2.53	0.42	-18.8
GM 217	57	8.8	5.3	20.4	5.54	27.9	2.75	0.57	-6.5
GM 218	30	6.7	63.3	21.8	6.72	31.2	2.93	0.52	-1.8

Family	# clones	% select. Clones	% Fol. Ret.	Yield Fresh (t/ha)	Yield DM (t/ha)	% DM	Plant Type (1-5)	HI (0-1) ¶	SI §
GM 236	36	11.0	30.6	24.2	7.05	29.6	2.65	0.65	5.9
GM 237	2	0.0	0.0	3.4	1.07	21.1	4.75	0.36	-61.9
GM 238	52	17.3	23.1	30.0	8.16	28.1	3.21	0.66	5.3
GM 239	16	37.5	50.0	28.0	8.58	31.1	2.34	0.57	11.3
GM 246	42	14.3	16.7	25.5	7.48	29.9	2.61	0.61	6.3
GM 247	16	37.5	37.5	22.2	6.62	29.6	2.81	0.67	3.2
GM 248	27	18.5	25.9	23.6	6.75	28.3	2.91	0.69	2.5
GM 249	43	16.8	46.5	22.1	6.89	30.7	2.81	0.54	-0.5
GM 250	29	10.3	10.3	25.7	7.45	29.6	2.78	0.58	3.2
GM 252	21	4.8	19.5	21.9	6.02	27.4	3.12	0.63	-5.7
GM 253	57	3.5	52.6	22.0	6.71	30.2	2.90	0.56	-1.4
GM 255	31	9.7	0.0	24.8	6.87	28.1	2.77	0.52	-3.6
GM 258	29	17.2	44.8	24.9	7.21	29.6	2.97	0.60	1.8
GM 259	55	21.8	1.8	26.7	7.44	27.7	2.70	0.63	3.4
GM 262	62	16.4	53.2	24.6	6.89	28.3	2.62	0.59	0.7
GM 266	61	6.6	32.8	23.4	6.56	28.3	2.75	0.58	-1.5
GM 273	28	17.9	0.0	26.6	7.74	29.6	2.75	0.62	6.2
GM 274	16	12.5	50.0	27.0	7.99	30.3	2.69	0.55	5.2
GM 280	9	0.0	0.0	9.31	2.53	25.3	3.33	0.57	-27.3
GM 281	39	0.0	18.0	14.0	3.30	23.0	3.04	0.62	-22.7
GM 282	76	2.6	56.6	19.4	5.11	27.2	2.82	0.59	-8.4
GM 287	24	8.3	29.2	24.3	6.47	27.4	3.08	0.63	-2.5
GM 288	46	26.0	32.6	27.4	8.04	29.5	2.63	0.58	6.1
GM 290	66	44.0	40.9	32.7	9.22	29.0	2.77	0.65	12.8
GM 291	31	12.9	12.9	26.7	7.12	26.6	2.95	0.70	2.4
GM 302	53	26.4	24.4	27.4	8.01	29.2	2.75	0.56	3.4

¶ HI = **Harvest Index** (Root production / total biomass).
§ SI = **Selection Index** (combines several variables of economic relevance)

The reason for selecting 277 clones has a justification. The next phase of selection will include 300 clones, which will be evaluated in three separate trials of 100 genotypes each. The 23 additional clones (300-277= 23) will be included to carry out an experiment to compare three different selection procedures:

a) A group of 21 clones with the best selection index.
b) A group of 21 clones with the highest root yield.
c) A group of 21 clones with the highest harvest index.

The best 21 clones (based on selection index) are, obviously among the 277 selected clones because that was precisely the selection criteria utilized. However, few clones (14 to be precise) where among the best 21 clones for yield or harvest index, but had not been selected by the selection index. These clones were included, therefore, to make this comparison possible. Table 2.13 presents the characteristics of the three groups that will be used for the contrasts of selection criteria. It should be pointed out that two or more of the groups shared some clones. Nine additional clones will be used as check, making up the total of 300 clones (277+14+9=300).

Table 2.13. Results of the selection from the *Clonal Evaluation Trials* (Santo Tomás, Atlántico), using three different criteria.

Parameters	Yield (t/ha)		Dr matter (%)	Plant type (1 a 5)	Harvest index (0 a 1)
	Fresh roots	Dry matter			
Statistics of the best 21 clones for Harvest Index					
Maximum	40.1	12.2	34.3	4.50	0.86
Minimum	11.5	2.3	18.8	1.00	0.62
Mean	26.22	6.97	26.75	3.43	0.77
St. Dev.	8.38	2.70	3.81	0.94	0.05
Statistics of the best 21 clones for Yield					
Maximum	68.6	19.6	32.8	4.00	0.79
Minimum	39.4	9.8	20.4	1.00	0.53
Mean	49.76	13.58	27.94	2.38	0.68
St. Dev.	6.72	2.46	3.24	0.74	0.06
Statistics of the best 21 clones for Selection Index					
Maximum	68.6	19.6	34.8	4.00	0.75
Minimum	31.6	10.7	27.7	1.00	0.54
Mean	46.01	14.13	31.40	1.81	0.66
St. Dev.	8.57	2.03	2.19	0.73	0.06

Table 2.14. Results of the diallel trials conducted in Pitalito y Santo Tomás (Atlántico). Each
F1 cross was composed by 30 clones. Three replications per location were used.

Cross	Trips (1 - 5)	Stakes/pl. (Number)	Fresh roots (t/ha)	Harvest index (0 - 1)	Dry matter (%)	Plant Type (1 - 5)	Root Type (1 - 5)
1x2	3.03	8.98	34.93	0.54	27.45	3.18	2.89
1x3	2.70	10.58	26.51	0.43	29.47	2.97	3.21
1x4	2.27	11.23	31.45	0.45	28.78	2.95	2.92
1x5	1.63	11.87	42.29	0.57	26.45	2.66	2.78
1x6	1.75	12.29	36.51	0.47	29.10	2.64	3.01
1x7	1.91	12.13	42.35	0.55	28.14	2.71	2.82
1x8	2.72	8.68	38.14	0.55	26.27	3.35	3.18
1x9	2.93	10.55	45.68	0.56	27.00	3.11	2.79
2x3	2.40	9.26	32.82	0.54	29.05	2.84	2.86
2x4	2.63	9.88	27.69	0.47	27.05	3.13	3.18
2x5	2.04	11.66	35.48	0.55	26.64	2.59	2.97
2x6	2.23	10.67	37.98	0.53	28.26	2.80	2.78
2x7	2.28	11.40	34.76	0.52	27.64	2.68	2.94
2x8	2.86	9.08	31.63	0.52	28.19	3.29	3.11
2x9	2.68	9.38	36.25	0.52	28.12	2.99	2.85
3x4	2.47	10.50	34.22	0.49	28.57	2.82	2.94
3x5	1.65	9.87	40.99	0.59	27.07	3.00	2.84
3x6	2.22	11.47	38.90	0.49	29.23	2.57	2.92
3x7	1.95	11.24	39.37	0.54	28.59	2.76	2.89
3x8	2.61	11.05	34.77	0.49	28.36	3.00	3.08
3x9	2.59	9.49	41.16	0.59	27.74	2.99	2.94
4x5	2.04	9.40	37.29	0.56	25.29	3.23	3.09
4x6	2.21	10.88	35.59	0.51	28.38	2.74	2.95
4x7	1.73	11.20	34.01	0.46	27.93	2.97	3.15
4x8	3.12	8.44	35.53	0.53	27.74	3.45	3.10
4x9	2.93	9.27	31.49	0.51	27.20	2.99	3.20
5x6	1.64	11.95	40.98	0.52	28.00	2.69	2.83
5x7	1.36	12.38	42.59	0.54	25.66	2.71	2.98
5x8	1.97	9.48	35.96	0.57	26.81	3.19	3.02
5x9	1.94	11.32	40.65	0.58	24.74	2.82	2.90
6x7	1.76	12.76	37.49	0.46	28.07	2.76	3.01
6x8	2.44	11.11	38.58	0.51	27.93	2.82	3.08
6x9	2.29	10.62	41.28	0.56	28.40	2.51	2.74
7x8	1.94	11.48	42.87	0.54	26.36	2.74	2.79
7x9	1.58	10.64	39.48	0.52	27.98	2.69	2.99
8x9	2.57	10.25	42.70	0.55	26.89	3.04	2.83
Max.	3.12	12.76	45.68	0.59	29.47	3.45	3.21
Min.	1.36	8.44	26.51	0.43	24.74	2.51	2.74
Mean	2.25	10.62	37.23	0.52	27.63	2.90	2.96
St.Dev.	0.46	1.14	4.39	0.04	1.11	0.23	0.13

It should be remembered that along with the *Clonal Evaluation Trial* an ambitious experiment of diallel crosses was also harvested in May this year. In Table 2.14 the averages (combined across the two locations) of the most important traits are presented.

From the breeding viewpoint, the results from the diallel study from nine parents are equivalent to the *Clonal Evaluation Trial*. The only difference being that, rather than having all the plants from a given clone planted in one-row plot, in the diallel experiment these plants were scattered in three replications at two locations. Also six rather than eight plants were used to represent each clone in the diallel. Best performing clones will also be included in *Preliminary Yield Trials*. The experiment was planted again this year.

The diallel experiment included 36 crosses from nine parents (9*8/2=36). Each cross was represented by 30 clones (with a few exceptions). The trials were planted in two locations (Santo Tomás and Pitalito), with three replications each.

Eleven of the 36 families yielded more than 40 t/ha. Progenitors 2 (CM 6754-8) and 4 (SM 805-15) did not participate in any of these high-yielding families, whereas parents 5 (SM 1565-17) and 9 (SM 1665-2) were involved in five of these eleven families with superior yields.

Regarding the reaction to trips, the best crosses (low average scores) involved parents 5 (SM 1565-17) and 7 (SM 1219-9). For dry matter content parents 3 (CM 8027-3) and 6 (SM 1411-5) were the best based on the averages of their progenies, whereas parent 5 (SM 1565-17) was characterized by progenies with low dry matter content in the roots.

Table 2.15. Results of all the crosses with a common parent from the diallel trial conducted in two locations in the Sub-Humid environment (Santo Tomás y Pitalito. Atlántico).

Progenitor	Trips score (1 - 5)	Stakes per plant (Number	Fresh roots (t/ha)	Harvest Index (0 - 1)	Dry matter (%)	Plant type (1 - 5)	Root type (1 - 5)
1=MTAI 8	2.37	10.79	37.2	0.51	27.83	2.95	2.95
2=CM 6754-8	2.52	10.04	33.9	0.52	27.80	2.94	2.95
3=CM 8027-3	2.32	10.43	36.1	0.52	28.51	2.87	2.96
4=SM 805-15	2.42	10.10	33.4	0.50	27.62	3.04	3.07
5=SM 1565-17	1.78	10.99	39.5	0.56	26.33	2.86	2.93
6=SM 1411-5	2.07	11.47	38.4	0.51	28.42	2.69	2.92
7=SM 1219-9	1.81	11.65	39.1	0.52	27.54	2.75	2.95
8=SM 1657-12	2.53	9.95	37.5	0.53	27.32	3.11	3.03
9=SM 1665-2	2.44	10.19	39.8	0.55	27.26	2.89	2.91
Maximum	2.53	11.65	39.8	0.56	28.51	3.11	3.07
Minimum	1.78	9.95	33.4	0.50	26.33	2.69	2.91
Mean	2.252	10.623	37.2	0.524	27.626	2.899	2.961
St. Dev.	0.291	0.636	2.34	0.020	0.651	0.130	0.053

The data for each cross can be further consolidated to produce the averages of all the crosses involving a given parent, which are presented in Table 2.15. As it is frequently the case results from Table 2.15 demonstrate the difficulties in producing a perfect genotype. For instance the progenies of parent 5 (SM 1565-17) were outstanding yield wise and showed excellent reaction to trips. However, they also showed the lowest dry matter content in the entire experiment. Progenies from parent 6 (SM 1411-5) had a superior plant type (the lowest average score = 2.69), the highest dry matter content (28.42%), but low harvest index (0.51). It is clear that the results presented in Table 2.15 agree with those from Table 2.14 with the averages of each individual F1 family.

It should be remembered that results from Table 2.14 are averages across 30 clones making up each F1 family, and those from Table 2.15 grouping together the averages of all the progenies with a parent in common. The range of variation among individual clones is much larger. For instance, the highest average for yield in Table 2.14 was 45.68 t/ha (cross 1 x 9), but the highest yield by an individual clone was equivalent to 134 t/ha (Santo Tomás trial). When we perform the analysis of the segregation within each family (that is, among the 30 clones from each F1 family), some interesting results are expected to surface.

Advantages of the new scheme: implications for industry

In the North Coast, the *Clonal Evaluation Trial* was handled in a particular fashion. Because of the bimodal distribution of rainfall, which begins end of April to early May, cassava is traditionally harvested in February or March. Plants harvested at this time cannot be used as seed source because the stakes have deteriorated by the time the rains arrive in May. Consequently, the *Clonal Evaluation Trial* (based on six plants) used to be evaluated during the dry season, using three plants. The remaining three plants were left as seed source, being cut in May.

This situation meant that seed (produced from only three plants) was limited and, as a result, the following evaluation stage could not be made with replicates. The previous year, for the first time, the procedure for conducting the *Clonal Evaluation Trial* was modified considerably. First, the number of plants representing each clone was increased to eight. Of these eight plants, two were harvested in March, mainly to measure dry matter content during the optimal time for taking this measure. When the rains arrive, the cassava plant reinitiates its growth, thus extracting energy that had been accumulated in the roots. As a consequence, dry matter content drops to the extent that starch and chip-drying industries usually either reject the roots or pay low prices for them.

The new procedure, for the *Clonal Evaluation Trial* requires the measuring of dry matter content in each clone on two occasions: during the dry season (March) and after the rains arrive (May). The previous year a group of 20 clones was selected because of their high dry matter content and capacity to maintain it after the rains arrive. These clones were planted again this cycle

Capacity of maintaining high dry matter content with the arrival of the rains
The new breeding scheme requires measuring dry matter content in the roots of each clone

twice: in the middle of the dry period and after the arrival of the rains. This situation offers the possibility of selecting clones that have the capacity of maintaining high dry matter content after the initiation of the rains. Last year a group of 20 clones was selected for this trait and they will continue to be evaluated and crossed among themselves to evaluate the possibility of improving this important trait.

Capacity of retaining leaves for a longer period of time in the absence of biotic or abiotic stresses.
Another significant result obtained from the *Clonal Evaluation Trial* from the previous year was the observation about the capacity of some genotypes to retain leaves for longer periods during plant growth observed by the end of October. At that time, the crop was 5½ months old and a differential capacity to retain leaves was already obvious.

Table 2.16. Effect of leaf retention in 5½-month-old cassava on traits measured 5 months later (at harvest) in the *Clonal Evaluation Trial*, Santo Tomás, Department of Atlántico, Colombia.

Leaf retention	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Dry matter content (%)	Plant type (1 - 5)	Harvest index (0-1)
Yes	26.62	7.74	29.59	2.72	0.60
No	23.05	6.63	28.84	2.80	0.57

During the current season the evaluation for leaf retention was conducted slightly later than the previous year, which proved to be undesirable because leaf senescence was already occurring in clones that obviously had retained the leaves for a longer period of time. The end result was that the data was probably not as reliable as in 2001. Still an clear effect on root yield could be observed. In Table 2.16 the mean performances of the clones that retained the leaves versus those that did not are presented. The former yielded more that three t/ha of fresh roots than the latter (about 1 t/ha of dry matter). Although the date for evaluation proved to be not at the optimum time, consolidating information on the positive effect of leaf retention on yield and other characteristics of agronomic relevance is gradually emerging.

The connection of leaf retention with the new breeding scheme originates in the number of plants representing each clone. In the new system eight plants (rather than one as was the case in the old system) are used to represent each clone. The differences in leaf retention became very apparent because there were eight identical plants showing the trait, and that helped to call the attention of the scientists when visiting the field.

Possibility of a gross estimation of General Combining Ability for progenitors.
One of the most important advantages of the new breeding scheme is that data from all progenies are taken and recorded from the very first evaluation stage. This produced a more balance data of all the progenies produced by a given progenitor, which is a valuable

approach for determining its breeding value.

Table 2.17. Progenitors of clones included in the *Clonal Evaluation Trial* (Santo Tomás, Atlántico) described in Table 2.10.

Progenitor	Number of clones produced	% of clones selected
CM 523-7	511	6.80
CM 6754-8	231	16.45
CM 8027-3	318	23.10
SM 805-15	289	9.24
SM 1219-9	465	13.19
SM 1411-5	305	25.70
SM 1565-17	399	12.24
SM 1657-12	155	13.20
SM 1665-2	400	15.43
SM 2192-6	465	19.24
MTAI 8	394	15.16
Total	3932	-.-
Mean	-.-	15.43

Table 2.17 is a summary of the information provided earlier in Table 2.12. In Table 2.17 the number of clones derived from a given progenitor and the proportion of them that were eventually selected is presented. Progenies of clone CM 523-7 (the old and venerable ICA-Catumare variety) where not competitive, since only 6.80% of them survived. This is not a surprise because CM 523-7 is adapted to the Acid Soil Savannas environment and adapts poorly to the Sub-Humid conditions. This clone was included as progenitor for this region, however, because of its known resistance to bacteriosis (*Xanthomonas axonopodis* pv. *manihotis*). This disease is not common in the drier environments but occasionally appears when rains are more abundant. The inclusion of CM 523-7, therefore, aimed at introgressing some level of resistance to bacteriosis in the germplasm adapted to the Sub-Humid conditions. Most of the progenies were rejected, but few (and that is enough) survived and passed to the following stage of evaluation.

SM 1411-5 and CM 8027-3 produced progenies that had a much higher chances of being selected. A very poor performance was shown by the progenies of SM 805-15 in addition to that of CM 523-7. It is this consolidated information that allows us to guide the decision of what parents to use or not to use in the production of a new generation of promising genotypes. For instance, SM 805-15 has not been included in the list of progenitors for the Sub-Humid environment (Table 2.1). SM 1411-5 has been included and current data supports that decision. On the other hand, we have contrasting results from other clones. For instance, SM 1565-17 was not very outstanding based on information from Table 2.17 (with only 12.24% of its progeny being selected). But this clone was highlighted for its high yield potential and reaction to trips in the diallel analysis (Table 2.15), but also because of its low dry matter content. That is probably the reason why a lower proportion of their progenies

was selected. Exactly the opposite situation is faced by CM 8027-3, with poor performance regarding reaction to trips and fresh root productivity (Table 2.15), but the highest dry matter content. Because of the high emphasis given to dry matter content by the Selection Index, it is, therefore, not surprising that a higher proportion of its progenies was selected. This clone has not been included in the list of parents for this year, but it will be re-introduced next year.

Table 2.18. Results of the best five out of 20 selected from the **Experiment 1** of the *Preliminary Yield Trials* conducted in Pitalito (Atlántico).The trials included three replications of 10-plant plots and 75 genotypes. In italics clones used as check.

Clones or parameters	Plant type (1-5)	Root type (1-5)	Harvest Index (0-1)	Dry matter content (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Selection Index
SM 2545-22	3.7	2.0	0.64	32.3	36.5	11.8	39.69
SM 2618-8	2.0	2.3	0.50	31.6	30.5	9.7	30.9
SM 2548-22	1.7	1.7	0.57	29.6	28.3	8.5	28.66
SM 2619-4	2.0	3.0	0.45	34.4	26.9	9.2	28.06
SM 2616-6	2.7	2.7	0.58	32.7	25.7	8.5	22.37
Statistics of the 20 clones selected.							
Maximum	4.0	3.0	0.6	34.6	36.5	11.8	39.69
Minimum	1.3	1.7	0.4	23.5	18.4	5.6	8.34
Mean	2.33	2.32	0.52	30.48	26.29	8.00	18.32
St.Dev.	0.72	0.41	0.06	2.97	4.07	1.29	8.47
CG 1141-1	*1.7*	*2.7*	*0.51*	*30.3*	*21.3*	*6.6*	*13.11*
CM 3306-4	*4.0*	*2.7*	*0.38*	*34.2*	*16.7*	*5.7*	*-6.45*
M TAI 8	*1.7*	*2.0*	*0.54*	*30.1*	*25.2*	*7.6*	*20.36*
Statistics of the 75 clones evaluated in Experiment 1.							
Maximum	4.3	4.0	0.6	34.6	36.5	11.8	39.69
Minimum	1.3	1.7	0.2	20.9	7.3	1.7	-41.41
Mean	2.73	2.76	0.46	29.29	20.51	6.03	0.00
St.Dev.	0.72	0.55	0.08	3.23	5.70	1.80	16.44

The best 225 clones selected from the *Clonal Evaluation Trial* harvested the previous year were planted in three different *Preliminary Yield Trials* identified as Experiments 1, 2 an 3. Each of these trials included 75 clones that were evaluated in 10-plants plots with three replications. The most relevant results of these trials are presented in Tables 2.18, 2.19, and 2.20. One of the advantages of using the selection index with standardized values, described above, is that by definition an average performance has a value of zero. This can be confirmed in the right column of these Tables, where the population mean of the 75 clones was, in every case, zero. A positive selection index suggests better than average performance. A negative value, on the other hand, indicates an undesirable general performance.

Table 2.19. Results of the best five out of 20 selected from the **Experiment 2** of the *Preliminary Yield Trials* conducted in Pitalito (Atlántico). The trials included three replications of 10-plant plots and 75 genotypes. In italic clones used as checks.

Clones or parameters	Plant type (1-5)	Root type (1-5)	Harvest Index (0-1)	Dry matter content (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Selection Index
SM 2771-5	2.7	1.7	0.49	34.3	46.2	15.9	42.94
SM 2773-32	2.3	3.7	0.64	39.0	25.5	10.0	31.10
SM 2775-4	2.3	1.7	0.55	33.8	32.0	10.8	25.60
SM 2773-21	1.7	2.7	0.52	34.5	28.0	9.6	25.13
SM 2629-36	2.3	2.3	0.58	33.4	29.7	9.9	22.52
Statistics of the 20 clones selected.							
Maximum	3.3	3.7	0.6	39.0	46.2	15.9	42.9
Minimum	1.0	1.3	0.4	24.5	20.5	7.1	11.4
Mean	2.28	2.20	0.53	33.17	28.66	9.47	18.72
St.Dev.	0.60	0.64	0.06	2.83	5.74	1.88	7.69
CG 1141-1	*2.7*	*2.0*	*0.48*	*32.3*	*27.5*	*8.9*	*9.96*
CM 3306-4	*3.7*	*2.0*	*0.44*	*32.7*	*21.4*	*7.0*	*0.08*
M TAI 8	*2.3*	*2.0*	*0.5*	*31.3*	*25.8*	*8.1*	*10.8*
Statistics of the 75 clones evaluated in Experiment 2.							
Maximum	4.00	3.67	0.71	39.05	46.20	15.86	42.94
Minimum	1.00	1.00	0.32	22.70	7.29	1.89	-40.50
Mean	2.71	2.40	0.49	30.51	23.36	7.17	0.00
St.Dev.	0.62	0.61	0.08	3.62	6.69	2.28	16.19

It can be observed in Table 2.18 that the average selection index of the 20 selected clones (out of the 75 evaluated) was clearly larger than zero (18.32), indicating the superiority of their performance compared with the rest of the experiment. The three checks included in the trial (CG 1141-1, CM 3306-4, and MTAI 8) had an average of 9.01. MTAI 8 was the best check with a selection index of 20.36, demonstrating that this excellent clone is still very competitive in the region. The best five clones, however, had an average selection index of 29.93. Yield wise the 20 selected clones produced an average of 8 t/ha of dry matter, whereas the three checks produced an average of 6.63 t/ha (20% superiority). Dry matter content of the best five and the 20 selected clones were, respectively 32.12 and 30.48%, whereas the average for the three checks was 31.53%.

For the **second experiment**, the results are presented in Table 2.19. The mean selection index for the 20 clones selected was 18.72, similar to the one in Experiment 1. The checks had a mean of 6.94. Yield in the second experiment was higher than in the first with an average of more that one t/ha of dry matter productivity, compared with Experiment 1. The mean dry matter productivity of the 20 clones selected was 9.47 t/ha, compared with 8 t/ha of the three checks (18% superiority). The best five clones yielded an impressive 11.2 t/ha of dry matter. Dry matter content of these clones was 35.0%, in comparison with 32.1% for the checks.

Table 2.20. Results of the best five out of 20 selected from the **Experiment 3** of the *Preliminary Yield Trials* conducted in Pitalito (Atlántico). The trials included three replications of 10-plant plots and 75 genotypes. In italic clones used as checks.

Clones or parameters	Plant type (1-5)	Root type (1-5)	Harvest Index (0-1)	Dry matter content (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Selection Index
CM 9456-21	2.7	1.3	0.54	29.9	37.19	11.16	29.54
SM 2781-6	1.0	1.3	0.51	27.4	35.36	9.43	29.04
CM 9456-12	2.7	1.7	0.66	31.3	30.57	9.55	28.46
SM 2783-26	1.3	1.0	0.46	29.0	35.59	10.38	28.35
SM 2782-4	2.7	1.3	0.57	31.5	32.71	10.23	27.81
Statistics of the 20 clones selected.							
Maximum	4.3	3.0	0.7	31.7	37.2	11.2	29.5
Minimum	1.0	1.0	0.4	22.9	22.3	6.9	10.3
Mean	2.37	1.68	0.54	29.44	30.63	8.96	20.29
St.Dev.	0.91	0.61	0.06	2.16	4.55	1.20	6.15
CG 1141-1	*2.3*	*2.0*	*0.48*	*31.0*	*15.99*	*5.03*	*-0.32*
CM 3306-4	*4.7*	*2.3*	*0.42*	*33.4*	*16.67*	*5.57*	*-9.2*
M TAI 8	*3.0*	*2.0*	*0.50*	*30.9*	*25.10*	*7.78*	*10.2*
Statistics of the 75 clones evaluated in Experiment 3.							
Maximum	5	3.33	0.66	33.42	37.19	11.16	29.54
Minimum	1	1	0.33	17.61	9.01	1.728	-39.00
Mean	3.05	2.09	0.49	28.11	22.45	6.34	0.00
St.Dev.	0.98	0.58	0.06	3.94	6.90	2.14	15.99

Results of **Experiment 3** are presented in Table 2.20. Mean performance of the 20 selected clones (average Selection Index = 20.29) was markedly superior to that of the three checks (average Selection Index = 0.23). The superiority of the selected material is mainly due to higher productivity of fresh roots (30.63 versus 19.25 t/ha). Dry matter content in the selected material, however, was lower than in the checks (29.44 versus 31.7 %).

The 20 selected clones from each experiment were pooled together in a 60-genotype *Advanced Yield Trial*, which will be planted in 25-plants plots with three replications at two locations (See Figure 2.1). Hopefully, the trial will be conducted for two consecutive years until their best entries are considered suitable for the last stage of selection (*Regional Trials*)

The *Advanced Yield Trial* stage was not conducted this year because of a flood in 1999 that created a vacuum in the evaluation process. Therefore, the next stage of the evaluation process (see Figure 2.1) is the *Regional Trials*. In this cycle, 40 clones were included and evaluated in up to 11 locations. Four in the truly sub-humid environments of Atlántico and Magdalena Departments; four in the more humid conditions of the Córdoba and Sucre Departments; and the remaining three locations in the Urabá region.

Table 2.21 summarizes the results of the *Regional Trial* in the Atlántico and Magdalena Departments (Pitalito, Baraona, Caracolí, and Santo Tomás). Averages of fresh root production, dry matter content and harvest index across the four locations are provided. Also, the ranking (based on the selection index) for each individual location is included in the right columns. The order in which the clones are presented was based on the selection index taking into account the averages across the four locations.

One striking feature of the data presented in Table 2.21 is the sharp contrasts in the ranking of each material in different locations. The best material (across locations) occupied the 17th, 30th, 1st, and 13th order in each of the four locations, respectively. This is a common feature of cassava, which frequently exposes large genotype by environment interaction. This is particularly true for those clones in the top of the table. At the bottom, however, the results are much more consistent. In general, results from Caracolí tended to be contrasting with those from the other three locations.

The best clone (across the four environments using the selection index) was SM 1759-29. This clone, however, showed the high interaction by environment interaction mentioned above. The same is true for the second clone (CM 3306-19), which ranked 34th, 1st, 22nd, and 23rd at each location. The third (CM4919-1) and fourth (SM 1411-5) clones had a more consistent performance across locations. With the exception of the first clone (SM 1759-29) all the clones on top of the table had been included as parents for the crossing nursery of this year (Table 2.1). Worth mentioning is the position occupied by MTAI 8. Until recently, this clone was second to none in the region. But the Table 2.21 ranked eight. It is not clear if the gradual decrease of the competitiveness of this clone is due to the genetic superiority of newer materials; a result of some decline of its performance due to contamination of non-pathogenic organisms; or a combination of these two reasons.

In Table 2.22, the results of the *Regional Trials* conducted in the more humid region of Córdoba and Sucre Departments are presented. The sharp changes in the ranking at different locations observed in the top of Table 2.21 is not seen here. The best clones (SM 1665-2) ranked 5th, 4th, 1st, and 11th in the four locations. Similar results were observed in the following top performing clones. This would suggest that genotype by environment in this region was not as important as in the previous one, therefore, allowing for an easier identification of the best clones. As was the case for the Atlántico and Magdalena Departments, the clones at the bottom of the Table showed a consistently poor performance also in the case of the Córdoba and Sucre Departments.

A different kind of genotype by environment can be observed when we compare the results from Tables 2.21 and 2.22. If the best ten clones from each location are taken into account, only four clones (CM 4919-1, CM 3306-19, SM 1669-7, and SM 1973-25) were shared by the two regions. The best clone from Table 2.21 occupied the 34th position in Table 2.22. Likewise, the best clone in Table 2.22 ranked 19th in Table 2.21. The situation on CM 6754-8, sencond in Córdoba-Sucre, was 27th in the Atlántico-Magdalena. This situation has been noticed before and used as an example of the genotype by environment interaction considering these large regions in the north coast of Colombia.

Table 2.21. Results of the *Regional Trial* for the sub-humid conditions in Atlántico and Magdalena Departments. Ranking of clones is based on selection index estimated from across locations average.

	Clone	Fresh roots (t/ha)	Dry matter (%)	Harvest index (0 a 1)	Ranking based on selection index at each individual location			
					Pitalito	Baraona	Caracolí	S.Tomás
1	SM 1759-29	32.49	29.06	0.58	17	30	1	13
2	CM 3306-19	25.72	28.04	0.68	34	1	22	23
3	CM 4919-1	24.71	27.79	0.69	3	7	18	5
4	SM 1411-5	27.92	27.28	0.60	4	21	8	3
5	SM 1565-17	30.24	25.55	0.64	21	10	16	29
6	CM 6119-5	22.36	29.32	0.62	15	5	11	2
7	SM 805-15	24.42	28.14	0.62	24	3	23	17
8	M TAI 8	25.85	27.27	0.64	10	6	26	18
9	SM 1669-7	21.31	29.89	0.59	7	23	2	7
10	SM 1973-25	21.94	29.49	0.58	1	9	14	19
11	SB 0216-9	25.74	27.50	0.60	39	2	19	20
12	SM 643-17	20.50	30.53	0.55	8	8	4	14
13	M VEN 25	27.33	26.79	0.58	9	26	7	4
14	CM 7514-8	22.79	27.92	0.65	20	11	24	8
15	SM 1511-6	23.50	27.33	0.65	12	15	5	6
16	SM 1650-7	19.28	30.20	0.58	35	13	6	1
17	CM 4843-1	23.76	27.10	0.64	29	12	21	22
18	SM 1778-45	23.51	27.85	0.59	18	16	25	9
19	SM 1665-2	23.68	26.30	0.69	31	4	33	27
20	MCOL 1505	23.36	27.58	0.59	19	17	30	16
21	SM 1669-5	21.92	27.99	0.61	14	14	28	15
22	CM 6740-7	24.94	26.44	0.61	2	33	38	10
23	M BRA 384	24.68	27.07	0.57	6	20	37	25
24	SM 1438-2	21.72	28.19	0.57	5	34	15	11
25	CM 8475-4	22.21	27.89	0.54	13	24	31	24
26	SGB 765-4	21.18	28.39	0.53	11	25	10	26
27	CM 6754-8	20.22	26.87	0.69	25	27	12	32
28	CM 523-7	19.04	28.52	0.58	16	32	32	12
29	SM 1778-53	21.11	27.42	0.53	27	22	35	28
30	CM 8027-3	19.56	27.03	0.61	26	35	13	34
31	MCOL 2215	16.19	28.80	0.59	23	31	3	36
32	CG 1141-1	20.06	27.44	0.54	36	19	9	38
33	SM 1627-16	19.25	27.59	0.54	33	29	17	30
34	CM 3306-4	17.25	28.44	0.53	28	28	27	33
35	SGB 765-2	17.07	27.55	0.59	22	18	20	37
36	SM 1973-23	18.63	25.67	0.61	32	36	36	31
37	CM 6758-1	17.81	26.34	0.53	30	37	39	21
38	SM 1624-2	14.86	28.00	0.43	37	39	34	35
39	M PER 183	19.74	22.61	0.54	38	40	40	39
40	SM 1657-14	15.96	25.39	0.45	40	38	29	40
Maximum		32.49	30.53	0.69	33.78	45.50	65.94	30.39
Minimum		14.86	22.61	0.43	14.17	16.22	13.67	9.50
Mean		22.09	27.61	0.59	23.90	26.28	19.49	18.71

Table 2.22. Results of the *Regional Trial* for the sub-humid to humid conditions in Córdoba and Sucre Departments. Ranking of clones is based on selection index estimated from across locations average.

	Clone	Fresh roots (t/ha)	Dry matter (%)	Harvest index (0 a 1)	Ranking based on selection index at each individual location			
					Sahagun	C. Oro	Corozal	La Unión
1	SM 1665-2	31.11	30.81	0.59	5	4	1	11
2	CM 6754-8	28.96	31.62	0.59	1	12	11	7
3	CM 4843-1	29.19	31.52	0.56	11	1	8	4
4	SM 1669-7	23.17	34.99	0.53	8	3	6	12
5	CM 3306-19	28.36	30.45	0.58	6	21	2	19
6	SM 1973-25	28.32	32.37	0.48	16	2	26	2
7	SM 1438-2	24.39	33.64	0.49	33	15	3	3
8	CM 4919-1	25.63	31.06	0.57	10	6	24	8
9	SM 643-17	19.86	35.55	0.52	27	9	9	6
10	SM 1973-23	23.53	32.68	0.53	13	16	13	10
11	M TAI 8	24.67	31.98	0.53	7	8	27	14
12	SM 1669-5	23.46	32.17	0.54	15	7	14	23
13	CM 523-7	23.00	32.53	0.54	19	14	4	20
14	CM 6119-5	20.50	33.82	0.53	12	13	10	27
15	CG 1141-1	20.92	33.59	0.53	25	10	16	13
16	SM 1565-17	31.14	26.75	0.56	3	28	7	35
17	M BRA 384	28.46	28.77	0.52	32	17	20	9
18	SM 1411-5	22.78	32.01	0.52	31	5	12	25
19	SB 0216-9	25.33	31.41	0.47	14	23	34	1
20	SM 1511-6	21.36	32.54	0.53	9	20	25	22
21	SM 1650-7	23.35	31.99	0.49	36	24	5	5
22	SGB 765-2	20.93	33.32	0.49	23	19	22	15
23	CM 8027-3	20.77	32.63	0.50	22	na	15	26
24	MCOL 2215	17.21	34.99	0.48	28	18	23	24
25	MCOL 1505	22.19	31.33	0.51	20	34	19	16
26	SM 805-15	23.31	30.73	0.50	2	31	32	31
27	CM 3306-4	18.18	35.21	0.42	26	25	18	21
28	SM 1778-53	20.36	33.21	0.43	18	27	21	32
29	CM 7514-8	17.96	33.56	0.47	29	22	31	17
30	SM 1627-16	20.49	32.25	0.46	4	36	33	28
31	CM 6758-1	21.15	32.14	0.44	21	29	17	33
32	M VEN 25	19.63	31.51	0.51	37	11	28	30
33	SM 1624-2	15.89	33.05	0.41	24	32	35	36
34	SM 1759-29	17.82	32.11	0.37	35	30	37	18
35	SM 1778-45	14.56	32.42	0.42	30	35	38	29
36	CM 6740-7	18.54	28.58	0.49	17	38	30	38
37	SGB 765-4	13.86	33.42	0.37	34	37	29	39
38	CM 8475-4	15.50	31.61	0.40	38	33	36	37
39	SM 1657-14	13.67	27.23	0.41	40	26	40	34
40	M PER 183	12.03	23.74	0.37	39	39	39	40
Maximum		31.14	35.55	0.59	29.28	38.11	37.61	41.56
Minimum		12.03	23.74	0.37	7.06	12.33	5.83	14.78
Mean		21.79	31.88	0.49	14.69	23.89	22.06	26.51

na = not available

Table 2.23. Results of the *Regional Trial* for the humid conditions in the Urabá Region. Ranking of clones is based on selection index estimated from across locations average.

	Clone	Fresh roots (t/ha)	Dry matter (%)	Harvest index (0 a 1)	Ranking based on selection index at each individual location		
					Carepa	Necoclí	Mutatá
1	SM 1565-17	40.68	33.83	0.67	1	23	26
2	SM 1511-6	35.62	37.02	0.63	7	14	3
3	SM 1411-5	37.31	35.68	0.65	25	1	11
4	SM 1669-7	31.39	38.72	0.64	18	19	2
5	SM 1438-2	33.95	38.36	0.57	11	6	7
6	SGB765-4	36.60	36.32	0.60	2	17	na
7	CG 1141-1	31.45	38.78	0.62	22	5	4
8	SM 1665-2	34.75	35.68	0.68	17	16	5
9	CM 7514-8	31.54	37.34	0.66	13	3	16
10	SM 1669-5	33.61	36.20	0.66	9	12	8
11	M VEN 25	34.26	36.96	0.57	10	7	20
12	CM 3306-4	31.05	37.78	0.63	23	18	6
13	CM 3306-19	36.23	33.66	0.71	21	10	10
14	SM 1973-25	31.82	37.70	0.59	4	24	15
15	SM 805-15	37.25	34.61	0.60	8	9	27
16	SM 1650-7	30.83	37.39	0.60	12	15	14
17	SM 1624-2	30.74	38.65	0.51	37	22	1
18	SGB 765-2	33.43	35.57	0.61	20	4	32
19	SM 1973-23	31.88	36.09	0.63	6	20	28
20	CM 4919-1	32.04	34.66	0.67	3	32	24
21	M COL 2215	27.72	37.88	0.60	5	11	35
22	SM 1627-16	28.46	37.38	0.60	31	8	13
23	SM 1778-53	29.85	37.40	0.54	29	21	9
24	SB 0216-9	31.67	36.13	0.53	32	2	37
25	SM 1759-29	28.70	37.37	0.54	15	35	17
26	M COL 1505	33.43	34.35	0.56	30	13	34
27	CM 6119-5	26.54	36.63	0.65	14	30	18
28	CM 6740-7	30.70	35.16	0.56	na	36	na
29	CM 8027-3	26.51	36.56	0.58	27	29	23
30	SM 643-17	26.08	37.30	0.54	19	38	30
31	CM 6754-8	27.31	34.87	0.62	26	39	25
32	M TAI 8	26.20	34.88	0.61	33	34	29
33	CM 8475-4	24.97	36.94	0.51	16	40	12
34	CM 523-7	28.80	34.35	0.53	36	33	19
35	CM 4843-1	27.47	33.69	0.59	28	25	40
36	SM 1657-14	26.70	34.99	0.49	35	31	33
37	CM 6758-1	27.16	35.11	0.43	40	26	31
38	SM 1778-45	22.59	35.86	0.54	34	28	39
39	M BRA 384	22.35	34.17	0.57	39	27	36
40	M PER 183	29.88	29.62	0.58	38	37	38
Maximum		40.68	38.78	0.71	73.15	42.22	30.56
Minimum		22.35	29.62	0.43	30.09	16.67	8.33
Mean		30.74	36.04	0.59	43.47	31.28	17.46

na = not available

It should be obvious that these interactions resulting in sharp contrasts in the rankings creates huge problems to the breeder. It becomes very difficult to decide which clone is really adapted to these conditions. However, some criteria are used for overcoming this problem. For instance three out of the four clones that were among the best ten in Tables 2.21 and 2.22 had been included as parents in the crossing nursery of this year (Table 2.21). CM 6754-8 has also been included as progenitor because of its known outstanding performance in the Córdoba-Sucre region, and in spite of its recognized lack of adaptation to the drier region of Atlántico-Magdalena.

Finally in Table 2.23, the results from the same *Regional Trials* conducted in the Urabá Region are presented. This area is an excellent environment for growing cassava and has an interesting future. The Urabá Region is known for its banana and plantain production, with large volume of it being exported. Recently, international regulations ruled out the possibility of stapling the boxes used for banana and plantain packaging. The industry, therefore, began importing large volumes of starch for gluing the boxes. Cassava offers an ideal alternative source for preparing these adhesives. Furthermore, CLAYUCA is conducting promising studies to produce the adhesives with flour (not starch) from cassava roots. The cassava flour should be a very competitive product against the imported starch.

As was the case in the Atlántico-Magdalena region, there was a sharp contrast in the ranking of clones at the top of the table, for the three locations where the trials were conducted. The best clone (SM 1565-17) was 1st, 23rd and 26th. The second best clone (SM 1511-6) was 7th, 14th, and 3rd. So defining the best clones for the region offers some difficulties, which are not present when deciding which are the clones poorly adapted. Only one clone (SM 1669-7) is among the ten-best clones in the three regions were these trials were conducted. The three remaining clones that were among the ten best in Tables 2.21 and 2.22 occupied the 13th (CM 3306-19), 14th (SM 1973-25) and 20th (CM 4919-1) rank in the Urabá region.

Table 2.24. Rankings of the eight clones selected for their good performance across the 11 locations where *Regional Trials* for the Sub-Humid environment were conducted.

Clone	Regions in the Northern Coast		
	Atlántico Magdalena	Córdoba Sucre	Urabá
SM 1411-5	4	18	3
SM 1669-7	9	4	4
SM 1665-2	19	1	8
SM 1438-2	24	7	5
SM 1565-17	5	16	1
SM 1973-25	10	6	14
CM 3306-19	2	5	13
CM 4919-1	3	8	20

In Table 2.24 we summarize the results of the *Regional Trials*. A group of eight clones has been selected based on its across-regions performance. In spit of the difficulties created by

the genotype by environment interactions, it should be clear that, eventually, it is possible to identify genotypes with an adequate and wide adaptation to the environments covered. All these clones (except for SM 1973-25) had been included as parents in the crossing nursery for this year. Many of these materials were also identified as genetically superior based on the peroformance of their progenies in the *Clonal Evaluation Trial* (Table 2.17) or in the Diallel Study (Table 2.15).

2.4.2 Selections for the Acid-Soil Savannas Environment

As for the Caribbean coastal eco-region, only the most relevant experiments conducted for this environment are described below (Table 2.25) followed by the respective results for each type of evaluation. As for Barranquilla, many of the improvement activities developed for the Villavicencio area also benefiting other regions.

The F1 stage was planted back in CIAT-Palmira, this a result of the measures taken to control both the white flies and frog skin disease. Many botanical seeds were planted but only 2267 produced vigorous enough seedlings to be transplanted to the F1 plot (Table 2.25). Plants that produce at least seven stakes in May next year, will be used for the *Clonal Evaluation Trial* of year 2003.

Table 2.25. Trials conducted in the Acid Soil Savannas in the 2001-2002 cycle[¶].

Trial	Site	N° of genotypes	N° of reps	**Observations**
F1	CIAT-Palmira	2267	1	See Table 2.26
Clonal Evaluation	La Libertad	1211 (7)	1	See Table 2.27 and 2.28
Diallel study	La Libertad	1360 (6)	3	See Tables 2.31-2.34
Preliminary Yield Trial	La Libertad	252 (10)	3	See Tables 2.35-2.37
Advanced Yield Trial	La Libertad	70 (25)	3	See Table 2.38
Regional Trials	La Libertad	32 (25)	3	See Table 2.39
	Santa Cruz	32 (25)	3	See Table 2.39
	Cabuyal	32 (25)	3	Harvesteed but not analyzed yet
	Camural	32 (25)	3	See Table 2.39
	Bca de Upia	32 (25)	3	See Table 2.39

[¶] Values in parentheses refer to the number of plants per plot. [§] Genotypes involved in the diallel experiment.

A description of the origin of the 1211 clones of the *Clonal Evaluation Trial* is provided in Table 2.26. In the year 2000, a total of 3572 botanical seeds had been planted for the Acid

Soil Savannas region. Many did not germinate or else, produced weak seedlings that died early before or soon after transplantation to the field. Eventually, only 1211 of the 1239 plants grown were vigorous enough to produce the seven stakes required for the *Clonal Evaluation Trial* planted for this cycle (Table 2.26). Most of the progenitors utilized to generate the *Clonal Evaluation Trial* have had an outstanding performance (as it will be demonstrated later in this Section); or else had been included for specific purposes.

Because of the prevalence of foliar diseases such as cassava bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*) and superelongation (induced by the fungus *Sphaceloma manihoticola*), evaluations must ensure optimal disease pressure. It is desirable to eliminate, as early as possible in the improvement process, those genotypes susceptible to these diseases. Thus, in the *Clonal Evaluation Trial*, the furrows were located, one behind each other, in a single row and separated by plants that served as spreaders of these diseases. These spreader plants permitted not only high pressure, but also ensured uniform distribution of the diseases. Planting material for spreader plants were stakes chosen from plants that had been discarded, precisely for being susceptible to these diseases, during the previous cycle. The high mortality of spreader plants observed 1-2 months after planting demonstrates that their spreading role has been adequately fulfilled early in the season. Figure 2.2 illustrates the way the spreader plants were located through the *Clonal Evaluation Trial*.

There is a slight difference in the way the *Clonal Evaluation Trial* is harvested in the Acid Soils Savannas, compared with the drier environment of the Northern Coast of Colombia. There is no marked period without rains, therefore, the harvest can be carried out in one step. This is the reason why only seven (rather than eight) plants were used to represent each clone. All the plants were harvested together in May. One other difference for this trial is that Plant Type incorporates a heavy component of reaction to foliar diseases mentioned above. The large number of progenies that could be evaluated in this trial are the results of the financial support by the Ministry of Agriculture of Colombia and the Poultry Growers Association of Colombia (FANAVI). It also reflects the relative success that the measures taken to control Frog Skin Disease at the F1 stage.

As in the case of the previous region, selection in the Acid Soils Savannas was also conducted through a selection index:

Selection index = [FRY * 10] + [DMC * 8] - [PT * 8] + [HI * 5]

where,   FRY = fresh root yield
           DMC = dry matter content
           PT = plant type using a 1(excellent) to 5 (very poor) visual scale
           HI = harvest index

The weight given to plant type has been increased to 8 (it was 5 in the sub-humid eco-region), because of the heavier pressure to select for materials resistant to foliar diseases present in the acid soils savannas.

Table 2.26. Origin of the 1239 plants from which 1211 clones were obtained for *the Clonal Evaluation Trial* harvested in May, 2002 at CORPOICA – La Libertad (Villavicencio, Meat Department). SM families have unknow fathers, but it is certain that they come from a selected group of clones in the policross plots

	Family	Mother	Father	Planted	Transplanted
1	CM 6787	CM 523-7	CM 2177-2	50	32
2	CM 9474	CM 6370-2	CM 6921-3	30	44
3	CM 9831	CM 2177-2	HMC 1	52	18
4	CM 9903	SM 1741-1	CM 6740-7	75	46
5	CM 9918	CM 7951-5	SM 1219-9	69	24
6	CM 9940	SM 1219-9	SM 653-14	87	33
7	CM 9953	SM 1741-1	SM 1219-9	75	33
8	GM 115	SM 2075-1	MPAN 135	100	30
9	GM 240	CM 7033-3	SM 1219-9	60	14
10	GM 276	SM 1565-15	SM 2219-11	65	36
11	SM 2366	CM 6934-4	Unknown	75	47
12	SM 2610	SM 1363-3	Unknown	100	40
13	SM 2632	CM 4574-7	Unknown	75	45
14	SM 2634	CM 6438-14	Unknown	75	48
15	SM 2640	SM 1543-17	Unknown	75	38
16	SM 2642	SM 1565-15	Unknown	75	24
17	SM 2649	CM 6370-2	Unknown	50	16
18	SM 2658	SM 1460-1	Unknown	75	15
19	SM 2739	SM 1822-5	Unknown	75	20
20	SM 2792	SM 1565-15	Unknown	75	13
21	SM 2841	CM 7073-7	Unknown	67	19
22	SM 2842	SM 1583-8	Unknown	87	22
23	SM 2844	SM 1779-8	Unknown	75	15
24	SM 2846	SM 1811-38	Unknown	81	32
25	SM 2847	SM 1812-56	Unknown	75	26
26	SM 2848	SM 1820-8	Unknown	88	25
27	SM 2850	SM 1855-21	Unknown	75	9
28	SM 2852	SM 1861-18	Unknown	76	7
29	SM 2853	SM 1862-25	Unknown	75	4
30	SM 2854	SM 1870-31	Unknown	78	28
31	SM 2855	SM 1871-26	Unknown	75	13
32	SM 2857	MCOL 2298	Unknown	72	6
33	SM 2870	SM 1741-1	Unknown	70	32
34	SM 2899	SM 2059-7	Unknown	72	38
35	SM 2965	CM 4574-7	Unknown	74	38
36	SM 2966	CM 6740-7	Unknown	67	12
37	SM 2968	SM 1219-9	Unknown	85	42
38	SM 2970	SM 1479-8	Unknown	78	19
39	SM 2971	SM 1690-13	Unknown	60	4
40	SM 2972	SM 1779-8	Unknown	57	20
41	SM 2973	SM 1822-12	Unknown	76	20
42	SM 2974	SM 1855-21	Unknown	84	29
43	SM 2976	SM 1861-18	Unknown	67	27
44	SM 2977	SM 1862-25	Unknown	77	21
45	SM 2978	SM 1870-31	Unknown	77	19
46	SM 2979	SM 1871-33	Unknown	60	8
47	SM 2980	SM 2219-11	Unknown	85	27
48	SM 2982	CM 2772-3	Unknown	75	43
49	SM 3022	CM 2772-3	Unknown	71	18
TOTAL				**3572**	**1239**
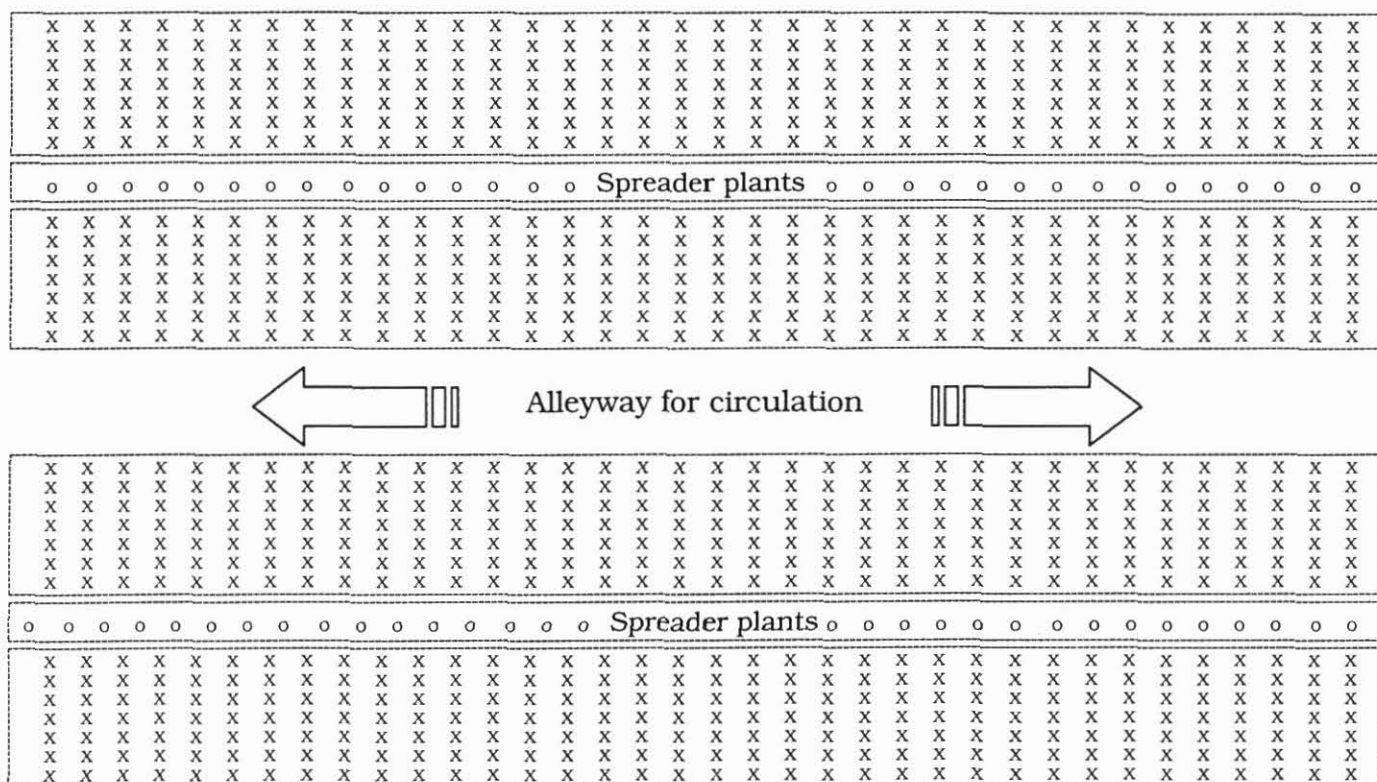
Figure 2.2. Illustration of the way spreader plants were positioned in the *Clonal Evaluation Trial* at CORPOICA – La Libertad, to provide uniform and high disease pressure.

Results of the *Clonal Evaluation Trial* are presented in Tables 2.27 and 2.28. Good development of leaf diseases could be observed early, together with a wide range of variation for both cassava bacterial blight and super-elongation. The fraction selected (Table 2.27), reacted well to leaf diseases (average for plant type = 2.53), compared with the average for the whole population (3.98). Similarly, good selection pressure was achieved for dry matter productivity (6.87 versus 3.06 t/ha), as for dry matter content (32.31% versus 28.71%), and harvest index (0.48 versus 0.38).

Data from Table 2.28 is useful for understanding why some families fail to contribute with clones worth to be selected, while others are outstanding. For instance, none of the 14 clones from family CM9733 was selected. These clones had a very low fresh-root yield (2.50 t/ha), and poor dry matter content (22.34%). Moreover, the average for plant type was 5.0. This means than all and every one of the clones was rated 5, suggesting that they had a very poor reaction to foliar diseases and/or very undesirable plant architecture. In contrast, the 38 clones from family GM 221 yielded 18 t/ha of fresh roots, with good level of dry matter content (32.80%). This family also showed the second best rating for plant type (2.66). All these factors contributing to the high proportion of selected clones from this family (84.2).

Table 2.27. Relevant results from the selection performed in the *Clonal Evaluation Trial* planted in CORPOICA – La Libertad(Villavicencio, Meta Department).

Clone or Parameter	Fresh roots (t/ha)	Dry matter (t/ha)	Harvest Index (0 a 1)	Plant Type [¶] (1 a 5)	Dry matter (%)
Results of the best 12 clones selected					
GM 235-55	36.14	12.59	0.55	1	34.8
GM 219-48	33.14	10.89	0.53	1	32.9
GM 233-72	31.14	11.11	0.58	2	35.7
GM 227-74	32.29	9.99	0.47	1	30.9
GM 229-73	25.14	8.75	0.53	1	34.8
GM 256-66	32.00	10.77	0.53	2	33.6
CM 9901-56	20.86	7.20	0.63	1	34.5
GM 223-39	34.14	10.20	0.53	2	29.9
GM 219-50	24.86	8.03	0.56	1	32.3
GM 240-48	35.71	10.15	0.47	2	28.4
GM 221-41	24.00	8.80	0.45	2	36.7
CM 9460-74	19.94	6.97	0.48	1	35.0
Statistics of the 172 clones selected.					
Maximum	36.14	12.59	0.66	5.00	37.63
Minimum	12.74	3.78	0.28	1.00	26.37
Mean	21.35	6.87	0.48	2.53	32.31
St. Dev.	5.00	1.58	0.06	0.87	2.19
Statistics of the 1211 clones evaluated					
Maximum	36.14	12.59	0.77	5.00	38.54
Minimum	0.03	0.01	0.00	1.00	16.49
Mean	10.10	3.06	0.38	3.98	28.71
St. Dev.	7.06	2.28	0.13	1.08	4.11

[¶] Leaf diseases and plant type were classified visually on a scale where 1 = excellent and 5 = very poor. A score of 3 represents a performance similar to the average of the population being evaluated.

The information from Table 2.28 can be used to produce information of all the progenies from a given parent (parents are used in generating more than one family). The averages of the progenies derived from each progenitor are presented in Table 2.29. Progenitors CM 4574-7, and SM 1565-15 are outstanding based on the excellent selection index averages of their progenies. Also, clones CM 7033-3 and SM 2219-11 produced progenies with a performance that was better than the average (selection indexes 2.31 and 2.64, respectively). Progenies from SM 1219-9 were marginally superior to the mean of the population (selection index= 0.44). The rest of the progenitors produced progenies that, across different variables introduced into the selection index, were mediocre. It was surprising to see that the progenies of CM 6740-7 had a poor performance (selection index = -4.22). This might be because this clone is better adapted to the conditions in the "*piedemonte*", than in the more stressful savannas where this trial was conducted.

Table 2.28. Results of the *Clonal Evaluation Trial* presented as average for each family represented in the trial. A few clones (15) were representing small families and have been omitted from this list.

Family	# clones	Selected Clones (%)	Fresh Roots (t/ha)	Dry Matter (t/ha)	Dry Matter (%)	Plant Type (1-5)	Foliage Yield (t/ha)	Harvest Index (0-1)	Frog Skin (#)	Selection Index
CM 8035	36	5.6	7.32	2.03	26.99	4.67	9.83	0.41	0	-9.85
CM 9460	41	34.1	11.96	3.56	28.52	3.00	18.34	0.37	14	8.67
CM 9642	38	15.8	5.76	1.59	23.38	4.37	10.77	0.26	1	-15.89
CM 9733	14	0.0	2.50	0.61	22.34	5.00	6.53	0.22	0	-27.73
CM 9901	30	10.0	8.66	2.67	30.04	3.63	11.87	0.39	0	-1.63
GM 219	36	75.0	16.31	5.18	31.31	2.39	20.24	0.42	0	24.62
GM 220	35	65.7	15.70	4.95	31.43	3.14	17.93	0.46	0	20.34
GM 221	38	84.2	17.92	5.89	32.80	2.66	22.40	0.45	1	28.77
GM 223	46	54.3	14.42	4.37	30.26	3.28	17.07	0.46	1	15.78
GM 224	28	28.6	11.06	3.35	29.02	4.43	18.60	0.33	0	-0.89
GM 225	31	6.5	5.91	1.73	26.75	4.29	13.41	0.27	0	-14.70
GM 226	38	26.3	10.62	3.31	30.67	3.79	15.64	0.38	10	2.60
GM 227	48	25.0	10.86	3.31	29.76	4.13	14.72	0.39	0	1.61
GM 229	46	30.4	11.71	3.64	30.56	3.57	20.20	0.35	0	5.54
GM 232	16	12.5	7.23	2.18	28.64	4.44	12.20	0.34	0	-10.17
GM 233	46	30.4	12.01	3.73	30.04	4.02	16.20	0.40	0	5.35
GM 234	8	12.5	5.37	1.39	26.48	4.63	9.14	0.25	0	-18.63
GM 235	27	7.4	6.21	1.92	26.68	4.44	9.25	0.33	0	-12.80
GM 240	28	14.3	9.93	2.98	29.41	3.68	14.57	0.39	1	1.03
GM 241	13	53.8	12.51	4.00	31.41	3.15	18.11	0.39	0	10.85
GM 243	27	18.5	8.92	2.60	27.76	4.52	13.51	0.35	0	-6.46
GM 245	10	10.0	5.24	1.47	27.59	4.70	9.69	0.29	0	-17.79
GM 256	40	42.5	13.80	4.39	31.40	3.58	18.99	0.41	0	12.48
GM 261	21	23.8	10.70	3.32	29.95	4.29	12.22	0.45	0	1.99
GM 263	42	19.0	10.70	3.23	30.32	3.86	12.51	0.46	0	3.88
GM 264	30	23.3	9.30	2.78	28.55	4.47	14.01	0.37	1	-4.63
GM 265	15	0.0	2.71	0.72	24.51	4.87	7.07	0.24	0	-25.94
GM 266	29	13.8	9.03	2.70	29.40	4.60	11.96	0.42	0	-3.55
GM 275	16	25.0	10.97	3.30	28.87	3.94	15.18	0.39	0	2.55
GM 276	33	21.2	10.70	3.34	30.94	3.73	14.87	0.41	0	3.74
GM 277	14	28.6	11.37	3.51	30.76	3.86	13.63	0.45	0	5.68
GM 278	25	0.0	5.13	1.32	25.19	4.84	11.33	0.30	0	-18.93
GM 279	26	11.5	8.35	2.39	27.65	4.50	13.96	0.35	0	-7.85
GM 298	28	14.3	8.70	2.54	28.43	4.25	10.23	0.44	0	-3.60
GM 299	27	14.8	8.32	2.30	26.82	4.63	10.25	0.41	0	-7.39
GM 300	19	5.3	3.49	0.84	22.92	4.79	7.94	0.24	0	-24.07
GM 301	21	4.8	7.53	2.11	26.79	4.43	8.39	0.44	0	-7.47
GM 303	26	42.3	15.00	4.39	29.13	3.81	16.53	0.47	1	14.59
GM 304	20	0.0	6.41	1.69	25.97	4.50	12.73	0.33	0	-13.58
GM 305	32	37.5	11.77	3.65	30.01	4.25	15.60	0.42	0	4.10
GM 307	26	0.0	2.72	0.61	21.54	4.96	7.31	0.24	0	-26.83

The performances of the progenies from HMC-1, MPER 183 and MTAI 8 were also very undesirable (selection indexes -6.11, -20.61, and -7.69, respectively). MTAI 8 had been included because of its high dry-matter productivity and dry matter content. It does not possess resistance to the foliar diseases common in the acid soil savannas. This probably contributed to the poor results from its progenies. This situation illustrates the difficulty for combining several desirable traits in one cassava clone. HMC-1 and MPER 183 were included as progenitors because of their good cooking quality characteristics. However, the results obtained are very discouraging regarding the real usefulness of these lines for this eco-region.

Table 2.29. Averages of all the progenies derived from a common progenitor based on the data produced by the *Clonal Evaluation Trial* at CORPOICA – La Libertad (Villavicencio, Meta Department).

Progenitor	Number of clones	Seleced Clones (#)	Selected Clones (%)	Plant type (1 - 5)	Fresh roots (t/ha)	Foliage yield (t/ha)	Harvest Index (0 - 1)	Dry matter (%)	Selection Index
CM 4574-7	293	141	0.481	3.37	12.99	17.95	0.39	30.10	10.65
CM 6740-7	300	68	0.227	4.02	8.86	13.63	0.34	28.23	-4.22
CM 7033-3	162	56	0.346	3.76	10.63	15.14	0.37	29.54	2.31
SM 1219-9	270	71	0.263	4.01	10.06	13.46	0.40	29.45	0.44
SM 1565-15	225	85	0.378	3.66	11.76	16.84	0.39	30.24	6.33
SM 2058-2	148	21	0.142	4.39	8.13	10.92	0.39	27.49	-6.88
SM 2219-11	300	86	0.287	4.02	10.96	14.36	0.41	29.20	2.64
HMC 1	183	37	0.202	4.44	8.78	12.32	0.36	27.51	-6.11
MPER 183	198	10	0.051	4.70	4.43	9.64	0.27	24.47	-20.61
MTAI 8	209	31	0.148	4.45	7.88	11.14	0.38	27.44	-7.69

Table 2.30 presents phenotypic correlation values among different relevant traits. Worth mentioning is the high correlation between root productivity and plant type (ρ= -0.607); foliage production (ρ= 0.796); and harvest index (ρ= -0.676). Plant type also had good correlations with foliage productivity (ρ= -0.544); Harvest Index (ρ= -0.372); and surprisingly, dry matter content (ρ= -0.460).

A second diallel study was conducted in the acid-soil savannas environment. The ten parents involved in this study were listed in Table 2.7. As for the diallel in the northern coast, each F1 cross was represented by 30 clones, two environments were used, with three replications each. The trials were both planted in CORPOICA-La Libertad, but with two very contrasting soil conditions. The results of these trials are presented in Table 2.31 and 2.32, for the high- and low-fertility environments, respectively. The differences in soil fertility resulted in little development of foliar diseases in the trial with good soil fertility, but excellent disease pressure in the trial planted in soils with edaphic limitations. Likewise, productivity was very contrasting with average fresh-root yields of 28.31 and 12.10 t/ha, respectively

Table 2.30. Phenotypic correlacions for relevant traits evaluated in the *Clonal Evaluation Trial* (CORPOICA – La Libertad) from 1211 genotypes.

	Fresh root production (t/ha)	Fresh foliage production (t/ha)	Harvest Index (0-1)	Dry matter content (%)
Plant type (1 a 5)	-0.607	-0.544	-0.372	-0.460
Fresh root production		0.796	0.676	0.544
Harvest Index				0.507

Looking at the best ten crosses (based on fresh-root yield) from Table 2.31, the parents that more frequently participated in these crosses were 7 (participating in five of those crosses) and 1 and 4 (related to four crosses each). Looking at the worst ten yielding crosses, frequent progenitors were number 5 and 8 (participating in four families each) and parent 10 (which produced three of these poor families). The average fresh-root production for the high-soil fertility trial was 28.31 t/ha, ranging from 37.51 (cross 7x10) down to 20.40 t/ha (cross 1x8). It must be remembered that these averages are coming from 30 plants in three replications (90 observations). Therefore an average of 37.51 t/ha is quite remarkable.

Results from the low-fertility trial (Table 2.32) are contrasting with the ones from the first environment. It is interesting to note how the soil fertility affected the capacity of the plants to protect themselves from the foliar diseases. It is well known that under good nutrition the plants can better react against biotic stresses, and that the contrary is also true. The two trials were less than a km away, so the differences in disease pressure can be reasonably explained by the soil fertility factor.

Some results are obvious from the summary presented in Table 2.32. Cross 8x9 had very little level of resistance to super-elongation disease (SED), with an average rating of 4.18. Cross 9x10 had also a high rating (4.12). One immediate conclusion is that, at least, parent 9 had very low levels of resistance to SED. On the other hand, progenitors 1 and 5 produced progenies with good reaction to the disease.

In relation to dry matter content, parent 5 was outstanding in the high-fertility soil trial participating in seven of the 12 best crosses. Parent 10 was the second best, participating in four of the best F1 crosses for this trait. On the other hand, dry matter content under low soil fertility favored crosses from parent 1 (five F1 crosses out of the 12 best); followed by parent 4 (participating in four outstanding crosses); and parents 2, 5 and 7 (with three families each). It is possible that the relatively better performance for the progenies from parent 1, under low-soil fertility roots is due to its outstanding reaction to diseases. If that were the case, this would be another evidence of how adaptation to a particular environment [in this case defined by the prevalent foliar diseases] results in higher dry matter content in the roots.

Table 2.31. Average of each F1-cross from the diallel study planted under high soil fertility conditions at CORPOICA-La Libertad (Villavicencio, Meta Department).

F1 Cross	Plant height (cm)	Plant type (1-5)	Root type (1-5)	Dry matter (%)	Harvest Index.	FR yield (t/ha)
1x2	301.42	2.81	3.08	32.71	0.44	32.68
1x3	296.97	2.94	3.36	30.95	0.43	27.22
1x4	311.28	2.96	3.04	32.82	0.46	32.09
1x5	267.90	3.18	3.69	34.30	0.39	21.77
1x6	268.33	3.01	3.04	31.50	0.48	36.44
1x7	314.33	2.72	2.80	31.26	0.46	33.72
1x8	258.50	3.51	3.76	32.11	0.38	20.40
1x9	286.42	3.59	3.14	31.28	0.41	31.91
1x10	272.22	3.17	3.47	32.08	0.45	30.97
2x3	303.64	2.70	3.35	31.85	0.41	27.15
2x4	293.11	3.15	3.57	32.18	0.40	26.68
2x5	269.89	3.14	3.06	33.66	0.37	26.61
2x6	284.83	3.11	2.88	30.29	0.43	31.05
2x7	294.39	2.98	3.01	31.59	0.45	34.60
2x8	254.86	3.56	3.61	31.70	0.44	26.44
2x9	292.89	3.28	3.43	29.96	0.42	28.73
2x10	242.72	3.26	3.22	33.11	0.46	30.44
3x4	259.25	2.93	3.26	32.15	0.46	26.78
3x5	265.89	3.13	3.18	33.39	0.39	27.66
3x6	259.51	2.64	2.87	30.09	0.45	31.66
3x7	237.22	3.37	3.28	31.14	0.47	24.42
3x8	258.20	3.49	3.75	29.99	0.39	21.82
3x9	279.25	3.18	3.10	30.42	0.42	29.08
3x10	219.22	3.41	3.20	31.78	0.52	26.12
4x5	262.47	3.24	3.31	31.71	0.45	30.12
4x6	257.39	3.04	2.83	31.44	0.51	34.19
4x7	243.50	3.14	2.83	32.47	0.54	34.19
4x8	241.00	3.53	3.63	33.56	0.42	21.88
4x9	276.17	3.14	3.56	31.05	0.41	27.41
4x10	256.28	3.22	3.22	33.57	0.49	32.88
5x6	256.28	2.97	3.24	34.64	0.40	24.21
5x7	257.53	3.10	3.57	33.83	0.42	23.20
5x8	225.42	3.52	3.47	33.19	0.48	27.57
5x9	244.39	3.01	3.44	31.08	0.41	26.22
5x10	241.78	3.43	3.43	33.18	0.37	20.56
6x7	269.11	2.86	3.41	31.60	0.45	22.12
6x8	238.36	3.59	3.25	33.23	0.45	25.00
6x9	284.61	2.88	3.23	30.06	0.43	31.38
6x10	249.67	2.94	3.03	32.92	0.49	31.96
7x8	244.08	3.58	3.16	31.53	0.47	33.76
7x9	263.44	3.40	3.22	31.25	0.41	29.54
7x10	256.22	2.97	2.72	34.10	0.48	37.51
8x9	268.47	3.51	3.35	31.10	0.46	27.20
8x10	220.53	3.79	3.53	32.48	0.46	24.32
9x10	283.89	3.31	3.66	31.34	0.36	22.26
Maximum	314.33	3.79	3.76	34.64	0.54	37.51
Minimum	219.22	2.64	2.72	29.96	0.36	20.40
Mean.	265.17	3.19	3.27	32.04	0.44	28.31
St.Dev.	23.25	0.28	0.27	1.23	0.04	4.47

Table 2.32. Average of each F1-cross from the diallel study planted under low soil fertility conditions at CORPOICA-La Libertad (Villavicencio, Meta Department).

F1 cross	SED (1-5)	CBB (1-5)	Plant type (1-5)	Dry matter (%)	Harvest Index (0-1)	FR yield (t/ha)
1x2	2.26	2.48	2.79	32.78	0.45	18.97
1x3	2.17	2.52	2.67	31.61	0.37	12.61
1x4	2.36	2.46	3.00	32.35	0.43	15.11
1x5	2.20	2.54	2.86	34.49	0.37	14.01
1x6	2.73	2.43	3.26	31.49	0.39	8.31
1x7	2.57	2.63	2.52	32.25	0.44	19.37
1x8	2.49	2.57	3.58	32.20	0.37	11.49
1x9	3.24	2.56	4.08	30.61	0.35	10.90
1x10	3.00	2.33	3.27	32.96	0.34	10.51
2x3	2.97	2.75	3.15	31.37	0.35	10.97
2x4	3.32	2.49	3.59	30.42	0.36	10.23
2x5	2.46	2.52	2.87	34.18	0.44	16.15
2x6	2.93	2.97	3.81	30.96	0.44	14.71
2x7	3.10	2.70	3.04	32.97	0.46	15.24
2x8	3.52	2.88	4.29	28.39	0.41	8.58
2x9	3.89	2.67	4.46	24.33	0.25	4.88
2x10	3.36	2.50	4.04	31.98	0.45	14.14
3x4	2.47	2.63	3.13	32.32	0.48	15.71
3x5	2.23	2.65	3.34	31.69	0.40	11.94
3x6	2.78	3.21	3.74	29.75	0.33	9.60
3x7	2.39	2.89	3.97	29.47	0.35	11.81
3x8	2.64	2.86	4.11	30.28	0.42	7.19
3x9	3.48	2.79	4.29	27.01	0.33	8.54
3x10	2.83	2.69	3.94	29.98	0.41	10.32
4x5	3.01	2.59	3.74	32.48	0.43	12.82
4x6	3.11	2.97	4.10	31.12	0.33	8.88
4x7	2.54	2.61	3.44	32.49	0.49	15.77
4x8	2.81	2.65	4.14	31.67	0.51	15.61
4x9	3.46	2.85	4.58	27.15	0.36	6.94
4x10	3.23	2.45	4.08	32.05	0.45	14.51
5x6	2.28	2.78	3.21	31.62	0.44	14.96
5x7	2.19	2.50	3.25	32.50	0.50	17.64
5x8	2.90	2.76	3.94	31.63	0.47	18.27
5x9	3.31	2.66	4.23	26.47	0.39	11.79
5x10	2.93	2.40	3.92	31.18	0.39	11.12
6x7	2.64	2.75	3.54	29.86	0.44	15.12
6x8	2.53	3.16	4.27	30.53	0.46	16.99
6x9	3.46	2.84	4.49	25.20	0.30	5.12
6x10	3.07	2.67	4.14	30.73	0.39	9.80
7x8	2.77	2.74	4.04	31.65	0.48	17.79
7x9	3.51	2.64	4.39	28.20	0.30	8.53
7x10	3.07	2.69	3.99	32.90	0.44	13.40
8x9	4.18	2.51	4.64	23.11	0.24	3.26
8x10	3.67	2.66	4.37	31.29	0.47	12.71
9x10	4.12	2.13	4.97	23.91	0.18	2.06
Maximum	4.18	3.21	4.97	34.49	0.51	19.37
Minimum	2.17	2.13	2.52	23.11	0.18	2.06
Mean	2.94	2.66	3.76	30.52	0.40	12.10
St.Dev.	0.52	0.20	0.59	2.65	0.07	4.15

Parent 4 produced families with excellent harvest indexes in the two environments where the diallel trials were planted. For the same trait parent 10 produced good progenies in the low-fertility field and parent 7 in the high-fertility field. Parent 9 produced five of the worst families for harvest index in the low-fertility trial, and parent 5 in the high-fertility conditions. This last observation is not surprising because parent 5 (SM 1565-15) is particularly well adapted to the savanna conditions where it shows excellent canopy development. When progenies from this clone are grown in high fertility soils the canopy development may prove to be too excessive, resulting in low harvest index.

For plant type, progenitors 1, 2, 3, 6, and 7 produced the best progenies in the high-fertility trial. In the low-fertility conditions, on the other hand, parent 1, followed by parents 2 and 5 had a superior performance based on the results of their progenies. The good level of resistance to SED found in parent 5 may have contributed to the good rating for plant type, as well.

Table 2.33 Averages of the nine F1-cross families produced by each of the ten parents evaluated in the diallel experiments conducted at CORPOICA – La Libertad (Villavicencio, Meta Department). For each parent, the first line presents the result of the low-fertility trial, and the second line for the high fertility conditions.

Progenitor	SED. (1 - 5)	CBB (1 - 5)	Plant type (1 - 5)	Root type (1 - 5)	Dry matter (%)	Harvest Index (0 - 1)	Fresh roots (t/ha)
1	2.56	2.50	3.11	3.45	32.30	0.39	13.47
CM 4574-7	-.-	-.-	3.10	3.26	32.11	0.43	29.69
2	3.09	2.66	3.56	3.68	30.82	0.40	12.65
CM 6740-7	-.-	-.-	3.11	3.25	31.89	0.42	29.38
3	2.66	2.78	3.59	3.68	30.39	0.38	10.96
CM 7033-3	-.-	-.-	3.09	3.26	31.31	0.44	26.88
4	2.92	2.63	3.76	3.61	31.34	0.43	12.84
SM 1219-9	-.-	-.-	3.15	3.25	32.33	0.46	29.58
5	2.61	2.60	3.49	3.34	31.80	0.43	14.30
SM 1565-15	-.-	-.-	3.19	3.38	33.22	0.41	25.32
6	2.84	2.86	3.84	3.68	30.14	0.39	11.50
SM 2058-2	-.-	-.-	3.00	3.09	31.75	0.45	29.78
7	2.76	2.68	3.57	3.43	31.37	0.43	14.96
SM 2219-11	-.-	-.-	3.12	3.11	32.08	0.46	30.34
8	3.06	2.75	4.15	3.69	30.08	0.43	12.43
HMC 1	-.-	-.-	3.57	3.50	32.10	0.44	25.38
9	3.63	2.63	4.46	4.25	26.22	0.30	6.89
MPER 183	-.-	-.-	3.26	3.35	30.84	0.41	28.19
10	3.25	2.50	4.08	3.76	30.78	0.39	10.95
MTAI 8	-.-	-.-	3.28	3.28	32.73	0.45	28.56

The information from Tables 2.31 and 2.32 has been consolidated in Table 2.33 where the averages of the nine F1 crosses derived from each of the ten progenitors is presented, individually for each environment. Each average is based theoretically on 810 data points (nine crosses, 30 genotypes per cross, and three replications). Therefore, these figures are very solid and reliable.

For resistance to SED, parents 1 (CM 4574-7), 5 (SM 1565-15), and 3 (CM 7033-3) showed the best reaction (low score). On the other hand, parents 8 (HMC-1), 2 (CM 6740-7), 10 (MTAI 8), and 9 (MPER 183) were mediocre based on the reaction of their progenies to the disease. Worth mentioning is that similar conclusions were drawn from the *Clonal Evaluation Trial* (Table 2.29). In was a surprise to find out that the progenies from MTAI 8 presented good levels of reaction to the bacterial blight (CBB), with a score of 2.50 also found in the first parent (CM 4574-7). M TAI 8 was developed in Thailand (where it was named Rayong 60) and it was not known to have resistance to the disease. This situation may be due to some sort of recessive resistance in MTAI 8 (already suggested in the literature) or else, because the levels of CBB were not has high as for SED, therefore, resulting in misleading results. Progenies from SM 2058-2 presented high ratings for CBB, suggesting that this clone is very susceptible to the disease. This would also support what was concluded from the data in Table 2.32 where, five of the worst crosses for CBB included SM 2058-2 as one of the progenitors.

Progenitors 7 (SM 2219), 5 (SM 1656-15), and 1 (CM 4574-7) produced the progenies with highest average productivity in the low-environment trial. MPER 183, on the other hand, produced progenies with very low root productivity (almost half as much as those from the previously mentioned parents). Poor performances in the low-fertility trial were shown by the progenies of MTAI 8 and CM 7033-3. In the high-fertility conditions the progenies from SM 2219-11 showed the highest root-productivity, followed by those from CM 4574-7, SM 1219-9, and CM 6740-7. In this environment the progenies from SM 1565-15, HMC-1 and CM 7033-3 yielded poorly. Many of these conclusions agree with those drawn from the *Clonal Evaluation Trial* (Table 2.29).

Table 2.34. Phenotypic correlations measured in the low-fertility diallel trial conducted at CORPOICA-La Libertad (Villavicencio, Meta Department). Correlations were estimated using averages for each family across the three replications.

	CBB (1-5)	Plant type (1-5)	Root type (1-5)	Fresh root yield (t/ha)	Dry matter (%)	Harvest index (0-1)
SED (1-5)	-0.10	0.77	0.77	-0.73	-0.74	-0.58
CBB (1-5)		0.20	0.01	0.07	-0.10	0.13
Plant type			0.69	-0.64	-0.73	-0.42
Root type				-0.94	-0.82	-0.83
FR yield					0.80	0.88
Dry matter						0.73

Taking advantage of the huge volume of information from these diallel trials the phenotypic correlations shown in Table 2.34 were obtained. Very high correlations with root productivity were found for root aspect score (ρ= -0.94), harvest index (ρ= 0.88), dry matter content (ρ= 0.80), reaction to SED (ρ= -0.73), and plant type score (ρ= -0.64). Negative correlations here are due to the fact that in the scores 1= excellent and 5=very poor performance.

It is also worth mentioning the little association between reaction to CBB and SED. Although there seems to be a negative association (ρ= -0.10), the relationship is weak enough to suggest that it is feasible to obtain genotypes with good levels of reaction to both disease, that is that the traits are likely to be independently inherited and controlled. CBB and root yield showed a low correlation probably because the disease level was not high enough this year.

Table 2.35. Results of the best five clones in **Experiment 1** of the *Preliminary Yield Trials* conducted at CORPOICA – La Libertad (Villavicencio, Meta Departament).

Clone or parameter	Plant type (1 - 5)	Fresh roots (t/ha)	Fresh foliage (t/ha)	Harvest index (0 - 1)	Dry matter (%)	Dry matter (t/ha)
SM 2786-10	2.67	34.58	23.54	0.60	35.48	12.24
SM 2636-6	1.00	24.64	16.09	0.61	32.68	8.12
SM 2636-26	2.00	37.40	30.47	0.55	31.22	11.70
SM 2638-20	1.67	25.94	26.25	0.50	35.28	9.17
SM 2632-4	1.33	23.02	23.75	0.50	34.68	8.07
Statistics of the best 20 clones selected						
Maximum	3.67	37.40	30.52	0.61	37.96	12.24
Minimum	1.00	16.04	14.06	0.46	31.22	5.65
Mean	2.45	25.96	23.57	0.53	34.01	8.81
St.Dev..	0.71	5.62	5.93	0.05	1.82	1.74
Statistics of the 84 clones evaluated						
Maximum	4.67	37.40	31.72	0.61	38.09	12.24
Minimum	1.00	2.40	2.92	0.22	23.13	0.82
Mean	3.13	16.28	16.97	0.47	31.88	5.32
St.Dev..	0.72	7.91	6.98	0.08	2.89	2.67
Average of four checks (Brasilera, Catumare, Reina y HMC 1)						
Mean	3.83	9.14	12.01	0.38	30.16	2.91

The second stage of evaluation according to the diagram illustrated in Figure 2.1 is the *Preliminary Yield Trial*. Similar to what was done in the northern coast the 240 clones selected the previous year from the *Clonal Evaluation Trial*, were split in three different experiments with 80 entries each. The trials had 10-plant plots and three replications each. Four checks (HMC 1, Brasilera, ICA-Catumare or CM 523-7 and CORPOICA-Reina or CM 6740-7) were also included in each trial. Tables 2.35, 2.36, and 2.37 present the most relevant results from these trials.

In **Experiment 1**, family SM 2636 with seven representatives had a prevalent presence among the 20 selected clones. This family comes from the little known clone SM 593-5. The average performance of the 20 selected clones was clearly superior to those of the four checks. For instance for dry matter production the averages were, respectively, 8.81 and 2.91 t/ha (Table 2.35). The selection index for the 20 selected clones was 29.13, whereas for the four checks the same parameter was very deficient: -27.00 (data not shown).

Results from **Experiment 2** are presented in Table 2.36. The full-sib family CM 9460 (derived from the cross CM 6740-7 x CM 4574-7) participated in six of the best 20 clones. Also, family SM 2792 (derived from SM 1565-15) was represented by four clones in the fraction of 20 selected. On average the 20 selected clones yielded almost 8 t/ha of dry matter, compared with 2.37 t/ha for the four checks. Selection indexes from the selected group versus the checks was also very contrasting: 24.47 versus –30.5 (data not shown).

Table 2.36. Results of the best five clones in **Experiment 2** of the *Preliminary Yield Trials* conducted at CORPOICA – La Libertad (Villavicencio, Meta Departament).

Clone or parameter	Plant type (1 - 5)	Fresh roots (t/ha)	Fresh foliage (t/ha)	Harvest index (0 - 1)	Dry matter (%)	Dry Matter (t/ha)
CM 9461-1	2.67	22.71	15.21	0.59	37.81	8.59
SM 2787-1	2.33	36.98	28.44	0.56	29.94	11.07
CM 9460-12	2.00	22.60	23.23	0.49	34.28	7.75
CM 9460-41	3.33	33.13	31.98	0.51	33.52	11.10
CM 9461-3	3.67	25.94	22.92	0.52	36.71	9.52
Statistics of the best 20 clones selected						
Maximum	4.00	36.98	31.98	0.66	37.81	11.10
Minimum	2.00	13.85	7.92	0.45	29.94	4.49
Mean	2.95	23.26	19.65	0.54	34.45	7.95
St.Dev..	0.60	5.69	5.56	0.06	2.14	1.67
Statistics of the 84 clones evaluated						
Maximum	5.00	36.98	31.98	0.68	37.81	11.10
Minimum	1.67	2.81	2.14	0.26	27.76	0.85
Mean	3.43	15.40	15.58	0.49	33.02	5.12
St.Dev..	0.73	7.24	6.90	0.08	2.30	2.47
Average of four checks (Brasilera, Catumare, Reina y HMC 1)						
Mean	4.42	7.24	9.69	0.42	31.72	2.37

The most relevant results from **Experiment 3** are presented in Table 2.37. Family CM 9464 (SM 1411-5 x CM 4574-7) contributed with six of the 20 selected clones. It is interesting to note that one of these parents (SM 1411-5) is not adapted to this eco-region, but has an outstanding performance in the sub-humid environment of the northern coast of Colombia. This result is useful to highlight the advantages frequently observed when crossing materials adapted to different eco-regions. Other family with good representation among the 20

selected clones was CM 9461 (derived from the cross CM 6921 x CM 4574-7). Four of the selected clones belong to this family.

As in the other two experiments, the selected fraction had a much better performance than the four checks. Dry matter yield was 7.25 t/ha versus 3.32 t/ha for the checks. Selection indexes were 24.84 and –10.31 for the selected fraction and the four checks, respectively.

Table 2.37. Results of the best five clones in **Experiment 3** of the *Preliminary Yield Trials* conducted at CORPOICA – La Libertad (Villavicencio, Meta Departament).

Clone or parameter	Plant type (1 - 5)	Fresh roots (t/ha)	Fresh foliage (t/ha)	Harvest index (0 - 1)	Dry matter (%)	Dry matter (t/ha)
CM 9462-17	2.67	34.90	30.31	0.53	32.15	11.27
SM 2727-31	3.67	31.88	20.63	0.60	35.10	8.95
CM 9464-29	3.00	24.17	20.21	0.54	34.97	8.01
CM 9464-19	1.67	14.17	10.00	0.58	31.39	3.22
CM 9461-15	3.33	26.88	17.29	0.60	31.44	7.43
Statistics of the best 20 clones selected						
Maximum	4.67	34.90	35.10	0.61	40.46	11.27
Minimum	1.67	14.17	10.00	0.46	27.10	3.22
Mean	3.35	23.64	21.32	0.52	33.43	7.25
St.Dev..	0.66	5.49	5.86	0.05	3.18	1.79
Statistics of the 84 clones evaluated						
Maximum	3.97	15.30	15.48	0.48	32.57	4.79
Minimum	5.00	34.90	35.10	0.66	40.46	11.27
Mean	1.67	3.33	2.71	0.25	24.59	0.88
St.Dev..	0.61	6.90	6.22	0.07	3.21	2.25
Average of four checks (Brasilera, Catumare, Reina y HMC 1)						
Mean	4.08	9.84	10.31	0.48	32.38	3.32

The results of an *Advanced Yield Trial* conducted at CORPOICA–La Libertad are presented in Table 2.38. Seventy genotypes (of which 5 were checks) were planted in plots of 25 plants each, with three replicates. The materials evaluated in this trial came from a *Preliminary Yield Trial* conducted the previous cycle. Excellent performance was shown by CM 6740-7 (CORPOICA-Reina), who occupied the fifth place, based on the selection index. The first and third clones belong to the same family: CM 8748 (derived from the cross between SM 494-2 and CM 2952-2). Having two sister clones occupying such a relevant position among 70 genotypes provides strong evidence that there is a good genetic material behind them. For a diversity of reasons, many of which are not clear, the general performance of this trial was poorer that those observed in other trials.

Table 2.38. Results from the *Advanced Yield Trial* conducted at CORPOICA – La Libertad (Villavicencio, Meta Department). The trial involved 70 genotypes grown in 25-plant plots and three replications.

Clone or parameter	Plant type (1 - 5)	Root type (1 - 5)	Harvest index (0 - 1)	Dry matter (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)
CM 8748-4	3.33	2.00	0.52	34.35	23.33	8.03
SM 2375-13	1.67	2.00	0.54	35.08	18.44	6.59
CM 8748-2	3.00	2.00	0.46	28.58	22.89	6.89
SM 2425-3	3.33	2.33	0.55	30.18	21.44	6.46
Reina	*2.33*	*2.33*	*0.49*	*35.83*	*17.56*	*6.30*
SM 2452-3	3.00	2.33	0.50	32.42	19.26	6.27
SM 1807- 1	2.67	2.67	0.50	36.52	16.44	6.06
SM 1773- 2	1.33	2.67	0.41	38.28	13.19	5.11
SM 2456-3	2.00	2.67	0.49	31.72	15.33	4.90
SM 1812-69	2.67	3.00	0.56	33.28	15.63	5.46
Statistics of the best 20 clones selected						
Maximum	3.33	3.67	0.56	38.28	23.33	8.03
Minimum	1.33	2.00	0.41	28.58	12.52	4.35
Mean	2.47	2.70	0.48	34.50	16.55	5.73
St.Dev.	0.67	0.52	0.04	2.59	3.10	0.91
Statistics of the 70 clones evaluated						
Maximum	4.67	4.33	0.61	42.05	23.33	8.03
Minimum	1.33	2.00	0.21	27.08	2.22	0.75
Mean	2.95	3.12	0.44	33.53	12.48	4.24
St.Dev.	0.76	0.57	0.09	2.56	4.13	1.41

Finally in Table 2.39 the results of the first of the four *Regional Trials* that have been harvested and analyzed (one remains to be harvested). CM 6740-7 (CORPOICA – Reina) and CM 4574-7 showed good performances at CORPOICA – La Libertad, but not in the remaining locations. On the other hand CM 523-7 (ICA- Catumare) had a much better adaptation to the first three locations (Cumaral, Barranca de Upía and Santa Cruz) characterized by strong edaphic limitations than at CORPOICA – La Libertad, which has milder problems of soil acidity.

As in the case of the sub-humid environment, the *Preliminary Yield Trials* planted this year will be used also in the acid-soil savannas to compare three criteria of selection: selection index, fresh root productivity and harvest index. Therefore, the *Preliminary Yield Trials* already planted and to be harvested in May 2003, have the following characteristics. Each trial has 64 genotypes, four of them are checks and the remaining 60 are experimental clones. Since there are three experiments, in total 180 experimental clones are under evaluation. Out of these 180 clones 172 had been selected as mentioned in Table 2.27. The remaining eight clones (180-172=8) have been included to carry out this comparison.

Table 2.39 Results from the *Regional Trials* conducted at four locations in the acid soil savannas (Meta Departament). The 32 genotypes are ranked according to their respctive selection index.

Clone	Plant type (1-5)	Harvest index (0-1)	Dry matter (%)	Fresh root (t/ha)	Dry matter (t/ha)	RANKINGS			
						Cumaral	Bca.Upia	Sta.Cruz	CORPOICA
SM 2219-11	2.17	0.65	26.58	35.76	12.65	8	5	1	9
CM 6921- 3	2.00	0.51	30.80	32.00	12.92	1	4	8	13
SM 1363-11	2.50	0.56	28.54	31.71	12.88	15	6	11	2
SM 1143-18	2.09	0.57	27.75	34.13	12.49	2	14	15	5
SM 667-1	2.08	0.55	25.21	37.46	11.92	7	9	6	15
SM 1405-5	2.33	0.53	29.75	23.88	9.50	17	7	3	20
BRASILERA	2.75	0.56	26.87	32.85	12.41	3	8	24	14
CM 6975-14	2.75	0.52	26.63	36.07	12.82	22	2	21	6
MBRA 502	2.83	0.53	26.86	33.89	12.23	13	10	7	23
SM 1864-10	2.08	0.56	27.04	29.82	10.65	4	22	20	8
CM 523- 7	2.50	0.49	27.30	23.83	8.92	14	1	10	29
SM 1353-3	2.92	0.60	26.99	33.06	12.18	10	30	12	3
CM 5306- 8	2.42	0.53	28.41	27.99	10.55	18	12	2	24
SM 1152-13	2.92	0.56	29.13	33.59	12.63	5	28	22	1
SM 1694-2	2.50	0.49	27.72	23.35	9.39	24	3	5	25
SM 1699-26	2.33	0.54	27.38	25.90	9.66	16	16	4	22
SM 1862-25	2.59	0.52	28.28	27.53	10.15	11	15	16	19
CM 4574-7	2.25	0.55	24.12	32.74	10.84	23	18	18	4
CM 6740-7	2.92	0.54	26.23	33.15	11.91	28	13	17	7
SM 1794-18	3.25	0.53	28.36	25.67	10.13	21	11	9	27
CM 6438-14	2.75	0.53	27.05	28.64	10.53	9	26	13	21
SM 1859-26	3.00	0.52	26.31	33.52	11.99	6	17	29	17
SM 1810-6	2.75	0.54	27.81	23.64	9.42	20	21	19	11
CM 7052- 3	2.34	0.50	26.92	22.04	7.78	12	29	26	18
CM 6055-3	3.00	0.58	24.47	26.52	9.46	26	24	25	12
SM 1241-12	3.00	0.59	24.00	26.72	8.40	27	31	14	16
SM 1854-23	3.17	0.58	26.04	24.68	8.68	19	23	23	26
SM 1697-1	3.58	0.56	23.21	29.63	9.93	30	27	31	10
SM 1565-15	3.42	0.47	26.58	20.32	7.67	25	19	28	30
SM 1483-1	2.58	0.42	21.99	11.85	3.76	31	20	27	31
CM 2177- 2	3.17	0.46	25.68	18.12	6.17	29	25	30	28
SM 1872-9	3.33	0.44	17.48	7.71	1.82	32	32	32	32
Parameters						**Dry matter yield statistics for each location**			
Maximum	3.58	0.65	30.80	37.46	12.92	24.12	18.30	8.79	16.39
Minimum	2.00	0.42	21.99	11.85	3.76	2.78	2.41	1.04	1.07
Mean	2.68	0.54	26.77	28.39	10.34	14.84	11.48	4.93	9.05
St. Dev.	0.42	0.04	1.88	5.86	2.16	5.12	3.73	2.14	3.89

Each criteria of selection will be represented by the best 20 clones (from the *Clonal Evaluation Trial* harvested this year) for Harvest Index, fresh root yield, and selection index. Because the selection criteria for the breeding project is the selection index, obviously, the 20

best clones for this criteria were included among the 172 clones. Also, many but not all of the best 20 clones for high yield and harvest index were among the 172 selected. Only eight were among the best 20 for harvest index or fresh root yield, but had not been selected for breeding purposes. They were included in the *Preliminary Yield Trial*, however, to carry out the comparison mentioned above. Table 2.40 describes the main characteristics of each of the three groups. Obviously, some clones participate in more than one of those three groups.

Table 2.40 Statistics of the three groups of 20 clones (some clones shared by more than one group) planted to compare three selection criteria.

Parameters	Plant type (1 - 5)	Fresh root yield (t/ha)	Harvest index (0 - 1)	Dry matter (t/ha)	Dry matter (%)	Selection index
Best 20 clones for Harvest Index						
Maximum	5.00	32.57	0.77	11.11	37.63	66.28
Minimum	1.00	5.71	0.57	1.78	27.12	3.20
Mean	3.40	18.61	0.61	5.86	31.36	29.35
St.Dev.	1.14	6.77	0.05	2.32	3.21	18.10
Best 20 clones for Fresh Root Yield						
Maximum	4.00	36.14	0.58	12.59	35.67	77.85
Minimum	1.00	27.43	0.35	7.87	26.37	31.53
Mean	2.37	30.66	0.50	9.60	31.33	50.54
St.Dev.	0.90	2.65	0.05	1.14	2.47	11.86
Best 20 clones for selection index						
Maximum	3.00	35.71	0.63	11.11	36.68	69.10
Minimum	1.00	17.14	0.42	6.13	28.41	48.62
Mean	1.63	27.18	0.50	8.96	33.20	55.51
St.Dev.	0.60	5.18	0.05	1.41	2.11	6.27

2.4.3. Selections for the Mid-altitude valleys

As for other eco-regions, the most relevant experiments conducted in the Valle del Cauca will be listed first (Table 2.41) followed by results specific to each type of evaluation. As for the other regions already discussed, many improvement activities developed here also benefited other areas. For the mid-altitude valleys the selection index utilized was as follows:

Selection Index = [Fresh root yield * 10] + [Dry matter content * 8] - [Plant type * 5] + + [Harvest Index * 5]

This selection Index is identical to the one used for the sub-humid environment in the northern coast.

Table 2.41. Trials conducted in the Department of Valle del Cauca, Colombia, during 2000-2001.

Trial	Site	N° of genotypes	N° of reps	Observations
F1	Cenicaña	1500	1	In the new selection scheme the plants are left growing in the field for 10 months.
Clonal Evaluation	Palmira	880 (7)	1	See Table 2.42 to 2.44
Diallel study	Jamundi Palmira			See Table 2.45 and 2.46 See Table 2.47 and 2.48
Preliminary Yield Trial	Palmira	110 (10)	3	See Table 2.51
Regional Trials	Buga	34 (25)	3	See Table 2.52
	Jamundí	34 (25)	3	See Table 2.52
	S.Quilichao	34 (25)	3	See Table 2.52
	Bocas Palo	34 (25)	3	See Table 2.52
	Vijes	34 (25)	3	See Table 2.52
	Morales	34 (25)	3	See Table 2.53
	La Cumbre	33 (25)	3	See Table 2.54

¶ Values in parentheses refer to the number of plants per plot. § Genotypes involved in the diallel experiment.

The problems of frogskin disease and whitefly

Most problems with frogskin disease are related to sources of inoculum and transmission by vector insect(s) from infected to healthy plants. Strategies for reducing problems aim to reduce sources of inoculum, on the one hand, and vector populations, on the other. Measures taken to control the disease are summarized below. Two years ago, CIAT initiated a process of *indexation* to confirm that the materials it held were free of viral diseases. For frog skin disease, indexation is carried out by grafting a bud of the hypersensitive variety 'Secondina' on to the variety to be indexed. *Secondina* is highly sensitive to the presence of the disease's causal agent, and the graft will detect any contaminated material. However, this technique is slow, requiring up to three months for final diagnosis, and is labor intensive. Even so, a very large proportion of the germplasm bank has already been indexed, together with the major varieties planted. The materials confirmed free of frogskin disease (and other viral diseases) were grown in isolated environments where only "clean" germplasm had been planted. Careful management for whitefly was carried out to prevent this vector introducing viral diseases. Fields were located where no commercial plantings of cassava were nearby.

Even if only indexed materials are planted at CIAT and careful management for whitefly populations is carried out, insects carrying disease may eventually arrive from nearby areas.

The decision has therefore been made not to extract vegetative seed from cassava without previously inspecting the roots to confirm that the plant is indeed free of symptoms. This will help eliminate any plant that may have been infected during growth at CIAT (except those cases where infection occurred late in the plant's development and symptoms have not had time to be expressed). This method has been confirmed as helping in reducing disease incidence to a certain degree.

The results from the *Clonal Evaluation Trial* harvested in June are presented in Table 2.42. The trials included as many as 880 genotypes represented by seven plants each. The trial was planted in CIAT Experimental Station in Palmira. At harvest time, 39 clones showed suspicious symptoms for frog skin disease, and were discarded as a preventive measure. This level of contamination represents about 4% of incidence, a much lower level than that found in previous years. One hundred and forty clones were selected from this trial. Average fresh root yield for the whole trial was 28.9 t/ha and for the selected fraction the average was an impressive 48.15 t/ha. Dry matter productivity for all the clones evaluated averaged 11.07 t/ha, whereas for the selected fraction it was 19.24 t/ha.

Table 2.42. The most relevant results from *Clonal Evaluation Trial* evaluated at CIAT-Palmira. Department of Valle del Cauca, Colombia, during August 2001 to June 2002.

Clone or parameter	Plant type (1 - 5)	Dry matter (%)	Fresh roots (t/ha)	Fresh foliage (t/ha)	Harvest Index (0 - 1)	Dry matter (t/ha)
GM 265-82	2	42.18	81.25	59.58	0.58	34.27
CM 9901-112	1	41.29	63.33	30.00	0.68	26.15
GM 311-42	2	41.55	79.58	69.79	0.53	33.06
GM 311-55	4	40.14	93.13	62.92	0.60	37.38
GM 260-32	2	40.90	66.67	38.54	0.63	27.27
GM 284-58	3	40.16	76.04	42.71	0.64	30.54
GM 292-46	1	39.91	58.96	30.42	0.66	23.53
GM 271-43	3	41.50	65.42	29.17	0.69	27.15
GM 234-119	1	39.09	66.67	50.83	0.57	26.06
GM 268-35	1	41.92	47.92	27.29	0.64	20.09
GM 312-65	1	38.05	68.54	52.71	0.57	26.08
GM 309-67	1	42.24	51.04	43.13	0.54	21.56
Statistics from the best 140 clones						
Maximum	5.00	45.50	93.13	101.25	0.80	37.38
Minimum	1.00	34.50	22.29	8.33	0.42	9.36
Mean	2.60	40.14	48.15	29.54	0.64	19.24
St. Deviation	0.92	1.85	13.75	17.21	0.07	5.28
Statistics from the 880 clones evaluated						
Maximum	5.00	45.53	93.13	157.08	0.83	37.38
Minimum	1.00	22.39	0.19	1.23	0.07	0.07
Mean	3.33	37.97	28.91	24.24	0.56	11.07
St. Deviation	0.98	2.94	16.39	19.18	0.13	6.40

Table 2.43 Averages of the families that participated in the *Clonal Evaluation Trial* conducted at CIAT – Palmira and harvested in June, 2002. The trial had 880 genotypes.

Family	# clones	Selection Index	Select. clones (%)	Plant type (1 - 5)	Fresh roots (t/ha)	Dry matter (%)	Harvest Index (0 - 1)	Frog skin (%)
CM 9733	26	-8.94	7.7	3.31	28.46	34.81	0.55	0.0
CM 9901	33	11.56	33.3	2.88	31.27	39.02	0.68	0.0
CM 9903	24	9.76	37.5	2.58	28.68	38.34	0.68	8.3
CM 9945	15	-0.42	6.7	3.60	34.13	37.59	0.52	13.3
CM 9946	29	-13.22	0.0	3.48	4.14	39.70	0.51	10.3
CM 9949	17	-18.58	0.0	3.44	3.97	37.66	0.51	0.0
CM 9953	20	2.75	0.0	3.30	23.99	38.03	0.70	5.0
GM 228	39	3.21	12.8	2.92	23.74	38.79	0.61	0.0
GM 230	36	6.92	16.7	3.29	30.56	39.06	0.62	0.0
GM 231	28	0.62	17.9	3.36	27.43	37.77	0.61	0.0
GM 234	41	8.54	19.5	2.83	35.06	38.14	0.60	0.0
GM 235	24	8.29	33.3	2.92	30.45	38.68	0.64	0.0
GM 254	25	11.33	28.0	3.17	28.71	40.09	0.68	0.0
GM 257	25	12.86	40.0	3.44	30.04	41.28	0.65	0.0
GM 260	16	-0.44	18.8	3.38	23.46	38.84	0.58	0.0
GM 264	23	5.82	17.4	3.30	30.98	38.27	0.65	4.3
GM 265	36	1.00	8.3	3.33	27.90	37.98	0.60	5.6
GM 268	17	3.54	29.4	3.38	26.84	38.62	0.64	5.9
GM 269	8	8.01	12.5	2.88	30.23	37.80	0.69	25.0
GM 271	14	2.50	28.6	3.64	34.32	36.92	0.65	7.1
GM 283	8	-1.31	12.5	3.75	25.23	39.33	0.54	0.0
GM 284	41	8.44	36.6	3.37	37.98	38.97	0.56	12.2
GM 292	17	5.31	17.6	3.41	32.48	38.43	0.61	5.9
GM 293	11	0.57	9.1	3.55	30.63	38.30	0.55	9.1
GM 294	10	-9.79	10.0	3.67	39.05	34.70	0.42	0.0
GM 296	18	-3.77	11.1	3.28	23.45	37.40	0.58	5.6
GM 297	8	-4.80	0.0	3.13	28.98	34.88	0.62	12.5
GM 298	35	-23.68	0.0	3.00	3.08	35.88	0.46	20.0
GM 306	9	-17.44	0.0	3.11	35.76	32.56	0.35	0.0
GM 308	21	-12.20	4.8	3.24	30.29	35.53	0.38	4.8
GM 309	41	-1.04	12.2	3.40	39.89	36.49	0.47	0.0
GM 310	36	-5.12	11.1	3.34	29.53	37.70	0.43	0.0
GM 311	32	-2.61	12.5	4.22	37.21	38.54	0.44	0.0
GM 312	42	-4.18	9.5	3.89	33.68	38.31	0.42	2.4
GM 313	37	-3.58	18.9	3.57	34.56	37.37	0.45	5.4
GM 314	18	-4.89	0.0	4.00	36.99	37.09	0.45	22.2
Total	880	-.-	-.-	-.-	-.-	-.-	-.-	39
Mean	24	0.0	0.16	3.34	28.70	37.75	0.56	0.04

The 140 clones selected from the *Clonal Evaluation Trial* will be planted in three different experiments, in this case with 49 clones each. The total number of clones to participate in these experiments, therefore, will be 147 (49 x 3). The difference between the 140 clones selected and the 147 clones that will make up the next phase of evaluation gives the possibility of incorporating seven checks. The number 49 allows for a square lattice design, which will be used in all the evaluations at the *Preliminary Yield Trial* level.

The *Clonal Evaluation Trial* is not only the source of promising clones for the next stage of evaluation. The information from the trials is used to determine the how good the progenitors used to generate new clones are. Therefore, the data from the trial has been condensed to produce the information presented in Table 2.43. In this table, the average of each family for the most relevant traits are provided.

Families CM 9945, GM 269, GM 284, GM 297, GM 298 and GM 314 presented an unquestionable incidence of frog skin disease. It is assumed (and there are good justifications for this assumption) that frog skin disease is not transmitted through the botanical seed. The stakes for this trial come from plants grown the previous cycle at CENICAÑA, in a relatively isolated field, surrounded by sugar cane. At the F1 stage the F1-plants from the same family are planted together one after the other in the same row. For logistic reasons, the clones from the same family at the *Clonal Evaluation Trial* are also planted one after the other. In this case, however, there were seven plants representing each clone (not one as in the F1 stage). Therefore, the seven plants of each clone were planted together in 1-row plot, and in the following plot there will be the second clone from the same family and so forth. This information is relevant to understand what may have happened with frog skin disease. A quick review of the levels of incidence for each family, would suggest that there were three or four foci where the disease was probably introduced. It is not possible, however, to determine if the infection took place at the F1 or at the *Clonal Evaluation* stages, or in both instances. This situation is another justification for the decision taken to split each family in three groups planted scattered through the *Clonal Evaluation Trial*. In this way, at least for the familiy unit, there will be the equivalent of three replications. Had we done this a year before we could have circumstantial evidence that the infection occurred at the F1 stage, for instance.

Few families had an outstanding performance, based on the proportion of their clones that had been selected (Table 2.43). CM 9901, CM9903, GM 235, GM 257, and GM 284 had the highest proportion of selected clones (> 30%) and the highest averages for selection index (> 8.0). In contrast, families CM 9946, CM 9949, CM 9953, GM 297, GM 306, and GM 314 had not a single clone selected from them which, with one exception, was associated with negative selection indexes.

The highest productivity was observed in family GM 284 with an average yield of almost 38 t/ha of fresh roots, and optimum level of dry matter content (38.97%). However, plant type may not be a strength for this germplasm (average score slightly worse than the average of the population). Family GM 257 had poor plant architecture (average score = 3.44) but was excellent for its dry matter content (41.28%). Although fresh root productivity in this family was not the highest, it presents the highest proportion of selected clones. Families CM 9901,

CM 9903, and GM 235 had excellent yields and good dry matter contents along with good plant type (average scores < 3.0).

All the families that failed to have at least one clone selected where characterized by their poor plant type (except for family GM 298). Yield productivity of CM 9946, CM 9949, CM 9953 and GM 298 was very low and can explain their failure in having some of their clones selected. GM 297 and GM 306 had much higher yields, but their dry matter content were too low (< 35%), and therefore, the selection index correctly rejected them. GM 314 presented good yields and high dry matter contents in the roots, but it had very poor plant type (average score = 4.0) and low harvest index (0.45). This family was also severely affected by frog skin disease.

The data from the *Clonal Evaluation Trial* can be further condensed by looking at the averages of all the progenies derived from a common progenitor (Table 2.44). Clones CM 6740-7 (CORPOICA-Reina), SM 1636-24, SM 1741-1 and, surprisingly, MTAI 8 are outstanding based on the proportion of their progenies being selected (> 20%).

Table 2.44. Averages for the progenies derived from a common parent. The information comes from the *Clonal Evaluation Trial* harvested in CIAT-Palmira in June 2002. A total of 880 had been evaluated.

Progenitor	Families (#)	Progeny size (#)	Select. clones %	Plant type (1 - 5)	Fresh roots (t/ha)	Dry matter (%)	Harvest Index (0 - 1)	Frog Skin (%)	Selection Index.
CM 6740-7	8	246	21.54	3.00	29.75	38.30	0.61	1.22	5.29
CM 6754-8	1	15	6.67	3.60	34.13	37.59	0.52	13.33	-0.42
SM 805-15	3	61	1.64	3.50	11.47	38.61	0.51	8.20	-11.57
SM 1219-9	8	219	19.63	3.27	30.63	38.57	0.61	1.83	5.30
SM 1278-2	6	139	18.71	3.20	27.95	38.48	0.59	2.88	2.76
SM 1411-5	1	29	0.00	3.48	4.14	39.70	0.51	10.34	-13.22
SM 1565-17	1	17	0.00	3.44	3.97	37.66	0.51	0.00	-18.58
SM 1636-24	5	142	25.35	3.57	33.81	39.32	0.56	3.52	5.80
SM 1673-10	8	149	15.44	3.57	30.17	38.13	0.54	2.68	-0.78
SM 1741-1	8	173	21.39	3.25	31.51	37.97	0.59	8.67	3.19
SM 2058-2	1	35	0.00	3.00	3.08	35.88	0.46	20.00	-23.68
SM 2219-11	1	35	0.00	3.00	3.08	35.88	0.46	20.00	-23.68
HMC 1	6	137	12.41	3.27	31.49	37.31	0.57	5.11	0.75
MECU 72	8	236	10.59	3.64	34.77	37.22	0.43	3.39	-4.74
MPER 183	6	103	9.71	3.37	30.77	36.00	0.56	3.88	-4.42
MTAI 8	1	24	33.33	2.92	30.45	38.68	0.64	0.00	8.29

In the case of MTAI 8 few clones were representing the parental properties of this clone, so the results should be taken with some caution. However, this result is another evidence of the advantage of making crosses among clones adapted to different eco-regions. That was the case from SM 1411-4 which, being adapted to the northern coast, produced good progenies for the acid soil savannas. As a matter of fact CM 6740-7 (released for the acid soil savannas) is another example of a clone from adapted to one eco-region producing good progenies adapted to a different environment.

The progenies from SM 1636-24 had the highest selection index average (5.80), except for MTAI 8 that had too few clones representing it. The average productivity of the progeny from this clone was very high (33.81 t/ha of fresh roots), second only to those from CM 6754-8 (34.13 t/ha), but with much higher dry matter content (39.32 versus 37.59%). In any case, CM 6754-8 was represented by only 15 clones. It is obvious that the major limiting factor for the progenies derived from SM 1636-24, is the plant architecture, which is also related to a relatively low harvest index.

It is interesting to note the poor performance of the progenies from MPER 183. This clone, per se, has excellent properties. However, it proved to be a very poor progenitor both, for the acid soil savannas and the mid-altitude valleys. SM 805-15 is another case of a good clone that is not a good progenitor. It should be remembered that the progeny from this clone was very mediocre when evaluated in the sub-humid conditions from the northern coast (Table 2.17). Both clones are going to be eliminated from the crossing blocks based on this consistent information.

In general, there is no clear trend regarding frog skin disease, emerging from Table 2.44. Only two progenitors (MTAI-8 and SM 1565-17) produced symptoms-free progenies, but they were small progenies and represented only one family in each case. The opposite happened with the cross between SM 2058-2 x SM 2219-11 which resulted in the highest frog skin incidence (20%). Having been represented by a single family of clones located in one part of the field, it is not justifiable to conclude that this family is particularly susceptible to frog skin. It may have been just the position where it was planted, rather than a higher tendency to get infected.

As was the case for the sub-humid and acid-soil savannas environments, a dialed study was also conducted for the mid-altitude valleys. A set of nine parents was used. As in the other diallels, 30 clones represented each F1 cross. Two locations with three replications in each were used to obtain the data. Results from the evaluation at CIAT-Palmira are presented in Tables 2.45 and 2.46, and those from AGROVELEZ-JAMUNDI are in Tables 2.47 and 2.48. The same variables are presented for the diallel studies at the two locations, with the exception of scoring for mites at CIAT-PALMIRA and for white flies at AGROVELEZ-JAMUNDI. The fact that no reliable data for white flies could be taken at CIAT-PALMIRA reflects the success of the measures taken to control this problem. In effect, white flies pressure at CIAT's experimental station in Palmira was very low during the reported period because of the interruption of cassava planting for one month every year. This measure disrupts white flies cycle and has resulted in a significant reduction of this problem in our plantings at this station.

Table 2.45 Average for the crosses (based on 30 clones) from a 9-parent diallel conducted at CIAT-PALMIRA.

Cross	Mites (1 to 5)	Plant Type (1 to 5)	Harvest Index (0 to 1)	Dry matter (%)	Fresh roots (t/ha)
1x2	3.34	3.15	0.55	35.29	50.88
1x3	3.07	3.18	0.50	35.92	36.17
1x4	3.09	3.22	0.47	36.15	46.15
1x5	3.83	3.59	0.54	36.11	36.63
1x6	3.13	2.94	0.54	36.03	46.65
1x7	3.85	3.11	0.54	34.25	41.02
1x8	2.63	2.39	0.44	36.19	56.16
1x9	3.70	3.24	0.46	37.17	34.81
2x3	3.90	3.45	0.55	35.55	40.58
2x4	3.69	3.26	0.54	33.80	37.84
2x5	3.56	3.19	0.58	36.35	45.41
2x6	3.92	3.15	0.54	35.67	43.28
2x7	4.17	3.32	0.55	35.46	44.64
2x8	2.69	2.61	0.52	34.32	61.72
2x9	3.71	2.91	0.53	34.74	64.74
3x4	4.36	3.23	0.49	34.38	41.66
3x5	3.75	3.35	0.52	36.11	31.39
3x6	3.80	3.24	0.59	35.39	41.01
3x7	4.20	3.52	0.52	34.24	34.04
3x8	3.24	3.09	0.46	34.97	45.12
3x9	4.04	2.99	0.49	34.05	48.98
4x5	3.95	3.52	0.50	36.44	36.06
4x6	3.79	2.87	0.50	36.34	44.74
4x7	4.15	4.11	0.48	33.34	29.85
4x8	2.98	2.92	0.43	35.72	49.00
4x9	3.90	3.08	0.52	34.70	61.58
5x6	3.86	3.29	0.62	37.46	51.56
5x7	4.27	3.66	0.52	34.96	37.10
5x8	2.75	3.08	0.46	35.66	50.22
5x9	4.06	3.43	0.50	33.89	46.20
6x7	4.01	3.30	0.57	35.68	42.99
6x8	2.66	2.84	0.52	35.00	44.47
6x9	3.71	2.69	0.58	35.19	57.94
7x8	3.67	3.08	0.47	34.04	47.64
7x9	4.23	3.11	0.54	33.97	47.63
8x9	2.59	2.76	0.43	33.40	52.79
Maximum	4.36	4.11	0.62	37.46	64.74
Minimum	2.59	2.39	0.43	33.34	29.85
Mean	3.62	3.16	0.52	35.22	45.24
St.Dev.	0.52	0.32	0.05	1.03	8.45

Table 2.45 shows the averages for each F1 cross in the diallel at Palmira. The average fresh root yield was 45 t/ha is excellent, considering that it involves 1080 new genotypes. Averages for F1 families ranged from 29.85 to 64.74 t/ha. Best yields were observed for crosses 2x9, 2x8, and 4x9, with mean yields of 64.74, 61.72, and 61.58 t/ha, respectively. Similarly, these crosses presented the highest dry matter yields exceeding 21 t/ha. High dry matter content in the roots was observed from crosses involving parents 5 (SM 1673-10) and 6 (SM 1741-1).

Results from the diallel at Palmira were consolidated to obtain the averages for the progenies of each progenitor (Table 2.46). The best two parents for fresh root yield were MPER 183 and MECU 72 with average yields above 50 t/ha. This reveals the advantages of having the access to materials from the germplasm bank (both progenitors are landraces from Peru and Ecuador, respectively). In addition, MECU 72, is not only a valuable source of resistance to white flies, but also posses good levels of resistance to mites. SM 1278-2 and HMC-1 were the worse progenitors based on the average root productivity of their progenies.

Table 2.46. Averages of all the progenies from each of the parents included in the diallel study conducted at CIAT-PALMIRA.

Progenitor	Mites (1 to 5)	Plant Type (1 to 5)	Harvest Index (0 to 1)	Dry matter (%)	Fresh roots (t/ha)
1= CM 6740-7	3.33	3.10	0.51	35.89	43.56
2 = SM 1219-9	3.62	3.13	0.55	35.15	48.63
3 = SM 1278-2	3.80	3.26	0.52	35.08	39.87
4 = SM 1636-24	3.74	3.28	0.49	35.11	43.36
5 = SM 1673-10	3.75	3.39	0.53	35.87	41.82
6 = SM 1741-1	3.61	3.04	0.56	35.84	46.58
7 = HMC 1	4.07	3.40	0.52	34.49	40.62
8 = MECU 72	2.90	2.85	0.47	34.91	50.89
9 = MPER 183	3.74	3.02	0.51	34.64	51.83
Maximum	4.07	3.40	0.56	35.89	51.83
Minimum	2.90	2.85	0.47	34.49	39.87
Average	3.62	3.16	0.52	35.22	45.24

SM 1673-10, CM 6740-7, and SM 1741-1 produced progenies with higher dry matter content based on the results from Palmira, whereas MPER 183, MECU 72, and HMC-1 generated progenies with low dry matter in their roots. HMC-1 also was characterized by progenies with higher susceptibility to mites (Table 2.46). The results so far analyzed from the diallel study illustrate, once amore, the difficulties in combining in one genotype desirable traits. For instance, MPER 183 has excellent levels of root productivity but very low dry matter contents (explaining the results from the *Clonal Evaluation Trial*). On the other hand, CM 6740-7 has high dry matter content, but its productivity was not as outstanding. HMC-1 also has high dry matter content, but low root productivity and clear susceptibility to mites.

The best crosses for dry-matter yield were 2x9 (22.49 t/ha), 4x9 (21.37 t/ha), 6x9 (20.39 t/ha), and 1x8 and 2x8 (both with 20.32 t/ha). Parent 9 is in three of these five crosses.

Table 2.47. Average for the crosses (based on 30 clones) from a 9-parent diallel conducted at AGROVELEZ-JAMUNDI.

Cross	White flies (1 to 5)	Plant Type (1 to 5)	Harvest Index (0 to 1)	Dry matter (%)	Fresh roots (t/ha)
1x2	3.07	3.54	0.40	29.95	50.43
1x3	2.56	3.31	0.42	35.02	49.12
1x4	2.71	2.89	0.42	31.60	48.35
1x5	2.94	3.08	0.40	32.93	49.29
1x6	3.09	3.06	0.45	33.30	53.45
1x7	3.18	3.21	0.47	32.62	55.94
1x8	1.73	3.19	0.38	30.34	50.71
1x9	2.97	2.92	0.38	33.69	41.26
2x3	2.87	3.18	0.50	33.50	56.47
2x4	3.38	3.34	0.35	27.95	35.46
2x5	2.65	3.23	0.46	32.38	52.17
2x6	3.83	2.99	0.46	34.15	46.20
2x7	2.99	3.12	0.41	31.00	44.95
2x8	1.61	3.48	0.41	28.61	59.26
2x9	2.67	2.69	0.42	31.27	60.18
3x4	4.13	3.04	0.44	32.81	42.96
3x5	3.37	3.08	0.42	33.85	40.26
3x6	3.75	3.29	0.41	34.51	37.60
3x7	3.46	3.15	0.46	34.00	39.27
3x8	2.06	2.97	0.39	32.89	46.92
3x9	3.11	3.15	0.39	32.48	49.91
4x5	3.00	3.08	0.37	32.49	41.54
4x6	3.60	3.22	0.38	32.92	43.76
4x7	3.47	3.26	0.38	29.74	31.38
4x8	2.00	3.12	0.35	31.20	50.07
4x9	3.67	2.84	0.43	31.04	57.41
5x6	3.48	3.13	0.52	35.15	56.92
5x7	3.21	3.05	0.44	32.66	43.09
5x8	1.69	3.18	0.36	30.44	42.48
5x9	3.24	3.08	0.37	30.30	42.58
6x7	3.57	3.23	0.48	33.55	47.80
6x8	3.02	3.34	0.37	30.28	38.42
6x9	3.73	2.81	0.44	32.41	56.67
7x8	2.05	3.35	0.41	30.88	52.76
7x9	3.45	2.84	0.46	31.44	64.42
8x9	1.82	2.88	0.34	28.89	46.87
Maximum	4.13	3.54	0.52	35.15	64.42
Minimum	1.61	2.69	0.34	27.95	31.38
Average	2.98	3.12	0.41	32.01	47.95
St. Dev.	0.66	0.19	0.04	1.80	7.59

Results from the diallel at Jamundí are presented in Tables 2.47 and 2.48. Although there was severe pressure from white flies, average fresh root yield were higher than at Palmira (47.95 versus 45.24 t/ha). Root dry-matter content in Jamundí, however, was lower than at Palmira (32.01 versus 35.22 %). Best crosses for fresh root production at Jamundí were 7x9, 2x9, and 2x8.with 64.4, 60.2, and 59.3 t/ha, respectively. Crosses 2x9 and 2x8 also had been among the highest yielding F1-cross families at Palmira.

Table 2.48. Averages of all the progenies from each of the parents included in the diallel study conducted at AGROVELEZ-JAMUNDI.

Progenitor	White flies (1 to 5)	Plant Type (1 to 5)	Harvest Index (0 to 1)	Dry matter (%)	Fresh roots (t/ha)
CM 6740-7	2.78	3.15	0.41	32.43	49.82
SM 1219-9	2.88	3.20	0.42	31.10	50.64
SM 1278-2	3.16	3.15	0.43	33.63	45.31
SM 1636-24	3.24	3.10	0.39	31.22	43.87
SM 1673-10	2.95	3.11	0.42	32.52	46.04
SM 1741-1	3.51	3.13	0.44	33.28	47.60
HMC 1	3.17	3.15	0.44	31.99	47.45
MECU 72	2.00	3.19	0.38	30.44	48.44
MPER 183	3.08	2.90	0.40	31.44	52.41
Maximum	3.51	3.20	0.44	33.63	52.41
Minimum	2.00	2.90	0.38	30.44	43.87
Average	2.98	3.12	0.41	32.01	47.95

Data from Table 2.48 suggest again a good performance of the progenies from MPER 183 with the highest levels of average fresh root production (52.41 t/ha), followed by SM 1219-9 (50,64 t/ha) and CM 6740-7 (49.82 t/ha). In relation to dry-matter contents, the progenies from SM 1278-2 and SM 1741-1 were the only to average above 33 %. As was observed in Palmira, the progenies from MPER 183 and MECU 72 had low dry matter contents (31.44 and 30.44 %, respectively).

SM 1741-1 proved to be susceptible to white flies (average score 3.51) and as expected, MECU 72 produced progenies with excellent reaction to this insect (average score 2.00). The progenies from CM 6740-7 and SM 1219-9 showed good levels of resistance to white flies (average scores < 3.00).

Table 2.49. Correlations for measurements taken at CIAT-PALMIRA and AGROVELEZ-JAMUNDI based on the F1-cross family averages.

Plant type 1-5	Root type 1-5	Root color 1-5	Pulp color 1-9	Commercial roots (No.)	Fresh foliage (kg/pl)	Dry matter (%)	Harvest index (0-1)	Fresh root Yield (t/ha)
0.05	0.53	0.97	0.91	0.28	0.81	0.57	0.69	0.68

The highest dry-matter yields observed at Jamundí were from crosses 7x9 (20.25 t/ha), 5x6 (20.01 t/ha), 2x3 (18.92 t/ha), 2x9 (18.82 t/ha), and 6x9 (18.37 t/ha). Two of these five families of crosses (2x9 and 6x9), also were among the highest yielding materials at Palmira.

The problem of combining different desirable traits into one genotype is one of the difficulties frequently encountered by plant breeders. A second other limitation occurs when clones with outstanding performance in one location have a poor one in a second location, resulting in high genotype by environment interactions. Table 2.49 shows the correlations for several variables measured at Palmira and Jamundí. Correlations are based on the average for each F1 cross (across three replications of each location). Fresh root at both locations showed a good correlation of 0. 68. In general all correlations were high (> 0.50) with the exception of plant type (ρ = 0.05) and number of commercial roots (ρ = 0.28). It is possible that the low correlation for plant type is a result of the two different insect pests (mites in Palmira and white flies in Jamundí) affecting the trials. The reaction to these pests has a strong effect on the plant aspect variable, as well.

A last result of the diallel study is presented in Table 2.50, where phenotypic correlations among variables at each location are summarized.

Table 2.50 Phenotypic correlations among variables measured in the diallel studies at AGROVELEZ-JAMUNDI (above diagonal) and CIAT-PALMIRA (below diagonal), using the averages of the 36 F1 families.

	Mites Palmira	Plant type	Root type	Root Color	Pulp Color	Commer. roots	Foliage (kg/pl)	Dry matter %	Harvest index	Fresh root Yield
White flies	-.-	-0.15	-0.04	0.56	0.06	-0.13	-0.59	0.43	0.46	-0.20
Plant type	0.65	-.-	0.50	-0.05	-0.14	-0.32	-0.29	-0.18	-0.11	-0.27
Root type	0.13	0.51	-.-	-0.25	0.25	-0.81	-0.31	-0.26	-0.61	-0.80
Root color	0.63	0.39	0.10	-.-	-0.50	0.17	-0.30	0.10	0.43	0.10
Pulp color	-0.01	0.02	-0.24	-0.48	-.-	-0.13	-0.13	0.09	-0.17	-0.28
Comm. Roots	-0.37	-0.81	-0.57	-0.09	-0.26	-.-	0.36	0.24	0.57	0.85
Foliage kg/pta	-0.61	-0.71	-0.23	-0.49	-0.02	0.65	-.-	-0.35	-0.35	0.56
Dry matter	-0.14	-0.05	-0.27	-0.19	0.38	-0.08	-0.17	-.-	0.62	0.05
Harvest index	0.41	0.17	-0.33	0.61	-0.17	0.08	-0.63	0.20	-.-	0.48
FR Yield.	-0.39	-0.74	-0.51	-0.07	-0.22	0.92	0.74	-0.10	0.03	-.-

At Palmira yield and reaction to mites had a correlation = -0.39, whereas at Jamundí the correlation between white flies and fresh root yield was -0.20. These values were negative because a small insect damage is scored as 1, and a severed damage with a score of 5. The correlation between yield and plant type was much higher at Palmira (ρ = -0.74) than at Jamundí (ρ = -0.27). Plant type at Jamundí was difficult to score because of the high soil fertility resulted in frequent lodging in several families. Also worth mentioning is the contrast in the correlations between harvest index and fresh root yield at Palmira (ρ = -0.03) and (ρ = 0.48).

It was not possible to establish a clear relationship between fresh root yield and dry matter content in the roots. Therefore, it should be possible to eventually generate high yielding clones with high dry-matter content in their roots. The fact that correlations are not necessarily evidence of a cause – effect relationship is supported by the meaningless correlations between root color and harvest index (ρ = 0.63 and 0.43) or between root color a reaction to insects (ρ = 0.56 and 0.63).

In Table 2.51, the most relevant results of the *Preliminary Yield Trial* are presented. This trial included 110 genotypes, planted in two, 5-plant row plots and with three replication at CIAT-Palmira. The clones and stakes for this trial came from a *Clonal Evaluation Trial* conducted the previous year at CEUNP about 15 km from CIAT headquarters, where any clone that had just one plant with even weak symptoms of frog skin was eliminated. Still the *Preliminary Yield Trial* resulted with a very high disease incidence. As much as, 60% of the clones showed symptoms of the disease. It is not clear what happened but it is also certain that for some reason the planting at CEUNP, resulted in high degree of frog skin incidence. This should be considered as a defeat imposed by this elusive disease. All the materials planted at CEUNP had been indexed to be frog-skin free. One mistake was committed: to allow sequential plantings, and therefore, there were 6-months old plants nearby other materials just planted. It was not surprising to see that the late plantings at CEUNP were the most severely affected by frog skin. We have learned from this experience: it is not allowed to plant sequentially in the same station for periods longer than 1-2 months. For instance, this year, all the plantings at CIAT – Palmira were done between August 15 and September 20.

In spite of this problem, the general results of the *Preliminary Yield Trial* was very encouraging (Table 2.51). The 25 selected clones have been moved out of CIAT-Palmira station because, even if they did not presented any symptoms of frog skin they could still be late-infected. It has been decided that only materials certified to be disease-free and that maintain that condition will be allowed to be grown at CIAT station. The 25 selected clones, therefore, were planted at Santander de Quilichao (an area where frog skin is endemic, and therefore is not going to create a new problem). Once we have the facilities in working conditions, these clones will be subjected to meristem culture to be cleaned from the disease, and re-enter the evaluation process. This may take as much as two years, and illustrates the complications that breeding cassava at CIAT faces if the pathogen (or pathogens?) involved in frog skin disease are not soon identified

Table 2.51. Results from the *Preliminary Yield Trial* conducted at CIAT-Palmira with 110 clones evaluated in 10-plant plots with three replications.

Clone or parameter	Fresh roots (t/ha)	Plant type (1 - 5)	Harvest index (0 - 1)	Dry matter (%)	Foliar retention (1 - 5)
SM 2580-24	64.1	4.00	0.67	37.8	1.67
SM 2795-15	47.5	2.67	0.69	38.7	2.33
CM 9504-1	41.3	3.00	0.60	41.0	2.00
SM 2652-11	40.1	1.33	0.59	38.7	2.00
SM 2588-14	46.0	2.67	0.60	38.7	2.00
SM 2659-3	34.9	2.67	0.55	40.4	1.67
SM 2799-17	22.0	1.67	0.60	43.4	2.33
SM 2795-13	34.0	2.33	0.61	40.2	2.33
SM 2800-3	31.9	1.67	0.65	37.8	2.33
SM 2580-29	36.4	2.00	0.62	37.8	2.67
Statistics of the 25 clones selected					
Maximum	64.08	4.00	0.69	43.36	3.33
Minimum	21.96	1.00	0.55	34.99	1.67
Mean	36.42	2.61	0.62	38.84	2.31
St. Deviation	8.31	0.80	0.04	1.68	0.44
Statistics of the 110 clones evaluated					
Maximum	64.08	5.00	0.75	43.36	3.67
Minimum	6.17	1.00	0.18	30.22	1.67
Mean	26.10	3.12	0.55	36.67	2.50
St. Deviation	9.73	0.91	0.10	2.40	0.48

Fresh root productivity for the selected fraction averaged 36.42 t/ha, compared with the population mean of 26.10 t/ha (Table 2.51). Dry matter content was excellent with 38.84% for the selected fraction and 36.67% for all the clones evaluated. Leaf retention was scored when this trial was about five-month of age. The results of the score are presented in the right column of Table 2.51. As it is usually the case the scale used was 1= excellent and 5= very poor.

Because of the availability of this variable the selection index for this trial was as follows:

Selection Index = [Fresh root yield * 10] + [Dry matter content * 8] - [Plant type * 5] + + [Harvest Index * 3] – {Foliar retention * 3]

As was the case in the northern coast, it was possible to detect a clear relationship between leaf retention at five-months of age and root productivity at ten- or eleven-months after planting (the correlation coefficient was = - 0.38). Leaf retention was scored under no disease or abiotic stress (i.e. water stress). Regression analysis suggests that for each unit increase for the score in foliar retention there will be a reduction in as much as six t/ha in root productivity (Figure 2.3)
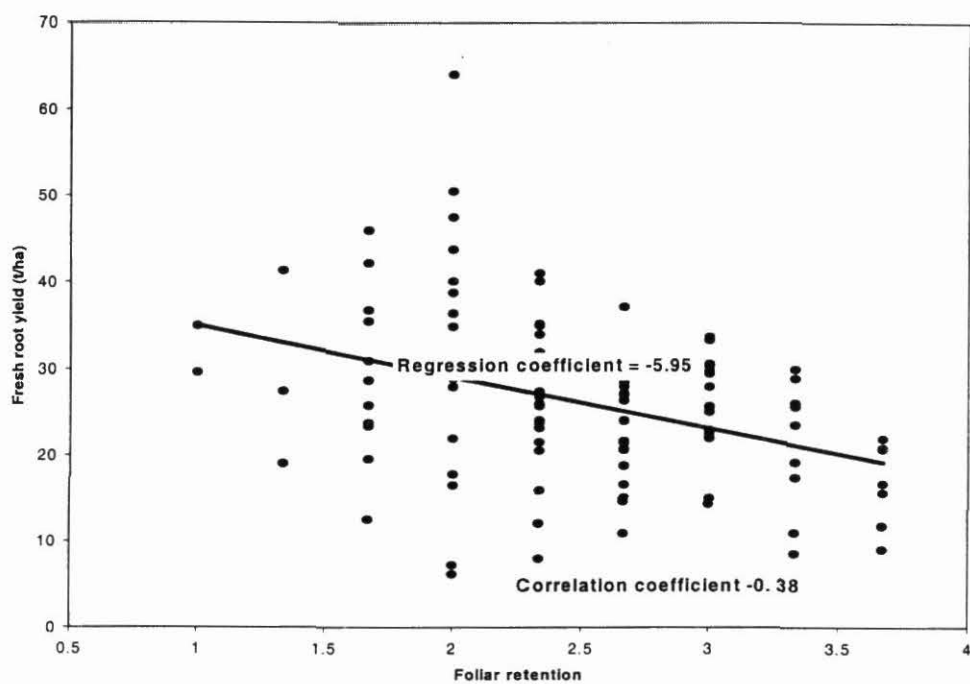
Figure 2.3. Relationship between foliar retention at five months and fresh root yield at 10 months after planting. Results from 110 genotypes evaluated at CIAT-Palmira in a trial with three replications.

Another interesting result from this trial was the possibility of determining the effect of frog skin infection on foliage productivity. Because there were cases were plants from a given clone were clean of symptoms in one or two replication and showing symptoms in the remaining replication(s) it was possible to measure the effects of frog skin in a few variables. For instance, on average plants with symptoms yielded about 20.15 t/ha of fresh foliage, whereas plants with no symptoms produced only 15.07 t/ha. In a way, this is not a surprise because frog skin prevents the translocation of starches from the leaves to the storage roots. Having more nutrients available, the above ground tissue develops more vigorously in the presence of the disease. Foliar retention was also affected by frog skin The average foliar retention score of clones that showed symptoms in the roots had been 2.48, whereas those clean of the disease had had a considerably worse score (3.32).

Table 2.52 presents the results of the *Regional Trials* conducted for the mid-altitude valleys environment. These trials were planted in five representative locations (Cauca and Valle del Cauca Departments), and included thirty genotypes, including two checks. Experimental plots had 25 plants and three replications per trial were used at each location.

The highest yields were obtained at Jamundí and Bocas de Palo, Buga and Vijes showed intermediate productivity, and S. de Quilichao had the lowest yields. The latter environment

is characterized by acid soils and high white flies pressure. The highest dry matter content was obtained at Vijes (37.67%).

Table 2.52 Relevant results from the *Regional Trials* conducted in Cauca and Valle del Cauca Departments for the mid-altitude valleys environment. At the bottom of each location their respective means for fresh root yield and dry matter content are provided. Highlighted in boldface are the two checks.

Clone	Fresh root (t/ha)	Dry matter (%)	Cooking quality (1 - 5)	HCN (1 - 9)	Ranking at each location				
					Boca Palo	Jamundi	Sder. Quilichao	Buga	Vijes
SM 1965-1	25.61	36.75	3.15	4.75	4	6	1	1	15
SM 2141-1	25.03	36.67	2.85	6.00	7	3	2	8	4
SM 1642-22	28.06	34.46	4.00	6.00	9	12	3	5	1
CM 8370-11	25.57	35.06	1.35	5.75	2	2	11	3	6
SM 1855-15	24.20	35.38	1.00	4.25	1	10	4	14	14
SM 2211-3	24.15	35.10	3.00	7.00	8	17	9	2	7
SM 2052-4	25.12	33.48	2.50	7.00	3	7	8	6	19
SM 2160-2	21.73	34.79	3.65	5.50	14	5	23	10	3
SM 1520-16	22.64	33.54	1.00	6.50	5	1	24	19	10
CM 8370-10	20.83	35.41	1.35	6.00	22	4	10	22	2
SM 2073-1	20.77	35.33	1.00	5.00	11	22	25	4	5
SM 1520-18	19.80	34.52	1.35	5.25	10	13	21	12	13
CM 6660-21	20.26	34.82	3.00	7.00	23	8	16	20	8
SM 1871-33	19.47	35.59	1.00	4.50	20	19	6	7	22
M TAI 8	18.50	35.08	4.15	7.50	19	14	7	24	12
SM 1779-7	21.31	34.13	1.65	4.75	18	26	5	23	11
SM 2058-2	20.86	33.43	4.00	7.25	15	11	18	18	21
SM 1660-4	21.32	32.58	3.00	5.75	21	9	19	25	17
SM 2085- 7	20.92	33.60	3.00	6.50	12	18	15	15	26
CM 523-7	*15.84*	*35.69*	*2.50*	*7.25*	*27*	*20*	*12*	*21*	*9*
SM 2198-4	17.37	34.85	3.50	8.00	16	16	28	17	20
SM 2119-1	15.57	35.77	2.00	5.50	26	21	22	13	16
SM 1788-11	16.62	34.96	3.50	5.00	24	28	14	9	24
CM 8759-14	19.98	32.52	2.85	6.25	17	23	26	26	18
CM 7463-2	16.68	33.13	2.15	3.00	13	25	29	16	23
SM 2030-5	13.93	34.56	4.00	6.25	6	30	17	28	30
SM 2052-3	13.49	33.76	2.15	3.25	25	15	30	29	25
MPER 183	*19.16*	*30.84*	*1.35*	*4.25*	*30*	*29*	*20*	*11*	*27*
SM 1788-13	18.21	31.74	3.85	4.75	28	27	13	27	28
SM 1959-1	13.52	31.05	2.35	5.50	29	24	27	30	29
Maximum	28.06	36.75	4.15	8.00	**Fresh root yield and dry matter content.**				
Minimum	13.49	31.05	1.00	3.99	24.09	25.58	13.77	17.19	20.45
Mean	20.22	34.29	2.54	5.71	33.97	35.59	31.04	33.17	37.67

¶ **HCN**: Cyanogenic potential (9=bitter, 1= sweet). **Cooking quality**: 1= excellent, 5= very poor.

Clones SM 1965-1, SM 2141-1, SM 1642-22, CM 8370-11 and SM 1855-15 had a consistently good performance across the environments where the trials had been conducted (Table 2.52). A rapid multiplication process will be started for these clones, as well as a meristem culture to produce frog-skin free vitroplant versions of them. The remaining clones had an erratic behavior. The clones used as checks (CM 523-7 and MPER 183) had a relatively poor performance, particularly for MPER 183.

Finally in Tables 2.53 and 2.54 the results of two *Regional Trials* for the high-altitude eco-region (> 1400 m.a.s.l.) are presented. Because of lack of sufficient planting material, not all the entries were planted in the two trials (only 17 of the clones can be found in the two trials). Yield of fresh roots was much higher at Morales (average 30.46 t/ha) that at La Cumbre (Average 11.32 t/ha). Clone 1058-13 had an excellent performance at Morales (Table 2.53, first place) and at La Cumbre (Table 2.54, third place). Clone SM 1053-23 had a good ranking from Morales (6th place), and also had an excellent performance at La Cumbre, but its ranking is irrelevant because at that location it was used as a check.

There were quite a few remarkable examples of genotype by environment interactions among the materials included in the two highland trials. Clone SM 1937-1 was fourth at Morales and second before the last in La Cumbre. MCOL 2261 showed a contrasting preference, it was 32nd at Morales and 10th at La Cumbre. SM 1933-5 was fifth at La Cumbre and 26th at Morales. A few clones, had a poor performance at both locations (SM 1940-3 and 1833-21).

2.4.4. Selections for the Middle Magdalena Region

CIAT recently began evaluating cassava elite materials in this region. Working in the mid-Magdalena River region is important because of the social relevance of cassava and because it combines characteristics of the sub-humid and the acid-soil savannas. One of the objectives for these evaluations was to determine the kind of germplasm that adapts better to this environment (south of Cesar and Bolivar Departments and Santander and Norte de Santander Departments).

Table 2.55 summarizes the results of four Regional Trials conducted in this eco-region (San Vicente, Barrancabermeja, Sabana de Torres, and Regidor). As in the previous cases, the ranking of each material was established based on a selection index. Most of the best performing clones are adapted to the sub-humid environment, although germplasm adapted to the acid-soil savannas and the mid-altitude valleys also was included. MTAI 8 was second only to SM 653-14, regarding fresh root productivity (> 30 t/ha). These two varieties showed excellent harvest indexes (> 0.70) and good dry matter content in the roots (> 35%), although they were not the best clones for this last trait.

Germplasm adapted to the acid-soil savannas or mid-altitude valleys (MPER 183, CM 523-7, HMC-1 and SM 1219-9) showed a mediocre performance in the region (Table 2.55), although CM 7951-5 performed relatively well.

Table 2.53. Results of the *Regional Trial* conducted in Morales (Cauca Departament) with materials adapted to the highland environment 1400 to1800 meters above sea level).

Clone or parameter	Fresh Roots (t/ha)	Dry matter (%)	Harvest Index (0 a 1)	Selection Index
SM 1058-13	43.73	39.03	0.61	35.02
SM 998-3	50.33	37.43	0.52	34.27
CM 8106-4	38.80	40.23	0.61	32.99
SM 1937-1	42.40	37.00	0.54	22.79
SM 2229-36	39.20	36.67	0.54	17.31
SM 1053-23	40.33	34.33	0.61	13.82
SM 1061-5	27.53	39.97	0.48	10.86
CG 402-11	35.80	33.97	0.61	6.33
CM 7438-14	34.87	35.00	0.56	6.32
SM 1703-17	31.27	38.30	0.40	5.99
SM 1495-22	30.27	35.97	0.58	4.45
CM 7138-12	29.87	38.43	0.39	4.32
SM 1946-2	32.27	35.90	0.50	3.64
SM 1835-28	29.07	37.43	0.47	3.06
SM 2227-21	23.83	37.13	0.63	2.50
SM 1944-10	27.93	37.30	0.50	2.47
CM 7190-2	29.23	37.20	0.43	0.81
SM 524-1	32.60	33.90	0.58	0.31
MCOL 2061	28.83	35.10	0.59	0.08
SM 1938-12	29.17	36.60	0.45	-0.45
SM 2226-48	27.37	34.67	0.61	-2.42
SM 1833-21	28.57	35.30	0.49	-4.09
SM 2233-11	33.30	32.87	0.50	-5.70
MCOL 1522	31.80	34.23	0.44	-5.81
SM 850-1	27.60	36.23	0.40	-6.09
SM 1933-5	27.47	35.30	0.38	-10.40
SM 1940-3	21.93	36.00	0.39	-15.16
SM 2311-3	24.53	32.53	0.45	-21.35
SM 2232-15	19.13	35.23	0.39	-21.66
MCOL 2740	28.93	31.03	0.36	-24.88
SM 2237-26	19.47	34.60	0.36	-25.01
MCOL 2261	19.30	32.70	0.45	-27.56
SM 2228-28	18.60	31.53	0.36	-36.76
Maximum	50.33	40.23	0.63	-.-
Minimum	18.60	31.03	0.36	-.-
Mean	30.46	35.73	0.49	-.-
St. Dev.	7.39	2.24	0.09	-.-

Table 2.54. Results of the *Regional Trial* conducted in La Cumbre (Valle del Cauca Departament) with materials adapted to the highland environment 1400 to1800 meters above sea level).

Clone or parameter	Mother	Father	Harvest Index (0 a 1)	Fresh roots (t/ha)	Dry matter (%)	Cooking quality (1-5)	Selection Index
SM 856-11	CG 402-11		0.55	20.22	34.7	3.0	41.41
SM 1269-6	CG 402-11		0.55	22.50	31.05	1.6	33.18
SM 1058-13	CG 402-11		0.47	15.79	33.9	1.0	25.52
CM 7438-6	CG 481-3	MCOL 2261	0.41	15.19	34.8	2.3	25.03
SM 1933-5	CG 402-11		0.33	16.06	33.95	2.3	20.61
SM 1716-1	SG 638-6		0.48	15.69	32.35	3.0	20.09
SM 1835-28	CG 481-3		0.32	15.24	32.85	3.0	14.41
SM 1707-2	CM 523-7		0.45	9.86	33.85	3.0	11.49
SM 524-1	MCOL 1522		0.41	9.26	34.15	1.6	9.64
MCOL 2261	Unknown		0.38	13.56	31.25	2.3	7.36
MCOL 2740	Unknown		0.36	14.31	30.65	2.3	6.02
CM 7595-1	MCOL 2261	CG 406-6	0.44	15.70	28.8	nd	5.61
SM 1710-3	MCOL 1522		0.43	13.40	29.7	1.0	3.42
SM 707-17	CG 402-11		0.35	10.33	31.4	1.6	-0.42
SM 709-1	CG 481-3		0.25	8.29	32.7	3.0	-4.25
SM 1002-1	MCOL 1413		0.32	15.09	28.4	3.0	-2.05
SM 850-1	MCOL 2257		0.31	10.00	30.4	3.0	-6.39
SM 1846-12	MCOL 647		0.30	10.09	29.65	3.0	-9.32
SM 1938-12	CM 723-3		0.38	6.11	30.55	3.0	-11.55
SM 1946-4	MCOL 2060		0.28	7.73	30.7	2.3	-11.51
SM 1495-22	CG 402-11		0.36	5.28	31.1	2.3	-12.20
SM 1061-5	CG 481-3		0.23	5.19	32.3	3.0	-13.33
SM 929-3	CG 358-3		0.24	11.50	28.6	3.0	-12.47
SM 1944-17	MCOL 717		0.24	6.57	30.55	3.0	-16.23
SM 1833-21	CG 401-6		0.24	7.36	29.85	2.3	-17.03
SM 1940-3	CM 4488-4		0.20	6.02	30.95	3.0	-17.62
SM 853-21	CG 358-3		0.28	9.65	27.95	5.0	-17.26
SM 1703-17	CG 501-2		0.24	10.65	27.15	1.0	-19.59
SM 1937-1	CG 1231-3		0.23	5.32	28.6	2.3	-26.45
SM 1946-2	MCOL 2060		0.24	7.69	27.15	1.0	-26.10
CG 402-11			0.47	22.10	na	na	28.87
SM 1053-23			0.52	19.30	na	na	24.75
SM 1944-10			0.45	19.03	na	na	21.31
Maximum			0.55	22.50	34.80	5.00	41.41
Minimum			0.20	5.19	27.15	1.00	-26.45
Mean			0.34	11.32	31.00	2.46	0.00
St. Dev.			0.10	4.55	2.21	0.87	17.92

na = not available

The first eight clones showed a more or less uniform performance with relatively consistent rankings at each of the four locations. The first clone showing important signs of genotype by environment interaction was SM 1557-17, which was 17th at San Vicente, but it was 3rd in Sabana de Torres. SM 1433-4 also showed notable changes in its ranking at different locations: it was the best clone at Sabana de Torres, but had mediocre performances in the other three locations. In spite of these two cases, results from the four locations were generally consistent suggesting that they offered relatively uniform environmental conditions for cassava growth.

Table 2.55. Results from four *Regional Trials* conducted in the Middle Magdalena River region.

Clone	Fresh root yield (t/ha)	Fresh foliage yield (t/ha)	Harvest index (0 - 1)	Dry matter (%)	Ranking at each location			
					San Vicente	B/meja	Sabana Torres	Regidor
SM 653-14	31.66	14.32	0.71	35.96	1	2	10	3
MTAI 8	31.25	12.58	0.72	35.40	7	6	2	2
CM 3306-4	26.42	14.33	0.66	36.69	2	7	16	1
CM 4843-1	32.08	17.30	0.64	34.30	5	4	7	11
CM 7514-8	22.98	17.73	0.56	38.61	13	5	6	5
CM 7951-5	23.58	10.65	0.69	36.41	3	9	5	9
CM 6119-5	25.67	14.28	0.64	36.12	6	3	11	6
SM 1741-1	26.22	14.94	0.64	35.17	8	11	8	4
SM 1557-17	29.03	14.07	0.67	33.08	17	10	3	10
CG 1141-1	23.40	13.66	0.62	35.70	18	1	12	8
MVEN 25	26.52	19.25	0.58	34.64	9	8	15	7
MBRA 383	25.78	18.02	0.59	34.57	10	12	9	12
SM 1219-9	24.91	12.41	0.66	33.31	11	13	4	16
SM 1433-4	17.88	16.28	0.52	38.06	14	15	1	14
HMC1	19.16	10.93	0.64	35.12	12	14	13	15
CM 849-1	21.52	19.13	0.53	34.11	16	17	14	17
CM 523-7	14.84	12.97	0.55	35.77	4	16	17	13
MPER 183	14.81	11.90	0.58	31.07	15	18	18	18
Maximum	32.08	19.25	0.72	38.61				
Minimum	14.81	10.65	0.52	31.07				
Mean	24.32	14.71	0.62	35.23				

During this year, we have introduced yet another modification in the region. As was indicated in Table 2.3 botanical seed for producing stakes for a *Clonal Evaluation Trial* targeting specifically this region had been planted. In Table 2.56 a general description of the materials included in this trial is provided.

Table 2.56. Description of the families and clones included in the first *Clonal Evaluation Trial* planted in the Middle Magdalena River eco-region.

	Family	Mother	Father	Seed planted	Clones in trial
1	CM 8335	MCOL 1505	CM 2177-2	50	28
2	CM 9614	CM 6740-7	MNGA 19	50	32
3	CM 9748	CG 1141-1	SM 653-16	37	26
4	CM 9772	CM 7073-7	MNGA 19	45	32
5	CM 9903	CM 6740-7	SM 1741-1	50	35
6	CM 9916	CM 7951-5	CM 6740-7	38	27
7	CM 9928	CM 8593-13	SM 206929	35	22
8	CM 9934	CM 8602-27	SM 2069-26	51	25
9	CM 9935	SM 2075-1	CM 8602-27	42	31
10	CM 9940	SM 1219-9	SM 653-14	50	33
11	CM 9953	SM 1219-9	SM 1741-1	50	37
12	CM 9965	SM 1741-1	SM 1565-17	47	20
13	CM 9985	SM 2075-1	SM 2102-36	33	27
14	GM 70	SM 494-2	MTAI 8	55	22
15	GM 212	CM 523-7	SM 805-15	50	26
16	GM 215	SM 1565-17	CM 523-7	50	21
17	GM 235	MTAI 8	CM 6740-7	37	23
18	GM 266	SM 1219-9	MTAI 8	43	18
19	SM 1521	CM 3299-4	Unknown	50	8
20	SM 2733	SM 1210-10	Unknown	50	22
21	SM 2826	CM 4365-3	Unknown	50	23
22	SM 2830	CM 7514-8	Unknown	50	20
23	SM 2832	SM 805-15	Unknown	50	14
24	SM 2859	CM 6740-7	Unknown	45	15
25	SM 2861	SM 643-17	Unknown	50	18
26	SM 2864	SM 1210-4	Unknown	50	14
27	SM 2882	CM 3372-4	Unknown	50	16
28	SM 2963	MTAI 8	Unknown	50	18
29	SM 2965	CM 4574-7	Unknown	50	26
30	SM 2967	CM 7033-3	Unknown	50	21
	TOTAL			**1408**	**700**

2.4.5. Selections for the Tolima-Huila Departments.

This region shares characteristics of the acid-soil savannas and the mid-altitude valleys. Two trials specifically targeting the region have been planted. A *Clonal Evaluation Trial* (see Table 2.3) and a *Preliminary Yield Trial* are described in Tables 2.57 and 2.58, respectively.

At the Tolima and Huila Departments two different Advanced Yield Trials were also planted during the present period, and will be harvested in 2003. Table 2.59 lists the clones that have been included in these two trials.

Table 2.57. Description of the families and clones included in the first *Clonal Evaluation Trial* planted in the Tolima-Huila Departments eco-region.

	Family	Mother	Father	Seed planted	Clones in trial
1	CM 8035	MTAI 8	HMC 1	50	21
2	CM 9642	CM 6740-7	MPER 183	50	17
3	CM 9733	HMC 1	MPER 183	31	4
4	CM 9765	CM 6754-8	SM 653-16	41	17
5	CM 9791	SM 1433-4	MNGA 19	25	28
6	CM 9912	SM 1433-4	CM 7514-8	50	32
7	CM 9914	CM 7514-8	SM 1565-17	50	22
8	CM 9926	SM 1565-17	CM 8027-3	50	9
9	CM 9961	SM 1433-4	MTAI 8	50	12
10	CM 9962	SM 1438-2	SM 1565-17	44	22
11	CM 9966	SM 1565-17	MTAI 8	32	15
12	GM 234	CM 6740-7	HMC 1	50	21
13	GM 265	SM 1219-9	MPER 183	50	38
14	SM 1521	CM 3299-4	Unknown	50	7
15	SM 2802	SM 1219-9	Unknown	50	23
16	SM 2804	SM 1565-17	Unknown	50	16
17	SM 2805	SM 1741-1	Unknown	50	29
18	SM 2826	CM 4365-3	Unknown	50	24
19	SM 2829	CM 7395-5	Unknown	50	13
20	SM 2834	SM 1411-5	Unknown	50	32
21	SM 2836	SM 1433-4	Unknown	50	34
22	SM 2839	SM 1565-17	Unknown	50	29
23	SM 2865	SM 1219-9	Unknown	50	40
24	SM 2866	SM 1460-1	Unknown	50	25
25	SM 2870	SM 1741-1	Unknown	50	18
26	SM 2871	MBRA 12	Unknown	50	20
27	SM 2873	CM 523-7	Unknown	50	12
28	SM 2882	CM 3372-4	Unknown	50	28
	Total			**1323**	**608**

Table 2.58. List of the clones included in the first *Preliminary Yield Trial* planted in the Tolima-Huila Departments eco-region.

SM 1778-50	SM 2097-4	SM 1646-5	SM 1955-7	CM 8803-7
SM 2095-12	SM 1785-10	SM 1789-52	SB 237-8	SM 2277-2
SM 1969-18	SM 1789-20	SM 1784-15	SM 1519-2	SM 1521-10
SM 643-17	SM 1910-5	SM 1652-21	SM 2090-7	CM 8288-46
SM 1627-16	SM 1431-2	SM 1657-14	SM 2085-7	CM 8472-5
MEX 95	SM 1948-29	SM 2090-1	SM 1791-40	MTAI 8
SM 2277-4	CM 6758-3	SM 1539-2	SM 2099-4	CM 3306-4
SM 1511-14	SM 2091-6	CM 3555-6	SM 2269-8	CG 1141-1
CM 4365-3	IND 39	CT 20-2	SM 1652-16	CM 4919-1
SM 1516-7	SM 1657-12	CM 8288-43	CM 8472-4	

Table 2.59. List of the clones included in the first *Advanced Yield Trial* planted in two locations in the Tolima-Huila Departments eco-region.

Natagaima (Tolima) VEREDA: Rincon de Anchique	Palermo (Huila) VEREDA: El Juncal
CG 1141-1	CM 660-21
CM 523-7	CM 7453-2
CM 849-1	CM 8370-10
CM 3306-4	CM 8370-11
CM 4843-1	SM 1520-16
CM 6119-5	SM 1520-18
CM 7514-7	SM 1642-22
CM 7951-5	SM 1660-4
SM 653-14	SM 1779-7
SM 1219-9	SM 1855-15
SM 1433-4	SM 2085-7
SM 1557-17	SM 2141-1
SM 1741-1	SM 2160-2
SM 1871-33	SM 2198-4
SM 1959-1	SM 2111-3
SM 1965-1	MTAI 8
SM 2052-4	MPER 183
SM 2058-2	CM 523-7
SM 2073-1	HMC 1
MTAI 8	MBRA 383
MVEN 25	CM 7951-5
MPER 183	SM 1219-9
HMC-1	
MBRA 383	

Activity 2.5 Research on post-harvest physiological deterioration

Rationale
One of the major constraints upon cassava production as a commercial crop is the perishability of its roots. Two basic types of deterioration have been identified: a physiological deterioration (PPD) which may begin within 24 hours of harvest in susceptible varieties, and a microbial deterioration which appears later and is affected by root damage, storage environment and genotype (Jennings and Hershey, 1985). PPD consists of discoloration of the parenchyma as blue-black vascular streaks. Because of PPD, cassava roots need to be consumed shortly after harvesting (van Oirschot, et al., 2000). The short post-harvest storage life of cassava is a characteristic that limits the marketability of the roots, implies high marketing margins and risks, and restricted management flexibility for farmers and processors. At least two factors have already been identified as affecting PPD: yellow roots with higher carotene contents tend to have delayed PPD onset, and high dry matter content accelerates PPD (CIAT, 2001). Any alternative aiming at reducing the PPD problem is strategic for CIAT's research on cassava, as well as for those industries processing cassava roots.

Specific Objectives
a) To develop facilities for a more precise evaluation of PPD.
b) To identify post-harvest treatments that would allow reducing or delaying the onset of PPD.
c) To determine cooking quality of ten clones with fresh roots and after different post-harvest treatments.
d) To evaluate root quality for the production of croquettes from elite germplasm

Materials and methods
Ten clones (CM 523-7, SM1520-16, MPER183, CM7463-2, CM8370-11, SM2073-1, SM1779-7, SM1871-33, SM1855-15, and SM1959-1) from the regional trials conducted through Valle del Cauca Department (Colombia) were identified for this evaluation. The clones were chosen based on the cooking quality of their roots. Roots from these ten clones were used for this experiment and after different alternative storage conditions were evaluated for PPD and cooking quality. The treatments considered in this trial were:

- Controlled temperature at 6°C, roots within plastic bags
- Controlled temperature at 6°C, roots in shelves
- Without control of temperature, roots within plastic bags
- Without control of temperature, roots in shelves

Each experimental unit was made up of ten commercial size roots for each of the treatments. PPD evaluations were made on each individual root, but statistical analysis was done on the

average PPD of the ten roots of each experimental unit. Data on dry matter content (%) and cooking quality were taken on the day roots were harvested and were used as check for further analysis. Results of the treatments were evaluated at 7, 14, 21 and 28 days after harvest and measured as PPD and cooking quality.

The regional trials were conducted at different locations and harvested at different time, one after the other. Differences among the different regional trials, therefore, reflect geographical differences and environmental conditions at the time of harvest. Regional Trial 1 was harvested in the middle of a lengthy dry spell. There were some roots affected by frog skin disease, which may have influenced on the evaluation of cooking quality. Trials 2 and 3 were harvested under normal rain conditions (no droughty weather) with temperatures ranging from 25 to 35 °C. Varieties, as expected, showed preferences from one location to the other, resulting in significant genotype by environment interactions.

Results
A chamber that allows evaluation under controlled temperature and moisture conditions was built by CONGELAGRO within the CIAT campus. It is large enough to allow for relevant field studies. Temperature ranges from 5°C to 35°C. Air relative moisture can be maintained at 60 to 70%. This chamber was utilized to carry out this research on genetic effect on PPD and alternative methods for post-harvest handling to manage the PPD problem.

CIAT continued the initial work by Zapata (2001). The main objectives were to identify different post-harvest treatments (controlled temperature and confinement within plastic bags) that would allow to reduce or delay the onset of PPD in cassava roots. For this work the recently built chamber was used with excellent results, which are under analysis and will be published during 2003.

The cooking quality of the roots from these trials was not always satisfactory. For this trait we employed a 1 to 5 scale (1=excellent and 5= very poor). The environmental conditions in the locations were the trials were conducted were not conducive to the production of roots with excellent cooking quality. The conditions at harvest time were not favorable either. Trial 1 was harvested just before the beginning of the rainy season. Dry matter content was higher than in the other trials and the cooking quality was good. For most varieties, the good cooking quality was maintained for several days after harvest and for a longer period than for Trials 2 and 3. The regional trials 2 and 3 were harvested when the rains had begun, and cassava plants had re-initiated their growth. When this occurs there is a marked reduction in dry matter content, because of the energy used by the plant to produce new foliage. It is clear that because of the conditions these trials were harvested, that dry matter content was much lower than in the first and so was the cooking quality of their roots (which had an average score = 3). The cooking quality decreased quickly (score = 5) for those roots maintained in conditions conducive to PPD.

There are several cassava-growing regions in Colombia. It is strategic for CONGELAGRO to identify the best varieties from each of the regions that may eventually provide with row material for its operations. It is also important for CIAT to identify those clones that produce

good quality roots for the processing industry. Within this project, activities oriented to answering these questions had been included.

From the different regions, Villavicencio in the eastern savannas was selected as the first option. Therefore root samples from 13 new clones were delivered to CONGELAGRO for their processing into croquettes. The clones evaluated were:, CM5306-8, CM7052-3, SM1810-6, CM6055-3, SM1405-5, SM1864-10, CM6438-14, SM1565-15, CM6740-7, SM1859-26, CM6975-14, BRASILERA, and NN (WIGASA).

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OUTPUT 3
Collaboration with other institutions.

Activity 3.1 Support national programs that have traditionally collaborated with CIAT in the development and improvement of cassava.

Rationale:
CIAT has the responsibility to contribute with cassava research worldwide. In the past, this was achieved through the collaboration of National Agriculture Research Programs (NARs), and in the case of Africa, with the valuable collaboration with IITA. This scenario has changed drastically through the last decade, when the NARs in most of the tropical countries weakened consistently. However, new institutions and partners are assuming a leading role and CIAT is actively searching for these new partners. In this activity, at least for Latin America, we are closely collaborating with CLAYUCA. In the implementation of industrial uses of cassava, because of the convenience of our location, most of the validation and adaptive research is carried out in Colombia. Once the technology (for instance, for the artificial drying of cassava roots) is evaluated and offers acceptable results, it can be moved out to other countries. This strategy implies that a considerable portion of our research is carried out in Colombia. However, this does not imply that cassava projects at CIAT are restricting their activities only to Colombia.

Specific Objectives:
a) *To promote the use of cassava and the adoption of new technologies and germplasm by cassava growing countries of the world.*
b) *To contribute to the training of personnel involved with cassava research.*
c) *To identify new partners in each country.*

Results
A major thrust in CIAT's strategy to achieve the stated objectives has been through training and visits to NARs, in addition to the provision of germplasm described in Output 2. In Table 3.1 a list of the most important events in which personnel from the project participated is provided. In these list different type of events were considered: technical visits to different NARs, scientific symposiums or congresses, training courses, planning workshops, etc.

Because of the vigorous support from the Colombian Government a large effort has been invested in training and promotion of cassava research and development in the country. In Table 3.2 a description of the different courses and workshops in which the cassava improvement project personnel participated is provided. In Table 3.3 a list of conferences and field trips in which this personnel participated is also provided. There are many more specific activities and contributions that cannot be mentioned because of their informal nature. An important activity in this regard is the continuous consulting from producers, students, researchers and processors from Colombia and other countries. An important amount of energy is dedicated to satisfy the demand for information and products through these requests.

Table 3.1 International events related to cassava research and development in which personnel from the project participated.

	Month	Event	Contact Institution(s)	Location
1	End of year 2001	II Latin American Roots and Tubers Symposium (three days)	INIA-CIP- Univ. La Molina	Lima - PERU
2	February	Planning workshop for research and development activities in Haiti (one week)	Hillside Agriculture Project	Port-au-Prince, HAITI
3	February	Visit to Cornell Univ.: collaboration in the area of cassava biotechnology (two days)	Cornell University	Ithaca - USA
4	April	Planning workshop for the Biofortification proosal (three days)	IFPRI	Houston - USA
5	May	Technical visit to interact with cassava researchers from Brazil (one week).	EMBRAPA – IAC – Universidad Sao Paulo	Bahia, Brasilia, Campinas. BRAZIL
6	June	Course on modern systems for cassava production and processing (three days)	Agropecuaria Mandioca INIA-IDEA-CLAYUCA	Anozoátegui - VENEZUELA
7	June	Course on modern systems for cassava production and processing (three days)	INIA – IDEA - CLAYUCA	Cojedes - VENEZUELA
8	June	Course on modern systems for cassava production and processing (three days)	INIA – IDEA - CLAYUCA	Zulia - VENEZUELA
9	August	Technical visits to Northern and Southern Haiti (two week)	Hillside Agriculture Project	Several locations in HAITI
10	August	Technical visit to Dominican Republic (three days)	IDIAF	Several locations in DOMINICAN REP.
11	September	Intl Workshop on Conservation, Evaluation an Use of Cassava Genetic Resources (three days)	INIA	Huaral - PERU
12	September	Visit to ETH: collaboration in the area of cassava biotechnology (two days)	ETH	Zurich - SWITZERLAND
13	October	The use of biotechnology for cassava improvement (three days)	Rockefeller Foundation	Bellagio - ITALY
14	October	Visit to cassava growing regions in Southern China (one week)	CATAS-GSCRI	Hainan–Nanning CHINA
15	October	7th Regional Workshop on Cassava Research in Asia (one week).	The Nippon Foundation	Bangkok THAILAND
16	November	Intl course for modern technologies for cassava production and processing (two weeks)	CIAT-CLAYUCA	Cali-COLOMBIA
17	November	Technical visits to Northern and Southern Haiti (one week)	Hillside Agriculture Project	Several locations in HAITI
18	November	Study visit to cassava production and processing facilities in Thailand (one week)	CIAT's Regional Office-CLAYUCA	Several locations in THAILAND
19	November	Planning workshop for bio-availability studies for carotenes from cassava roots (two days).	IFPRI and several NGOs operating in Haiti.	Port-au-Prince HAITI

Table 3.2. Training courses and workshops to promote cassava and the use of modern technologies conducted during the year 2002 in Colombia.

	Month	Topic	Participants (No.)	Location
1	February	Integrated pest management practices for white flies in cassava	20	S.Quilichao, Cauca
2	February	Rapid multiplication systems for the production of vegetative seed.	25	CIAT-Palmira
3	February	Integrated pest management practices for white flies and frog skin disease.	8	Mondomo, Cauca
4	February	Traditional and modern systems for cassava production.	25	Municipio Tibú, Cauca
5	March	Mechanization of cultural practices for cassava production	30	CIAT-Palmira
6	March	Integrated pest management practices for white flies in cassava	20	San Luis, Tolima
7	March	Production and handling of stakes. Processing of fresh cassava roots.	10	Montería, Córdoba
8	April	Potential of cassava for the Middle Magdalena River Region.	15	Puerto Boyacá, Magdalena.
9	May	Production and handling of stakes. Rapid multiplication schemes.	40	Villavicencio, Meta.
10	May	Analysis of the problems related to the industry of fermented starches.	25	CIAT-Palmira
11	June	Training on techniques for chipping and natural drying of cassava roots.	10	Sabana Torres, Magdalena
12	June	Cassava production with special emphasis for industrial uses.	27	CIAT-Palmira
13	June	Cassava potential as an industrial crop. Relevance for the Santander region	45	El Zulia, Norte de Santander.
14	July	New varieties of cassava for the industry in the sub-humid environment.	15	Polonuevo, Atlántico
15	July	Production and handling of stakes. Rapid multiplication schemes.	na	S.Quilichao, Cauca
16	July	Follow up on the analysis of the problems for the industry of fermented starches.	20	CIAT-Palmira
17	August	Integrated pest management practices for white flies and frog skin disease.	9	S. Quilichao, Cauca
18	August	Identification and management of super elongation disease and bacterial blight.	12	Montería, Córdoba.
19	September	Training course: modern technologies for cassava production and processing.	40	Bucaramanga, Santander.
20	September	Cassava potential as an industrial crop. Relevance for the coffee growing region	35	Manizales, Caldas
21	September	Planning meeting for the creation of "trapiches yuqueros" in the Cesar.	12	Valledupar, Cesar.
22	October	Training course: modern technologies for cassava production and processing.	30	Yopal, Arauca

Table 3.3. List of conferences and field trips in which personnel working in the cassava improvement project participated.

	Month	Topic	Participants (No.)	Location
1	March	Cassava research for the sub-humid environment.	10	Barranquilla, Atlántico
2	April	Production and handling of stakes. Rapid multiplication schemes.	20	Cereté, Córdoba
3	April	Research for the genetic improvement of cassava.	15	CIAT-Palmira
4	May	Traditional and modern systems for cassava production.	35	Villavicencio, Meta.
5	May	Modern techniques for cassava production.	30	Villavicencio, Meta.
6	May	Production and handling of stakes. Rapid multiplication schemes.	40	Villavicencio, Meta.
7	May	Production and handling of stakes. Rapid multiplication schemes.	50	S. Quilichao, Cauca
8	June	Production and handling of stakes. Rapid multiplication schemes.	12	Villavicencio, Meta.
9	June	Description of new cassava varieties for the processing industry.	10	Villavicencio, Meta.
10	June	Modern techniques for cassava production.	11	Villavicencio, Meta.
11	June	Evaluation of production costs for cassava in different areas of the country.	27	CIAT-Palmira
12	July	Production and handling of stakes. Rapid multiplication schemes.	45	Montería, Córdoba.
Field visits with farmers				
1	March	Field visit to the nurseries in the sub-humid environment	12	Barranquilla, Atlántico
2	March	Field visit to the nurseries in the humid environment	12	Montería, Córdoba
3	June	Field visits to well managed production plots in the acid-soil savannas.	10	Yopal and Aguazul, Arauca
4	October	Field visits to production plots and processors in the acid-soil savannas	na	Arauca, Meta and Casanare

Figure 3.1 illustrates some of the events that have taken place during the period covered in this report. Whenever possible training courses will include field visits taking advantage of the nurseries scattered across Colombia.
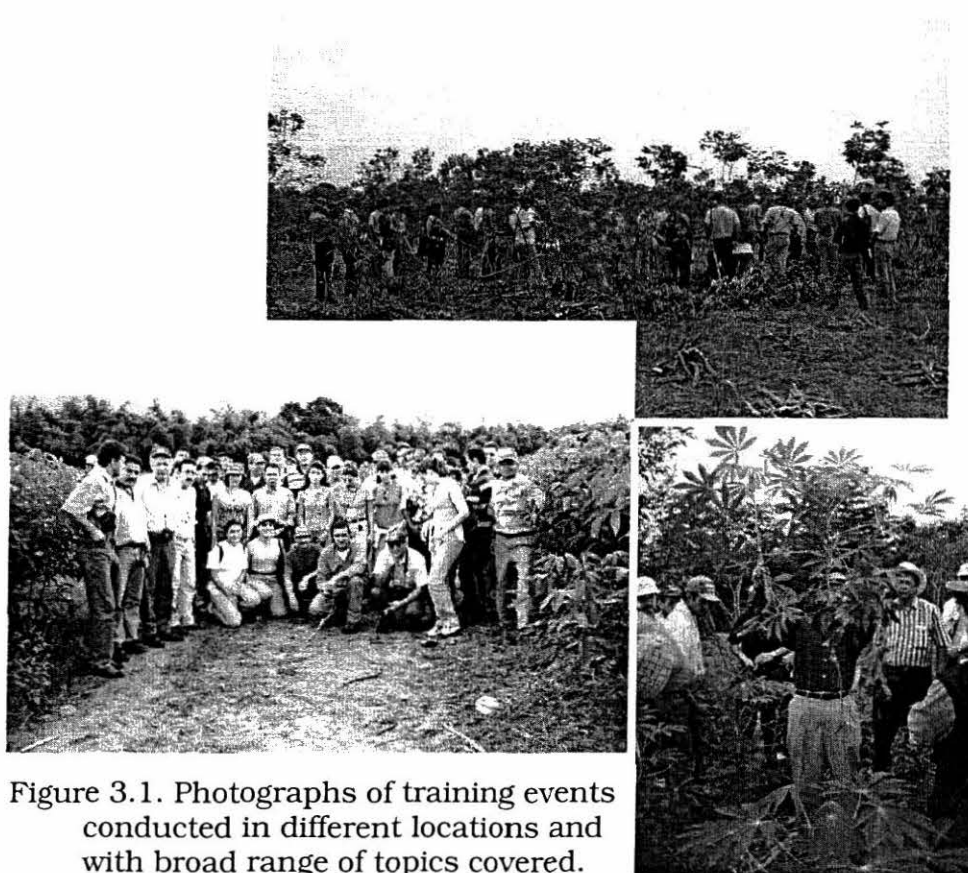
Figure 3.1. Photographs of training events conducted in different locations and with broad range of topics covered.

Activity 3.2 Publication of a book with updated information on modern cassava production and processing techniques.

Rationale:
Considerable progress has been achieved in the last 20 years in cassava technologies. The economic conditions have changed dramatically in many tropical countries resulting in a renewed interest in the industrial uses of cassava roots and foliage. The last publication covering a broad range of topics from cassava breeding to utilization was already outdated.

Specific Objectives:
a) To publish a book with updated information for cassava production and processing.

Results
A huge effort has been made jointly between CIAT and CLAYUCA for the publication of this text, which demanded an active participation of many scientists for almost two years. The final product is highly satisfactory (Figure 3.1) and has begun to be distributed in all Spanish-speaking countries.
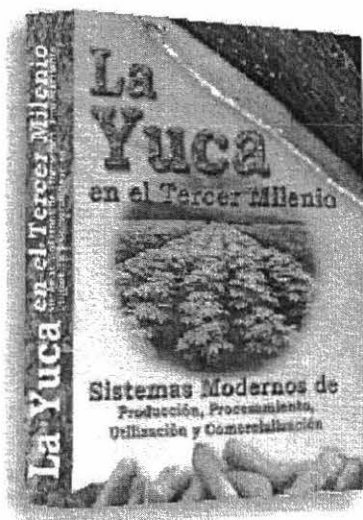
Figure 3.2. The most recent publication on cassava by CIAT and CLAYUCA. The text goes with a booklet with the illustration of the most important problems by diseases insects and nutritional disorders.

Activity 3.3 Promotion of cassava for new uses in the International Agriculture Exposition at Bogota.

Rationale:

Every other year an important agriculture exposition takes place for a week in Bogotá. People related to agriculture production and processing agriculture products from all Latin American countries as well as North America and Europe attend to this meeting. It is considered strategic to have a presence in the exposition to promote the new potential of cassava products.

Specific Objectives:

a) To participate with a stand in the International Agriculture Exposition.

Results

In Figure 3.3 several photographs of the stand that CIAT and CLAYUCA jointly prepared for the Exposition. It is estimated that more than 100,000 people attend this Exposition. Several industries are present as well.

For the stand there was a need for the presence of 3 or 4 people continuously for the week the exposition lasts. A major coordination effort was made by CLAYUCA to have this stand set up. During the event large number of publications and hand outs were sold and given, respectively.
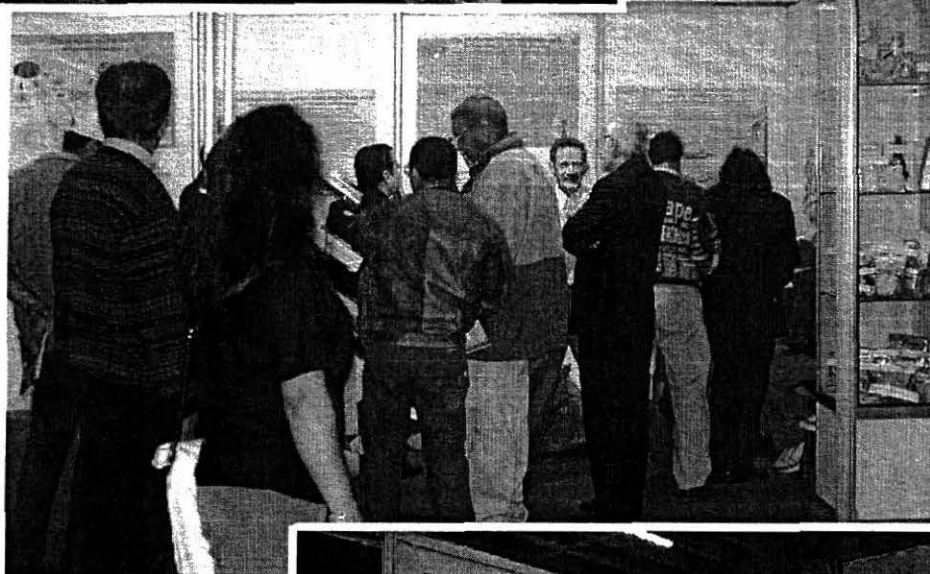
Figure 3.3.Cassava
stand in **AGROEXPO**,
Bogotá. August, 2001

Activity 3.4 Participation in the training of young scientists from different regions of the world.

Rationale:
There is always a need to train young scientists in cassava research. Training is envisioned as a fundamental activity to guarantee adequate human resources for the future of cassava and an ideal way for establishing close links with NARs.

Specific Objectives:
a) To facilitate and finance training of young scientists interested in cassava research..

Results
Whenever there was a possibility and resources to train young scientists the project responded positively. Table 3.4 provides a summary of the young scientists that have visited our project during 2002.

Table 3.4. Young scientists visitors that have spent from a few weeks to several months of training and research at CIAT – Palmira, during period covered by this report.

	Name	Country	Activity
1	Thi Cach Nguyen	Vietnam	Ph.D. Thesis of cassava breeding.
2	Henry Ojulong	Uganda	Ph.D. Thesis on cassava genetics
3	Jorge E. Gonzalez	Colombia	Young scientist program from COLCIENCIAS
4	Prapit Wongtiem	Thailand	Cassava biotechnology
5	Chommanat Kerdkhong	Thailand	Cassava biotechnology
6	María Cuevas	Dominican Rep.	Cassava production and processing
7	Armando Gerst	Venezuela	Cassava production and seed multiplication
8.	Emmanuel Okogbenin	Nigeria	Cassava biotechonology
9.	Luis Montes	Guatemala	Cassava biotechnology
10.	Elizabeth Okai	Ghana	Cassava biotechnology
11.	Charles Buitrago	Colombia	Cassava biotechnology

A major factor contributing to the success in the area of collaboration with NARs from Latin American Countries has been the complementation and collaboration between CIAT and CLAYUCA, which is dully acknowledged here.

OUTPUT 4

Developing a new approach for cassava breeding integrating biotechnology tools.

Rationale

Cassava improvement has not been as consistent and efficient as in other crops due to many constraints. A typical scheme implies crossing elite clones to produce segregating families (Figure 4.1). Each individual produced is highly heterozygous. Once a superior genotype is identified (a process that requires about six years), it is vegetatively multiplied to take advantage of the reproductive habits of this crop. This system (except for the vegetative multiplication) has similarities with the ones used for autogamous crops (beans, wheat, rice, etc.) as well as for the hybrid maize industry. However, there is a major difference because cassava is never pushed to produce inbred (homozygous) lines from the segregating progenies of a given cross. The system also bears some similarities with recurrent selection used in allogamous crops (maize), but there is a significant difference because in cassava there is not a clearly defined population whose allelic frequencies are modified through evaluation and selection, as in true recurrent selection schemes.

Pedigree selection [¶]	Current system in cassava	Recurrent selection in alogamous crops	Semester
A x B	A x B	Original cycle (C0) of the population	1
F1		200 families evaluated	2
F2		Best 20 families selected and recombined (C1)	3
F3	A progeny of size "n" is evaluated in successive stages. Simultaneously, the number of plants representing each clone is increased.	200 new families (C1) evaluated	4
F4		Best 20 families selected and recombined (C2)	5
F5		200 new families (C2) evaluated	6
F6 ⇒ C		Best 20 families selected and recombined (C3)	7
		200 new families (C3) evaluated	8
C x Y		Best 20 families selected and recombined (C4)	9
F1		200 new families (C4) evaluated	10
F2		Best 20 families selected and recombined (C5)	11
F3		200 new families (C5) evaluated	12
F4	Best clone selected ⇒ C	Best 20 families selected and recombined (C6)	13
F5	C x Y	200 new families (C6) evaluated	14

[¶] Used in autogamous crops and for the production of inbred lines in hybrid crops such as maize.

Figure 4.1. Illustration (highly simplified) of the differences in breeding systems employed for the genetic improvement of different type of crops.

From the simplified information provided in Figure 4.1, it is apparent that a major drawback of cassava breeding is also the length of each breeding cycle. For the reasons below, cassava breeding is slow and inefficient.

a. Because no inbreeding is carried out at any stage of cassava breeding, a sizable genetic load (undesirable or deleterious genes) is expected to prevent the crop fully achieving its actual yield potential (Hedrick, 1983).

b. There are no clearly defined populations (as defined by quantitative genetics), allelic frequencies cannot be efficiently modified. Cassava breeding in this regard resembles more the selection of segregating progenies from two parental lines in autogamous crops.

c. Because the highly heterozygous nature of the crop, dominance effects are likely to play a very important role in the performance of materials being selected. The current scheme can exploit dominance effects because, once an elite clone is identified, it can be propagated vegetatively, therefore carrying along the combination of genes responsible for dominance effects (Lamkey and Staub, 1998). However, selection of progenitors for the production of new segregating material is based on their *per se* performance. In that case, the current procedure has a bias because the breeding values of these clones are unlikely to be well correlated with their performance, precisely because of the distorting effects of dominance.

d. Production of recombinant seed is cumbersome in cassava. On average, only 0.6 viable seeds per pollination are produced. It takes about 16-18 months since a given cross is planned until an adequate amount of seed is produced.

e. When a desirable trait is identified, it is very difficult to transfer it from one genotype to another (even if a single gene controlled the trait). The back-cross scheme, one of the most common, successful and powerful breeding schemes for cultivated crops (Allard, 1960) is not feasible in cassava, because of the constant heterozygous state used throughout the breeding process.

f. Maintenance of genetic stocks is expensive and cumbersome. The only proven methods for long term storage of germplasm is through tissue culture procedures, which is expensive and requires several months to recover plants for planting in the field. The other alternative is maintaining representative plants in the field, which is also expensive when a large number of genotypes need to be maintained year after year, and also the stocks are vulnerable to gradual contamination by pathogens.

g. The occasional exchange of germplasm among cassava breeding programs in different countries is restricted to a few plants from few genotypes. Cassava breeding projects, effectively work in isolated conditions.

h. Lack of inbreeding in cassava implies that there are few opportunities for identifying useful recessive traits, which could have huge beneficial effects on the crop. For instance, acyanogenesis in the roots has been identified as a very desirable trait. It has been postulated that this trait may be recessive. Also worth mentioning are the several starch mutations that are generally recessive in most crops (Neuffer et al., 1997).

These may all be a valid explanation for the limited genetic gains for higher productivity observed in the crop, compared with that of other crops such as maize or rice. It should be emphasized that because the highly heterozygous condition of cassava in every stage of the breeding process, consolidation of genetic gains is difficult, due to the inherent genetic instability of heterozygosity.

From the practical point of view, implementing a traditional recurrent selection method in cassava offers some problems. Pollinations are slow and inefficient. It takes about 16-18 months since a given cross is planned until the recombinant seed is finally obtained (usually field operations have to adjust to the occurrence of the rainy season, and if that is the case, then planting can only be done 24 months after planning the cross). The third year would be used to grow the plant from the botanical seed. During the fourth year, a clonal evaluation could finally be carried out. Therefore a typical recurrent selection method would require no less than six to seven years (Figure 1). In this case, however, no selfing for reducing genetic load would have been included.

Sspecific Objectives
a) *To develop a methodology that will allow overcoming some of the most important drawback in the current cassava-breeding scheme.*
b) *To incorporate biotechnology tools into routine cassava breeding activities.*

Materials and methods

Advantages of inbreeding
When an elite clone is self-pollinated two important events occur: **a)** the unique, specific combination of genes present in the genotype is broken, therefore loosing the agronomic superiority that the clone might have. **b)** self pollination forces an average of half of the loci to become homozygous, thus facilitating the elimination of undesirable, deleterious alleles present in the original clone but hidden because of its predominant heterozygosity. In other words, selfed progenies allow for a reduction of the genetic load originally present in the clone, therefore becoming better progenitors themselves. In a way, selfing allows to "*concentrate*" the desirable genes originally present in the elite clone.

If inbreeding was pursued until near or complete homozygosity, then the transfer of desirable traits through the back-cross scheme becomes feasible. Also homozygosity "*captures*" genetic superiority because of its inherent genetic stability. Therefore each cassava improvement cycle would be a consolidated step that could help further progress in a more consistent and predictable way. On the other hand, each time a hybrid is used as parent, the process goes back to the initial step because of the genetic instability of the heterozygous material. In the latter case, progress cannot be easily consolidated or sustained through time, but in a rather inefficient way.

From the quantitative point of view the variability of a given population has traditionally been split into two major components: additive and dominant effects (Bos and Caligari, 1995; Hallauer and Miranda, 1988). Additive effects are very important because they define the breeding value of an individual, that is, its relative merit based on the quality of the progeny they produce. Dominance effects are also very important in plant breeding. They are the main contributors to the heterosis or hybrid vigor observed in hybrid cultivars, including

cassava. However, contrary to the additive effects, the dominance cannot be transmitted to the progeny. This means that dominance effects cannot be effectively exploited in a breeding program, unless a sophisticated breeding scheme (reciprocal recurrent selection) is employed (Hallauer and Miranda, 1988).

Table 4.1. Distribution of additive and dominance genetic variance among and within full-sib and inbred line families (Hallauer and Miranda 1988).

Type of family	Among families		Among plants within families	
	Additive variance	Dominance variance	Additive variance	Dominance variance
Half-sib family	1/4	0	3/4	1
Full-sib family	1/2	1/4	1/2	3/4
Inbred lines	2	0	0	•0

The current breeding scheme is based on the selection of individual genotypes within half- or full-sib families. Table 1 illustrates how the total genetic variance and its components (additivity and dominance) are partitioned among and within different types of families. It is clear that all genetic effects will influence the selection of plants from half- or full-sib families. That is, 100% of additive variance and 100% of dominance variance are exploited during the selection process (this is so because the breeder selects the best families, and then, the best genotype within the best family). This is a convenient situation because, once a given clone is selected, both the additive and dominance effects determining its good performance can be perpetuated because of the vegetative reproduction of the crop. The specific combination of genes present in a clone can be maintained unaltered generation after generation, as long as there is only vegetative reproduction.

However, only the additive portion of the total genetic variance can effectively be passed on to a next generation when the same clone is used as parent in a breeding project. It is important to recognize that dominance strongly influences the selection of the best clones but has no effect on their breeding value. In other words, dominance effects can be beneficial for the *per se* performance of a clone, but it has a confounding effect of its actual value as progenitor.

Inbreeding is advantageous because it erases the dominance effects from the selection process. The resulting inbred lines do not possess any dominance effects, and therefore, there will be no heterosis or hybrid vigor expressing in their performance. That is precisely why inbred lines are inferior, agronomically speaking, compared with the non-inbred cassava materials. A striking feature of the data presented in Table 4.1 is that inbred lines show twice the additive variance originally present. In other words, when selecting among inbred lines the additive variance originally present (among F2 plants from an F1-hybrid) has been expanded, thus greatly facilitating the selection process of those additive effects that are precisely the only ones that define a superior progenitor.

Inbred lines are better material for selecting progenitors because, by definition, they carry lower levels of genetic load, no confounding dominance effect influence the selection process and because the additive genetic effects are expanded considerably, making the selection

more efficient. Also if a breeding process is based on the use of inbred lines the transfer of valuable traits is greatly facilitated because the back-cross scheme becomes feasible.

The availability of inbred lines in cassava would also benefit other areas in addition to breeding. Genetic and molecular marker analysis would be facilitated if homozygous lines were produced. The only way to maintain germplasm in cassava is either by growing the plants in the field or by tissue culture. Inbred lines could be maintained and shipped in the form of botanical seed. Phytosanitary problems could be reduced or eliminated if maintenance and/or multiplication of genetic stocks were partially based on botanical seed. Germplasm exchange among the few cassava-breeding projects in the world would be enhanced because it would be based on botanical seed, rather that vitro-plants. Finally, clones could be reproduced by sexual means. Although time – consuming, the first stage of evaluation could be based on many plants produced by the crossing of selected inbred lines. Currently the evaluation process takes three years to reach a stage for selection based in just 30 plants.

The problems of inbreeding
Cassava, being an out-crossed crop, abhors inbreeding and shows severe depression. As was the case of temperate maize in the early 1900s and tropical maize by the 1970s, cassava will need to be improved for its tolerance to inbreeding depression. A few recurrent selection cycles (selfing each elite clone down to the S2 level, and recombining the surviving progenies) should prepare elite cassava populations for the trauma of total homozygosity.

A recurrent selection involving the production of inbred lines would be difficult to implement because of the length of each cycle of selection. It is estimated that no less than **nine years** will be required from the time a group of elite clones are selected until recombinant seed from their inbred lines was obtained. Therefore, if a breeding scheme using inbred lines is to be implemented, a way to reduce the time required for each cycle of selection is urgently needed.

Doubled haploids have been produced and have benefited breeding efforts in many crops (Griffing, 1975). Upon producing an F1 plant, tissue culture techniques are applied to the reproductive tissue (typically anther culture). This process produces a haploid tissue that, quite frequently, doubles spontaneously to produce the doubled-haploid tissue, which by definition, is homozygous. There are other alternatives for producing similar materials (i.e. using inter-specific crosses). However these methods have seldom been incorporated as a routine tool in breeding projects.

If an efficient protocol for the production of doubled-haploids were available, it could be incorporated into the cassava breeding process with the advantages that inbred lines offer as explained above. From the practical point of view the protocol for the production of doubled-haploids would allow shortening the time required to produce hybrids from inbred lines down to **three years**.

Expected responses to selection with alternative breeding methods.
Breeding projects always search for maximizing the gains from selection. The genetic progress (**GP**) after one cycle of recurrent selection can be estimated as follows (Hallauer and Miranda, 1988; Simmonds and Smartt, 1999):

$$GP = i\,\sigma^2_A\,/\,\sigma_F$$

Where i is a factor related to the intensity of selection (proportion of the population selected to be parent for the next cycle); σ^2_A is the additive component of the genetic variance measured in the parental population and σ_F is the phenotypic standard deviation of the parental population. These σ^2_A and σ_F parameters will vary depending on the selection unit used (i.e. individual plant, mean family performance, mean of clones across replicated trials in different locations, etc.).

In turn, the σ_F can be defined as:

$$\sigma_F = \sqrt{\sigma^2_A + \sigma^2_D + \sigma^2_E}$$

Where σ^2_A and σ^2_D are the additive and dominance components of the genetic variance and σ^2_E is the environmental effect of the evaluation of the respective selection unit. It should be clear that to increase **GP** there are only few alternatives: **a)** Increase the value of i (that is increase the selection intensity); **b)** Increase the additive component of the genetic variance exploited by selection and/or **c)** Reduce one or all components of the phenotypic standard deviation. Changes in the selection intensity do not depend in the breeding scheme employed. On the other hand the proportion and magnitude of the additive component of the genetic variance exploited and the size of the phenotypic variance depend heavily on the breeding method implemented, as illustrated below.

The phenotypic standard deviation in phenotypic mass selection is very large because of the environmental component associated with single plant evaluations. In the clonal selection used in cassava, although single genotypes are selected (as in mass selection), the environmental variance is reduced because "**n**" plants representing the genotype are used in the evaluation and selection process. However, all the dominance effects remain as component of the denominator of the formula. Therefore, clonal selection would maximize **GP** when dominance effects are negligible, which is not the case of cassava.

When doubled-haploids are used, two important modifications are introduced into the formula for **GP**: **a)** The additive component in the numerator and denominator of the formula is now twice as large as before (but in the denominator the square root of the additive component is used); and **b)** The dominance component of the phenotypic variance disappears.

$GP_{(Phenotypic\ mass\ selection)}$	=	$\dfrac{i\,\sigma^2_A}{\sqrt{\sigma^2_A + \sigma^2_D + \sigma^2_E}}$

$GP_{(Clonal\ selection)}$	=	$\dfrac{i\,\sigma^2_A}{\sqrt{\sigma^2_A + \sigma^2_D + (\sigma^2_E\,/n)}}$

$GP_{(Doubled-Haploids\ selection)}$	=	$\dfrac{i\,2\,\sigma^2_A}{\sqrt{2\,\sigma^2_A + (\sigma^2_E\,/n)}}$

The information provided illustrates the advantage of clonal evaluation over phenotypic mass selection. It also suggests that selection based on doubled-haploids would be better than the clonal evaluation (based on F1s) traditionally used for cassava breeding. However, because of the inbreeding depression observed in this crop, doubled-haploids would perform very poorly compared with the full vigor clones (F1s).

Months	Activity	Action
0	Elite clones are selected and planted	
6-8	Plants from elite clones start to flower	
24	DH obtained from selected clones. Ten vitro-plants per DH	σ^2_A increases and σ^2_D disappears from differences among clones
34	DH evaluated for many agronomic traits based on *per se* performance	Selection operating in $2\sigma^2_A$
52	Seeds from crosses among selected DH obtained (no less than 10 seed / cross)	A controlled recovery of σ^2_D
62	Selection of F1 crosses based on 10-plants plots in target environments	Selection on high-h^2 traits and multiplication of planting material
68	Planting of nursery for next cycle of recurrent selection	Shortening of the duration of each selection cycle
74	Evaluation and selection of hybrids from DH lines based on 100 plants	Selection for dominance effects (heterosis) and low-h^2 traits
74	Best DH lines selected and heterotic patterns among them identified	Capture of genetic superiority (including that from dominance)
84	Seed from crosses to further improve DH lines obtained	Initiation of a new cycle of (reciprocal) recurrent selection
0	Beginning of a new cycle of selection	

Figure 4.2. Illustration of a breeding scheme based on the production, evaluation and selection of doubled-haploid cassava lines to exploit additive and dominance effects in the production of superior hybrid clones.

It is difficult to make a fair comparison between the traditional and the new proposed scheme, because the latter introduces an intermediate stage when selection in the homozygous stage is conducted on the *per se* performance of the doubled haploids. This is the point where twice as much additive variance can be exploited. The second stage in the new scheme would be reconstituting the dominance effects in a controllable and predictable

way, by crossing specific pairs of doubled haploids lines. Hybrids developed from inbred cassava clones should perform better than current hybrids because: **a)** the elimination of deleterious genes through homozygosity; **b)** easier identification of "complementing parents" for the production of hybrids with maximized heterozygosity or hybrid vigor (dominance effects); and **c)** the possibility of building up, over several recurrent selection cycles, the dominance effects.

Results

A drastic change in the way cassava breeding is achieved should be introduced for taking advantage of the benefits of inbreeding. Figure 4.2 illustrates the general features of the proposed scheme.

Production of doubled-haploid (DH) lines

The process starts with the selection of elite clones themselves or after improvement for tolerance to inbreeding following the S2-recurrent selection described above. Once the planted material begins flowering, tissue will be taken for the induction of doubled haploidy through tissue culture protocols developed specifically for that purpose in cassava.

Upon the production of **DH** tissue or embryos, in vitro multiplication of each line will be carried out, to produce at least 10 hardened plants ready for transplantation to the field. This would take place at the end of the second year of activities.

Selection of doubled-haploid lines

Several **DH** lines will be produced and the ten plants representing each of them will be planted in a *Clonal Evaluation Trial* in the proper target environment. Hopefully these trials will involve at least 200 **DH** lines. Selection of these lines will be conducted for relevant characteristics with moderate to high heritability: resistances to diseases and/or insects, plant architecture, root dry matter content, root and parenchyma color, harvest index, etc. The selection at this stage operates with twice the additive genetic variance expected to be found in the original population under random mating conditions. Therefore, it is expected that large contrasts will be apparent at this stage. Lines surviving to this stage will have, by definition, reduced genetic load compared with the elite lines from which originated.

While the field evaluation is conducted lab analyses can be simultaneously carried out to obtain the molecular fingerprinting of each line. This will allow for further selection of characteristics difficult or impossible to determine from the field trials. For instance, marker assisted selection for CMD (Cassava Mosaic Disease) could be implemented in Colombia, although the disease is not present in this country. Also genetic distances among the lines could be determined to facilitate the following stage within the recurrent selection cycle.

Production of hybrids from selected DH lines

The following stage in the selection process involves the production of hybrids among the surviving **DH** lines. It is expected that from the 200 or more **DH** lines at least 30 will reach this stage. Although it is clear from the literature that genetic distances have failed to explain satisfactorily the heterosis among inbred lines in maize (Lamkey and Staub, 1988), genetic distances measured through molecular markers can be used at least to orient the crosses

that deserve some priority. This could be justified until an adequate definition of heterotic patterns is eventually reached. Since the parental materials (**DH** lines) are homozygous just a few seeds per cross are required at this stage. The only justification for obtaining more than one seed would be to accelerate the time required for evaluation with large number of plants representing each hybrid or clone.

With the production of hybrids from the selected **DH** lines, dominance effects (heterosis) are generated and, because of the breeding scheme proposed, will be fully exploitable by the cassava-breeding project.

Evaluation of **DH**-derived hybrids.

Depending on the number of hybrid seed produced the previous stage the evaluation and selection of hybrids can be conducted in two successive steps or just one growing cycle. In Figure 4.2, it is assumed that only ten plants from each cross can be obtained from botanical seed, and therefore that the evaluation and selection is conducted in two consecutive growing cycles.

The *first selection* is performed on all the hybrids produced and based on the 10 plants representing each hybrid clone. Because there is no replication, selection will be based only on high–heritability traits, and in the proper target environment, to allow for the pressure from biotic and abiotic limiting factors. The same evaluation plots are used as seed multiplication plots.

The *second stage* of selection and evaluation is conducted with about 100 plants (i.e. two replications at two locations with 25-plant plots). Only hybrids that survived the selection process the previous year will be included in this evaluation. Low-heritability traits are incorporated as selection criteria at this stage. Only a few clones will survive this selection and they will be included in *Regional Trials* for their eventual release as has been traditionally done up to now.

Preparation of nurseries for next cycle of recurrent selection

While the evaluation of hybrids is conducted, their parental **DH** lines will be planted in the field in such a way that they are about six-month old when the results of the hybrid trials become available. As soon as the hybrid trials yield results regarding the best **DH** progenitors and the identification of eventual heterotic patterns, they will be crossed to generate new genetic material for the following cycle of selection. The only purpose of these crosses will be to generate F1 plants from which to extract flower tissues for the production of a new generation of **DH** lines. Crosses will be made among DH lines from the same heterotic group. It is expected that the definition of heterotic patters will initially be weak (precisely because no breeding has been made to strengthen them), but as the process develops they will become stronger and clearer.

Hybrid trials will not only generate elite clones to be included in *Regional Trials* and eventually be released as new varieties, but also provide important information about the **DH** lines that generated the hybrid clones. This information will be used to determine lines with good general combining ability (i.e. that generate progenies with performances that are better than the mean of all the hybrids evaluated) as well as detecting heterotic patterns. This information is fundamental for deciding the kind of crosses that will be made for the next selection cycle.

Advantages of the proposed scheme

The capacity to produce inbred lines in cassava through the use of a dynamic process allows to drastically change the breeding process: **a)** the emphasis will shift from producing vast number of hybrids hoping that one (or few) will be genetically superior, towards the production of parental lines that will allow 'to design' outstanding hybrids in a gradual, consistent and reliable fashion; **b)** genetic loads will be quickly reduced in elite cassava populations; **c)** hybrids produced from inbred lines will be better than hybrids produced from non-inbred progenitors because genetic load is reduced and because the system allows building up dominance effects; **d)** germplasm exchange will be greatly facilitated (botanical seed of outstanding parents) with obvious advantages for the cassava research community; **e)** gene exchange will also be greatly facilitated (currently it is very difficult to transfer one valuable gene from its source into an agronomically superior clone: the availability of inbred lines would make the back-cross scheme feasible for cassava); **f)** inbred materials are genetically stable, they allow the breeder to capture and efficiently exploit the genetic superiority contained in them, therefore, guaranteeing a sustainable and consistent genetic progress that cannot be observed nowadays; **g)** once a given combination of inbred lines is found (good performing hybrids) the same genotype could be produced at first using botanical seed, and from there by *vegetative means*. This implies not only a faster multiplication rate but also cleaner genetic stocks (from the phytosanitary point of view); **h)** the system allows for the identification of useful recessive traits.

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OUTPUT 5
Activities related with the maintenance of the germplasm bank of cassava and other *Manihot* species.

CIAT has been trusted with the maintenance of the cassava world germplasm bank, which includes more than 6000 accessions of *Manihot esculenta* and other *Manihot* species. In the following pages a summary of activities related to the germplasm bank will be described.

Activity 5.1. Maintenance of Manihot germplasm bank in the field.

Rationale

The Genetic Resources Unit is officially in charge of the maintenance of the cassava germplasm bank, both *in vitro* and in the field. However, for practical reasons, the field operations are coordinated by IP3 project. Since year 2000 an extensive activity to clean up from frogskin disease, the germplasm bank has been carried out. Plots from the germplasm bank maintained in the field, because of its very nature, could not be eliminated even if frogskin disease appeared in some of the plants. Eventually the incidence of the disease increased to unacceptable levels.

Specific Objectives:
a) *To contribute with the maintenance the cassava and related wild species germplasm bank in the field.*
b) *Implementation of scheme for reducing incidence of frogskin disease in germplasm bank and elite clones.*

Materials and methods

Drastic measures were taken to reduce the level of incidence of frogskin disease back to acceptable levels. The strategy implies four main elements:

Regeneration of each accession from the in vitro collection. From each accession, a plant from the *in vitro* collection was regenerated and indexed to certify it is free of diseases. Plants passing this first test are then hardened in conditions that do not allow for the presence of white flies, and therefore, minimizes the possibility of acquiring the frogskin disease agent again.

Planting of disease free plantlets outside CIAT, in isolated fields. Because of the higher incidence of frogskin disease at CIAT (mainly at the germplasm bank collection in the field), plants that are certified to be disease free, or those developed from botanical seeds (which do not transmit viral agents to the plants germinating from them), were planted outside CIAT in isolated plots (CEUNP). Only virus-free plants were planted in those isolated plots. In the meantime, plantings at CIAT were reduced as a higher proportion of the cassava germplasm is being certified to be disease-free. In short the outside plantings were certified to be *"clean"*, whereas the plantings at CIAT were not. This situation was maintained until the middle of

2001, when materials not certified to be disease free moved out of CIAT, and those that are *clean*, came back to the station.

Breaking the life cycle of the white flies at CIAT. In addition of maintaining an ideal reservoir for the agent of the frogskin disease in the germplasm bank, there is a second factor that facilitated the spread of the disease. In effect, the white flies problem has increased considerably during the last few years. A major factor for this increment has been the continuous planting of cassava year round. The insects, therefore, had an ideal condition for maintaining high population densities. Between July 8 and August 9, 2001, there was no cassava plant in the field at CIAT's station in Palmira. It is expected that this measure will reduce population densities for the insect, and in turn, will reduce to a minimum the already inefficient transmission of the frogskin disease agent to healthy plants.

Harvest of stakes only from asymptomatic plants. A common procedure to harvest cassava is to first take the stakes (vegetative *"seed"*) out of the field, and then harvest the roots. In fact this practice prevents the elimination of stakes from diseased plants, because when the roots are evaluated for symptoms, the stakes from each plant has already been mixed with other stakes from different plants. Starting in this year, the harvest protocol has been changed slightly. The whole plant is first taken out of the ground, so before taking the stakes the roots can be inspected to make sure they are asymptomatic. Stakes are taken only from plants that do not show the symptoms. This practice will reduce to a very minimum the "seed" transmission of the disease to only two possible cases: **a)** when the worker fails to recognize the symptoms; or **b)** when the plant has been infected late in the season and, therefore, it does not show the symptoms but the disease will be transmitted through its stakes.

Results

All the activities were carried out as expected. A large proportion of accessions from the germplasm bank was evaluated for frogskin disease and, if clean, planted in isolated conditions. Sequential plantings were performed as the plants were certified to be disease-free. Therefore, harvest of these plants was also done sequentially. The levels of frogskin were very low, as expected. However, given the results from the previous year, when higher than acceptable levels of frogskin disease were observed, it has been decided not to plant the entire germplasm bank in the field, until the vector(s) and pathogen(s) are clearly determined.

During the current year the possibility of a viroid related to frogskin disease was discarded. Currently a viral agent and/or a phytoplasm have been postulated as the causal agent of this elusive disease (See Output 7). Scientists working in cassava entomology are also carrying out studies to determine which species may be acting as a vector of the disease. Although it has always been suspected that whiteflies may be involved, now the possibility of a cycadelid is also considered.

A major achievement was in relation to the management of the whiteflies problem. By interrupting the breeding cycle of the insect by eliminating all cassava plants in the field for one month, we have achieved excellent results. By November, cassava plantings in the station were still remarkably clean from whiteflies and only at the end of the season we could see some population growth. As a result, we have seen, for the first time in many years, other arthropods pest coming back. That was the case of mites and mealybugs. This is, in a way, an evidence of the success we have had reducing the problem of the whiteflies that reached

such a high level of incidence that prevented other pest colonizing cassava plantings at CIAT-Palmira.

Activity 5.2.Evaluation of M. esculenta and related species from the germplasm collection for useful traits, particularly for the natural occurrence of apomixis.

Rationale

Apomixis is a highly desirable trait for cassava. This mode of reproduction would facilitate germplasm exchange because the shipment of botanical seed implies much lower phytosanitary risks than vegetative propagules. Apomictic seed could also be used to conserve germplasm for longer period of time a much lower costs. Also, depending on the genoytpe, apomixis could greatly increase multiplication rate once the "seed" of an elite clone needs to be increased.

Specific Objectives:
a) To search for the natural occurrence of apomixis in the germplasm bank collection.
b) To carry out collaborative research with other institutions in the area of apomixis.

Materials and methods

Apomixis has always been an interesting process to cassava breeders. There have been some reports of apomixis occurrence in the *Manihot* genus (Nassar et al., 1998. Genetics and Molecular Biology 21:527-530). This reproductive abnormality is likely to occur in cassava germplasm introgressed with wild species. The germplasm bank includes accessions collected in areas where the likelihood of natural crossing between *M. esculenta* and other *Manihot* species is high (especially from the Amazon basin). Therefore, we have already started to bag clusters of female flowers, searching for a genotype that will produce seed without pollination. Having apomictic cassava would greatly simplify maintaining genetic stocks unchanged, and also facilitate the exchange of germplasm almost without the risks of introducing diseases.

Results

So far the process has yielded no positive results, but only a fraction of the entire germplasm bank collection has been screened, because of the transitional stage most of the accessions were going through to be cleaned from frogskin disease. This activity, which has low probability of success, will be maintained until the entire collection has been properly tested.

Activity 5.3.Evaluation of M. esculenta and related species from the germplasm collection for novel types of starches and other traits of economic relevance.

Rationale

The starch industry has always requested for different types of starches in cassava roots. No extensive evaluation has been made on starch properties of each accession in the germplasm bank. Novel starch types have large economic relevance for the industry.

Protein levels in cassava roots are low. This situation results in the price of flour or chips from dried cassava roots being about 70% of that of field maize. Increasing protein levels in cassava roots is therefore an important objective for the industrial processing of cassava for animal feed, and certainly a critical goal for those areas where cassava is important as a food security crop.

Specific Objectives:
   a) To screen the germplasm bank in search for the natural occurrence of novel starch types
   b) To search for high-protein cassava roots.

Results
We have begun a systematic characterization of the starch properties in the roots of the accessions from the germplasm bank. Every year up to 2000 accessions are evaluated.

Also based on the results from a group of 600 genotypes we have determined the possibility of higher protein levels in cassava roots originated from Meso-America, particularly Guatemala. We are recovering clones from that region of the world to confirm this hypothesis.

OUTPUT 6
Breeding for insect and other arthropods resistance and development of alternative methods for their control

Activity 6.1. Evaluation of cassava germplasm for resistance to whiteflies (*Aleurotrachelus socialis*) during 2002.

Rationale

As direct feeding pests and virus vectors, whiteflies cause major damage in cassava based agroecosystems in the Americas, Africa and to a lesser degree in Asia. Eleven species have been identified feeding on cassava in the Neotropics but two predominate, *Aleurotrachelus socialis* in the Northern region of South America (Colombia, Venezuela and Ecuador), while *Aleurothrixus aepim* is the major species causing crop damage in Brazil. In Africa *Bemisia tabaci* is the vector of African Cassava Mosaic Disease (ACMD) while in recent years *Bemisia afer*, originally from East Africa, has now been found in the Americas (Perú) and may pose as a future threat to the cassava crop if rapid adaptation and dissemination occurs.

Both *A. socialis* and *A. aepim* cause direct damage to cassava by feeding on the phloem of leaves, causing chlorosis and leaf fall, which can result in considerable crop loss. Neither species is known to transmit virus diseases. Research at CIAT to control whiteflies has concentrated on a combination of host plant resistance (HPR) and biological control (BC), with initial emphasis being given to HPR (See PE-1 Annual Report for research activities with BC). Although whitefly resistance in agricultural crops in general is rare, several good sources of resistance have been identified in cassava and high-yielding, whitefly resistant cassava hybrids are being developed. One of these CG 489-31 (CIAT Breeding Code) is being released officially by CORPOICA (Colombia, MADR) in November of 2002.

A continued high incidence of frog skin disease at CIAT during the 2001-2002 growing season, deterred much of our efforts to carry out pest research on the CIAT station. There was a constant shortage of disease free germplasm that is needed to carry out many of the pest resistance mechanism studies that confirms and explains much of our field results and observations. Several of the planned studies had to be postponed due to lack of planting material of several of the resistant genotypes. Even some of the off station plantings, that had previously supplied germplasm, became infested with frog skin, limiting access to planting materials.

Materials and methods

Whitefly evaluations of cassava germplasm were done at principally two sites, at the CIAT farm in Santander de Quilichao and at CORPOICA, Nataima, Tolima. At CORPOICA Nataima, 722 hybrids of the family CM 8996 were evaluated. This family is from a cross of MEcu 72 x MCol 2246 (whitefly resistant x whitefly susceptible). The objective of this on-going research is to study the genetics of resistance of whitefly (*A. socialis*) in cassava. The same cultivars were planted in March 2002 at Santander de Quilichao, and although some whitefly damage and population data has been taken, yield data will be known when the trial is harvested in March 2003. The CORPOICA, Nataima planting was done in May 2001, and harvested in March of 2002 so that yield data is available.

Results

CORPOICA, Nataima: Field evaluations of *A. socialis* damage and populations were carried out three times during the crop cycle, using a 1 to 6 damage and whitefly population scale (Table 6.1). Whitefly (*A. socialis*) populations were low throughout the crop cycle and this resulted in low damage ratings for the genotypes evaluated (Figure 6.1). Neither whitefly populations nor damage reached the 4.0 level on the 1-6 scale and only on a few cultivars did the levels reach 3.0. The low whitefly incidence makes it nearly impossible to separate the resistant from susceptible cultivars, and this trial will need to be repeated. Interestingly, the local farmers cultivar, Aroma, which is highly susceptible to whiteflies, was planted throughout the field as a local control. Whitefly populations on Aroma were relatively higher than on the CM 8996 family (Figure 6.2), reaching the 3.0 to 4.0 level on a majority of the sites where planted in the fields. The difference in whitefly populations and damage between the CM 8996 progeny and the control may, at least in part be due to the resistance conferred by MEcu 72.

Table 6.1. Population and damage scales for evaluating cassava germplasm for resistance to whiteflies.

Population Scale (Nymphs and Pupae)
1 = no whitefly stages present
2 = 1-200 individuals per cassava leaf
3 = 201-500 per leaf
4 = 501-2000 per leaf
5 = 2001-4000 per leaf
6 = > 4000 per leaf

Damage Scale
1 = no leaf damage
2 = young leaves still green but slightly flaccid
3 = some twisting of young leaves, slight leaf curling
4 = apical leaves curled and twisted; yellow-green mottled appearance
5 = same as 4, but with "sooty mold" and yellowing of leaves
6 = considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems.

At harvest, the exceptional vigor of the genotypes from the CM 8996 family was notable (Photo 6.1). Both parents of this family are vigorous varieties and combined with the whitefly resistance conferred by MEcu 72, these results are not unexpected. Harvest data shows a range in plant yield from 4.5 to 86.5 T/ha, and numerous cultivars with high harvest index, high dry matter, good root color and cooking quality. 75 sister cultivars were selected for high yield, between 20 and 86.5 T/ha, such as cultivars CM 8996-342 and Cm 8996-54 respectively (Figure 6.3). Dry matter content was as high as 42% in genotype CM8999-314 (Table 6.2). These 75 genotypes will be evaluated in more detail, with increased replications in future trials.
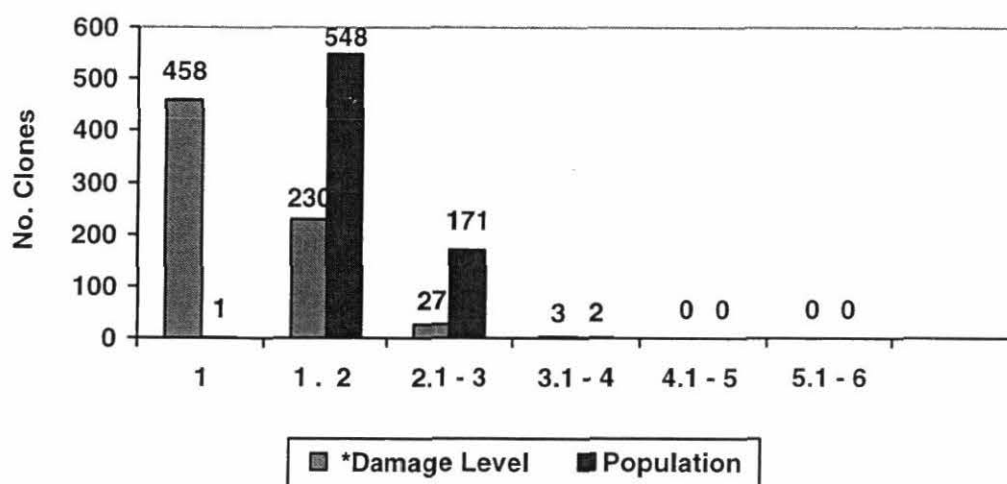
* Four cultivars not evaluated due to extreme stunting of plants.

Figure 6.1. Cassava damage and whitefly population ratings due to whitefly (*Aleurotrachelus socialis*) feeding on clones from the family CM 8996 (MEcu 72 x MCol 2246) at CORPOICA, Nataima (Tolima, 2002).
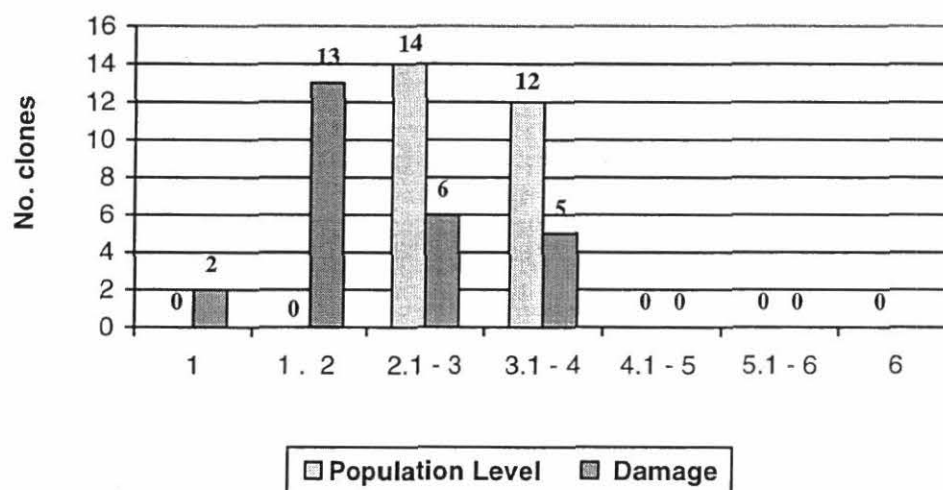
Figure 6.2. Whitefly (*Aleurotrachelus socialis*) populations and damage ratings on the variety Aroma (local farmers variety) at CORPOICA, Nataima (Tolima, 2002).
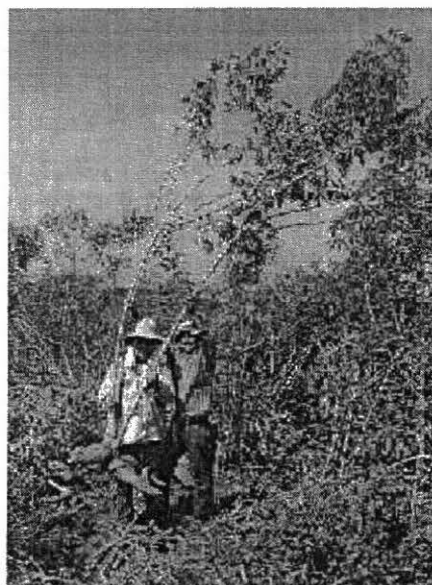
Photo 6.1. Genotypes of family CM 8996 (MEcu 72 x MCol 2246) harvested at CORPOICA, Nataima; Note exceptional vigor of plants.

This family in its entirety has also been replanted in CORPOICA, Nataima for a second cycle of evaluations. Whitefly damage and population data will be taken throughout the year and the trial will be harvested during early 2003.

Results

Santander de Quilichao. At this site, as at the Tolima site, initial whitefly populations were lower than expected. However populations' levels did reach 4.0 on about 30 cultivars (Figure 6.4). *A. socialis* populations started early in the crop cycle but did not increase greatly over time and other pests, notably mites and thrips, occurred and their populations quickly increased. 326 of 756 clones (43%) presented moderate level of damage to thrips and 302 (40%) had high or severe levels of damage (Figure 6.5). Only 128 (17%) had low levels of damage (1-2 on a 1 to 6 damage scale). Since MEcu 72, the female parent, is highly resistant to thrips, and MCol 2246 is susceptible this kind of segregation in the progeny is not unexpected. Of the 128 clones displaying resistance to thrips, 38 belong to the group of selected cultivars from Tolima, as previously described (Figure 6.6). Of the 75 clones selected in Tolima (Figure 6.3), 33 (44%) are susceptible to thrips and 38 or about 50% are resistant. The cultivar CMC-40 was planted at Santander de Quilichao as the control, and is highly susceptible to thrips and gave thrips damage ratings above 4.0, on 100% of the plants evaluated, often reaching 5 or 6 on the damage scale, again indicating the high level of thrips population.
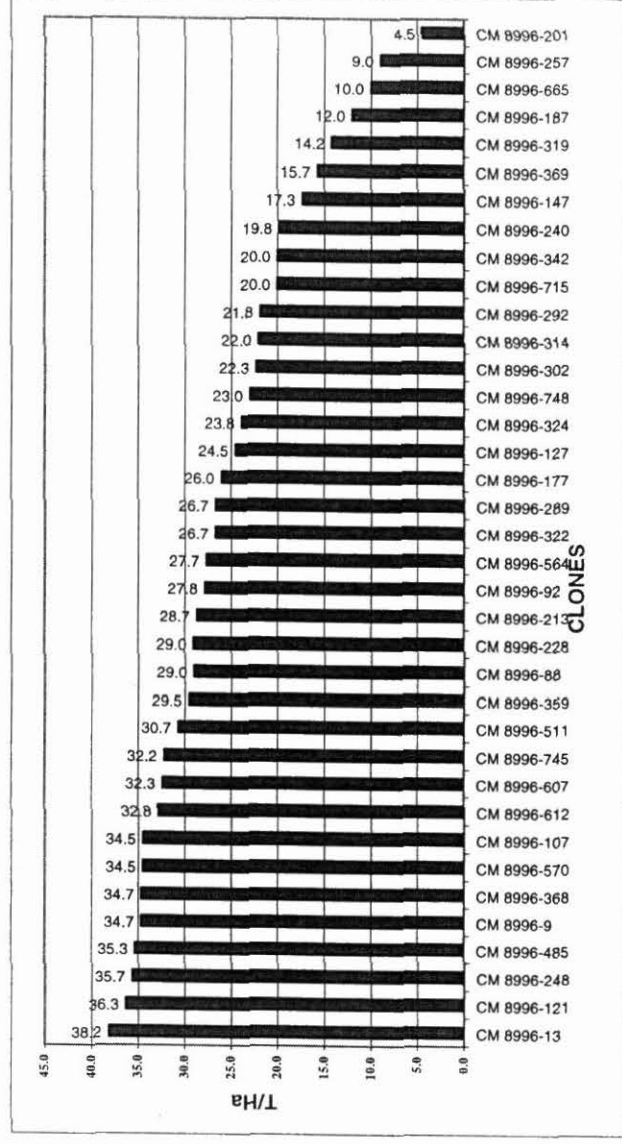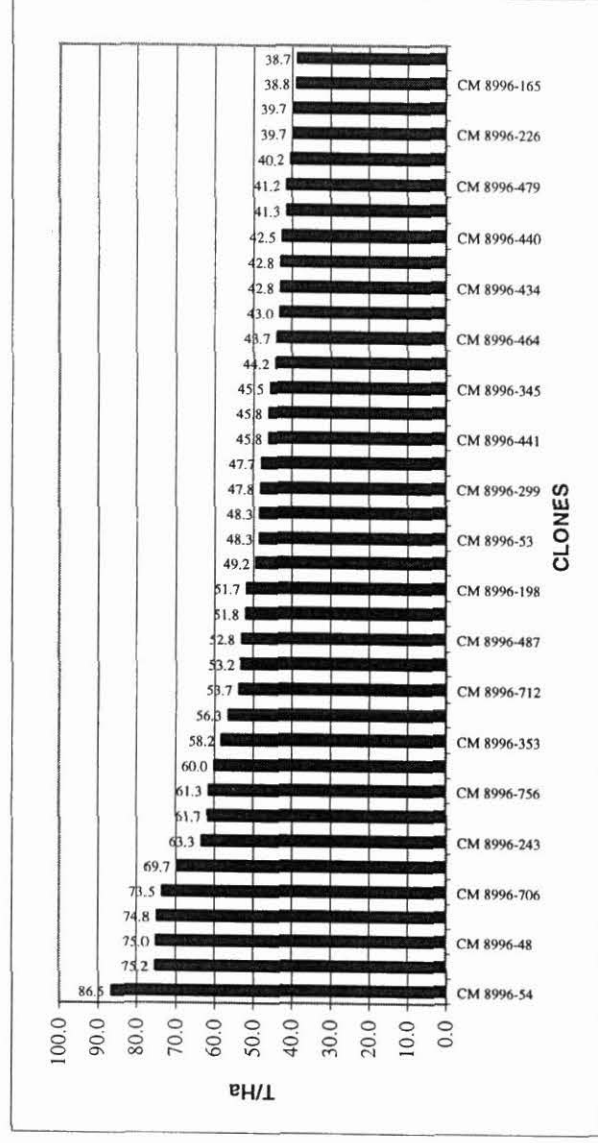
Figure 6.3. Range in yield of 75 cassava clones selected for whitefly resistance at CORPOICA, Nataima, 2002.

Table 6.2. Harvest index, total yield and % dry matter content of 75 selected cassava progeny from a MEcu 72 x MCol 2246 cross and evaluated for resistance to the whitefly, *Aleurotrachelus socialis* at CORPOICA, Nataima.

Clone	Harvest Index	Total Yield	Dry Matter
CM 8996-314	0.59	22.0	42.0
CM 8996-257	0.45	9.0	40.2
CM 8996-665	0.62	10.0	38.8
CM 8996-511	0.50	30.7	38.7
CM 8996-201	0.52	4.5	38.5
CM 8996-189	0.56	41.3	37.5
CM 8996-302	0.50	22.3	37.3
CM 8996-88	0.55	29.0	36.8
CM 8996-319	0.49	14.2	36.8
CM 8996-261	0.61	40.2	36.6
CM 8996-324	0.58	23.8	36.6
CM 8996-369	0.38	15.7	36.5
CM 8996-292	0.53	21.8	36.4
CM 8996-147	0.40	17.3	36.3
CM 8996-368	0.57	34.7	36.3
CM 8996-213	0.44	28.7	36.2
CM 8996-208	0.60	49.2	36.2
CM 8996-177	0.59	26.0	36.2
CM 8996-210	0.68	45.8	36.2
CM 8996-748	0.71	23.0	36.0
CM 8996-92	0.55	27.8	35.9
CM 8996-358	0.57	38.7	35.9
CM 8996-342	0.51	20.0	35.8
CM 8996-187	0.46	12.0	35.7
CM 8996-228	0.48	29.0	35.5
CM 8996-248	0.54	35.7	35.5
CM 8996-289	0.54	26.7	35.5
CM 8996-243	0.66	63.3	35.4
CM 8996-127	0.67	24.5	35.4
CM 8996-479	0.65	41.2	35.4
CM 8996-715	0.62	20.0	35.4
CM 8996-322	0.49	26.7	35.3
CM 8996-745	0.44	32.2	35.2
CM 8996-485	0.56	35.3	35.2
CM 8996-353	0.56	58.2	35.1
CM 8996-107	0.65	34.5	35.0
CM 8996-226	0.61	39.7	35.0
CM 8996-607	0.53	32.3	34.8
CM 8996-199	0.61	39.7	34.8
CM 8996-570	0.50	34.5	34.8
CM 8996-564	0.51	27.7	34.7
CM 8996-165	0.58	38.8	34.6
CM 8996-53	0.57	48.3	34.5
CM 8996-9	0.41	34.7	34.5
CM 8996-359	0.51	29.5	34.3

| Clone	Harvest Index	Total Yield	Dry Matter
CM 8996-612	0.71	32.8	34.3
CM 8996-434	0.56	42.8	34.2
CM 8996-299	0.64	47.8	34.2
CM 8996-13	0.56	38.2	34.2
CM 8996-121	0.54	36.3	34.1
CM 8996-42	0.54	56.3	34.1
CM 8996-101	0.52	44.2	34.0
CM 8996-712	0.65	53.7	34.0
CM 8996-217	0.59	51.8	34.0
CM 8996-441	0.55	45.8	33.9
CM 8996-214	0.62	53.2	33.6
CM 8996-178	0.65	42.8	33.6
CM 8996-345	0.50	45.5	33.5
CM 8996-467	0.61	48.3	33.5
CM 8996-306	0.53	43.0	33.4
CM 8996-198	0.53	51.7	33.3
CM 8996-240	0.58	19.8	33.3
CM 8996-440	0.71	42.5	33.2
CM 8996-714	0.72	47.7	33.2
CM 8996-616	0.59	69.7	32.9
CM 8996-487	0.66	52.8	32.7
CM 8996-756	0.60	61.3	32.4
CM 8996-706	0.66	73.5	32.3
CM 8996-464	0.51	43.7	32.3
CM 8996-643	0.61	61.7	32.3
CM 8996-298	0.55	60.0	31.9
CM 8996-216	0.54	74.8	31.3
CM 8996-54	0.63	86.5	29.2
CM 8996-323	0.65	75.2	29.0
CM 8996-48	0.61	75.0	28.6
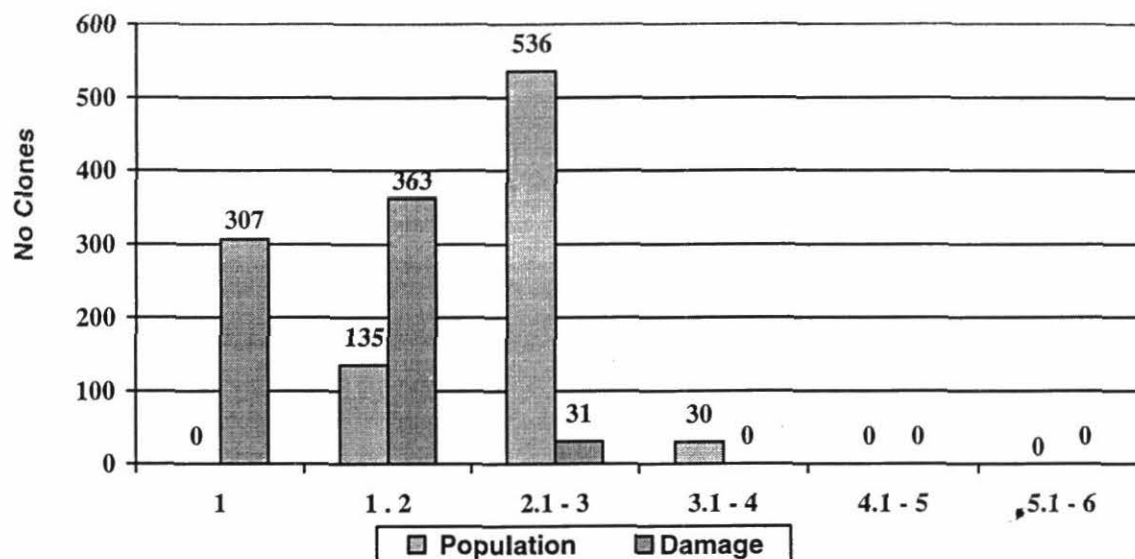
Figure 6.4. Whitefly (*Aleurotrachelus socialis*) populations and damage ratings on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) (originating from CORPOICA, Nataima) at Santander de Quilichao (Cauca, 2002).
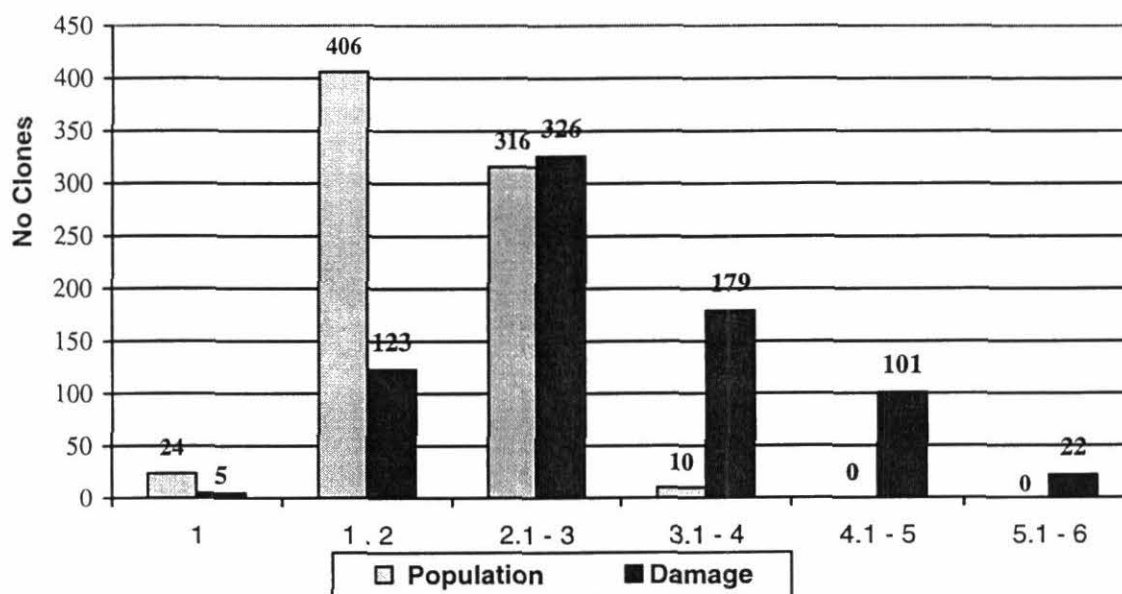
Figure 6.5. Thrips (*Frankliniella williamsi*) population and damage ratings on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).
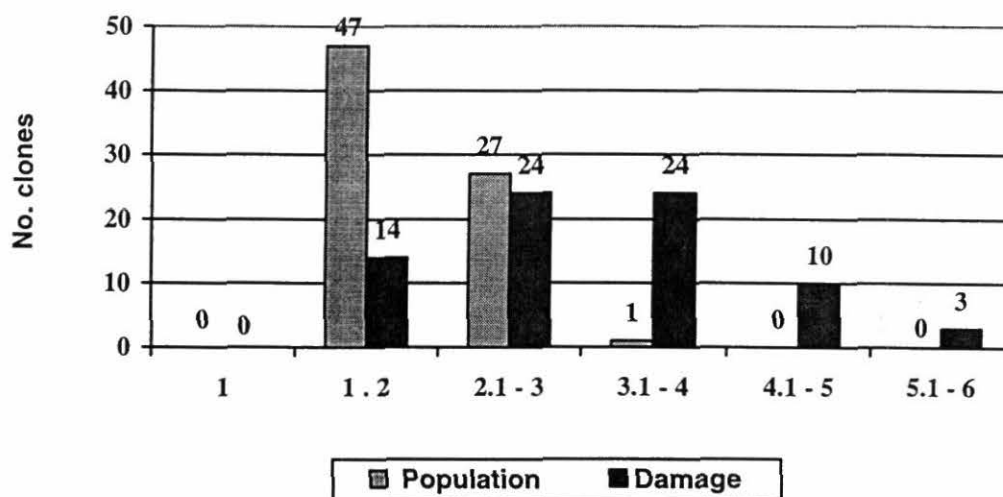
Figure 6.6. Thrips (*Frankliniella williamsi*) population and damage in selected cultivars (from CORPOICA, Nataima) of family CM 8996, at Santander de Quilichao (Cauca, 2002).

Mite (*Mononychellus tanajoa*) attacks also occurred during the dry season in this trial and high populations were evident. 705 of the 706 (99.8%) clones evaluated resulted in high damage levels, above 3.1 on the 1 to 6 damage scale. Only 1 clone (0.14%), CM8996-641, presented low or no damage (Figure 6.7). The mite damage on the susceptible control, CMC-40 was above 4.0 on 72.5% of the plants and where low damage was observed, thrips attack was so severe that it masked mite populations and damage (Figure 6.8). Of the 75 selected clones from the Tolima planting, none showed any resistance to mites, all having damage ratings above 3.0 (Figure 6.9).
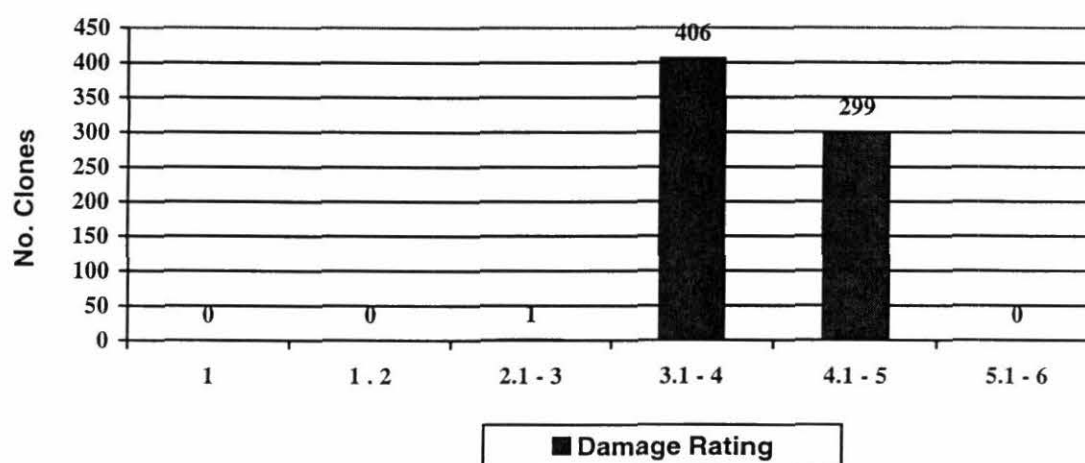
Figure 6.7. Mite (*Mononychellus tanajoa*) population and damage rating on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).
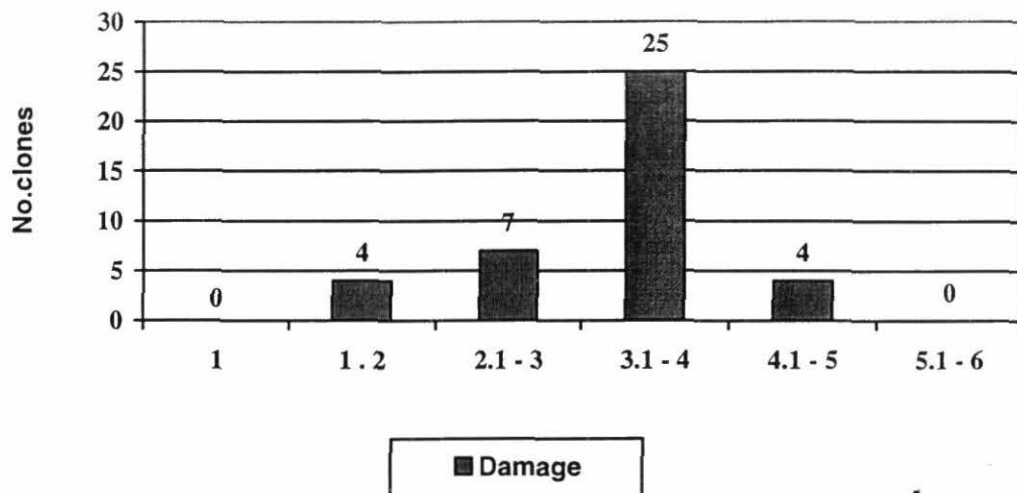
Figure 6.8.   Mite (*Mononychellus tanajoa*) damage ratings on CMC-40 (control plots) at Santander de Quilichao (Cauca, 2002).
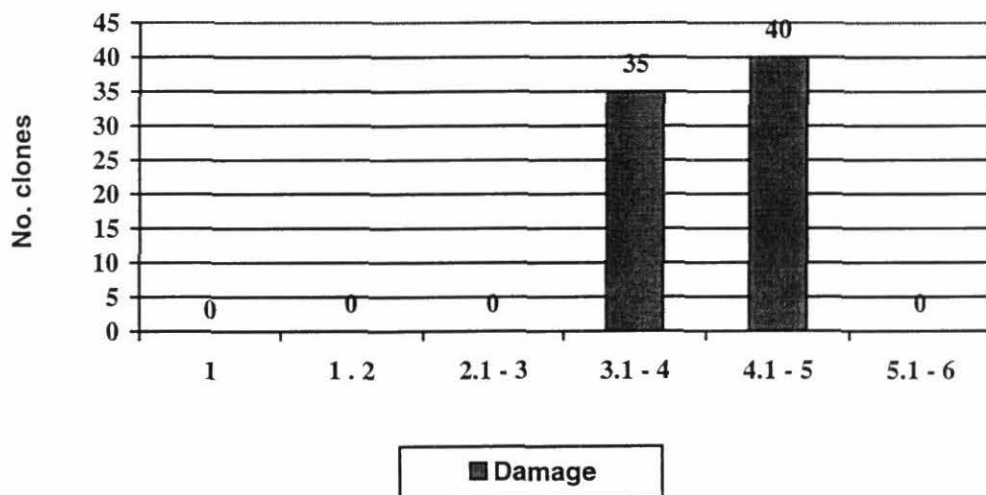
Figure 6.9. Mite (*Mononychellus tanajoa*) damage ratings in selected cultivars (from CORPOICA, Nataima) from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Cauca, 2002).

Of the 75 clones selected in Tolima for yield and low whitefly damage, all responded well at Santander de Quilichao with low whitefly populations (Figure 6.10). Both damage ratings and whitefly populations were low.
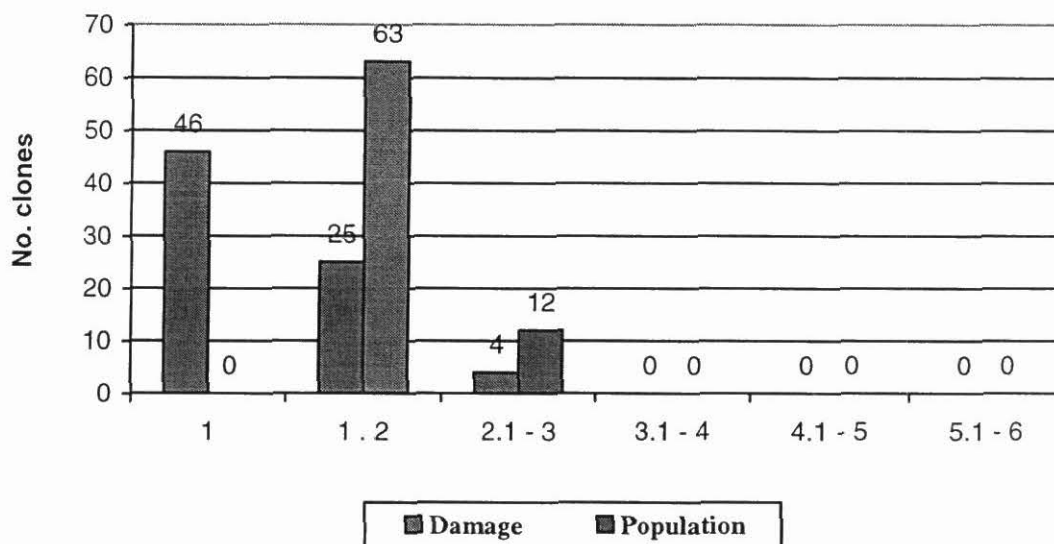
Figure 6.10. Whitefly (*Aleurotrachelus socialis*) population and damage rating on cassava clones from the family CM 8996 (MEcu 72 x MCol 2246) at Santander de Quilichao (Valle del Cauca), previously selected at CORPOICA, Nataima (2002).

In a breeding or germplasm development program it is always advantageous to identify cultivars that exhibit multi resistance, that is resistance to several pests in a single cultivar. Table 6.3. Lists the reaction of several cultivars to thrips and whitefly attack, where whitefly is the key pest.

Table 6.3. Cultivars from family CM 8996 (MEcu 72 x MCol 2246) showing low whitefly and thrips damage ratings at CORPOICA, Nataima.

Clone	Thrips		Whitefly	
	Population	Damage	Population	Damage
CM 8996-107	1.5	1.5	2.0	1.0
CM 8996-322	1.5	1.5	2.2	1.0
CM 8996-48	2	1.5	2.4	1.0
CM 8996-121	2	2	2.4	1.0
CM 8996-198	2	2	2.2	1.0
CM 8996-199	2	2	2.2	1.0
CM 8996-213	2	2	2.2	1.0
CM 8996-228	1.5	2	2.4	1.0
CM 8996-243	3	2	3	1.0
CM 8996-306	2	2	2.6	1.0
CM 8996-324	2	2	2.4	1.0
CM 8996-369	1.5	2	2	1.0
CM 8996-487	1.5	2	2.2	1.0
CM 8996-712	2	2	2.2	1.0
CM 8996-189	2	2.5	2.2	1.0

Clone	Thrips		Whitefly	
	Population	Damage	Population	Damage
CM 8996-214	1.5	2.5	2.2	1.0
CM 8996-248	2	2.5	2.4	1.0
CM 8996-342	2.5	2.5	2.4	1.0
CM 8996-353	2	2.5	2.2	1.0
CM 8996-440	2.5	2.5	3	1.0
CM 8996-464	2	2.5	3.2	1.0
CM 8996-607	2	2.5	2.6	1.0
CM 8996-612	2	2.5	2.4	1.0
CM 8996-745	2	2.5	2	1.0
CM 8996-9	2	2.5	2.6	1.0
CM 8996-147	2	3	3	1.0
CM 8996-240	2.5	3	2.4	1.0
CM 8996-257	2	3	2.2	1.0
CM 8996-261	2	3	2.2	1.0
CM 8996-302	2	3	3	1.0
CM 8996-319	2.5	3	2.6	1.0
CM 8996-345	2	3	2.4	1.0
CM 8996-358	2	3	2.6	1.0
CM 8996-359	2.5	3	2.4	1.0
CM 8996-42	2	3	1.8	1.0
CM 8996-467	3	3	3.2	1.0
CM 8996-570	2	3	2.2	1.0
CM 8996-88	2	3	2	1.0

Activity 6.2. Evaluation of cassava dialel crosses for whitefly (A. socialis) resistance/ susceptibility at Jamundi, Valle del Cauca (2002).

During the first semester of 2002, cassava breeding field trials were carried out to determine the combining ability (general or specific) (Dialelics) of different genotypes. Since a heavy whitefly (primarily *A. socialis*) attack occurred, an evaluation of whitefly populations and damage was done on these genotypes. A 1 to 6 damage and population scale were used to evaluate 36 families, each containing 30 sister progeny grown in replications (Table 6.4) (see whitefly section Activity 6.1).

Table 6.4.   Thirty-six cassava families of 30 plants each in a dialelic cross evaluated for whitefly (*A. socialis*) damage and populations (Jamundí, Valle del Cauca, 2002).

Crosses	Female	Male
CM 9901	CM 6740-7	SM 1219-9
GM 228	CM 6740-7	SM 1278-2
GM 230	CM 6740-7	SM 1636-24
GM 231	CM 6740-7	SM 1673-10
CM 9903	CM 6740-7	SM 1741-1
GM 234	CM 6740-7	HMC 1
GM 308	MEcu 72	CM 6740-7
CM 9642	CM 6740-7	MPer 183
GM 254	SM 1219-9	SM 1278-2

Crosses	Female	Male
GM 257	SM 1219-9	SM 1636-24
GM 260	SM 1219-9	SM 1673-10
CM 9953	SM 1219-9	SM 1741-1
GM 264	SM 1219-9	HMC 1
GM 309	MEcu 72	SM 1219-9
GM 265	SM 1219-9	MPer 183
GM 267	SM 1278-2	SM 1636-24
GM 268	SM 1278-2	SM 1673-10
GM 269	SM 1278-2	SM 1741-1
GM 270	SM 1278-2	HMC 1
GM 310	MEcu 72	SM 1278-2
GM 271	SM 1278-2	MPer 183
GM 283	SM 1636-24	SM 1673-10
GM 284	SM 1636-24	SM 1741-1
GM 285	SM 1636-24	HMC 1
GM 311	MEcu 72	SM 1636-24
GM 286	SM 1636-24	MPer 183
GM 292	SM 1673-10	SM 1741-1
GM 293	SM 1673-10	HMC 1
GM 312	MEcu 72	SM 1673-10
GM 294	SM 1673-10	MPer 183
GM 296	SM 1741-1	HMC 1
GM 313	MEcu 72	SM 1741-1
GM 297	SM 1741-1	MPer 183
GM 314	MEcu 72	HMC 1
CM 9733	HMC 1	MPer 183
GM 306	MEcu 72	MPer 183

Results

A total of 990 genotypes were evaluated (not all plants from the crosses were viable and some could not be evaluated. 131 genotypes (13.2%) showed no whitefly damage and 301 or 30.4% had damage ratings of 2.0 indicating high levels of resistance to whiteflies (Figure 6.11). 558 genotypes had damage ratings above 3.0 and 285 of these above 4.0, indicating that there was high selection pressure during the trial (Figure 6.11). The 432 cultivars that received ratings of 2.0 or lower are available upon request. It can be noted in this list that the cultivar GM 309-1 (MEcu 72 x SM 1219-9) had no damage and few or no whiteflies present. In this list, it can also be noted that numerous genotypes had very low damage and correspondingly low populations of whiteflies, between 1.0 and 1.7. These materials are very promising for whitefly resistance and should be maintained and re-evaluated.
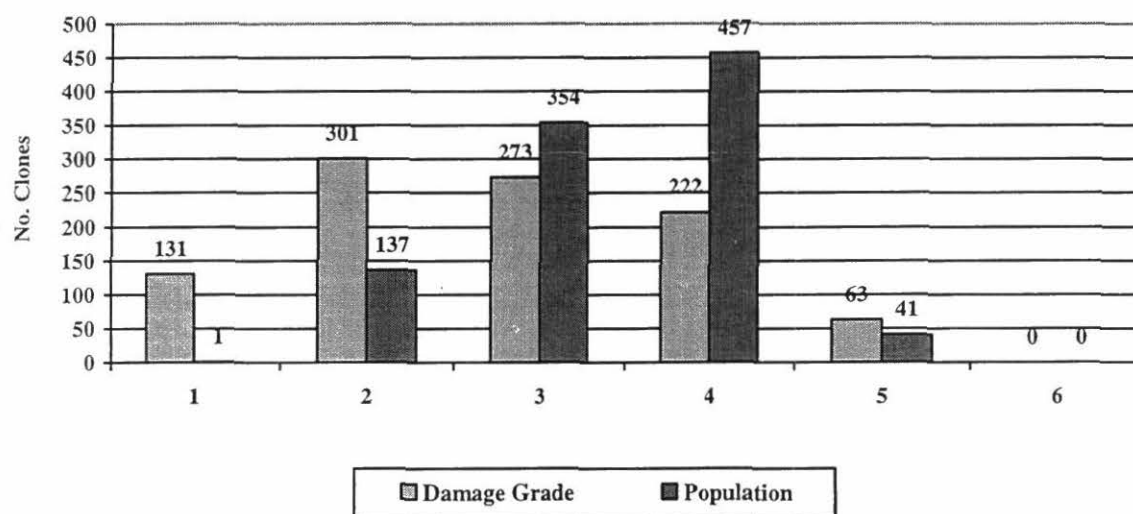
Figure 6.11. Cassava damage grade and whitefly (A. socialis) population levels in 990 progeny from a dialelic cross of 36 families (Jamundí, Valle del Cauca, 2002).

As a result of these evaluations, 141 genotypes were selected as most promising for whitefly resistance, without taking into account yield data. Of these 141genotypes 32 clones or 22.7% resulted having Frog Skin Disease. It was also observed that some families did not show frog skin symptoms. These included GM 294 (SM 1673-10 x MPer 183) and GM 297 (SM 1741-1 x MPer 183). At harvest (Breeding section) in the family GM 309 (MEcu 72 x SM 1219-9), 27 of the 30 sister plants had frog skin disease (Table 6.5).

Table 6.5.   Proportion of selected cassava families from a dialelic cross diagnoses with frog skin disease (Jamundí, Valle del Cauca, 2002).

Family	Parents	No. Plants with Frog Skin	% Frog Skin
GM 268	SM 1278-2 x SM 1673-10	0	0.0
GM 270	SM 1278-2 x HMC 1	0	0.0
GM 294	SM 1773-10 x MPer 183	0	0.0
GM 297	SM 1741 – 1 x MPer 183	0	0.0
GM 296	SM 1741 – 1 x HMC 1	1	3.3
GM 271	SM 1278 – 2 x MPer 183	2	6.7
GM 284	SM 1636 – 24 x SM 1741 - 1	2	6.7
GM 264	SM 1219 – 9 x HMC 1	3	10
GM 306	MEcu 72 x MPer 183	3	10
GM 231	CM 6740 – 7 x SM 1278 - 2	4	13.3
GM 228	CM 6740 – 7 x SM 1278- 2	5	16.7
GM 234	CM 6740 – 7 x HMC 1	5	16.7
GM 230	CM 6740 – 7 x SM 1636 - 24	7	23.3
GM 265	SM 1219 – 9 x MPer 183	7	23.3
GM 269	SM 1278 – 2 x SM 1741 – 1	7	23.3
GM 283	SM 1636 – 24 x SM 1673 – 10	7	23.3
GM 311	MEcu 72 x SM 1636 - 24	11	36.6
GM 314	MEcu 72 x HMC 1	11	36.6
CM 9642	CM 6740 – 7 x MPer 183	11	36.6

GM 312	MEcu 72 x SM 1673 - 10	12	40.0
GM 308	MEcu 72 x CM 6740 - 7	13	43.3
GM 310	MEcu 72 x SM 1278 – 2	13	43.3
CM 9901	CM 6740 – 7 x SM 1219 - 9	15	50.0
GM 313	MEcu 72 x SM 1741 – 1	17	56.7
CM 9903	CM 6740 – 7 x SM 1741 - 1	22	73.3
GM 257	SM 1219 – 9 x SM 1636 - 24	22	73.3
GM 309	MEcu 72 x SM 1219 - 9	27	90.0

Activity 6.3. Evaluation of whitefly (*Aleurotrachelus socialis*) cassava clones for resistance to *Bemisia tabaci*.

Whiteflies are reported feeding on cassava in nearly all cassava growing regions of the tropics. Eleven species have now been identified globally. In the neotropics *Aleurotrachelus socialis* predominates in Northern South America, while *Aleurothrixus aepim* is the major species in Brazil. *Bemisia tabaci*, a pantropical species, predominates in Africa where it is the vector of Africa Cassava Mosaic Disease (ACMD). Until recently, *B. tabaci* biotypes found in the neotropics did not feed on cassava, and it has been speculated that the absence of ACMD in the Americas may be related to the inability of *B. tabaci* to colonize cassava. During the last decade a new biotype (B) of *B. tabaci* has been collected feeding on cassava in the neotropics.

The appearance of Biotype B is cause for concern as it is now considered that ACMD poses a more serious threat to cassava production in the Americas as most traditional varieties in the neotropics are highly susceptible to the disease. Therefore a project is now underway to identify possible *B. tabaci* resistance in cassava germplasm.

The CIAT cassava germplasm bank of more than 6000 accessions is continually being screened for resistance to arthropod pests, especially whiteflies. Over a period of more than 15 years several clones have been selected as sources of resistance to the whitefly species *Aleurotrachelus socialis*. The clone MEcu 72 has consistently expressed the highest levels of resistance. Additional cultivars expressing moderate to high levels of resistance in field trials include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365. Whitefly resistance hybrids from a MEcu 72 x MBra 12 cross have been produced and evaluated; the progeny CG 489-31 is being released by the Colombian MADR to cassava producers in Nov. 2002.

The objectives of this research project is to determine if the *A. socialis* resistant sources will also express resistance to the B biotype of *Bemisia tabaci*.

Methodology

The stock of Biotype "B" of *B. tabaci* to initiate a colony on cassava came from the CIAT Bean Improvement Project (IP-1). *B. tabaci* adults, harvested from the bean colony, were allowed to first oviposit on poinsettia (*Euphorbia pulcherrima*). After five generations established on poinsettia, the colony was transferred to Jatropha (*Jatropha gossypiifolia*), where it has been established for 12 generations. The colony established on Jatropha was then transferred to both *Manihot esculenta* and *Manihot carthaginensis* (Figure 6.12). The "B" biotype of *B. tabaci* colony has now been reared for two generations on *M. esculenta* (Var. MCol 2063) and 3 generations on *M. carthaginensis*. Colonies are maintained at CIAT in growth rooms under controlled conditions: 12 hrs. photoperiod, 25 ± 2°C and 50-80 % RH. This methodology was

designed in order to gradually or progressively adapt *B. tabaci* from beans to *Manihot* species by passing it through related species of the Euphorbiaceae family.

Bean plants are held in the screen house for 17 days; poinsettia and Jatropha for 40 to 50 days and cassava 30 to 40 days, before being exposed to the *B. tabaci* colony. Plants are grown in 15cm diameter plastic pots, watered daily and receive no fertilizer nor pesticide during their development. Plants are placed in fine nylon meshed wooden cages (1m ht x 1m width), where *B. tabaci* infestation takes place. Whitefly adults are harvested with a pipette suction devise, from the colony and released in the experimental cages. Fresh plants of each species are supplied for each of the colonies on a regular basis; usually no more than two generations of *B. tabaci* are reared on the same plant.
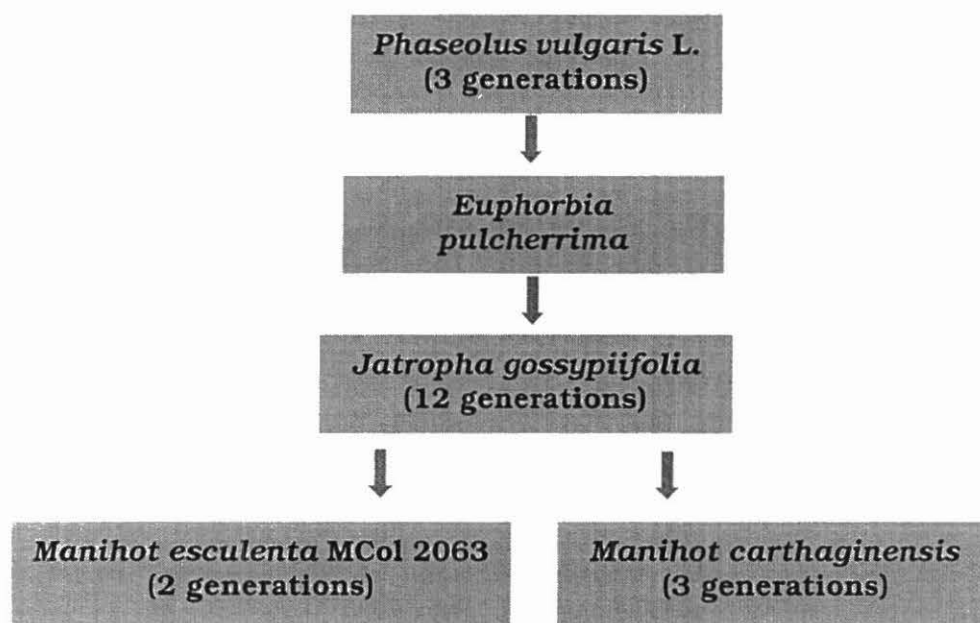
Figure 6.12. Plant species sequence for adapting the whitefly species *Bemisia tabaci*, B biotype, from beans (*P. vulgaris*) to *Manihot esculenta* and *M. carthaginensis*.

The cassava variety MCol 2063 was selected as a host plant because it is recognized as being very susceptible to whiteflies, especially to *A. socialis*. *M. carthaginensis*, also known as "yuca de Cartagena" is a wild species that grows naturally on the North Coast of Colombia and is being used in genetic improvement programs because of its high protein content, considerably higher than cultivated species of *M. esculenta*.

Preliminary studies were done with four wild species, *Manihot flabellifolia, M. peruviana, M. tristis* and *M. carthaginensis*, to evaluate their potential to host the B biotype of *B. tabaci*. *B. tabaci* adapted best to *M. carthaginensis* so this species was selected to establish a *B. tabaci* colony.

B. tabaci became established on MCol 2063 after 2 generations and on *M. carthaginensis* after 3 generations. The evaluation of cassava genotypes for resistance to *B. tabaci* was first done by exposing them to the *M. carthaginensis*, since this host showed the best adaptation.

Every 3 months a molecular identification of the *B. tabaci* colony is carried out using RAPD-PCR to assure that contamination with the "A" biotype has not occurred.

The initial three *M. esculenta* genotypes selected to be evaluated are CMC-40 MEcu 72 and CG 489-34. CMC-40 is highly susceptible to *A. socialis*; the *A. socialis* colony is maintained on this genotype. MEcu 72 is highly resistant to *A. socialis*; evidenced by low populations, no damage symptoms and high nymphal mortality in laboratory studies. CG 489-34 is a hybrid progeny of a MEcu 72 x MBra 12 cross, displaying moderate levels of resistance to *A. socialis*.

Experimental Procedures: HPR experiments with the three cassava genotypes were done in growth chambers under controlled temperature, humidity and photoperiod conditions. MEcu 72, CG 489-34 and CMC-40 plants were multiplied through vegetative cuttings and planted in 15cm diameter plastic pots. Plants were placed in a screen house. Experiments were initiated by selecting plants that possessed 4 to 6 true leaves and approximately 30 to 50 cm high. Biotype "B" *B. tabaci* recently emerged adults were harvested from the *M. carthaginensis* colony by using a pipette aspirator and a glass vial with a perforated lid and 40 pair were sexed. Each pair was placed within a 2.5cm diameter leaf-0clip cage (Figure 6.13) on a cassava leaf. Every 48 hours the adult pair was moved to new area of the leaf, until the female died. Fecundity was measured by counting eggs oviposited by each female during the 48 hr. period.
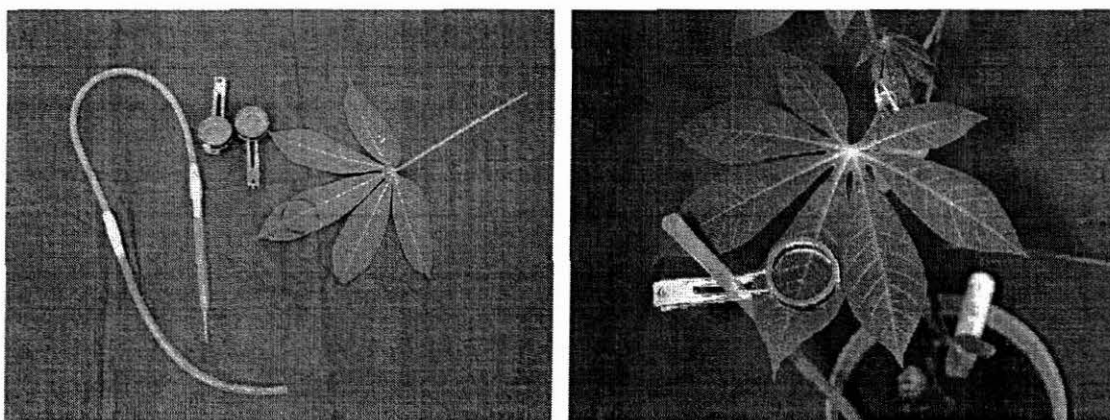
Figure 6.13. Methodologies for installing male and female biotype B of *Bemisia tabaci* on cassava (*Manihot esculenta*) leaves.

Experiments to determine development time, rate of survival and proportion of *B. tabaci* ("B") females developed were carried out under the previously described conditions. 40 adults *B. tabaci* "B" biotype were placed in the leaf cages on the leaf underside of each genotype. Adults were removed after 6 hours and 200 eggs were selected. Egg hatch and nymphal development or mortality were observed until the adult stage and the proportion of emerged females was noted (Figure 6.14).

Demographic parameters were determined using a methodology described by Manzano (2000). Data on development time and survival of immatures were combined with experimental reproduction data "lxmx," to create life tables and calculate demographic parameters for *B. tabaci* "B." For each experiment the following parameters, defined by Price (1975) were calculated: net reproduction rate (Ro, this represents the number of females

produced by each female in one generation), generational time (T = the average time span required between the birth of the parents and the birth of their progeny. The intrinsic rate of increase of a population (r_m) for B. tabaci "B" is estimated using the equation proposed by Carey (1993):

$$\sum exp(-r_m x) l x m x = 1$$

Where:
$\quad$ x $\quad$ = age
$\quad$ Lx $\quad$ = age of specific survival
$\quad$ Mx = proportion of the females of the progeny of a female at age x.

To calculate the r_m values, the corrected age x + 0.5 was used (Carey, 1993).
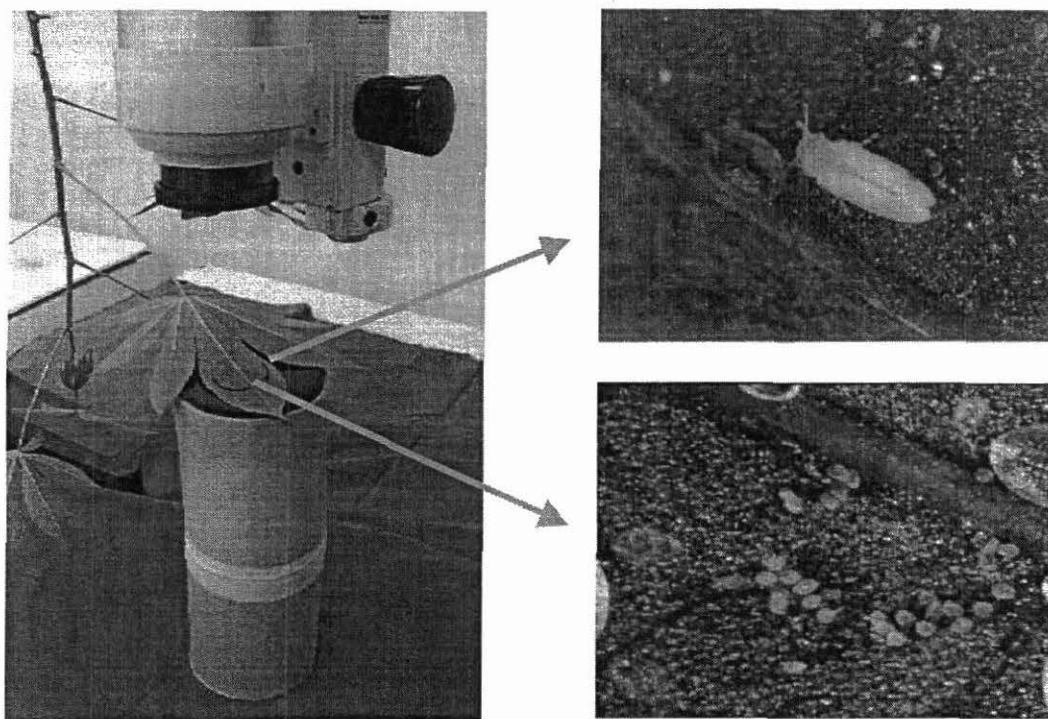
Figure 6.14. Techniques for recording egg and adult populations of biotype B of *Bemisia tabaci* on *cassava leaves*.

Data Analysis:

- Significant differences between the longevity values and fecundity on each cassava genotype were determined with an analysis of variance using the Kruskal-Wallis test.

- Values for oviposition rates were determined using an ANOVA analysis. For the three parameters, multiple comparisons were done using the Student-Newman-Keuls test.

- The differences in development time between cassava genotypes were evaluated with an analysis of variance using the Kruskal-Wallis test. Comparisons of survival rates were done using the x^2 test.

Results

A. Adaptation of *B. tabaci* "B" on several hosts.

Mean longevity of *B. tabaci* biotype "B" was highest on cassava when females originated from poinsettia (*E. pulcherrima*), when compared Jatropha (*J. gossypiifolia*) and beans (*P. vulgaris*). Average longevity differed significantly between the three hosts, except for the longevity values of *J. gossypiifolia* and *P. vulgaris* (Student Newman-Keuls $P < 0.05$, after Kruskal-Wallis $P < 0.0001$). Longevity of adults on cassava, originating from the 3 hosts is shown in Figure 6.15. Average fecundity was also significantly different between the three hosts (Kruskal-Wallis $P < 0.0001$), except for the values obtained between *E. pulcherrima* and *J. gossypiifolia* (Student Newman-Keuls $P < 0.05$) (Table 6.6). Reproduction curves indicated by daily oviposition (Figure 6.16) resulted in higher oviposition on *J. gossypiifolia* although not over a long time period.

The average ovipositioned rate (eggs per female over two days) was higher for *J. gossypiifolia* (2.64) (Table 6.6). Average ovipositional rate was significantly different between treatment (ANOVA, $P < 0.0001$); comparisons between females originating from the three hosts show no significant differences in average ovipositional rate (student Newman-Keuls $P < 0.05$).
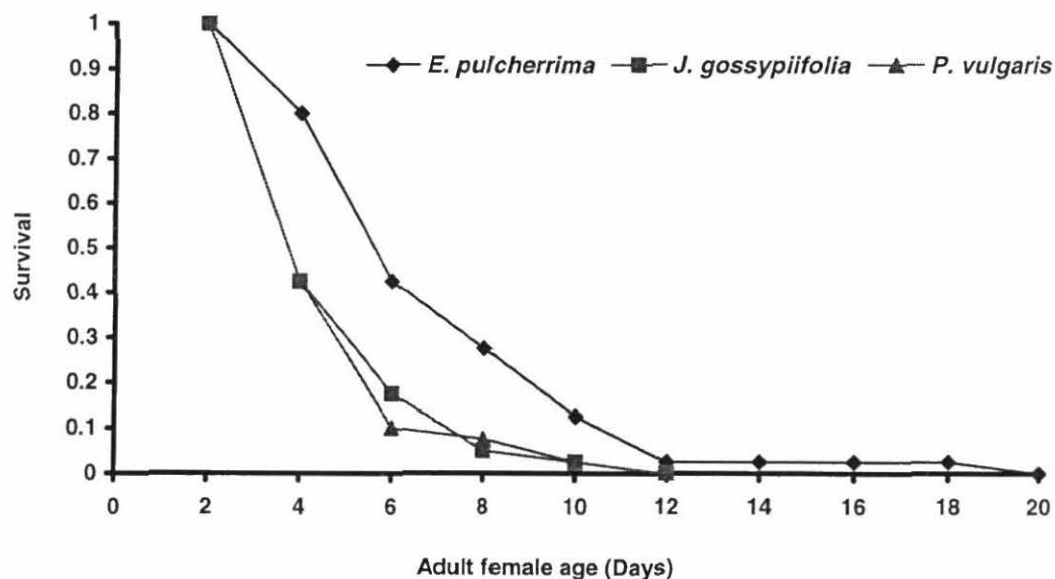
Figure 6.15. Survival curves of biotype B of *Bemisia tabaci* adult females, that originated from three separate hosts, (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).
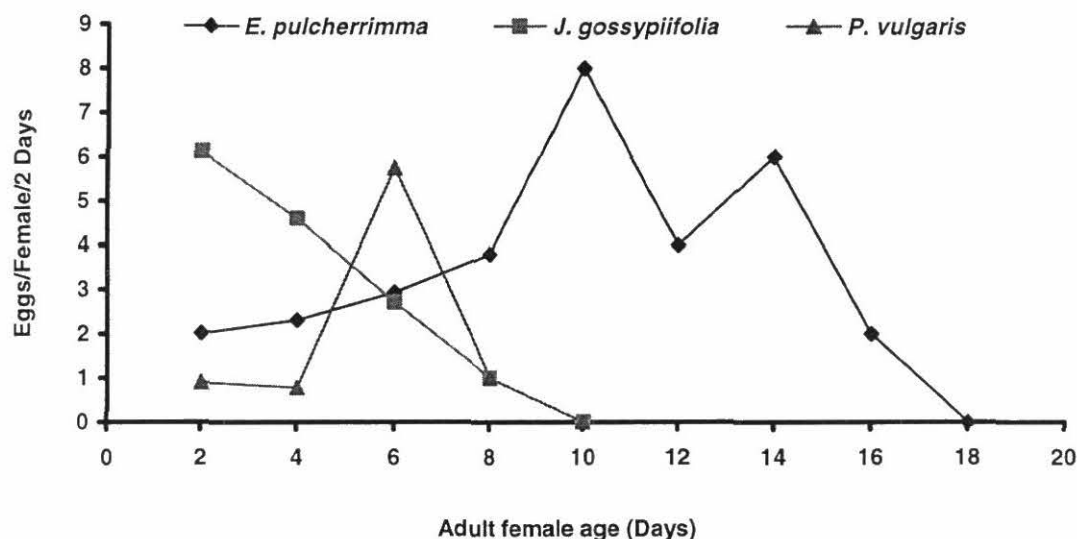
Figure 6.16. Reproduction curves of biotype B of *Bemisia tabaci* females that originated from three separate hosts (*Phaseolus vulgaris*, *Euphorbia pulcherrima* and *Jatropha gossypiifolia*).

Table 6.6. Mean longevity (d), mean fecundity and rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on cassava from females originating from 3 host populations.

Parameter	*J. gossypiifolia*	*E. pulcherrimma*	*P. vulgaris*
Mean Longevity*	3.25 a	5.6 b	3.1 a
Range	2-10	2-18	2-10
# Insects	40	40	40
Mean Fecundity*	8.6 a	7.65 a	1.82 b
Range	1-41	1-48	1-19
Mean Rate Oviposition ϕ	2.64 a	1.36 b	0.58 c
Range	0.5-8	0.4-3	0.5-3.5

Figures followed by different letters across columns indicate significant differences.
* Kruskal-Wallis $P < 0.0001$, Student-Newman-Keuls method $P < 0.05$.
ϕ One-way ANOVA $P < 0.0001$, Student-Newman-Keuls method $P < 0.05$.

Development time for progeny from females from *E. pulcherrima* (50 days) and *P. vulgaris* (49.5) were similar; the lowest development time was from progeny from *J. gossypiifolia* females (44.4 days). The rate of survival for immatures was significantly different for *J. gossypiifolia* with respect to the other two hosts. The proportion of females was equal for all three hosts (50%) Table 6.7.

Discussion: The intrinsic rate of growth (r_m) of *B. tabaci* "B" originating from each of the hosts and developing on *M. esculenta* (MCol 2063), permits determining that *J. gossypiifolia* was best adapted in that the *B. tabaci* "B" population had the highest intrinsic rate of growth,

exceeding *E. pulcherrima* by 8.3% and *P. vulgaris* by 58.3%. In addition it presented the lowest generational time, 44.76 days from generation to generation (Table 6.7).

Survivorship of the immature states of *B. tabaci* "B" was 27.5% on *M. esculenta/J. gossypiifolia* relationship, considerably higher than the other two hosts. When this is expressed together with other demographic parameters (Table 6.7) the adaptation advantage that *B. tabaci* "B" has when population originate on *J. gossypiifolia* is obvious when compared to the other hosts.

Table 6.7. Demographic parameters from biotype B of *Bemisia tabaci* on *Manihot esculenta*; adult females originating from three separate plant host species.

Parameter	J. gossypiifolia	E. pulcherrima	P. vulgaris
Development time (d)	44.41	50.60	49.50
Rate of survival (%)	27.50	3.00	2.00
Proportion females (%)	50.90	50.00	50.00
Intrinsic rate of increase	0.048	0.044	0.020
(r_m)	8.63	11.60	1.82
Net reproductive rate (Ro)	44.76	56.03	51.30
$\sum lxmx$			
Generation time (T)			

B. Evaluation of resistance/tolerance of cassava genotypes B. tabaci "B."

The low intrinsic rate of increase values (r_m) and the demographic values of *B. tabaci* "B" on *M. esculenta* (MCol 2063) indicate that population is low on this host and that there is not a significant increase in one generation to another. Because of these results, and additional host was sought to adapt populations of *B. tabaci* "B" to *M. esculenta*. *M carthaginensis*, because it is closely related to *M. esculenta* was chosen as the population source for evaluation of *M. esculenta* genotypes (complete data on *M. carthaginensis* not yet available) (Figure 6.17).
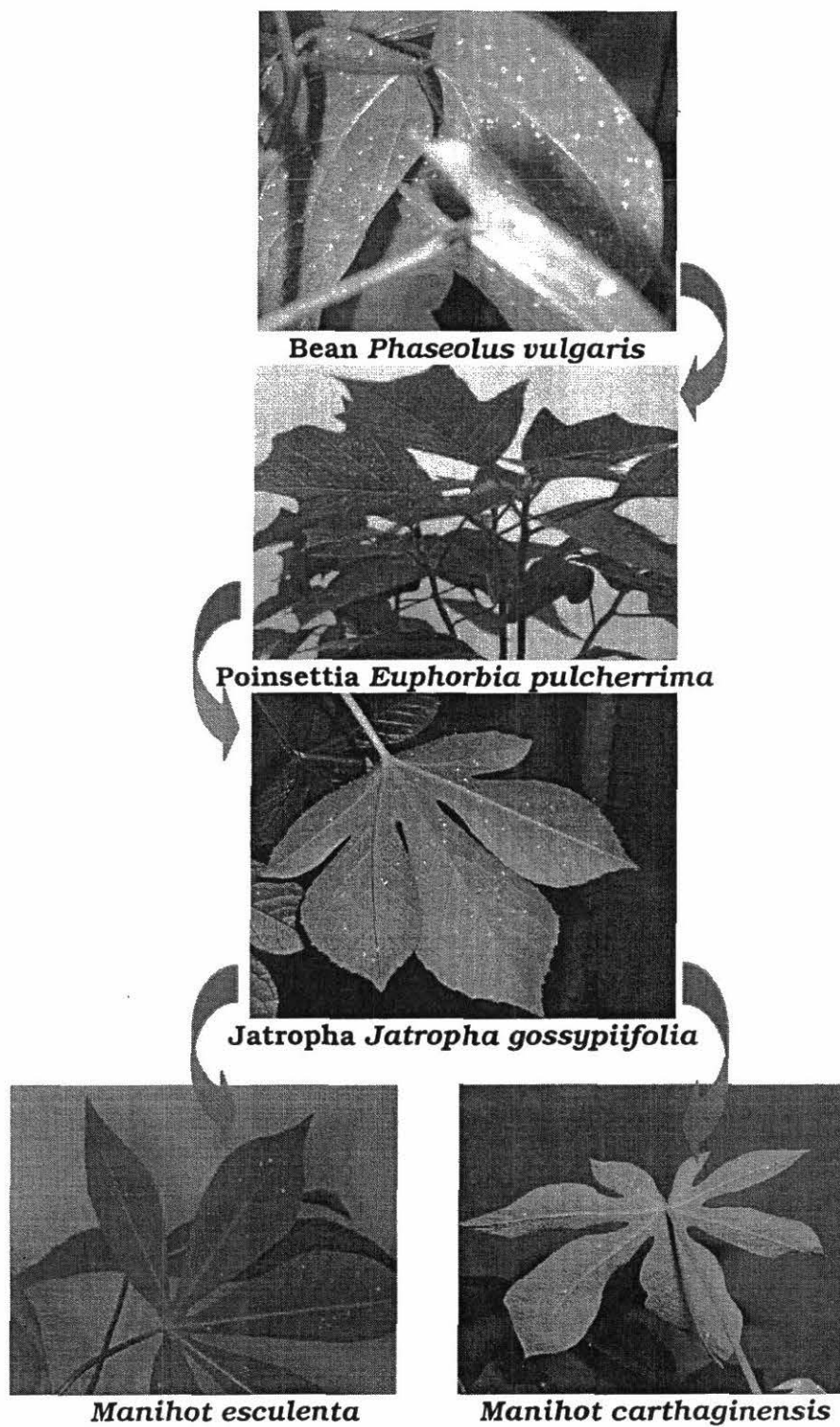
Figure 6.17. Photo scheme of plant species sequence for adapting *Bemisia tabaci*, biotype B from beans to *Manihot esculenta* and *M. carthaginensis*.

Preliminary results indicate that adult longevity on the three genotypes, CMC-40, MEcu 72 and CG 489-34 ranged from 2 to 20 days, but was longest on MEcu 72 (Figure 6.18). Average longevity was not significantly different between genotypes (Kruskal-Wallis, P=0.0809). The fecundity range for the three genotypes was 1 to 40 eggs per female over 2 days. The average fecundity was significantly different between genotypes (Kruskal-Wallis, P < 0.0001), except for MEcu 72 values (6.3) and CG 489-34 (5.07) (Student-Newman-Keuls, P < 0.05) (Figure 6.19). Oviposition was lowest on CMC-40.
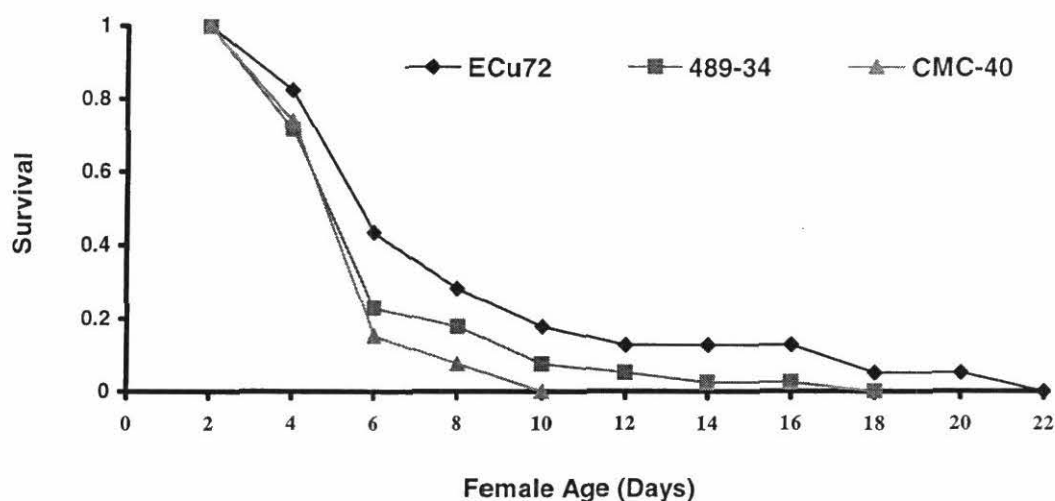
Figure 6.18. Survival curves of biotype B of *Bemisia tabaci* on three cassava genotypes: MEcu 72, CG 489-34 and CMC-40.
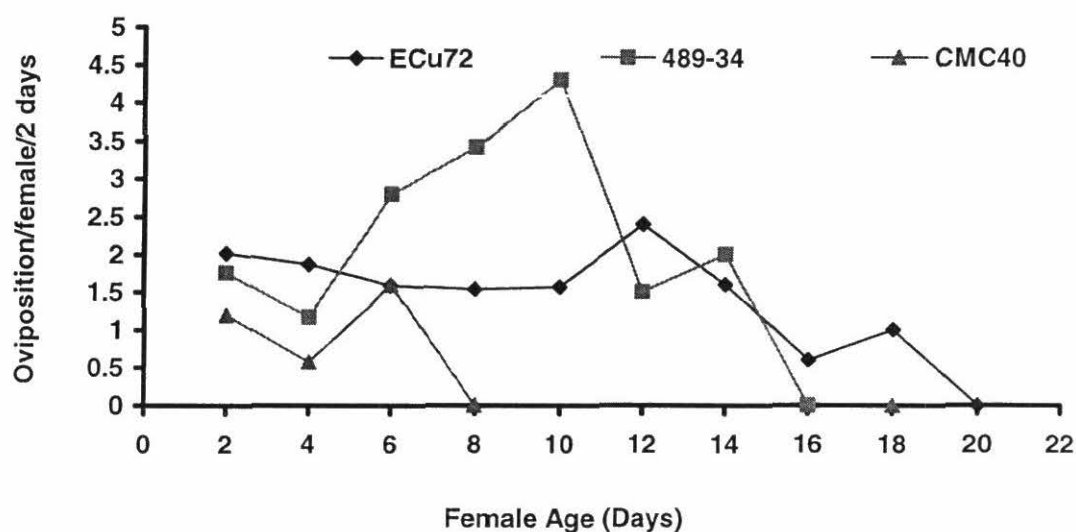
Figure 6.19. Reproduction curves of biotype B of *Bemisia tabaci* on three genotypes of cassava: MEcu 72, CMC-40 and CG 489-34.

The average ovipositional rate (eggs/female/2 days) increased from 0.49 in CMC-40 to 0.89 in MEcu 72. The average rate of oviposition was significantly different for the 3 genotypes (One-Way ANOVA P < 0.0001), except for the comparison between MEcu 72 and CG 489-34 (Student-Newman-Keuls, P < 0.05) (Table 6.8).

Table 6.8. Mean longevity (days), Mean fecundity (eggs) and mean rate of oviposition (eggs/female/2 days) of biotype B of *Bemisia tabaci* on three cassava genotypes.

Parameter	CG 489-34	CMC-40	MEcu-72
Mean longevity *	5.07a	3.9a	6.3a
Range	2-16	2-8	2-20
# Insects	39	39	39
Mean fecundity *	4.35a	1.89b	5.61a
Range	1-24	1-12	1-40
Mean Rate of oviposition	0.86a	0.49b	0.89a
φ Range	0.25-1.56	0.25-2.75	0.25-3.8

Figures followed by different letters across columns indicate significant differences.
* Kruskal-Wallis $P < 0.0001$, Student-Newman-Keuls method $P < 0.05$.
φ One-Way ANOVA $P < 0.0001$, Student-Newman-Keuls method $P < 0.05$.

Development time for MEcu 72 was 55.1 days, (Table 6.9) indicating a very low level of adaptation for this *B. tabaci* "B" on this genotype. On genotypes CMC-40 and CG 489-34 *B. tabaci* "B" did not complete its cycle of egg to adult, only permitting nymphal development to the third instar (Figure 6.20).
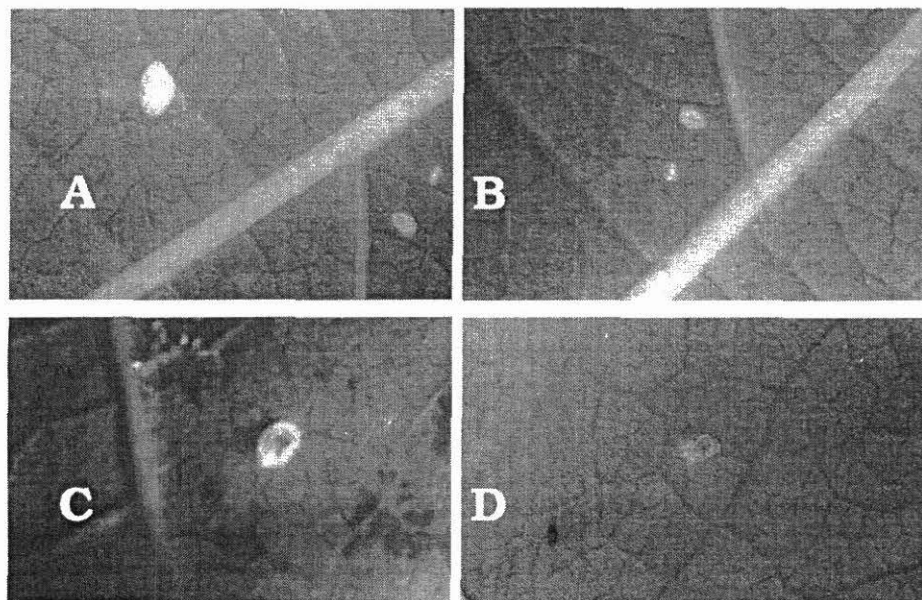
Figure 6.20. Development stages of biotype B of *Bemisia tabaci*: A and B. N1, N2 and N3 (30 days) on MEcu 72; C. N2 and eggs (30 days) on CG 489-34, and D. N2 (30 days) on CMC-40.

These preliminary results indicate that none of the three cassava genotypes are adequate hosts for *B. tabaci* "B" as it only reached the adult stage on MEcu 72 with a low survival of only 3% (Table 6.9).

In addition, since *B. tabaci* "B" females oviposited on all 3 genotypes, oviposition is not a good indication of adaptability or host acceptance since nymphal survival did not occur on two genotypes and was very low on the third. It should also be noted that MEcu 72 is resistant to *A. socialis*, CG 489-34 is moderately resistant and CMC-40 is susceptible. Results expressed in these experiments, although not conclusive, are different.

Table 6.9. Demographic parameters of biotype B of *Bemisia tabaci* on genotype MEcu 72.

Parameter	MEcu 72
Development time (d)	55.1
Rate of survival (%)	3
Proportion of females (%)	33
Intrinsic rate of increase (r_m)	0.2958345
Net reproduction rate (Ro) $\sum lxmx$	5.61
Generation time	58.33

Activity 6.4. Evaluation of thrips (*Frankliniella williamsi*) damage on dialel crosses (36 families at Pitalito, Atlántico, Colombia, 2002).

Thrips (*F. williamsi*) is a dry season pest that can cause sever damage to cassava if a prolonged attack occurs and susceptible varieties are being grown. Thrips resistance is common in cassava germplasm, approximately one-half the cultivars are resistant. Pubescent cultivars express resistance and this is a heritable trait. If non-pubescent varieties are grown in seasonally dry areas, severe damage and yield losses can occur, often requiring pesticide applications. Yield losses of 25 to 30% on susceptible varieties is common.

Several trials managed by the breeding section were evaluated at Pitalito, Atlántico. These included dialelic crosses, observation fields, yield trials and others. From an entomological perspective, these trials are evaluated for pest resistance/susceptibility to the several pests that might occur during the cropping cycle in this agroecosystem.

A dialelic cross of 36 families, with 30 plants per family in three replications (total 3240 plants) were evaluated individually (each plant) for thrips damage. A 1.0 to 6.0 damage scale (1.0 = no damage/resistant; 6.0 = severe damage/susceptible). The highest damage rating of the three replications was noted, resulting in 1047 cultivars rattled.

Results show a high level of thrips resistance in these families (Figure 6.21). 483 of the 1047 clones (46%) presented no thrips damage (grade 1.0) and 385 (37%) had a low rating of 2.0, indicating that 83% of the cultivars evaluated are resistant to thrips. 58 cultivars (5.5%) had

intermediate damage ratings (3.0) and 121 (11.5%) presented high to severe damage and are classified as susceptible. Dialelic crosses, because of the quantity of possible crosses can increase the number of resistant germplasm.
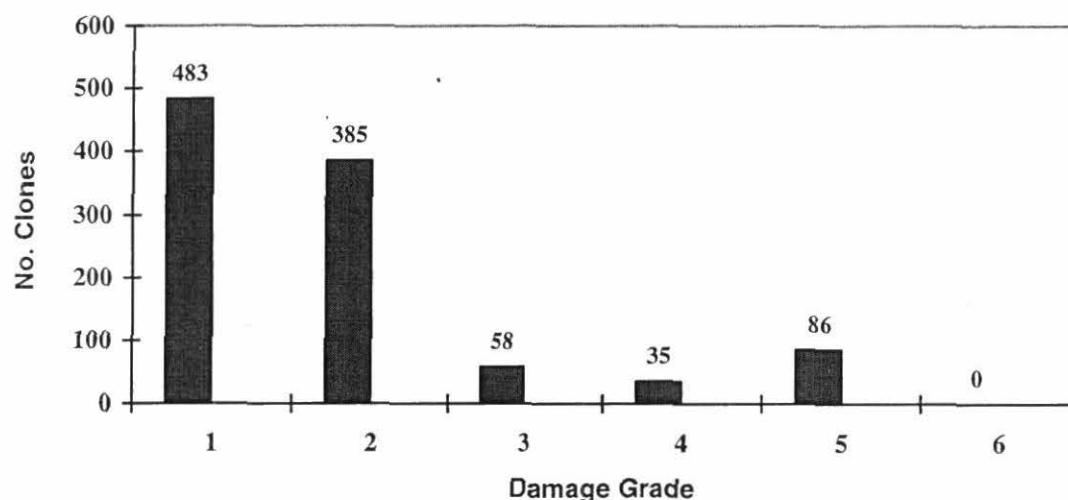

Figure 6.21. Evaluation of thrips (Frankliniella williamsi) damage on cassava dialelic crosses 36 families (Pitalito, Atlántico, Colombia, 2002).

Activity 6.5. Evaluation of cassava germplasm for resistance to the cassava green mite, Mononychellus tanajoa.

Rationale

Mites are a universal pest of cassava although the species complex may differ between regions. More than 40 species of mites have been reported feeding on cassava; the most frequent are *Mononychellus tanajoa*, *M. caribbeanae*, *Tetranychus cinnabarinus* and *T. urticae* (also reported as *T. bimaculatus* and *T. telarius*. Serious yield losses due to mites are reported only from the Americas and Africa. Cassava is the major host for the *Mononychellus* species, whereas the *Tetranychus* species have a wide host range. Numerous species of mites are reported as feeding on cassava in Asia, but none appear to be causing serious damage nor yield losses.

The cassava green mite (CGM), *M. tanajoa* is the most damaging species and is reported causing crop losses in the Americas and Africa, especially in the seasonally dry regions of the lowland tropics. *M. tanajoa* is a native to the neotropics, first reported from Brazil in 1938. More recent evidence indicates that its origin (as well as the genus *Mononychellus*) may have been originated in the northern part of South America (Colombia and Venezuela). It first appeared in Africa (Uganda) in 1971 and by 1985 it had spread across most of the cassava belt occurring in 27 countries and causing estimated root yield losses of 13 to 80%. Yield losses in the Americas due to *M. tanajoa* range 15 to 73% depending on length of dry period and varietal susceptibility.

In the Americas; cassava agroecosystems can be characterized by several major factors in their relation to the management of cassava green mites.

1. The length and severity of the dry period is important in determining the extent of the pest populations build up over time and the ability for the plant to recover.

2. A large complex of natural enemies, especially the predatory mites (Fam.: Phytoseiidae) has been identified associated with CGMs. Research has shown that some key species, or a combination of them can effectively reduce CGM populations down to or below economic injury level.

3. Cassava germplasm in the neotropics have been shown to contain low to moderate levels of resistance to *M. tanajoa*. This resistance or tolerance can complement host plant resistance and make an important contribution to managing CGM populations.

4. Pesticide use for control of mites (or other pests such as thrips, whiteflies and stemborer) can disrupt this system, especially the existing biological control. CGM predators are very sensitive to chemical pesticides, and even a low doses they can easily destroy the predatory biocontrol mite and cause a serious phytophagous or CGM outbreak.

The sensitivity of CGM biocontrol agents to pesticide use is ample justification to give considerable support to a strategy to identify, develop and deploy cassava germplasm with, at least, low levels of resistance or tolerance to CGM as a complementary system. CIAT's mite resistance research has been on-going for numerous years, having evaluated more than 5000 accessions from the CIAT Cassava Germplasm Bank. About 6% or about 300 cultivars have been identified as having low to moderate levels of resistance. CIAT's mite resistance has traditionally been carried out at two sites: (1) CIAT Palmira, located in the mid-altitude (1000 m) Andean Highlands, where mite populations are usually moderate; and (2) the Magdalena region of the Colombian Atlantic Coast, in the lowland tropics with a prolonged (4 to 6 months) dry season and high mite populations. Low to moderate levels of resistance are indicated by a 0-3.5 damage ratings on a 0-6 evaluation scale. Due to security problems in recent years the evaluation site in the Magdalena regions (i.e. Pivijay) has often been inaccessible, and therefore much of our resistance research has shifted to CIAT, with an increase in greenhouse and laboratory studies. In addition the problem with frog skin disease on the CIAT station and the inability to move germplasm has also hindered germplasm evaluation.

Since about 1996, whitefly populations in CIAT cassava fields increased dramatically, while mite populations (and to a certain degree thrips populations) decreased and no field evaluations were done during this periods. During 2000 a "veda" (prohibition) on cassava growing was imposed for one month on the CIAT farm. Whitefly populations in plantings after the veda were considerably lower, never attaining the level prior to the veda. A result of this practice was the subsequent increase in the populations of other pests, especially mites and thrips. The increase in mite (*M. tanajoa*) populations were undoubtedly aided by the unfortunate decision to apply pesticide to control thrips during the Dec. 2001 to Feb. 2002 dry period. These pesticides applications probably reduced natural enemy populations of mites and other pests. A sampling of CIAT cassava fields at that time disclosed that very few phytoseiid mite predators had survived pesticide applications. This, combined with a reduced whitefly competition, resulted in CGM outbreak.

All of the evaluations of field trials described below were done in collaboration with cassava breeders and genetists as part of an on-going activity to evaluate all cassava germplasm, including hybrids for CGM resistance.

Mite (CGM) Field Evaluations: The CGM outbreak resulted in considerable mite damage to most of the cassava trials planted at CIAT. An evaluation of all of this germplasm was carried out using a 1 to 6 damage scale (Table 6.10). A total of 8368 genotypes were evaluated. These included the following trials GY200156, GY4200172, GY200126, GY200127, GY200130, GY200132, GY200173 and GY200180. The damage rating for 5128 of these genotypes can be observed in Figure 6.22. These results show the high CGM field populations in that 3759 genotypes or 73% had high damage ratings of 4 or above.

Table 6.10.   Damage evaluation scale for cassava green mite, *Mononychellus tanajoa.*

|	
1.0	No damage to growing point of cassava plant.
2.0	Shoots, and/or adjacent leaves with a few yellowish spots.
3.0	Shoots and /or adjacent leaves with many yellowish spots.
4.0	General yellowing of apical part of the plant; a slight reduction in shoot size.
5.0	Severe yellowing of apical shoots and leaves, shoot deformation and reduction in size.
6.0	Shoot totally reduced, no leaves on apical part of plant; yellowing and defoliation of intermediate part of the plant. Shoots may die.
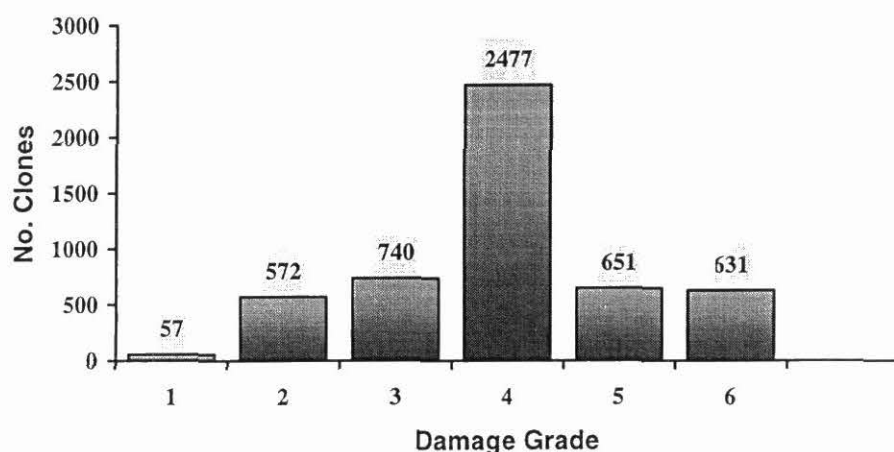
Figure 6.22. Evaluations of 5218 cassava genotypes for damage/resistance caused by CGM (*Mononychellus tanajoa*) feeding; trials planted at CIAT during 2002 season.

Most of the cultivars in the CIAT cassava germplasm bank, over the years, have been evaluated for CGM damage in the field so any evaluations done at this time can be compared to previous evaluations. In field evaluations, there is always the possibility of "gaps" since mite behavior tends towards focal points of high populations, and gaps, or lower populations between these "center of action" or core population points. The continued evaluation of germplasm, whenever outbreaks occur can help eliminate these "escapes."

Trials GY200156 and GY200172 are genotypes from the germplasm bank. 2359 genotypes were evaluated (Figure 6.23), 2135 or 90.5% had damage ratings of 4.0 or above, indicating a high selection pressure. No genotype was rated 1.0, or no damage and this is consistent with previous evaluations and confirms that CGM resistance in cassava is at low to moderate levels. 224 genotypes did result in ratings of 3.0 or below. Of these 27 were previously selected as resistant and these results confirm those evaluations (made at CIAT, Palmira and Pivijay, Magdalena) (Table 6.11).
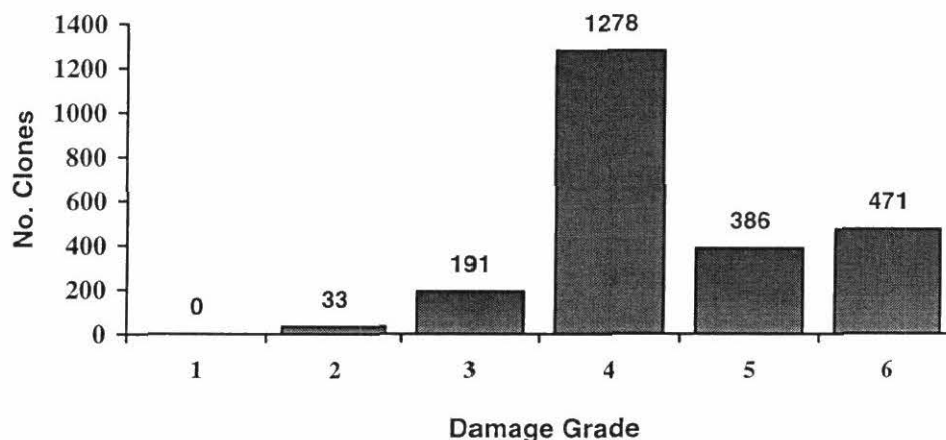

Figure 6.23. Evaluation of damage caused by the CGM, *Mononychellus* socialis on cassava genotypes; GY200156 and GY200172 trials at CIAT, Palmira (2002).

Table 6.11. Twenty seven (27) genotypes selected for resistance to the CGM (*Mononychellus tanajoa*) from an evaluation of 2359 clones from the cassava germplasm bank planted at CIAT during 2002.

CG 489 -1	MCol 2179
CG 502-1	MEcu 85
CM 3456-3	MMex 71
CM 6070-1	MPer 255
CM 6173-8	MPer 265
MBra 93	MPer 315
MBra 245	MPer 317
MBra 276	MPer 322
MBra 391	MPer 560
MBra 410	MVen 121
MBra 411	MVen 133
MCol 1254	MVen 174
MCol 1522	MVen 276
MCol 1856	

Observations Fields, GY200126: This field of 872 genotypes, 7 plants to each genotype. 507 genotypes or 58% had damage ratings of 4.0 or above (Figure 6.24).
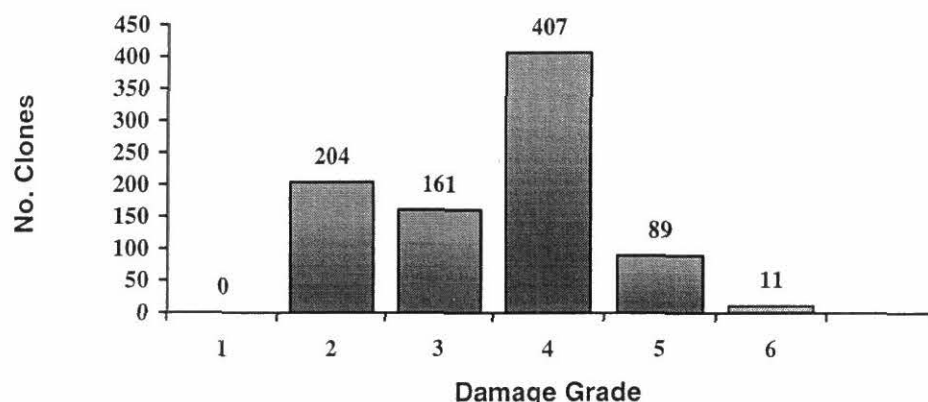
Figure 6.24. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field Trial GY200126, (CIAT, Palmira, 2002).

The 42% of the genotypes with a 2.0 or 3.0 damage rating is possibly and indication of good mite resistance contained in these genotypes. However these relatively low ratings could also be due to escapes and since this was the first time these genotypes had been evaluated for mite resistance, they should be reevaluated in future trials.

Yield Trials, 1st cycle, GY200127: 110 genotypes with 10 plants in three replications were evaluated. Mite damage was very high, 96 or 87% had damage ratings of 4.0 or above (Figure 6.25). The remaining 14 genotypes that had damage ratings of 2.0 or 3.0 indicate the possibility of good resistance levels and should be reevaluated. These consist of the following 14 genotypes.

SM 2576-5	SM 2653-6
SM 2580-4	SM 2655-3
SM 2584-5	SM 2655-6
SM 2584-21	SM 2659-2
SM 2588-6	SM 2659-12
SM 2649-3	SM 2663-5
SM 2649-13	SM 2799-17

Observation Fields, GY200132. These are FICI's and 546 genotypes, each containing 6 plants were evaluated. 317 or 58% had damage ratings above 4.0 and can be discarded as susceptible to CGM (Figure 6.26). A relatively high number, 229 genotypes (42%) were evaluated as "promising," also indicating that mite populations may not have been sufficiently high nor evenly spread. These 229 genotypes need to be re-evaluated.
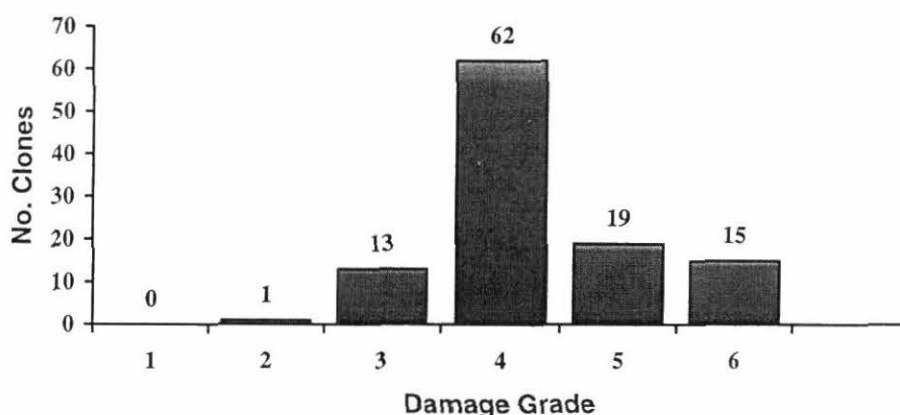
Figure 6.25. Evaluation of damaged caused by CGM *Mononychellus tanajoa* on cassava germplasm; Yield Trial, CY 200127 (CIAT, Palmira, 2002).
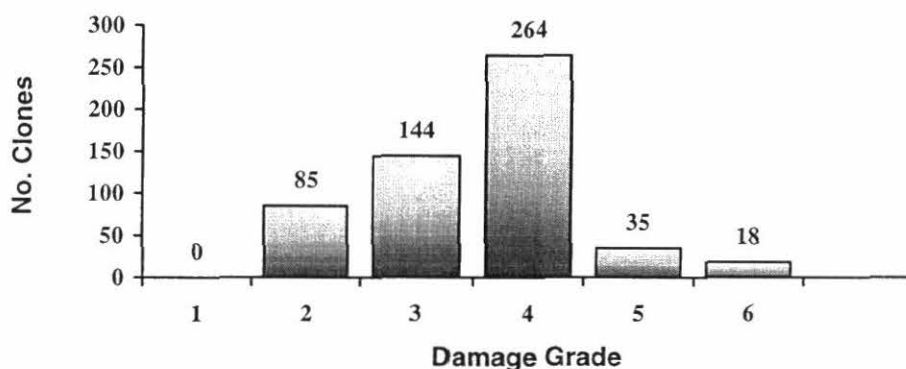

Figure 6.26. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field Selections, FICI GY200132 (CIAT, Palmira, 2002).

Observation Field GY200173: This trial was designed to study the genetic composition and quality of 604 genotypes (6 plants of each clone) resulting from crosses of 40 families. The grouping of these genotypes, based on the evaluations made, is different from the previous trials that have been described (Figure 6.27). 325 genotypes, or nearly 54% had damage ratings in the 1 to 3 range. The distinct characteristic of this trial is that 5 families originated from the wild genotype MFLA 437-7, having this parent in its background. The use of wild species as a source or resistance to cassava pests, especially mites has always been a distinct possibility. These results, which need to be verified in future trials, indicate that the wild species should be considered as a resistance source for cassava arthropod pests.
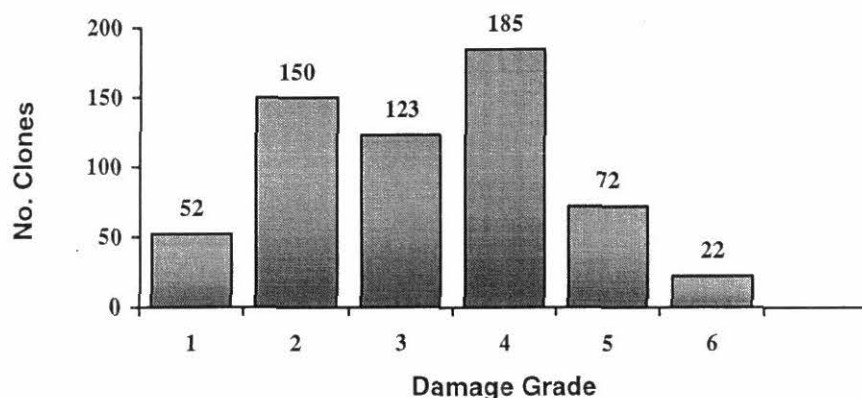
Figure 6.27. Evaluation of damage caused by the CGM, *Mononychellus tanajoa*, on cassava genotypes; Observation Field, Genetic Quality CG 200173 (CIAT, Palmira, 2002).

Observation Field, self GY200180: The genotypes that were selfed in this trial were MCol 72, MCol 1505, HMC1, MTAI-1, MVen 77, CM 849-1. The total number of genotypes from these self-crosses was 455 progeny of which 152 (34.4%) resulted in damage ratings of 2.0 or 3.0 (Figure 6.28). The genotype selfed that had the greatest number of progeny with low damage rating was CM 489-1, with 61.8% of the progeny evaluated in the 2.0 to 3.0 range.
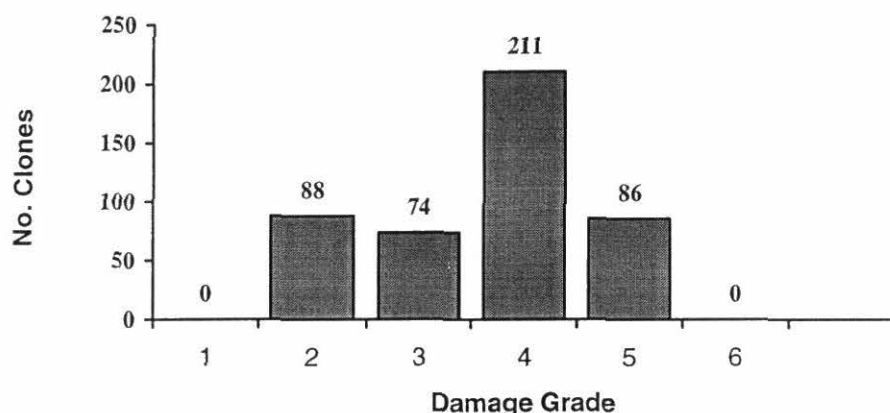

Figure 6.28. Evaluation of damage caused by the CGM, *Mononychellus tanajoa* on cassava genotypes; Observation Field self crosses GY200180 (CIAT, Palmira, 2002).

Dialelic cross/CIAT (ZEC-04) GY200130: This trial was planted to study the genetic segregation of the progeny of 36 crosses. The evaluations were made to determine the reaction of the genotypes to CGM attack. 149 genotypes resulted in a damage rating between 2.0 and 3.0 in the replications; 113 genotypes of the selected progeny (low damage ratings) corresponded to the CM 306, CM 308, CM 309, CM 310, CM 311, CM 312 and CM 313 crosses, all of which have MEcu 72 as the female parent. These results present an interesting case in that MEcu 72 is resistant to whiteflies, mites and thrips. Crosses made with this genotype should or could be used to obtain resistance to pests across ecosystems.

Unfortunately in the CIAT planting there was a high incidence of Frog Skin Disease and this impedes the continued evaluation of the selected genotypes in the several trials. An effort

needs to be made to recuperate some of the more elite materials in terms of resistance to mites. More importantly, we are now are knowledgeable of which crosses may result in the best mite resistant progeny. There is a need to follow up on this. Particular attention should be given to crosses including wild Manihot species and the self crosses.

Activity 6.6. Phytophagous mite identification, cassava and other crops.

For more than 25 years CIAT has collected and maintained a collection of phytophagous mites. This collection is updated with new additions every year and information stored in a database is available to collaborators around the Globe (i.e. recent request from The Colombia Federation of Rice Growers (FEDEARROZ), the Instituto Colombiano Agropecuario (ICA), Instituto Nacional de Investigaciones Agropecuarias (INIA), Venezuela, El Instituto de Investigación Agropecuaria de Panamá (IDIAP) and Exportadores de Bananos del Ecuador (DOLE).

By constantly adding to the collection and database, through our surveys and travels to other countries and regions, we are able to map more accurately the distribution of these pests and their natural enemies. This is valuable information for IPM, Biological Control or Germplasm Development Programs. During 2002, phytophagous mites were collected from cassava, rice, uchuva, sorghum, banana and cratylia from Colombia, Panamá, Ecuador and Venezuela (Table 6.12).

Table 6.12. Phytophagous mite species collected from cassava and other host during 2001-2002 and added to CIAT collection.

Sample	Country	Dept.	Site	Host	Species
2567	Colombia	Atlántico	Polonuevo	Cassava	*Tetranychus tumidus* *Oligonychus gossypi**
2568	Colombia	Atlántico	Pitalito	Cassava	*Oligonychus gossypi** *O. peruvianus**
2569	Colombia	Atlántico	Baranoa	Cassava	*Mononychellus tanajoa*** *M. caribbeanae**
2574	Colombia	Valle	Palmira, CIAT	Rice	*Schizotetranychus paezi*
2576	Colombia	Cundinamarca	Granada	Uchuva	*Eriophyidae's mites* *Tarsonemidae's mites*
2577	Colombia	Casanare	Nunchia	Rice	*S. paezi*
2580	Colombia	Tolima	CORPOICA, Nataima	Cassava	*M. tanajoa* *M. mcgregori*
2581	Colombia	Valle	Palmaseca	Cassava	*T. urticae*
2583	Venezuela	Anzoategui	Frailes	Cassava	*M. caribbeanae*
2584	Venezuela	Anzoategui	Pariaguan	Cassava	*M. tanajoa* *M. caribbeanae*
2585	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2586	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2587	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2588	Venezuela	Anzoategui	El Tigre, INIA	Cassava	*M. caribbeanae*
2589	Venezuela	Anzoategui	El Tigre, INIA	Cratylia	*M. planki*
2590	Venezuela	Anzoategui	El Tigre, INIA	Sorghum	*O. grypus*
2591	Venezuela	Cogedes	Tinoco	Cassava	*M. tanajoa*

Sample	Country	Dept.	Site	Host	Species
2592	Colombia	Valle	Palmaseca	Cassava	*M. tanajoa***
2593	Ecuador	El oro	Pasaje	Banana	*Tetranychys sp.*
2594	Panamá	Cocle	Anton	Cassava	*M. caribbeanae***
2595	Panamá	Herrera	La Asunción	Cassava	*M. tanajoa*
2596	Panamá	Chiriqui	Siogui abajo	Cassava	*Calacarus guerreroi*
2597	Colombia	Cauca	Quilichao, CIAT	Cassava	*M. tanajoa*
					M. mcgregori
2598	Colombia	Valle	Palmira, CIAT	Banana	*O. yothersi*

* *Neozygites* pathogen infesting tetranichid mites.
** High incidence of *Neozygites fungus*.

Activity 6.7.Arthropod taxonomic activities on cassava and other crops.

Rationale

The IPDM project provides a service of identifying arthropod pests collected from different crops, but especially those crops related to CIAT's mandate and activities. These collections also include natural enemies related to crop pests, and much of this information is found in the preceding activities. A database is maintained of all collections and this is available to collaborating institutions and national research and extension programs.

One of the activities of the CIAT convened "Global Whitefly IPM Project" is to provide taxonomic support for whiteflies and their natural enemies collected from the different agroecosystems of Latin America (Neotropics). Project collaborators located in the numerous countries involved in the project (about 16 in Latin America) continue to send shipments of specimens collected for processing, monitoring and identification. These identifications are of vital importance for the development and implementation of IPM projects in these countries.

Collected specimens are conserved on microscope slides and documented in the whitefly database that is accessible through "Access." This service is also extended to parasitoids as well as other species collected from associated crops (i.e. crops associated with cassava) and made available to all collaborating institutions and countries. During the past year training in collecting, monitoring, and identification of whiteflies has been extended to scientists, students and collaborators from numerous institutions.

In addition to conventional morphological taxonomy techniques, during this year we have implemented in the cassava entomology laboratory, the application of molecular techniques based on PCR, especially for the identification of whiteflies and their parasitoids. These techniques offer a rapid, relatively low cost method for identifying critical species or complexes that are often morphologically indistinguishable during one of their life stages. These techniques or tools can help classify very small insects that often require fixation and microscopic mounting for identification.

In addition during the past year we initiated the collecting and identification of homopterous (Order: Homoptera) species associated with the cassava crop as possible vectors of Cassava Frog Skin Disease. Studies were also initiated to collect and identify "fruit-flies" associated with various tropical fruit crops in a collaborative project with the Tropical Fruits Project.

Project I. Whiteflies

Objective
Process and identify whitefly species collected in Nicaragua, El Salvador, Brazil, Colombia, Ecuador (etc.) from several crops. The materials will be organized within the reference collection and registered in the data bank. Molecular techniques (PCR) will be used in the identification and personnel from national institutions will be trained.

Methodology
Whitefly samples are sent by collaborators in alcohol in vials. Permanent mounts are made in Canadian balsam; specimens are identified and stored in the collection at CIAT, and registered in the database. Parasites are sent for identification to Dr. Gregory Evans (University of Florida) and Dr. Mike Rose (Montana State University).

Molecular techniques using DNA extraction, amplification (PCR) and RAPD's were used for identification of some whitefly species and two parasitoid species (*Eretmocerus mundus* and *E. eremicus*).

Training in these techniques was offered to ICA personnel (Turipaná) and postgraduate students from "La Escuela Politécnica del Ejército," Ecuador.

Results
Whitefly specimens sent from El Salvador, Nicaragua and Brazil were identified to species (Table 6.13). Specimens were collected from numerous crops and at least six different whitefly species were identified.

Table 6.13. Whitefly species collected from several host in 5 countries (El Salvador, Brazil, Nicaragua, Panama and Colombia).

Country	Host	Species	No. of Samples
El Salvador	Pipian, Chile tomato, cucumber, squash, bean, eggplant, col, cowpea, radish, loroco, watermelon, sweet pepper, guisquil, cauliflower, soybean	*B. tabaci* (Gennadius)	60
El Salvador	Potato	*Trialeurodes vaporariorum* (Westwood)	2
Brazil	*Manihot esculenta*	*Aleurothrixus* sp. pos. aepim (Goeldii)	4
Nicaragua	Green pepper, tomato	*B. tabaci* (Gennadius)	17
Panamá	*Manihot esculenta*	*Trialeurodes variabilis* (Quaintance) *Aleurotachelus socialis* Bondar.	2
Colombia	*Musa acuminata*	*Trialeurodes abutiloneus* Haldeman	1

The parasitoids collected from *B. tabaci* were identified by Dr. G. Evans. Two species *Encarsia tabacivor* and *E. nigricephala* were identified. We are waiting confirmation on other specimens sent to the two above-mentioned taxonomists.

Molecular Techniques: The technique of RAPD-PCR has been used to generate molecular markers that are useful in the identification of various groups of insects. The RAPDs-PCR for *B. tabaci* is with the primer OPC-04 (it showed polymorphism between the two populations, indicating a clear separation). These bands permitted distinguishing the two different biotypes for the *B. tabaci* population (which are morphologically identical). These amplified DNA fragments for sample A (biotype A) corresponding to 1636 pb, 890 pb and 469 pb, which are absent in the B samples (Biotype B). In this case two fragments at approximately 1327 pb and 1018 pb were observed; in addition similar bands appear for both biotypes (Figure 6.29).
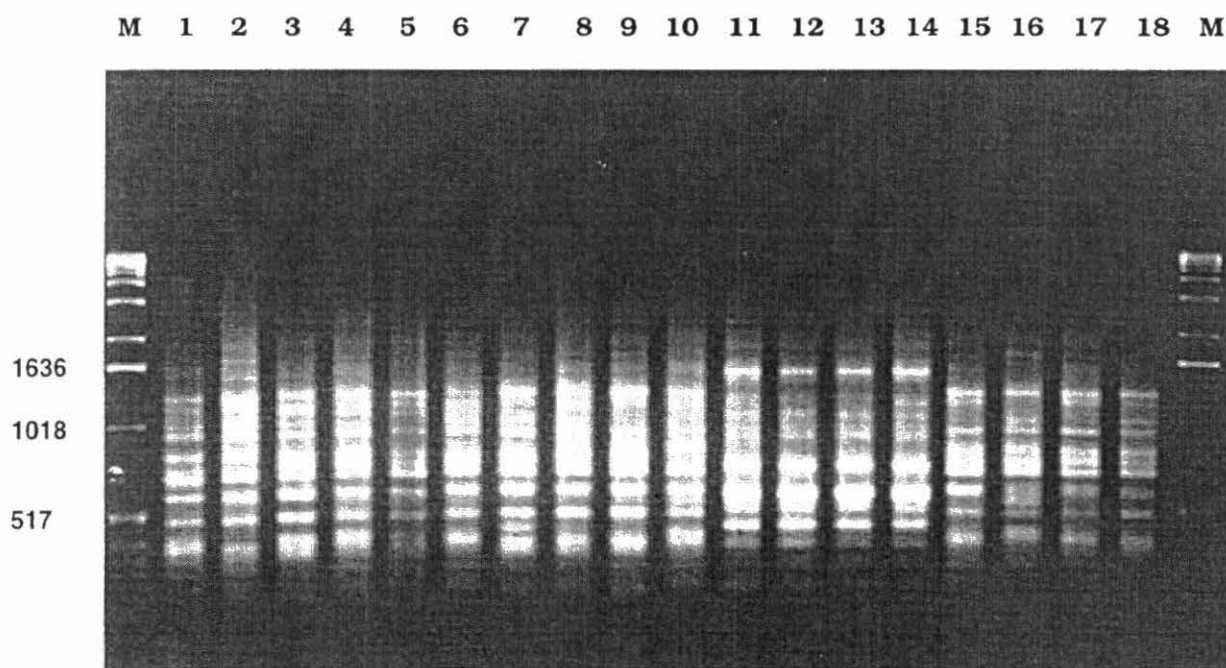

Figure 6.29. Identification of A & B biotypes of *B. tabaci* from RAPDs with primer OPC-04. Biotype B, lines 1-10 and 15-18, Biotype A, lines 11-14. M=Kb Marker.

This technique permits using dead insects, preserved in 70% alcohol, but must be dried before they are homogenized. Results are the same as when fresh individuals are used. This technique is relatively low cost and widely used, but has the drawback of not being easily reproducible and may be inconsistent. Therefore it is suggested that numerous replications be used, and to maintain reference populations as controls, since they can be contaminated by parasites or other organisms present in the insect (i.e. whitefly).

PCR's using the ITS primers (developed by J.L. Cenis, CIDA, Murcia, España) specific for the parasitoid *Eretmocerus*, produced 1 band that is used to differentiate the two populations collected. The bands present a molecular weight of approximately 700 pb for populations of *E. eremicus* and of 600 pb for *E. mundus* (Figure 6.30). The use of specific markers as in the ITS (Internal Transcribed Spacer) case for *Eretmocerus* are costly and require time to

determine their sequences, but they are very sensitive for accurate diagnosis. The species can be identified by size of the amplified product and visualized on the agarose gel.
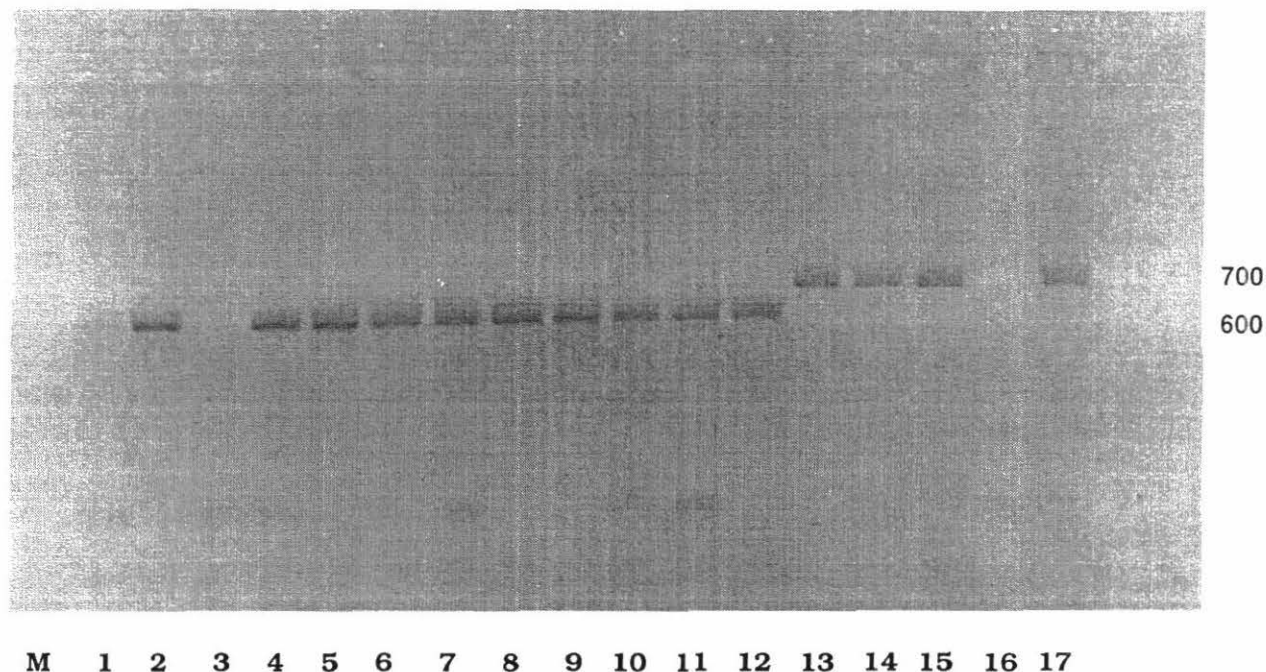

Figure 6.30. Band patterns generated by PCR with the sequence marker ITS for *Eretmocerus mundus* (lines 1-12), of ≈ 600 pb and *E. eremicus* (lines 1317) of ≈ 700 pb.

II. Homopterans/Frog Skin Disease

The Insect Order Homoptera, is often referred to as "true bugs." It contains the families of leafhoppers, (Cicadellidae), plant hoppers (Fulgoridae, Delphacidae, Cixidae), treehoppers (Membracidae), spittlebugs or froghoppers (Cercopidae), as well as the whiteflies (Aleyrodidae). A characteristic that several of the species in this order have in common is their ability to transmit plant virus diseases or phytoplasms.

The cassava frog skin disease (CFSD) is causing considerable crop losses in Colombia and other countries and is hindering the movement and evaluation of germplasm. The long held assumption that CFSD was whitefly vectored, is presently being challenged. Other possible vectors among the aforementioned homopterans are now being considered, especially Cicadellidaes and Delphacidaes.

With the exception of whiteflies, other homopterans have not been identified as major pests of cassava, and their populations, when present are usually very low. A determined effort has been initiated to collect and identify the Homoptera species associated with cassava. Collections are being carried out at several different localities, especially where CFSD is prevalent. Numerous species have now been identified (Table 6.14); the species *Scaphytopius* pos. *fuliginosus* (Cicadellidae) is most frequently collected and from several sites, including Valle del Cauca, Cauca and Tolima. Several species that have been collected, lack identification and will be sent to appropriate taxonomists.

These homopterans are most frequently found on cassava during the early morning hours and usually on young plants, 2 to 6 months. They are difficult to find on older plants. In addition, it has been observed that weedy cassava plots contain greater species diversity, indicating that many of these species may not be feeding on cassava, but rather on the associated weeds. Eventually, selected species will be reared under controlled conditions and vector pathogenicity tests carried out.

Table 6.14. Homopteran species collected from cassava plants at several locations in Colombia.

Department	Municipality	Site	Family	Species	Observations
Valle del Cauca	Palmira	CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus* Osborn	
Cauca	Santander de Quilichao	Hacienda Bariloche	Cicadellidae	Scaphytopius *pos*.fuliginosus	2 months field plot
			Delphacidae	1 species	
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus*	Some plants with frog skin
Cauca	Santander de Quilichao	Granja CIAT	Cicadellidae	*Scaphytopius* pos.*fuliginosus* 5 species unidentified	Weedy plot
			Delphacidae	1 species	
Quindío	La Tebaida		Cixiidae	1 species	Weedy plot
			Cicadellidae	5 species	
Risaralda	Morelia	Santa Rita	Cixiidae	1 species	Weedy plot
			Cicadellidae	4 species	
	Cerritos		Cixiidae	1 species	4 month field
			Cicadellidae	2 species	
Tolima	Espinal-Chicoral	Granja Nataima	Cicadellidae	*Scaphytopius* pos. *fuliginosus* 1 species*	Some plants with frog skin
	Gualanday		Cicadellidae	*Scaphytopius* pos.*fuliginosus* 1 species*	Non-weedy plot
	Ambalema	Vía Ambalema	Cicadellidae	1 species*	Weedy plot
	Espinal	San Francisco	Cicadellidae	1 species*	Weedy plot

* Similar Cicadellidae collected from the three sites (not identified, but appear to be an Empoasca).

Activity 6.8. Studies on the natural resistance of four wild Manihot species (Manihot spp) to three arthropod pests (Mononychellus tanajoa, Aleurotrachelus socialis and Phenacoccus herreni), under greenhouse conditions.

Rationale

Cassava arthropod pests have been shown to significantly reduce root yield through both direct (the cassava root) and indirect (leaves and stems) feeding. Control of cassava pests relies mostly on a combination of host plant resistance (HPR) and biological control (BC). These two complementary systems can be low cost to the farmer and environmentally sound as well as effective in reducing damage in an integrated pest management (IPM) program. There is a large complex of arthropod pests attacking cassava, especially in the neotropics, the regions of origin of the cassava crop.

The objective of this research is to evaluate four species of wild *Manihot* as a potential source of resistance to three of the major pests of cassava, mites (*Mononychellus tanajoa*), whiteflies (*Aleurotrachelus socialis*) and mealybugs (*Phenacoccus herreni*). Mites, whiteflies and mealybugs cause significant yield losses in cassava in the Americas, Africa and Asia.

This research is divided into two parts; the first consists in the acquisition and establishment of vegetative materials of the *Manihot* species. Different methodologies are used, including rooting techniques, soil-sand mixtures, soil source (site or location). The second part consists of infesting the different wild species, as well as control genotypes with the aforementioned arthropods, and carrying out an evaluation of infestation levels (population dynamics, behavior and damage.

Introduction

Manihot spp (Euphorbiaceae) is a genus native to the neotropics with a wide range of habitats, that extend from the south of Arizona (USA) to Argentina (Rogers and Appan, 1973). Species within this group are perennials and vary from short-stemmed bushes to 10 to 12 meter high trees. The majority of the species have tuberous roots and some can accumulate large quantities of starch, as is the case with cassava (*Manihot esculenta*), an important tropical crop that is the major calorie source for more than 500 million persons (Allem, 1992; Best and Henry, 1992).

In spite of cassava's importance, certain aspects such as crop origin and its phytogenetic relationship with other species within the genus have not been well clarified. Several different sites have been proposed as the center of origin within the neotropics, with diverse evidence; the South of Mexico and Guatemala (Rogers, 1963; Renvoise, 1972), the Caribbean Coast of Colombia and Venezuela (Saur, 1952), and the Amazon Basin of Brazil (Decandalle, 1967; Nassar, 1978; Allem, 1987, 1994) are areas proposed to be the center of origin and domestication in accordance with linguistic, anthological, archaeological, taxonomic and geographic information.

From a morphological point of view, independent taxonomic studies indicate diverse wild taxa of the genus as most closely related to cassava, in general dividing between species native to North and Central America and species of South America origin. Rogers and Appan (1973) propose *M. aesculifolia*, the Central American species closest to cassava. Allem (1987) disagrees and proposes *M. tristis*, native of South America as the closest relative to cassava. He has also reported that in collecting trips in Brazil (1992, 1994), he has found two wild

morphological variations of the cultivated species; a glabrous *M. esculenta* subspecies *flabellifolia*, and a pubescent one, *M. esculenta* subspecies *peruviana*.

It has only been in recent years that wild *Manihot* species have been incorporated into characterization, conservation and use by phytogenetic resources. Although considerable variability and heterozygosity is assumed in the wild species, owing to their wide geographic distribution cross polinization and the presence of different characteristics that would be desirable in the crop, studies have not been done to confirm these attributes. At CIAT, the small collection of wild germplasm suffers from complete documentation and is poorly represented geographically. There does not exist, at present, a regular characterization and evaluation of wild germplasm (Roa, 1997).

Bertran (1993) proposes that *M. aesculifolia* is the species closest to cassava and that *M. carthaginensis*, a species distributed on the Caribbean Colombo-Venezuelan coast, is one of the closest parents to the cultivated species. It is clear that a sufficient genetic diversity or variation needs to be maintained of these wild species to insure future adaptation, and methods should be developed that permits estimating the diversity present in a species (Roa, 1997).

I. Multiplication of wild *Manihot* species: The species selected for this phase of the project were *M. carthaginensis*, *M. esculenta* subsp. *flabellifolia*, *M. esculenta* subsp. *peruviana*, and *M. tristis*. These were selected for various reasons, including their relationship to the cultivated species, their centers of origin or domestication in relation to cassava, similarity to cassava in morphological characteristics and in vivo or in vitro availability (Table 6.15).

Table 6.15. Distribution and ecology of *Manihot* species included in the insect resistance study.

	M. brachyloba	M. carthaginensis	M. esculenta subsp. flabellifolia	M. esculenta subsp. peruviana	M. tristis
Distribution	Bolivia, Brazil, Colombia, Ecuador, Costa Rica, Guyana, Perú, Venezuela[1]	Colombia, Venezuela, Brazil, Dominican Republic, Trinidad y Tobago	Brazil, Venezuela, Surinam, Guyana[2]	Brazil, Peru[2]	Brazil, Venezuela, Surinam[1]
Ecology	Common in zones of secondary growth and under slight shade around river banks[1]	Xeric forests and growing in limestone, in open areas and costal zones[1]	Dry semi-deciduous forests (Campo Cerrado, Brazil) and disturbed Amazon forest[2]	Disturbed Amazonian forests[2]	Grown on poor rock or granite soils[1]

[1] Rogers and Appan, 1973.
[2] Allem, 1994.

Numerous plantings of the above mentioned species were made in an attempt to multiply and maintain adequate number of plants within each species to evaluate against the target arthropod species. At least 12 attempts were made over the past 9 months to get good plant establishment. Stem cuttings were first sown in propagation chambers or in pots with mixtures of sand and soil (3:1 proportion). Few cuttings germinated. In some cases fungicides and rooting hormones were added to the soil mixtures. Attempts were also made to root stem cuttings in liquid (water) solution, but this resulted in a high percentage of stem rotting causing plant mortality. Rotting and high plant mortality also occurred when plant shoots were placed in sand-soil mixtures or in distilled water. The addition of an antifungal, anti rotting agent (Banrot) did not prevent rotting and high mortality. The success rate for establishing a consistent supply of these five wild Manihot species was very low (Table 6.16). Some success was achieved with *M. carthaginensis* and *M. tristis*, but the percent survival was low.

More recently, the last batch of *M. esculenta* subsp. *flabellifolia* and *M. peruviana* received from plantings in Santander and in-vitro multiplied materials have germinated and progressing well. 100% of these plants have survived (Table 6.16).

In conclusion of this phase I, 44 genotypes of Manihot, including wild, domesticated and cultivated; 17 of these did not survive, 13 presented very low levels of survival (< 25%) and three genotypes between 25 and 50% and only 1 at 50 to 75%. In the latter plantings (Sept 14, 2002) 10 genotypes had survival rates between 75-100% but these were recently planted and their long term survival is not yet proven. Our experience up to this point indicates that very high humidity is detrimental to the establishment (rooting and survival) of these wild *Manihot* species.

Due to the difficulty in establishing sufficient wild species, it has not been feasible to establish colonies of mites, whiteflies and mealybugs on wild *Manihot*. There is no assurance that we will be able to accomplish this establishment in the near future. It is recommended that the pest species be established for 5 generations on wild *Manihot* before evaluating germplasm. Give the life cycle of each of the arthropod species this calculates to 2 months for mites, four months for mealybugs and nearly 6 months for whiteflies. The lack of establishment of the wild Manihot species has deterred initiating the second part of this project. Hopefully these problems will be overcome in the near future.

Table 6.16. Plant survival of *Manito* species evaluated in insect resistance study.

Species	Genotype	No. Plants Sown	No. Plants Survived	% Survival
Manihot carthaginensis	30-1	30	1	3.33
Manihot carthaginensis	30-4	30	0	0
Manihot carthaginensis	30-5	30	1	3.33
Manihot carthaginensis	31-1	30	2	6.66
Manihot carthaginensis	37-8	48	3	6.25
Manihot carthaginensis	160-5	220	2	0.90
Manihot carthaginensis	160-4	160	16	10
Manihot flabellifolia	180-2	21	0	0
Manihot flabellifolia	213-7	355	0	0
Manihot flabellifolia	225-2	105	0	0
Manihot flabellifolia	230-2	110	4	3.63
Manihot peruviana	240-3	106	5	4.71
Manihot peruviana	241-3	161	0	0
Manihot peruviana	248-1	85	0	0
Manihot peruviana	254-1	42	0	0
Manihot peruviana	266-4	25	0	0
Manihot peruviana	269-1	13	0	0
Manihot tristis	130-3	365	1	0.27
Manihot tristis	132-36	326	27	8.28
Manihot tristis	144-2	30	0	0
Flores	Domesticated	30	17	56.66
Santa Catalina	Domesticated	27	13	48.14
Ibacaba	Domesticated	23	1	4.34
Siringa	Domesticated	18	4	22.22
Lapa Blanca	Domesticated	26	20	76.92
Yuca de agua	Domesticated	14	0	0
Inayá	Domesticated	10	0	0
Nupara	Domesticated	20	15	75
Pintadillo	Domesticated	15	0	0
Tresmesina dulce	Domesticated	4	0	0
Abeja	Domesticated	9	2	22.22
Dulce Cucura	Domesticated	7	0	0
Wasoco	Domesticated	9	0	0
Pupuña	Domesticated	22	0	0
Manihot flabellifolia	439	27	27	100
Manihot flabellifolia	443	23	23	100
Manihot esculenta sub. Flabellifolia	444-002	10	10	100
Manihot peruviana	414	17	17	100
Manihot peruviana	417-003	7	7	100
Manihot peruviana	417-005	28	28	100
Manihot esculenta	MBra 12	12	12	100
Manihot esculenta	CM 7395	17	17	100
Manihot esculenta	CMC 40	15	8	53.3
Manihot esculenta	MEcu 72	15	15	100

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Output 7
Disease Resistance in Cassava

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

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

Specific objectives

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

Materials and methods

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

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

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

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

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

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

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

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

Results

On analyzing the genetic variability of 85 isolates of *Xam* through the AFLP technique, three groups could be distinguished (Figure 7.3). The first group clustered at a similarity level of 0.6, and is formed of isolates from different localities in Colombia. The second group clustered at 0.7, and comprises 81% of the Venezuelan isolates included in this study, and 4 Brazilian isolates. The third group clustered at 0.4, and is formed by most of the Brazilian isolates, 3 isolates from Venezuela, 1 from Cuba, and 3 from Colombia. In this group, clusterings below the 0.4 similarity level also occurred, indicating great genetic variability within the Brazilian locations, possibly related to the also high level of genetic diversity observed for the host plant (Roa et al. 1997; Sánchez et al. 1999). When new pathogen strains are introduced into a given area, the genetic diversity already found within the pathogen population is increased, thereby favoring the development of new pathotypes (Restrepo 1999).
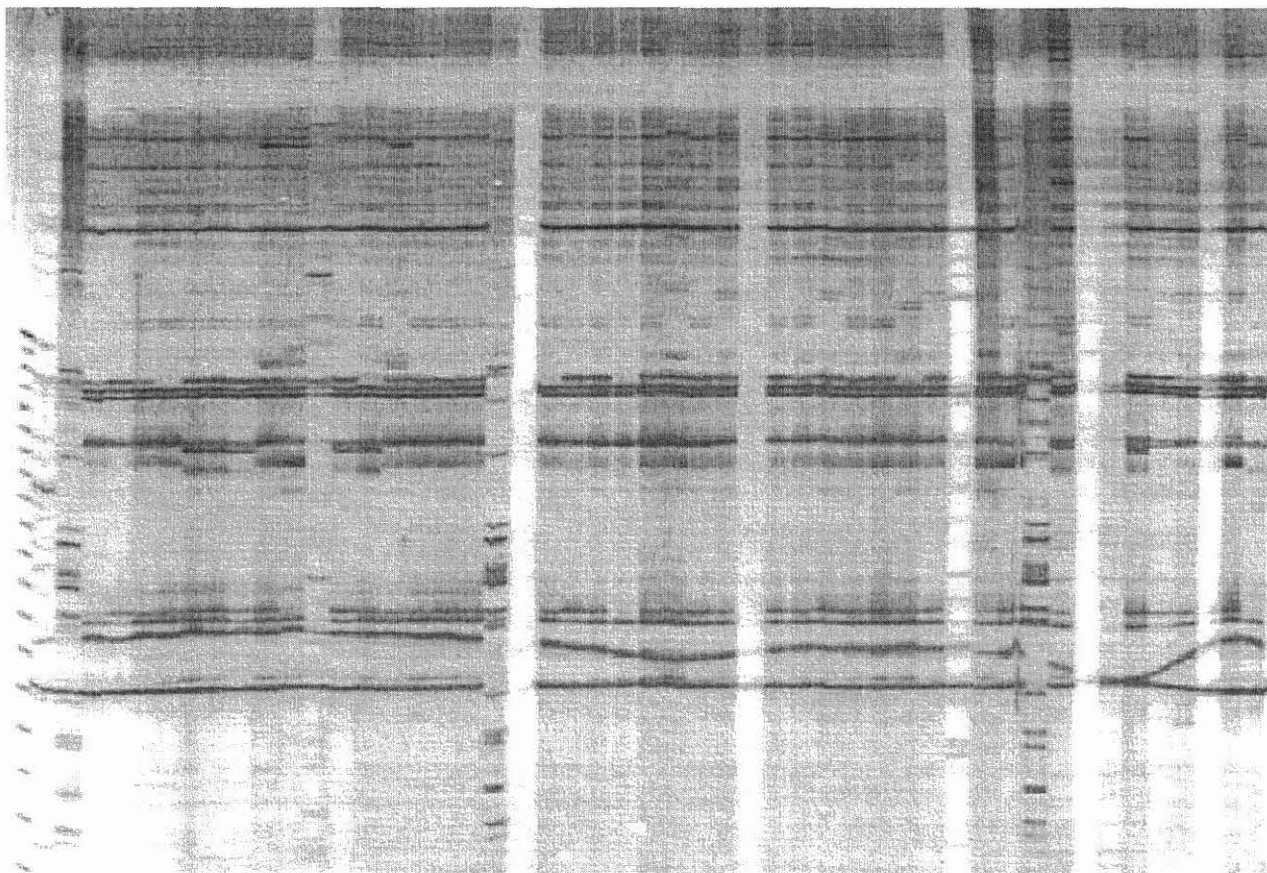
Figure 7.1. Patterns of AFLP for thirty eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.
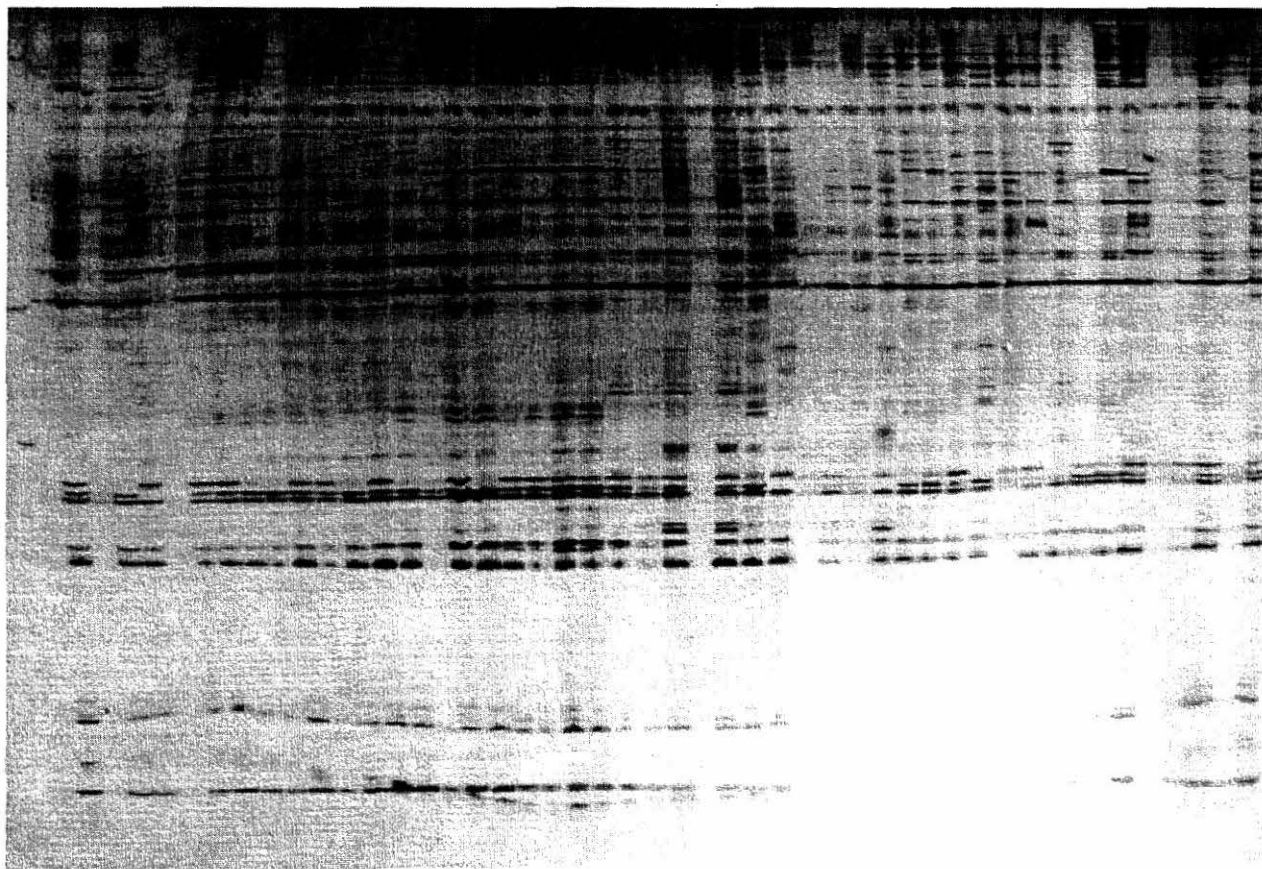
Figure 7.2. Patterns of AFLP for forty eight isolates of *Xanthomonas axonopodis* using EC/MA primer combination (Lines 2-49), line 1 is a ladder 30-330.

Cluster analysis led to the formation of three groups of isolates that could be separated by country (i.e., Brazil, Colombia, and Venezuela). Within these large groups, subgroups can be found, based on different areas within the countries. The cluster of these *Xam* populations showed high variability—a significant finding, because a population with high genetic variability can adapt faster to antibiotics and resistant hosts. Several evolutionary factors can affect a population's genetic structure, including size and number of individuals, gene flow, and host selection (Mejía 2002).
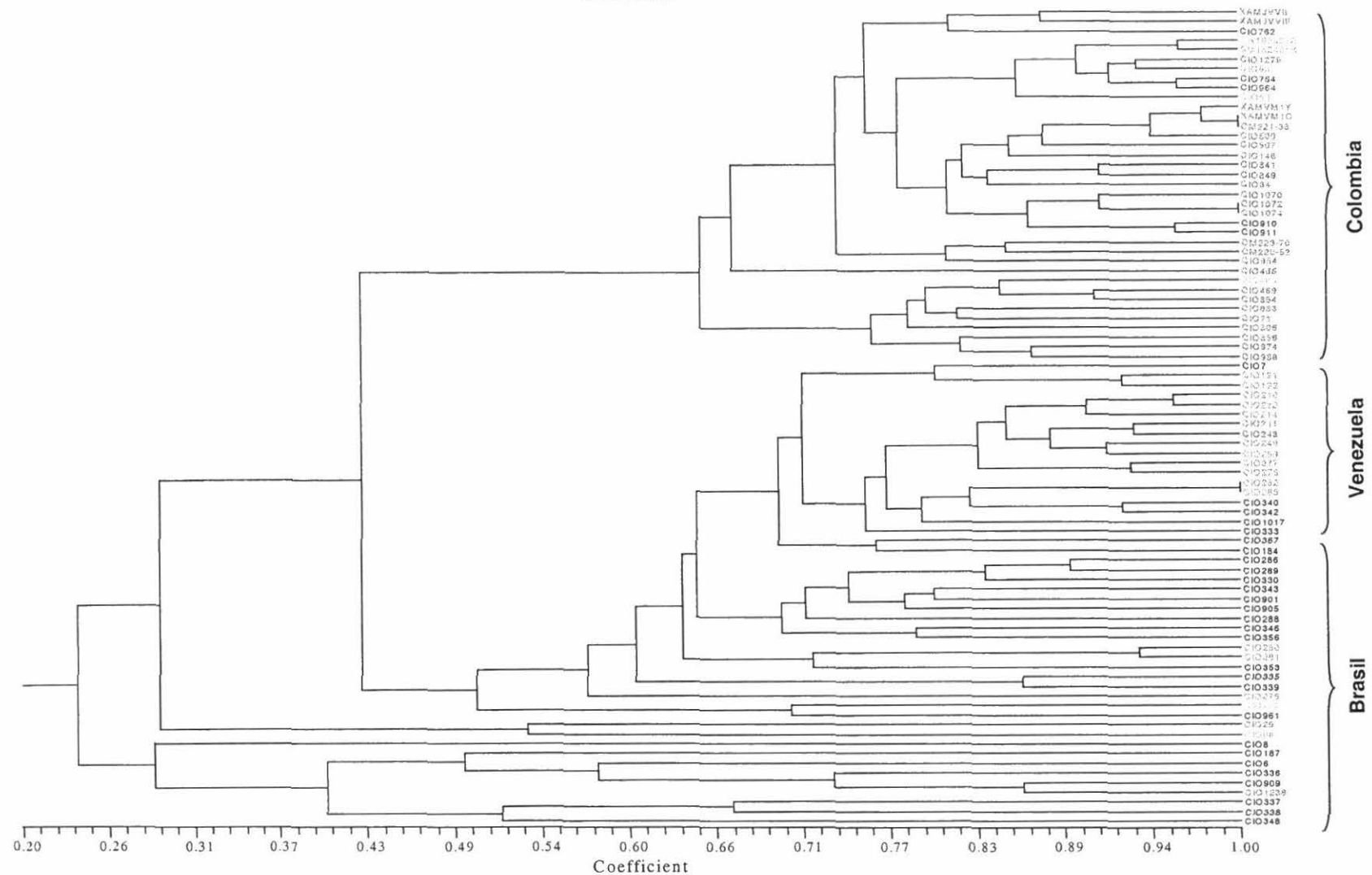
Figure 7.3.Similarity dendrogram of 85 isolates of *Xanthomonas axonopodis* pv. *manihotis*, based on AFLP analysis, using the unweighted pair-group method with arithmetic averaging (UPGMA) program of NTSYS-pc 2.02 (FJ Rohlf, Exeter Software, New York).

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

Specific objectives

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

Methodology

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

Results

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

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

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

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

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

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

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

Table 7.3 (cont.)

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

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

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

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

Table 7.6 (cont.)

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

Table 7.6 (cont.)

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

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

Methodology: North Coast

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

Results: North Coast

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

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

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

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

Methodology: Cauca

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

Treatments

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

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

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

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

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

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

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

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

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

Results: Cauca

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

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

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

a. 30 days after planting.

Methodology: Quindío

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

Treatments for the first trial

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

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

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

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

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

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

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

Treatments for the second trial

The second trial was planted in August 2001:

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

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

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

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

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

Results: Quindío

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

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

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

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

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

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

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

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

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

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

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

Specific objectives

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

Methodology

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

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

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

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

Results

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

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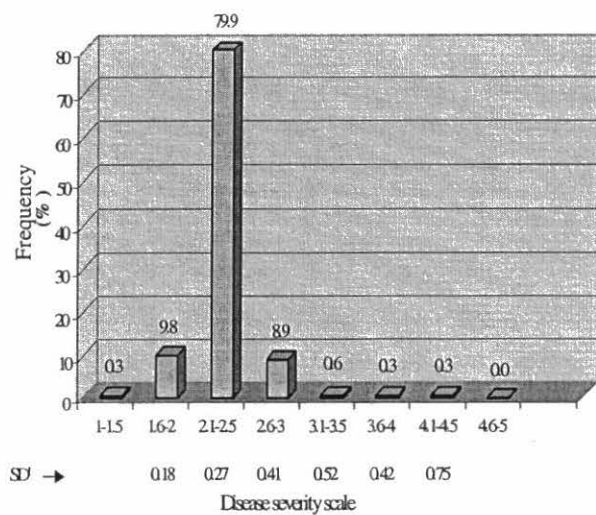
(A)
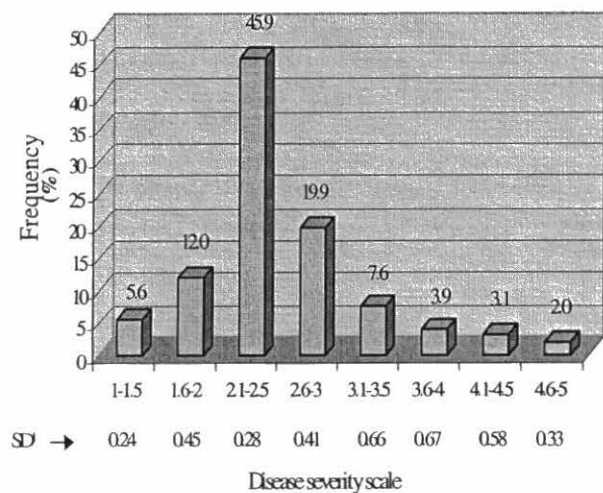
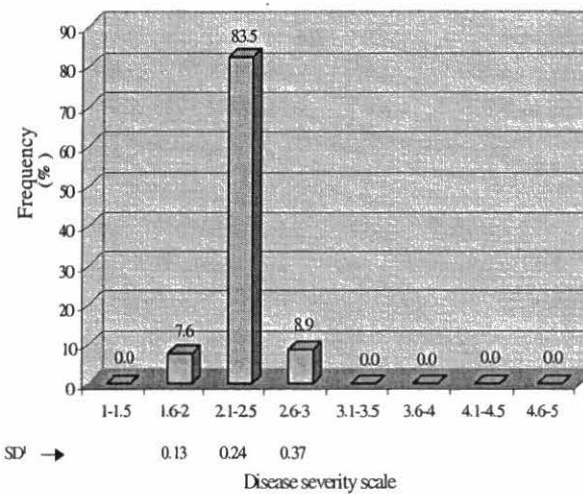
(B)
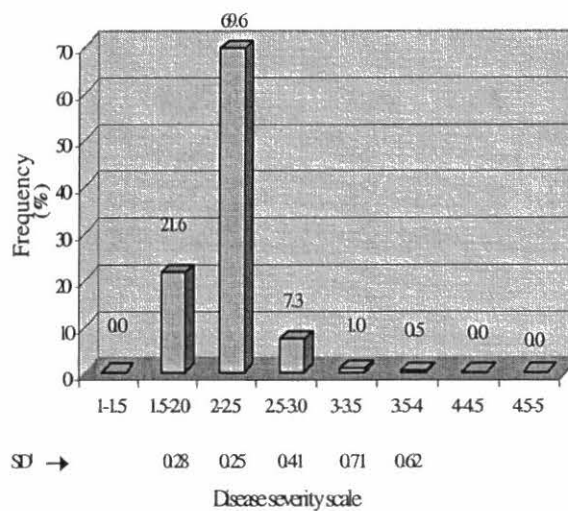
(C)

(D)

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

Activity 7.6.   Evaluating simple sequence repeat markers linked to bacterial blight resistance in cassava

Specific objectives

1. To evaluate 486 SSR primers in a BC1 family by bulked segregant analysis (BSA).
2. To identify SSR markers associated with disease resistance in the field.

Methodology

According to the results obtained in *Activity 7.5*, the selected family GM 315, a backcross between M Nga 19 and CM 9208-13, itself a progeny of M Nga 19, was evaluated, using SSR markers to search for an association between CBB resistance and segregating bulks. Parents of this family are both resistant and heterozygotic.

Bulking. The evaluation method involves comparing two pooled DNA samples of individuals from a segregating population originating from a cross. Within each pool, or bulk, the individuals are identical for the trait or interest gene but are arbitrary for all other genes. Two pools that contrast for a trait (e.g., resistant versus susceptible to a particular disease) are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. Bulked segregant analysis provides a method of focusing on regions of interest or areas sparsely populated with markers (Michelmore, 1991).

The GM 315 progeny that appeared highly resistant or highly susceptible according to the severity scale for CBB (Figure 7.4A) was selected to form two contrasting bulks. Individuals from resistant bulk scored 1.0 to 2.0 on the severity scale, while susceptible ones scored between 4.0 and 5.0.

DNA extraction. DNA, concentrated at 10 ng/μL, was extracted from selected individuals forming the bulks, using the Gilbertson-Dellaporta protocol (Dellaporta et al. 1983; Table 7.10

SSR markers. M Nga 19 and bulks, comprising resistant and susceptible individuals, were evaluated, using 486 microsatellite primers, which were separated into two groups based on DNA used to design them. 153 NS primers, based on cDNA, and 345 SSRY primers, based on genomic DNA, were used.

Each PCR reaction was performed in 25-μL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 mM MgCl$_2$; 1.5 U of *Taq* polymerase, 1 μM primer; 2.5 μL 10X *Taq* polymerase buffer; and 50 ng *template* DNA. Amplification with NS and SSRY was carried out, first in a Robocycler 96 (Stratagene) thermal cycler programmed for 2 min at 95°C; 30 cycles of denaturing for 40 s at 95°C, annealing for 1.30 min at 55°C, and extension for 2 min at 72°C; and a final extension for 5 min at 72°C. Amplification continued in a second thermal cycler (MSJ-Research PTC-200) for 2 min at 95°C; 30 cycles of denaturing for 30 s at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C; and a final extension for 5 min at 72°C.

Table 7.10. Average and range of disease severity for cassava bacterial blight within each plot of individuals from each bulk (resistant and susceptible) in the cassava family GM 315.

	Resistant bulk			Susceptible bulk	
Individual code no.	Disease severity		Individual code no.	Disease severity	
	Range	Average		Range	Average
210	1.0-1.5	1.2	45	4.0-5.0	4.6
131	1.0-2.0	1.5	51	4.0-5.0	4.3
5	1.0-2.0	1.2	114	4.0-5.0	4.4
295	1.0-1.5	1.5	357	4.0-5.0	4.4
261	1.5	1.5	36	4.0-5.0	4.4
153	1.5-2.0	1.6	246	4.0-5.0	4.4
281	1.0-1.5	1.2	79	4.5-5.0	4.9
116	1.0-2.0	1.6	223	4.0-5.0	4.4
169	1.0-2.0	1.5	200	4.5-5.0	4.9
185	1.5-2.0	1.8	345	4.5-5.0	4.7
46	1.5-2.0	1.6	265	4.0-5.0	4.5

The PCR product was electrophoresed in 6% polyacrylamide gel, where flowering time and mite resistance from another project is also presented.

Opening Bulks
Individuals from Resistant and susceptible Bulks were evaluated with candidate primers, which showed polymorphism between bulks. The marker's presence or absence in each individual can help us to confirm association between SSR marker and CBB resistance in the field.

Results
Bulked segregant analysis of cassava family GM 315 detected polymorphism between each bulk and/or between evaluated parent and bulks (Figure 7.5).

Results suggested that some primers could be chosen as candidate primers to be evaluated as SSR marker associated with CBB resistance in the field. Results from bulked segregant analysis in cassava family GM 315 are presented in the following list:

SSR primers	Number
Total primers evaluated	486
Polymorphic in bulks	28
Candidates as SSR markers	13

To confirm that association, 13 SRR primers were evaluated in each individual that forms part of resistance and susceptible bulks (Figure 7.6)

Primer SSRY65 showed differences between resistant and susceptible individuals and can be considered SRR marker, it can be evaluated in the whole population. Two resistant genotypes did not show resistant marker, probably because of recombination, which could cause loss of some genomic regions.
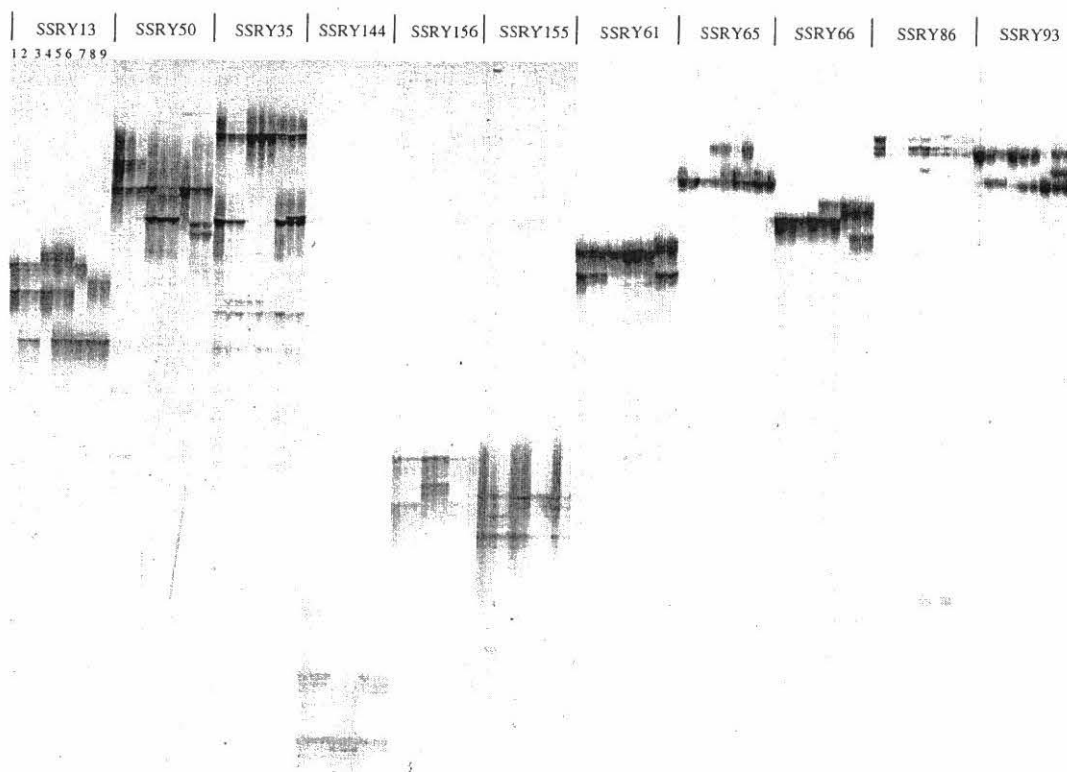
Figure 7.5. Eleven SSRY primers used to identify SSR markers by bulked segregant analysis. Nine samples were evaluated with each primer. The first three wells for each primer are from a population where flowering time is studied (lane 1 = early flowering male parent; lane 2 = early flowering bulk; lane 3 = late flowering bulk). The next three wells are from a GM 315 family where resistance to cassava bacterial blight is studied (lane 4 = resistant male parent; lane 5 = resistant bulk; lane 6 = susceptible bulk). The last three 7 = resistance male parent; lane 8 = resistant bulk; lane 9 = susceptible bulk).
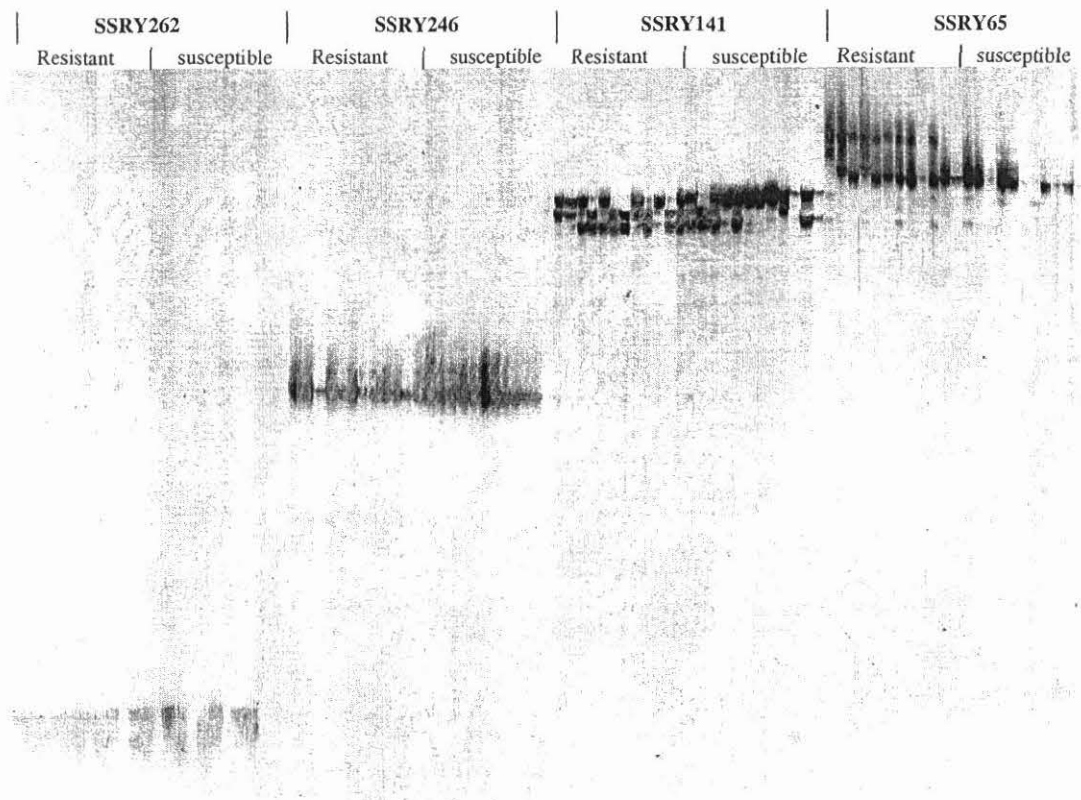
Figure 7.6.   Four candidate primers evaluated in each individual that forms the bulks in cassava family GM 315. Each lane has an individual and they are separated in resistant and susceptible ones.

References

Dellaporta SL; Wood J; Hicks JR. 1983. A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19.

Michelmore, R. W., Paran, I., and Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregant populations. Proc. Natl. Acad. Sci. USA. Vol 88: 9828-9832, November 1991. Genetics.

Activity 7.7. Evaluating a diallel assay in Villavicencio

Specific objectives
   1. To evaluate a diallel study for resistance to CBB and SED

Methodology
A 10 × 10 diallel study, comprising 45 families with 30 plants each, was evaluated for the plants' reaction to CBB and SED under natural disease pressure and according to a disease severity scale of 1 to 5, where 1 = no symptoms and 5 = plant death.

The diallel was planted with three replicates at two sites at CORPOICA's "La Libertad" station. Only one site had disease pressure, so the other was not evaluated for disease. The 10 genotypes conforming the diallel were:

CM 4574-7	CM 6740-7	CM 7033-3	SM 1219-9	SM 1565-15
SM 2058-2	SM 2219-11	HMC-1	M Per 183	M Tai 8

Results
The severity average for the families oscillated between 2.1 and 3.2 for CBB and between 2.2 and 4.2 for SED. Greater severity and variation among plants in each family was observed for SED than for CBB (Table 7.11), probably because CBB pressure was not high.

Table 7.12 presents averages for CBB and SED severity for all progenies obtained from each parent (810 individuals: 9 crosses, 30 plants/cross, 3 replicates). According to the data, CM 4574-7, SM 1565-15, and CM 7033-3 had progenies with the greatest resistance to SED, whereas the CM 4574-7 and M Tai 8 progenies had the highest CBB resistance. CM 4574-7 tended to have the highest general combining ability for resistance to these diseases. M Per 183, HMC-1, M Tai 8, and CM 6740-7 had progenies with the lowest resistance.

Table 7.11. Range, average, and standard deviation (SD) of severity[a] of cassava bacterial blight (CBB) and superelongation disease (SED) for each diallel crossing in cassava, Villavicencio, Colombia.

Cross[b]	Statistic	CBB	SED	Cross[b]	Statistic	CBB	SED	Cross[b]	Statistic	CBB	SED
1 × 2	Range	2.0-3.5	1.0-4.0	2 x 9	Range	2.0 - 4.0	2.0 - 4.5	5 x 6	Range	2.0 - 4.0	1.0 - 4.5
	Avg	2.5	2.3		Avg	2.7	3.9		Avg	2.8	2.3
	SD	0.3	0.6		St dev	0.6	0.5		St dev	0.5	0.6
1 × 3	Range	2.0-3.5	1.0-4.0	2 x 10	Range	2.0 - 3.5	2.5 - 4.5	5 x 7	Range	2.0 - 3.5	1.0 - 4.0
	Avg	2.5	2.2		Avg	2.5	3.4		Avg	2.5	2.2
	SD	0.3	0.6		St dev	0.4	0.6		St dev	0.3	0.5
1 × 4	Range	2.0-3.5	1.5-5.0	3 x 4	Range	2.0 - 4.0	1.0 - 5.0	5 x 8	Range	2.0 - 4.0	1.5 - 4.0
	Avg	2.7	2.5		Avg	2.6	2.4		Avg	2.8	2.9
	SD	0.3	0.7		St dev	0.5	0.7		St dev	0.5	0.7
1 × 5	Range	2.0-3.5	1.0-4.0	3 x 5	Range	2.0 - 4.0	1.5 - 5.0	5 x 9	Range	2.0 - 4.0	2.0 - 4.5
	Avg	2.5	2.4		Avg	2.6	2.2		Avg	2.7	3.3
	SD	0.3	0.7		St dev	0.5	0.7		St dev	0.5	0.7
1 × 6	Range	2.0-4.5	1.0-4.5	3 x 6	Range	2.0 - 5.0	1.5 - 5.0	5 x 10	Range	2.0 - 3.5	1.5 - 4.5
	Avg	2.5	2.6		Avg	3.2	2.7		Avg	2.4	2.9
	SD	0.5	0.9		St dev	0.9	0.8		St dev	0.4	0.7
1 x 7	Range	2.5 - 3.5	1.5 - 4.0	3 x 7	Range	2.0 - 4.5	1.0 - 4.0	6 x 7	Range	2.0 - 4.0	1.0 - 4.0
	Avg	2.6	2.6		Avg	2.9	2.4		Avg	2.8	2.6
	St dev	0.4	0.7		St dev	0.7	0.7		St dev	0.5	0.6
1 x 8	Range	2.0 - 3.5	1.0 - 4.0	3 x 8	Range	2.0 - 4.0	1.5 - 4.5	6 x 8	Range	2.0 - 4.5	1.5 - 4.0
	Avg	2.6	2.5		Avg	2.9	2.7		Avg	3.2	2.5
	St dev	0.4	0.5		St dev	0.7	0.8		St dev	0.5	0.6
1 x 9	Range	2.0 - 4.0	2.0 - 4.0	3 x 9	Range	2.0 - 4.0	1.5 - 4.5	6 x 9	Range	2.0 - 4.0	2.0 - 4.5
	Avg	2.6	3.2		Avg	2.8	3.5		Avg	2.8	3.5
	St dev	0.3	0.6		St dev	0.6	0.6		St dev	0.6	0.6
1 x 10	Range	2.0 - 4.0	1.5 - 5.0	3 x 10	Range	2.0 - 4.0	1.5 - 4.5	6 x 10	Range	2.0 - 4.0	1.0 - 4.5
	Avg	2.3	3.0		Avg	2.7	2.8		Avg	2.6	3.1
	St dev	0.4	0.8		St dev	0.6	0.7		St dev	0.6	0.8
2 x 3	Range	2.0 - 4.0	1.5 - 4.5	4 x 5	Range	2.0 - 4.0	2.0 - 4.5	7 x 8	Range	2.0 - 4.5	1.5 - 4.5
	Avg	2.8	3.0		Avg	2.6	3.0		Avg	2.8	2.7
	St dev	0.6	0.8		St dev	0.5	0.6		St dev	0.6	0.7
2 x 4	Range	2.0 - 4.0	2.0 - 5.0	4 x 6	Range	2.0 - 4.0	1.5 - 4.5	7 x 9	Range	2.0 - 4.0	2.0 - 4.5
	Avg	2.5	3.3		Avg	3.0	3.1		Avg	2.6	3.5
	St dev	0.5	0.7		St dev	0.7	0.6		St dev	0.6	0.6

2 x 5	Range	2.0 - 4.0	1.0 - 4.5	4 x 7	Range	2.0 - 4.0	1.5 - 4.0	7 x 10	Range	2.0 - 4.0	1.0 - 4.0
	Avg	2.5	2.5		Avg	2.6	2.6		Avg	2.7	3.1
	St dev	0.4	0.7		St dev	0.4	0.6		St dev	0.6	0.7
2 x 6	Range	2.0 - 4.5	1.0 - 4.0	4 x 8	Range	2.0 - 3.5	1.0 - 4.0	8 x 9	Range	2.0 - 4.0	3.0 - 5.0
	Avg	3.0	2.9		Avg	2.6	2.8		Avg	2.5	4.2
	St dev	0.7	0.8		St dev	0.4	0.7		St dev	0.6	0.4
2 x 7	Range	2.0 - 4.0	1.5 - 4.5	4 x 9	Range	2.0 - 4.0	2.0 - 4.0	8 x 10	Range	2.0 - 3.5	1.5 - 4.5
	Avg	2.7	3.1		Avg	2.9	3.5		Avg	2.7	3.7
	St dev	0.4	0.7		St dev	0.6	0.5		St dev	0.5	0.6
2 x 8	Range	2.0 - 4.5	1.5 - 5.0	4 x 10	Range	2.0 - 3.5	1.0 - 4.0	9 x 10	Range	2.0 - 3.5	3.0 - 4.5
	Avg	2.9	3.5		Avg	2.5	3.2		Avg	2.1	4.1
	St dev	0.7	0.8		St dev	0.5	0.8		St dev	0.3	0.3

[a]Severity on a scale from 1 to 5, where 1 = no symptoms and 5 = plant death.
[b]Parents of each cross are identified in Table 7.12.

Table 7.12.   Disease severity average for progenies of each parent in a dialed study of cassava, Villavicencio, Colombia.

Parent		CBB[a]	SED[b]
Code no.	Genotype		
1	CM 4574-7	2.50	2.54
2	CM 6740-7	2.66	3.09
3	CM 7033-3	2.76	2.66
4	SM 1219-9	2.63	2.92
5	SM 1565-15	2.60	2.61
6	SM 2058-2	2.86	2.84
7	SM 2219-11	2.68	2.76
8	HMC-1	2.75	3.06
9	M Per 183	2.63	3.63
10	M Tai 8	2.50	3.25

a.   CBB = cassava bacterial blight.
b.   SED = superelongation disease.

Activity 7.8.   ***Evaluating probes of resistance gene analogs in cassava parentals of different crosses for resistance to Phytophthora root rot***

Specific objective

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

Methodology

DNA was extracted from leaf tissues of five cassava parental genotypes—M Nga 2, CM 2177-2, M Bra 1045, M CR 81, and M CR 54—using the Gilbertson-Dellaporta protocol (Dellaporta et al. 1983). M Nga 2 is intermediately resistant to *Phytophthora tropicalis* and susceptible to *Phytophthora* isolate MTR6, whereas CM 2177-2 is susceptible to *P. tropicalis* and resistant to isolate MTR6. Genomic restriction with the enzymes *Eco*RI, *Eco*RV, *Hae*III, *Hind*III, *Dra*I, and *Taq*I was done after gel depurination and denaturation. The digested DNA was transferred overnight to a Hybond N+ membrane, using 10X SSC (NaCl and trisodic citric acid) as transferring solution. The DNA was fixed on the membrane by ultraviolet light in a Stratalinker.

Cells of *Escherichia coli*, strain DH5-α, were transformed by electroporation, introducing pGEM-T Plasmid Vector System (Promega), containing 10 disease resistance gene analogs (RGAs) isolated from maize and rice. Transformed cells were kept at –80°C in glycerol 30%. Minipreparations were prepared with Concert Rapid Plasmid Purification Systems (Gibco-BRL) from transformed cells. A PCR, using primer pairs T7/SP6, M13F/M13R, and T3/T7, was done to amplify inserts, which were then used as probes by marking with 32P[dATP] to hybridize them with genomic restrictions of the cassava parents described above.

Results

The 10 RGAs were successfully multiplied in the DH5-α *E. coli* strain, using Cell-Porator® Voltage Booster from Gibco-BRL at 2.4 kV/cm². The transformants were selected on blue/white color screening by plating on LB/ampicillin/IPTG/X-gal media. The complete digestion of genomic DNA was observed, using the six enzymes indicated above.

Southern blot analysis for each enzyme and genotype was performed. All filters were then hybridized with seven different probes from rice and maize, labeled with 32P. The probe Pic 15, a NBS gene from maize, showed bands with very low hybridization to both parents, at different molecular weight with *Eco*RV (1500 bp for CM 2177-2 and 1600 bp for M Nga 2), *Hind*III (1600 bp for CM 2177-2 and 1500 bp for M Nga 2), *Dra*I (1400 bp for CM 2177-2 and 1500 bp for M Nga 2) (Figure 7.7). The process was repeated, but no hybridization was achieved, probably because of technique sensibility (Figure 7.8 B). Hibridization of a cassava probe used as control, to cassava genome restricted with the mentioned enzymes, is showed in Figure 7.8.A. In conclusion, these monocotyledonous probes have too low homology with cassava DNA. We are therefore continuing with degenerated primers based on disease resistance genes from crops other than cassava.
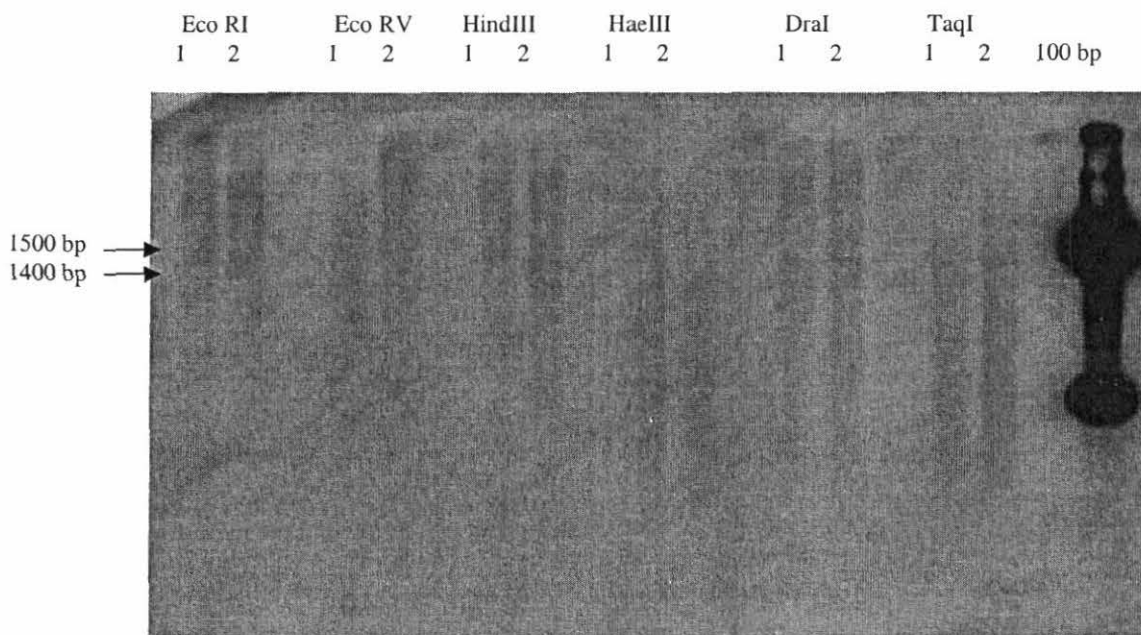
Figure 7.7. Hybridization of probe Pic 15 from maize to DNA digested with six enzymes from CM 2177-2 (1) and M Nga 2 (2), parents of the K family of cassava.

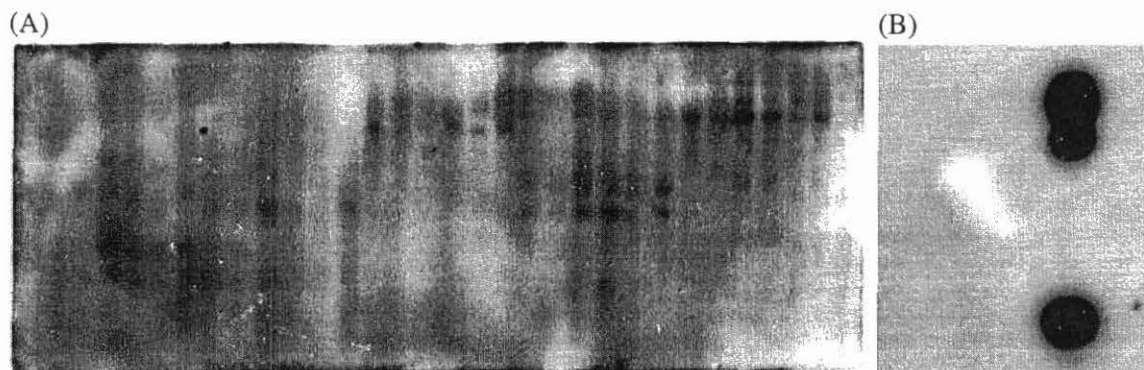(A)   (B)

Figure 7.8. **(A)** Hybridization of a control probe from the cassava genetic map to different cassava genotypes. **(B)** Hybridization of the monocotyledonous probe Pic 21 to a 100-bp ladder on a membrane where family K parents DNA was transferred.

Reference

Dellaporta SL; Wood J; Hicks JR. 1983. A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19.

Activity 7.9.   ***Using PCR with degenerated primers to search for resistance gene analogs associated with resistance to cassava bacterial blight***

Specific objective

1. To develop molecular markers associated with resistance to CBB.

Methodology

A set of five primers used in rice by Chen et al. (1998) and corresponding to conserved domains in disease resistance genes were used to amplify similar sequences in cassava DNA from CBB-resistant genotypes.   Each PCR reaction was performed in 25-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 mM MgCl$_2$; 0.25X Q solution (QIAGEN kit for PCRs); 1.5 U of *Taq* polymerase; 1 µM primer; 2.5 µL 10X *Taq* polymerase buffer; and 150 ng template DNA. For control reactions, template DNA was substituted by sterilized distilled H$_2$O.

Amplification of NBS, Pto, WipK, and XLLR was carried out in a MSJ-Research PTC-100 thermal cycler programmed for 5 min at 94°C; 45 cycles with denaturing for 1 min at 94°C, annealing for 1 min at 45°C, and extension for 2 min at 72°C; and a final extension for 7 min at 72°C. For amplification with the KSU primer, the same program was used, but changing the annealing temperature to 42°C and the final extension time to 10 min.

The primer NBS is a sequence from conserved motifs of the nucleotide-binding site in tobacco N and *Arabidopsis* RPS2 gene (Yu et al. 1996); XLRR is a sequence based on the leucine-rich

repeat region of the RPS2 and Xa 21 from rice (Chen et al. 1998); Pto is a sequence for potato kinase (Leister et al. 1996); WipK amplifies the conserved region of MAK kinase from parsley (Y12875), tobacco (D61377), *Arabidopsis* (MPK3), and *Medicago sativa* (MMK4) (Ligterink et al. 1997); and KSU is a sequence recommended by Dr Hulbert Scot, Kansas State University.

Primers used were:

XLRR f: 5'-CCGTTGGACAGGAAGGAG-3'
XLRR r: 5'-CCCATAGACCGGACTGTT-3'

WipK 1: 5'-GGTCGTGGTGCTTATGGAAT-3'
WipK 2: 5'-CCATGAAGATGCAACCGAC-3'

NBS f1: 5'-GGAATGGGNGGNGTNGGNAARAC-3'
NBS r1: 5'-YCTAGTTGTRAYDATDAYYYTRC-3'

Pto 1: 5'-ATGGGAAGCAAGTATTCAAGGC-3'
Pto 2: 5'-TTGGCACAAAATTCTCATCAAGC-3'

KSU f: 5'-GGIGGIGTIGGIAAIACIAC-3'
KSU r: 5'-ARIGCTARIGGIARICC-3'

DNA (150 ng) from three cassava genotypes resistant to CBB (CM 6438-14, CM 7772-13, and CM 3311-4) and from one susceptible genotype (M Bra 1045) was amplified with the primers described. The PCR product was electrophoresed in 1.8% agarose gel in 0.5X TBE buffer. A 100-bp DNA ladder was used to estimate the size of each amplified DNA fragment.

The PCR product was purified, using QIAquick PCR purification kit (QIAGEN). To search for sequences associated with NBS genes, well-defined bands between 350 and 800 bp, obtained by PCR with the degenerated NBS primer, were eluted from agarose gel, using QIAquick Gel extraction kit (QIAGEN).

PCR products and eluted bands were introduced into the DH5-α *E. coli* strain, 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 and conserved in glycerol at -80°C.

Different sized bands were observed by restriction with enzyme *Eco*RI from the vector and electrophoresed in 1.5% agarose gel in 0.5X TBE buffer.

Some clones will later be sequenced to search for homologies with disease resistance genes reported in GenBank (www.ncbi.nlm.nih.gov) and, using sequence-matching resistance genes, primers will be designed to amplify DNA from a segregant population, using SSCP analysis.

Results
Cloning. PCR products from three cassava genotypes resistant to CBB (CM 6438-14, CM 7772-13, and CM 3311-4) and from one susceptible genotype (M Bra 1045) were amplified with the primers described above (Figure 7.9). The PCR product was electrophoresed in 1.8%

agarose gel in 0.5X TBE buffer. A 100-bp DNA ladder was used to estimate the size of each amplified DNA fragment.

(A)

(B)
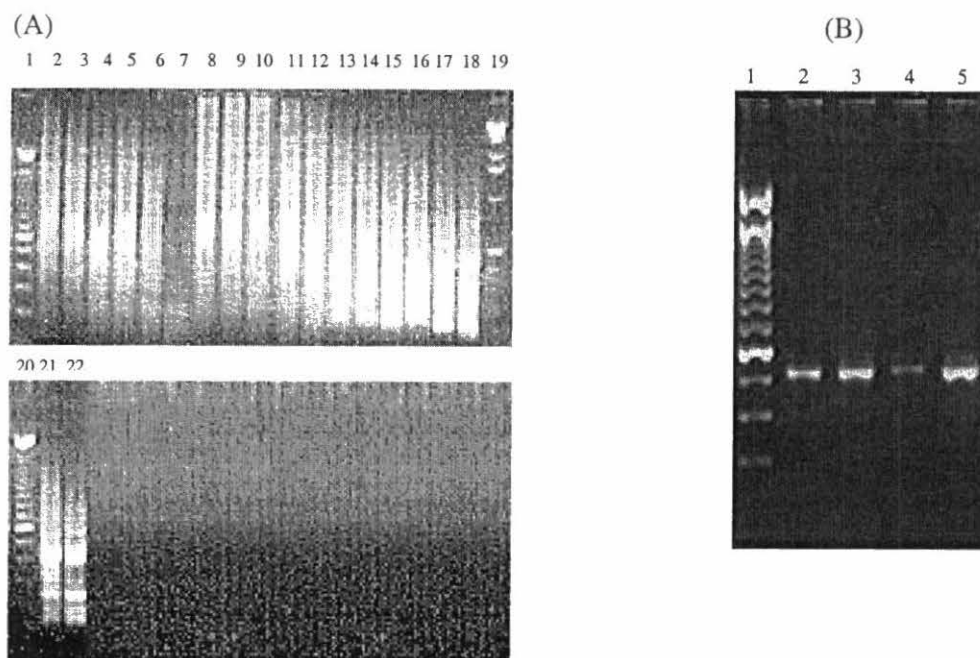
Figure 7.9.   DNA from CM 6438-14, CM 7772-13, CM 3311-4 and M Bra 1045 amplified with primers NBS, Pto, WipK, and XLLR. **(A)** *Primer NBS:* lane 1 = 100 bp; lane 2 = CM 6438-14; lane 3 = CM 7772-13; lane 4 = CM 3311-4; lane 5 = M Bra 1045; lane 6 = positive control (M Cr 81); lane 7 = negative control. *Primer Pto:* lane 8 = CM 6438-14; lane 9 = CM 7772-13; lane 10 = CM 3311-4; lane 11 = M Bra 1045; lane 12 = positive control (M Cr 81). *Primer WipK:* lane 13 = CM 6438-14; lane 14 = CM 7772-13; lane 15 = CM 3311-4; lane 16 = M Bra 1045. *Primer XLLR:* lane 17 = CM 6438-14; lane 18 = CM 7772-13; lane 19 = 1 kb; lane 20 = 100 bp; lane 21 = CM 3311-4; lane 22 = M Bra 1045. **(B)** *Primer KSU:* lane 1 = 100 bp; lane 2 = CM 7772-13; lane 3 = CM 3311-4; lane 4 = CM 6438-14; lane 5 = M Bra 1045.

Band elution and cloning. Well-defined bands between 350 and 800 bp were obtained by PCR with a degenerated NBS primer. These were then eluted from agarose gel (Figure 7.5, inside circle, lane 2).

The number of clones obtained with the primers 6 (WipK), 13 (XLLR), 7 (Pto), and 1 (NBS) from DNA amplification of resistant genotypes CM 6438-14, CM 7772-13, and CM 3311-4 (Table 7.13).

Table 7.13. Clones obtained by PCR from CM 6438-14, CM 7772-13, and CM 3311-4 with primers NBS, Pto, WipK, and XLRR.

Clone	Primer	Size (bp)	Genotype	Clone	Primer	Size (bp)	Genotype
N36	NBS	600	CM 7772-13	X1	XLRR	530	CM 3311-4
P30	Pto	350	CM 3311-4	X2	XLRR	700	CM 3311-4
P31	Pto	390	CM 3311-4	X3	XLRR	750	CM 7772-13
P32	Pto	420	CM 3311-4	X4	XLRR	310	CM 7772-13
P33	Pto	330	CM 3311-4	X5	XLRR	1150	CM 7772-13
P34	Pto	380	CM 3311-4	X6	XLRR	390	CM 7772-13
P35	Pto	580	CM 3311-4	X7	XLRR	370	CM 6438-14
P36	Pto	530	CM 3311-4	X8	XLRR	500	CM 6438-14
W1	WipK	1200	CM 3311-4	X9	XLRR	400	CM 6438-14
W2	WipK	480	CM 3311-4	X10	XLRR	450	CM 6438-14
W3	WipK	420	CM 3311-4	X11	XLRR	280	CM 6438-14
W4	WipK	230	CM 3311-4	X12	XLRR	600	CM 6438-14
W5	WipK	610	CM 7772-13	X13	XLRR	580	CM 6438-14
W6	WipK	300	CM 6438-14				

Different sized bands were observed by restriction with enzyme *Eco*RI from the vector and electrophoresed in 1.5% agarose gel in 0.5X TBE buffer (Figure 7.11).
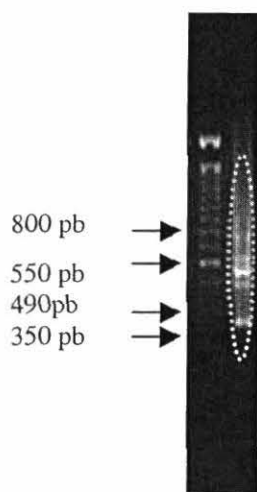

800 pb →
550 pb →
490pb →
350 pb →

Figure 7.10. Bands obtained by PCR with a degenerated NBS primer and eluted from agarose gel (inside circle, lane 2). Lane 1 = 100 bp; lane 2 = CM 7772-13 amplified with NBS.
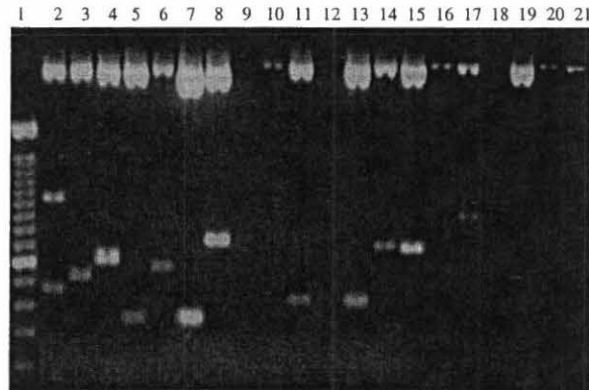
Figure 7.11. Different-sized bands observed by restriction with enzyme *Eco*RI from pGEM-T
Easy vector. Lane 1 = 100 bp; lanes 2-8 = different CM 3311-4 clones; lane 9 =
CM 7772-13 amplified with WipK; lanes 10-12 = different CM 6438-14 clones
amplified with WipK; lanes 13-20 = different CM 3311-4 clones amplified with
XLLR; lane 21 = CM 7772-13 amplified with XLLR.

Clones N-36, P-36, W-1, W-2, W-5, X-1, X-3, X-5, X-8, X-9, X=10 and X-12 were sequenced
from the plasmid, using T7 and SP6 primers. No homologies were found in GenBank
database to any disease resistance gene.

References

Chen XM; Line RF; Leung H.1998. Genome scanning for resistance gene analogs in rice,
    barley, and wheat by high-resolution electrophoresis. Theor Appl Genet 97:345-355.
Leister D; Ballvora A; Salamini F; Gebhardt CA. 1996. PCR-based approach for isolating
    pathogen resistance genes from potato with potential for wide application in plants.
    Nature Genet 14:421-429.
Ligterink W; Kroj T; Zur Nieden U; Hirt, H.; Scheel D. 1997. Receptor-mediated activation of a
    MAP kinase in pathogen defense of plants. Science 176:2054-2057.
Yu YG; Buss GR; Saghai Maroof MA. 1996. Isolation of a superfamily of candidate disease-
    resistance genes in soybean based on a conserved nucleotide-binding site. In: Proc
    Natl Acad Sci (USA) 93:11751-11756.

Activity 7.10.   ***Using PCR with degenerated primers to search for resistance gene
analogs associated with resistance to Phytophthora Root Rot***

Specific objective

   1. To develop molecular markers associated with resistance to PRR.

Methodology

Three sets of primers used in rice by Chen et al. (1998) (*see* Activity 7.8), corresponding to
conserved domains in disease resistance genes, were used to amplify similar sequences in

cassava DNA from genotypes resistant to *Phytophthora* spp. As in Activity 7.8, each PCR reaction was performed in 25-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 mM MgCl$_2$; 0.25X Q solution (QIAGEN kit for PCRs); 1.5 U of *Taq* polymerase; 1 µM primer; 2.5 µL 10X *Taq* polymerase buffer; and 150 ng template DNA. For control reactions, template DNA was substituted by sterilized distilled H$_2$O.

Amplification was carried out in a MSJ-Research PTC-100 thermal cycler programmed for 5 min at 94°C; 45 cycles with denaturing for 1 min at 94°C, annealing for 1 min at 45°C, and extension for 2 min at 72°C; and a final extension for 7 min at 72°C. For amplification with KSU primer, the same program was used, but changing the annealing temperature to 42°C and the final extension time to 10 min.

The primer NBS is a sequence from conserved motifs of the nucleotide-binding site in tobacco N and *Arabidopsis* RPS2 gene (Yu et al. 1996); Pto is a sequence for potato kinase (Leister et al. 1996); KSU is a sequence recommended by Dr Hulbert Scot, Kansas State University.

Primers used were:

    NBS f1: 5'-GGAATGGGNGGNGTNGGNAARAC-3'
    NBS r1: 5'-YCTAGTTGTRAYDATDAYYYTRC-3'

    Pto 1: 5'-ATGGGAAGCAAGTATTCAAGGC-3'
    Pto 2: 5'-TTGGCACAAAATTCTCATCAAGC-3'

    KSU f: 5'-GGIGGIGTIGGIAAIACIAC-3'
    KSU r: 5'-ARIGCTARIGGIARICC-3'

DNA from three cassava varieties resistant to *Phytophthora* spp. (M Bra 1045, M CR 81, and M Bra 532) was amplified with the primers described. The PCR product was electrophoresed in 1.8% agarose gel in 0.5X TBE buffer. A 100-bp DNA ladder was used to estimate the size of each amplified DNA fragment.

To search for sequences associated with NBS genes, well-defined bands between 350 and 800 bp, obtained by PCR with the degenerated NBS primer, were eluted, using a QIAGEN kit. PCR products and eluted bands were ligated in a pGEM-T Easy vector, which was introduced into the DH5-α *E. coli* strain by electroporation at 2.4 kV/cm². 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 to sequence by automated dideoxy sequencing (ABI Prism 377-96 DNA sequencer) and analyzed with Sequencher 4.1 software. Sequences were matched by nucleotide-protein sequence homology, using Blastx, a tool in GenBank (www.ncbi.nlm.nih.gov). Primers were designed, using Primer 3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

Results

Cloning. PCR products (Figure 7.12) were ligated in PGEM-T Easy vector. Transformant strains of *E. coli* DH 5-α were obtained by electroporation and conserved in glycerol at -80 °C. Different size bands were observed by restriction with enzyme *Eco* RI from the vector and electrophorized in 1.5% agarose gel (Figures 7.13. and Table 7.14). PCR was done in some recombinant plasmids with double fragment observed when restricted by *Eco* RI. A total of 27 clones were obtained with NBS primer, two clones with Pto primer by DNA amplification of resistant genotypes M Bra 1045 and M Cr 81 and one with KSU primer by DNA amplification of resistant genotype M Bra 532.

(A)   (B)

100 bp   1 2 3 4 5 6 7 8 9 10 11 12 13 14 15   100 bp 16 17 18   19 20 NC
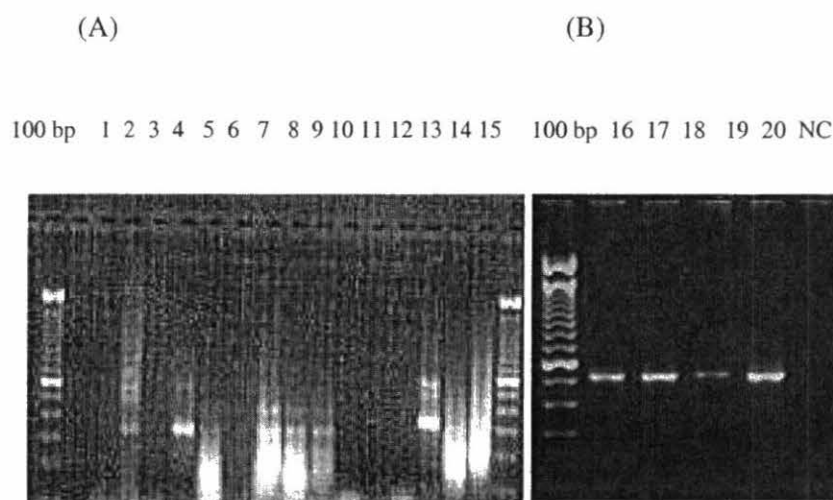
Figure 7.12. DNA from cassava genotypes (M Bra 1045 and M CR 81) resistant to Phytophthora root rot and amplified with primers NBS, Pto, and KSU. **(A)** *Primer NBS:* lane 1 = M Bra 1045; lane 2 = M CR 81; lane 3 = M Bra 1045; lane 4 = M CR 81; lane 6 = negative control; lane 9 = M Bra 1045; lane 10 = M CR 81; lane 11 = M Bra 1045; lane 12 = M CR 81. *Primer Pto:* lane 5 = M Bra 1045; lane 7 = M CR 81; lane 8 = M CR 81; lane 13 = M Bra 1045; lane 14 = M CR 81; lane 15 = M CR 81. **(B)** *Primer KSU:* lane 16 = CM 7772-13; lane 17 = CM 3311-4; lane 18 = CM 6438-14; lane 19 = M Bra 1045; lane 20 = M CR 81; NC = negative control.

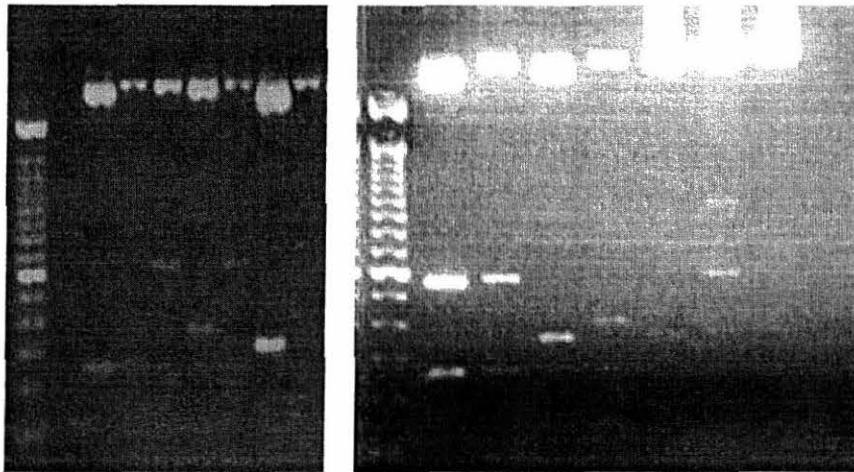100 bp 1 2 3 4 5 6 7   100 bp 8   9   10   11   12   13   14

Figure 7.13. Inserts obtained from M CR 81 and M Bra 1045 by PCR with a degenerated NBS primer, cloned in pGEM-T Easy vector and excised with *EcoRI*. Lane 1 = clone 1-2; lane 2 = clone 1-3; lane 3 = clone N-32; lane 4 = clone N-33; lane 5 = clone 2-3; lane 6 = clone N-34; lane 7 = clone 4-1; lane 8 = clone 1-4; lane 9 = clone 1-5; lane 10 = clone 2-4; lane 11 = clone 2-5; lane 12 = clone 2-6; lane 13 = clone 4-3; lane 14 = clone 4-4.

Table 7.14. Clones obtained by PCR from cassava varieties M CR 81 and M Bra 1045 with NBS and Pto primers.

Clone	Genotype	Primer	Size (bp)	Clone	Genotype	Primer	Size (bp)
N-13	M Bra 1045	NBS	131	N-35	M Cr 81	NBS	1100
N-15	M Bra 1045	NBS	300	N-37	M Bra 1045	NBS	325
N-16	M Bra 1045	NBS	866	N-38	M Bra 532	NBS	474
N-17	M Bra 1045	NBS	280	1-2	M Cr 81	NBS	270
N-18	M Bra 1045	NBS	873	1-4	M Cr 81	NBS	800
N-20	M Bra 1045	NBS	120	1-5	M Cr 81	NBS	800
N-21	M Bra 1045	NBS	120	1-5-1	M Cr 81	NBS	800
N-22	M Bra 1045	NBS	500	1-5-2	M Cr 81	NBS	800
N-23	M Bra 1045	NBS	464	2-3	M Bra 1045	NBS	650
N-24	M Bra 1045	NBS	893	2-5	M Bra 1045	NBS	400
N-30	M Cr 81	NBS	950	4-1	M Cr 81	NBS	300
N-31	M Cr 81	NBS	950	4-3	M Cr 81	NBS	1600
N-32	M Bra 1045	NBS	650	P-10	M Bra 1045	Pto	400
N-33	M Bra 1045	NBS	502	P-24	M Bra 1045	Pto	440
N-34	M Cr 81	NBS	320	K-1	M Bra 532	KSU	496

Band elution and cloning. Figure 7.14 shows eluted bands, which were obtained by PCR of M CR 81 DNA with a degenerated NBS primer. No positive clones were obtained after cloning in *E. coli.*

Sequencing. Some clones were sequenced to search for homologies with disease-resistance genes reported in GenBank (www.ncbi.nlm.nih.gov). With sequence-matching resistance genes, primers were designed to amplify DNA from a segregant population. The sequence obtained from clones N-23, N-33, N-37, N-38 and K-1 with T7 primer are reported in Figure 7.15, and their matching scores are described in Table 7.15, where homology with diverse disease resistance gene products is presented. Table 7.15 thus shows a list of main resistance gene analogs to sequenced clones N-23, N-33, N-37, N-38 and K-1, which were matched by nucleotide-protein sequence homology, using Blastx, a tool in GenBank. Sequences obtained matches with NBS, NBS/LRR, NBS/Toll and disease resistance genes reported for different species.
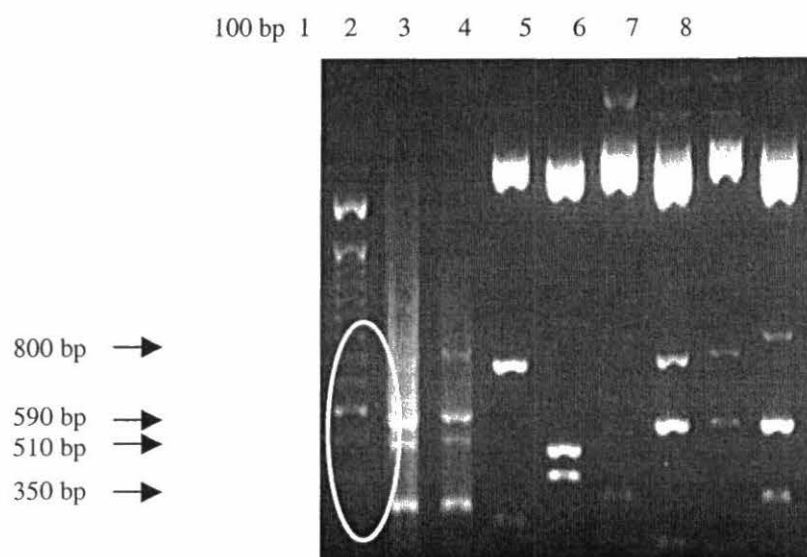
Figure 7.14.   Bands obtained by amplification of DNA from M CR 81 with a degenerated NBS primer and eluted from agarose gel (inside circle, lane 2). Clones in the pGEM-T Easy vector were restricted with *Eco*RI (lanes 3-8).

(A)

```
1   GAATGGGGGT CGTCGGGAGAACAACTATTGC TAGACKDGGMTRTSAGCMACTATCCYCTC
61  AATWWGAAGGTAGCAGCTTTCTTKCAAATGTTAGAGAAGKTGGGGAGAAGTATGGTTWGG
121 WYYCTTWACAAAAACAGMTGCTTACTGCAATTTTAATTGATCCGKACATATCTATTTGCG
181 ATGCTCATAGTGSAKCTGATGAGGWCASAAKTGGGCTACMTGGGAAAAAAGKYCTARTMR
241 TKCTGGATGATGTCTGCCRATTGGACCAGTTAAAMTTWTTMRCTGGCATGCATGATWCGG
301 ATMSGGAATGGAAGCAAGGTAATCMTSACRACTNNAAATCMCTAGYKAAYTCACGGYCGY
361 CTGCANGTSCTCCATRTSSGAKAKCTCCCAACGCGCCKRTGCATMCCTTGAGTTTMTATA
421 TNGCCCCTACATAGCTTGACGTARTCATGGTCATAGGCGCTCCT
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(B)

```
1   GGAATGGGAGGGGTGGGAAAGACAACTTTAGCTCAGCTTCTYTTTAATGAAGCCAAACTG
61  AACTTTGATTTAACGGCTTGGGTTTTAKTTGGKGATGATTTTGACGTTTTCARAATCTCC
121 CAAACGATTTTCCAGTGGTTTGGGGGGRRATTTTGATGGCAAARATTTGAATTTGCTTYAA
181 GTAAGATTGAAGGAAAAGCTKTCCCATAARAAATTTTTGATTGKCCYGGATGACCTTTGG
241 AATGARAAGTWTGAGGRTTGGAATCYYTTTYGNGGGCCYTTTGAAWWTGGGGCAAGAGGA
301 ASCMGGGTAATCNTTCCMMCTAGRAATCMCTNTTTGAATTNNCGGCCNCCTGCAGGTCNA
361 CCATNTGGGANAGCNCCCMMCCCSGTTGGATGCNTANCTNGAGTNTTYTATATGGNCCCY
421 CAAWAGCTTKGCSTAATCATGGGCATAGCTGTTTCYGTGTGAAAATGKTATTCCCTCCCA
481 ATTYCMCMCAACAWNCCAGCCC
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(C)

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1   GGAATGGGGGGCGTAGGGAAGACAACTCTAGCTCAACTAGTCTACAATGATCCCATGTTG
61  GAGTTTGATTTAAAAGCCTGGGTGTCTGTTGGTGAAGATTTTGATGTTTCCAGGGTCACA
121 AAAACATTTCTTCTTCAACTGGGTGATGGCGGT0GATGATAAAGATTTGAATCTGCTTCAG
181 GTAAAATTGAAGCAAAAGTTGTCTGGGAAGAAGTTTTTAGTTGTCCTAGATGATGTCTGG
241 ACCCAGAACTATGAAGAATGGGCTCTATTTTGGGGTCCTTTTGAAGCAGGGGCTCCTCAA
301 AGCAAGATCATCATCACAACTAGAA
```

(D)

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1   AGGGCTAGGGGGAGGCCAGGGCTAGGGCTAGGGCTAGGGGGAGGCCATTGGCGTAATCTA
61  CCACCTTACATGACAACCCCATATATTCTTTTGAAGGATGGTGATTTTGGAAGGCCCTTA
121 GACTTAAAAGTTGAAGGCCTTCAATATCATCTAGTCCTTTAGCCTTGGATATTTGATCCA
181 CTCCATGACCATTAAGCAAATGCTCATCTCTAGTAGTGATAAGAATTCTACTCCCTGGGC
241 CAAACCAATCATTTTTTCCGGCTAGTTTTTGCAACTGATTTAGTTCATTCACATCATCCA
301 TAACAATCAAGACCCTTTTGAGGCGAAGCTTCCTTTTTATCATGTCCATTCCATTATATA
361 TATTCCATATTTTAACCCTTTTCTCCGTAAGAATTTCTGAAAGAAGTTGTTCTTGTAAAG
421 AGACTAGGCCACCTTTAGATGAAACTTTCAMTAAACACTGGGAAAGGAAGGCYC
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(E)

```
1   GGGGGGGGTGGGGAAGACGACTTTTATACCAAGGATATATGCGGCCTCCCCCTAGCCCTAG
61  CCTGGGGGGGGTGGGGAAGACSACCATTGCAAGAGCTTAATACAATTCCGTATCTTATCAT
121 CAGTTTGAGGGTAAGGCCTTCCTTTCCAGTGTTAGAGAAGTTTCATCTAAAGGTGGCCTA
181 GTCTCTTTACAAGAACAACTTCTTTCAGAAATTCTTACGGAGAAAAGGGTTAAAATATGG
241 AATATATATAATGGAATGGACATGATAAAAAGGAAGCTTCGCTTCAAAAGGGTCTTGATT
301 GTTATGGATGATGTGAATGAACTAAATCAGTTGCAAAAACTAGCCGGAAAAAATGATTGG
361 TTTGGCCCAGGGAGTAGAATTCTTATCACTACTAGAGATGAGCATTTGCTTAATGGTCAT
421 GGAGTGGATCAAATATACAAGGCTAAAGGACTAGATGATATTGAAGGCCTTCAACTTTTA
481 AGTCTAAGGGCCTTYC
```

Figure 7.15. Sequences of cassava clones isolated from M Bra 1045 and M Bra 532 by PCR with degenerated NBS primers; (A) clone N-23 (464 bp); (B) clone N-33 (502 bp); (C) clone N-37 (325 bp); (D) clone N-38 (474 bp), isolated from MBRA 532 with a NBS primer; (E) clone K-1 (496 bp), isolated from MBRA 532 with KSU primer. D: A, G or T, K: G or T, M: A or C, N: any base, R: A or G, S: G or C, W: A or T, Y: C or T. Underlined bases show where each primer starts amplification.

Table 7.15.   Main resistance gene analogs matching with clones 2-4, N-23, and N-33 isolated by PCR with a degenerated NBS primer from cassava genotype M Bra 1045.

Protein matching in GenBank	Matching species	Homology score (bits)	Prob. of higher scores	Identities (%)[a]	Positives (%)[b]
Clone N-23 (primer T7)					
NBS-kinase protein Z2	*Solanum tuberosum*	56.2	1e -07	35	47
Putative disease resistance gene analog NBS-LRR	*Malus prunifolia*	54.3	5e –07	40	47
Disease resistance-like protein	*Glycine max*	52.8	1e -06	35	45
Putative resistance gene homolog	*Cucumis melo*	51.6	3e –06	32	45
NBS-2	*Cucumis melo*	51.2	4e -06	32	45
Resistance-gene protein	*Vigna unguiculata*	50.4	7e –06	34	44
Resistance-like protein KNBS2	*Glycine max*	48.9	2e -05	35	43
Resistance-like protein KNBS3	*Glycine max*	48.9	2e -05	34	45
Putative resistance protein	*Glycine max*	48.9	2e -05	31	41
Resistance-gene protein	*Vigna unguiculata*	48.9	2e -05	33	44
Clone N-33 (primer T7)					
NBS/LRR resistance protein-like protein	*Theobroma cacao*	103	1e -21	44	59
Resistance protein candidate	*Lactuca sativa*	96.7	1e -19	46	60
Probable resistance protein-soybean (fragment)	*Glycine max*	93.2	1e -18	43	59
Disease resistance-like protein	*Glycine max*	92.4	2e -18	45	60
NBS-LRR resistance-like protein J78	*Phaseolus vulgaris*	92.0	2e -18	41	57
Disease resistance protein I2	*Lycopersicon esculentum*	88.2	4e -17	42	55
Putative resistance protein KNBS4	*Glycine max*	87.8	5e -17	41	58
Resistance gene analog	*S. phureja* × *S. stenotomum*	84.3	5e -16	40	57
Clone N-37 (primer T7)					
NBS/LRR resistance protein-like protein	*Theobroma cacao*	92.4	1e -18	44	53
Disease resistance protein homolog	*Vigna unguiculata*	86.7	5e -17	44	53
RGA-B protein	*Cicer arietinum*	82.8	8e -16	43	51
NBS-LRR resistance-like protein J78	*Phaseolus vulgaris*	80.5	4e -15	41	49
Clone N-38 (primer T7)					
Putative disease resistance-like protein NBS-LRR	*Malus domestica*	126	8e -29	46	70
Putative disease resistance protein OB8	*Phaseolus vulgaris*	125	2e -28	45	66
Putative disease resistance gene analog NBS-LRR	*Malus prunifolia*	125	2e -28	45	68
Putative NBS-LRR type disease resistance protein	*Pisum sativum*	125	2e -28	47	70

Putative resistance gene homologue	*Cucumis melo*	124	4e -28	43	64
R-gene homolog, similar to St334	*Solanum tuberosum*	120	4e -27	46	71
NBS-LRR-Toll resistance gene analog protein	*Medicago sativa*	110	8e -24	42	67
Clone K-1 (primer T7)					
Putative disease resistance gene analog NBS-LRR	*Malus prunifolia*	146	1e -34	52	72
Resistance-like protein KNBS3	*Glycine max*	142	2e -33	50	74
Resistance-gene protein	*Vigna unguiculata*	135	2e -31	52	72
Putative disease resistance-like protein NBS-LRR	*Malus domestica*	132	1e –30	48	73
Resistance protein analog	*Phaseolus vulgaris*	133	9e –31	49	66
NBS-LRR-Toll resistance gene analog protein	*Medicago sativa*	132	2e –30	50	72

a. Identities = matching gene products.
b. Positives = matching nucleotide.

Primer design. Based on the sequences obtained, primers were designed, using Primer 3 software (wwwgenome.wi.mit.edu/cgibin/primer/primer3_www.cgi). Primer characteristics are presented in Table 7.16. In Figure 7.15, underlined bases show where each primer starts amplification.

Table 7.16. Primers designed to amplify disease-resistant sequences from cassava K family.

Primer	Base starting	Length bp	Melting temp. °C	GC %	Product size (bp)
From clone N-23					
CGTCGGGAGAACAACTATTGC	11	21	61.91	52.4	453
GGAGCGCCTATGACCATGA	463	19	62.15	57.9	
From clone N-33					
TGATTTAACGGCTTGGGTTT	66	20	59.45	40.0	390
GAAACAGCTATGCCCATGATT	455	21	59.06	42.9	
From clone N-37					
GGGAAGACAACTCTAGCTCAACT	16	23	58.24	47.8	325
GATGATGATCTTGCTTTGAGGA	315	22	59.27	40.9	
From clone N-38					
GCCATTGGCGTAATCTACCA	44	20	60.86	50.0	400
TTCATCTAAAGGTGGCCTAGTCTC	443	24	60.15	45.8	
From clone K-1					
CCATTGCAAGAGCTTAATACAATTC	83	25	60.37	36.0	351
TTTGATCCACTCCATGACCA	433	20	59.89	45.0	

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Activity 7.11. ***Evaluating cassava genotypes and families for resistance to Phytophthora root rot***

Specific objectives
1. *To evaluate Phytophthora sp. resistance of a group of cassava landraces from Mitú.*
2. *To evaluate Phytophthora sp. resistance of some elite cassava cultivars.*
3. *To evaluate Phytophthora sp. resistance of two cassava families.*

Methodology: Mitú landraces

Roots from 24 cassava landraces collected from indigenous settlements at Mitú, Vaupés, and 3 genotypes adopted by indigenous farmers through participatory research were inoculated with fungal discs of the *Phytophthora* sp. isolate MTR4, also collected in Mitú. Root damage was determined by measuring width and length of lesions 7 days after inoculation. Rotten area of roots was calculated.

Methodology: elite genotypes

Ten elite genotypes and some commercial and local varieties from the Department of Quindío and Santander de Quilichao (Department of Cauca) were inoculated with fungal discs of *Phytophthora tropicalis*. Root damage was determined 5 days after inoculation by measuring width and length of lesions in transverse cuttings by the inoculation hole.

Methodology: cassava families CM 9582 and CM 9600

Cassava families CM 9582 (M Bra 1045 × M CR 81) and CM 9600 (M CR 81 × M CR 54), comprising 28 and 17 genotypes respectively, were characterized by root rot resistance after inoculation of fresh roots with fungal discs of *Phytophthora tropicalis*. Root damage was determined by measuring width and length of lesions 5 days after inoculation. A frequency analysis and graph with resistance distribution were completed.

Table 7.17. Twenty-eight cassava genotypes from Mitú, Vaupés, characterized for their resistance to *Phytophthora* sp., isolate MTR4.

Variety	Rotten area (cm^2)	Root rot (%)	Variety	Rotten area (cm^2)	Root rot (%)
Abeja	1.04	8.21	Siringa	4.28	18.02
Abiyú	2.32	11.04	Totuma	0.78	3.50
Dulce Cucura	2.07	10.33	Tres Mesina Dulce	4.06	17.02
Flores	1.17	7.35	Wasoco	0.25	1.41
Guaracú	2.00	8.92	Yuca de Agua	2.27	8.99
Hoja de Plátano	0.71	6.84	Yuca de Garza	3.18	18.22
Ibacaba	1.96	12.19	Yuca de Mico (white)	2.94	7.33
Inayá	1.49	10.06	Yuca de Mico (red)	3.65	14.43
Lapa Blanca	5.15	17.53	Yuca de Piña	3.18	15.05
Mirití	2.95	6.06	Yuca de Rana	2.92	14.62
Nupará	6.65	39.68	CG 165-7	3.39	27.30
Pintadillo	3.60	14.01	M Bra 12	5.65	18.89
Pupuña	3.23	8.83	M Bra 1044	3.08	9.04
Santa Catalina	0.70	5.00	M Ven 25	2.33	6.30
Duncan 5%	3.04	11.77	Duncan 5%	3.04	11.77

Results: Mitú landraces

Varieties Wasoco, Totuma, Santa Catalina, and Mirití were highly resistant to the pathogen, with the percentage of rot ranging from 1.4% to 6.05%, whereas 'Nupará', 'Yuca de Garza', and 'Siringa' were the most susceptible, having areas of rotten root similar to those of the susceptible control (M Bra 12), with percentages ranging from 18% to 39.7%. The control varieties—M Ven 25, M Bra 1044, and CG 165-7—adopted by indigenous farmers through participatory research, had rot percentages of 6.3%, 9.04%, and 27.3%, respectively (Table 7.17).

Results: elite genotypes

Of the 10 elite genotypes, M Bra 71, M Bra 1045, M Bra 532, CM 6438-14, and CM 3311-4 were the most resistant genotypes, with the percentage of rotten root area ranging from 7.17% to 15.3%. 'Manzana', a local variety from the Department of Quindío, had 28.11% of root area affected, as opposed to its performance in the field, where it was highly susceptible. 'Verde', a local variety from Santander de Quilichao (Department of Cauca), had 23.24% root area rotten. CM 7772-13, CM 523-7 (ICA Catumare), CM 2177-2 (ICA Cebucán), SM 1210-4, and M Col 2066 (Chiroza) were the most susceptible genotypes, with the percentage of rot ranging from 28% to 47.8% (Table 7.18). M Col 2066 in the field is susceptible to root rot.

Table 7.18. Characteristics of some commercial, elite, and local varieties of cassava genotypes for their resistance to *Phytophthora tropicalis* by artificial inoculation of roots in the laboratory.

Genotype	Rotten area (cm^2)	Root rot (%)	Genotype	Rotten area (cm^2)	Root rot (%)
Commercial genotypes			Genotypes for resistance studies		
CM 523-7	8.09	29.44	CM 7514-7	4.75	21.23
CM 2177-2	12.81	28.88	CM 7772-13	6.07	47.84
CM 3306-4	4.86	22.62	M Bra 12	5.81	26.59
HMC-1	4.86	22.77	M Bra 71	1.48	7.18
CM 3311-4	5.64	15.30	M Bra 532	1.25	10.29
			M Bra 1045	3.76	8.75
Elite Genotypes			M Nga 2	7.61	19.10
CM 2772-3	9.26	25.49			
CM 5655-4	8.13	17.07	Local varieties		
CM 6438-14	3.46	10.64	M Col 2066	10.71	28.10
M Bra 383	5.90	19.97	Manzana	3.44	24.82
M Per 183	7.34	19.84	Verde	8.75	23.24
SM 1210-4	5.45	28.11			
SM 1557-17	6.19	18.57			
SM 1741-1	5.22	16.03			
SM 653-14	3.67	16.33			
SM 909-25	6.81	26.60			
Duncan 5%	2.86	10.37	Duncan 5%	2.86	10.37

Results: cassava families CM 9582 and CM 9600

Disease severity was low, compared with the previous year's evaluation. Frequency analysis resulted in groupings according to percentage of root rotten area, as shown in the following list:

Group	CM 9582 family	CM 9600 family
1	0-6.8	0-10.5
2	6.8-13.5	10.5-21.0
3	13.5-20.3	21.0-31.5
4	20.3-27.0	31.6-42.0
5	27.1-33.8	42.1-52.5

As shown in Figure 7.16, 3.7% of individuals from the CM 9582 family are in groups 1 and 2, while 81.4% of individuals are in groups 3 and 4. Of the individuals in the CM 9600 family, 14.8% are in groups 1 and 2, while 25.9% are in group 5.
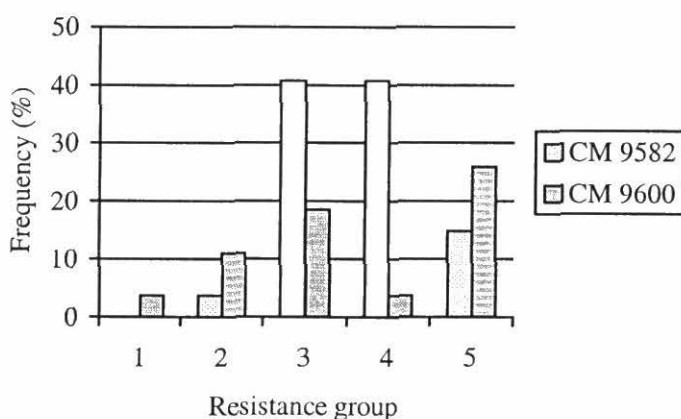
Figure 7.16. Breakdown of cassava families CM 9582 and CM 9600, inoculated with *Phytophthora tropicalis* on roots, according to their resistance groups, based on frequency analysis.

Activity 7.12.   *Genetics of Resistance to Rot Caused by Phytophthora tropicalis in Two Segregating Populations of Cassava (Manihot esculenta Crantz)*

Rationale

In Brazil, of the *Phytophthora* spp., *P. drechsleri* Tucker most severely attacks cassava (Albuquerque and Figueiredo 1968). This species has been identified in Colombia (Oliveros et al. 1974), together with *P. nicotianae* var. *nicotianae* (Sánchez 1998; Lozano and Loke 1994;

Soto et al. 1988). Other species reported as cassava pathogens in different countries are *P. erythroseptica* (Fassi 1957), *P. cryptogea* (CIAT 1991), *P. meadii* and *P. arecae* (Alvarez et al. 1997; Barragán et al. 1998), and *P. tropicalis* (which is similar to *P. capsici*).

The development of *Phytophthora* spp. is favored by use of inadequate agronomic practices and ineffective fungicides, transport of material from affected areas to those free of the pathogen, and by planting in compact or very clayey soils (Takatsu and Fukuda 1990).

Currently, CIAT selects for resistance to *Phytophthora* spp. under greenhouse conditions, inoculating shoots and roots with isolates that were previously identified by sequencing the ITS region in the rDNA.

Molecular techniques are increasingly being used to decipher the genetic base of complex agronomic traits. Genetic improvement for disease resistance can be achieved more quickly and effectively by using molecular markers.

To better understand the genetics of resistance to *Phytophthora* spp., this study evaluates individuals from the cassava populations K family and CM 9582 for their reaction to root rot caused by *P. tropicalis*.

Materials and Methods

Plant materials

In 2000 and 2001, 1-year-old roots of 69 cassava genotypes belonging to the K family grown at CIAT's experiment station at Santander de Quilichao (Department of Cauca) were inoculated and evaluated. In July-August 2001, 1-year-old roots of 43 cassava genotypes belonging to the CM 9582 population were harvested at the Centro de Investigación de la Caña de Azúcar de Colombia (CENICAÑA, Florida, Department of Valle) and evaluated. Also included in the study were four genotypes from CIAT's Quilichao station: one resistant (M Bra 1045) and three susceptible (M Col 2066, CM 2177-2, and M Nga 2) to *Phytophthora tropicalis* (*Pt*):

Parent	Origin	Reaction to *Pt*
K family		
M Nga 2	Nigeria	Susceptible
CM 2177-2	Hybrid, CIAT	Susceptible
CM 9582		
M CR 81	Costa Rica	Susceptible
M Bra 1045	Brazil	Resistant

For the QTL analysis, roots of 92 genotypes of the K family were harvested at CIAT in 2000. The roots were then washed with drinking water and detergent, and disinfected, first with 1% hypochlorite, then with 30% ethanol, each for 10 min. The roots were then dried with sterilized paper towels. The material that was disinfected but not inoculated the same day was stored (for a maximum of 24 h) in a cold room at 4°C until inoculated.

The pathogen

As inoculum, isolate 71 was used. It was identified, through sequencing the ITS region of ribosomal DNA, as *P. tropicalis* (which is similar to *P. capsici*). This isolate was found in cassava infected with root rot in Barcelona (Department of Quindío). The inoculum was cultured in medium prepared with oat agar (2% Quaker® oats, 2% agar) and antibiotics (penicillin at 900 mg/mL; rifampicin, 0.2 g/mL; and ampicillin, 750 mg/mL). Incubation was carried out at temperatures between 20°C and 26°C for 4 to 11 days for the K family and 6 to 7 days for the CM 9582 population.

Inoculation

Within an isolation chamber, in front of a burner, a piece of cassava root, about 15 mm long, was extracted with a punch, 7 mm in diameter. At the bottom of the perforation left behind, a piece of the fungus, also extracted with a punch, 5 mm in diameter, was deposited. The extracted piece of cassava root was replaced and secured with masking tape. Each genotype was also inoculated with a negative control, that is, the medium of oat agar and three antibiotics, but no *P. tropicalis*. Once inoculated, the treated cassava roots were deposited in plastic bags, containing moist, sterilized, paper towels. The closed bags were then placed in plastic trays, and left at 22°C in darkness for 7 (K family) or 5 days (CM 9582).

Evaluation

From each cassava root, a cross section was taken at the point where the inoculum was deposited. The height and width of both wound and entire cross section were measured, together with root length, and depth of inoculum in the root. The type of rot was also evaluated: 1 = soft/moist; 2 = dry; 3 = soft/dry; 4 = soft/moist and dry. These data were recorded and processed through *Excel's* calculation program.

Data analysis

The experimental unit was the root. For the K family, the following were taken into account: (1) genotypes with fewer than five (2000) or six (2001) roots were excluded; (2) roots with an average diameter of less than 3 cm were discarded; (3) slices of root with wounds wider than 7 (2001) or 8 cm (2000) were considered as having 100% of their area infected, and values for roots wider than 7 (2001) or 8 cm (2000) were converted for 7 or 8 cm, respectively.

For the CM 9582 population, the following were taken into account: (1) genotypes with fewer than four replicates were excluded; (2) roots with an average diameter of less than 3 cm were eliminated; (3) slices of root with wounds wider than 6 cm were considered as having 100% of their area infected, and values for roots wider than 6 cm were converted for 6 cm.

QTL analysis

One framework map was used for QTL analysis, based on the segregation of molecular markers in a population from a cross between two heterozygous parents: M Nga 2 (female) and CM 2177-2 (male). The female-derived map was based on the segregation of female alleles, corresponding to 192 markers that compromised RFLP, random amplified polymorphic DNA (RAPD), isoenzymes, microsatellites, expressed sequence tags (ESTs) and known genes (Fregene 2002, in preparation).

A significant association between a DNA marker and *Phytophthora* resistance was declared if the probability was more than 0.005 to minimize the detection of false positives. The degree of phenotypic variance explained by each marker was obtained from the regression coefficient (r^2 values). Total r^2 values from each QTL were computed as:

$$(sum\ of\ squares\ for\ each\ marker)/(total\ sum\ of\ squares)$$

All data were analyzed with Q-Gene on McIntosh.

Results

The roots of 69 individuals of the K family (M Nga 2 × CM 2177-2) and of 43 individuals of the CM 9582 population (M Bra 1045 × M CR 81) were inoculated with *P. tropicalis* to determine the genetic base of these populations' resistance to Phytophthora root rots (PRR).

The K family genotypes evaluated in 2000 and 2001 showed 30%-70% of areas continuously infected (Figure 7.17). Some genotypes that, in 2000, had intermediate resistance to *P. tropicalis* tended to become susceptible in 2001 and vice versa.
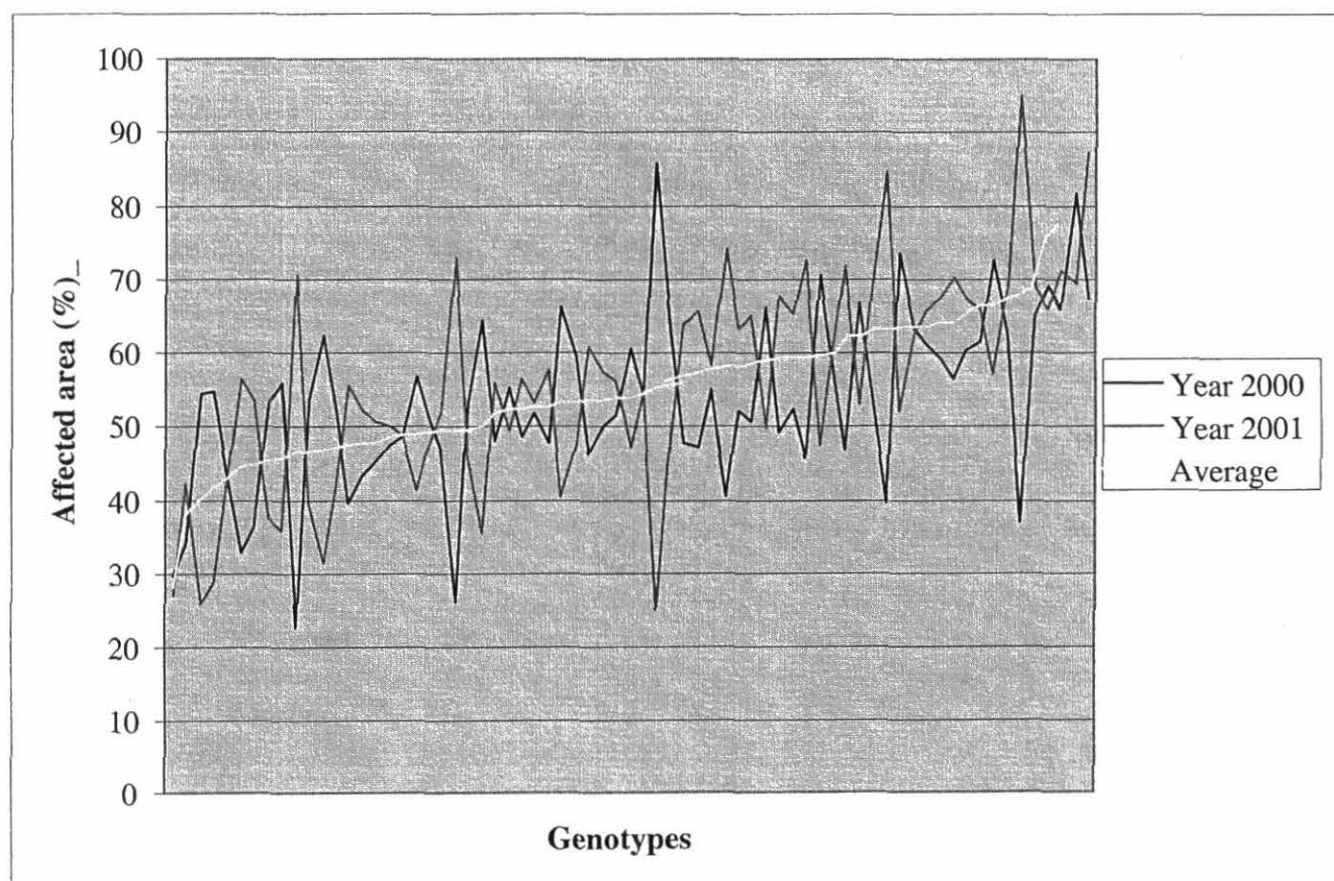
Figure 7.17.   Percentage of area affected by the root rot fungus *Phytophthora tropicalis* across genotypes of the cassava K family for years 2000 and 2001.

Certain genotypes with intermediate resistance in 2000 continued presenting intermediate resistance in 2001, showing 30%-60% of area infected. Genotypes from the CM 9582 population (2001) maintained 70%-90% of areas continuously infected. Few genotypes had intermediate resistance (Figure 7.18). Figures 7.19 and 7.20 show the distribution of individuals by group, according to the degree of resistance to the pathogen. Resistant materials were not detected.
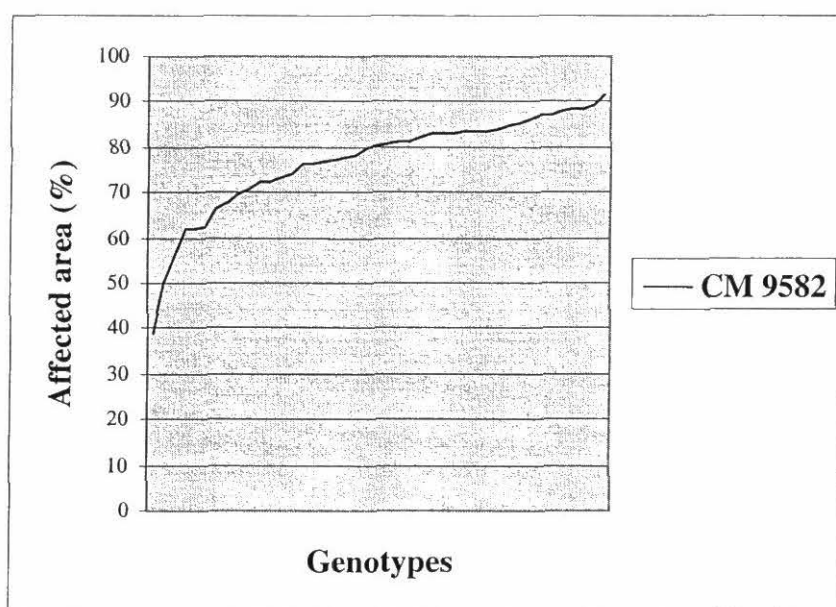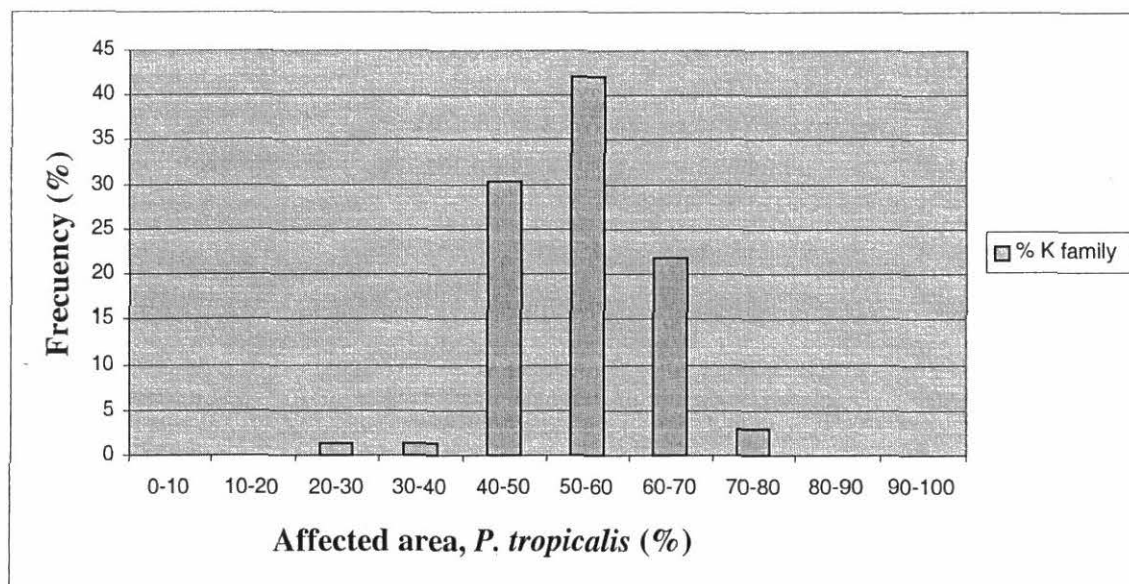

Figure 7.18.   Percentage of area affected by the root rot fungus *Phytophthora tropicalis* across genotypes of the cassava CM 9582 population for year 2001.

Figure 7.19. Distribution of the frequency of genotypes from the cassava K family according to the percentage of area affected by the root rot fungus *Phytophthora tropicalis.*
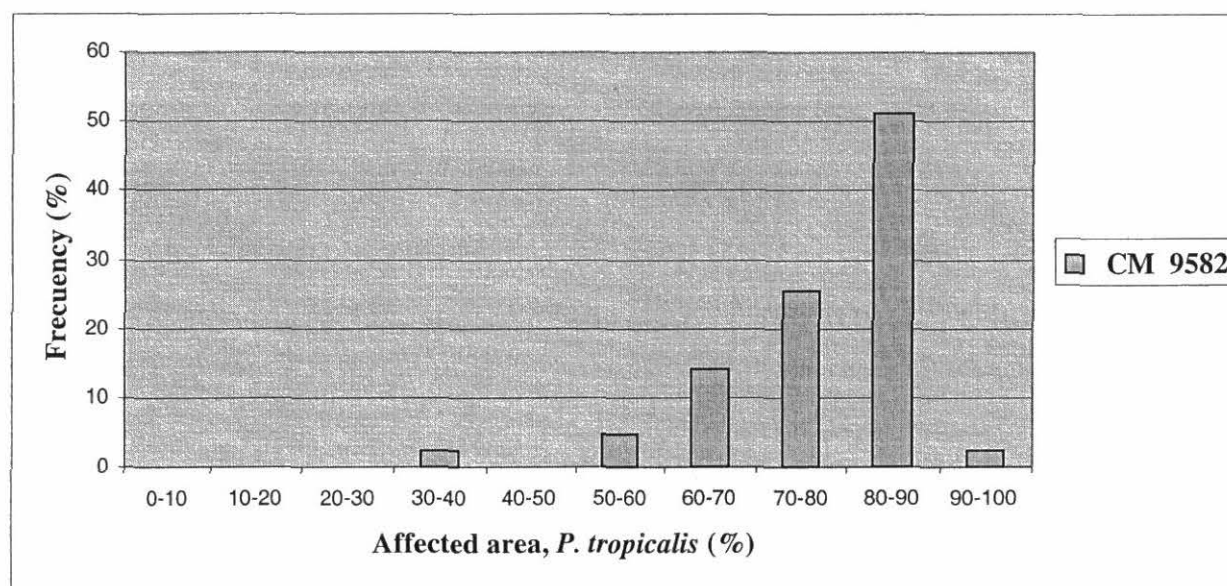

Figure 7.20. Distribution of the frequency of genotypes from the cassava CM 9582 population according to the percentage of area affected by the root rot fungus *Phytophthora tropicalis.*

For the K family across 2000 and 2001, the 10 genotypes with the highest intermediate resistance to *P. tropicalis* had values that ranged between 28% and 47%; and the 10 most susceptible genotypes averaged between 63% and 77%. For the CM 9582 population, the figures were, respectively, 35% and 69% and 84% and 88% (Table 7.19).

Table 7.19. Phenotypic evaluation of two cassava populations segregating for resistance to root rot (RR) caused by the fungus *Phytophthora tropicalis*.

Population or genotype	Reaction to RR			Population or genotype	Reaction to RR	
	Year				Year	
	2000	2001	Average		2001	CV (%)
	Family K			CM 9582		
	Intermediate (I) to resistant (R):					
K 19	29.7	27.1	28.4	136	35.2	21.3
K 110	34.2	42.1	38.2	148	49.6	57.3
K 88	54.4	26.0	40.2	150	54.9	25.6
K 98	54.8	28.8	41.8	133	60.3	20.5
K 69	44.2	41.8	43.0	151	61.2	21.0
K 114	32.8	56.6	44.7	121	61.6	16.5
K 79	36.3	53.6	44.9	71	66.7	6.9
K 66	53.2	37.8	45.5	115	67.8	6.2
K 30	55.9	35.8	45.9	140	68.9	13.3
K 81	22.8	70.5	46.7	47	69.1	14.7
Average	41.8	42.0	41.9		59.5	20.3
Correlation between years			-0.67			
	Susceptible (S):					
K 9	60.2	67.3	63.8	42	84.4	5.4
K 57	61.6	65.9	63.8	68	84.7	8.4
K 92	72.7	57.3	65.0	172	85.2	7.5
K 148	62.7	69.4	66.0	153	85.4	13.5
K 39	37.1	95.2	66.1	62	85.9	8.9
K 35	64.9	69.0	66.9	45	86.2	4.4
K 6	69.2	65.9	67.5	52	86.2	9.7
K 122	65.9	71.0	68.5	163	87.5	6.5
K 145	81.6	69.4	75.5	78	87.9	13.2
K 64	67.3	87.2	77.3	91	88.0	5.3
Average	64.3	71.7	68.0		86.3	8.3
Correlation			-0.66			
			General			
Average	53.6	56.0	54.8		76.0	
Correlation			-0.15			
			Parents			
M Nga 2	66.3	56.7	61.5	M CR 81	--	
CM 2177-2	69.6	83.9	76.7	M Bra 1045	46.1	19.6
			Checks			
M Bra 1045 (R)	11.6	51.5	31.5	M Col 2066 (S)	70.9	11.6
M Col 2066 (S)	70.5	86.2	78.3	M Nga 2 (S)	55.4	19.1
				CM 2177-2 (S)	68.3	7.4

Of the 10 intermediately resistant genotypes from the K family, six (K19, K88, K98, K69, K66, and K30) presented very low intermediate resistance during 2000, increasing toward the end of the year. The other four genotypes (K81, K79, K110, and K114) had higher intermediate resistance in 2000 than in 2001.

Of the 10 K family genotypes showing susceptibility in 2001, seven (K9, K57, K148, K39, K35, K122, and K64) were less susceptible in 2000 than in 2001, with the other three being more susceptible.

On average, the 10 intermediately resistant genotypes from the K family were more resistant than the 10 intermediately resistant genotypes from the CM 9582 population. Likewise, on average, the 10 most susceptible genotypes of the CM 9582 population were more susceptible than the 10 susceptible genotypes of the K family.

The coefficient of variation calculated for the CM 9582 population was 12.6%, indicating that the study was reliable with a margin of relatively low experimental error.

For genotypes from the K family with intermediate resistance, the distribution of frequency of genotypes against area infected by *P. tropicalis* presented a curve similar to that of a normal distribution (Figure 7.19). In contrast, for the most susceptible genotypes from the CM 9582 population, the curve was rising (Figure 7.20), with M Bra 1045 showing 56.3% of area infected.

The correlation between root length and area infected was –0.30 for the CM 9582 population, indicating that the longer the root, the less disease found.

Table 7.20 shows the results of the single-marker regression analysis of percentage of infected area in roots inoculated in the laboratory. Markers defined eight QTLs located on linkage groups C, H, J, N, Q, and V (Table 7.20). The QTLs explain between 1.3 and 9% of the variance, the most significant QTL being no. 7, located in linkage group V (chromosome no. 22) of the female-derived framework map.

Table 7.20. QTLs explaining the highest values of variance for resistance in cassava, as described by the percentage of root area infected. Values in bold are significant at $P = 0.05$.

Linkage group (female map)	Markers (position in cM)[a]	F[b]	V[c] (%)	P[d]	QTL no.
C (3)	RGY172	0.029	5.4	<0.0500	1
H (8)	SSRY178	0.315	1.3	<0.0500	2
J (10)	CDY76	0.163	4.0	<0.0500	3
	K2a	0.040	8.6	<0.0500	4
N (14)	SSRY13	0.078	4.2	<0.0500	5
Q (17)	SSRY911	0.047	5.7	<0.0500	6
V (22)	NS911	0.007	9.0	0.0070	7
	GY153	0.049	4.5	<0.0500	8

a. Distance from the first marker noted (o).
b. *F* statistics from analysis of variance.
c. Percentage of variance explained (from r^2 coefficient of regression).
d. Probability of *F* statistic.

Discussion and Conclusions

No reports exist on the genetic basis of resistance to root rot caused by *P. tropicalis* in cassava. Hence, this resistance was evaluated phenotypically in two populations: K family and CM 9582.

K family

Some of the genotypes evaluated from the K family expressed intermediate resistance to *P. tropicalis*, with some presenting intermediate resistance in 2000 but susceptibility in 2001. The opposite also occurred, where some susceptible genotypes in 2000 presented intermediate resistance in 2001.

Such changes may have been triggered by changes in the soil, environmental conditions, or use of chemical products (e.g., fertilizers). These factors indirectly affect partial resistance to pathogens—as corroborated by a study on the partial resistance of maize to *Puccinia sorghi*—and affect QTL expression (Lübberstedt et al. 1998).

Other factors may include the long vegetative cycle, vegetative propagation without quality control of planting stakes, and changes in populations of microorganisms (either beneficial or detrimental) in the rhizosphere and roots. Variability in resistance across years may indicate a polygenic nature of the K family, although the environment usually influences phenotypical expression, generating variation. It is important to note that certain genotypes of the K family with intermediate resistance in 2000 continued expressing it in 2001.

Although both parents of the K family are susceptible to *P. tropicalis*, a group of genotypes from this family showed intermediate resistance. This indicates that the parents are heterozygotes (Fregene et al. 1997) and that they both have resistance genes.

CM 9582 population

The CM 9582 population is obtained by crossing M Bra 1045 with M CR 81. In previous studies, M Bra 1045 has shown resistance to *P. tropicalis*, but in this study, it is susceptible, probably because of changes in environmental factors, as explained above. The genetic base of M Bra 1045 can be assumed to be polygenic, and to have epistasis in this crossing.

The two populations

On comparing the intermediate resistance presented by the K family and CM 9582 population, we found that the CM 9582 population had few genotypes with intermediate resistance to *P. tropicalis*. That is, the 10 most resistant genotypes of the K family had a higher degree of resistance than did the 10 most resistant genotypes of the CM 9582 population. The differences probably lie in the genetic crossings between the parents, which differ for the two populations.

Although the populations differed in their genetic base of resistance to *Phytophthora*, the levels of resistance observed were not sufficiently high to warrant use in genetic improvement programs. Hence, identifying new parents and developing new populations are desirable.

QTLs

Results show that resistance to *Phytophthora* root rot is polygenic in the K family. Results also suggest that the parameters measured for resistance are different and may represent different components of resistance. The occurrence of individuals more resistant than the two parents and the detection of QTLs associated with molecular markers from the female-derived map show that resistance alleles coming from both parents contribute to resistance in the progenies (transgressive segregation). Such characteristics are well known in heterozygous species and are useful for combining resistance genetic factors in the same cultivar (Jorge et al. 2001).

Genotypes classified as resistant in 2000 and susceptible in 2001, and vice versa, can be explained by the effect of environmental factors on the biochemical composition of inoculated cassava roots. Such a hypothesis, however, has to be proved.

Future research, ideally, should include:

- Inoculation of each root with a negative control and the pathogen, thereby reducing the probability of evaluating false positives.

- Use of roots without frogskin disease and with diameters measuring 4 to 7 cm.

- Study of factors influencing the expression of resistance.

- Evaluation of roots from different localities, such as Quindío and Cauca.

- Study of *Phytophthora* pathogenesis in cassava roots and resistance mechanisms.

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Activity 7.13. Super Elongation Disease: Development of hot water treated of cassava cuttings in the greenhouse at CIAT and in the field on the Colombian North Coast and Llanos Orientales.

Greenhouse CIAT

As series of control practices for Super Elongation Disease (SED, causal agent *Sphaceloma manihoticola)* were evaluated in a greenhouse at CIAT. All treatments were applied to two varieties 'Brasilera' (M Col 2737) and 'La Reina' (CM 6740-7). The treatments were:

Treatments
1. Thermotherapy, stem cuttings immersed in a water bath at 49°C during 49 min.
2. Stem cuttings immersed for 5 min in Score® (difenoconazole, 2.5 cc/L of the commercial product)
3. Stakes immersed for 5 min in Kocide® (copper hydroxide, 5g/L of the commercial product)
4. Stakes immersed for 5 min in water
5. Untreated stakes of the variety M Tai 8 was used as a susceptible control.

The experimental design was a randomized complete block design, with seven replicates and 20 plants per treatment for each variety. Each plant was protected with a plastic cover to avoid contamination by spores between the treatments.

Table 7.21.   Effect of stake treatments on Super Elongation Disease.

Treatment	SED	
	Germination[a]	AUDPC[b]
CM 6740-7		
Thermotherapy	80	7.0
Score®	95	15.1
Kocide®	85	4.4
Water	85	24.5
M Tai 8	95	0
M Col 2737		
Thermotherapy	100	11.1
Score®	100	19.6
Kocide®	85	7.6
Water	100	20.1
M Tai 8	100	0
M Col 2737 and CM 6740-7		
Thermotherapy	90	10.5 cc
Score®	97	18.3 b
Kocide®	85	6.3 d
Water	92	22.3 a
M Tai 8	97	0 e

[a]Percentage of germination, number of germinated plants.
[b]Average of the Area Under the Disease Progress Curve.
[c]Duncan's multiple range test, alpha <= 0.05.

The highest infections (average AUDPC of 22,3) occurred in the varieties M Col 2737 and Cm 6740-7 treated with water (Table 7.21). This confirms that the stem cuttings, obtained in the field, were highly infected by SED. The highly susceptible variety M Tai 8 did not show any disease symptoms, which indicates that cross contamination did not occur.

Kocide®, a protectant in the case of stem cuttings, provided the best control with an average AUDPC of 6,3. We suggest that foliar applications during the periods when inoculum pressure is great could provide better control. The thermotherapy The AUDPC of 10,5 in thermotherapy treatments was similar to that of water only. Hence we suggest that higher temperatures and longer exposures be tested.

Stem cuttings treated with Score®, which is known to be systemic, had an AUDPC of 19,6. This is a low level of control and for future experiments the immersion time will be prolonged and foliar applications will be made. At the moment in the Atlantic Coast (Sincelejo, Sucre) stem treatment and foliar applications of Score® (2.5 cc/L) and Kocide® (5 g/L) are being tested on susceptible varieties. Stem cuttings were also treated before planting using the

same doses of Score®. This fungicide was more effective than Kocide. Foliar applications appear to be more effective then treatment of stem cuttings. Leave rapidly absorb the product, which affects the subcuticular growth the hyphae of *Sphaceloma manihoticola.* Although the mode of action of the Score® is both has protective and curative, applications should be initiated when the first symptoms of the disease appears (label information, Score®).

Score®® and Kocide® do not persist for long on the leaves, whereas other products like Daconil® (chlorothalonil) which have adherents persist for longer periods and can be applied less frequently then Score®® and Kocide®. This product and others will be tested to optimize disease control.

Field evaluations at the Colombian North Coast

Field tolerance to SED, Cassava Bacterial Blight (CBB) and other disease control practices were evaluated in field trials in the municipality of Sincelejo (Sucre).

The incidence and severity of SED under various treatments (Table 7.22) are currently being evaluated in Sucre with the variety 'Venezolana' (M Ven 25). The regional variety 'Venezolana' was planted with vegetative seed obtained from a farm at Sincelejo, where SED was present. The experimental design was a randomized complete block design, with three replicates and 45 plants per treatment.

Table 7.22. Evaluation of the germination of control practices on SED at the Colombian north coast.

Treatment	Germination (%)[a]
	Sincelejo (Sucre)
Fertilized[b]	
Stake selection	98
Thermotherapy[c]	100
Kocide®, Sistemin®[d]	99
Score®, Sistemin®[e]	100
Control, traditional farmer's practice	100
Without fertilizer	
Stake selection	100
Thermotherapy[c]	100
Kocide®, Sistemin®[d]	100
Score®, Sistemin®[e]	100
Control, traditional farmer's practice	100

[a]Percentage of germination, number of germinated plants.
[b]Sucre: 15-15-15 (NPK) 300 kg/ha.
[c]Stakes immersed in water heated over a wood fire to 49°C for 49 min.
[d]Stakes immersed for 5 min. in Kocide® (copper hydroxide, 5 g/L) and Sistemin® (dimethoato, 3cc/L).
[e]Stakes immersed for 5 min in Score® (difenoconazol, 2.7 cc/L) and Sistemin®. Score® was applied four months after planting.

The following genotypes were planted at Sucre to evaluate resistance to SED and CBB (120 plants/genotype): SM 6758-1, SM 1665-2, CM 6119-5, SM1565-17, CM 4843-1, CM 6754-8,

CM 4919-1, SM 1438-2, M Tai 8 and the local variety Venezolana (M Ven 25). Control measures were not applied.

Germination of cassava plants for the control practices did not present significant differences (Table 7.22). The planted genotypes showed germination percentage from 77% for SM 1665-2 and 100% for M Ven 25 (Table 7.23).

Table 7.23.   Evaluation the germination of 10 different cassava genotypes planted at Sincelejo (Sucre).

Genotype	Germination (%)
Sincelejo (Sucre)	
SM 1565-17	99
SM 1438-2	97
CM 4919-1	87
CM 6119-5	86
SM 1665-2	77
CM 6754-8	98
CM 4843-1	74
CM 6758-1	92
M Ven 25	100
M Tai 8	92

Activity 7.14   ***Detection of a Phytoplasma Associated with Frogskin Disease in Cassava (Manihot esculenta Crantz) in Colombia***

Introduction
Frogskin disease (FSD) was first reported in 1971, in the Department of Cauca, southern Colombia, apparently originating from the Amazon region of either Brazil or Colombia (Pineda and Lozano 1981). The disease has since spread throughout Colombia (Atlantic Coast, and Departments of Cauca, Valle de Cauca, Vaupés, and Putumayo), Venezuela (States of Amazonas, Aragua, Barinas, Cojedes, Monagas, and Portuguesa) (Chaparro-Martínez and Trujillo-Pinto 2001), and Brazil.

Frogskin disease directly affects root production, causing yield losses of 90% or more. Although symptoms vary according to temperature and genotype, the roots become thin and woody, and starch content is very low. The causal agent has not been identified, although research so far suggests that FSD may have a viral etiology and may be transmitted by an aerial vector.

Frogskin disease can be controlled by using tolerant varieties, healthy vegetative planting materials, and adequate plant health management.

Materials and methods

Plant tissue
Several molecular and microscopy staining techniques were applied to detect phytoplasmas in plant tissues from cassava (*Manihot esculenta*, 10 samples), periwinkle (*Catharanthus roseus*, 4 samples), and naranjilla or *lulo* (*Solanum quitoense*, 2 samples). Vegetative tissues from the following FSD-infected cassava varieties were used: CM 849-1, SM 1219-9, Parrita, and M Bra 383, all harvested at Jamundí, Valle de Cauca, Colombia. The plants used were about 12 months old. The roots were severely infected by FSD. The leaves and flowers did not show visible symptoms caused by phytoplasmas (such as witches' broom) or viruses. Samples of healthy 'Secundina', obtained by *in vitro* culture of meristem tips, were used as negative control. Infected plants from plots at CIAT (Palmira) were also included in the analysis.

Microscopy
Two staining methods were used: DAPI (4,6 diamidine 2-phenylindole), which stains the phloem (Sinclair et al. 1989); and Dienes' stain, which metabolizes and produces a blue color (Deeley et al. 1979).

DNA extraction
Total DNA was extracted as described by Gilbertson and Dellaporta (1983) from samples of each of the following tissues: roots, stems, petioles, leaf midribs, and flowers of FSD-infected and healthy cassava plants. DNA was also extracted from the leaves of naranjilla and periwinkle, infected by phytoplasmas. DNA was diluted in sterilized deionized water to a final concentration of 20 ng/μL.

Direct and nested PCR. DNA samples were amplified in a nested PCR. For the first amplification, we used the primer pairs P1/P7 or R16mF2/R16mR1 (Table 7.24) under the following conditions: 120 ng of diluted DNA, 1X buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 μM of each primer, and 1U *Taq* polymerase. Thirty-five cycles were conducted in a PTC–100 thermocycler (Programmable Thermal Controller, MJ Research, Inc., Watertown, MA) as follows: 1 min (2 min for the first cycle) denaturation step at 94°C, annealing for 2 min at 55°C, and primer extension for 3 min (10 min in final cycle) at 72°C. PCR products were diluted at 1:10 with sterilized deionized water. For the nested PCR, we used 2 μL of diluted PCR to amplify with the primer pair R16F2n/R16R2 as described above, but using an annealing temperature of 50°C. PCR products were analyzed by electrophoresis on 1.2% agarose gels and photographed, using an Eagle Eye II I image analyzer (Stratagene, La Jolla, CA).

Table 7.24. Primers used for PCR amplification and sequencing of 16S rRNA genes of plant pathogenic phytoplasmas.

Primer	Sequence 5' - 3'	Reference
R16R2	TGACGGGCGGTGTGTACACCCG	Gundersen and Lee (1996)
R16mF2	CATGCAAGTCGAACGGA	Gundersen and Lee (1996)
R16mR1	CTTAACCCCAATCATCGAC	Gundersen and Lee (1996)
R16F2n	GAAACGGCGGTGTGTACAAACCCCG	Gundersen and Lee (1996)
P1	AAGAGTTTGATCCTGGCTCAGGATT	Deng and Hiruki (1991)
P7 (23S)	CGTCCTTCATCGGCTCTT	Smart et al. (1996)

RFLP analyses

The nested-PCR products of the controls, and the 16S rDNA sequences of cassava, periwinkle, and naranjilla were amplified with primer pair R16F2n/R2. A 5-μL aliquot of each PCR product (1.2 kb) was digested with each of the restriction endonucleases *AluI* and *RsaI* according to manufacturer's instructions (Promega, Madison, WI). The restriction products were then analyzed on a 2% agarose, visualized, and saved in a gel documentation system (Eagle Eye II, Strategene). The restricted-DNA patterns of infected cassava, periwinkle, and naranjilla were compared with the RFLP patterns produced by the control strains.

Cloning, transformation, and sequencing of DNA

Six PCR products were sequenced directly, using a DNA-sequencing kit from Applied Biosystems, with 3 μL water, 1 μL primer, 4 μL mix from kit, and 1 μL DNA. The PCR products were purified, using the QIAquick PCR Purification Kit (QIAGEN), 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 blue/white color screening by plating on LB/ampicillin/IPTG/X-gal media. Plasmids were extracted with a Plasmid Miniprep System Kit (Gibco-BRL). Positive inserts were observed by plasmid restriction with *EcoRI* 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, with 3 μL water, 1 μL primer, 4 μL mix from kit, and 1 μL DNA. Sequences were analyzed with Sequencer 4.1 software and matched by nucleotide, using the Blastn tool in GenBank (www.ncbi.nlm.nih.gov).

Results and discussion

CIAT's Cassava Pathology programmed a series of activities aimed at identifying the possible causal agent of FSD in cassava. The principal advances are summarized below.

For many crops, the causal agents of similar diseases were considered to be viruses. However, over the last 20 to 25 years, the causal agents were found to be phytoplasmas. For example, lethal yellowing disease in the coconut palm was reported by Nutman and Roberts (1955) as being viral, whereas Beakbane et al. (1972), Heinze et al. (1972), Plavsic-Banjac et al. (1972), and (Mariau et al. 2002) all identified the causal agent as being a phytoplasma.

In this study, we present evidence that FSD is associated to a phytoplasma and that, by applying molecular tools and microscopy, we successfully detected phytoplasmas in FSD-infected cassava roots, leaf midribs, petioles, and peduncles.

The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were successfully used in a nested-PCR assay to detect and confirm that phytoplasmas were associated with FSD.

To detect and subsequently classify the phytoplasmas, two pairs of universal primers (P1/P7 and R16F2n/R2) were used to amplify the 16S rDNA gene. A 1.2-kb fragment was amplified from all samples, including infected roots (Figure 7.21). This fragment was present only in

samples collected from plants showing visible external symptoms in the roots. Direct PCR, using the primers R16mF2 and R16mR1 also detected phytoplasmas.

The presence of phytoplasmas in roots, stems, petioles, leaf midribs, and flowers was confirmed by DAPI and Dienes' stain by microscopy (Figures 7.22 and 7.23).

Sequence analysis of the cloned fragment (Figure 7.24) revealed that the cassava phytoplasma was similar to the chinaberry yellows phytoplasma (GenBank acc. no. AF495657, 16SrXIII Mexican periwinkle virescence group) and cirsium white leaf phytoplasma (GenBank acc. no. AF373106, 16SrIII X-disease group), both with a sequence homology of 100% and 99% in two partial fragments with a total of 1.01 kb (Table 7.25). The sequence length was 1202 bp (Table 7.26).

According to the RFLP patterns with *RsaI*, the cassava phytoplasma was similar to that for naranjilla, whereas that for periwinkle was different again. *AluI* did not highlight differences among the samples (Figure 7.25). Future research will involve evaluation with another group of enzymes, and sequence analysis will be carried out to classify the phytoplasmas.

We have already started studies on the transmission of the causal agent of FSD. Remission experiments, using chlortetracycline, with cassava, periwinkle, and poinsettia are being conducted, and we will need to determine the role of phytoplasmas in this destructive disease.

This is the first report of phytoplasmas being associated with FSD in cassava. Future research topics will include the development of molecular detection methods, vector identification, and classification of phytoplasmas associated with FSD. The design of novel approaches to achieve effective control will remain a constant goal.
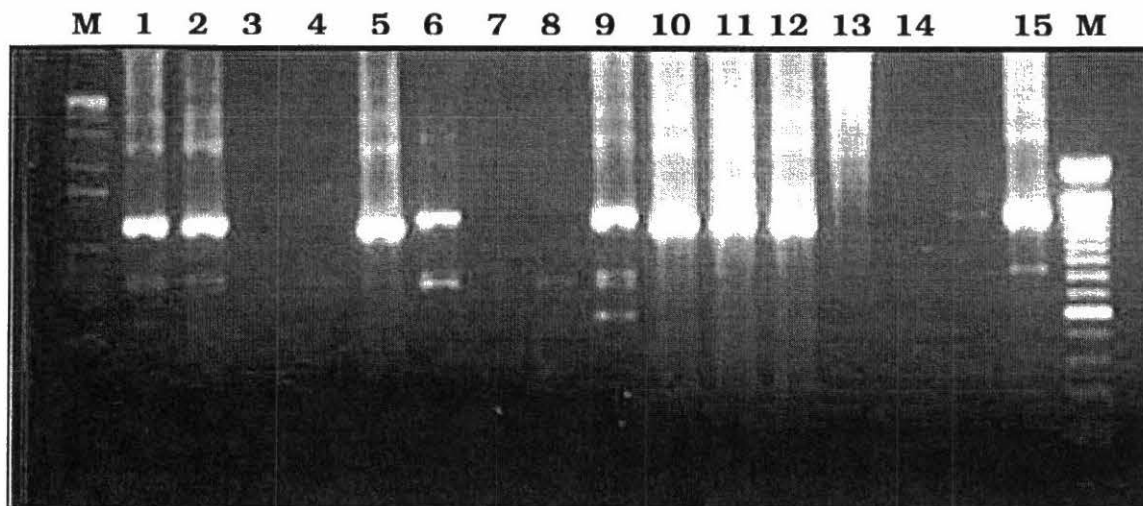
Figure 7.21. Nested PCR of infected and healthy plant tissues, using primers R16MF2, R16MR1/R16F2N, R16FR2. Lanes 1 and 2 = infected cassava roots (pulp); lanes 3 and 4 = leaves and shoot from healthy cassava plants; lane 5 = stem tissue from an infected cassava plant; lane 6 = petiole from an infected cassava plant; lanes 7 and 8 = peel from infected cassava roots; lanes 9 and 10 = infected cassava roots; lanes 11 and 12 = leaf tissue from naranjilla and periwinkle, respectively; lane 13 = degraded DNA from periwinkle; lane 14 = negative control without DNA; lane 15 = positive control; lane M = bp ladder.
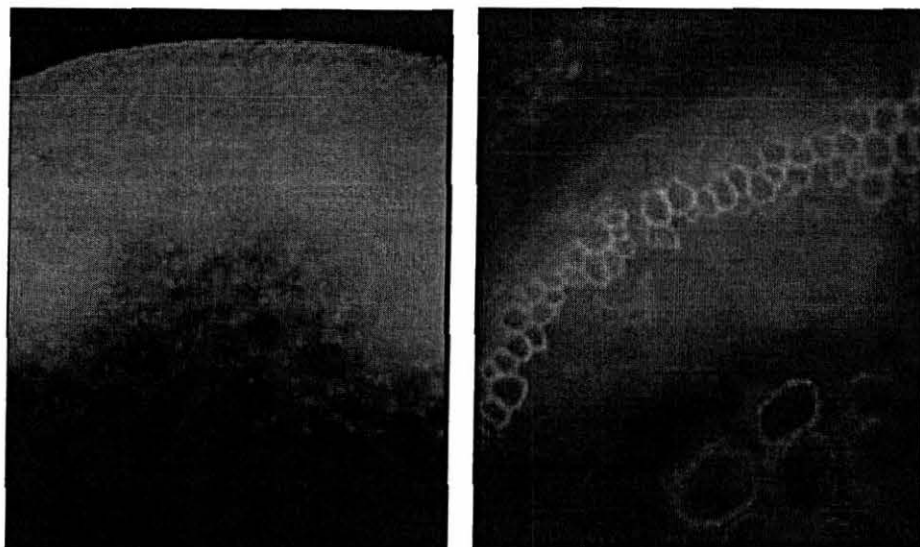
A   B

Figure 7.22. DAPI stain of healthy (A) and infected (B) cassava leaf tissue.
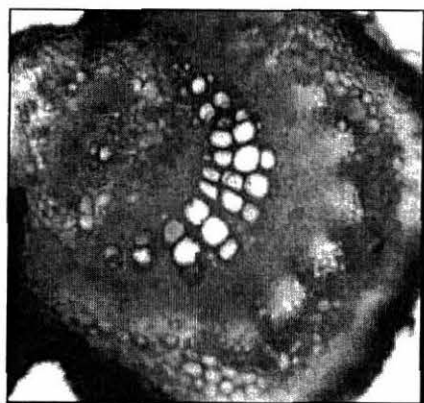
A   B

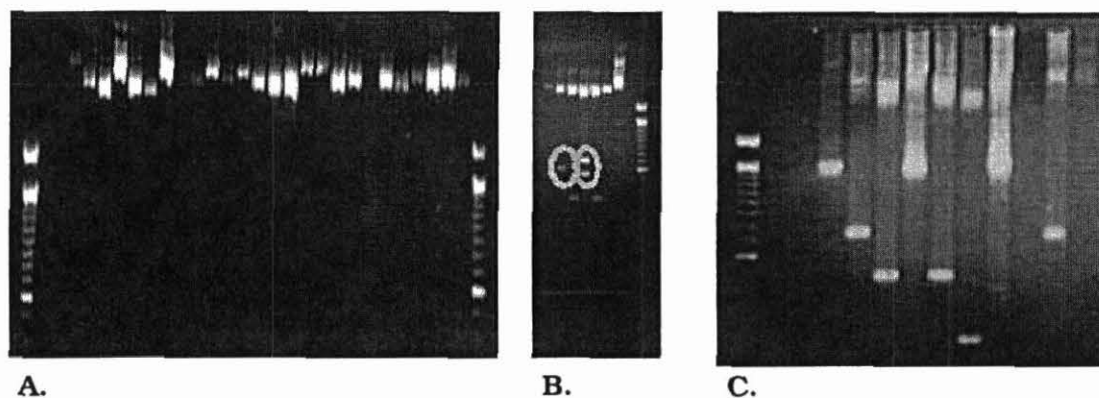Figure 7.23. Dienes' stain of healthy (A) and infected (B) cassava leaf tissue.

Figure 7.24. Cloning and restriction with *Eco*RI: (A) plasmids; (B) their restriction; and (C) PCR to confirm presence of inserts in the plasmids.
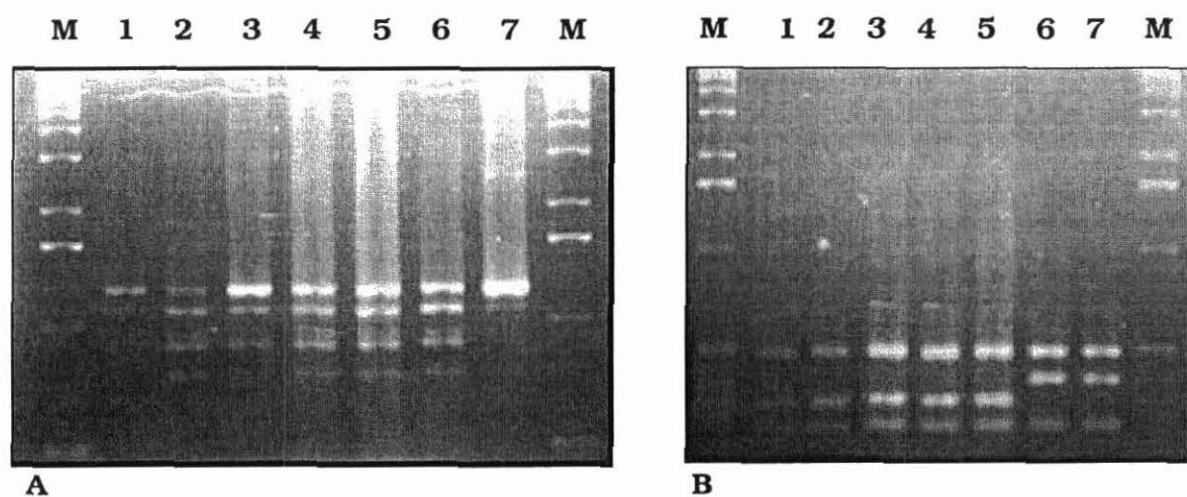

Figure 7.25. RFLP patterns obtained: (A) *AluI*; (B) *RsaI*. Lanes 1-3 = cassava; lanes 4 and 5 = naranjilla; lanes 6 and 7 = periwinkle; lane M = bp ladder.

Table 7.25. Homology found between DNA obtained from cassava infected by FSD by nested PCR and phytoplasma sequences reported in GenBank.

Matching in GenBank	GenBank number	Sense	Homology score (bits)	Probability of higher homology	Homologued fragment (bp)	Identities Absolute	(%)
Cirsium white leaf phytoplasma rRNA operon B	AF373106.1	5'- 3'	1084	0.04	546	546	100.0
		3'- 5'	856	0.0	465	459	98.7
Chinaberry yellows phytoplasma 16S rRNA gene	AF495657.1	5'- 3'	1084	0.0	546	546	100.0
		3'- 5'	872	0.0	465	461	99.1
Chayote witches' broom phytoplasma ChWBIII strain 16S rRNA gene, 16S–23S rRNA intergenic	AF147707	5'- 3'	1076	0.0	546	545	99.8
		3'- 5'	856	0.0	465	459	98.7
Poinsettia branch-inducing phytoplasma rRNA operon B	AF190223	5'- 3'	1068	0.0	546	544	99.6
		3'- 5'	856	0.0	465	459	98.7
Gaillardia phyllody phytoplasma 16S rRNA gene	AY049029	5'- 3'	1060	0.0	542	540	99.6
		3'- 5'	872	0.0	465	461	99.1
Dandelion virescence phytoplasma rRNA operon B	AF370120.1	5'- 3'	1045	0.0	546	541	99.1
		3'- 5'	864	0.0	465	460	98.9

Table 7.26.   Sequences of a phytoplasma obtained from cassava infected by frogskin disease.

Identification	Size (bases)	Sense	Sequence
PCR-6RF Phytoplasma	546	Forward	TTGAAGGTATGCTTAAGGAGGGGCTTGCGACACATTAGTTAGTTGGCAGGGTAAAGGCCT ACCAAGACTATGATGTGTAGCTGGACTGAGAGGTTGAACAGCCACATTGGGACTGAGAC ACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGGCAATGGAGGAAACTCT GACCGAGCAACGCCGCGTGAACGATGAAGTACCTCGGTATGTAAAGTTCTTTTATTAAGG AAGAAAAAAGAGTGGAAAAACTCCCTTGACGGTACTTAATGAATAAGCCCCGGCTAATTAT GTGCCAGCAGCCGCGGTAATACATAAGGGGCGAGCGTTATCCGGAATTATTGGGCGTAA AGGGTGCGTAGGCGGTTTAATAAGTCTATAGTTTAATTTCAGTGCTTAACGCTGTTGTGCT ATAGAAACTGTTTTACTAGAGTGAGTTAGAGGCAAGCGGAATTCCATGTGTAGCGGTAAA ATGCGTAAATATATGGAGGAACACCAGAGGCGTAGGCGGCTTGCTGGGACTTTACTGAC GCTGAGGC
PCR-6RR2 Phytoplasma	593	Reverse	CGAAATGCTGATTCGCGATTACTAGCGATTCCAACTTCATGAAGTCGAGTTGCAGACTTCA ATCCGAACTGAGATTGATTTTGTGAGATTGGCTAAGAACTCGCGTTTCAGCTACTCTTTGT ATCAACCATTGTATCACGTTTGTAGCCCAGATCATAAGGGGCATGATGATTTGACGTAATC CCCACCTTCCTCCAATTTTTCATTGGCAGTCTCGTTAAAGTCCCCATCATTACATGCTGGC AATTAACGACAAGGGTTGCGCTCGTTTTAGGACTTAACCTAACATCTCACGACACGAGCT GACGACAACCATGCACCACCTGTTTTCCTGATAACCTCCATTATATTTCTATAACTTCGCA AGAAAATGTCAAGACCTGGTAAGGKTTTTCGTGTATTCTTCGAAATTAAACAACATGGATC CACCGCTTGTGCGGAGTCCCGTCAATTCCTTTAAGTTTCATACCTTGCGTAACGGNACTA CTCAGGCGGGAGGACTTAATGGTGTTAAACTTTCAANAAACCGGGGTTTACCCGGAACAC YTTAANTACCTCAATTCGGTTTACGGGNGGTKGGGACCTACCCAGGG

Acknowledgements

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Activity 7.15.   *Evaluating Thermotherapy and Biological Products for Controlling Frogskin Disease in Cassava*

Objectives
Frogskin disease (FSD) in cassava is transmitted by planting contaminated stakes. No effective method exists for disinfecting stakes and preventing the disease's dissemination. In the following experiments we aim to develop a methodology that includes products and processes for disinfecting cassava stakes of FSD.

Methodology

Experiment 1
Stakes of the cassava variety Parrita—a Chiroza type for the fresh market—were collected from a highly infected commercial cassava crop at Jamundí (Department of Valle, Colombia). Stakes were selected at harvest time to ensure FSD was present. About 80% of the plants presented symptoms, many very severely. The stakes were planted into pasteurized soil, free of FSD, in pots placed in an isolated greenhouse at CIAT.

Before planting, the cassava stakes were treated with one of the following: thermotherapy; immersion in fresh, whole, cow's milk; and immersion in gliricidia (*Gliricidia sepium*) leaf extract. The methodology for thermotherapy, involving hot water, was based on that developed to control *Phytophthora* species (sp. *tropicalis* and others) and *Xanthomonas axonopodis* pv. *manihotis*. Cow's milk has been reported to denaturalize tomato virus and gliricidia is well known in organic agriculture as a viricide.

Treatments:

1   No treatment

2   *Trichoderma* sp., strain 14PDA-4 (1×10^6 conidia/mL); stakes immersed for 30 min and solution applied to soil at planting time

3   Tachigaren® (hymexazol, granular presentation), 0.75 g/L; stakes immersed for 30 min and solution applied to soil at planting time

4   Whole, pure, fresh, cow's milk; stakes immersed and not rinsed

5   Gliricidia (leaf extract, 100 g/L in 50% alcohol, blended, 1 night fermentation), stakes immersed and solution applied to soil at planting time

6 to 20   Hot water therapy:
- immersion in hot water for 49 min at 49°C (no pretreatment)
- pretreatment for 20 min at 49°C followed by 1, 2, or 3 h at each of 49°C, 51°C, 53°C, 55°C, 57°C

Experiment 2

Three genotypes from Jamundi were used: M Bra 383, CM 849-1, and SM 1219-9. Incidence of FSD was between 10% and 70%, according to genotype. Experimental conditions and characteristics were similar to those of Experiment 1.

Treatments:

A. Stakes planted vertically:
1   Gliricidia (50% ethanol, no filtering), stakes immersed, applications to soil
2   Milk (pure), stakes immersed and not rinsed
3   Stakes immersed in water
4   No treatment

B. Stakes planted horizontally:
1   Control
2 to 17   Hot water therapy:
- immersion in hot water for 49 min at 49°C (no pretreatment)
- pretreatment for 20 min at 49°C followed by 1, 2, or 3 h at each of 49°C, 51°C, 53°C, 55°C, 57°C

Experiment 3

See Experiment 2 for the genotypes and experimental design used. Cassava stakes were prepared either with or without a longitudinal perforation that extended from the top of the stake to the center of the medula, using a small drill. After treatment, the top of the stakes were covered with paraffin.

Treatments:

1 and 2   Chlortetracycline (100 ppm or 1000 ppm), stakes immersed and applications (at 1000 ppm) to foliage on a weekly basis

3 and 4   Chloramphenicol (100 ppm or 1000 ppm), stakes immersed and applications (at 1000 ppm) to foliage on a weekly basis

5   Chlortetracycline (1000 ppm), stakes immersed, and alternating weekly foliar applications with either chloramphenicol or chlortetracycline at 1000 ppm

Results and discussion

Although the experiments are still continuing, we found that germination rates of cassava after the stakes were treated with milk and gliricidia improved by rinsing the stakes (milk) or filtering the extract (gliricidia).

Figure 7.26.   Effect of hot water treatment, Tachigaren® (Ta), *Trichoderma* (Tr) isolate 14PDA-4, milk (M), and gliricidia (G) on germination rates of stakes from the cassava variety Parrita infected with FSD.

Stakes of the variety Parrita treated with hot water for 3 h at 55°C and for any time at 57°C did not germinate (Figure 7.26). Germination rates after the other temperatures (49°C, 51°C, and 53°C) were highly acceptable. Germination rates after 1 or 2 h at 55°C were 88% and 78%, respectively. These treatments included a pretreatment of 20 min at 49°C, 24 h before the main treatment.

Hot water treatment of stakes from M Bra 383, CM 849-1, and SM 1219-9 at 53°C (including pretreatment) improved germination (average of 80.6%), compared with no treatment (75.6%). Germination after pretreatment for 10, 20, or 30 min and followed by a main treatment at 53°C for 1 h was, respectively, 91.7%, 77.8%, and 86.1%. Where no pretreatment was given, germination was 66.7%, thus highlighting the importance of applying a pretreatment to improve germination.

Activity 7.16.   *Promoting the adoption of CIAT cassava genotypes among indigenous groups of Mitú, Department of Vaupés, Colombia*

CIAT genotypes CM 2772-3, ICA Catumare, M Bra 1044, and M Bra 97 were adopted by women farmers of the Tukano indigenous group in Mitú, Department of Vaupés, Colombia. Women from five indigenous communities planted these varieties, together with native varieties, in several of their *chagras* (small farming plots). They followed traditional planting arrangements, which consisted of 3 to 30 or more varieties associated with other crops. Some CIAT varieties were also being grown in *chagras* of communities outside the project's area of influence. So far, their performance has been acceptable, with yields similar to those of native ones, and the quality of their starch and flour is appropriate for preparing traditional foods.

Activity 7.17.   Collecting cassava landraces around Mitú

A native cassava collection was conformed and planted at departmental farm of Vaupés (Mitú), with 23 varieties collected from chagras in different Tukanoan indigenous communities settled close the Vaupés river and the road Mitú – Monfort (Table 7.27).

Table 7.27.   Native cassava varieties collected in different Tukanoan communities in Mitú (Vaupés) and planted at Vaupés departmental farm (Mitú).

Variety	Tukanoan name	Root pulp color	Variety	Tukanoan name	Root pulp color
Castaña	Castaña ducú	Yellow	Yuca de Chicharra	Ñairoa ducú	White
Gallineta	Ajá ducú	Yellow	Yuca de Cuya	Bajato ducú	White
Inayá	Isquí ducú	White	Yuca de güío	Piró ducú	Yellow
Mirití	Neé ducú	Yellow	Yuca de maíz	Jocó ducú	White
Patabá	Ñumú ducú	White	Yuca de Mico	Asque ducú	Yellow
Siringa	Waso ducú	White	Yuca de Mojarra	Varía ducú	Cream
Tucunaré	Buú ducú	Yelow	Yuca de paca	Semé ducú	Cream
Vapu	Váspu ducú	Cream	Yuca de Pato	Patu ducú	Cream
Wasaí	Mispï ducú	Yellow	Yuca de Puño	Buú ducú	White
Wansoco	Utañimí ducú	White	Yuca de uva	Usé ducú	White
Yuca de Abejorro	Veranu ducú	White	Yuca roja	Soarí ducú	White
Yuca de algodón	Busá ducú	Yellow			

Activity 7.18.   Multiplying promising cassava genotypes to ensure sufficient planting material for both greenhouse and field experiments

A total of 159 promising cassava genotypes are being propagated in a farm located in Rozo, Palmira (Department of Valle del Cauca, Colombia) for greenhouse experiments on varietal resistance, genetic studies, and disease management.

Activity 7.19.   Using meristem culture to clean cassava cuttings of frogskin disease

Table 7.28 shows the number of cassava genotypes being cleaned of FSD for use by CIAT's Cassava Pathology Program for experiments on varietal resistance, genetic studies, and disease management. By thermotherapy, meristem culture, and grafting with 'Secundina', 33 genotypes have been cleaned. A total of 93 genotypes are kept *in vitro*, with 8 clones (plants) each one, corresponding to 744 clones.

Table 7.28. Cassava genotypes cleaned of frogskin disease for use in different cassava pathology research projects at CIAT.

Step	Genotypes in process	Clones (no.)	Results
Thermotherapy	19		
Meristem propagation in vitro	93	8	744 clones in 4E, 17N, and 8S culture media
ELISA assay	26	100	CCMV = 3 positive clones CsXV = 6 positive clones
In screenhouse, no grafting	5	5	
Grafting with 'Secundina'	33	87	FSD = 2 positive clones

The 'Secundina' genotype has been propagated *in vitro* to obtain disease-free plants for use as an FSD indicator in assays searching for the virus vector and disease management. A total of 84 plants, 40 in the screenhouse and 44 *in vitro*, were obtained last month.

Activity 7.20. ***Training farmers, technicians, and extension agents in participatory research, cassava management, oil-palm cultivation, and disease control strategies***

Courses
- Fundamentals in molecular biology for plant pathologists
    For ASCOLFI (Colombian Association of Plant Pathology and Related Sciences), September 2001

- Modern systems of cassava production and processing in Colombia: cassava disease management
    El Espinal, 20-22 November 2001

- Cassava production: integrated disease management
    Three courses for 137 participants
    Venezuelan municipalities of El Tigre (Anzoátegui), San Carlos (Cojedes), and Maracaibo (Zulia), 4-15 May 2002

- Modern production, processing, and utilization systems
    For Technicians and farmers. CLAYUCA, 25-28 June 2002

- Integrated management of cassava pests and diseases
    For Master Science students from Escuela Politécnica del Ejército, ESPE, Ecuador 10-12 September 2002

Seminars

- Management of major cassava diseases in the North Coast, with emphasis on superelongation disease
  Sincelejo, 19 November 2001

- Molecular biology techniques applied to crop pathogen identification and characterization. Seminar given during a workshop for an international course on "Tropical hortifruticulture with emphasis on organic production and biological management"
  For 22 participants from Latin America, with two participants from the Cabildo de Guambía (Silvia, Cauca)
  CIAT, 15-16 November 2001

- Advances in cassava pathology research
  For 30 students from the Universidad de Caldas
  Manizales, 20 December 2001

- Cassava disease management
  For Alejandro Larios, starch producer from Caicedonia (Valle)
  CIAT, 7 February 2002

- Superelongation disease management in cassava. Seminar given during the I Regional Workshop on Fast Propagation (*In Vitro*) and Genetic Transformation
  32 participants from Brazil, Venezuela, Ecuador, and Colombia
  CIAT, 26 February 2002

- Advances in cassava pathology research
  For 34 students from the Universidad de Caldas
  Manizales, 18 April 2002

- Cassava diseases: diagnosis and control
  For 4 researchers from Haiti
  15 May 2002

- Cassava pathogens
  For 30 bacteriology students from the Universidad del Valle
  Cali, July 2002

- Integrated management of cassava diseases
  For Manuel Naranjo and Jorge Peña, cassava agronomists from Casanare
  5 August 2002

- Advances in the knowledge and management of rose mildews
  For ASOCOLFLORES
  Bogotá, 29 August 2002

- Principal cassava pathogens. Seminar given during the III International Congress of the National College of Bacteriologists (CNB)

Universidad del Valle, Cali, 1-4 November 2002

- Advances in cassava pathology research
    For 32 students from the Universidad de Caldas
    Manizales, 26 September 2002

Training
- Three courses on soil management and integrated pest and disease management
    242 indigenous women farmers of the communities of Cucura, Bocas del Yí, and
    Macaquiño, Colombia
    October-December 2002

- Establishing Local Agricultural Research Committees
    15 indigenous farmers and local technicians
    April 2002

- Isolating *Sphaceloma manihoticola* and understanding superelongation disease
    Juan Manuel López, Professor of Genetics, Universidad de Sucre, Colombia
    April 2002

- Isolation and inoculation of *Phytophthora* sp. in soybean
    Ana Claudia Gordillo, CORPOICA "La Libertad", Villavicencio
    April 2002

- Isolation and inoculation of *Phytophthora* spp., *Pythium* sp., *Fusarium* spp.
    Alexandra Delgado, Hacienda San José, Palmira
    April-September 2002

- Soilborne pathogens in cassava and sugarcane
    Mariela Becerra, Universidad Francisco de Paula Santander Facultad de Ciencias
    Agrarias y del Ambiente
    Cúcuta, May-June 2002

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and
    water in crops affected by *moko*
    Luz Piedad Estrada, ICA—Quindío
    June 2002

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and
    water in crops affected by *moko*
    Ana Lucía Gaviria and Yaneth Rivera, Universidad del Quindío, Armenia
    June 2002

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue, soil, weeds, and
    water in crops affected by *moko*, and management of *moko* by disinfection of soil and
    tools

Rosinelly Pérez, Especial, La Tebaida, Quindío
July 2002

- Isolation of *Ralstonia solanacearum* from plantain and banana tissue and soil in crops affected by *moko*
  Carlos Aníbal Montoya, ICA—Palmira
  16 July 2002

- Cassava disease management
  Norman Pérez, Chemonics, Putumayo
  18 July 2002

- Molecular and traditional characterization of *Ralstonia solanacearum.*
  Abraham Oleas, Ecuador
  16-17 September 2002

- Biological controllers
  César Cano, Perkins
  July-August 2002

- *Phytophthora* spp. culture management
  Alejandro Corredor, Universidad de Caldas, Manizales
  9 August 2002

Publications, Awards, Meetings, and Theses

Publications

Caracterización genética y patogénica en Colombia de *Sphaerotheca pannosa* var. *rosae*, agente causal del mildeo polvoso en rosa.
> E Alvarez, JL Claroz, JB Loke, C Echeverri. Fitopatología Colombiana 25(1-2):7-14, 2001.

Control del mildeo polvoso en cultivos de rosa por aplicación de un lixiviado de compost de plátano.
> E Alvarez, CX Grajales, J Villegas, GA Llano, JB Loke. Poster presented at the XV Muestra Agroindustrial y Empresarial. Universidad La Gran Colombia, Armenia, Quindío, 1-3 November 2001.

Desarrollo de prácticas ecológicas de manejo de pudrición radical (*Phytophthora* spp.) en yuca (*Manihot esculenta*).
> E Alvarez, JB Loke, GA Llano. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Control de mildeo polvoso en rosa (*Sphaerotheca pannosa* var. *rosae*) por aplicación de lixiviado de compost de raquis de plátano.
> E Alvarez, CX Grajales, J Villegas, JB Loke. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Evaluación de dos inductores de resistencia para el control de pudrición del cogollo en palma de aceite *Elaeis guineensis* en los Llanos Orientales de Colombia.

    GA Llano, E Alvarez, MC Feris, ML Hernández, SM Rodríguez. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Caracterización molecular y clasificación de fitoplasmas asociados con la palma de aceite.

    E Alvarez, JL Claroz. Paper presented at the XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Enfermedades del cultivo de la yuca y métodos de control.

    E Alvarez, GA Llano. Chapter in: Cultivo de la yuca en el tercer milenio. Sistemas modernos de producción, procesamiento utilización y comercialización. Edited by B. Ospina and H. Ceballos. P. 131 – 147. CIAT, Cali, 2002.

Guía práctica para el manejo de las enfermedades, las plagas y las deficiencias nutricionales de la yuca.

    E Alvarez, GA Llano. Chapter in pocketbook. P. 19 – 40. CIAT, Cali, 2002.

Evaluación de la adaptación de variedades de yuca con resistencia a *Phytophthora* spp., mediante investigación participativa en comunidades indígenas de Mitú (Vaupés, Colombia).

    GA Llano, E Alvarez, JB Loke, R Madriñán, JA Restrepo, JR Mora. Revista Acta Agronómica Vol 51 (1/2): 31-39, 2001 - 2002. Universidad Nacional de Colombia.

Selecting *Phytophthora*-resistant cassava, using participatory methodology, for conditions at Mitú, northeastern Amazon, Colombia.

    GA Llano, E Alvarez. Agren (submitted).

Two brochures in process:

    Añublo bacterial de la yuca

    Superalargamiento de la yuca

Control del mildeo polvoso (*Sphaerotheca pannosa* var. *rosae*) en rosa (*Rosa* sp.), usando un lixiviado de compost del raquis de plátano (*Musa* AAB).

    E Alvarez, CX Grajales, J Villegas, JB Loke. Revista ASOCOLFLORES # 62: 41 – 47. January –June, 2002.

Validación de un biofungicida y fertilizantes foliares como alternativa de control del mildeo polvoso en cultivos de rosa.

    E Alvarez, C Echeverri, JB Loke. Revista ASOCOLFLORES (in press).

Awards

Second place, XV Muestra Agroindustrial y Empresarial, Universidad La Gran Colombia, Armenia, Quindío, 1-3 November 2001, for:

    Control del mildeo polvoso en cultivos de rosa por aplicación de un lixiviado de compost de plátano.

      E Alvarez, CX Grajales, J Villegas, GA Llano, JB Loke.

Nomination for indigenous communities from Vaupés for the 2002 Equator Prize of UNDP for:

Participatory research on the control of Phytophthora root rots in cassava, conservation of native cassava varieties, and agroecosystem sustainability.
E Alvarez, GA Llano.

Meetings attended

XXIII Congreso Nacional de Fitopatología, held by ASCOLFI, Bogotá, 3-6 July 2002.

Perspectivas de la producción ecológica para productos hortifrutícolas, held by Fundación Centro de Investigiación Hortofrutícola de Colombia – CENIHF. And Corporación Autónoma Regional del Cauca CVC. Roldanillo, Valle, 26-27 July 2002.

Bachelor theses presented

Juan Fernando Mejía. 2002. Caracterización molecular y patogénica de aislamientos de *Sphaceloma manihoticola* del sur y centro de Brasil. Universidad Nacional de Colombia—Palmira.

César Andrés Ospina. 2002. Caracterización poblacional de *Colletotrichum* spp., agente causal de la Antracnosis de cítricos en el núcleo productor de occidente. Universidad Nacional de Colombia—Palmira.

Claudia Ximena Grajales and Jimena Villegas. 2002. Control de *Sphaerotheca pannosa* var. *rosae* en rosas mediante la utilización de lixiviado de compost de raquis de plátano. Universidad de San Buenaventura—Cali.

Theses for Master of Sciences and Philosophy Doctor degrees in progress

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

Germán A. Llano. Evaluación de la asociación de sondas heterólogas y genes análogos con la resistencia de yuca a *Phytophthora* spp. For a Master of Agrarian Sciences in plant breeding at the Universidad Nacional de Colombia—Palmira.

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

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Linkages with CIAT's Partner Institutions

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Instituto Agronómico de Campinas (IAC)
Instituto de Investigaciones de Viandas Tropicales (INIVIT, Cuba)
Instituto Tecnológico de Roldanillo (for use of vegetal extracts in grape)
IPRA (based at CIAT, Colombia)

Secretaría de Agricultura del Vaupés (at Mitú)
UMATAs (Mitú, Santander de Quilichao, Buenos Aires, Caicedonia, La Tebaida, and Montenegro)
Universidad Nacional de Colombia—Palmira (Valle del Cauca, Colombia)

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OUTPUT 8
Development and use of biotechnology tools for cassava improvement

Cassava is a remarkable crop with many advantages over other crops, particularly in relation to stability of performance, capacity to achieve acceptable productions in low fertility soils, and general tolerance to biotic and abiotic stresses. However, the crop also has some clear disadvantages. Among some of the limitations, the low reproductive rate and the length of each cycle of selection slows down the genetic progress achieved and limits the amount of genetic information available for the species. New biotechnology tools can help in overcoming or reducing some of these problems specific to cassava.

Activity 8.1. Marker-Assisted Selection (MAS) for Breeding Resistance to the Cassava Mosaic Diseae (CMD) at CIAT

Collaborators: Dr Alfred Dixon (IITA), Emmanuel Okogbenin, Edgar Barrera, Jaime Marin, Martin Fregene (CIAT)
Funding: The Rockefeller Foundation

Important Outputs
1) *Implementation of MAS for CMD resistance at CIAT*
2) *Proof of concept that the SSR marker NS158 can be used to predict CMD resistance in different crosses for resistance breeding.*
3) *More than 7000 seeds have been obtained for the second phase of the MAS project.*

Rationale
The absence of cassava mosaic disease (CMD), the most important production constraint in Africa and India, limits the usefulness of CIAT cassava germplasm in those areas. With the discovery of a dominant CMD resistance gene, *CMD2*, and 3 molecular markers tightly associated with it, it is now possible to breed for CMD resistance at CIAT. A pilot experiment was set up together with IITA in 2000, as a proof of concept of the utility of molecular markers in CMD resistance breeding. Six crosses, and reciprocals, were made between TME3 and TME9, two cassava land races from Nigeria that carry *CMD2*, and: a susceptible Nigerian land race and 2 elite cassava varieties from IITA, one tolerant and the other susceptible to CMD. We describe here findings of that experiment.

A second phase of molecular breeding for CMD resistance breeding at CIAT has also been initiated. CMD resistant progenies derived from TME3 that were obtained from IITA in 2000 were crossed to elite parents of CIAT's cassava gene pools, and to high carotene or high protein content genotypes. Seeds harvested from the crosses were germinated *in vitro* from embryo axes, to permit sharing of the CMD resistant genotypes with collaborators in Africa and India, in preparation for MAS. These plants will also be the basis of breeding for CMD resistance in CIAT cassava gene pools..

Methodology

The MAS crosses were made in 2000 and a seedling nursery was established at the IITA Mokwa sub-station, a low CMD pressure site in Nigeria, in June 2001. The crosses were harvested December last year and re-established as a clonal observation trial at the IITA headquarters in Ibadan, a high CMD pressure area. Table 8.1 summarizes the families that are in the clonal observation trial. The plants were evaluated at 3, 4 and 6 months after planting for resistance to CMD.

Table 8.1 Crosses from TME3 and TME 9 planted in IITA for to test markers associated with *CMD2* for molecular marker-assisted selection

Family name	Female	Male	Seeds harvested	Plants in field	Total plants in field
M1	TME 3	TME 117	36	18	
M2	TME 117	TME 3	220	95	113
M5	TME 3	91934	103	49	
M6	91934	TME 3	60	12	61
M7	TME 3	30572	70	49	
M8	30572	TME 3	846	791	840
M17	TME 9	TME 117	368	309	
M18	TME 117	TME 9	174	107	416
M21	TME 9	91934	370	282	
M22	91934	TME 9	27	12	294
M23	TME 9	30572	264	214	
M24	30572	TME 9	700	552	766
				Grand Total	2490

DNA was isolated from 1-2g of young leaves and dried for 24h in an oven at 48°C. The dried leaves were ground using a power drill and washed sand. DNA isolation was from 200mg leaf tissue using a miniprep version of the Dellaporta (1983) protocol. Molecular marker analysis was at CIAT. A single dilution, 10X, was employed for all samples. The samples were analyzed with the SSR marker NS158, the closest marker found to date to *CMD2*. PCR analysis and acrylamide gel analyses were carried out as described by Akano et al. 2002. Gel image was captured by scanning and transferred to a Microsoft Excel file for the inclusion of resistance data and interpretation.

A large number of crosses were made between CMD resistant progenies, introduced from IITA to CIAT, and elite parents of CIAT cassava genepools, high beta-carotene varieties and wild *Manihot* accesions and inter-specific hybrids with high protein and dry matter content (Table

8.2). To permit for sharing of this invaluable germplasm with collaborators in India and Sub Saharan Africa as well as keeping a copy here for breeding at CIAT, the seeds are being germinated from embryo axes. Once germinated, the plantlets will be multiplied, molecular-assisted selection (MAS) will be performed using the marker NS158, and CMD resistant genotypes will be shipped to collaborators.

Results

DNA isolation using dried leaves, a power drill and the mini Dellaporta protocol allowed for the processing of 130-150 samples daily. Yield of DNA was between 10-20ug/ 200mg of leaves, which provides enough DNA for more than 200 PCR reactions. This DNA also stores very well and can be used at again at a later time. For more routine MAS work, DNA extraction using the protocol currently used for bean MAS at CIAT will be tested (CIAT 2001).

Molecular marker analysis, using the NS158 SSR marker that is tightly associated to *CMD2*, revealed the marker to be an excellent prediction tool for CMD resistance in some crosses but to a lesser extent in others (Figure 8.1 and Table 8.2). Scrutiny of the data reveals a problem of shared allele sizes in the molecular marker NS158. Examination of allele sizes for marker NS158 in the parental genotypes TME3, TME9, CMD resistant genotypes, and TME117 and TM91924, the susceptible genotypes, reveals they have the same allele size for the allele that is associated with resistance in TME3 and TME9. Although NS158 is tightly linked to *CMD2*, less than 1 cM, it has the same allele size for certain resistant and susceptible genotypes. This could be due to a close relationship of these genotypes and also highlights the need to develop a marker that is truly unique to CMD2. Efforts are underway to clone, by positional cloning, *CMD2* and to develop an allele specific PCR fragment for use as a sequence characterized amplified region (SCAR) marker.

The predominance of genotypes designated as resistant from field evaluation but without the resistant allele, termed as resistant "recombinants", underscores a fundamental problem in field evaluation of disease resistance, i.e uneven pathogen pressure. The crosses employed to test the MAS concept have been in the field for only 6 months and uneven disease pressure can lead to susceptible genotypes being erroneously scored as resistant.

Table 8.2. Summary of the molecular marker analysis of the crosses and recombinants.

Cross	No. genotypes	Recomb. (R)	Recomb. (S)	% total R.
TME3xTME117 (+reciprocals)	110	31	0	28.1
TME3xTMS91934 (+reciprocals)	61	15	0	24.6
TME3x TMS30572 (+reciprocals)	815	40	4	5.3
TME9xTMS91934 (+reciprocals)	223	44	4	22.5
TME9xTMS30572 (+reciprocals)	733	32	3	4.7
TME9xTME117(+reciprocals)	395	93	4	23.5

In an earlier study where a similar set of crosses had been in a field in Uganda for 2 years under heavy disease pressure there was no recombinants (CIAT 2001). This strengthens the case for MAS in CMD resistance breeding.
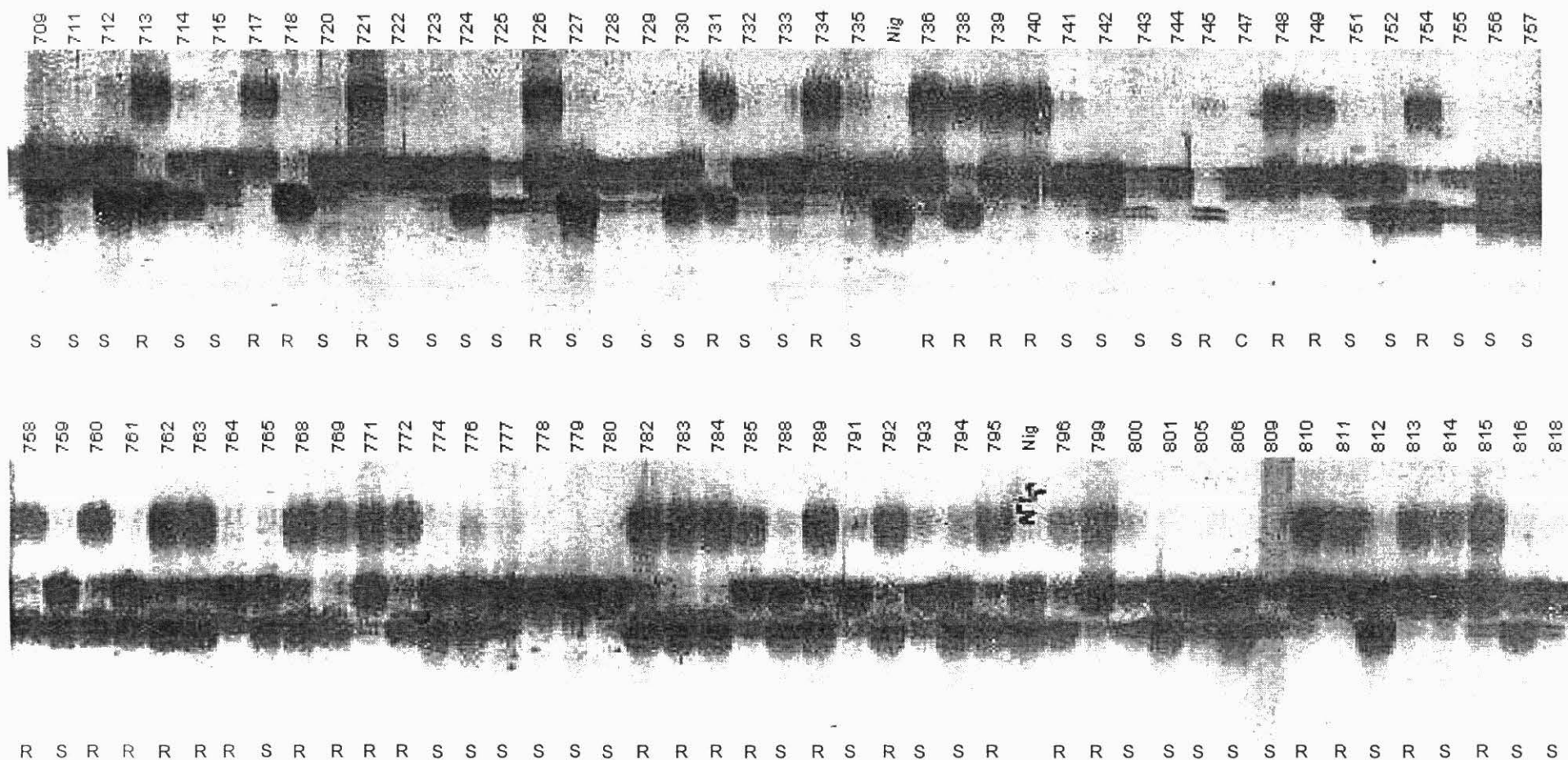
Figure 8.1. Silver stained gel polyacrylamide gel showing PCR analysis of SSR marker NS158 in the cross TME3 x TMS30572 and field resistance data of resistant (R and susceptible (S) genotypes. The topmost alleles are associated with resistance. Three recombinant genotypes can be observed (in red).

More than 7000 seeds were obtained from crosses between the CMD donor parents and elite parents of CIAT's cassava gene pools (Table 8.3). These seeds are being germinated *in vitro* and MAS will be employed to select resistant genotypes for shipment to collaborators in India and Africa.

Table 8.3. List of seeds obtained this year from genetic crosses between CMD donor parents and parents at CIAT for multiple purposes

Mother	Father	Fuente	Purpose 1	Purpose2	No. of Seeds
CMD donor parents x elite parents of agro-ecology zone one (tropical lowlands)					
CG 1141- 1	C 413	GY200122	Z01	ACMD	10
CM 3306- 4	C 4	GY200122	Z01	ACMD	56
CM 3306- 4	C 18	GY200122	Z01	ACMD	11
CM 3306- 4	C 33	GY200122	Z01	ACMD	30
CM 3306- 4	C 39	GY200122	Z01	ACMD	8
CM 3306- 4	C 243	GY200122	Z01	ACMD	5
CM 3306- 4	C 413	GY200122	Z01	ACMD	24
CM 6754- 8	C 33	GY200122	Z01	ACMD	1
SM 1411- 5	C 33	GY200122	Z01	ACMD	1
C 4	MTAI 8	GY200122	ACMD	Z01	470
C 33	CM 3306- 4	GY200122	ACMD	Z01	34
C 33	CM 6754- 8	GY200122	ACMD	Z01	18
C 33	MTAI 8	GY200122	ACMD	Z01	9
C 39	CM 3306- 4	GY200122	ACMD	Z01	19
C 127	MTAI 8	GY200122	ACMD	Z01	54
C 243	MTAI 8	GY200122	ACMD	Z01	8
C 413	MTAI 8	GY200122	ACMD	Z01	6
MTAI 8	C 4	GY200122	Z01	ACMD	28
MTAI 8	C 18	GY200122	Z01	ACMD	6
MTAI 8	C 33	GY200122	Z01	ACMD	32
MTAI 8	C 39	GY200122	Z01	ACMD	35
MTAI 8	C 243	GY200122	Z01	ACMD	44
MTAI 8	C 413	GY200122	Z01	ACMD	33
				SUBTOTAL	943

Table 8.3 (cont.)

CMD donor parents x elite parents of agro-ecology zone 2 (acid savannas)					
CM 523- 7	C 4	GY200122 Z02	ACMD		11
CM 523- 7	C 33	GY200122 Z02	ACMD		138
CM 523- 7	C 39	GY200122 Z02	ACMD		79
CM 523- 7	C 243	GY200122 Z02	ACMD		26
CM 4574- 7	C 18	GY200122 Z02	ACMD		1
SM 909- 25	C 4	GY200122 Z02	ACMD		95
SM 909- 25	C 18	GY200122 Z02	ACMD		4
SM 909- 25	C 33	GY200122 Z02	ACMD		58
SM 909- 25	C 39	GY200122 Z02	ACMD		37
SM 909- 25	C 413	GY200122 Z02	ACMD		16
SM 1219- 9	C 243	GY200122 Z02	ACMD		3
C 4	CM 523- 7	GY200122 ACMD	Z02		16
C 4	CM 4574- 7	GY200122 ACMD	Z02		8
C 4	SM 909- 25	GY200122 ACMD	Z02		18
C 4	SM 1219- 9	GY200122 ACMD	Z02		1
C 18	CM 4574- 7	GY200122 ACMD	Z02		2
C 33	CM 523- 7	GY200122 ACMD	Z02		3
C 33	CM 4574- 7	GY200122 ACMD	Z02		31
C 33	SM 909- 25	GY200122 ACMD	Z02		7
C 39	CM 4574- 7	GY200122 ACMD	Z02		9
C 39	SM 1219- 9	GY200122 ACMD	Z02		2
C 243	CM 4574- 7	GY200122 ACMD	Z02		2
C 243	SM 1219- 9	GY200122 ACMD	Z02		26
					596

CMD donor parents x elite parents of agro-ecology zone 4 (mid-altitude Andean)					
CM 7951- 5	C 4	GY200122 Z04	ACMD		44
CM 7951- 5	C 18	GY200122 Z04	ACMD		13
CM 7951- 5	C 33	GY200122 Z04	ACMD		82
CM 7951- 5	C 39	GY200122 Z04	ACMD		27
CM 7951- 5	C 243	GY200122 Z04	ACMD		22
CM 7951- 5	C 413	GY200122 Z04	ACMD		8
SM 1741- 1	C 4	GY200122 Z04	ACMD		61
SM 1741- 1	C 18	GY200122 Z04	ACMD		8
SM 1741- 1	C 33	GY200122 Z04	ACMD		133
SM 1741- 1	C 39	GY200122 Z04	ACMD		28
SM 1741- 1	C 413	GY200122 Z04	ACMD		26
C 4	AM 244- 31	GY200122 ACMD	Z04		6
C 4	SM 1741- 1	GY200122 ACMD	Z04		5
C 4	MCOL 1734	GY200122 ACMD	YRT		135
C 4	MCOL 2206	GY200122 ACMD	YRT		195
C 18	SM 1741- 1	GY200122 ACMD	Z04		2
C 33	SM 1741- 1	GY200122 ACMD	Z04		31
C 39	SM 1741- 1	GY200122 ACMD	Z04		4
C 127	SM 1741- 1	GY200122 ACMD	Z04		6
C 127	MCOL 1734	GY200122 ACMD	YRT		28
C 243	SM 1741- 1	GY200122 ACMD	Z04		5
					869

Table 8.3 (cont.)

CMD donor parents x wild species (high protein content and CGM resistance)					
OW 183- 4	C 127	GY200122 ALW	ACMD		28
C 4	CW 66- 60	GY200122 ACMD	CGM		2
C 4	CW 66- 73	GY200122 ACMD	CGM		12
C 4	CW 67- 42	GY200122 ACMD	CGM		3
C 4	OW 280- 1	GY200122 ACMD	PTN		13
C4	OW230-3	GY200123 ACMD	PTN		159
C4	OW 231- 4	GY200122 ACMD	PTN		183
C 243	OW 280- 1	GY200122 ACMD	PTN		12
					412

CMD donor parents x high beta carotene content varieties					
C 243	MCOL 1734	GY200122 ACMD	YRT		2
C 243	MCOL 2206	GY200122 ACMD	YRT		13
C 18	MCOL 2056	GY200122 ACMD	YRT	•	4
C 33	MCOL 2056	GY200122 ACMD	YRT		19
C 33	MCOL 2206	GY200122 ACMD	YRT		16
C 127	MCOL 2206	GY200122 ACMD	YRT		47
MBRA 1A	C 18	GY200122 YRT	ACMD		4
MBRA 1A	C 39	GY200122 YRT	ACMD		10
MCOL 1734	C 4	GY200122 YRT	ACMD		54
MCOL 1734	C 18	GY200122 YRT	ACMD		5
MCOL 1734	C 33	GY200122 YRT	ACMD		48
MCOL 1734	C 127	GY200122 YRT	ACMD		37
MCOL 2206	C 4	GY200122 YRT	ACMD		7
MCOL 2206	C 18	GY200122 YRT	ACMD		22
MCOL 2206	C 127	GY200122 YRT	ACMD		8
MMAL 66	C 18	GY200122 YRT	ACMD		30
MTAI 2	C 18	GY200122 YRT	ACMD		13
Total					338

Open pollinated seeds of CMD donor parents			
C 4	GY200122 ACMD		4200
C 18	GY200122 ACMD		20
C 127	GY200122 ACMD		100
C 243	GY200122 ACMD		50
	Total:		4370
	Grand Total:		7527

Future perspectives

1. The need to develop a marker that is truly unique to CMD2 to eliminate confounding effects of alleles from susceptible genotypes having the same size with the allele associated with CMD resistance

2. MAS of the new crosses

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Activity 8.2. Development of Expressed Sequence Tags (ESTs) from TME3, the source of CMD2, the Dominant Cassava Mosaic Disease (CMD) Resistance Gene ,

Collaborators: Dr Ryohei Terauchi, Dr Hideo Masamura (IBRC, Kitakami, Japan), Martin Fregene (CIAT)

Important Outputs

1) *Four thousand expressed sequence tags (ESTs) developed from the CMD resistant genotype, TME3.*

b) *Annotation of several hundreds of the ESTs and their use in annotation of SAGE tags differentially expressed in TME3 under heavy disease pressure.*

Rationale

Attempts to clone genes expressed down stream of the single dominant gene, designated *CMD2*, that confers high levels of resistance to the cassava mosaic disease (CMD) by the serial analysis of gene expression (SAGE) has led to identification of many differentially expressed tags – 11bp cDNA sequences. Two methods were employed to annotate the tags obtained, PCR amplification of a cDNA library, using the tag sequence as sense primer and a primer designed from the 3' end of the multiple cloning site of the vector (pYES, Invitrogen Inc.), and ESTs from CMD resistant genotypes. We describe here the generation of 4000 ESTs expressed in a CMD resistant genotype challenged with the virus.

Methodology

A cDNA library was constructed in pYES (Invitrogen Inc.) using mRNA from the CMD resistant bulk. Two microlitre of the cDNA library was electroporated into 40ul of *E.Coli* HB101 cells (Gibco BRL) and plated on LB agar plates + ampicillin (100ug/ml). A total of 5,000 colonies were picked into 70ul of LB media + ampicillin (100ug/ml) in 384 well plates. Plasmid isolation was by the MONTAGE 96-well plate system (Millipore Inc), 4 96-well plates or 384 clones were processed at a time. The 3' end sequencing of the cDNA clones was with

a primer designed from the 3' end of the multiple cloning site of pYES (Invitrogen Inc.) and 5ul of plasmid miniprep. Sequencing PCR reaction was with the big dye terminator kit (Applied Biosystems) on a 9600 Perkin Elmer Machine or an MJ Research DNA engine (Tetrad). The sequence reaction was cleaned using the multi screen 96-well plate format (Millipore Inc.) and analyzed on a Shimadzu RISA 384-capillary sequencing machine. Sequences obtained were cleaned from vector sequences by eye and combined into one single text file using a program written in perl, running on a SunSparc Station (Sun Microsystems Inc.). A program was written in perl to perform batch BLAST (Altschul et. al at 1990) similarity searches for sequence identification using the CIAT local BLAST site (http://gene2/BLAST/inicio.htm).

Results

The 3' end sequencing of about 5000 cDNA clones generated a total of 4000 ESTs. Homology with known genes and proteins deposited in public data bases were sought using the local BLAST (Altschul et. al at 1990) at CIAT, the identity of about 2500 sequences could be ascertained with a good confidence level which corresponds to about 800 unique sequences. Redundancy found in sequences of known functions was about 30%. The ESTs were used for tag annotation and results are summarized Table 8.4. The most abundant tags were easily annotated, for example, identity of the ten genes that make up 5% of all expressed transcripts were found by ESTs, but annotation of less abundant tags is not as efficient. This suggests that the PCR method of tag annotation is a more powerful route to annotating SAGE tag compared to ESTs from regular cDNA libraries. On the other hand ESTs from a normalized cDNA library may be a more efficient means of tag annotation compared to non-normalized libraries. EST data will be be submitted to the Gene Bank.

Future Perspectives
1. Submission of the ESTs to GeneBank.

Table 8.4. Putative identity of SAGE tags annotated by cassava ESTs.

Tag Seq.	Suscep	Resist	Total	Putative identity
CCAGGTTGT	88	72	160	Chlorophyll A-B binding protein type II 1B, chloroplast precursor
CTGCAATGG	58	59	117	NONSPECIFIC LIPID-TRANSFER PROTEIN PRECURSOR (LTP) (ALLERGEN PYR
TTTGGATTC	58	37	95	Chlorophyll A-B binding protein type II 1B, chloroplast precursor
TTTGGGTGC	34	31	65	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR
GATTTCATT	29	26	55	Photosystem I reaction center subunit X, chloroplast precursor
ATGATATCA	18	23	41	THIAZOLE BIOSYNTHETIC ENZYME, CHLOROPLAST PRECURSOR.
GATTTGTGT	25	18	43	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR
AACTCCTTT	13	18	31	HISTONE H2B
TTCTTGTAT	33	16	49	CHLOROPHYLL A-B BINDING PROTEIN 7 PREC
TTCTGTTGA	24	16	40	Chlorophyll A-B binding protein 151, chloroplast precursor
TAGTCTTAT	18	14	32	PROBABLE NONSPECIFIC LIPID-TRANSFER PROTEIN AKCS9 PRECURSOR (LTP).
GCGTTGGTG	15	12	27	CYTOCHROME C OXIDASE POLYPEPTIDE III
AATGACCTT	1	12	13	TUBULIN BETA CHAIN
CGCCAGACA	3	11	14	ELONGATION FACTOR 1-ALPHA (EF-1-ALPHA).
CATTGTACA	8	11	19	Chlorophyll A-B binding protein 4, chloroplast precursor (LHCII
ATGTGGTCT	6	11	17	GERMIN-LIKE PROTEIN 1 PRECURSOR.
AAGAAGCTC	6	11	17	40S RIBOSOMAL PROTEIN S15A (PPCB8).
CGTAATCAG	30	10	40	PROBABLE NONSPECIFIC LIPID-TRANSFER PROTEIN AKCS9 PRECURSOR (LTP).
CCTGACCTC	23	9	32	Chlorophyll A-B binding protein 151, chloroplast precursor (LHCII
TTAATATGG	1	6	7	CYCLIN A/CDK2-ASSOCIATED PROTEIN P19
TACTTTGTA	13	5	18	Carbonic Anhydrase, Chloroplast Precursor (Carbonate Dehydratase).
GGTGTCTCT	13	5	18	40S RIBOSOMAL PROTEIN S4.
CGATTAAAA	1	5	6	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (PPIASE) (ROTAMASE)
TTGGATCTT	0	4	4	HYPOTHETICAL 59.9 KD PROTEIN IN SGA1-KTR7 INTERGENIC REGION.
TAGAATCTT	1	4	5	superfamily: myrosinase-associated protein MyAP;
GCACAACAC	8	4	12	CHLOROPHYLL A-B BINDING PROTEIN 4 PREC
AGAACCACT	1	4	5	ELONGATION FACTOR TU, CHLOROPLAST PRECURSOR (EF-TU).
AATTTGATG	1	4	5	SUCCINATE DEHYDROGENASE [UBIQUINONE] I
AAGTGGTGC	0	4	4	60S RIBOSOMAL PROTEIN L17/protein tyrosine phosphatase e
GTGGTGGTA	0	3	3	60S RIBOSOMAL PROTEIN L2 (L8) (RIBOSOMA
GCTTCATTA	0	3	3	UBIQUITIN-CONJUGATING ENZYME VARIANT M...
CCTCAATCC	0	3	3	cholecystokinin B receptor - rat
ATTCCTGAT	0	3	3	putative protein [Arabidopsis thaliana]
AGGGAGGCA	0	3	3	PHOTOSYSTEM II CORE COMPLEX PROTEINS PSBY PRECURSOR (L-ARGININE
AAATTGAAA	0	3	3	unknown [Euphorbia esula]./recA protein A.thaliana

Activity 8.3. Simple Sequence Repeat (SSR) Marker Diversity in Cassava (Manihot esculenta Crantz) Landraces from Nigeria

Collaborators: Dr Alfred Dixon (IITA), Ms Adebola Raji (IITA, and Ph.D. student University of Ibadan, Ibadan, Nigeria), Mr Jaime Marin, Martin Fregene (CIAT)

Important Outputs
1) *Completion of the Nigerian country study, 270 land races with 31 SSR markers and and observation of high geneic diversity.*
2) *Confirmation of moderate to high genetic differentiation between land races from Nigeria and Guatemala and also observation of secondary structure of genetic diversity in Nigerian collection that may represent heteroic groups.*

Rationale
Nigeria is the world's largest producer of cassava with an annual production of 32 million tons a year (Nweke et al. 2001). This is a more than 200% increase from production twenty years ago. Reasons for the leap in production have been attributed to government policies that favor cassava, population increase and the adoption of improved cassava varieties (Nweke et al. 2001). Nigeria has the highest area in Africa planted to improved varieties, 60%, and cassava is more of an urban staple and cash crop than a food security crop. The impact of the increased commercialization of cassava in Nigeria is expected to lead to an even greater adoption of improved varieties and an erosion of land races and the inevitable loss of diversity. A high level of genetic diversity of cassava has been demonstrated in land races found in traditional Ameri-Indian and African farming communities (Doyle 1997; Fregene et al. 2002), principally a product of the allogamous nature of cassava, agricultural practices, natural and farmer selection. This genetic diversity is an important resource and needs to be collected and preserved for future use. Besides a study of genetic diversity might reveal genetic differentiation amongst accession that might represent heterotic pools. A study to assess the genetic diversity of cassava land races in Nigeria was initiated in July 2000, we describe here completion of the SSR characterization component and present insights gained from the study.

Specific Objectives
a) *To study the genetic diversity of cassava land races on a country-wide basis.*
b) *Assess genetic differentiation between the Nigerian and Latin American accessions, for example accessions from Guatemala.*
c) *Genetic crosses between Nigerian and Neo tropical land races that are highly differentiated to test for heterotic groups.*

Methodology
The original study plan was to characterize by SSR markers the 148 accessions held at the cassava germplasm banks of the National Root Crop Research Institute (NRCRI) and the International Institute of Tropical Agriculture (IITA). The IITA collection was made during the collaborative study of cassava in Africa (COSCA) from 65 villages in the entire country. The lack of passport data for more than half of the NRCRI collection and the incomplete IITA

COSCA collection lead to a decision to conduct a fresh collection in all 65 Nigerian villages surveyed by the COSCA study. The collection was carried out by 3 teams from IITA during the period of May through June 2001. All 65 COSCA villages were visited and an average of 4-5 of the most commonly grown varieties were collected. Farmers were also asked questions on where they got their varieties, disease and pest incidence and end uses. A total of 285 accessions were collected. The names and passport data of the land races collected can be seen at the following URL: http://www.ciat.cgiar.org/Molcas, under Nigeria country study. The collection was planted at IITA, Ibadan and will be maintained there.

DNA was isolated from young leaf tissue harvested from field plants according to a modified miniprep method of Dellaporta et al. (1983) at the biotechnology research unit, IITA, Ibadan. DNA was quantiated by flourimetry and shipped to CIAT for molecular analysis. A student from IITA participated in the molecular analysis as a means of transferring the technology to Nigeria. A set of 36 SSR markers, two from each of the 18 linkage groups of the cassava genome, selected based on their clear banding patterns and robustness across several SSR diversity studies, had been selected earlier, this set of markers was employed in characterizing the land races. PCR amplification, gel electrophoresis, and silver staining were as described earlier for these SSR markers (Fregene et al 2002). A previous study had shown high differentiation between African and Guatemalan land races, a set of 13 land races from Guatemala was therefore included to confirm the earlier observation. SSR allele data captured off the gels using the computer software "Quantity One" (Bio-Rad Inc.) Genetic distance, based upon the proportion of shared alleles (PSA), was obtained using the computer program "microsat" (Minch 1993). The distance matrix obtained was displayed graphically using a principal component analysis (PCA) using the computer program JMP (SAS Institute 1997). Parameters of genetic diversity and differentiation were calculated from allele data using the computer packages GENSURVEY (Vekeman et al 1997) and FSTAT (Goudet 1990).

Results
Data from a total of 31 unlinked SSR loci was available for statistical analysis the other 5 markers had poor overall data quality requiring their elimination. Genetic diversity parameters, including total heterozygosity (Ht) and genetic differentiation (Gst) ranged widely from locus to locus (Table 8.5). The average number of alleles for each locus was roughly four and is similar to that found for a study of land races from Tanzania and 7 Neo-tropical countries (Table 8.6). The probability that 2 randomly selected alleles in a given accession are different, average gene diversity, was 0.5832 ± 0.0482 and it is quite high as also found for the previous study (Fregene et al. 2002). However, average gene diversity was higher for land races from Guatemala (0.62 - 0.650) compared to those from Nigeria 0.49 - 0.57). Cassava land races from the humid and sub humid regions of Nigeria had higher average gene diversity compared to those from the semi-arid region of the country. The results found here buttress earlier findings that agricultural practices of cassava farmers and the allogamous nature of cassava produces a large pool of volunteer seedlings that natural and human selection acts upon to maintain a high level of land race diversity of a clonally propagated crop (Doyle et al. 2001; Fregene et al. 2002).

Unique alleles were found in the Guatemalan accessions and pair-wise comparison of the genetic differentiation estimator F_{ST} revealed moderate to high genetic differentiation between the Nigerian and Guatemalan land races (Table 8.7). Of particular interest are Guatemalan land races from the town El Progresso.

Genetic distances between all pairs of individual accessions was calculated by the 1-proportion of shared alleles (1-PSA) and presented graphically by a principal coordinate analysis (PCA) (Figure 8.2). The PC1 and PC2 accounted for 26% and 16% of the total variance respectively. The PCA clearly separates the accessions from Guatemala from those from Nigeria, but it also reveals a sub-structure in the accessions from the Semi-arid region of Nigeria. The presence of a defined sub-structure in the genetic relationship of cassava land races from Africa has been demonstrated before in Tanzania (Fregene et al. 2002). It is yet to be understood the underlying basis for the sub-structure. These results also agree with a previous AFLP marker study of 29 African and 11 Neo-tropics land races that placed African and Neo-tropics land races in two distinct cluster with a sub structure for the African accessions (Fregene *et al.* 2000).

The differentiation amongst land races from Guatemala and Nigeria observed in a previous study (Fregene et al. 2002) and confirmed here may well represent heterotic pools as have been found for maize (Shull *et al.* 1952). One of the principal reasons for•this study was to assess genetic diversity in cassava land races as a first step to delineating heterotic pools for a more systematic improvement of combining ability via recurrent reciprocal selection. Activities ongoing include diallel crosses of representative land races from Nigeria and Guatemala.

Future perspectives
1. Genotype a larger land race collection from Guatemala with the 36 SSR markers
2. Analyze the SSR marker results
3. Genetic crosses between Nigerian and Guatemalan land races.

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Table 8.5. Parameters of Genetic diversity, Ho, Hs, Ht, Dst, Gst and Gst' (correction for differences in sample size) by SSR locus

LocName	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'
SSRY4	0.874	0.729	0.751	0.022	0.027	0.756	0.029	0.036
SSRY12	0.751	0.678	0.691	0.014	0.017	0.695	0.02	0.024
SSRY19	0.538	0.606	0.708	0.102	0.127	0.734	0.144	0.174
SSRY20	0.878	0.812	0.821	0.009	0.011	0.823	0.01	0.013
SSRY21	0.692	0.534	0.555	0.021	0.026	0.56	0.038	0.047
SSRY34	0.328	0.412	0.45	0.039	0.049	0.46	0.086	0.106
SSRY38	0.125	0.152	0.171	0.019	0.024	0.176	0.112	0.136
SSRY51	0.34	0.652	0.655	0.002	0.003	0.655	0.004	0.005
SSRY52	0.61	0.612	0.653	0.041	0.051	0.663	0.062	0.077
SSRY59	0.526	0.646	0.716	0.07	0.088	0.733	0.098	0.119
SSRY61	0.482	0.527	0.548	0.021	0.026	0.553	0.038	0.048
SSRY63	0.264	0.523	0.503	-0.02	-0.025	0.498	-0.04	-0.05
SSRY64	0.621	0.643	0.663	0.02	0.025	0.668	0.03	0.037
SSRY69	0.707	0.645	0.68	0.034	0.043	0.688	0.051	0.062
SSRY82	0.775	0.725	0.779	0.055	0.068	0.793	0.07	0.086
SSRY10	0.823	0.703	0.752	0.049	0.061	0.765	0.065	0.08
SSRY10	0.507	0.472	0.501	0.029	0.036	0.508	0.057	0.071
SSRY10	0.303	0.36	0.373	0.013	0.016	0.376	0.034	0.043
SSRY11	0.284	0.314	0.315	0.001	0.001	0.315	0.003	0.004
SSRY13	0.798	0.585	0.634	0.05	0.062	0.646	0.078	0.096
SSRY14	0.626	0.571	0.634	0.063	0.079	0.65	0.099	0.121
SSRY15	0.837	0.744	0.789	0.045	0.056	0.8	0.057	0.07
SSRY15	0.605	0.551	0.624	0.073	0.091	0.642	0.117	0.142
SSRY16	0.873	0.589	0.711	0.122	0.152	0.741	0.172	0.206
SSRY16	0.5	0.655	0.718	0.063	0.078	0.734	0.087	0.107
SSRY16	0.621	0.496	0.502	0.006	0.008	0.504	0.012	0.015
SSRY17	0.12	0.251	0.325	0.074	0.093	0.343	0.228	0.269
SSRY17	0.793	0.637	0.702	0.065	0.081	0.718	0.092	0.113
SSRY17	0.817	0.767	0.814	0.047	0.059	0.826	0.058	0.072
SSRY18	0.726	0.678	0.75	0.072	0.09	0.768	0.096	0.117
SSRY18	0.807	0.725	0.79	0.065	0.082	0.807	0.083	0.101
Overall	0.598	0.58	0.622	0.041	0.052	0.632	0.067	0.082

Table 8.6. Intra-population and inter-population estimates of genetic diversity parameters of cassava land races from different agro-eclogies of Nigeria and Guatemala

Population	n	#loc.	#l oc_P	PLP	K	K_P	HO_p	HE_p	HEc_p
Nig-Humid	50	31	30	96.8	4.3	4.4	0.5823	0.5683	0.5742
Nig-Semi Arid	111	31	29	93.5	4.2	4.4	0.5517	0.4972	0.4995
Nig-Sub humid	81	31	30	96.8	4.5	4.5	0.576	0.5677	0.5713
GUA-pro	5	31	30	96.8	2.9	3.0	0.678	0.558	0.6212
GUA-otro	6	31	31	100.0	3.5	3.5	0.6044	0.5977	0.6501
Mean :				96.77	3.9	3.97	0.5985	0.5578	0.5832
std				2.28	0.64	0.66	0.0482	0.037	0.0573

n: number of genotypes per sample
#loc: number of SSR loci;
#loc_P: number of polymorphic loci
PLP: percentage pf polymorphic loci
K: average number of allele per locus
K_P: average number of allele per polymorphic loci
Ho: observed heterozygosity
He: Average gene diversity
Hec_p: Average gene heterozygosity corrected for small samples size

Table 8.7. Pair-wise estimates of genetic differentiation estimated by F_{ST} (theta) between cassava land races from the humid, sub humid and semi-arid regions of Nigeria and 2 regions of Guatemala

	Nig.Sub-humid	Nig. Semi Arid	Nig. Humid	Gua-pro	Gua-otro
Nigeria Sub humid	0	0.0715	0.0026	0.1287	0.0843
Nigeria Semi Arid	0.0715	0	0.0511	0.1741	0.1133
Nigeria Humid	0.0026	0.0511	0	0.1288	0.0844
Guatemala-pro	0.1287	0.1741	0.1288	0	0.0047
Guatemala-otro	0.0843	0.1133	0.0844	0.0047	0
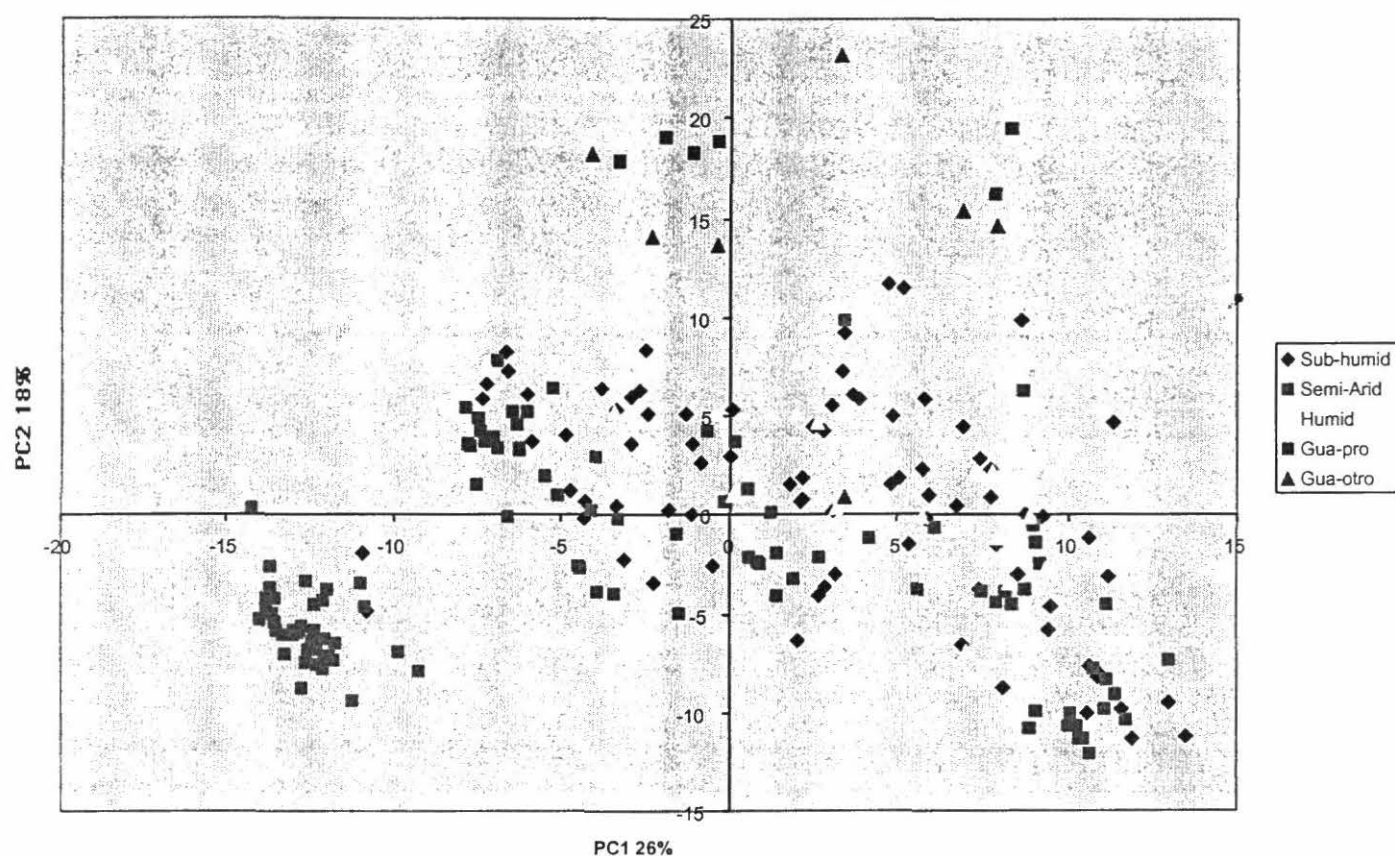
Figure 8.2 PCA of cassava land races from Nigeria and Guatemala based on genetic distances (1-proportion of shared alleles) from 31 SSR markers

Activity 8.4. Simple Sequence Repeat (SSR) Marker Assessment of Genetic Diversity of Cassava Land Races from Guatemala

Collaborators: Dr Cesar Azudia Luis Monte (Facultad de Agronomia, Universidad de San Carlos de Guatemala), Dr Daniel Debouck, Martin Fregene (CIAT)

Important Outputs
1) *Collection and establishment of 128 cassava accessions from all over Guatemala*
2) *Characterization of the collection with 30 SSR markers to date*

Rationale

Two primary centers of diversity, one in South America and the other in Meso-America have been postulated for the genus *Manihot* (Roger and Appan 1973). Although several studies have demonstrated a likely South American origin for the crop (Allem, 1994; Fregene et.al 1994; Roa et al. 1997; Olsen and Schaal 1999), the diversity of cassava and its wild relatives in Meso- America is great enough to suggest it as a second center. Besides, the potential of Meso-American diversity in cassava improvement has not been properly assessed. Three recent studies of genetic diversity in land races from South America and Meso-America (Chavariagga et. al. 1999; Fregene et. al. 2002; Raji et. al. unpublished data) have revealed unique alleles in land races from Guatemala at a frequency high enough to suggest a Meso American center of cassava diversity. The results of the three studies were based upon 6, 4, and 13 Guatemalan land races. The small sample size of the previous study could distort the allele frequencies and lead to wrong conclusions. A larger collection and SSR characterization of land races from Guatemala was therefore planned to confirm preliminary data of a Meso-American center of diversity and to secure the largely untapped diversity in Guatemala before it becomes extinct. In addition, a selection from the Guatemalan collection will be crossed to CIAT elite parents to evaluate the utility of the Meso-American diversity in cassava breeding.

The present study was to confirm the high genetic differentiation between cassava land races from Guatemala and Nigeria, Brazil, and Colombia. If the uniqueness of the Guatemalan germplasm is confirmed, genetic crosses to CIAT's elite breeding lines will be made to test hybrid vigor and delineate heterotic pools. Plant materials are a collection of cassava from all over Guatemala and a representative group used in previous studies from Nigeria, Colombia and Brazil to confirm earlier results. It is hoped that results of the uniqueness and the utility of the Guatemalan germplasm will give collection and conservation of this germplasm in regions of Meso-America high priority (Azurdia and Gomez 2002)

Methodology

A collection of cassava land races was carried out all over Guatemala in May this year (Azurdia and Gomez 2002). A total of 128 accessions were collected in the departments of Baja Verapaz, Quiche, Huehuetenango, Alta Verapaz, San Marcos, Escuintla y Santa in Guatemala (Figure 8.3). For comparison with results of previous studies, DNA from 6, 11 and 12 cassava land races from Nigeria, Colombia y Brazil respectively were included. DNA from the Guatemalan accessions was isolated at the Facultad de Agronomia, Universidad de

San Carlos de Guatemala using a micro-prep protocol of the Dellarporta (1983) methodology and transferred to CIAT. DNA from the other accessions was obtained from previous studies at CIAT.

Figure 8.3. Collection sites of cassava germplasm in Guatemala May 2002.

The concentration and quality of DNA samples was accessed by flourimetry and agarose gel electrophoresis respectively. The DNA samples were diluted to 10 ng/ml for subsequent PCR analysis. A set of 36 SSR markers, carefully chosen to represent a broad coverage of the cassava genome with moderate to high polymorphism information content (PIC) and robust amplification, were used in this study. SSR diversity studies PCR amplification, polyacrylamide gel electrophoresis, and silver staining were as described earlier for these SSR markers (Fregene et al 2002).

Results

A total of 30 SSR markers have been analyzed to date in the Guatemalan germplasm. Results so far reveal a number of unique alleles in the Guatemalan accessions not found in those from other regions (Figure 8.4). The allele data was captured using the program "Quantity One" (Bio-Rad Inc) and entered directly into EXCEL (Microsoft Inc) in preparation for statistical analysis (Figure 8.5). Statistical analysis to be carried out include: principal component analysis (PCA) of a distance matrix based upon 1-proportion of shared alleles, and parameters of genetic diversity and differentiation as described in Fregene et al (2002).
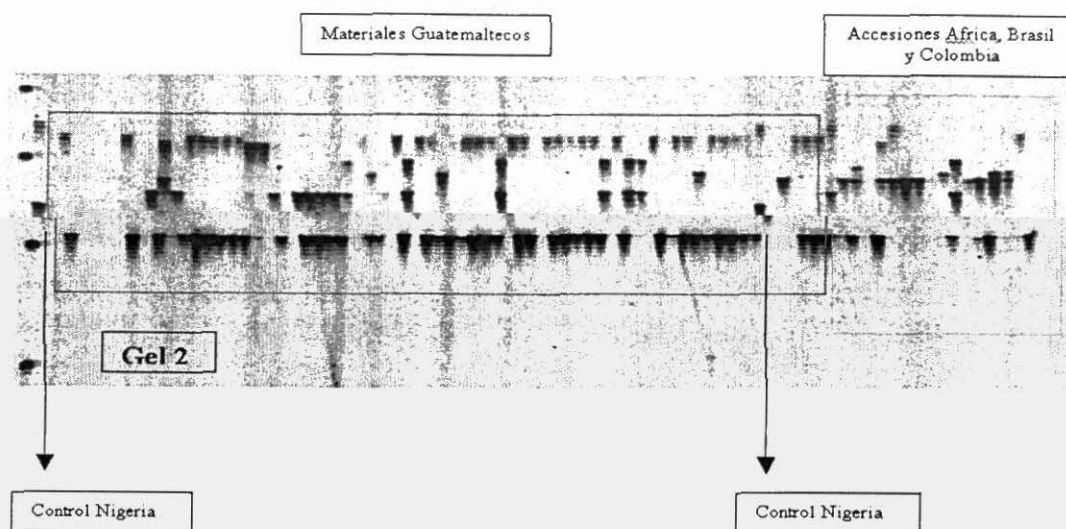
Figure 8.4. Silver-stained polyacrylamide gel of PCR amplification of cassava accessions from Guatemala, Nigeria, Colombia and Brazil with primers of SSR marker SSRY20. A unique allele can be observed in the Guatemalan accessions with a high frequency.
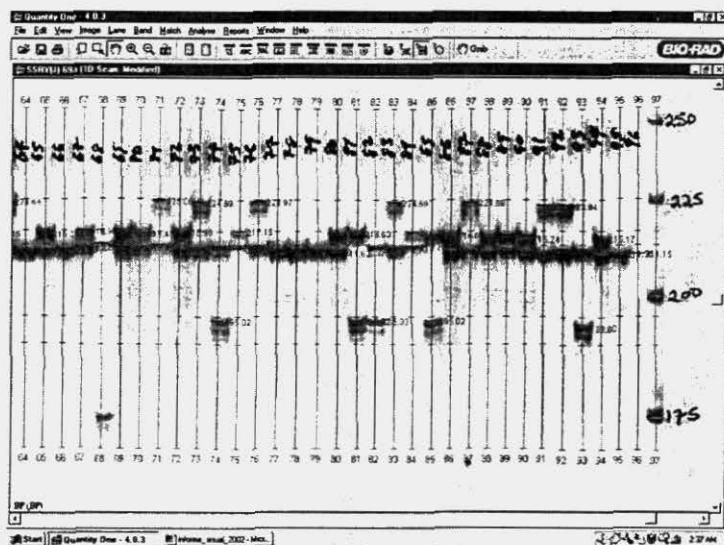
Figure 8.5 . Determination of SSR allele sizes on silver-stained polyacrylamide gels using the software "Quantity One" (Biorad)

Future Perspectives
1. Statistical analysis of the SSR data to estimate genetic diversity and differentiation
2. Genetic crosses between representative accessions from Guatemala and elite cassava parents at CIAT.

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Activity 8.5. Report on the Molecular Characterization of Ghanaian cassava (Manihot esculenta Crantz) Land races and Predictability of Heterosis.

Collaborators: Elizabeth Okai, Dr John Otoo (Crop Research Institute, CRI, Kumasi, Ghana) Martin Fregene (CIAT) Dr Alfred Dixon (IITA)

Funding: IPICs, University of Uppsala, Sweden

Important Outputs

1. Collection of more than 300 cassava land races from 62 villages in the major cassava growing regions of Ghana.
2. Establishment of the collection at the University of Legon and IITA Head quarters, Ibadan.

Rationale

Cassava is an important food crop in developing countries where it is the fourth source of calories, after rice, sugarcane and maize, for more than 400 million people (El-Sharkawy, 1993). Africa is now the largest producer of cassava with a production of 90million metric tones in 1999(FAO 2000). It is cultivated mainly for its storage roots, which provide 390-400 calories/10g dry matter. The leaves when consumed as vegetable provide 7g protein per 100

g edible portion (IITA 1991). The collaborative study of cassava in Africa (COSCA) revealed that cassava serves as a family food staple, a famine reserve crop, and a cash crop.

Cassava was introduced from Brazil, its country of origin, to the tropical areas of Africa, the Far East and the Caribbean Islands by the Portuguese during the 16th and 17th centuries (Jones, 1959). In Ghana, the then Gold Coast, the Portuguese grew the crop around their trading ports, forts and castles. It was a principal food eaten by both the Portuguese and the slaves. By the second half of the 18th century, cassava had become the most widely grown and used crop of the people of the coastal plains (Adams 1957)

The spread of cassava from the coast into the hinterlands was very slow. It reached Ashanti region, Brong Ahafo and the northern Ghana, mainly around Tamale in the 1930.Until the early 1980s,the Akans of the forest belt preferred plantain and cocoyams and sorghum and millet in the north. Cassava became firmly established in most areas after the serious drought of 1982/83 when all other crops failed completely (Korang-Amoakoh,Cudjoe and Adams 1987).

Cassava ranks first in the area under cultivation and utilization. Cassava contributes 22% of the agricultural gross domestic product AGDP compared to 5% for maize, 2% for rice, and 14% for cocoa (Al-Hassan, 1989;Dapaah, 1996). According to the Ghana Living Standards Survey (GLSS), for 1.73 million sampled households 83% were found engaged in cassava production. The spread of cassava into the upper west and upper east of Ghana is an indication of growing trend in cassava production through area expansion(MOA, 1990).

In the traditional bush-fallow system, some cassava plants are allowed to grow during the fallow period, which is long enough to allow cassava to flower and set seeds. The usual out-crossing habit of cassava leads to the production of numerous heterozygous gene pools, which create phenotypic diversity and new hybrid combinations from self-sown seed from which farmers select and propagate desirable types. This process creates pools of new land races, which are adapted to the different agro-ecological zones of Ghana. Coupled with this are the several names that farmers give to cassava as they distribute among themselves. Several land races have been found with the same name and morphological characteristics yet genetically different and the same land races could have different names in several places (Fregene et al., 2000). Doku (1969) recorded 30 such named local varieties in 1930 and by 1960 the number had increased to over 90. Selection for desirable traits has been done by farmers over 1000s of years. Hence the landraces possess higher frequencies of genes required for adaptation to biotic and abiotic stresses, food quality characteristics than unadapted materials. Vegetative propagation also leads to the accumulation of pest and diseases and good varieties susceptible to these biotic stresses disappear. These factors lead to a fairly high turnover of varieties and has implications for gene pool structure of cassava in any center of diversity. Selection is one of the principal factors at work in cassava's gene differentiation in Africa. Evidence for genetic drift has not been demonstrated given that cassava is vegetatively propagated crop, however the use of spontaneous sexual seeds by farmers has been documented (Fregene et al. 2002). High heterosis for yield components, starch, and number of roots have been observed in cassava, and hence considered a promising method of genetic improvement (Easwari Amma and Sheela, 1996). Heterotis groups identified in maize in the early 20th century (Shull et al 1952) have been the basis of a very successful hybrid seed industry.

Specific Objectives

a) The objective for this study is to assess the genetic diversity in Ghanaian landraces

b) To detect heterotic patterns in the collection and between the Ghanaian collection and land races from other countries and regions.

c) To generate hybrids between the Ghanaian land races and genotypes from putative heterotic groups and select together with farmers superior hybrids from the crosses.

Methodology

In January 2002 a collection of cassava land races from all the agro ecological zones in Ghana was done. A total of 45 villages visited during the collaborative study on cassava in Africa (COSCA) were visited. Another 28 villages, important for cassava production, were also visited. Farmers were assembled and asked to share information on cassava varieties grown by them, characteristics of their varieties, and reasons for keeping them. Farmers volunteered to give mature cassava stems, which were labeled.

Fresh young leaf samples of the accessions were collected on ice and used for DNA extraction. An amount of 0.1g of the fresh young leaf was ground in liquid nitrogen and the DNA extracted using the Qiagen kit. The extraction was carried out in IITA, Ibadan, Nigeria. The DNA was carried in absolute ethanol to CIAT. DNA quantification was done using the fluorometer. The DNAs were diluted to 10ng/ul and used for SSR reactions. A sub-set of 36 SSR markers, two from each of the 18 linkage groups of the cassava genome, was employed to obtain an estimate of genetic diversity and differentiation in the land races. PCR amplification, gel electrophoresis, and silver staining was as described earlier (Fregene et al 2002). An internal control of 10 genotypes was included to permit comparison between this study and other ones. The PAGE gels containing SSR data will be scanned and allele sizes determined using the computer software "Quantity One" (Bio-Rad Inc.) based upon an internal gel molecular marker size standard. Genetic distance, based upon the proportion of shared alleles (PSA), will be obtained from the raw allele size data using the computer program "microsat"of Eric Minch (http://www.lotka.stanford.edu/microsat.html). Distances between the accessions will be subjected to principal component analysis (PCA) using JMP (SAS Institute 1995) to obtain a structure of relationship between the land races. Parameters of genetic diversity and differentiation will be calculated from allele data using the computer packages GENSURVEY (Vekeman et al 1997) and FSTAT (Goudet 1990).

Results

A total of 320 landraces were collected including 18 genotypes with yellow roots. Farmers who responded were predominantly women. Among the land races were very early bulking ones 3-9 months after planting. The various local names given suggest a lot of useful traits farmers had associated with the cultivars. Cassava hard wood stems were cut to 20-30cm sizes and planted in plastic pots in a nursery. These were sent to the field after 4weeks and planted in an irrigated field at the Ashiaman office of the Ghana Irrigation Authority. A copy of the collection was packaged and sent to IITA. Accessions were planted in single rows at 1m x 1m spacing with improved varieties as checks.

To date, seventeen out of a set of thirty-six primers used routinely for SSR characterization of cassava genetic diversity have been analyzed. The rest of the analysis is on going. Once the SSR marker analysis is completed, genetic distance and estimates of genetic diversity and differentiation will be calculated. A principal component analysis (PCA) will also be carried

out to graphically display the genetic distance matrix. Based upon clusterings obtained above, genotyopes representative of the clusters will be selected as parents for a diallel experiment to search for heterotic patterns.

Future Perspectives
1. Complete SSR marker analysis of the entire collection
2. Obtain estimates of genetic diversity and differentiation from the SSR data
3. Test heterotic patterns present within the collection or between the collection and others.

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Activity 8.6. A Web-Based Data Base of Simple Sequence Repeat Characterization of Genetic Diversity of Cassava Land Races.

Collaborators: Charles Buitrago, Fernando Rojas, Danny Mauricio Montero, Martin Fregene (CIAT)

**Funding:** IPICs, University of Uppsala, Sweden

Important Output
1. A web-based data base to share results of SSR diversity studies of cassava with the cassava research community.

Rationale
One of the objectives of the cassava molecular diversity network (MOLCAS) is to make available to cassava researchers everywhere results of the molecular characterization of cassava genetic diversity. With the completion of the Peruvian, Nigerian and Tanzanian study, a web-based database was constructed in Oracle to accommodate the results of the above and other studies. Data available for viewing include passport data of the accession, raw SSR gel data, allele sizes, SSR locus information, parameters of genetic diversity and differentiation and principal component analysis (PCA) of genetic distances. The database will be updated as other country studies become available.

Results
The database is organized according to country studies, the Peruvian, Tanzanian and Nigerian country studies are the available ones at the moment. The first page of each country study has links to raw marker data namely, allele sizes per accession and genome location of the marker, where available (Fig 1). Other links are intra-population estimates of genetic diversity, gel images of individual markers and appendixes of additional information from the country study (Figure 8.6). The URL for the database is: http://www.ciat.cgiar.org/Molcas. Since the inception of the database in June, average monthly hits for the first three months averaged 2500 hits per month (Table 8.8). The high number of visits to the site confirms the importance of the MOLCAS database for the cassava community.
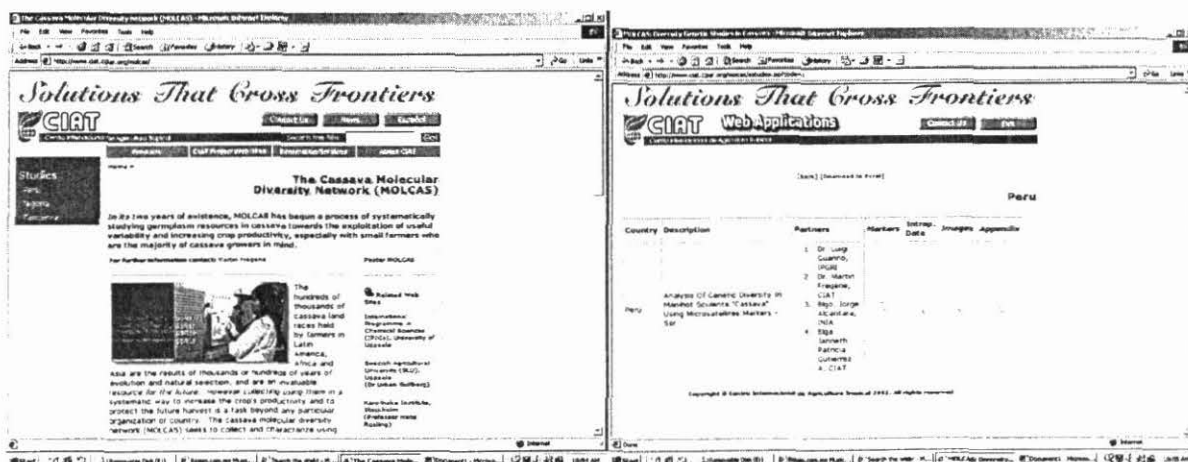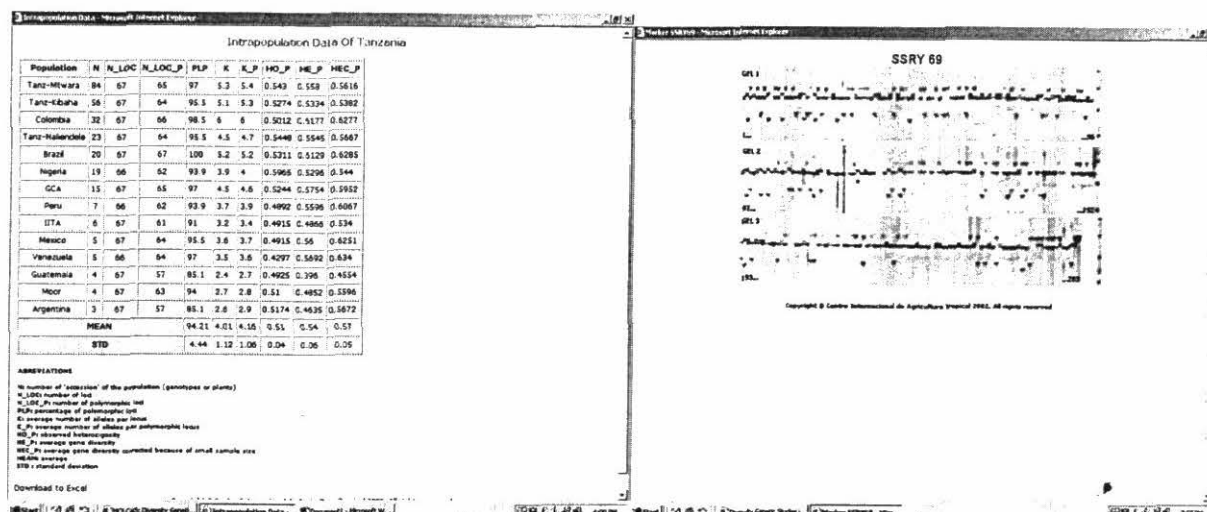
Figure 8.6. An illustration of the different pages of the SSR diversity data-base on MOLACS web site **http://www.ciat.cgiar.org/molcas**.

Table 8.8. Monthly hits at the MOLCAS web site **http://www.ciat.cgiar.org/molcas** for June, July and August and the total for the 3 months.

Request	Aug 2002	Jul 2002	Jun 2002	Total Hits
/molcas/imagen.jsp	1,033	723	816	2,572
/molcas/locus.jsp	982	743	713	2,438
/molcas/alelosp.jsp	556	84	486	1,131
/molcas/	123	114	94	350
/molcas/markers-det.jsp	110	45	63	220
/molcas/estudios.jsp	96	93	0	196
/molcas/studies.jsp	35	16	67	119
/molcas/intrap_data2.jsp	55	37	22	114
/molcas/pcr_cond.jsp	50	22	20	93
/molcas/imagenbioquim.jsp	50	25	0	75
/molcas/appendix1.jsp	44	19	0	65
/molcas/appendix2.jsp	45	10	0	55
/webapps/molcas/	4	2	46	52
Total	3,183	1933	2327	7,480

Future Perspectives
1. Include results of other country studies as they become available
2. Seek for a way to unite data sets from diverse studies.

Activity 8.7. Mining the Primary and Secondary Gene Pool: Protein and Dry Matter Yield Genes from Wild Manihot Species

Collaborators: Nelson Morantes, Teresa Sanchez, Martin Fregene (CIAT)
Funding: CIAT core funds

Important Outputs
1. Second year evaluation of wild and inter-specific accessions for the AB-QTL scheme
2. Identification of an inter-specific hybrid with a putative yield of 114t/ha

Rationale
The advanced back cross QTL (AB-QTL) identification and introgression of favorable alleles of gene for high protein and dry matter content, pest resistance and starch quality in cassava is in its second year. During the first year more than 1,200 accessions of wild species and inter-specific hybrids representing 7 wild *Manihot* species were evaluated for the above traits and genotypes with high protein and dry matter content, excellent resistance to white flies and very high amylopectin content were identified (CIAT 2001). The best genotypes were selected for second year evaluation of six plants (clonal observation or single row trial, SRT) and also planted in the hybridization block for genetic crosses to elite CIAT parents. Genetic crosses between the selected wild accessions and CIAT elite parents will provide F_1 families to initiate the AB-QTL scheme. This year we report on the SRT trial, 6 plants as against 1 last year, of the wild accessions and also on genetic crosses made.

A major problem this year was the high incidence of frog skin disease (FSD) in the clonal observation trial, more than 70%, which has lowered considerably dry matter content and affected protein content in an unknown way. Some genotypes turned out with an exceptionally high amount of protein, while others showed a significant reduction compared to last year's result. FSD infected materials that showed high values for the above traits have been re-planted in Santander the Quilichao pending a virus clean-up of these genotypes by tissue culture and thermotherapy. Nevertheless, seeds obtained from crosses to the selected wild accessions are supposedly virus free and have been planted in the seedling nursery for transfer to the field and trait evaluation at harvest.

Methodology
Accessions of inter-specific hybrids and wild *Manihot* species with high protein and dry matter content, good resistance to white flies, and high amylopectin content starch identified from last year's evaluation were established at CIAT Palmira this year as a clonal observation trial. A selection index program developed by the cassava breeding unit (CIAT annual report 2000) was used to select the best 12 genotypes for protein content, dry matter content, and white fly resistance and the best 4 genotypes low amylose content, a total of 145 wild accessions and 343 inter-specific hybrids for genetic crosses. Due to very poor germination of a majority of the wild species planted directly in the fields from woody stakes, a principal problem with wild species, it was necessary to plant these accessions again in bags in the green house. The stakes were treated with growth hormones to aid germination and transferred to the field after 2 months in the green house. For some materials, the problems with poor germination continued and open pollinated seeds obtained last year from these

genotypes had to be planted to obtain information on the trait of interest.

At 10 months after planting, all six plants were harvested and measured for fresh root yield, dry matter content, foliage weight, harvest index, culinary quality, starch content/quality, and frog skin disease according to standard CIAT procedures. Dried roots from genotypes that had high protein content last year were sent for total protein determination at the CIAT analytical service lab. Seeds from the genetic crosses were also collected and germinated in a seedling nursery in preparation for transfer to the field. Due to the lateness in field establishment some of the wild accessions, particularly the high protein content genotypes, mature seeds could not be harvested before the end of the hybridization season, the immature seeds were therefore harvested and germinated from embryo axes (see activity 6 for more details).

Results

The second year evaluation of inter-specific hybrids and wild accessions selected for high protein and dry matter content, and other traits of interest confirmed the stability of the trait value across years (Table 8.9). However, the experiment was greatly inhibited by the difficulty of establishing the wild accessions from woody stakes.

Table 8.9. Crude protein percentage of dry root scored over 2 years in wild *Manihot* accessions with high protein content. Data from 2002 is based on 6 plants.

Genotype	Mother	%Protein 2001	%Protein 2002	FSD
OW 284- 1	TST XXX- 77	7.00		Infected
OW 131- 2	TST XXX- 2	7.13	9.48	Infected
OW 134- 1	TST XXX- 8	7.26		Infected
OW 136- 3	TST XXX- 13	8.22		Infected
OW 230- 2	FLA 441- 5	9.20		Infected
OW230-3	FLA 441- 5	10.50	9.63	
OW230-4	FLA 441- 5	10.34	6.99	
OW 230- 5	FLA 441- 5	9.14		Infected
OW 231- 3	FLA 444- 7	7.16	5.24	Infected
OW 231- 4	FLA 444- 7	11.00	7.69	Infected
OW 231- 6	FLA 444- 7	8.27		
OW 280- 1	TST XXX- 51	7.24	7.28	Infected
OW181-2	FLA 423- 6	5.89		Infected
OW132-2	TST XXX- 3	11.71	9.48	

Note: Missing values are due to no storage roots, a effect of severe FSD infection

The inter-specific hybrids fared much better, an observation worthy of mention is a genotype, CW67-30, from an inter-specific hybrid family between cassava and its progenitor, *M. esculenta* sub species *flabellifolia* that had a fresh root yield yield of 114 t/ha or 39t/ha dry matter yield (Table 8.10). The genotype CW67-30 showed very vigorous growth and profuse production of foliage, it has been planted again with more replications to examine if the extraordinary yield will be repeated. Also planted again are the above best 25 inter-specific hybrids.

A large number of crosses were made between wild accessions and inter-specific hybrids having high protein and dry matter content, waxy starch, and pest resistance and more than 2000 seeds in total were obtained (Table 8.11). Good size populations exist for the different traits and for the identification of F_1 genotypes for the AB-QTL scheme. The seeds have been planted at CIAT Palmira and they will be evaluated for the relevant traits at 9-10 months after planting.

Table 8.10. Dry matter yield and yield components of the best 25 inter-specific hybrids from 4 families having the same *M. esculenta* sub spp *fabellifolia* accession as male parent.

Clone	Mother	Father	Harvest index	% Dry matter	Yield t/ha	Dry matter yield (t/ha)	Starch	Taste
CW 67-30	MFLA 437- 007(3)	MCOL 2215	0.46	34.08	114.42	39.00	1.00	4.00
CW 66-10	MFLA 437- 007(3)	CM 2766- 5	0.33	38.40	49.00	18.82	2.00	2.00
CW 67-33	MFLA 437- 007(3)	MCOL 2215	0.36	36.73	44.92	16.50	5.00	5.00
CW 65- 75	MFLA 437- 007(6)	CG 501-16	0.50	23.66	61.60	14.58	1.00	4.00
CW 67-24	MFLA 437- 007(3)	MCOL 2215	0.50	33.76	40.92	13.81	2.00	5.00
CW 67-116	MFLA 437- 007(6)	MCOL 2215	0.49	32.57	40.58	13.22	3.00	5.00
CW 66-28	MFLA 437- 007(3)	CM 2766- 5	0.45	34.62	36.00	12.46	3.00	3.00
CW 67- 40	MFLA 437- 007(6)	MCOL 2215	0.43	31.71	36.83	11.68	3.00	3.00
CW 66-76	MFLA 437- 007(3)	CM 2766- 5	0.36	35.40	29.83	10.56	1.00	4.00
CW 67-124	MFLA 437- 007(6)	MCOL 2215	0.62	33.48	28.00	9.38	2.00	5.00
CW 67-55	MFLA 437- 007(6)	MCOL 2215	0.27	36.31	25.75	9.35	1.00	2.00
CW 67-152	MFLA 437- 007(6)	MCOL 2215	0.51	36.00	25.83	9.30	2.00	3.00
CW 66-61	MFLA 437- 007(3)	CM 2766- 5	0.28	35.37	26.17	9.25	3.00	5.00
CW 67-136	MFLA 437- 007(6)	MCOL 2215	0.25	34.71	26.40	9.16	3.00	4.00
CW 67-18	MFLA 437- 007(3)	MCOL 2215	0.24	34.90	26.20	9.14	1.00	3.00
CW 67-77	MFLA 437- 007(6)	MCOL 2215	0.21	32.14	26.33	8.46	4.00	5.00
CW 67-98	MFLA 437- 007(6)	MCOL 2215	0.35	29.70	27.17	8.07	3.00	5.00
CW 64-7	MFLA 437- 007(6)	CG 487-2	0.22	29.70	27.17	8.07	3.00	5.00
CW 65-79	MFLA 437- 007(6)	CG 501-16	0.20	33.72	23.92	8.06	1.00	4.00
CW 66- 35	MFLA 437- 007(3)	CM 2766- 5	0.31	35.87	21.50	7.71	1.00	3.00
CW 67-129	MFLA 437- 007(6)	MCOL 2215	0.71	35.87	21.33	7.65	3.00	5.00
CW 67-121	MFLA 437- 007(6)	MCOL 2215	0.30	28.22	25.25	7.12	3.00	3.00
CW 66-21	MFLA 437- 007(3)	CM 2766- 5	0.24	33.64	21.00	7.06	3.00	3.00
CW 67-44	MFLA 437- 007(6)	MCOL 2215	0.40	31.13	22.60	7.04	1.00	4.00
CW 67-126	MFLA 437- 007(6)	MCOL 2215	0.62	31.13	22.00	6.85	3.00	5.00
CW 66-49	MFLA 437- 007(3)	CM 2766– 5	0.43	20.03	27.00	5.41	3.00	4.00

Table 8.10 (cont.)

Statistics of best 25 inter-specific hybrids evaluated								
Maximum				0.71	38.40	114.42	39.00	1.00	3.00
Minimum				0.20	20.03	21.00	5.41	5.00	5.00
Average				0.39	32.80	33.76	11.07	2.35	3.96
Standard Dev.	Statistics of 343 inter-specific hybrids evaluated			0.14	4.06	19.16	6.54	1.09	1.00
Maximum				0.71	55.88	114.20	39.00	1.00	5.00
Minimum				0.01	16.30	5.80	1.19	5.00	2.00
Average				0.16	27.59	9.66	2.70	2.80	4.10
Standard Dev.				0.12	7.17	9.90	3.46	1.37	1.00

Table 8.11. Summary of sexual seeds obtained from crosses between inter-specific hybrids and wild *Manihot* accessions high in protein, dry matter content, and waxy starch.

	Family	Mother	Father	Purpose of cross		No. of seeds
	Protein					
175	CW 179	OW 132- 2	MTAI 8	PTN	Z01	21
178	CW 185	OW 180- 1	MTAI 8	PTN	Z01	9
186	CW 204	OW 231- 3	AM 244- 31	PTN		14
187	CW 205	OW 231- 3	MTAI 8	PTN	Z01	11
188	CW 206	OW 280- 1	AM 244- 31	PTN		64
189	CW 207	OW 280- 1	MTAI 8	PTN	Z01	291
190	CW 207	MTAI 8	OW 280- 1	Z01	PTN	16
						426
	Protein and yellow roots					
221	CW 73	CM 1585- 13	OW 284- 1	YRT	PT-MS	15
222	CW 177	OW 132- 2	CM 1585- 13	PTN	YRT	128
223	CW 184	OW 180- 1	MCOL 1734	PTN	YRT	13
224	CW 186	OW 181- 2	CM 1585- 13	PTN	YRT	13
225	CW 188	OW 181- 2	MCOL 1734	PTN	YRT	23
226	CW 207	OW 280- 1	CM 1585- 13	PTN	YRT	215
227	CW 212	OW 284- 1	MCOL 1734	PT-MS	YRT	13
228	CW 251	MCOL 1734	OW 189- 1	YRT	PT-MS	18
229	CW 256	MCOL 1734	OW 280- 1	YRT	PTN	13
						451
	Dry matter and yellow roots					
21	CW 69	CM 1585- 13	CW 30- 29	YRT	DMC	6
22	CW 69	CW 30- 29	CM 1585- 13	DMC	YRT	6
23	CW 70	CM 1585- 13	OW 234- 2	YRT	DMC	58
24	CW 71	CM 1585- 13	OW 240- 8	YRT	DMC	19
25	CW 72	CM 1585- 13	OW 280- 2	YRT	DMC	1
26	CW 100	CW 30- 29	MCOL 1734	DMC	YRT	3
27	CW 100	MCOL 1734	CW 30- 29	YRT	DMC	4
28	CW 101	CW 30- 31	CM 1585- 13	DMC	YRT	9
29	CW 115	CW 30- 73	CM 1585- 13	DMC	YRT	1
30	CW 127	CW 30- 73	MCOL 1734	DMC	YRT	1
31	CW 127	MCOL 1734	CW 30- 73	YRT	DMC	46
32	CW 147	CW 47- 3	MCOL 1734	DMC	YRT	3
33	CW 147	MCOL 1734	CW 47- 3	YRT	DMC	11

Table 8.11 (cont.)

34	CW 153	CW 48- 1	MCOL 1734	DMC	YRT	54
35	CW 154	MCOL 1734	CW 48- 1	YRT	DMC	66
36	CW 155	CW 56- 5	CM 1585- 13	DMC	YRT	23
37	CW 166	CW 56- 5	MCOL 1734	DMC	YRT	38
38	CW 181	OW 146- 1	MCOL 1734	DMC	YRT	11
39	CW 211	OW 280- 2	MCOL 1734	DMC	YRT	11
40	CW 249	MCOL 1734	CW 28- 38	YRT	DMC	34
41	CW 250	MCOL 1734	CW 30- 65	YRT	DMC	44
42	CW 252	MCOL 1734	OW 234- 2	YRT	DMC	16
43	CW 253	MCOL 1734	OW 240- 6	YRT	DMC	113
44	CW 254	MCOL 1734	OW 240- 8	YRT	DMC	16
45	CW 255	MCOL 1734	OW 269- 4	YRT	DMC	71
						665

	Dry matter					
46	CW 82	CM 7951- 5	OW 240- 8	Z02	DMC	22
66	CW 111	CW 30- 31	MTAI 8	DMC	Z01	16
82	CW 128	CW 30- 73	MTAI 8	DMC	Z01	4
90	CW 137	CW 30- 87	MTAI 8	DMC	Z01	4
107	CW 167	CW 56- 5	MTAI 8	DMC	Z01	168
108	CW 169	CW 60- 7	SM 1036- 8	DMC		5
109	CW 170	CW 60- 7	MTAI 8	DMC	Z01	27
112	CW 180	OW 146- 1	AM 244- 31	DMC		70
118	CW 197	OW 213- 4	MTAI 8	DMC	Z01	71
121	CW 216	SM 1219- 9	CW 48- 1	Z02	DMC	11
122	CW 221	SM 1460- 1	CW 30- 65	Z02	DMC	3
123	CW 222	SM 1460- 1	CW 48- 1	Z02	DMC	19
124	CW 228	SM 1460- 1	OW 240- 8	Z01	DMC	6
125	CW 262	MTAI 8	OW 234- 2	Z01	DMC	83
126	CW 263	MTAI 8	OW 240- 6	Z01	DMC	4
127	CW 264	MTAI 8	OW 240- 8	Z01	DMC	33
						546

	Waxy starch					
2	CW 143	CW 39- 2	MTAI 8	ALW	Z01	5
5	CW 191	OW 183- 4	MCOL 1734	ALW	YRT	1
						6

Grand total						**2094**

Notes: PTN-protein; ZO-agro-ecological zone; YRT-yellow root; DMC/MS- dry matter content

Future Perspectives
1. Evaluate the inter-specific hybrids generated for the target traits
2. Continue making crosses of

References
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8.8 Mining the Primary and Secondary Gene Pool: Resistance Genes for Green Mites from *Manihot esculenta* sub species *flabellifolia*

Collaborators: Nelson Morantes, Jose Maria Guerrero, Anthony Bellotti, Martin Fregene (CIAT)
Funding: CIAT core funds

Important Outputs
1. Discovery of a very high level of resistance to CGM in inter-specific hybrids of *M. esculenta* sup spp *flabellifolia*
2. A putative marker associated with a high level of resistance to the cassava green mite

Rationale
The cassava green mites (*Mononychellus tenajoa*) is a biotic stress of cassava that becomes prominent during periods of prolonged dry periods. In East Africa, overlapping outbreaks of CMD and CGM during the dry season tend to result in very heavy losses and a severe reduction in farm profits (Legg et al. 1998). January this year at CIAT Palmira was particularly dry and, not surprisingly, a very heavy incidence of mites was recorded on the station. The CIAT cassava entomology group conducted a thorough evaluation of cassava plants in the field and while most plants had damage ratings of 4 on the CIAT scale of 1-5, where 1 is no symptoms, and 5 is severe leaf damage and stunted growth, 4 inter-specific hybrid families from the wild *Manihot* accession MFLA 437- 007 showed an almost equal number of susceptible(score of 3-4) and resistant (score of 1-2) genotypes.

The very high level of resistance found in the inter-specific hybrids and the almost equal number of susceptible and resistant genotypes suggests a simple mode of inheritance of the resistance gene(s), which makes deployment of this source of mite resistance very attractive. Bulk segregant anlysis (BSA) using 500 SSR markers was quickly used to identify molecular markers associated with resistance. At the same time, highly resistant hybrids were crossed to CMD resistant parents, to combine CMD and CGM resistance for Africa, and also to elite cassava parents at CIAT.

Methodology
A clonal observation trial of 6 plants per genotype of inter-specific hybrids between the cassava varieties CG487-2, CG501-16, MCol2215, and CM2766-5 and the *M. esculenta* sup spp *flabellifolia* accession MFLA 437- 007, designated CW68, CW65, CW67, and CW66 respectively were planted at CIAT Palmira August last year. They were evaluated for resistance to mites during a very heavy mite infestation January this year. A high level of resistance was observed in about half of the inter-scpefic hybrids. It was thought desirable to transfer this high level of resistance to elite parents of the cassava gene pools. Genetic crosses where therefore made to elite parents of the cassava gene pool.

Following the interesting distribution of resistant genotypes observed in the families, the family CW67 was chosen for bulk segregant analysis of CGM resistance. Ten resistant and ten susceptible genotypes were used for molecular analysis. DNA was isolated from 1-2g of young leaves and dried for 24h in an oven at 48°C. The dried leaves were ground using a

power drill and washed sand and DNA was isolated from 200mg leaf tissue using a miniprep version of the Dellaporta (1983) protocol. DNA from the cassava parent Mcol2215 was also isolated for inclusion in the analysis. The wild parent no longer exists it was eliminated from the field in 2000 during an eradication of the wild *Manihot bank*, many of which were contaminated with frog skin disease, the crosses were made in 1995. DNA from the bulks and parent was genotyped with the 500 available cassava SSR markers. Markers that were polymorphic in the bulks were analyzed in individual genotypes that make up the bulks.

Results

Resistance response to CGM in the families CW65, 66, 67 and 68 was qualitative, i.e., all 6 plants of resistant genotypes showed no visible symptom, while all plants of susceptible genotypes were always heavily infected. The percentage of resistant to susceptible plants was about the same (Figure 8.12). A chi square of the ratio of resistant to susceptible plants was not significantly different from a 1:1 ratio at a probability level of 0.05 for CW65, 66, and 68. This fits the expected segregation ratio for a single dominant gene heterozygous in the wild accession. BSA revealed an allele of the SSR marker, SSRY330, is present in the resistant parent and in the resistant bulk but absent in the susceptible bulk and the susceptible parent (Figure 8.13).
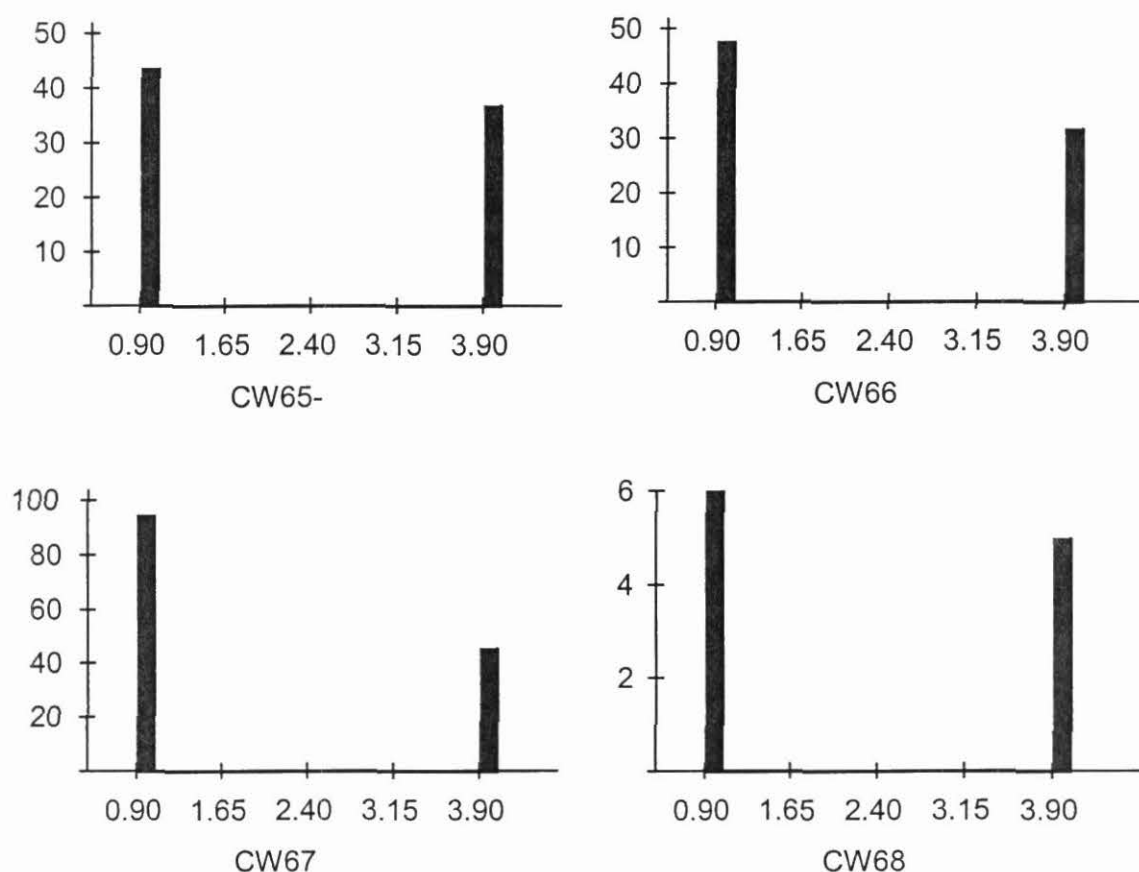
Figure 8.12. Distribution of response to CGM in 4 inter-specific hybrids derived from a single accession of *M. esculenta* sup spp *flabellifolia* MFLA 437- 007 as father.

The polymorphism was confirmed when the individuals of the bulks were screened with the SSR marker although 3 and 1 recombinant could be observed in the resistant and susceptible bulk respectively (Figure 8.13). The SSR marker is currently being analyzed in all individuals of CW67, as well as those of the other three families.

Genetic crosses have been made between inter-specific hybrids having high CGM resistance and elite parents of CIAT gene pools, a total of 832 seeds were obtained (Table 8.7). This cross can be loosely described as a back cross and it is the second step of the AB-QTL scheme. Seeds obtained have been planted at CIAT Palmira and they will be evaluated for the relevant traits at 9-10 months after planting.

F R S | R.individuals | S individuals |
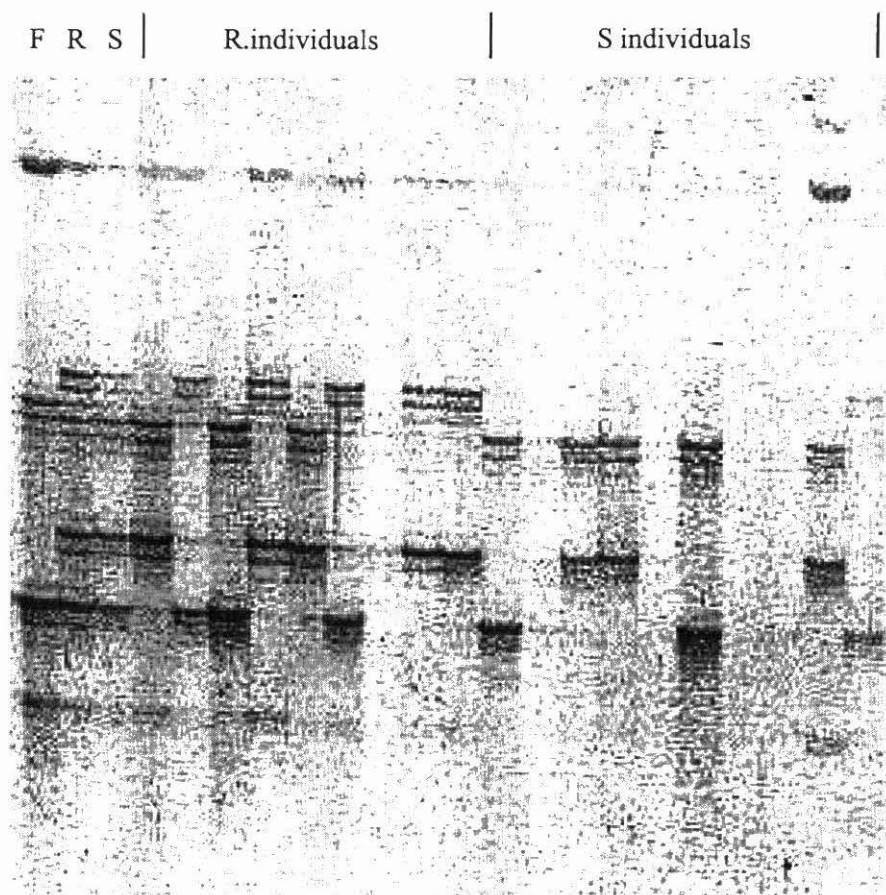
Figure 8.13. Silver stained polycrylamide gel of bulk segregant analysis (BSA) of CGM resistance in the CW67 family, the topmost fragment segregates with resistance. Three and one recombinants respectively can be observed in the CGM resistant and susceptible varieties. F stands for father, the male parent (MFLA 437- 007), while R and S stands for the resistant and susceptible bulk respectively.

Future Perspectives
1. Analyze the marker SSRY330 in the entire individuals of all 4 families namely, CW65, CW66, CW67, and CW68.
2. Second year evaluation of the inter-specific hybrids in Santander the Quilichao.

Table 8.7. Seeds obtained from crosses between the inter-specific hybrids with high levels of resistance to CGM and elite parents of cassava gene pools at CIAT.

Family	Mother	Father	No. of seeds
CW 75	CM 3306- 4	CW 66- 60	8
CW 76	CM 3306- 4	CW 68- 3	12
CW 77	CM 7951- 5	CW 65- 77	7
CW 78	CM 7951- 5	CW 66- 19	4
CW 79	CM 7951- 5	CW 66- 62	2
CW 80	CM 7951- 5	CW 67- 42	5
CW 81	CM 7951- 5	CW 67- 98	4
CW 213	SM 805- 15	CW 67- 39	1
CW 214	SM 805- 15	CW 67- 87	11
CW 215	SM 909- 25	CW 66- 60	10
CW 217	SM 1219- 9	CW 65- 77	23
CW 218	SM 1219- 9	CW 66- 73	24
CW 219	SM 1219- 9	CW 66- 74	4
CW 220	SM 1219- 9	CW 67-123	15
CW 223	SM 1460- 1	CW 66- 19	18
CW 224	SM 1460- 1	CW 66- 60	22
CW 225	SM 1460- 1	CW 66- 62	42
CW 226	SM 1460- 1	CW 66- 73	16
CW 227	SM 1460- 1	CW 68- 3	3
CW 229	SM 1511- 6	CW 67- 87	35
CW 230	SM 1565- 15	CW 66- 19	5
CW 231	SM 1565- 15	CW 66- 60	27
CW 232	SM 1665- 2	CW 66- 19	31
CW 233	SM 1665- 2	CW 66- 60	4
CW 234	SM 1665- 2	CW 66- 74	49
CW 235	SM 1665- 2	CW 67- 87	129
CW 236	SM 1669- 5	CW 66- 19	58
CW 237	SM 1669- 5	CW 66- 60	12
CW 238	SM 1669- 5	CW 66- 62	2
CW 239	SM 1669- 5	CW 66- 73	7
CW 240	SM 1669- 5	CW 66- 74	36
CW 242	SM 1669- 7	CW 67- 87	13
CW 241	SM 1669- 5	CW 67-123	8
CW 243	SM 1741- 1	CW 66- 19	9
CW 244	SM 1741- 1	CW 66- 60	18
CW 245	SM 1741- 1	CW 66- 62	3
CW 246	SM 1741- 1	CW 67- 91	12
CW 247	SM 1778- 45	CW 66- 19	4
CW 248	SM 1778- 45	CW 67- 45	4
CW 257	MTAI 8	CW 65- 77	33
CW 258	MTAI 8	CW 66- 60	31
CW 259	MTAI 8	CW 66- 73	59
CW 260	MTAI 8	CW 66- 74	6
CW 261	MTAI 8	CW 67-123	5
			832

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Activity 8.9 Construction of an SSR Map of Cassava Based upon Linkage Analysis in a F₂ Cross Derived from Non-Inbred Parents and QTL Mapping of Early Bulking.

Collaborators: Jaime Marin, Emmanuel Okogbenin, Nelson Morante, Martin Fregene (CIAT)
Funding: **The Rockefeller Foundation**

Important Outputs
1. Construction of a SSR linkage map of cassava
2. Discovery of QTLs, consistent across different generation with additive or dominant/recessive gene action for dry root yield, fresh foliage weight and harvest index.

Rationale
The QTL mapping early bulking at CIAT has identified a number of major QTLs for this important trait (Okogbenin and Fregene 2002). Last year, a new F₂ mapping population was developed from an F₁ individual to validate the authenticity, magnitude, and action of these QTLs. Quantitative trait loci (QTL) mapping in single generation, full-sib pedigrees of allogamous crops is complicated by the inability to access all information on the genetic architecture of the quantitative trait in question. Furthermore, marker genotype in these mapping populations result from the independent meioses and crossovers in the maternal and paternal parents leading to separate maps for each parent which alters QTL mapping by redefining mating type at a locus level rather than all loci in both parents. (Groover et al. 1994; Van Eck et al. 1994; Grattapaglia et al. 1994).

The new genetic map of cassava was constructed using SSR markers, a relatively easier to use marker system. The use of SSR markers have considerably cut down the time required for the development of a cassava map compared to earlier efforts, from 3 years to a little over a year. Although, 3 F₂ crosses were developed, only one, the cross with the highest number of heterozygous markers in the F₁ parents, was chosen for map development.

Methodology
The F₂ population obtained from an F₁ progeny, K150, of the cassava map population was selected as our F₂ mapping population because of the large number of markers that were heterozygous in K150, more than 60%, and the relatively large number of progeny, 372. Due to poor seedling development of certain genotypes (resulting in senescence in some few cases), only 268 plants of initial 372 in the F₂ population were used for genotyping. The F₂ seedlings were initially germinated in the screen house at CIAT headquarters in Palmira under intensive management and care in February 2000. Seedlings were later transplanted to the field in July 2000 and harvested for planting materials at 11 months after planting (MAP) the following year (2001). Of the 268 genotypes used for mapping analysis, only 207 genotypes with relatively sufficient stem cuttings (12 stakes) of about 25 cm long each could be planted for QTL mapping experiment at Santa Elena, a location 25 Km from CIAT headquarters. Individual genotypes were sown on May 18, 2001 in single row plots of 6 plants each in randomized complete block design of two replications. Plants were planted at 0.8 cm between plants and 1m between rows. All plants were harvested 7 months after planting (MAP). Traits measured include: dry root yield, fresh foliage and harvest index.

DNA was extraction from individual genotypes of the F_2 population has been described earlier (CIAT 2001). For the parental survey, 186 SSR markers developed by Mba et al. (2001), 132 SSR markers from a cassava root and leaf cDNA library (Mba et al., unpublished data), and 154 SSR markers generated by Fregene et al. (unpublished data), a total of about 500 markers, were used. PCR amplification and PAGE gel electrophoresis were as described by Mba et al (2001). For linkage analysis, individuals of the F_2 population were by the three different genotypic classes expected for a F_2 population and Chi square values were computed to test for significant deviations from expected ratios (segregation distortion). Linkage analysis was using MAPMAKER/EXP 3.0 (Lander et al., 1987). Recombination fractions were converted to map distances, centiMorgan (cM), using Kosambi mapping function..

The mean of each genotype over two replications was used for the correlation analysis and QTL mapping. Phenotypic correlations coefficients between yield and components were estimated and tested for significance (P< 0.05). Type III mean squares from ANOVA based on General Linear Model procedure (Proc GLM; SAS 1996) was used to calculate broad sense heritability estimates for each trait.

The locations of putative QTLs were determined by interval mapping analysis using MAPMAKER/QTL 1.1b (Patterson et al. 1988). Maximum likelihood estimates of both additive (a) and dominance (d) effects were calculated simultaneously during the genome scan for QTLs as performed by MAPMAKER/QTL. The gene action of a QTL, largely additive, dominant or recessive, can be determined by evaluating the relative likelihood of gene models. To test for additional QTLs, we fixed the position and effect of one QTL, the single QTL model (SQM), then re-scan the genome searching for other QTLs, using a two-QTL model (TQM). Two-QTL map allows each locus to control its fraction of the variance while at the same time estimating the effect of the other. The multi-locus model was used explain how much of the phenotypic variance among the F_2 population for each trait was explained by fitting in the model, QTLs identified in SQM and other additional QTLs detected in TQM.

Results
A total of 122 SSR markers, or 25%, were polymorphic in the F_1 parent and could be scored in the F_2 mapping population (Fig 1). This number is close to what is expected, that is 50% x 50% (average percent polymorphisms of SSRs in cassava x percent polymorphisms expected in a F_1 genotype). Most of the markers surveyed had the expected segregation, a ratio of 1:2:1, for homozygous for parent A, heterozygous and homozygous for parent B respectively. Deviation from the expected 1:2:1 genotype frequency was significant (P ≤ 0.05) for 33 (27%) of the 122 markers scored. The 122 markers were employed in constructing a linkage map (Fig2). The linkage map consists of 100 markers, 22 markers remained unlinked. The presence of so many unlinked markers suggests that the available SSRs still do not cover the entire cassava genome. The number of linkage groups in this map (22) exceeds the haploid number of chromosomes for cassava, indicating that the map is also unsaturated.

Phenotypic data for DR, FF and HI showing means, standard deviation, kurtosis, skewness, and W-test are summarized in Table 8.14. Data range for each trait measured revealed wide variation in the F_2 population as equally observed in the F_1. All of the traits studied showed continuous distribution as expected for quantitative traits. The heritability (H^2) estimates in the F_2 were 66% for dry root yield (DR), 68% for Fresh foliage weight (FF) and 78% harvest index (HI). The relatively high heritability of the three traits is in agreement with high estimates obtained in the F_1 (Okogbenin and Fregene 2002). A total of nine QTLs (LOD> 2.0)

(three QTLs each) influencing FF. DR and HI were identified by interval mapping analysis on seven linkage groups (Table 8.15). Results revealed that two QTLs (*Dr3* and *Ff3*) fall within a single interval (NS 928 – SSRY 153) separated by only 4 cM (Table 8.15). The direction of the genetic effects of these two QTLs are similar, suggesting that they are probably not different QTLs, providing evidence for gene pleiotropy for DR and FF at this locus.

Seven highly significant two-QTL interactions were identified for FF, and 4 each for DR and HI (Table 8.16). Phenotypic variance (PV) explained for these interactions varied from 11 to 36% with LOD scores ranging between 2.74 and 8.97. Some of the QTLs identified in the single QTL model (SQM) significantly interacted with each other. In some instances, interactions led to highly significant increase in LOD and PV explained. For example, *Dr1* significantly interacted with *Dr13* resulting in LOD of 5.14 and explained PV of 17.2%, which were higher than the sum of LOD scores and PVE for both QTLs under the SQM. All additional QTLs identified in the two-QTL model (TQM) were fitted along with those identified in the SQM in a multi-locus model to determine total phenotypic variance explained among the F_2 progeny for each trait. The total PV explained based on multiple QTL model are 33% for foliage, 44% for DR and 37% for HI.

The gene action of individual QTLs was evaluated by comparing the fits of individual QTL models (Lander and Botstein, 1989). The three QTLs detected for FF revealed different gene actions: *Ff3* had a recessive gene model, *Ff5* dominance, while *Ff9* exhibited additive gene action. Two of the QTLs identified for DR (*Dr3* and *Dr13*) were consistent with a recessive gene model. Two other QTLs, *Hi2* and *Hi9* exactly fit a pure additive model. The additive effect, which is the measurement of the change in a population mean when an allele of a QTL is substituted, showed that *Hi9* increased harvest index while *Hi2* decreased HI. The third QTL for HI (*Hi12*) was recessive.

A comparison was made of QTLs identified in the experiments using F_1 and F_2 crosses. Results reveal that 1, 7, and 3 common QTLs were detected for fresh foliage, dry matter yield and harvest index respectively (Table 8.17).

Future Perspectives
1. Test QTLs that are stable across generations and thatexplain substantial phenoytpic variance (>20%) in a different genetic background.

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Table 8.14 Performances of three traits evaluated in the F_2 population

Trait	Range	Mean	Standard deviation	Skewness	Kurtosis	W-statistic
Fresh foliage (g)	112.50-3900.00	1152.15	583.26	1.08	2.34	0.94*
Fresh root (g)	213.24	213.24	120.62	0.27	-0.43	0.96*
Harvest Index	0.38	0.38	0.14	-0.4	-0.28	0.96*

Table 8.15. QTLs associated with dry root yield (DR),fresh foliage(FF) and harvest index (HI) in the F_2 population.

Trait	QTL	Flanking markers	Length (cM)	Linkage group	LOD	QTL position (cM)	PVE	a	d	d/a	Mode
FF	Ff3	NS 928 – SSRy 153	16.3	3	2.95	0.0	7.6	-87.84	274.90	-3.13	R
	Ff5	SSRy 35 – SSRy 284	28.1	5	2.26	10.0	31.1	-414.21	-556.36	1.34	D
	Ff9	SSRy 12 – SSRy 91	31.0	9	2.16	38.0	5.5	-211.98	-21.83	0.10	A
DR	Dr1	NS 911-NS 847	17.7	1	2.18	12.0	9.2	0.3	73.70	245.67	DR
	Dr3	SSRy 928 - SSRy153	16.3	3	2.14	4.0	7.3	-21.53	52.40	-2.43	R
	Dr16	NS 33 - SSRy 100	16.3	13	2.25	18.0	6.0	47.35	-64.61	-1.36	R
HI	Hi2	NS 149 - SSRy83	7.3	2	6.67	0.0	15.0	-0.08	0.00	0.05	A
	Hi9	SSRy52 - NS 340	3.1	9	2.25	0.0	54.3	0.05	0.00	0.00	A
	Hi12	NS 74 - NS 389	44.4	12	2.10	0.0	4.9	0.01	-0.06	-6.00	A

Individual QTL loci are named by trait (abbreviation indicated in titles) and linkage groups. The LOD score (LOD) and percent phenotypic variance explained (PVE) by the QTLs are presented from the single-QTL model with unconstrained gene action. The additive effect (a) dominance deviation (d), and ratio of dominance to additivity (d/a) for each QTL are presented in their original units. The possible pure modes of gene action (Mode) for each QTL are indicated based on testing of additive (A) and dominant (D, R) models as described in Materials and methods (if d = 0, then A, if d = a then D, if d = -a then R). If a model reduced likelihood by 10-fold or more, it was deemed unlikely. When two pure modes of gene action could not be deemed unlikely, the more likely mode was listed first (e.g for Dr1, dominance (D) was most likely but recessivity (R) could not be deemed unlikely, thus the mode for this locus is denoted DR. QTL position is position of LOD peak given as distance from the first marker listed in the interval.

Table 8.16 Two-QTL interactions affecting Dry root yield, fresh foliage and harvest index.

Trait	LG	Interval 1	QTL	QTL position (cM)	Interval 2	QTL	QTL position (cM)	LG	PVE	LOD
FF	9	SSRY 12 – SSRy 91	Ff9	38.0	NS 717 – SSRy 3	Ff4a	14.0	4	12.0	3.07
	9	SSRY 12 – SSRy 91	Ff9	38.0	NS 217 – NS74	Ff12	0.0	12	11.1	4.02
	9	SSRY 12 – SSRy 91	Ff9	38.0	SSRy50 – SSRy 281	Ff15a	40.0	15	11.9	2.74
	3	NS 928 – SSRy 153	Ff3	0.0	NS 717 –SSRy 3	Ff4a	14.0	4	16.0	3.97
	3	NS 928 – SSRy 153	Ff3	0.0	SSRy12 – SSRy91	Ff9	38.0	9	13.2	4.98
	3	NS 928 – SSRy 153	Ff3	0.0	SSRy 50 – SSRy281	Ff15b	28.0	15	15.4	3.56
	5	SSRy 35 – SSRy 284	Ff5	10.0	NS 717 – SSRy 3	Ff4b	8.0	4	36.0	3.48
DR	3	NS 928 – SSRy 153	Dr3	4.0	NS 717 – SSRy 3	Dr4	16.0	4	13.2	3.47
	16	NS 33 – SSRy 100	Dr16a	18.0	NS 74 – NS 319	Dr12	20.0	12	14.6	3.30
	16	NS 33 – SSRy 100	Dr16a	18.0	NS 33 – SSRy 100	Dr16a	12.0	16	15.4	3.23
	1	NS 911 – NS 847	Dr1	12.0	NS 33 – SSRy 100	Dr16b	18.0	16	17.2	5.14
HI	2	NS 149 – SSRy 83	Hi2	0.0	SSRy 182 – SSRy 148	Hi8	8.0	17	22.1	8.97
	9	SSRy 52 – NS 340	Hi9	0.0	NS 149 – SSRy 83	Hi2	0.0	2	17.0	7.64
	12	NS 74 – NS 389	Hi12	0.0	NS 267 – SSRy 1	Hi18	26.0	18	13.1	2.76
	12	NS 74 – NS 389	Hi12	0.0	NS 149 – SSRy 83	Hi2	0.0	2	18.3	8.25

In cases where multiple QTLs affecting a trait were found along the same linkage group, the QTLs are distinguished by letters indicating the temporal order in which they were discovered (e.g. Ff15a and Ff15b). The LOD score (LOD) and percent phenotypic variance explained (PVE) by the QTLs are presented from the two-QTL model with unconstrained gene
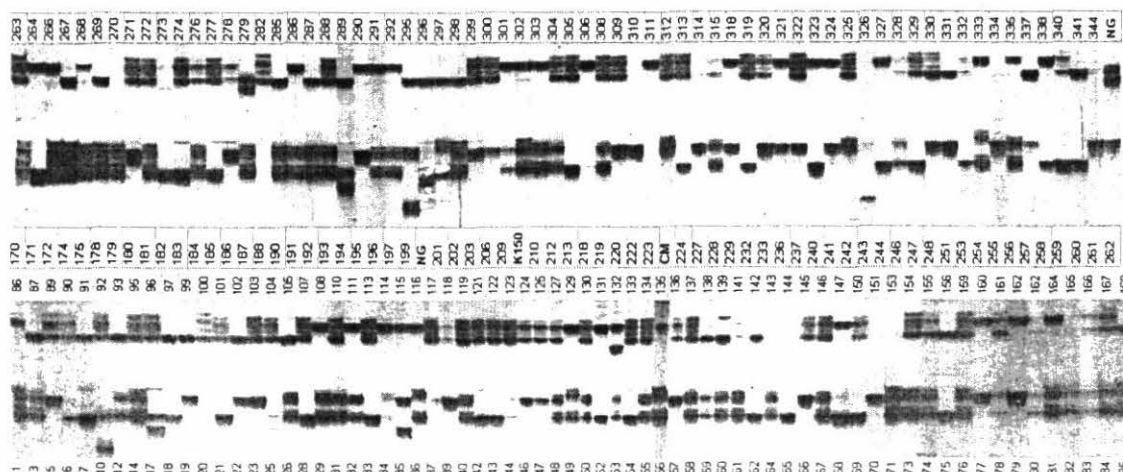
Figure 8.8. Silver stained polyacrylamide gel showing segregation of SSRY marker SSRY 105 in invididuals of the F$_2$ mapping population.
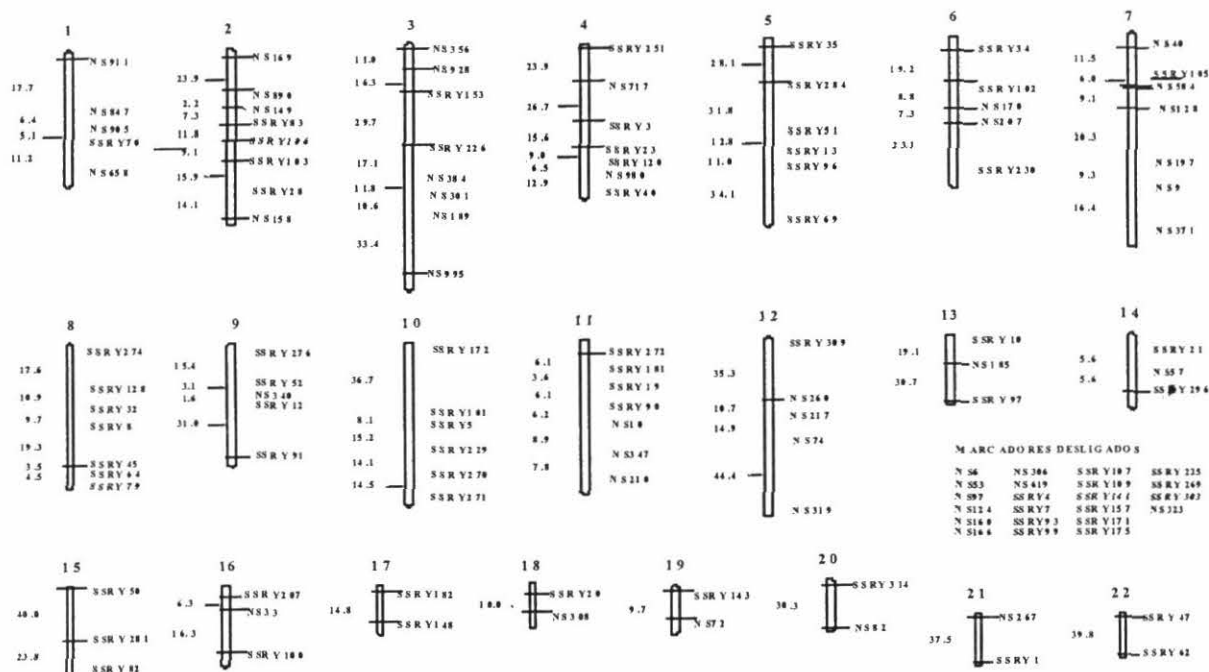
Figure 8.8. A linkage map of cassava (*Manihot esculenta* Crantz) based upon a F₂ cross and SSR markers.
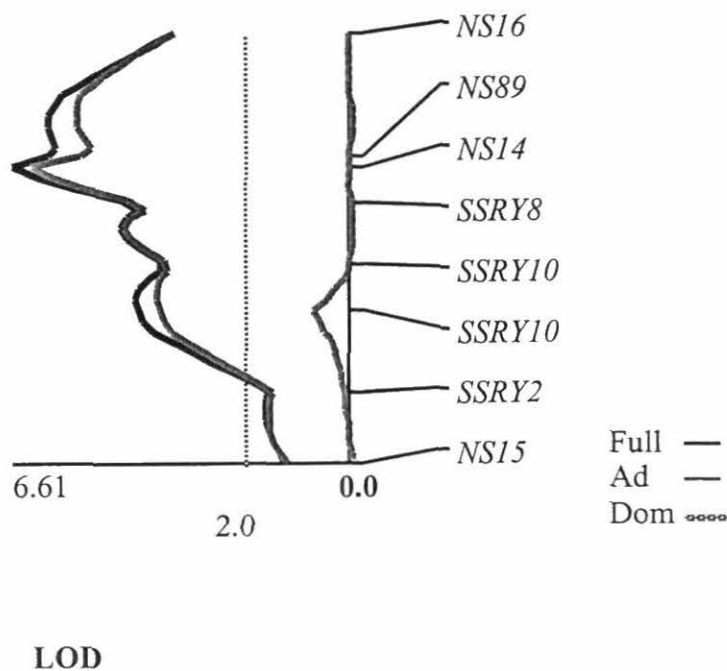
Figure 8.9. A first order QTL for harvest index detected by interval mapping.

Table 8.17. Putative QTLs that were common in the F_1 y F_2 mapping populations for fresh foliage, dry matter yield and harvest index

Trait	QTL F_1	QTL F_2
F.F	GP R (GY48)	GP 22 (SSRY47-SSRY62)*
DR	GP S (GY153-GY212)	GP 1 (NS911-NS847)
	GP G (GY6)	GP 3 (NS928-SSRY53)
	GP D (GY181-GY42)	GP 4 (NS 717-SSRY3)*
		(SSRY3-SSRY23)*
	GP J (K10)	GP 10 (SSRY5-SSRY229)*
	GP-L (CBB1; CDY131)	GP 18 (SSRY20-NS308)*
	GP UD (GY24)	GP 2 (NS149-SSRY83)
HI	GP E (NGY162)	GP 12 (NS74-NS319)*
	GP A (rBEST)	GP 20 (SSRY 314-NS82)*
	GP J (GY34)	GP 17 (SSRY182-SSRY148)

FF = fresh foilage yield; DR = Dry matter yield; HI = Harvest index. GP = Linkage group. The marker interval where the QTL was found is indicated in parenthesis.

Activity 8.10 QTL Mapping of Cyanogenic Potential (CNP) in Cassava

Collaborators: Elizabeth Kizito (Ph.D. Student, Swedish Agricultural University, SLU, Uppsala, Sweden) Dr Urban Gullberg (SLU, Uppsala, Sweden) Dr Thomas Egwang (Medical Biotech Laboratories MBL, Kampala, Uganda), Dr Anton Bua (NARO, Namulonge, Uganda), Martin Fregene (CIAT)
Funding: **BIOEARN, SAREC,Stockholm, Sweden**

Important Outputs
1. Establishment of a SSR marker lab at the Medical Biotech Laboratories (MBL) for the genotyping of segregating populations.
2. Generation and establishment of cyanogenic mapping populations adapted to Uganda, particularly the cassava mosaic disease (CMD).

Rationale
Cassava produces cyanogenic glucosides which is often rightly or wrongly seen as a health hazard to consumers, particularly in the very poor segment of the population that depend on cassava as a staple. Conventional breeding for low cyanogenic potential (CNP) is fraught with the confounding effect of the environment and developmental stage at which CNP is measured. A project has been initiated in Uganda to map the genes controlling cyanogenic potential (CNP) in cassava with funding from the Swedish project for Biotechnology and Biosafety Research Network in East Africa, (BIOEARN). The identification of molecular

marker associated with CNP will increase the efficiency and the cost-effectiveness of breeding low CNP cassava varieties for Uganda. The BIOEARN project is being conducted as a joint project between the MBL, Swedish University of Agricultural Sciences (SLU), Uppsala, NARO, Namulonge, and International Centre for Tropical Agriculture (CIAT). Activities in the project so far include the development of mapping populations and their establishment at National Agricultural Research Organization (NARO), Namulonge. In addition, facilities for simple sequence repeat (SSR) genotyping of the mapping population have been made available at the MBL. A meeting of partners under the project was planned for mid-may to

a) Resolve bottlenecks in the molecular marker activity
b) Appraise progress made so far and write a short report
c) Make recommendations on future activities

Methodology

Two key requirements for the genetic mapping of CNP are segregating populations with a simple pedigree and easily assayed molecular markers on a genome wide basis. For cassava two kinds of markers are currently available on a genome-wide basis: simple sequence repeat (SSR) markers and restriction fragment length polymorphism (RFLP) markers. The SSR are highly informative and polymerase-chain-reaction (PCR)-based markers making them most appropriate for genetic mapping. The RFLP markers are also informative, but tedious to use, they are therefore being converted to PCR-based markers known as single strand conformation polymorphism (SSCP) markers.

To implement the SSR marker technology at the MBL for genetic mapping of CNP, 8 SSR markers from the Cassava MapPairs were amplified by PCR in 7 parental genotypes, 2 grand parental and 5 parental genotypes, of some of the F_2 mapping progenies using a Perkin Elmer 9700 PCR machine. The PCR reaction was electrophoresed on 6% polyacrylamide gels and visualized by silver staining.

The original mapping population for the study was several F_2 families derived from selfing F_1 progeny from the Gomani x Mbundumali cross. Initial *in vitro* establishment of the sexual seeds from embryo axes was conducted at the tissue culture facility of the Namulonge station using 30 seeds from the F_2 family GMM13. Poor growth of the majority of the cultures was observed. It was then decided to continue establishment in pots filled with sterile soils. An issue raised with the use of the Gomani x Mbundumali F_2s is their poor adaptation to Uganda, particularly adaptation to the devastating cassava mosaic disease (CMD). It was therefore decided to create back-up F_2 families generated from local varieties. A very bitter local variety known as Tongolo was crossed to two CMD resistant varieties, SS4 and TME4. The F_1 progenies were planted directly in the field March 12 and are now 10 weeks old.

Results

The parental survey of parents of the mapping population with more than 500 SSR markers have begun. Results obtained so far reveal a good level of polymorphisms in the parents (Figure 8.10). A summary of families and number of plants expected are shown in Table 8.18. Not all families will be established due to their small sizes.

Table 8.18. Summary of the total number of seeds, viable seeds and expected plants from Gomani x Mbundumali F₂ families.

Code	Total No. of seeds	Total No. of viable seeds	Plants expected (80% germination)
GMM3	333	221	160
GMM96	266	173	138
GMM66	151	121	96

Table 8.19 summarizes the F1 and reciprocals of the Tongolo crosses currently growing in the field at NARO, Namulonge.

Table 8.19. Summary of the total number of plants from the Tongolo x SS4 and Tongolo x TME4 and their reciprocals.

Code	Cross	Number of plants
To be determined	Tongolo x SS4	170
To be determined	SS4 x Tongolo	73
To be determined	Tongolo x TME4	102
To be determined	TME4 x Tongolo	37

It is necessary to keep the field weed free and watered during the oncoming dry season that begins in June. The plants also will greatly benefit from some fertilizer application to ensure that woody stakes can be obtained by December when they will be cloned. At the moment, the crosses have not been given a code, neither have the individual plants been labelled, it is necessary that this be done within the next two months to avoid a mix-up later in the experiments.

Future Perspectives
1. Parental survey of Mbundumali, Gomani, Tongolo, SS4, TME 4 and selected progenies using SSR and SSCP markers. To be included are 5 parents and 7 progenies (a total of 12 samples) to be analyzed with all available markers
2. Harvest of two roots from the new CNP crosses at 7 months after planting (MAP), evaluation for CNP using the picrate method and generation of F2 families for mapping
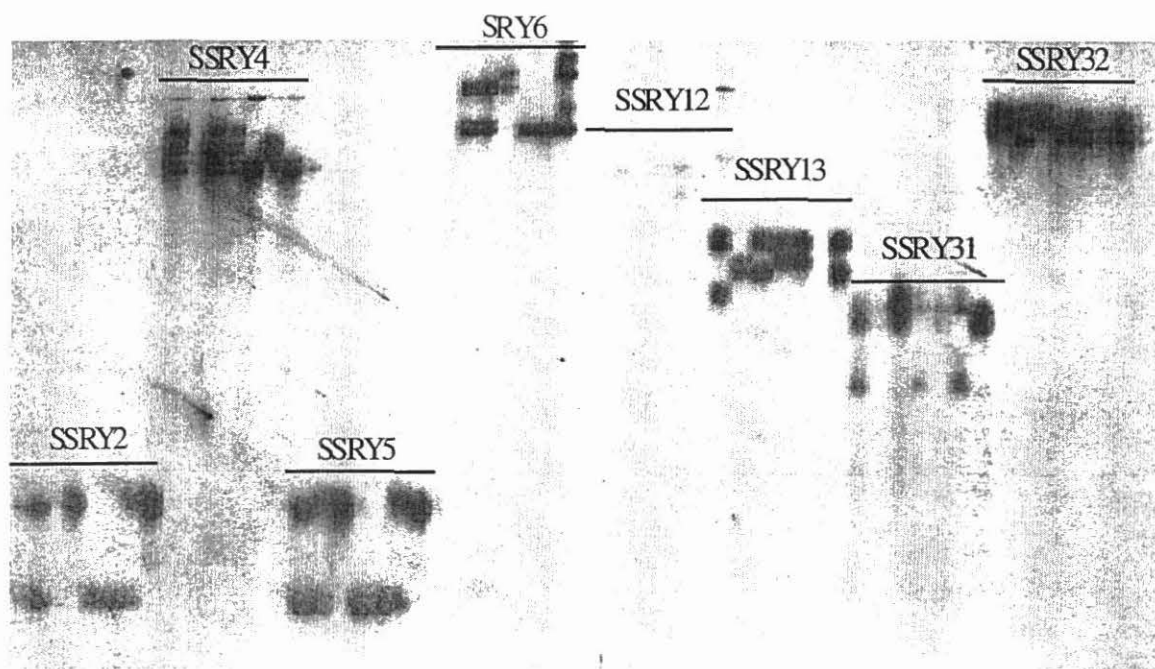
Fig 1. Polyacrylamide silver stained gel of 8 SSR markers and parental genotypes of the CNP mapping F2 populations. Orders of the parents are: Gomani, Mbundumali, GMM46, GMM66, GMM88, GMM91, and GMM96.

Activity 8.11 Gene Tagging of Beta-Carotene Content in Cassava

Collaborators: Nelson Morante, Teresa Sanchez, Alba Lucia Chavez, Hernan Ceballos, Martin Fregene (CIAT)
Funding: **CIAT**

Important Outputs
1. Identification of an ideal gene tagging population for beta-carotene
2. A scheme to increase content of beta-carotene by plant breeding and MAS.

Rationale
Occurrence of vitamin A deficiency and other nutritional problems overlaps with areas where cassava is an important staple food. Efforts have therefore been made to realize the potential

of cassava to improve the vitamin A consumption of people living in the tropical belt, where it is a predominant crop. Both roots and leaves of cassava contain considerable amounts of vitamin A precursors (β-carotene), 0.102 to 1.040 mg/100 g fresh roots and 12.05 to 96.42 mg/100 g fresh leaf weight (CIAT 2001). Several studies have highlighted the possibility of increasing available content of beta-carotene in both leaves and roots (Iglesias et al 1997, CIAT 2002, this report). Furthermore, the high β- carotene content is not often combined with high dry matter yield, resistance to pests and diseases and acceptable root quality. Crosses between selected sources of cassava with high nutritional quality and adapted local will need to be made.

Given the long growth cycle of cassava, improvement of a trait that is expressed only late in the growth cycle of the crop, such as β carotene, will benefit from marker-assisted selection. The inheritance of β- carotene in cassava has been demonstrated to be controlled by 2 genes (Iglesias et al. 1997). It is therefore a relatively simple trait to identify markers for. Molecular markers associated with β-carotene will allow for its selection early in the breeding cycle. We describe here discovery of a S_1 cross from the cassava land race MCol72 with a wide spectrum of segregation for beta-carotene from pure white to pink color. This family is appropriate for the development of markers for beta-carotene, the precursor of vitamin A.

Methodology
As part of an initiative to develop cassava populations tolerant to inbreeding, 14 genotypes commonly used as parents in the CIAT breeding were selfed and the seeds established at the experimental station of CENICAñA last year. This year, a clonal observation experiment of 10 plants per genotype, was set up at CIAT Palmira. At 10 months after planting the experiment was harvested. It was observed that in the S_1 family of 38 plants from the Colombian land race MCol72, a wide segregation was observed in root color from white to pink. The roots were scored qualitatively using the usual CIAT scale of 1 (white) to 8 (pink); pictures were also taken of a cross section of the root. Two genotypes with the deepest pink coloration were selected for quantitative determination of carotenes in the roots using high performance liquid chromatography. The parental genotype MCol72 has a root color that is normally described as cream colored or 4 on the CIAT scale.

The discovery of a wide segregation for root color in an S_1 cross from a cream colored variety provides an ideal population for bulk segregant analysis (BSA) of β carotene content and to identify markers associated with genes controlling the above trait. For DNA isolation, 1-2g of young leaves were harvested from all 38 genotypes into small paper envelopes and dried for 24h in an oven at 48°C. The dried leaves were ground using a power drill and washed sand and DNA was isolated from 200mg using a miniprep version of the Dellaporta (1983) protocol. Two bulks of 6-10 DNA sample from genotypes with pure white and pink roots respectively were created. DNA from the bulks and parent will be genotyped with the 500 cassava SSR markers; markers polymorphic in the bulks will be employed to analyze the entire population. Markers associated with yellow/pink color will be determined by a simple linear regression of phenotypic data on marker genotype marker class means (single point analysis) using the computer package Q-GENE 2.30B (Nelson, 1997). The amount of phenotypic variance explained by each marker will be considered significant if the probability of observing an R^2 value is less than 0.005.

Results

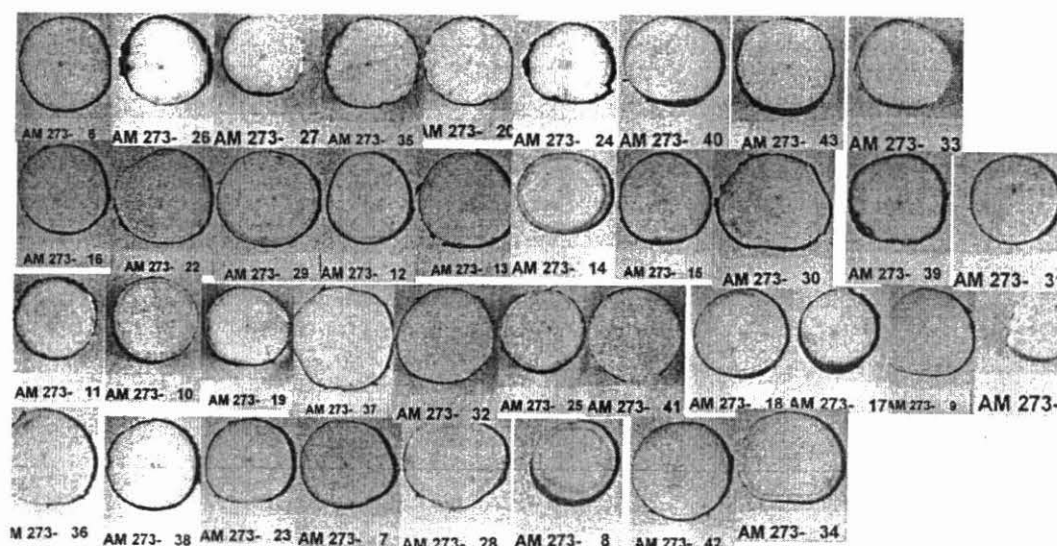The S_1 cross showed a wide spectrum of segregation for root color (Figure 8.11).

Figure 8.11. Cross section of root parenchyma of 38 genotypes of the S1 family derived from MCol72. A wide spectrum of variation in color can be seen from pure white to pink. Also to be noted is the pattern of deposition of beta-carotene.

The evaluation of two genotypes with pink roots revealed total beta-carotene content of 1.69mg/100g fresh weight (AM273-23) and 1.38100g fresh weight (AM273-7) respectively. The value obtained from AM273-23 is the highest to date from the characterization of the CIAT germplasm bank for beta-carotene. These results reveal the potential to increase beta-carotene in the roots. A series of experiments have therefore been planned to:

a) Generate a larger S_1 family from Mcol72 for gene tagging of beta-carotene.
b) Generate additional S_1 families from other cream, yellow and pink varieties and tag the genes involved
c) Determine if alleles of genes that control beta-carotene content from different varieties are complementary by making crosses between genotypes that carry them.

Genotypes for crosses to generate additional S_1 families can be observed in Table 8.20.

Table 8.20. Summary of genotypes established at CIAT Palmira this year to identify favorable alleles of genes controlling beta-carotene content.

Genotype		No. of plants
Cream or yellow varieties		
1	CM 507-34	10
2	CM 996- 6	10
3	SM 526- 3	10
4	MCOL 144	10
5	MCOL 721	10
6	MCOL 1530	10
7	MCOL 1721	10
8	MCOL 2435	10
9	MCR 54	10
10	MGUA 29	10
11	MPER 572	10
Deep yellow or pink varieties		
1	MCR87	10
2	MBRA337	10
3	COL 2199	10
4	MCOL 2318	10
5	MPER297	10

Future Perspectives

1. Bulk segregant analysis (BSA) of beta carotene content in cassava
2. Generation of additional S_1 families from other cream, yellow and pink varieties and BSA analysis to detect additional alleles that may be of a complementary nature

References

Iglesias, C., Mayer J., Chávez A.L., Calle, F., 1997. Genetic potential and stability of carotene content in cassava roots. Euphytica 94:367-373.

Activity 8.12 Production of Waxy Cassava Starch via the Down Regulation of GBSSI Gene.

Collaborators: Gina Jazbleidi, Paul Chavariagga, Chikelu Mba, Martin Fregene (CIAT)
Funding: Ministerio de Agricultura y Desarollo Rural de Colombia.

Important Outputs

1. Isolation of a complete cDNA clone of the GBSS gene from cassava and construction of a transformation cassette with the GBSS gene in the anti-sense orientation
2. Initiation of transformation activities

3. Training of a Colombian undergraduate project student, Gina Jazbleidi, in the tools and methodology of genetic transformation in cassava

Rationale
Higher incomes from cassava in marginal areas of the developing world where the crop is generally found requires the industrialization of the crop and the development of novel industrial products for cassava with the aid of modern biotechnology. There are several novel products that can be produced from cassava. They include modified starches, such as 100% amylopectin or 100% amylose starches, from the down regulation of the granule bound starch synthethase (GBSS) gene, or the starch branching enzyme (SBE) gene. The industrial applications of either pure amylopectin or pure amylose starches, such as the production of high value biodegradable polymers from pure amylose starches or the use of 100% amylopectin in thickeners, pastes, and glues, is a market with unlimited growth potential.

With funds from the Ministerio de Agricultura y Desarollo Rural of Colombia a project has been initiated to genetically engineer industrial varieties with an anti-sense construct of the GBSSI. The granule bound synthethase (GBSS), is the predominant starch synthase gene, and catalyses the conversion of ADP-glucose to amylose through the linkage of a ADP glucose to a pre-existing glucan chain. Anti-sense disruption of the GBSSI gene has been employed to create potato transformants with 70-100% amylopectin via the down-regulation of the GBSSI gene Salehuzzaman et. al. (1993).

Methodology
Isolation of a cassava GBSS cDNA clone
More than 87, 000 clones of a cassava root and leaf cDNA library cloned in the vector pCMV SPORT (GIBCO BRL inc. USA) has been gridded onto high density filters (Mba et al. 2000, unpublished data). The library was screened using a potato GBSS cDNA clones, a kind gift of Dr Christine Gebhardt, Max Planck Institute, Cologne, Germany. The potato GBSS gene was labeled with [^{32}P] dATP by random primer labelling and hybridized overnight to the cDNA filters according to standard protocols for Southern hybridization used in cassava (Fregene et al. 1997). The filters were washed 2 times with 2XSSC + 0.1%SDS for 5 minutes at 60°C and autoradiography was at –80°C using 2 intensifying screens.

Construction of transformation cassettes.
Primers were designed from published sequences of a full length cassava cDNAs of the GBSSI gene (Salehuzzaman et. al. 1993) that incorporate *BamHI* and *XbaI* restriction enzyme recognition sites to enable sub-cloning of the cDNA clone in the anti-sense orientation into the multiple cloning site (MCS) of the vector pRT101.). The primers were used to amplify the cDNA clone obtained above and the PCR product was cleaned using the QIAGEN PCR clean up kit (QIAGEN Inc., Los Angeles, California), digested with the appropriate enzymes. A 2.1kb *BamHI/XbaI* fragment was subcloned in the sense and anti-sense between the 35S promoter and the 35S poly adenylated terminator region of vector pRT101, a kind gift of Dr Ryohei Terauchi, Iwate Biotechnology Research Center, Kitakami, Japan. The 35S promoter, GBSS gene, in anti-sense orientation, and the termintor region were excised using the restriction enzyme Hind *III* , separated on a special gel, symergel (Diversified Biotech Inc., USA), eluted and cloned into the Hind *III* site of the binary vector pBIG101 having the GUS-intron and *nptII* reporter genes, a gift of Dr Richard Sayre, Ohio State University, Columbus Ohio.

Genetic Transformation

Genetic transformation was by particle bombardment and *Agrobacterium* transformation of friable embryo callus (FEC) cultures. About 20µg of the pBIG101 constructs plasmid was coated onto gold particles and used in the helium gun bombardment of new FEC suspensions of the model variety for cassava transformation TMS60444 according to standard protocols established for cassava at CIAT (CIAT2001). For *Agrobacterium* transformation, the pBIG101 construct was transformed into strain EHA105 according by electroporation and transformed clones were selected on LB media plates plus Kanamycin (50ug/ml final). After 2 days, white colonies were picked and incubated in 10ml LB + Kanamycin (50ug/ml final) for another two days at 28°C. Bacteria was collected by centrifugation at 3000rpm for 20 min in a table top centrifuge and re-suspended in 500ul of solution containing 10mM MgCl2, 1omM MES, and 100uM Acetosyringone. Cassava FEC was co-cultured with the *Agrobacterium* suspension according to standard protocols at CIAT (CIAT 2001)

Results

Three GBSS cDNA clone obtained from screening the cassava library were sequenced and one clone was found to be a complete cDNA clone. The cDNA clone has the ATG start codon 81 base pairs down stream from the beginning of the cDNA sequence and a stop codon about 100 base pairs from the poly A tail. PCR amplification with the designed primers yielded a fragment about 2.1kb in size that corresponds to the full length GBSS cDNA clone (Figure 8.12).
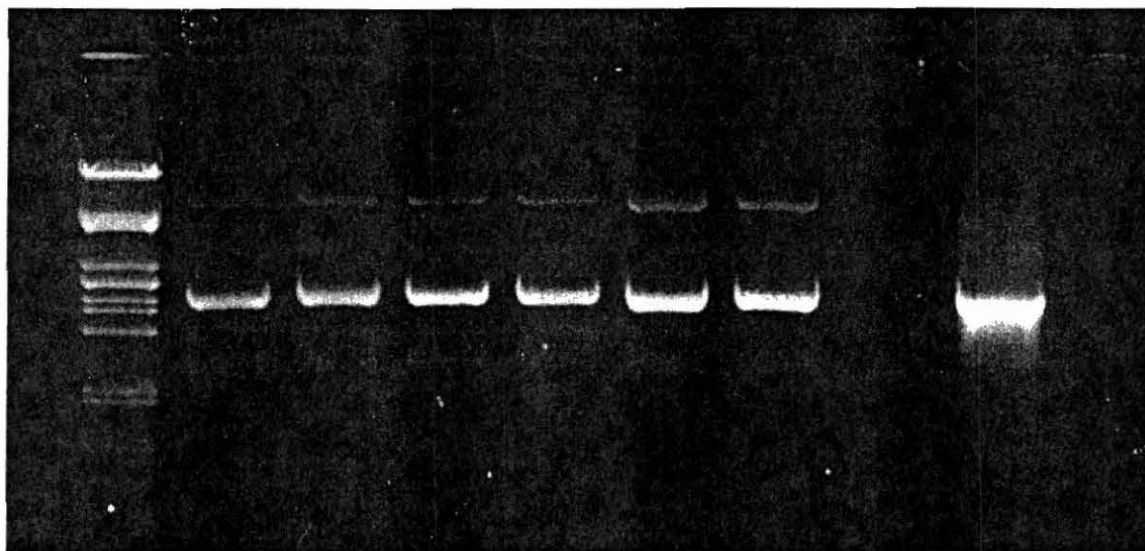
Figure 8.12.   PCR amplification of the GBSS cDNA clone using primers designed to introduce restriction enzyme sites at the ends of the gene. The first lane by the right is molecular weight marker Lambda DNA digested with *HindIII* , the next six lanes are PCR amplification of the GBSS gene, the last lane is a control, PCR product of the GBSS potato gene.

The resulting PCR fragment was digested with *BamHI* and *XbaI* restriction enzyme digestion and cloned into the MCS of pRT101. The GBSS gene, promoter and terminator sequences were excised with *Hind III*, and the two resulting fragments of sizes 2.7 and 2.6 kb were separated by electrophoresis (Figure 8.13). The bigger fragment was eluted and cloned into the *HindIII site* of pBIG101. This is the construct that was used in the particle gun and *Agrobacterium* mediated transformation.
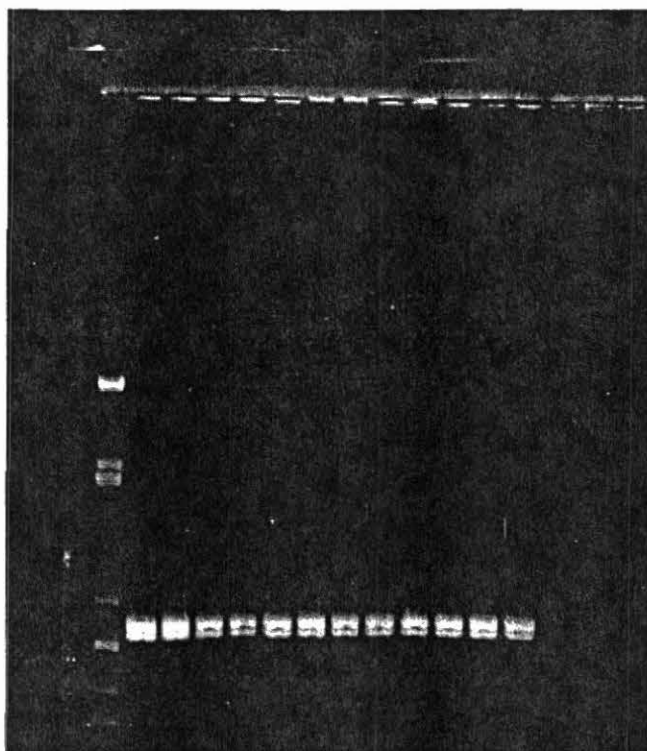

Figure 8.13. *HindIII* digested pRT101 plasmid containing the cassava GBSS gene in anti-sense orientation. The fragments of about 2.7kb and 2.6kb in size respectively represent the GBSS gene flanked by the 35S promoter and the polyadenylated terminator sequence and the rest of the pRT101 plasmid.

The transformation experiments are ongoing and conclusive results of reporter gene assays are expected at the end of December. Once the transformed calli has been revealed to have stable incorporation of the construct, regeneration of the transgenic calli will be initiated.

Future Perspectives

1. Agrobacterium transformation and regeneration of the industrial cassava variety "Reina" with the anti-sense construct of GBSSI cloned in pBIG101.

References

CIAT, (2001). Annual Report Project SB2, Assessing and Utilizing Agrobiodiversity through Biotechnology, CIAT, Cali, Colombia, pp 202-205.

Fregene M, Angel F, Gomez R, Rodriguez F, Chavarriaga P, Roca W, Tohme J, Bonierbale M (1997) A molecular genetic map of cassava (*Manihot esculenta Crantz*). Theor Appl Genet 95: 431-441

Salehuzzaman, S.N.I.M, Jacobsen, E., and Visser, R.G. F. (1993) Isolation and characterization of a cDNA-encoding granule-bound starch synthase in cassava (Manihot esculenta Crantz) and its antisense expression in potato. Plant Mol. Biol. 23:947-962.

8.13 Transient Transformation Assay of a Cassava Mosaic Disease (CMD) Candidate Resistance Gene Differentially Expressed During Host Plant Disease Resistance Response

Collaborators: Dr Ryohei Terauchi, Dr Hiromasa Saitoh, Ms Shizuko Fujisawa (IBRC, Kitakami, Japan), Janneth Patricia Gutierrez, Martin Fregene (CIAT)
Funding: The Rockefeller Foundation.

Important Outputs

1. Establishment of a system for the transient assay of foreign genes (luciferase) in cassava using a biolistic method.
2. Biolistic- inoculation of a CMD resistant and susceptible cassava genotypes with infectious virus DNA clones.
3. Co-transformation of a CMD susceptible genotype with a full length beta-tubulin gene and infectious virus DNA clones
4. Agro-inoculation (infiltration) of *N. tabaccum* and *N. benthamiania* with infectious virus clones and with a full length beta-tubulin gene.

Rationale

High levels of resistance to the cassava mosaic disease (CMD), the crop's most important production constraint in Africa, is mediated by a single dominant genes designated *CMD2*. The serial analysis of gene expression (SAGE) has been employed to identify many genes differentially expressed in resistant genotypes in response to heavy disease pressure of the African Cassava Mosaic Virus (ACMV) and the East African Cassava Mosaic Virus. The most differentially expressed gene was a beta-tubulin gene found to be expressed 12 times in CMD resistant genotypes compared to susceptible genotypes. Tubulin are the building block of

microtubules the main component of celluar cytoskeleton and they have been implicated in cell-to-cell progression and cytoplasm-to-nucleus movement of viruses.

To further understand the role beta-tubulin plays in the molecular basis of resistance, an experiment was designed to transiently co-transform a CMD susceptible cassava genotype and other host of the virus, for example *Nicotiana benthamiana* and *Nicotiana tabacum*, with infectious viral clones and the beta-tubulin gene. Infectious DNA clones of two strains of the virus were obtained from Drs John Stanley and Rob Briddon, John Innes Center, Norwich UK, and a license to work with them was obtained by the Iwate Biotech Research Center (IBRC). Method of transient transformation was by agro-infiltration, *N. benthamiana* and *N. tabaccum*, or biolistic inoculation, cassava. A CMD susceptible cassava variety, TMS30555, and the cassava land race that is the original source of CMD2, the single dominant CMD resistance gene, TME3, were used for the experiments.

Methodology
Plant materials were the CMD resistant variety TME3 and the susceptible variety TMS30555. Both genotypes were obtained as tissue culture plantlets from IITA and transferred to pots with soil for the experiments. A full length beta-tubulin cDNA clone was PCR amplified using primers that contain the recognition site for the appropriate restriction enzymes and cloned into PRT101 (35S promoter), for biolistic inoculation, or into the XVE inducible binary expression vector or into the binary vector pBIN m-gfp-ER, for agro-inoculation. The PCR fragment was cleaned and digested with the appropriate enzyme and eluted from a 1.5 % agarose gel. The purified fragment was then cloned into appropriate vector and transformed by electroporation into *E.Coli* strain HB101. A 1:50 dilution of plasmid preparations from an overnight culture of a single *E.Coli* colony was analyzed by PCR, using the original beta-tubulin primers, to confirm success of the cloning experiment. To that ensure that a full length beta-tubulin protein will be expressed in the transient assay, 3 clones from each cloning experiment was sequenced with 4 primers that covers the entire length of the gene.

Infectious DNA clones from two virus strains were employed in the transient assay experiment, partial repeats of the A and B genome of the Sri-Lanka Cassava Mosaic Virus (SLCMV A and SLCMVB) cloned into the binary vector pBIN PLUS, and full length clones of the African Cassava Mosaic Virus (ACMV) A and B genome cloned into pUC19 (ACMV A and ACMVB). Infectivity of cassava by virus partial repeats in a binary vector clones and agro-inoculation has not been demonstrated, therefore all agro-inoculation experiments were with *N. benthamiana* and *N. tabaccum*. But cassava has been successfully infected by biolistics, therefore the biolistic experiments were with cassava and *N. tabaccum*, as control.

A preliminary biolistic experiment was conducted to standardize the conditions for the BIO-RAD particle gun bombardment and to ensure expression of the beta-tubulin gene cloned into PRT101. Leaf discs of about 5cm^2 from the cassava genotype TME3 and *N. tabaccum* were used for the preliminary experiment. There were three treatments: bombardment with the PRT101 plasmid containing the luciferase gene alone, the luciferase construct combined with a PRT101 plasmid in which the luciferase gene has been replaced with a beta-tubulin gene having the 11bp truncation and the non-truncated beta-tubulin gene. Three leaf discs were used for each treatment and per crop. Particle gun bombardment was according to standard procedures established at the IBRC (Terauchi, 2002, personal communication). One 1 month-old live plant each was included for both cassava and *N. tabaccum* in the luciferase treatment as control. Leaf discs were bombarded once with 2.5ug of DNA from the

respective clones, while whole plants were bombarded 3 times. After bombardment, leaves were placed on water in a petri dish an incubated at 25°C for two days.

Two days later, total proteins were isolated from leaf discs transiently transformed with the beta-tubulin gen, to confirm the expression of the beta-tubulin gene using standard procedures established at the IBRC (Saitoh, 2002 personal communication). Exactly 3.6ug of total protein lysate from all leaf discs from treatments 2 and 3, and 2 non-bombarded controls were separated on a SDS-PAGE gel. Western blot analysis, using an anti-body raised to purified rat brain beta-tubulin, was carried out as described in the manufacturer's product sheet (SIGMA Inc, St. Louis, USA).

Following the preliminary experiment, a biolistic experiment was designed to inoculate the CMD resistance genotype TME3 and the CMD susceptible variety TMS30555 with the ACMV A and B infectious clones. A second treatment was co-bombardment of the ACMV clones and the beta-tubulin gene into the CMD susceptible genotype TMS30555. Three one-month old plants of TME 3 were used in the first treatment, while 2 one-month old plants of TMS30555 were each used in the first and second treatments. Plants were bombarded twice with 2.5ug of DNA from the respective clones as described earlier. The inoculated plants were transferred to a virus containment area in a secure green house.

For the agro-inoculation by infiltration experiment, the SLCMV A and SLCMV B clones were transformed into agro-bacterium strains MOG and EHA105. Similarly the beta-tubulin gene in the binary vectors XVE and pBIN m-gfp-ER respectively was transformed into agro-bacterium strains MOG and EHA105. Agro-infiltration was according to standard methods established at the IBRC (Saitoh 2002, personal communication). There were 4 treatments: the virus alone (a mixture of SLCMV A and SLCMV B), the virus and the beta-tubulin gene (in XVE), the beta-tubulin gene alone, and a dilution of the beta-tubulin gene to reflect the concentration in the mixture with viral clones. Three plants of *N. benthamiana* and *N. tabaccum* each were used per treatment. Agro-infiltrated plants were transferred to a virus containment area in a secure green house. After two days, the beta-tubulin gene will be induced by treatment of agro-infiltrated plants with the animal steriod, estradiol. Plant tissue will be collected from all plants and stored for Western analysis of gene expression using a conjugated mouse beta-tubulin antibody or antibodies raised to the coat protein of ACMV. Agro-inoculation of cassava could not be achieved due to poor infiltration, cassava leaves are covered with a thick cuticle.

Results
The full length of the beta-tubulin gene is 1667bp, the translated portion of the gene corresponds to 1341bp or 447 amino acid motifs. The beta tubulin protein showed more than 80% homology with similar genes from rice and arabidopsis. Comparison of the complete sequence of the beta-tubulin gene with ESTs generated for tag annotation during the SAGE experiment revealed two beta-tubulin molecules, one transcript had an 11bp truncation in the 3' untranslated end of the cDNA molecule. This molecule was also the most abundant from the EST project, 4 as against 1 of the non-truncated. It is not clear if this molecule plays any role in the over-expression of beta-tubulin, therefore the molecules were separated in at least one of the transient assay experiments, the biolistic inoculation.

Results of the biolistic experiment to standardize the conditions for the BIO-RAD particle gun bombardment for expression of the beta-tubulin gene particle gun revealed good expression

of the full length beta-tubulin gene in all leaf disc by western blot (Fig 1). Similar results were also obtained with non-bombarded controls suggesting the detection of endogenous beta-tubulin.   There is therefore a need to separate the confounding influence of endogenous tubulin and transient expression via the use of a non-plant secondary antibody fused to the tubulin protein. The luciferase assay was conducted on leaf discs from all treatment using a rapid enzyme substrate assay and a flourescence reader according to standard methods in use at the IBRC (Terauchi et al. 2002, personal communication). Results revealed good enzyme activity on the average for all samples, although samples from the co-inoculation (luciferase and beta-tubulin) had a 5 to 10 magnitude reduction. This may be an effect of the quantity of leaf samples, as more than half of the leaf disc had already been used for the Western blot analysis. The luciferase activity of the bombarded cassava and *N. tabaccum* whole plants were the highest for all samples.

The western blot analysis of the tobacco plants agro-infiltrated with the infectious virus clones or beta-tubulin gene or both remain to be carried out. The plant tissue is stored at – 80oC pending a trip to Japan to conclude the above studies.   ⸰

Future Perspectives
1. Western blots of agro-bacterium and particle gun infected plans to assess expression of the beta-tubulin gene and the infectious viral clones
2. Additional co-transformation experiments to better synchronize expression of the beta-tubulin gene and infection by viral clones.

8.14 Progress Towards a PCR-Marker Based Map of Cassava

Collaborators: **Angela Zarate, Edgar Barrera, Martin Fregene (CIAT)**
Funding: **CIAT**

Important Outputs
1. Implementation of the SSCP technique for the analysis of sequence tagged sites (STS) in cassava
2. Design of primers for another 141 SSR markers for cassava

Rationale
Progress towards a PCR-based map of cassava for gene tagging and marker-assisted selection (MAS) has crossed a significant mile stone with the report of a SSR only map of cassava (CIAT 2002, this report). However, 20% of SSR markers evaluated remained unlinked and denotes that the map is not complete and more markers need to be developed. At the same time, saturation of the RFLP genetic map of cassava has continued with 57 SSR markers from genomic sequences (Zarate et al 2002, unpublished data) and another 45 SSR markers from cDNA sequences (Garcia et al 2002, unpublished data) added to the map.

To ensure that a PCR-based map of cassava can be achieved within the near future efforts have been geared this year to converting RFLP markers on the genetic map of cassava to single sequence conformation polymorphism (SSCPs). Furthermore, additional SSR markers

have been designed from cassava genomic sequences reported in GeneBank, with particular reference to BAC end sequences developed at the Clemson University Genome Institute (CUGI).

Methodology

More than 100 RFLPs from the molecular genetic map of cassava have been sequenced. Primers were designed from 2 RFLP clones, CYP79D1 and CYP79D2 and used to amplify total genomic DNA from the two parents of the mapping population and a sub-set of 20 progenies. PCR amplifications were carried out in 12.5-μl reactions containing 100 ng of DNA, 0.5μM of each primer, 10 X of *Taq* polymerase buffer (500mM KCl, 100mM Tris-HCl (pH 8.5), and 1 mg/ml gelatin), 2mM of $MgCl_2$, 0.5mM of dNTPs and 0.25 U of *Taq* polymerase. The PCR profile was: 94°C for 2 min, followed by 30cycles of 95°C for 1 min, 55 °C for 2 min and 72 °C for 2min. A final extension step of 72 °C for 10 min was added at the end. The PCR amplification was cleaned with the QIAGEN PCR clean-up kit (QIAGEN Inc? Los Angeles, CA) and digested to completion with 10U of Hinf I restriction enzyme for 3h at 37 °C according to the manufacturer's instruction (New England Biolabs, Cambridge, MA). The digestion product was electrophoresed on 6% polyacrylamide-MDE gels (Cambrex Bio Science Inc, Baltimore, MD) made up of 10% glycerol, 6ml 10XTBE, 25ml MDE gel solution, 65ul TEMED, 500ul APS, and 60ml deionized water, at 40Watts for 48h. The gel was stained as described by Slaubaugh et al (1997). Briefly, the gel was fixed for 3 min in 10% ethanol, 0.5% acetic acid, stained for 5 min in fixing solution plus 0.2% silver nitrate , washed in water for 1 min and developed for approximately 10-20 min in 3% NaOH and 0.27 % formaldehyde in water. Following staining, the gel was fixed for a further 5 min and washed in water.

The construction of a bacterial artificial chromosome (BAC) library from the white fly resistance variety MOLC72 has been described earlier (Tomkins et al 2001). A total of 2301 BAC ends were sequenced and 1755 good sequences were deposited in gene bank (Tomkins et al 2002. Unpublished data). The BAC end sequences were downloaded from GeneBank and the following SSR motifs: (AT), (GA), (CA), (GC), (GT), (TCT), (GCT), (AGC), (GCC), (GGT), (ATT), (GGA), (TATG), and (GTGA) were searched for in the sequences using the DNAMAN software. The local BLAST facility at CIAT (http://gene2/BLAST/inicio.htm) was used to compare the sequences with each other to eliminate duplicated sequences. Primer design was using Primer 3.0, the primer picking software found at http://waldo.wi.mit.edu/cgi-bin/primer/primer3.

Results

SSCP analysis of the parents of the cassava mapping population and a sub-set of 20 genotypes of the same population revealed polymorphisms that segregated as single dose restriction fragment (SDRF) polymorphism. The entire mapping population is being analyzed at the moment. Primers will be designed from other sequenced RFLP markers that are currently on the map to also convert them to PCR-based markers. A draw back of the SSCP methodology is the need to always purchase costly MDE gel solutions (Cambrex Bio Science Inc, Baltimore, MD). Efforts are also being made to try normal polyacrylamide gel solutions in an effort to reduce costs. The sequenced RFLP markers being converted to SSCP markers can also be described as sequence tagged sites (STSs). These have the advantage that once they have been analyzed in any population the map location as well as the sequence is known.

A total of 141 BAC end sequences were found to contain SSR motifs. The distribution of SSR sequences was as follows: 67 dinucleotide, 46 trinucleotides, 4 tetranucleotide, and 24 other (mixture of different repeats) motifs, a mixture of two or three motifs (Table 8.21). Average length of the dinucleotide repeats was 8 repeats for the dinuleotide, 6 for the tri and 4 for the tetras. Primers designed will shortly be sent for synthesis and once available they will be placed on the existing map of cassava beginning with the parental survey.

Table 8.21. Di, tri, tetra nucleotide, and other SSR repeats found in the BAC end sequences.

Type of SSR	Number	Percentage
AT	38	27
GA	24	17
CA	4	3
GC	1	0.7
TCT	11	8
GCT	3	2
AGC	3	2
GGT	4	2
ATT	12	8
GGA	13	9
TATG	4	2
Others	24	17
Total	141	

Future perspectives
1. Continue the SSCP analysis for the genetic mapping of STSs in cassava
2. Parental survey of the additional 141 primers and mapping of polymorphic ones.

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Activity 8.15 Development of Populations Tolerant to Inbreeding Depression in Cassava

Collaborators: Nelson Morante, Teresa Sanchez, Hernan Ceballos, Martin Fregene (CIAT)
Funding: CIAT

Important Outputs
1. Development of populations tolerant to inbreeding depression.
2. Generation of S_2 families from 6 elite cassava genotypes

Rationale
Cassava is an allogamous tetraploid that accumulates a significant genetic load that is released on inbreeding. The average yield of selfed lines was observed to be about half the average yield of parental genotypes and the degree of inbreeding varied greatly amongst different genotypes (Kawano et al. 1978). Inbreeding depression primarily affects the general growth and vigor of the plant, reflected directly in lower root yield, and therefore no inbreeding is carried out at any stage of traditional cassava breeding. Cassava breeders in general strive to select progenitors and make crosses that maximize heterozygosity and minimize inbreeding

But inbreeding is desirable for cassava for the reduction of the genetic load currently carried by many elite clones which in turn permit the use of valuable breeding schemes such as back-cross breeding. But more importantly inbreeding is important because it eliminates the confounding effect of dominance process and maximizes the additive gene variance on selection (Ceballos et al 2002). Inbred lines also have the advantage that they can be shipped and stored as botanical seed, facilitating the exchange of germplasm which, at the moment can only be done via expensive tissue culture shipments. A selection program was therefore set-up for tolerance to inbreeding in 1999, at the moment more than 300 S_1 lines have been produced from 5 elite clones and S_2 lines were produced this year (see net section of this report). We describe here evaluation of the S_1 families

Methodology
Fourteen cassava genotypes were chosen for the development of populations tolerant to inbreeding. The genotypes were chosen due to their good general combining ability performance for yield, dry matter yield or root quality. They include the following lines: MCO122, CM523-7, MCOL1684, MBRA12, MCOL2060, MVEN77, MCOL1522, MTAI1, MPAN51, MECU169, MCOL1468, MCOL72, CM849-1, HMC1. More than 300 pollinations were made per genotype and between 30-150 seeds were obtained per genotype. The seeds were planted at the CENICAñA experimental station October 2000.

At 10 months after planting, harvested August 2001, all plants were harvested and measured for fresh root yield, dry matter content, foliage weight, harvest index, culinary quality, starch content/quality, and frog skin disease according to standard CIAT procedures. Six families were selected to continue the process of inbreeding based on their average yields and flowering. The families were also planted in an observational trial, six plants in single row replication, in October 2001 at CIAT Palmira and harvested in May 2002. The traits described

above were evaluated for all plants.

Results

Inbreeding depression was severe in many of the genotypes, particularly in MBRA12, MCOL1684, MCOL1468 and MCOL1522, where inbreeding depression of more than 70% was observed. Very poor vigor and sufficient stakes could not be obtained for further experiments and were therefore eliminated. Six families showed better tolerance to inbreeding depression as observed from their yield data (Table 8.22), highlighting the great differences in cassava to inbreeding depression. Maximum yield of these families were on an average of 18 tons/ha of fresh root with the exception of family AM312 that had 35t/ha, this family also had the largest standard deviation. Average root yield was less than half of the average yield of the parents which is in agreement with earlier observations of inbreeding depression in cassava. The 2002 experiment, the clonal observation experiment agreed quite closely with data from the seedling trial (data not shown). Distribution of dry matter content and harvest index from the clonal observation revealed normal distribution of the traits in all families (Figure 8.23.

Table 8.22. Summary of fresh root yield from a seedling trial of S_1 families conducted at the CENICAñA experimental station in 2001.

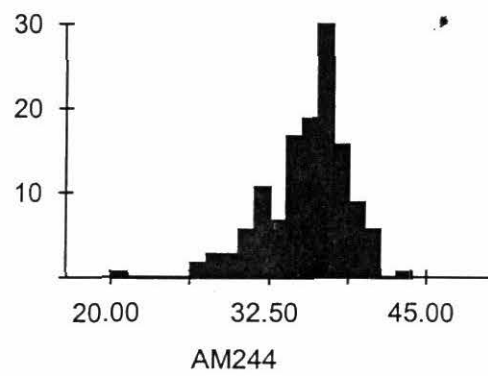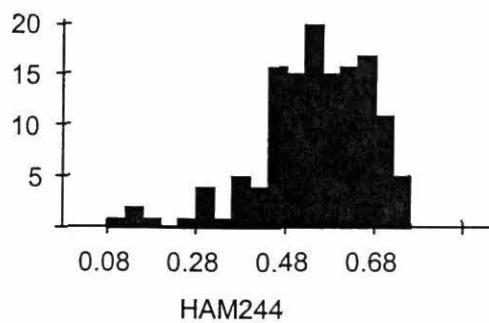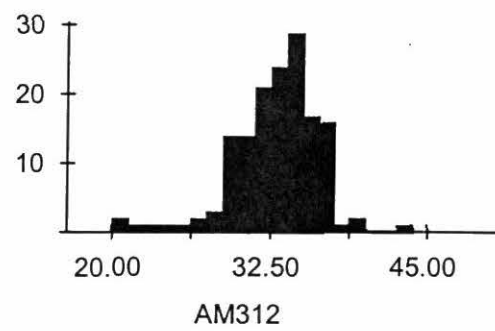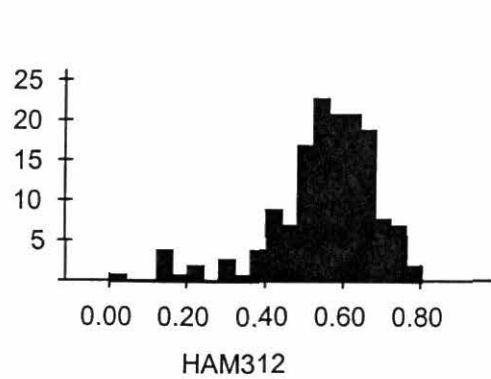| Family name	Parent	Maximum fresh root yield t/ha	Minimum fresh root yield t/ha	Average root yield t/ha	Standard deviation
AM244	MCOL1505	18.5	0.00	7.58	5.46
AM266	HMC-1	18.5	0.00	8.31	4.17
AM273	MCOL72	14.7	0.00	7.0	4.38
AM277	MTAI1	18.0	0.00	6.64	5.96
AM278	MVEN77	15.0	0.00	3.86	5.38
AM312	CM849-1	35.5	0.00	6.04	6.04
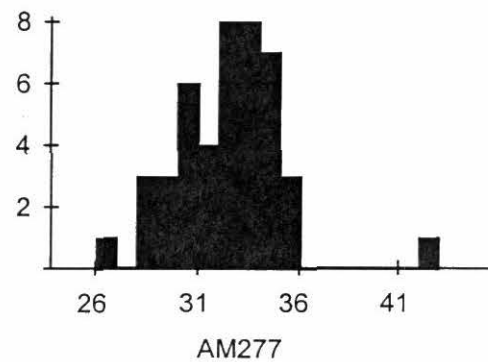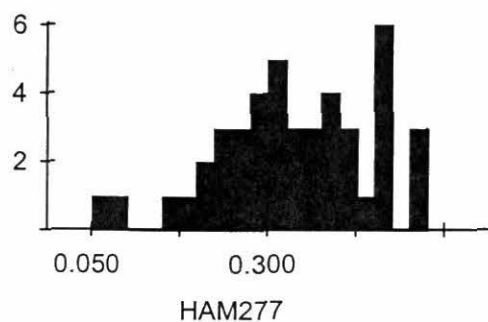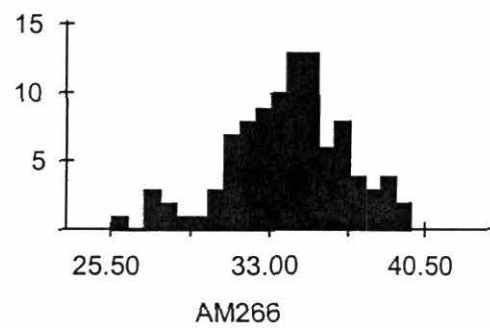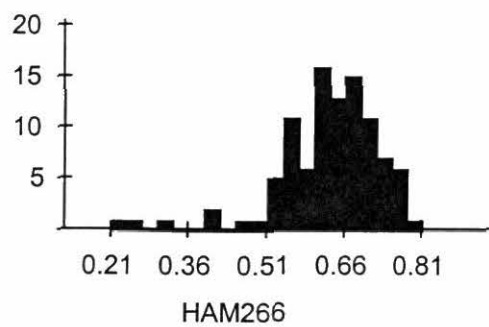
Figure 8.14. Distribution of harvest index (prefix H-) and dry matter content in 4 S_1 families

Table 8.23 Best 25 genotypes for dry matter yield and their yield components in six S_1 families.

Genotype	Parent	FY t/ha	HI	% DMC	DMY t/ha	Starch	Taste
AM 266- 108	HMC 1	98.00	0.66	33.58	32.91	1	1
AM 266- 103	HMC 1	91.20	0.69	35.73	32.59	1	1
AM 312- 18	CM 849- 1	78.25	0.56	37.32	29.20	2	3
AM 266- 21	HMC 1	77.88	0.63	38.68	30.12	1	1
AM 244- 79	MCOL 1505	75.17	0.54	37.14	27.92	2	3
AM 312- 15	CM 849- 1	72.75	0.65	35.57	25.88	2	2
AM 266- 58	HMC 1	72.08	0.53	33.34	24.03	1	2
AM 266- 24	HMC 1	71.63	0.68	37.66	26.97	1	1
AM 312- 103	CM 849- 1	68.17	0.63	37.14	25.32	2	5
AM 266- 18	HMC 1	66.80	0.60	36.40	24.31	1	1
AM 312- 32	CM 849- 1	66.17	0.62	37.48	24.80	1	2
AM 266- 113	HMC 1	65.83	0.64	34.84	22.93	1	1
AM 312- 130	CM 849- 1	62.83	0.67	34.08	21.41	3	4
AM 312- 92	CM 849- 1	60.42	0.54	36.53	22.07	3	5
AM 266- 82	HMC 1	60.00	0.64	39.47	23.68	1	1
AM 244- 39	MCOL 1505	58.75	0.69	40.03	23.52	2	2
AM 312- 49	CM 849- 1	58.38	0.73	32.10	18.74	3	3
AM 266- 87	HMC 1	58.20	0.71	35.55	20.69	1	1
AM 312- 138	CM 849- 1	58.17	0.58	30.55	17.77	2	5
AM 266- 31	HMC 1	58.13	0.62	37.00	21.51	1	1
AM 244- 53	MCOL 1505	58.10	0.58	33.76	19.62	1	3
AM 244- 129	MCOL 1505	58.00	0.71	31.64	18.35	2	2
AM 277- 29	MTAI 1	57.83	0.45	34.40	19.89	4	4
AM 266- 19	HMC 1	57.50	0.76	33.51	19.27	2	2
AM 266- 17	HMC 1	57.33	0.61	36.68	21.03	1	2
Statistics of best 25 inter-specific hybrids evaluated							
Maximum		98.00	0.76	40.03	32.91	1.00	1.00
Minimum		57.33	20.03	30.55	17.77	4.00	5.00
Average		66.70	0.63	35.61	23.78	1.68	2.32
Standard Dev.		10.90	0.07	2.41	4.29	0.85	1.38
Statistics of 343 inter-specific hybrids evaluated							
Maximum		98.00	0.80	43.69	32.90	1.00	1.00
Minimum		0.50	0.03	18.69	0.00	5.00	5.00
Average		27.37	0.53	33.42	9.45	1.96	2.78
Standard Dev.		16.68	0.15	3.63	6.14	1.08	1.35

FY: Fresh root yield; DMY: Dry matter yield; HI: Harvest Index; DMC: Dry matter content

Table 8.24. Best 25 genotypes for dry matter content in six S_1 families.

Genotype	Parent	FY t/ha	HI	% DMC	DMY t/ha	Starch	Taste
AM 312- 140	CM 849- 1	32.75	0.56	43.69	14.31	3	5
AM 244- 95	MCOL 1505	34.75	0.68	42.93	14.92	1	2
AM 277- 44	MTAI 1	37.08	0.32	42.07	15.60	5	5
AM 244- 146	MCOL 1505	37.20	0.51	41.01	15.25	1	2
AM 244- 82	MCOL 1505	25.75	0.63	40.84	10.52	1	2
AM 244- 164	MCOL 1505	36.42	0.58	40.73	14.83	1	1
AM 244- 81	MCOL 1505	45.17	0.46	40.22	18.16	1	2
AM 244- 101	MCOL 1505	39.00	0.50	40.08	15.63	1	2
AM 244- 39	MCOL 1505	58.75	0.69	40.03	23.52	2	2
AM 244- 33	MCOL 1505	52.25	0.64	39.68	20.73	1	1
AM 312- 17	CM 849- 1	54.50	0.65	39.62	21.59	2	5
AM 244- 133	MCOL 1505	22.75	0.59	39.61	9.01	1	1
AM 266- 82	HMC 1	60.00	0.64	39.47	23.68	1	1
AM 244- 62	MCOL 1505	21.92	0.46	39.47	8.65	1	2
AM 244- 154	MCOL 1505	48.08	0.52	39.43	18.96	1	2
AM 312- 33	CM 849- 1	42.42	0.66	39.33	16.68	1	2
AM 244- 75	MCOL 1505	49.67	0.62	39.21	19.48	2	2
AM 244- 56	MCOL 1505	50.00	0.46	39.16	19.58	1	2
AM 244- 96	MCOL 1505	44.40	0.58	39.12	17.37	5	5
AM 266- 92	HMC 1	39.50	0.70	39.02	15.41	1	1
AM 244- 98	MCOL 1505	28.17	0.62	38.95	10.97	1	2
AM 244- 88	MCOL 1505	30.08	0.50	38.84	11.68	1	3
AM 266- 21	HMC 1	77.88	0.63	38.68	30.12	1	1
AM 266- 64	HMC 1	35.33	0.61	38.65	13.66	1	2
AM 244- 124	MCOL 1505	29.92	0.58	38.64	11.56	1	1
Statistics of best 25 inter-specific hybrids evaluated							
Maximum		77.88	0.70	43.69	30.12	1.00	1.00
Minimum		21.92	0.32	38.64	8.65	5.00	5.00
Average		41.35	0.57	39.94	16.48	1.52	2.24
Standard Dev.		13.09	0.09	1.31	5.05	1.16	1.33
Statistics of 343 inter-specific hybrids evaluated							
Maximum		98.00	0.80	43.69	32.90	1.00	1.00
Minimum		0.50	0.03	18.69	0.00	5.00	5.00
Average		27.37	0.53	33.42	9.45	1.96	2.78
Standard Dev.		16.68	0.15	3.63	6.14	1.08	1.35

FY: Fresh yield; DMY: Dry matter yield; HI: Harvest Index; DMC: Dry matter content

Two families, AM266 and AM312 account for 83% of the best 25 genotypes for fresh yield in the clonal observation trial (Tables 8.23 and 8.24). A genotype from AM266 had maximum yield of 98 t/ha, this same family produced the genotype with the highest yield in the seedling trial albeit different genotypes, underscoring the big error that can occur when evaluation is based on a single plant and the effects of inter-genotypic competition. The family AM312 accounted for 67% of the best 25 genotypes for dry matter content. The trend

observed in the data from the second year evaluation that individuals from certain S_1 families tend to dominate the group of best genotypes for certain traits highlights the important role additive genes play in the expression of these traits.

Future Perspectives
1. Establishment and evaluation of S_2 progenies

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Activity 8.16. *Culture of Embryo Axes of Immature and Mature Sexual Seeds for the Establishment of Genetic Stocks and Breeding Populations*

Collaborators: Luis Guillermo Montes, Nelson Morante, Martin Fregene
Funding: The Rockefeller Foundation

Important Outputs
1. Recovery of S_2 plants from immature seeds by embryo culture
2. Optimization of protocols for embryo culture of mature and immature seeds.

Rationale
To safeguard some of the very valuable cassava genotypes infected by the frog skin disease (FSD) during the last growing season, the construction of a tissue culture facility for clean-up of the most valuable FSD infected lines via tissue culture and thermo-therapy was approved by CIAT management. The tissue culture facility, completed late August, consists of a growth room and a bio-safety hood room. It is available to the CIAT cassava community for tissue culture clean-up of FSD infected materials and propagation of clean planting materials.

Another use to which the facility has been put is embryo rescue of immature and mature sexual seeds. Interventions to control the escalating problems of white flies at the CIAT experimental station, namely a compulsory one-month "zero cassava" period at CIAT has meant that all plants other than plants in the hybridization block need to be harvested at 11 months after panting. This year, poor flowering of S_1 genotypes in the hybridization block and profuse flowering of S_1 plants in clonal observation trials (COT) implied that the COT plants had to be used for making genetic crosses to generate S_2 families. Because these

plants must be removed in less than a year, the fruits were harvested less than 90 days after pollination, in other words before maturity. The embryos of the immature seeds from the S_2 families were rescued by embryo culture according to protocols developed at CIAT (Fregene 1999).

The facility was also used for establishment, from embryo axes, of mature seeds of breeding populations for CMD resistance. A key reason for CMD breeding at CIAT is to develop Latin America cassava gene pools adapted to the disease should in case it makes its debut in the region. A second important objective is to facilitate germplasm shipment of CIAT's elite cassava germplasm to regions, such as India and Sub Saharan Africa, where CMD is endemic, through the introgression of CMD resistance into CIAT's elite germplasm. To permit marker-assisted selection (MAS) of CMD resistance at CIAT for Latin America and at the same time fulfill plant quarantine conditions for the shipment of the CMD resistant CIAT germplasm to India and Africa, it is necessary to germinate and maintain in vitro breeding populations. This year more than 3000 controlled crosses, and >4000 open pollinated crosses were made involving CMD resistant parents introduced from IITA. The seeds were harvested as mature or immature seeds and are being germinated from embryo axes.

Specific **Objectives**
a) *To rescue embryos of immature seeds of S_2 families*
b) *To germinate from embryo axes of seeds from CMD breeding populations*
c) *To rescue embryos from crosses between high root protein content accessions of wild Manihot species and elite cassava parents*

Methodology
Immature fruits, the fruits were cut open using a sharp knife and the seeds removed and tested for viability (ability to sink in water). A total of 446 viable S_2 seeds were obtained. Another 3600 matured seeds were obtained from crosses between CMD resistance and elite parents of CIAT cassava gene pools, wild accessions with high protein and high beta-carotene varieties. More than 1000 immature seeds were obtained from crosses between high root protein content accessions of wild *Manihot* species and elite cassava parents and also required embryo rescue.

Immature or mature seeds were removed and surface-sterilized by immersion in 70% alcohol for 1 min, followed by immersion in 0.5% sodium hypochlorite for 6 min, and then rinsed three times with sterile water. Under aseptic conditions, the seeds were split along the longitudinal axis utilizing sterile pliers and the embryonic axes were removed with sterile forceps and scapel. Excised embryonic axes were placed radicle down on 1/3 MS medium, supplemented with 0.01 mgl^{-1} NAA, 0.01 mgl^{-1} GA3, 1.0 mgl^{-1} thiamine-HCL, 100 mgl^{-1} inositol, 2% sucrose, 0.7% agar (Sigma Co.) and 25 mgl^{-1} of a commercial fertilizer containing: N 10, P 52, K 10, pH 5.7-5.8 (Roca 1984). This medium is also known as 17N. The embryo cultures were incubated under an alternate temperature regime of 35°C for 16 h and 25°C for 8 h, in darkness for the first 5 days, to promote growth of the radicle, then under continuos illumination from a 40 W fluorescent bulb (5,000 μmol m^{-2} s^{-1}) for the next 5 days. The cultures were then transferred to a growth chamber with a 12h photoperiod (illumination, 5,000 μmol m^{-2} s^{-1}) at 27°C and grown for 40 to 45 days.

Over time, several modifications were made to the protocol to reduce the difficulty of opening up immature fruits, given the large number of fruits to be handled, and also to reduce damage to the embryos during removal. The first modification was to cut the fruit in half, remove the seed, disinfect and place directly on 17N media. The temperature and light regime was also modified for better germination as follows: a constant temperature regime of 28-30°C and a 12/12h photo-period and the use of a piece of dark cloth for 10 days to reduce influx of light, after which the cultures were fully exposed to light.

Problems with bacterial contamination were solved by adding the antibiotic rinfampicilin to the media at a concentration of 50mg/l. Fungal contamination on the other hand was eliminated by increasing the concentration of sodium hypochlorite to 5% and extending incubation times up to 10 min. Due to continued problems with poor germination, a modification of the seed scarification and surface-sterilization was added for mature seeds. Seeds were soaked in 50% sulfuric acid for 30 minutes followed by immersion in sterile water for 30 min, then a 5 min wash in alcohol, and incubation in 5% sodium hypochlorite for 6 min, followed by 3 final rinses with sterile water. The softened testa was then scrapped off with a knife and the whole seed, cotyledons and embryos, was placed on the media.

Results
The 446 immature S_2 seeds were harvested the same day and stored at room temperature. Embryos were extracted and cultured over a period of 2 weeks, with an average of 40 seeds processed every day. During the first week, problems of fungal infection, apparently due to storage at room temperature, lead to a huge loss of seeds. This problem was eventually solved, but the 8-14 day storage of the immature fruits, reduced the viability of the seeds considerably. Previous experiences revealed that air drying excised immature seeds at room temperature drastically reduced germination rates (Fregene 1999). It appears that storage of immature fruits at room temperatures had the same debilitating effect as air drying immature seeds. Immature seeds from the inter-specific crosses were therefore harvested and embryos cultures on a daily basis. Only 67 plants or 15.02% could be recovered from the 446 embryos cultured. The S_1 genotypes from which S_1 plants cold be recovered include AM244-35, AM244-38, AM244-39, AM244-64, AM244-101, AM244-109, AM244-135, AM244-164, AM266-21, AM266-41, AM266-50, Y AM266-76. These S_1 plants have been multiplied in preparation for transfer to the green house and to the field the following year.

Results of more than 500 immature seeds from inter-specific crosses processed so far have revealed more than 80% germination rates, confirming the damaging effects of storing immature seeds before embryo culture. So far, more than 100 mature seeds from the crosses generated for marker-assisted breeding of CMD resistance were cultured from embryo axes. Germination rates were higher than 90%. Results were much better here presumably due to the maturity of the seeds and the sulphuric acid treatment. No effect on germination and growth was observed of changing the temperature regime from the previous regimes described earlier for embryo rescue (Fregene et al 1997) to a constant 28-30°C day and night.

Future Perspectives
Completion of the embryo culture of the CMD resistance breeding populations and the inter-

specific hybrids for higher protein content.

References

Fregene, M.; Ospina J. A. Y Roca, W. M. (1999). Recovery of cassava (*Manihot esculenta* Crantz) plants from culture of inmature zygotic embryos. En: *Plant Cell Tissue and organ culture,* 55: 39-43

Roca, W. M. (1984). Cassava. En: Sharp W. R.; Evans,D. A.; Ammirato, P. V. Y Yamada, Y. (Eds). Handbook of Plant Cell Culture; 2: Crop Species. MacMillan, New York. p 269-301.

Activity 8.17 TRIPS

1. Visit to the Iwate Biotechnology Research Center (IBRC), Kitakami, Iwate, Japan. Transient assay of a CMD candidate gene for resistance to the African Cassava Mosaic virus. March 23-April 19, 2002
2. Visit to Uganda. Set-up of a SSR marker lab in Kampala (medical biotech labs) and visit to NARS partners. May 15-May 228 2002
3. Visit to India. Visit to CTCRI on the MoU between CIAT and CTCRI on germplasm transfer and training July 20 to July 30, 2002.
4. Attend the Meeting on Biotechnology, Breeding and Seed System s for African Crops in Entebbe Nov 3-7.

Activity 8.18 TRAINING

Visiting Researchers
1. Ms Cach, Vietnam (BSA of flowering)
2. Ms E.lizabeth Okai, Ghana (Genetic diversity)
3. Mr Luis Monte, Guatemala (Genetic diversity)
4. Ms Prapit, Thailand (BSA dry matter)
5. Ms Chommanat, Thailand (Genetic diversity)
6. Luis Guillermo, Colombia (Tissue culture)
7. Charles Buitrago, Colombia (Web-based data base management)
8. Mr Emmanuel Okogbenin, Nigeria (Fine mapping).

Students
1. Jaime Marin, undergraduate student Universidad de Tolima, Ibague (QTL mapping of early bulking)
2. Angela Zarate, undergraduate student Universidad de Tolima, Ibague (Conversion of RFLP to SSCP markers)
3. Martha Isabel Moreno, Post graduate (M.Sc.) student Universidad de Valle, Cali (Gene cloning of CMD2)
4. Gina Puentes Jazbleidi, undergraduate student Universidad Nacional, Sede Palmira (Waxy cassava starch)
5. Paula Andres, undergraduate student, Universidad Javeriana, Bogota (Gene tagging of CBB resistance)

PUBLICATIONS

Refereed journals
Akano A., Barrera E., Dixon A.G.O., Fregene M. (2002). Molecular Genetic Mapping of Resistance to the African Cassava Mosaic Disease. Theor and Appl Genet 105:521-525.

Okogbenin E. and Fregene M (2001) Genetic Analysis and QTL Mapping of Early Bulking in an F₁ Segregating Population from Non-inbred Parents in Cassava (*Manihot esculenta* Crantz) (Theor and Appl Genet published online September 10)

Book chapter

Fregene M., Tohme J., Roca W., ChavariaggaP., Escobar R., and Ceballos H. (2002) Biotechlogia para la Yuca. In: Ospina and Ceballos (eds). La Yuca en el tercer Milenio. Sistemas Modernos de produccion, Procesamiento, Utilization y Comercializacion. CIAT. Cali, Colombia