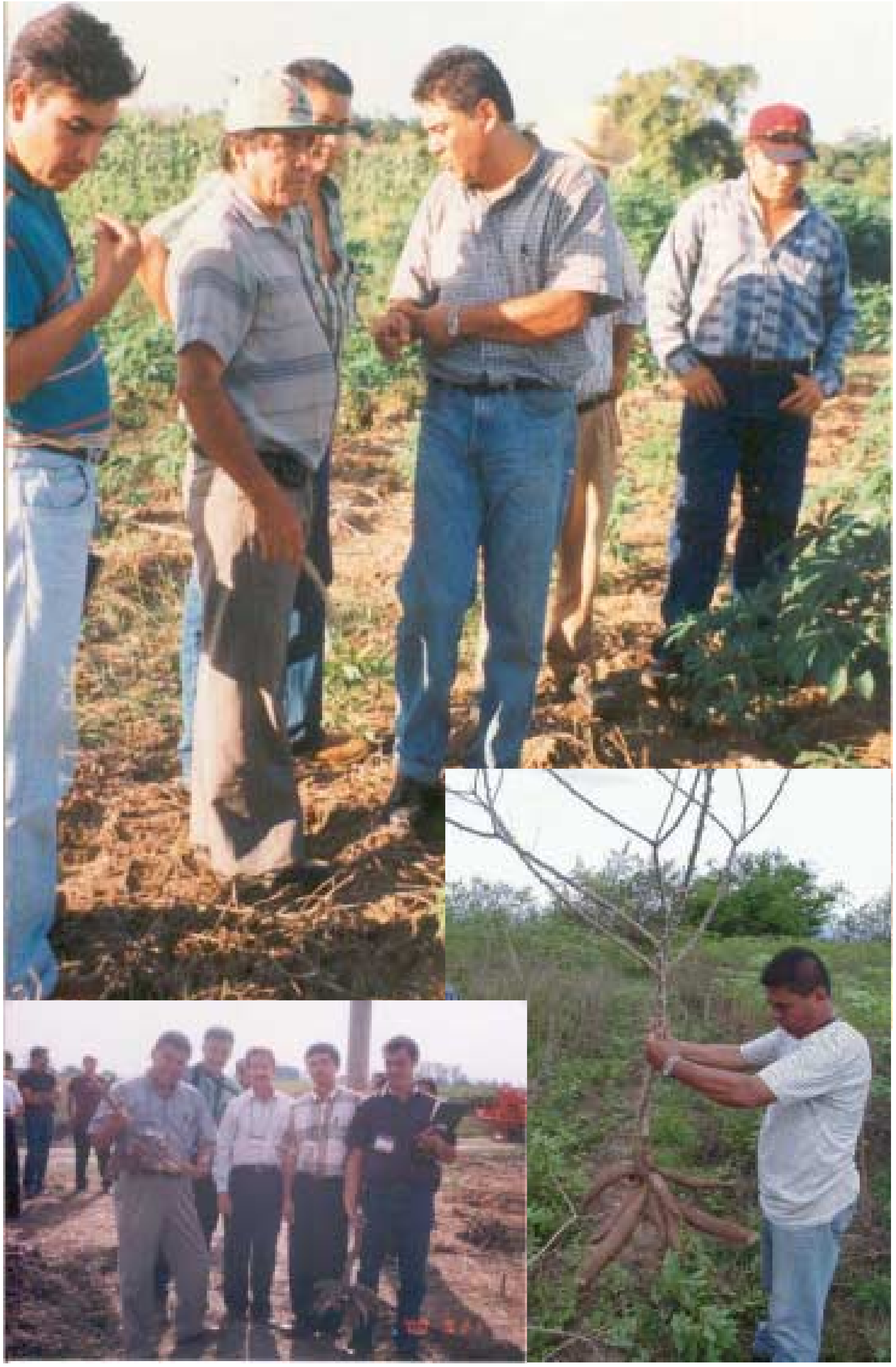
Improved cassava for the developing world

Project IP-3 Annual report 2001

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

In memoriam
JAIRO BEDOYA LOZANO:



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Highlights from IP3 Project

Whiteflies

The combination of very high whitefly (especially the species *Aleurotrachelus socialis*) populations and the increasing incidence of Frogskin Disease have made it nearly impossible to carry out research trials on cassava and maintain the germplasm bank in the field at the CIAT station. A decision was taken to suspend cassava planting at CIAT for a one-month period. The frequent (nearly monthly) and staggered plantings of cassava on the CIAT station, combined with a favorable rainfall pattern, results in a constant supply of new cassava growth, which is conducive to a build up of whitefly populations. It was therefore hypothesized that a defined period with no cassava at CIAT would “break” the whitefly cycle by and reduce pest populations in subsequent plantings. Since *A. socialis* has no known additional hosts, a pause in cassava plantings will remove its food source and deny the migration of pest populations from older to recently planted cassava fields.

Initial results indicate that this pest management practice has been effective. Post fallow cassava plantings have not attracted high numbers of whitefly adults, resulting in reduced oviposition and hopefully, lower whitefly populations. It is also calculated that this and other management practices (i.e. disease free planting material) will also reduce the incidence and severity of Frogskin Disease. These crops management practices will need to be implemented on a continued (yearly) basis in order to insure minimal pest and disease incidence and provide satisfactory conditions for maintaining cassava germplasm and conducting research trials on the CIAT station.

Diseases

Phytophthora species are soilborne pathogens causing root rots. The search for cassava cultivars with resistance to Phytophthora has gone on continuously since de mid 1990s. The availability of tolerant breeding lines, which appear more durable in the face of pathogenic diversity, provides cassava breeders with new opportunities. Additional security might be achieved by incorporating major resistance genes into a tolerant genetic background. Useful resistance to root rot has been detected in clones from the Amazon basin. Additional sources of resistance have now been identified in CIAT’s germplasm bank. Combining the resistance from these two sources will lead to a more stable management of the disease, for which no resistance had been detected previously. New cassava cultivars with superior disease resistance are available and they have been adopted by women of the Tukano indigenous group in Mitú (Vaupés, Colombia). Some progress is underway that may result in the identification of the causal agent of Frogskin disease.

Record yield

A new generation of clones is being developed as result of the new breeding scheme and the modification of breeding objectives. The higher emphasis on varieties that are particularly targeting industrial processes (feed and starch industries) requires high dry matter productivity, regardless of certain root quality traits such as color, cyanogenic potential, etc. A commercial yield of 84 t/ha of fresh roots was attained in a 9.5 ha planting at Ciénaga de Oro (Department of Córdoba) with clone SM 1433-4. This is probably a yield record, at least for Colombia, and demonstrates the enormous potential of this crop.

Advantages of the new breeding scheme

Modifications introduced in the breeding scheme resulted in advantages that had not been unforeseen before their implementation. In addition to shortening the length of the breeding cycle (by more than a year), and increasing the heritability of traits we select for, the new method has allowed for the identification of a few traits that may be key for cassava breeding in the near future. We have found, for instance, that the ability to retain leaves for a longer period of time (at five months of age) results in a yield increase of seven tons of fresh root (or two tons of dry matter) per hectare. We have also identified a group of clones that exhibit the capacity to maintain dry matter content upon the arrival of the rains. The new breeding scheme allows for an estimate of *general combining ability* effects for those traits that are agronomically important. This, in turn, will help identifying germplasm with high *breeding value*. We have also planted three sets of diallel crosses that will produce basic genetic information that is still missing in this crop.

Hidden treasures in the germplasm bank

We continue to explore the germplasm bank in search of useful traits. As already stated, sources of resistance to root rot diseases has been recently found. A few years ago we also found resistance to whiteflies. We are currently screening the germplasm bank in search of genotypes that are micronutrient dense (i.e., carotenes), or that offer novel starch types. We are also screening the bank for other traits more difficult to obtain but that would have a huge positive effect on the way we breed, handle and culture cassava. Two of those traits are apomixis and natural occurrence of resistance/tolerance to herbicides. Although preliminary, some interesting differential reaction to two different commercial herbicides has been observed.

Dealing with African Cassava Mosaic

The discovery of a qualitative and high level of resistance to the devastating cassava mosaic disease (CMD) and molecular markers linked to it have made conceivable marker-assisted breeding for CMD resistance at CIAT. Progress made this year includes the establishment of a sexual hybridization scheme between resistant donor lines received last year from IITA and CIAT elite parents. Included for genetic crosses are high carotene lines, to combine high carotene and CMD resistance, targeted to sub-Saharan Africa. Furthermore, field experiments in Uganda have revealed that the novel source confers resistance against the Ugandan variant (UgV), an aggressive recombinant strain of the virus that caused a disease epidemic that swept through Uganda and is now spreading into the Democratic Republic of Congo, Kenya, Tanzania and Rwanda. A marker-assisted selection (MAS) scheme has also been initiated at IITA for the rapid verification of CMD resistant selections in resistance breeding to contain the spread of the epidemic. Progress is also being made on the identification of candidate genes that may mediate the molecular basis of CMD resistance.

Taking advantage of wild relatives

The first year evaluation of more than 1000 genotypes of 7 wild Manihot species and inter-specific hybrids has identified accessions with very high protein and dry matter content, waxy starches (low level of amylose), and white fly resistance. A modified advanced back cross QTL (ABC-QTL) identification and introgression scheme has been initiated to transfer these genes to cassava. The F1 families obtained from above will be evaluated twice at the seedling and clonal observation trial stage. The goal of the ABC-QTL scheme is high yielding cassava lines with high protein, dry matter and carotene content for distribution to collaborators at the International Institute for Tropical Agriculture (IITA), Ibadan and NARs in Latin America and Asia.

Dealing with post-harvest physiological deterioration

Post-harvest physiological deterioration (PPD) results in a very short shelf life of cassava roots after they have detached from the plant at harvest. Because of PPD, large amount of cassava roots get spoiled. The commercialization of roots is cumbersome and expensive. Basic research to understand the biochemical pathway of PPD is currently carried out through a collaborative project with the University of Bath (England). At the same time more applied research has been conducted to deal with the problem by modifying the way the roots are harvested and handled afterward. In collaboration with a pre-cooked, frozen croquettes producer (McCain Foods) we have made substantial progress that will allow this industry to reduce by 30% the cost of the raw material employed in the production of croquettes. Basically our results allow transport and storage (until processing) without the need of freezing the roots, as done up to now.

👍 *Towards a reduction of rural poverty*

Most of the highlights listed above have a direct bearing on improving the socio-economic conditions of rural communities that depend partially or totally on cassava. The ultimate goal of our project is to attain this objective through alternative strategies that result in increasing cassava productivity, the value of the harvest (i.e. harvesting foliage), and/or reducing production costs. For farmers that do not sell their products to the market, we also deliver materials whose production is more reliable. We are also, as in the past, actively searching and promoting for alternative uses for cassava, as well as for novel processing techniques. Our concerns also is on the environmental impact of cassava production and processing. As an example, CLAYUCA and CIAT are also working in association with the private sector in the development of methodologies for dry extraction of starch. This would immediately benefit the environment because there will be no production of effluents that one way or another affects the environment.

👎 *A frustrating problem that remains unsolved*

Frogskin disease has been a frustrating problem for many years. In spite of significant efforts and investments, the causal agent remains unknown as well as the transmission mechanisms. It has always been considered that frogskin disease was induced by a viral agent. Likewise we have always suspected that whiteflies were involved in the transmission of the disease from plant to plant. However, we have not been able to positively identify any viral particle, nor demonstrate that the whiteflies are indeed the vectors. We are now evaluating a new hypothesis that a viroid is, indeed, the pathogen associated with this disease. Until a definitive identification of the causal agent and development of practical diagnostic methods, multiplication of cassava vegetative seed will remain a vulnerable process.

👎 *A significant loss for cassava research at CIAT*

For many years the cassava projects at CIAT benefited from the presence of a numerous and highly qualified personnel from IRD-France. This personnel, however, was recently relocated back in France. For CIAT this has been a great loss in human resources, which will be very difficult to overcome. People involved had integrated very well with CIAT community and their absence is and will be noticed. To them and IRD, our words of appreciation.

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

The overall objective of this project 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 leaves and roots. Collaborative project with IFPRI and the University of Adelaide.

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. Ascorbic acid and carotene are known to have antioxidant properties. Therefore, PPD was measured in a sample of genotypes to evaluate the potential correlation between these two vitamins and PPD. A major problem regarding PPD is obtaining reliable data, because the 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 total carotene contents in both tissues*;
c) *to correlate vitamin contents with physiological post-harvest deterioration.*
d) *to search for genotypes with higher protein content in the roots*.

Materials and methods
Harvesting and sampling. 682 genotypes of cassava were selected to represent the wide variability in carotene content and PPD susceptibility from the CIAT germplasm collection (6000 genotypes). Three plants per cultivar were harvested, all at 9-12 months of maturity.

Carotene concentration measurements**.** The extraction procedure outlined by Safo-Katanga et al. (1984) was modify 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 ºC 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.

Post-Harvest Physiological Deterioration (PPD) measurements**.** Five commercially sized roots (minimum length 18 cms) 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.

A total of 682 accessions of the Cassava CIAT collection was evaluated with respect to carotene content in roots and leaves and PPD deterioration.

Results

Analysis of carotene content. Carotene concentration in leaf tissue ranged from 18.71 mg/100 g FW to 96.2 with a mean of 50.32 mg/100 g FW and a standard deviation of 10.43 (Table 1.1). These values are similar to those found in the group of 500 accessions evaluated the previous year (see 2000 annual report). The carotene distribution showed a symmetric tendency (skewness = 0.2). Carotene concentration in roots ranged from 0.13 y 0.92 mg/100 g FW with a mean of 0.23 and a standard deviation of 0.10 (Table 1.1). This distribution showed a strong skewness with long right tails (skewness = 2.83). Significant correlation (0.84) was observed for carotene content and color intensity of cassava roots. Carotenes concentrate much more on leaves than in roots, illustrating, once again, the excellent nutritive value of cassava leaves. There was no correlation (0.02) between carotene concentration in leaves and roots.

Correlations among vitamins contents and PPD. The correlation between PPD and carotene content in cassava roots was -0.07. (Figure 1.1) This relationship is similar to the one reported previously (-0.13). Even though a high correlation between carotene content and PPD was not observed, it was previously noted (Chávez el al., 2000) that at carotene concentration in roots higher than 50 mg carotene/100 FW, PPD did not exceed 20%, suggesting a threshold effect (Table 1.2). However, results illustrated in Figure 1.1 clearly contradict previous findings. PPD studies are affected by large experimental errors arising from environmental variation at the time the evaluations are carried out. Because of logistic limitations, germplasm bank accessions must be harvested gradually through time. Therefore, the PPD evaluations are carried out at different times and are seriously affected by environmental factors (particularly temperature). Because of this situation CIAT built a large chamber were temperature and relative moisture can be controlled. In the future all the PPD evaluations, will be performed under uniform environmental conditions.

The project will continue screening cassava landraces from CIAT's germplasm collection, exploring the genotypes with higher carotene contents. However, only cassava with non-white roots will be evaluated in the future. The objective is to identify a group of clones with highest carotene content to help in the plant breeding approach for improving the micronutrient content of this staple crop.

Table 1.1. Carotene concentration in leaves and roots of 682 cassava accessions from CIAT's Germplasm Bank Collection.

Data from leaves		**Data from roots**	
Range (mg/100 FW)	Frequency	Range (mg/100 FW)	Frequency
18.71-26.46	6	0.13-0.21	437
26.47-34.22	38	0.22-0.30	166
34.23-41.98	94	0.31-0.39	29
41.99- 49.74	197	0.40-0.48	19
49.75-57.5	184	0.49-0.57	18
57.6-65.35	115	0.58-0.66	9
65.36-73.11	35	0.67-0.75	2
73.12-80.87	10	0.76-0.84	2
80.88-88.63	2	0.85-0.93	1
>88.64	1	>0.93	0
Minimum	18.71	Minimum	0.13
Maximum	96.2	Maximum	0.92
Median	57.45	Median	0.525
Skewness	0.2	Skewness	2.83
Mean	50.32	Mean	0.23
SD	10.43	SD	0.10

Table 1.2. Cassava genotypes with high carotene content and low PPD.

Genotype	**PPD (%)**	**Carotene mg/ 100g FW**
MCOL 2086	5.7	0.51
CM 3199 - 1	19.5	0.51
MCOL 676	19.0	0.51
MCOL 2068	5.7	0.53
MBRA 487	20.0	0.55
MCOL 2498	4.3	0.57
MCOL 2099	9.5	0.57
CM 5655 - 1	4.3	0.58
MBRA 465	10.9	0.63
MCOL 2410	8.1	0.65
MCOL 2459	4.3	0.66
MCOL 2412	7.9	0.70
MBRA 206	6.7	0.72
MCOL 2436	8.3	0.83
MCOL 2439	11.4	0.92

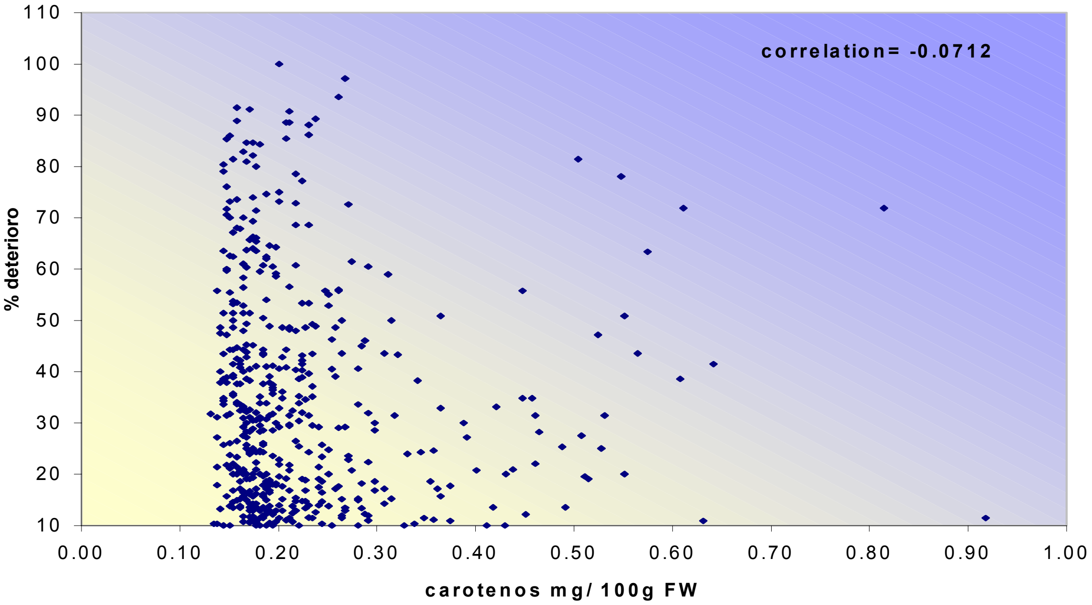

Figure 1.1. Relationship between PPD and carotene content in cassava roots

The project will also evaluate the expression and stability of high concentrations of carotene in elite genotypes under different environmental conditions. Although cassava genotypes with high concentrations of total carotenoids have been identified, it is necessary to determine the proportion of carotenoids with pro-vitamin A activity present in cassava roots and leaves.

Activity 1.2. Genetic variation in minerals concentration of cassava genotypes and its relationship with post-harvest physiological deterioration.

Rationale

Deficiencies of vitamin A, iron and zinc are widespread in sub-Saharan Africa and in many tropical areas where the diets of poor human populations are mainly plant-based and the intake of animal derived products are low. Since cassava is a staple food in regions where there are severe deficiencies of micronutrients, the crop can be used as a vehicle to deliver vitamins and minerals in higher concentrations.

In the field of human and animal nutrition there is an increasing amount of evidence of a synergistic effect between vitamins and certain minerals. Preliminary results suggest that iron and zinc contents in the diet increase vitamin A absorption and vice versa. Therefore, for the study of micronutrients availability from cassava roots and leaves, it is also important to measure mineral contents.

Objective

The objective of this investigation was to evaluate the extent of genetic variation of minerals concentrations in 411 genotypes from the cassava world germplasm bank held at CIAT.

The overall objective of this project is to improve the nutritional status of people living in marginal environments of the tropics, by selecting and promoting cassava genotypes with high bioavailability of micronutrients and vitamins.

Methods

Mineral concentration measurement. Leaves and roots were collected (sampling is the same done for the evaluation of carotenes content), dried, ground to powder and sent to the Analytical Laboratory of University of Adelaide were the samples were analyzed by inductively coupled plasma atomic emission spectrometry. All sample processing was carried out to avoid contamination from soil, wich has mineral concentrations higher than that of vegetal tissues.

Carotene concentration measurements. The extraction protocol for leaves and roots is described in the report "Evaluation of genetic diversity for total carotenes content in cassava leaves and roots" (2001 annual report).

Post-Harvest Physiological Deterioration (PPD) measurements**.** PPD was measured seven days after harvest on genotypes whose concentration of carotenes in roots were measured simultaneously ("Evaluation of genetic diversity for total carotenes content in cassava leaves and roots" (2001 annual report).

Results

Trace mineral concentrations (all measurements in mg/kg) Roots averaged 14.88, of iron, 8.15 of zinc, and 803.11 of calcium on a dry matter basis (Table 1.3). Mineral concentrations in the leaves were much higher, averaging 281.61 of iron, 47.53 of zinc and 13760 of calcium. These leaf concentration figures are much higher than those in most staple foods. Although the leaves are eaten as a vegetable with high water content and low mineral density, they supply high leaves of mineral per calorie.

Correlations among minerals of roots and leaves. A high positive correlation between minerals content in roots and leaves was observed for Mn (0.508) and K (0.407). The accumulation of the others minerals in roots and leaves seems to be rather independent.

Correlations among minerals and carotene contents**.** In cassava roots a very weak relationship between total carotenes content and Zn, and Ca was found (correlation coefficients of 0.005 and 0.1792, respectively), for Fe there was no correlation. For leaves, the correlations were significants for Mn (0.150), Na (-0.148) y Ca (0.146).

Correlation among minerals in roots and PPD. In general, correlations between PPD and mineral concentrations in cassava roots were low. The higher correlation coefficient found were negative: K (-0.2936, Figure 1.2) and Fe (0.1854, Figure 1.3). It seems that an inverse relationship between these minerals and the Post-harvest Physiological Deterioration process may exist.

Cluster Analysis. This study contructs groups (clusters) wich have common interesting characteristics for the identification of elite genotypes for breeding programs. Fifteen clusters were determined and among them the genotypes that have the higher concentrations of minerals were MBRA 11 and CM 6068-3. SM 722-13 present low potasium concentration and high PPD. MCOL 2436, MCOL 2439, MCOL 2459 and MBRA 206 present low PPD and higher carotene content. Table 1.4 summarizes these data.

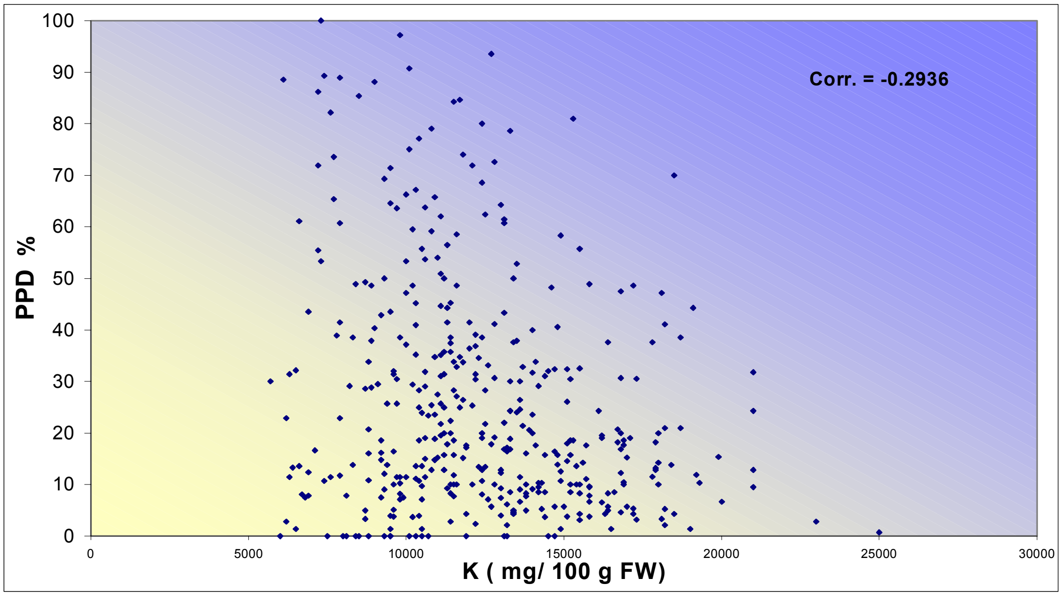

Figure 1.2. Relationship between PPD and Potassium concentration in cassava roots

Table 1.3. Simple descriptive statistics (minimum, maximum, and mean) for mineral concentrations (mg/kg) of 411 genotypes of cassava.

Mineral	**Leaves**			**Roots**		
	Min	Max	Mean	Min	Max	Mean
Iron	120.63	950.00	281.61	5.99	75.92	14.88
Manganese	18.67	200.00	61.39	0.47	5.00	1.47
Boron	4.02	31.16	14.04	1.14	3.44	1.95
Copper	2.81	12.36	7.39	0.49	40.31	6.78
Zinc	15.14	150.47	47.53	2.63	37.52	8.15
Calcium	6300	32000	13760	303	2500	803.11
Magnesium	2600	11300	4710	520	2400	1015.11
Sodium	10.27	113	29.47	18.56	1230	135.5
Potassium	8700	23000	15148	5700	25000	12381
Phosphorus	2300	7600	3901	980	3200	1720.07
Sulphur	2400	5200	3319	123	550	280.7
Aluminum	59.5	880	211	4.42	43.95	8.77

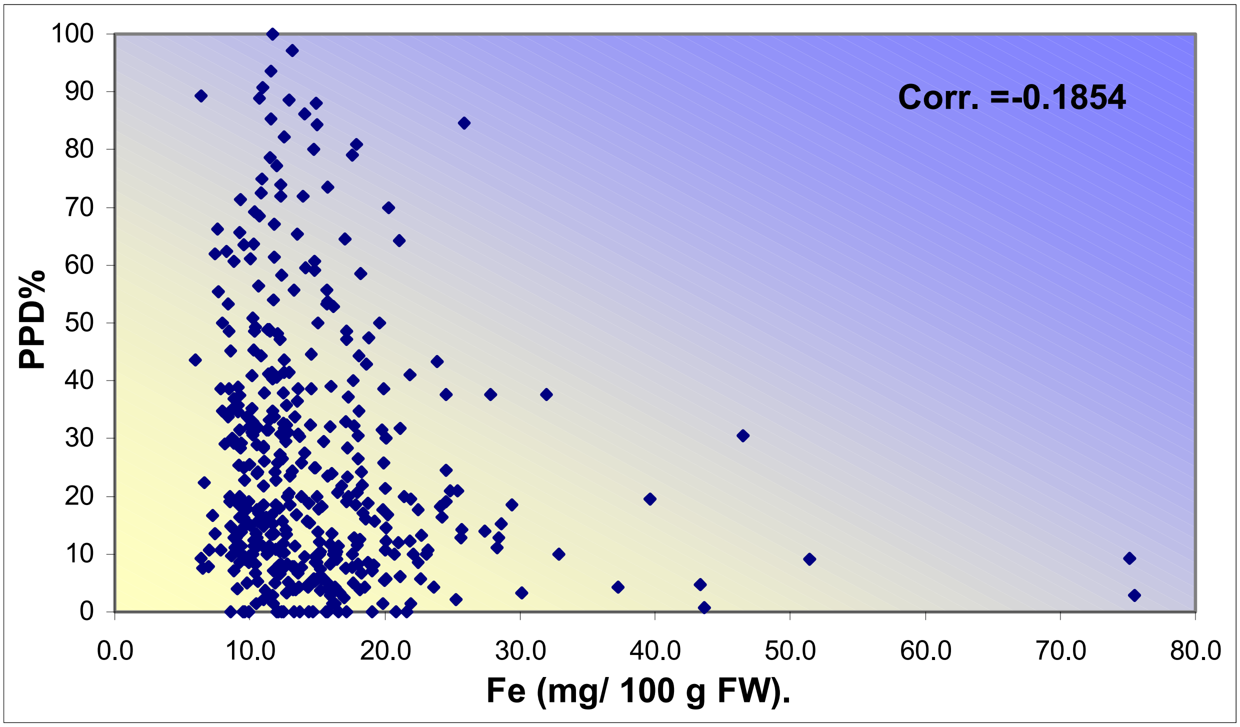

Figure 1.3. Relationship between PPD and Fe concentration in cassava roots

These results demonstrate that there is a substantial genetic variation that can be exploited to improve cassava micronutrient density. New varieties with superior concentration of iron, zinc and pro-vitamin A, can be identified to exploit the fact that a synergy may operate in absorption, internal transport, and function among these micronutrients. The project will continiue searching for a group of varieties with higher micronutrients content to help in the plant breeding approach to improve the micronutrient content of this staple crop. Also, the expression and stability of minerals in elite genotypes under different environmental conditions will be evaluated.

Activity 1.3. 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.

Objective

1) To determine the proportion of different carotenoids making up the “total carotenes” measurement obtained in this output.
2) To produce preliminary data on how constant (across different genotypes) is the proportion of the different carotenoids make up the total carotene measurement.

Results

At this point the HPLC process has been fine tuned. An specific column was purchased and preliminary runs have been made until the proper β-carotene and α–carotene standards arrive. The preliminary results suggest (as expected) that a large proportion of the total carotene measurement is, indeed, β-carotene.

Table 1.4. Genotypes with common characteristics identified from the cluster analysis.

Genotypes	Characteristic
MBRA 11	High Fe,
CM 6068-3	High Zn,
	High Na, K, Cu, P, S and Al
SM 722-13	Low K
	High PPD
MCOL 2436	High carotene on roots
MCOL 2439	High K
MCOL 2459	High B
MBRA 206	Low PPD
CM 5460-5, MBRA 49	
MBRA 88, MBRA 89	Low K
MBRA 90, MBRA 103	Low B
MBRA 104, MBRA194	High PPD
MCOL 480, MCOL 847	
MCOL 2456, MECU 6	
MCOL 2389, MCUB 35	
MBRA 122, MBRA 465	
MBRA 468, MCOL 1995	High carotene on roots
MCOL 2272, MCOL 2363	High Ca
MCOL 2412, MCOL 2528	High Mg
MBRA 214, MCOL 2289	

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OUTPUT 2
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 workplan. 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 parental material based on previous cycle results, and the information obtained from the 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 usually employed 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.

Specific Objectives

a) *Based on information of evaluation trials at several locations, and the new objectives defined for the project, a set of elite clones was identified for recombination, to start a new cycle of selection.*
b) *For this particular cycle, the parental genotypes have included for each agro-ecological zone, at least two genotypes with high-carotene, yellow roots.*

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.

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 variety crossed to genotypes with specific traits; or requests from CIAT scientists that want to pyramid genes, or develop segregating progenies for gene tagging.

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, 2001 through March, 2002. F1 plants will grow until the planting of the trials early in 2003.

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. An additional advantage of the new selection scheme is that it permits estimating what is known as the *general combining ability*, which, defined simply, is the capacity of an individual to produce good progeny.

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

#	**Sub-humid tropics**	**Inter-regional**	**Acid-soil savannas**
1	SM 643-17		SM 909-25
2	SM 805-15		SM 1219-9
3	SM 809-9		SM 1363-3
4	SM 1068-10	Rayong 60 (MTAI 8)	SM 1363-11
5	SM 1181-3	CM 4919-1	SM 1460-1
6	SM 1201-5	CM 6754-8 ¶ →	SM 1543-17
7	SM 1210-10	SM 1411-5	SM 1565-15
8	SM 1411-5	SM 1438-2	SM 1741-1
9	SM 1433-4		SM 1822-5
10	SM 1438-2		CM 507-37 ¶
11	SM 1511-6		CM 2177-2 ¶
12	SM 1600-4		CM 2772-3 †
13	SM 1656-7		CM 4574-7
14	SM 1665-2		CM 6438-14 ¶
15	SM 1669-5		CM 6740-7
16	SM 1669-7		CM 6921-3
17	SM 1778-45		CM 6934-4
18	CM 3306-4		CM 7951-5
19	CM 4365-3		MECU 64 §
20	CM 4919-1	CM 6740-7	MECU 72 §
21	CM 6119-5 † ←	SM 1219-9	MPER 183
22	CM 6754-8 ¶	CM 7951-5	MPER 335 §
23	CM 7395-5	MBRA 383	MCOL 2737
24	CM 7514-8	CM 6438-14	MBRA 383
25	CG 1141-1		MBRA 387
26	Rayong 60 (MTAI 8)‡		MBRA 502
27	Kasetsart 50 ‡		

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

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 is done to date.

During this year, the 53 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, and .3* for more detail). The agronomic performance of some of these materials *per se* is also described below. The seed produced from the current crossings will be harvested between September 2001 and March 2002. Planting materials were also selected from these parents to seed the *F1* in July 2001.

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 the high-altitude tropics, crosses will be carried out within a group of three clones recently identified as excellent, of good acceptability to farmers, and carrying sources of resistance to whitefly (MECU 72, MECU 64, and MPER 335). This pest has become the one true constraint to cassava cultivation in that region of Colombia.

Finally, a group of genotypes adapted to the sub-humid environment that performed very well in terms of maintaining high levels of dry matter content after the rains begin were brought back to CIAT, Palmira, for further evaluation. These materials are briefly described on Table 2.11.

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**. 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**).

Results

A total of 189,278 recombinant cassava seeds was produced at CIAT's Experiment Station, Palmira, during June 2000 to November 2001 (Table 2.2). From each cross, a given number of botanical seed was obtained to initiate selection (stage *F1* in Figure 2.1). About 10% (21,163) of the produced botanical seed was planted (Table 2.3) to initiate stage *F1*. Of these, only 83% (i.e., 17,614) could be transplanted, because either some seed did not germinate or emerged plantlets were too weak to survive transplanting.

Table 2.2. Production of recombinant cassava seed at CIAT, Palmira, Valle del Cauca, Colombia, between June 2000 and November 2001.

Purpose of cross	Controlled Crosses	Poli-crosses	TOTAL
Wide adaptation	3340		3340
Tolerance to inbreeding depression	3320		3320
Basic genetic studies	3476		3476
Specific adaptation to:			
Sub-humid environments	17008	56632	73640
Acid soil savannas	13275	38744	52019
Mid-altitude valleys	11771	36565	48336
Resistance to:			
Bacterial blight	2846		2846
Whiteflies	1655		1655
Physiological deterioration	86		86
Root rots	560		560
TOTAL	57337	131941	189278

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 contamination from frogskin disease was very difficult. 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 or viroid, is not transmitted through botanical seed.

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.

Type of cross	Germinated seed	Transplanted seedlings
Molecular genetic map	1339	548
Resistance to whiteflies	1339	1086
Studies on physiological deterioration of roots	84	74
Resistance to root rots	251	189
Tolerance to inbreeding depression	2956	2021
Resistance to bacterial blight	2007	1835
Adaptation to sub-humid environments	5251	4718
Adaptation to acid soil savannas	4603	4128
Adaptation to mid-altitude valleys	3333	3015
TOTAL	21163	17614

Activity 2.3. Generation and distribution of advanced breeding materials for Asian National Programs and Africa (through collaboration with IITA).

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 Asian 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 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 2000 through September 2001.

Continents	Genotypes in-vitro	Crosses (families)	Plants (in-vitro)	Seeds in the shipment
Latin America				
In-vitro	76		6439	
Hybrid seed		137		6955
Asia				
In-vitro	282		598	
Hybrid seed		300		16106
Africa				
In-vitro	15		106	
Hybrid seed		437 ¶		21580 ¶
Europe + USA				
In-vitro	15		75	
Hybrid seed		2		100
Total				
In-vitro	388		7218	
Hybrid seed		876		44741

¶ Hybrid seed from crosses with wild relatives.

Results

A considerable fraction of the seed produced by the projec has been transferred to National Programs in different regions of the world. As shown in Table 2.2, close to 189,278 recombinant seeds were produced in 2000 and 2001 and about 25% of that seed 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. However, it will take longer for Asian National Programs to receive materials from CIAT. 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.

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 5000 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) *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:

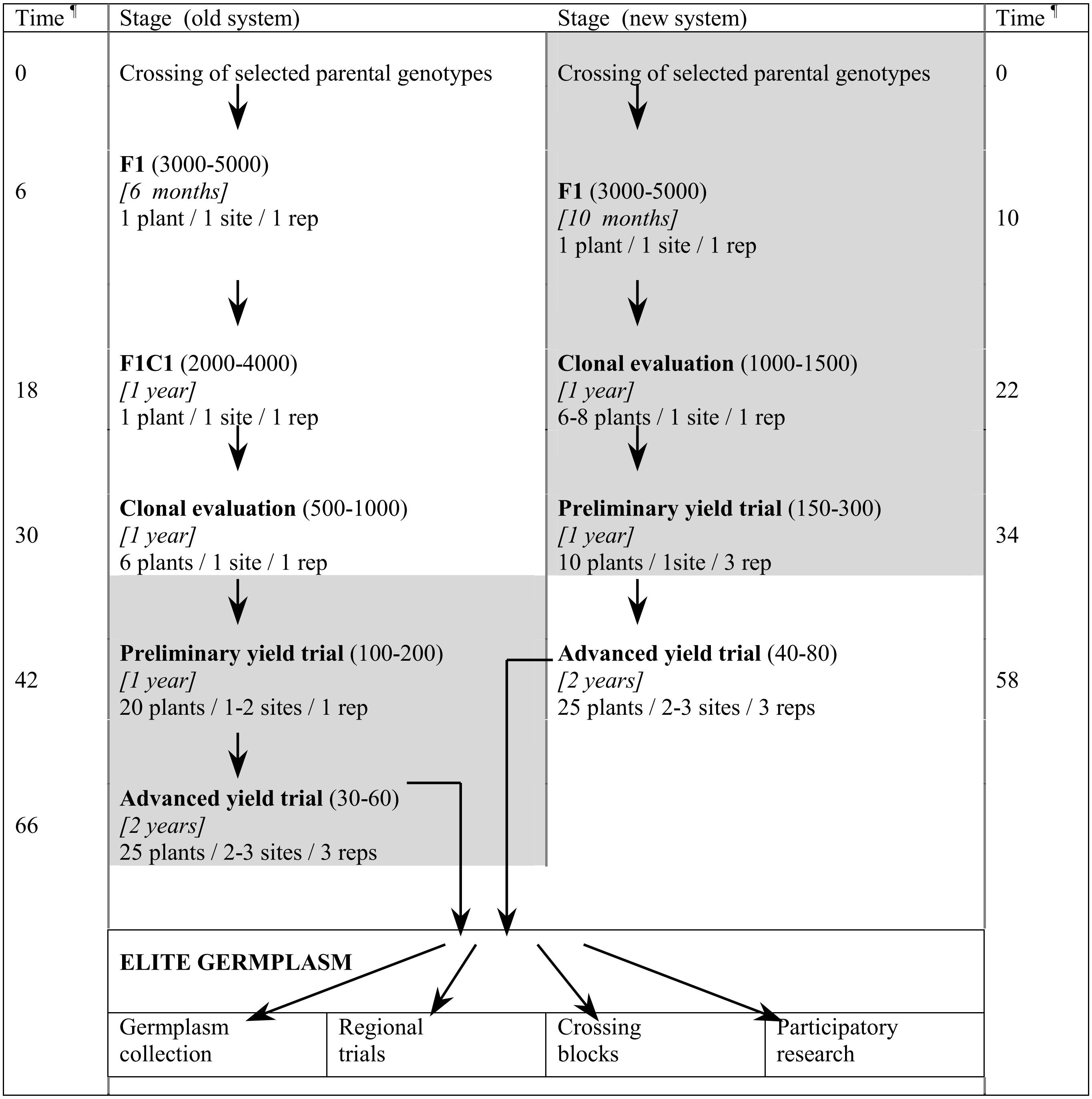
1) The *F1* plants will be grown for 10 months rather than 6. At that age they can produce up to 8-10 stakes. The stakes will be sent to the proper evaluation site for the *Clonal Evaluation*. This implies that the *F1C1* stage is eliminated and that no duplicate of each genotype is necessarily maintained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

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

Description	Representative Countries / Regions	Evaluation Sites
Sub-humid tropics (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 (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 (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 (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



¶ 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 2001, and approximately 10,000 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.

Preparing new clonal evaluation trials following a diallel mating design

Two important aspects of the hybridizaton strategy executed 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. This diallelic analysis will produce information essential for understanding the heredity mode of many traits of economic importance in the crop, and thus fundamental to more efficient genetic improvement in the future. In addition, it will help train a postgraduate student from Vietnam.

The *F1* plants were recently harvested for use as planting materials for the North Coast (Table 2.6), for the Acid Soil Savannas (Table 2.7) and inter-Andean valleys (Table 2.8). As a result, the *Clonal Evaluation* stage for March-May 2001 to March-May 2002 for the three environments will be represented by these relevant studies.

For scientific validity, a singular planting is being 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 will be used to plant the trials and the others will be planted in nurseries to serve as seed source. The six stakes for the trials will be distributed in three replicates located at two representative sites. This way, every family of crosses is one of full sibs (i.e., both parents known) and will be made up of 30 genetically different individuals. Each individual will be 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.

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:

$$\text{Selection index} = [FRY * 10] + [DMC * 8] + [PT * 3] + [HI * 3]$$

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.

Table 2.6. Number of transplanted plants (below diagonal) in CENICAÑA for the diallel experiment for the sub-humid environment of the North Coast of Colombia. The code assigned to each cross is above the diagonal [¶].

Parent	MTAI-8	CM 523-7	CM 6754-8	CM 8027-3	SM 805-15	SM 1565-17	SM 1411- 5	SM 1219-9	SM 1657-12	SM 2192-6	SM 1665-2
MTAI-8	-.-	CM 8209	CM 9106	CM 9148	CM 9178	CM 9966	CM 9958	GM 266	GM 289	GM 302	GM 291
CM 523-7	80	-.-	GM 210	GM 211	GM 212	GM 215	GM 214	GM 213	GM 216	GM 218	GM 217
CM 6754-8	55	24	-.-	CM 9921	CM 9945	CM 9907	CM 9954	GM 236	GM 237	GM 239	GM 238
CM 8027-3	76	93	63	-.-	CM 9703	CM 9926	CM 9923	GM 246	GM 247	GM 249	GM 248
SM 805-15	88	86	76	53	-.-	CM 9949	CM 9946	GM 250	GM 251	GM 253	GM 252
SM 1565-17	89	49	96	68	84	-.-	CM 9957	CM 9952	GM 280	GM 282	GM 281
SM 1411-5	100	79	51	82	41	81	-.-	GM 255	GM 272	GM 274	GM 273
SM 1219-9	103	90	81	82	76	78	61	-.-	GM 258	GM 262	GM 259
SM 1657-12	27	40	35	80	51	43	34	80	-.-	GM 288	GM 287
SM 2192-6	86	34	17	49	91	81	19	69	68	-.-	GM 290
SM 1665-2	92	73	84	82	85	91	56	98	71	77	-.-

[¶] 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 2.7. Number of transplanted plants (below diagonal) in CENICAÑA for the diallel experiment for the acid-soil savannas in the Easter plains of Colombia. The code assigned to each cross is above the diagonal [¶].

Parent	HMC-1	MTAI-8	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	105	-.-	CM 9127	GM 226	GM 235	GM 307	GM 266	GM 279	GM 301	GM 305
CM 7033-3	37	85	-.-	GM 219	GM 227	GM 245	GM 240	-.-	GM 241	GM 243
CM 4574-7	61	86	74	-.-	CM 9460	GM 225	GM 220	GM 221	GM 222	GM 223
CM 6740-7	70	77	95	84	-.-	CM 9642	CM 9901	GM 229	GM 232	GM 233
MPER-183	71	92	67	72	87	-.-	GM 265	GM 278	GM 300	GM 304
SM 1219-9	68	75	69	76	76	59	-.-	GM 256	GM 261	GM 263
SM 1565-15	61	86	-.-	74	85	83	82	-.-	GM 275	GM 276
SM 2058- 2	79	89	66	18	55	78	78	61	-.-	GM 298
SM 2219-11	85	91	74	80	88	72	88	90	114	-.-

[¶] 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. Number of transplanted plants (below diagonal) in CENICAÑA for the diallel experiment for the mid-altitude valleys of Colombia. The code assigned to each cross is above the diagonal [¶].

Parent	CM 6740-7	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	78	-.-	CM 9953	GM 254	GM 257	GM 260	GM 264	GM 265	GM 309
SM 1741-1	86	71	-.-	GM 269	GM 284	GM 292	GM 296	GM 297	GM 313
SM 1278-2	100	75	68	-.-	GM 267	GM 268	GM 270	GM 271	GM 310
SM 1636-24	82	66	88	12	-.-	GM 283	GM 285	GM 286	GM 311
SM 1673-10	85	69	60	67	42	-.-	GM 293	GM 294	GM 312
HMC-1	87	64	70	18	16	48	-.-	CM 9733	GM 314
MPER-183	87	76	51	53	30	51	68	-.-	GM 306
MECU-72	71	86	71	75	66	75	65	51	-.-

[¶] 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*.

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, 2001 are summarized below.

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 ecoregion are described in Tables 2.9 to 2.15. Table 2.9 lists all trials, whereas the other tables show results specific to each one.

A total of 3798 botanical seeds (targeting this environment) were planted, of which 1080 constitute the ***diallel trial*** that would also serve to identify superior clones. Stakes from those *F1* plants that were not part of the *diallel* experiment were planted in an ordinary *Clonal Evaluation Trial,* similar to that described below.

Table 2.9. Trials conducted in the subhumid ecosystem (North Coast of Colombia) in the 2000-2001 cycle¶.

Trial	Site	N° of genotypes	N° of reps	Observations
F1 (diallel)	Cenicaña	3798 (1080)§	1	In the new selection scheme the plants are left growing in the field for 10 months.
F1 C1	Santo Tomás	1024 (1))	1	Clones that produced < 8 stakes the previous year are given a second chance.
Clonal Evaluation	Santo Tomás	1350 (8)	1	See Table 2.10
Preliminary Yield Trial	Santo Tomás	57 (20)	1	Eliminated: flood in 1999 resulted in poor quality of seed and lack of uniformity in plant density.
Advanced Yield Trial	Santo Tomás	88 (25)	3	See Table 2.13
Regional Trials	Pitalito	60 (25)	3	See Table 2.14
	Santo Tomás	60 (25)	3	See Table 2.14
	Molineros	60 (25)	3	See Table 2.14
	C. de Oro	60 (25)	3	See Table 2.15
	Corozal	60 (25)	2	See Table 2.15
Seed Multiplication	Various	52.9 ha	-.-	

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

Table 2.10. Results of the selection carried out in the *Clonal Evaluation Trial* at Santo Tomás, Department of Atlántico, from 1350 families evaluated during May 2000 to May 2001.

Parameter or	**Yield (t/ha)**		**Harvest Index**	**Plant type**	**Dry matter content (%)**	
Genotye	Fresh roots	Dry matter	(0 to 1)[¶]	(1 to 5)[§]	March	May
		Results from the 1350 clones evaluated				
Minimum	0.00	0.00	0.00	1.00	0.00	0.00
Maximum	57.02	18.15	0.84	5.00	45.96	36.76
Mean	20.23	5.77	0.45	3.02	32.41	26.74
		Results from the 215 clones selected				
Minimum	19.76	6.23	0.39	1.00	25.96	16.64
Maximum	57.02	18.15	0.84	5.00	45.96	36.76
Mean	32.20	9.76	0.54	2.59	34.41	29.05
		Results from the 8 checks included in the trial				
Minimum	6.90	1.84	0.40	3.00	0.00	21.83
Maximum	34.17	11.02	0.69	5.00	37.45	36.76
Mean	23.77	7.29	0.55	3.88	31.62	29.25
Best 10 clones selected across the three strata for high, medium and low areas in the field						
SM 2546-44	53.09	18.15	0.64	2.00	34.31	35.00
SM 2546-32	54.88	16.24	0.61	2.00	32.95	28.53
SM 2771-5	51.55	16.21	0.64	2.00	35.49	30.12
SM 2615-13	47.62	14.82	0.48	4.00	38.57	28.22
SM 2615-28	51.31	14.77	0.64	2.00	35.12	26.73
SM 2629-36	38.09	12.17	0.60	2.00	37.07	30.21
SM 2621-1	34.29	11.34	0.45	2.00	38.38	31.33
SM 2769-15	39.64	10.95	0.62	3.00	31.81	26.19
SM 2775-2	41.43	9.71	0.69	3.00	27.08	22.20
SM 2769-11	30.24	8.64	0.63	1.00	33.04	27.09

[¶] 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.

Table 2.10 shows the results of the *Clonal Evaluation Trial* harvested in early 2001. This experiment was large because 1350 genotypes were evaluated, each represented by eight plants. This contrasts with the previous year when only 220 genotypes had been evaluated, each being represented by only six plants. The contributions of the Ministry of Agriculture and Rural Development 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.

Another reason for the reduced number of families evaluated in the previous cycle was the presence of frogskin disease, which led to the early elimination of many families. The fact that so many families could be evaluated this year with almost zero incidence of the disease (only two of a theoretical total of 10,800 plants had suspicious but non-definitive symptoms) attests to the efficiency of the preventive measures that were taken.

The experimental plot's large size (>1 ha) and the observation that cassava families planted in high, intermediate, and lower areas of the plot developed differently led to stratifying the plot into three. The genotypes located in the intermediate area performed notably less than those located in either the higher or lower areas. Selection was carried out separately for each "stratum", with the best 16% of materials in each being selected. With this procedure, we could reduce the environmental effect of terrain.

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 deteriorate by the time the rains arrive in May. Consequently, the *Clonal Evaluation Trial* (based on six plants) was 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. This 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 remaining six plants stayed in the field until the rains arrived and were finally harvested in mid-May, at which time root yield and dry matter content were measured again. Despite the duplication of work, the advantages of this procedure are great:

- ☞ Six plants are available as seed source, making available sufficient stakes for the following evaluation to be conducted with three replicates.
- ☞ Genotypes whose dry matter content does not diminish drastically upon the arrival of the rains can be identified. This has enormous impact for areas with bimodal rainfall distribution.
- ☞ Since data is taken and recorded on all genotypes (not only selected ones) it is possible to estimate *general combining ability* effects.

Table 2.10 summarizes the results of the *Clonal Evaluation Trial*. For yield, data from the March (two plants) and May (six plants) harvests were combined. Plant type was classified on a visual scale where 1 = plant with outstanding traits and 5 = highly undesirable plant. Yield of dry matter content, averaged across the experiment (1350 clones), was 5.77 t/ha, and across the selected fraction (215 genotypes) was 9.76 t/ha. To compare, the average dry matter yield of eight checks (including the best clone for the area, MTAI 8) was 7.29 t/ha. The best check yielded a maximum of 11.02 t/ha, while the best of the selected clones produced 18.15 t/ha.

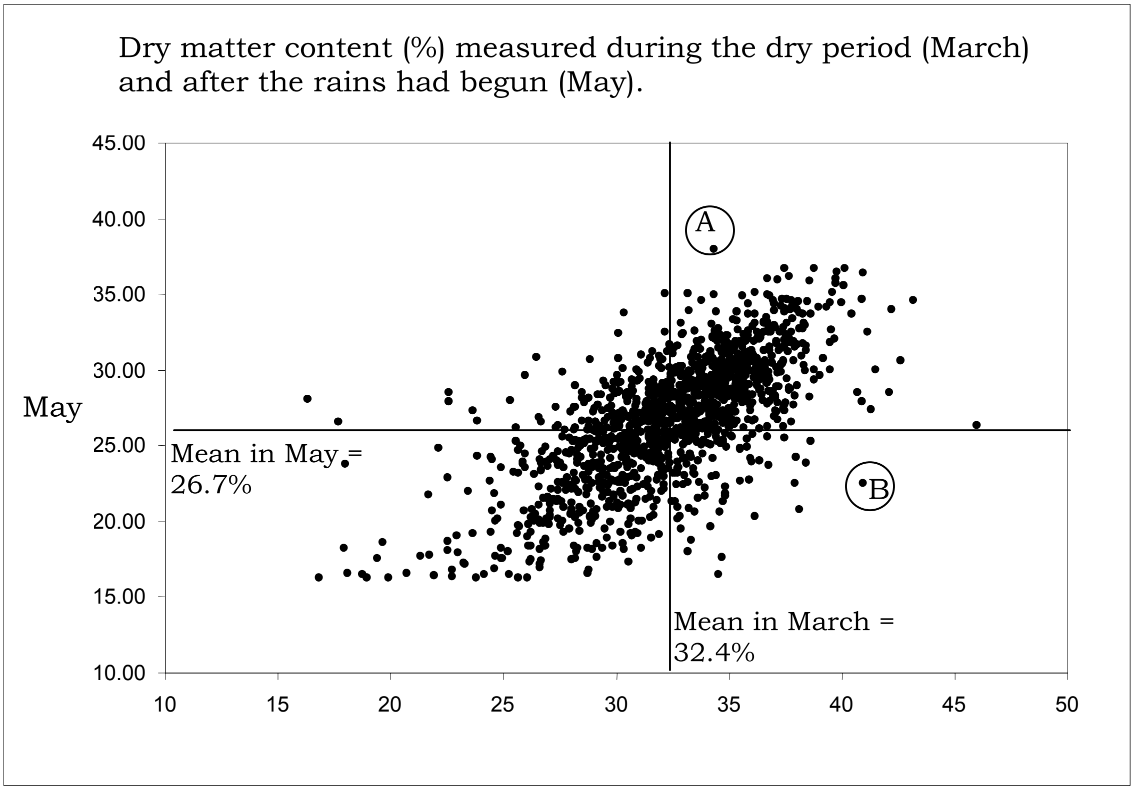


Figure 2.2. Dry matter content (%) measured for 1350 genotypes in March and again in May. To provide points of reference, averages are marked, together with a group of materials that maintained dry matter content of more than 33% after the rains arrived. Clones A and B are discussed in the text.

The final section of Table 2.10 presents the results of the best 10 clones across the three strata. As mentioned above, clones located in intermediate areas presented a markedly lower performance than those of the upper and lower areas. The last clone listed in Table 2.10 came from intermediate areas of the plot, thus showing a poorer performance. Some materials with relatively low dry matter content were selected because they could meet certain needs of the fresh market, which usually requires intermediate levels of dry matter content.

The new procedure, for the *Clonal Evaluation Trial* permitted the measuring of dry matter content in each clone on two occasions: during the dry season (March) and after the rains arrive (May). Figure 2.2 illustrates the relationship between dry matter content in March and that in May for the 1350 genotypes evaluated.

From the information provided in Figure 2.2, one concludes that a close relationship exists between the two sets of dry matter contents, corroborated by a correlation coefficient of 0.689. However, observations made in March do not allow us to efficiently predict those materials that will present high dry matter content in May. For example, clone "A" in Figure 2.2 had high dry matter content in March (> 40%), but very low content in May (< 25%). In contrast, clone "B" showed a mediocre performance in March (< 35%), but it was outstanding (about 37.5%) after the rains arrived**.**

Figure 2.3. Differences in leaf retention as observed in the *Clonal Evaluation Trial* at Santo Tomás, Department of Atlántico. At 5½ months, some families were retaining leaves while others were letting them fall.

Because the crop needs to maintain high dry matter content after the rains arrive to supply the industrial sector of the North Coast or similar regions in the world, we proceeded to select a group of materials that stood out for this trait. Many were already among the 215 genotypes selected by their good general performance, but others were characterized only for their high dry matter content, even after the rains arrived (Table 2.11). The average dry matter content across all clones evaluated was 32.41% and 26.74% for March and May, respectively, thus indicating the significant impact of the arrival of the rains on this trait.

Another significant result obtained from the *Clonal Evaluation Trial* 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. Although in most materials (1225 or 90.7%), leaf abscission had already occurred in the lower 2/3 of the plant, the remaining 125 (or 9.3%) clones had still retained their leaves (Figure 2.3). Leaf retention capacity was recorded at that time.

Table 2.11. Dry matter content as measured in March and May 2001, of a group of clones selected for their good performance in this trait from the *Clonal Evaluation Trial*.

Pedigree	**Dry matter content (%)**		**Percentage of Retention** ¶
	In March	**In May**	
SM 2545-20	39.73	35.77	0.90
SM 2546-52	37.16	35.96	0.97
SM 2546-54	37.36	34.74	0.93
SM 2618-16	40.13	36.70	0.91
SM 2619-1	37.97	34.00	0.92
SM 2619-5	39.78	36.52	0.92
SM 2619-6	38.46	34.58	0.90
SM 2619-12	37.68	34.02	0.90
SM 2621-4	43.16	34.60	0.80
SM 2621-14	39.54	35.16	0.89
SM 2621-25	37.45	33.85	0.90
SM 2621-28	38.74	36.71	0.95
SM 2622-1	40.92	36.46	0.89
SM 2623-1	37.01	33.92	0.92
SM 2772-2	40.41	33.74	0.83
SM 2772-7	38.54	35.89	0.93
SM 2772-8	39.30	34.20	0.87
SM 2773-46	34.31	37.98	1.11
SM 2775-17	38.07	34.54	0.91
SM 2603-9	40.86	34.67	0.85
Mean	38.83	35.20	0.91
Minimum	34.31	33.74	0.80
Maximum	43.16	37.98	1.11

¶ Ratio between measurement in may over that of march.

Table 2.12. 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	Dry matter content (%)		Harvest index (0 to 1)		Fresh roots yield (t/ha)		Dry matter yield (t/ha)		Fresh root yield¶
	March	May	March	May	March	May	March	May	(t/ha)
Yes	32.15	28.51	0.55	0.50	27.05	24.12	9.16	6.95	24.96
No	31.48	26.27	0.48	0.44	21.91	18.89	7.08	5.10	19.75

¶ Weighted average of fresh roots yield taking into account the number of plants harvested in March and May.

Table 2.13. The best 15 materials and 5 principal checks from an *Advanced Yield Trial* in which 76 experimental clones and 12 checks were evaluated, Santo Tomás, Department of Atlántico.

Rank	**Clone**	**Harvest Index (0 to 1)** [¶]	**Dry matter Content (%)**	**HCN (1 to 9)**	**Yield (t/ha)**		**Selection Index**
					Fresh roots	**Dry matter**	
			Performances of the best 15 experimental clones				
1	CM 8288-46	0.62	31.7	8.0	62.8	19.9	31.44
2	CM 8803-7	0.64	34.3	8.5	48.0	16.4	25.50
3	SM 2085-7	0.56	30.9	8.5	59.2	18.2	22.38
4	CM 4843-1	0.68	33.8	8.5	44.1	14.9	21.42
5	SM 1789-52	0.57	31.5	7.0	54.6	17.2	19.99
6	SM 1427-1	0.65	33.6	8.5	43.4	14.5	18.72
7	CM 6182-8	0.68	37.1	8.0	30.9	11.4	18.66
8	SB 0216-9	0.59	32.1	9.0	47.4	15.2	15.30
9	SM 1759-29	0.55	34.6	4.0	40.1	13.9	14.34
10	SM 1438-2	0.59	35.8	8.5	34.6	12.4	14.31
11	SM 2081-34	0.57	34.2	8.5	39.9	13.7	13.63
12	SM 1619-3	0.49	35.4	9.0	38.5	13.7	12.78
13	SM 1669-7	0.6	35.6	7.5	33.3	11.9	12.70
14	SM 1411-5	0.58	33.8	9.0	38.5	13.0	11.24
15	CM 8288-43	0.51	35.3	9.0	35.6	12.5	10.25
	Mean	0.59	34.0	8.1	43.4	14.6	17.51
			Performances of the best five checks				
18	PAN 135	0.59	34.1	8.5	35.6	12.1	9.63
37	MTAI 8	0.53	35.2	8.5	26.7	9.4	1.40
39	VEN 169	0.56	32.3	8.0	34.7	11.2	1.32
41	COL 2215	0.57	35.6	5.5	23.2	8.3	0.78
42	CM 3306-4	0.51	37.2	5.5	20.5	7.6	0.77
	Mean	0.52	33.0	7.5	26.9	8.8	-6.22
			Results from the complete data set				
Mean	0.53	33.6	7.5	30.5	10.2	-0.2	0.53
Minimum	0.35	27.60	4.00	7.00	1.90	-48.4	0.35
Maximum	0.68	40.3	9.00	62.8	19.9	31.44	0.68
C.V. (%)	17.3	3.5	-.-	32.8	33	-.-	17.3
L.S.D.(5%)	0.2	2.7	-.-	17.3	5.8	-.-	0.2

[¶] Harvest index is obtained by dividing the production of commercial roots by total biomass (roots + aerial parts). Harvest indexes higher than 0.5 are preferred.

Table 2.12 presents the averages of different traits for the 1225 clones that did not retain their leaves and for the 125 that did. The notable difference observed between the performances of the two groups suggested that the capacity to retain leaves at 5 months of age (at a time when no marked water stress has yet occurred in the region) has, indeed, a profound effect on overall performance measured at 10 months of age. The materials that retained leaves yielded, on the average, 26% more fresh roots (24.96 versus 19.75 t/ha), which represents an addition of about 2 t/ha of produced dry matter. Furthermore, leaf retention was also observed to associate with higher dry matter content (between 1% and 2% more, depending on when it was measured) and with a higher harvest index (by about 10%). These results are significant in that a trait has been identified that is most likely to be of high heritability (i.e., easy to select and fix in populations adapted to the North Coast) and has a profound effect on the agronomic performance of cassava in this region.

Note the worsening performance overall that occurs as a result of regrowth, once the rains begin (Table 2.12). This situation, however, is temporary and the plants rapidly recover. The new evaluation procedure for *Clonal Evaluation Trial* permits identifying suitable clones for eventual release as elite varieties for the farmer. A significant change introduced this year was to measure and take data of *all* clones included in this type of trial. This permits obtaining averages (based on several clones) of the progenies of a given progenitor. The relative performance of all progeny of a father (or mother) is of great importance in identifying those materials that tend to produce good progenies. As has been explained, not all superior clones have the capacity to produce good progeny. The information used from these trials (technically called *general combining ability*) permits a more precise and scientifically sound response to the most relevant question for the plant breeder: What progenitors to use? This subject has already been mentioned and will be described in more detail in the section on selection for Acid Soil Savannas.

As reported in June 2000, rainfall in October and November 1999 was heavy in the North Coast. One consequence of this atypical phenomenon was that, in Caracolí, Atlántico, part of the plot carrying the nurseries was flooded. This situation not only affected the quality of the data of some of that year's trials, but also the physiological state of the resulting seed. The preliminary yield trials planted with that seed (57 genotypes in plots of 20 plants each) were discarded because of low and irregular germination. The stakes produced this year will be used for evaluating these genotypes again, hopefully in trials with more uniform plant densities.

From a selection and its seed, produced in Caracolí, an *Advanced Yield Trial* was planted (Table 2.13) This evaluation constituted 88 genotypes, each planted in plots of 25 plants each, with three replicates. The materials evaluated included 12 checks (MTAI 8 among them). The average dry matter yield of the 15 best materials (14.6 t/ha) was notably higher than that of the checks (8.8 t/ha). The ranges of variation for dry matter content were similar for both experimental clones and checks, although the average of the best 15 clones was slightly higher (34%) than that of the checks (33%). According to the selection index, clone SM 1438-2 occupied 10th place, SM 1669-7 13th, and SM 1411-5 14th—all were used as parents in the crossing blocks for this year (Table 2.1). In contrast, the excellent clone MTAI 8 occupied 37th place. This suggests that the excellent performance of the new materials will relegate MTAI 8 to a less important position, even though, until recently, MTAI 8 had no rival in this ecoregion. Incidentally, several of these clones are, in fact, progenies from MTAI 8. These same results are confirmed by other evaluations described below.

Table 2.14 presents data from three *Regional Trials* conducted in three environments of the sub-humid Caribbean: Pitalito, Santo Tomás, and Molinero. Fortunately, at the first two sites, the crop developed very well, without major biotic or abiotic problems. Molinero, however, suffered an intense drought from October 2000 to April 2001 and crop yields were severely affected.

These *Regional Trials* constituted the final stage of selection, and from these materials were selected those that will eventually be released by the Corporación Colombiana de Investigación Agropecuaria (CORPOICA). These trials evaluated 60 genotypes, including 4 checks. Of the 60 clones evaluated, only the results of the 15 best, together with four checks, are presented (Table 2.14). The order in which the materials are presented is established by averaging performance across the three sites according to the corresponding selection index. Of the best 15 clones, 10 were used as parent this year (Table 2.1).The yield of fresh roots at Molinero (13.1 t/ha) was notably lower than that at the other two sites (33.7 and 26.8 t/ha for Pitalito and Santo Tomás, respectively). The performance of the new materials was, on the average, superior to that of what had been the best clone in the North Coast (MTAI 8). Indeed, this excellent material occupied 10th place in this evaluation.

Table 2.14. Results from three *Regional Trials* conducted in the sub-humid Caribbean region. Sixty genotypes were evaluated, but only the 15 best performing clones are listed along with four checks. Clones have been listed according to their ranking (combined across the three sites).

Clone	**Pitalito (Department of Atlántico)**					**Stanto Tomás (Department of Atlántico)**					**Molinero (Department of Atlántico)**				
	Yield (t/ha)		**% dry matter content**	**Selection Index**	**Rank**	**Yield (t/ha)**		**% dry matter content**	**Selection Index**	**Rank**	**Yield (t/ha)**		**% dry matter content**	**Selection Index**	**Rank**
	Fresh Roots	**Dry matter**				**Fresh Roots**	**Dry matter**				**Fresh Roots**	**Dry matter**			
SM 1438- 2	52.8	19.8	37.6	34.9	1	38.7	14.2	36.9	20.3	4	18.4	6.4	34.1	22.7	4
SM 1665- 2	49.9	17.3	34.7	22.9	5	47.7	15.7	32.9	23.9	1	19.2	5.5	28.7	16.3	9
SM 1669- 7	37.4	14.1	37.7	23.2	4	30.3	12.0	39.5	17.5	7	17.2	5.7	32.6	21.1	6
SM 1778-45	41.2	14.4	34.9	14.9	9	36.3	12.5	34.1	16.0	10	16.7	4.9	29.2	12.4	13
CM 4919-1	37.0	13.0	35.1	17.0	7	34.0	12.0	35.2	18.2	5	12.3	3.9	31.9	7.1	21
SM 1669- 5	31.5	11.6	36.9	8.4	16	33.8	12.3	36.5	16.8	8	15.1	4.6	30.9	15.2	10
SM 1411- 5	34.9	12.5	35.4	7.7	17	33.1	11.1	33.5	8.7	18	22.9	7.0	30.5	32.1	2
SM 1565-17	48.6	15.4	31.6	9.9	14	36.3	10.5	29.2	8.3	21	23.3	6.2	26.5	24.3	3
SM 1511- 6	34.9	12.3	35.2	7.5	18	29.9	11.5	38.5	14.6	12	15.8	4.6	29.2	14.7	11
M TAI 8	33.3	11.8	35.6	7.2	20	33.0	11.6	35.1	15.9	11	14.9	4.6	31.1	10.6	16
CM 6119-5	30.6	11.5	37.6	12.6	11	29.1	11.0	37.6	13.2	15	12.7	3.8	29.8	2.6	27
CM 3306-19	42.7	12.7	29.8	-1.5	34	33.4	10.3	30.9	8.7	19	23.8	7.6	32.2	39.0	1
SM 1778-53	34.0	11.9	35.0	3.0	23	23.5	8.5	36.3	1.2	32	19.9	5.8	29.2	21.2	5
SM 1973-25	43.6	16.7	38.1	26.0	3	36.0	13.3	37.6	16.5	9	10.2	2.8	27.4	-15.4	50
M VEN 25	32.0	11.3	35.0	0.7	31	40.8	14.4	35.2	22.3	2	12.2	3.8	30.7	1.3	29
Mean [1]	39.0	13.7	35.3	13.0	14.2	34.4	12.1	35.3	14.8	11.6	17.0	5.1	30.3	15.0	13.8
St. Dev.	7.1	2.5	2.3	10.2	10.0	5.6	1.8	2.8	6.0	8.3	4.3	1.3	2.0	13.2	13.3
CG 1141-1	24.0	8.7	36.2	-7.2	43	22.0	7.6	34.7	-5.7	42	14.7	4.2	29.0	6.5	23
CM 3306-4	30.4	11.1	36.3	1.4	28	20.4	7.5	36.6	-10.7	48	12.2	3.7	30.8	-3.2	36
MCOL 1505	28.3	9.6	33.9	-10.7	46	30.0	10.2	34.1	1.9	31	11.2	3.0	27.3	-7.0	41
MCOL 2215	21.5	7.9	36.8	-11.8	49	19.8	7.3	36.4	-9.6	47	12.1	4.0	33.4	6.10	24.0
Mean [2]	27.2	9.7	35.6	-5.5	39.4	26.6	9.4	35.4	-0.4	34.0	12.5	3.8	30.2	0.7	30.6
St. Dev.	4.4	1.5	1.2	6.2	9.3	8.9	3.0	1.1	13.6	19.1	1.3	0.4	2.3	5.9	7.8
Mean [3]	33.7	11.7	34.8	0.0	30.5	26.8	9.3	34.0	0.0	30.5	13.1	3.8	29.1	0.0	30.5
St. Dev.	8.3	3.0	2.0	14.7	17.5	8.6	3.0	5.1	18.0	17.5	4.3	1.4	3.0	16.0	17.5

[1] Mean and standard deviation of the 15 best performing clones, [2] checks (including those among the 15 best clones), and [3] of the 60 materials evaluated.

Table 2.15. Results from two *Regional Trials* conducted in the humid Caribbean region. Sixty genotypes were evaluated, but only the 15 best performing clones are listed along with four checks. Clones have been listed according to their ranking (combined across the two sites).

	Corozal (Department of Sucre)				**Ciénaga De Oro (Department of Córdoba)**			
Clone	**Fresh root yield (t/ha)**	**Dry matter content (%)**	**Dry matter yield (t/ha)**	**Rank**	**Fresh root yield (t/ha)**	**Dry matter content (%)**	**Dry matter yield (t/ha)**	**Rank**
CM 4919-1	22.72	35.61	8.09	8	28.39	36.24	10.29	6
SM 1973-23	25.39	34.91	8.86	3	24.28	36.96	8.97	16
CM 7514-8	32.67	36.93	12.07	1	23.33	37.08	8.65	18
M BRA 384	24.11	34.38	8.29	6	27.22	34.04	9.27	14
SM 1669- 7	18.06	36.56	6.60	18	27.72	38.53	10.68	4
SM 1669- 5	25.89	35.69	9.24	2	23.44	35.61	8.35	20
CM 6758-1	21.06	34.78	7.32	11	25.39	37.53	9.53	11
SM 1665- 2	22.83	29.82	6.81	15	29.00	34.42	9.98	9
CM 6754-8	20.67	31.86	6.58	20	29.72	33.96	10.09	8
CM 6758-3	22.44	32.89	7.38	9	22.22	36.77	8.17	21
CG 1141-1	17.83	34.58	6.17	25	26.94	38.16	10.28	7
SM 1778-53	19.72	34.03	6.71	17	22.72	38.73	8.80	17
SM 1650-7	15.50	35.69	5.53	35	27.06	40.78	11.03	1
SM 1565-17	23.39	30.21	7.07	13	27.78	28.39	7.89	26
SM 1433- 4	27.72	31.63	8.77	4	19.61	35.89	7.04	36
CM 6119-5	19.78	36.40	7.20	12	19.56	39.64	7.75	29
SM 1759-29	14.56	34.04	4.95	39	27.44	39.01	10.71	2
SM 1438- 2	13.67	33.36	4.56	42	28.50	37.48	10.68	3
M TAI 8	18.72	30.90	5.78	32	26.44	35.08	9.28	13
SM 1973-25	15.89	34.62	5.50	36	24.61	39.12	9.63	10
Minimum	6.11	6.26	1.88	1	0.00	0.00	0.00	1
Maximum	32.67	41.91	12.07	60	29.72	40.78	11.03	60
Mean	17.52	32.88	5.78	30.5	20.35	35.15	7.29	30.5
St. Deviation	5.33	2.59	1.88	17.46	6.12	5.20	2.30	17.46

Clone:
SM 1433-3

Fresh Roots Yield:
84 t/ha

Site:
Ciénaga de Oro
(Córdoba, Colombia)

Area:
9.5 ha

Figure 2.4. Photograph of a proud farmer that harvested about 84 t/ha of fresh roots with the experimental clone SM 1433-4. Ciénaga de Oro, Department of Córdoba, Colombia.

Table 2.14 shows a phenomenon that frequently occurs: related genotypes occupying significant positions (e.g., SM 1778-45 and SM 1778-53, and SM 1669-5 and SM 1669-7). The same group of materials was also evaluated in the humid Caribbean (Departments of Córdoba and Sucre). Table 2.15 presents the most relevant results of trials conducted in Ciénaga de Oro and Corozal. As in previous years, a strong genotype-by-environment interaction was observed, since the performance of the clones varied drastically, depending on whether they were evaluated in the sub-humid Caribbean (Departments of Atlántico and Magdalena) or humid (Departments of Córdoba and Sucre). Clone CM 6754-8, traditionally, has performed well in this latter environment, while its performance in drier conditions is mediocre. The opposite occurs with clone MTAI 8, as corroborated by the current evaluations. However, some materials have also shown an acceptable performance in both environments, as in the case of clones CM 4919-1, SM 1665-2, SM 1669-7, SM 1669-5, SM 1438-2, and SM 1778-5. Sister clones such as SM 1665-2 and SM 1665-5 also perform well. Most of these high-performing clones have been included as parental materials for the next crosses (Table 2.1).

It was in this region (Ciénaga de Oro, Department of Córdoba) where a record in cassava productivity was observed. A new clone (SM 1433-4) yielded 84 t/ha in a commercial field of 9.5 ha. The maternal parent of this outstanding clone is MTAI 8.

2.4.2. Selections for the Acid Soil Savannas Environment

As for the Caribbean coastal ecoregion, only the most relevant experiments conducted for this environment are described below (Table 2.16) followed by the respective results for each type of evaluation. As for Barranquilla, many of the improvement activities developed for the Villavicencio area also benefited other regions.

Table 2.16. Trials conducted in the Acid Soil Savannas in the 2000-2001 cycle[¶].

Trial	Site	N° of genotypes	N° of reps	Observations
F1 (diallel)	Cenicaña	3402 (1080)[§]	1	In the new selection scheme the plants are left growing in the field for 10 months.
F1 C1	La Libertad	1178 (1) (1))	1	Clones that produced < 7 stakes the previous year are given a second chance.
Clonal Evaluation	La Libertad	1350 (7)	1	See Table 2.17 and 2.18
Preliminary Yield Trial	La Libertad	57 (20)	1	See Table 2.19
Advanced Yield Trial	La Libertad	72 (25)	3	See Table 2.20
		84 (25)		See Table 2.21
Regional Trials	La Libertad	27 (25)	3	See Table 2.22
	Matazul	27 (25)	3	See Table 2.22
	Restrepo	27 (25)	3	See Table 2.22
Seed Multiplication	Various	25.8 ha	-.-	

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

A total of 3402 botanical seeds resulting from crossings specifically directed to the Acid Soils Savannas Region was planted in *F1* (Table 2.16). Of this seed, 1080 constituted a diallelic trial. As already explained, this trial will not only provide valuable information on the heredity of traits important to this region, but will also enable clones to be selected, as normally happens in field observation stage. The *F1* material that will not be part of the diallelic study will be planted in a *Clonal Evaluation Trial* similar to that described below. Even though the *F1C1* evaluation was eliminated in favor of the new type of *Clonal Evaluation Trial* (Figure 2.1), when *F1* plants did not succeed in producing sufficient stakes (seven per clone in the case of the Acid Soil Savannas), they were nevertheless used and planted in their respective areas of adaptation. Seed from these plants was obtained and included in the *Clonal Evaluation Trial* planted in July 2001.

Table 2.17 shows the most relevant results from the *Clonal Evaluation Trial* in the Acid Soils Savannas Region, for which 1525 clones were planted, each represented by seven plants. 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 be carried out to ensure optimal pressure from these diseases 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. Figure 2.5 shows the *Clonal Evaluation Trial* and surroundings at about 1½ months after planting. 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 observable in Figure 2.5, demonstrates that their spreading role had been adequately fulfilled early in the season.

Good development of leaf diseases could be observed early, together with a wide range of variation for both cassava bacterial blight and superelongation (Figure 2.5). The fraction selected (Table 2.17), as a result, reacted well to leaf diseases (average of 2.61), compared with the average for the whole population (2.92). Similarly, the dry matter productivity improved (8.57 versus 5.47 t/ha), as did dry matter content (32.33% versus 30.41%), plant type (2.67 versus 3.64), and harvest index (0.50 versus 0.43). Finally, although, for this trial, checks were not inserted among the clones evaluated, the variety Reina (CM 6740-7) was planted in lateral furrows and in one central throughout the experiment. The general performance in the trial compared favorably with the performance of Reina.

Table 2.17. The most relevant results from *Clonal Evaluation Trial* evaluated at CORPOICA—La Libertad, Villavicencio, Department of Meta, Colombia, during June 2000 to March 2001.

Data set	**Disease Ratings (1 to 5)**	**Plant type[¶] (1 to 5)**	**Yield (t/ha)**		**Dry matter Content (%)**	**Harvest Index (0 to 1)**	**Selection Index**
			Fresh roots	Dry matter			
			Results from the selected fraction (240 clones)				
Mean	2.61	2.67	26.67	8.57	32.33	0.50	26.07
Std.Dev.	0.77	0.92	4.15	1.10	2.41	0.06	5.88
Minimum	1.00	1.00	17.71	5.89	24.23	0.38	18.54
Maximum	4.00	5.00	43.06	11.61	47.82	0.66	46.72
			Results from the complete data set (1525 clones)				
Mean	2.92	3.64	17.74	5.47	30.41	0.43	0.00
Std.Dev.	0.80	1.13	6.57	2.16	3.22	0.09	20.07
Minimum	1.00	1.00	0.00	0.00	0.00	0.00	-124.37
Maximum	5.00	5.00	43.06	11.61	47.82	0.66	46.72

[¶] 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.

Advantages of the new scheme: implications for the crop's genetics

The concept of the *general combining ability*, mentioned above, is illustrated in Table 2.18. A group of families is chosen ("SM" with only one progenitor in common, "CM" with two progenitors in common) in terms of the number of clones that constitute each family and of each family's contrasting responses to variables of agronomic importance. Of the 1525 clones evaluated, 240 were selected, that is, about 16%. Any family presenting an index higher than 0.16 of selected clones probably performed better than the overall population. As a result, the progenitor that identifies them had a better performance (in terms of its progeny) than did the average progenitor.

Those families with a higher proportion of selected clones were CM 9459 (i.e., CM 6370-2 × CM 4574-7), CM 9460 (CM 6740-7 × CM 4574-7), CM 9461 (CM 6921-3 × CM 4574-7), and SM 2786 (CM 6438-14). Note that among the parents is clone CM 6740-7, recently released as 'CORPOICA Reina'. These parents tend to produce progeny superior to that of the other progenitors in the same evaluation. Precisely this information led to the inclusion of all these materials (except clone CM 6370-2) as parents for crossings to be carried out during the current agricultural year (Table 2.1). Likewise, those clones whose progeny did not stand out (SM 1210-10, CM 8275-2, SM 1282-2, SM 673-1, SM 737-38, and CM 3997-1 were excluded. MPER 183 was included as progenitor because of the good culinary quality of its roots. Such a trait may have good industrial potential.

This type of analysis can give rise to additional information. For example, with regard to reaction to leaf diseases, the progenies of MPER 183, SM 1282-2, SM 1210-10, and SM 1411-5 (a clone adapted to coastal conditions, where disease pressure is not as heavy as in the Acid Soil Savannas) were clearly more susceptible than the overall population. Contrasting progenies were those of SM 1565-15 and

CM 6438-14—the latter is to be released soon and will be precisely characterized for its tolerance of diseases *per se*). Similar comparisons can be done for other variables, such as dry matter yield or dry matter content.

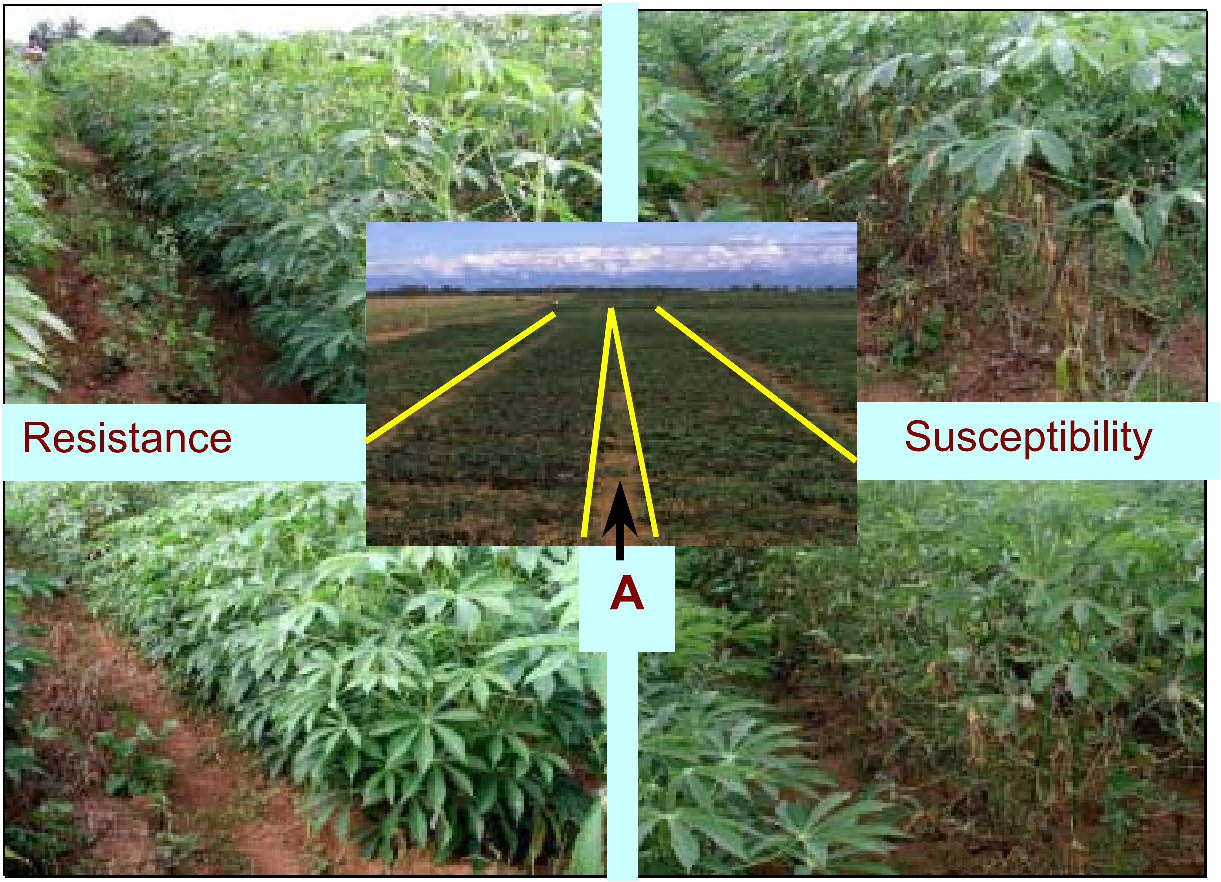


Figure 2.5. *Clonal Evaluation Trial* at CORPOICA—La Libertad, Villavicencio, Department of Meta, Colombia, 1½ months after planting (center). A = positions of disease-spreader plants. To the left and right, five-month-old clones illustrating contrasting phenotypes for disease reaction (resistant and susceptible, respectively).

The most relevant results of the *Preliminary Yield Trial*, in which 55 clones were evaluated, including several checks are presented on Table 2.19. The best check for the Acid Soil Savannas (Brasilera) occupied 9th place in this trial, according to the selection index. The best cultivar in this trial was 'CORPOICA Reina' (CM 6740-7).

Table 2.18. Averages of some families (with one (SM) or two (CM) progenitors in common) that participated in the *Clonal Evaluation Trial* evaluated at CORPOICA—La Libertad, Villavicencio, Department of Meta, Colombia. Contrasting families were selected to illustrate the concept of *general combining ability*.

Family	Nº of clones	Mother (father)	Proportion selected (0 to 1)¶	Disease rating (1 to 5)	Plant type (1 to 5)	Dry matter content (%)	Yield (t/ha)		Harvest Index (0 to 1)	Selection Index
							Fresh roots	Dry matter		
SM 2743	15	MPER 183	0.00	3.88	4.59	24.02	10.82	2.84	0.35	-35.80
SM 2733	46	SM 1210-10	0.00	3.07	4.70	26.46	10.82	3.01	0.34	-29.27
SM 2725	35	CM 8275-2	0.00	2.95	4.48	27.67	16.14	4.59	0.48	-8.35
SM 2609	38	SM 1282-2	0.03	3.36	4.48	28.59	12.06	3.61	0.41	-18.34
SM 2607	18	SM 1210-10	0.00	3.56	4.22	28.89	12.60	3.72	0.41	-17.08
SM 2220	12	SM 673-1	0.25	2.92	3.35	28.89	20.40	5.85	0.51	6.26
SM 2600	30	SM 737-38	0.00	2.77	4.11	28.89	12.95	3.88	0.40	-13.33
CM 9459	24	CM 6370-2 (CM 4574-7)	0.50	3.12	3.13	29.50	24.66	7.22	0.50	13.63
SM 2590	31	CM 3997-1	0.00	3.04	4.26	31.02	14.01	4.43	0.43	-7.71
CM 9449	12	CM 4574-7 (CM 4365-3)	0.25	3.01	3.09	31.02	18.98	5.91	0.44	6.08
SM 2792	75	SM 1565-15	0.21	2.34	3.13	31.02	18.63	5.85	0.41	5.88
SM 2632	26	CM 4574-7	0.23	2.51	2.91	31.32	20.22	6.35	0.42	8.86
CM 9460	42	CM 6740-7 (CM 4574-7)	0.40	2.89	3.20	31.32	23.24	7.28	0.43	11.79
CM 9456	16	SM 1411-5 (CM 4365-3)	0.13	3.45	3.24	31.63	15.61	5.03	0.46	0.55
SM 2634	21	CM 6438-14	0.24	2.48	3.64	31.63	20.22	6.35	0.42	6.52
SM 2635	12	CM 6740-7	0.25	3.24	3.68	31.63	22.00	6.89	0.47	10.15
SM 2636	44	SM 593-5	0.30	2.74	3.20	31.93	19.69	6.29	0.43	7.88
CM 9461	56	CM 6921-3 (CM 4574-7)	0.39	2.98	3.42	31.93	22.18	7.06	0.43	10.91
SM 2791	17	SM 1562-11	0.29	3.18	3.42	31.93	17.39	5.58	0.48	5.34
SM 2642	34	SM 1565-15	0.12	2.54	2.84	31.93	18.27	5.85	0.37	5.20
CM 9464	41	SM 1411-5 (CM 4574-7)	0.22	3.33	2.58	32.54	20.58	6.67	0.41	10.27
SM 2786	19	CM 6438-14	0.37	3.07	3.06	33.45	19.51	6.51	0.46	12.86
Mean of all progenies			0.16	2.92	3.64	30.41	17.74	5.47	0.43	0.00

¶ Proportion of selected clones over the total number of clones making up a given family.

The results of an *Advanced Yield Trial* conducted at CORPOICA–La Libertad are presented in Table 2.20. Seventy-two genotypes, 11 of which 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 and consisted of 150 clones. In both, the previous and this year, excellent performance was seen for a group of clones belonging to the SM 2361 family and derived from clone CM 4574-7, which, as already mentioned, is notable for its outstanding progeny (Table 2.18). The materials were ranked according to their respective selection indexes (first two columns, Table 2.20).

Table 2.19. Results of the *Preliminary Yield Trial* conducted at CORPOICA–La Libertad between June 2000 and March 2001, Villavicencio, Department of Meta, Colombia. The 15 best-performing clones are listed, together with 5 checks.

Rank	**Clone**	**Harvest index (0 to 1)**	**Dry matter content (%)**	**Yield (t/ha)**		**Selection index**
				Fresh roots	**Dry matter**	
			Results of the 15 best-performing clones			
1	CM 6740-7	0.58	31.3	39.6	12.4	40.45
2	SM 2455-5	0.49	32.0	38.8	12.4	39.04
3	SM 2366-32	0.45	27.7	29.6	8.2	11.57
4	SM 2558-1	0.52	27.0	29.6	8.0	11.32
5	SM 2552-8	0.47	33.3	27.1	9.0	26.94
7	SM 2561-11	0.34	26.8	26.7	7.1	1.57
8	SM 2552-12	0.42	26.2	25.4	6.7	0.18
10	SM 2555-11	0.56	33.0	24.2	8.0	24.66
11	SM 2552-5	0.46	29.0	22.1	6.4	5.98
12	SM 2555-2	0.35	30.9	21.7	6.7	8.43
13	SM 2465-4	0.50	29.2	21.3	6.2	6.70
14	SM 2559-6	0.57	30.6	21.3	6.5	13.26
15	SM 2559-3	0.58	31.2	20.8	6.5	14.82
16	SM 2554-4	0.50	30.0	20.4	6.1	8.09
17	SM 2552-2	0.37	31.1	20.0	6.2	7.36
	Mean	0.48	29.95	25.91	7.76	14.69
			Results from the five checks			
6	COL 1468	0.50	24.3	27.1	6.6	-1.38
9	Brasilera	0.48	32.9	24.6	8.1	22.57
19	Catumare	0.42	27.2	20.0	5.4	-3.84
27	COL 1684	0.54	28.8	18.3	5.3	2.52
32	Cebucán	0.42	26.3	16.3	4.3	-11.74
	Mean	0.47	27.90	21.26	5.94	1.63
			Results from the 57 genotypes evaluated			
	Mean	0.408	29.398	17.814	5.261	-0.01
	Minimum	0.110	22.000	2.500	0.700	-42.25
	Maximum	0.58	33.3	39.6	12.4	40.45
	St. Dev.	0.104	2.467	7.431	2.292	15.55

Table 2.21 presents the most relevant results of a second *Advanced Yield Trial*, also conducted at CORPOICA—La Libertad, with 84 genotypes. The table shows the best 15 clones, among which the variety "Brasilera" (MCOL 2737) stood out. This variety had been included as progenitor for the crossings (Table 2.1). Its performance was also significant, according to data in Tables 2.19 (9th place) and 2.20 (8th place). Because the clones came from different experiments, coincidence was unlikely to have influenced the results of the two *Advanced Yield Trials*, suggesting the true genetic superiority for this material.

Table 2.20. Results of an *Advanced Yield Trial* conducted at CORPOICA–La Libertad, Villavicencio, Department of Meta, Colombia, during June 2000 to March 2001. Boldface indicates the clones of the SM 2361 family. Only the results of the 15 best-performing clones are presented.

Rank	**Clone**	**Harvest index (0 to 1)**	**Dry matter content (%)**	**Yield (t/ha)**		**Selection index**
				Fresh roots	**Dry matter**	
			Results of the 15 best-performing clones			
1	SM 2366- 30	0.59	30.9	31.3	9.7	29.16
2	SM 2425- 3	0.59	27.5	37.1	10.2	28.86
4	**SM 2361- 34**	**0.53**	**33.6**	**25.6**	**8.6**	**25.06**
5	SM 2375- 13	0.63	30.5	28.7	8.8	24.61
6	SM 2458- 9	0.50	34.7	23.5	8.1	23.58
7	SM 2452- 6	0.45	35.0	23.2	8.1	22.07
9	SM 2454- 6	0.50	33.4	22.7	7.6	17.90
10	**SM 2361- 29**	**0.46**	**34.6**	**20.4**	**7.0**	**15.99**
11	SM 2380- 1	0.47	32.9	23.0	7.6	15.68
12	**SM 2361- 25**	**0.57**	**31.0**	**23.6**	**7.3**	**14.50**
13	CM 8288- 46	0.57	28.6	27.3	7.9	13.56
14	SM 2458- 1	0.46	32.3	22.6	7.3	12.61
15	SM 2461- 4	0.42	33.9	20.6	7.0	12.55
16	SM 2454- 1	0.42	33.2	21.2	7.0	11.39
17	**SM 2361- 30**	**0.50**	**33.1**	**19.6**	**6.5**	**11.21**
Mean 15 best clones		0.51	32.35	24.69	7.91	18.58
			Results from the five best checks			
3	CM 6740- 7	0.50	34.3	25.6	8.8	26.16
8	Brasilera	0.55	32.4	24.3	7.9	19.55
42	VEN 77	0.51	27.6	23.2	6.4	0.44
63	CM 2177- 2	0.36	28.8	14.0	4.0	-18.46
64	COL 1684	0.48	27.1	14.0	3.8	-19.32
Mean of 11 checks		0.45	28.6	14.8	4.4	-14.12
			Results from the 72 genotypes evaluated			
Mean		0.46	30.4	19.1	5.9	0.3
Minimum		0.28	24.60	3.50	0.90	-46.40
Maximum		0.63	35.00	37.10	10.20	29.16
C.V. (%)		12.9	4.8	23.1	24	
L.S.D. (5%)		0.1	3.4	7.7	2.5	

The results from *Regional Trials* conducted at three sites in the Department of Meta are presented in Table 2.22. As in the previous cases, the clones within each experiment were ranked according to their selection index at each site. From the rankings at each site, the average ranking across the three sites was obtained (second column, Table 2.22). The averages for the materials listed in the table were then ordered. Checks are underlined. Clone CM 523-7 is variety '*ICA-Catumare'*, released more than 10 years ago by ICA. Clones CM 6740-7 ('*CORPOICA Reina'*) and CM 6438-14 ('*Vergara*') both performed well. Several of the best genotypes listed in Table 2.22 had been included as progenitors for crossings this year (Table 2.1). Figures 2.6 and 2.7 show photographs of these two excellent materials that, doubtlessly, will have great impact on plains agriculture in the next few years.

Table 2.21. Performance of the best fifteen (out of 84 genotypes evaluated) in a second *Advanced Yield Trial* conducted at CORPOICA–La Libertad, Villavicencio, Department of Meta, Colombia, between June 2000 and March 2001.

Clone	**Dry matter content (%)**	**Plant type (1 to 5)**	**Yield**		**Harvest index (0 to 1)**	**Selection index**
			Fresh roots (t/ha)	**Dry matter (t/ha)**		
Brasilera	33.59	3.33	34.54	11.60	0.57	38.51
SM 1363-11	33.19	2.67	32.59	10.86	0.56	37.17
CM 8748-2	32.01	2.67	35.00	11.20	0.53	35.89
SM 1807-1	31.64	2.67	31.94	10.11	0.53	29.53
SM 1794-18	34.97	1.67	25.56	8.94	0.45	27.28
SM 2202-11	35.95	3.00	25.46	9.18	0.50	26.78
SM 1812-69	32.77	2.67	26.20	8.57	0.53	22.64
SM 2068-3	31.60	2.67	27.59	8.71	0.53	21.94
SM 2182-3	31.39	3.33	26.39	8.29	0.61	21.08
SM 1960-1	31.48	2.33	26.02	8.21	0.54	20.89
SM 2288-3	33.89	3.00	23.52	7.99	0.50	17.78
SM 2058-2	32.59	3.33	24.91	8.14	0.55	17.76
CM 8748-4	30.48	3.67	29.54	8.99	0.52	16.71
SM 1697-1	30.71	2.67	27.69	8.50	0.48	16.25
SM 2217-8	31.60	2.67	26.11	8.24	0.48	15.80
Results from all the materials evaluated in this trial						
Mean	30.20	3.34	21.18	6.46	0.47	0.00
Minimum	20.78	1.67	1.16	0.27	0.19	-3.62
Maximum	35.95	5.00	35.00	11.60	0.62	1.93
Results from the materials used as checks in this evaluation						
Mean	30.77	3.17	23.64	7.52	0.48	10.33
Minimum	20.78	1.67	1.16	0.27	0.19	-3.62
Maximum	35.95	5.00	35.00	11.60	0.62	17.78

Training and crop promotion events

Several activities were also carried out for training and crop promotion for the Acid Soil Savannas region. A highly important regional event—the successful release of variety *CORPOICA Reina*—was officiated on 10 November 2000 by the Minister of Agriculture and Rural Development Dr. Rodrigo Villalba (Figure 2.8) and supported by this project. Another relevant activity for the region was the execution of an intensive, 5-days course on modern cassava production and processing. Forty-eight technicians attended (Figure 2.9).

Table 2.27. Results from *Regional Trials* conducted in three representative locations of the Acid Soil Savannas ecoregion in the Department of Meta, in Colombia.

Clone	Rank	La Libertad				Matazul				Restrepo			
		Yield (t/ha)		% dry matter content	Harvest index (0-1)	Yield (t/ha)		% dry matter content	Harvest index (0-1)	Yield (t/ha)		% dry matter content	Harvest index (0-1)
		Fresh roots	Dry matter			Fresh roots	Dry matter			Fresh roots	Dry matter		
SM 1363-11	2.67	27.78	8.94	32.19	0.37	27.04	10.73	39.69	0.52	18.52	7.03	37.95	0.64
SM 1152-13	7.00	25.46	7.66	30.08	0.41	25.83	10.18	39.45	0.55	20.37	7.43	36.50	0.66
SM 1794-18	6.00	24.54	8.36	34.08	0.38	28.33	11.12	39.28	0.57	13.70	4.78	35.07	0.56
CM 6438-14	***12.33***	***15.19***	***4.94***	***32.52***	***0.44***	***23.33***	***8.86***	***37.96***	***0.48***	***23.24***	***8.66***	***37.23***	***0.65***
SM 1821-7	6.67	22.69	6.87	30.28	0.34	34.91	12.86	36.83	0.59	12.22	4.22	34.54	0.66
SM 1143-18	13.33	21.76	5.92	27.21	0.52	27.96	10.13	36.23	0.56	15.93	5.27	33.11	0.69
SM 1854-23	12.33	19.91	5.68	28.53	0.45	32.96	11.47	34.74	0.62	13.43	4.51	33.56	0.66
MBRA 502	13.00	20.65	7.04	34.10	0.46	17.41	5.98	34.34	0.40	26.57	8.88	33.29	0.61
CM 6921- 3	14.67	18.80	6.08	32.34	0.34	26.94	10.17	37.67	0.54	9.72	3.38	34.49	0.57
CM 6740-7	***12.67***	***18.98***	***6.24***	***32.90***	***0.47***	***23.15***	***8.25***	***35.63***	***0.59***	***14.07***	***4.77***	***34.04***	***0.61***
Brasilera	12.33	27.04	8.98	33.20	0.43	21.20	7.99	37.70	0.53	8.15	2.51	30.80	0.61
CM 4574-7	13.00	20.56	6.67	32.45	0.53	19.07	6.87	36.01	0.46	18.52	6.19	33.65	0.60
SM 1483-1	11.00	29.44	8.81	29.91	0.38	26.85	9.64	35.91	0.53	12.50	3.81	30.39	0.55
SM 2219-11	13.67	26.57	8.06	30.32	0.34	26.20	8.65	32.93	0.63	12.13	3.80	31.20	0.62
CM 6975-14	10.67	19.44	6.49	33.38	0.36	22.78	8.39	36.82	0.48	15.19	5.21	34.21	0.56
SM 1241-12	18.33	23.70	6.91	29.17	0.46	19.26	6.21	32.36	0.59	13.70	4.35	31.71	0.69
CM 523- 7	18.33	9.44	2.84	30.09	0.52	21.76	8.12	37.25	0.52	12.41	4.30	34.75	0.59
SM 1862-25	19.00	22.96	6.62	28.84	0.46	17.59	6.28	35.71	0.51	10.65	3.77	35.37	0.55
SM 1697-1	17.33	26.39	7.90	29.93	0.46	25.56	8.90	34.74	0.49	9.03	2.57	28.59	0.49
CM 7052- 3	19.67	21.02	6.14	29.19	0.44	24.07	8.15	33.83	0.51	13.89	3.95	28.55	0.61
SM 1812-69	18.33	25.09	7.69	30.66	0.52	22.78	7.75	34.16	0.59	6.20	1.70	27.33	0.57
SM 1694-2	22.33	20.19	6.80	33.69	0.42	13.61	4.74	34.74	0.40	9.44	3.23	34.21	0.50
SM 1565-15	17.00	16.94	5.06	29.84	0.38	23.06	8.54	36.99	0.47	11.76	3.86	32.43	0.58
CM 2177- 2	22.33	19.07	5.32	27.87	0.32	20.28	7.46	36.80	0.46	9.44	3.05	32.21	0.58
SM 1674-1	20.67	12.59	3.63	28.81	0.59	24.81	9.14	36.83	0.53	7.31	2.30	31.54	0.47
SM 1859-26	20.00	19.54	5.14	26.33	0.43	25.00	8.35	33.41	0.56	12.78	3.92	30.69	0.62
CM 7073- 7	20.67	18.61	6.04	32.44	0.49	15.83	5.65	35.52	0.48	7.87	2.55	32.39	0.45
CM 5306- 8	24.33	22.41	6.52	29.08	0.46	18.06	6.42	35.34	0.44	4.07	1.35	32.89	0.37
SM 2068-3	20.00	25.65	7.69	29.98	0.47	19.26	6.35	32.95	0.45	6.11	1.77	29.00	0.44
SM 1881-17	25.33	24.63	7.00	28.43	0.53	12.50	4.23	33.75	0.30	4.44	1.09	24.56	0.44
Mean	15.50	21.57	6.60	30.59	0.44	22.91	8.25	35.85	0.51	12.45	4.14	32.54	0.57
Minimum	2.67	9.44	2.84	26.33	0.32	12.50	4.23	32.36	0.30	4.07	1.09	24.56	0.37
Maximum	25.33	29.44	8.98	34.10	0.59	34.91	12.86	39.69	0.63	26.57	8.88	37.95	0.69

Figure 2.6. Cassava clone CM 6740-7 (‘*CORPOICA Reina’*), CORPOICA—La Libertad, Villavicencio, Department of Meta, Colombia.

Figure 2.7. Cassava clone CM 6438-14 (‘Vergara’), CORPOICA—La Libertad, Villavicencio, Department of Meta, Colombia.

Figure 2.8. Field day at CORPOICA—La Libertad, during the official release of cassava variety *CORPOICA Reina* (CM 6740-7), Department of Meta, Colombia.

Figure 2.9. Field activities during an intensive course on modern cassava production and processing conducted in collaboration with CLAYUCA and CORPOICA, La Libertad, Department of Meta, Colombia.

2.4.3. Selections for the Mid-altitude valleys

As for other ecoregions, the most relevant experiments conducted in the Valle del Cauca will be listed first (Table2.23) 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.

Table 2.23. Trials conducted in the Department of Valle del Cauca, Colombia, during 2000-2001.

Trial	**Site**	**N° of genotypes**	**N° of reps**	**Observations**
F1 (diallel)	Cenicaña	3402 (1080)[§]	1	In the new selection scheme the plants are left growing in the field for 10 months.
F1 C1	CEUNP	1178 (1))	1	Clones that produced < 7 stakes the previous year are given a second chance.
Clonal Evaluation	CEUNP	654 (5)	1	See Table 2.24
Preliminary Yield Trial	Palmire	123 (20)	1	See Table 2.25
Advanced Yield Trial	Palmira	60 (25)	3	See Table 2.26
	Palmira	61 (25)	3	See Table 2.27
Regional Trials	Palmira	27 (25)	3	See Table 2.28
	S.Quilichao	13 (25)	2	See Table 2.29
Seed Multiplication	Various	54.8 ha	-.-	-.-

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

Since year 2000, a process of cleaning materials at CIAT was initiated to eliminate or minimize the incidence of frogskin disease, possibly of viral or viroid origin. Figure 2.10 illustrates the symptoms of this disease.

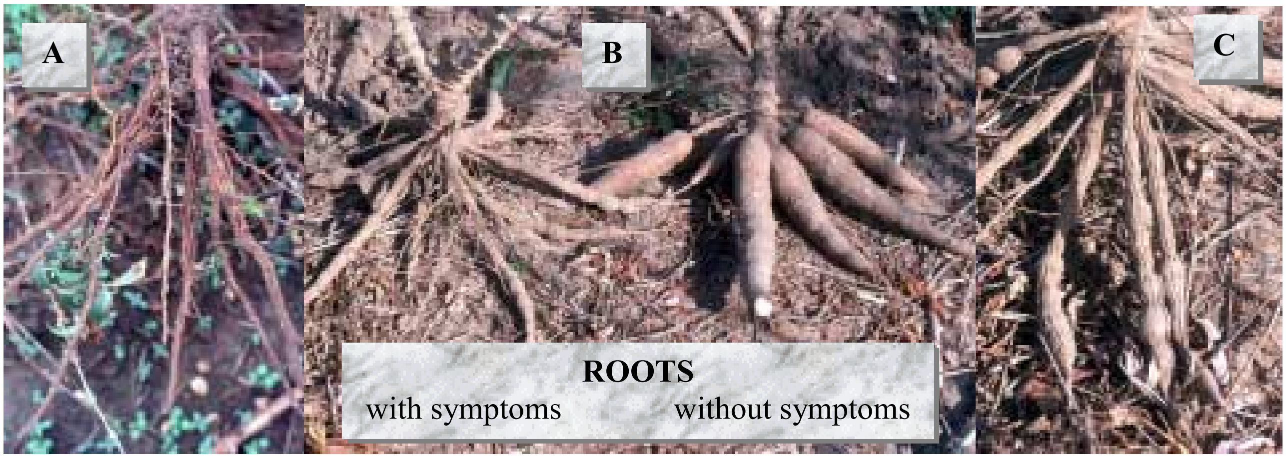


Figure 2.10. Symptoms of frogskin disease on cassava roots (A and C). Compare with the symptomless roots (B).

The problems of frogskin disease and whitefly

Most problems with frogskin disease are related to sources of inoculum and transmission by vector insects 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, as from 9 July of the current year, 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 highly efficient in reducing disease incidence, as described below.

Taking advantage of the presence of materials with variable levels of incidence of frogskin disease at the Agrovélez Farm (Jamundí, Valle del Cauca), the disease was monitored in three seed plots. The roots of each plant were carefully inspected to determine the presence or absence of disease. The locations of infected plants in the field were also established, because the spatial distribution of these plants can be highly useful in determining the relative importance of disease transmission. These data were taken from each plot of seed during harvest in February-May 2000, thereby eliminating plants that presented symptoms. The resulting seed was planted and harvested the following March-April 2001. The results for the 2 years are presented below for each variety.

Clone SM 1219-9: This clone presented intermediate levels of infection during the evaluation year 2000 (Figure 2.11). Of 4830 plants evaluated, 135 presented symptoms (2.8%) and were eliminated as seed source. The results for this year (Figure 2.12) show that of 1480 plants evaluated only 6 presented symptoms (0.004%). Four of the six plants were distributed more or less randomly in the field but two neighboring plants were observed to present symptoms. The probability that these neighboring plants were the result of contaminated cuttings that were then planted side by side is very low, suggesting that one of these plants may have been contaminated by its diseased neighbor.

Clone M Bra 383: Of the evaluated clones, MBRA 383 presented the least incidence of frogskin disease. Of 6800 plants inspected during the 2000 harvest, only 68 presented symptoms (1.0%). These plants were distributed more or less randomly in the field (Figure 2.13). During harvest on 10 May 2001, 1012 plants were evaluated, of which 11 presented symptoms, that is, about 0.011%. Again, the distribution of some sick plants suggested disease transmission, even though frequencies were considerably low (Figure 2.14).

Clone SM 1543-16: Of all the varieties, clone SM 1543-16 presented the highest level of incidence, initially, at 4.39% (159 plants of 3625 evaluated during the March 2000 harvest). After eliminating plants that were clearly or suspected to be contaminated, levels of disease incidence dropped markedly. During the March 2000 harvest, 32 of 10,452 plants evaluated were found to have symptoms (0.003%). These results demonstrate the effectiveness of inspecting roots before cutting stakes from each plant. Note that this system was applied to commercial plantings, implemented by company field workers, thereby demonstrating that the system is not only efficient, but also simple to implement.

The results from the *Clonal Evaluation Trial* harvested by the end of July are presented in Table 2.24. Due to lack of space in this out of station location only five plants per plot were planted in this trial. There was a remarkable variation for yield potential and a good pressure by white flies. It was on this trail that an experiment for a non-destructive electric technique for measuring root volume was used (see *Section 2.5.5.*)

Table 2.24. The most relevant results from *Clonal Evaluation Trial* evaluated at CEUNP, Cavasa. Department of Valle del Cauca, Colombia, during September 2000 to July 2001.

Data set	**White flies (1 to 9)**	**Plant type[¶] (1 to 5)**	**Yield (t/ha)**		**Dry matter content (%)**	**Harvest index (0 to 1)**	**Selection Index**
			Fresh roots	**Dry matter**			
			Results from the selected fraction (240 clones)				
Mean	4.15	2.49	55.37	21.19	38.54	0.59	23.23
Std.Dev.	0.87	0.75	12.02	4.16	2.26	0.07	7.88
Minimum	1.50	1.0	34.38	13.50	33.31	0.38	14.95
Maximum	5.50	4.0	104.69	39.53	43.88	0.72	57.62
			Results from the complete data set (1525 clones)				
Mean	4.43	3.13	37.48	13.83	36.87	0.53	0.00
Std.Dev.	0.70	1.00	14.00	5.20	2.80	0.10	16.60
Maximum	5.50	5.0	104.69	39.53	43.88	0.74	57.62
Maximum	5.50	5.0	104.69	39.53	43.88	0.74	57.62
FAMILY			Mean performances for each family (≈ GCA)				Success rate [§]
SM 2588	3.86	2.83	42.80	14.98	35.00	0.55	4 (18) = 0.22
SM 2661	5.17	3.06	37.46	14.30	38.17	0.53	2 (18) = 0.11
SM 2658	5.00	3.76	40.75	14.89	36.54	0.58	4 (21) = 0.19
SM 2659	4.04	2.50	52.23	20.68	39.59	0.49	8 (14) = 0.57
SM 2653	4.25	2.71	37.37	14.44	38.64	0.48	7 (14) = 0.50
SM 2647	4.63	3.93	25.79	9.91	38.42	0.37	0 (15) = 0.00
SM 2802	4.21	3.18	36.62	13.24	36.16	0.61	1 (28) = 0.04

[¶] 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.

[§] Number of selected clones out of the total number of clones making up each family (within parenthesis) = proportion of the total number of clones that were selected.

At the end of Table 2.24, an example of contrasting families has been included. Family SM 2588 had a much lower mean rating for white flies (3.86) compared with families SM 2661 and SM 2658 (with ratings of 5.17 and 5.00, respectively). Other examples of the usefulness of the concept of general combining ability are families SM 2659 and SM 2653 whose clones had about 50% of chances of being selected. On the other hand, families SM 2647 and SM 2802 scarcely had any of their clones selected. Looking at the common progenitor for each family, therefore, can be the best way of learning about the breeding values of those progenitors.

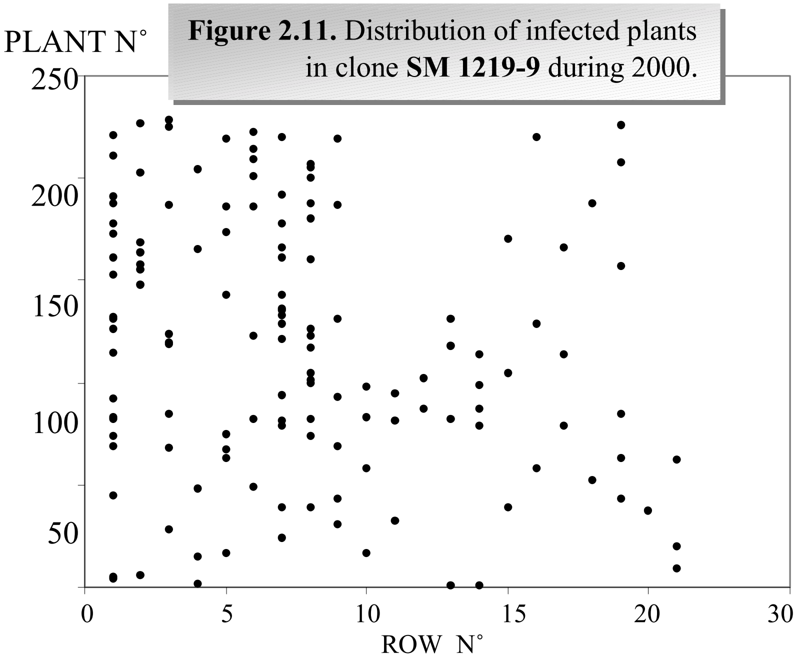


Figure 2.11. Distribution of infected plants in clone **SM 1219-9** during 2000.

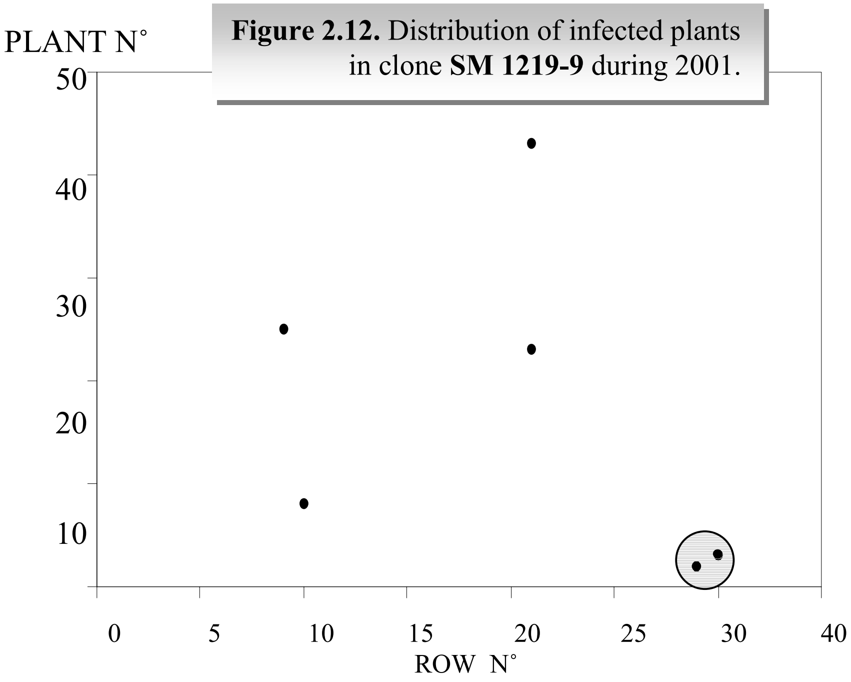


Figure 2.12. Distribution of infected plants in clone **SM 1219-9** during 2001.

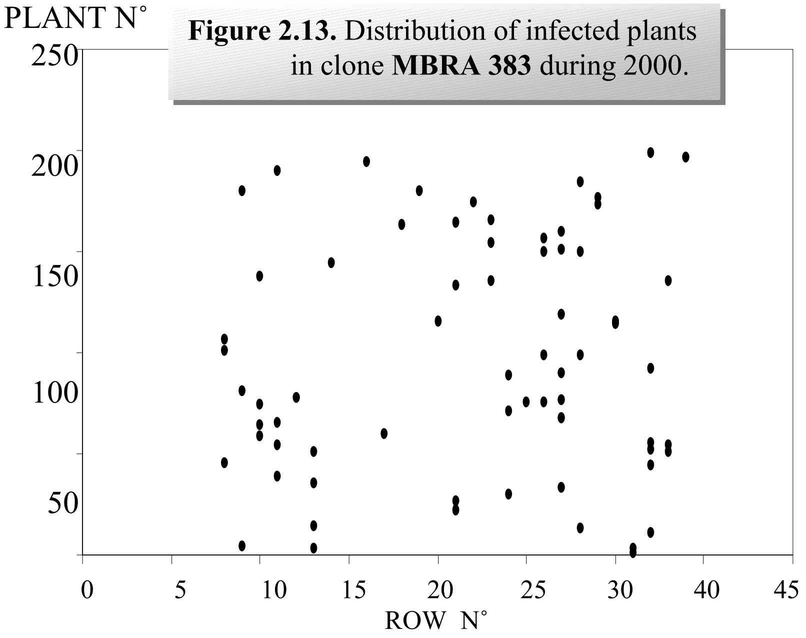


Figure 2.13. Distribution of infected plants in clone **MBRA 383** during 2000.

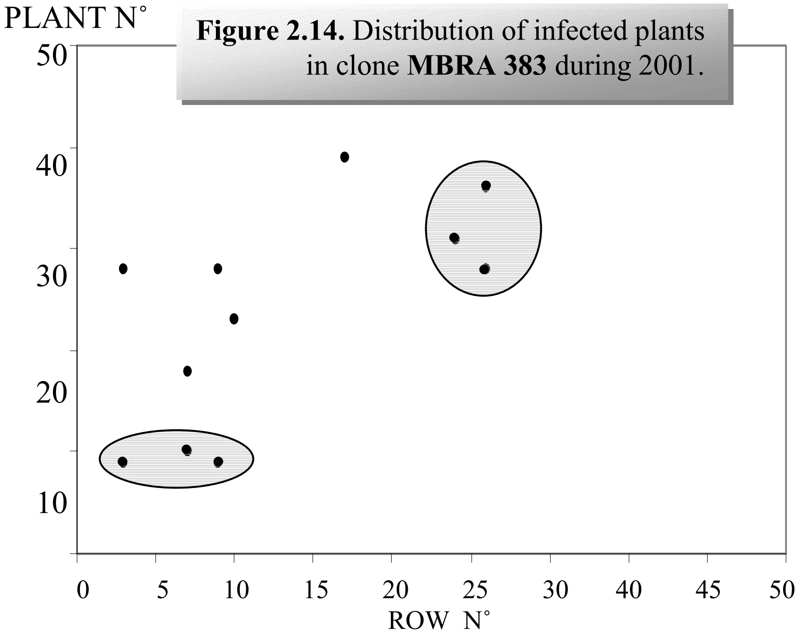


Figure 2.14. Distribution of infected plants in clone **MBRA 383** during 2001.

Results relevant to different trials.

Table 2.25 presents results of the *Preliminary Yield Trial* in which 117 genotypes and 6 checks were evaluated in plots of 20 plants each, with one replicate. As in the other trial report, materials were ranked according to their selection indexes. The best genotype did not yield the most dry matter per hectare, but occupied first position because its selection index was favored by its high dry matter content, which was notably superior (at 47.0%) to that of the remaining genotypes, which averaged across the trial at 33.5%. (This is a good example of how the selection index functions). The performance of the experimental clones was better compared with that of the checks used in the experiment.

The most relevant results of the *Advanced Yield Trial*, based on 60 genotypes, including experimental and check clones, planted in plots of 25 plants each, with three replicates are presented in Table 2.26. Again, the best genotype (according to the selection index) was not that which presented the highest yields. Clone SM 1778-44 occupied first place because it had the highest dry matter content across the entire experiment, a fact which was duly taken into account by the selection index. As in the previous trial, the experimental clones performed very well, compared with that of the checks.

Table 2.25. Results of the *Preliminary Yield Trial* harvested at CIAT, Palmira, Valle del Cauca, Colombia, where 123 genotypes were evaluated. The 15 best-performing clones are listed, together with six checks.

Rank	**Clone**	**Harvest index (0 to 1)**	**Dry matter content (%)**	**Yield (t/ha)**		**Selection index**
				Fresh roots	**Dry matter**	
			Results of the 15 best-performing clones			
1	CM 9024-13	0.47	47.0	19.5	9.2	48.33
2	SM 2361-18	0.53	34.7	38.5	13.4	39.20
3	SM 2555-9	0.59	35.0	35.5	12.4	37.28
4	SM 2448-19	0.56	37.1	30.8	11.4	36.25
5	SM 2462-3	0.58	33.6	33.3	11.2	29.54
6	SM 2540-3	0.52	36.4	25.5	9.3	25.47
7	SM 2366-35	0.79	33.2	25.0	8.3	21.51
8	SM 2452-11	0.46	38.5	19.2	7.4	21.41
9	SM 2551-5	0.43	33.7	28.5	9.6	19.25
10	SM 2552-6	0.41	35.3	24.8	8.8	18.36
11	SM 2463-2	0.53	35.7	21.2	7.6	17.36
12	SM 2382-5	0.51	32.8	27.7	9.1	17.28
13	SM 2548-6	0.44	34.8	24.2	8.4	16.69
14	SM 2458-13	0.38	38.2	17.8	6.8	16.50
15	SM 2554-4	0.45	33.0	27.5	9.1	16.14
	Mean	0.51	35.93	26.60	9.47	25.37
			Results from the best 3 performing checks			
51	MPER 183	0.54	31.2	20.8	6.4	3.14
54	MBRA 12	0.57	30.1	21.9	6.6	2.07
103	MVEN 77	0.41	30.0	12.4	3.7	-15.86
	Mean of all six checks	0.44	30.28	13.65	4.15	-12.41
			Results from the 117 genotypes evaluated			
	Mean	0.39	33.53	16.09	5.80	0.00
	Minimum	0.13	28.40	1.30	1.90	-27.64
	Maximum	0.79	47.00	38.50	13.40	48.33

Table 2.26. Relevant results of the *Advanced Yield Ttrial* carried out at Palmira, Valle del Cauca, Colombia. The 15 best-performing clones (out of 60 evaluated) are listed, together with 3 checks.

Rank	**Clone**	**Harvest index (0 to 1)**	**Dry matter content (%)**	**Yield (t/ha)**		**Selection index**
				Fresh roots	**Dry matter**	
		Results of the 15 best-performing clones				
1	SM 1778-44	0.52	40.0	27.7	11.1	36.00
2	CM 8370-11	0.52	37.6	31.9	12.0	35.80
3	SM 1779-7	0.49	38.6	29.3	11.3	33.23
4	SM 2141-1	0.46	38.9	26.7	10.4	28.51
5	SM 1855-15	0.52	36.3	28.5	10.3	25.95
6	SM 2058-2	0.53	33.5	32.5	10.9	24.56
7	SM 2211-3	0.45	36.8	24.5	9.0	17.85
8	SM 2082-1	0.45	35.1	25.1	8.8	13.63
9	SM 1778-53	0.40	38.3	19.9	7.6	12.60
10	CM 8774-1	0.41	37.8	20.2	7.6	11.97
11	CM 8370-10	0.42	36.6	21.6	7.9	11.06
12	CM 8759-14	0.51	33.3	25.3	8.4	10.79
13	SM 2198-4	0.38	37.8	20.1	7.6	10.61
14	SM 2052-4	0.52	33.6	24.3	8.2	10.40
15	SM 2202-1	0.48	34.8	23.0	7.9	10.29
	Mean	0.47	36.60	25.37	9.27	19.55
		Results from the best 3 performing checks				
18	HMC 1	0.53	34.9	20.3	7.1	7.95
32	MPER 183	0.54	31.5	22.7	7.1	1.96
53	MBRA 12	0.39	31.7	14.1	4.5	-18.14
	Mean of all six checks	0.43	31.22	15.03	4.77	-16.51
		Results from the 60 genotypes evaluated				
	Mean	0.43	34.5	18.7	6.5	0
	Minimum	0.28	28.9	7.2	2.7	-33.99
	Maximum	0.57	40.0	32.5	12.0	36.00
	C.V. (%)	11.0	3.2	20.7	21.2	-.-
	L.S.D. (5%)	0.1	2.9	7.0	2.5	-.-

Results of a second *Advanced Yield Trial*, in which 61 genotypes were evaluated, are presented in Table 2.27. The average dry matter yield of the best 15 clones was almost double (8.03 t/ha) than that of the check clones (4.67 t/ha)—a similar situation to that observed in Table 2.26. Table 2.28 shows the results of the regional trial conducted at CIAT, in which 27 elite clones were evaluated. Clone CM 8370-11 demonstrated high yield potential. This clone also had outstanding performance in other trials, such as described in Table 2.26 (first *Advanced Yield Trial*) in which it occupied 2nd place, even though it showed again the highest dry matter yield. This clone was not included in the second *Advanced Yield Trial* (Table 2.27), meaning that its performance cannot be compared with that of the genotypes that participated in the evaluation. Another, related clone (CM 8370-10) also appeared among the best clones in Tables 2.26 and 2.28. Finally, clone SM 2141-1 performed well in the *Advanced Yield Trial* (Table 2.26) and the *Regional Trial* (Table 2.28).

Table 2.29 gives the complete results of the second *Regional Trial* conducted in Santander de Quilichao (Department of Cauca), in which a smaller number of genotypes (21, including three checks) were evaluated. Because they came from different evaluations, little coincidence exists among the materials evaluated in the two *Regional Trials*. From the present cycle, we will start consolidating the group of materials that have reached the advanced stages of selection, so that they will be integrated into a single *Regional Trial* that will be planted in up to 12 different environments. These materials had been severely affected by two whitefly attacks, both in Palmira and Santander de Quilichao.

Table 2.27. The most relevant results of the second *Advanced Yield Trial* conducted at CIAT, Palmira, Valle del Cauca, Colombia. The 15 best-performing clones (out of 61 evaluated) are listed, together with 6 checks.

Rank	Clone	Harvest Index (0 to 1)	Dry matter Content (%)	Yield (t/ha)		Selection Index
				Fresh roots	Dry matter	
		Results of the 15 best-performing clones				
1	SM 2375-3	0.47	37.9	30.5	11.6	36.51
2	SM 2371-6	0.50	36.5	24.9	8.9	22.63
3	SM 2349-19	0.45	37.3	23.6	8.8	21.28
4	CM 9021-4	0.46	39.0	19.9	7.7	20.72
6	CM 9021-1	0.54	39.5	16.6	6.6	19.20
7	SM 2448-7	0.44	33.8	28.8	9.7	18.44
8	SM 2352-14	0.47	38.1	19.2	7.3	16.73
9	SM 2349-10	0.52	38.8	16.1	6.4	15.22
10	CM 8740-4	0.41	36.8	20.6	7.5	12.78
11	SM 2352-10	0.49	36.0	20.4	7.3	12.44
12	SM 2375-10	0.40	36.4	21.3	7.7	12.34
13	SM 2382-8	0.56	33.4	23.6	7.9	11.78
14	SM 2456-3	0.44	37.3	18.4	6.9	11.53
15	SM 2365-18	0.47	35.0	21.6	7.6	10.52
16	CM 8658-1	0.56	29.9	29.0	8.6	9.64
	Mean	0.48	36.38	22.30	8.03	16.78
		Results from the best 3 performing checks				
5	MPER 183	0.52	37.5	21.1	7.9	19.84
25	MBRA 12	0.43	36.0	17.1	6.2	4.40
32	MVEN 77	0.39	36.9	12.6	4.7	-2.06
Mean of all six checks		0.40	35.63	12.90	4.67	-5.37
		Results from the 61 genotypes evaluated				
	Mean	0.40	35.30	16.40	5.80	0.00
	Minimum	0.09	29.00	2.80	1.00	-33.17
	Maximum	0.56	39.80	30.50	11.60	36.51
	C.V. (%)	12.20	8.60	18.30	20.00	-.-
	L.S.D. (5%)	0.10	7.90	5.30	2.10	-.-

Table 2.30 presents results of sequential samplings, starting at 7 months of age, of 15 elite materials for the edaphoclimatic conditions of Valle del Cauca, and Northern Cauca Deparpents. This comparison of clone performance over time was carried out to identify early maturity, estimate other agronomic parameters, and determine degree of resistance to pests and diseases under different pressures. The most notable clones in terms of potentially high fresh yield (>40 t/ha) and dry matter production (>14 t/ha) are CM 7951-5; MPER183; SM 1460-1; SM 1741-1; MBRA 383; SM 1543-16, and SM 1219-9. Most of these clones are early maturing, together with clones CM 3306-4 and SM 1557-17. These results corroborate what is reported for 1999-2000. Close correlation was observed for fresh root yield between the experimental data obtained so far and estimated data obtained at the commercial level—42 and 35 t/ha for the two harvests carried out so far. All these clones (except for SM 1557-17) have been included as progenitors in the crossings (Table 2.1).

Table 2.28. The most relevant results of the *Regional Trial* conducted at CIAT, Palmira, Valle del Cauca, Colombia, in which 27 genotypes were evaluated in plots of 25 plants each, with three replicates. Only the 10 best-performing clones are listed, together with 5 checks.

Rank	Clone	Harvest Index (0 to 1)	Dry matter Content (%)	Yield (t/ha)		Selection Index
				Fresh roots	Dry matter	
		Results of the 10 best-performing clones				
1	CM 8370-11	0.55	36.6	34.5	12.6	35.14
2	SM 1855-15	0.61	36.9	22.1	8.1	17.67
3	CM 5655-4	0.54	35.2	25.1	8.8	15.63
4	SM 1871-33	0.44	36.0	24.5	8.8	14.34
5	SM 2141-1	0.45	37.1	21.9	8.2	13.65
6	CM 3306-4	0.37	40.8	14.4	5.9	10.29
7	SM 1642-22	0.59	34.4	22.4	7.7	10.28
8	CM 7514-7	0.38	41.0	13.0	5.3	8.89
9	SM 2160-2	0.46	37.5	17.7	6.6	8.33
11	SM 1210-4	0.44	37.0	16.7	6.2	4.71
Mean of best10 clones		0.483	37.25	21.23	7.82	13.89
		Results from the best 3 performing checks				
10	CM 523-7	0.56	34.7	19.6	6.8	5.84
15	MPER 183	0.52	32.5	19.7	6.4	-1.52
17	MCOL 1505	0.38	35.8	15.0	5.4	-3.15
Mean of all six checks		0.488	32.96	15.38	5.12	-7.99
		Results from the 27 genotypes evaluated				
Mean		0.45	35.3	17.1	6.1	0.00
Minimum		0.22	29.5	7.6	2.3	35.14
Maximum		0.62	41	34.5	12.6	-30.78
C.V. (%)		31.7	3.7	23.8	24.4	-.-
L.S.D. (5%)		0.2	3.9	6.1	2.2	-.-

Overcoming the whitefly problem

In recent years, whitefly populations have increased significantly in the farm fields of northern Cauca, greatly increasing production costs and severely reducing yields to such a point that many crops were abandoned. To solve this threat, the subcommittee proposed, at a recent meeting, the following points:

☞ Trials on various white flies control and management measures should be intensified.

☞ Varietal improvement at CIAT should be intensified and accelerated to obtain clones resistant to the pest.

☞ The ICA should conduct campaigns to disseminate information on whitefly control and management.

We have implemented a quarantine for cassava plantings in our Palmira station. From July 8 to August 9 there was no cassava plant left in the field. This measure was taken to brake the reproductive cycle of the different white fly species that affect our plots at this location. By November, when the new plots were almost four months old, it was obvious that this practice has resulted in a noticeably lower pest pressure.

Table 2.29. The most relevant results of a second *Regional Trial* conducted at Santander of Quilichao,Department of Cauca, Colombia, in which 21 genotypes were evaluated. Boldface indicates those three clones used as checks.

Rank	**Clone**	**Harvest index (0 to 1)**	**Dry matter content (%)**	**Yield (t/ha)**		**Selection index**
				Fresh roots	**Dry matter**	
1	SM 2061-12	0.49	34.4	24.0	8.3	26.99
2	SM 2219-11	0.63	32.0	24.5	7.8	26.73
3	CM 8658-1	0.61	31.1	22.9	7.1	19.92
4	MCOL1505	0.61	32.7	17.6	5.8	12.81
5	BRA 489	0.43	37.3	13.9	5.2	10.71
6	CM 6740-7	0.53	33.0	16.7	5.5	8.59
7	SM 2069-1	0.58	30.6	18.5	5.7	7.71
8	CM 849-1	0.58	30.4	18.7	5.7	7.59
9	SM 1881-9	0.54	31.6	16.5	5.2	4.60
10	SM 1871-45	0.53	30.6	17.2	5.3	2.93
11	SM 1245-25	0.52	30.8	17.0	5.2	2.67
12	SM 1860-27	0.53	26.1	21.4	5.8	-0.53
13	SM 1862-16	0.53	31.3	14.7	4.6	-0.57
14	SM 825- 9	0.53	30.6	15.2	4.6	-1.45
15	SM 2065-4	0.56	27.6	17.9	4.9	-2.82
16	SM 1870-24	0.57	25.1	20.7	5.2	-3.34
17	SM 1673-5	0.61	27.3	16.8	4.5	-4.15
18	SM 1460-1	0.54	29.8	14.0	4.2	-5.94
19	SM 1812-56	0.54	29.7	13.0	3.8	-8.42
20	CM 523-7	0.45	31.1	12.3	3.8	-9.48
21	SM 1557-26	0.37	32.0	9.7	3.1	-15.73
22	CM 2177- 2	0.56	25.6	10.8	2.8	-24.01
23	CM 6438-14	0.29	30.5	8.6	2.6	-25.44
24	CM 6850-2	0.51	26.7	7.8	2.1	-29.41
Mean		0.53	30.30	16.30	5.00	0.00
Minimum		0.29	25.10	7.80	2.10	-29.41
Maximum		0.63	37.30	24.50	8.30	26.99
Meanof xhecks		0.54	29.80	13.60	4.10	-6.89
C.V. (%)		11.20	5.90	28.90	29.00	-.-
L.S.D. (5%)		0.10	7.70	10.60	3.30	-.-

2.4.4. Selections for the humid Caribbean Region (Departments of Sucre and Córdoba)

The activities developed for this region are very much integrated with those of Barranquilla. For example, the results of the *Regional Trials* conducted in Sucre and Córdoba have already been described in Table 2.15 because they include the same genotypes used in the trials for the sub-humid Caribbean (Departments of Atlántico and Magdalena). The activities described here serve the humid agro-ecosystem, including the banana-producing region of the Urabá Antioqueño. In this region there is a growing need for starch production (recently a ban in the use of staples in the packages used for banana transport became effective, therefore creating a demand for glues that was not present short time ago).

In addition to the evaluation plots (namely *Regional Trials*), the activities in the region concentrate in seed multiplications and training. A total of 40 ha have been dedicated to the multiplication of seed of those clones that perform particularly well in this kind of environment. This region also was privileged with the official release of four cassava varieties by COORPOICA-Turipaná on 7 July 2000. Numerous farmers, researchers, and entrepreneurs of the region attended the event. The released varieties were *CORPOICA Rojita, CORPOICA Caribeña, CORPOICA Colombiana, and CORPOICA Sucreña*. The last two clones are, respectively, CM 3306-19 and CM 3555-6, genotypes that are related to other materials of broad dissemination in the North Coast and of proven agronomic virtues. These materials developed first through evaluations carried out by CIAT's genetic improvement project, then through evaluations and selections by CORPOICA. The first two clones are, respectively, SGB 765-4 and SGB 765-2, and were developed by CORPOICA, using participatory research methodologies. The botanical seed and *F1C1* plants that gave rise to these varieties were produced by CIAT. The event for releasing these varieties was financed by CIAT.

Eight training and/or promotion events took place in the region. In addition to the field day organized for the release of the four new varieties, five workshops were carried out jointly with CLAYUCA for appropriate seed production technologies and CIAT actively participated in the meetings for the "*Cadena Productiva Avícola*" (productive chain for the poultry sector). The area is an important producer of maize and cassava for Colombia.

2.4.5. Selections for the Middle Magdalena River Region

CIAT initiated its cassava activities in this region during 1999-2000. Progress was slow because the agreement initially proposed with the NGO "*Plan de Paz del Magdalena Medio*" could not be realized, and 8 months were lost. Finally, a research assistant was contracted directly to assume responsibility for the activities in this region. Because CIAT's previous experiences in this area were practically nil, CIAT began by first identifying the type of cassava that adapted well to the prevailing conditions.

Despite the delay in activities, four regional trials have been planted and will be harvested by the end of November 2001. These evaluations will help identify, on a sounder basis, those clones that are adapted to the region and have high and stable yields. For the rest of 2001, extensive plantings in support of initiatives of the poultry production sector are being carried out. The region may also be favored with significant support from the Colombian Government, who is seeking ways of relieving its serious problems of public order.

As in the case of the humid Caribbean region, the main activity here (in addition to *Regional Trials*) are seed multiplication and training. Up to 4.2 ha of multiplication nurseries were planted and several training events took place, starting with a two-days workshop on November 2000 when the Regional Trials were planted as part of the activities, and finishing with another two-days workshop by November 2001 at the time these trials are harvested.

This region is strategic for two main reasons: **a)** Bucaramanga concentrates a large proportion of the Colombian poultry industry, which urgently needs a local source of energy for their diets. **b)** The region is under tremendous social pressure, with strong presence of illegal crops farming and the presence of violent groups. Large poultry businesses established in this region, for the first time ever, started to grow their own cassava as a result of the promotion that CIAT and CLAYUCA have made. This has been a breakthrough development that only after three years of research and workshops could materialize. The production plots are located in the Middle Magdalena River Region and in the Córdoba – Sucre Departments (Humid Caribbean Region).

Table 2.30. Results of sequential harvests on the best genotypes available for the Mid-altitude valleys region. Agrovélez Farm (Jamundí, Valle del Cauca, Colombia).

Clones	**Dry matter content (%)**				**Fresh root yield (t/ha)**				**Dry matter yield (t/ha)**				**Fresh Forage (t/ha) ¶**	**HCN (1-9) §**	**Cooking quality (1-5) †**
	Months after planting				**Months after planting**				**Months after planting**						
	7	8	9	10	7	8	9	10	7	8	9	10			
CM 7951-5	36. 5	34. 8	36. 3	39. 8	40. 5	41. 1	57. 3	63. 0	14. 8	14. 3	20. 8	25. 0	5.8	4.5	1.0
MPER 183	28 .5	34. 9	30 .1	30. 1	33 .9	38 .4	36 .0	50. 8	9. 7	13 .4	10 .8	15. 2	6.6	3.0	3.0
SM 1543-16	34 .8	33 .2	34 .9	35. 8	32 .3	26 .8	36 .0	49. 3	11. 2	9. 0	12 .6	17. 6	4.6	6.0	4.3
SM 1460-1	34 .1	34 .5	35 .0	35. 2	38. 1	32 .8	38 .1	46. 5	13. 0	11 .3	13 .3	16. 4	5.3	6.0	3.0
SM 1741-1	36 .7	31 .2	37 .0	38. 8	45. 1	35 .3	38 .3	44. 4	16. 5	10 .8	14. 2	17. 2	5.3	5.0	3.0
SM 1557-17	34 .8	33 .0	35 .4	34. 6	37. 0	39 .8	36 .6	41. 5	12. 9	13 .1	13. 0	14. 3	4.1	4.5	3.0
MBRA 383	28 .5	34 .9	36. 8	38. 7	33 .9	38 .4	34 .5	41. 3	9. 7	13 .4	12. 7	16. 0	7.5	3.0	3.0
SM 1219-9	34 .0	32 .3	32 .4	36.3	34 .5	44 .4	33 .5	39. 8	11 .7	14 .3	10. 8	14. 4	5.9	7.5	5.0
SM 909-25	34 .7	30 .9	35. 9	37. 8	33. 8	34 .8	42 .5	39. 4	11. 7	10 .5	15. 2	14. 9	5.6	2.0	3.0
CM 7514-7	39 .1	38 .5	40. 7	41. 2	29. 1	29 .6	28 .4	36. 3	11. 4	11 .4	11. 5	14. 9	5.7	4.0	1.6
CM 3306-4	37 .4	36 .6	38 .0	39. 1	35 .1	29 .8	30 .5	34. 8	13. 1	10 .9	11. 6	13. 6	6.4	3.0	2.3
CM 6740-7	32 .6	33 .4	34 .4	36. 3	23 .9	37 .5	34 .0	29. 1	7. 8	12. 5	11. 7	10. 6	7.7	3.5	2. 3
CM 523-7	34 .4	34 .0	35 .7	34. 8	29 .6	33 .0	36 .1	23. 8	10 .2	11. 2	12. 9	8. 2	5.6	4.5	2.3
CM 849-1	33 .9	32 .5	33 .8	35. 5	19 .9	25 .1	21 .3	22. 0	6. 7	8. 3	7. 2	7. 7	7.2	5.5	5.0
SM 653-14	32 .6	16 .9	35 .0	36. 4	23 .9	33 .9	22 .5	19. 8	7. 8	5. 8	7. 8	7. 3	7.2	2.5	1.6
MEan	34. 8	33.4	35 .4	36. 7	32. 2	33. 6	35 .0	38. 8	11. 2	11. 2	12. 4	14. 2	6.0	4.3	2.9

¶ Yield of fresh forage averaged over the four harvest times.

§ Measured at ten months of age 1= low and 9= high cyanogenic potential.

† Evaluated at 10 months of age 1= excellent, 5= very poor.

2.4.6. Selections for the Tolima – Huila Departments.

The activities developed for this region cannot be described without first giving a heartfelt homage to our friend and colleague Jairo Bedoya who was responsible for this region until his premature death in December 2000. This lamentable event significantly affected CIAT's activities in this area, as it occurred while several agreements were being realized. These agreements could not be formalized and were eventually lost through lack of records. Nevertheless, seedbeds had been planted and several training and crop promotion events were carried out during 2000. Jairo's position has already been filled out.

During each visit to the Tolima – Huila Departments, the research assistant visits between 20 and 25 cassava farmers. He also keeps permanent contact with the personnel of the Cooperativa Agroindustrial del Tolima (COOPALTOL, Tolima), the Procesadora y Comercializadora de Alimentos, S.A. (PROCEAL, Neiva), Universidad Surcolombiana (USCO), CORPOICA, and the technical personnel of the municipal units for agricultural technical assistance or '*UMATAs*'.

This area is important for cassava production. There is a large fish industry localized in the region. Fish feed made out of cassava seems to perform better because it remains floating for a longer period of time. Also, a significant proportion, particularly in the Department of Huila, is very dry (much dryer than the Barranquilla region), therefore providing us with an ideal testing environment for drought, relatively close to Headquarters and with good access, and adequate infrastructure particularly roads wise.

2.4.7. Selections for the High Altitude Tropical Ecosystem.

The agroecosystem of high-altitude tropics is located in that part of the Department of Cauca where an important industry of cassava sour starch extraction operates. The region is characterized by low-fertility soils, mountainous topography, land tenure, and the presence of indigenous ethnic groups. Altitude ranges between 1400 and 1800 m above sea level. Although cassava production and processing in this type of environment is highly limited (less than 5% of national area planted), it has enormous social impact for the inhabitants. Although, as a result, genetic improvement activities are not on the same scale as those carried out for other ecosystems, they are still considerable, taking into account the area planted with this type of germplasm. Similar environments can be found in other countries in South America as well as in certain areas of Africa.

Table 2.31 describes the nurseries and seedbeds planted with cassava clones adapted to high-altitude tropics. Most of these plantings are located in the Department of Cauca, which is severely affected by whiteflies. This, in turn, has led to a high incidence of frogskin disease. In general, this environment itself leads to comparatively poor yields, because of the prevailing low temperatures, reduced heliophany (i.e. effective sunshine hours) and low soil fertility. The crop receives few or no inputs at all. Most activities are developed in the municipalities of Morales and Pescador.

Morales is a municipality of the Department of Cauca and is representative of the tableland conditions of Popayán. It was selected for having many adverse factors and stresses that would guarantee the production of improved cassava germplasm for high-altitude tropics (1400–1800 m above sea level), with resistance to *Phoma* spp., cassava bacterial blight, adaptation to low temperatures, and high dry matter yield for starch production. Table 2.32 gives the results obtained in the *Advanced Yield Trial* for this site, indicating the excellent performance of clones CM 7190-2, CM 7438-14, SM 850-1, SM 998-3, SM 1053-23, SM 1058-13, SM 1703-17, SM

1946-2, and MCOL 2740 ('*Sata*'), with yields (fresh weight) of more than 25 t/ha and dry matter yields of more than 11 t/ha. Pescador is also a municipality in the Department of Cauca, and is characterized by being an important cassava-producing area for the sour starch industry. It is located at 1500 m above sea level, has highly undulating topography, and low-fertility acid soils that are especially low in phosphorus. This project, carried out by FIDAR with support from CIAT, was to evaluate, multiply, and deliver to farmers three new improved cassava clones, developed by the cassava improvement program at CIAT. The check was the clone *'Algodona'*, widely disseminated in the region.

Table 2.31 Experimental nurseries planted for materials adapted to high-altitude tropics (1400-1800 m above sea level).

Site¶	Nº of clones	Type of nursery	Area (ha)
Morales	28	Yield trial	0.4
Morales	34	Yield trial	0.4
Morales	36	Yield trial	0.5
Jamundí	16	"Regional Trial"	1.0
Jamundí	8	Elite clones trial	0.5
El Tambo	5	Seed bank	1.0
El Tambo	5	Seed bank	1.0
Various	Several	Seed multiplication	6.48

¶ All sites are in the Department of Cauca, except for Jamundí, which is in the Department of Valle del Cauca.

Although, in this harvest, yield of the regional clone *Algodona* was heavily affected by frogskin disease, contracted by infected seed from farmers' fields, the results presented in Tables 2.33 and 2.34 show the farmers' order of preferences, after the methodologies developed by PRGA project on participative plant breeding.

The improved clones SM 707-17, SM 853-21, and SM 352-1, especially the first two, can be recommended for planting in Caucan hillsides and other similar ecosystems with altitudes as high as 1400-1800 m above sea level. These clones are superior to the regional *Algodona* for their (1) higher potential for yield and good starch content; (2) good plant type and seed production; (3) acceptable culinary quality and possibility for use in the region's fresh-root markets; (4) earlier maturity, making their harvest possible in a shorter time; and (5) resistance to diseases, especially those caused *Phoma* spp. and cassava bacterial blight. Farmers' preferences were determined by following the guidelines for participatory research developed at CIAT.

The farmers of northern Caucan hillsides, located at more than 1400 m above sea level face the problem that the few varieties of cassava adapted to their conditions are not resistant to cassava bacterial blight, a disease that can cause heavy losses, both in root yield and starch content and quality. To solve this problem, CIAT's cassava improvement program decided to hybridize clones from the Acid Soil Savannas, including MCOL 647 and SG 638-6, as sources of resistance to the disease. After many years of evaluations and selections, and with special thanks to the Ministry of Agriculture and Rural Development for its support in maintaining these trials, the following clones have been identified as being adapted to hillsides at altitudes of more than 1400 m above sea level, as having resistance to cassava bacterial blight, and as having excellent agronomic qualities: CG 402-11, CM 7138-7, SM 524-1, SM 707-17, SM 850-1, SM 856-11, and SM 1846-12.

Table 2.32. Results of the *Advanced Yield Trial* conducted at Morales, Department of Cauca, with materials adapted to high-altitude tropics¶.

Clone	Fresh root yield (t/ha)	Dry matter content (%)	Dry matter yield (t/ha)	Cooking quality (1 to 5)
CM 6748-3	17.3	40.1	6.9	1.0
CM 7138-12	22.3	37.5	8.3	5.0
CM 7190-2	27.3	40.4	11.0	4.3
CM 7438-6	15.5	38.8	6.0	2.3
CM 7438-14	27.4	38.0	10.4	3.6
CM 8106-4	21.1	41.6	8.8	2.3
SM 709-1	16.6	38.0	10.3	1.0
SM 850-1	27.6	37.6	10.5	1.0
SM 998-3	41.6	38.3	15.9	5.0
SM 1053-23	32.8	36.4	11.9	3.0
SM 1058-13	26.7	40.7	10.8	2.3
SM 1061-5	21.4	40.0	8.56	5.0
SM 1269-6	18.4	34.8	6.4	1.0
SM 1495-22	21.4	40.2	8.6	5.0
SM 1501- 23	17.3	38.5	6.6	5.0
SM 1703-17	31.7	40.3	12.7	1.0
SM 1833-21	26.0	39.2	10.1	1.6
SM 1834-40	16.7	36.6	6.1	4.3
SM 1835-28	22.6	38.8	8.7	5.0
SM 1933-5	27.8	37.5	10.4	3.0
SM 1937-1	30.9	39.5	12.2	5.0
SM 1938-12	24.7	38.8	9.58	3.6
SM 1940-3	28.7	38.0	10.9	3.0
SM 1944-10	26.6	39.1	10.4	2.3
SM 1946-2	25.9	37.9	9.8	3.0
SM 1946-4	23.6	38.8	9.1	1.0
Mean	24.61	38.67	9.65	3.06
Minimum	15.50	34.80	6.00	1.00
Maximum	41.60	41.60	15.90	5.00
		Check included in the evaluation		
MCOL 1522	28.9	35.6	10.2	3.6
MCOL 2061	11.3	37.8	4.2	3.0
MCOL 2261	25.3	35.2	8.9	1.0
MCOL 2740	28.6	36.6	10.4	3.0
CG 402-11	20.5	35.6	7.2	1.0
SM 524-1	17.4	36.9	6.4	5.0
Mean	22.00	36.28	7.88	2.77
Minimum	11.30	35.20	4.20	1.00
Maximum	28.90	37.80	10.40	5.00

¶ Culinary quality on a scale where 1 = excellent and 5 = very poor.

Table 2.33. Morphological traits of clones adapted to high-altitude tropics, Department of Cauca.

Clone	Plant height (cm)	Height 1st branch (cm)	Number of branching	Root shape	External root color	Internal root color	Constrictions in roots	Stakes / Plant (Nº)
Algodona	220	75	4	Cylindrical	Intermediate dark	White	Few	9
SM 707-17	170	65	3	Conic-cylindric	Dark	White	Few	6
SM 853-21	255	65	3	Irregular	Dark	White	Some	7
SM352-1	170	60	3	Irregular	Intermedoiate dark	White	Some	8

Table 2.34. Agronomic traits of clones adapted to high-altitude tropics, Department of Cauca ¶.

Clone	**Harvest Index (0 to 1)**	**Fresh root yield (t/ha)**	**Dry matter content (%)**	**Dry matter yield (t/ha)**	**HCN (1 to 9)**	**Cooking quality (1 to 5)**
Algodona	0.26	9.4	35.4	3.32	4	1.6
SM 707-17	0.56	34.3	35.5	12.1	4	3.0
SM 853-21	0.48	34.5	35.1	12.1	2	2.8
SM352-1	0.51	31.8	33.9	10.7	3	2.0

¶ Local check *Algodona* shows the detrimental effects of frog skin disease.

Activity 2.5. Other Research Initiatives

The CIAT cassava genetic improvement project conducts other activities that benefit, directly or indirectly, from the contributions of the Ministry of Agriculture and Rural Development and FENAVI-FONAV. These activities and their importance are summarized below.

2.5.1. Controlling physiological deterioration.

Rationale

Postharvest physiological deterioration (PPD) represents a major problem of storage and trade in cassava roots. Once harvested, cassava roots are difficult to manage. The lack of infrastructure to control PPD and the roots' high water content hamper transportation and raise costs, making the product more expensive. CIAT therefore initiated a search for alternative storage methods that would attenuate PPD under controlled conditions. One of the major constraints for research on PPD is the large experimental errors affecting its measurement.

Specific objectives

a) *Determine the effects of temperature and gas characteristics of the atmosphere on PPD.*
b) *Develop a chamber that will allow, under controlled environmental conditions, a better determination of the reaction to PPD of different cassava genotypes.*
c) *Ultimately, define environmental conditions (different from freezing) for delaying or reducing PPD.*

Materials and methods

Two contrasting varieties were evaluated for their reaction to PPD: MPER 183 (tolerant) and CM 523-7 (susceptible). The methodology used permitted the (1) evaluation of increase in shelf life of cassava roots when stored under low temperatures, and (2) design, on an experimental scale, a chamber for controlled atmospheres to evaluate PPD incidence under three distinct mixtures of gases with compositions low in O_2 and high in CO_2 (Figure 2.15). The trials were carried out in paired observations so that one treatment comprised controlled storage (positive control) and another was the check, with storage under environmental conditions (negative control). When storage times for both treatments were completed, each were analyzed and evaluated according to three variables: percentage of dry matter in roots on the day of harvest, PPD, and percentage of dry matter on the day of evaluation.

Figure 2.15. A chamber for controlled atmospheres designed to carry out applied research on the control of postharvest physiological deterioration in cassava.

Results

The evaluations showed that roots stored at temperatures between 8ºC and 10ºC, with a relative humidity at 80%, had delayed appearance of PPD, increasing shelf life from 2-4 days to at least 14 days (Figure 2.16). The controlled atmosphere treatments, with gas mixtures in compositions different from that of air, established two mixtures that delayed the appearance of PPD symptoms for 96 h. These mixtures were 5% O_2, 5% CO_2, and the balance in N_2; and 2.5% O_2, 5% CO_2, and the balance in N_2. The knowledge generated by this may reduce costs of raw material to the precooked and frozen croquette industry by 30% to 40%. This work constitutes a Thesis research proposal by Gloria Zapata Otalvaro, who recently obtained her degree with distinction.

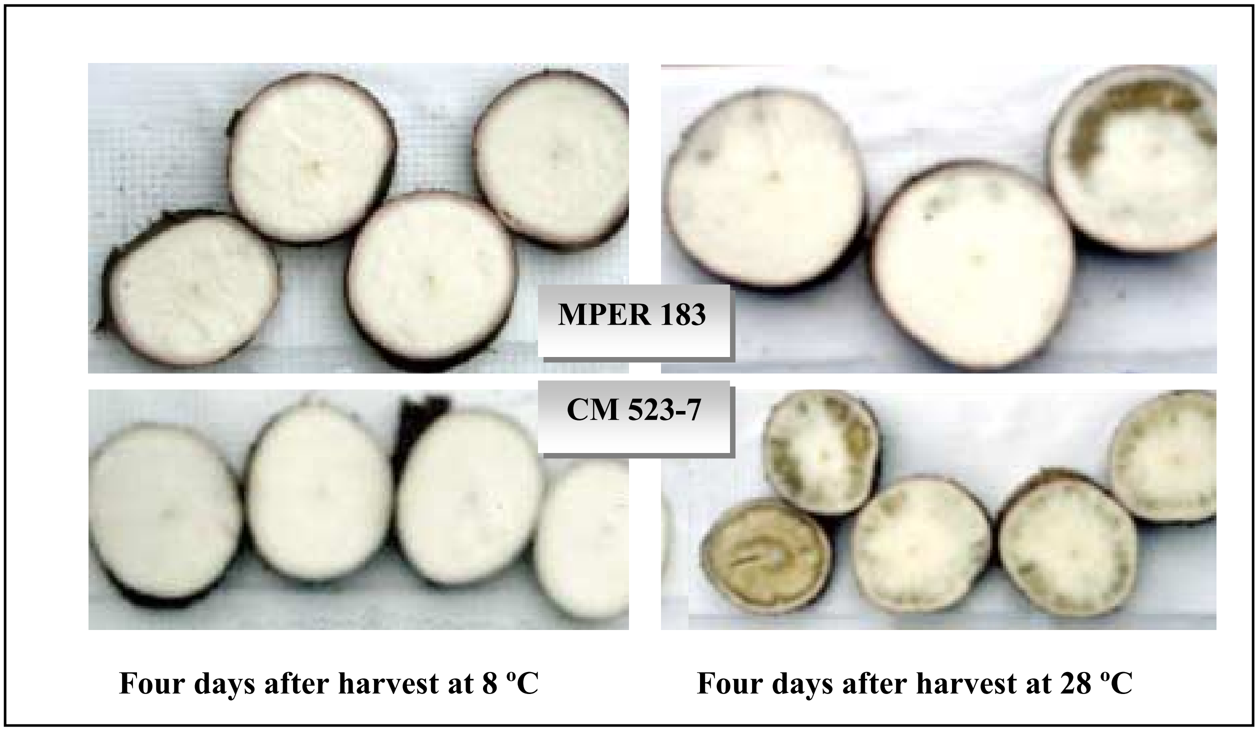


Figure 2.16. The effect of controlled environment on postharvest physiological deterioration of roots in cassava varieties MPER 183 (tolerant) and CM 523-7 (susceptible).

2.5.2. Development of progenies segregating for the trait 'root bullking capacity.

Rationale

An interesting trait, which perhaps bears a more obvious relationship to yield, is the capacity to accumulate starch in the roots. In the same crossing, one can observe that some clones present a normal root development, whereas others do not accumulate starch (Figure 2.17). Obviously, the incapacity to accumulate starches in roots is a highly undesirable trait. Nevertheless, this '*mutation*' offers an interesting option, because we can eventually identify the gene(s) responsible for starch accumulation in roots. A major achievement of the genetic improvement of crops has been to increase what is known in physiology as the '*sink strength'*. Briefly, this concept defines a plant's productivity in terms of (1) its capacity to photosynthesize at the *source* (i.e., leaves) of photosynthates, and (2) the *demand* for photosynthates by storage organs (e.g., ears, spikes, and roots). In many cases, limited crop productivity has been demonstrated to reside, *not* in its capacity to produce photosynthates, but in the ability of the storage organs to demand and absorb them.

Many factors affect the bulking capacity in cassava, even when proper canopy has developed (i.e. absence of foliar diseases and pests, or environmental stresses such as drought). Frog skin disease is known to restrict the flow of photosynthesis products to the root system. Also, some plants in segregating progenies have been observed to lack the bulking capacity required for economic yields in cassava. These plants are the subjects of our current interest.

Specific objectives

a) *Identify progenies with large number of individual genotypes in which a few show lack of bulking capacity.*
b) *Recover stakes from each member of such family and plant them again to confirm preliminary observations.*
c) *Ultimately, if segregation of bulking capacity is confirmed, develop populations for molecular markers.*

Figure 2.17. Segregation of cassava clones for the capacity (+) or lack thereof (-) to store starch in roots in different types of crosses.

Results

The practical usefulness of this study is the possibility that it offers for identifying, through molecular markers, the gene (or genes) responsible for defining *sink strength* in roots (or bulking capacity) so they accumulate starch. Although this allows the elimination of plants that do not have the molecular marker(s) that identify them as having sufficient *sink strength*. More interesting is the possibility of inserting multiple copies of the gene(s) within an individual with the expectation that this will result in increased *sink strength*.

Currently, segregating families have been selected from *Clonal Evaluation Trials* harvested in the first semester of 2001, and planted again. After repeating observations in the current planting for the incapacity to accumulate starch in roots, we will initiate crossings for the respective genetic studies.

2.5.3 Development of progenies segregating for the 'leaf retention' trait.

Rationale

From the *Clonal Evaluation* stage onward, it could clearly be observed that an obvious segregation existed for certain traits of great relevance to cassava productivity. It was already been mentioned the effect of leaf retention by a 5-6-month-old plant has on its root yield at 10-11 months old (Table 2.12). In the diallel studies to be conducted this year, special attention will be paid to this trait to elucidate its mode of heredity. Eventually, we will identify a group of clones from a single cross that will present an interesting segregation for this trait, that is, some will present good foliar retention whereas others will not.

With this type of segregating material, we will identify, using molecular markers, the gene (or genes) responsible for the heredity of this trait. Identifying a molecular marker for this trait will facilitate elimination of plants that will not retain leaves even while they are still seedlings, and before they are transplanted to F1 nurseries. In other words, instead of having only about 125 materials capable of retaining their leaves, as happened in the *Clonal Evaluation Trial* for the sub-humid Caribbean (Table 2.12), we will have 1500 clones expressing this trait. This increases immensely the possibility of identifying high productivity genotypes.

Specific objectives

a) *Confirm that leaf retention at 5-6 months of age is indeed closely related to yield potential.*
b) *Develop segregating populations for the genetic study of the trait.*
c) *Ultimately, develop molecular markers for the trait (particularly if it is based on one or few genes).*

Results

The most important step has already been given when the trait was found to have a potential role in defining yield potential. Results already found strongly support this hypothesis (Table 2.12). In the diallel studies already planted, families exposing a good segregation for this trait will be identified. The relationship of the leaf retention trait will then be reasserted but now in a common genetic background. If results are positive, the different plants of these segregating families will be used for molecular markers studies.

2.5.4. Preparing a manual on modern cassava production, processing and use.

Rationale

Several breakthrough events took place on cassava research and development in the past few years which have not been properly publicized and promoted. Both CIAT and CLAYUCA felt the need to summarize all recent developments in a publication that would serve the need of producers, processors and scientists involved in cassava research, production and use.

Specific objectives

a) *Write the different chapters of the book.*
b) *Contribute with the editorial process.*
c) *Find financial support for the publication of the book.*

Results

Since May 2000, most of the personnel of CIAT cassava genetic improvement project and and CLAYUCA's, has been preparing manuscripts for the manual on *Cultivo de la yuca en el tercer milenio: Sistemas modernos de producción, procesamiento y utilización* [Cultivating cassava in the third millennium: modern systems of production, processing, and use] to be presented at AGROEXPO 2001. The manual will have about 600 pages of text and an inside pocket book for identifying symptoms of nutritional deficiencies, insect pests, and diseases. The project's personnel have helped prepare the following chapters:

☞*La yuca en Colombia y el mundo: Importancia económica y usos*
[Cassava in Colombia and the world: its economic importance and uses]

☞*Morfología y taxonomía de la planta*
[Plant morphology and taxonomy]

☞*Semilla y métodos de multiplicación rápida*
[Seed, and methods for rapid multiplication]

☞ *Los recursos genéticos de* Manihot *en el Centro Internacional de Agricultura Tropical*
[The genetic resources of *Manihot* held at the International Center of Tropical Agriculture]

☞*Control de malezas*
[Weed control]

☞*Mejoramiento genético de la yuca*
[Cassava genetic improvement]

☞*Biotecnología de la yuca*
[Cassava biotechnology]

The external financial resources have been found and CIAT Publication's office has contributed with the time of their personnel for edition and design. This is yet another example of the synergistic and positive relationship between CIAT and CLAYUCA. The book is due for publication before the end of 2001.

2.5.5. Evaluation of a non-destructive, electric system for measuring root development.

Rationale

Cassava is a perennial crop. There is no physiological development that determines when the crop is ready for harvest similar, for instance, to the '*black layer*' in then maize kernels. In other words the crop can be harvested any time, as long as there has been enough time for starch accumulation in the roots. The crop typically is harvested at about 10 months of age, but in many areas particular genotypes can be harvested as early as at six month of age. Several factors determine the age the crop is harvested: **a)** enough time must be given for the plant to store commercial yields; **b)** depending of the time of the year, environmental conditions severely affect dry matter content of the roots (see Tables 2.10 and 2.11); **c)** Environmental conditions and the age of the plant also influence HCN content in the roots. **d)** In subsistence farming, cassava is left as a reserve source of food and is harvested only when needed.

There is plenty of evidence that large genetic variation exists regarding early bulking capacity. However, because of logistic reasons evaluation trials are harvested at 10 months of age, therefore missing the possibility of identifying those early producing clones. One problem is that harvest means destruction of the plant and, usually loosing the vegetative seed that, at this early stage of breeding, is very valuable and scarce. Developing a non-destructive method for monitoring root bulking is, therefore, highly desirable.

Specific objectives

a) *Evaluate the electric capacitometer and correlate its measurements with root production at different plant ages and on different genotypes*.

b) *Determine the best measuring procedures*.

c) *Determine factors potentially affecting the correlation between electric measurement and root mass*.

Materials and methods

Two experiments were carried out. In the first one four different varieties, thought to have contrasting early bulking capacities were planted, so that sequential harvest could be performed starting at three months of age hrough the tenth month. The second experiment was done on 361 segregating clones from the *Clonal Evaluation Trial* planted at CEUNP (see Table 2.24), all harvested at 10 months of age. Based on previous report on this subject (Dalton, 1995; Mualem and Friedman, 1991; van Been, 1997) the plots were dully irrigated before capacitance measurements and harvest, so these activities were carried with the soil at field capacity (or very close to this condition).

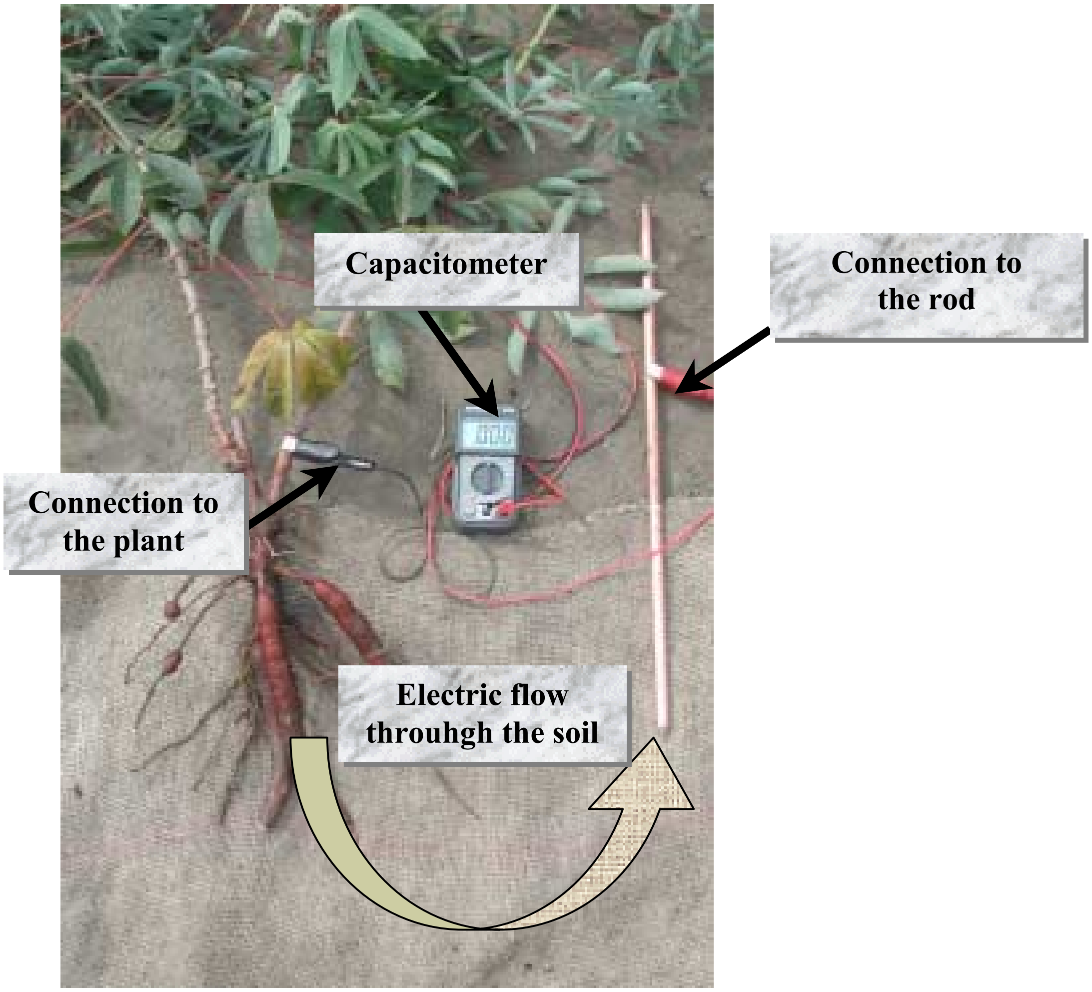


Figure 2.18. Illustration of the way the electric capacitance measurement works in the field. Measurements are taken to the plants still in the field (not harvested).

Results

This research is also part of a Thesis work by a student (Jorge Eliécer González G.) from National University of Colombia (at Palmira) who successfully presented his thesis. Figure 2.18 shows the system as used in the field, with the exception that plants mere measured for the electric capacitance while standing in the field prior to harvest. For the first experiment two of the four varieties chosen presented varying degrees of frogskin disease and were, therefore, not considered for the study. Figures 2.19 and 2.20 illustrate the relationship between fresh root yield and the capacitance measurement for MPER 183 and SM 1557-17, respectively.

First experiment.

Some interesting results were observed. The general correlation between fresh root yield and capacitance, across genotypes and harvesting time, was 0.63 and statistically highly significant. Similar results were obtained for dry matter yields. However, there was a considerable variation in the value of the correlation coefficients along time (Table 2.35). High correlations were found either at the beginning or at the end of the season for MPER 183, but somewhat lower values for the eight and ninth months. The second variety (SM 1557-17) had much higher values at all times, except for the last harvest (tenth month).

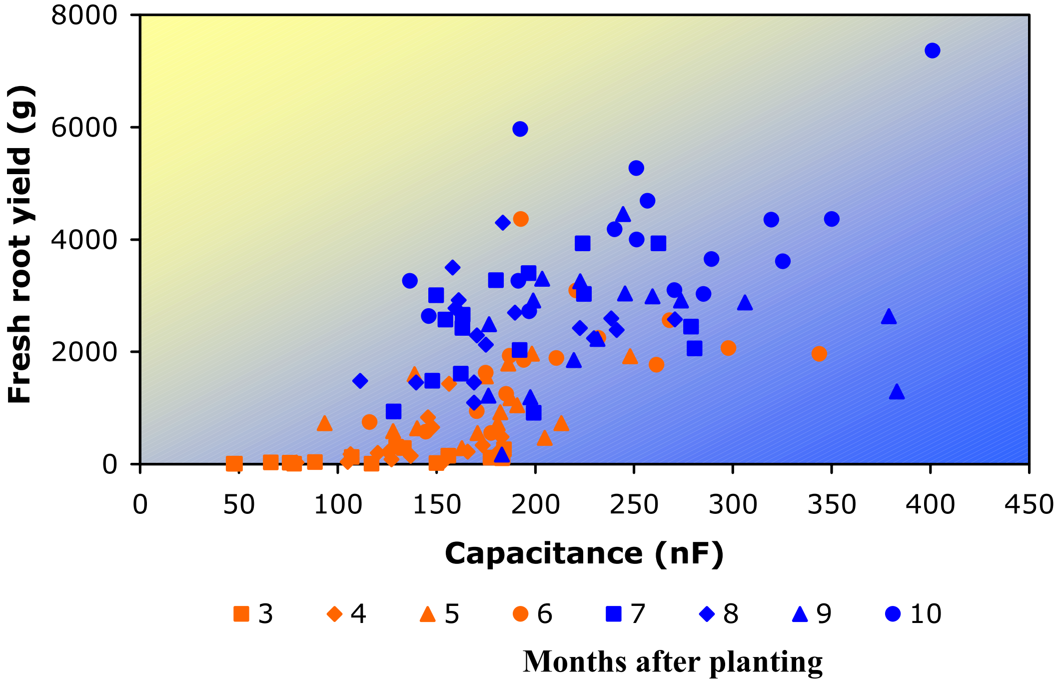


Figure 2.19. Relationship between fresh root production and electric capacitance in clone MPER 183 in successive harvest from the third to the tenth month of age.

Several alternative regression models were considered. Globally (data from both clones and at all harvesting times), the best model was the exponential ($Y= 28.972\ e^{0.019x}$) with a R^2 value of 0.43. The exponential relationship is reasonable because at early stages (months 3, 4 and 5) there will be very little amount of harvested roots, but yet the root system will be extensive enough for the capacitometer to measure values different from zero.

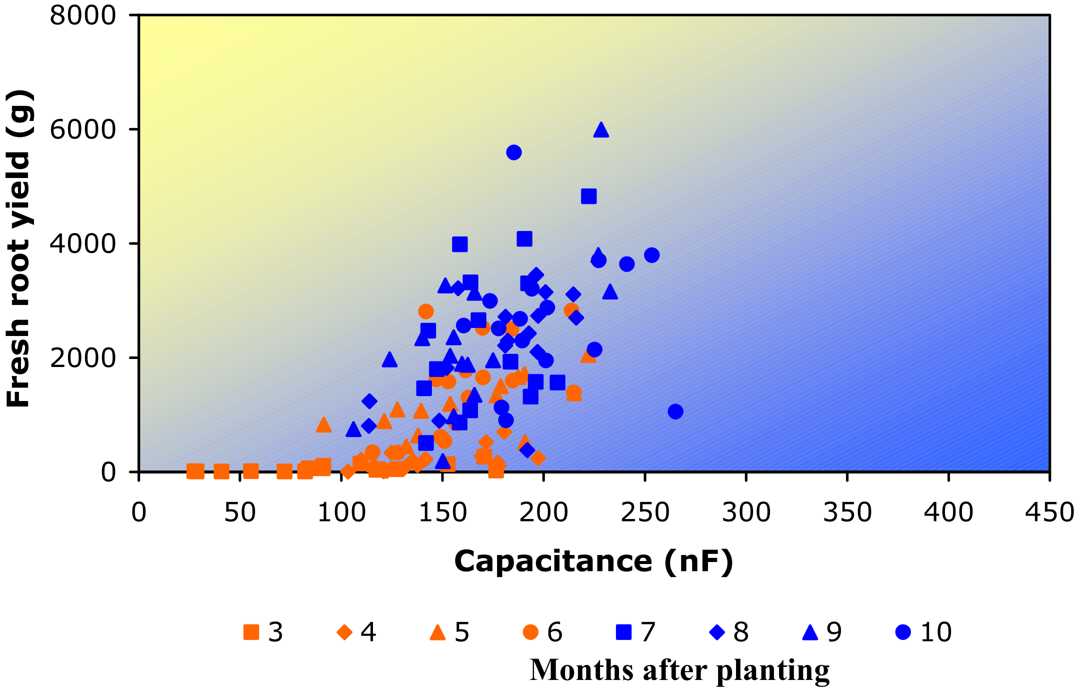


Figure 2.19. Relationship between fresh root production and electric capacitance in clone SM 1557-17 in successive harvest from the third to the tenth month of age.

Table 2.35. Correlation coefficients and statistical probabilities (within parenthesis) between fresh root production and electric measurement on two different cassava clones from the third to the tenth month of age.

Clone	**Age (in months) of harvested plants**							
	3	4	5	6	7	8	9	10
MPER 183	0.56	0.38	0.38	0.39	0.37	0.23	0.14	0.50
	(0.024))	(0.150)	(0.149)	(0.133)	(0.160)	(0.398)	(0.618)	(0.048)
SM 1557-17	0.60	0.54	0.71	0.55	0.41	0.60	0.71	0.06
	(0.014)	(0.031)	(0.002)	(0.028)	(0.116)	(0.015)	(0.002)	(0.838)
Both clones together	0.59	0.38	0.51	0.45	0.36	0.40	0.30	0.50
	(0.000)	(0.033)	(0.003)	(0.010)	(0.046)	(0.024)	(0.094)	(0.004)

Second experiment.

The nature of the second experiment was very different from the first. Basically a much larger number of clones was evaluated, with a larger contrast in yield potential than that observed in the first experiment. Also, only one harvest time was used this time. This experiment simulated the way the system would be used for detecting early bulking germplasm.

For all the 361 clones electric capacitance was measured on four plants (the first plant was avoided). Harvest and yield measurement was carried out individually for each of the four harvested plant in the first 78 clones. For the remaining 283 clones the roots of the four harvested plants was pooled together for measuring yield. The correlation coefficient between the individual plant measurements in the first 78 clones was 0.52, whereas the correlation between pooled yield (combining roots from the four plants), and the average capacitance (also across the four plants of each plot) was 0.50. Furthermore the data set was divided according to dry matter content. Table 2.36 shows the results of this analysis. The degree of association between yield potential and the capacitance measurement tends to be stronger in low dry matter content conditions. This may be a result of the better electric conductivity of water over starch.

All this results, although preliminary, are very promising (see also Figure 2.20). There is a need for further refinement of the measuring method, including measurements realized at frequencies different to the one used (1 kHz), and improving the electric contact, particularly between the wire and the stem of the plant. Also it is fundamental that soil moisture is uniform and at field capacity.

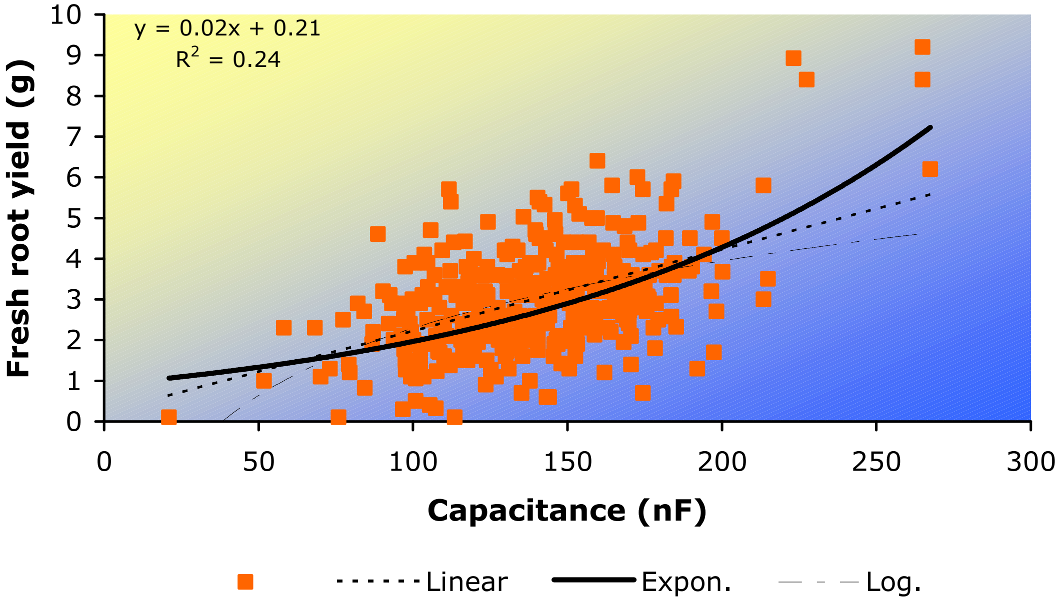


Figure 2.20.Relationship between capacitance (nF) and fresh root production (g) in all the measurements obtained from the *Clonal Evaluation Trial* at CEUNP (second experiment). Out of several alternative regression models evaluated the simple linear model was the best, with a determination coefficient R^2=0.24.

Table 2.36. Correlation coefficients between electric capacitance and root yield in 361 genotypes grouped according to their dry matter content in the roots.

Dry matter content (%)	Correlation	Probability¶	Nº of clones
30.0 - 31.9	0.70	0.0075	13
32.0 – 33.9	0.81	0.0001	32
34.0 – 35.9	0.45	0.0008	52
36.0 – 37.9	0.42	0.0001	106
38.0 - 39.9	0.26	0.0082	102
40.0 - 41.9	0.59	0.0001	42
42.0 – 43.9	0.19	0.5195	14

¶ Probability of correlation coefficient being equal to zero.

2.5.6. Evaluation of phytohormones in their capacity to alter plant architecture.

Rationale

Plant architecture in cassava is as important as in any other crop, with the addition that this trait has an effect on the capacity of the plant to produce stakes, a situation unique for cassava and observed but in few other crops. The architecture of the plant depends greatly on the flowering of the plant. In effect, when the plant flowers a terminal inflorescence appears at the end of the stem effectively breaking the apical dominance of the stem. Two or more branches will then develop. Depending on the number of flowering stages the plant goes through, the number of branching levels that the plant will have. Some genotypes do not flower, and therefore only one or two single stems develop. These erect types are preferred by many farmers because they facilitate greatly the labors to take care of the crop, particularly weeding. They also produce large amount of stakes. One problem for this plant type is that since they are the result of the absence of flowering, eventually they end up being a biological dead end (they could not be used in breeding nurseries due to their inability to flower). Other varieties tend to flower late in the season and the angle of the branches is reduced, therefore also yielding an erect type. Yet other varieties will flower early (low branching), profusely (many branching levels) and/or with wide angles of branching. Depending on the final result of these variables the plant type will result in an unacceptable phenotype, or a bushy plant type that may be interesting for two reasons: they produce a lot of forage and they may be more efficient in the use of soil nutrients. This plant type, however, has the serious problem of not producing large amount of stakes. Figure2.21 illustrates some contrasting plant types from the north coast.

Modification of plant architecture using exogenous applications of plant hormones is appealing for two reasons: **a)** it may lead to the possibility of forcing flowering in those erect types that are preferred by most farmers; **b)** it may be the only alternative for commercial production of bushy cassava types.

Specific objectives

a) *Determine the potential of different doses of plant hormones in modifying flowering habits in cassava.*

Materials and methods

Gibberellic acid (GA_3) was applied on three different cassava genotypes with contrasting plant architecture (CM6754-8, CM8027-3, and CM 849-1). For each clone up to 240 stakes were planted at normal plant densities. From a total of 720 plants, 216 were treated and the remaining 504 plants served as border plants. Three different doses were utilized (0 ppm; 50 ppm; and 100 ppm) on 24 plants of each clone. The exogenous application of GA_3 was done at 64 and 115 days after planting, early in the morning, spraying the solution particularly over the bottom of the leaves. Statistical analysis consists of a split-plot design, with four replications. (main plots are the clones and the sub-plots are the different doses of GA_3).

Figure 2.21. Cassava clones illustrating different plant architecture types, from the erect type on the left (MTAI 8) to the bushy type on the right (CM 6754-8) which is particularly suited for forage production in the norther coast of Colombia.

Results

This work is also carried out as a Thesis for an undergraduate student (Carmen Tulia Potosí Guampe) of the National University of Colombia (at Palmira). The last measurement obtained from this experiment was at 162 days after planting. Significant differences were observed for plant height depending on the dose. Dose by genotype interactions were also significant.

2.5.7. Estimation of the costs involved in a rapid multiplication scheme based on the use of micro-stakes.

Rationale

The low multiplication rate of cassava has been listed as one of the most important constraints for cassava development. Several techniques have been developed for overcoming this problem, including faster tissue culture or the use of micro-stakes alternatives. Although the protocols have been worked out, seldom these alternative multiplication rates have been applied at large scale. Therefore there is a need to scale up the process both to evaluate its viability when large volume of vegetative material is handled, and to estimate the costs of production. This information can be used by the private sector if the interest eventually develops for the creation of commercial seed production endeavors.

Specific objectives

a) *To set up a facility for a large production of vegetative material through the use of micro-stakes*.
b) *To complete the system with an anti-white flies screenhouse*.
c) *To produce clean vegetative propagules of elite clones*.
d) *Estimate the costs of production*.

Materials and methods

Several key elite clones were selected for multiplication using this system. Most of them are adapted to the mid-altitude valleys, where the common presence of white flies has resulted in a higher incidence of frog skin disease. The process starts with the indexation of "*mother plants*" to make sure they are free of frog skin and various virus that affect cassava. The long stems obtained from these disease-free plants were cut into micro-stakes, about 5-cm long and with two buds (Figure 2.22), which were then put to sprout in moist chambers. Every two weeks the resulting shoots are "harvested" and put to produce roots in glass containers with plain water. Once they produce roots the plantlets are transferred to plastic bags with soil and eventually moved to the screen-house (Figure 2.23). for hardening and further grow (about 1-2 months) before being transplanted to the field (Figure 2.24).

Figure 2.22. Planting of the micro-stakes used in the rapid multiplication system using moist chambers.

Results

The process has been used to multiply SM 909-25 (1355), SM 1219-9 (1213), SM 1460-1 (450), SM 1741-1 (272), CM 7514-7 (975), CM 7951-5 (490), MBRA 383 (998). The number of plants produced for each clone and already transplanted to the field is mentioned within parenthesis. These plants are certified to be disease free and constitute an important set of elite clones for this environment. It should be pointed out that the multiplication process begun while the indexation took place (frog skin disease indexation requires a grafting with the highly susceptible '*secondina*' clone, which may take up to three months for results). As the results from the indexation came, several plants were found to be contaminated and, therefore, the plantlets obtained from them had to be discarded. Otherwise, the system proved to be operational and able to produce consistently large number of vegetative material with few problems to be solved now and then.

The system implemented allowed two people to produce about 10,000 plantlets in a period of three months. Costs have been estimated for a period of continuous production for two years (8 batches of 10,000 plants each). The total for infrastructure costs (mainly the moist chambers) was therefore divided by eight in the estimate of costs presented in Table 2.37. The cost of the special screen house was not included in this cost estimates because it is only necessary in areas where white flies are an important vector for cassava diseases.

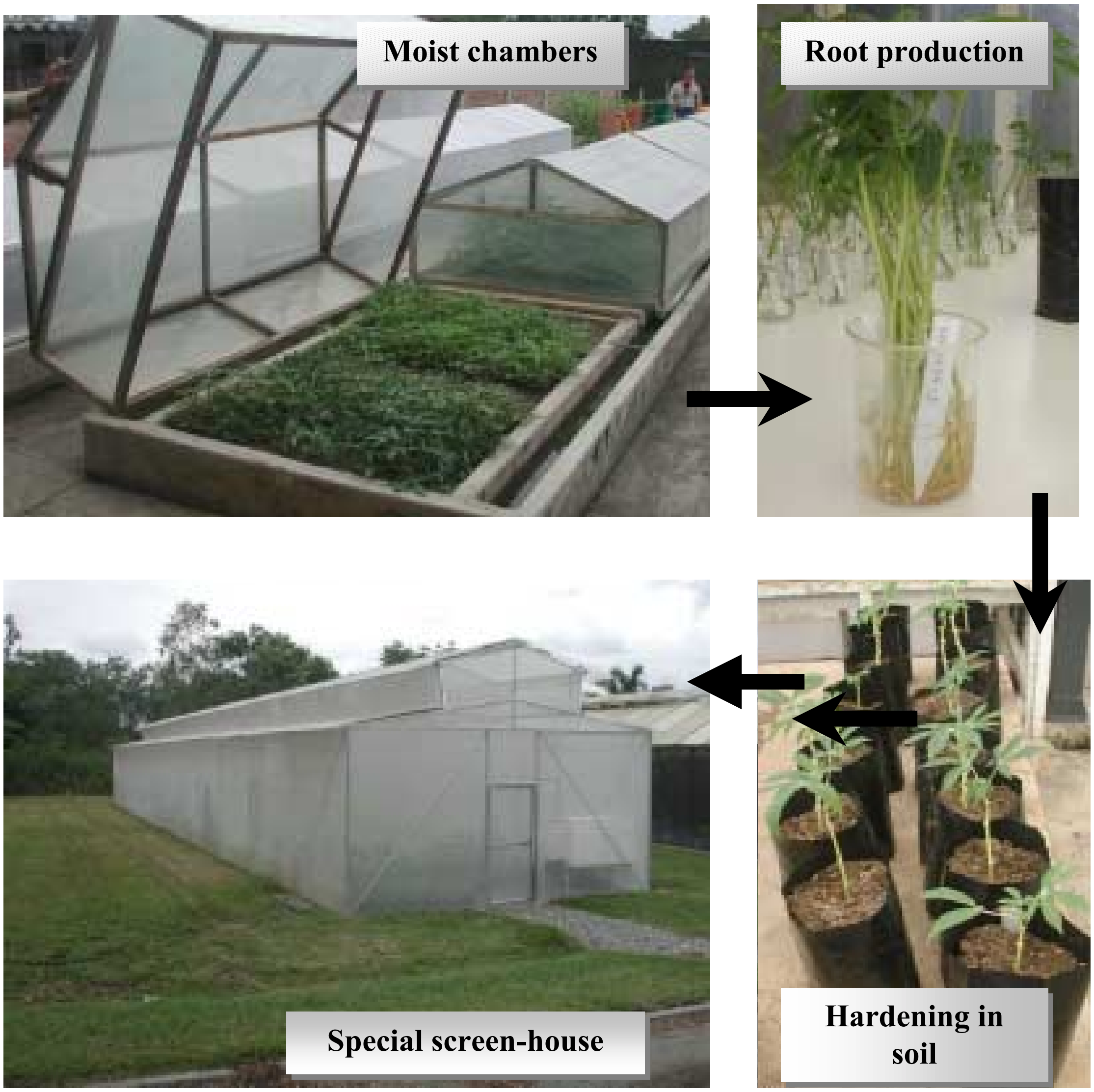


Figure 2.23. General scheme for rapid multiplication of cassava plantlets using the micro-stakes approach.

Table 2.37. Distribution of costs (US dollars[¶]) for the production of a batch of 10,000 plantlets using the micro-stakes multiplication system.

ITEM	COST
Agrochemicals, plastic bags, razor blades, detergent, fertilizers, etc.	253.59
Infrastructure (distributed in 8 batches)	112.28
Labor (two people)	909.09
TOTAL	1274.9

[¶] 1 US$ -= .200 Colombian pesos

Figure 2.24. Plantets produced by the micro-stakes multiplication system transplanted into the field in Santa Helena, Valle del Cauca, Colombia.

The price per plantlet using this system was 0.127 US$, which is slightly more than 10 times the cost of an ordinary stake, obtained from a commercial plot, and with no sanitary certification. Because of their cost and the cumbersome process of transplanting (rather that placing stakes into the soil) it is unlikely that these plantlets will be used directly in a commercial planting,. The most possible avenue for implementing this system would include one further stage of field multiplication. In other words, the 10,000 plantlets produced per batch are transplanted into the field (1 ha) and after 10-12 months stakes are ‘harvested’ from the fully-grown plants. In this way the initial cost of each plantlet can be divided by the number of stakes obtained from each plant (10-15 if proper care is taken), with the additional income derived from the roots harvested along with the stakes. It should be pointed out that the field stage of the multiplication scheme is also done in other crops such as potato. It is also important to note that the field multiplication should be carried out in isolated plots (no large cassava production around) and with a close monitoring of eventual pests and/or diseases that may appear. Control of white flies, is in this context, a fundamental requisites.

2.5.8. Development of a table for adjusting missing plants in evaluation trials.

Rationale

Uniform competition in cassava is fundamental for an acceptable experimental error in evaluation trials. However, it not always feasible to achieve a perfect plant stand. As a matter of fact irregular sprouting of stakes is a common event and often result in high experimental errors in our evaluation plots. Therefore, a table to adjust agronomic traits, depending on the number of missing plants is highly desirable.

Specific objectives

a) *To develop a table for adjusting trial results on number of missing plants using modern cassava varieties.*

Materials and methods

Evaluations were carried out in three different environments, all in Colombia: sub-humid environment of the Northern Coast, acid soils in the Eastern Savannas and the mid-altitude valleys. In the first two environments only one variety was used (MTAI 8 and CM 6740-4, respectively). In the latter environment, two different clones were used (CM523-7 and MPER 183). Experimental plots were based on 25 plants (see scheme in Figure2.25).

0	0	0	0	0
0	1	2	3	0
0	4	5	6	0
0	7	8	9	0
0	0	0	0	0

Figure 2.25. Scheme illustrating the identification of each plant inside experimental plots designed for measuring the effect of missing plants in evaluation trials.

The nine inside plants were numbered as illustrated in Figure 2.25. For each variety eight different treatments were applied, by removing one, two, etc. or up to eight of the numbered plants of each plot. The localization of the plant(s) to be removed was dully randomized. Three replications per treatment were available. This procedure allowed for measuring agronomic traits in plots with only one plant up to plots with perfect plant stands (nine plants). The information that we are ultimately interested in is developing a chart that will allow us to adjust the results of relevant agronomic traits in evaluation trials to the number of missing plants.

Results

Preliminary results obtained are presented in Table 2.38. As expected, yield reduction as the number of harvested plants drops is not noticeable until more than four plants are missing. Only when more than four plants are missing the performance of most agronomic traits start to change drastically.

Table 2.38. Preliminary results of the experiment to evaluate the effect of missing plants in evaluation trials of cassava.

Harvested plants (#)	Fresh root yield per plant (t/ha)	Fresh root yield per plot (t/ha)	Forage yield per plot (t/ha)	Harvest index per plot
	CLONE CM 523-7 EVALUATED IN PALMIRA			
1	47.33	5.26	3.70	0.55
2	55.17	12.26	8.37	0.60
3	41.22	13.74	8.33	0.62
4	51.08	22.70	12.30	0.65
5	37.40	20.78	12.30	0.62
6	51.50	34.33	19.70	0.64
7	36.95	28.74	16.89	0.63
8	31.83	28.30	16.74	0.63
9	31.89	31.89	20.44	0.60
	CLONE MPER 183 EVALUATED IN PALMIRA			
1	68.00	7.56	4.30	0.59
2	80.33	17.85	9.56	0.65
3	74.56	24.85	11.85	0.68
4	53.50	23.78	14.26	0.62
5	47.73	26.52	15.15	0.64
6	47.72	31.82	14.63	0.69
7	47.19	36.70	21.82	0.63
8	46.08	40.96	22.04	0.65
9	32.11	32.11	16.00	0.66
	CLONE CM 6740-7 EVALUATED IN VILLAVICENCIO			
1	21.67	2.41	2.96	0.45
2	23.75	5.28	5.19	0.50
3	25.28	8.43	7.13	0.54
4	19.38	8.61	8.52	0.50
5	19.67	10.93	10.28	0.51
6	18.47	12.31	12.22	0.50
7	18.69	14.54	14.07	0.51
8	15.52	13.80	14.35	0.49
9	15.65	15.65	17.13	0.48

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Carlos Reyes (Almidones Nacionales, Colombia)
Sergio Rodríguez (INIVIT, Cuba)
Diego Miguel Sierra (Federación Nacional de Avicultores – Colombia)
Erwin Silva (Industrias Protón Ltda., Colombia)
Watananonta, Watana (Rayong Field Crops Research Center, Thailand)

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Ministerio de Agricultura (Perú)
NIPPON Foundation (Japan)
USAID (United States of America)

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.*

☞ ***Collaboration with Universidad Nacional de Colombia with emphasis in the area of cassava physiology***. There has always been a very productive association between CIAT and the *Universidad Nacional de Colombia*, including an agreement that allows professors from the University to invest part of their working hours at CIAT. The agreement allows them to use the physiology laboratory and to develop special projects to take advantage of this well equipped facility. Undergraduate students are carrying out the physiological studies as part of their thesis research projects. The areas of research and collaboration materialized through thesis research projects as follows:

- *Photosynthesis enzymatic activity measurements in 30 germplasm bank accession:* At this point data from this study are being processed and analyzed. The thesis will be submitted by the end of the year.
- *Monitoring root growth using a capacitance meter for a non-destructive evaluation of productivity through time:* Thesis successfully presented by the end of October. Results had already been presented in *Section 2.5.5*.
- *Induction or inhibition of flowering of cassava through hormonal treatments.* Research still underway. Some preliminary results had been presented in *Section 2.5.6*.

☞ ***Official release of two cassava varieties for the acid-soil savannas.*** The release of two varieties for the acid soil savannas was initiated by CORPOICA with cultivar Reina or **CM 6740-7** (see Figure 2.8), which is specifically adapted for the transition environment between non-acidic fertile soils and the severely acidic infertile soils of the savannas *("Piedemonte")*. The identification of this variety should be familiar to the reader, because it was depicted in Figure 2.6, This clone performs well in the acid soil savannas but also in the coffee growing area. The other genotype (**CM 6438-14**) is more rustic and outperforms other clones when tested in the marginal soils of the acid soil savannas. A photograph of this material was included in Figure 2.7. The official release of CM 6438-14 will take place as soon as there is enough planting material probably during the first semester of 2002.

☞ ***Seed shipment to Haiti and training of Haitian scientists***. As part of the international efforts aiming at improving the conditions in Haiti, CIAT is actively promoting the introduction in that country of new germplasm from several crops, including cassava. Three Haitian scientists were trained in cassava breeding and agronomy during a week at CIAT headquarters. Three visits to the country by CIAT senior staff were also paid, for better determining the needs for improving cassava production in the country. Large numbers of vitro-plants from elite germplasm identified for the conditions and uses of cassava in Haiti were introduced into the country. Unfortunately, the initial steps suffered from lack of experience in hardening large number of vitro-plants (up to 4000 per batch), and many died in the process. The initial problems are currently in the process of solution, including the visit to CIAT headquarters of a collaborator (from *Double-Harvest*, an NGO actively working in Haiti) who will contribute in the hardening of vitro-plants in the future.

☞ ***Collaboration with Asian Countries***. As mentioned above, a vietamese scientist will come to carry out her Ph.D. thesis analyzing one of the three diallel data sets. As part of our activities in Asia a senior scientist visited the region for a month early in 2001, visiting Thailand, Vietnam, Indonesia and India. As a result of this visits a large shipment of botanical seed was arranged (see Table 2.4), as well as delivery of a set of 31 elite clones with outstanding adaptation to the three main environmental conditions in Colombia. CIAT continued delivering the core collection from the cassava germplasm bank, so a duplicate of this valuable collection is available to Asian scientists. The duplicate will be planted at Rayong Farm in Thailand. Also a formal agreement between CIAT and CTCRI (Central Tuber Crops Research Institute) at Kerala state in India was signed. The agreement will facilitate the exchange of germplasm and information between the two institutions. CIAT was also represented at the 25th Meeting of the International Vitamin A Deficiency Meeting, held at Hanoi, Vietnam during February, 2001.

Collaboration with Latin American countries. Colombia has become a demonstration case for the potential of cassava in the tropics. There is a valuable commitment from the Colombian government and private sector to finance research on cassava. This contribution resulted not only in the development of high-yielding germplasm (see Figure 2.4), but also in new uses of cassava. This year we evaluated for the first time a prototype for the mechanical harvest of cassava foliage. Considerable work has been done in collaboration with CLAYUCA, in alternative uses of cassava, particularly in the areas of animal feed and novel starch extraction methodologies. Most of these activities aim at the idea of demonstrating in our host country the potential and diversity for cassava. Visitors from other Latin American countries are exposed to the experiences we have in Colombia. Scientists, entrepreneurs, and farmers from Argentina, Brazil, Costa Rica, Dominican Republic, Ecuador, Haiti, Mexico, Panama, Peru, and Venezuela have been exposed to these experiences. In October a senior representative of CIAT attended to the launching of the Caribbean Roots and Tuber Network (CAROT). This network is part of the PROCICARIBE project, which also has many other networks in the region. An important player is also CARDI (Caribbean Agriculture Research and Development Institute). The meeting took place in Dominica, attended by about 30 people representing mainly the English-speaking countries (Dominican Republic being the only exception): Dominica, Barbados, Jamaica, Antigua & Barbados, Grenada, Monserrat, St. Kitt/Nevis, St. Lucia, St. Vincent & the Grenadines, Trinidad & Tobago, and Guyana.

☞ ***Cassava Biotechnology Network (CBN) Meeting.*** In November 2001, the project attended to this important meeting held in St. Louis (Missouri , USA). The meeting is a result of the collaboration with ILTAB at Donal Danforth Plant Science Center and constitutes a relevant gathering of all scientist doing research on cassava, particularly in the area of biotechnology.

☞ ***Global Cassava Strategy***. CIAT is part of the Coordinating Group of the Global Cassava Strategy initiative by FAO, together with other important actors for cassava research and development (IITA, CIRAD, EMBRAPA, IFAD, and NRI). We held a meeting for continuing the activities of this initiative, which involved the acceptance of ILTAB's proposal to boceme part of the Global Cassava Strategy.

Activity 3.2. Collaborate with the financial consolidation and expansion of CLAYUCA through the inclusion of new countries.

Rationale

As a result of the changes that took place during the last decade in most of the Latin American countries, the traditional partners with whom CIAT interacted in relation to cassava changed considerably. CLAYUCA came into existence, in part, to fill up some of the spaces that have been left empty and contributes considerably to the transference of technology and germplasm developed at CIAT into the affiliated countries. As a new institution CLAYUCA is already playing a key role in cassava research and development in the region. CIAT and CLAYUCA have developed a close and complementary research agenda that strengthens both institution on one hand, and avoids duplication of efforts on the other.

Specific Objectives

a) *To help CLAYUCA consolidate and expand during its second year of existence.*
b) *To carry out join research activities with CLAYUCA.*

Results

☞ Mechanization. It has been interesting to see the evolution of this concept, first mentioned in the 1999 Annual Report. Since then, planting and harvesting implements for the mechanization of cassava cultural practices, have been introduced and successfully used in several regions of Colombia. Harvesting equipment ha been bought and evaluated to determine their efficiency, operational costs, and amount and quality of their output.

CLAYUCA and CIAT have recently evaluated, for the first time, the prototype for the mechanical harvest of cassava fresh foliage (Figure 3.1). The nutritive quality of cassava leaves was described in the Output 1, so it is not necessary to explain here the economic and biological relevance of developing a system for the mechanical harvest of the foliage. Two alternatives are being evaluated: **a)** High-density cassava planting for the periodic harvesting of the foliage produced with a lower priority for root production which is harvested at about two years after planting. **b)** Harvest of leaves just before the normal harvest of roots. In this case the priority is root production, with an additional value for the foliage harvested. At this point we built and introduced a foliage-harvesting machine that will be adapted for both alternatives of foliage harvest. We are also collaborating to carry out a large experiment for the evaluation of foliage production in the Córdoba Department. Three varieties (MTAI8, CM 4919-1, and CM 4843-1) will be planted at three different plant densities (112,000; 62,500; and 40,000 pl/ha) with excellent results.

☞ *Artificial drying of cassava*. A project was presented by CLAYUCA and funded by the Colombian Ministry of Agriculture to build a pilot plant to conduct research in the area of artificial drying of cassava tissue (both roots and leaves). The plant is already functioning. Project IP3 contributed with the evaluation process of the products of the plant. As a result of this research we can now conclude that artificial drying of cassava tissue is feasible from the economic point of view and that the product has cyanide levels below the maximum tolerated. All the adjustments of the pilot plant so far made (or to be made in the future) were based, in part, on the close monitoring of the quality of the cassava flour produced. A major achievement of this process is that it produces no water effluent. Further perspectives in this area involve analysis of the potential for a dry extraction of "starch" from the flour produced by the plant. A project to evaluate the potential of the flour produced by the drying plant for production of adhesives, which is in great demand after the ban of the use of staples in the boxes containing banana and other produces for human consumption.

Figure 3.1. The foliage mechanical harvester in action. Cassava tender canopy is cut and stored in a trailer.

☞ *Training and production of a cassava manual*. A considerable effort has been made jointly between CIAT and CLAYUCA for training, particularly in Colombia. About eleven workshops (from two to five days long) were organized in different regions of Colombia to promote modern cassava production and processing. Up to 50 people attended each of these events. The ultimate goal of this activity is to promote the vision of cassava as an industrial crop with many alternative uses. The workshops stress strategies for increasing productivity and reducing production costs, which are fundamental to reach a competitive cost of the raw material for the industry. The cassava manual will incorporate all the information presented in these workshops, and was described in *Section 2.5.4*.

Achievements for this activity (CLAYUCA 2000 and 2001 Annual Reports) can be summarized as follows: 1) CLAYUCA consolidated and expanded; 2) Very important complementary research activity between CIAT and CLAYUCA resulted in increased impact for the cassava sector; 3) It demonstrates, once again, the feasibility and benefits of this type of consortium; and 4) Important steps given to expand the area of cassava utilization.

Activity 3.3. Interaction with the private sector for animal feed, starch and processed cassava for human consumption, for the success of their operations.

Rationale

Given the new political and economic environment in most of the tropical countries, it is clear that there has been an increased presence of the private sector, even in activities that had been predominantly carried out by the government. NARs have weakened and also realized that it was necessary to establish linkages with the productive sector. CIAT has been sensitive to these changes and, therefore, has strengthened its association with the private sector, being careful of avoiding any bias or preference to a particular sector (i.e. starch, animal feed, processed food for humans, etc.) or a particular enterprise.

This approach has many advantages. Because of the vacuum left by the NARs in many cases it is the private sector that had to take up the responsibility of promoting the crop and carry out extension work with personnel of their own. This logistic infrastructure is very convenient for channeling the technologies developed by CIAT. During the VI Asian Cassava Research Workshop that took place in Vietnam the presentation of each country made it clear that every success story for cassava occurred when there was a close collaboration between the farmers, the researchers and the consumers of the roots. This is also true for Latin America.

Specific Objectives:

a) *To establish close relationship with the productive sector for better understanding the needs that can be satisfied through cassava breeding.*
b) *To develop a working relationship that allows CIAT to take advantage of the logistic infrastructure of the productive sector*.
c) *To promote the use of cassava for industrial purposes*.

Results

CIAT is gradually learning to interact with different industries that process cassava one way or another. The creation of CLAYUCA has greatly facilitated this interaction. We have already some benefits from this interaction and below some examples are presented.

- *Processed cassava for human consumption*. These high value-added products (frozen croquettes or fried cassava chips), require very specific products. We are carrying out a collaborative work to determine the best characteristics in cassava roots for their use in the production of fried cassava chips for the snacks market. This work will include the use of yellow (high carotene) cassava roots as well as strategies for reducing the effect post-harvest physiological deterioration on the cost of the raw material for this industry (see *Section 2.5.1*).

  Another product is the fried cassava chip, which is currently sold in the U.S. snacks market (although restricted to areas with strong presence of Latin American people). The advantage of this activity lies on its potential for growth, its need for a constant supply of raw material which would help stabilizing the price of cassava, the demand of labor for processing the products in rural areas (before the roots are sent to the final processing plant), the creation of a relatively large alternative market for cassava, and the increase of demand for a high quality product. We are developing a research agreement with a processing company to carry out research on the traits that characterize the best root type for the fried cassava chip industry.

- *Starch industry*. One of the challenges for industrial uses of cassava is the constant supply of roots. This may be a difficult task in areas with long dry periods when planting is not possible for a period of 3—4 months. That is the case of the northern coast of Colombia. An important starch factory is based nearby Barranquilla, and we collaborate closely with this company. In addition to providing seed for their operations, we have contributed with them in training of their personnel in the agronomy of cassava. This is another way that the project is using to consolidate the idea of cassava as an industrial crop. Visitors from other countries have had the opportunity to visit the northern coast of Colombia and see the results of this collaboration. Such an initiative is very relevant because it demonstrates the renewed interest of the industry in this crop.

- *Feed industry*. We have received strong support by poultry associations in different countries of the region. Their interest is in the use of dried cassava roots in up to 20-30 % of the diets. It has been a very productive and intense collaboration. During the year, every single issue of their monthly publication (“Avicultores”) has had an article on the use of cassava in the poultry business. Several of them originated in CIAT. Again, the creation of CLAYUCA has been fundamental to facilitate this interaction, also resulting in several research projects approved, and partially funded, by the Poultry Associations. Valuable information has been produced to demonstrate the feasibility of feeding poultry with a diet that includes not only cassava roots but also foliage.

CIAT and CLAYUCA are currently submitting research projects to the Swine Growers Association and the Cattle Raising Growers Associations of Colombia. In this case the target will be the promotion of cassava silage as animal feed in different regions of Colombia.

OUTPUT 4
Participative plant breeding with women and small farmers in Africa and Latin America.

Researches: Luis Alfredo Hernández R, Hernán Ceballos (Cassava improvement, IP-3, CIAT), Antonio López (CORPOICA – Turipaná, and Marco Jaramillo (CORPOICA – Carmen de Bolivar)

Rationale

In Northern Colombia, cassava is a major staple crop and provides an important linkage for small farmers to urban and processing markets. Close to 70% of the root production is used for direct human consumption. Therefore, cassava varieties need to produce well and meet stringent consumer preferences. This area was selected for a special project for applying the tools of participatory plant breeding.

Specific Objectives

a) *To develop impact-oriented breeding methods that can deliver positive benefits in marginal areas to the rural poor and particularly to women farmers.*
b) *To carry out analysis of user groups.*
c) *To identify types and compare diagnostic methods.*
d) *To target accurately and disseminate more acceptable and productive varieties for poor women and men farmers.*
e) *To evaluate diverse genotypes through farmers, intermediaries and consumers.*

Materials and methods:

Study area

The study area included the provinces of Córdoba, Sucre, Bolívar and Atlántico in the Colombian Caribbean region. The townships Ciénaga de Oro in the Province of Córdoba and Los Palmitos in the Province of Sucre were selected as representative of cassava and starch production and the natural drying of the roots. The cities of Barranquilla (Atlántico), Cartagena (Bolívar), Magangué (Bolívar), Sincelejo (Sucre) and Montería (Córdoba) were defined as the most important end markets in the region. The activities corresponding to concentrated feeds, fresh consumption and middlemen (criteria and characterization) were carried out there.

The general utilization of the farmers-end users was updated doing a Rapid Rural Appraisal in the project area. To identify user types, a Cluster analysis (Ward's minimum variance, 1963), was applied using the preference criterion elicited in a previous survey.

The comparison of methods was based on two criteria as follows: **a)** a variable named *"consideration",* which determines if a preference criterion was taken into account in each diagnostic method evaluated in the project. This variable was measured in a scale from 0 (no consideration or no answer) to 4 (100% of people interviewed answered positively). **b)** a variable named *"grade"*. This variable measured the quantity and quality of information for each diagnostic method. For grade, a scale from 0 = total absence of a criteria; 1 = criteria without reason; 2 = criteria with reasons but without cause-effect relationship; and 3 = criteria with a cause-effect relationship. With this data, a multiple correspondence analysis was carried out to identify differences between diagnostic methods used in the preferences' elicitation phase.

Activity 4.1. Comparative analysis of end-user groups.

The project goal is to develop impact-oriented breeding methods that can deliver positive benefits in marginal areas to the rural poor, particularly to women farmers. The study was divided into two phases:

- ☞ *Identification of the different users who are involved in the production-consumption chain for cassava in the region.*
- ☞ *Characterization and grouping of users and comparison of diagnostic methods.*

Analysis of user groups to identify types and preferences. Phase I consisted of two activities:

- ☞ Revision of secondary sources of information: Corporación Colombia Internacional (1997), Municipio de Los Palmitos (1998), Municipio de Ciénaga de Oro (1994) and Janssen (1986).
- ☞ Verification in the field and in the end markets of the existence of different types of users through exploratory surveys (including visits to the Municipal Technical Assistance Units (UMATAS) and Provincial Offices of Agriculture in Córdoba, Sucre and Bolívar).

In Phase II the different types of users identified were grouped and characterized on the basis of preferences with respect to the traits of cassava and the criteria used to make decisions at their respective level in the chain. In the case of the producers, information was obtained regarding their preferences at six stages of the crop: (1) from planting material to planting time, (2) up to 60 days after planting (DAP), (3) 60 to 120 DAP, (4) 120 to 180 DAP, (5) 180 DAP to harvest, and (6) from harvesting to sale of the roots. For the chippers-dryers, starch manufacturers and manufacturers, the preferences were obtained for each of the stages of the process—from the purchase to the packing of the cassava.

Results

Production chains that involve cassava in the Caribbean region. There are three production chains that involve cassava in the Caribbean region: producer-fresh consumption, producer-starch industry and producer-feed concentrate industry.

Marketing channels. In the fresh market chain there are two marketing channels between the producer and the household consumer:

- The producer sells to the local middleman, who sells to a final middleman in the main cities, who in turn sells to the wholesale supply centers, the supermarkets and to retailers in the end market.
- The producer sells to the local middleman who sells directly to retailers in the local and end markets in the cities.
- The starch chain is characterized by three channels. In the chain of the feed concentrates industry, there are two channels:
  - ☞ A shorter one, with a middleman between the producers and the industry.
  - ☞ There is a local middleman between the drying plant and the producer.

Activity 4.2. Comparison of tools used for obtaining the information

The main activity was based on a survey. This tool was used for all the user groups. It is necessary to prepare a well-structured instrument beforehand in order to gather the necessary information, precisely and in a form that is easily analyzable.

Advantages

- It is a rapid method, requiring little time to apply.
- It is economical.
- The interviewer does not have to be an expert in research.
- Reasonable volumes of information are managed because more precise and concrete answers to the topics are obtained.
- Systematization of the information is easy.
- It does not require many technical resources—just the instrument and a pencil—to obtain the information.
- The interviewee does not have to be literate.

Disadvantages

- Preparation of the instrument requires time and knowledge.
- It is schematic and pigeonholes data, making it the least flexible of the tools used for obtaining information.
- Although the interviewer does not need to be an expert in the subject, he/she should have some knowledge of the same and great skill in applying the instrument. In some cases previous training is required.
- In the specific case of this research, a difficulty was traveling to the sites where the interviewees were found given the long distances, the poor conditions of the dirt roads and the social unrest reigning in the zone.
- It is considered the least reliable of the tools because the interviewees can say anything and the interviewers may not have the capacity to confront them on the spot.
- The situation of social unrest in the region makes it more difficult to obtain information through this instrument as the interviewees are reluctant to give out information of any kind.
- Knowledge of the zone is required to get to the sites where the interviewees are found; otherwise a guide is required.

In-depth interview. For this study the interviews were recorded. Starch manufacturers, chippers, wholesalers and manufacturers and in some cases producers and consumers were interviewed.

Advantages

- It establishes a relationship of greater trust between the interviewer and interviewee.
- It is possible to obtain a greater volume of information.
- It is more flexible than the survey.
- It does not require many technical resources.
- It is more reliable than the survey.
- Although it is more complicated to systematize and analyze the information, it provides a sounder basis for the same.
- The fact that the interviewee is literate or not is irrelevant.
- It facilitates not only the gathering of the interviewees' knowledge (facts and concepts) but also their perceptions, feelings and opinions.

Disadvantages

- It requires more time to implement.
- It is slightly more costly than the survey due to the fact that fewer interviews can be done in the same amount of time.
- It requires more time and willingness on the part of the interviewee; thus on some occasions several visits have to be made.
- It requires previous knowledge of the interviewee.
- The interviewer needs more preparation.
- The systematization of the information is more complicated.
- It requires an atmosphere conducive for conducting the interview.

Focus groups

Advantages

- The opinions and perceptions of the participants are confronted so that these can be discussed and socialized in the group.
- The information that is obtained is more objective because it is subjected to discussion and confrontation; whereas in the individual tools, the information is more subjective.
- It provides an opportunity to debate the different opinions of the participants extensively.
- The level of the participants' schooling does not matter because the discussions are written down and taped by the facilitators.

Disadvantages

- It is a tool that requires extensive preparation time.
- It is more costly than surveys.
- Systematization of the information is more complicated because more disperse information is gathered and adequate methodologies for systematization are not available.
- It is an appropriate tool for homogeneous groups and/or with similar thematic, economic, social and cultural interests.
- To convene the focus groups, it is necessary to have the backing of institutions from the zone because it is not easy to bring people together, much less in zones where there is social unrest.
- Its implementation requires the presence of more than one person for orienting the discussion, who should have great skills for taking notes, gathering the information and keeping the discussions on track.

Interactive workshops. This tool, which requires from 5-7 h, was used with producers, middlemen and chippers. The minutes from the workshop were transcribed from the tapes, materials prepared by participants were systematized, information was classified and categorized, and informal and descriptive analyses were made.

Advantages

- In contrast with the other tools including the focus groups, the interactive workshops permit the active participation of everyone because they are not only subjects from whom information is extracted, but they also receive information from the workshop facilitators.
- The participants are free to decide on the methodology, time and tools used in the workshops.
- Overall, the tool is the most participatory, reliable and flexible of all those used in this study.
- The group of participants does not have to be homogeneous with respect to economic, social or cultural interests.

Disadvantages

- It requires the most preparation. In addition to the workshop guide for extracting the information, the facilitators present themselves through a technical talk on some topic related to the area of research, which sets the stage for beginning the dialogue with the participants.
- It was the tool that had the highest average costs per participant.
- Systematization of the information is complicated because the information gathered is more disperse and expressed in diverse forms, which complicates their homogenization.
- In order to extend invitations to participate in the focus groups, it is necessary to have the backing of institutions present in the zone because it is not easy to get the people to attend meetings, especially in zones where there is so much social unrest.
- The technical presentation of the facilitators must be of high quality in order not to lose credibility with the participants.

. Overall conclusions

- From the producer of cassava to the end consumption of the fresh or processed roots, there is more than one marketing channel, which offers different options when the harvest is to be sold. Between the producer and the end consumer, there are other users who impose preferences established by the end consumers; moreover, they also establish other arbitrary standards (for their own benefit) at the moment of the purchase.
- In each group of users, there are different types with some preferences in common and others that are very specific to each one.
- The manufacturers that used cassava in a greater number of production lines had more requirements and preferences than those who specialized in the production of balanced feeds for poultry.
- The open-ended questions on the survey made it possible to obtain more information at a lower cost for both producers, middlemen and consumers; the in-depth interview was best for the chippers-dryers and starch manufacturers.
- The interactive workshop and the focus groups were the most costly, and the amount and quality of information obtained was less than for the other tools when applied to producers and chippers-dryers.

- The QDI of a variable is a good indicator for characterizing the variables mentioned by the groups, given the numeric variability among them. It is also a good parameter of the quality of the information and the discriminatory power of the variables.
- The DIT index constitutes a good measure of the amount and quality of the information obtained in a given diagnostic tool.

One of the objectives of this project financed by DFID refers to the organization in Colombia of what a workshop on participatory plant breeding attended by the farmers participating in the process (October 30 through November 1, 2001). It is expected that through the project a working capacity based on basic breeding concepts will remain upon its conclusion. To fulfill this objective a publication will be written and printed, with the following content: **a)** Genetics and breeding; **b)** Reproductive systems in plants; **c)** Varietal evaluation and selection. With the feedback obtained through this workshop we expect to improve the document, so the publication will be useful for facilitators in other similar events

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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. Research progress on the micro-nutrient and starch properties of hundreds of genotypes from the germplasm bank was reported as Output 1. In the following pages a summary of other 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 (also see Activity 2.6*).

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 at CEUNP. Sequential plantings were performed as the plants were certified to be disease-free. Therefore, harvest of these plants was also done sequentially. The first plantings were, as expected, free of frogskin symptoms. However, later plantings started to show occasional plants with clear symptoms. These late plantings were discarded regardless the presence or absence of symptoms. It was a drawback to find plants with symptoms of the disease. Several alternative explanations for this fact are: a) the indexation procedure allows for false negatives; b) in spite of the isolated location chosen for this planting, the disease was introduced relatively early, so that by the end of the season there was already a solid presence in the bank accessions; and c) the causal agent of frogskin is not a virus, but a viroid.

Although we had to discard some material because we were not certain to be disease free, a large proportion of the germplasm bank is back in the station and, hopefully, free of frog skin. A set of preliminary results would indicate that the causal agent of frogskin, may eventually be a viroid. This would explain several perplexing and frustrating results observed previously. However, much more research is required to confirm this hypothesis.

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.

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.

This activity also includes a collaborative effort with Professor Nagib Nasser from the Universidade de Brasilia (Brazil). As a kind gesture, professor Nasser shared with us F1 seed of his inter-specific crosses with wild relatives, a material that according to his publications has shown the occasional occurrence of apomixis. Unfortunately only three botanical seeds germinated and the resulting seedlings died soon after germination.

Activity 5.3. Evaluation of **M. esculenta** *and related species from the germplasm collection for the natural occurrence of resistance or tolerance to herbicides.*

Rationale

One of the major problems for cassava cultivation is the frequent soil degradation associated with it. Soil degradation occurs because cassava is generally planted in the most vulnerable soils (low fertility and slopped land) and farmers do not apply fertilizers to cassava as they do with other crops. The soil degradation is not, therefore, caused by cassava itself, but ultimately originate in farmers decisions. There is, however, one reason for which cassava can be held responsible for soil degradation: the slow cover of the soil by the canopy. In effect, cassava will require between one and two months to cover the soil, leaving it exposed to erosion. Because of this, different CIAT projects have been looking for alternative cultural practices to overcome this soil vulnerability at early stages of cassava growth. Minimum or zero tillage practices are a common recommendation for reducing soil erosion. However, weed control within this type of tillage becomes a major issue. The natural occurrence of resistance or tolerance to herbicides or its induction for traditional breeding in different crops has been reported in the literature (Dyer, 1996, Shaner et al., 1996).

Specific Objectives:

a) *To screen the germplasm bank in search for the natural occurrence of resistance to two different commercial herbicides.*

Materials and methods

Each accession from the germplasm bank is represented by only four plants. Therefore not all the vegetative stakes produced at CEUNP are used as part of the normal planting of the germplasm bank. It was decided that two sets of a large sample of material (1560 genotypes) would be used to screen for the natural occurrence of resistance/tolerance to herbicides. Each genotype was represented by only one plant in each set. Because the source of seed for this planting varied in age, the materials for each set was planted sequentially through a two months period in three different batches. Each set will be treated with one of two commercial herbicides. The first two batches of each set have already been treated. The third and last batch will be treated at the end of November. The following herbicides and doses will be used for this activity:

	Commercial name	Technical name	Chemical name	Dose
1	Basta or Finale	Glufosinate ammonium	4-[hydroxy-(methyl) phosphinoyl]-D, L-homoalanine (left isomer)	1.5 l/ha
2	Round-Up	Glyphosate	N-phosphonomethyl-glycine	2 l/ha

Results

Figure 5.1 illustrates some interesting, yet preliminary, results. The application of herbicide was followed, three hours later, by a heavy rain. Some of the chemical may have been washed out, but obvious differential reactions could be observed for both herbicides.

Figure 5.1. Differences in reaction to herbicides Round-Up® (left) and Basta® (right), observed within representatives of the cassava germplasm bank at CIAT.

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

An important feature of the IP3 project relates to the integration of breeding, entomology, plant pathology and the development and use of tools from biotechnology. In spite of the "divisions" created by the project structure, these four scientific areas have maintained as much a close relationship as possible. In Output 6, the progress related to insect and other arthropods is summarized. A major issue in relation to integrated pest management has been the implementation of a quarantine at CIAT when there was no cassava plant in the field for a month. This facilitated the management of the white flies problem as reported in *Section 2.4.3*.

Activity 6.1. Evaluation of cassava germplasm for resistance to the whitefly, Aleurotrachelus socialis during 2001.

Rationale

The high incidence of frog-skin disease in CIAT cassava germplasm during the 2000-2001 growing season had a direct influence on our ability to evaluate cassava germplasm and carry out experiments on the CIAT station. The constant build up of frog skin disease, combined with increasingly high whitefly populations made it impossible to work with cassava genotypes and maintain the cassava germplasm bank on the CIAT station. The decision was therefore taken to suspend cassava planting at CIAT, resulting in a 2 month period, once existing fields and germplasm was harvested, when there was no cassava being grown at CIAT. It was calculated that this would reduce frog skin disease and whitefly incidence in subsequent plantings. Steps were also taken to clean germplasm of frog skin disease by using the tissue culture method to propagate germplasm bank accessions and other elite materials.

The implementation of these measures resulted in the need to identify additional sites off of the CIAT station to maintain cassava germplasm, carry out experiments and multiply elite materials or varieties needed to maintain arthropod colonies for research purposes. The site chosen to grow-out much of this material, free of frog skin disease, was the Experimental Farm of the "Universidad Nacional de Colombia/Palmira (CEUNP), located in the municipality of Candelaria, Valle del Cauca, and at CENICAÑA, municipality of Florida, Valle del Cauca. In addition, the cassava entomology section rented small (<1ha.) of land at two localities (one about 7kms from CIAT, the second about 25 km.) where there are few cassava plantings and therefore, hopefully, a reduced incidence of whiteflies and the absence of frogskin disease. This provided sites where elite genotypes for arthropod, especially whitefly, resistance could be multiplied, and available for greenhouse and field experiments.

Materials and Methods

The majority of the evaluations done for whitefly resistance during the 2000-2001 growing cycle were done at CEUNP and in close collaboration with the cassava plant breeding and genetics sections. Five groups of genotypes were evaluated for *A. socialis* damage/resistance using both a damage and population scale (Table 6.1):

1. Genotypes from the CIAT cassava germplasm bank, 2117 accessions.
2. Genotypes corresponding to controlled crosses (CW), including wild species and clones from *Manihot esculenta*; 321 clones.
3. Open crosses (OW), non-controlled crosses; 606 cultivars.
4. Family K, that correspond to crossed between M Nig 2 x CM 2177-2 (Cebucan). This family is utilized for studies on the genetic map; 103 clones in the family.
5. *Clonal Evaluation Trial* (CET) of plant breeding program. Materials evaluated for Interandean valley ecosystems, 500 to 1200 m.a.s.l.; 653 cultivars.

A total of about 3800 clones were evaluated during 2001 at the CEUNP location. Both the damage scales and population scales are based on a 1 to 6 rating, with 1=to the absence of damage and no whitefly immature or pupae present; a 6 rating expresses maximum damage and whitefly population (Table 6.1). A 1 or 2 rating is considered highly resistant; a 2.5 to 3.5 is moderate to low levels of resistance, and a 4 or above rating in either damage or population is considered susceptible. Those genotypes or cultivars receiving a 1 to 3.5 rating should be re-evaluated in subsequent plantings. A low rating could indicate an "escape," in that, by chance whitefly populations were not high on a particular genotype. Often numerous evaluations (4 to 7) are required to identify a resistant genotype, when natural field infestations are used.

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.

Results

Nearly 3800 genotypes were evaluated consisting of the five aforementioned groups. The overall evaluation indicates that whitefly populations were high and resulted in significant selection pressure (Figures 6.1 and 6.2). 2508 cultivars, or 63.0% of the total germplasm evaluated had a damage rating of 4.0 to 6.0 and these are considered susceptible and can be eliminated from any future need for screening or evaluation (Figure 6.1). At the other extreme, 212 clones (5.6%) expressed no damage symptoms (1 rating), indicating possible high levels of resistance. 586 clones (15.4%) had ratings from 1.5 to 2.5. In this case damage symptoms and whitefly stages were present but both at low levels, indicating a moderate level of resistance. These evaluations, therefore, resulted in about 21%, or 798 clones being rated below 2.5, indicating the possibility of high to moderate levels of resistance. These clones need to be reevaluated in subsequent years and "escapes" need to be identified.

About 492 clones, or 13.0%, had a rating of 2.5 to 3.5; these clones may have some moderate to low levels of resistance. However, many may also have considerably favorable agronomic qualities, i.e. high yield or dry matter, that could prove valuable in a hybridization program and crossing with genotypes with higher levels of resistance or higher yielding.

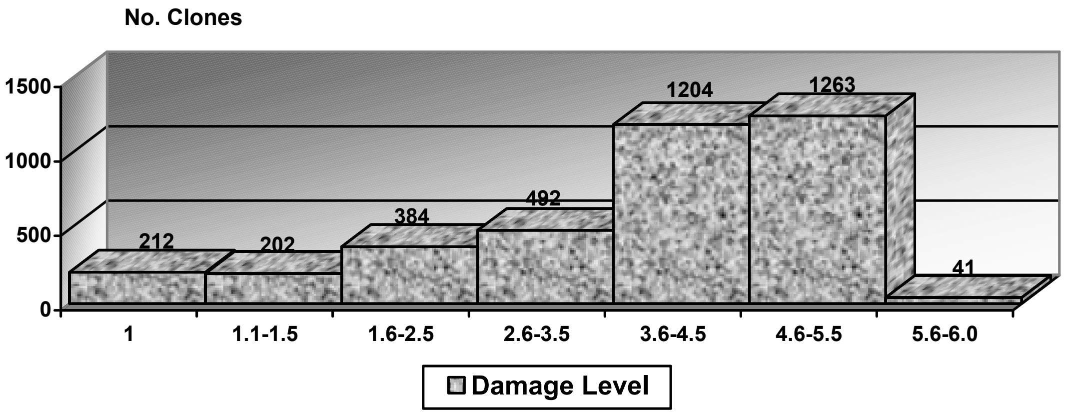

Figure 6.1. Whitefly damage ratings on all cassava germplasm (3798 clones) evaluated at CEUNP, CIAT, 2001, for *Aleurotrachelus socialis* resistance.

The first objective in a mass screening or evaluation of germplasm for arthropod pest resistance is to identify susceptible germplasm and this can be done on the first evaluation. There is no need to reevaluate susceptible genotypes, because there are no, by definition, "escapes." As cited previously, we can therefore eliminate > 2500 clones or 63% of those evaluated as susceptible to *A. socialis*.

Whitefly population ratings were correspondingly high; 2640 clones (69.6%) had high populations, 3.6 or above on the 1 to 6 evaluation scale (Figure 6.2), indicating good selection pressure. Only 25 clones, or 0.7%, were void of whiteflies. Again, these may be escapes.

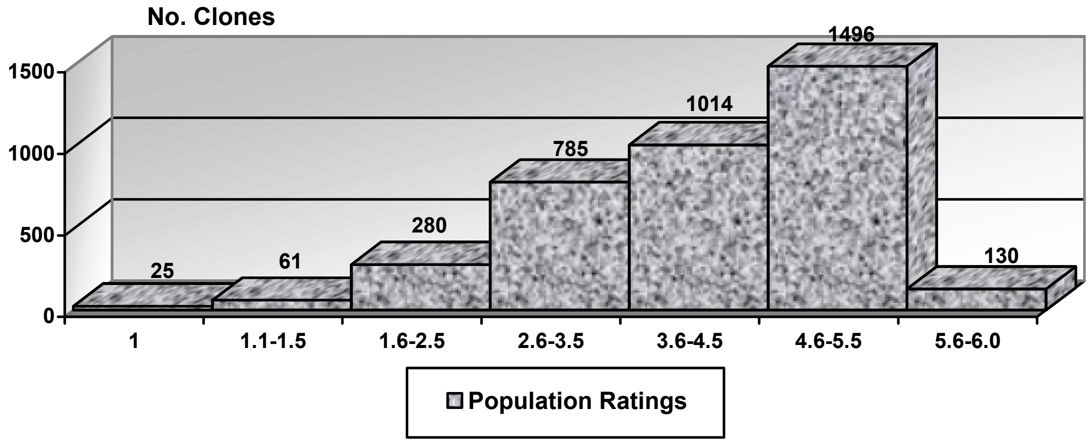

Figure 6.2. Whitefly population (nymphs and pupae) on all cassava germplasm (3791 clones) evaluated at CEUNP, CIAT, 2001, for resistance to *Aleurotrachelus socialis*.

Of the elite genotypes from the germplasm bank, evaluated a CEUNP, 2117 accessions, 1136 or 53.7% were highly susceptible (damage rating of 3.6 to 6) (Figure 6.3), while 187 clones (8.8%) had a damage rating of 1, and 461 (21.8%) a rating of 1.1 to 2.5. The corresponding populations of *A. socialis* can be observed in Figure 6.4. 1887 clones, or 89.2%, had population ratings above 2.6; 9 clones (0.4%) were void of whiteflies and 228 clones (10.8%) had populations below 2.5. These results indicate an overall moderate to high populations and well distributed throughout the field.

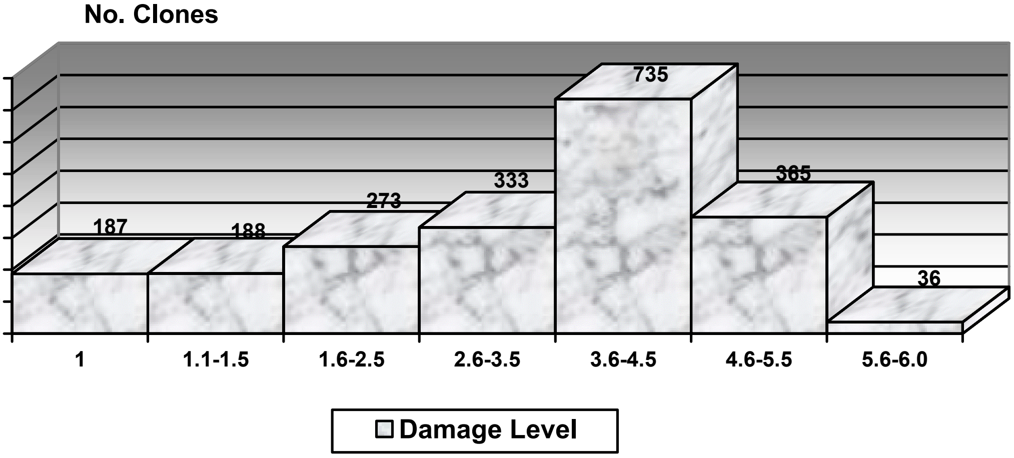

Figure 6.3. Evaluation of elite cassava germplasm (2117 clones) for resistance to whitefly (*Aleurotrachelus socialis*) at CEUNP/CIAT during 2001.

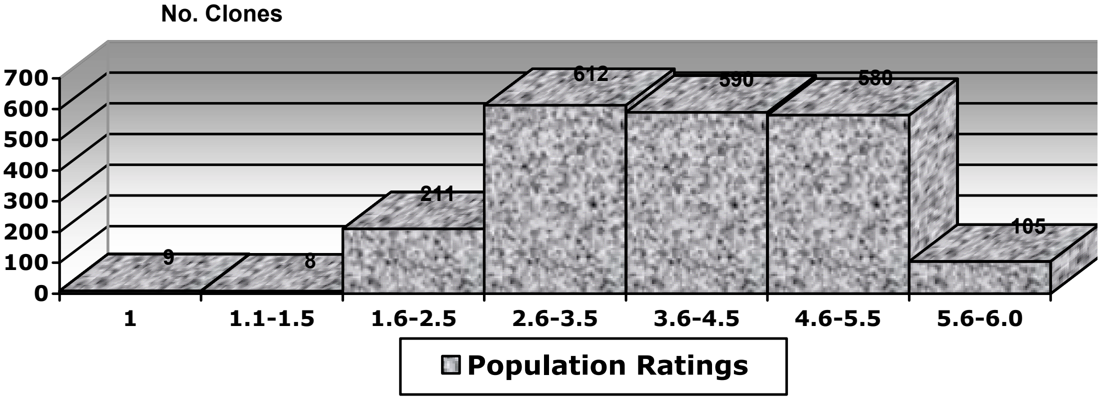

Figure 6.4. Whitefly population (nymphs and adults) ratings on 2117 cassava clones being evaluated for resistance/damage by *Aleurotrachelus socialis* at CEUNP/CIAT during 2001.

Figures 6.5, 6.6, 6.7, and 6.8 show the damage ratings for the different groups of materials evaluated (see materials and methods section). For those genotypes corresponding to the controlled crosses (CW), 72 clones, or 22.4% had damage ratings below 2.5, and 224 (69.8%) above 2.6 (susceptible) (Figure 6.5).

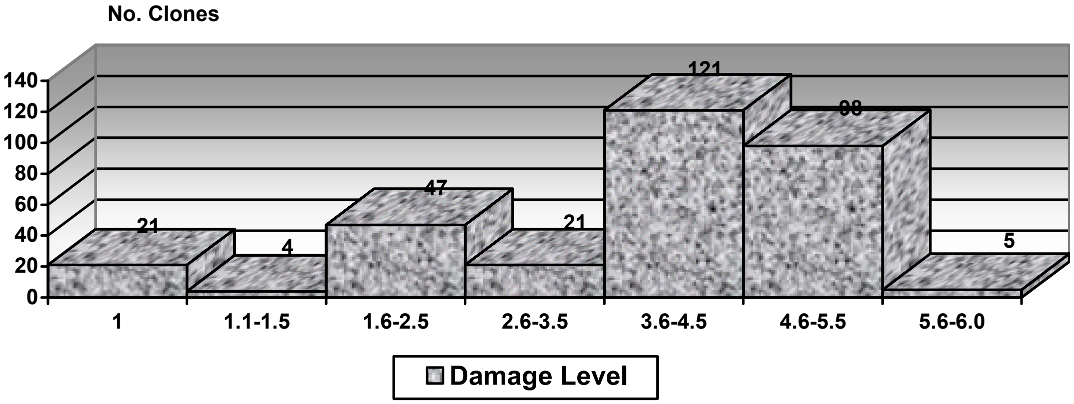

Figure 6.5. Evaluation of cassava germplasm (controlled crosses = CW) for resistance/ damage to the whitefly, *Aleurotrachelus socialis*, at CEUNP/CIAT during 2001.

For those genotypes corresponding to the open crosses (OW), 79 clones, or 13.0% had damage ratings below 2.5, and 527 (87.0%) above 2.6 (Figure 6.6). The family K crosses (MNig-2 x CM 2177-2), resulted in nearly all progeny being highly susceptible to *A. socialis* (Figure 6.7). No cultivars received a damage rating below 2.5, only 4 cultivars had a rating below 3.5 and 99 or 96% received a rating above 3.6 and therefore susceptible. Since neither of the parents have show *A. socialis* resistance, it is not surprising that no resistance is indicated in the progeny.

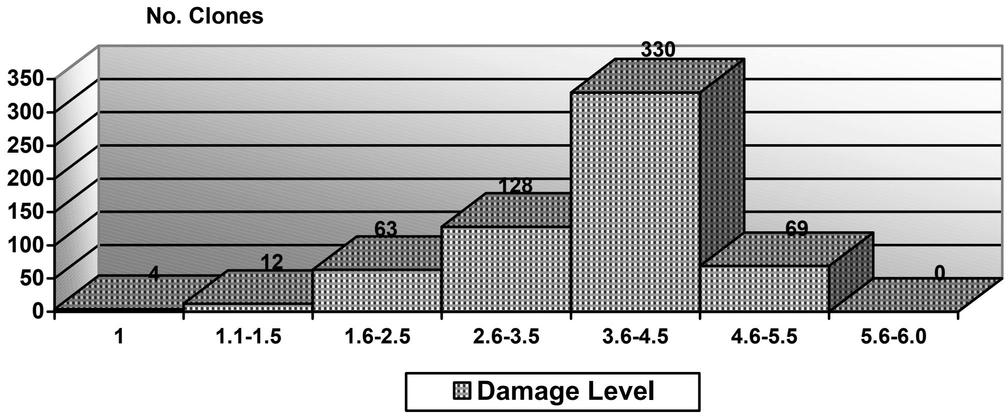

Figure 6.6. Evaluation of cassava germplasm (open crosses = OW) for resistance/damage to the whitefly, *Aleurotrachelus socialis* at CEUNP/CIAT during 2001.

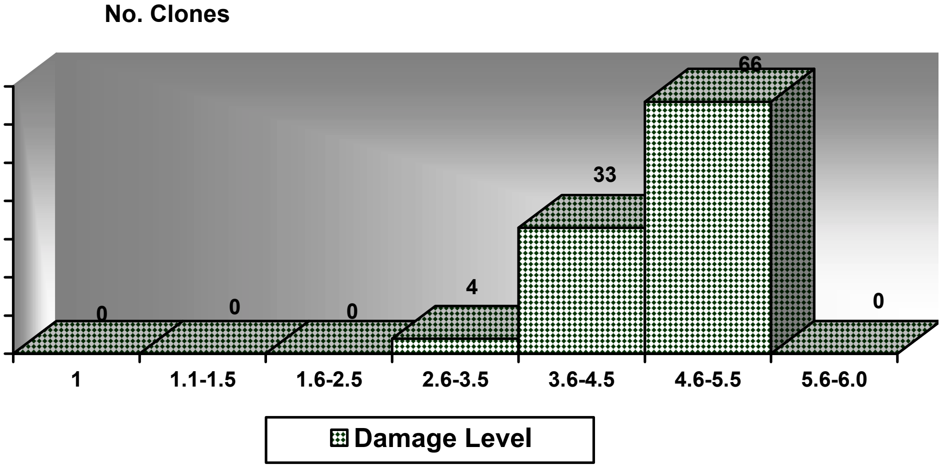

Figure 6.7. Evaluation of cassava germplasm (Family K) for resistance/damage to the whitefly, *Aleurotrachelus socialis* at CEUNP/CIAT during 2001.

Results from the *Clonal Evaluation Trial* were similar; only 13 clones or 2%, were evaluated below a 2.5 rating (Figure 6.8), while 88% (555 clones) had damage ratings above 3.6. About 10% (64 clones) did have ratings between 2.6 to 3.5, indicating there may be some low to moderate levels of *A. socialis* resistance in this germplasm.

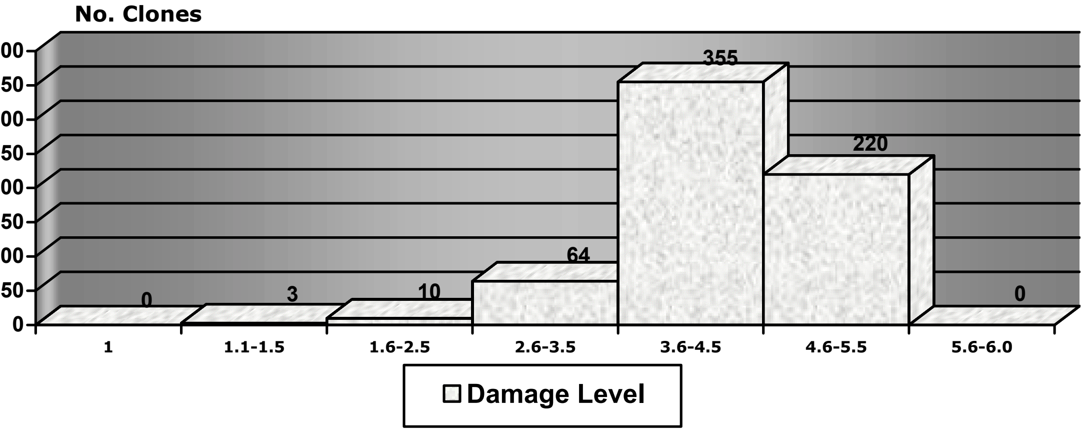

Figure 6.8. Evaluation of cassava germplasm (clonal evaluation trial= OC) for resistance/ damage to the whitefly *Aleurotrachelus socialis* at CEUNP/CIAT during 2001.

Table 6.2 summarizes the damage and population ratings for all of the genotypes evaluated. As can be observed both damage and population levels were consistently high indicating that this (CEUNP) was a good site for *A. socialis* evaluation on cassava germplasm.

Table 6.2. Whitefly (*Aleurotrachelus* socialis) damage and population ratings for germplasm groups evaluated at CEUNP during 2001.

Group	**No. of**	**% Clones by Damage Rating**				**% Clones by Population Rating**			
Germ.	**clones**	**1.0**	**1.5-2.5**	**2.6-3.5**	**4.0-6.0**	**1.0**	**1.5-2.5**	**2.6-3.5**	**4.0-6.0**
E.G	2117	8.8	21.8	15.7	53.7	4.3	10.4	30.0	60.3
C.W	317	6.6	16.1	6.6	70.7	3.4	18.4	12.5	65.7
O.W	606	0.7	12.4	21.1	65.8	0.8	10.7	22.7	65.7
Fam K	103	0.0	0.0	3.9	87.4	0.0	0.0	4.9	95.1
CET	652	0.0	2.0	9.8	88.2	0.0	1.7	8.9	89.4

E.G = Elite Germplasm
CET = Clonal Evaluation Trial

Table 6.3. A list of the best cassava genotypes selected from germplasm evaluations for whitefly (*Aleurotrachelus socialis*) resistance at CEUNP, Valle del Cauca, during 2001.

(1)		**(2)**		**3)**	**(4)**	**(5)**
Elite (CGB) (1.0)		**CW (< 2.0)**		**OW (< 2.0)**	**Family K (< 3.5)**	**CO (< 2.5)**
PER 320	PER 602	CW 14-8	CW 39-8	OW 106-3	K-108	SM 2649-4
PER 594	SG 250-3	CW 14-9	CW 14-1	OW 229-5	K-41	SM 2653-5
CM 1288-17	SG 638-6	CW 14-11	CW 14-5	OW 105-6	K-58	SM 2588-5
CM 2298-3	COL 183	CW 14-12	CW 40-13	OW 238-1	K-73	SM 2649-5
BRA 759	COL 2016	CW 14-13	CW 40-3	OW 153-2	K-6	SM 2652-9
BRA 785	COL 2379	CW 14-14	CW 57-1	OW 189-1	K-15	SM 2589-28
COL 304	ECU 72	CW 14-16	CW 58-1	OW 228-3	K-38	SM 2589-31
COL 1722	PER 380	CW 20-1	CW 58-2	OW 179-1	K-51	SM 2649-3
CM 4013-1	BRA 859	CW 21-3	CW 58-4	OW 101-7		SM2652-10
COL 2260	COL 403	CW 21-5	CW 58-5	OW 105-7		SM 2652-12
CM 2146-3	COL 774	CW 14-7	CW 39-1	OW 108-4		SM 2653-6
CM 2766-4	BRA 1123	CW 21-1	CW 39-3	OW 228-4		SM 2663-5
BRA 860	PER 421	CW 21-2	CW 39-5	OW 240-2		SM 2575-7
ECU 108	SG 787- 10	CW 14-3	CW 39-9	OW 252-3		
COL 2156	COL 327	CW 14-6	CW 39-11	OW 103-8		
PER 534	COL 875	CW 20-2	CW 41-1	OW 252-4		
COL 225	COL 1467	CW 14-2	CW 41-2			
BRA 627	COL 1503	CW 14-4	CW 41-3			
COL 561	COL 1509	CW 21-4	CW 41-4			
SM 536-8	COL 2032	CW 14-10	CW 57-2			
COL 2653		CW 14-17	CW 28-38			
COL 2656		CW 40-2	CW 28-34			
PER 554		CW 39-7				

*
(1)=Elite accessions from germplasm bank.
(2)=Genotypes corresponding to controlled crosses.
(3)=Open crosses
(4)=Family K, controlled cross between Mnig 2 x CM 2177-2
(5)= Observation field, plant breeding section.

Table 6.3 is a listing and summary of all of the genotypes evaluated, and represents the best materials selected in each of the groups of genotypes evaluated. In group (1) consisting of the elite accessions from the Cassava Germplasm Bank (CGB), 187 accessions had a damage rating of 1.0. The 43 accessions listed in table 6.3 also had a population rating below 2.0, and for that reason are listed as the best materials.

Activity 6.2. Evaluation of cassava germplasm for resistance to arthropod pests at several locations on the Colombia North (Atlantic) Coast.

Rationale

Cassava originated in the Neotropics although its exact center of origin is a moot issue; consequently, the greatest diversity of arthropods reported attacking the crop is from the Americas. An estimated 200 species have been reported, many of which are specific to cassava and have adapted in varying degrees to an array of natural biochemical defenses that include laticifers and cyanogenic compounds. The pest complex varies greatly over the main cassava growing areas and recent explorations in the Neotropics indicate that the arthropod pest complex is not geographically uniform.

When the cassava crop is grown under conditions of irregular, limited rainfall as in the lowland sub-humid and semiarid tropics, arthropod pest populations increase, causing considerable yield reduction. In these agroecosystems, a complex of pests attack the crop over its 8 to 18-month growing cycle. In these seasonally dry regions, it is common to observe populations of mites, mealybugs, lacebugs, whiteflies, stemborers, thrips and minor pests infesting cassava plants.

The Colombia North Coast, represented by the Departments of Atlántico and Sucre, contain seasonally dry or semiarid agroecosystems where a complex of pests can be observed feeding on cassava. In collaboration with the cassava Germplasm Development Project, arthropod pest evaluations were done at five localities.

These include Santo Tomás (Pitalito and Caracolí), Molineros (Loruaco), Cantangallo (Corozal) and Ciénaga de Oro. Yield trials involving a selection of cassava germplasm was evaluated at 8 sites.

Materials and Methods

Approximately 60 cultivars were evaluated at each of the 8 sites, measuring the arthropod damage and populations. Each plot (cultivar) consisted of 20 plants and evaluations were made on the 6 central plants of each plot. A 1 (absence of damage or pest population) to 6 (maximum damage or pest population) rating scale was used to record damage and pest population levels. Using this method pest populations and damage could be measured at each site and compared to other sites. This leads to a descriptive analysis of the pest complex at each site and the levels of damaged being caused. In addition it also allows for the resistance /susceptible evaluation of each genotype. These evaluations also allow for the detection of any “new” pests that might appear in this ecosystem. The plant breeding program carries out the evaluations on vigor, yield, harvest index, and additional agronomic qualities of the cultivars.

Results

The pest complex observed across the different sites included the mite, *Oligonychus peruvianus*, the stembororer, *Chilomima clarkei*, the whitefly species, *Aleurothachelus socialis*, *Aleurodichus dispersus*, *Trialeurodes* sp, and *Tetraleurodes* sp. In addition an unidentified lepidopteran (Fam: Piralidae) species, the larvae of which attacks leaves to its body and feed on the stem surface and buds, but des not penetrate into the stem.

Overall the pest found in highest incidence was the mite *O. peruvianus*. This species is an often observed pest of cassava, however it is not usually found in high populations. The mite species more commonly observed in high populations in this agroecosystems are *Mononychellus tanajoa* and *M. caribbeanae*. Table 6.4 is a composite of the evaluations at each site. As can be observed, *O. peruvianus* was present in moderate to high populations at all sties except Molineros, and causing considerable damage to cassava leaves. Highest levels were at Santo Tomás (Atlántico) at the Caracolí site where in four breeding trials 86.4% and 87.5% of the clones in a *Yield Trial* (ER), and *Preliminary Yield Trial* (EPR) were infected with damage levels at 3.6 to 6.0 on the 1 to 6 damage scale. High *O. peruvianus* populations and damage was also observed at Cantagallo (Sucre) with 81.7% of the clones infested and equally high damage ratings.

Figures 6.9 through 6.12 express the damage and populations ratings of *O. peruvianus* at Caracolí, Santo Tomás for yield trials. It can be observed that populations were high at all sites, with no cultivars escaping damage nor infestations, and only one cultivar with a damage rating below 2.5 (CG 1141-1). This same clone however in a yield trial at a different site (Caracolí) had a damage and population both at 4.0. Individual ratings for each clone evaluated are available in the cassava arthropod resistance database. Other clones that present relatively lower ratings included MCol 2215 (3.0 damage rating), MTai-8 (3.0), SM 2440-9 (3.0) and SM 2440-5 (3.5), all had populations rating 4.0 or above, indicating high levels of susceptibility and considerable mite populations pressure at the site.

The stemborer, *C. clarkei*, presented a low to moderate level of incidence in the Santo Tomas (Caracolí) zone, in all of the trials evaluated (Table 6.4). No pest presence was observed in Molineros, Cantagallo, nor Ciénaga de Oro. The lepidopteran (Piralidae) was only observed in low incidence in Santo Tomas and Cantagallo, and in higher incidence in Ciénaga de Oro.

Whitefly populations, especially *A. socialis* were observed on all clones evaluated at all 8 of the yield trial sites (Table 6.4). However populations were very low, seldom higher than 2.0 (only one as high as 3.0), and damage ratings seldom exceeding 1.5. *Trialeurodes* sp. populations were highest at Molineros (Loruaco) with population ratings fluctuating between 2.0 and 3.0, but damage ratings not exceeding 1.5. At Cantagallo (Sucre) and Ciénada de Oro, *Trialeurodes* sp. was observed only in very low populations, not exceeding a 1.5 rating on any of the clones and no noticeable damage. The other whitefly species, *A. disperses* and *Tetraleurodes* sp. were observed in several of the sites but only at very low incidence.

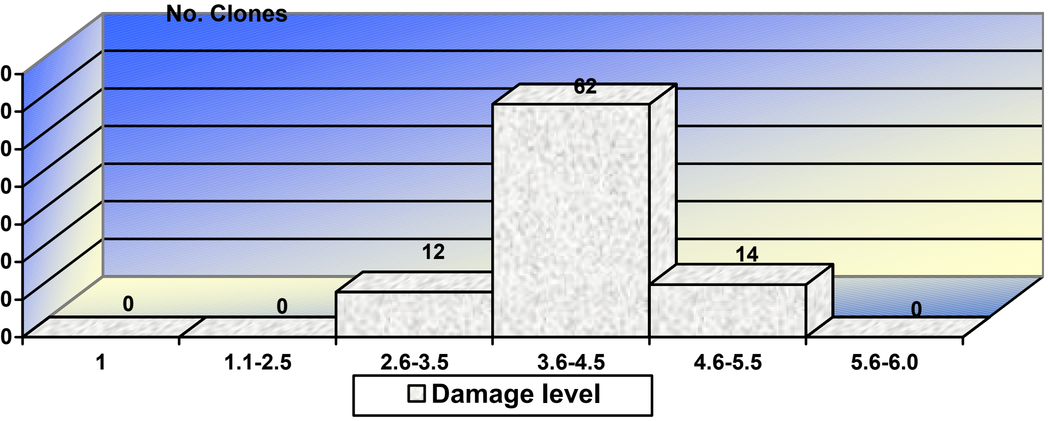

Figure 6.9. Evaluations of cassava clones for *Oligonychus peruvianus* mite damage in yield trials at Caracolí, Santo Tomás, Atlántico, during 2001.

Table 6.4. Evaluation of selected cassava germplasm (60 clones) for arthropod pest damage and populations at 8 sites on the Colombian North Coast during 2001.

Locality and trial[1]	PESTS													
	O. Peruvianus				***A. Socialis***		***C. Clarkei***			**Pyralidae**			**Trialeurodes**	
	% Damage grade*		**% Population grade***		**% Damage grade**	**% Population grade**	**% Population grade**			**% Population grade**			**% Damage grade**	**% Population grade**
	2.6-3.5	3.6 - 6	2.6 -3.5	3.6 - 6	1-3.5	1-3.5	1.0	2-3	4-6	1.0	2-3	4-6	1-3.5	1-3.5
Santo Tomás ER SEL.EPR Caracolí	14.3	85.7	3.0	97.0	100	100	62.9	37.1	0.0	-	-	-		
Santo Tomás EPR Caracolí	48.4	51.6	37.5	65.5	100	100	70.3	26.6	3.1	*	-	-		
Caracolí ER	13.6	86.4	1.1	98.9	100	100	52.3	47.7	0.0	*	-	-		
POT.R.V+N Pìtalito	50.0	50.0	20.0	80.0	100	100	43.3	55.0	1.7	*	-	-		
Santo Tomás EPR. V+N	58.3	36.7	26.7	73.3	100	100	30.0	61.7	8.3	15.0		1.7		
Molineros EPR	-	-	-	-	100	100	-	-	-	-	-	-	100	100
Cantagallo EPR	18.3	81.7	60.0	40.0	100	100	-	-	-	*	-	-	100	100
Ciénaga de Oro EPR	73.3	26.7	23.3	76.7	100	100	-	-	-	8.3	88.3	3.4	100	100

* % Damage grade; based on 1= no damage, to 6= severe damage.

% Population grade; based on 1= no pest presence, to 6=high pest populations.

[1] ER = Yield trial.

EPR = Preliminary yield trial.

U = Old plantings.

N = New plantings.

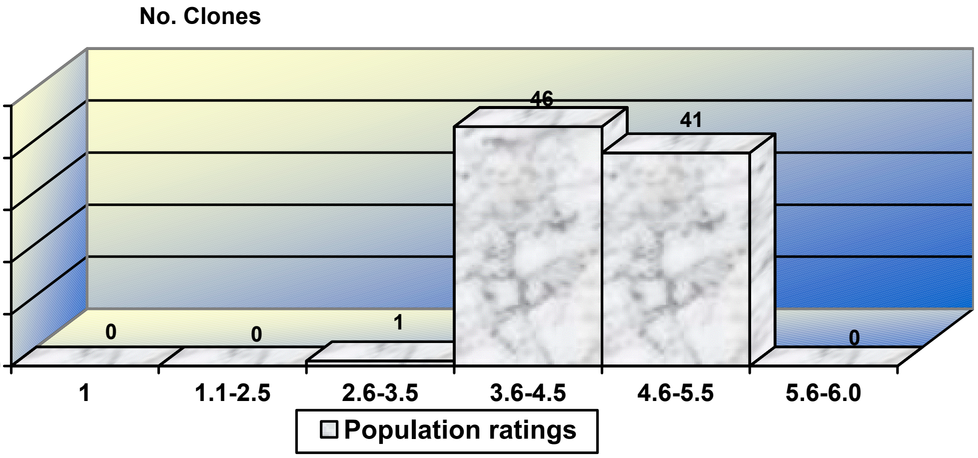

Figure 6.10. Evaluation of cassava clones of *Oligonychus peruvianus* mite populations in yield trials at Caracolí, Santo Tomás, Atlántico, during 2001.

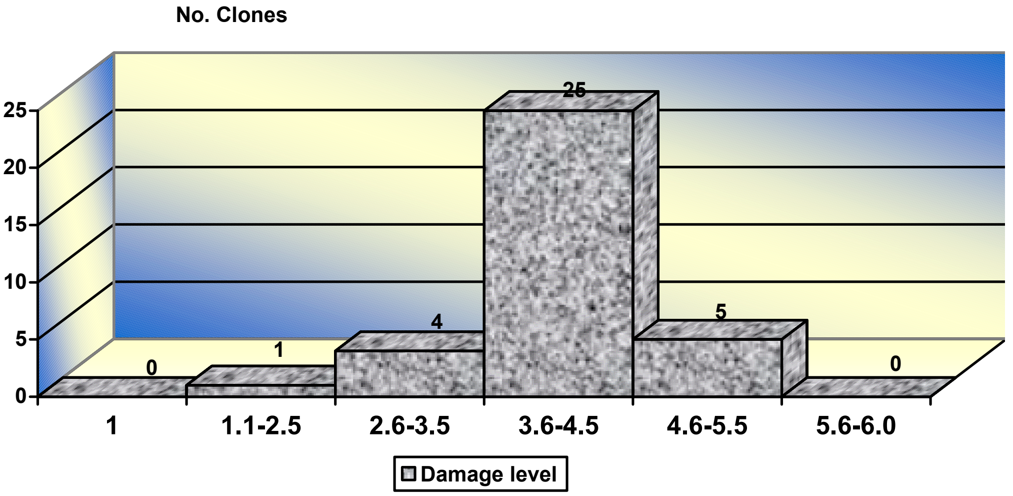

Figure 6.11. Evaluation of cassava clones of *Oligonychus peruvianus* mite damage/ resistance in preliminary yield trials at Santo Tomás, Atlántico, during 2001.

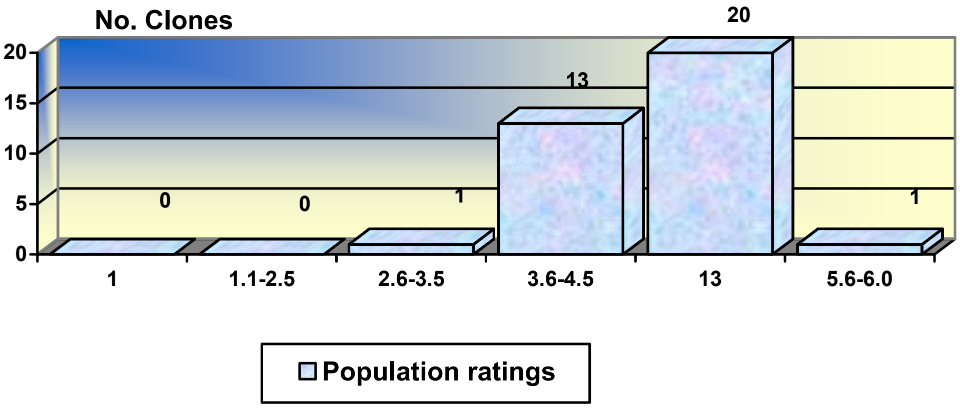

Figure 6.12. Evaluations of cassava clones for *Oligonychus peruvianus* populations in preliminary yield trials at Santo Tomás, Atlántico, during 2001.

Activity 6.3. CORPOICA evaluations of cassava varieties and hybrids resistant to the whitefly, Aleurotrachelus socialis, in the Upper Magdalena Valley.

Rationale

The whitefly, *Aleurotrachelus socialis* is the principal insect pest limiting cassava production in the department of Tolima, a sub-region of the Upper Magdalena Valley. Cassava yield losses in this region, due to whitefly attack have been reported as high as 68%. Farmers in the region often resort to the use of agrochemicals to combat whitefly attacks. However pesticide use is often ineffective, costly and harmful to natural enemies and the environment. In addition whiteflies easily acquire resistance to pesticides, if used continually or indiscriminately.

Host plant resistance (HPR) offers an effective, economically feasible and environmentally sound alternative for whitefly (*A. socialis*) control. HPR studies were initiated by CIAT and CORPOICA more than 15 years ago and more than 5000 varieties from the CIAT cassava germplasm bank have now been evaluated at either the CORPOICA (El Espinal, Tolima) or the CIAT (Palmira) sites. The consistently high *A. socialis* populations at the former site have made it an ideal location to accomplish HPR field evaluations over a prolonged time period.

Early on in these evaluations, a source of *A. socialis* resistance was observed in the clone MEcu 72. This clone has consistently expressed high levels of resistance during numerous evaluations, for several years and across several agroecosystems. MEcu 72 and MBra 12 (an agronomically desirable clone with some field tolerance to whiteflies) were used in a crossing program to provide high yielding whitefly resistant clones. 127 Progeny from this cross were evaluated at El Espinal over several growing cycles and four hybrids (progeny) were chosen for their resistance to *A. socialis* and yield and consumer quality characteristics.

Complimentary investigations in greenhouse and field trials showed that *A. socialis* feeding on resistant clones had less oviposition, longer development periods, reduced size and higher mortality than those feeding on susceptible one. In addition whitefly resistant clones showed little or no, significant difference in yield between insecticide-treated and non-treated plots. The progeny selected from the MEcu 72 x MBra 12 cross, CG 489-34, CG 489-4, CG 489-31, and CG 489-23, have consistently displayed moderate levels of resistance.

CORPOICA, Nataima, El Espinal, has been evaluating these materials, parents and hybrids for several years with the prospects of varietal release of whitefly (*A. socialis*) resistant varieties.

Materials and Methods

Two "Evaluations of Agronomic Efficiency" were set up to evaluate whitefly resistant and susceptible germplasm by CORPOICA; one at the Nataima, El Espinal Station in Tolima, and the second at the Granja El Juncal at the South Colombian University in Neiva, Huila. Whiteflies are a problem at both sites.

The experimental design used completely randomized blocks with four repetitions and the treatments were 10 cassava genotypes (Table 6.5). Each experimental block was 36m^2, six rows of six plants, leaving the 16 center plants for sampling. 20cm long cuttings, with at least four nodes were treated with a 5-minute insecticide/fungicide dip. Stake cuttings were planted vertically on ridges, and a herbicide (Diurón + Alaclor) was applied immediately after planting. Weeds were controlled manually during the growth cycle of the experiment. An application of *Bacillus thuringiensis* for cassava hornworm (*Erinnyis ello*) control was also made.

Table 6.5. Origin of cassava genotypes evaluated by CORPOICA, Nataima, El Espinal, Tolima.

Genotype	Origin or Source
MBra 12	Brazil
MEcu 72	Zamora, Chinchipe, Ecuador
CG 489-4	MEcu 72 x MBra 12 CIAT
CG 489-23	MEcu 72 x MBra 12 CIAT
CG 489-31	MEcu 72 x MBra 12 CIAT
CG 489-34	MEcu 72 x MBra 12 CIAT
CMC 40	Campinas, Brazil, Mantequierá, MCol 1468, Manihoica P-11
MCub 74	Señorita, Cuba
CM 4365-3	CM 976-15 x MCol 2207 CIAT
Aroma	El Guamo, Tolima, Colombia

Table 6.6. Whitefly adult and nymph population scales.

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

Monthly evaluations of whitefly (*A. socialis*) damage and populations were carried out using standard damage and whitefly population scales (Tables 6.6 and 6.7). Prior to harvest, and during harvest, data was collected on morphological and agronomic characteristics of the varieties or genotypes involved in the evaluation. These data included, yield, harvest index, dry matter, and culinary qualities, such as flavor, texture (root parenchyma), fiber, digestibility, HCN content and physiological deterioration.

Table 6.7. Cassava/whitefly damage ratings.

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.

Results

6.3.1 Nataima A site.

Significant differences in whitefly (*A. socialis*) damage and infestations were recorded on the cassava genotypes evaluated at the Nataima site (Figure 6.13) (Tukey Test). The genotypes MEcu 72 and CG 489-31 had damage levels of 1 (no damage), while CMC-40, the susceptible control, and Aroma, the regional (farmers) variety recorded damage levels of 3.57 and 2.53 respectively. Damage levels on CMC-40 and Aroma eventually reached 4.38 during the forth month of growth (Figure 6.14). These data indicate that both varieties are susceptible to *A. socialis*. Other genotypes with low damage ratings were the hybrids (MEcu 72 x MBra 12), CG 489-34, CG 489-23 and CG 489-4 with ratings of 1.09, 1.16 and 1.17 respectively, again indicating high levels of resistance (Table 6.8).

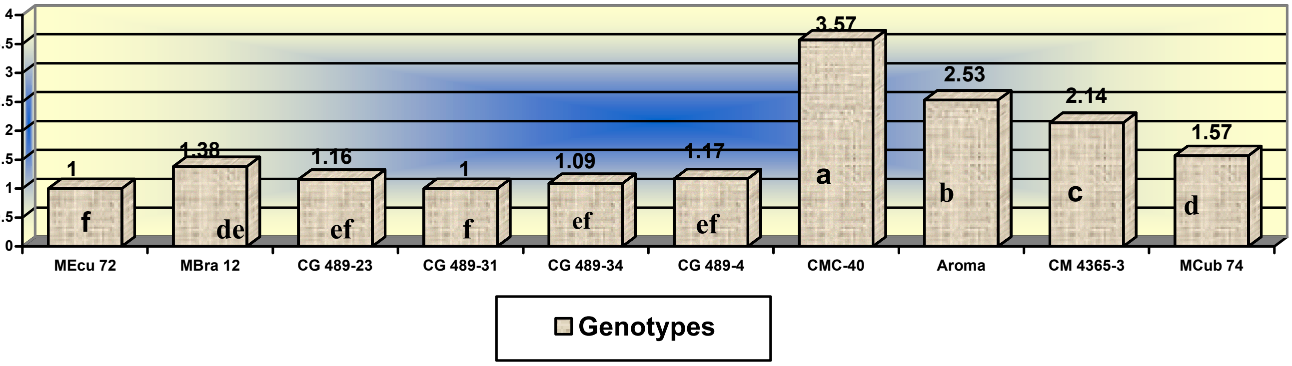

Figure 6.13. Average whitefly (*Aleurotrachelus socialis*) damage levels for 10 cassava genotypes at CORPOICA, Nataima using a 1 (no damage) to 6 (severe damage) scale.

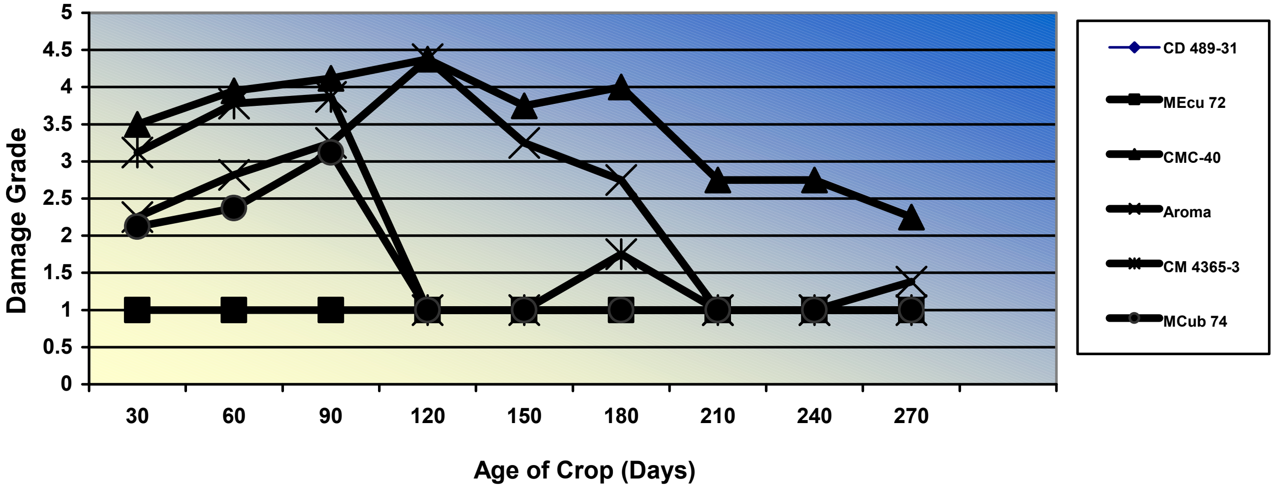

Figure 6.14. Monthly whitefly (*Aleurotrachelus socialis*) damage ratings for 6 cassava genotypes recorded at CORPOICA, Nataima.

Table 6.8. Whitefly (*Aleurotrachelus socialis*) damage ratings on 10 cassava genotypes during a 9-month growth cycle at Nataima, Tolima.

	Age of Crop (Months)/Damage Rating[1]									
Genotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Average**
CG 489-31	1	1	1	1	1	1	1	1	1	1
CG 489-34	1	1	1.75	1	1	1	1	1	1	1.08
CG 489-23	1.37	1.37	1.75	1	1	1	1	1	1	1.17
CG 489-4	1	1.5	1.87	1	1	1	1	1	1	1.17
MBra 12	1	2.62	2.25	1	1.02	1	1	1	1	1.38
MEcu 72	1	1	1	1	1	1	1	1	1	1
MCub 74	2.12	2.37	3.12	1	1	1	1	1	1	1.57
CMC-0	3.5	3.95	4.12	4.375	3.75	4	2.75	2.75	2.25	3.57
CM 4365-3	3.12	3.78	3.87	1	1	1.75	1	1	1	2.14
Aroma	2.25	2.82	3.25	4.375	3.25	2.75	1	1	1.38	2.53

[1] Damage rating: 1 = No plant damage.
2 = Severe damage, leaf necrosis, stems thin and weakened, considerable sooty mold.

The results also show that whitefly damage was highest during the first 5 months of the growth cycle and reduced during the remaining 4 months (months 6 through 9) (Table 6.8). On CMC-40 damage rating remained relatively high throughout the growth cycle and with the regional variety Aroma, tapered off during the final three months (7 through 9). CMC-40 and Aroma were the only two cultivars that had damage ratings above four (4) during the growth cycle. Plant damage at this level is expressed by severe leaf distortion, and yellow to green speckling on apical and mid leaves with a presence of sooty mold. CMC-40 also showed some leaf necrosis during the third to 6th month of crop growth.

There is a correlation, as expected, between plant damage and whitefly populations. The genotypes CG 489-31 and MEcu 72 had low pupal populations of 28 and 46 average pupae per lower leaf evaluated (Figure 6.15). The pupal population is an indication of how many whitefly individuals are able to complete their life cycle on a given genotype. The genotypes CMC-40 and Aroma, the susceptible control and the farmers regional variety, had 485 and 461 pupae per lower leaf respectively (Figure 6.15). (Significant difference, <1%). In addition pupal populations on the middle third of the leaves were highest on CMC-40 (419 per leaf) and Aroma (272) and lowest on MEcu 72 (2) and CG 489-31 (6) (Table 6.9). Pupal populations were also comparatively and significantly lower on the three additional progeny CG 489-34, CD 489-23 and CG 489-4 (Table 6.9). Nymphal populations were lowest on CG 489-31 (8) and MEcu 72 (14), intermediate on the hybrids and highest on CMC-40 (192) and Aroma (158). MEcu 72 and CG 489-31 had about a 96% lower nymphal population than the two susceptible genotypes.

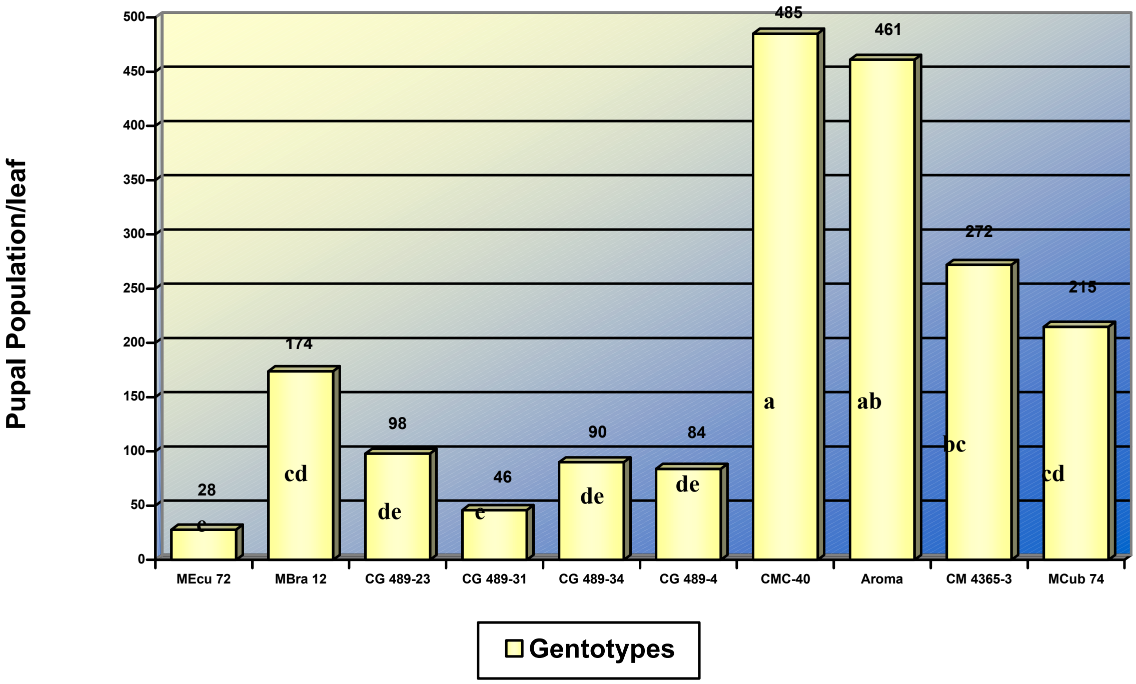

Figure 6.15. Whitefly (*Aleurotrachelus socialis*) pupal populations per lower (1/3) leaf recorded in efficacy trials of 10 cassava genotypes at CORPOICA, Nataima, El Espinal, Tolima.

Adult populations on the 10 genotypes gave similar results; adult populations, observed mostly on the upper leaves of the plant, for MEcu 72 and CG 489-31 were 5.8 and 3.6 adults per leaf respectively, they were intermediate on the progeny CG 489-34 (7.8), CG 489-23 (8.2) and CG 489-4 (11.0), and highest on CMC-40 (79.4), CM 4635-3 (74.0) and Aroma (68.6) (Figure 6.16). Egg population (oviposition) was lowest on MEcu 72 (5.8) and CG 489-31 (7.2), intermediate in the hybrids, and highest in CMC-40 (485) and Aroma (389) (Table 6.9).

Table 6.9. Populations of different whitefly (*Aleurotrachelus socialis*) stages on 10 resistant and susceptible cassava genotypes at CORPOICA, Nataima, Tolima.

Genotype	***Pupae/Leaf*** **Lower Third**	**Nymphs/Leaf Middle Third**	**Pupae/Leaf Middle Third**	**Adults/Leaf Upper Third**	**Egg/Leaf Upper Third**
CG 489-31	46 e	8 f	6 ef	3.6 e	7.2 e
CG 489-34	90 d	26 ef	20 ef	7.8 cde	15.8 d
CG 489-23	98 d	76 de	56 de	8.2 cde	14.8 d
CG 489-4	84 d	28 f	24 ef	11.0 bcd	18. d
MBra 12	174 c	90 cd	96 cd	15.0 b	153.2 c
MEcu 72	28 e	14 f	2 f	5.8 de	5.8 f
MCub 74	215 c	84 cd	76 cd	13.8 dc	95.6 c
CMC-40	485 a	192 a	419 a	79.4 a	485 a
CM 4365-3	272 b	134 bc	128 c	74.0 a	332 b
Aroma	461 a	158 ab	272 b	68.6 a	389 a
Signific.	**	**	**	**	**
CV (%)	13.96	8.01	9.56	7.62	7.91

** Significant differences, 1% level, Tukey.

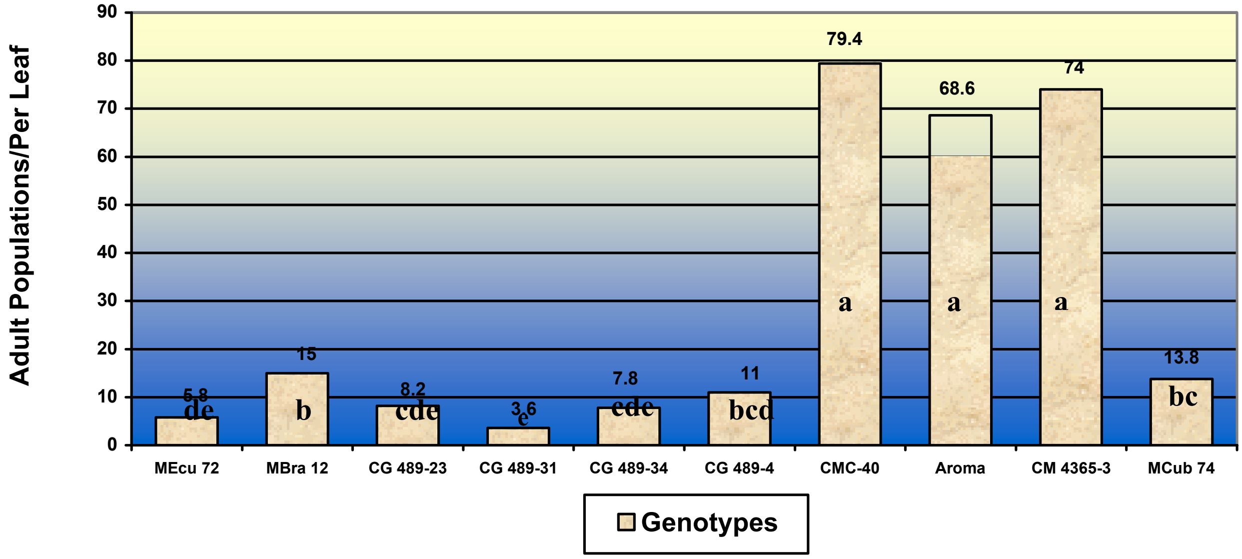

Figure 6.16. Whitefly (*Aleurotrachelus socialis*) adult per leaf populations on 10 cassava genotypes at CORPOICA, Nataima, El Espinal.

MBra 12, the male parent in the cross with MEcu 72, was usually intermediate in terms of whitefly populations and damage, but higher than any of the progeny. For example it had 174 adults, 90 nymphs, 15 adults, and 153 eggs, per leaf (Table 6.9), all higher than MEcu 72 and the four progeny, but not nearly as high as the susceptible clones CMC-40 and Aroma. These results indicate that the hybrid progeny have inherited considerable resistance from the female parent, MEcu 72. These results also reinforce the presence of an antibiosis mechanism involved in the resistance. The low numbers of adults and oviposition on the resistant genotypes may also signify an Antixenosis mechanism for oviposition.

Yields were highest on the clones MCub 74, CM 4365-3, MBra 12 and CG 489-34 with 28.8, 26.8, 25.4 and 23.5 tons per hectare respectively (Table 6.10). CM 4365-3, Aroma, CG 489-23 and CG 489-31 had the highest dry matter content with 40.49%, 38.1%, 36.0% and 39.94% respectively. The lowest yields were Aroma (12.4 T/ha.), CG 489-4 (3.3 T/ha.) and MEcu 72 (17.1 T/ha.). The low yield of CG 489-4 is mainly attributed to the high incidence of root rot, greater than 50% of the roots. Yield difference for all of the genotypes with the exception of CG 489-4, were not significant.

Whitefly populations, it should be noted, did not remain high on MCub 74 and CM 4365-3 (Figure 6.14) throughout the 9-month growth cycle. Whitefly populations were high only during the first three months and therefore not during the root bulking stage of the plant. This may partially explain the higher yields with these two genotypes.

There was no significant difference in the number of stakes (cuttings) produced per plant (Table 6.10). The culinary quality evaluations show that all genotypes are sweet, or low in HCN content, good cooking quality, soft, low fiber content and cream or cream to white in color.

Table 6.10. Agronomic characteristics, including yield and dry matter content, of 10 cassava genotypes evaluated under whitefly pressure at CORPOICA, Nataima, El Espinal.

Genotype	Total Root Yield (T/ha.)	Harvest Index	Dry Matter %	Dry Matter Yield (Kg/ha.)	Cuttings/Plant
CG 489-31	18.2 AB	0.55 AB	35.94 C	6525 AB	12.6 A
CG 489-34	23.5 AB	0.69 A	32.99 EF	7685 AB	11.7 A
CG 489-23	18.5 AB	0.60 AB	36.00 C	6710 AB	12.0 A
CG 489-4	3.3 B	0.19 C	35.2 CD	1168 A	13.7 A
MBra 12	25.4 A	0.65 AB	35.37 CD	8952 A	15.2 A
MEcu 72	17.1 AB	0.57 AB	33.90 DE	5803 AB	11.3 A
MCub 74	28.8 A	0.64 AB	33.43 E	9666 A	17.0 A
CMC-40	19.5 AB	0.51 B	31.61 F	6108 AB	15.0 A
CM 4365-3	26.8 A	0.68 A	40.49 A	10862 A	13.2 A
Aroma	12.4 AB	0.48 B	38.10 B	4721 B	14.4 A
	**	**	**	**	N.S
Probab. %	0.97	0.01	0.01	0.66	44.5

** Significant differences at 5% level, Tukey Test.
NS = No significant differences.
Averages with the same letter are not significantly different.

6.3.2. El Juncal, Neiva (Huila) site.

Whitefly populations at the El Juncal site were very low throughout the duration of the trial. The damage level on all 10 genotypes remained at 1.0, and pupae populations ranged from 1.01 to 1.05 on the lower third of the leaves. There were no significant differences in pupal, nymphal and damage grades.

The lack of whitefly pressure was reflected in yield results; the highest yielding genotype was CMC-40, a vigorous, high yielding variety but very susceptible to pest damage, especially whiteflies. CMC-40 yielded 27.4

T/ha., MCub 74, 23.9 and CG 489-34, 23 T/ha. Aroma the regional or farmers variety yielded only 14.7 T/ha. (Table 6.11). There were, however no significant differences between yield of the 10 genotypes, with the exception of CMC-40 and MEcu 72, which yielded only 11.8T/ha. A significant aspect of this data, is that in the absence of whitefly pressure, high yielding, but susceptible cultivars, such as CMC-40, can out-yield resistant cultivars. However under heavier whitefly pressure, CMC-40 is very susceptible to yield reduction, as was observed in the Nataima trial, where it was one of the lowest yielding varieties. It can also be noted from these data that MEcu 72, although highly resistant to whiteflies, is not a high yielding variety and therefore needs to be included in a breeding program where yields can be increased while its high resistance to whiteflies (as well as mites and thrips) can be retained.

6.3.3. Nataima B.

The second trial planted at Nataima during the second semester of the year using the same genotypes and aforementioned methodologies.

Whitefly populations during this trial were higher than in the El Juncal, Neiva, but not as high as in the Nataima A experiment. However populations were sufficiently high to result in significant differences in damage and yield. Whitefly damage ratings were highest on CMC-40 (3.48), followed by Aroma (2.31) the regional variety, and CM 4365-3 (2.23). Damage was lowest on MEcu 72 (1.0 = no damage), CG 489-31 (1.0) CG 489-4 (1.01), CG 489-34 (1.02) and CG 489-23 (1.03) (Table 6.12). Damage was notably more severe on CMC-40 with severe leaf distortion, yellow mottling of upper leaves, sooty mold on the middle and lower leaves and some necrosis and defoliation of lower leaves. MEcu 72 and the four hybrid progeny were free of any of these damage symptoms, reinforcing the presence of considerable whitefly resistance in these genotypes. MBra 12 and MCub 74 showed some tolerance to whiteflies with low damage levels 1.20 and 1.25 respectively.

Table 6.11. Agronomic characteristics, including yield and dry matter content, of 10 cassava genotypes evaluated under whitefly presence at El Juncal, Neiva, by CORPOICA.

Genotype	Total root yield (T/ha.)	Harvest index	Dry matter %	Cuttings/plant
CG 489-31	17,708 AB	0.43 CD	29.3ABC	15.1 A
CG 489-34	23,041 AB	0.55 AB	22.8 D	18.5 A
CG 489-23	20,227 AB	0.50 BC	27.3 BCD	13.3 A
CG 489-4	18,742 AB	0.49 BC	28.9 ABC	14.0 A
MBra 12	20,145 AB	0.52 BC	26.5 CD	11.8 A
MEcu 72	11,820 B	0.36 D	27.4 BCD	15.7 A
MCub 74	23,879 AB	0.59 AB	28.6 ABC	12.8 A
CMC-40	27,445 A	0.63 A	24.6 CD	11.7 A
CM 4365-3	22,227 AB	0.57 AB	33.2 A	11.7 A
Aroma	14,691 AB	0.41 CD	32.4 AB	12.2 A
Signific.	*	**	**	NS
CV %	26.7	8.8	7.9	29.9

* Significant differences at 5% level.
** Significant differences at 1% level.
NS = No significant differences.
Averages with the same level are not significantly different.

Table 6.12. Whitefly (*Aleurotrachelus socialis*) populations and damage ratings on 10 cassava genotypes during efficacy trials at CORPOICA, Nataima, El Espinal, Tolima.

Genotype	**Damage Rating[1]**	**Lower Third**		**Mid Third**				**Upper Third**			
		Pupae Rating	**Pupae/ Leaf**	**Nymph Rating**	**Nymphs/ Leaf**	**Pupae Rating**	**Pupae/ Leaf**	**Adult Rating**	**Adults /Leaf**	**Ovipositio n Rating**	**Eggs /Leaf**
CG 489-31	1.00 c	1.05 e	10	1.02 c	4	1.03 d	6	1.05 d	1	1.10 d	2
CG 489-34	1.02 c	1.19 de	32	1.27 c	54	1.19 cd	38	1.13 cd	2.6	1.32 cd	6.4
CG 489-23	1.03 c	1.42 cde	84	1.19 c	38	1.28 cd	56	1.21 cd	4.2	1.51 bcd	10.2
CG 489-4	1.01 c	1.21 de	42	1.17 c	34	1.21 cd	44	1.21 cd	4.2	1.42 bcd	8.4
MBra 12	1.20 c	1.82 bcd	164	1.63 bc	126	1.58 bcd	116	1.44 bc	8.8	2.18 b	3.6
MEcu 72	1.00 c	1.06 e	12	1.08 c	16	1.05 d	10	1.15 cd	3	1.16 d	3.2
MCub 74	1.25 c	1.96 bc	192	1.73 bc	146	1.58 cd	116	1.42 c	8.4	2.16 bc	52
CMC-40	3.48 c	3.10 a	650	2.63 a	389	2.86 a	458	2.04 a	28	3.55 a	365
CM 4365-3	2.23 c	2.06 bc	218	2.00 ab	200	1.91 bc	182	1.90 a	18	3.04 a	212
Aroma	2.31 c	2.32 b	296	2.26 ab	278	2.30 a	290	1.75 a	15	3.30 a	260
Signific.	**	**		**		**		**		**	
CV %	20.68	16.53		18.29		19.1		9.24		16.79	

* Significant different at 5% level, Tukey Test.
** Significantly different at 1% level, Tukey Test.
No significant difference.
Averages with the same letter are not significantly different.
[1] See Table 6.3.

As expected, whitefly populations followed a similar pattern to that of the damage scores (Table 6.12). Pupae levels were lowest on MEcu 72 (1.06, see table 6.6), CG 489-31 (1.05), CG 489-34 (1.19) and CG 489-4 (1.21) and highest on CMC 40 (3.10), Aroma (2.32) and CM 4365-3 (2.06). This ranking remained the same for pupal populations on the lower and middle third of the leaves although populations were higher on the lower leaves (Table 6.12). In actual numbers, CG 489-31 and MEcu 72 had 10 and 12 pupae per leaf respectively while CMC-40 and Aroma had 650 and 296 respectively on lower leaves. These same differences proportionally occurred for pupal populations on middle leaves.

Nymphal populations followed a similar pattern to those of pupae. Lowest whitefly nymphal populations were on CG 489-31 (1.02 rating) and MEcu 72 (1.08) and highest on CMC-40 (2.63), Aroma (2.26) and CM 4365-3 (2.0). There was a significant difference in *A. socialis* nymphal rating between MEcu 72 and the four hybrid progeny, and CMC 40, Aroma and CM 4365-3 (Table 6.12). In actual numbers, CG 489-31 had only 4 nymphs per leaf, while CMC-40 had 389.

Adult *A. socialis* populations were highest on CMC-40 (2.04), CM 4365-3 (1.9) and Aroma (1.75) and significantly different than all other genotypes (Table 6.12). Lowest adult populations were on the four hybrids and MEcu 72. In actual numbers, an average of only 1 whitefly per leaf was observed on CG 489-31, and only 3 per leaf on MEcu 72. 28 adults per leaf were collected, on average, from CMC-40. Oviposition was corresponding low on the hybrids and MEcu 72 (3.2 eggs per leaf) and highest on CMC- 40 (365 eggs per leaf) Aroma (260) and CM 4365-3 (212).

These results confirm the moderate to high levels of resistance to *A. socialis* that has been observed, and previously recorded, in the variety MEcu 72, and the four hybrid progeny.

Yield were highest on CM 4365-3 (35.0 T/ha.), CG 489-31 (33.4 T/ha.), MBra 12 (33.9 T/ha. And CG 489-4 (33.0 T/ha.) (Table 6.11). Yields were lowest on MEcu 72 (19.4 T/ha.), CG 489-23 (20.5 T/ha.), Aroma (21.7 T/ha.) and CMC-40 (22.4 T/ha.). The difference in yield between the susceptible control CMC-40 and the best hybrid, CG 489-31, was 33%. Compared to the regional farmers variety, Aroma, the difference was nearly 36%. In addition, CMC-40 has a very low dry matter, 26.7%, the lowest of any of the genotypes evaluated. Dry matter yield differences between CMC 40 and CG 489-31 was 41%.

The highest dry matter content were Aroma at 35.2%, CM 4365-3 (33.8%) and CG 489-23 (33.2%) while the lowest after CMC 40 were MEcu 72 (28.8%) and CG 489-34 (29.9%). Stake production was highest with MEcu 72 (23.7 per plant) and lowest with CG 489-23 (11.9), Aroma (12.5), and CMC-40 (12.8). Root rots incidence was lower in this trial than the Nataima A trial. For example CG 489-4 had only 4.9 % root rot while in the Nataima A trial it was 50.4%. However MEcu 72, CMC-40, and Aroma all suffered between 7 to 9% root rot. MEcu 72 had a low number of plants harvested slightly more than 50% over the maximum and this may account for its lower yield. Several other genotypes, especially CG 489-34 and CMC-40, also suffered plant loss. This may be due to poor germination, or robbery; the Nataima site has historically been prone to this latter problem.

A combined analysis of the three trials shows that CM 4365-3 had the highest total yield at 28.0 T/ha., followed by MBra 12 (26.5 T/ha.), MCub 74 (26.1 T/ha.) and CG 489-34 (24.1 T/ha.) (Table 6.12). The lowest combined yields were MEcu 72 (16.1 T/ha.) and Aroma (16.2 T/ha.) (Figure 6.17). Significantly higher yields were obtained in the Nataima B trial when compared to the other two trials (Table 6.13) and the Nataima A trial had higher combined yields of the 10 genotypes than the Neiva trial in commercial root production.

Table 6.11. Agronomic characteristics, including yield, harvest index and dry matter content, of 10 cassava genotypes evaluated under whitefly (*Aleurotrachelus socialis*) pressure at CORPOICA, Nataima (B), El Espinal.

Genotype	Total Root Yield T/ha.	Harvest Index	Dry Matter %	Cuttings/Plant
CG 489-31	33.4 a	0.56 ab	31.0 abcd	18.7 ab
CG 489-34	25.7 a	0.64 a	29.9 bcd	14.4 b
CG 489-23	20.4 a	0.53 ab	33.2 abc	11.9 b
CG 489-4	33.0 a	0.56 abc	32.7 abc	14.9 b
MBRA 12	33.9 a	0.63 a	31.0 abcd	13.9 b
MECU 72	19.3 a	0.43 c	28.8 cd	23.7 a
MCUB 74	25.8 a	0.49 bc	30.8 abcd	18.9 ab
CMC 40	22.4 a	0.57 ab	26.7 b	12.8 b
CM 4365-3	35.0 a	0.57 ab	33.8 ab	18.3 ab
AROMA	21.7 a	0.45 bc	35.2 a	12.5 b
Signific.	**	**	**	**
C.V. %	0.97	0.01	0.01	44.5

** Significantly difference at 5% level, Tukey Test.

Average with the same letter are not significantly different.

Table 6.12. Total root yield of 10 cassava genotypes at three localities in the Upper Magdalena Valley, conducted by CORPOICA.

Genotypes	Total root yield t/ha.	Total root yield t/ha.	Total root yield t/ha.	Total root yield	
	Nataima A	**Nataima B**	**Neiva B**	**Average**	**Nataima A-B**
CG 489-31	18.2	33.4	17.7	23.1 ab	25.8
CG 489-34	23.5	25.7	23.0	24.1 ab	24.6
CG 489-23	19.6	20.5	20.2	19.7 ab	19.5
CG 489-4	33.3	33.0	18.7	18.3 ab	18.1
MBra 12	25.4	33.9	20.1	26.5 a	29.7
MEcu 72	17.1	19.6	11.8	16.1 c	18.2
MCub 74	28.8	25.8	23.9	26.1 a	27.3
CMC-40	19.5	22.4	27.4	23.1 ab	21.0
CM 4365-3	26.8	35.0	22.2	28.0 a	30.9
Aroma	12.4	21.7	14.7	16.3 b	17.0
Average/Locality	19.4	27.1	20.04	22.1	23.2

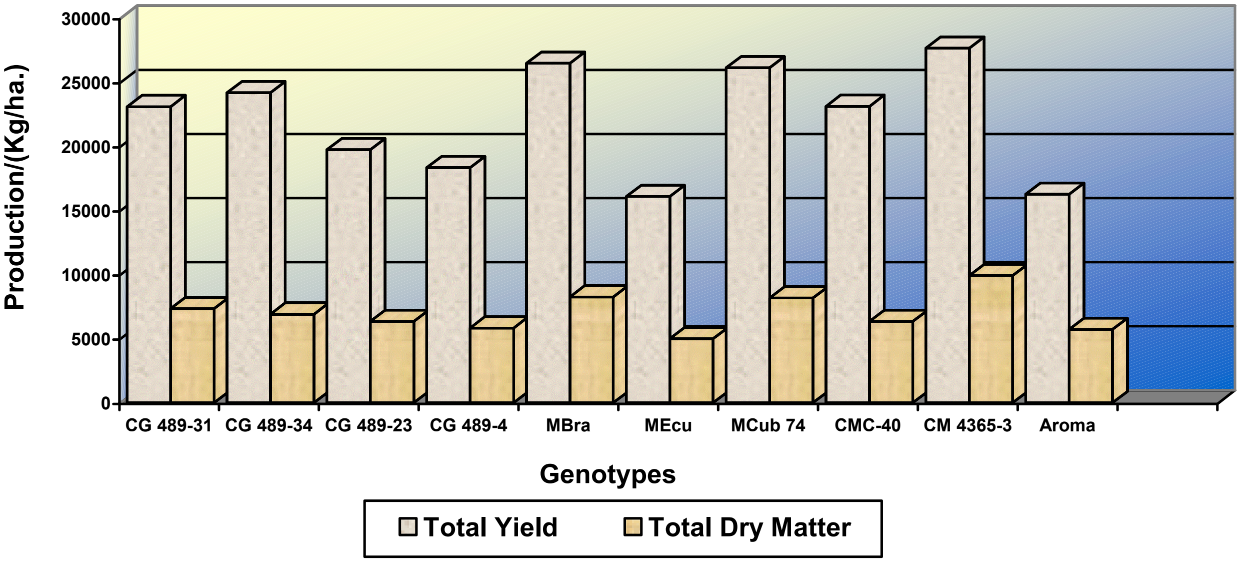

Figure 6.17. Average total yield and dry matter content; combined data from three whitefly resistance efficacy trial of 10 cassava genotypes at their localities in the Upper Magdalena Valley.

Table 6.13. Combined analysis for yield characteristics from efficacy trials of 10 cassava genotypes at three locations in the Upper Magdalena Valley.

Locality	Yield commercial Root (Kg/ha.)	Total Root Yield (Kg/ha.)	Harvest Index	Dry Matter %	Total Dry Matter Production (Kg/ha.)	Cuttings/ Plant	Leaf Weight
Neiva 99 A	10169 c	20011 b	0.50 b	28.10 c	5623 b	13.7 a	5715 a
Nataima 99 A	16553 b	19352 b	0.56 a	35.30 a	6831 b	13.8 a	1272 b
Nataima 99 b	19967 a	26968 a	0.54 a	31.30 b	8445 a	15.5 a	5518 a

Average with same letter are not significantly different, Tukey Test.

If we look at the two Nataima trials, where whitefly populations were highest, CM 4365-3 (30.9 T/ha.) and MBra 12 (29.7 T/ha.) were the highest and Aroma (17.0 T/ha.) was the lowest. These results indicate that the farmers regional variety has consistently yielded lower than all of the genotypes tested, although much of this data is not significantly different. In addition it can also be noted that CMC-40 when grown where there is little or no whitefly pressure will yield as high or higher than the other whitefly resistant genotypes. However under high whitefly pressure, CMC-40 is quite susceptible and yields diminish, and are lower than the best resistant hybrids.

Although all of the hybrids yielded higher than Aroma, the regional variety, two hybrids CG 489-31 and CG 489-34 (Table 6.14) gave considerably higher yields. These two whitefly resistant genotypes would be recommendable under conditions of high whitefly pressure.

CORPOICA, Nataima, has indicated that the hybrid CG 489-31 (Table 6.14) is the best candidate for release to farmers in the region. They cite CG 489-34 high yield and dry matter combined with a high level of whitefly resistance, and its adaptations to the ecosystem as important characteristics for varietal release.

Table 6.14. Morphological characteristics of two cassava genotypes, CG 489-31 and CG 489-34.

Plant	***CG 489-31***	**CG 489-34**
Plant Height	250,4 (236,2 - 262,5) cm	231.4 (222 - 245) cm
Height, first branching	1400 (115,2 - 145,3) cm	112.8 (103 - 124) cm
Levels of branching	2	2.5 (2 - 3)
Branches at each level	3	02.8
Leaves		
Color: growing point	Light green	Light green
Pubescence: growing point	High	Intermediate
Shape: central leaf lobe	Lanceolada lanceolate	Elliptical elliptic
Leaf Color	Dark green	Dark green
Vein Color	Light green	Light green
Petrole Color	Dark red	Yellow-red
Leaf weight (kg/ha.)	4517	3236
Stem		
Epidermis (color)	Dark brown	Cream
Colenquima (color)	Dark green	Light green
External color	Dark reddish brown	Yellowish-green-brown
Nodes	Prominent	Prominent
Cutting production/plant	15	14.8
Roots		
Form	Conical cylindrical	Conical cylindrical
Peduncle	Intermediate	Intermediate
External (outer) color	Dark brown	Cream (light brown)
Peel color	Rosy/pinkish	Rosy/pinkish
Pulp color	White	White to cream

Acknowledgements
Activity 6.3 data and text was condensed from the article “Evaluación de Variedades de Yuca Resistentes a Mosca Blanca en el Valle Alto del Magdalena.”

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Activity 6.4. Evaluation of cassava genotypes for a resistance to the B. biotype of Bemisia tabaci.

Rationale

Whiteflies are a major pest of many agricultural crops in many areas of the world, but especially in the tropics and subtropics. Yield losses are estimated in the hundreds of millions of dollars. Many whitefly species cause crop losses through direct foliar feeding, while others are very efficient vectors of numerous economically important plant viruses. There are approximately 1200 species with a wide host range, including legumes, fruits, root and tuber crops, vegetables, cotton and ornamentals.

Whiteflies cause considerable losses in cassava agroecosystems, both as direct feeding damage and as vectors of virus diseases. *Aleurochachelus socialis* and *Aleurothrixus aepim* reduce cassava yields considerable due to direct feeding damage in the neotropics. A single whitefly species, *Bemisia tabaci* (this is actually a complex of biotypes and may include the species *B. argentifolii*), is the vector of Africa Cassava Mosaic Disease (ACMD), Bean Golden Mosaic, Tomato Yellow Leaf Curl (TYLC) and at least 30 other geminiviruses of important food crops. This pantropical pest feed on cassava throughout Africa, several countries in Asia and more recently in the Neotropics.

Prior to 1990, the *B. tabaci* biotypes found in the Americas did not feed on cassava. It has been speculated that the absence of ACMD in the Americas was related to the inability of its vector, *B. tabaci*, to colonize cassava. Since the early 1990s a new biotype (B) of *b. tabaci*, considered by some a separate species (*B. argentifolii*), has been found feeding on cassava in the Neotropics. It is considered that ACMD now posses a more serious threat to cassava production given that most traditional cultivars in the Neotropics are highly susceptible to the disease. In addition to *B. tabaci* biotype complex is the vector of several viruses often grown in association with cassava or near it, especially in traditional cropping systems in the tropics. The possibility of virus diseases moving among these crops on the appearance of new viruses represents a potential threat.

Host plant resistance (HPR) studies initiated at CIAT > 15 years ago, are systematically evaluating the 6000 cultivars in the germplasm bank for whitefly resistance, especially to *A. socialis*. Several sources of resistance to *A. socialis* have now been identified (See Annual Report, CIAT, IP-2, 1998-2000). The clone MEcu 72 has consistently expressed the highest levels of resistance. Additional cultivars expressing high to moderate levels of resistance include MEcu 64, MPer 335, MPer 415, MPer 317, MPer 216, MPer 221, MPer 265, MPer 266 and MPer 365. MEcu 72 and MBra 12 (an agronomically desirable clone with field tolerance to whiteflies) were used in a crossing program to provide high-yielding, whitefly-resistant clones that showed no significant differences in yield between insecticide-treated and non-treated plots. The progeny CG 489-34 from this cross has demonstrated moderate to high levels of resistance in field and laboratory trials.

The purpose of the current research is to evaluate the resistance in cassava to the whitefly species, *A. socialis*, against the B biotype of *Bemisia tabaci*.

Materials and Methods

The establishment of *B. tabaci* colony on cassava

In 1997, the CIAT Bean Improvement Project (IP-1) established a colony of the B. biotype of *B. tabaci* on beans (*Phaseolus vulgaris*, var. ICA Pijao). This colony provided the stock for initiating a B biotype of *B. tabaci* colony on cassava. *B. tabaci* individuals (adults) were harvested from the bean colony and allowed to oviposit on poinsettia (*Euphorbia pulcherrima*), located in fine nylon meshed, wooden cages (1m height x 1m wide). The objective here was to first establish a colony of *B. tabaci*, biotype B, on a species related to *Manihot esculenta*, as *B. tabaci* did not transfer successfully from beans directly to cassava. *E. pulcherrima*, like cassava, is a Euphorbiaceae. Five potted plants were located in each cage.

The colony of *B. tabaci* (B) once established on poinsettia, after 5 generations, was transferred to Jatropha (*Jatropha gossypiifolia*), a closely related species to *M. esculenta*. After 5 generations on Jatropha, the colony was transferred to cassava (var. MCol 2063) (Figure 6.18). To initiate all colonies, plants with 4 to 6 leaves (about 30 to 50 cm in height) and insect free, were utilized. Infestations were done using the pupal stage, adults about to emerge.

To secure the identification of *B. tabaci,* B. biotype, the RAPD-PCR technique was used (Figure 6.19). In this study, the monitoring of the B. biotype was done with H9 primer.

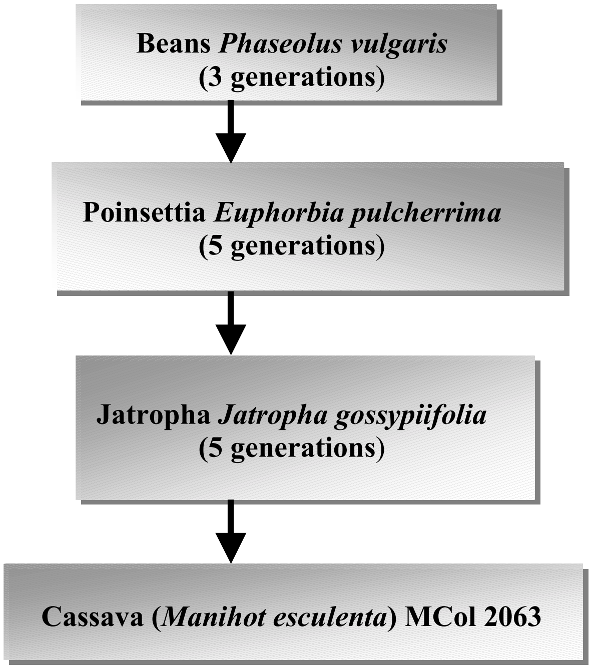

Figure 6.18. Plant species sequence for adapting the whitefly species, *Bemisia tabaci*, B. biotype from beans (*P. vulgaris*) to cassava.

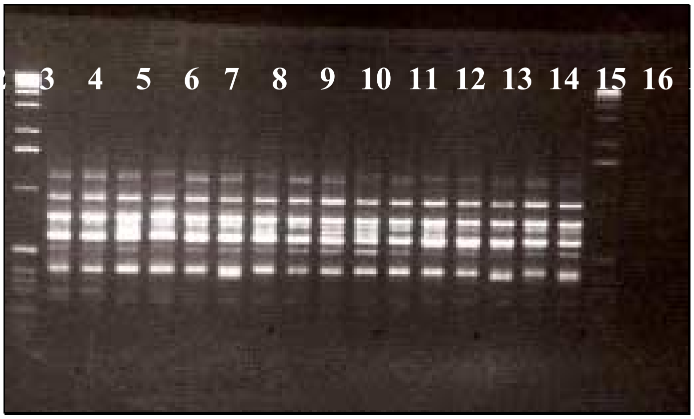

Figure 6.19. Amplification bands obtained using the H9 primer on *Bemisia tabaci*, B biotype individuals collected from different host plants; 1-4 biotype "B," 5-7 poinsettia, 8-10 bean, 11-13 Jatropha, and 14-16, cassava.

Results

Biotype B of *B. tabaci*, reared originally on beans, easily adopted, first to poinsettia and then on Jatropha. The colony remained stable on *J. gossypiifolia*, facilitating the establishment of the colony on cassava, variety MCol 2063. By first adopting to Jatropha, B. biotype of *B. tabaci* adults easily took to cassava, their populations multiplying rapidly (Figure 6.20).

The intrinsic rate of increase of B. biotype of *B. tabaci* populations on J. *gossypiifolia*

These studies were carried out in CIAT growth room (25±2°C, 70±5% RH, with 12 hrs. light). Adults utilized had been reared on *J. gossypiifolia* for 5 generations. Recently emerged *B. tabaci* adults were collected from the colony, sexed (1:1, M:F) and placed in small leaf snap cages (2.5cm diameter). At 24-hour intervals, adults were removed and transferred to another area of the leaf, until female death. Fecundity was estimated by the number of eggs oviposited in each 24 hour period.

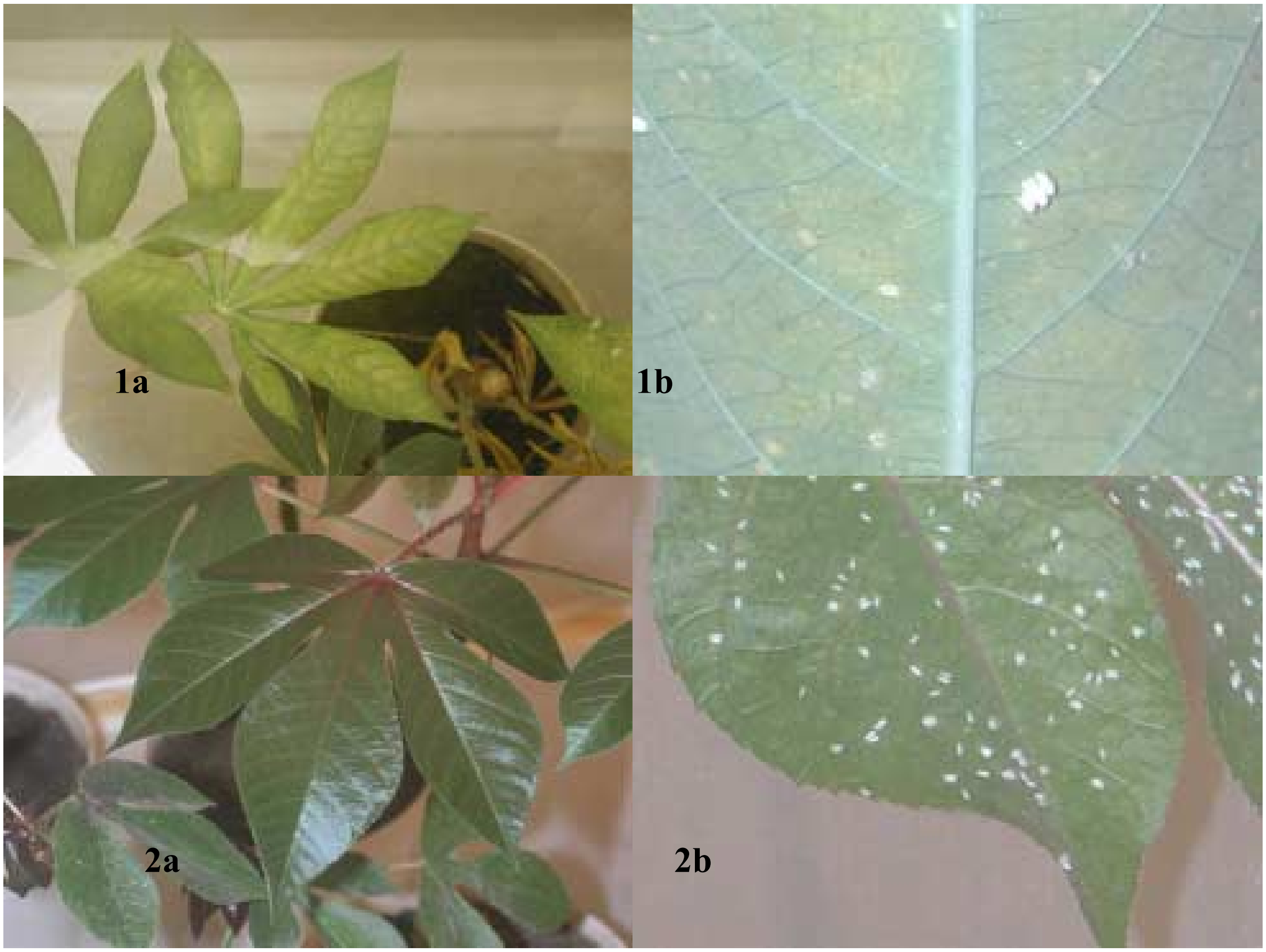

Figure 6.20. Adults and nymphs of "B" biotype of *B. tabaci* on cassava variety, MCol 2063 (1a and 1b), and Jatropha (2a and 2b).

Development time, survival rate, and female/male ratio were studied by placing 40 *B. tabaci* adults in leaf snap cages and allowing them to oviposit on the underside of Jatropha leaves. After 6 hours, adults were removed and 200 eggs of *B. tabaci,* B biotype were selected and allowed to develop. The development time and survival rate of immatures was recorded. By combining these data, the demographic parameters or life tables were developed. The net reproduction rate (Ro) represents the number of descendent females that females produce in each generation, and the generational time (T), is the period of time from parent birth to birth of the offspring. The intrinsic rate of increase of the population (IM) for the B biotype of *B. tabaci* was calculated using Carey's formula (1993).

∑exp(-ɼmx)lxmx=1 Where: x = age
lx = age, specific survival
mx = proportion of female progeny of one female at age x

To calculate ɼm values, the age corrected as x + 0.5 was utilized.

Results and Discussion

Adult female longevity of *B. tabaci* on *J. gossypiifolia* was 30 days and fecundity was 23.8 eggs (Figures 6.21 and 6.22). The medium rate of oviposition (eggs/female/day) was 11.9.

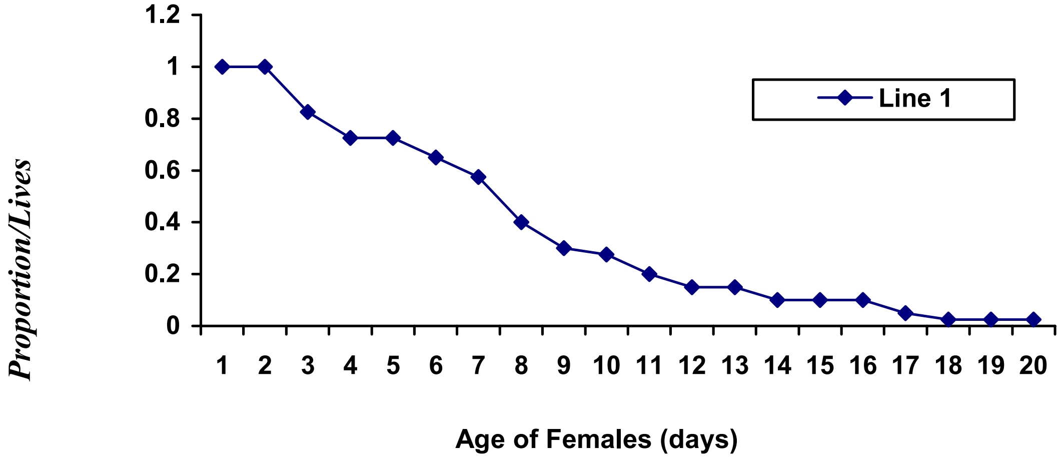

Figure 6.21. Survival curve of female whiteflies, biotype "B" of *Bemisia tabaci* on *Jatropha gossypiifolia.*

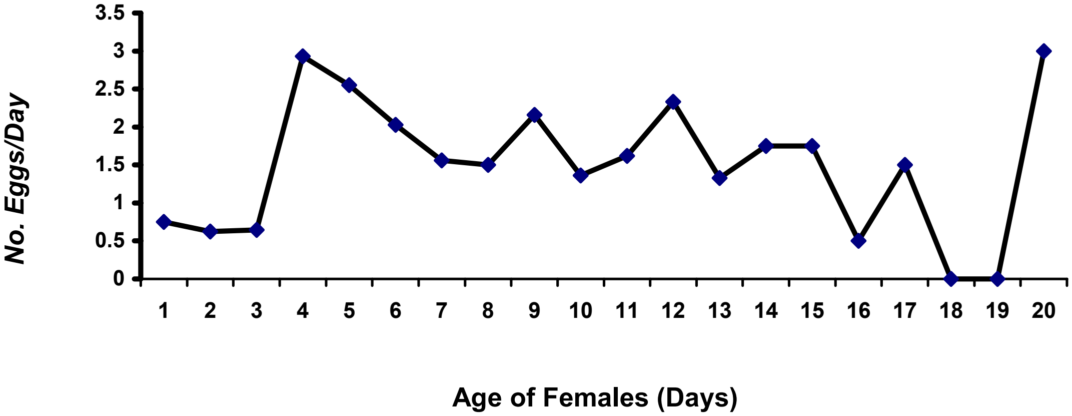

Figure 6.22. Reproduction curve of adult whitefly females of Biotype "B" of *Bemisia tabaci* feeding on *Jatropha gossypiifolia.*

Development time of B. biotype of *B. tabaci* on *J. gossypiifolia* was 32.5 days, and the survival rate of immatures was 46.5%. The female/male emergence was 49.46 and 50.54% respectively (Table 6.15).

Table 6.15. Demographic parameters (life table) of Biotype "B" of *Bemisia tabaci* whiteflies feeding on *Jatropha gossypiifolia*.

Parameters	J. gossypiifolia
	HR: 70±5% T: 25±2°C
Development time (Days)	32.5
Survival rate (%)	46.5
Proportion of females (%)	49.46
ɼm, Intrinsic rate of increase of population (Days)	0.066
Ro, Net reproduction rate	66.298
∑lxmx	
T, generational time (Days)	37.474

Future Projection

The next stage of this research will be to determine the intrinsic rate of increase and demographic parameters (life stable) of B. biotype of *B. tabaci* on the cassava genotypes MEcu 72 (whitefly, *A. socialis*, resistant), CG 489-34 (moderately resistant) and CMC 40 (susceptible).

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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, these four scientific areas have maintained as much a close relationship as possible. In Output 7, the progress related to cassava diseases is summarized.

Activity 7.1. Characterization of 50 genotypes under greenhouse conditions regarding their reaction to 12 different isolates of common bacterial blight (CBB).

Specific objectives

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

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

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

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

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

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

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

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[a] Disease reaction: resistant (R) = from 1.0 to 2.0; intermediate (I) = from 2.5 to 3.0; susceptible (S) = from 3.5 to 5.0.

[b] Isolates: See Table 7.1.1.

[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 (VM2 and VM7) in the field and greenhouse. Correlation was carried out with genotypes evaluated in the field in each zone.

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

Specific objectives

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

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

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

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

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

[a] CBB = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.0; and susceptible, from 3.5 to 5.0.
SED = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.5; and susceptible, from 4.0 to 5.0.

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

CBB pressure was lower in Matazul than in Villavicencio, and 31 genotypes showed resistance to the disease, 2 showed intermediate resistance, and 4 were susceptible, whereas 23 genotypes were resistant to SED, 13 showed intermediate resistance, and 1 was susceptible. Genotypes Brasilera, Ica Catumare, Ica Cebucán, La Reina, SG 104-74, SM 1468-9, SM 1555-17, SM 1855-9, and SM 2069-1 were evaluated at both sites.

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

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

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

Genotype	CBB	SED	Genotype	CBB	SED	Genotype	CBB	SED
Brasilera	4.5	4.0	CM 9472-4	4.0	2.5	SM 2641-2	4.0	2.0
Catumare	3.0	3.0	CM 9472-7	3.0	2.5	SM 2641-7	2.5	2.0
HMC-1	4.5	4.0	CM 9483-4	2.5	3.5	SM 2641-9	2.5	3.0
La Reina	4.0	2.5	SM 1812-92	2.5	2.0	SM 2641-11	2.5	2.5
CM 8746-1	2.5	3.0	SM 2220-18	4.0	2.0	SM 2642-3	3.5	2.5
CM 8747-5	2.5	4.0	SM 2220-19	4.0	2.5	SM 2642-17	2.5	2.0
CM 9449-2	2.5	3.0	SM 2220-20	2.5	2.0	SM 2642-24	2.5	3.0
CM 9449-6	2.5	2.5	SM 2366-44	3.5	2.0	SM 2642-27	2.5	2.5
CM 9449-8	2.5	3.5	SM 2366-45	3.5	2.5	SM 2644-1	3.5	3.0
CM 9450-5	4.0	4.0	SM 2366-46	2.5	4.0	SM 2644-3	2.5	2.5
CM 9451-1	2.5	2.0	SM 2366-49	4.0	2.0	SM 2644-4	4.0	4.0
CM 9452-6	3.5	4.0	SM 2366-50	4.5	2.5	SM 2644-5	4.0	4.0
CM 9452-11	2.5	2.5	SM 2366-57	4.0	2.5	SM 2645-1	4.0	4.0
CM 9452-13	4.0	4.0	SM 2452-13	2.5	3.0	SM 2646-2	3.0	3.5
CM 9452-15	2.5	3.0	SM 2561-32	3.5	2.5	SM 2724-9	3.5	4.0
CM 9456-26	2.5	2.5	SM 2592-14	4.0	3.0	SM 2724-15	3.5	4.0
CM 9456-40	3.5	2.5	SM 2593-21	4.0	2.5	SM 2724-18	3.0	2.5
CM 9459-1	4.5	2.5	SM 2594-16	4.0	3.5	SM 2726-4	4.0	3.0
CM 9459-2	2.5	3.0	SM 2599-25	3.0	4.0	SM 2726-17	3.5	2.5
CM 9459-6	3.0	3.0	SM 2599-41	4.0	3.0	SM 2727-1	4.0	3.0
CM 9459-10	2.5	3.0	SM 2599-49	3.5	3.5	SM 2727-9	3.0	2.5
CM 9459-11	3.0	2.5	SM 2601-22	4.0	3.5	SM 2727-12	4.0	2.5
CM 9459-12	2.5	2.0	SM 2601-23	4.0	3.0	SM 2727-20	4.0	4.0
CM 9459-13	2.5	2.0	SM 2601-27	4.0	4.0	SM 2727-23	4.0	4.0
CM 9459-15	3.0	2.0	SM 2601-30	3.5	3.0	SM 2727-26	4.0	2.5
CM 9459-18	2.5	3.0	SM 2601-31	4.0	3.0	SM 2727-27	3.5	2.5
CM 9459-21	3.0	2.0	SM 2601-39	4.0	3.0	SM 2727-31	3.5	3.0
CM 9459-22	4.0	2.0	SM 2601-44	2.5	2.5	SM 2727-36	4.0	3.0
CM 9459-24	3.5	2.0	SM 2601-55	4.0	2.5	SM 2727-42	2.5	4.0
CM 9460-1	2.5	2.5	SM 2601-56	4.0	3.0	SM 2727-43	4.0	4.0
CM 9460-3	3.0	4.0	SM 2603-23	4.0	4.0	SM 2728-9	4.0	3.0
CM 9460-9	2.5	3.0	SM 2606-25	4.0	4.0	SM 2730-1	4.0	2.5
CM 9460-12	3.0	2.0	SM 2606-27	2.5	3.0	SM 2730-8	2.5	3.0
CM 9460-13	2.5	3.0	SM 2608-27	3.0	3.0	SM 2730-12	3.0	2.5
CM 9460-15	2.5	3.0	SM 2609-54	4.0	2.5	SM 2730-26	3.0	4.0
CM 9460-16	2.5	2.5	SM 2612-29	4.0	2.5	SM 2730-42	4.0	3.0

Continuation Table 7.4.

CM 9460-17	3.0	2.5	SM 2632-2	3.5	2.5	SM 2730-43	4.0	4.0
CM 9460-25	3.0	2.0	SM 2632-4	3.0	2.0	SM 2738-1	2.5	3.5
CM 9460-34	2.5	2.5	SM 2632-5	2.5	3.5	SM 2739-1	4.0	3.0
CM 9460-35	3.0	2.5	SM 2632-15	3.0	2.5	SM 2739-4	3.5	3.0
CM 9460-37	3.5	3.0	SM 2632-17	2.5	2.5	SM 2786-1	2.5	2.5
CM 9460-38	2.5	2.0	SM 2632-22	3.5	3.0	SM 2786-5	3.5	2.0
CM 9460-39	2.5	4.0	SM 2633-3	2.5	3.0	SM 2786-7	3.0	2.5
CM 9460-40	3.5	3.0	SM 2633-10	3.0	3.0	SM 2786-9	2.5	2.0
CM 9460-41	2.5	2.5	SM 2634-4	3.5	2.0	SM 2786-10	2.5	2.0
CM 9460-42	4.5	3.0	SM 2634-7	2.5	2.0	SM 2786-15	2.5	2.5
CM 9461-1	4.0	2.5	SM 2634-8	3.0	2.0	SM 2786-18	3.5	3.0
CM 9461-2	4.0	2.0	SM 2634-9	3.5	2.5	SM 2787-1	3.0	2.5
CM 9461-3	2.5	3.0	SM 2634-13	3.5	2.0	SM 2787-4	3.0	2.5
CM 9461-5	2.5	2.0	SM 2635-4	2.5	2.0	SM 2787-5	4.5	2.5
CM 9461-6	3.0	3.0	SM 2635-6	3.5	3.0	SM 2787-13	3.5	3.5
CM 9461-7	2.5	2.0	SM 2635-12	3.5	4.0	SM 2790-2	4.5	2.0
CM 9461-8	2.5	3.0	SM 2636-4	3.5	3.5	SM 2790-17	4.0	2.5
CM 9461-10	2.5	2.5	SM 2636-5	2.5	2.5	SM 2790-18	3.0	3.0
CM 9461-11	2.5	3.0	SM 2636-6	2.5	2.0	SM 2790-27	4.0	2.5
CM 9461-12	2.5	2.5	SM 2636-10	2.5	2.5	SM 2790-28	4.0	2.5
CM 9461-13	4.0	2.5	SM 2636-14	2.5	2.0	SM 2790-32	4.0	2.5
CM 9461-14	4.0	2.5	SM 2636-18	2.5	2.0	SM 2791-2	4.0	2.5
CM 9461-15	3.5	3.0	SM 2636-19	4.0	4.0	SM 2791-5	3.5	2.0
CM 9461-17	3.0	2.5	SM 2636-20	4.0	2.0	SM 2791-12	3.0	3.0
CM 9461-18	2.5	3.0	SM 2636-26	2.5	2.0	SM 2791-16	4.0	3.5
CM 9461-21	4.0	2.5	SM 2636-29	4.0	3.5	SM 2791-17	3.0	4.0
CM 9461-32	5.0	3.0	SM 2636-30	4.0	3.5	SM 2792-3	3.0	2.0
CM 9461-35	3.0	2.5	SM 2636-42	2.5	2.0	SM 2792-6	4.0	3.5
CM 9461-36	4.0	3.0	SM 2636-44	2.5	2.5	SM 2792-11	2.5	2.5
CM 9461-51	2.5	2.5	SM 2638-6	3.0	4.0	SM 2792-12	4.0	2.5
CM 9461-53	2.5	3.0	SM 2638-10	2.5	2.0	SM 2792-14	5.0	4.0
CM 9461-56	3.0	2.5	SM 2638-11	3.5	3.0	SM 2792-16	3.0	2.5
CM 9462-17	3.0	3.0	SM 2638-12	3.0	2.5	SM 2792-28	4.0	3.0
CM 9463-2	2.5	2.5	SM 2638-13	2.5	3.0	SM 2792-31	3.0	3.0
CM 9463-10	2.5	2.5	SM 2638-17	3.5	2.0	SM 2792-32	2.5	4.0
CM 9463-15	3.5	3.0	SM 2638-20	3.5	2.0	SM 2792-36	3.0	2.5
CM 9463-19	4.0	2.5	SM 2638-23	2.5	2.5	SM 2792-37	4.0	2.5
CM 9464-1	2.5	2.5	SM 2638-27	3.5	3.0	SM 2792-38	3.0	4.0
CM 9464-3	3.5	3.0	SM 2638-40	2.5	2.0	SM 2792-42	4.0	3.0
CM 9464-19	2.5	4.0	SM 2638-44	2.5	2.0	SM 2792-43	4.0	2.0
CM 9464-26	2.5	2.5	SM 2640-1	3.5	2.5	SM 2792-50	3.5	2.5
CM 9464-27	2.5	2.5	SM 2640-6	4.0	2.5	SM 2792-52	4.0	3.0
CM 9464-29	2.5	3.0	SM 2640-7	3.0	2.5	SM 2793-7	2.5	2.5
CM 9464-30	2.5	2.5	SM 2640-8	2.5	2.0	SM 2794-2	2.5	2.5
CM 9464-33	2.5	3.5	SM 2640-9	3.0	2.0	SM 2794-18	3.5	3.0
CM 9464-36	2.5	4.0						

[a] CBB = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.0; susceptible, from 3.5 to 5.0.
SED = Resistant, from 1.0 to 2.0; intermediate, from 2.5 to 3.5; susceptible, from 4.0 to 5.0.

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

Specific objectives

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

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

Department of Cauca

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

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

Treatment

1 2.5 t/ha chicken manure + 300 kg/ha of the chemical fertilizer Agropremix® (15% N, 10% P_2O_5, 12% Zn, 2% B, 0.75% Cu, 3% S, and 0.01% of Mo)
2 2.5 t/ha chicken manure + potassium sulfate (180 kg/ha K_2O)
3 2.5 t/ha chicken manure + potassium chloride (180 kg/ha K_2O)
4 2.5 t/ha chicken manure + thermotherapy (stakes immersed in water heated over a wood fire to 49°C for 49 min)
5 *Trichoderma* strain 14PDA-4 (1×10^4 conidia/mL)
6 *Trichoderma* strain 19TSM-3A (1×10^4 conidia/mL)
7 Cassava variety La Reina (CM 6740-7)
8 Stake selection
9 2.5 t/ha chicken manure (traditional farmer's practice)

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

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

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

Plant height and stake production per plant were greatest when the trial was fertilized with Agropremix. Table 7.5 shows the effect of the treatments on yield and incidence of rotten roots. All treatments surpassed the control in stake production per plant. Yield under all treatments in San Jerónimo was very low because of low-fertility soil and the plot's history of six cassava crops previous to the trial. Chemical fertilization did not increase yield, whereas treatments with *Trichoderma* 14 PDA-4 and selection of stakes improved yields by 33.6% and 25.8%, respectively, although root-rot incidence was higher than for the control. In contrast, *Trichoderma* 19 TSM-3A helped reduce root rots. Potassium sources also helped reduce rots. The variety La Reina showed no root rots.

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

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

Treatment	Plant height (m)	Stake production per plant	Yield (T/ha)	Root rot disease		
				Incidence (% affected plants)	Severity (Kg affected roots/ha)	Percentage of affected roots
Agropremix	2.1	10.2	3.63	14	183	4.8
K_2SO_4	1.9	8.4	3.2	5	50	1.5
KCl	2	8.5	3.6	5	67	1.8
Stake selection	2	9.4	4.38	4	150	3.3
Thermotherapy	2	8.2	3.95	23	150	3.7
Control, traditional farmer's practice	1.9	7.9	3.48	16	100	2.8
Trichoderma strain 14PDA-4	2	8.6	4.65	17	175	3.6
Trichoderma strain 19TSM-3A	2	9.1	3.15	5	33	1.0
Cassava variety Reina (CM 6740-7)	2.8	8.4	5.15	19	0	0.0

Department of Quindío

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

Treatment

1 Fertilization with KCl (180 kg/ha K_2O).
2 Fertilization with K_2SO_4 (180 kg/ha K_2O).
3 Farmer fertilization: Farm "El Jardin" applied 350 kg/ha of a mixture of ammonium sulfate and borax at a rate of 50:1.5; Farm "Guayaquil" applied 500 kg/ha of a mixture of Nitrax-DAP-KCl at a rate of 1:2:2. Fertilizers were applied 45 days after planting.
4 Stakes given thermotherapy (49°C for 49 min).
5 Stakes immersed for 5 min in Orthocide® (captan, 4 g/L of the commercial product) and Ridomil® (metalaxyl, 3 g/L of the commercial product).
6 Stakes immersed in Lonlife® (ascorbic acid) at 4%.
7 Biological control: stakes immersed for 10 min in a suspension of *Trichoderma* (1×10^4 conidia/mL), strains 19TSM-3A and 41 PDA-3A. The area around the stake was treated with 100 mL/plant of the fungal suspension.
8 Varietal resistance, using genotypes 'HMC-1', 'ICA Catumare', 'M Per 183' ('Peruana'), and the local variety 'Chiroza' (M Col 2066).

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

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

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

Control practices	**Plant height (m)**[b]	**Root yield (t/ha)**	**Stakes per plant**	**Bacterial blight**		**Root Rot Disease**		
				Incidence (% affected plants)	**Severity (%)**	**Incidence (% affected plants)**	**Severity (T affected roots/ha)**	**% affected roots**
Variety HMC-1								
Thermotherapy[c]	1.73	62 a	36 a	21 a	89	2 a	3.7 a	5.6
Biocontrol with Trichoderma spp.[d]	1.89	63 a	36 a	16 a	89	2 a	1.8 a	2.8
Micobiol®[e]	2.31	60 a	37 a	12 a	56	1.3 a	0.2 a	0.3
Ridomil (metalaxyl)	1.91	70 a	39 a	16 a	89	1.7 a	1.0 a	1.4
Potassium chloride (KCl)	1.90	70 a	37 a	18 a	100	2 a	0.3 a	0.4
Potassium sulfate (K_2SO_4)	1.90	80 a	38 a	24 a	100	2 a	1.1 a	1.4
Local varieties								
Manzana	1.93	41 a	36 a	21 a	100	2 a	7.1 a	14.8
HMC-1	1.86	51 a	37 a	22 a	100	1.8 a	6.1 a	10.7

a. Duncan's multiple range test, alpha <= 0.05.
b. At 7 months after planting.
c. Oil drum on wood fire, with the water's temperature at 49ºC for 49 min.
d. Strain 14 PDA-4.
e. Contains *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thuringiensis.*
f. Duncan's multiple range test, alpha <= 0.05.
g. At 7 months after planting.
h. Oil drum on wood fire, with the water's temperature at 49ºC for 49 min.
i. Strain 14 PDA-4.
j. Contains *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thuringiensis.*

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

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

Treatment	**El Jardín**		**Guayaquil**		**Average**	
	Plant height (m)	**No. of stakes/ plant**	**Plant height (m)**	**No. of stakes/ plant**	**Plant height (m)**	**No. of stakes/ plant**
Fertilization						
KCl (180 Kg/ha K_2O)	1.81	8.4	2.14	9.9	1.98	9.2
K_2SO_4 (180 Kg/ha K_2O)	1.92	10.3	1.92	7.1	1.92	8.7
Control farmer[a]	1.88	11.8	1.86	8.9	1.87	10.4
Control without fertilization	1.89	10.1	1.89	9.2	1.89	9.7
Stake treatment						
Thermotherapy (49°C during 49 min)	1.92	9.7	1.86	6.5	1.89	8.1
Orthocide® (4 g/L) + Ridomil® (3 g/L) [b]	1.74	9.0	1.85	8.7	1.80	8.9
Lonlife® 4%	-	-	2.14	8.5	2.14	8.5
Biological control						
Trichoderma strain 41PDA-3A	1.75	8.2	2.07	7.7	1.91	8.0
Trichoderma strain 19TSM3A	1.88	10.7	1.92	6.9	1.90	8.8
Varietal resistance						
Chiroza	2.59	18.3	2.34	17.2	2.47	17.8
HMC-1	1.81	10.7	2.30	10.5	2.06	10.6
Ica Catumare	2.03	11.0	2.80	13.9	2.42	12.5
M Per 183	1.82	10.2	-	-	1.82	10.2

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

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

Treatment	**El Jardín**			**Guayaquil**			**Average**		
	Root yield (t/ha)	**Roots affected by Root Rot (kg/ha)**	**(%)**	**Root yield (T/ha)**	**Roots affected by Root Rot (kg/ha)**	**(%)**	**Root yield (t/ha)**	**Roots affected by Root Rot (kg/ha)**	**(%)**
Fertilization									
KCl (180 Kg/ha K_2O)	42.6	0	0.0	23.4	439	1.8	33	220	0.7
K_2SO_4 (180 Kg/ha K_2O)	29.9	0	0.0	22.3	0	0.0	26.1	0	0.0
Farmer's control[a]	50.5	0	0.0	23	1869	7.5	36.8	935	2.5
No fertilization	47.9	0	0.0	19.2	575	2.9	33.6	288	0.8
Stake treatment									
Termotherapy (49ºC/ 49 min)	35.1	123	0.3	20.8	1768	7.8	28	946	3.3
Orthocide® (4 g/L) + Ridomil® (3 g/L)[b]	37.3	0	0.0	27.9	514	1.8	32.6	257	0.8
Lonlife® 4% + (ascorbic acid)	-	-	-	23.4	114	0.5	23.4	114	0.5
Biocontrol with Trichoderma									
Strain 41PDA-3A	28.3	0	0.0	28.9	247	0.8	28.6	124	0.4
Strain 19TSM 3A	32.4	0	0.0	14.7	41	0.3	23.6	21	0.1
Varietal resistance									
Chiroza	38.6	0	0.0	15.5	3086	16.6	27.1	1543	5.4
HMC-1	38.7	0	0.0	25.2	24	0.1	32	12	0.0
ICA Catumare	59.5	597	1.0	28.9	1028	3.4	44.2	813	1.8
M Per 183	32	3009	8.6	-	-	-	32	3009	8.6

[a] Farm "El Jardin": ammonium sulfate + borax (50:1.5) at 300 kg/ha. Farm "Guayaquil": Nitrax-DAP-KCl (1:2:2) at 500 kg/ha.

[b] At Farm "Guayaquil", Orthocide® was replaced by copper oxychloride.

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

At Farm "El Jardin", 65-day-old plants were affected by the bacterium *Xanthomonas axonopodis* pv. *manihotis* in some treatments. The bacterium was not present in treatments with K_2SO_4, thermotherapy, nor in the genotypes 'ICA Catumare' and 'Chiroza', which have shown acceptable resistance to the disease, whereas 'HMC-1' and 'M Per 183' are susceptible. As the crop aged, incidence of the bacterium became insignificant.

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

Comparing Departments

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

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

Treatment	**Root yield (T/ha)**						
	Quindío				**Cauca**		
	Montenegro (Cantores)	**Montenegro (Guayaquil)**	**La Tebaida (El Jardín)**	**Average**	**Santander de Quilichao (San Jerónimo)**	**(El Turco)**	**Average**
Thermotherapy	62	21	35	39.3	4	15	11.5
Trichoderma	63	22	30	33.5	3.9	-	3.9
KCl	70	23	43	45.3	4	-	4
K_2SO_4	-	22	30	26	-	9	9
Manzana	41	-	-	41	-	-	-
Chiroza	-	15	39	27	-	-	-
La Reina (CM 6740-7)	-	-	-	-	5	-	5
Ica Catumare	-	29	59	44	-	-	-
HMC-1	-	25	39	32	-	-	-
M Per 183							
Farmer[a]	51	23	51	41.7	4	15	9.5

[a] Montenegro and La Tebaida: HMC-1; Santander de Quilichao: Verdecita

Activity 7.4. Characterization of F_1 progeny and parental material of families K (M Nga2 x CM 2177-2) and CM 9582 (M Bra 1045 x M Cr 81) regarding their resistance to Phytophthora root rot.

Specific objectives

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

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

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

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

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

[a] Origin of isolates: 44, Quindío, Colombia; P4, Colombia; P12, Brazil; 69, Colombia.

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

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

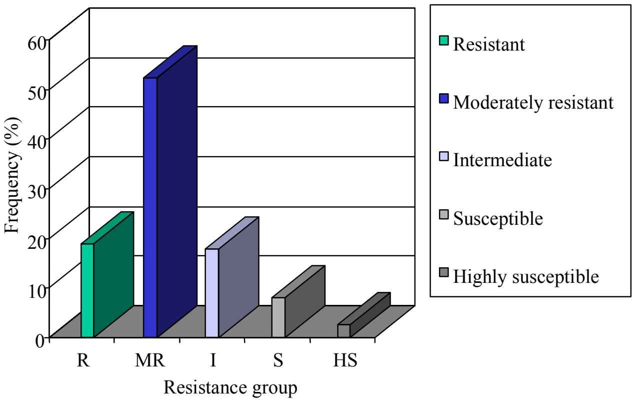


Figure 7.1. Breakdown of family CM9582 (M Bra 1045 x M Cr 81) according to degree of resistance to *Phytophthora tropicalis* inoculated on roots.

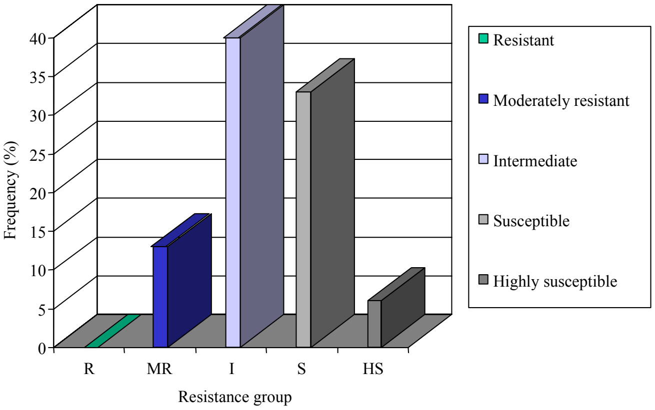


Figure 7.2. Breakdown of family K (M Nga 2 x CM 2177-2), inoculated with *Phytophthora tropicalis* on roots, according to degree of resistance.

Activity 7.5. Evaluation of probes of resistance gene analogs in individuals of the K family regarding resistance to __Phytophthora root__ rot.

Specific objectives

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

Materials and methods

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

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

Results

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

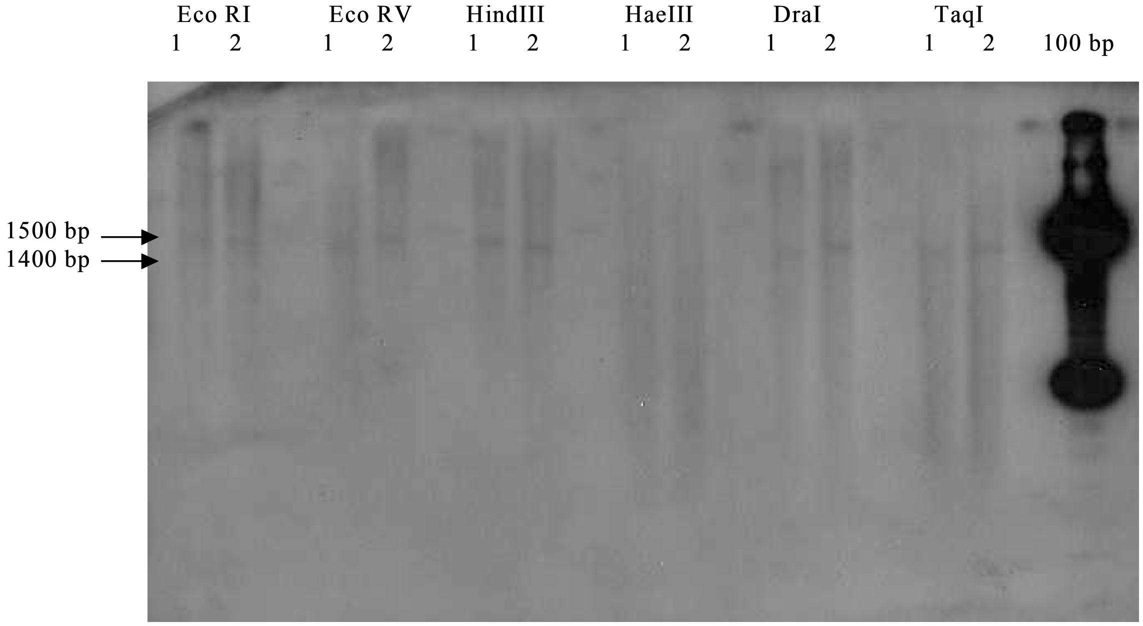


Figure 7.3. Hybridization of probe Pic 15 from maize to DNA digested with six enzymes from M 2177-2 (1) and M Nga 2 (2), parents of the K family of cassava.

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

Specific objectives

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

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

Five set of primers used in rice by Chen et al. (1998), corresponding to conserved domains in disease resistance genes, were used to amplify similar sequences in cassava DNA from genotypes both resistant and susceptible to *Phytophthora* spp. Each PCR reaction was performed in 25-µl volume consisting of 0.2 mM dATP, dCTP, dGTP and dTTP each; 2.5 mM $MgCl_2$; 0.25 X Q solution (Qia Gen kit for PCRs); 1.5 U of *Taq* polymerase, 1 µM primer; 2.5 µl 10X *Taq* polymerase buffer; and 100 ng template DNA. For control reactions, template DNA was substituted by sterile distilled H_2O. Amplification was carried out in an MSJ-Research PTC-200 thermal cycler programmed for 5 min at 94 °C, 1 min at 45 °C, and 2 min at 72 °C. A 2.5-min ramp time was used between the 94 °C denaturation and the 45 °C annealing steps.

The primer NBS is a sequence from conserved motifs of the nucleotide-binding site in tobacco N and *Arabidopsis* RPS2 gene (Yu et al., 1998). XLRR is a sequence based on the leucine-rich repeat region of the RPS2 and Xa 21 from rice (Chen et al., 1998). Pto is a sequence for potato kinase (Leister et al., 1996). Pox amplifies an intron region of a peroxidase gene in tomato. WIPK amplifies the conserved region of MAK kinase from parsley (Y12875), tobacco (D61377), *Arabidopsis* (MPK3), and *Medicago sativa* (MMK4) (Ligterink et al., 1997).

Primers used are:

XLRR f: 5'- CCGTTGGACAGGAAGGAG- 3'
XLRR r: 5'- CCCATAGACCGGACTGTT-3'

WIPK 1: 5'- GGTCGTGGTGCTTATGGAAT-3'
WIPK 2: 5'-CCATGAAGATGCAACCGAC-3'

NBS f1: 5'- GGAATGGGNGGNGTNGGNAARAC-3'
NBS r1: 5'- YCTAGTTGTRAYDATDAYYYTRC-3'

Pto 1: 5'- ATGGGAAGCAAGTATTCAAGGC-3'
Pto 2: 5'- TTGGCACAAAATTCTCATCAAGC-3'

Pox 1f: 5'- GGAGCTTCTCTCATTCGTCT-3'
Pox 1r: 5'-TAGCAGAATACCTCCATCAC-3'

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

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

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

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

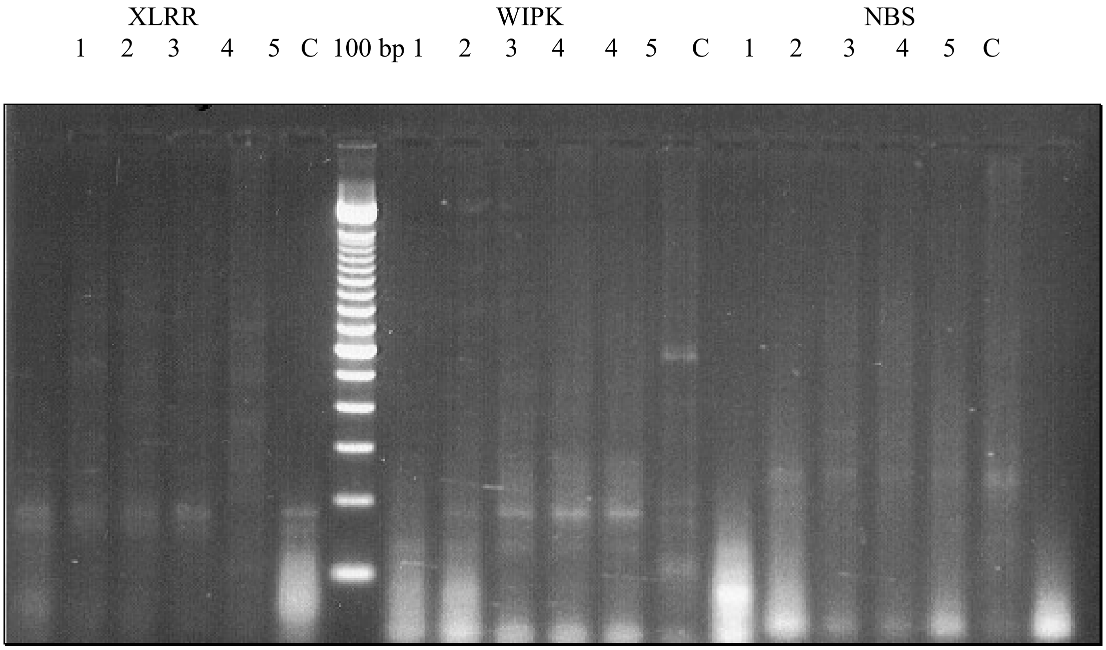


Figure 7.4. DNA amplification of resistant and susceptible cassava varieties with primers XLRR, WIPK and NBS, corresponding to conserved domains related to disease resistance. Cassava varieties used were M Bra 1045 (1), M Nga 2 (2), M Cr 81 (3), and M Cr 54 (4). Rice (5) was included as control. C stands for the PCR control.

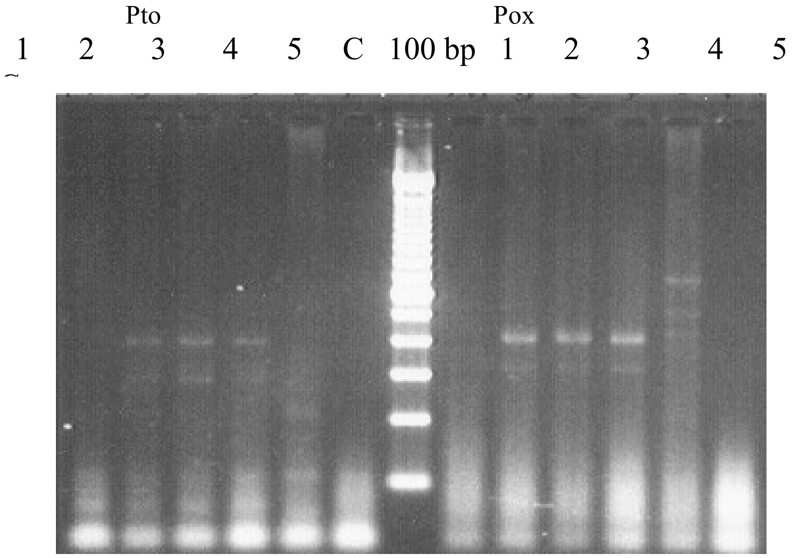


Figure 7.5. DNA amplification of resistant and susceptible cassava varieties with primers Pto and Pox, corresponding to conserved domains related with disease resistance. Cassava varieties used were M Bra 1045 (1), M Nga 2 (2), M Cr 81 (3), and M Cr 54 (4). Rice (5) was used as control. C stands for the PCR control.

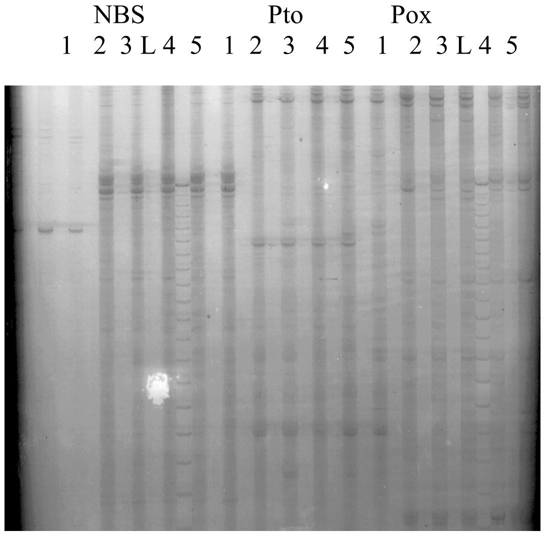


Figure 7.6. DNA amplification of resistant and susceptible cassava varieties with primers NBS, Pto and Pox, corresponding to conserved domains related with disease resistance. Cassava varieties used were M Bra 1045 (1), M Cr 81 (2), K 64 (3), M Col 2066 (4), and K 142 (5). L stands for the 330-bp ladder.

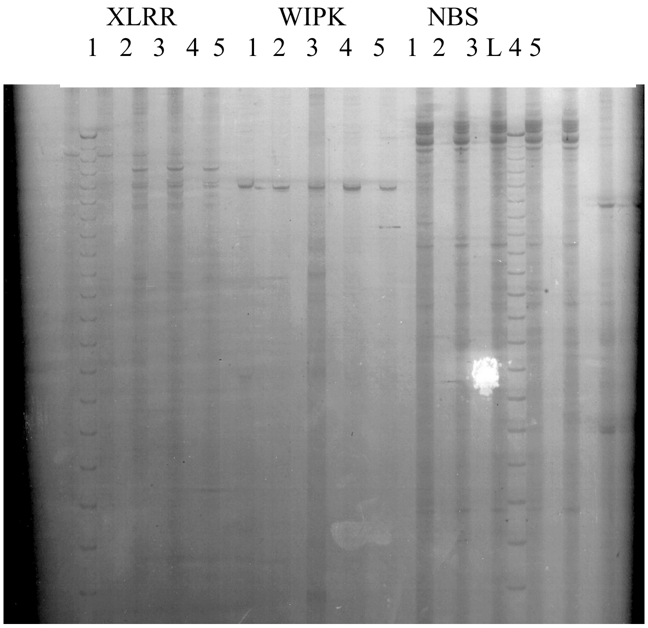
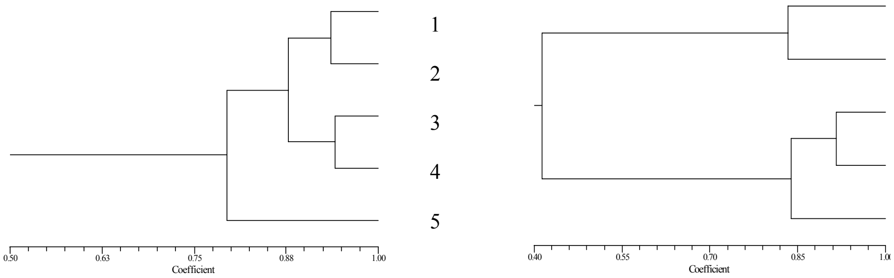
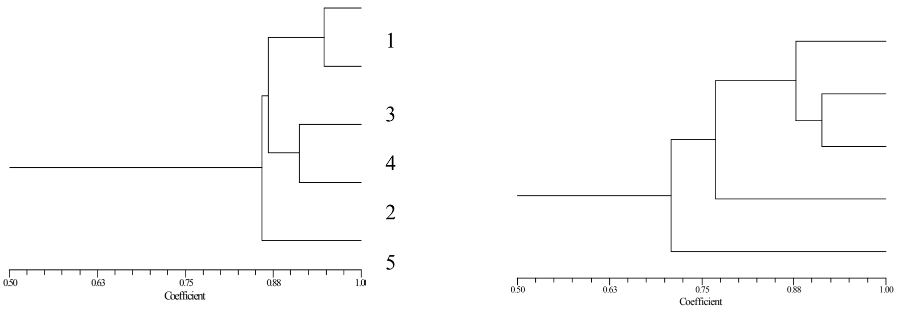


Figure 7.7. DNA amplification of resistant and susceptible cassava varieties with primers XLRR, WIPK, and NBS, corresponding to conserved domains related with disease resistance. Cassava varieties used were M Bra 1045 (1), M Cr 81 (2), K 64 (3), M Col 2066 (4), and K 142 (5). L stands for the 330-bp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



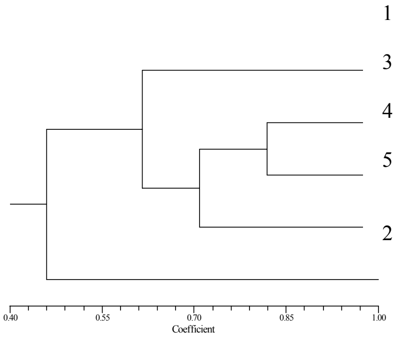
NBS

XLRR



Pox

Pto



WIPK

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[a] CBB = common bacterial blight; SED = superelongation disease; *Phytophthora* resistance in F_1 of CM 9582 (M Bra 1045 x M Cr 81); CM 9600 (M Cr 81 x M Cr 54).

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

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

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

Seminars

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

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

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

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

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

Training

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

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

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

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

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

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

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

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

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

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

Publications

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

Ongoing thesis work

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

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

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

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

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

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

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

Donors

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Ministry of Agriculture and Rural Development, Colombia
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Kansas State University (S. H. Hulbert and J. Leach)
Michigan State University (K. Lamour)
Palmas de Casanare
The Royal Veterinary and Agricultural University, Copenhagen, Denmark (D. Collinge)
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Secretary of Agriculture, Mitú, Colombia (G. Arbeláez)
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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 breeding of resistance to CMD in Latin American cassava gene pools

Rationale

In order to protect cassava production in Latin America and Asia from an accidental introduction of the Cassava Mosaic Disease (CMD), and to continue to provide improved cassava germplasm for gene pool enhancement in Sub-Saharan Africa, a marker-assisted CMD resistance breeding project was initiated two years ago at CIAT. There are two sources of resistance to CMD, the currently deployed source from *M.glaziovii* and a novel source controlled by a single dominant gene, *CMD2,* found in Nigerian cassava land races. The older source is already available at CIAT, having been introduced more than a decade ago, while progeny bearing the new source were introduced last year from IIITA. In addition, last year, an SSR and RFLP markers were identified that flank *CMD2* at 8 and 9cM respectively (Akano et al., 2001, CIAT2000). Efforts have continued to seek for additional SSR markers from two newly available sets of SSR markers and also to begin generating breeding populations for MAS.

The two principal tools for marker-assisted breeding of CMD resistance are a reliable source of the trait and easily assayed markers tightly associated with genes controlling the traits. Both are now available therefore setting the stage for MAS for CMD resistance at CIAT. Particular emphasis has been placed on combining CMD resistance with high carotene content (yellow cassava) for shipment to collaborators in IITA and India.

Materials and methods

Twenty CMD resistance cassava lines from an F_1 mapping population derived from TME3, one of the land races with the new source of CMD resistance, were shipped to CIAT from IITA as *in vitro* plantlets with permission from the Colombian plant quarantine authorities. The plantlets were sub-cloned and tested for the presence of virus by PCR, using gemini virus specific primers and ELISA (CIAT 2000). All tests were negative for the presence of virus. With the permission of the plant quarantine authorities, the plants were transplanted to the screen house, and after inspection by the plant quarantine officials, they were transferred to the field for genetic crosses.

Discovery of an SSR marker, from a set of 186 SSR markers developed at CIAT (Mba et. al 2000), linked to *CMD2* have been described earlier (Akano et al. 2001, CIAT 2000). A second set of 132 SSR markers, obtained from a cassava root and leaf cDNA (Mba et al., 2000 unpublished data), and a third set of 154 SSR markers, generated from the previous genomic library by another round of screening (Fregene et al., 2001 unpublished data) have become available recently. Bulk segregant analysis, using the CMD susceptible and resistant parents, and two pools of 40 susceptible and 40 resistant genotypes from the mapping progeny, was conducted with the two new sets of markers. PCR amplification and gel analysis were as described by Mba et al.,, 2000.

Results
More than 50 plants representing 20 progeny of TME3 introduced from IITA have been established in the CIAT hybridization block in preparation for genetic crosses. The CMD resistant lines will be crossed to elite parents of the respective gene pools from 3 agro-ecologies and high carotene lines (Table 8.1). Genetic crosses are expected to begin the early part of next year and seeds will be ready by the middle of next year. Seeds harvested from inter-population improvement of the ACMD populations will be shared with IITA and Indian collaborators and the rest will be planted in germination trays and transferred to the field. Marker genotyping will be carried out on these plants and only those with the marker alleles tightly linked to *CMD2* will be transferred to the regular CIAT selection scheme.

Table 8.1. List of CMD resistance donor lines from IITA, elite CIAT parental lines and high carotene content lines for marker-assisted breeding of CMD resistance.

	Genotype	**No. of plants**		**Genotype**	**No. of plants**
	CMD Resistant donor lines from IITA derived from the TME3 land race				
1.	C 6	2	11.	C 127	3
2.	C 18	10	12.	C 227	5
3.	C 19	6	13.	C 243	10
4.	C 22	0	14.	C 373	10
5.	C 24	4	15.	C 377	10
6.	C 35	4	16.	C 400	1
7.	C 39	4	17.	C 413	2
8.	C 43	10	18.	C 4	10
9.	C 54	2	19.	C 33	10
10.	C 101	4	20.	C 41	8
Elite Parental Lines for crosses to CMD resistant lines					
	Clones for the Lowland Humid Agro-Ecology:				
1.	CM 3306- 4	20			
2.	CM 6754- 8	20			
3.	SM 1411- 5	20			
4.	MTAI 8	20			
Clones for the Acid Savannah Agro-Ecology					
1.	CM 523- 7	20			
2.	CM 4574- 7	20			
3.	CM 6740- 7	20			
4.	SM 1821- 7	20			
Clones for the Mid Altitutde Agro-Ecology					
1.	CM 7951- 5	20			
2.	SM 909- 25	20			
3.	SM 1219- 9	20			
4.	SM 1741- 1	20			
Yellow cassava lines low in cyanogenic potential for crosses to CMD resistant lines					
1.	CM 489- 1	20	7.	MCOL 2056	20
2.	CM 2772- 3	20	8.	MCOL 2061	20
3.	CM 3750- 5	20	9.	MCOL 2206	20
4.	SM 1433- 4	20	10.	MCOL 2316	20
5.	MBRA 1A	20	11.	MCOL 2564	20
6.	MCOL 1734	20	12.	MMAL 66	20
			13.	MTAI 2	20

BSA with the two new sets of SSR markers identified two markers NS158, from the enriched genomic library, and SSRY339, from the cDNA library, associated with CMD resistance. The two markers were analyzed in the 80 individuals of the resistant and susceptible bulks and 1 recombinant was found for NS158 and 10 for SSRY339. Although these results are preliminary and need to be confirmed in a large size progeny, they suggest that NS158 is much closer to CMD2 than SSRY28, the closest marker identified to date. The precise distance of NS158 is being determined in a large progeny of about 2500 genotypes, developed at IITA for comparison between marker assisted selection and conventional breeding of CMD resistance (see *Section 8.2*).

Future plans include the following activities:

☞ Genetic crosses between CMD donor lines from IITA and CIAT elite parental lines as well as high carotene lines.
☞ Marker genotyping of populations with the SSR marker NS158 and selection of lines carrying the resistant allele for introduction into the regular cassava breeding program
☞ Sharing of seeds with IITA, particularly those from crosses with high carotene content.

Activity 8.2. A Comparison of marker assisted selection (MAS) and conventional selection for the rapid deployment of the novel CMD resistance gene (CMD2) in cassava gene pools.

Rationale

The principal objective of the project “Molecular Mapping of Genes Conferring Resistance to the Cassava Mosaic Disease (CMD) in African Cassava Germplasm” funded by the Rockefeller foundation is to identify markers tightly linked to different sources of CMD resistance for efficient and cost-effective deployment of resistance genes. Molecular markers linked to a novel source with high level of resistance, designated *CMD2* (Akano et al., 2001), have been identified. The effectiveness of *CMD2* against a wide spectrum of strains of the virus in sub Saharan Africa, including the aggressive Ugandan variant (UgV) (see section 1.4, this report), makes its deployment very appealing in protecting cassava production against the ravages of CMD both in Africa and Latin America. Of urgent importance is containing the rapidly advancing front of UgV that has now reached the Democratic Republic of Congo, Kenya, and Tanzania.

Conventional breeding for CMD resistance involves at least 4 cycles of selection for resistance at the seedling, clonal and preliminary and advanced yield trial stages. Disease pressure, which may vary from year to year, may lead to escapes that are carried along, in certain cases, up to the third cycle. Genetic markers for CMD resistance enables the elimination of susceptible genotypes at the seedling stage and reduces significantly, 50% in the case of *CMD2*, the materials to be evaluated in the field at the crucial single row (clonal) trial stage, where more than 95% of genotypes are eliminated. Markers are even more important when two or more genes/traits are involved, the reduction in progenies to be evaluated becomes even higher. In many cassava production scenarios, CMD resistance has to go hand-in-hand with cassava bacterial blight (CBB) resistance.

A MAS project for CMD resistance was therefore initiated with IITA to enable us to test the fidelity of CMD markers developed at CIAT, a very convenient approach considering that phenotypic and molecular data can be obtained at the same time and compared. The MAS project is also necessary to work out the details for routinely using these markers in cassava breeding.

Matherials and methods

The original group of CMD resistant land races for the study consists of TME3, TME4, TME28, and TME9. TME28 was dropped out due to very poor flowering, while seeds obtained from crosses with TME4 are not considered here due to its very close genetic similarity with TME3. Only crosses to TME3 and TME9, crossed to elite IITA parental lines, TMS30572, TMS91934, and TME117, a land race favored for his good cooking roots, are reported here. TMS30572 is moderately resistant to CMD, while the other two are susceptible. TMS91934 has a very high level of resistance to the all known strains of CBB (Verdier 1999, pers communication) and can serve as a source of CBB resistance in progenies of the highly CBB susceptible land races TME3 and TME9. The seedling nursery and field establishment were at the IITA sub-station in Mokwa located in the Guinea Savannah agro-ecology of Nigeria. This site is characterized by low CMD disease pressure and ideal for the multiplication of cuttings from CMD susceptible lines.

Before transplanting, 2 young leaves were harvested from each genotypes for DNA isolation and SSR analysis. The leaves were bagged in small plastic sample bags and carried on ice to IITA head quarters, Ibadan for DNA isolation. DNA isolation was by a miniprep isolation procedure of the Dellaporta et al., (1983) protocol using 100-200mg of fresh leaf tissue and a twenty fold reduction in volumes of the isolation buffers and reagents. DNA isolated was shipped to CIAT head quarters for SSR marker analysis. DNA was not quantified for marker analysis, 5ul of a 10X dilution was used in PCR reactions. All the genotypes were analyzed with SSR markers tightly linked to *CMD2*, SSR118 and NS158. SSR marker analysis were as described by Akano et al., (2001).

Results

Sexual seeds obtained from crosses to the CMD resistant land races and plantlets, transplanted to the field are summarized in Table 8.2, a total of 2490 genotypes are currently growing in the field. Harvesting of two young leaves from plantlets just before transplanting was initially thought to be stressful to the young plants, however more than 99% of transplanted genotypes survived and only 2 were lost. This is an important observation as it suggests that molecular assisted selection can be done even while the plants are in the seedling nursery.

Table 8.2. Summary of seeds and plants obtained from reciprocal crosses of TME3 and TME 9.

Family name	Female progenitor	Male progenitor	Number of seeds harvested	Plants in the field	Total number of 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 successfully isolated from all 2488 genotypes and the parental lines. The SSR markers, SSRY28 and NS158 have been analyzed in close to half of the genotypes and marker analysis is still ongoing. The large number of plants obtained from crosses from both TME3 and TME9 makes them ideal not only for making comparisons between MAS and conventional selection, and for marker-fidelity studies, but also for fine mapping markers linked to CMD resistance. Great care was therefore exercised in relating plants in the field to raw SSR data, a special template was set up in microsoft excel for this purpose.

Once molecular marker data becomes available for all genotypes, those with marker alleles linked to *CMD2* will be selected and five 20cm long woody stakes harvested for field establishment in a high CMD pressure location, in this case, IITA Ibadan. CMD resistance will be evaluated at 3 and 6 months after planting to confirm the results of marker analysis and for comparison with an unselected population of all genotypes also to be established at IITA, Ibadan.

In the near future we plan to: a) Evaluate all 2490 genotypes for CMD resistance under heavy disease pressure, and b) .Compare selection efficiency of marker-aided selection against phenotypic selection

Activity 8.3. Annotation of SAGE tags (Transcripts) differentially expressed in CMD resistant and susceptible genotypes

Rationale

The objective of the serial analysis of gene expression (SAGE) of CMD resistance in cassava is to identify candidate genes that are expressed in the *CMD2* – mediated response to the cassava mosaic virus (CMV). These candidate genes may be the novel dominant gene or genes expressed down stream that maybe the molecular basis of resistance. SAGE of CMD resistant and susceptible progeny from an F_1 mapping progeny yielded 12,700 15bp tags (representing expressed genes), divided into 5733 and 7053 tags for the resistant and susceptible genotypes respectively (CIAT 2000; Fregene et al., 2001 in preparation). One hundred and seventy five transcripts were expressed 3 to12 times more in the resistant bulk compared 94 transcripts found 3-5 times in the susceptible bulk.

The next step of the SAGE experiment is to identify the transcripts represented by the 15bp tags, a task complicated by the scanty EST data available for cassava. A PCR approach was therefore employed to amplify longer fragments using the 15bp tags as primers and a cDNA library from resistant genotypes as template. At the same time an EST project was initiated in collaboration with the Iwate Biotech Research Center, Kitakami, Japan.

Materials and methods

A cDNA library had earlier been constructed in pYES (Invitrogen Inc.) using mRNA from the CMD resistant bulk (Fregene and Terauchi 2000 unpublished data). The 13 or 15 bp SAGE tags served as forward primers and pYES vector sequences as reverse primer in PCR of a dilution of the cDNA library. PCR primers were synthesized for 28 tags expressed four times or more in the resistant bulk compared to the susceptible. PCR reaction conditions was 10 mM Tris-HCl, 50 mM KCl, 2.5mM $Mgcl_2$, 200µM each dNTPs,10pmol of the forward and reverse primer, and 2.5U of “hot star” Taq polymerase (Qiagen Gmbh) in a 50µl volume. DNA template was 1µl of a 10X dilutions of the cDNA library from the CMD resistant bulk. Thermal cycling conditions were 35 cycles of 95°C 4min, 94°C 30sec, 45°C 1min, 70°C 1min, and a final extension cycle of 70°C for 5min. PCR product was cloned into the PGEMT vector (Promega Inc.) transformed into *E.Coli* by electroporation and sequencing was off the purified plasmid template using the T7 forward primer.

To identify associations between differentially expressed genes and the dominant CMD resistance gene, cloned fragments from the tag PCR amplification were screened as RFLP probes in Southern blots of DNA of the parents and bulks of the CMD mapping progeny using 4 restriction enzymes namely: *Eco*RI, *Eco*RV, *Hind*III, and *Hae*III. DNA isolation, filter preparation and Southern hybridization were as described by Fregene et al., (1997).

Transcripts found to be polymorphic in the parental survey were analyzed in the 80 individuals of the resistant and susceptible bulks and if found polymorphic, will be analyzed in a large mapping progeny of about 2500 individuals to identify the precise position relative to the CMD resistance gene.

For EST generation, 2ul 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 using the MONTAGE 96-well plate system (Millipore Inc), four 96-well plates or 384 clones were processed at a time. PCR sequence reaction was with the 3' end primer designed from pYES (Invitrogen Inc.) and 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. A target of 3000 ESTs have been set for tag annotation.

Results

Of the 28 tag PCR, 24 gave good PCR products that could be cloned and sequenced, and 18 of these gave good and long enough sequence for BLAST (Atschul 1990) sequence similarity searches. Most of the tags were about 150-300bp long and the tag primer was found for 16 of the 18 sequenced tags, about 40bp from the 5' end. The putative identities of these transcripts and their tag primers are shown in Table 8.3. Parental and bulk filters were screened with all 24 tag PCR products. Results revealed polymorphism in 2 of the tags, 25 and 11. Tag 25 is a transcript showing homology to WPKY transcription factor, while tag 11 shows similarity to a bHLH transcription factor GBOF-1. These tags are being analyzed further in a larger population.

So far tag annotation has identified genes known to be involved in systemic acquired resistance (SAR) response to disease in plants. They include a WRKY transcription factor, catalases, a pectin-esterase and reductases. Other genes were also found implicated in plant response to disease but are part of the cell mechanism known to aid virus replication including elongation factor alpha-1. Elongation factor 1-alpha (EF1alpha), is an essential component of the translation machinery that delivers aminoacyl-tRNA to ribosomes. Virus proteins such as HIV-1 Gag polyprotein that play key functions at almost all stages of the viral and the conserved 3'-terminal stem-loop (3' SL) of the West Nile virus can bind to EF1alpha and incorporate it into the virus replication machinery (Blackwell and Brinton 1997; Cimarelli and Luban J 1999).

The 3' end sequencing of cDNA clones is ongoing and is expected to provide 3000 ESTs for tag annotation. Homology with known genes and proteins deposited in public data bases are being sought for as sequences are produced. Preliminary results reveal that that transcripts known to be abundant in cells such as ribosomal and chloroplast sequences constitute only about 10% of all sequences suggesting the cDNA library provides a good representation of genes expressed. Sequence data generated will be also be submitted to the Gene Bank.

Immediate activities to follow up with this study are: a) Annotate many more tags using 3' end ESTs, and b) Determine the function of some differentially expressed genes by over-expressing them in susceptible cassava plants and challenging them with infectious ACMV clones.

Activity 8.4. The novel CMD resistance gene (CMD2) confers high levels of resistance to the aggressive Ugandan strain (UgV)

Rationale:

There are at least three geminivirus species that are causal agents of CMD in Africa and one in India: the African cassava mosaic virus (ACMV), the East African mosaic virus (EACMV), and the South African cassava mosaic virus (SACMV), and the Indian cassava mosaic virus (ICMV) in the Indian sub-continent. In addition some strains of EACMV are recombinant like the one associated with the Ugandan epidemic; which consists of the

EACMV containing the coat protein of ACMV (Zhou et al., 1997). This is the virus, the Ugandan variant, (UgV) causing the current epidemic that swept through Uganda and is now spreading into the Democratic Republic of Congo, Kenya, Tanzania and Rwanda.

The new source of CMD resistance controlled by a single dominant gene, *CMD2*, has been shown to confer high levels of resistance to ACMV and EACMV (Akano et al., 2001, Ogbe 2000, personal communication). The necessity to evaluate the new resistance of CMD against more strains, particularly the aggressive Ugandan variant (UgV) before deployment in African and Latin American genepools led to the shipment of *in vitro* plants of 3 CMD mapping populations to Uganda. The mapping populations have been evaluated for CMD resistance over a period of one year in the field.

Table 8.3. BLAST sequence homology of 28 differentially expressed transcripts in bulks of CMD resistant and susceptible cassava genotypes.

Tag No.	**Sequence**	**No. of transripts in resistant bulk**	**No. of transcripts in resistant bulk**	**BLAST homology of amplified cDNA fragment**
1	CTAGAATGACCTTGT	12	1	Cytoskeleton related protein
2	CTAGCGCCAGACAGT	11	3	Elongation factor 1-alpha
3	CTAGCTCTGTGTATC	8	2	N/A
4	CTAGCAAAGCAGCGC	7	0	Pectin-esterase
5	CTAGGAAACAATCCT	7	1	Photosystem I chain II precursor
6	CTAGTACACAATGTA	7	1	N/A
7	CTAGCTCGCCGTAAG	6	0	Histone
8	CTAGTTAATATGGTA	6	1	N/A
9	CTAGTTCAAAGGAAG	6	1	Ribosomal protein
10	CTAGTTAAAATGTGA	5	0	Catalase
11	CTAGAGCTTTTCACT	5	1	bHLH transcription factor GBOF-1
12	CTAGCCGGATCTCCT	5	1	N/A
13	CTAGCGATTAAAAAA	5	1	Rubredoxin
14	CTAGTGGAGCAATAC	5	1	DNA binding regulatory protein
15	CTAGTTGCTTTGCAC	5	1	Initiation factor 3k (A. thaliana)
16	CTAGAAGTGGTGCTT	4	0	Nuclear import protein
17	CTAGACTGAAGTCAG	4	0	Hypothetical protein (A. thaliana)
18	CTAGAGCACGAGT	4	0	Ubiquinol--cytochrome c reductase
19	CTAGATAATAAAAGG	4	0	N/A
20	CTAGATCCTTGCCTT	4	0	No significant similarity
21	CTAGGCAGGATCAAG	4	0	N/A
22	CTAGCTGAATTATAG	4	0	N/A
23	CTAGGCAGCCGCCGC	4	0	Catalase
24	CTAGGGATTATTCAT	4	0	N/A
25	CTAGGTGGACGAGAC	4	0	Transcription factor WRKY
26	CTAGTAATCGCTCAG	4	0	40S ribosomal protein
27	CTAGTTGGATCTT	4	0	N/A
28	CTAGTTGGATTCTTT	4	0	N/A

N/A cDNA sequence data not yet available

Materials and methods

Three CMD resistance mapping populations, including two with the novel source of resistance, were multiplied *in vitro* and shipped to IITA Eastern regional center sub-station at the NARO station in Namulonge in early 2000, at least 3 plantlets were shipped per genotype. The plantlets were hardened and transferred to the field in June 2000. CMD resistance evaluation was carried out at 3 and 6 months after planting by staff of IITA East and South Africa regional center, and jointly with Lee Calvert and Martin Fregene at 12 months after planting in June 2001. Disease pressure is very high in Namulonge and susceptible genotypes are easily noticed by to their stunted growth and general deformation. The severe symptom is usually attributed to the Ugandan variant (UgV variant).

To confirm that *CMD2* gene also confers resistance against UgV, SSR analysis was conducted on leaf tissue harvested from 40 resistant and 40 susceptible progeny of the C population (TME3 X TMS30555) in the field. DNA isolation was by a miniprep version of Dellaporta et al., (1983) using about 200mg of leaf tissue. DNA isolation was conducted at the Medical Biotech Laboratories, Kampala, Uganda and shipped to CIAT. The SSR markers, SSRY28, and NS158 were analyzed in the 80 genotypes as described by Akano et al., 2001.

Results

Variation in CMD resistance in the B and C mapping population was qualitative, i.e. all plants of resistant genotypes showed no visible symptom, even on regrowth, while all plants of susceptible genotypes were heavily infected. The chi square of the ratio of resistant to susceptible plants gave a value of 1.1. This is not significantly different from a 1:1 ratio at a probability level of 0.05. This fits the expected segregation ratio for a single dominant gene heterozygous in the CMD-resistance parent, as was observed from earlier evaluations in Nigeria. SSR marker analysis revealed an allele of SSRY28 was present in the resistant progeny but absent in the susceptible progeny. (Figure 8.1) as found earlier for SSR analysis of evaluations done in Nigeria. Analysis with NS158 again showed similar results with the exception that no recombinant was found, with SSRY28 one recombinant was observed among the resistant genotypes.

The discovery that *CMD2* confers resistance to UgV is of strategic importance in efforts to contain the Ugandan epidemic that has now spread to the Democratic republic of Congo, Kenya, Tanzania and other regions around the great lakes of East Africa. A concerted effort should be made to deploy *CMD2* in local germplasm from these and sorrunding areas. IITA is currently distributing seeds to NARs in the region obtained from crossing TME 3, and other donor parents, to local cultivars, this process can be made more efficient by adding a marker pre-screening step of the germinated seeds to ensure that only CMD resistant lines are introduced into the breeding scheme and subsequent multiplication exercises. This is particularly important in adjacent countries with low disease pressure where the UgV has not made its debut but may arrive in the near future.

For the near future we plan to carry out the following activities:

- ☞ **Work with IITA to deploy *CMD2* in cassava gene pools around the great lakes of East Africa under the auspices if the RF CMD project.**
- ☞ **Evaluate TME3 and its progeny in India against the ICMV.**

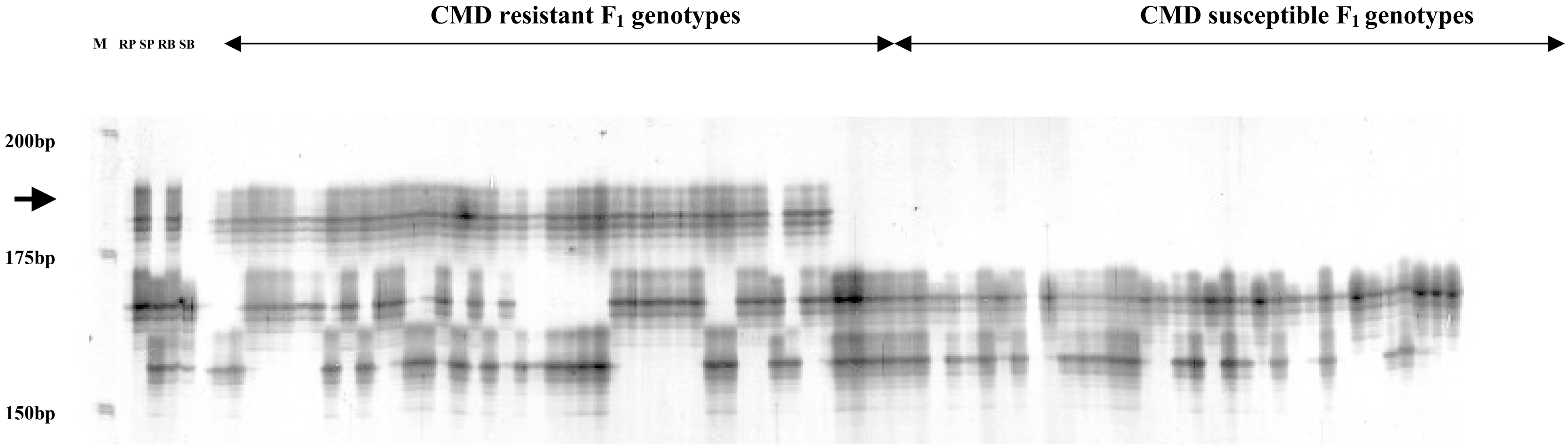

Figure 8.1. Polyacrylamide gel image of SSR marker SSRY28 analyzed in the CMD resistant parent (RP), susceptible parent (SP), resistant bulk (RB), susceptible bulk (SB), 40 resistant and 40 susceptible genotypes from Uganda. Arrow points to the SSR allele that is associated with CMD resistance. Molecular weight marker (M) is 25bp ladder.

Activity 8.5. Transformation and regeneration of some CIAT elite cultivars: towards testing candidate CMD resistance genes.

Rationale

Following the discovery of a single dominant gene(*CMD2*) that controls the novel form of resistance to CMD and efforts to identify candidate genes by the serial analysis of gene expression (SAGE), the stage is set for gene function analysis of *CMD2*. However genetic complementation of gene function is severely hampered by a lack of routine and robust transformation and regeneration methods for Latin American cultivars, the target group of CMD susceptible cultivars. A series of experiment was therefore set up in collaboration with the Danforth center to examine and refine the different steps for cassava regeneration and transformation.

The first step in the development of tissue for transformation and regeneration experiments is the induction of embryogenesis. Cassava varieties are known to respond differently to embryogenesis. There is therefore a need to first screen for responsive lines and to identify optimal conditions for embryogenesis. A set of elite materials from CIAT were selected for the experiments based on their success with farmers and as parents for cassava breeding.

Materials and methods

A list of the seven CIAT cassava elite lines used in this study can be seen in Table 8.4. The lines were multiplied in-vitro in MS2 (Murashige and Skoog's basal medium supplemented with 2%sucrose) medium to generate large quantities of plantlets. This was to facilitate production of young leaf lobes for explanting to perform primary embryogenesis experiment. Unopened young leaf lobes were excised from these plantlets at 4weeks old and placed on Murashige and Skoog's media supplemented with 2% sucrose and 50Mm Picloram(MS2,50P). Alongside this work, explants from 4 week-old plantlets from model cultivar for cassava embryogenesis, TME 60444 were cultured on MS2, 50P and placed at different light intensity. This was to identify the optimal condition for embryogenesis, since light is known to be a crucial factor. Three light levels were tested-dark, low light (0.42 µmol m^{-2} s^{-2}) and high light (7.2 µmol m^{-2} s^{-2}). Globular embryogenic structures from model cultivar 60444 and the 4 responsive lines from CIAT were excised and placed on Gresshorf and Doy (1974) medium, supplemented with 2% sucrose, 50Mm Picloram. These cultures were in culture rooms and at 3-4 weeks, the new globular embryogenic structures produced were transferred to fresh GD2, 50P medium.

Results

The result of the embryogenesis experiment in the CIAT lines are summarized in Table 8.4. Three CIAT lines, CM523-7, CM6740, CM2177-2 had a conversion rate to embryogenic structures from explant comparable to the control cultivar TMS60444. Pretreating *in vitro* plants in different light regimes did not have a significant influence on the induction of embryogenic tissues. However, when young unopened leaf lobes were explanted onto Murashige and Skoog basal media supplemented with 2% w/v sucrose and 50 µM picloram, low light was found to be significantly superior no light and high light treatments. Low light lead to an increase in the formation of embryogenic structures by a factor of two.

After two cycles FECs were produced in the model cultivar. These were then multiplied to generate more target tissues for transformation experiment. After 3 cycles FECs were generated in 3 out of the 4 lines from CIAT. Work is on going to increase the conversion rate among the CIAT lines and also produce more OES from explants in order to increase the generation of FEC

In the near future we will be doing the following activities: **a)** Conduct transformation and regeneration efficiency experiments, with FEC from model cassava cultivar TME 60444 as target tissues, to compare particle gun bombardment and *Agrobacterium* mediated transformation; and **b)** Translate the findings from above to elite lines from CIAT from which FEC have been generated to achieve a transgenic lines with genes of interest.

Table 8.4. Conversion rate of explants from CIAT's elite cassava lines to embryogenic structures

Serial number	Genotype	Percent putative embryogenic tissue	Percent organized embryogenic tissue	No of explant used
1	CM523-7	20	70	80
2	CM6740	20	50	90
3	CM2177-2	20	75	80
4	CM4574-7	1	1	80
5	TAI1	1	1	80
6	TAI8	0	60	80
7	MBRA 383	5	1	80
8	Control TME60444	20	75	

Activity 8.6. Simple Sequence Repeat (SSR) marker diversity in cassava (Manihot esculenta **Crantz***) landraces: genetic diversity and differentiation in a predominantly ssexually propagated crop.*

Rationale

The study of cassava land races from two Southern Tanzanian districts reported last year was extended to assessing genetic diversity and differentiation of cassava land races from 5 countries in South America, 2 in Central America, and 2 in Africa. A number of elite lines developed at CIAT and IITA were included in the analysis to evaluate the effect of breeding on genetic diversity. SSR marker variation at 67 loci was assessed in 314 accessions of cassava land races from Brazil, Colombia, Peru, Venezuela, Argentina, Guatemala, Mexico, Tanzania, and Nigeria. Accessions from the Neo Tropics were from the CIAT germplasm collections and those from Tanzania were the same field collection made in 1999 in a key introduction point of cassava into Africa (South Western Tanzania) and described in the annual report last year. The Nigerian Land races were from a collection held at IITA, Ibadan.

The main reason for the assessment of genetic diversity and differentiation found in cassava land races is to delineate heterotic pools for a more rational approach to choosing parents for cassava improvement and the exploitation of combining ability via recurrent reciprocal selection (Keeratinijakal and Lamkey 1993). The heterotic patterns found in maize populations at the turn of the century is the basis of a very successful maize hybrid industry and has raised maize yields 500% since 1928 (Shull 1952, Tomes 1998), a high level of genetic differentiation , as revealed by molecular markers, were later found between these populations (Melchinger et al., 1990).

Materials and methods

Plant materials, DNA isolation, and SSR marker analysis have been described elsewhere (CIAT, 2000; Fregene et al., 2001). Genetic diversity within and among accessions was estimated by the software package GEN-SURVEY (Vekemans and Lefebvre 1997) using the following statistics: percentage of polymorphic loci, mean number of alleles per polymorphic loci, average observed heterozygosity, H_o, and the average gene diversity, H_e (Nei, 1978). For all loci and for all accessions the total heterozygosity, (H_T) and the proportion of among accession differentiation (G_{ST}) were estimated according to Nei, 1978. Standard deviations for the above parameters were estimated over loci and samples by Jackknife (Quenoille, 1956; Efron, 1982). Given the small evolutionary divergence times for the accessions, the infinite alleles model (IAM) (Goldstein et al., 1995) was assumed for all calculations.

Genetic differentiation was quantified by the F statistics estimator F_{ST} (theta) (Wright 1951) as described by Weir and Cockerham (1984) using FSTAT 2.9 (Goudet 1998). G_{ST} gives the same estimate of genetic differentiation as F_{ST} but takes into account variation in sample sizes, as is the case in this study. Confidence intervals were calculated per locus over samples, and over loci by Jacknife, and by bootstrapping over loci. Pairwise values of F_{ST} between samples (land race group) was also estimated and the pairwise matrix analyzed by cluster analysis, using Ward's hierarchical clustering of JMP (SAS Institute 1995)

To assess if random genetic recombination created by farmer selection from spontaneous seedlings have played a part in the evolution of genetic diversity, parent-offspring relations were sought in the SSR data from the Southern Tanzanian collection using the computer program CERVUS (Marshall et al., 1998). CERVUS simulates a maternal and a paternal genotype from allele frequencies observed in the study population, and derives an offspring genotype by Mendelian sampling of the parental alleles. The simulation also alters the genotypic data to reflect the existence of un-sampled males, missing loci and incorrectly typed loci, according to the values of the simulation parameters. Each candidate parent is considered in turn as the alleged father, and LOD scores are calculated for all males for whom genetic data exists. Once all males have been considered, the most likely and second most likely males are identified and the Delta score (difference in LOD scores) calculated. The final stage of the simulation is to find critical values of Delta so that the significance of Delta values found in paternity inference in the study population can be tested.

Results

The large number of unlinked SSR loci employed in this study enabled a rigorous estimation of genetic differentiation and diversity structure of cassava land races from the primary and secondary center of diversity not previously carried out for cassava. The reliability of estimates for genetic variation, such as H_e, H_o, F_{ST} and genetic distances, depend more on number of loci than the number of individuals sampled (Baverstock and Moritz 1996). Estimates of genetic differentiation ranged widely from loci to loci, underscoring the danger of assessing SSR diversity using a small set of SSR markers. The genetic diversity of maize as a sub-set of diversity found in its *teosinte* progenitors vary from 25% to 75% based upon what location of the genome the diversity analysis was based on (Eyre-Walker et al., 1998).

Principal findings of the study is genetic diversity, as assessed by the average gene diversity, H_e, was high in all countries with an average heterozygosity of 0.5358±0.1184. (Table 8.5). Highest genetic diversity was found in Brazil and Colombian, although genetic diversity between Latin American and African land races is comparable. No unique alleles with a frequency of more than 25% was found within country samples with an exception of Guatemala and Nigeria. The genetic differentiation estimator F_{ST} (theta), revealed a low level of differentiation (F_{ST}=0.091±005) between country samples compared to the average for crop species - F_{ST} =0.34 (Hammrick and Godt 1997). Nonetheless pair-wise F_{ST} data between countries reveals high genetic differentiation (F_{ST} =0.26) between accessions from Nigeria and Guatemala, and a moderate to high differentiation between country accessions of the primary and a secondary center of diversity (Table 8.6 and Figure 8.2).

Table 8.5. Genetic diversity within groups of cassava land races classified according to country of origin. Standard deviations were estimated by jack-knife over loci. Ht, Hs, Dst and Gst are given over loci and over populations (country collections).

Population	Sample size	Number of Loci	Polymorphic Loci (No.)	Polymorphic Loci (%)	Mean N°. of alleles per loci	Mean N°. of alleles per polymorphic loci	Ho [¶]	He [§]	He(p) [†]	Fis_p
Argentina	3	67	57	85.1	2.6	2.9	0.5174	0.4635	0.5672	0.0596
Brazil	20	67	67	100	5.2	5.2	0.5311	0.6129	0.6285	0.146
Colombia	32	67	66	98.5	6	6	0.5012	0.6177	0.6277	0.1875
GCA	15	67	65	97	4.5	4.6	0.5244	0.5754	0.5952	0.1072
Guatemala	4	67	57	85.1	2.4	2.7	0.4925	0.396	0.4554	-0.1269
Mexico	5	67	64	95.5	3.6	3.7	0.4915	0.56	0.6251	0.1987
Peru	7	66	62	93.9	3.7	3.9	0.4892	0.5596	0.6067	0.1771
Venezuela	5	66	64	97	3.5	3.6	0.4297	0.5692	0.634	0.2975
Tanz-Mtwara	84	67	65	97	5.3	5.4	0.543	0.558	0.5616	0.0295
Tanz-Naliendele	23	67	64	95.5	4.5	4.7	0.5448	0.5545	0.5667	0.034
Tanz-Kibaha	56	67	64	95.5	5.1	5.3	0.5274	0.5334	0.5382	0.0144
Nigeria	19	66	62	93.9	3.9	4	0.5965	0.5296	0.544	-0.1245
IITA	6	67	61	91	3.2	3.4	0.4915	0.4866	0.534	0.061
Moor	4	67	63	94	2.7	2.8	0.51	0.4852	0.5596	0.0572
Mean				94.23	4.03	4.17	0.5136	0.5358	0.5745	0.0799
Std. Dev.		4.45	1.11	1.06	0.0378	0.0602	0.0495	0.1184		
	Ht[‡]	**Hs**[Δ]	**Dst**[Ω]	**Gst**[∫]						
Mean	0.6499	0.5812	0.0687	0.1075						
Std. Dev.	0.1595	0.147	0.0318	0.0565						
95% CI	0.61	0.5463	0.0621	0.0953						
99% CI	0.6871	0.6136	0.0758	0.1195						

[¶] **Ho** Average observed heterozygosity within country. [§] **He** Average expected heterozygosity within country. [†] **He(p)** Average expected heterozygosity within country corrected for small sample sizes (Nei 1978).

[‡] **Ht** Total Heterozygosity in the entire data set. [Δ] **Hs** Gene diversity within country averaged over the entire data set [Ω] **Dst** Average gene diversity between populations. [∫] **Gst** Coefficient of gene differentiation.

Table 8.6. Pairwise estimator of Fst (theta) between pairs of country groupings of cassava land races.

Population	**Argentina**	**Brazil**	**Colombia**	**GCA**	**Guatemala.**	**Mexico**	**Peru**	**Venezuela.**	**Tanz-Mtwara**	**Tanz-Naliendele**	**Tanz-Kibaha**	**Nigeria**	**IITA**	**Moor**
Argentina	0	0.063	0.071	0.068	0.236	0.043	0.128	0.028	0.145	0.127	0.1654	0.2067	0.1447	0.163
Brazil	0.063	0	0.057	0.044	0.145	0.040	0.112	0.030	0.079	0.0787	0.115	0.1076	0.1296	0.105
Colombia	0.071	0.057	0	0.011	0.112	0.038	0.078	0.012	0.092	0.0827	0.129	0.129	0.1237	0.114
GCA	0.068	0.044	0.011	0	0.123	0.028	0.097	0.012	0.081	0.0682	0.1077	0.1388	0.1275	0.112
Guatemala	0.236	0.145	0.112	0.123	0	0.147	0.191	0.121	0.168	0.1638	0.2103	0.2696	0.2689	0.251
Mexico	0.043	0.040	0.038	0.028	0.147	0	0.085	-0.006	0.098	0.082	0.1185	0.1405	0.117	0.112
Peru	0.128	0.112	0.078	0.097	0.191	0.085	0	0.053	0.142	0.1212	0.1529	0.1877	0.1687	0.161
Venezuela	0.028	0.030	0.012	0.012	0.121	-0.006	0.054	0	0.057	0.0476	0.0839	0.1147	0.0795	0.059
Tanz-Mtwara	0.145	0.079	0.092	0.081	0.168	0.098	0.142	0.057	0	0.0076	0.0452	0.1402	0.118	0.108
Tanz-Naliendele	0.127	0.079	0.083	0.068	0.164	0.082	0.121	0.048	0.008	0	0.0097	0.1358	0.0888	0.092
Tanz-Kibaha	0.165	0.115	0.129	0.108	0.210	0.119	0.153	0.084	0.045	0.0097	0	0.1625	0.1121	0.122
Nigeria	0.207	0.108	0.129	0.139	0.270	0.141	0.188	0.115	0.140	0.1358	0.1625	0	0.1605	0.114
IITA	0.145	0.130	0.124	0.128	0.269	0.117	0.169	0.080	0.118	0.0888	0.1121	0.1605	0	0.018
Moor	0.163	0.105	0.114	0.112	0.251	0.112	0.161	0.059	0.108	0.0919	0.1215	0.1142	0.0182	0

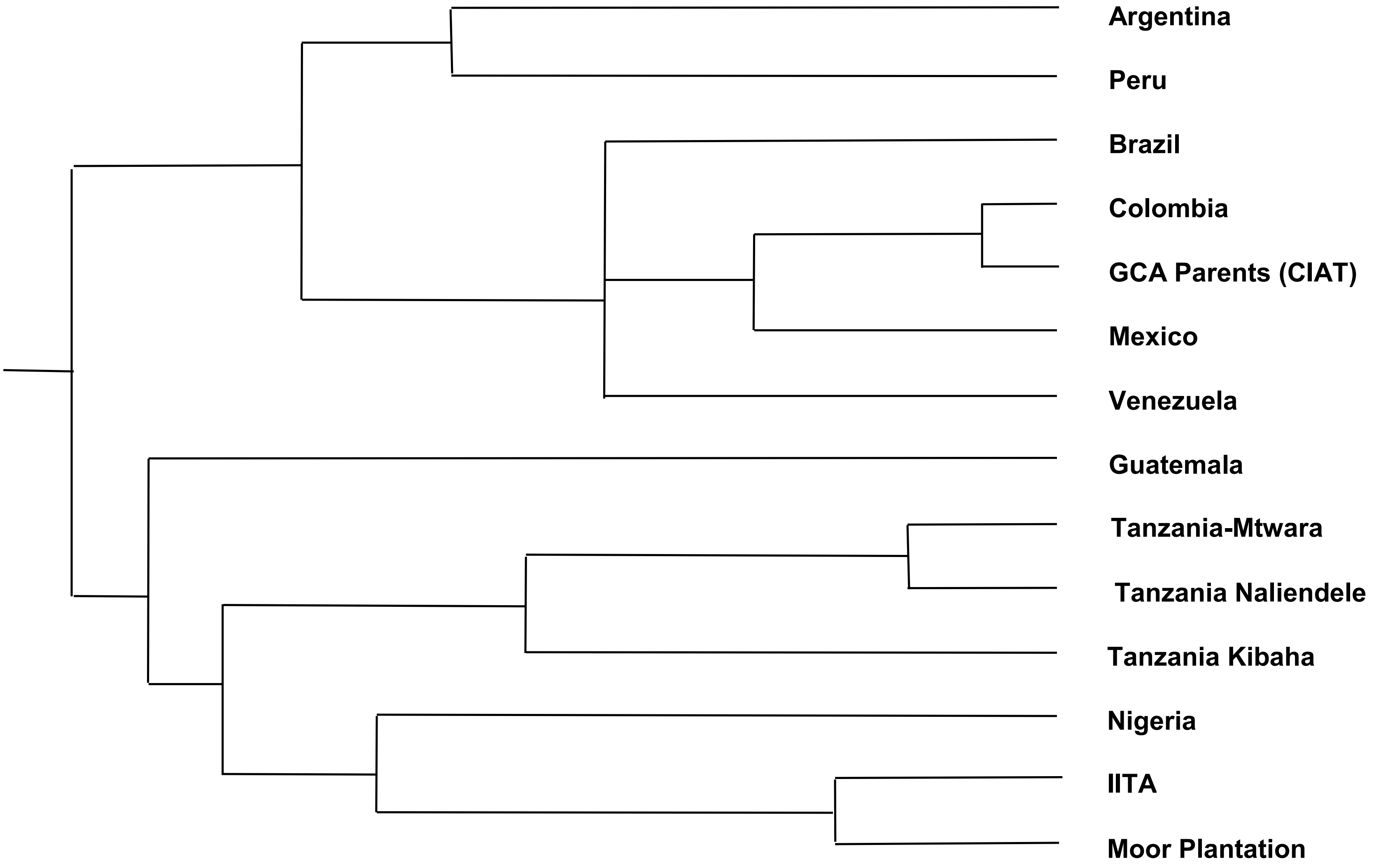

Figure 8.2. Hierarchical clustering (Ward) of Fixation Index (F_{ST}) pair-wise distances between cassava land races, grouped by country, and elite parents. by source.

A total of 51 parent-offspring relationships were found in the 96 accessions collected from Southern Tanzania using a delta threshold level of 1.0 (Fregene et al., 2001, Appendix2). Analysis of parent-offspring are confounded by closely related offspring, the statistic delta calculated by CERVUS compares LOD scores of the two best putative parents to reduce the confounding effects of full- or half-sibs. Results of the parent-offspring relationship successfully identified a known parent of TMS 30572, an improved line from IITA, which was included as an internal control. The genotype 58308 from the Moor plantation, Ibadan, Nigeria, breeding program of the 1950s, served as a parent source of cassava mosaic disease resistance (CMD) for TMS30572.

The overall low level of genetic differentiation in cassava is comparable with that found in perennial forest trees, 0.084 on an average (Hamrick and Godt 1996; Le Corre et al., 1997). Forest trees have experienced many foundation events after the expansion from a few Southern refuges 15,000 years ago after the last glacial period (Huntley 1990). Austerlitz et al., (2000) demonstrated that the unexpected low differentiation and high genetic of trees events can be explained by high gene flow, both seed and pollen flow, and the length of their juvenile phase. Cassava was likely domesticated from populations of *M.esculenta* sub spp *flabellifolia* along the Southern rim of the Amazonian basin within the last 10,000 years ago (Olsen et al., 1999). Its expansion into other regions of Latin America, Africa and Asia would have led to founders effect of reduced diversity and an increase in genetic differentiation. The unexpected low level of genetic differentiation and the high genetic diversity of cassava land races in all countries may therefore be due to high genetic diversity of original populations, extensive movement of germplasm and spontaneous genetic recombination. The common practice of using volunteer plants and the circulation of woody planting material, often to replace varieties destroyed by herbivores, biotic and abiotic stresses would have lead to highly heterogeneous cassava fields after domestication. Diversity found in a single farmer's field has also been shown to be equal to the core-of-the core collection of 38 accessions representative of the world cassava collection at CIAT (Elias et al., 2000).

Future plans for this activity include the evaluation of A larger sample set of land races from Nigeria and Guatemala regions will be analyzed to confirm results obtained here. Genetic crosses between and within Guatemalan land races and Nigerian land races to test correlation between differentiation and heterosis.

Activity 8.7. Assessment of genetic diversity among African cassava cccessions resistant to the cassava mosaic disease using SSR markers

Rationale

The cassava mosaic virus disease (CMD) is considered the most devastating disease of cassava in Africa causing severe yield losses ranging from 20-95% (Thresh et al., 1994). It is caused by the cassava mosaic begmoviruses, which are transmitted by the whitefly (*Bemisia tabaci* Genn) and spread through propagation of infected vegetative propagules. It is estimated that total crop yield losses due to CMD cost the African continent about $2 billion per annum (IITA, 1997). The most effective means of controlling CMD is by host plant resistance and resistance was first identified in third back cross derivatives between cassava and its wild relative *Manihot glaziovii* Muller von Argau (Nicholas, 1947). Despite the progress made in resistance breeding, there still is the need to increase the levels of resistance, particularly against aggressive recombinant strains that can spontaneously occur (Zhou et al.„ 1997). Recently a novel source of resistance controlled by a single dominant gene was found in some Nigerian land races (Mignouna and Dixon 1996) and this has lead to a more systematic evaluation of African land races.

To facilitate choice of parents for breeding more durable CMD resistance while maintaining a good level of genetic diversity, 18 SSR markers were used to evaluate genetic diversity within a collection of 78 African cassava accessions resistant and susceptible to cassava mosaic virus disease (CMD). The accessions include 5 improved accessions, 68 resistant and 10 susceptible land races A total of 18 SSR markers were employed to determine genetic relationships. The second objective of this study was to predict possible novel sources of resistance to CMD based upon SSR marker clustering which can then serve as a basis for further genetic studies.

Materials and methods

The cassava accessions and their source used in this study are shown in Table 8.7. The land races had previously been evaluated in several location and years for their reaction to CMD based on their phenotypic expression of symptom severity using the standard five point scoring scale system for CMD (IITA, 1990). DNA isolation was from 1-3 g of young leaves per accession after Dellaporta et al., (1983). Thirty-six SSR markers, two each from 18 linkage groups of the cassava genetic map (Fregene et. al 1997; Mba et al., 2000) (Table 8.8), were employed in the initial SSR analysis. SSR analysis was as described in Mba et al. (2000). Individual accessions were scored as diplotypic data “0102” and as haplotypic data “1” presence of a band, and “0” absence of a band for the SSR data. individually and the different alleles were recorded for each sample screened.

The haplotypic data was used to calculate genetic distances between pairs of cassava accessions, using the Dice algorithm, and to draw a dendogram using the unweighted pair-group mean average (UPGMA) cluster method of Nei’s genetic distances (Sneath and Sokal 1973). The genetic distances and dendogram were computed with the NTSYS-PC computer programme, ver. 2.02 (Rohlf 1997). The diplotypic data was employed to calculate estimates of genetic diversity estimates: percentage of polymorphic loci, mean number of alleles per polymorphic loci, average observed heterozygosity, H_o, and the average gene diversity, H_e (Nei 1978), using the computer program Gen-Survey (Vekemans and Lefebvre, 1997). Confidence intervals, at the 95% level, were obtained through 200 bootstraps over loci for the means of the above parameters.

Results

The overall level of polymorphism, 92%, is better than that found a previous AFLP study of CMD resistance and susceptible land races, 69%, (Fregene et al., 2000) confirming the superiority of SSR markers for genetic diversity studies. A dendogram of genetic distances grouped the 78 accessions into 5 groups at coefficient of similarity of 0.4 (Figure 8.3). The first group has nine members including the line 58308, the principal parental line for the *M.glaziovii* source of CMD resistance, and its top progeny TMS 30572, the improved accession 91/02324, four resistant and one susceptible land race (see Table 8.7 for accession groupings). The next group, which was the largest, was made up of two improved accessions M94/0583 and 29 land races, including one susceptible accession. All the resistant land races from the Republic of Benin and the majority of resistant land races from Nigeria and the Togo were in this group. The group also included a resistant land race from Angola and one from Ghana. The third group consists of the improved accession TMS30001, 17 resistant and one susceptible land races. Group four was made up of seven susceptible and five resistant land races, and group five, made up of the improved accession M94/0121and eight other resistant land races. Duplicates were detected between some of the Nigerian land races such as TME581 and TME12, TME5 and TME3, TME62, TME6 and tME4, between TME242 and TME240, TME435 and TME288, TME479 and TME470 and between TME480 and TME225. The clustering pattern of the land races and the level of duplication is in agreement with the AFLP study of Fregene et al., (2000).

Overall genetic diversity of the land races was high, 0.512, comparable to that described for a larger set of land races from 7 African, South and Central American countries, although the large difference in number of markers makes this comparison inadequate (M. Fregene et al., 2001, CIAT 2001, this report). Gene diversity was highest among the land races and accessions in cluster group 3 followed by those in group 4 then group 2 and the lowest was detected in cluster group 1 (Table 8.8). Of the total genetic diversity, 0.47 was due to within cluster diversity and genetic differentiation between cluster was low (Gst = 0.096). The amount of genetic differentiation which has been reported for cassava, (G_{ST}=0.43, Fregene et al., 2000) is higher than that found in this study. Discrepancies in gene diversity estimates have been attributed to nature of markers systems (Djé, et al, 2000), but it may also be due to the small set of African land races and an inclusion of Latin American land races in the Fregene et al., (2000) study.

Results of this study reveal a substantial amount of genetic diversity in CMD resistance germplasm appropriate for genetic improvement of CMD resistance as well as other traits, particularly yield. It also suggest that there may be other sources of resistance to CMD other than the known ones based on the clustering pattern of the resistant accessions. The Nigerian land races that have the novel source of resistant cluster together away from land races from other African nations and from the older source of resistance, 58308. This result suggests that resistance to CMD may have arisen independently several times in the past. This result will be confirmed by genetic analysis of crosses between resistant and susceptible land races from clusters other than those with land races bearing currently known sources o resistance.

The future plans for this activity include Marker-assisted genetic analysis of crosses between resistant and susceptible land races from clusters other than those with land races bearing currently known sources o resistance.

Table 8.7. List of accessions their pedigree/local name (or assigned code by Country collectors), country of orign CMD status (R= resistant, S= susceptible) and assigned cluster group of genetic similarity.

Accession	Pedigree/Local name	Country	Status	Group
58308	M.esculenta x M. glaziovii	IITA	R	1
M94/0583		IITA	R	2
I30001		IITA	R	3
91/02324	TME1 OP	IITA	R	1
M94/0121		IITA	R	5
TME638	EJ 79	Ghana	R	4
TME635	MNN 55	Ghana	R	4
TME631	SE 210	Ghana	R	3
TME630	Amin	Ghana	R	4
TME581	Oke Local	Nigeria	R	2
TME572	Udoh Local	Nigeria	R	5
TME568	Mundele Paco (ANG-3)	Angola	R	4
TME565	Prescose de Angola (ANG-4)	Angola	R	3
TME546	SS4 (T8)	Uganda	R	4
TME526	Ka13 (Kenya Ostrom)	Côte d'Ivoire	R	1
TME498	R.A 16	Nigeria	R	2
TME480	RB92/0119	Benin	R	3
TME479	Agric	Benin	R	2
TME478	RB92/0123	Benin	R	3
TME477	RB92/0104	Benin	R	3
TME474	CAP94/064	Benin	R	5
TME470	CAP94066	Benin	R	2
TME461	RB92/0188	Benin	R	3
TME456	CAP94062	Benin	R	3
TME455	RB92/0116	Benin	R	2
TME451	CAP94067	Benin	R	2
TME449	RB92/0182	Benin	R	2
TME446	RB92/0204	Benin	R	2
TME443	CAP94090	Benin	R	2
TME437	RB92/0103	Benin	R	5
TME435	RB92/0175	Benin	R	2
TME434	RB92/0155	Benin	R	5
TME431	MAIN 11	Togo	R	1

Table 8.7 (cont.)

Accession	Pedigree/Local name	Country	Status	Group
TME429	MAIN 4	Togo	R	1
TME419	Gbazekoute	Togo	R	3
TME379	Ofegbe	Nigeria	R	2
TME288	Akano	Nigeria	R	2
TME287	Power	Nigeria	R	2
TME282	Alice Local	Nigeria	R	2
TME279	Obasanjo	Nigeria	R	2
TME278	Oko Warangbala	Nigeria	R	2
TME258	25	Ghana	R	1
TME243	Toma 26	Togo	R	3
TME242	Toma 76	Togo	R	2
TME241	Toma 136	Togo	R	3
TME240	Toma 75	Togo	R	2
TME236	Toma 37	Togo	R	5
TME232	Toma 63	Togo	R	5
TME230	Toma 36	Togo	R	5
TME229	RB92/0130	Benin	R	5
TME228	Toma 97	Togo	R	2
TME225	92/0099	Togo	R	3
TME209	1254(880887)	Cameroon	R	1
TME204	RB98/0113	Benin	R	3
TME199	RB89/59	Benin	R	3
TTME5	Bagi Wawa	Nigeria	R	2
TME62	Yau Rogor	Nigeria	R	2
TME13	MS-20	Nigeria	R	2
TME12	Tokunbo	Nigeria	R	2
TME11	Igueeba	Nigeria	R	3
TME9	Olekanga	Nigeria	R	3
TME8	Amala	Nigeria	R	3
TME7	Oko-Iyawo	Nigeria	R	2
TME6	Lapai-1	Nigeria	R	2
TME4	Atu	Nigeria	R	2
TME3	2ND Agric	Nigeria	R	2
TME1	Antiota	Nigeria	R	2
I30572	58308 x Branca de Sta Caterina OP	IITA	R	1
TME401	Toma 141	Togo	S	4
TME59	Dandualla-2	Nigeria	S	4
TME60	Darazo Rogor	Nigeria	S	2
TME104	Rogor-5	Nigeria	S	3
TME107	Danwara	Nigeria	S	4
TME117	Isunikankiyan	Nigeria	S	4
TME123	Panya	Nigeria	S	4
TME218	881260(882160)	Cameroon	S	4
TME382	Suleja-5(92/0163)	Nigeria	S	4
TME557	Lossakpleh	Côte d’Ivoire	S	1

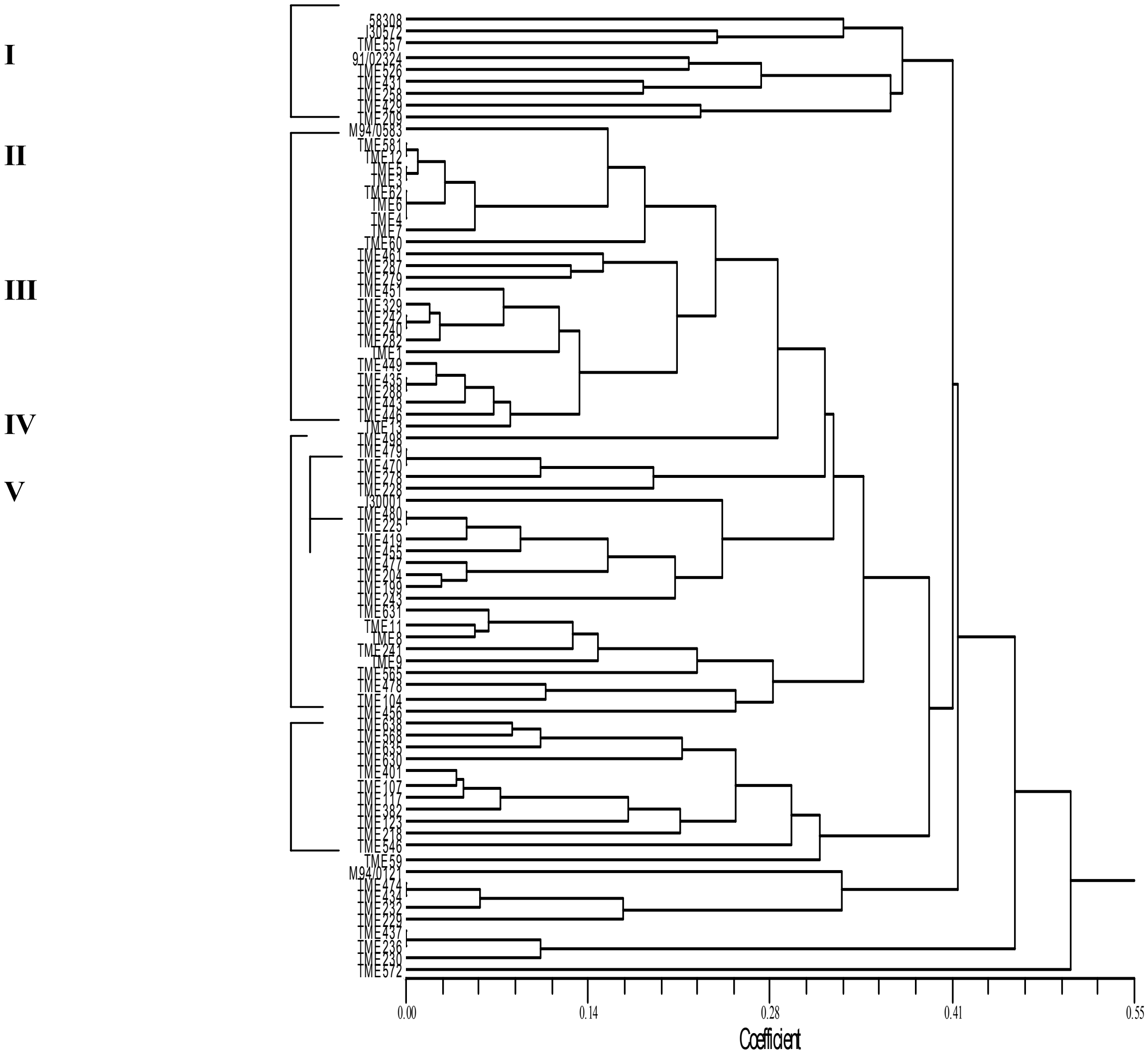

Figure 8.3**.** Dendrogram of genetic distance showing the association between the 78 cassava accessions based on SSR using UPGMA cluster analysis.

Table 8.8. Gene diversity analysis within and among cassava accessions by cluster group

Cluster Group	PLP[a]	A[b]	A_P[c]	H_o[d]	H_E[e]	H_{EC}[e]
Cluster Group 1	88.9	2.7	2.9	0.4979	0.4165	0.444
Cluster Group 2	94.4	2.6	2.6	0.6431	0.4369	0.4459
Cluster Group 3	100	2.8	2.8	0.6446	0.4828	0.5006
Cluster Group 4	94.4	2.7	2.7	0.6034	0.4378	0.4605
Cluster Group 5	100	2.7	2.7	0.5663	0.4624	0.5193
Mean	95.56±4.65	2.68±0.06	2.73±0.09	0.591±0.061	0.4473±0.026	0.4741±0.034
	Ht	Hs	Dst	Gst	Dm	Rst
Mean	0.514	0.466	0.048	0.096	0.0634	0.1417
Std. Dev.	0.138	0.135	0.041	0.083		
95% CI	0.446	0.404	0.032	0.062		
95% CI	0.583	0.533	0.065	0.135		

[a] Percentage of polymorphic loci at the 5% level within accessions

[b] Mean number of alleles per locus within accessions

[c] Mean number of polymorphic alleles per locus within accessions

[d] Average observed heterozygosity

[e] Average gene diversity within accessions corrected for small populations

Activity 8.8. Analysis of genetic diversity in cassava landraces from the Coastal, Andean and Forest region of Peru

Rationale

Peru is considered one of several countries with enormous amounts of biodiversity and it is a center of diversity for numerous cultivated crop, including cassava. The National Genetic Resources and Biotechnology Program (PRONIRGEB, its Spanish Acronym) of the National Institute for Research (INIA, its Spanish acronym) has as its principal objectives the conservation of native germplasm for use by the scientific community and local farmers. PRONIRGEB has two cassava germplasm banks located in Donoso, Lima, with 240 accessions, and El Porvenir, Tarapoto, forest agroecology, having 260 accessions. These accessions are kept as field collections, with a significant portion as tissue culture collections, and they have been characterized morphologically.

Under the project “Models of diversity and genetic erosion of traditional cultivars in Peru: rapid assessment and early detection of risks using GIS tools” funded by the BMZ and executed by IPGRI and INIA, genetic diversity is being assessed using molecular tools combined with GIS methods to provide indicators of genetic erosion. In the first phase of this project a national laboratory for characterizing genetic resources has been set up and training of personnel, in the area of molecular markers for genetic diversity assessment, is being implemented to run the laboratories. The objective of this study was to train a national scientist from INIA in simple sequence repeat (SSR) analysis in cassava, one of crops addressed under the BMZ project.

Materials and methods

One hundred accessions from the PRONIRGEB-INIA cassava germplasm collection was used in this study. The accessions were selected based upon the place collected: coastal, Andean or forest, to provide a representative sample of cassava grown in Peru (Table 1). About 100mg of young leaf tissue obtained from field grown plants was used to isolate DNA using a mini CTAB preparation (CIP1997). Eighteen SSR markers, one from each linkage group, and selected for to their high heterozygosity in a previous SSR study of cassava land races (Fregene et al., 2001, CIAT 2001, this report) were used for SSR analysis as described by Mba et al. (2000). Gel analysis of PCR amplification product is also as described by Mba et al., (2000).

Raw SSR data was scored as "1" and "0" for presence and absence of DNA bands respectively or haplotype data. The bands were then numbered and the data transformed by Excel to "0102" or diplotype data.. The haplotype data was used to calculate genetic distances between pairs of cassava accessions, using the Dice algorithm, and to derive principal components (PC) (Sneath and Sokal 1973). The first and second components were presented in a graphical form using Excel. The genetic distances were computed with the NTSYS-PC computer programme, ver. 2.02 (Rohlf 1997), while the PC analysis was done using SAS (SAS Institute). The diplotype data was employed to calculate estimates of genetic diversity: percentage of polymorphic loci, mean number of alleles per polymorphic loci, average observed heterozygosity, H_o, and the average gene diversity, H_e (Nei 1978), using the computer program Gen-Survey (Vekemans and Lefebvre, 1997). Confidence intervals, at the 95% level, was obtained through 200 bootstraps over loci for the means of the above parameters.

Results

Average genetic diversity was very high 0.68, and there was no significant difference between diversity found in the three regions (Table 8.9). Genetic differentiation as estimated by Gst was very low (0.0074) and confirms the same pattern found in the study of cassava land races from 7 countries (CIAT 2001, this report) although the large difference in markers makes this comparison ineffective. PCA of land races from the coastal, Andean and forest region also did not reveal any distinct clustering pattern among the land races with the exception that a single accession from Brazil included in the analysis was separated from the Peruvian accessions (Figure 8.4).

The present study reveals an unexpected low level of genetic differentiation and high genetic diversity of cassava land races in regions as diverse as the Andean, coastal and forest region of Peru (Table 8.10). It strongly supports the hypothesis of extensive movement of germplasm between regions, the high genetic diversity of original populations, as well as the wide adaptation of cassava.

Future plans for this activity include:

- ☞ A set of 36 primers, a gift from CIAT, will be used at PRONIRGEB-INIA to continue SSR analysis of all germplasm accessions held in the National collection.
- ☞ Conduct a comparative analysis between Peruvian germplasm held at PRONIRGEB-INIA and at CIAT for exchange of land races currently not present in either collection.

Table 8.9. List of Cassava Accessions from the PRONIRGEB-INIA Germplasm Collection and Source

It.	Códi.	Región	Departamento	It.	Códi.	Región	Departamento
1.	181	Selva	Ucayalí	51.	020	Sierra	Cusco
2.	145	Selva	San Martín	52.	001	Sierra	Amazonas
3.	174	Selva	Ucayalí	53.	037	Sierra	Huánuco
4.	049	Sierra	Junín	54.	002	Selva	Amazonas
5.	085	Costa	Lima	55.	024	Sierra	Huánuco
6.	163	Selva	Sap Martín	56.	035	Sierra	Huánuco
7.	101	Costa	Lima	57.	033	Sierra	Huánuco
8.	157	Selva	San Martín	58.	038	Sierra	Huánuco
9.	180	Selva	Ucayalí	59.	034	Sierra	Huánuco
10.	153	Selva	San Martín	60.	029	Sierra	Huánuco
11.	143	Costa	Piura	61.	030	Sierra	Huánuco
12.	108	Costa	Lima	62.	026	Sierra	Huánuco
13.	104	Costa	Lima	63.	027.	Sierra	Huánuco
14.	082	Costa	Lima	64.	043	Sierra	Junín
15.	048	Sierra	Junín	65.	022	Sierra	Cusco
16.	139	Costa	Piura	66.	042	Sierra	Junín
17.	112	Costa	Lima	67.	031	Sierra	Huánuco
18.	117	Costa	Lima	68.	044	Sierra	Junín
19.	097	Costa	Lima	69.	023	Sierra	Cusco
20	138	Costa	Piura	70.	036	Sierra	Huánuco
21.	165	Selva	San Martín	71.	004	Selva	Amazonas
22.	175	Selva	Ucayalí	72.	003	Selva	Amazonas
23	089	Costa	Lima	73.	027	Sierra	Huánuco
24.	142	Costa	Piura	74.	045	Sierra	Junín
25.	148	Selva	San Martín	75.	046	Sierra	Junín
26.	179	Selva	Ucayalí	76.	113	Costa	Lima
27.	155	Selva	San Martín	77.	116	Costa	Lima
28.	160	Selva	San Martín	78.	121	Costa	Lima
29.	144	Costa	Piura	79.	125	Costa	Lima
30.	047	Sierra	Junín	80.	129	Costa	Lima
31	094	Costa	Lima	81.	131	Selva	Loreto
32.	183	Selva	Ucayalí	82.	135	Selva	Loreto
33.	178	Selva	Ucayalí	83.	133	Selva	Loreto
34.	173	Selva	Ucayalí	84.	134	Selva	Loreto
35.	182	Selva	Ucayalí	85.	136	Costa	Piura
36.	158	Selva	San Martín	86.	137	Costa	Piura
37.	164	Selva	San Martín	87.	140	Costa	Piura
38.	176	Selva	Ucayalí	88.	141	Costa	Piura
39.	086	Costa	Lima	89.	146	Selva	San Martín
40.	172	Selva	Ucayalí	90.	149	Selva	San Martín
41.	040	Sierra	Huánuco	91.	150	Selva	San Martín
42.	032	Sierra	Huánuco	92.	151	Selva	San Martín
43.	019	Sierra	Cusco	93.	152	Selva	San Martín
44.	005	Selva	Amazonas	94.	154	Selva	San Martín
45.	025	Sierra	Huánuco	95.	156	Selva	San Martín
46.	018	Sierra	Cusco	96.	159	Selva	San Martín
47.	081	Costa	Lima	97.	161	Selva	San Martín
48.	028	Sierra	Huánuco	98.	162	Selva	San Martín
49.	039	Sierra	Huánuco	99.	199		Bolivia
50.	021	Sierra	Cusco	100.	228		Brasil

Table 8.10. Gene diversity analysis of 100 accessions from the Peruvian Coastal, Andean and Forest regions.

Region	N°	PLP^{a}	A^{b}	A$_{P}^{c}$	H$_{o}^{d}$	H$_{E}^{e}$	H$_{EC}^{e}$
Coast	27	100	5.2	5.2	0.7259	0.7031	.7165
Forest	39	100	5.2	5.2	0.6178	0.6683	0.6770
Highland	30	100	4.9	4.9	0.6879	0.6879	0.6994
Mean		100	5.13 ±0.18	5.13±0.18	0.6718 ± 0.0540	0.6864 ± 0.0174	0.6976 ± 0.0198
		Ht	Hs	Dst	Gst		
Mean		0.7032	0.6980	0.0052	0.0074		
Std.Dev.		0.0865	0.0866	0.0086	0.0124		
95% CI		0.6573	0.6506	0.0009	0.0012		

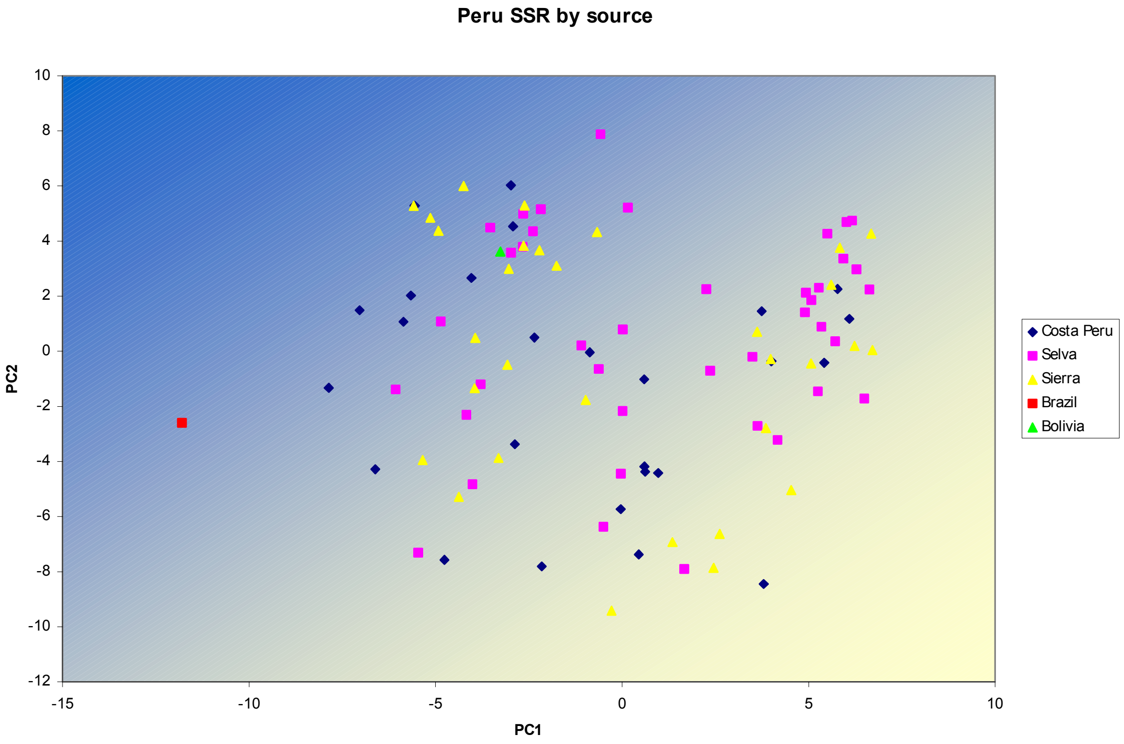

Figure 8.4. Principal component analysis (PCA) showing the genetic relationship between 100 cassava accessions from the coastal, Andean and Forest regions of Peru.

Activity 8.9. Root quality and pest resistance genes from wild relatives of cassava for broadening the crop genetic base

Rationale

As a major staple food crop across the tropics, cassava can serve as a cheap means of deploying adequate protein requirement amongst the poor and for feeding animals. But cassava's starchy roots are very low in protein compared to other crops; less than 2% dry matter of protein in cassava compared to 9.1% in potato, there is therefore a need to increase the protein content in roots of cassava. Cassava is also an important source of starch, 70-90% of cassava dry root matter is starch, the rest being fibers. Raw or unmodified cassava starches are increasingly important raw materials in textile, alcohol, animal and human food industries world-wide and this is expected to grow (Henry 1995). An increase therefore in starch dry matter content (equivalent to starch content) translates into higher income per unit land, per unit labor (investment) for farmers growing cassava

Several wild relatives of cassava are known to possess up to 15% protein and more than 50% dry matter in their roots. These germplasm resources are a useful source of genes for the improvement of protein in cassava. Reports of crosses between cassava and *M. tristis* revealed root protein content of more than 8% in F_1 hybrids (Bolhuis 1953; Asiedu et al., 1992). Unfortunately the high protein content was lost during back crossing to recover the desired characteristics and high root yield of cassava (Asiedu 1992). For several years now, it has been shown that the "tremendous genetic potential locked up in germplasm banks can be released by shifting the paradigm from searching for phenotypes to searching for superior genes using molecular genetic maps and an advanced back cross mapping scheme (Tanksley and McCouch, 1997). An evaluation of protein and dry matter content, amylose/amylopectin ratio and white fly resistance was therefore conducted on germplasm resources of 7 wild *Manihot* species held at CIAT genetic resources unit (GRU).

Materials and methods

More than 800 sexual seeds representing accessions of *M. esculenta* sub spp *flabelifolia*, *M. esculenta* sub spp *peruviana*, *M. tristis*., *M.carthaginensis*, *M. walkerae, M.brachyloba and M.fomentosa* were planted in seedling trays at CIAT. Of these number, 695 accessions were transplanted to the field at the Centro Experimental de la Universidad Nacional, Palmira (CEUNP). From six month after planting, sequential evaluation of white fly resistance was conducted on all genotypes. At 8 months after planting 3 roots were milked from 678 accessions and evaluated for protein content, dry matter percentage, amylose/amylopectin ratio, and storage root size according to standard procedures established at CIAT.

Another set of 400 sexual seeds of inter-specific hybrids between cassava and *M. esculenta* sub spp *flabelifolia*, *M. esculenta* sub spp *peruviana*, *M. tristis*., *M.carthaginensis*, *M. chlorosticta and M. pseudo glaziovii* were germinated. A total of 322 were successfully transplanted to the field and 3 roots evaluated at 8 months after planting for the above traits. To confirm results obtained in the first year, 6 woody stakes were obtained from wild species accessions and inter-specific hybrids high in protein, dry matter, white fly resistance or low in amylose/amylopectin ratio, and planted in single row clonal observation plots at CIAT. At the same time 6-10 stakes of a selected sub set of these genotypes were planted in a hybridization block to initiate the advanced back cross QTL marker scheme to introgress favorable genes for the above traits into cassava. Due to the poor germination of some accessions, stakes were planted in the green house before transfer to the field.

Result

The first year evaluation of more than 1000 genotypes of 7 wild *Manihot* species and inter-specific hybrids revealed a moderate to very high levels for protein and dry matter content, waxy starches (low level of amylose), and white fly resistance. Table 8.11 shows the data for genotypes with the highest protein content. The best genotypes for white fly resistance were found in inter-specific hybrids with *M. chlorosticta*. (Table 8.12). A second year evaluation of six plants from the top genotypes will be conducted, but at the same time selections have been made from the top genotypes for genetic crosses. 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 (Table 8.13). Although only 4 genotypes from each group will be used for crosses a larger number was selected to accommodate variation that may occur due to the environment. Evaluation for the above traits will therefore be conducted before crosses are made to cassava). At least 100 seeds are expected for each of the 48 families (cross combination). The cassava parents for crosses are the elite parents of the 4 agro-ecological gene pools (Table 8.14). Some of these lines are high carotene lines eg. SM 1433-4, and will lead to a combination of high carotene with high protein content.

Table 8.11. Best genotypes of wild *Manihot* accessions for protein content from evaluation of 3 roots.

Accession number	**Mother plant**	**Bulking ¶·**	**% dry matter**	**% crude protein**	**%crude fiber**	**% ash**	**% amylose**
OW 27-1	CTH XXX-1	3	10.17	7.00	4.27	3.10	10.33
OW 62-2	FLA 433-2	0	27.04	13.08		2.74	
OW 130-4	TST XXX-42	2	17.92	11.77		3.56	
OW 132-2	TST XXX-3	4	22.40	11.71	4.69	3.14	13.98
OW 132-4	TST XXX-3	1	43.82	13.12	12.52	1.85	16.31
OW 139-1	TST XXX-38	4	29.59	11.69	8.06	1.07	18.61
OW 141-1	TST XXX-41	0	30.43	12.15	14.72	3.08	
OW 143-1	TST XXX-54	0	34.30	12.17		4.79	
OW 145-2	TST XXX-77	0	24.30	13.58		5.41	
OW 145-3	TST XXX-77	0	34.88	14.71	22.91	2.99	
OW 153-4	CTH 409-1	0	16.94	13.17		3.45	
OW 170-3	CTH XXX-106	1	29.03	12.12		2.65	
OW 172-1	CTH XXX-121	1	6.47	18.99		6.59	
OW 180-4	FLA 423-5	4	27.47	13.50	5.38		14.56
OW 183-4	FLA 423-8	2	21.44	14.59	3.61	3.32	9.71
OW 185-2	FLA 423-10	1	28.97	11.77	6.97	3.56	17.19
OW 231-3	FLA 444-7	4	30.31	11.84	3.15	1.98	13.98
OW 235-1	FLA 508-1	1	35.46	12.07	5.84	1.91	13.94
OW 276-1	TST XXX-26	1	30.48	17.34	25.53	2.64	
OW 278-1	TST XXX-40	1	41.69	16.33	36.39	1.57	
OW 284-2	TST XXX-77	2	17.15	13.45	13.78	4.62	

¶·Bulking: **1**=Fibrous roots, **2**=Poor storage root formation, **3** = good but small sized storage root formation, **4** = Commercial sized storage roots, and **5** = Very big commercial sized roots.

The advanced back cross QTL identification and introgression scheme to be followed is briefly described. The F$_1$ families obtained from above will be evaluated twice at the seedling and clonal observation trial stage. The best 10 inter-specific hybrids, 3 for protein, dry matter content, white fly resistance, and one for low amylose, will be selected for each agro-ecology and backcrossed to their respective recurrent parent. At least 200 BC$_1$ seeds will be generated per family, or a total of 6000 seeds from 30 families. The BC$_1$ will be evaluated twice as above and marker genotyping will be for the best BC$_1$ families from each agro-ecology for each trait for QTL analyisis. BC$_1$ lines for the different agro-ecologies bearing favorable QTLs will be inter-crossed with one another. Genotypes from the BC$_1$F$_1$ will be planted and evaluated in six-plant rows as described above. Lines found to have high protein and dry matter content will be selected and introduced into the normal breeding program at CIAT. More crosses will be made from the best families identified and sexual seeds will be shared with the International Institute for Tropical Agriculture (IITA), Ibadan and NARs collaborators in Latin America and Asia.

Future plans for this activity include the genetic crosses of wild *Manihot* species accessions and inter-specific hybrids to elite parents of gene pools by agro-ecology. Also, a second year evaluation of protein, dry matter, white fly resistance or low in amylose/amylopectin ratio in wild *Manihot* species accessions and inter-specific hybrids.

Table 8.12. Best 21 genotypes for white fly resistance evaluated in inter-specific hybrids. All hybrids are with *M.chlorosticta*.

Accession	**Extent of white fly on leaves**				**Extent of Damage** ¶.		
Number	ADULTS	EGGS	NYMPH	PUPAE	UPPER	MED.	LOWER
CW 14- 2	1.5	1.5	1.5	1.5	1.0	1.0	1.0
CW 14- 3	1.0	1.0	1.0	1.5	1.0	1.0	1.0
CW 14- 4	2.0	2.0	2.0	1.0	1.0	1.0	1.0
CW 14- 6	2.0	1.5	1.5	1.0	1.0	1.0	1.0
CW 14- 7	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 8	2.0	2.0	1.0	1.0	1.0	1.0	1.0
CW 14- 9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 10	2.0	2.0	2.0	2.0	1.0	1.0	1.0
CW 14- 11	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 12	2.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 13	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 15	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 16	2.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 14- 17	2.0	1.5	2.0	2.0	1.0	1.0	1.0
CW 20- 1	2.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 20- 2	2.0	1.5	1.5	1.0	1.0	1.0	1.0
CW 21- 1	2.0	1.5	1.5	1.0	1.0	1.0	1.0
CW 21- 2	1.0	1.0	1.0	1.5	1.0	1.0	1.0
CW 21- 3	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CW 21- 4	2.0	2.0	1.5	1.5	1.0	1.0	1.0
CW 21- 5	1.0	1.0	1.0	1.0	1.0	1.0	1.0

¶. Extent of damage and white fly on leavesis in a scale of 1-5, 1 being no damage or presence and 5 being maximum damage.

Table 8.13. Genotypes of wild *Manihot* accessions and inter-specific hybrids with high protein, dry matter content, low amylose (Waxy) and good white fly resistance selected for crosses to elite parents of cassava genepools.

Trait Waxy	Accession Number	Mother genotype	Number of plants
	OW 30- 3	CTH XXX- 62	10
	OW 179- 7	FLA 423- 4	10
	OW 183- 4	FLA 423- 8	8
	OW 262- 4	PER 416- 1	10
	Protein and Dr Matter (combined)		
	OW 186- 2	FLA 426- 3	10
	OW 186- 5	FLA 426- 3	8
	OW 189- 1	FLA 427- 3	10
	OW 230- 3	FLA 441- 5	10
	OW 230- 4	FLA 441- 5	10
	OW 231- 2	FLA 444- 7	10
	OW 240- 7	PER 406- 2	10
	OW 257- 1	PER 413- 5	10
	OW 261- 1	PER 415- 4	4
	OW 262- 7	PER 416- 1	10
	OW 263- 4	PER 416- 2	8
	OW 263- 9	PER 416- 2	10
	OW 284- 1	TST XXX- 77	10
	Protein		
	OW 66- 5	FLA 430- 5	10
	OW 132- 2	TST XXX- 3	10
	OW 139- 1	TST XXX- 38	10
	OW 179- 1	FLA 423- 4	10
	OW 180- 1	FLA 423- 5	10
	OW 180- 4	FLA 423- 5	10
	OW 181- 2	FLA 423- 6	10
	OW 181- 3	FLA 423- 6	10
	OW 182- 8	FLA 423- 7	10
	OW 230- 6	FLA 441- 5	10
	OW 231- 3	FLA 444- 7	10
	OW 231- 4	FLA 444- 7	5
	OW 235- 3	FLA 508- 1	5
	OW 236- 2	FMT XXX- 4	6
	OW 248- 3	PER 411- 5	10
	OW 280- 1	TST XXX- 51	10
	OW 284- 2	TST XXX- 77	10
	Dry Mattter content		
	OW 95- 1	PER 412- 4	10
	OW 146- 1	TST XXX- 12	10
	OW 213- 4	FLA 437- 1	10
	OW 95- 1	PER 412- 4	10
	OW 146- 1	TST XXX- 12	10
	OW 213- 4	FLA 437- 1	10
	OW 213- 5	FLA 437- 1	10
	OW 234- 2	FLA 496- 1	10
	OW 240- 6	PER 406- 2	10
	OW 240- 8	PER 406- 2	10
	OW 248- 7	PER 411- 5	10

Table 8. 3 (cont.)

OW 252- 2	PER 412- 4	10
OW 262- 3	PER 416- 1	10
OW 262- 5	PER 416- 1	9
OW 269- 4	PER 417- 6	10
	White fly resistance	
OW 36- 2		10
OW 61- 4		10
OW 95- 2	PER 412- 4	10
OW 96- 2	PER 412- 8	10
OW 100- 2		4
OW 103- 8		10
OW 105- 6		5
OW 105- 7		10
OW 238- 1		10

Table 8.14. Elite parents of cassava gene pools for advanced backcross QTL mapping scheme

Elite Parents by their prospective agro-ecologies
CLONES FOR THE LOWLAND HUMID AGRO-ECOLOGY:
MTAI -8
SM 1411-5
CM 3306-4
SM 1433-4
CLONES FOR THE ACID SAVANNAH AGRO-ECOLOGY
CM 523-7
CM 6740-7
CM 4574-7
SM 1821-7
CLONES FOR THE MID ALTITUTDE AGRO-ECOLOGY
MBRA383
CM 7951-5
SM 909-25
SM 1219-9

Activity 8.10. Progress towards a PCR-marker based map of cassava and its relatives in cassava breeding.

Rationale

Gene mapping projects in cassava at CIAT have proliferated in the past 2 years from a single mapping population to about 5 at the moment. Genotyping these mapping population in a realistic manner, with respect to time and costs, require molecular markers other than RFLPs, currently the most predominant marker on the cassava map. The need for a PCR-based map of cassava is all the more urgent considering that an advanced back cross QTL (ABC-QTL) mapping project has been initiated to introgress higher protein and dry matter content from wild *Manihot* species into cassava. The success of any ABC-QTL relies heavily on a reliable frame-work map amongst other criteria.

A concerted effort to develop SSR markers was initiated 3 years ago and has yielded more than 500 markers at the moment. Mba et. al described the development of 186 SSR markers from genomic libraries and another 132

SSR markers were obtained from a cassava root and leaf cDNA (Mba et al., 2000 unpublished data). A third set of 158 SSR markers was also generated from the previous genomic library by another round of screening (CIAT 2000, Fregene et al., 2001 unpublished data). We describe here the genetic mapping of 58 SSR markers from the third set of 158 markers polymporphic in the parents of the F_1 mapping progeny. In addition we report on the progress in converting mapped RFLP markers to sequence tagged sites.

Materials and methods

The development of the enriched SSR library, screening, sequencing of SSR containing clones, and primer design for the third set of SSR markers have been described elsewhere (CIAT 2000). The 158 primers were screened in the parents, TMS 30572, and CM2177-2, of the mapping progeny using standard methods for cassava (Mba et al., 2000). Polymorphic markers were then analyzed in the entire progeny of 147 individuals. The source of plant tissue for DNA isolation of the mapping progeny was exclusively from a set of *in vitro* cultures obtained from the CIAT genetic resources unit or a copy kept in the green house. Linkage analysis of the SSR markers will be with the MAPMAKER computer software as described by Fregene et al., (1997).

Due to cost considerations, particularly the cost of sequencing kits at CIAT, the sequencing of RFLP clones for the development of STSs clones was contracted out to the Washington University Genome Sequencing Center. Bacteria clones of RFLP probes were cultured overnight in LB media in special culture plates (COSTAR Inc, California USA) with the appropriate antibiotic. Culture plates were sealed and shipped on dry ice to the Washington University Genome Sequencing for sequencing

Results

A total of 58 SSR markers were found to be polymorphic between the parents of the mapping progeny. At the moment 33 SSR markerss have been scored in the F_1 mapping progeny of 147 individuals. The gel images of the SSR analysis was captured and stored as JPG files for onward transfer to the cassava genome data base in the ACDB format. The raw SSR data was entered in Microsoft Excel text files in preparation for chi square analysis of segregation ratios and eventual linkage analysis using MAPMAKER.

Results from sequencing of the RFLP clones, contracted out to the Washington University Genome Sequencing Center, is being expected. Once received primers will be designed and synthesized from close to the 3' and 5' ends. Genetic mapping of the STSs will be by straight-forward PCR amplification, cleaved amplified product polymorphisms (CAPs) and single sequence conformation polymorphism (SSCP).

Future plans for this activity include a linkage analysis of single dose SSR markers scored in the mapping population; a complete sequencing of mapped RFLP markers; and the implementation of a single sequence conformation polymorphism (SSCP) protocol for the mapping of sequence tagged sites (RFLP markers) and cDNAs in cassava.

Activity 8.11. Down regulation of GBSSI gene in cassava for the production of waxy 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 modest 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.

Materials and methods

Primers were designed from published sequences of a full length cDNAs of the GBSSI gene (Salehuzzaman et al., 1993). Primers were used to amplify the homologous region of the GBSSI gene in cassava, cultivar MNg2, as described by Munyikwa et al., 1998. The genomic fragment of GBSSI was then used to screen a cassava, cultivar MNg2, root and leaf cDNA library, cloned in the GIBCO BRL vector pCMV.SPORT as described by Munyikwa et al., 1998. More than 87,000 clones from this library have been picked and arrrayed on high density filters (Mba 2000, unpublished data). The 3' and 5' end of the cDNA clone will be sequenced and restriction site from the sequence data identified. The GBSSI gene will be liberated using the appropriate enzymes and subcloned, in the antisense orientation, into the pCAMBIA13 plasmid containing the reporter genes coding for GUS and NPT both under the control of the CaMV 35S promoter and terminated by the CaMV poladenylated region.

Transformation of FECs from the model cultivar TMS60444 will be by *Agrobacterium* transformation. Regeneration and hardening of transformed cultures will be according to standard protocols established at CIAT.

Results

The project began in earnest September this year with the appointment of a undergraduate project student, Gina Jazbleidi and the arrival of GBSSI primers. The corresponding fragment of the GBSSI gene has been amplified from total genomic DNA of MNg2 and work is ongoing to screen the MNg2 library with the PCR amplification product.

Future plans for this activity include: **1)** Sub clone the GBSSI cDNAs identified into pCAMBIA13 and check expression of the gene; and **2)** *Agrobacterium* transformation of TMS60444 with the anti-sense construct of GBSSI cloned in pCAMBIA13.

Activity 8. 12. QTL mapping in an F_1 population from non-inbred parents in cassava (Manihot esculenta **Crantz***) I: yield, yield related and root quality traits.*

Rationale

Most characteristics of agronomic importance such as yield and related characters as well as quality traits are inherited quantitatively. The number of genes and their interactive effects controlling expression of quantitative traits are poorly understood. The observed phenotypes of these traits are also influenced by the environment. Mapping genes controlling quantitative characters may elucidate the genetics of these traits and help design rationale breeding schemes. Studies on mapping quantitative trait loci (QTL) have been conducted in a number of crop species and have helped to dissect the inheritance of complex traits (Paterson et al. 1988; Stuber et al. 1992).

Cassava breeding strategies can be made more efficient if the inheritance of complex yield and yield related traits are known, particularly in the choice of parents for this long growth cycle crop. An F_1 population intra-specific cross from non-inbred parents that has been analyzed with more than 400 RFLP, SSR, and RAPD markers was evaluated in replicated multi-location trials for yield, yield related, and quality traits over a period of two years in two environments. We describe here the final results of QTL analysis of these traits and discuss insights gained from the study.

Materials and methods

Twelve agronomically important traits were evaluated in the F_1 mapping population (CM7857) of 144 progeny at the CIAT research station in Palmira and Quilichao in 1998 and 1999. Traits evaluated include fresh root yield (FRY), dry root yield (DRY) and starch yield (STY). Yield related characters evaluated were dry matter percentage (DMC), fresh shoot weight (FSW), harvest index (HI) number of storage roots (NSR), starch content (STC), and leaf area index (LAI). Quality traits such as culinary quality (CQ), amylose content (AML) and post harvest physiological deterioration (PHD) were also scored. The experimental design in both locations was a partially balanced triple lattice design, with three replicates of twelve blocks each, and twelve plots per block (12 by12), with 144 individuals of the F_1 population. The plot size of 20 m^2, comprising of twenty plants, was arranged in five rows of four plants each, resulting in fourteen border plants and six central plants. The trials were planted on ridges in 1 x 1 m arrangement for a population density of 10,000 plants ha-^{1}. Field experiments were not fertilized, but were kept free of weeds and insects as much as possible. Phenotypic evaluation of the 11 traits is as described in Okogbenin and Fregene (2001). These traits were measured on six central plants in a plot and values were taken per plot, with means calculated over replications. All evaluations were at eleven months after planting (11 MAP), in both locations.

Nine genotypes in the F_1 mapping population provided insufficient amount of good quality stem cuttings due to the poor plant vigor of the mother plants from which planting stakes were made and resulted in missing data. Our data were therefore analyzed as RCB experiment (Cochran, 1957). Combined analysis of variance across trials in the two environments and years was calculated on phenotypic data of the F_1 progeny. The analysis was done considering all variables as random. Variation in the traits measured in two years, were partitioned into sources attributable to genotype, location, year, replications, interactions and error by analysis of variance (ANOVA), according to the model:

$$\boxed{Y_{ijkl} = \mu + \gamma_i + \in_j + \gamma\in_{ij} + r(\gamma\in)_{ijk} + g_l + \gamma g_{il} + \in g_{jl} + \gamma g\in_{ijl} + \varepsilon_{ijkl}}$$

Where μ is the population mean; γ_i is the effect of the ith year; $\in_j$ is the effect of the jth environment; $\gamma\in_{ij}$ is the effect associated with the interaction of the ith year and jth environment; $r(\gamma\in)_{ijk}$ is the effect of the kth replication within the interaction of the ith year and jth environment; g_l is the effect of the lth genotype; γg_{il} is the effect of the interaction of the ith year and lth genotype; $\in g_{jl}$ is the effect associated with the interaction of the jth environment and lth genotype; $\gamma g\in_{ijl}$ is the effect of the interaction between the ith year, lth genotype and jth environment; and ε_{ijkl} is the error associated with measuring the phenotype, Y, of the genotype lth genotype grown in the kth replication of the jth location in the ith year; i = 1, 2; j = 1, 2; k = 1, 2, 3; l = 1,2 3, ……144. Tests of significance of F-ratios were obtained using SAS procedures GLM (SAS Institute, 1996). Type III sums of squares were used in these analyses because our data was unbalanced. Traits evaluated in one year (CQ, NR, STY, STC LAI and AML) were analyzed in a combined analysis using a similar model above excluding year as a variable.

Genetic variances were calculated by solving the expected mean squares from ANOVA, and then used in estimating heritability according to the formula of Fehr (1987, p. 257). Pearson correlation coefficients were calculated for each trait/environment combination.

QTL analyses were performed on untransformed data. Normalizing data through transformation may misrepresent differences between individuals by pulling skewed tails toward the center of the distribution. For QTL analysis, we used two framework linkage maps that were derived from the segregation of gametes in the female and male parents of 150 individuals. The female derived map (1208.3 cM) is based on the segregation of

143 molecular markers in the gametes of the female parent, while the male derived map spanning 1475 cM in length is based on the segregation of 135 markers in the male gametes (Fregene et al., 1997). Association between markers and trait was 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) running on a G3 Power Mcintosh. The amount of phenotypic variance explained by each marker was considered significant if the probability of observing an R^2 value was less than 0.005 (Lander and Bostein, 1989). This stringent threshold was adopted in order to reduce Type 1 errors (Dudley, 1993). We also re-analyzed our data using the PGRI computer package based on the t-test conditioning analysis (Liu, 1995) to identify more potential loci associated with evaluated traits. Where three or more linked makers were found significantly associated to a trait, such markers were subjected to further analysis in a multiple regression model according to the equation:

$$\boxed{Y_{ij} = \mu + A_i + B_i + C_i + \ldots + Z_i + \varepsilon_{ij}}$$

Where Y_{ij} is the trait value of phenotype j with marker score i, μ is the overall mean for the trait, $A_i + B_i + C_i + \ldots$ and Z_i represent the linked markers associated with the trait loci and ε_{ij} is the random error. Using multi-locus model can reduce co-linearity among markers.

Results

Mean phenotypic values, standard errors and ranges for the 11 traits are shown in Table 8.15. Analysis of variance revealed significant differences between the genotypes for all traits with the exception of PHD (Table 8.16). Significant year by environment interaction was observed for five (FRY, DRY, HI, DMC and FSY) of the six traits evaluated in two years (Table 8.16). All six traits (STY, ST%, NR, LAI, AML and CQ) which were evaluated only in one season, showed significant genotype by environment interaction. Broad sense heritability estimates were 87% for HI, 71% for FSW, 70% for AML, 62% for DMC, 60% for DRY, 50% for FRY, 36% for NR, 44% for STY, 30% for ST% and 7% for PHD. A low heritability estimate for PHD suggests high non-genetic influence in the expression of PHD in the population, which is in agreement with our observation of non-significant variation between the genotypes for PHD. Heritability was not estimated for culinary quality because it was qualitatively scored.

Table 8.15. Performances of the twelve traits in different trials.

Trait[a]	Trial[b]	Mean	SD	Range	Kurtosis	Skewness	W -statistic
DRY	Q-98	925.55	267.15	1480.46	0.35	0.12	0.99 ns
	P-98	1179.73	343.50	1922.31	0.41	-0.12	0.99 ns
	Q-99	465.45	178.40	917.39	-0.12	0.13	0.98 ns
	P-99	388.92	157.99	700.69	-0.26	0.46	0.96*
DM%	Q-98	32.99	3.50	16.75	-0.34	0.00	0.98 ns
	P-98	38.16	3.40	19.58	1.06	-0.64	0.97*
	Q-99	31.15	3.39	19.41	1.63	-0.94	0.95*
	P-99	32.77	2.30	10.71	0.08	-0.50	0.96*
FRY	Q-98	3089.42	741.63	4962.5	1.08	-0.20	0.99 ns
	P-98	2778.50	874	5043.33	0.19	0.00	0.99 ns
	Q-99	1462.93	571.79	2811.11	0.05	-0.29	0.98 ns
	P-99	1088.36	470.06	2100	-0.17	0.44	0.96
NSR	Q-99	9.06	3.22	16.56	0.21	-0.10	0.98 ns
	P-99	5.75	1.84	10.27	0.60	0.32	0.98 ns
FSW	Q-98	1165.13	425.13	2181.94	-0.18	0.48	0.97*
	P-98	2757.15	825.33	4454.17	1.01	0.58	0.97*
	Q-99	903	391.32	2067.78	0.16	0.42	0.98 ns
	P-99	904.8	526.14	2456.94	0.84	1.05	0.91*
HI	Q-98	0.71	0.06	0.31	0.51	-0.70	0.96*
	P-98	0.52	0.08	0.43	0.16	-0.22	0.98 ns
	Q-99	0.60	0.10	0.60	2.15	-1.10	0.94*
	P-99	0.53	0.11	0.56	0.11	-0.55	0.96*
LAI	P-99	1.23	0.49	2.39	-0.08	0.34	0.98 ns
ST%	Q-98	82.92	1.79	9.67	-0.03	-0.02	0.98 ns
	P-98	82.87	2.29	11.33	-0.29	-0.15	0.98 ns
STY	Q-98	768.86	226.20	1222.64	0.27	0.14	0.98 ns
	P-98	977.17	283.38	1624.29	0.39	-0.15	0.99 ns
AML	Q-98	14.98	0.98	4.61	-0.26	-0.04	0.98 ns
	P-98	13.08	1.24	8.95	3.30	0.83	0.97*
CQ	Q-98	3.91	0.66	3.11	-0.49	-0.40	0.95*
	P-98	1.94	0.76	4	1.80	1.29	0.89*
PHD	Q-98	9.26	9.38	48.1	2.60	1.60	0.81*
	P-98	37.50	17.34	97	0.37	0.47	0.98 ns
	Q-99	9.51	9.90	47.18	4.43	2.12	0.74*
	P-99	10.23	13.69	84.45	3.72	1.56	0.89*

[a] DRY,dry root yield; DMC, dry matter content, FRY, fresh root yield, NSR, number of storage roots; FSW, fresh shoot weight; HI, harvest index; LAI, leaf area index (5 MAP), STC, starch percentage content; STY, starch yield; AML, amylose content; CQ, culinary quality; PHD, post harvest deterioration.

[b] Q = Quilichao, P = Palmira, 98 = 1998.

ns Distribution is not significantly different from normal.

* Distribution is significantly different from normal.

A positive and significantly high correlation was found between dry root yield and fresh root yield (r = 0.91 to r = 0.97). Fresh root yield was positively correlated with fresh shoot yield (r = 0.51 to r = 0.67). This positive correlation is in agreement with findings that top growth is critical for yield production (El-Sharkawy and Cock,). Negative correlation was found between fresh shoot yield and harvest index (r = -0.45 to –0.58) indicating that, the shoot system is a major sink which could strongly compete with storage roots for available carbohydrate in the plant. Higher number of storage roots resulted in higher dry root yield (r = 0.76 to r = 0.84) in agreement with previous findings that root yield is associated with number of roots (Pelliet and El-Sharkawy, 1994). Number of roots was also positively and significantly correlated with dry matter percentage (r = 0.50 to r = 0.53), dry root yield (r = 0.74 to r = 0.79) and fresh shoot weight (r = 0.41 to r = 0.49). Starch yield was highly correlated as expected with dry root yield (r = 0.99). Starch is the major component of yield and constitutes about 70% to 85% (on dry weight basis) of root yield (IITA, 1994; Rickard et al., 1991). Starch yield was negatively and significantly correlated with culinary quality. High culinary quality (given as low scores in the evaluation scale) was associated with increase in starch. Thus, it should be possible to increase starch yield and improve culinary attributes of cassava simultaneously. Culinary quality declined with increase in post harvest deterioration resulting in a negative correlation (r = -0.24 to r = -0.44). Post harvest deterioration was found to be positively correlated with dry matter percentage (r= 0.40 to r = 0.50). This suggests that while high dry matter percentage (increase in starch) is important to improvement in culinary quality, it is undesirable for post harvest deterioration. Leaf area index was significantly and positively correlated with DMP (r = 0.32), DRY (r = 0.38), FRY (r = 0.41), FSW (r = 0.33) and NSR (0.40). The growth, development and final yield of the cassava plant, is dominated and determined by, the relation of LAI to total biomass produced and its distribution (Cock and El-Sharkawy, 1988).

Table 8.16. Mean squares from the analysis of variance of six traits evaluated both in 1998 and 1999 in two locations.

Source of variation[¶]	df[†]	Fresh root yield (t/ha)	Dry matter (%)	Dry root yield (t/ha)
Yr	1	462919875(d)	7481.5(d)	155989869(d)
Loc.	1	243704324(a)	5729.7(d)	2190308(d)
Yr x Loc	1	259387114(d)	660.5(d)	12599434(d)
Rep x (Yr x Loc)	8	6169239(d)	49.5(d)	662439(d)
Gen.	143	2169912(d)	63.6(d)	321104(d)
Yr. x Gen[§]	142	432370	18.0(b)	102450
Loc x Gen[§]	143	622342	18.1(b)	128509
Yr x Loc x Gen	[c]	520630(d)	11.7	107287(d)
R^2		0.83	0.73	0.82

Source of variation[a]	df[†]	Fresh shoot yield (t/ha)	Harvest index (0 to 1)	PPD (%)
Yr	1	1150135473(d)	0.965(d)	94971(a)
Loc.	1	1816531(d)	6.393(d)	236704(c)
Yr x Loc	1	52433740(d)	1.118(d)	79882(a)
Rep x (Yr x Loc)	8	6849731(d)	0.072(d)	18940
Gen.	143	2804802(d)	0.060(d)	14818
Yr. x Gen[§]	142	769424	0.011(d)	14481
Loc x Gen[§]	143	800730	0.007	14575
Yr x Loc x Gen	[c]	803714(d)	0.005(c)	15335
R^2		0.85	0.83	0.35

[¶] Abbreviations under sources of variation are: Yr (year), Loc. (location), Rep. (replication), and Gen (genotype).

[§] Yr x Gen. and Loc. x Gen were tested using Yr x Locx Gen as the error term under random model used for ANOVA.

[†] The degrees of freedom *df* was 136 for fresh root fresh shoot yield and harvest index, 129 for dry matter yield and dry matter content and 135 for post-harvest physiological deterioration.

(a), (b), (c), (d) F-test significant at the 0.05, 0.01, 0.001and 0.0001 probability levels.

Table 8.17. Summary of single marker analysis , percent phenotypic variance attributable to a marker, linkage group membership of markers in an F_1 segregating population from the cassava CM 7857cross (TMS 30572 x CM 2177-2).

Trait[a]	QTL[b]	Map	Linkage group	Marker[c]	Trial	% PVE[d]	Effects	Probability
DRY	dryD.1	Female	D	GY 42	Q-98	15	-199.90	0.0005
	dryD.2	Female	D	GY 181	Q-98	12	-179.34	0.0018
	dryG.1	Male	G	GY 6	Q-99	11	-121.60	0.0040
	dryJ.1	Male	J	GY 34-1	Q-99	17	-152.62	0.0030
	dryUM.1	Female	UM	Ai18b	Q-98	10	-85.76	0.0044
	dryUM.2	Female	UM	ri14b	P-98	15	-263.85	0.0025
DMC	dm%A.1	Male	A	GY28	Q-98; P-99	16	-0.87	0.0017
	dm%D.1	Female	D	GY 50	P-98	10	-2.19	0.0030
	dm%D.2	Female	D	GY 42	Q-99	12	-2.22	0.0.003
	dm%J.1	Male	J	rM8b	Q-99	12	-1.31	0.0020
	dm%J.2	Male	J	GY34-1	Q-99	11	-1.20	0.0021
	dm%L.1	Female	L	rAF14a	Q-98	11	-2.34	0.0047
	dm%M.1	Female	M	rGY 192	P-98; Q-99	9	2.16	0.0004
	dm%O.1	Male	O	GY29	Q-98	11	-2.35	0.0034
	dm%UM.1	Female	UM	ONI.C2	P-98	6	-1.81	0.0024
FRY	fryD.1	Female	D	GY42	Q-98	10	-327.67	0.0051
	fryE.1	Male	E	r14a	P-98	10	267.23	0.0044
	fryG.1	Male	G	GY6	Q-99	11	-327.67	0.0050
	fryH.1	Male	H	GY77	Q-98	15	327.67	0.0004
	fryJ.1	Male	J	GY7	Q-99	12	-327.67	0.0020
	fryM.2	Female	M	rGY215	Q-99	6	-277.74	0.0040
	fryN.1	Male	N	GY109-1	Q-99	10	-327.67	0.0050
	fryUM.1	Female	UM	r14b	P-98	18	-327.67	0.0008
NSR	nsrC.1	Female	C	GY23	Q-99	10	2.06	0.0050
	nsrG.1	Male	G	rGY62	P-99	10	-0.55	0.0046
	nsrJ.1	Male	J	GY7	P-99; Q99	14	-1.38	0.0010
	nsrJ.2	Male	J	GY34-1	Q-99	19	-2.88	0.0001
FSW	fswC.1	Male	C	rGY89-1	Q-99	13	-279.27	0.0010
	fswC.2	Male	C	GY23-1	Q-99	12	-274.94	0.0040
	fswD.1	Female	D	rGY180	P-98	11	327.67	0.0001
	fswF.1	Female	F	GY203	Q-98	8	238.46	0.0008
	fswG.1	Male	G	rCDY16-1	P-98, Q-98	14	-327.67	0.0009
	fswG.2	Male	G	GY41	P-98	18	327.67	0.0001
	fswH.1	Male	H	GY77	Q-98	12	280.58	0.0019
	fswI.1	Female	I	rD5a	Q-98	11	260.82	0.0040
	fswJ.1	Female	J	rCDY74	P-99	12	-327.67	0.0040
	fswN.1	Male	N	GY20	Q-99	14	283.94	0.0010
	fswP.1	Female	P	rGY220	Q-99	11	247.05	0.0042
HI	hiC.1	Male	C	GY81-1	P-98	13	0.05	0.0026
	hiD.1	Female	D	rGOT-2	P-98	11	-0.04	0.0029
	hiF.1	Female	F	GY 194	P-99	11	-0.07	0.0001
	hiUM.1	Female	UM	GY 212	Q-99	7	-0.05	0.0020
	hiUM.2	Female	UM	GY 142	Q-99	7	-0.06	0.0028
	hiUM.3	Female	UM	ri14b	P-98	19	-0.07	0.0005

Table 8.17 (cont.)

Trait[a]	QTL[b]	Map	Linkage group	Marker[c]	Trial	% PVE[d]	Effects	Probability
LAI	laiA.1	Female	A	ACP-1	P-99	11	0.44	0.0020
	laiC.1	Female	C	rO11a	P-99	20	-0.33	0.0001
	lai.C2	Male	C	rGY18	P-99	11	-0.19	0.0043
	laiG.1	Female	G	AGPaseB	P-99	13	0.15	0.0028
	laiG.2	Female	G	AM18	P-99	18	-0.26	0.0001
STC	st%A.1	Male	A	rGY75-1	Q-98	12	-1.22	0.0039
	st%C.1	Male	C	rK9a	P-98	12	1.53	0.0022
	st%F.1	Male	F	GY80	Q-98	11	-1.17	0.0032
	st%O.1	Male	O	rGY91	Q-98	15	-1.44	0.0004
STY	styD.1	Female	D	GY42	Q-98	15	-169.09	0.0006
	styD.2	Female	D	GY181	Q-98	12	-152.83	0.0017
	styUM.1	Female	UM	ri14b	P-98	15	-218.45	0.0022
	styUM.2	Female	UM	Ai18b	Q-98	10	-144.19	0.0048
AML	amlD.1	Female	D	GY50	P-99	7	-0.66	0.0014
	amlD.2	Female	D	GY179	P-99	7	-0.65	0.0016
	amlI.1	Female	I	rD5a	Q-99	11	-0.69	0.0044
	amlK.1	Male	K	GY82	Q-99	12	0.78	0.0025
	amlN.1	Female	N	rGY145	Q-99	7	0.53	0.0015
CQ	cqA.1	Male	A	GY28	Q-98	14	0.49	0.0033
	cqD.1	Female	D	GY50	Q-98	8	0.35	0.0010
	cqE.1	Female	E	rS2	Q-98	12	-0.45	0.0017
	cqE.2	Female	E	rGY118	P-98	14	0.46	0.0010
	cqF.1	Female	F	GY196	P-98	6	-0.36	0.0044
	cqH.1	Male	H	rQ11	Q-98	16	0.55	0.0008
	cqP.1	Male	P	r13b	P-98	12	-0.52	0.0016
	cqUM.1	Female	UM	rONI.C2	Q-98	6	0.32	0.0031
PHD	phdD.1	Female	D	O19	P-99	15	-10.80	0.0006
	phdE.1	Female	E	rS2	P-99	11	9.18	0.0040
	phdE.2	Female	E	GY217	P-99	10	-9.11	0.0050
	phdF.1	Male	F	rV20a	Q-99	11	7.22	0.0050
	phdG.1	Female	G	rK16d	Q-99	10	-8.42	0.0007
	phdL.1	Female	L	CDY131	P-98	12	13.84	0.0025
	phdL.2	Male	L	rCDY131-1	P-99	12	9.55	0.0040
	phdO.1	Female	O	GY138	Q-98	8	5.23	0.0024
	phdUM.1	Male	UM	GY120	P-98	11	-11.36	0.0028
	phdUM.2	Female	UM	Ai18b	P-99	13	-5.25	0.0014
	phdUM.3	Female	UM	GY202	P-98	6	8.05	0.0049

[a] DMC = dry matter content, DRY = dry root yield, FRY = fresh root yield, FSY = fresh shoot yield, NR = number of storage roots per plant, HI = harvest index, STC = starch content in the roots, STY = starch yield, CQ = culinary quality, AML = amylose content (%), PHD = post harvest deterioration; LAI = leag area index.

[b] Individual QTLs are designated with Q indicating QTL, abbreviation of the trait name and the linkage group. When more than one QTL affecting a trait was identified on the same linkage group, they are distinguished by different number.

[c] Marker significantly associated with trait variation

[d] Percentage phenotypic variance explained by the individual marker locus-trait associations based on regression analysis

[e] Significance levels determined for F-tests based on each pairwise comparison of a quantitative trait and marker locus ($p< 0.005$)

Because all the traits, with the exception of PHD, exhibited highly significant genotype by environment interaction or genotype by location by year interaction, QTL analysis was done separately for each trial. Putative QTLs for each trait are listed in Table 8.17. A total of eighty-one QTLs were found for the twelve traits analyzed. QTLs detected in this study were found distributed in all but two linkage groups (B and Q). Between four and eleven QTLs were detected per trait, with each explaining between 6 and 19 % of the observed phenotypic variances. The number of QTLs detected should be taken as the minimum. The stringent threshold chosen ($p < 0.005$) while reducing the chance of Type-I errors (false positives), would have also led to the possibility of Type-II errors (not detecting valid QTLs) (Dudley, 1993). Though QTL-marker linkages have been found to remain reproducible across environment-specific QTLs are often expressed under particular environmental conditions (Patterson et al, 1988). Our results showed that majority of the QTLs associated with traits were found in one trial. This could be due to the very significant genotype x environment interaction observed in our experiment across sites and years for most of the traits evaluated.

Table 8.18. Marker loci associated with pleiotropic/linkage effects.

Marker	**Linkage group**	**QTL[a]**	**No. of traits**
GY28	A	dm%A.1, cqA.1	2
GY42	D	dryD.1, fryD.1, styD.1	3
GY181	D	DryD.2, styD.2	2
GY50	D	dm%D.1, amlD.1, cqD.1	3
rS2	E	cqE.1, phdE.1	2
GY6	G	dryG.1, fryG.6	2
GY77	H	fryH.1, fswH.1	2
rD5a	I	fswI.1,amlI.1	2
GY7	J	fryJ.1, nsrJ.1	2
GY34-1	J	dryJ.1, dm%J.2, nsrJ.2	3
r14b	UM	dryUM.2, fryUM.1, styUM.2	3
Ai18b	UM	dryUM.2, styUM.1, phdUM.2	3
ONI.C2	UM	dm%UM.1, cqUm.1,	2

[a] Individual QTLs are designated with Q indicating QTL, abbreviation of the trait name and the linkage group. When more than one QTL affecting a trait was identified on the same linkage group, different numbers are used to distinguish them. DMC, dry matter content; DRY, dry root yield, FSW, fresh shoot weight; NSR, number of storage roots per plant; HI, harvest index; STC starch content in the roots; STY, starch yield; AML, amylose content; PHD, post-harvest deterioration; CQ, culinary quality.

Thirteen QTLs (16%) were found in common for with yield and related traits that were correlated (Table 8.18). The observed congruence of some of the QTLs, in classical quantitative genetics, is assumed to be due to pleiotropy or close linkage. Zhuang et al (1997) explained that if pleotropism is the major reason for the coincidence of the QTLs, for related traits, the directions of their phenotypic effects could be expected. However, if close linkage was the major reason, the direction of the effects of the QTLs may be different, although the coincidence of the locations of the QTLs can still be expected. A general coincidence of the locations and directions of the effects of the QTLs for related traits were observed in this study suggesting that pleiotropism rather than close linkage of the QTLs might be the reason for the correlation of related traits. The distinction between linkage and pleiotropy is important for breeding purposes as well as for scientific reasons.

QTLs analysis of important agronomic traits provides an insight into the inheritance of these traits that can guide breeding decisions. However the usefulness of this information depends on how good the QTLS study was and what percentage and magnitude of key QTLs were successfully detected The mating design and method of analysis becomes important here. Single marker analysis used in our study could inflate the phenotypic effects of the QTLS if the genes are very closely linked to the markers, or could underestimate the effects if these QTLs lie far away from the marker locus associated them. The approach does not define the likely position of a QTL, and cannot distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect. Further activities have been initiated to improve QTL mapping studies of these traits in cassava. The activities include the use of F_2 populations and adding more highly polymorphic markers to the genetic map to increase saturation. Markers such a simple sequence repeats (SSR) are particularly useful because of their high levels of polymorphisms.

Further plans for this activity include testing the effectiveness of other kinds of crosses, including F2 populations and wide crosses, involving wild relatives, in detecting QTLs.

*Activity 8.13. QTL mapping in an F_1 population from non-inbred parents in cassava (***Manihot esculenta** *Crantz) II: morphological traits.*

Rationale

Plant architecture is an important agronomic feature in cassava production particularly under particular farming systems and cultural practices such as in intercropping. This is most evident in sub-Saharan Africa where monoculture is rarely practiced due to the security and efficient use of space and resources crop diversification provides small-scale holder farmers. Plant architecture has implications for yield and weed control through canopy information, incident light absorption by leaves, and branching habit (Nweke et al., 1994). A strong association has also been found between length of stem with leaves and dry matter yield in certain genotypes (see Table 2.12, in *Section 2.4.1*).

The use of the linkage map of cassava to study the inheritance of morphological traits of cassava, such as plant height, branching height and levels, leaf morphology (shape and size), and length of stem with attached leaves is described here. A number of these traits have been observed to possess high broad sense heritability and some are thought to be controlled by single genes. Finding molecular markers tightly associated with genes controlling these traits will permit marker-assisted negative selection at the seedling stage to "weed" out undesirable morphologies and make the selection at consequent stages more cost-effective and efficient. Negative selection at the seedling stage to eliminate inferior genotypes, combined with indirect selection for yield, using harvest index, is most the efficient breeding scheme for yield in cassava (Kawano et al., 1998).

Materials and methods

The 144 individuals of the mapping progeny were evaluated for morphological traits in a partially balanced triple lattice design in CIAT headquarters at Palmira and at another CIAT station in Satander de Quilichao. The study was conducted over a period of two years, 1998 and 1999. The F_1 population was planted on ridges, in plot sizes of 20 m^2 (of 5 rows x 4 columns), by this arrangement, each plot had fourteen border plants and six central plants. The six central plants in each plot were evaluated for plant height (PH), length of stem with attached leaves (LSL), and Leaf shape (LS, a ratio of leaf length LL to leaf width LW). Other traits include first branching height (BH), branching levels (BL), leaf area index (LAI) and the derived trait, branching index (BI) expressed as the ratio of BH to PH.

Separate analyses of variance for progeny data were conducted, for all traits evaluated. The final data were analyzed as in RCB experiment due to missing data in a random effects model. Combined analysis of variance (SAS ANOVA procedure) was as described earlier for yield and yield related traits (CIAT 2001, this report). H^2, on entry mean basis, was estimated for each trait using the variance components of the expected mean squares

(Fehr, 1987, p. 257) from our analysis of variance of the F_1 population. Spearman's rank correlation coefficients were calculated for each trait/location combination based on progeny data. Phenotypic data were subjected to QTL analysis using untransformed data based on markers from the male- and female- derived maps of the F_1 mapping population as described earlier. A significant association between the traits and the marker ($p < 0.005$) was considered an evidence of a QTL in the region of the marker.

Results

With the exception of leaf shape, where a bimodal distribution pattern was observed, all other traits analyzed exhibited continuous distribution in the F_1 population, typical of quantitative traits. The correlation between the two environments for the traits ranged from 0.38 in 1998 and 0.57 in 1999 for length of stem with leaves to 0.97 for leaf shape in 1998 (Table 8.19). Analysis of variance detected significant differences between the genotypes for each trait (Table 8.20). Analysis of variance showed that, of the four traits evaluated in two years (PH, BH, BL and LSL), only LSL was not significant for genotype by location by year interaction (Table 8.21). It would suggest that PH, BH, and BL are much more variable traits compared with LSL across locations and or years.Broad sense heritabilty estimates based on plot entry means were 91% for plant height, 83% for branching levels, 94% for branching height, 80% for leaf length, 90% for leaf width and 27% for length of stems with leaves.

Table 8.19. Phenotypic correlation coefficients of morphological traits across environments.

Quantitative trait	**Correlation coefficient between sites**	
	1998	1999
Leaf length (LL)	0.71***	-
Leaf width (LW)	0.90***	-
Leaf area (LA)	0.70***	-
Leaf shape (LS)	0.97***	-
Plant height (PH)	0.55***	0.76***
Branching height (BH)	0.83***	0.86***
Branching levels (BL)	0.46***	0.67***
Length of stem with leaves (LSL)	0.38***	0.57***

[1] Heritability estimate was based on data from two locations (Palmira and Quilichao) in two years except for leaf width and length evaluated in one year.

*** Indicates that the correlation coefficient (r) is significant at $P < 0.001$.

Leaf shape was measured only in 1998 and a marker locus, GY99, explained phenotypic variation of 61% for LS suggesting it to be a major gene. This result was confirmed in a BC_1 cross derived from the mapping population. (Okogbenin and Fregene, 2001). Earlier reports have described leaf shape as being controlled by a single gene with broad leaf shape recessive gene to narrow leaf shape effect at this locus (Graner 1942). With the female derived map, no significant effect was found for leaf shape at this locus, but with male derived map, this locus explained 61% of the observed phenotypic variance for leaf shape. This observation underscores the need to analyze both male- and female-derived maps QTL mapping experiments in F_1 progeny from non-inbred parents to capture the effects of dominant genes. QTLs found for the other morphological traits are summarized in Table 8.21.

Results of QTL analyses show that these morphological traits, with the exception of LS, are mainly under quantitative genetic control as expected. The small QTL effects found for most of the traits, particularly for stem with leaves (STL), a trait with a suggested simple mode of inheritance, disagrees with the high broad sense heritabilities and suggests the CM6857 mapping progeny may not be the most appropriate cross for QTL mapping of these traits or the population structure is inadequate. Another factor that could confound QTL analysis is the low level of polymorphism between genomes of the parents, major QTLs in a monomorphic region of the genome will fail to be detected. These factors will be addressed in subsequent studies.

Future plans for this activity include: a) Development of new crosses with CM6754-8, SM1068-10, and SM1257-7, three genotypes that show a simple mode of inheritance for stem with leaves (STL) in previous crosses; and b) Analysis of some of these traits in inter-specific crosses with a higher level of genome level polymorphism.

Table 8.20. Mean squares from the analysis of variance of four traits evaluated both in 1998 and 1999.

Source of variation[a]	**df**	**Plant height**	**Branching height**	**Branching levels**	**Length of stems with leaves**
Yr	1	695180.53****	264118.87****	33.45****	30488.08****
Loc.	1	119310.32****	8.40^{ns}	2.60**	280233.17****
Yr x Loc	1	893041.08****	87469.82****	51.84****	29362.45****
Rep x (Yr x Loc)	8	8376.52****	1564.48****	1.57****	3219.11****
Gen.	143	7457.05****	5121.28****	4.72****	1038.00****
Yr. x Gen[b]	142	701.09 ^{ns}	533.74^{ns}	0.50^{ns}	601.25^{ns}
Loc x Gen[b]	143	710.00 ^{ns}	201.50^{ns}	0.79**	694.01^{ns}
Yr x Loc x Gen	137[c]	716.56****	409.01****	0.50****	660.50^{ns}
R^2		0.90	0.93	0.78	0.56

[a] Abbreviations under sources of variation are: Yr (year), Loc. (location), Rep. (replication), and Gen (genotype).

[b] Yr x Gen. and Loc. x Gen were tested using Yr x Locx Gen as the error term under random model (McIntosh, 1983) used for ANOVA.

[c] The degrees of freedom *df* for plant height, branching height, branching levels was 137, but 136 for length of stems with leaves.

*, **, **** F-test significant at the 0.05, 0.01, and 0.0001 probability levels

^{ns} not significant

Table 8.21. List of putative QTLs detected from data collected in the F_1 mapping trials at Palmira and Quilichao in two years

Trait[a]	QTL[b]	Map	Linkage group	Marker	Trial				% PVE[c]	Effects[d]	P-value[e]
					Q-98	P-98	Q-99	P-99			
LW	lwB.2	Female	B	GY197	ns	*	-	-	6	0.35	0.0040
	lwL.1	Female	L	CDY131	*	*	-	-	12	0.27	0.0017
	lwH.1	Male	H	rGY99	*	*	-	-	61	-0.76	0.0000
LL	llH.1	Male	H	rGY99	*	*	-	-	15	2.00	0.0005
LA	laF.1	Female	F	GY37	*	ns	-	-	8	2.59	0.0011
	laH.1	Male	H	rGY99	*	*	-	-	33	-12.98	0.0000
	laJ.1	Female	J	CDY76	ns	*	-	-	12	-7.36	0.0023
	laL.1	Female	L	CDY131	ns	*	-	-	11	7.73	0.0037
	laN.1	Female	N	rGY148	*	ns	-	-	7	-2.59	0.0010
	laN.2	Female	N	AD4b	*	ns	-	-	10	-3.28	0.0038
	laO.1	Female	O	GY138	*	ns	-	-	8	2.65	0.0009
LS	lsD.1	Male	D	ri18b	*	*	-	-	13	0.026	0.0035
	lsH.1	Male	H	rGY99	*	*	-	-	61	-0.10	0.0000
	lsL.1	Female	L	rAF14a	*	ns	-	-	11	0.04	0.0037
	lsL.2	Female	L	CBB1	*	ns	-	-	8	0.019	0.0050
PH	phD.1	Female	D	rGY180	*	ns	ns	ns	6	15.56	0.0031
	phF.1	Female	F	GY196	ns	ns	ns	*	8	14.45	0.0008
	phF.2	Female	F	GY203	ns	*	ns	*	7	16.44	0.0010
	phG.1	Male	G	rCDY16-1	ns	ns	ns	*	14	-19.90	0.0017
	phG.2	Male	G	GY121	ns	ns	ns	*	9	-8.92	0.0030
	phI.1	Female	I	R1	ns	ns	*	ns	10	21.30	0.0050
	phJ.1	Female	J	rCDY74	ns	ns	ns	*	14	-20.24	0.0010
	phJ.2	Male	J	GY34-1	ns	ns	*	ns	11	-21.66	0.0040
	phN.1	Male	N	GY10	ns	ns	*	ns	11	11.82	0.0044
BH	bhA.1	Female	A	GY28	ns	*	*	*	7	-16.06	0.0030
	bhJ.1	Male	J	GY34-1	ns	ns	*	ns	13	-18.87	0.0031
	bhM.1	Female	M	rGY192	ns	*	ns	ns	7	16.32	0.0028
BI	biD.2	Female	D	rGY167	ns	*	*	ns	8	-0.03	0.0008
	biD.3	Male	D	rGY57	*	ns	*	ns	14	0.08	0.0017
	biJ.1	Male	J	K10	ns	*	ns	ns	10	-0.08	0.0042
	biM.1	Female	M	GY154	ns	ns	ns	*	6	0.05	0.0035
BL	BlD.1	Female	D	rGY167	ns	*	ns	ns	7	0.38	0.0026
	blD.2	Female	D	rGY180	*	ns	ns	ns	6	0.34	0.0040
	blD.3	Male	D	GY57	ns	*	*	ns	11	-0.49	0.0002
	blG.1	Male	G	GY97	*	ns	ns	ns	10	0.45	0.0043
	blO.1	Male	O	nGY138	ns	ns	*	ns	12	-0.43	0.0020
	blR.1	Male	R	AE2	*	*	ns	ns	10	-0.44	0.0030
	blS.1	Female	S	GY142	*	ns	ns	ns	12	0.46	0.0028

Table 8.21 (cont.)

Trait[a]	QTL[b]	Map	Linkage group	Marker	Trial				% PVE[c]	Effects[d]	P-value[e]
					Q-98	P-98	Q-99	P-99			
LSL	lslC.1	Male	C	GY23-1	ns	ns	ns	*	12	-8.40	0.0050
	lslF.2	Female	F	GY196	*	ns	ns	ns	8	2.64	0.0009
	lslG.1	Female	G	rGA-131	ns	ns	ns	*	11	4.26	0.0048
	lslG.2	Male	G	GY41	ns	ns	ns	*	10	7.60	0.0050
	lslI.1	Female	I	rD5a	*	ns	*	ns	13	1.96	0.0007
	lslM.1	Female	M	rGY192	ns	ns	*	*	7	1.38	0.0008
	lslUM.1	Female	UM	r14	ns	ns	ns	*	12	8.21	0.0030
	lslUM.2	Female	UM	GY212	*	ns	ns	ns	6	1.29	0.0015

[a] LL, leaf length; LW, leaf width, LA, leaf area; LS, leaf shape; PH, plant height; BH, branching height; BI, branching index; LSL, length of stem portion with leaves.

[b] Individual QTLs are designated with Q indicating QTL, abbreviation of the trait name and the linkage group. When more than one QTL affecting a trait was identified on the same linkage group, they are QTLs are distinguished by different number.

[c] Phenotypic variance explained.

[d] Phenotypic effects of the QTL, being the difference between homozygote and heterozygote genotypes at a locus.

[e] Probability of the association between a QTL and marker. When a QTL – marker association is significant at more than one trial the most significant P value is declared and corresponding PVE and phenotypice effects of QTLs are given.

Activity 8.14. Marker fidelity study of QTLs identified for early bulking in cassava

Rationale

The QTL mapping early bulking at CIAT has identified a number of major QTLs for this important trait (Okogbenin and Fregene 2001; CIAT 2000). Comportment and inheritance of tentative markers identified to be associated with QTLs for early bulking would need to examined in advanced breeding populations and different genetic backgrounds to propose a model of genetic control and estimate genetic interactions. This will lead to better exploitation of existing variability and fine mapping of earlier mapped QTLs. To validate the authenticity, magnitude and action of these QTLs a new F_2 mapping population was developed from F_1 individuals bearing positive alleles of the identified QTLs. This population is being genotyped with SSR toward the development of a framework genetic map for QTL analysis. A field trial to evaluate early bulking in the F_2 population has also been set up.

Based on asymptotic theory, the type of progeny developed in an experiment will affect the power to identify QTL using marker trait methods (Beavis, 1998). An F_2 population has increased power for QTL mapping of traits as well as elucidating genetic action and interactions of genes involved. In addition, the development of F_2 population is a step further at improving QTL mapping through use of more advanced statistical tools such as interval mapping and composite interval mapping in detecting the precise locations of QTLs, and their genetic effects actions and effects.

Materials and methods

To confirm putative QTLs for early bulking and to determine gene actions involved in the control of this trait, 3 F_2 populations were developed by selfing three profusely flowering genotypes in the F_1 population in the last quarter of 1999. The individuals selected for -self-pollination, were CM 7857-68, CM 7857-145 and CM 7857-150 resulting in 396, 725 and 519 seeds respectively. Seeds from CM 7857-145 were established from embryo axes as described earlier (CIAT 2000). Seeds from CM 7857-68 and CM7857-150 were however planted in germination trays in the screen house to reduce cost associated with *in vitro* culture. A total of 473 plantlets were obtained for CM 7857-145. Of the 396 seeds for CM 7857-68, 245 or 62% germinated, while 72% (or 372 seeds) of the total 519 seeds for CM 7857-150 germinated. Resulting seedlings were then transferred to the field for planting after two months in the screen house. Results from a preliminary evaluation of traits related to early bulking indicate that CM 7857-150 showed high wide variability for these traits and therefore only F_2 progeny from this individual were selected for further marker analysis and phenotypic evaluation.

For SSR marker analysis, total genomic DNA was isolated according to a miniprep version of the Dellaporta (1983) extraction procedure. Total DNA was extracted from fully expanded young leaves of green house-or field grown plants of a total of 268 genotypes of the F_2 population produced by selfing CM 7857-150. Leaf samples of 0.15 – 2.0g was powdered in liquid nitrogen using a mortar and pestle. The powder was transferred to a frozen 1.5ml eppendorf tubes using a frozen spatula. The powder was resuspended in 800 µl of extraction buffer (100mM Tris-HCl, 50mM EDTA and 500mM Nacl) and 50 µl of 20% SDS. The ground tissue was shaken vigorously in the buffer and then vortexed intermittently for 15min at 65^{o}C. To this solution, 250 µl of ice-cold 5M potassium acetate was added and homogenised by gently inverting 5-6 times. The mixture was incubated in ice for 20 minutes and then centrifuged at 12000rpm for 10 min. The aqueous solution was transferred to a new 1.5ml eppendorf tube and the nucleic acids was precipitated by adding one volume of ice-cold isopropanol (approximately 700µl), and then mixed by gently inverting 8-10 times before incubating at –80^{o}C for one hour, followed by centrifugation at 12000rpm for 10 min. The resulting supernatant was poured off and the pellet resuspended in 500µl of 50mM Tris-HCl/10Mm EDTA. The precipitation process is then repeated for a second time to eliminate salts in the DNA Electrophoresis and fluorometry were used in determining DNA quality and DNA concentration respectively.

The development of 186 SSR markers for cassava have been described by Mba et al., 2000. Another 132 SSR markers were obtained from a cassava root and leaf cDNA (Mba et al., 2000 unpublished data). A third set of 154 SSR markers were also generated from the previous genomic library by another round of screening (Fregene et al., 2001 unpublished data). A total of more than 500 SSR markers now exist for cassava and were the source of markers for genotyping the F_2 population. SSR Markers were first of all screened in the parents of the F_1 mapping progeny and 3 F_1 progenies - parents of the F_2 families, if this information was not already available from previous studies. PCR amplification and PAGE gel analysis were as described by Mba et al., (2000) .

A field trial of the selected F_2 populations was established at Santa Elena on 18 May 2001, in an RCB design of single row plots of 6 plants each and two replications. Of the 268 genotypes of the F2 population, only 207 genotypes had sufficient stem cuttings (12 stakes) could be planted. Germination percentage ranged from 16 – 100% amongst the genotypes. The trial will be harvested at 7 MAP (December 2001) and evaluated for early bulking by measuring dry matter yield, dry weight of foliage, harvest index and number of roots.

Results

Of the 500 SSR markers results reveal that 200 are heterozygous in CM7857-150 and will segregate in the F_2 population. One hundred markers have so far been screened in the F_2 population. Seventy three markers (73%) segregated as single dose markers and have yielded segregation data in the F_2 progeny. Eight markers (8%), which were polymorphic in the F_1 parents and K150, did not segregate in the F_2 progeny, revealing these markers as double dose markers. Results also revealed two markers, which though were heterozygous in K150, segregated into two genotypic classes only such markers are not useful in the construction of a F_2 framework map. Nine other markers, which though were polymophic in the F_1 parents and K150, selectively amplified only few individuals in the F_2 progeny. Such markers are being repeated with changes in $MgCl_2$ concentrations and annealing temperatures to enhance DNA amplification in the F_2 progeny. Only markers with goodness of fit to the expectation of 1:2: 1 will be utilized for map construction using the computer package MAPMAKER. Marker genotyping of the F_2 population continues and a target of 180 markers have been set. The phenotypic evaluation of the cross later in the year will provide the other component required for QTL analysis.

Future plans for this activity include: 1) Completion of marker genotyping of the F_2 population; and 2) QTL analysis of the F_2 population

Activity 8.15. QTL mapping of cyanogenic potential (CNP) in cassava

Rationale

The food safety of cassava is compromised by the presence of cyanogenic glucosides that break down on mechanical damage of cassava tissues and results in the release of a poison, hydrogen cyanide (HCN). Cassava varieties vary in their potentials to produce HCN, a fact that could be exploited to control its level through breeding. The cyanogenic potential (CNP) of a cassava genotype is assessed at the end of its growth cycle and it is affected by the environment. Improvement of CNP will therefore benefit from marker assisted selection via a reduction in the time for breeding and an increase in the selection efficiency. The goal of this project is to develop markers for marker assisted breeding for reduced cyanogenic glucoside content in cassava.

Materials and methods

CNP is a quantitative trait and many genes are expected to control the biosynthesis, transport and storage of cyanogenic glucosides. Quantitative Trait Loci (QTLs) mapping will be therefore be used to identify markers associated with key genes controlling CNP as has been described for casssava (Okogbenin and Fregene 2001, CIAT2001, this report). A high CNP cassava line Gomani from Malawi was crossed as pollen parent to a low CNP line Mbundumali as staminate parent and about 100 F_1lines were generated. After SSR marker analysis to confirm true hybrid nature of progeny, 3 F_1 lines were selfed to obtain about 200seeds each. Seeds were tested for viability by soaking in water. After the viability test, embyro culture was carried out for 600 seeds in the 17N culture medium (1/3 medium, supplemented with 0.01 mg l^{-1}NAA, 0.01 mg l^{-1} GA_3, 1.0 mg l^{-1} thiamine-HCL, 100 mg l^{-1} inositol, 2% sucrose, 0.7% agar (Sigma Co.) and 25 mg l^{-1} of a commercial fertilizer containing: N 10, P 52, K 10, pH 5.7-5.8. Culture of embryo axes are as described by Fregene et al., (1998). The embryo cultures were incubated in darkness for three days to promote radicle growth and then transferred to growth chambers with a 12hr photoperiod. A total of 240 progeny were hardened and established in the field.

Three F_2 populations segregating were obtained by selfing 3 F_1 progenies obtained from a cross between high and low CNP lines. About 200 seeds were obtained from each cross and embryo axes from each seed cultured in vitro. The seeds were cultured in the Med Biotech Laboratories, Kampala, and will be transferred to the green house at NARO, Namulonge after 1 month. They will the be taken to the field in a low pressure site in Uganda for the production of woody stakes.

The following year the F_2 populations will be evaluated for CNP in a replicated fashion. At the same time an SSR marker survey of the Mbundumali and Gomani grand parents, and F_1 parents, using all 500 available SSR markers will be conducted at CIAT. Polymorphic markers from the parental survey will be used to genotype an F_2 population selected based upon the highest number of heterozygous markers in the F_1 parent. Single marker and interval marker analysis will be conducted to identify markers associated with genes controlling CNP using the computer software packages Q-gene and PRGI. Molecular markers found associated with CNP will be tested in a marker-fidelity study to evaluate its usefulness for cassava breeding.

Future plans include the clonning of the F_2 family and establish a replicated CNP trial in the field and the production of a map with 120 genome-wide markers selected from the cassava genetic map available at the International Center for Tropical Agriculture (CIAT).

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