Improved Cassava for the Developing World

Annual Report 2008

Project SBA-2

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

ANNUAL REPORT 2008

Outcome Line

SBA-2

Improved Cassava for the Developing World

TABLE OF CONTENTS

CHAPTER 1

GENETIC BASE OF CASSAVA AND RELATED *MANIHOT* SPECIES EVALUATED AND MADE AVAILABLE FOR CASSAVA IMPROVEMENT: NUTRITIOANL QUALITY

The overall objective of this output is to generate genetic stocks and knowledge about genetic variability for root quality traits in cassava, with a particular emphasis of nutritional quality and special traits to make processing cassava more competitive. The main activities focus in developing and identifying cassava germplasm whose roots have higher carotene contents. Protein, Zn and Fe contents are also important targets. The scope of research does focus on nutrients concentrations, related agronomic characteristics and the effect of processing. In addition, there is a need for a better understanding of the biochemical and genetic basis of these high nutritional quality traits.

Because of the nature of the research described in this output, it is one of the many collaborative activities between projects **SB2** and **IP3,** as well as the **HarvestPlus** Challenge Program, which also involves EMBRAPA from Brazil and IITA. To maintain some coherence through this report some of the activities reported herein may also be reported by **SB2** and/or **HarvestPlus.**

1. DEVELOPING SOURCE CASSAVA GERMPLASM FOR THE HIGH-CAROTENOID TRAIT

In May 2005 a clearly defined goal of 15 µg of β-carotene per gram of fresh root was established from the nutritional point of view. CIAT cassava breeding project reacted to the establishment of these goals by initiating a rapid cycling recurrent selection scheme" in which crosses among high-carotenoids content genotypes are crossed, the botanical seed produced germinated and the resulting seedlings transplanted to the field for evaluation at the proper age (9-11 months of age). The best genotypes are then immediately incorporated into the crossing blocks and within two years progenies from these elite genotypes (which would be a new cycle of recurrent selection) can be harvested and screened. Traditional recurrent selection in cassava normally requires about 6-8 years for completion.

The main objectives of the cassava-breeding project at CIAT therefore are:

a. Obtain cassava germplasm that meets the nutritional objective of µg of β-carotene per gram of fresh root (through the rapid cycling process described above).
b. High-carotenoids genotypes are evaluated for their per se agronomic performance and join the mainstream breeding process.
c High-carotenoids genotypes are routinely crossed with elite germplasm cassava that is then evaluated for their agronomic performance.
d. High-carotenoids genotypes are also crossed with sources of resistance to ACMD to combine the two traits.

e. In vitro plants of the high-carotenoids genotypes are produced for their shipment to Africa (IITA) so they can be used in the crossing blocks with locally adapted high-carotenoids clones.
f. Contribute to the introduction of high-carotenoids, drought-tolerant germplasm developed or identified by EMBRAPA-Brazil

NEW CROSSING BLOCKS TO PRODUCE RECOMBINANT SEED (OCTOBER 2007-DECEMBER 2008).

Every year the best group of genotypes, based on nutritional quality and other desirable traits is planted to make crosses among them (or in special cases, make self-pollinations) and obtain botanical seed. In October 2007 the following genotypes were included in the crossing blocks to increase one or more nutritional traits and/or combine them with good agronomic performance, including crosses with sources of resistance to Cassava Mosaic Disease (**Table 1.1**).

Table 1.1 Parental lines to be used in crosses for their high carotenoids content potential.

High carotenoids progenitors from the germplasm collection					
CM 9816-2	GM 893-4	MBRA 253	MBRA 1321	MCOL 2199	MCOL 2489
GM 708-63	GM 893-5	MBRA 496	MCOL 2070	MCOL 2318	MCOL 2547
GM 734-57	GM 893-16	MBRA 502	MCOL 2141	MCOL 2436	MPER 297
GM 849-33	MBRA 1A	MBRA 1107	MCOL 2175	MCOL 2459	
High carotenoids progenitors from Brazil					
CB 4-4	CB 4-28	CB 5-9	CB 46-3	CB 12-10	SB 325-38
CB 4-10	CB 5-5	CB 5-14	SB 325-32	CB 19-10	SB 326-24
CB 4-25	CB 5-6	CB 7-9	SB 325-35	CB 44-15	SB 326-31
High carotenoids progenitors from selections of F1 in 2005					
GM 905-3	GM 905-56	GM 905-69	SM 3306-13	SM 3308-48	SM 3309-46
GM 905-21	GM 905-57	SM 3306-1	SM 3308-16	SM 3308-49	
GM 905-37	GM 905-60	SM 3306-4	SM 3308-24	SM 3308-63	
GM 905-43	GM 905-66	SM 3306-5	SM 3308-27	SM 3308-150	
GM 905-52	GM 905-68	SM 3306-7	SM 3308-45	SM 3308-156	
High carotenoids progenitors from selections of F1 in 2006					
AM 689-24	AM 690-38	AM 702-41	GM 1548-20	GM 1551-20	GM 1560-10
AM 689-42	AM 702-14	AM 720-43	GM 1548-33	GM 1551-36	GM 1561-8
AM 690-5	AM 702-27	GM 1546-7	GM 1550-15	GM 1556-4	GM 1561-11
AM 690-9					
High carotenoids progenitors from selections of F1 in 2007					
AM 625-5	GM 1515-17	GM 1521-10	GM 1530-4	GM XXX-6	SM 3372-5
AM 625-37	GM 1517-19	GM 1521-12	GM 1530-5	GM XXX-9	
AM 625-27	GM 1517-42	GM 1521-18	GM 1530-6	SM 3355-42	
GM 1471-24	GM 1520-12	GM 1521-3	GM 1532-5	SM 3372-29	

GM 1471-32	GM 1520-15	GM 1521-5	GM 1534-11	SM 3372-35	
GM 1514-19	GM 1520-3	GM 1521-9	GM 1554-5	SM 3372-43	

For the work CIAT is conducting to develop cassava germplasm as source of the high-carotenoids trait in addition to this trait per se, to be useful particularly in Africa it has to be combined with resistance to the Cassava Mosaic Disease and, hopefully, also to its vector the white flies. Because of the uses in human consumption include boiling roots, it is also desirable, for this particular form of consumption, to combine high carotenoids content with low cyanogenic potential (HCN). **Table 1.2** provides information of the different progenitors that have been and will be used to combine the high carotenoids trait with these additional traits.

Table 1.2. Parental lines to be used in crosses to combine high nutritional quality with tolerance to relevant pests and diseases.

Resistance to CMD					
C 4	C 19	C 39	C54	C 227	C 243
C 6	C 24	C 41	C 101		
C 18	C 33	C 43			
Low HCN					
MBRA 924	MCOL 1030	MCOL 1185	MCOL 1516	MPAN 100	
MCOL 304	MCOL 1132	MCOL 1458	MECU 141A		
Resistance to white flies					
CG 48931	MECU 72	MPER 331	MPER 459	MPER 497	MPER 545
MECU 64	MPER 321	MPER 390	MPER 461	MPER 504	MPER 564

BIOAVAILABILITY STUIDES CONDUCTED IN IOWA WITH HIGH-CAROTENE CASSAVA ROOTS.

In November 2008 samples of cassava roots from genotype GM 905-66. This genotype produces roots with average total carotenoid content around ten micrograms and beta-carotene around 7.5 micrograms.

Results of this study were not released to CIAT yet. But as an accidental outcome of this activity, an interesting characteristic of the roots from this clone was tentatively established. Two roots of this genotype were left by mistake in an office through the holidays at the end of 2008. In February 2008 the roots were cut and, surprisingly, they did not show any symptom of post-harvest physiological deterioration or PPD. Cassava roots spoil one or two days after harvest as a result of this PPD which is an induced reaction from the plant and not the result of infectious agents. PPD remains a major problem for cassava because it means that it needs to be processed or consumed 2-3 days after harvest. In

practice this means high marketing prices for cassava roots, and frequent losses that for the poor farmers mean a major setback.

Figure 1.1 illustrates the sections of the roots from GM 905-66. It had been reported that high-carotene roots tend to have delayed or reduced PPD (Sánchez et al, 2005). However these earlier reports only reported the possibility of at best few days. The roots of GM 905-66 were stored at room temperature for two months, an unprecedented finding. As it can be seen there is no symptom of PPD. The color of the root has faded away. When harvested these roots were considerably yellower. It has been known already that carotenoids oxidize through time, so this change is not surprising.

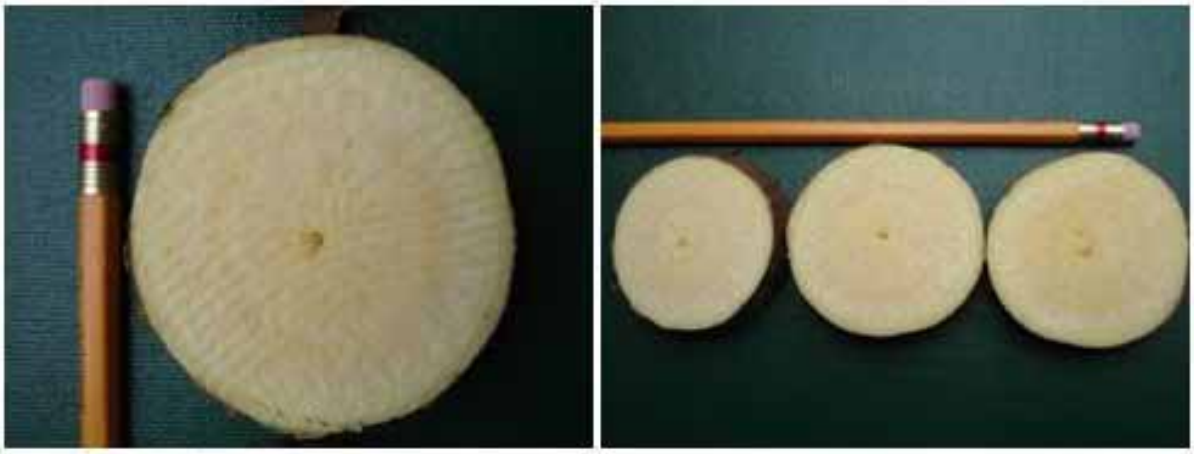


Figure 1.1. Section of roots from yellow-rooted genotype GM 905-66 two months after harvest.. Roots were left in an office and maintained at room temperature. Two months after harvest these roots did not show any symptom of post-harvest physiological deterioration.

SAMPLING VARIATION SUDY.

As stated above in May 2005 a high nutritional goal of 15 μg of β-carotene per gram of fresh root was established within the HarvestPlus Challenge Program. CIAT cassava research shifted, therefore, its goals to produce source materials that reached this nutritional level. A drastic change in the breeding methodology was implemented for a faster genetic gain. As described in Chapter 3, a normal breeding cycle in cassava lasts abut 6-8 years since the botanical seed of segregating progenies are germinated until the best genotypes among these segregating progenies can be definitively identified. This long breeding cycle is required because most relevant variables are quantitatively inherited and are strongly affected by the environment, showing large genotype by environment interaction effects. Because of the low multiplicative rate of planting material in cassava (1:7 ratio) it takes several years until enough planting material is available for multi-location trials. These

multi-location trials are required for the proper selection for traits heavily influenced by the environment.

In the case of carotenoids content, however, evidence of high heritability for carotenoids content in cassava roots has been provided (CIAT 2008). Because of this special feature of the main breeding objective of this challenge program, CIAT implemented a rapid cycling scheme to shorten the breeding cycles and, therefore, speed up the rate of genetic gains time wise. Figure 1.2 illustrates the differences between the traditional selection cycle for overall agronomic performance versus the rapid cycling selection for high-carotenoids content. The rapid-cycling scheme depicted in **Figure 1.2** has been implemented since 2004 at CIAT and rapid progress has been achieved as illustrated in **Figure 1.3**.

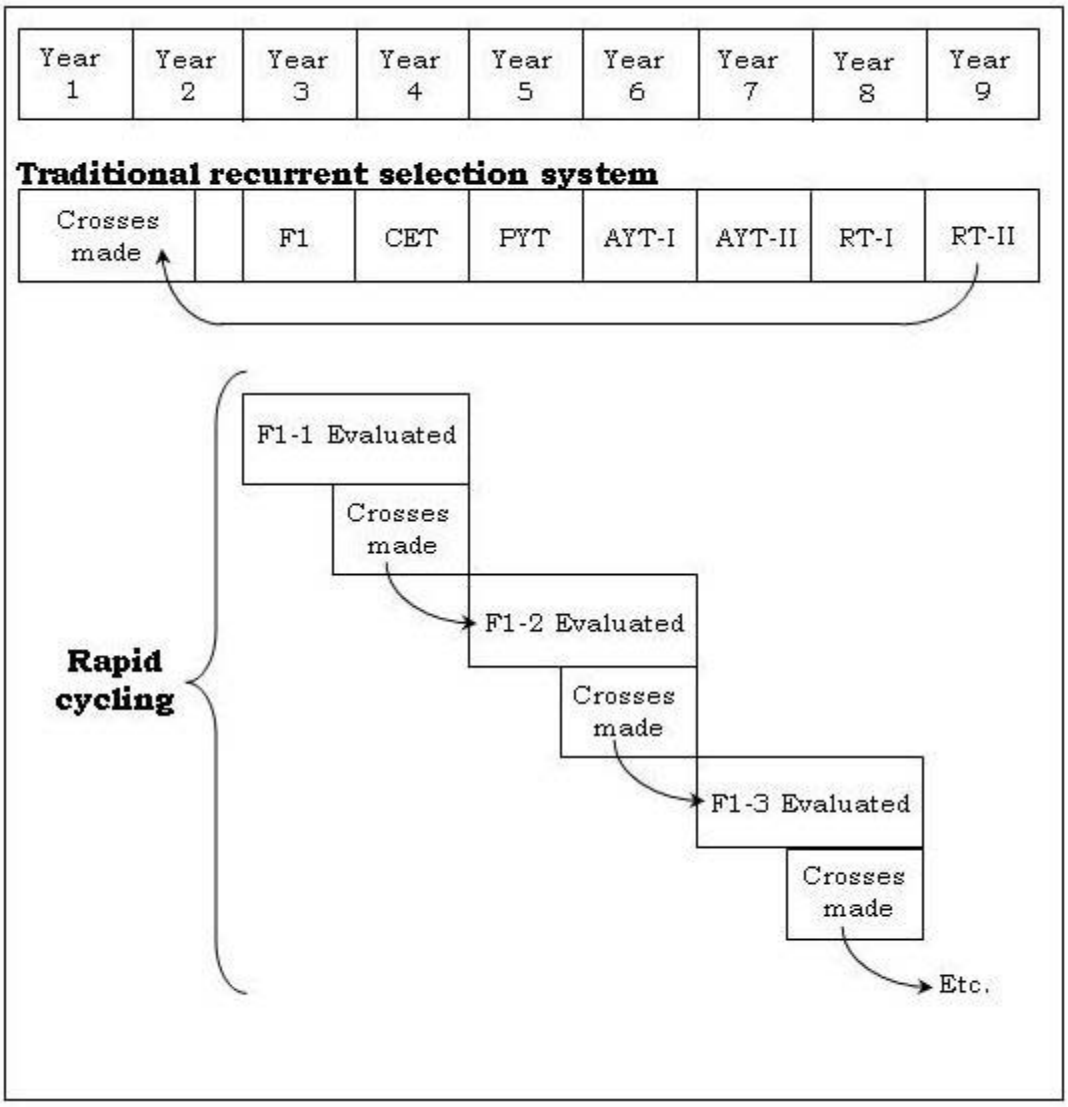


Figure 1.2. Illustration between the traditional and rapid-cycling recurrent selection systems. While the F1 generation is evaluated in the field, high-carotene genotypes (represented by a single plant)

are selected. Trial/nurseries are left for a longer period of time to allow selected F1 plants to flower and to make crosses among them to initiate a next cycle of selection.

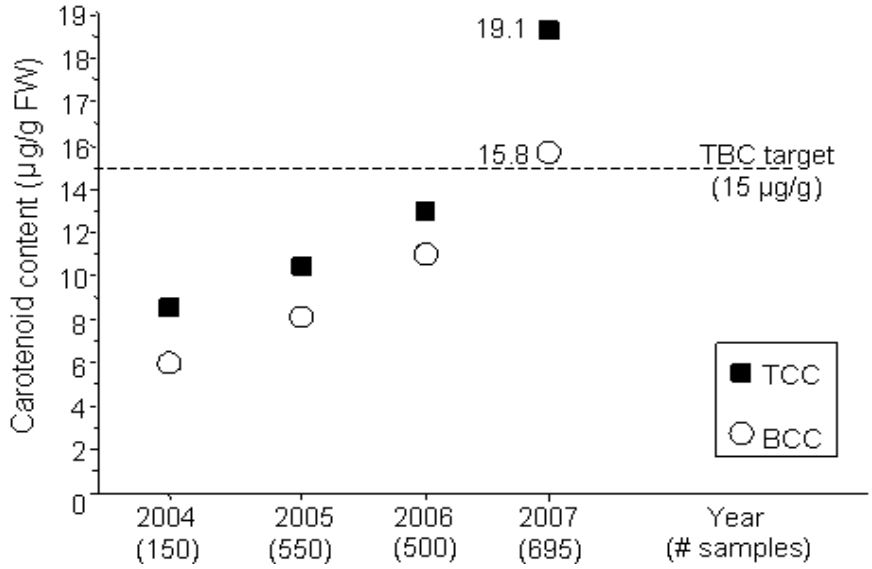


Figure 1.3. Progress over the last three years in increasing total carotenoids content (TTC) and β-carotene content (BCC) in the cassava project at CIAT. Data presented illustrates the maximum levels measured in non-replicated quantifications.

The rapid-cycling system (**Figure 1.2**) relies on single plant evaluation and immediate crosses among selected genotypes to initiate a new recurrent selection cycle. During 2008 a large experiment was conducted to evaluate the carotenoids content based on cloned plants and not based on plats derived from botanical seed (F1 plants in Figure 1.2 also known as ***seedling*** plants). This experiment was conducted to confirm the relationship between the seedling and cloned plant. A second purpose of this experiment was to measure again sampling variation among plants of the same genotype, among roots from the same plant, and aliquots from the same root. Several years ago a sample like this was conducted but when carotenoids levels were much lower (CIAT, 2003; Chávez et al., 2008). The project considered important to make sure that quantifications in the seedling plants would be maintained once the selected genotypes were cloned and roots were obtained from plants that had been asexually propagated. Cloned plants allow for the availability of several plants per genotype.

In November 2008 a set of 35 genotypes were harvested and their carotenoids content quantified. These genotypes were selected because they ranged widely in their carotenoids content (based on the seedling plant evaluations). Three roots from two plants were harvested from each genotype. Two quantifications per root (aliquots) were made, for a total of 12 measurements in most genotypes. **Figure 1.4** illustrates the kind of variation for each quantification and each genotype. Results were encouraging regarding the relationship between quantifications in the seedling and in the cloned plants. In general quantifications

in the seedlings were about 1-2 μg higher than in the cloned plants. It is not clear if this is because of differences in the age of the plant, changes in the laboratory making the quantifications or environmental factors.

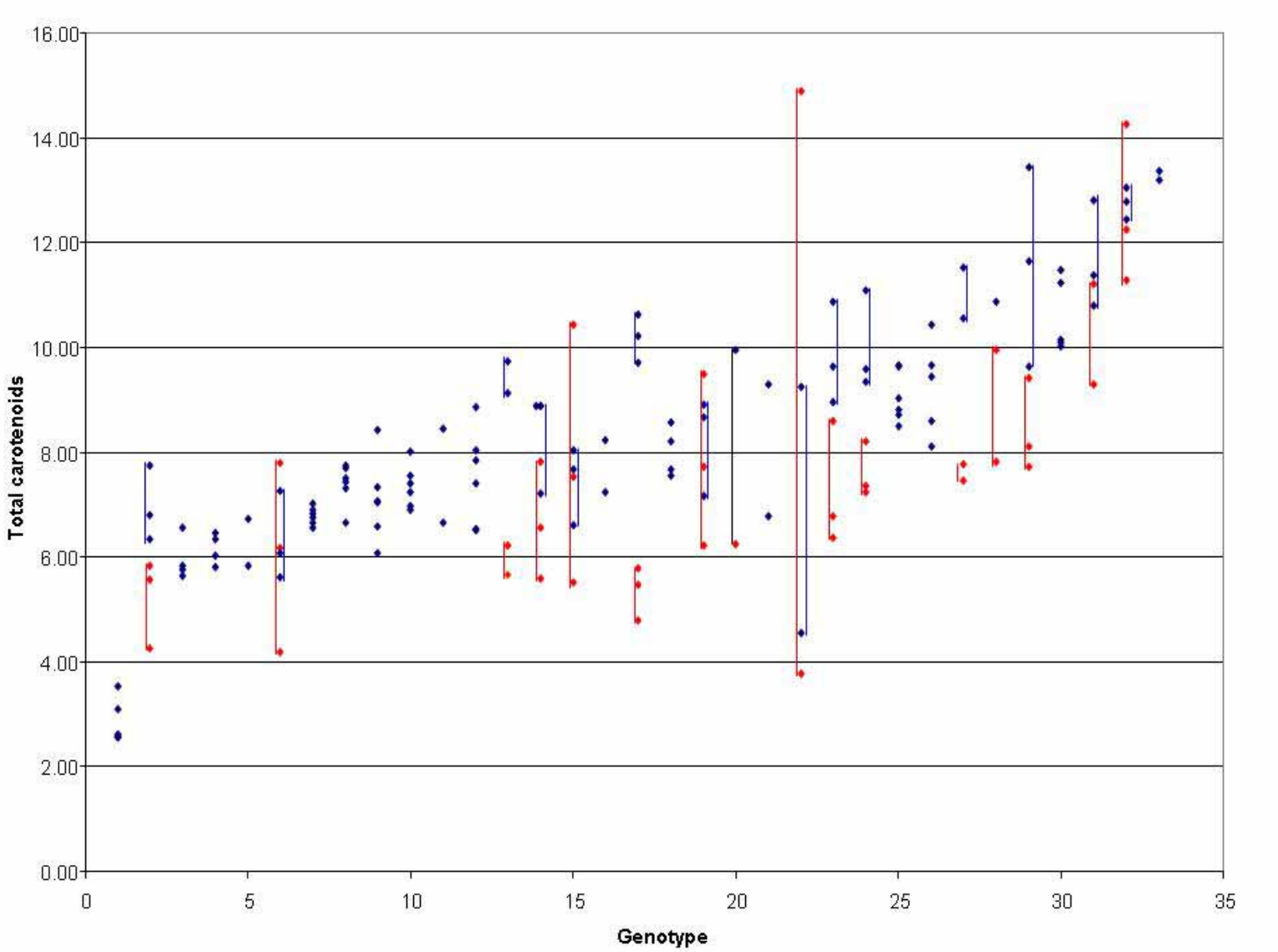


Figure 1.4. Variation in total carotenoids content (TCC) in 33 genotypes. Three roots from each of two plants were harvested to represent each genotype. In some cases plant to plant (red versus blue) variation was important. Sampling variation, does not seem to increase with higher TCC values.

An undergraduate thesis (Moralba Domínguez García) has been submitted to Universidad del Valle using the information of this section. The topic of this thesis is "Evaluation of carotenoids content in different cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomea batatas* L.) varieties.

2. Developing source cassava germplasm for the high-Protein trait

Several years ago different sources of cassava cultivars that had higher-than-normal levels of protein were identified and reported in the literature (Ceballos et al., 2006). Hundred of crosses among these materials have been made and are currently being made. Table 1.3 lists a group of improved germplasm and landraces that have in common the high-protein trait. These materials were included in a crossing block (October 2007-December 2008). A large proportion of the seed produced in this nursery will be shared with partners and colleagues from Africa, Asia and Latin America and the Caribbean.

Table 1.3. Parental lines to be used in crosses for their high protein content potential.

CM 696-1	SM 1406-1	MBRA 900	MCOL 2199	MCR 142	MMEX 108
CM 3199-1	MBRA 26	MBRA 1384	MCOL 2436	MGUA 33	MPAN 7
CM 3236-3	MBRA 101	MCOL 219	MCOL 2459	MGUA 76	MPER 243
CM 5620-3	MBRA 158	MCOL 226B	MCOL 2493	MGUA 79	MPER 286
CM 7310-1	MBRA 162	MCOL 678	MCOL 2532	MGUA 86	MPTR 49
SM 629-6	MBRA 300	MCOL 689B	MCOL 2694	MGUA 91	MVEN 134
SM 673-1	MBRA 435	MCOL 1563	MCR 38	MMAL 13	
SM 734-5	MBRA 890	MCOL 1734	MCR 136	MMEX 95	

3. Biofertilization study.

CIAT planted a trial aiming at quantifying the possibility of increasing Fe and Zn contents trough adequate applications of fertilizers. This is the coherent and logical outcome of previous work where Zn and Fe contents in cassava roots were shown to be heavily dependent of edaphic conditions (pH in addition to Fe and Zn contents). Therefore, for the delivery of Fe and Zn to human populations, in addition to the possibility of a genetic component, a cultural practice approach (which would not be suitable for TCC and BCC) is under investigation. **Table 1.4** lists the type and quantity of fertilizers applied in the experiment. Four edaphic environments (three contrasting soils at CIAT and the acidic soil of Santander de Quilichao) were used, with three replications per location. Two varieties were used (HMC-1 and CM 4919-1). Experimental plots had three plants. Each experimental plot was surrounded by non-treated plants from the same variety. **Table 1.5** provides information about the environmental conditions where the four evaluations were conducted.

Trials were harvested at the proper age and samples taken. As all the trials were consolidated a total of 384 samples were prepared and shipped to Australia for their analysis at Waite Laboratory at the University of Adelaide. The analysis and interpretation of the data is being done in collaboration with Dr. Graham Lyons. Data will be reported after its analysis.

Table 1.4. Description of the different fertilizers included in a "*biofertilization*" trial to quantify the impact of Fe, Zn, Se and I availability in the soil (or after foliar applications) in the content of these elements in the root at harvest time.

	Treatment	**Dosis**	**Source**
1	0 Kg Zn/Ha	0 kg	ZnSo4
2	5 Kg Zn/Ha	5 kg	ZnSo4
3	10 Kg Zn/Ha	10 kg	ZnSo4
4	20 Kg Zn/Ha	20 kg	ZnSo4
5	Foliar ZnSo4 45 / 90 dap	2%	ZnSo4
6	10 Kg Zn/ha (as ZnSo4) + Foliar ZnSo4 45 / 90 dap	10 KG + 2%	ZnSo4
7	0 Kg Fe/Ha	0 kg	FeSo4
8	5 Kg Fe/Ha	5 kg	FeSo4
9	10 Kg Fe/Ha	10 kg	FeSo4
10	20 Kg Fe/Ha	20 kg	FeSo4
11	Foliar FeSo4 45 / 90 dap	2%	FeSo4
12	10 Kg Fe/ha (as FeSo4) + Foliar FeSo4 45 / 90 dap	10 KG + 2%	FeSo4
13	5 Kg Zn/ha + 5 Fe/ha	5KG	ZnSo4+ FeSo4
14	15 Kg Zn/ha + 15 Fe/ha	15 KG	ZnSo4+ FeSo4
15	Se	150 GR	Na2O4Se
16	I	115 GR	KH(IO3)2

dap = days after planting

Table 1.5. Edaphic characteristics of the four locations where the bio-fertilization trials were grown.

Location	Plantig Date	Organic Matter(%)	pH	Ca	Mg	K	P	Zn	Fe	Texture
				me/100gr			(ppm)			
Ciat I1A	Aug.17 2007	3.2	7.0	19.8	12.0	.89	62.0	4.8	1.9	Clay
Ciat I1B	Aug.18 2007	2.6	7.2	14.9	12.9	.74	42.0	4.4	1.6	Clay
Ciat H1N	Aug.19 2007	2.5	7.3	15.7	12.6	.68	47.0	4.2	1.3	Clay
Quilichao	Oct.03 2007	6.2	4.5	1.95	.82	.22	10.5	2.1	31.5	Clay loam

4 GENETIC MAPPING OF BETA-CAROTENE CONTENT FROM MULTIPLE SOURCES IN CASSAVA.

A project to fortify cassava varieties grown by smallholder farmers with high carotenoids content has been launched as means to combat micronutrient deficiencies in areas where cassava is widely grown and consumed. Knowledge of the inheritance and gene action of beta-carotene accumulation in cassava can be used to guide the breeding process and also to combine favorable alleles at multiple loci that control beta-carotene content in cassava. The objective of this study was to identify simple sequence repeat (SSR) markers associated with beta carotene content in cassava through the bulked segregant analysis (BSA) of F_1 and S_1 families segregating for beta-carotene content.

To select the best families for the marker-aided analysis of the inheritance of beta-carotene content in cassava, TCC and TBC were evaluated in 800 individuals from 46 F_1 families having white, cream, and yellow colored root parenchyma. Three families, namely GM708, GM 734, and CM 9816 were selected for further study. In addition, eleven S_1 families, obtained from self-pollinating high, medium, and low genotypes from the three selected F_1 families, were also chosen for validation of associations between SSR markers and high carotenoids and/or β-carotene content (**Table 1.6**).

The extraction and quantification of total carotenes in fresh cassava roots was conducted following the established procedures (Safo-Katanka et al. 1984). Two or three plants are harvested per genotype and 5 of the best roots selected. The roots are cleaned, chopped up into small cubes, mixed, and a sample of 5g drawn. Simple statistics, for example average, median, standard deviation, maximum, minimum, was calculated for TCC and TBC. A frequency distribution of total carotenes in each family was also drawn as a preliminary assessment of gene control of β-carotene content in cassava.

Table 1.6. S_1 families obtained from F_1 genotypes with high, medium, and low beta carotene content to validate SSR marker association with beta carotene content in cassava roots.

Father	Mother	Cross	No. of S_1 genotypes
CM9816-1	CM9816-1	AM-689	70
CM9816-2	CM9816-2	AM-690	68
CM9816-5	CM9816-5	AM-691	68
CM9816-6	CM9816-6	AM-692	46
GM893-5	GM893-5	AM-710	26
GM893-8	GM893-8	AM-712	49
GM893-16	GM893-16	AM-718	32
GM893-18	GM893-18	AM-720	34
GM708-63	GM708-63	AM-702	20
GM708-20	GM708-20	AM-697	35
GM708-27	GM708-27	AM-698	30

DNA extraction was using the Dellaporta (1983) protocol as modified for cassava; the quality of the DNA was verified using agarose gel (0.8%) electrophoresis stained with ethidium bromide (0.5ug/ml) and quantified using a DNA flourometer (DYNA Quant, Hoefer). Dilutions of each DNA sample were made to a final concentration of 10ng/ul. For bulked segregant analysis (BSA), bulks of high and low beta-carotene content were constituted for each family. Between 10 and 20 individuals with high beta-carotene content, 7-11µg/g, were selected as the high bulk and, 10-20 genotypes with beta-carotene content lower than 2µg /g were selected as low bulks.

The bulks and parents were evaluated with 140 SSR markers that have earlier been selected from the genetic map of cassava to cover the entire cassava genome at a marker density of one marker every 10-20cM. To identify association between molecular markers associated with TCC or TBC content after the BSA, a correlation and simple regression analysis was conducted, considering the marker genotypic classes as independent variable and content of total carotenes as dependent variable. Markers that explain large amounts of phenotypic variation for total carotenes were evaluated in the S_1 families for further marker validations. Frequency distribution of TCC in the three families tends to follow normal distribution, suggesting that several genes control TCC (**Figure 1.5**). Markers polymorphic were evaluated in the individuals of the bulks (**Figure 1.6**) and those that showed consistency with results from analysis of the bulks were analyzed in all progenies.

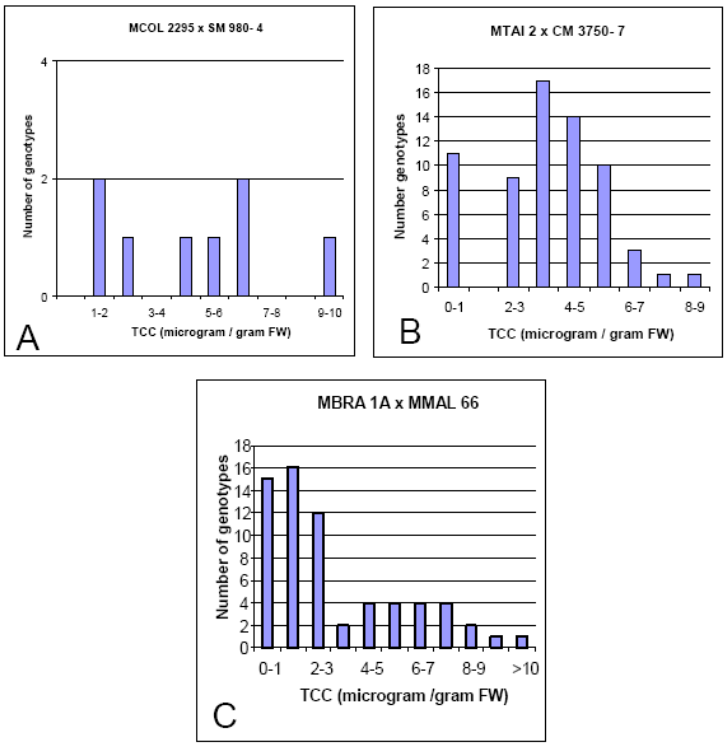


Figure 1.5. Distribution frequencies of TCC values (µg/g) in the three families evaluated. A. CM9816, B. GM-734 y C. GM708.

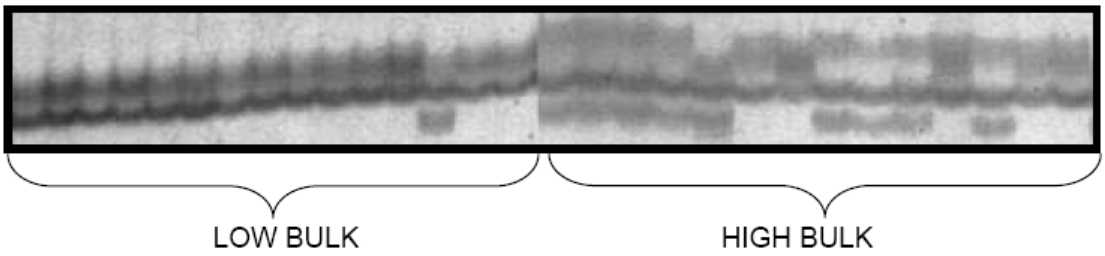


Figure 1.6. Evaluation of SSR marker SSRY-313 in high and low bulks of GM 708.

Single marker analysis, by simple regression, of association between the polymorphic markers and total carotene content revealed a number of major quantitative trait loci (QTL) controlled beta-carotene content in cassava (**Table 1.7**). The QTLs explained up to 26% of phenotypic variation. Five QTLs were identified on linkage group D for all of the 3 families, suggesting major QTLs that go across different sources of enhanced beta-carotene content reside on this linkage group (**Table 1.7**). Families GM708 and CM9816 also had QTLs on linkage group G (**Table 1.7**). Six other QTLs were unique amongst the 3 families.

Table 1.7. Association between SSR markers and beta-carotene content in the families GM 708, GM 734 and CM 9816 as revealed by single marker analysis (simple regression).

Family	SSR Marker	Correlation	Regression	Linkage Group
GM708	SSRY 178	0.31	0.1	H
	NS267	0.26	0.07	R
	SSRY313	0.44	0.19	D
	SSRY226	0.23	0.05	G
	SSRY88	0.31	0.1	K
GM734	SSRY250	0.51	0.26	L
	SSRY242	0.31	0.1	A
	SSRY21	0.28	0.08	D
	NS717	0.41	0.17	D
CM9816	SSRY49	0.42	0.18	C
	SSRY195	0.42	0.18	F
	SSRY330	0.37	0.14	N/A
	SSRY324	0.23	0.05	D
	NS158	0.43	0.18	G
	SSRY172	0.33	0.11	J
	SSRY313	0.47	0.22	D

Single marker analysis, by simple regression, of association between the polymorphic markers and total carotene content in families S_1 confirmed the existence of major quantitative trait loci (QTL) controlled beta-carotene content in cassava. The QTLs explained up to 32% of phenotypic variation (**Table 1.8**).

Table 1.8. Association between SSR markers and beta-carotene content in the families S_1 revealed by single marker analysis (simple regression).

Family	SSR Marker	Correlation	Regression	Linkage Group
AM-689	NS-158	0.44	0.19	G
	NS-717	0.48	0.23	D
	SSRY-172	0.41	0.17	J
	SSRY-195	0.34	0.12	F
	SSRY-21	0.49	0.24	D
	SSRY-324	0.47	0.22	D
	SSRY-330	0.44	0.19	D
	SSRY-49	0.46	0.21	C
AM-690	NS-158	0.39	0.15	G
	NS-717	0.47	0.22	D
	SSRY-172	0.36	0.13	J
	SSRY-195	0.34	0.12	F
	SSRY-21	0.44	0.19	D
	SSRY-313	0.38	0.14	D
	SSRY-324	0.38	0.15	D
	SSRY-330	0.43	0.18	D
	SSRY-49	0.39	0.15	C
AM-691	NS-717	0.32	0.1	D
	NS-158	0.33	0.11	G
	SSRY-195	0.45	0.2	F
	SSRY-21	0.36	0.13	D
	SSRY-324	0.34	0.12	D
	SSRY-330	0.36	0.13	D
	SSRY-49	0.33	0.11	C
AM-692	SSRY-172	0.39	0.15	J
	SSRY-324	0.44	0.19	D
	SSRY-330	0.47	0.22	D
	NS-158	0.46	0.21	G
	NS-717	0.44	0.19	D
	SSRY-195	0.32	0.1	F
	SSRY-21	0.46	0.21	D
AM-697	SSRY-178	0.45	0.2	H
	NS-158	0.34	0.11	G
	SSRY-49	0.36	0.2	F
	SSRY-226	0.37	0.13	G
	SSRY-88	0.32	0.1	K
AM-698	NS-158	0.32	0.1	G
	SSRY-178	0.34	0.11	H
	SSRY-49	0.39	0.15	F
	SSRY-226	0.33	0.11	G
	SSRY-88	0.32	0.1	K

Table 1.8 cont.

Family	Correlation	Regression	Linkage Group	SSR Marker
AM-702	SSRY-178	0.48	0.23	H
	NS-158	0.46	0.21	G
	SSRY-49	0.47	0.22	F
AM-710	NS-158	0.42	0.18	G
	SSRY-195	0.33	0.11	F
	SSRY-21	0.48	0.23	D
	SSRY-330	0.46	0.22	D
	SSRY-31	0.32	0.11	F
	SSRY-226	0.4	0.16	G
AM-712	NS-158	0.49	0.24	G
	SSRY-195	0.35	0.12	F
	SSRY-21	0.48	0.23	D
	SSRY-313	0.49	0.24	D
	NS-717	0.54	0.29	D
	SSRY-226	0.45	0.2	G
	SSRY-31	0.35	0.13	F
AM-718	NS-158	0.48	23	G
	SSRY-195	0.33	11	F
	SSRY-21	0.57	32	D
	SSRY-313	0.51	26	D
	NS-717	0.51	26	D
	SSRY-226	0.49	24	G
AM-720	NS-717	0.36	0.13	D
	SSRY-21	0.36	0.13	D
	SSRY-226	0.34	0.11	G
	NS-158	0.36	0.13	G
	SSRY-178	0.36	0.13	H

In a previous study of the mode of inheritance and the number of genes involved in determination of yellow root color in a S1 family (AM320) derived from the Thai variety MTAI8, 3 polymorphic markers were found to be associated with root color and controlled between 30 and 40% of phenotypic variance (CIAT 2005). All of the markers - SSRY 313, NS251, and NS717 - are located on linkage group D and are similar to those found in these studies.

These results reveal that regions of the cassava genome controlling beta-carotene content are common for different sources of increased beta-carotene content but also unique with respect to source. Gene action for all aforementioned QTLs are thought to be additive in nature but confirmation will come from subsequent marker-validation studies already being conducted. Association of molecular markers and beta-carotene content was initially conducted with regression analysis. there is a need to conduct the analysis with other more powerful forms of analysis including interval and composite interval analysis. Markers that explain large amounts of phenotypic variation identified in this study will be validated in additional families having genetic backgrounds different from those used in this study

Ana Cruz Morillo Coronado, and Yacenia Morillo Coronado (two Ph.D. female students have been involved in this work.

5. REFERENCES

Ceballos H., T. Sánchez, A. L. Chávez, C. Iglesias, D. Debouck, G. Mafla and J. Tohme (2006). Variation in crude protein content in cassava *(Manihot esculenta* Crantz) roots. Journal of Food Composition and Analysis 19:589-593.

Chávez, A. L., H.Ceballos, D. B. Rodriguez-Amaya, J. C. Pérez, T. Sánchez, F. Calle, and N. Morante (2008). Sampling variation for carotenoids and dry matter contents in cassava roots. Journal of Root Crops 34(1). 43-49.

CIAT (2003). Centro Internacional de Agricultura Tropical. Project IP3, Improved Cassava for the Developing World, Annual Report 2002. Apartado Aéreo 6713, Cali, Colombia.

CIAT (2008). Centro Internacional de Agricultura Tropical. Project IP3, Improved Cassava for the Developing World, Annual Report 2007. Apartado Aéreo 6713, Cali, Colombia.

Sánchez, T., A.L. Chávez, H. Ceballos, D. B. Rodriguez-Amaya, P. Nestel and M. Ishitami (2005). Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content. Journal of the Science of Food and Agriculture 86(4): 634-639.

CHAPTER 2

GENETIC BASE OF CASSAVA AND RELATED *MANIHOT* SPECIES EVALUATED AND MADE AVAILABLE FOR CASSAVA IMPROVEMENT: HIGHER COMMERCIAL VALUE

2.1. FIELD EVALUATION OF ACCESSIONS FROM THE GERMPLASM COLLECTION

Because of problems related to the accumulation of diseases the germplasm collection is no longer routinely grown in the field. The collection had become a focus of diseases, particularly frog skin disease (FSD), because given its importance no genotype could be eliminated if found to have symptoms of the disease. However, the phenotypic characterization of the collection has not been completed. Therefore, during a considerable part of 2006 plantlets from the in vitro collection were obtained, hardened and transplanted to the field where they were grown during 2007 in an isolated field in CENICAÑA. These hardened plants were used to characterize phenotypically these materials; extract self-pollinated seed used in the screening for useful recessive traits; and obtain planting material for their formal evaluation in different environments. Plants in CENICAÑA were harvested by mid 2008 and from each genotype 14 cuttings were obtained. For each genotype six cuttings were planted in Palmira (Valle del Cauca) and Santo Tomás (Altántico) the two remaining stakes were planted in the eastern acid-soil savannas environment in the Meta Department.. Not all accessions could be planted in the three environments. The nursery in Palmira (Table 2.1) was planted in an isolated field at CIAT to prevent, as much as possible, contamination with FSD (frog skin disease).

Table 2.1. Country of origin, number of accessions and number of plants grown in the field (CIAT, Palmira) from the recoveries of in vitro germplasm collection of cassava.

Country	# accessions	# plants	Country	# accessions	# plants
ARGENTINA	38	228	NIGERIA	3	18
BRASIL	507	3042	PANAMA	9	54
COLOMBIA	534	3204	PARAGUAY	45	270
COSTA RICA	44	264	PERU	88	528
CUBA	33	198	FILIPINAS	1	6
ECUADOR	23	138	PUERTO RICO	1	6
FIDJI	3	18	TAILANDIA	2	12
GUATEMALA	21	126	USA	2	12
INDONESIA	11	66	VENEZUELA	39	234
MALASIA	24	144	ELITES	152	912
MEXICO	34	204	Total	**1614**	9684

The materials described in Table 2.1 will be handled as a CET. However, many more activities will be carried out on that field. A full phenotypic characterization will be made to complete the database of the Genetic Resources Unit on the Manihot collection. These plants are better suited to provide more reliable data than those planted the previous season at CENICAÑA because the latter came from hardened plants from the in vitro collection and there is some remnant effect of that drastic change. Data for completion of the collection will, therefore, be obtained for all the morphological descriptors. An important advantage is that phenotypic data will be available from the simultaneous evaluations conducted in the mid-altitude valleys (Palmira), sub-humid (Santo Tomás) and acid-soil savannas (Puerto López) environments.

In addition since six plants are available from each genotype, self-pollinations will be made in as many genotypes as possible. Some of that self-pollinated seed (20-30 seeds per accession) will be germinated next year and their respective plants screened in search of useful recessive traits. The rest of the seed will be used to start a novel approach of conservation of Manihot genetic resources, serving as a back-up for the in vitro collection. Rather than preserving the genotypes themselves this approach aims at preserving the genes present in each genotype.

During the second semester of 2009 the materials will be harvested and fully characterized, including for their agronomic performance. As it has been observed some landraces can occasionally turn out to have an outstanding performance and can be directly used by farmers. That is the case, for example of MPER 183, widely grown in the Valle del Cauca region. Screening of these materials will also concentrate in the search for germplasm with intense yellow pigmentation that would contribute to the HarvestPlus initiative to produce cassava germplasm with increased levels of pro-vitamin A carotenoids. The evaluation in Palmira will allow for screening of different pests that may affect cassava. This location is particularly suitable for evaluation to white flies.

Table 2.2. Country of origin, number of accessions and number of plants grown in the field from the recoveries of in vitro germplasm collection of cassava. Trial planted in Puerto López (Meta Department) a representative location of the acid-soil eastern savannas of Colombia.

Country	# accessions	# plants	Country	# accessions	# plants
ARGENTINA	25	50	NIGERIA	2	4
BRASIL	514	1028	PANAMA	5	10
COLOMBIA	547	1094	PARAGUAY	34	68
COSTA RICA	41	82	PERU	91	182
CUBA	32	64	FILIPINAS	1	2
ECUADOR	23	46	PUERTO RICO	1	2
FIDJI	3	6	TAILANDIA	2	4
GUATEMALA	19	38	USA	3	6
INDONESIA	11	22	VENEZUELA	40	80
MALASIA	25	50	ELITES	50	100
MEXICO	29	58	**Total**	**1498**	**2996**

Table 2.2 lists the materials and number of plants that were also planted in the acid-soil savannas environment at Puerto López, Meta Department. That particular environment is ideal to screen for resistance to bacterial blight (CBB) and super-elongation disease (SED), in addition to the adaptation to acid soil conditions typical of that region.

The same set of accessions from the germplasm collection was also planted in the sub-humid environment at Santo Tomás (Atlántico Department). Table 2.3 describes the number of accessions and total number of plants of this large evaluation (about one hectare in size). This environment provides ideal conditions for the evaluation of reaction to different pests, particularly mites and thrips. Given the lengthy rainless period that characterizes this environment, drought tolerant genotypes may have a better chance to have an outstanding performance in these conditions.

Table 2.3. Country of origin, number of accessions and number of plants grown in the field from the recoveries of in vitro germplasm collection of cassava. The trial was planted in Santo Tomás (Atlántico Department) which is a location representative of the sub-humid environment.

Country	# accessions	# plants	Country	# accessions	# plants
ARGENTINA	30	180	NIGERIA	3	18
BRASIL	529	3174	PANAMA	7	42
COLOMBIA	566	3396	PARAGUAY	41	246
COSTA RICA	42	252	PERU	101	606
CUBA	34	204	FILIPINAS	1	6
ECUADOR	30	180	PUERTO RICO	1	6
FIDJI	3	18	TAILANDIA	2	12
GUATEMALA	23	138	USA	3	18
INDONESIA	11	66	VENEZUELA	40	240
MALASIA	26	156	ELITES	66	396
MEXICO	31	186	**Total**	**1590**	**9540**

2.2. CHARACTERIZATION OF STARCH QUALITY TRAIS FROM A LARGE SAMPLE OF CASSAVA GERMPLASM

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of commercial production of starch in tropical and subtropical countries [1]. About 73.7 to 84.9% of dry root weight of cassava is starch [2]. Compared with other root and tuber tropical crops, cassava starch and its biosynthesis have been well studied [1, 3-9]. The starch granules are generally round (oval), with a flat surface on one side (truncated) and size of individual granules ranges from 5 to about 40 µm, with reported averages varied from 5.4 to 17.2 µm [1, 3, 8-9]. However, recently a mutation with considerably smaller starch granule size has been reported [10].

There is a widespread variation of starch biochemical and functional properties reported in the literature. Amylose content, for example, has been reported to range from 18.6 to 23.6% [3; 11]; 17 – 25 % [12]; 18 to 25% [1]; 15.9 to 22.4% [9] or 13.6 - 23.8% [2]. An amylose-free

natural mutation has also been reported recently [13] as well as a high amylose/small granule induced mutation [10]. Earlier, N. Zakhia and co-workers [14] reported root and starch quality traits from the cassava core germplasm collection of CIAT (from 502 to 565 genotypes depending on the trait). Average amylose content was around 22%. Most reports in the literature are based on starch samples for just a single or few genotypes. Perhaps the only exception is the 22.7-32.4% range of variation reported from CIAT germplasm [14], 160 Indonesian genotypes [15]. When characterizing cassava starch, therefore, it is difficult to define what a "typical" or "normal" cassava starch is.

The cassava-breeding project at the International Center for Tropical Agriculture (CIAT) has implemented several strategies to develop high-value cassava clones to take advantage of the new opportunities opened to cassava by the globalization of the economies in many tropical countries [16-18]. The main objective is to develop not only clones with high and stable productivity, but also with root characteristics that better fit the needs of the different industries. For the feed industry high-protein clones have been identified [19]. For the starch industry, different approaches to develop and identify clones with novel starch properties have been gradually introduced in the cassava-breeding project [17, 18]. In addition, the identification of those genotypes where interesting starch quality variations are expressed requires the availability of special tests. As a result of these activities two commercially relevant mutations have been recently reported [10, 13] and valuable information on starches from a large number of cassava genotypes has been collected.

CIAT holds in trust the worldwide cassava germplasm collection with more than 6000 accessions. Three main types of accessions can be mentioned: a) wild relatives of cassava within the *Manihot* genus; b) traditional landraces grown by farmers in Africa, Asia or Latin America and the Caribbean (LAC); and c) improved cassava germplasm produced by breeding projects in the continents mentioned above. The collection is maintained *in vitro* at CIAT experimental station located in Palmira, Valle del Cauca, Colombia [20].

The following information has been consolidated in a research manuscript and submitted for its publication in Starch/Stärke. The objective of this study was to analyze the starch characteristics of more than four thousand cassava clones (landraces and improved varieties) and few wild species, which had been evaluated in the root quality laboratory at CIAT. The financial support of National Starch Company for the early phases of this research is hereby acknowledged.

2.2.1 Materials and methods

As part of the project to identify high-value cassava germplasm, with special emphasis in starch quality and other root-traits, CIAT initiated the screening of starch quality traits in accessions from the germplasm collection as well as improved cassava varieties developed by CIAT, the International Institute of Tropical Agriculture (IITA) or two breeding projects in Thailand at Department of Agriculture and Kasetsart University [17, 18].

The first step was to retrieve the germplasm from the *in vitro* condition and grow the plants in the field for starch extraction and root evaluation. Plantlets from the *in vitro* collection were hardened in greenhouse conditions following standard protocols [21]. After a week of gradual hardening, plantlets were transferred to green house conditions for a period of about two months and then transplanted to the field. Initially five plantlets per genotypes initiated the hardening process. But, unavoidably, some of these plants were lost in the process.

Therefore, analyses reported herein are based on 1 to 5 plants per genotype. Plants were harvested at 10-12 months of age from 1999 to 2003 near Palmira, Colombia (1000m above sea level). **Table 2.4** provides information about the origins of the cassava genotypes evaluated in this study. The variation in the number of clones from different countries is representative of the diversity of germplasm in the collection. As expected, most of the material comes from LAC, given the American origin of the genus *Manihot* [22, 23]. The most common origins were Colombia and Brazil with 1230 and 919 clones, respectively. Peru and Venezuela were represented by more than 100 genotypes. On the other hand, only two clones represented Chile and China. Among the materials evaluated there were 12 genotypes representing three different wild *Manihot* species *(M. chlorosticta, M. carthaginensis*, *M. esc. flabellifolia* and *M. filamentosa*). Root and starch analyses follow the general protocols described in [24].

Table 2.4. Summary of the origins from the materials evaluated in this study and the number of clones representing them.

Country	# clones	Country	# clones	Country	# clones
Argentina	42	Fiji	5	Thailand	12
Bolivia	4	Philippines	5	USA	7
Brazil	919	Guatemala	61	Venezuela	158
Chile	2	Indonesia	33	Vietnam	4
China	2	Malaysia	46	CG [1]	46
Colombia	1230	Mexico	66	CM [1]	364
Costa Rica	86	Nigeria	13	GM [1]	188
Cuba	64	Panama	30	SG [1]	28
Dominican Rep.	4	Paraguay	94	SM [1]	138
Ecuador	83	Peru	281	Other. [2]	12
El Salvador	5	Puerto Rico	9	*Manihot* sp	12

[1] Improved clones developed at CIAT. CG, CM and GM identify clones whose mother and father are known. For SG and SM only the mother is known.

[2] Mostly a group of improved clones not developed at CIAT.

Root and starch moisture content. Up to five roots from the same plant were peeled and immediately cut into small pieces and mixed. Moisture content was determined after drying 50 g of sample (freshly cut pieces or starch) at 60 °C for 48h [25].

Flour extraction. Freshly cut pieces from the harvested peeled root(s) were lyophilized during 24 hours at – 30 °C and then ground. A FreeZone Stoppering Tray Drier - Model 79480 and a 6 Liter Freeze Dry System – Model 77530 (Labconco Corporation, Kansas City, USA) were used. The flour thus obtained was stored in plastic bags for total and reducing sugar and starch analyses.

Starch isolation. Freshly cut pieces of peeled roots were suspended in tap water and crushed in an Osterizer blender. The slurry was filtered through a 100µm sieve. The starch was allowed to settle and the supernatant decanted off and dried in an oven with fan-forced ventilation at 40°C during 48h

Total and reducing sugars. Content of total and reducing sugars were determined according to Cronin et al. [27]. Sugars were extracted from 2 g of root flour using an 80% ethanol solution, a Fehling reagent and a glucose standard curve. A Cecil spectrophotometer model CE 2021-Series 2000 (Cambridge, UK) was used in the determination.

The following characteristics were quantified using the standard procedures reported in the literature (27, 28, 29, 30, 31): determination of starch content paste clarity, colorimetric amylose determination, pasting properties using a Rapid Viscoanalyzer, and total cyanide content.

2.2.2 Results and Discussion

The wild Manihot species reported variation which fell within the range of values for *Manihot esculenta* accessions and therefore no distinction has been made between them and cultivated cassava.

Table 2.5 summarizes the results of the most relevant root quality traits. Average dry matter content was 33.6%, with a range of variation from 14.28 to 48.12%. There was a slight asymmetry in the frequency distribution with a tendency of longer tails to the left (skewness -0.40). Cyanogenic potential ranged widely from 14 to 3274 ppm, with an average of around 327 ppm. Distribution was highly asymmetrical (skewness 2.96) with a long tale to the right (**Figure 2.1**). Total and reducing sugars also showed an asymmetrical distribution with longer right tales, particularly in the case of reducing sugars (skewness 1.76 and 3.08, respectively). Average values were 3.75 and 1.31% for total and reducing sugars, respectively. Average starch content was 84.5%, with a tendency of values to concentrate towards the higher values (skewness -0.65), which accentuated a similar tendency observed for dry matter content.

Table 2.5. Root quality traits from more than 4000 cassava genotypes expressed in Dry Basis.

Parameter	Dry matter content (%)	Cyanogenic potential (ppm)	Sugars content		Starch content (%)
			Total (%)	Reducing (%)	
Maximum	48,1	3274	18,8	15,7	91,0
Minimum	14,3	14	0,2	0,0	65,0
Average	33,6	327,4	3,8	1,3	84,5
St.Deviation	6,47	397,7	2,32	1,43	3,34
Skewness	-0,40	2,96	1,76	3,08	-0,65
Count	4051	4050	4049	4049	4049

Amylose content ranged from 15.2 to 26.5% with an average of 20.7% (**Table 2.6**). The average amylose content of 20.7% is a very robust estimate since it is based in such a large sample of genotypes. Distribution was practically normal with a very slight asymmetry towards a longer right tale (**Figure 2.2**). No more than 1.5% of the samples had amylose values below 17.5 or above 24.5%. Water solubility and swelling power showed a more asymmetrical distribution with skewness values ranging from 1.53 to 1.77 (longer right tales). Average paste clarity was 45.2% with a large variation ranging from 12.5 to 96.6%

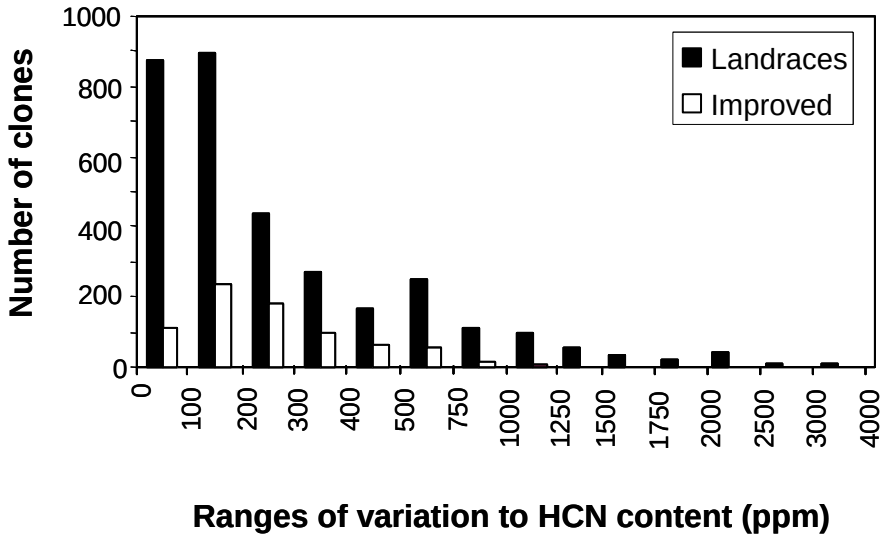


Figure 2.1. Histogram illustrating distribution of cyanogenic potential (HCN in ppm) of landraces and improved cassava. There is a clear asymmetry with a long tale to the right and the distribution of improved clones tends to be more concentrated around lower HCN values.

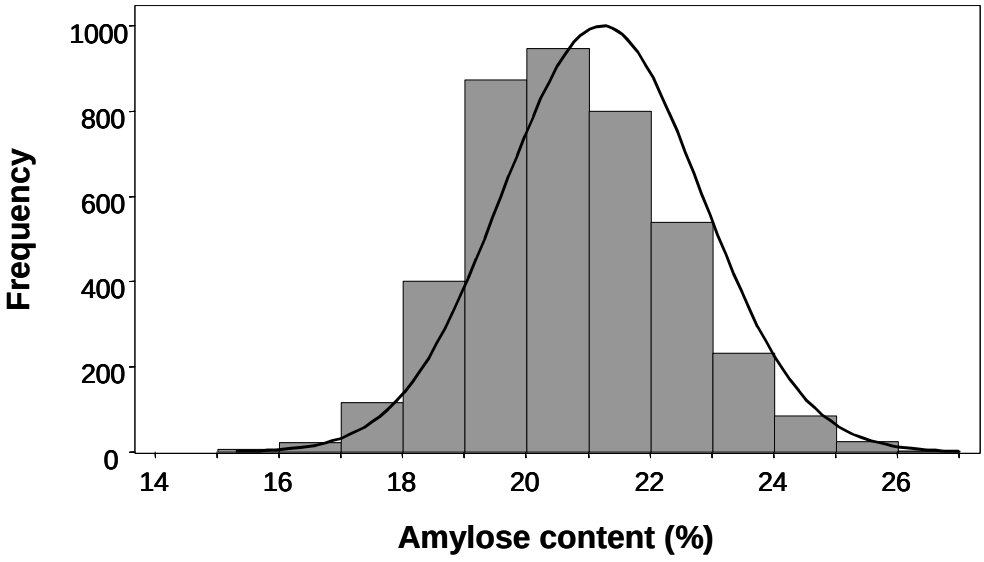


Figure 2.2. Histogram illustrating distribution of amylose (% of total starch) in starch samples from 4046 different cassava accessions. Frequency distribution is close to that expected from the normal distribution curve (provided in the Figure). The right tale tends to be slightly longer.

Table 2.6. Starch quality traits from more than 4000 cassava genotypes.

Parameter	Amylose Content (%)	Water Solubility (% db)	Swelling Power (% g/g)	Paste Clarity (%)
Maximum	26,5	16,6	15,5	96,6
Minimum	15,2	0,2	0,8	12,5
Average	20,7	2,2	4,6	45,2
St. Dev.	1,61	1,59	2,31	10,54
Skewness	0,22	1,77	1,53	-0,30
Count	4042	4050	4050	4044

Pasting properties of the samples analyzed are described in **Table 2.7**. Average pasting temperature was 65.3 ^{o}C and ranged from 58.8 to 71.2. Distribution frequency was relatively symmetrical as for the other parameters described in Table 4. Maximum viscosity averaged at 777.5 cP, with a wide range of variation form 146 up to 1505 cP. Breakdown ranged from 28.1 to 859.0 cP with an average around 298.1 cP. Consistency ranged from 626 down to 0 cP with an average of 155.8 cP. Finally average setback was -144.5 cP with a minimum of -702.0 and a maximum observed value for 273.0 cP.

Table 2.7. Pasting properties from starches of more than 4000 cassava genotypes.

Parameter	Pasting Temperature (^{o}C)	Maximum Viscosity (cP)	Breakdown (cP)	Consistency (cP)	Setback (cP)	Ease of Cooking (min)
Maximum	71,2	1505,0	859.0	626.0	273.0	5,6
Minimum	58,8	146,0	28.1	0.0	-702.0	1,1
Average	65.3	777,5	298.1	155.8	-144.5	2,8
St.Dev.	1.75	165,03	107.1	57.8	96.2	0,72
Skewness	-0,13	0.22	0.81	0.94	-0.38	0,33
Count	4051	4051	4051	4051	4051	4051

When clones were grouped by country or region of origin some weak trends could be observed in some cases. For example, clones from Brazil and Indonesia had the highest averages for HCN (508 and 408ppm, respectively), whereas Ecuador, Peru as well as improved clones, showed the lowest averages (158, 162 and 219 ppm, respectively). Wild relatives of cassava tended to have higher levels of total sugars (average 4.80%), while that for improved clones was 4.05%. The Peruvian clones showed the lowest average for total sugars (3.22%), along with non-Indonesian clones from Asia (3.24%), which also showed the lowest averages for reducing sugars (0.98%). Wild cassava relatives also had high levels of reducing sugars (2.09%). Water absorption tended to be higher in South American clones (4.79; 4.81; 4.84 and 5.05% for Brazil, Peru, Colombia and Ecuador, respectively) than in Central (3.59%) and North America (3.62%) as well as clones from Indonesia (3.52%). A

similar trend was observed for swelling power. Starches from clones originating in the southern region of South America tended to have low averages for maximum viscosity (727 cP) and breakdown (257 cP), whereas clones from Peru and Ecuador tended to have the highest averages (>830 cP for maximum viscosity) and 362 cP for breakdown of clones from Ecuador. Finally, wild relatives tended to have low averages for consistency values (131 cP), and ease of cooking (2.53 min).

Table 2.8 presents relevant information for the contrast between landraces and improved clones. For most variables there is no much difference between these two groups of cassava genotypes. Perhaps the only cases where some interesting differences could be observed were for dry matter content 32.8 versus 36.7% for landraces and improved clones); and cyanogenic potential (340 versus 267 ppm respectively for landraces and improved clones). In an earlier study, Bokanga [32] analyzed 881 genotypes from IITA's collection, 559 accessions from CIAT core collection, 184 landraces from Cameroon and 144 Nigerian local cultivars and in no case found acyanogenic cassava. Amylose content averages were very similar (20.89 and 20.02 %) as where most of the other starch characteristics including pasting temperature and maximum viscosity. Clarity was slightly lower for landraces (44.5%) compared with improved clones (48.1%).

Table 2.8. Comparison between landraces and improved clones root quality traits.

	3272 landraces			772 improved clones			
Parameters	Mean	St. Dev.	Min.	Max.	Mean	St. Dev.	Min.	Max.
Root traits								
Dry matter (%)	32.8	± 6.4	14,3	47.9	36.7	± 5.9	14,7	48,1
HCN content (ppm)	340	± 428	14	3274	267	± 212	28	2147
Flour traits								
Total sugar (%)	3,68	± 2.33	0,20	18,8	4,06	± 2.26	0,63	13,45
Reducing sugars (%)	1,25	± 1.43	0	15,7	1,56	± 1.41	0,01	8,24
Starch (%)	84,5	± 3.4	65,0	91,0	84,4	± 3.0	73,0	91,0
Starch traits								
Amylose (%)	20,89	± 1.6	15,15	26,46	20,02	± 1.44	15,75	25
Water solub. (% db)	2,15	± 1.51	0,2	12,3	2,25	± 1.85	0,24	16,6
Swell. Power (%g/g)	4,57	± 2.35	0,79	15,4	4,65	± 2.14	2,08	14,7
Clarity (%)	44,5	± 10.7	12,5	86,3	48,1	± 9.25	21,7	96,6
Pasting temp. (°C)	65,2	± 1.75	58,7	71,1	65,4	± 1.69	60,0	69,7
Maximum viscosity	776	± 169	152	1401	783	± 148	146	1505
Break down (cP)	300	± 109	35	859	290	± 99	28	795
Consistency (cP)	158	± 59	0	626	147	± 52	1	394
Setback (cP)	-142	± 95	-702	273	-153	± 102	-584	103
Ease of cook. (min.)	2,76	± 0.68	1,14	5,61	2,98	± 0.84	1,14	4.89

The results presented in this article are exploratory. Most cases samples were un-replicated. However when unusual data was observed, because of the particular interest in identifying unusual starch types, it prompted a second analysis to confirm the data. This approach, although it is limited from the experimental point of view, allowed the analysis of such a large number of genotypes, and provides very reliable information since at least outlaying data

points were confirmed. One problem that remains unsolved is the possibility of a plant, for unknown reasons, producing starch samples with characteristics that may not be representative of that genotype. By and large, however, average values presented in this study should be very robust and properly represents starch characteristics of cassava. Range of variation is also useful to provide an idea of what traits may offer alternatives for further genetic improvement.

Although this study was not set up for comparing landraces and improved clones, the fact that the latter show a relatively higher dry matter content (36.7%) compared with landraces (32.8%) is highly suggestive, as it is the reduced cyanogenic potential (267 versus 340 ppm). These two characteristics have been key breeding objectives over the years and the differences observed would reflect the efficiency of breeding approaches, and the responsiveness of cassava to them. In other words, heritability of these traits must have been appropriate for allowing these genetic improvements.

On the other hand, there have been no efforts to change starch characteristics (until now) in cassava breeding projects. The only successful approach in that area has been the genetic transformation to produce amylose-free cassava [33] and along with the discovery of amylose-free and high-amylose mutations [10,13] open up a new world of opportunities for the cassava and starch sectors. But the germplasm analyzed in this study was not affected by these recent discoveries. The fact that there is no much difference between averages for landraces and improved clones is not irrelevant. After four decades of genetic improvement of cassava some changes could have occurred if, in fact these parameters were negatively (or positively) and strongly correlated with agronomic performance. The absence of significant shifts in the averages for these traits, even if they were not selected criteria, would indicate that they are relatively neutral for agronomic performance. In other words, there is no indication that these traits have been selected for indirectly through any association between them and agronomic performance.

For some traits (water solubility, swelling power, paste clarity, paste breakdown, consistency, and setback) there is large range of variation. It is very interesting to examine the relationships of these attributes from data of all evaluated clones (eg breakdown versus swelling power). More investigation and comparison on starch structure of clones with a great difference in functionalities are of great interest.

2.2.3 Conclusion

Analysis of a large sample of cassava (including few wild relatives) has been conducted. The size of this analysis provides reliable information regarding average and ranges of distributions for the most relevant starch and root parameters. Average dry matter content was found to be 33.6% of which an average of 84.5% was starch. However average dry matter content of landraces and wild relatives, was lower (32.8%) compared with that of improved clones (36.7%). Average amylose content across the entire evaluation was 20.7% with no major difference between landraces and improved clones. Maximum viscosity was 777.5 cP, breakdown was 298.1 cP, consistency was 155.8 cP and setback was -144.5 cP. The large sample of starches analyzed provides very robust information regarding the actual characteristics of cassava starch. The ranges of variation for different variables suggest that there may be possibility for further improvements such as the one observed for dry matter content.

2.3 EVALUATION OF SELF-POLLINATED PROGENY FROM MATERIALS OF THE GERMPLASM COLLECTION

One of the major changes that cassava research at CIAT has had in the past few years is the systematic introduction of partial inbreeding throughout the germplasm to allow for the expression (and therefore identification) of recessive traits. Most of these recessive traits result in undesirable characteristics as it happens in other crops, but few offer interesting advantages. As a result of this systematic self-pollination and evaluation of the partially inbred S1 progenies significant discoveries have been made. In March 2006 an amylose-free mutations was discovered and reported in the prestigious Journal of Agricultural and Food Chemistry (13). That same month a second mutation was also identified. This mutation was identified in the self-pollinated progeny of a mutagenized population. The phenotype is characterized by a small granule size and higher-than-normal levels of amylose.

The report of this mutation was published last year also in the Journal of Agricultural and Food Chemistry (10). These discoveries are important not only because it allowed ciat to identify sources of high-value traits but also because it demonstrates that the technical approaches suggested by the cassava team at CIAT were sound. The predictions made several years ago regarding the hopes of identifying sources for high-value traits have now been totally confirmed and proven.

During 2008 a new generation of self-pollinated germplasm was evaluated in the field. It was very apparent in one family composed from 16 genotypes that the parental progenitor (MVEN 331) carried a recessive trait. Half of the 16 plants showed a distinctive feature: leaves without petioles. More over several of these plants had a unique and distinctive phenotype (**Figure 2.3**) with absence of branching (at least for the first 6-8 months of age).

The phenotype depicted in Figure 2.1 offers interesting commercial applications. The most immediate one would be for the production of dried cassava foliage. One of the bottlenecks for this new market for cassava is the costs involved in the harvesting of the foliage. The only practical approach is a mechanical harvest that would also carry a considerable amount of young stems and petiole tissue. It should be easy to envision a system whereas the kind of plants shown in Figure 1 are harvested and since there is no petiole, nor any branching the leaves could be easily peeled off the stem. The result would be a reduce cost of harvest and, because the reduced proportion of petiole and young stems, a better quality of the foliage with reduced fiber content. This last characteristic would be fundamental for the use of dried foliage in the composition of diets for the poultry industry.

A second important potential application of this mutation would be the possibility of drastically increasing plant densities in commercial planting of cassava. It should also be easy to accept the idea that this new plant type could allow for higher plant densities in cassava fields. Perhaps as many as 30,000 plants per hectare could be used. This concept is important because most of the genetic gains achieved through the last century relates to modifications in plant architecture. The use of semi-dwarf wheat and rice varieties lead to the highly successful green revolution. In the case of maize, if there is a single characteristic that can explain the consistent gains observed after the first introduction of commercial hybrids is reduced plant height with increased tolerance to higher plant densities. Today modern hybrid maize is planted at much higher densities than 40-50 years ago. So, this mutation observed in cassava may lead to a new plant type and, perhaps, a green revolution for this crop. It should be mentioned that the petioles mutation had been known in cassava for a long time.

There are few accessions in the collection with this trait (in addition to the progenitor which does not have the mutation in homozygous condition, MVE332 shows the petioles phenotype). What this mutation brings in is the a certainty of the genetic nature of this mutation, the reasonable hypothesis that it is the result of a single recessive mutation and, more importantly, the combination of the petioles trait with short plant height and non-branching architecture.

Figure 2.3 Illustration of a plant type mutation resulting in a petioles leaf and very erect architecture. This plant type offers interesting possibilities for the future of cassava.

Crosses have been or will be made among these mutant plant (in few of them there was a very late flowering) with those accessions in the germplasm collection that show the petioles trait to initiate a breeding with this gene pool characterized by the petioles, non-branching phenotype. These crosses will recover the vigor lost as a result of the inbreeding depression typical of S1 genotypes.

2.4 Field evaluation of the core collection

During the past few years as part of the strategy to evaluate and characterize the accessions for the germplasm collection the Core collection was evaluated in the three environments were the current evaluation is conducted as described in Section 2.1.

In **Table 2.9** the main results of the evaluation of the core collection across these environments are presented. Only the average for accessions from different countries can be presented. This is a summary of a very large data set that has been transferred to the Genetic Resources Unit to be incorporated into the information made publicly available on the collection.

Table 2.9. Averages of accessions evaluated in CETs in the sub-humid, acid-soil savannas and mid-altitude valleys environments.

Accession	FRY	FFY	H.I.	DMC	DMY	S.I.
Argentina	15,5	13,6	0,56	30,4	4,8	16,5
Bolivia	10,4	21,2	0,45	24,3	3,0	-13,4
Brasil	14,1	18,9	0,47	28,8	4,1	6,4
China	27,3	20,8	0,54	26,1	7,0	18,0
Colombia	10,5	19,5	0,42	28,3	3,0	-3,5
Costa Rica	8,6	20,3	0,33	27,6	2,3	-10,9
Cuba	12,2	17,1	0,46	27,9	3,5	0,6
Dominicana	15,2	19,8	0,50	31,2	4,8	12,3
Ecuador	7,2	18,8	0,32	26,8	2,0	-15,0
Fji	5,7	22,3	0,22	31,6	1,8	-7,3
Guatemala	13,2	22,1	0,37	27,4	3,8	-3,8
India	12,8	17,2	0,45	27,6	3,4	0,4
Malasia	10,1	22,5	0,36	29,1	3,0	-1,6
Mexico	14,3	20,2	0,42	27,6	4,1	0,3
Nicaragua	7,3	10,4	0,49	30,5	2,1	-2,5
Panamá	8,6	19,8	0,36	27,8	2,5	-9,2
Paraguay	10,0	10,1	0,52	28,6	2,9	1,8
Perú	13,2	19,2	0,43	25,4	3,5	-6,3
Philipinas	16,5	13,4	0,57	29,5	4,7	13,6
Puerto Rico	14,3	20,5	0,44	29,7	4,3	9,7
Tailandia	20,4	15,7	0,54	32,4	6,8	24,1
Usa	15,5	11,8	0,58	29,5	4,6	14,2
Venezuela	12,3	20,5	0,40	29,5	3,7	1,6
Landraces	**12,8**	**18,1**	**0,40**	**28,6**	**3,7**	**2,0**
Improved	**16,9**	**20,2**	**0,49**	**30,3**	**5,1**	**16,4**
All	**12,2**	**18,8**	**0,43**	**28,2**	**3,5**	**0,0**

FRY: Fresh root yield (t/ha); FFY: fresh foliage yield (t/ha); H.I. Harvest index (0-1); DMC: Dry matter content (%); DMY: dry matter yield (t/ha) and S.I.: selection index

It is interesting to note the improved performance of improved clones which yielded and average of 4 t/ha more than non-improved landraces. Harvest index improved considerably (from 0.40 to 0.49) and dry matter content is also higher (28.6 versus 30.3%).

2.5 References

[1] S. N. Moorthy: Tropical sources of starch, in *Starch in food* (Ed. A. C. Eliasson). CRC Press, Boca Ratón, Fl, 2004.

[2] J. E. Rickard, M. Asaoke, J. M. V. Blanshard: The physico-chemical properties of cassava starch. *Trop. Sci.* 1991, *31,*189-207.

[3] R. Hoover: Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate polymers* 2001, *45,* 253-267.

[4] Y. Baguma, S. Chuanxin, S. Ahlandsberg, J. Mutisya, S. Palmqvist, P. R. Rubaihayo, M. J. Magambo, T. G. Egwang, H. Larsson, C. Jansson: Expression patterns on the gene encoding starch branching enzyme II in the storage roots of cassava (*Manihot esculenta* Crantz). *Plant Sci.* 2003, *164,* 833-839.

[5] T. R. I. Munyikwa, S. Lageveld, S. N. I. M. Salehuzzaman, E. Jacobsen, R. G. F Visser: Cassava starch biosynthesis: new avenues for modifying starch quantity and quality. *Euphytica* 1997, *96,* 65-75.

[6] S. N. I. M. Salehuzzaman, E. Jacobsen, R.G. F. Visser: Isolation and characterization of a cDNA-encoding granule-bound starch synthase in cassava (*Manihot esculenta* Crantz) and its antisense expression in potato. *Plant Mol Biol* 1993, *23,* 947-962.

[7] K. Sriroth, V. Santisopasri, C. Petchalanuwat, K. Kurotjanawong, K. Piyachomkwan, C. G. Oates: Cassava starch granule structure-function properties: influence of time and conditions at harvest on four cultivars of cassava starch. *Carbohydrate Polymers* 1999, *38,*161-170.

[8] F. H. G. Peroni, T. S. Rocha, C. M. L. Franco: Some structural and physicochemical characteristics of tuber and root starches. *Food Sci. Tech. Int.* 2006, *12(6),*505-513.

[9] A. L. Charles, Y. H. Chang, W. C. Ko, K. Sriroth, T. C. Huang: Influence of amylopectin structure and amylose content on the gelling properties of five cultivars of cassava starches. *J. Agric. Food Chem.* 2005, *53,* 2717-2725.

[10] H. Ceballos, T. Sánchez, A. P. Tofiño, E. A. Rosero, K. Denyer, K., A.M. Smith, D. Dufour, N. Morante, J. C. Pérez, B. Fahy: Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). *Journal of Agric. Food Chem.* 2008, *56(16),* 7215-7222.

[11] I. Defloor, I. Dehing, J. A. Delcour: Physico-chemical properties of cassava starch. *Starch/Stärke* 1998, *50,* 58-64.

[12] A. Fernandez, J. Wenham, D. Dufour, C. C. Wheatley: The influence of variety and processing on the physicochemical and functional properties of cassava starch and flour *in Cassava flour and starch: progress in research and development* (Ed. D. Dufour, G. M. O'Brien, R. Best) CIRAD/CIAT Montpellier France/ Cali Colombia. 1996.

[13] H. Ceballos, T. Sánchez, N. Morante, M. Fregene, D. Dufour, A. M. Smith, K. Denyer, J. C. Pérez, F. Calle, C. Mestres: Discovery of an amylose-free starch mutant in cassava (*Manihot esculenta* Crantz). *Journal of Agricultural and Food Chemistry* 2007, *55(18),* 7469-7476.

[14] N. Zakhia, C. Wheatley, G. O'Brien, D. Dufour: Screening of CIAT cassava germplasm diversity: the relationship between cyanogenic potential and cassava starch functional properties, in *The cassava biotechnology network. Proceedings of the second international scientific meeting, 1994/08/22-26, Bogor, Indonesia* CIAT Document n°150, 823-830, 1995.

[15] E. Sudarmonowati, N. S. Hartati, L. Sukmarini: Amylose content variation of Indonesian cassava genotypes and its correlation with RAPD and AFLP markers, in *Proceedings of the First International Meeting on Cassava Breeding, Biotechnology and Ecology.* Brasilia, 2006.

[16] H. Ceballos, C.A. Iglesias, J. C. Pérez, A. G. O. Dixon: Cassava breeding: opportunities and challenges. *Plant Molecular Biology* 2004, *56,* 503-515.

[17] H. Ceballos, M. Fregene, Z. Lentini, T. Sánchez, Y. I. Puentes, J. C. Pérez, A. Rosero, A. P. Tofiño: Development and Identification of High-Value Cassava Clones. *Acta Horticulturae* 2006, *703,* 63-70.
[18] H. Ceballos, M. Fregene, J. C. Pérez, N. Morante, F. Calle: Cassava Genetic Improvement, in *Breeding Major Food Staples* (Ed. M. S. Kang, P. M. Priyadarshan). Blackwell Publishing. Ames, IA. 2007.
[19] H. Ceballos, T. Sánchez, A. L. Chávez, C. Iglesias, D. Debouck, G. Mafla, J. Tohme: Variation in crude protein content in cassava *(Manihot esculenta* Crantz) roots. *Journal of Food Composition and Analysis* 2006, *19,* 589-593.
[20] http://isa.ciat.cgiar.org/urg/main.do?language=es
[21] R. J. Segovia, A. Bedoya, W. Triviño, H. Ceballos, G. Gálvez, B. Ospina: Metodología para el Endurecimiento de 'vitroplantas' de yuca, in *La Yuca en el Tercer Milenio.* (Ed. B. Ospina H. Ceballos). CIAT, Cali, Colombia. 2002.
[22] H. Brücher: Useful plants of neotropical origin and their wild relatives. Springer-Verlag. Berlin and New York 1989.
[23] A. Allen-C. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). *Genetic Resources and Crop Evolution* 1994, *41,* 133-150.
[24] J. Aristizábal, T. Sánchez: Guia técnica para producción y análisis de almidón de yuca. Boletin de Servicios Agrícolas de la FAO. Roma, Food and Agriculture Organization of the United Nations. 2007.
[25] M. L. Prieto, C. E. Valencia: Obtención de un método patrón y desarrollo de un método rápido y práctico para la determinación del contenido de humedad en yuca (*Manihot esculenta* Crantz). Universidad del Valle and Universidad Nacional de Colombia. Degree Thesis. 1988, 101 pp (Available at CIAT, Apartado Aéreo 6713, Cali. Colombia).
[26] D. A. Cronin, S. Smith: A simple and rapid procedure for the analysis of reducing, total and individual sugars in potatoes. *Potato Research* 1979, *22(2),* 99-105.
[27] I. L. Batey: Starch analysis using thermostable a-amylases. Starch/Starke 1986, *34,* 125-128, modified by Mestres as reported in: C. Mestres Gélification d'amidons de maïs modifiés thermiquement. Application à la fabrication de pâtes alimentaires sans gluten. Ph. D. Thesis, France,1982.
[28] S. A. S. Craig, C. C. Maningat, P. A. Seib, R. C. Hoseney: Starch paste clarity, *Cereal Chem*, 1989, *66(3),* 173-182.
[29] ISO 6647 (F). Riz: Détermination de la teneur en amylose, 1987.
[30] C. Mestres, M. Nago, N. Akissië, F. Matencio: End use quality of some African corn kernels. Cooking behavior of whole dry-milled maize flours; incidence of storage. *J Agric Food Chem* 1997, *45,* 565-571
[31] S. A. J. A. Essers, M. Bosveld, R. M. Der VanGrift, A. G. J. Voragen: Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *J. Sci. Food Agric.* 1993, *63 (3),* 287 – 296.
[32] M. Bokanga: Distribution of cyanogenic potential in cassava germplasm, in *International Workshop on Cassava Safety.* IITA, Ibadan, Nigeria. *Acta Horticulturae* 1994, *375,* 117-123
[33] C. J. J. M. Raemakers, E. Sofiari, N. J. Taylor, G. G. Henshaw, E. Jacobsen, R. G. F. Visser: Production of transgenic cassava plants by particle bombardment using luciferase activity as the selection marker. *Mol. Breeding,* 1996, *2,* 339-349.

CHAPTER 3

DEVELOPMENT OF NEW GENETIC STOCKS AND IMPROVED GENE POOLS FOR THEIR EVALUATION IN KEY TARGET ENVIRONMENTS

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

3.1 SELECTION OF PROGENITORS BASED ON PREVIOUS CYCLE RESULTS AND INFORMATION FROM OTHER OUTPUTS (I.E., RESISTANCE/TOLERANCE, ROOT QUALITY TRAITS, ETC.)

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

The main objectives of this activity are: **1)** To identify, a set of elite clones, based on information from evaluation trials at several locations, and new objectives defined for the project. These clones are recombined to start a new cycle of selection. **2)** To include as progenitor, for each agro-ecological zone, at least one genotype with high-carotene, yellow roots; and **3)** To base the selection of parental lines increasingly on information from the performance of their progenies (≈ general combining ability or breeding value).

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, clones with outstanding performance for the most important agronomic traits are selected. After the analysis of results 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, landraces or already released cultivars that can contribute special features to the progenies generated are also included. Lately, thanks to the modifications

introduced to the evaluation process selection of parents is greatly affected by data of the progenies they produce (≈ general combining ability). It is envisioned that about 15-20% of the parental lines will be changed, eliminating those with poor general combining ability and introducing new clones that have had outstanding performance *per se* in *Advanced Yield Trials* to assess their breeding value. The information provided by pathologists, entomologists and quality specialists in relation to sources of resistance or special traits is used to select genotypes for controlled crosses. These controlled crosses are developed upon specific requests from National Programs that want their main landrace, or released varieties, crossed to genotypes with specific traits; or requests from CIAT scientists that want to pyramid genes, or develop segregating progenies for gene tagging.

As will be described below, one of the major changes introduced in the cassava breeding scheme at CIAT has been to take and record data on all progenies starting at the first evaluation stage (*Clonal Evaluation Trials*). The kind of information obtained allows a gross estimation of *general combining ability* (simply defined, it is the capacity of an individual to produce a good progeny) of parental lines employed in generating the clones included in those trials. This information is increasingly influencing the decisions of materials that will continue to be used as parents and those that will not. Significant changes were introduced during the 2002-2003 growing season by blocking the Clonal Evaluation Trials to reduce the large effects that the environmental variation within these large trials had on the average performance of each family. Basically these changes follow the ideas described by Gardner in 1961 for stratified phenotypic mass selection.

The parents selected for the development of gene pools targeted to specific ecosystems is presented in **Table 3.1.** The agronomic performance of these materials is described further down in this document. Seed will be harvested from July, 2008 through December, 2008. F1 plants will grow until the planting of the trials early in 2009. A major decision to take in the genetic improvement of crops is how to choose materials for use as parents that will produce new varieties with increased production potential and adequate adaptation to the environmental conditions under which they will be cultivated.

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

Table 3.1 list the clones selected as progenitors. 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*. The agronomic performance of some of these materials *per se* is also described. These tables also mention the parental lines for special purpose crosses. The seed produced from the current crossings will be harvested until December 2009.

Table 3.1. Parental lines to be used in crosses for different ecosystems, relevant for cassava production in the world.

Progenitors adapted to sub-humid environments.					
CM 9067-2	GM 273-57	SM 1511-6	SM 2615-25	SM 2769-11	SM 2782-4
CM 9560-1	GM 248-71	SM 1759-29	SM 2619-4	SM 2772-5	SM 2834-31
CM 9912-11	GM 259-167	SM 2081-34	SM 2619-12	SM 2773-32	SM 3061-31
CM 9913-11	GM 273-61	SM 2545-22	SM 2620-1	SM 2775-2	SM 3106-14
CM 9924-19	GM 451-36	SM 2546-32	SM 2621-29	SM 2779-56	MTAI 16
CM 9946-108	GM 521-26	SM 2546-40	SM 2629-36	SM 2780-17	
Progenitors adapted to acid-soil conditions					
CG 165-7	CM 9460-9	CM 9953-76	SM 2610-43	SM 2727-12	SM 2658-26
CM 1335-4	CM 9461-1	SM 1821-7	SM 2632-47	SM 2730-1	SM 2965-29
CM 2509-1	CM 9940-2	SM 1859-26	SM 2636-6	SM 2739-4	SM 2977-6
CM 2766-3	CM 9463-19	SM 2219-11	SM 2638-13	SM 2786-10	Cantaclaro 5
CM 4729-4	CM 9464-25	SM 2610-57	SM 2640-21	SM 2792-31	MCOL 638
CM 7596-5	CM 9474-42	SM 2601-44	SM 2642-35	SM 2852-5	SM 2658-26
Progenitors adapted to mid-altitude valleys					
CM 7951-5	GM 234-106	GM 297-89	SM 1965-1	SM 2860-10	SM 3047-19
CM 8370-11	GM 254-89	GM 374-32	SM 2052-4	SM 2864-17	SM 3087-3
CM 9733- 91	GM 265-4	GM 555- 3	SM 2058-2	SM 2869-9	SM 3090-8
CM 9903-107	GM 269-14	SM 1642-22	SM 2211-3	SM 2871-23	HMC 1
CM 9953-121	GM 294-24	SM 1855-15	SM 2858-31	SM 2913-4	MBRA 12

Planting materials were also selected from these parents to seed the **F1** in July 2008. In addition to crossing, some of these clones were also self-pollinated to begin an S_2 recurrent selection scheme to improve each of them for tolerance to inbreeding. The justification for this approach is given later when the description of a cassava-breeding scheme based on the production of doubled-haploids or partially inbred materials such as the activities described in the chapter "Cassava Genetic Improvement" by H. Ceballos, M. Fregene, J. C. Pérez, N. Morante and F. Calle**.**. **In:** Breeding Major Food Staples (M.S. Kang and P.M. Priyadarshan Eds.). 2007. p. 365-391, Blackwell Publishing. Ames, IA. USA.

3.2 ESTABLISHMENT OF CROSSING BLOCKS AND PRODUCTION OF RECOMBINANT SEED FROM PREVIOUSLY ESTABLISHED BLOCKS

Populations developed for specific ecosystems represent the basis for our cooperation with National Programs and **IITA** (International Institute of Tropical Agriculture, Ibadan, Nigeria). The development of genetic stocks is gaining importance through the years. Genetic stocks are produced based on the recombination of a set of genotypes that excel for a particular trait, and we would like to upgrade that trait beyond its natural range of variation (i.e. look for transgressive segregation in broader adaptation). Stocks developed for inheritance studies or to support molecular mapping of specific traits are constructed by the recombination of contrasting genotypes (i.e. resistance to CMV, 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. The specific objective of this activity is 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.

For polycrosses we use the design developed by Wright 1965 for polycrosses in forage species. For this type of design there is a need to have a number of clones equal to a prime number minus one (i.e. 12, 16, 18, etc.). The design allows for each genotype to have the same probability of being surrounded by any other genotype of the selected group. Knowledge on flowering capacity is important in order to select a group of materials with synchronized flowering. When there are considerable differences we have to implement delayed planting and/or pruning of the earliest flowering genotypes. At harvest the seed from different plants of the same genotype are combined together and named as a half-sib family (**SM**). For controlled crosses, we plant 10 to 20 plants depending on the flowering capacity of the genotype in question. The fruit developed from each flower has the potential to produce 3 seeds, but in average we obtain no more than 1 seed per pollination. This is due to the sensitivity of the stigma to the manipulation during pollination. Seeds from the same cross are mixed together and name as a full-sib family (**CM**). Because the number of CM families produced in the last few years has reached 10,000, we began utilizing a new code for full-sib families (**GM**).

Table 3.2. Production of recombinant cassava seed at CIAT, Palmira, Valle del Cauca, Colombia, between October 2007 and December 2008.

Purpose of crosses	**Controlled crosses**	**Polycrosses**	**Total**
Self-pollinations (S1)	688		
Self-pollinations (S2)	93		
High carotenoid content	2990	1521	
High carotenoid content x CMD	472		
High protein	3958		
High carotenoid content x high protein	151		
High protein x low cyanide	899		
Tolerance to PPD	910		
Tolerance to PPD x Low Starch (sugary?)	544		
Starch quality mutants	3008		
Seed for Tailand (TTDI)	17102		
Specific adaptation to:			
Sub-humid environment	298	4387	
Acid soil savannas	158	3256	
Mid-altitutde valleys	196	3121	

Total	**31467**	**12285**	**43752**

About 44,000 recombinant cassava seeds were produced at CIAT's Experiment Station, Palmira, between October 2007 and December 2008 (**Table 3.2**). Although the recombinant seed was produced at CIAT, the generated seedlings used to be transplanted to fields outside the Experiment Station and under conditions of isolation from other cassava crops. Thus, the generated **F1** plants grew and were maintained under conditions where possibilities of contamination from frogskin disease were minimized. This strategy, as can be seen in the description of results from different **Clonal Evaluation Trials**, has been 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 phytoplasm, is not likely to be transmitted through botanical seed.

3.3 GENERATION AND DISTRIBUTION OF ADVANCED BREEDING MATERIALS FOR NATIONAL PROGRAMS

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

For Africa, our breeding efforts have been traditionally channeled through our collaboration with the International Institute of Tropical Agriculture (**IITA**) in Nigeria. As a result extensive germplasm with Latin American "blood" has been introduced to Africa in a long introgression project financed by the International Fund for Agriculture Development (**IFAD**). The purpose of this special project was, among several others, to introgress Latin American cassava germplasm into Africa, in order to increase the genetic base of the crop in that continent, particularly for drought tolerance. This introgression process requires crosses to combine the desirable traits of Latin American germplasm, with resistance to the African Cassava Mosaic Virus (**ACMV**) disease. More recently, with resources provided by the Rockefeller Foundation the introgression of new genetic variability for cassava breeding in Africa has focused in Eastern Africa (particularly in Tanzania)

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. We have benefited from the availability of molecular markers to select "embryo-rescued" tissue cultured germplasm that had been already been selected for the presence of the marker related to CMD. This is only for the materials

developed for their shipment, introduction and evaluation in Africa in collaboration with IITA and/or NARs. In this process we have also benefited from the valuable contribution of IITA who kindly provided cassava germplasm carrying new, dominant and effective sources of resistance to the virus.

A considerable fraction of the seed produced by the project has been transferred to National Programs in different regions of the world. As shown in **Table 3.3**, In the future, we foresee that the flux of improved germplasm between CIAT-HQ, and the Thai and other Asian breeding programs will continue, and it will be through CIAT that other National Programs will receive progenies involving the latest selections of elite germplasm from Asia.

Table 3.3. Shipments of cassava germplasm to collaborators in Africa, Asia and Latin America and the Caribbean regions, as well as to advanced laboratories in Asia, Europe and/or North America (Januray – December 2008).

Continents	Genotypes in-vitro	Crosses (families)	Plants (in-vitro)	Seeds in the shipment
Latin America				
In-vitro	456		2010	
Hybrid seed		23		4234
Asia				
In-vitro	12		442	
Hybrid seed		55		17522
Africa				
In-vitro	2055		10035	
Hybrid seed		992		50518
Europe + USA				
In-vitro	118		688	
Total				
In-vitro	**2641**		**13175**	
Hybrid seed		**1070**		**72274**

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, the project has set up a tissue culture laboratory that produces large quantities of vitroplants for our colleagues. The Genetic Resources Unit previously carried out this activity but the number of clones to be produced and shipped far exceeds the capacity and function of that Unit. 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. A total of 2641 genotypes were shipped during the past year as in vitro plantlets.

3.4. SELECTION OF RECOMBINANT PROGENIES FOR BROAD AND SPECIFIC ADAPTATION WITHIN MAJOR AGRO-ECOSYSTEMS

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 3.4**. 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.

The specific objective of this activity is to develop and evaluate superior germplasm adapted to particular ecosystems and develop genetic stocks useful for other CIAT projects as well as for our collaborators in Asia, Africa and Latin America and the Caribbean Regions.

For each of the zones we conduct a recurrent selection program, with a progressive set of stages as described in **Figure 3.1.** 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).

The current evaluation system is described in **Figure 3.1**. This scheme has incorporated several modifications implemented and tested over the years. The main objective of such modifications were to reduce the selection of materials based on single-plant and/or non-replicated evaluations and to obtain data in the first selection stage as an approximation to the general combining ability of the parents used in the crossing nurseries. This information, in turn is used to decide what parents can continue for another cycle in the breeding nurseries as elite parents or dropped because of the poor performance of the progeny it produces. A description of the advantages of such a method has been published (Ceballos et al., 2004).

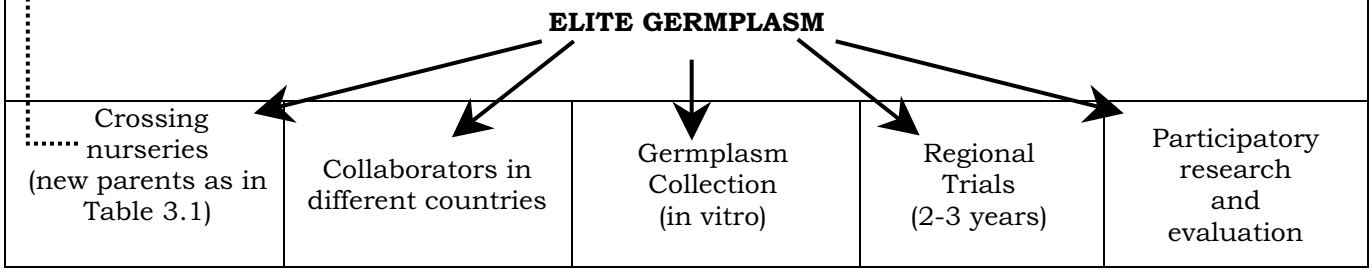
One important modification introduced at the clonal evaluation trials or **CETs** follows the idea of stratification suggested by Gardner in the 1960s. The field for the **CET** (usually a large field 1-2 ha) is divided in three “blocks” of about equal size. All the clones from a given family are then randomly allocated to one of these “blocks”. This modification allows for a replicated presence for each family. The individual clones, of course, cannot be replicated. On the other hand, the family means are based on three replications and therefore, more precisely estimated. Selection of individual clones was done within each “block”, following the ideas behind stratified mass selection proposed by Gardner in the 1960s.Therefore, to a certain extent selection of individual clones is more precise. The increase in precision is inversely proportional to the variation between the conditions in each of the three “blocks”.

Table 3.4. Main ecosystems for cassava production, representative production regions, and main breeding sites. Our efforts currently contenctrate on the sub-humid tropics, acid-soil savannas and mid-altitude valleys.

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

masl; meters above sea level

Time		Number of genotypes	Plot size (# of plants)	Number of Replications	Number of Locations
	Crossing of selected parental genotypes	Around 80 elite clones (see **Table 3.1**)			
10	**F1**	3000-5000	1	1	1
22	Clonal evaluation trial (**CET**)	1000-2000	7-8	1§	1
34	Preliminary yield trial (**PYT**)	100-300	10	3	1
46-58	Advanced yield trial (**AYT**)	40-100	20-25	3	2-3



¶ Time in months after germination of botanical seed.
§ One replication for clones within each "block" but three replications for families.

Figure 3.1. Basic scheme used for the evaluation and selection of segregating progenies. The number of genotypes varies according to year, general performance of germplasm, quality of data and experiments. Each year similar schemes are used for different environments.

Preparing new F1 field

About 19332 recombinant, botanical seeds were germinated early in 2007, and approximately 12169 of the resulting plantlets were transplanted at CIAT Experimental Station in Palmira (**Table 3.5**). This material represents the F1 stage described in **Figure 3.1**.

3.5 THE USE OF SELECTION INDEX

A Selection Index integrating the most relevant variables is used to facilitate selection in different trials. To avoid the problems related to the magnitudes used to measure different variables, the index is constructed using standardized deviation units (Steel and Torrie, 1960). As an example the typical selection index used for the Acid Soil Savannas environment of Colombia is presented below:

$$SI = (FRY * 10) + (DMC*8) + (HI*5) - (PT\ 3) - (SED3)$$

where SI is the selection index; FRY = fresh root yield; DMC = dry matter content; HI = harvest index; PT = rating for plant type or architecture; and SED = rating for super elongation disease. The relative importance of each trait is weighted, as shown in the formula above, by a subjective assessment by the breeder. Negative signs are used for those variables where lower values represent most desirable phenotypes. Harvest index has been consistently favored as one relevant variable to be included in early stages of selection such as CET trials (Kawano et al., 1998). Plant architecture also plays an important role in early stages of selection (Hahn et al, 1979). Since the SI is estimated using the standardized values, a positive SI means a performance better than the average, while a negative one means a poor performance.

Table 3.5. Cassava seed processed for producing F1 plants for various purposes at CIAT, Palmira, Valle del Cauca, Colombia. F1 nursery was transplanted in June 2007.

Purpose of cross	Germinated seed	Transplanted Seed
Sub-humid environments	4584	3568
Acid-soil savannas	3295	2221
Mid-altitude valleys	2952	1628
High carotenoids	3091	2175
High protein	1352	705
Starch quality traits	1578	540
Self pollinatios (Germoplasm collection)	2480	1332
Total	**19332**	**12169**

3.6 REFERENCES

Ceballos, H., Iglesias, C. A., Pérez, J. C. and Dixon, A.G.O. 2004. Cassava breeding: opportunities and challenges. Plant Mol. Biol. 56:503-515.

Ceballos, H., M. Fregene, J. C. Pérez, N. Morante and F. Calle**.** 2007. Cassava Genetic Improvement. **In:** Breeding Major Food Staples (M.S. Kang and P.M. Priyadarshan Eds.). p. 365-391, Blackwell Publishing. Ames, IA. USA.

Gardner, C.O. 1961. An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yields of corn. Crop Sci. 1:241-245.

Hahn, S.K., Terry, E.R., Leuschner, K., Akobundu, I.O., Okali, C. and Lal, R. 1979. Cassava improvement in Africa. Field Crops Research 2: 193-226.

Kawano, K., Narintaraporn, K., Narintaraporn, P., Sarakarn, S., Limsila, A., Limsila, J., Suparhan, D., Sarawat, V. and Watananonta, W. 1998. Yield improvement in a multistage breeding program for cassava. Crop Sci 38 (2): 325-332.

Steel, R.G.D. and Torrie, J.H. 1960. Principles and procedures of statisitics. McGraw-Hill Book Company. New York. USA., pp 39-40.

Wright, C.E. 1965. Field plans for a systematically designed polycross. Record of Agricultural Research 14: 31-41.

CHAPTER 4

DEVELOPMENT OF GENETIC STOCKS AND IMPROVED GENE POOLS ADAPTED TO THE SUB-HUMID ENVIRONMENTS.

This output relates to the efforts directed to the creation and identification of germplasm adapted to the sub-humid environment found in Colombia's Caribbean coast. The environment is characterized by a long dry spell without rains (January through April), low fertility soils and the common severe problems of pests (particularly thrips and different mite species). There are no major problems with diseases although super-elongation disease may because some occasional damage.

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, **Table 4.1** lists the most relevant trials, whereas the other tables show results specific to each one.

As mentioned in the previous Chapter (**Table 3.5**) a total of 4584 seeds were germinated and 3568 seedlings from these botanical seeds (targeting this particular environment) were transplanted at CIAT-Palmira in an isolated field. The planting of the current *F1* stage is isolated to reduce as much as possible infection by diseases that can be found at later stages of the evaluation process. Seedlings from botanical seed are considered to be disease-free and efforts are made to maintain this condition for as long as it can possibly be done. The germinated seedlings were then transplanted to the field were a total of 2105 plants survived. In April 2008 these plants will be harvested and selections made for those genotypes capable of producing at least 8 vegetative cuttings to plant the *Clonal Evaluation Trial*, which will be grown from April-May 2008 through March 2009 (see **Figure 3.1**). This *CET* will be planted in the Atlántico Department.

In June 2007 a new *Clonal Evaluation Trial (CET)* was planted in the Atlántico Department with a total of 1302 genotypes. *CETs* are large experiments around one hectare in size. A major constraint in their evaluation is the experimental error associated with the unavoidable variation in environmental conditions in such a large experimental plot. Because this is the first evaluation and selection stage (See Chapter 3) only 8 stakes are available from each genotype. Replication of each clone, therefore, is difficult to implement. On the other hand clones are grouped in either full- or half-sib families. Since many clones are generally available from each family they are randomly allocated in one of three blocks in which the field is divided. In other words instead of planting all the clones from a given family together one after the other, they are split in three groups, which are planted in the three blocks the entire evaluation is divided into (**Figure 4.1**). This approach allows for two interesting

advantages:

a) There is a replication effect for the families because all the clones from a given family are scattered in three "repetitions" in the field. The averages from all these clones are less affected by the environmental variation in such a large experiment.

b) Selection is made within each block. This is similar to the stratified mass selection suggested by Gardner (See Activity 3.1, page 3.2). This approach effectively overcomes the environmental variation that can be measured by comparing the means of each block.

Table 4.1. Trials conducted in the sub-humid ecosystem (North Coast of Colombia) in the 2007-2008 cycle.

Trial	**Location**	**Genotypes (# plants/rep)**	**Reps**
Trials conducted in Atlántico Department			
Clonal Evaluation Trial	Santo Tomás	1302	1
Preliminary Yield Trial (*PYT*-1)	Santo Tomás	60	3
Preliminary Yield Trial (*PYT*-2)	Santo Tomás	60	3
Preliminary Yield Trial (*PYT*-3)	Santo Tomás	60	3
Preliminary Yield Trial (*PYT*-4)	Santo Tomás	40	3
Preliminary Yield Trial (*PYT*-5), Core collection	Santo Tomás	54	3
Advanced Yield Trial (*AYT-I*)	Santo Tomás	80	3
Advanced Yield Trial (*AYT-II*)	Santo Tomás	35	3
Regional Trial *(RT-I)*	Santo Tomás	36	3
Regional Trial *(RT-II)*	Baranoa	30	3
Additional *AYT* Clones	Santo Tomás	15	3
Special selection trial	Santo Tomás	30	3
Trials conducted in Sucre Department			
Results from the Regional Trial *(RT-I)*	La Unión	30	3
Regional Trial *(RT-II)*	Toluviejo	30	3
Regional Trial *(RT*-II)	Sincelejo	30	3

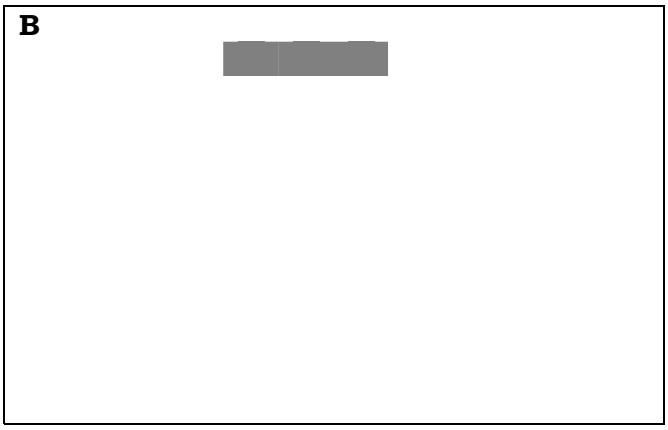


Figure 4.1. Advantage of splitting each family of clones in three groups that were randomly assigned to each of three blocks in the *CET*. (A= current procedure; B= previous situation). Because all the clones from the *CET* were divided, the average performance of each family were more precisely estimated, since each family was scattered in three different parts of the field, whereas before it was concentrated in just one sector (**Figure 4.1**). As a consequence, the estimates of GCA for each family are much more precise.

Tables 4.2 and 4.3 describe the most relevant results of *CET* from which the analysis of progenitors described in **Tables 4.4 and 4.5** were made. **Table 4.2** presents the results of the three blocks in which *CET* was divided. Maximum, minimum, average and standard deviation values for all clones evaluated and for the selected group are provided. Differences in the average performance of all the clones in each block give an idea of the variation in the field that has been controlled by splitting the *CET* into the three blocks. Average fresh root yield were 17.4, 16.1 and 15.5 t/ha respectively for Blocks 1, 2 and 3. If stratification of the *CET* were not made the tendency would have been to select clones located in the first block, and reject those in the third block. The stratification, however, eliminated this tendency. The average fresh root yield (FRY) of selected clones in Block 1 was 26.6, with an average dry matter content (DMC) of 35.9%, which yielded an average of 9,5 t/ha of dry matter (DMY). The average FRY of selected clones in Block 2 was 23.9, with an average DMC of 35.8%, which yielded an average of 8.5 t/ha of DMY. For Block 3 the average FRY was 24.9, with an average dry matter content of 34.7%, which yielded an average of 8.6 t/ha of dry matter.

Table 4.3 presents the performance of the best ten clones of each of the three blocks in *CET*. There were families that had several clones among the best ten mentioned in **Table 4.3**. Families GM 1100 and SM 3256 were represented by three clones scattered in Blocks 1, 2 or 3. Two clones represented families GM 1089; GM 11340; SM 3252, SM 3257, SM 3260, SM 3261, and SM 3265. In general the performance of these outstanding genotypes is excellent with average dry matter yields frequently above 10 t/ha. A matter of concern in the same type of trial harvested in the 2006-2007 season was the relatively low dry matter content observed in these progenies. Because of our interest in preserving planting material in the evaluation process, harvest takes place at the end of the dry season, just prior to the initiation of the rains. This strategy, unfortunately and unavoidably, exposes the trials to some early rains and most likely the low DMC observed in these trials is the result of plants starting to re-initiate their growth taking advantage of these early rains. In the future trials will be harvested two week earlier. Whereas in 2006-2007 average dry matter contents ranged between 27 and 28% the earlier harvest taking place during the 2007-2008 season drastically increased dry matter contents to desirable levels (ranging from 32 to 33%).

Tables 4.4 and 4.5 provide valuable information that consolidates all the performances of genotypes evaluated in blocks 1, 2 and 3 from *CET*. **Table 4.4** presents all the families involved in such trial. Each family, as explained above, is divided in three groups of genotypes of about equal size. Each group is then randomly allocated to blocks 1, 2 or 3 in the field. After harvest data of the three groups of each family is combined together for the information presented in **Table 4.4**. For instance the first family (GM 943) was represented by 12 clones. These 12 clones were equally divided in a group of 4 clones that went to Block 1. Another group of four clones went to Block 2 and the last group of four clones went to Block 3. As it can be seen, five of these 12 clones were selected: a much higher success rate (42%) that the average of the entire trial (16%). A simple way of assessing the value of each family is the average selection index. An index of around cero means that the family had an average performance. Positive selection indexes mean above average performance, the higher

the more outstanding. Negative selection indexes (as in the case of this first family) imply a below average performance.

Table 4.2. Results from the *Clonal Evaluation Trial* (*CET*) divided into three blocks and conducted in Santo Tomás (Atlántico Department). Statistics of the 68 clones selected in each block and all the clones evaluated in each block are presented. Evaluation was conducted using single-row plots with eight plants per genotype.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	t/ha	%	(%)
Block 1: Parameters of 68 selected clones (≈15.00%)							
Maximum	4	35,9	39,4	0,72	12,6	42,5	46,6
Minimum	1	15,5	12,0	0,42	6,1	31,3	19,5
Average	2	26,6	21,6	0,55	9,5	35,9	27,8
St. Deviation	1	4,5	5,7	0,06	1,4	2,1	6,0
Block 1: Parameters of 425 clones evaluated							
Maximum	5	35,9	48,8	0,72	12,6	43,5	46,6
Minimum	1	0,5	0,6	0,09	0,2	22,1	-76,0
Average	3	17,4	20,4	0,45	5,9	33,4	0,0
St. Deviation	1	7,1	7,4	0,10	2,5	3,2	20,5
Block 2: Parameters of 68 selected clones (≈15.00%)							
Maximum	5	37,8	35,5	0,67	12,8	39,8	46,12
Minimum	1	15,9	8,1	0,42	6,1	30,0	18,99
Average	2	23,9	20,2	0,55	8,5	35,8	26,15
St. Deviation	1	4,6	6,2	0,06	1,5	2,1	6,52
Block 2: Parameters of 422 clones evaluated							
Maximum	5	37,8	43,3	0,7	12,8	39,8	46,12
Minimum	1	2,7	0,6	0,0	0,6	17,3	-81,23
Average	3	16,1	18,2	0,5	5,4	33,0	0,00
St. Deviation	1	6,4	7,0	0,1	2,3	3,5	20,29
Block 3: Parameters of 68 selected clones (≈15.00%)							
Maximum	5	43,4	70,2	0,7	14,7	38,6	57,47
Minimum	1	14,1	8,9	0,4	5,4	28,2	19,77
Average	3	24,9	22,8	0,5	8,6	34,7	27,49
St. Deviation	1	6,4	10,4	0,1	2,0	2,1	6,87
Block 3: Parameters of 455 clones evaluated							
Maximum	5	43,4	70,2	0,7	14,7	38,6	57,47
Minimum	1	0,8	2,7	0,1	0,2	17,9	-67,99
Average	3	15,5	18,9	0,4	5,1	32,4	0,00
St. Deviation	1	6,7	8,1	0,1	2,3	3,2	19,87

Table 4.3. Results from the best ten clones per block in the Clonal Evaluation Trial (*CET*) ranked according to their selection index values, Santo Tomás (Atlántico). Harvested in March, 2008. Evaluation was conducted using single-row plots with eight plants per genotype.

Clon	Mother	Father	Plant Type	Yield (t/ha)		Harvest	Dry matter		Selection
			(1-5)	Root	Foliage	Index	t/ha	%	Index
Block 1									
SM 3252-3	CM 9067-2		1	33,8	19,4	0,64	12,3	36,4	46,6
SM 3260-8	SM 1759-29		1	31,6	19,1	0,62	11,6	36,7	44,0
SM 3256-15	SM 1511-6		2	33,8	13,0	0,72	11,8	34,8	43,1
SM 3254-9	SM 1427-1		3	33,1	27,0	0,55	12,6	38,2	41,8
GM 1100-8	CM 7514-8	CM 9067-2	2	35,9	20,6	0,64	12,3	34,3	40,2
SM 3256-7	SM 1511-6		3	20,3	22,7	0,47	8,6	42,5	33,7
SM 3265-1	SM 2629-36		2	24,7	14,1	0,64	9,2	37,2	33,7
SM 3256-18	SM 1511-6		2	28,6	20,6	0,58	10,3	36,1	33,2
GM 1155-7	CM 8475-4	SM 2782-4	1	27,5	23,4	0,54	10,0	36,3	33,1
SM 3260-13	SM 1759-29		2	29,2	20,5	0,59	10,4	35,7	33,1
Block 2									
GM 1089-13	CM 6758-1	SM 1521-10	2	37,8	24,1	0,61	12,8	33,7	46,1
GM 1067-22	CM 4365-3	CM 9067-2	3	31,6	20,6	0,60	11,9	37,6	44,2
GM 1132-5	CM 8027-3	SM 1973-25	3	30,8	34,2	0,47	11,9	38,6	39,7
GM 1130-5	CM 8027-3	SM 1656-7	3	26,4	15,5	0,63	10,1	38,3	39,4
GM 1089-9	CM 6758-1	SM 1521-10	2	29,5	15,2	0,66	10,3	34,8	38,7
GM 1208-6	SM 1433-4	SM 1650-7	2	32,2	31,3	0,51	11,5	35,9	38,7
GM 1130-8	CM 8027-3	SM 1656-7	1	28,9	27,7	0,51	10,4	36,0	37,0
GM 1067-28	CM 4365-3	CM 9067-2	2	27,5	25,3	0,52	10,3	37,3	36,1
GM 1100-24	CM 7514-8	CM 9067-2	1	27,3	20,2	0,58	9,6	35,3	35,6
SM 3259-17	SM 1669-7		3	30,8	20,8	0,60	10,8	35,1	35,5
Block 3									
SM 3267-20	MTAI 16		3	41,3	24,8	0,62	14,7	35,6	57,5
SM 3262-48	SM 2081-34		2	32,7	27,2	0,55	11,7	35,9	44,9
SM 3265-19	SM 2629-36		4	33,6	27,3	0,55	12,3	36,5	42,4
SM 3261-30	SM 1778-45		3	34,5	31,4	0,52	11,8	34,2	38,3
GM 1100-37	CM 7514-8	CM 9067-2	3	30,5	23,9	0,56	10,7	35,2	37,1
SM 3257-40	SM 1521-10		1	24,4	18,8	0,57	8,7	35,8	36,3
SM 3261-36	SM 1778-45		3	36,9	39,4	0,48	12,2	33,0	36,0
SM 3252-36	CM 9067-2		3	28,1	18,6	0,60	9,9	35,3	36,0
SM 3257-32	SM 1521-10		1	26,6	23,9	0,53	9,4	35,2	35,9
SM 3266-3	SM 2782-4		5	38,6	55,8	0,41	13,4	34,8	34,8

Families GM 943, GM 1067, GM 1081, GM 1082, GM 1100, GM 1130, GM 1132, GM 1148, SM 3252, SM 3257, SM 3258 and SM 3265 had excellent average selection indexes and higher than average selection within their respective progenies. However few of them (for example GM 1081 and GM 1082) were represented by few clones (three or two) so the assessment for their overall performance should be taken with caution. Similarly, families GM 1088, GM 1093, GM 1094, GM 1198, GM 1203 and GM 1232 were characterized by a

generally unacceptable performance with negative average selection indexes and no or few proportion of their clones selected.

Table 4.4. Family size, number of selected clones in each family and average selection index values for the progenies evaluated in *CET*. Data combines results from the three blocks in which the trial was divided.

Family	Size	Selected	Sel. Index	Family	Size	Selected	Sel. Index
GM 943	12	5	13,2	GM 1191	7	1	0,0
GM 1067	44	10	7,0	GM 1195	13	0	-4,6
GM 1069	17	0	-3,7	GM 1198	21	0	-8,3
GM 1070	24	4	-3,4	GM 1201	17	0	-6,9
GM 1072	10	0	2,6	GM 1203	33	3	-10,3
GM 1077	22	2	1,7	GM 1208	10	3	6,2
GM 1079	4	0	-2,9	GM 1209	19	1	-2,5
GM 1081	3	1	10,8	GM 1229	31	3	1,3
GM 1082	2	0	11,9	GM 1232	17	0	-11,9
GM 1084	6	1	2,3	GM 1238	3	0	-1,3
GM 1088	40	0	-9,5	GM 1245	15	2	5,1
GM 1089	20	4	3,2	SM 3252	42	14	9,6
GM 1092	38	6	-5,7	SM 3253	32	1	-4,4
GM 1093	21	0	-2,6	SM 3254	56	11	2,3
GM 1094	15	0	-1,9	SM 3255	42	5	-6,5
GM 1100	55	20	9,9	SM 3256	54	12	5,4
GM 1106	9	2	6,9	SM 3257	45	10	6,1
GM 1109	15	3	-6,4	SM 3258	22	7	6,2
GM 1129	25	2	-5,7	SM 3259	39	2	-3,4
GM 1130	11	4	9,7	SM 3260	44	9	3,2
GM 1132	10	4	11,9	SM 3261	41	8	-3,0
GM 1141	4	0	-5,5	SM 3262	62	3	-6,3
GM 1144	2	0	6,7	SM 3263	53	6	-2,9
GM 1148	11	4	6,2	SM 3264	46	4	-3,7
GM 1155	25	5	2,2	SM 3265	20	9	9,5
GM 1161	19	3	3,8	SM 3266	4	1	2,7
GM 1181	1	0	-2,7	SM 3267	21	7	4,7
GM 1182	28	2	-6,1	**Total/Mean**	1302	204	-0,022

As explained in chapter 3 during the crosses among elite germplasm a given elite clone may be used more than once in the production of new segregating families. **Table 4.5** summarizes the result of all the progenies derived from each progenitor whose progenies had been evaluated in the *CET* during the 2007-2008 season. For example, CM 8067-2 was used as progenitor in as many as seven families, whereas SM 2929-6 was used only once. The information provided in **Table 4.5** is an excellent approximation to general combining ability effects and is directly related to the breeding value of each progenitor. Progenitors SM2629-6, CM9067-2, CM7514-8, SM1669-5, MTAI16, CM8027-3 and SM1656-7 had an outstanding performance as evaluated through their respective progenies, who tended to have higher than normal success rates. In contrast progenies from parental clones SM2619-4, SM1669-7, SM1565-17, CM9560-1, SGB765-2 and SGB765-4 had a below-average performance, low

selection proportion and generally low to negative selection indexes, suggesting poor breeding values for these parents.

Table 4.5. Relative performance of progenitors involved in generating the progenies evaluated in *CET*. The performance of the progenitors is assesses through the average performance of all the progenies each progenitor produced.

Progenitor	# fam.	Selec. (%)	Pl. Type (1-5)	FRY (t/ha)	H.I. (0-1)	DMC (%)	DMY (t/ha)	Sel. Ind.
SM2629-6	1	45,0	3	21,3	0,48	33,1	7,1	10,1
CM9067-2	7	25,8	3	17,3	0,49	34,3	5,9	7,2
CM7514-8	3	31,6	3	18,1	0,48	33,9	6,2	6,5
SM1669-5	1	31,8	3	17,9	0,47	33,8	6,1	6,3
MTAI 16	1	33,3	3	18,7	0,45	33,6	6,4	4,7
CM8027-3	4	25,9	4	18,2	0,48	33,5	6,3	4,5
SM1521-10	3	17,7	3	20,0	0,50	31,3	6,2	4,0
SM1511-6	3	16,9	3	16,7	0,47	33,7	5,7	3,2
CM8475-4	4	21,4	3	16,9	0,43	33,6	5,8	2,2
CM4365-3	5	14,7	3	14,3	0,44	34,8	5,0	1,9
CM6754-8	6	9,8	3	15,9	0,46	33,5	5,3	1,4
SM1650-7	3	13,3	3	15,4	0,43	34,0	5,3	0,8
SM1759-29	2	19,1	3	14,9	0,46	33,7	5,1	0,8
SM1433-4	3	12,9	3	18,9	0,42	32,1	6,1	0,4
SM1427-1	2	15,5	3	15,4	0,45	33,4	5,2	-0,5
SM1656-7	3	20,5	3	18,3	0,44	32,2	6,0	-0,6
SM2546-32	3	9,4	3	16,5	0,49	31,7	5,3	-1,5
SM1637-22	2	10,0	4	15,6	0,50	32,5	5,2	-1,6
SM2619-4	5	6,5	3	13,5	0,46	33,8	4,6	-2,0
SM1973-25	2	12,9	4	15,5	0,46	33,2	5,3	-2,0
SM1778-45	1	19,5	3	15,7	0,41	33,0	5,5	-2,7
SM1669-7	1	5,1	3	15,6	0,45	32,5	5,1	-3,4
SM1565-17	3	5,9	3	19,7	0,49	29,5	5,8	-3,7
SM2782-4	5	15,1	3	15,9	0,43	32,5	5,2	-3,9
CM9560-1	1	3,1	3	14,8	0,46	31,9	4,8	-4,3
CM6758-1	5	7,5	3	14,4	0,44	32,7	4,8	-4,5
SM2081-34	3	8,4	3	15,7	0,42	31,8	5,1	-5,5
SGB765-2	2	6,9	4	15,1	0,44	32,5	5,0	-5,8
SGB765-4	7	6,3	4	14,2	0,42	32,8	4,7	-6,4
SM1438-2	1	11,9	3	14,4	0,42	32,4	4,8	-6,5

Genotypes that were selected in *CET*s during the 2006-2007 were evaluated in *PYT*s during the 2007-2008 season. Each genotype in a *PYT* is planted in three replications. Experimental units (plots) have two rows each with five plants for a total of ten plants per plot. Separation between rows within a plot is only 80 cm. On the other hand, a row is left empty to separate rows belonging to different plots. Therefore, plots are separated by 160cm space (Figure 4.2). This strategy is followed to favor within-family competition and not as much between-family competition. One of the concerns that breeders have when evaluating cassava genotypes in this kind of trial is that tall, vigorous genotypes (not necessarily the best and preferable ones)

tend to compete more favorably against smaller short plant types. This competition is not desirable.

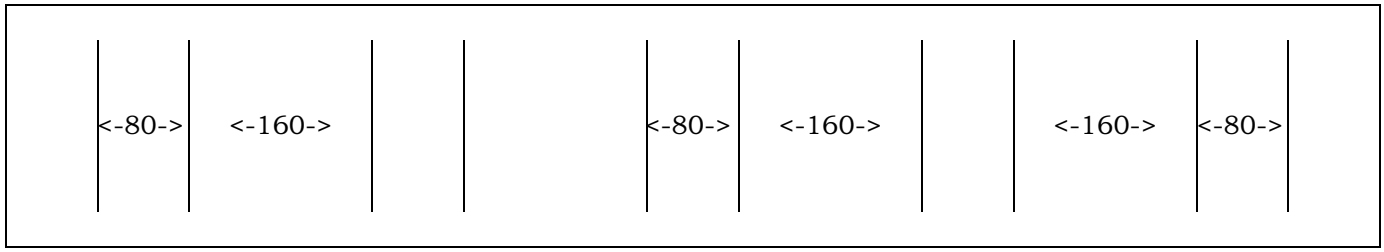


Figure 4.2. Illustration of the row separation within plots (80 cm) and between plots (160 cm) typically used in Preliminary Yield Trials (*PYT*s)

During the 2006-2007 season, two different type of *CET*s were conducted. In one case (*CET*-1) the standard 1 row with eight plants was used. In the second type of trial (*CET*-2) two replications with four plants each were used. Comparison of results from *CET*-1 and *CET*-2 were irrelevant at that stage because they had been physically planted in different plots. However, we were interested in learning the relative efficiency of each method of analysis later when comparisons in the same trial could be made. *PYT*s presented in **Tables 4.6, 4.7 and 4.8** combine progenies from *CET*-1 and *CET*-2 of the 2006-2007 season. It is recognized that the clones evaluated in these *CET*-1 and *CET*-2 were not the same, although their origin arose from the same families. It is assumed however, that progenies from each family planted in *CET*-1 and *CET*-2 had equal probabilities of being agronomically superior. If the progenies from *CET*-2 consistently provide a better average performance in the *PYT* compared with those from *CET*-1 it would suggest that the selection based on two replications of four plants each was more efficient in identifying better performing clones than the traditional system used in *CET*-1 (one replication with eight plants).

Table 4.6 presents the result of the first *PYT* evaluation. A total of 60 clones were evaluated (30 from *CET*-1 and 30 from *CET*-2). Commercial checks included in *PYT-1* were MTAI 8, Corpoica-Ginés and Corpoica-Verónica, who were ranked in the 1st, 12th and 27th, respectively. In this, excellence performance of new experimental clones is highlighted. Very high dry matter contents (above 35% in many cases) highlight the increased emphasis in this variable. Average dry matter yield of the 18 selected clones was 7.5 t/ha and the average dry matter content was above 35%. At the bottom of **Table 4.6** the averages of the 30 clones derived from *CET*-1 (1 rep with eight plants) and the 30 clones derived from *CET*-2 (2 reps with four plants each) are presented. Expectations are that averages would not differ significantly. Expectations were that if there was to be a clear difference would be in favor of clones selected from *CET*-2. However our results in this trial would suggest that it is not even convenient to complicate the clonal evaluation trials dividing them into two replications.

Table 4.7 presents the result of *PYT-2* evaluation. A total of 60 clones were evaluated (30 from *CET*-1 and 30 from *CET*-2). The same commercial checks were included in *PYT-2* but their ranking changed rather drastically. Corpoica-Ginés, MTAI 8, and Corpoica-Verónica, ranked in the 12th, 23h and 30th, respectively. This indicates that most selected clones were

superior to the three commercial checks. MTAI8 which had excellent performance in *PYT-1* had an slightly better than average performance in this *PYT-2*. The new experimental clones showed high dry matter contents (above 35% in many cases). Average dry matter yield of the 18 selected clones was 8.6 t/ha and the average dry matter content was above 35.7%. The changes in the performance of commercial checks, the only material that is evaluated simultaneously in different trials highlight the high frequent environmental influence on the performance of cassava. A problem that is difficult to address and justify the partitioning of large experiments into smaller ones. At the bottom of **Table 4.7** the averages of the clones from *CET*-1 and *CET*-2 are provided. In this trial, there was a clear advantage in favor of the clones selected based on the two reps with four plants each.

Table 4.6. Results from the *Preliminary Yield Trial* (*PYT-1*) evaluated in Santo Tomás (Atlántico Department). A total of 60 clones were evaluated, from which 18 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	t/ha	
Parameters of the best 10 selected clones							
SM 3128-14	4	27,6	20,3	0,59	36,0	9,9	33,8
SM 3195-3	2	22,9	16,1	0,59	36,2	8,3	33,1
CM 9962-27	2	28,6	25,7	0,52	34,9	10,0	29,3
SM 3196-1	2	19,0	16,1	0,55	37,9	7,2	27,8
GM 976-9	2	21,9	20,2	0,52	35,9	7,9	23,4
GM 955-3	2	22,5	22,9	0,49	36,0	8,0	21,6
GM 976-8	2	18,5	21,2	0,46	36,7	6,9	15,7
CM 9962-26	3	28,6	24,1	0,54	32,5	9,3	15,4
SM 3109-17	2	19,9	16,1	0,56	34,9	6,9	13,6
SM 3182-27	2	21,8	24,7	0,47	35,3	7,7	13,4
Commercial checks							
TAI 8	1	27,7	21,7	0,57	35,0	9,7	36,4
Ginés	4	23,1	22,3	0,51	35,4	8,2	12,9
Verónica	2	17,6	19,1	0,48	34,5	6,1	3,2
Parameters of 18 clones selected							
Maximum	4	28,6	29,2	0,59	38,8	10,0	33,8
Minimum	2	12,5	13,7	0,44	32,5	4,8	8,0
Average	3	21,7	21,1	0,51	35,7	7,7	17,1
St. Deviation	1	3,8	4,0	0,04	1,4	1,2	8,7
Parameters of 60 clones evaluated							
Maximum	4	28,6	33,9	0,59	38,8	10,0	36,4
Minimum	1	8,2	11,4	0,30	31,1	2,9	-44,8
Average	3	18,7	21,0	0,48	34,5	6,5	0,0
St. Deviation	1	4,7	5,1	0,06	1,8	1,6	17,1
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	2,7	18,4	20,2	0,48	34,5	6,4	0,391

CET-2	3,0	18,6	21,8	0,46	34,4	6,4	-2,379

Table 4.8 presents the result of *PYT-3* evaluation. As for the previous *PYT* evaluations, a total of 60 clones were evaluated (30 from *CET-1* and 30 from *CET-2*) and the same commercial checks were included. The ranking changed rather drastically. Corpoica-Ginés, Corpoica-Verónica and MTAI 8, ranked 8th, 11h and 28th, respectively. This indicates that many selected clones were superior to the three commercial checks. MTAI8 which had excellent performance in *PYT*-1 was the worst among the three commercial checks. Average dry matter yield of the 18 selected clones was 9.2 t/ha and the average dry matter content was 35.6%. As in the case of *PYT*-1 there is no evidence that planting trials using two replications with four plants each (*CET-2*) offers any advantage as can be observed at the end of **Table 4.8.**

Table 4.7. Results from the *Preliminary Yield Trial* (*PYT-2*) evaluated in Santo Tomás (Atlántico Department). A total of 60 clones were evaluated, from which 18 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	t/ha	
Parameters of the best 10 selected clones							
SM 3195-15	1	23,4	25,0	0,48	37,8	8,8	29,5
SM 3191-9	2	34,2	19,3	0,64	31,9	10,9	28,8
GM 942-12	2	22,7	22,4	0,50	36,4	8,3	20,2
SM 3190-34	3	26,5	26,9	0,50	35,7	9,5	19,0
CM 9958-108	3	26,8	31,4	0,47	35,5	9,5	17,9
GM 924-2	3	22,2	20,7	0,53	36,1	8,0	15,9
SM 3109-19	2	20,5	18,1	0,54	36,2	7,4	14,9
SM 3193-10	2	27,0	29,1	0,50	34,0	9,2	14,6
SM 3191-24	2	21,4	21,7	0,50	36,4	7,8	14,4
GM 976-10	3	20,8	24,1	0,46	37,5	7,8	13,6
Commercial checks							
Ginés	4	27,3	24,9	0,52	34,7	9,5	13,5
Tai 8	2	19,9	20,0	0,50	35,2	7,0	6,6
Verónica	2	19,7	18,6	0,52	34,3	6,7	4,3
Parameters of 18 clones selected							
Maximum	4	34,2	38,2	0,64	38,5	10,9	29,5
Minimum	1	16,9	17,0	0,41	31,5	6,5	9,1
Average	3	24,3	25,6	0,49	35,7	8,6	15,0
St.Deviation	1	4,7	6,6	0,06	2,1	1,2	6,1
Parameters of 60 clones evaluated							
Maximum	5	34,2	44,2	0,64	38,5	10,9	29,5
Minimum	1	11,6	12,0	0,26	29,7	4,1	-38,0
Average	3	20,5	24,3	0,46	34,8	7,1	0,0
St.Deviation	1	4,8	6,7	0,07	2,0	1,6	14,9
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	2,8	19,6	23,3	0,46	34,5	6,7	-3,421

CET-2	2,9	21,2	25,7	0,46	35,2	7,5	2,897

Table 4.8. Results from the *Preliminary Yield Trial* (*PYT-3*) evaluated in Santo Tomás (Atlántico Department). A total of 60 clones were evaluated, from which 18 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
SM 3193-33	4	42,8	44,1	0,48	36,2	15,7	40,9
CM 9962-36	3	31,0	25,5	0,55	35,4	11,0	28,4
GM 961-9	3	31,1	29,9	0,51	35,7	11,2	27,7
SM 3190-42	3	29,0	35,1	0,45	36,9	10,7	23,3
CM 9962-40	2	29,0	29,9	0,49	35,0	10,2	20,6
GM 671-5	2	18,8	20,1	0,48	39,1	7,3	19,5
GM 279-64	2	23,5	36,0	0,40	36,0	8,5	10,6
GM 226-75	2	25,7	26,8	0,49	33,1	8,5	10,3
CM 9958-102	3	23,1	37,2	0,39	36,8	8,5	9,2
CM 9962-56	4	25,1	33,2	0,43	36,0	9,1	9,0
Commercial checks							
Ginés	4	27,0	26,5	0,50	35,1	9,4	12,0
Verónica	1	20,4	22,1	0,48	35,2	7,2	10,2
Tai8	2	23,0	26,9	0,46	33,6	7,7	3,2
Parameters of 18 clones selected							
Maximum	4	42,8	44,1	0,55	39,1	15,7	40,9
Minimum	1	18,5	20,1	0,36	32,1	6,8	4,4
Average	3	25,7	29,9	0,46	35,6	9,2	14,0
St.Deviation	1	5,8	6,4	0,05	1,8	2,1	10,3
Parameters of 60 clones evaluated							
Maximum	5	42,8	46,2	0,58	39,1	15,7	40,9
Minimum	1	8,6	8,6	0,22	26,6	3,0	-29,4
Average	3	20,8	25,8	0,45	34,4	7,1	0,0
St.Deviation	1	6,1	7,4	0,07	2,5	2,1	14,4
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	2,7	20,4	23,4	0,47	34,4	7,0	1,929
CET-2	3,3	20,9	28,6	0,42	34,3	7,2	-3,085

Table 4.9 presents another *Preliminary Yield Trial* (*PYT-4*) that was generated with segregating materials that had been previously evaluated outside the standard selection process. A total of 40 clones were evaluated and only six were selected. Four commercial checks were included MVEN 25 (3rd ranking), CORPOICA-Verónica (10th ranking), CORPOICA-Ginés (14th ranking) and MTAI 8 (24th ranking). Emphasis in the selection was for

high dry matter content (average of selected clones 36.2%). Average dry matter yield was 7.4 t/ha.

Table 4.9. Results from the *Preliminary Yield Trial* (*PYT-4*) evaluated in Santo Tomás (Atlántico Department). A total of 40 clones were evaluated, from which 6 were selected. Performance of these 6 selected clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the 6 clones selected							
CM 9962-58	2	22,3	18,3	0,55	36,8	8,2	38,00
GM 976-13	2	25,3	17,1	0,60	34,9	8,9	36,30
SM 3181-16	2	20,7	26,6	0,45	36,5	7,6	24,59
SM 3182-35	2	16,3	18,3	0,47	36,6	6,0	19,46
CM 9958-103	2	16,5	26,2	0,38	37,6	6,2	18,88
SM 3197-9	1	21,1	25,8	0,45	34,5	7,3	18,48
Commercial checks							
VEN25	3	25,8	24,0	0,52	34,4	8,9	27,38
Verónica	2	16,6	15,5	0,52	35,2	5,9	16,81
Ginés	4	17,1	16,3	0,52	35,2	6,0	9,06
TAI8	3	14,5	13,0	0,50	33,0	4,9	-5,68
Parameters of 6 clones selected							
Maximum	2	25,3	26,6	0,60	37,6	8,9	38,00
Minimum	1	16,3	17,1	0,38	34,5	6,0	18,48
Average	2	20,4	22,1	0,48	36,2	7,4	25,95
St.Deviation	0	3,5	4,6	0,08	1,2	1,1	8,97
Parameters of 40 clones evaluated							
Maximum	4	25,8	33,0	0,60	37,9	8,9	38,00
Minimum	1	4,2	4,7	0,27	29,9	1,5	-38,91
Average	3	14,9	17,5	0,47	34,1	5,1	0,00
St.Deviation	1	5,2	6,4	0,07	1,9	1,9	18,42

An important area of research that was not conducted systematically in the past is the evaluation of materials from the germplasm collection. It has been a consistent experience of the project that many landraces held in the collection can offer outstanding agronomic performances and be suitable for their official release as varieties. In the 2006-2007 season, the core collection was evaluated as a *CET* in the Cesar Department. About 600 genotypes were evaluated and 50 were selected for further evaluation in *PYT*s. **Table 4.10** presents the result of this trial. Four commercial checks were included Venezolana (25th ranking), MVEN 25 (24th ranking), Cubanita (21th ranking), and Costeña (4th ranking). The best performing landrace was MARG 7 with more than 10 t/ha of dry matter, although the dry matter content was rather low (29.9%). An old cross from the breeding project (SG 455-1) was the second best performing clone with much higher dry matter yield (11.7 t/ha) and content (34.7%) but very poor plant type (4.0). The third best performing clone was also an improved genotype

(CM 4729-4) with a similar performance (good dry matter yields and contents but poor plant type).

Table 4.10.Results from the *Preliminary Yield Trial* (*PYT-5*) evaluated in Santo Tomás (Atlántico Department). A total of 54 clones from the core collection originally selected from an evaluation conducted during 2006-2007 in Barrancas (Cesar Department) were evaluated. A group of 16 of these genotypes (most landraces) were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
ARG 7	2	34,2	24,5	0,58	29,9	10,3	31,56
SG 455-1	4	34,4	30,9	0,54	34,7	11,7	27,70
CM 4729-4	4	35,6	35,0	0,51	30,2	10,7	19,06
VEN 185	3	27,5	24,0	0,53	32,5	8,9	17,00
BRA 191	3	28,1	33,1	0,45	34,3	9,7	16,98
CM 849-1	3	28,0	19,2	0,60	28,5	8,0	14,35
MEX 92	2	27,9	21,9	0,57	25,9	7,2	12,54
BRA 759	2	27,1	27,0	0,50	29,4	8,0	10,95
BRA 781	3	31,2	23,9	0,57	25,9	7,9	10,93
COL 978	4	16,1	12,9	0,56	36,1	5,8	8,63
Commercial checks							
Venezolana	4	13,9	12,3	0,54	36,8	5,1	3,44
MVEN25	3	18,0	15,2	0,55	32,0	5,8	3,63
Cubanita	2	16,5	15,8	0,51	32,9	5,4	4,41
Costeña	2	24,7	17,3	0,59	34,8	8,6	26,89
Parameters of 16 clones selected							
Maximum	4	35,6	35,0	0,69	36,1	11,7	31,56
Minimum	2	16,1	11,5	0,44	18,3	5,8	5,42
Average	3	27,2	23,1	0,54	30,1	8,1	12,87
St.Deviation	1	5,5	6,8	0,07	4,4	1,8	7,96
Parameters of 54 clones evaluated							
Maximum	4	35,6	35,0	0,69	39,1	11,7	31,56
Minimum	1	7,9	7,3	0,41	18,3	2,1	-30,93
Average	3	20,0	17,0	0,54	29,0	5,8	0,00
St.Deviation	1	7,0	6,8	0,06	4,5	2,3	15,32

Gentoypes that were selected from *PYT*s harvested in April 2007 were combined in a single Advanced Yield Trial (*AYT*) which was planted as first cycle (*AYT-1*) in two locations (Santo Tomás and at La Sierra in the Atlántico Department). A total of 80 clones were evaluated. **Table 4.11** presents the result of the evaluation in the Santo. Unfortunately, several trials that had been planted in collaboration with Industrias del Maíz Andina in La Sierra were stolen and could not be harvested nor data obtained. *AYT* are large experiments based on

three replications and 25-plant plots of which only the nine central plants are harvested for analysis. Five commercial checks were included CORPOICA-Verónica (27th ranking), Costeña (35th ranking), TAI8 (40th ranking), MVEN 25 (43rd ranking) and CORPOICA-Ginés (72nd ranking). Results from this first cycle of advanced yield trials were outstanding with excellent levels of dry matter productivity and dry matter contents.

Table 4.11. Results from the *Advanced Yield Trial* (*AYT-I*) evaluated in Santo Tomás (Atlántico Department). A total of 80 clones were evaluated. Performance of the best 10 clones is presented.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
SM 3060-34	2	34,5	26,9	0,56	37,5	12,9	36,09
SM 3112-60	1	33,8	25,7	0,57	36,8	12,4	33,76
CM 9912-166	3	35,1	30,7	0,53	36,8	12,9	27,15
CM 9954-7 4	2	30,0	29,7	0,51	38,0	11,4	27,01
SM 3065-10	2	38,1	20,3	0,65	33,6	12,8	23,87
SM 3060-59	3	34,4	27,6	0,55	35,9	12,4	21,90
CM 9912-150	2	39,1	28,1	0,58	33,7	13,2	20,02
SM 3154-12	3	29,2	26,7	0,52	37,1	10,8	18,40
GM 848-13	3	28,9	26,7	0,52	37,1	10,7	18,36
SM 3060-34	3	41,6	22,9	0,64	32,6	13,5	16,17
Commercial checks							
Verónica	1	23,2	19,2	0,55	35,5	8,3	5,57
Costeña	2	20,7	19,5	0,51	36,4	7,5	1,54
Tai	2	26,1	26,1	0,50	34,5	9,0	-1,07
M Ven 25	2	24,1	24,4	0,50	35,2	8,5	-1,68
Ginés	3	23,5	22,4	0,50	34,2	8,0	-15,33
Parameters of 80 clones evaluated							
Maximum	4	41,6	37,8	0,68	38,7	13,5	36,09
Minimum	1	13,5	15,6	0,33	32,0	5,0	-33,82
Average	2	25,4	25,3	0,50	35,4	8,9	0,00
St.Deviation	1	5,8	4,7	0,07	1,5	1,9	14,15

As in the case of *AYT-I* unfortunately a second-cycle trial (*AYT-II*) planted in La Sierra was also stolen and data could not be obtained. **Table 4.12** presents the result of this trial evaluated in Santo Tomás. A total of 35 clones were evaluated and they showed also excellent performance. Several commercial checks were included. The best performing check was MTAI 8 (ranked 6th); MVEN25 (ranked 9th) and Costeña (ranked 12th). Several of the best performing clones yielded above 12 t/ha of dry matter. Dry matter contents were also outstanding with several of the best performing clones showing more than 36%. It is indeed very unfortunate

that data from only one location is available but this kind of limitations illustrate the kind of environments and unusual circumstances that cassava breeding implies.

Table 4.12. Results from the *Advanced Yield Trial* (*AYT-II*) evaluated in Santo Tomás (Atlántico Department). A total of 35 clones were evaluated. Performance of the best 10 clones is presented.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
GM 291-90	2	47,8	20,8	0,70	34,9	16,8	35,33
CM 9924-19	2	40,7	19,6	0,67	35,2	14,3	23,07
GM 273-59	1	37,7	32,5	0,54	37,1	14,0	22,54
SM 3106–29	3	39,0	28,4	0,58	37,3	14,6	22,41
SM 3106-14	1	38,1	25,1	0,60	35,4	13,5	17,61
GM 273-82	3	34,2	23,1	0,60	36,3	12,4	11,88
GM 273-60	2	33,0	21,0	0,61	35,9	11,8	10,97
CM 9955-15	2	29,0	19,6	0,59	37,1	10,8	10,03
GM 451-36	2	32,7	23,1	0,59	35,4	11,6	7,10
CM 9912-107	3	37,3	21,9	0,63	34,5	12,8	6,59
Commercial checks							
MTAI 8	2	41,1	33,1	0,56	34,9	14,4	13,81
M VEN 25	2	42,1	30,4	0,58	34,3	14,4	10,49
ICA Costeña	2	30,9	22,5	0,58	36,3	11,3	6,68
Caiseli	1	23,2	21,3	0,52	37,6	8,8	4,77
Verónica	1	26,7	25,2	0,54	34,7	9,3	-7,36
Ginés	4	32,4	28,8	0,54	35,1	11,3	-7,73
Ica Negrita	4	25,4	28,0	0,48	36,8	9,4	-11,43
SM 1411-5	2	29,7	29,0	0,51	33,6	10,0	-16,80
Parameters of 35 clones evaluated							
Maximum	4	47,8	37,9	0,70	37,6	16,8	35,33
Minimum	1	14,7	11,7	0,44	31,9	4,8	-38,88
Average	2	31,9	24,8	0,56	35,2	11,2	0,00
St.Deviation	1	6,8	5,2	0,06	1,6	2,4	15,66

The following step in the evaluation process (see Figure 3.1) is the Regional Trial which also takes place in two cycles. *Regional Trial-I* is the first cycle of this kind of trial. Selected clones are then evaluated in *Regional Trial-II* or second cycle. **Table 4.13** describes the result of a *RT*-I evaluated in Santo Tomás (Atlántico Department) in which a total of 36 clones were evaluated, including five checks. As in the case of the Advanced Yield Trials a second location where this trial was planted was stolen (La Sierra). The best performing checks were MTAI8 (7th ranking), CORPOICA-Verónica (14th ranking) and ICA-Costeña (16th ranking). As in the advanced yield trials, very high dry matter yields (in many cases above 12 t/ha) and excellent levels of dry matter content (frequently above 36%) can be observed in data from **Table 4.13**.

Table 4.13. Results from the *Regional Trial (RT*-I) evaluated in Santo Tomás (Atlántico Department). A total of 36 clones were evaluated. Performance of the best 10 clones is presented.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
CM 9912-11	3	36,3	24,2	0,6	36,4	13,2	22,42
CM 9924-6	3	41,1	29,9	0,6	35,7	14,7	21,75
GM 462-4	2	30,2	22,9	0,6	37,9	11,5	18,82
SM 3067-16	2	35,7	21,4	0,6	34,0	12,1	13,96
SM 2779-56	3	36,8	23,5	0,6	34,7	12,7	13,08
GM 213-56	2	36,0	26,8	0,6	35,3	12,9	12,60
GM 214-62	3	27,6	20,3	0,6	37,6	10,4	8,90
CM 9946-68	3	31,8	25,7	0,6	36,3	11,5	8,65
SM 2779-59	3	34,0	23,1	0,6	35,1	11,9	7,51
CM 9957-35	2	32,2	21,6	0,6	34,2	11,0	6,71
Commercial checks							
MTAI8	2	41,5	29,4	0,6	32,6	13,5	10,05
Verónica	2	24,4	11,1	0,7	34,9	8,5	5,81
Costeña	2	25,4	15,9	0,6	35,8	9,1	3,56
Negrita	4	26,7	27,4	0,5	37,9	10,2	0,15
Ginés	4	31,2	23,7	0,6	34,3	10,7	-7,12
Parameters of 36 clones evaluated							
Maximum	4	44,9	31,8	0,7	37,9	14,7	22,42
Minimum	1	18,5	11,1	0,4	29,4	6,4	-25,72
Average	3	29,6	22,6	0,6	35,0	10,3	0,00
St.Deviation	1	6,2	5,0	0,1	1,8	2,0	11,72

Table 4.14 presents the results of the second-cycle regional trial *(RT-II*). As previous cases this trial had been planted in another location (La Sierra) but was stolen and data could not be collected. These trials were also planted in a different Department (Sucre) and data from this location will be presented in a different table. Thos regional trial was planted in a different location (Baranoa) and this reflects in the considerably lower averages compared with trials conducted at Santo Tomás. A total of 30 clones were evaluated, including five commercial checks. The best check (CORPOICA-Caiseli) ranked 3rd and the second best check (ICA-Costeña) ranked 5th. Dry matter contents were very high (most of selected clones ranging from 36 to 38%) but fresh root yields were low in this location (around 14 t/ha) which resulted in an average dry matter yield of about 5 t/ha).

The same trial was planted also in two locations in Sucre Department. The combined analysis of these two locations is presented in **Table 4.15**. CORPOICA-Caiseli and ICA-Costeña were the two best performing clones in the combined analysis (ranking third and fourth, respectively).
Three clones were in common among the best five experimental materials in **Tables 4.14** and **4.15** (CM 9560-1; SM 2775-4 and CM 9456-12) suggesting an excellent and stable performance of these materials.

Results from the two trials in Sucre Department were much better than that from Baranoa with high dry matter contents (average 35.1%) and good fresh root productivity (22.9 t/ha) which resulted in an average dry matter yield of 8.0 t/ha). These materials are going to be further analyzed in semi-commercial plots in Atlántico, Sucre and Córdoba Departments for one or two additional years and, depending on their performance may be officially released by the National Program in Colombia (CORPOICA).

Table 4.14. Results from the *Regional Trial (RT-II*) evaluated in Baranoa (Atlántico Department). A total of 30 clones were evaluated. Performance of the best 10 clones is presented.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
CM 9560-1	3	18,2	12,4	0,6	37,1	6,8	31,47
CM 9456-12	3	18,4	11,5	0,6	36,5	6,7	29,34
SM 2775-4	2	17,6	14,0	0,6	36,6	6,5	25,80
SM 2769-11	2	16,6	13,6	0,5	35,2	5,8	17,50
SM 2546-40	3	18,0	12,7	0,6	34,2	6,1	15,96
SM 2545-22	3	19,8	12,0	0,6	32,6	6,4	12,97
SM 2773-32	3	11,0	10,3	0,5	38,8	4,3	11,81
SM 1511-6	3	15,6	12,5	0,6	34,6	5,4	7,22
SM 2620-1	2	13,2	11,4	0,5	35,6	4,7	4,88
SM 2623-6	3	12,9	11,2	0,5	36,0	4,7	4,06
Commercial checks							
CAISELI	2	14,8	12,9	0,5	38,4	5,7	26,52
COSTEÑA	2	14,1	10,5	0,6	37,7	5,3	24,09
TAI 8	3	13,7	11,3	0,5	33,4	4,7	-9,34
VERONICA	4	12,8	10,2	0,6	34,0	4,3	-9,40
GINES	4	15,8	13,5	0,5	32,7	5,2	-10,16
Parameters of 30 clones evaluated							
Maximum	4	20,9	14,0	0,6	38,8	6,8	31,47
Minimum	2	7,5	9,7	0,4	31,0	2,5	-41,41
Average	3	14,1	11,9	0,5	34,7	4,9	0,00
St.Deviation	1	3,2	1,1	0,0	1,9	1,1	18,94

Table 4.15. Results from the *Regional Trial (RT-II*) evaluated in two locations in Sucre Department (Sincelejo and Toluviejo). A total of 30 clones were evaluated. Performance of the best 10 clones is presented.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the best 10 selected clones							
SM2775-4	2	30,2	17,7	0,6	37,2	11,3	29,76
CM9560-1	3	27,7	15,9	0,6	36,6	10,1	21,84
SM2773-32	3	17,7	11,5	0,6	39,2	6,9	10,60
SM2775-2	3	36,7	15,6	0,7	30,0	11,0	10,00
CM9456-12	3	22,7	10,5	0,7	35,8	8,1	9,49
SM1438-2	3	24,6	16,4	0,6	36,9	9,2	7,58
SM2545-22	2	30,4	17,5	0,6	32,6	10,0	7,31
SM2546-40	2	22,7	11,8	0,7	34,8	7,9	6,73
SM2769-11	2	25,4	15,5	0,6	33,9	8,5	5,26
SM1511-6	3	23,0	14,9	0,6	35,7	8,3	4,27
Commercial checks							
CAISELI	2	23,7	16,2	0,6	37,8	8,9	20,85
COSTEÑA	2	23,7	12,2	0,7	36,0	8,5	14,50
VERÓNICA	2	23,7	11,6	0,7	35,1	8,4	10,24
ORENSE	3	21,9	16,0	0,6	38,4	8,4	9,00
TAI	2	25,3	14,3	0,6	34,5	8,8	7,83
Parameters of 36 clones evaluated							
Maximum	4	36,7	17,9	0,7	39,2	11,3	29,76
Minimum	2	11,4	10,5	0,5	30,0	4,0	-39,61
Average	3	22,9	14,3	0,6	35,1	8,0	0,00
St.Deviation	1	4,8	2,2	0,0	1,8	1,6	15,70

Table 4.16 presents results of a special *Advanced Yield Trial* evaluated in Santo Tomás (Atlántico Department). A similar trial had been planted at Industrias del Maíz Andina in La Sierra, but had the same fate as the other trials and was illegally harvested before data could be obtained. This trial focuses particularly on the high and stable dry matter content. A first harvest took place at the normal harvesting time at the end of the dry season. It was based on nine central plants as any ordinary *AYT*. As it can be seen average dry matter content in the first harvest was relatively high (36%). The second harvest was based on 3 plants and took place after the return of the rains. Both harvests were based on three replications. Dry matter content on the second harvest, however, was considerably lower (31%) than in the first harvest. This is so because with the arrival of the rains, the plant reinitiates its growth drawing energy from the roots. The specific objective of this trial was to identify clones with good fresh root productivity, high dry matter contents in the normal harvesting time (first harvest) which are capable of maintaining as much as possible a high dry matter content

upon the arrival of the rains. Some of the selected clones had an outstanding yield potential after the arrival of the rains (above 15 t/ha of dry matter), and maintained or recovered adequate levels of dry matter content. These materials are a direct answer to the needs expressed by the starch industry in the region.

Table 4.16. Results from the special *Advance Yield Trial* (*AYT*) evaluated in one location in Santo Tomás (Atlántico Department) and harvested at two different dates. A total of 15 clones were evaluated. The first harvest follows the standard nine-plant harvest of *AYT*s. The second harvest was based on the harvest of three plants. Three replications were used for each harvesting date.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter		Ranking
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	(1-15)
Parameters of the 7 clones selected (first harvest data)							
GM 466-54	2	21,9	17,2	0,56	37,4	8,2	2
GM 437-20	3	25,0	32,2	0,44	38,2	9,5	4
GM 466-56	2	25,8	37,3	0,40	36,7	9,5	10
SM 3148–21	2	19,7	25,7	0,43	38,9	7,6	5
GM 466-57	2	21,3	22,4	0,49	36,6	7,8	9
SM 3153-47	3	25,8	25,7	0,50	37,4	9,7	3
CM 9912-134	3	18,6	22,1	0,46	36,5	6,8	14
Parameters of the 7 clones selected (second harvest data)							
GM 466-54	2	49,7	49,0	0,50	32,9	16,3	2
GM 437-20	3	52,1	71,1	0,43	34,6	18,0	5
GM 466-56	2	56,9	61,4	0,48	32,4	18,7	3
SM 3148–21	2	22,6	32,6	0,41	35,9	8,1	9
GM 466-57	2	35,8	39,3	0,47	32,4	11,6	8
SM 3153-47	3	29,6	33,5	0,46	30,8	9,5	13
CM 9912-134	3	26,1	34,7	0,43	34,6	9,0	10
Commercial checks (First harvest)							
MTAI8	1	33,8	26,6	0,57	34	11,6	1
Caiseli	1	19,6	18,4	0,51	36	7,0	12
Verónica	1	18,6	14,1	0,57	36	6,7	8
Commercial checks (Second harvest)							
MTAI8	1	73,9	57,8	0,57	27	20,2	1
Caiseli	1	35,0	37,6	0,48	34	12,0	4
Verónica	1	29,0	26,5	0,52	29	8,6	11
Parameters of 15 clones evaluated (first harvest)							
Maximum	4	33,8	37,3	0,57	39	11,6	15
Minimum	1	18,6	14,1	0,40	34	6,7	1
Average	2	23,8	24,5	0,50	36	8,6	8
St.Deviation	1	4,9	6,5	0,05	1	1,6	4
Parameters of 15 clones evaluated (second harvest)							
Maximum	4	73,9	71,1	0,57	36	20,2	15
Minimum	1	20,8	18,6	0,39	27	6,1	1
Average	2	41,4	45,0	0,47	31	13,0	8

St.Deviation	1	16,4	15,4	0,05	3	4,7	4

Table 4.17. Results from the *Regional Trial (RT-II*) evaluated in Santo Tomás (Atlántico Department). A total of 30 clones were evaluated. Performance of the seven clones selected is presented. This is a combination of different genotypes that were included in a common trial after their selection from different type of studies, including Diallel crosses made several years ago.

Parameter	Plant type	Fresh Root Yield (t/ha)		Harvest Index	Dry matter content		Selection Index
	(1-5)	Root	Foliage	(0-1)	%	(t/ha)	
Parameters of the 7 clones selected							
SM 3112-48	3	35,7	22,0	0,62	33,5	12,0	33,26
SM 3061-29	3	24,1	17,2	0,58	33,4	8,1	14,96
GM 280-15	2	24,2	17,3	0,58	33,1	8,0	14,87
GM 258-3	2	29,3	17,1	0,63	30,8	9,0	13,56
GM 410-22	2	25,5	18,6	0,58	32,1	8,2	10,51
CM 9926-24	3	25,4	21,6	0,54	31,7	8,0	3,24
GM 410-24	2	19,3	18,2	0,51	33,0	6,4	0,60
Commercial checks							
TAI 8	2	37,3	20,9	0,64	33,0	12,3	42,23
COSTEÑA	2	24,7	24,1	0,51	32,2	8,0	6,02
NEGRITA	3	18,5	21,1	0,47	34,9	6,5	2,44
VERONICA	2	13,4	12,7	0,51	30,2	4,1	-23,00
GINES	3	29,7	24,6	0,54	31,5	9,3	5,54
Parameters of 30 clones evaluated							
Maximum	4	37,3	24,6	0,64	37,8	12,3	42,23
Minimum	2	9,7	12,7	0,43	30,0	3,3	-25,52
Average	3	20,9	19,1	0,52	32,9	6,9	0,00
St. Deviation	1	6,6	3,2	0,06	1,7	2,1	16,31

The last experiment to describe includes a regional trial described in **Table 4.17.** This is a trial that included several different materials that were identified because of particular characteristics in earlier evaluations, including the Diallel Studies carried out several years ago. TAI 8 (one of the commercial checks was the best clone among the 30 evaluated. No experimental clone provided a performance that was attractive. Dry matter contents in the experimental clones was no particularly high, compared with the commercial checks. Unfortunately this is the only location when this trial could be harvested.

CHAPTER 5

DEVELOPMENT OF GENETIC STOCKS AND IMPROVED GENE POOLS ADAPTED TO THE ACID-SOIL SAVANNAS ENVIRONMENT

This output relates to the efforts directed to the creation and identification of germplasm adapted to the acid-soil savannas environment found in Colombia's eastern plains. The environment is characterized by acid soils with toxic levels of Al and Mn and severe deficiency of P and Ca. In addition, bacterial blight (CBB) and super-elongation disease (SED) are very common, inducing severe disease levels in materials that lack resistance or tolerance to them. Disease pressure is natural but in the nurseries, particularly at *CETs* stakes from diseased plants are taken and planted to serve as spreader of the diseases.

5.1. EVALUATIONS AND SELECTIONS IN THE ACID SOILS ENVIRONMENT

Activities developed for the acid-soil savannas environment were centralized initially in CORPOICA – La Libertad in Villavicencio and in the experimental farm Cantaclaro, property of Sumprocol (a member of the Petrotestig Group) in Puerto López, both in Meta Department. The latter has become a strong supporter of cassava research and production, aiming at the processing of cassava roots into bio-ethanol. The company is currently leading a project to develop an adequate cultural practices package for the competitive production of cassava in the acid soil savannas. The farm has a good fleet of machinery to assure the proposed goal of establishing up to 1,200 ha of cassava during the 2007-2008 period. **Table 5.1** lists the most relevant trials, whereas the other tables show results specific to each one. As mentioned in **Table 3.5** a total of 3295 seeds were germinated and 2221 seedlings from these botanical seeds (targeting this particular environment) were transplanted at CIAT-Palmira in an isolated field. The planting of the current *F1* stage is isolated to reduce as much as possible infection by diseases that can be found at later stages of the evaluation process. Seedlings from botanical seed are considered to be disease-free and efforts are made to maintain this condition for as long as it can possibly be done.

Clonal Evaluation Trials (CET) are very large experiments around one hectare in size. A major constraint in their evaluation is the experimental error associated with the unavoidable variation in environmental conditions in such a large experimental plot. Because this is the first evaluation and selection stage (See Chapter 3) only 8 stakes are available from each genotype. Replication of each clone, therefore, is difficult to implement. On the other hand clones are grouped in either full- or half-sib families. Since many clones are generally available from each family they are randomly allocated in one of three blocks in which the field is divided. In other words instead of planting all the clones from a given family together one after the other, they are split in three groups, which are planted in the three blocks the entire evaluation is divided into (**Figure 4.1**).

Table 5.1. Trials conducted in the acid-soil savannas environment during the 2007-2008 cycle.

Type of Trial	Genotypes (# plants)	Reps	Observations
Clonal Evaluation Trial	1213 (8)	1	Tables 5.2-5.5
Field observation CORE	434 (6)	1	Tables 5.6
Results from the *Preliminary Yield Trial* (*PYT*-1)	64 (10)	3	Table 5.7
Results from the *Preliminary Yield Trial* (*PYT-2*)	64 (10)	3	Table 5.8
Results from the *Preliminary Yield Trial* (*PYT-3*)	64 (10)	3	Table 5.9
Results from the *Advanced Yield Trial* (*AYT-I*)	65 (25)	3	Table 5.10
Results from the *Advanced Yield Trial* (*AYT-II*)	30 (25)	3	Table 5.11
Results from the *Regional Trial* (*RT-I*)	21 (25)	3	Table 5.12
Results from the *Regional Trial* (*RT-II*)	16 (25)	3	Table 5.13
Results from the *Regional Trial* (*RT-III*)	21 (25)	3	Table 5.14

Table 5.2. Results from the first *Clonal Evaluation Trial* divided into three blocks and conducted in Puerto López (Meta Department). Statistics of the 60 clones selected and all the clones evaluated in each block are presented.

	Plant type (1-5)	Fresh root yield (t/ha)	Foliage Yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **60** selected clones from Block-1							
Maximum	4	37,5	33,3	0,71	41,8	14,6	70,2
Minimum	2	20,8	9,8	0,50	32,1	7,0	22,6
Average	3	23,0	15,9	0,60	36,2	8,3	40,6
St. Deviation	0	2,4	4,6	0,06	2,9	1,1	7,3
Performance of the **410** clones evaluated in Block -1							
Maximum	5	37,5	33,3	0,71	43,2	14,6	70,2
Minimum	2	2,5	2,1	0,28	17,2	0,5	-39,8
Average	4	9,0	10,5	0,44	31,6	3,1	0,0
St. Deviation	1	6,4	4,6	0,10	6,1	2,4	22,0
Statistics of the **60** selected clones from Block-2							
Maximum	4	23,8	18,3	0,76	43,2	9,5	56,2
Minimum	1	10,6	7,1	0,53	32,6	4,2	27,3
Average	3	22,0	12,4	0,64	37,0	8,1	42,1
St. Deviation	1	1,7	2,8	0,05	2,7	0,8	6,4
Performance of the **402** clones evaluated in Block -2							
Maximum	5	23,8	20,0	0,76	43,3	9,5	56,2
Minimum	1	2,3	1,5	0,28	19,5	0,4	-41,9
Average	4	8,8	9,7	0,45	32,8	3,1	0,0
St. Deviation	1	6,1	3,3	0,11	5,9	2,4	22,4
Statistics of the **60** selected clones from Block-3							
Maximum	4	26,0	22,3	0,71	42,4	10,2	59,1
Minimum	1	10,6	10,0	0,50	32,3	4,3	25,6
Average	3	22,4	15,3	0,59	36,1	8,1	38,3
St. Deviation	1	2,0	2,2	0,04	2,6	0,9	7,4
Performance of the **401** clones evaluated in Block -3							
Maximum	5	26,0	22,3	0,71	43,1	10,2	59,1
Minimum	1	2,5	2,1	0,28	17,5	0,5	-45,8
Average	4	9,5	11,3	0,43	33,5	3,3	0,0
St. Deviation	1	6,0	3,6	0,10	5,6	2,3	21,7

Results from the CET evaluation (single row plots with eight plants) are presented in **Table 5.2**. The 1213 clones included in the *CET* were planted in three blocks with 410, 402 and 401 clones each one, respectively. Checks were also included in each block. Table 5.2 provides information on the averages for each of the three blocks. The variation among these three blocks is an error that eventually affects the selection process. By selecting within each block, however, this environmental effect could be effectively eliminated. Since selection indexes were calculated within each block there is no major variation for this variable across blocks. On the other hand the average fresh root yields were 9.0, 8.8 and 9.5t/ha respectively for Blocks 1, 2 and 3. This highlights the large environmental variation that is overcome by stratifying the selection within each block. Average dry matter yields in the selected fractions were excellent ranging from 8.1 to 8.3 t/ha.

Table 5.3. Description of the best five clones at each of the three blocks in the first CET harvested in April 2008 (based on single row plots with eight plants per plot).

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage Yield (t/ha)	Harvest Index (0-1)	Dry matter (%)	Dry root yield (t/ha)	Selection Index
Performance of the best ten clones from Block-1							
CM 9464-36	2	37,5	33,3	0,53	39,0	14,6	70,2
SM 1511-6	2	24,6	12,5	0,66	37,4	9,2	54,9
CM 9464-36	2	23,5	14,2	0,62	38,7	9,1	52,9
SM 2219-11	3	23,3	10,8	0,68	37,9	8,8	48,4
CM 9464-36	3	24,8	15,6	0,61	38,7	9,6	48,1
SM 1565-15	3	22,9	10,4	0,69	37,8	8,7	47,9
CM 9464-36	3	22,1	12,9	0,63	41,0	9,1	47,8
SM 1821-7	3	23,1	16,0	0,59	41,1	9,5	47,4
SM 2219-11	3	25,6	10,4	0,71	32,7	8,4	46,7
SM 1565-15	3	22,5	15,0	0,60	40,7	9,1	46,4
Performance of the best ten clones from Block-2							
CM 6921-3	1	21,7	11,5	0,65	39,4	8,5	56,2
SM 1565-15	2	21,0	11,0	0,66	43,2	9,1	54,7
CM 9464-36	2	22,9	11,7	0,66	38,5	8,8	51,8
SM 1565-15	3	22,5	9,6	0,70	42,0	9,4	51,7
CM 9464-36	3	22,5	8,8	0,72	41,1	9,2	51,3
SM 1565-15	3	22,1	7,1	0,76	39,6	8,7	50,3
CM 6921-3	3	20,8	9,4	0,69	43,0	9,0	49,8
CM 2177-2	2	21,7	12,7	0,63	39,5	8,6	49,6
SM 1565-15	3	23,5	12,1	0,66	40,4	9,5	49,5
CM 9464-36	3	22,3	11,3	0,66	41,6	9,3	49,1
Performance of the best ten clones from Block-3							
SM 2730-1	1	25,4	12,7	0,67	34,7	8,8	59,1
SM 1565-15	3	25,8	10,6	0,71	39,2	10,1	55,7
CM 9464-36	3	24,8	12,7	0,66	41,0	10,2	54,2
SM 2730-1	2	22,3	13,3	0,63	37,0	8,2	48,8
CM 9464-36	3	24,0	15,2	0,61	39,9	9,5	48,6
CM 9464-36	2	21,7	16,3	0,57	39,1	8,5	48,0
CM 6740-7	2	21,9	12,9	0,63	36,1	7,9	46,9
CM 9460-41	2	20,8	12,9	0,62	37,4	7,8	46,5
SM 2730-1	2	21,3	15,2	0,58	38,0	8,1	46,3
MCOL 2737	4	26,0	11,0	0,70	36,9	9,6	46,2

This *CET* at the acid-soil savannas environment allowed a drastic selection within each block. This is illustrated by the average superiority of the selected fraction mentioned above and highlighted by the very high selection indexes of the best clones in the evaluation. In general selection indexes tend to have a larger range of variation in this kind of location probably as the combined effect that resistance of diseases have through the plant type score (direct effect) and through fresh root yield and dry matter contents (indirect effects). **Table 5.3** describes the performances of the best clones in *CET*. Dry root yields ranged among the 30 clones described in that table from 7,8 to 14.6 t/ha, which is an excellent performance given the harsh environmental conditions where this materials are evaluated.

Table 5.4. Family size, number of selected clones in each family and average selection index values for the progenies evaluated in CET. Data combines results from the three blocks in which the trial was divided.

Family	Size (#)	Selected (#)	Sel. Index	Family	Size (#)	Selected (#)	Sel. Index
GM 677	11	3	5,5	GM 1226	3	0	-11,4
GM 679	8	0	-19,7	GM 1268	30	7	1,1
GM 934	6	1	4,8	GM 1277	16	1	-7,5
GM 956	42	2	-3,4	GM 1278	3	0	-17,9
GM 957	19	2	-6,8	GM 1280	15	0	-7,0
GM 1063	1	0	-9,3	GM 1281	3	0	2,4
GM 1064	30	3	0,0	GM 1283	1	0	-16,0
GM 1065	8	2	7,8	GM 1303	27	13	19,7
GM 1066	4	1	10,7	GM 1305	10	3	12,1
GM 1085	12	2	4,7	GM 1313	4	1	7,6
GM 1114	1	0	-2,3	GM 1317	1	0	-19,8
GM 1115	2	0	-25,3	GM 1320	1	0	-0,7
GM 1116	2	1	17,1	GM 1321	8	0	-15,9
GM 1120	12	4	7,6	GM 1322	9	3	7,6
GM 1121	3	0	-4,4	SM 3216	19	11	23,1
GM 1124	2	0	-2,9	SM 3217	36	5	-7,5
GM 1164	5	5	37,4	SM 3220	52	9	1,7
GM 1165	5	1	6,5	SM 3221	36	4	-4,5
GM 1167	3	0	9,5	SM 3231	83	2	-15,2
GM 1168	2	1	19,8	SM 3268	24	1	-3,9
GM 1170	31	5	12,7	SM 3269	14	1	-6,1
GM 1171	22	5	14,4	SM 3270	45	5	-8,1
GM 1172	45	7	4,8	SM 3271	37	10	6,6
GM 1173	25	3	5,7	SM 3272	7	1	14,1
GM 1174	61	19	14,5	SM 3273	4	1	-7,7
GM 1176	9	2	9,3	SM 3274	33	4	1,3
GM 1177	8	1	15,4	SM 3275	53	2	-12,1
GM 1178	9	0	-16,1	SM 3276	28	1	-5,5
GM 1193	1	0	-25,6	SM 3277	51	2	-5,5
GM 1215	1	1	54,9	SM 3278	8	0	-23,5
GM 1222	18	2	-14,0	SM 3279	51	4	1,7
GM 1224	31	8	14,4	SM 3280	26	3	-15,8
GM 1225	10	1	3,1	**Total/Mean**	**1213**	**180**	**0,000**

In **Table 5.4** the size (number of clones) and the number of selected clones from each family has been consolidated. Average selection index for each family is also provided. This data has been obtained by combining information of the three blocks in which the *CET* was divided into. The use of selection index has been already described in Output 3.

Table 5.5. Relative performance of progenitors involved in generating the progenies evaluated in CET. The performance of the progenitors is assesses through the average performance of all the progenies each progenitor produced.

Progenitor	# fam.	Selec. (%)	Pl. Type (1-5)	FRY (t/ha)	H.I. (0-1)	DMC (%)	DMY (t/ha)	Sel. Ind.
SM 1511-6	1	100	2,00	24,58	0,66	37,40	9,19	54,89
CM 9460-15	2	67	3,44	17,89	0,56	33,73	6,05	24,18
CM 9460-12	2	60	3,20	16,05	0,52	34,37	5,53	22,00
CM 6740-7	1	58	3,05	16,46	0,53	34,03	5,63	23,13
SM 2219-11	6	33	3,61	12,97	0,48	33,78	4,52	11,69
CM 4574-7	1	27	4,18	12,10	0,42	35,03	4,31	5,49
CM 8370-11	1	27	3,76	11,45	0,47	32,84	3,91	6,56
CM 9464-29	6	26	3,49	11,52	0,47	34,54	4,09	10,70
CM 9463-19	1	25	3,75	8,02	0,46	27,24	2,68	-7,74
SM 2782-4	1	25	4,25	12,66	0,52	33,12	4,18	7,60
SM 1363-11	3	24	3,62	10,73	0,47	37,85	4,14	12,80
SM 1665-2	1	23	3,93	12,00	0,47	29,14	3,74	1,11
CM 9464-36	11	18	3,53	9,85	0,45	35,32	3,60	7,49
SM 1219-9	1	17	4,08	9,99	0,45	33,03	3,48	1,71
CM 9460-41	4	17	3,21	10,30	0,46	36,39	3,74	12,42
CM 7951-5	10	16	4,43	9,24	0,43	31,76	3,09	-4,69
CM 6758-1	2	15	3,69	9,74	0,44	34,07	3,43	4,18
CM 523-7	3	14	3,58	8,11	0,46	33,58	2,99	2,46
SM 1565-15	6	13	3,75	8,55	0,44	34,12	3,11	1,94
SM 1812-69	1	12	3,70	9,02	0,44	32,67	3,08	1,35
CM 2177-2	10	12	4,01	9,01	0,43	34,12	3,18	0,85
CM 9461-15	3	11	3,85	8,90	0,44	33,97	3,12	1,63
CM 6921-3	1	11	4,11	7,08	0,42	30,82	2,37	-8,08
MCOL 638	7	11	4,22	7,38	0,42	27,57	2,26	-12,84
SGB 765-2	1	11	4,68	8,19	0,42	33,11	2,88	-6,82
SM 2730-1		10	3,44	9,06	0,44	33,41	3,14	3,69
SM 1460-1	2	10	4,24	8,53	0,43	30,85	2,81	-6,01
SGB 765-4	1	10	4,19	8,88	0,44	33,63	3,05	-0,83
SM 2727-12	5	8	4,27	6,71	0,42	32,43	2,39	-7,46
CM 2772-3	1	7	3,86	8,29	0,43	29,41	2,56	-6,05
SM 1821-7	1	6	4,44	6,80	0,42	33,13	2,42	-7,49
SM 2452-6	2	4	3,90	7,20	0,41	31,94	2,39	-5,45
SM 1859-26	1	4	4,15	6,76	0,42	28,34	2,06	-12,05
SM 1864-10	5	3	3,99	7,31	0,40	31,97	2,42	-6,10
MCOL 2737	1	2	4,59	6,15	0,41	29,21	1,96	-15,22
CM 8027-3	1	0	5,00	4,10	0,31	29,27	1,21	-25,25
SM 2792-32	1	0	4,20	5,54	0,40	28,42	1,66	-14,81

Several families such as GM 1164, GM 1171, GM 1174, GM 1268, GM 1303, SM 3216 and SM 3271 had a high proportion of their clones selected (considerably higher percentage of selected clones above the average of about 15-16%). As a matter of fact, every member of family GM 1164 was selected, although this particular family was relatively small with only five segregating progenies. Other interesting families were GM 1303 and SM 3216, which were relatively large and about 50% of their progenies were selected.

The worst performing families can be easily identified because of their negative average selection indexes. No progeny from GM 679, GM 1178, GM 1280, GM 1321 and SM 3278 was selected. In some cases these families were small and failing to have a member selected is more understandable. But family GM 1280 had 15 clones representing it an none was selected. The information from **Table 5.4** can be further consolidated around the average performance of each progenitor used to generate the *CET*. This is so because each progenitor can be used to produce more than one family. **Table 5.5** presents the average performance of the progenies from each progenitor of the clones evaluated in the *CET* this year. The order in this table was based on the average selection index of all the progenies from a given progenitor. The information from Table 5.5 identifies the most important characteristics of the progenies from each parent. This information is very closely related to the GCA estimates and reflects the breeding value of each progenitor. This information is very useful for defining the parents to be included in the crossing nurseries in the future.

Progenitor SM 1511-6 produced only one progeny which was selected. This is why 100% of its progeny was selected. This is irrelevant information regarding the true combining ability of this progenitor and only when more clones from it are evaluated its true merits will be determined. Progenies from CM 9460-15 were distributed in two families. Six of the nine progenies from this particular clone were selected. An almost identical situation defined the progenies from CM 9460-12 (distributed in two families and six out of ten clones were selected). Progenies from SM 2219-11 were distributed in six different families, and 47 out of a total of 144 clones were selected.

The best progenies regarding plant type score where those from clone SM 1859-26 (average score = 1.9) and those from progenitor M Bra 97 were very undesirable with an average score for plant type of 3.9. The second worst progeny, regarding plant type score, was that from MCOL 2758 with and average of 3.5 followed by those from CM 2967-8 and SM 1862-25, with an average of 3.2. It is clear that in general progenies from clone CM 2967-8 were outstanding regarding fresh root productivity and dry matter content of the roots, but were not, generally speaking, characterized by a nice plant architecture and/or plant health.

As explained in Chapter 2, there is a systematic evaluation of the germplasm collection accessions aiming at their full characterization but also in search of potentially useful clones. **Table 5.6** presents the results of germplasm of the Core Collection. These materials have been now evaluated in the mid-altitude valleys; sub-humid, and now acid-soil savannas environment. The analysis combined across these environments was presented in Section 2.4. Table 5.6 presents the data of the evaluation performed in Puerto López, Meta Department. This evaluation followed the typical structure of *CETs.* As can be observed several of the landraces that make up the core collection had an excellent performance in this kind of environment. Several accessions from Brazil and Peru were among the best performing clones. A total of 65 genotypes were selected and will be evaluated next season as a *PYT* evaluation.

Table 5.6. Results from the evaluation of the Core collection as a *Clonal Evaluation Trial* Puerto López (Meta Department). Statistics of the 65 clones selected and all the clones evaluated are presented.

	Plant type (1-5)	**Fresh root yield (t/ha)**	**Foliage Yield (t/ha)**	**Harvest Index (0-1)**	**Dry matter (%)**	**Dry root yield (t/ha)**	**Selection Index**
Statistics of the **10** best accessions							
BRA 258	1,0	28,9	25,3	0,5	36,8	10,7	64,89
BRA 472	2,0	39,8	37,2	0,5	31,2	12,4	64,55
PER 368	3,0	43,8	43,1	0,5	30,5	13,4	63,52
BRA 315	1,0	30,0	30,6	0,5	35,9	10,8	62,51
PER 281	3,0	40,2	26,6	0,6	28,3	11,4	60,59
PER 293	3,0	38,0	25,9	0,6	27,8	10,5	56,29
COL 2192	4,0	31,3	17,5	0,6	33,3	10,4	54,66
BRA 885	2,0	31,2	21,6	0,6	29,1	9,1	53,77
VEN 210	3,0	30,8	20,9	0,6	31,8	9,8	53,58
COL 1505	50	35,7	28,8	0,60	33,3	11,9	50,99
Performance of the **65** clones selected							
Maximum	5	43,8	43,1	0,74	36,9	13,4	64,89
Minimum	1	6,0	2,5	0,42	22,2	2,2	20,27
Average	3	19,5	16,0	0,56	31,1	6,0	36,04
St. Deviation	1	8,9	8,1	0,07	3,3	2,7	12,69
Statistics of the **434** clones evaluated							
Maximum	5	43,8	43,1	0,74	36,9	13,4	64,89
Minimum	1	0,6	0,6	0,16	16,8	0,1	-26,66
Average	4	6,6	6,7	0,49	25,7	1,8	0,00
St. Deviation	1	7,5	7,2	0,09	4,8	2,3	19,93

As explained in Chapter 3 (**Figure 3.1**) the step after *CET* in the selection process is the ***Preliminary Yield Trial*** *or* ***PYT***. Clones evaluated in these trials are those selected during the *CET* conducted the previous year. The eight plants from the *CET* produce more than 30 stakes. Therefore, the *PYT* are planted with three replications of 10-plant plots. Each experimental plot consists of two rows with five plants each. Since selections at the *CET* stage are conducted in three different blocks selections within each block generate a respective *PYT*. The clones allocated to each block at the *CET* (and selected) are therefore, competing among themselves also at the *PYT* phase. The reasons for this are: a) This approach maximized the genetic variability within each *PYT* by maximizing the number of families present in it; b) The performance of the cassava plant depends heavily on the quality of the stake from which it grew, and the quality of the stakes, in turn, depends on the environmental conditions in which the mother plant grew. By keeping together in the same *PYT* trial the clones that grew together at the *CET* a better uniformity of the quality of the stakes is achieved and, therefore, the experimental error at the *PYT* is somewhat reduced. Plots are made up of two rows with five plants each. Row spacing is 80cm within plots and 160cm between plots to favor within-family competition and discourage between-family competition.

Three different *PYT*s were planted this year (**Tables 5.7**, **5.8** and **5.9)**. Each *PYT* had a total of 64 genotypes of which only 12 were selected. The previous season (2006-2007) two types of *CET* were planted. Half of the new germplasm (545 genotypes) were evaluated in the typical arrangement (*CET-1:* single-row plots, 8 plants per plot and no replication). The other half (516 genotypes), however, were planted following a new approach in which the eight plants of each genotype were split into two replications. This second type of evaluation *(CET-2)* was therefore planted using a two replications design in which each plot had four plants. Each type of *CET* was divided in three blocks. Materials selected in the first block of *CET-1* and *CET-2,* were included in *PYT-1.* Selected clones from the second block of *CET-1* and *CET-2* were included in *PYT-2.* Similarly those materials selected in the third block of *CET-1* and *CET-2* the previous season, were included in *PYT-3* during 2007-208.

Results from *PYT-1* are presented in Table 5.7. A total of 64 clones were evaluated, including four checks. Of the sixty experimental clones 30 come from the first block of *CET-1*, following the traditional 1 replication x eight plants the previous season. The other 30 clones were evaluated in the first block of *CET-2*, using a different approach based on two replications with four plants each. Of the 60 experimental clones, 12 were selected. Table 5.7 presents the performance of the best ten clones as well as those of the four commercial checks. The only commercial check with positive selection index was CM 4574-7. Because of the harsh environmental conditions in Puerto López, compared with those of CORPOICA La Libertad, only this clone which is particularly well adapted to the open savannas had a performance above average. In milder environmental constraints, the other checks may have a better performance.

The limiting environmental conditions of Puerto López are highlighted by the relatively low dry matter yields. However the performance of the experimental clones was markedly superior to the commercial checks, including CM 4574-7.

At the bottom of Table 5.7 a comparison of the 30 clones selected from Block 1 *CET-1* versus those from Block 1 from *CET-2* is provided. It is accepted that this is not a perfect comparison between the two selection criteria (1 replication x 8 plants versus 2 replications x 4 plants) because the actual genotypes evaluated in each type of CET was different, although belonging to the same families. However, it is expected that on average they are similar. It is expected, therefore, that the performance of the 30 clones selected in CET-2 (which is more complicated from the logistical point of view) would be slightly better. This is in fact the case for *PYT-1*, where the average selection index of the 30 clones from CET-2 was positive (2,23), whereas those from CET-1 was negative (-1,35).

Table 5.8 presents the results of *PYT-2*, which followed the same criteria as that for *PYT-1*. the performance of the commercial checks was better that in the previous trial. Selection indexes of the four checks were all positive indicating they all had a performance above average. CM 4574-7 remained the best check. Most experimental clones selected were clearly superior to the four commercial checks. In this case the average performance of clones from CET-1 (selection index = 5.21) was clearly superior to those from CET-2 which was markedly negative (-6,57). **Table 5.9** presents the results from *PYT-3*. Results follow the same trend a the previous two trials. CM 4574-5 was the best commercial check and the other three had below average performance. As in *PYT-2* the average performance of progenies from CET-1 (selection index = 7,82) was clearly better than those from CET-2 (selection index = -10,19)

Table 5.7. Results from the *Preliminary Yield Trial* (*PYT-1*) divided into three blocks and conducted in Puerto López (Meta Department). Half of the experimental clones were selected from *CET-1* (one replication x eight plants) and the other half from *CET-2* (two replications x four plants).

	Plant type (1-5)	**Fresh root yield (t/ha)**	**Foliage yield (t/ha)**	**Harvest Index (0-1)**	**Dry matter (%)**	**Dry root yield (t/ha)**	**Selection Index**
Statistics of the best 10 clones							
SM 3212-2	1	19,7	14,8	0,57	30,8	6,0	44,93
SM 3202-15	2	18,9	14,6	0,57	35,1	6,7	44,80
SM 3205-6	3	24,9	12,8	0,66	30,6	7,6	42,72
GM 956-3	3	20,6	20,3	0,50	36,2	7,5	40,28
GM 971-3	2	18,5	9,3	0,67	29,8	5,6	33,16
GM 937-7	3	17,3	7,2	0,70	33,3	5,9	31,36
GM 688-14	3	16,7	9,8	0,64	30,8	5,2	26,37
SM 3213-35	3	19,2	14,4	0,57	29,5	5,7	24,45
SM 3209-5	3	20,1	12,3	0,62	29,9	6,1	23,11
SM 3201-6	3	15,2	10,3	0,60	30,4	4,7	19,38
Performance of four commercial checks							
CM 523-7	4	6,4	6,7	0,49	30,6	1,9	-19,25
CM 4574-7	3	14,3	8,5	0,64	31,3	4,4	22,52
CM 6438-14	4	6,3	8,4	0,44	31,9	2,0	-22,21
CM 6740-7	4	9,2	8,4	0,52	33,0	3,0	-7,42
Statistics of the **12** selected clones							
Maximum	3	24,9	20,3	0,70	36,2	7,6	44,93
Minimum	1	14,1	7,2	0,50	29,1	4,7	17,74
Average	3	18,5	12,4	0,60	31,6	5,9	30,63
St. Deviation	1	2,8	3,6	0,06	2,4	1,0	10,39
Performance of the **64** clones evaluated							
Maximum	5	24,9	30,7	0,70	36,2	7,6	44,93
Minimum	1	3,2	3,1	0,34	24,4	0,8	-46,54
Average	3	12,4	11,9	0,53	30,0	3,8	0,00
St. Deviation	1	4,2	5,1	0,08	2,6	1,4	20,84
Average performance of the 30 clones from CET-1 and the 30 clones from CET-2							
CET-1	3,3	11,9	12,4	0,52	30,1	3,6	-1,35
CET-2	3,2	13,4	11,9	0,53	29,6	4,1	2,23

Table 5.8. Results from the *Preliminary Yield Trial (PYT-2*) divided into three blocks and conducted in Puerto López (Meta Department). Half of the experimental clones were selected from *CET-1* (one replication x eight plants) and the other half from *CET-2* (two replications x four plants).

	Plant type (1-5)	**Fresh root yield (t/ha)**	**Foliage yield (t/ha)**	**Harvest index (0-1)**	**Dry matter content (%)**	**Dry root yield (t/ha)**	**Selection Index**
Statistics of the **best 10** clones							
SM 3205-4	1	20,0	12,0	0,63	30,6	6,1	52,87
SM 3208-16	1	24,3	26,4	0,48	30,2	7,3	48,60
SM 3212-3	3	20,8	15,5	0,57	34,1	7,1	41,70
SM 3203-37	3	16,4	15,4	0,52	33,8	5,5	30,73
GM 971-2	3	16,0	13,4	0,54	33,5	5,3	26,78
SM 3209-17	3	14,4	9,9	0,60	31,3	4,5	25,76
SM 3208-12	3	13,0	10,4	0,56	34,6	4,5	25,58
SM 3199-8	3	15,0	11,8	0,56	32,5	4,9	23,90
GM 935-2	3	12,3	8,7	0,59	32,9	4,1	17,76
GM 687-2	3	14,5	12,0	0,55	30,9	4,5	17,43
Performance of four commercial checks							
CM 4574-7	3	13,3	9,7	0,58	32,0	4,3	24,58
CM 6438-14	4	12,4	7,1	0,64	32,7	4,0	17,80
CM 523-7	3	7,0	5,9	0,56	32,0	2,2	8,79
CM 6740-7	3	13,4	11,8	0,53	28,6	3,8	7,07
Statistics of the **12** selected clones							
Maximum	3	24,3	26,4	0,63	34,6	7,3	52,87
Minimum	1	12,3	8,7	0,48	28,5	3,7	13,53
Average	3	16,2	13,3	0,56	31,8	5,2	28,22
St. Deviation	1	6,1	9,2	0,07	3,0	1,8	19,88
Performance of the **64** clones evaluated							
Maximum	5	24,3	26,4	0,64	34,6	7,3	52,87
Minimum	1	3,3	2,6	0,36	23,5	0,8	-55,73
Average	3	10,3	9,3	0,52	29,6	3,1	0,00
St. Deviation	1	4,8	4,2	0,06	2,8	1,6	21,62
Average performance of the 30 clones from CET-1 and the 30 clones from CET-2							
CET-1	3,2	12,1	10,4	0,53	29,6	3,7	5,21
CET-2	3,3	8,6	8,5	0,51	29,4	2,6	-6,57

Table 5.9. Results from the *Preliminary Yield Trial* (*PYT-3*) divided into three blocks and conducted in Puerto López (Meta Department). Half of the experimental clones were selected from *CET-1* (one replication x eight plants) and the other half from *CET-2* (two replications x four plants).

	Plant type (1-5)	**Fresh root yield (t/ha)**	**Foliage yield (t/ha)**	**Harvest index (0-1)**	**Dry matter content (%)**	**Dry root yield (t/ha)**	**Selection index**
Statistics of the **best 10** clones							
SM 3212-7	2	20,2	15,5	0,57	33,5	6,8	43,51
SM 3029-43	3	19,0	12,4	0,61	33,4	6,3	30,08
SM 3213-52	3	20,1	14,6	0,58	33,3	6,7	29,69
SM 3208-24	3	20,1	16,7	0,55	34,4	6,9	29,61
SM 3209-58	3	20,2	15,6	0,56	34,9	7,0	28,76
GM 688-12	3	19,5	14,4	0,58	33,3	6,5	28,55
SM 3203-24	3	19,6	14,6	0,58	33,2	6,5	28,38
SM 3208-23	3	19,6	15,6	0,56	35,2	6,9	27,96
SM 3206-26	3	20,4	15,1	0,58	32,5	6,6	27,94
SM 3202-22	3	18,9	14,8	0,57	33,3	6,3	26,76
Performance of four commercial checks							
CM 523-7	4	7,3	4,2	0,64	31,6	2,3	-31,95
CM 4574-7	3	20,2	12,3	0,62	30,9	6,2	8,91
CM 6438-14	3	16,1	9,5	0,63	32,7	5,3	-0,30
CM 6740-7	3	15,0	8,8	0,64	31,4	4,7	-6,71
Statistics of the **12** selected clones							
Maximum	3	20,4	16,7	0,61	35,2	7,0	43,51
Minimum	2	18,0	12,4	0,55	32,5	6,0	26,19
Average	3	19,6	14,7	0,57	33,6	6,6	29,48
St. Deviation	1	1,2	2,1	0,03	1,3	0,5	9,20
Performance of the **64** clones evaluated							
Maximum	5,0	20,4	16,7	0,7	35,2	7,0	43,5
Minimum	1,7	3,7	2,2	0,4	19,9	0,8	-47,9
Average	3,5	9,9	8,3	0,5	30,6	3,2	0,0
St. Deviation	0,7	6,8	4,5	0,1	3,4	2,4	22,4
Average performance of the 30 clones from CET-1 and the 30 clones from CET-2							
CET-1	3,2	11,4	10,0	0,52	32,1	3,8	7,82
CET-2	3,8	7,7	6,6	0,54	29,0	2,4	-10,19

Based on the relative performances of clones selected using the traditional scheme (one replication x eight plants) compared with those from *CET-2* (two replications x four plants) and the fact that the latter is much more complicated to conduct, the conclusions based on the *PYT* evaluations in the acid soil savannas would support the same finding from the sub-humid environment: it is not justified to use the alternative approach and the traditional system (1 replication x eight plants) seems to be consistently equal or superior.

Table 5.10. Results from the *Advanced Yield Trial-First Cycle* (*AYT-I*) divided into three replications and conducted in Puerto López (Meta Department).

Clone or parameter	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **best 10** clones							
SM 3171-13	3	24,5	14,3	0,63	31,4	7,7	51,85
SM 3176-3	1	17,7	17,3	0,51	33,5	5,9	28,44
SM 3176-5	3	20,4	12,9	0,61	29,1	5,9	25,53
SM 3077-51	2	18,3	13,6	0,58	30,4	5,6	24,66
GM 517-36	2	18,4	14,3	0,56	31,6	5,8	24,36
GM 541-23	2	16,9	14,5	0,54	34,1	5,8	23,85
SM 3176-17	3	19,9	15,7	0,56	31,3	6,2	23,19
SM 3077-75	2	16,8	10,2	0,62	30,4	5,1	22,64
SM 3169-16	3	19,1	14,4	0,57	30,1	5,8	18,24
GM 543-78	3	17,5	11,1	0,61	31,0	5,4	18,06
Performance of five commercial checks							
CM 4574-7	2	18,5	12,9	0,59	30,1	5,6	23,19
BRASILERA	3	15,3	11,3	0,58	31,8	4,9	6,99
CM 6438-14	3	15,7	13,2	0,54	30,2	4,8	1,58
CM 6740-7	3	15,3	13,7	0,53	26,8	4,1	-13,55
CM 523-7	4	9,9	8,5	0,56	30,0	3,0	-30,45
Performance of the **65** clones evaluated							
Maximum	5	24,5	17,3	0,63	34,8	7,7	51,85
Minimum	1	8,2	6,3	0,47	20,4	2,3	-48,75
Average	3	15,2	12,3	0,55	30,2	4,6	0,00
St. Deviation	1	2,6	2,1	0,04	2,5	0,9	17,85

Clones selected at the *PYTs* are grouped together in an **Advanced Yield Trial** or ***AYT***, which can be planted in more than one location and in 20-plant or 25-plant plots (Figure 3.1). When plots have 20 plants, the six central plants are harvested for evaluation and the remaining 14 plants of the periphery are left as source of planting material. Harvested plants, therefore, grow surrounded by plants of the same genotype and, therefore, harvest data is taken only from plants that have competed with sister plants. In the case of 25-plant plots the nine central plants are harvested and the surrounding 16 are left as source of planting

material In this environment *AYTs* are conducted for two consecutive years with some selection exerted in the first year. Therefore there are two types of *AYTs*. **First year (*AYT-I*)** evaluates the best performing clones emerging from the *PYTs* conducted the previous year. **Second year (*AYT-II*)** evaluates the best performing clones emerging from the *PYTs* conducted two years before and after a mild selection at the *First year AYT-I*.

Table 5.11. Results from the *Advanced Yield Trial-First Cycle* (*AYT-II*) divided into three replications and conducted in Puerto López (Meta Department).

Clone or parameter	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **best 10** clones from the 20 selected							
GM677-8	3	20,0	11,5	0,63	31,1	6,2	26,86
GM693-3	3	19,0	12,8	0,60	31,8	6,1	25,08
SM3119-24	1	18,0	14,9	0,55	31,3	5,6	24,71
GM536-79	3	18,8	13,2	0,59	31,8	6,0	23,04
SM3033-4	3	19,1	14,6	0,57	31,1	6,0	19,20
GM536-88	2	16,5	18,1	0,48	33,4	5,5	16,71
SM3070-20	3	16,5	13,0	0,56	32,1	5,3	14,94
GM536-64	2	17,6	16,3	0,52	30,4	5,3	13,38
GM220-81	2	17,4	16,6	0,51	29,8	5,2	9,37
CM9638-5	3	20,6	14,0	0,59	26,9	5,5	6,06
Performance of five commercial checks							
CM 4574-7	2	18,3	14,8	0,55	30,0	5,5	13,80
BRASILERA	3	16,5	12,9	0,56	30,5	5,0	5,79
CM 6740-7	4	14,5	11,4	0,56	31,5	4,6	-6,51
CM 6438-14	4	12,5	9,0	0,58	30,6	3,8	-10,91
CM 523-7	4	10,5	9,6	0,52	28,6	3,0	-38,45
Performance of the **18** clones selected							
Maximum	4	20,6	18,1	0,63	33,4	6,2	26,86
Minimum	1	10,5	9,0	0,48	26,9	3,0	-38,45
Average	3	17,1	13,5	0,56	30,7	5,2	9,54
St. Deviation	1	2,8	2,5	0,04	1,5	0,9	17,25
Performance of the **30** clones evaluated							
Maximum	5	20,6	19,4	0,63	33,7	6,2	26,86
Minimum	1	10,3	7,2	0,44	26,0	2,7	-45,92
Average	3	15,8	13,4	0,54	30,3	4,8	0,00
St. Deviation	1	2,6	2,8	0,05	1,8	0,9	18,26

The clones selected in the *PYTs* planted in May 2006 were then planted in May 2007 in the *AYT-I (1st cycle)* trial whose results are presented in **Table 5.10**. A total of 65 clones and five commercial checks were evaluated in this trial and the best 25 genotypes were selected to move on as *AYT (2nd cycle)*. The trial was conducted in Puerto López. The average fresh root yield of the 65 clones was 15.2 t/ha with values ranging from 25.4 down to 8.2 t/ha. Dry matter content ranged from 34.8% to 20.4%, with an average of 30.2%. Average dry matter yield was 4.7 t/ha ranging from 7.7 down to 2.3 t/ha.

There were five commercial checks planted in *AYT-I*. As it was the case of previous trials CM 4574-7 was still the best check under the severe acid-soil conditions of Puerto López. Average dry matter yield of this clone was above 5.6 t/ha. Among the experimental clones SM 3171-13, SM 3176-3 and SM 3176-5 had an excellent performance with dry matter yields > 5.9 t/ha. From this *AYT* a group of 25 clones was selected for a second cycle (*AYT-II*) currently in the field. Two families had more than one clone represented among the best ten clones listed in Table 5.10: SM 3176 (2nd, 3rd and 7th) and SM 3077 (4th and 8th).

Table 5.11 presents the results of *AYT-II* evaluated also at Puerto López. As it was the case of *AYT-I*, the best two checks (out of the five included) were again CM 4574-7 and Brasilera (actually a Colombian landrace in spite of its name). The 18 clones selected in this *AYT-II* will be included in a Regional Trial for the following and final cycle of evaluations.

Regional Trials (*RT*) are experiments in which a group of varieties is evaluated using a common and uniform technology in a wide range of representative environments (including soil characteristics and prevalent diseases and pests). The ultimate objective is to identify genotypes that are genetically superior to available local commercial checks. Regional trials identify germplasm that will be officially released as new varieties. Clones that are selected in *AYT-II* are combined in *RT* that are evaluated for 2-4 years. In the 2007-2008 season three types of *RT* were established.

RT-I originated in the selected clones in *AYT-II* from the 2006-2007 season grown in two different and contrasting environments at CORPOICA La Libetad (Loma and Porcinos) and the summary of this trial is presented in **Table 5.12**. A total of 21 clones were evaluated, including the five commercial checks listed in the table. As it is always the case in this kind of environment, CM 4574-7 was the best commercial check followed by CM 6348-14 and Brasilera. A total of 14 clones will be evaluated in additional *PR* the following years.

RT-II originated in the selected clones in *PR-I* from the 2006-2007 season grown in two different and contrasting environments at CORPOICA La Libetad (Loma and Porcinos). The summary of this trial is presented in **Table 5.13**. A total of 16 clones were evaluated, including the five commercial checks listed in the table. For the first time CM 4574-7 was not the best commercial check, but the second after Brasilera. In this particular evaluation only one experimental clone was superior to all five commercial checks. Unfortunately, the lack for resources for more extensive evaluations prevented planting these regional trials in other locations, which would have produced more reliable data. This trials, therefore (as was the case for the other regional trials), would have to be grown again and, hopefully, in more than one location during the next season.

Table 5.12. Results from the *Regional Trial-First Cycle* (*RT-I*) evaluated in Cantaclaro, Puerto López, Meta Department.

Clone or parameter	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **best 5** clones from the 20 selected							
GM 536-13	3	19,7	17,2	0,53	32,5	6,4	22,10
GM 220-79	3	21,5	18,1	0,55	26,0	5,6	20,15
SM 3077-21	2	18,0	21,2	0,46	32,2	5,8	18,96
GM 536-20	2	17,1	19,6	0,47	34,1	5,8	17,55
GM 536-49	3	16,7	18,1	0,48	34,1	5,7	8,92
Performance of five commercial checks							
CM 4574-7	3	19,1	18,0	0,51	28,1	5,4	10,73
CM 6438-14	3	18,4	18,4	0,50	30,5	5,6	9,71
BRASILERA	3	17,4	18,0	0,49	31,6	5,5	6,92
CM 6740-7	3	17,3	18,3	0,49	28,0	4,8	-1,25
CM 523-7	4	12,6	14,4	0,47	29,6	3,7	-30,58

Table 5.13. Results from the *Regional Trial-Second Cycle* (*RT-II*) evaluated in Cantaclaro, Puerto López, Meta Department.

Clone or parameter	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the 6 clones selected							
SM2632-47	3	18,8	14,8	0,56	29,2	5,5	32,17
SM2642-35	3	15,3	15,7	0,50	30,9	4,7	16,24
SM2658-26	4	13,2	12,9	0,51	30,8	4,1	0,74
SM2965-29	4	12,5	10,7	0,54	30,1	3,8	0,36
SM2610-57	3	13,8	13,8	0,50	29,3	4,0	-1,61
SM2977-6	3	13,9	14,1	0,50	27,1	3,8	-8,75
Performance of five commercial checks							
BRASILERA	3	16,4	15,3	0,52	31,2	5,1	23,36
CM 4574-7	3	18,1	15,9	0,53	27,4	5,0	14,24
CM 6740-7	3	14,2	13,2	0,52	28,1	4,0	-2,31
CM 6438-14	4	12,6	11,7	0,52	29,5	3,7	-6,05
CM 523-7	4	10,0	12,1	0,45	27,9	2,8	-37,16

Data presented in Table 5.14 comes from the third regional trial (*RT-III*) which originated in the selections made from *RT-II* evaluated at Loma and Porcinos (CORPOICA – La Libertad). A total of 21 clones were evaluated (including the standard five commercial checks). The performances of these checks were different, relatively speaking, to those in other trials. Experimental clones in this trial were clearly superior to the commercial checks. Very relevant and unusual is the fact that five of the best six clones in the entire evaluation belong to the same family (CM 9460). When this family was first evaluated in a CET (harvest of March 2004) there were 12 members and seven of them were selected. Through the years five of these seven clones managed to survive all the way to this regional trial and they still show excellent performance.

Table 5.14. Results from the *Regional Trial-First Cycle* (*RT-III*) evaluated in Cantaclaro, Puerto López, Meta Department.

Clone or parameter	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **best 10** clones from the 20 selected							
CM 9460-12	3	22,0	13,9	0,61	28,9	6,4	24,87
CM 9460- 9	3	23,1	10,8	0,68	25,4	5,9	23,60
CM 9460-15	2	18,5	15,8	0,54	32,3	6,0	22,49
CM 9461- 1	4	17,6	12,6	0,58	32,8	5,8	15,21
CM 9460-13	3	18,3	12,8	0,59	30,3	5,5	16,69
CM 9460-40	2	15,6	14,2	0,52	32,6	5,1	16,56
CM 9464-36	3	13,8	13,8	0,50	31,8	4,4	4,43
SM 2792-31	4	12,1	9,1	0,57	31,1	3,8	0,18
CM 9464-29	3	13,9	12,2	0,53	29,6	4,1	3,19
CM 9461- 5	4	11,4	8,7	0,57	26,8	3,1	-8,73
Performance of five commercial checks							
CM 6740-7	2	15,0	12,1	0,56	27,6	4,1	8,67
CM 6438-14	3	14,0	9,8	0,59	27,8	3,9	3,44
CM 523-7	4	9,9	9,6	0,50	30,4	3,0	-8,10
CM 4574-7	3	14,8	11,9	0,56	27,5	4,1	3,23
BRASILERA	3	14,9	12,4	0,55	32,7	4,9	11,40
Performance of the **21** clones evaluated							
Maximum	5	23,1	15,8	0,68	32,8	6,4	24,87
Minimum	1	4,1	2,4	0,05	2,9	1,2	-28,52
Average	3	13,5	10,3	0,54	27,5	3,9	2,59
St. Deviation	1	5,2	3,5	0,11	6,0	1,6	15,71

CHAPTER 6

DEVELOPMENT OF GENETIC STOCKS AND IMPROVED GENE POOLS ADAPTED TO THE MID-ALTITUDE VALLEYS ENVIRONMENT

Activities developed for the Mid-altitude Valleys environment were centralized initially in CIAT Experimental Station, in Palmira Valle del Cauca Department. Because of the problems of Frog Skin Disease (**FSD**) that CIAT has failed to overcome a decision has been taken to move the evaluations of materials outside the Experimental Station. Therefore germplasm targeting this environment is now evaluated not only in CIAT experimental station but, preferably in other farms isolated from other cassava fields. It is in CIAT Experimental Station where crossing blocks are planted to produce the botanical seed of segregating families targeting not only mid-altitude valleys but all the other environments as well. Therefore it is at Palmira that all botanical seed is produced. In addition it is in Palmira where that seed is germinated and F1 nurseries (See Figure 3.1) are planted. The plants in the F1 nurseries are used as source of vegetative cuttings for the Clonal Evaluation Trials that are then shipped to the target environments. Finally it is also in Palmira where the root quality laboratory conducts all the screening of quality traits that has been so productive in recent years.

6.1. EVALUATIONS AND SELECTIONS IN THE VALLE DEL CAUCA DEPARTMENT

Table 6.1 lists the most relevant trials, whereas the other tables show results specific to each one. As mentioned in Chapter 3 (**Table 3.5**) a total of 2952 seeds were germinated and 1628 seedlings from these botanical seeds (targeting this particular environment) were transplanted at CIAT-Palmira in an isolated field in May 2008. The planting of the *F1* stage is isolated to reduce as much as possible infection by diseases that can be found at later stages of the evaluation process. Seedlings from botanical seed are considered to be disease-free and efforts are made to maintain this condition for as long as it can possibly be done.

Table 6.1. Trials conducted in the Mid-altitude Valleys environment during the 2007-2008 cycle.

Type of Trial	Genotypes (# plants)	Reps	Observations
Clonal Evaluation Trial	2111	1	Tables 6.2-6.5
Preliminary Yield Trial (*PYT-1*)	64	3	Table 6.6
Preliminary Yield Trial *(PYT-2*)	64	3	Table 6.7
Preliminary Yield Trial (*PYT-3*)	64	3	Table 6.8
Advanced Yield Trial (*AYT-1*)	55	3	Table 6.9
Advanced Yield Trial (*AYT-2*)	44	3	Table 6.10
Regional Trial (*RT-1*)	15	3	Table 6.11
Regional Trial (*RT-I1*)			Table 6.12

The F1 nursery planted in June 2007 was harvested in April 2008. Enough vegetative cuttings from 1628, 10-months old plants, from that nursery could be obtained and planted in the *CET* for the mid-altitude valleys (Valle del Cauca Department) on May, 2008. The trial will be harvested in April 2009.

Clonal Evaluation Trials are very large experiments around one hectare in size. A major constraint in their evaluation is the experimental error associated with the unavoidable variation in environmental conditions in such a large experimental plot. Because this is the first evaluation and selection stage (See Chapter 3) only 7-8 stakes are available from each genotype. Replication of each clone, therefore, is difficult to implement. On the other hand clones are grouped in either full- or half-sib families. Since many clones are generally available from each family they are randomly allocated in one of three blocks in which the field is divided. In other words instead of planting all the clones from a given family together one after the other, they are split in three groups, which are planted in the three blocks the entire evaluation is divided into (**Figure 4.1**).

The highest fresh root yield was observed in the third block in CET with 15.0 t/ha, and the lower yield in a clone from block 2 with as little as 1.0 t/ha (**Table 6.2**). Average fresh root yield was around 20 t/ha with a range of variation between 19.0 (Block 2) and 21.4 t/ha (Block 1). Since selection is made within each block this kind of environmental variation does not influence selection. This is precisely the advantage of stratified selection as indicated in Chapter 4. Average dry matter content was 35-36% These values for dry matter content are considered adequate ranging from intermediate to high.

For each block a group of 60 clones was selected. Parameters of all the clones evaluated in each of the three blocks, as well as for those 60 clones selected are provided in **Table 6.2**. Dry matter yield was very high illustrating the high yield potential of cassava for this kind of environment. Average dry matter yield of the selected fractions were 13.0; 13.9; 14.6 for blocks 1, 2 and 3, respectively. Overall the selected population presented fresh root yields around 31.0 t/ha, average dry matter content above 39%.

The performance of the best ten clones out of the 60 selected from each block in CET is presented in **Table 6.3**. In general the most important traits showed excellent levels in genotypes presented in that table. Fresh root yield, dry matter content and their combination (dry matter yield) are very high as to be expected for non-replicated data such as this one.

Four clones from family SM 3208, three clones from families GM 1267 and SM 3284 and two clones from families SM 3286, SM 3287 and SM 3294 were among the best ten clones throughout the three blocks of this *CET* (**Table 6.3**). Selected clones from each block were combined together in a PYT which will be harvested in April 2009.

Table 6.4 provides a summary of the overall performance of the families included in the Clonal Evaluation Trail. In addition to size of each family Table 6.4 provides information on the proportion of selected clones as well as the average selection index for each family. The overall selection pressure was 14.7%. Families that had a percentage of selected clones above 15% therefore, are considered to offer a performance above the average, which should be generally related to a positive average selection index. Families GM 1060; GM 1117, GM

1267, GM 1275, SM 3192, SM 3207, SM 3283, SM 3284 SM 3289 and SM 3292 had a performance above the average following these two criteria. Similarly GM 936, and SM 3200 were represented by several clones and none were selected. They had negative average selection indexes. Other families failed to get clones selected (GM 750, GM 763, GM 1061 and SM 3148) but they were very small with less that four clones in every case.

Table 6.2. Results from the *Clonal Evaluation Trial* (*CET*) divided into three blocks and conducted in CIAT Experimental Station (Valle del Cauca Department). In this trial each genotype was planted in a single row with eight plants (no replications).

Parameters	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Statistics of the **60** selected clones from Block-1							
Maximum	3	36.3	38.4	0.72	42.9	15.0	38.77
Minimum	1	26.0	12.4	0.46	35.2	10.1	25.42
Average	2	32.7	26.8	0.55	39.8	13.0	29.19
St. Deviation	1	2.8	6.0	0.05	1.6	1.1	3.22
Performance of the **409** clones evaluated in Block -1							
Maximum	5	36.3	50.8	0.72	42.9	15.0	38.77
Minimum	1	3.3	9.8	0.17	26.8	1.0	-56.81
Average	3	21.4	22.6	0.47	36.2	7.9	0.00
St. Deviation	1	10.7	8.7	0.11	3.0	4.3	23.05
Statistics of the **60** selected clones from Block-2							
Maximum	3	32.9	35.4	0.73	42.1	13.9	48.36
Minimum	1	24.6	11.2	0.44	35.8	9.7	26.22
Average	3	30.1	25.1	0.55	39.6	11.9	31.67
St. Deviation	1	2.2	5.0	0.05	1.3	1.0	4.02
Performance of the **406** clones evaluated in Block -2							
Maximum	7	32.9	51.3	0.73	42.1	13.9	48.36
Minimum	1	6.6	10.9	0.22	26.8	2.0	-48.74
Average	4	19.0	24.1	0.42	36.5	7.0	0.00
St. Deviation	1	9.4	6.0	0.11	2.8	3.7	22.71
Statistics of the **60** selected clones from Block-3							
Maximum	3	36.3	36.8	0.74	43.2	14.6	45.51
Minimum	1	26.4	12.4	0.44	33.3	10.7	28.20
Average	3	32.9	26.5	0.56	39.6	13.0	33.25
St. Deviation	1	2.5	4.9	0.05	1.9	0.9	3.78
Performance of the **409** clones evaluated in Block -3							
Maximum	5	36.3	52.7	0.74	43.2	14.6	45.51
Minimum	1	3.1	9.4	0.13	27.4	1.0	-58.60
Average	4	20.2	21.8	0.46	35.0	7.3	0.00
St. Deviation	1	10.6	8.3	0.10	3.3	4.3	24.30

Table 6.3. Results of the best six clones in each of the three blocks from the *Clonal Evaluation Trial* (*CET*) conducted in CIAT Experimental Station (Valle del Cauca Department). In this trial each genotype was planted in a single row with eight plants (no replications).

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter content (%)	Dry root yield (t/ha)	Selection Index
Performance of the best **10** clones from Block-1							
SM 3185-27	2	34.6	32.2	0.52	42.9	14.8	38.77
SM 3287-10	3	32.6	12.6	0.72	40.9	13.3	36.50
SM 3208-69	3	35.2	26.4	0.57	42.5	15.0	36.26
SM 3285-15	1	36.3	32.2	0.53	39.1	14.2	35.51
SM 3214-29	2	31.6	26.2	0.55	42.1	13.3	35.30
SM 3227-58	2	35.7	23.2	0.61	39.1	13.9	33.76
SM 3294-8	3	35.2	20.8	0.63	40.5	14.2	33.47
SM 3208-67	1	26.3	20.6	0.56	41.1	10.8	32.91
GM 1267-3	2	33.9	26.3	0.56	40.1	13.6	32.85
SM 3292-2	2	33.5	29.4	0.53	40.8	13.6	32.75
Performance of the best **10** clones from Block-2							
SM 3289-7	2	32.9	20.2	0.62	42.1	13.9	48.36
SM 3226-69	1	30.3	11.2	0.73	36.9	11.2	40.93
SM 3284-27	3	32.9	25.0	0.57	41.7	13.7	39.59
SM 3286-59	1	25.1	24.3	0.51	41.3	10.4	38.33
GM 1267-9	2	32.9	18.5	0.64	37.8	12.5	37.04
GM 1323-5	2	31.0	21.6	0.59	39.3	12.2	36.98
SM 3224-94	2	29.0	25.0	0.54	40.6	11.8	36.37
SM 3287-16	3	32.9	24.3	0.58	40.2	13.2	35.62
SM 3284-32	3	32.3	27.2	0.54	40.6	13.1	34.53
SM 3284-31	2	27.9	18.8	0.60	39.5	11.0	34.48
Performance of the best **10** clones from Block-3							
GM 1275-3	2	32.9	19.9	0.62	42.1	13.9	45.51
SM 3286-75	2	32.8	26.0	0.56	42.1	13.8	42.05
GM 1267-13	1	33.1	30.9	0.52	40.2	13.3	40.89
SM 3204-14	1	34.5	26.2	0.57	38.1	13.1	39.64
SM 3208-16	3	32.6	27.3	0.54	43.2	14.1	38.61
SM 3192-68	2	32.8	26.0	0.56	40.7	13.3	38.59
SM 3208-12	3	30.6	12.4	0.71	40.3	12.3	38.10
SM 3294-43	2	36.3	22.0	0.62	37.3	13.6	36.99
SM 3198-80	2	35.2	27.9	0.56	39.1	13.7	36.87
SM 3231-95	2	32.2	19.3	0.63	38.8	12.5	36.81

Table 6.4. Family size, number of selected clones in each family for the progenies evaluated in *CET*. Data combines results from the three blocks in which the trial was divided.

Family	Size (#)	Selected (%)	Selection Index	Family	Size (#)	Selected (%)	Selection Index
GM 750	1	0.0	-17.82	SM 3226	39	12.8	-3.76
GM 763	3	0.0	-4.43	SM 3227	48	16.7	-6.03
GM 936	21	0.0	-5.76	SM 3231	38	15.8	-1.69
GM 938	31	12.9	8.68	SM 3232	12	8.3	6.15
GM 982	10	10.0	2.44	SM 3280	13	15.4	-9.91
GM 1060	17	29.4	7.35	SM 3281	3	33.3	10.25
GM 1061	4	0.0	13.45	SM 3282	78	7.7	-8.07
GM 1117	1	100.0	28.05	SM 3283	31	22.6	13.65
GM 1267	16	37.5	12.43	SM 3284	49	22.4	8.54
GM 1275	4	25.0	5.11	SM 3285	50	16.0	9.47
GM 1323	11	9.1	-18.45	SM 3286	93	16.1	4.65
SM 3148	1	0.0	-5.00	SM 3287	39	12.8	-3.49
SM 3185	20	10.0	1.14	SM 3288	35	8.6	-4.82
SM 3192	32	21.9	6.04	SM 3289	13	30.8	9.40
SM 3197	72	15.3	-4.36	SM 3290	2	0.0	4.80
SM 3200	16	0.0	-1.87	SM 3291	27	14.8	1.88
SM 3204	66	10.6	-6.55	SM 3292	13	30.8	10.08
SM 3207	48	20.8	6.37	SM 3293	23	13.0	-6.10
SM 3208	59	18.6	0.43	SM 3294	49	18.4	-4.14
SM 3214	30	10.0	-2.93	SM 3226	39	9.6	-3.76
SM 3224	54	5.6	-6.17	**Total/average**	**1224**	**14.7**	**-0.05**

Elite germplasm is frequently used as progenitor in more than one cross to produce more than one family. **Table 6.5** consolidates the information from **Table 6.4** grouping together all the progenies with a common parental clone. For example SM 1660-4 was used as progenitor in four different families and GC 489-31, SM 1779-7 and SM 1871-33 were used as progenitor in three. About 25% of the progenies from GC 489-31 (combined across the three families were it was used) were selected. This is a much higher degree of success than the average of about 15% across the whole *CET*. It is, therefore, reasonable to assume that CG 481-31 is a progenitor with excellent breeding value. In contrast, progenies from SM 1779-7, also grouped in three different families had a success rate of only 7.4% and a negative average of selection index. This would suggest that this progenitor had a breeding value below average. None of the progenies from AM 266-50 (grouped in two families) were selected and the average selection index was -17.82, indicating also a below average performance. Other progenitors had above average percentage of selected clones (GM 295-5, GM 297-110, SGB 765-2, SM 1642-22 and SM 2985-11) but they were used only in the composition of just one family. These results are used to take decisions regarding the new set of progenitors to be used in the production of germplasm adapted to the mid-altitude valleys environment.

Table 6.5. Relative performance of progenitors involved in generating the progenies evaluated in *CET*. The performance of the progenitors is assesses through the average performance of all the progenies each progenitor produced.

Progenitor	# fam.	Selec. (%)	Pl.Type (1-5)	FRY (t/ha)	H.I. (0-1)	DMC (%)	DMY (t/ha)	Sel. Ind.
AM 244-31	1	0.0	5	14.3	0.52	33.7	4.8	-17.82
AM 244-86	1	0.0	4	19.0	0.47	36.1	6.8	-4.43
AM 266-50	2	0.0	5	17.8	0.48	35.5	6.3	-7.78
CG 489-31	3	25.0	3	22.6	0.49	37.1	8.6	8.73
CM 523-7	1	18.6	4	19.2	0.43	36.2	7.1	-2.19
CM 3306-4	1	0.0	5	12.5	0.54	38.9	4.9	-5.00
CM 4365-3	1	37.5	3	25.1	0.50	36.8	9.5	12.43
CM 4574-7	1	0.0	4	19.8	0.44	35.4	7.2	-1.87
CM 7514-8	1	10.0	4	20.3	0.48	35.8	7.4	1.14
CM 7951-5	4	10.1	4	19.6	0.44	35.6	7.1	-2.15
CM 8370-10	1	0.0	4	18.4	0.46	34.8	6.5	-5.76
GM 284-87	1	7.7	4	16.1	0.45	35.4	5.8	-8.07
GM 295-5	1	22.6	3	27.0	0.50	36.6	10.0	13.65
GM 297-110	1	22.4	3	23.4	0.47	36.7	8.7	8.54
GM 308-85	1	16.0	3	24.4	0.48	36.5	9.0	9.47
GM 374-22	1	16.1	3	23.0	0.46	36.0	8.4	4.65
GM 509-18	1	12.8	4	18.1	0.45	35.4	6.6	-3.49
SGB 765-2	1	100.0	2	35.9	0.58	37.3	13.4	28.05
SGB 765-4	1	12.9	3	24.2	0.50	36.5	8.9	8.68
SM 1460-1	1	20.8	3	22.2	0.45	36.9	8.3	6.37
SM 1565-15	1	18.6	3	20.2	0.42	36.2	7.5	0.43
SM 1565-17	1	21.9	3	23.5	0.48	36.2	8.7	6.04
SM 1642-22	1	29.4	3	21.3	0.49	37.1	8.1	7.35
SM 1660-4	4	16.9	3	20.9	0.46	36.0	7.8	1.77
SM 1779-7	3	7.4	4	18.7	0.45	35.2	6.8	-4.24
SM 1871-33	3	16.1	4	19.9	0.47	35.7	7.3	0.00
SM 2052-4	1	16.7	4	17.4	0.45	35.4	6.4	-6.03
SM 2058-2	1	0.0	4	20.9	0.48	37.0	7.5	4.80
SM 2085-7	1	9.1	4	12.7	0.42	34.1	4.5	-18.45
SM 2211-3	1	14.8	3	20.9	0.43	36.4	7.8	1.88
SM 2985-11	1	30.8	3	24.4	0.48	37.0	9.2	10.08
SM 3085-16	1	13.0	4	19.0	0.42	35.2	7.0	-6.10
SM 3091-4	1	18.4	4	17.6	0.42	36.3	6.6	-4.14
SM 3096-6	1	9.6	3	19.8	0.44	36.0	7.3	-0.53
HMC 1	1	9.1	4	12.7	0.42	34.1	4.5	-18.45
MCOL 2737	2	13.2	4	19.1	0.42	35.9	7.0	-2.24
MCOL 638	1	15.4	4	17.4	0.44	34.4	6.2	-9.91
MECU 72	1	8.3	4	23.8	0.48	36.3	8.9	6.15
MTAI 8	1	10.3	4	17.1	0.41	35.4	6.2	-7.57

As explained in Chapter 3 (**Figure 3.1**) the step in the selection process after the *CET* is the ***Preliminary Yield Trial*** or ***PYT***. Clones evaluated in these trials are those selected during the *CET* conducted the previous year. The seven or eight plants from the *CET* produce more than 30 stakes. Therefore, they are planted with three replications of 10-plant plots. Each experimental plot consists of two rows with five plants each. Since selections at the *CET* stage are conducted in three different blocks selections within each block generate a respective *PYT*. The clones allocated to each block at the *CET* (and selected) are therefore, competing among themselves also at the *PYT* stage. The reasons for this are:

a) This approach maximized the genetic variability within each by maximizing the number of families present in it;

b) The performance of the cassava plant depends heavily on the quality of the stake from which it grew, and the quality of the stakes, in turn, depends on the environmental conditions in which the mother plant grew. By keeping together in the same trial the clones that grew together at the *CET* a better uniformity of the quality of the stakes is achieved and, therefore, the experimental error at the *PYT* is somewhat reduced.

During the previous cycle two different types of CET were conducted. *CET-1* followed the traditional design of one replication, in which each genotype was planted in a single-row plot with eight plants. Because of the concerns of lack of replication and the size of these trials it is relevant to have an idea of the convenience of introducing replicated evaluation even at this early stage of the evaluation process. Therefore, *CET-2* was planted using a different design in which the eight plants traditionally planted in a single row were split in two replications with four plants each. This followed the same ideas applied to the other two major environments (Sub-humid and acid-soils). Selections made using the two types of CETs were combined in a common *PYT*.

Clones that were selected from *CET-1* and *CET-2* for the Mid Altitude Valleys in May 2007 were planted in *PYTs* in June 2007 and harvested in May 2008. **Tables 6.6 to 6.8** provide the most relevant information for *PYTs* 1, 2 and 3, respectively. Comparison of the mean performance of each trial across **Tables 6.6** through **6.8** reveals the kinds of environmental variation that can be found, which is effectively controlled by growing three separate trials. Average fresh root yields were 24.4, 19.5, and 19.1 t/ha respectively for PYT *1, 2 and 3.*

Results from the first *PYT* are summarized in **Table 6.6**. A total of 60 experimental clones had been evaluated, as well as four commercial checks planted for comparison purposes. Three replications were used and plot size was the standard for this kind of trial based on ten plants (two rows with five plants each). As explained above, 30 of the experimental clones were selected in the first Block of *CET-1* (1 rep x eight plants) and the other 30 experimental clones from Block 1 in CET-2 (2 reps x four plants).

The best commercial check was HMC-1 and the only one with positive selection index. The other three checks had negative selection indexes (below average performance). Each and every selected clone was superior to the best commercial check and most of them produced more than 10 t/ha of dry matter. Average dry matter yield of the selected clones was, in fact, 11.7 t/ha. Average dry matter content of the selected fraction was 38.0%, which is an excellent value. Fifteen of the experimental clones included in *PYT1* were selected for evaluation in *AYT-I*. At the bottom of Table 6.6 the average of the 30 clones selected using the

1 rep x 8 plants approach versus the 2 reps x 4 plants are presented. Based on the average selection index of each group of 30 clones, the conclusion to draw from this experiment is that the additional complications to include two reps no only was not justifiable but it had negative effects.

Table 6.6. Results from the *Preliminary Yield Trial* (*PYT-1*) evaluated in Palmira (Valle del Cauca Department). A total of 84 clones were evaluated, from which 17 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Performance of the best **10** clones							
GM 397-2	3	39.5	28	0.6	38.5	15.2	39.01
SM 3091-64	2	33.1	17	0.7	37.9	12.6	33.14
SM 3093-37	2	41.7	34	0.6	35.6	14.8	31.58
SM 3226-32	2	25.2	19	0.6	40.2	10.1	26.07
SM 3226-8	4	29.8	18	0.6	39.9	11.9	24.94
SM 3221-26	2	31.5	26	0.5	37.7	11.9	22.99
GM 982-2	2	31.2	31	0.5	37.5	11.7	21.95
SM 3225-10	3	28.5	32	0.5	40.3	11.5	21.23
SM 2747-1	2	22.8	19	0.5	39.7	9.0	19.23
SM 3220-29	3	31.1	17	0.7	36.4	11.5	16.19
Performance of the four commercial checks							
HMC 1	4	31.1	17.6	0.64	35.7	11.1	7.24
M PER 183	4	27.2	26.6	0.52	34.2	9.3	-9.38
CM 7951-5	4	14.4	11.6	0.56	37.4	5.4	-11.57
MCOL 1505	4	13.3	12.5	0.52	36.1	4.8	-23.53
Performance of the **15** clones from selected							
Maximum	4	41.7	33.7	0.67	40.3	15.2	39.01
Minimum	2	22.8	16.9	0.46	35.5	9.0	13.96
Average	3	30.9	25.0	0.56	38.0	11.7	21.89
St. Deviation	1	4.8	6.1	0.06	1.6	1.6	7.85
Performance of the **64** clones evaluated							
Maximum	5	41.7	40.8	0.67	40.3	15.2	39.01
Minimum	2	12.0	8.4	0.25	32.4	4.4	-43.49
Average	3	24.4	24.1	0.50	36.6	9.0	0.00
St. Deviation	1	6.9	7.3	0.09	1.8	2.6	18.53
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	2.9	25.5	23.4	0.52	36.4	9.3	3.596
CET-2	3.3	23.7	25.7	0.48	36.8	8.8	-2.355

Results from the second *PYT* are summarized in **Table 6.7**. As in the first trial a total of 60

experimental clones had been evaluated: 30 of these experimental clones were selected in the second Block of *CET-1* (1 rep x eight plants) and the other 30 experimental clones from Block 2 in CET-2 (2 reps x four plants). HMC 1 was again the best commercial check and had already a negative selection index. It can be concluded that the four checks had below average performance. Average dry matter yield of the 15 selected clones was 9.4 and their average dry matter content was 37.7%. In this particular trial, materials selected last year CET-2 (2 reps x 4 plants) performed slightly better than those selected using the traditional system of 1 rep with eight plants.

Table 6.7. Results from the *Preliminary Yield Trial* (*PYT-2*) evaluated in Palmira (Valle del Cauca Department). A total of 84 clones were evaluated, from which 17 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Performance of the best **10** clones							
GM 982-30	3	28.7	18.1	0.61	40.0	11.5	35.76
GM 982-12	2	24.3	18.4	0.57	38.4	9.3	25.92
SM 3226-33	2	19.0	16.8	0.53	40.9	7.8	25.86
GM 397-5	3	24.0	19.9	0.55	38.8	9.3	23.05
SM 3229-18	3	25.4	23.2	0.53	38.8	9.9	21.95
SM 3227-17	2	20.5	13.1	0.60	38.4	7.9	21.40
SM 3226-13	3	20.3	16.6	0.55	40.9	8.3	21.39
SM 3228-35	1	28.4	19.9	0.58	33.6	9.6	20.71
CM 9884-3	3	31.5	24.5	0.57	35.6	11.2	20.49
GM 982-31	3	24.1	18.6	0.57	37.7	9.1	19.93
Performance of the four commercial checks							
MCOL 1505	4	11.9	9.3	0.55	34.9	4.3	-17.58
M PER 183	4	26.0	19.7	0.56	32.8	8.7	-6.37
HMC 1	4	20.4	14.4	0.59	35.3	7.2	-3.98
CM 7951-5	4	10.9	8.2	0.54	36.8	4.1	-14.53
Performance of the **15** clones from selected							
Maximum	3	31.5	24.5	0.71	40.9	11.5	35.76
Minimum	1	19.0	11.6	0.45	33.6	7.5	16.14
Average	3	25.0	18.5	0.58	37.7	9.4	21.60
St. Deviation	1	3.9	3.7	0.06	2.3	1.2	4.92
Performance of the **64** clones evaluated							
Maximum	4	31.5	29.4	0.71	40.9	11.5	35.76
Minimum	1	4.7	5.7	0.22	31.5	1.7	-44.75
Average	3	19.5	17.3	0.52	36.0	7.1	0.00
St. Deviation	1	6.6	5.6	0.10	2.0	2.4	18.85
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	3.2	19.8	17.2	0.52	35.6	7.1	-1.259

CET-2	3.1	19.5	18.0	0.51	36.6	7.1	2.674

Table 6.8. Results from the *Preliminary Yield Trial* (PYT-3) evaluated in Palmira (Valle del Cauca Department). A total of 84 clones were evaluated, from which 17 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 10 plant-plots.

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Performance of the best **10** clones							
GM981-3	2	26.3	28.2	0.48	39.7	10.4	33.49
GM982-15	3	34.2	27.6	0.57	38.0	13.0	33.38
SM3224-47	3	32.0	23.9	0.57	37.9	12.1	30.14
SM3226-44	3	23.2	18.8	0.56	40.2	9.3	29.85
SM3221-40	2	29.7	20.1	0.60	36.0	10.7	27.75
SM3227-23	3	32.6	18.5	0.64	36.2	11.8	26.82
SM3217-8	3	33.1	25.1	0.57	37.3	12.3	26.59
GM982-14	3	26.9	18.2	0.58	37.7	10.2	25.62
SM3232-20	2	34.9	36.2	0.49	34.9	12.2	25.46
SM3099-57	2	23.5	26.6	0.47	38.3	9.0	23.62
Performance of the four commercial checks							
CM 7951-5	3	17.9	13.4	0.57	37.2	6.8	6.72
HMC 1	4	22.4	17.1	0.57	35.4	7.9	0.30
M PER 183	3	20.6	22.4	0.48	33.4	6.9	-9.15
MCOL 1505	4	10.8	15.3	0.38	34.1	3.6	-25.83
Performance of the **15** clones from selected							
Maximum	4	34.9	36.2	0.64	40.2	13.0	33.49
Minimum	2	19.4	18.2	0.42	34.9	7.6	16.48
Average	3	27.5	23.8	0.54	37.8	10.3	25.24
St. Deviation	1	5.2	4.9	0.06	1.5	1.7	5.28
Performance of the **64** clones evaluated							
Maximum	5	34.9	47.9	0.64	40.2	13.0	33.49
Minimum	2	5.0	13.1	0.15	32.0	1.6	-53.10
Average	3	19.1	24.0	0.43	36.4	7.0	0.00
St. Deviation	1	7.6	6.7	0.12	1.9	2.8	20.99
Average performance of the 30 clones from *CET*-1 and the 30 clones from *CET*-2							
CET-1	3.1	19.7	24.8	0.42	36.1	7.2	-0.338
CET-2	3.2	18.7	24.1	0.43	36.8	6.9	1.271

Table 6.8 presents the result of *PYT-3.* Check CM 7951-5 was in this case the best, followed by HMC 1. Both had positive selection indices. Average dry matter productivity of the 15 selected clones was 7.0 t/ha and their dry matter content was 36.4%. Clones selected from *CET-2* performed slightly better than those from *CET-1*, although this difference is rendered insufficient to justify the additional logistic complications that *CET-2* implied.

Table 6.9 presents the result of the *Advanced Yield Trial – First Cycle* (*AYT-I)* combining the 17 selected clones from PYT1, PTY2 and PYT3 from last years evaluations. A total of 51 experimental clones and four checks were evaluated in this *AYT-I*. CM 7951-5 and M COL 1505 were the best two checks but both had negative selection indices. This means that the four checks had below average performance. A total of 22 clones were selected and they showed an average of 11.4 t/ha of dry matter and an outstanding 40.1% dry matter content. These 22 clones will be evaluated in a *AYT-II* to be harvested in April 2009.

Table 6.9. Results from the *Advanced Yield Trial* (*ATY-I*) conducted in Palmira (Valle del Cauca Department). A total of 55 clones were evaluated, from which 22 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 25 plant-plots.

Clone	Plant type (1-5)	Fresh root yield (t/ha)	Foliage yield (t/ha)	Harvest Index (0-1)	Dry matter content (%)	Dry root yield (t/ha)	Selection Index
Performance of the best **10** clones							
SM 3042-15	3	36.9	19.8	0.67	38.6	14.2	70.37
GM 297-137	2	26.6	18.7	0.58	36.9	9.9	32.64
GM 309-128	2	26.9	22.3	0.55	35.9	9.7	31.37
SM 3096-53	3	21.4	16.4	0.58	39.6	8.4	25.09
GM 570-38	3	17.7	12.8	0.60	40.0	7.1	21.33
SM 3042-3	3	24.8	20.4	0.53	38.4	9.6	20.98
SM 2911-3	2	23.5	21.7	0.52	36.7	8.6	20.57
CM 9953-254	3	22.1	15.6	0.59	37.8	8.4	20.40
CM 9953-252	2	21.9	15.8	0.58	36.7	8.0	19.35
GM 568-28	1	19.1	16.9	0.54	37.1	7.1	17.72
Performance of the four commercial checks							
CM 7951-5	4	17.1	13.8	0.56	37.3	6.4	-3.55
M COL 1505	4	22.3	16.2	0.59	33.8	7.6	-3.86
HMC-1	3	15.9	14.7	0.53	34.1	5.4	-17.96
M PER 183	3	19.6	18.0	0.53	32.3	6.3	-18.05
Performance of the **22** clones from selected							
Maximum	4	34.3	31.7	0.73	43.6	13.5	31.58
Minimum	2	23.9	9.6	0.46	36.6	9.8	3.38
Average	3	28.4	19.9	0.60	40.1	11.4	14.09
St. Deviation	1	3.0	5.7	0.07	1.6	1.1	8.64
Performance of the **55** clones evaluated							
Maximum	4	36.9	26.8	0.67	40.0	14.2	70.37
Minimum	1	12.2	12.8	0.43	32.3	4.1	-43.76
Average	3	19.2	17.1	0.53	35.9	6.9	0.00
St. Deviation	1	3.8	2.7	0.05	1.9	1.6	18.79

The *Advanced Yield Trial – Second Cycle (AYT-II*) described in **Table 6.10** was derived from an *AYT-I* harvested in May 2007. This trial included four commercial checks and 40 experimental clones. Every commercial check had a negative selection index. The average dry matter yield of the 20 selected clones was 7.4 t/ha, considerably higher that the checks. Dry matter content of selected progenies was 34.1%

Table 6.10. Results from the *Advanced Yield Trial* (*ATY-II*) conducted in Palmira (Valle del Cauca Department). A total of 44 clones were evaluated, from which 20 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 25 plant-plots.

Clone	Plant Type (1-5)	Fresh root yield (t/ha)	Foliage Yield (t/ha)	Harvest Index (0-1)	Dry matter content (%)	Dry root yield (t/ha	Selection Index
Performance of the best **10** clones							
CM9911-7	2.7	29.8	14.2	0.7	34.5	10.3	42.5
GM474-27	2.3	29.6	23.1	0.6	35.4	10.5	39.7
CM9911-13	2.7	31.3	19.2	0.6	33.2	10.4	35.8
CM9920-23	3.0	27.8	17.0	0.6	34.8	9.7	34.1
SM3043-10	2.3	19.9	13.6	0.6	36.4	7.3	27.7
SM2803-67	3.0	24.2	15.0	0.6	33.9	8.2	23.2
SM2805-47	1.3	21.3	19.6	0.5	33.9	7.2	20.5
SM2801-56	3.0	22.2	16.6	0.6	34.6	7.7	19.4
CM9907-110	4.0	27.9	20.3	0.6	33.1	9.2	17.8
CM9912-117	3.0	23.6	14.7	0.6	32.7	7.7	16.8
Performance of the four commercial checks							
M COL 1505	3.3	17.2	12.8	0.6	32.6	5.6	-0.6
M PER 183	3.0	18.8	16.6	0.5	31.0	5.8	-4.5
CM 7951-5	3.3	17.3	17.4	0.5	33.1	5.7	-3.3
HMC-1	4.0	17.5	13.4	0.6	30.0	5.2	-15.4
Performance of the **20** clones from selected							
Maximum	4	31.3	23.1	0.68	36.4	10.5	42.45
Minimum	1	16.2	13.6	0.45	31.4	5.6	1.59
Average	3	21.8	17.0	0.56	34.1	7.4	16.83
St. Deviation	1	5.0	2.6	0.06	1.2	1.7	13.18
Performance of the **44** clones evaluated							
Maximum	4.0	31.3	23.1	0.7	38.1	10.5	42.5
Minimum	1.0	9.7	11.2	0.4	27.8	3.2	-32.2
Average	3.1	17.8	16.4	0.5	33.0	5.9	0.0
St. Deviation	0.8	5.3	3.0	0.1	1.9	1.9	19.5

At the end of the evaluation cycle surviving clones are evaluated in Regional Trials (**Figure 3.1**). The first cycle regional trial described in **Table 6.11** included 15 genotypes (11 experimental clones and four checks). Seven clones were selected and all of them had a better performance than the best commercial check. Average dry matter yield was 6.7 t/ha with an average dry matter content of 32.9%. **Table 6.12** summarizes the result of a second cycle regional trial. M COL 1505 was the best commercial check and the best fourth ranking genotype. Five genotypes were selected and will be evaluated again during the 2008-2009 season. In previous years there has been frequent flooding at CIAT experimental station and this resulted in several trials being lost. The lack of resources to conduct multi-location trials combined with reduced availability of planting material results in these trials not being planted in several locations as would be desirable.

Table 6.11. Results from the *Regional Trial-First Cycle* (*RT-I*) conducted in Palmira (Valle del Cauca Department). A total of 15 clones were evaluated, from which 7 were selected. Each genotype was planted in three replications with 25 plant-plots.

Clone	Plant Type (1-5)	Fresh root yield (t/ha)	Foliage Yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root Yield (t/ha)	Selection Index
Performance of the **7** clones selected							
GM 297-89	2	26.1	18.2	0.59	36.5	9.5	53.44
GM 555-3	2	22.6	14.7	0.61	32.0	7.2	24.40
GM 374-22	2	21.7	19.2	0.53	33.1	7.2	21.81
SM 2985-8	3	19.1	15.7	0.55	33.3	6.4	14.27
SM 3096-20	3	20.3	20.9	0.49	32.1	6.5	6.30
SM 3087-3	3	14.8	7.4	0.67	31.8	4.7	4.26
SM 3090-8	2	17.3	19.1	0.47	31.7	5.5	1.83
Performance of the four commercial checks							
MCOL 1505	3	15.8	14.0	0.53	33.1	5.2	3.10
HMC-1	4	15.6	10.7	0.59	30.9	4.8	-11.62
M PER 183	4	14.0	16.9	0.45	31.3	4.4	-17.84
CM 7951-5	4	14.3	12.2	0.54	29.5	4.2	-20.31
Performance of the **7** clones from selected							
Maximum	3	26.1	20.9	0.67	36.5	9.5	53.44
Minimum	2	14.8	7.4	0.47	31.7	4.7	1.83
Average	2	20.3	16.5	0.56	32.9	6.7	18.04
St. Deviation	0	3.7	4.5	0.07	1.7	1.5	17.85
Performance of the **15** clones evaluated							
Maximum	4	26.1	24.1	0.67	36.5	9.5	53.44
Minimum	2	10.5	7.1	0.37	29.5	3.4	-36.90
Average	3	16.7	15.5	0.52	32.0	5.4	0.00

St. Deviation	1	4.4	4.7	0.09	1.7	1.7	22.58

Table 6.12. Results from the *Regional Trial – Second Cycle* (*RT-II*) conducted in Palmira (Valle del Cauca Department). A total of 44 clones were evaluated, from which 20 were selected. Performance of the best 10 clones is presented. Each genotype was planted in three replications with 25 plant-plots.

Clone	Plant Type (1-5)	Fresh root yield (t/ha)	Foliage Yield (t/ha)	Harvest Index (0-1)	Dry matter Content (%)	Dry root yield (t/ha)	Selection Index
Performance of the **5** selected clones							
SM 2858-31	2	21.6	23.0	0.48	33.6	7.3	25.95
SM 3047-19	4	19.9	20.6	0.49	37.1	7.4	25.78
SM 2858-2	3	20.3	22.5	0.47	33.4	6.8	15.92
GM 297-67	1	15.7	16.1	0.49	32.3	5.1	7.51
SM 2869-9	4	20.7	22.4	0.48	31.3	6.5	5.24
Performance of the four commercial checks							
M COL 1505	3	21.4	22.4	0.49	30.3	6.5	8.49
CM 7951-5	3	17.8	21.8	0.45	31.2	5.6	-7.74
M PER 183	3	17.2	24.7	0.41	30.8	5.3	-17.25
HMC-1	4	14.7	18.2	0.45	29.9	4.4	-29.48
Performance of the **5** clones from selected							
Maximum	4	21.6	23.0	0.49	37.1	7.4	25.95
Minimum	1	15.7	16.1	0.47	31.3	5.1	5.24
Average	3	19.6	20.9	0.48	33.5	6.6	16.08
St. Deviation	1	2.3	2.8	0.01	2.2	0.9	9.78
Performance of the 11 clones evaluated							
Maximum	4	21.6	24.7	0.50	37.1	7.4	25.95
Minimum	1	13.6	16.1	0.38	29.9	4.1	-32.97
Average	3	18.3	21.1	0.46	32.0	5.9	0.00
St. Deviation	1	2.7	2.5	0.04	2.1	1.1	20.16

6.2. CONTRIBUTION TO THE STUDIES OF GENE FLOW IN CASSAVA

CIAT is coordinating a World Bank supported project related to bio-safety issues involving several crops and countries. On of the objectives of this project is to assess the possibility of undesirable gene flow from one cassava genotype to another. This is particularly relevant as background information required for the release of genetically modified cultivars. Very little information has, so far, been generated in this area and the project is collaborating in the implementation of such pioneering work. For an undesirable cross between a genetically modified cassava (GMC) and a recipient cassava cultivar (landrace) to be successful several steps are required. The first step is the need for pollen from one of these genotypes to be able to fertilize a female flower from the other cultivar. Then the botanical seed will have to be able to produce a "seedling plant" (originating in a botanical seed not from a vegetative cutting) that will survive and competes with the normal cassava crop planted the following season.

Two different studies were initiated during the second semester of 2008 to answer some of these questions.

6.2.1 Quantification of natural crosses in cassava

Because there is a large area of cassava planted with the recently discovered waxy starch mutant, these plants offer an ideal material to quantify the frequency of crosses between different cassava cultivars. Waxy starch is a recessive trait. Botanical seed has been collected in the waxy starch mutant plot and will continue to be harvested through the first semester of 2009. The seed will then be germinated and non-waxy "contamination" will be easily quantified by growing the harvested seed and making an early assessment of starch quality in branches or stems of these plants at three months of age using the iodine solution

6.2.2 Survival of seedling volunteers

Cassava seeds produce seedling plants that are generally weaker than plants derived from vegetative cuttings. Two separate fields were identified in the Valle del Cauca Region that had been planted with a variety that tends to flower considerably. These fields were planted again with cassava providing an ideal condition for measuring the surviving capacity of these seedling plants. It has been observed already that most of the volunteer seedling plants are eliminated in the normal agronomic practices conducted by the farmer. The few surviving plants are weak because they are coming from botanical seed (vegetative cuttings produce much more vigorous plants), they are the result of self-pollinations happening during the previous cycle and therefore show considerable inbreeding depression, and also because the practices of directed application of fertilizers and insecticides tend to favor plants from the cuttings against the seedling plants. These studies and new ones to be developed during 2009 will provide valuable information. A full report will be prepared and presented elsewhere.

6.3. Adjustment of a rapid multiplication methodology to be applied to those F2 plants that produce waxy starch

The original idea of rapid multiplication of cassava planning material based on sigle bud/leaf is based on an earlier publication by Chant and Marden (1958). This method was later modified in 1972 by Klopenburg and co-workers (Department of Tropical Crops, Wageningen, The Netherlands) and by Sykes and Harney (Guelph University, Canada). The method was further improved by Pateña, Barba and Estrella (1979) at the Plant Breeding Institute from the University of the Philippines at Los Baños. CIAT presented this methodology (Tontyaporn, Peña and Zuraida) during a training course in 1979 in Palmira, Colombia. Following these earlier works Roca and collaborators further tested and improved this method to make it more efficient.

This work aims at recovering the original information, validate earlier results and make further improvements in the technology so that it can be applied for the rapid multiplication of genotypes that are found to produce waxy starch in a large F2 nursery to be transplanted in May 2009. Incidentally the method can be also applied for the rapid multiplication of any germplasm that TTDI finds strategic for its operations.

Studies were conducted to validate and improve a methodology to recover plants from explants that carry a single leaf and a single bud. The following varieties were used in the study: CM 2177-2, MCOL 1505, CM 4574-7, MPER 183 and TAI8 (= Rayong 60).

6.3.1 MATERIALS AND METHODS:

Figure 6.1 illustrates the conditions in which tissue was maintained to induce the production of roots (rooting chamber). It is a metal table with a iron structure (can preferably be aluminum) to support the plastic that maintains high moisture conditions inside. This particular table was 2 x1 m and its main surface was at 0.7 m from the floor. The height of the iron frame was 1 m with length and width similar to those of the table below. The top of the iron frame allowed for a two-slope "roof" for the chamber. The lateral plastic walls of the frame worked as curtains so the movement of samples was easy to conduct. Inside the chamber, and at mid-height, two small nebulizers were placed.

Figure 6.1. General view of the rooting chamber used in this study.

Two different media were used to induce the production of roots:

a) Sand substrate: Sand (mid-particle size) was sterilized with steam. The sand was then placed in the boxes shown in **Figure 6.1**. The height of these boxes was 20 cm. Every 10 cm wires were placed transversally on the sand surface.

b) Distilled water in glass flasks: This is the most common method used to induce rooting in the two-buds ex plant approach. Cupper oleate 2cc/lt) was used to prevent the growth of algae in the water.

Ex plants were first treated with a solution combining a chemical insecticide (Dimetoato (Sistemin) 2.0 cc/lt) and a fungicide (Orthocide 3grs/lt). In addition to the two rooting media described above each was used with or without the addition of hormones.

Adicionalmente, para los dos medios de enraizamiento, se utilizaron dos tratamientos con hormona y sin hormona. En el caso de los tratamientos utilizando como medio de enraizamiento agua destilada se adicionó oleato de cobre (2cc/lt) para evitar el crecimiento de algas en el medio.

☞ Identification of healthy and vigorous plants from each of the varieties listed above. These plants were not mature but only five month old, anticipating that the procedure will be used in young plants as soon as the test for the identification of those F_2 plants producing waxy starch during the second semester of 2009.

☞ With a sharp knife, previously disinfected with sodium hypochlorite, all leaves in a selected stem were extracted through a cutting just below the bud located at the base of each petiole. Care was given to assure that a section of hardened stem was also taken with the leaf. Excised leaves were then immediately placed in a recipient with distilled water with the proximal portion at the bottom.

☞ The distal portion of the leaves was cut so that only 30% of their surface remained. The leaves treated this way were randomly allocated to the different treatments.

☞ In the case of the treatments using the sand, small rows were marked in the sand so that the leaves could be placed with an inclination of about 30° from the vertical. The wires previously positioned helped to achieve this inclination (**Figures 2 and 3**). Once the cut leaves were properly positioned the bottom was covered with sand and a light pressure was exerted to facilitate contact between the tissue and the substrate.

☞ Ex plants were maintained under high moisture conditions using the nebulizers for 8 hours/day. The plastic covering the rooting chamber was left open in the lower 20 cm, so there was some airflow through the surface of the sand.

Figure 6.2: Illustration of the wires and rows plants marked on the sand

Figure 6.3. Illustration of the way the ex were placed in the rooting chamber

Every day the different leaves maintained under the different treatments were controlled. In the case of those treatments using distilled water, water was added as required to maintain the levels at the adequate levels (**Figure 6.4**): the level of the water was such at the level of the bud of each ex plant. The final evaluation and assessment of the different treatments took place 15 days after the beginning of the evaluation.

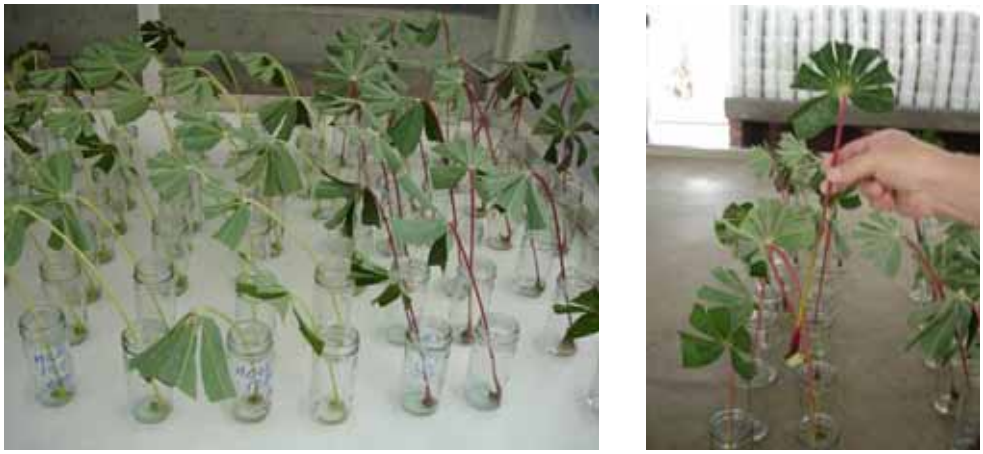


Figure 6.4. Treatment using distilled water.

6.3.2 RESULTS.

Table 6.13 summarizes the most important results of this initial evaluation using sand, with or without the addition of hormones for each of the five clones used. It can be observed that there were important differences in the way buds sprouted, depending if treatments included or not the addition of hormones. There were also some varietal differences. Regarding the development of roots it can be said, as a general rule that, whenever the bud sprouts, there will be a parallel initiation of roots in the scar tissue.

Results using distilled water as a medium are presented in **Table 6.14**. Compared with the sand treatment and irrespective of the addition or not of hormones there was a much lower sprouting in most varieties. In the case of CM 2177-2, MCOL 1505 and MPER183 some ex plants (without the addition of hormones) showed some incipient sprouting of their buds. **Figures 6.5 and 6.6** provide information regarding the variation in temperature and relative humidity respectively. According to this information the high day temperatures, associated with low relative moisture could perhaps explain the poor results observed when ex plants were placed in distilled water.

Figures 6.7 and 6.8 are presented to illustrate the way the different tissue evolved using different treatments. As stated above, the treatment with sand was better than that of distilled water. Leaves maintained in sand, frequently sprouted (**Figure 6.7**) and vigorous shoots were produced as the old leaves died out. In contrast the frequency of sprouting of

leaves maintained in the distilled water media (with or without hormones) was very low (**Figure 6.8**).

Table 6.13. Results of the treatments with sand with or without the addition of hormones.

Variety	With Hormone			Without Hormone		
	Rep.	# of explants		Rep.	# of explants	
		Planted	Sprouted		Planted	Sprouted
1. M Per 183	1	5	2	1	6	6
2. MTAI 8	1	5	0	1	5	5
3. CM 4574-7	1	7	2	1	5	4
4. MCOL 1505	1	7	7	1	6	5
5. CM 2177-2	1	7	5	1	8	7
1. M Per 183	2	6	1	2	5	5
2. MTAI 8	2	4	1	2	4	4
3. CM 4574-7	2	8	1	2	4	1
4. MCOL 1505	2	6	6	2	7	7
5. CM 2177-2	2	8	7	2	6	5
1. M Per 183	3	5	5	3	5	5
2. MTAI 8	3	6	2	3	5	5
3. CM 4574-7	3	7	7	3	4	2
4. MCOL 1505	3	6	6	3	6	6
5. CM 2177-2	3	8	2	3	7	7

Table 6.14. Results of the treatments with distilled water, with or without the addition of hormones.

Variety	With Hormone			Without Hormone		
	Rep.	# of explants		Rep.	# of explants	
		Planted	Sprouted		Planted	Sprouted
1. M Per 183	1	5	0	1	5	3
2. MTAI 8	1	5	0	1	5	0
3. CM 4574-7	1	5	0	1	5	0
4. MCOL 1505	1	5	0	1	5	3
5. CM 2177-2	1	5	0	1	5	4
1. M Per 183	2	5	0	2	5	1
2. MTAI 8	2	5	0	2	5	0
3. CM 4574-7	2	5	0	2	5	0
4. MCOL 1505	2	5	0	2	5	5
5. CM 2177-2	2	5	0	2	5	3
1. M Per 183	3	5	0	3	5	3
2. MTAI 8	3	5	0	3	5	1
3. CM 4574-7	3	5	0	3	5	1
4. MCOL 1505	3	5	0	3	5	5
5. CM 2177-2	3	5	0	3	5	3

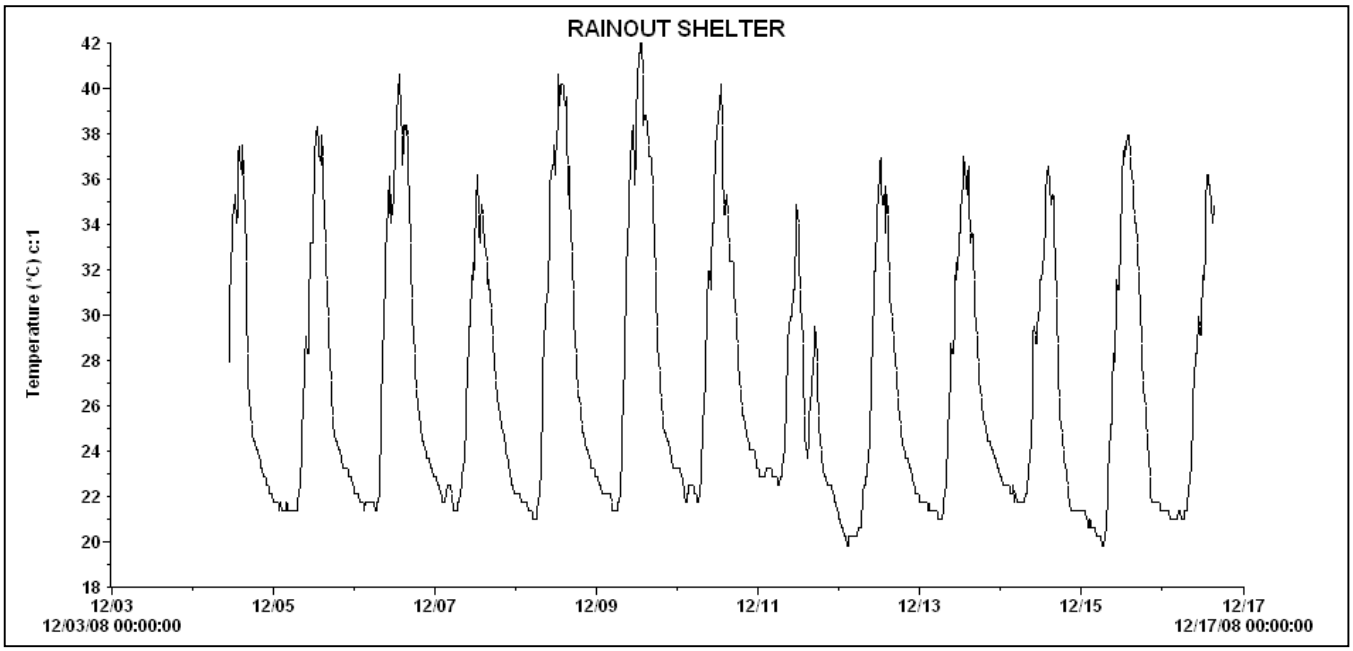


Figure 6.5. Variation in temperature in the rooting chamber for the ex plants maintained with distilled water.

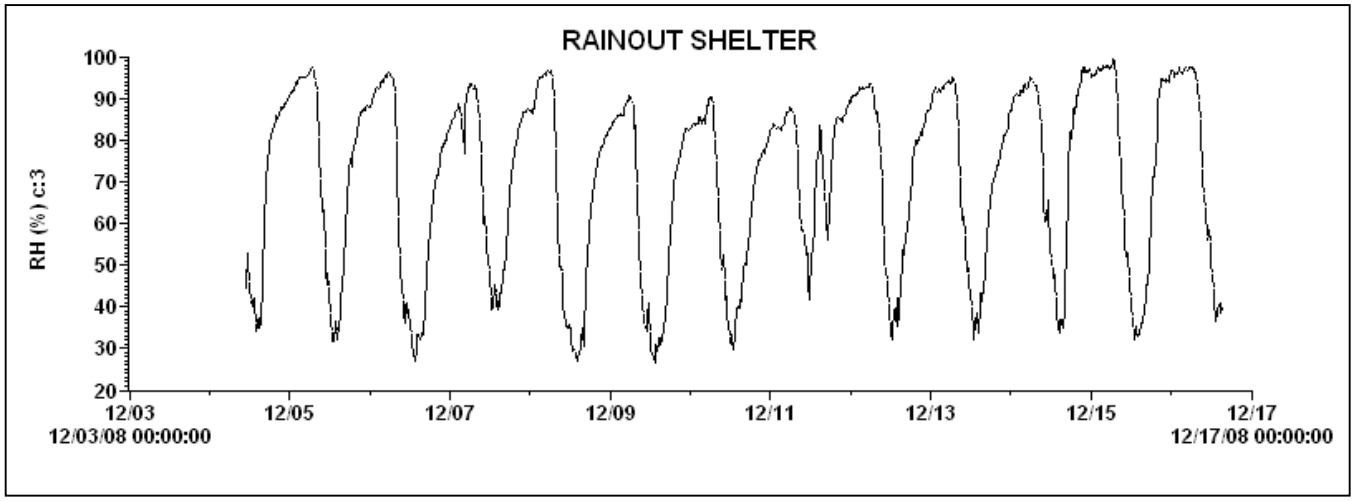


Figure 6.6. Variation in relative humidity in the rooting chamber for the ex plants maintained with distilled water.

6.3.3. ACTIVITIES TO BE CONDUCTED IN JANUARY-FEBRUARY 2009.

Based on the results of the first study the following actions will be conducted starting in January 2009. This will be a second step in the process of developing a suitable methodology of the method to be implemented by August-September 2009 in Thailand for the selected F_2 plants:

a. Establishment of a new trial using only sand with improvement of the mechanism to control relative humidity in the air and better control of the temperature.

b. Evaluate the advantages of producing ex plants that includes the whole section of the stem where the leaf is attached (rather than a section of it, as made in the present study). This would therefore be a one-bud micro-stake.

c. Evaluate if there is any effect of the positioning of the ex plant along the stem on its ability to sprout and initiate roots.

Figure 6.7. Sprouting responses in ex plants placed in sand medium. (A) Overall photograph illustrating the way the buds sprout as the old leaves die; (B) Sprouting of ex plants in sand without hormone; (C) Sprouting of ex plants in sand without hormone with the old leaves still attached; (D) Ex plants treated with hormones.

Figure 6.8. Sprouting responses in ex plants placed in distilled water with very poor results and seldom sprouting of the bud.

6.3.4. References

Cock, J. H.; J. L. Toro; and W. M. Roca. 1982. Multiplicación acelerada de material genético promisorio de yuca. Guía de estudio. Centro internacional de Agricultura Tropical (CIAT). Cali, Colombia. P 16-23.

Pateña, L. F. Barbara; R. C. and Estrella, J. B. 1979. New rapid methods of cassava propagation by leaf-bud and steam cuttings. IPB Circular. Institute of Plant Breeding, University of the Philippines at Los Baños.

CHAPTER 7

BREEDING FOR INSECT AND OTHER ARTHROPODS RESISTANCE AND DEVELOPMENT OF ALTERNATIVE METHODS FOR THEIR CONTROL

7.1 WHITEFLIES AND PARASITOIDS ASSOCIATED TO CASSAVA CULTIVATION IN SOUTH AMERICA.

This section was prepared by **Maria del Pilar Hernandez and Bernardo Arias**. Whiteflies (Hemiptera: Aleyrodidae) represent on of the most important pests in cassava. In Latin America the generalized lack of understanding and knowledge about the different species feeding in cassava grown in different regions and the poor management of this problem by farmers has lead to losses as high as 80% in root yields and even to farmers abandoning their crops.

For more than ten years the Cassava Entomology Team at CIAT, in collaboration with other research centers, particularly those in Ecuador (INIAP), Venezuela (CENIAP-INIA) and Brazil (EMBRAPA), have conducted a systematic and continuous screening and identification of a large sample of specimens collected from different cassava growing regions of South America.This systematic work, which also benefited from explorations, field work, and shipments made by technicians and farmers, resulted in a large and unique collection of whiteflies and the parasitoids found on them. These specimens have been received at CIAT, prepared in Canadian balsam for its identification. Frequently the material was then sent to Dr. Gregory A. Evans (Systematic Entomology Laboratory, USDA).

Aleyrodides from 17 locations in Colombia, 7 from Ecuador, 5 from Brazil and 10 from Venezuela. Different specimens were classified within the two existing sub-families Aleyrodinae or Aleurodicinae (**Figure 7.1**). It is important to emphasize that specimens used for identification were pupae (**Figure 7.2**). Mounted specimens are currently deposited at the Central Insects Collection at CIAT.

A total of 13 different Aleyrodidae were identified (**Table 7.1**). Colombia and Ecuador present the highest number of species (9 and 10 respectively). Six different species were found from Brazil samples and five in samples from Venezuela. *Aleurodicus dispersus, Aleurotrachelus socialis, Bemisia tuberculata* and *Trialeurodes variabilis* were the predominant species in every country. However only the last three are considered of economic importance in Colombia (Bellotti, *et a*l., 2005). *A. dispersus* is economically important in Asia and Africa in spite of its Caribbean origin. . *Aleurotrixus aepim* was found in the North East of Brazil and in the coastal regions of Ecuador. *Bemisia tabaci* was found in low population frequencies only at two Ecuadorian locations, although it is one of the most devastating cassava pests in Africa and parts of Asia and causes huge losses by vectoring different viruses, particularly CMD. Species from *Tetraleurodes* and *Aleuroglandulus* genera were found at low frequencies

in Colombia, Ecuador and Venezuela. *T. vaporariorum* was found in large numbers in higher elevation regions of Ecuador. On the other hand, *Aleuronudus, Paraleyrodes* and *Aleurodicus flavus* are found only occasionally and do not seem to represent a major problem for the crop.

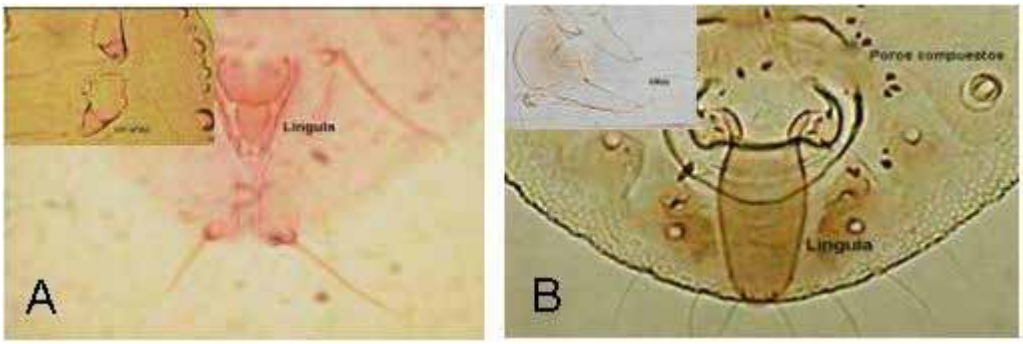
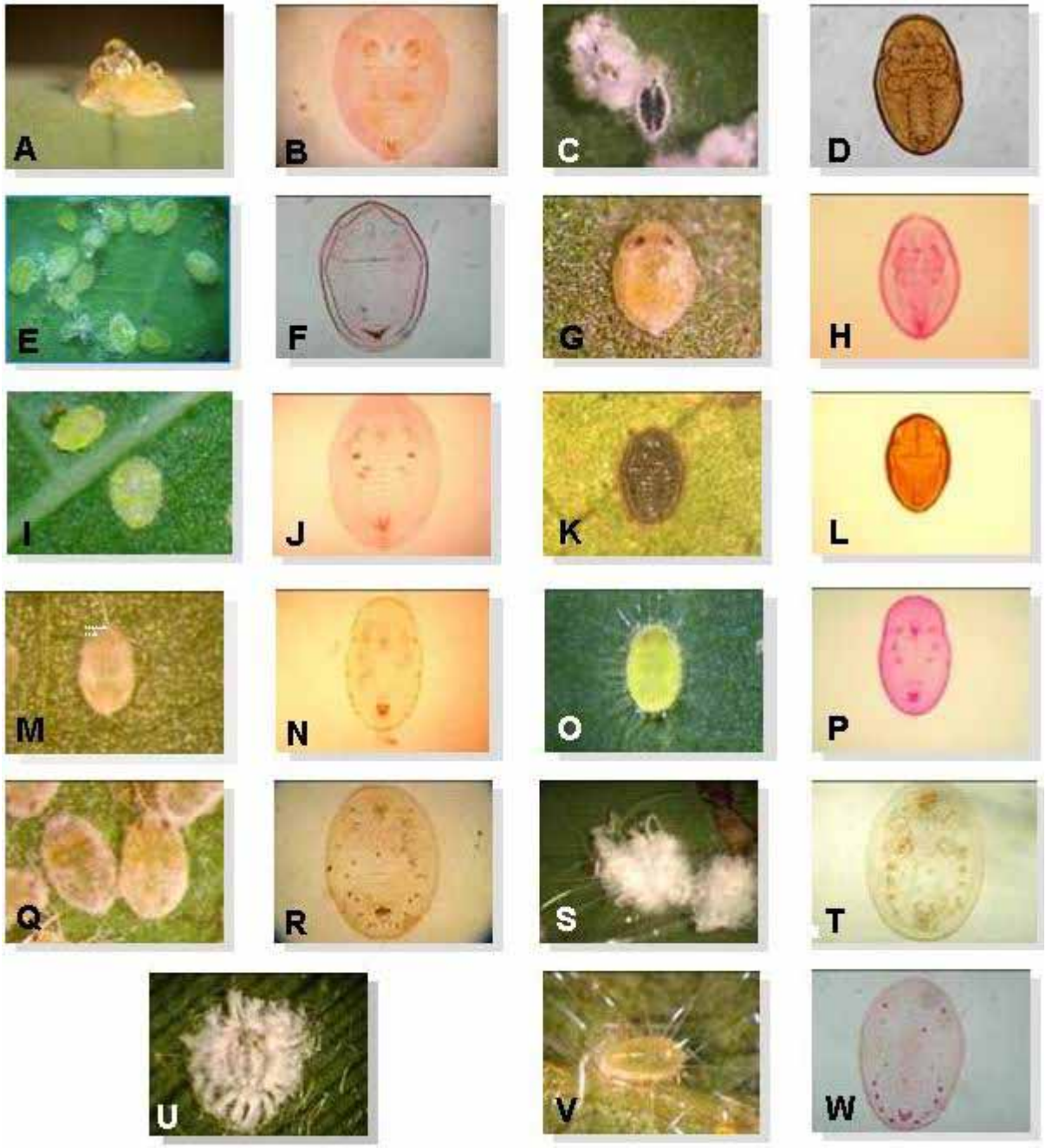


Figure 7.1. Specific characteristics of the two Aleyrodidae sub-families. A. Aleurodinae: legs with adhesive pads without nails. Lingula inserted inside the vasiform operculum. No composite pores. B. Aleurodicinae: legs with nails, long, exerted, lingula with two pairs of setae. Composed pores.

Figure 7.2 (next page). Whiteflies affecting cassava. Photographs A-P Subfamily Aleyrodinae. Photographs Q-W Subfamily Aleurodicinae.
Aleuroglandulus malangae pupae; **A:** General appearance. **B:** Close up of a mounted pupae showing glandular pores and abdominal segment;
Aleurotrachelus socialis pupae. **C)** General appearance of *p*upae showing black capsule surrounded by waxy scretions; **D)** Trachea-shaped abdominal segments.
Aleurothrixus aepim pupae. **E)** Pupal capsule showing long waxy filaments; **F)** Submarginal truncated suture with small vasiform orifice.
Bemisia tabaci pupae. **G)** Ovoid pupae; **H)** Triangular, vasiform orifice longer than the caudal ridge.
Bemisia tuberculata pupae. **I)** Ovoid pupae; **J)** Granulated dorsum with vasiform orifice of about the same length as the caudal ridge.
Tetraleurodes spp pupae. **K)** Pupae are shiny black; **L)** Margins are dented and submargins show a row of small glands.
Trialeurodes vaporariorum pupae. **M)** Pupae are ovoid and present setae; **N)** Papillae present in the submargin. Lobulated lingula and coxae without setae.
Trialeurodes variabilis pupae. **O)** Pupae are ovoid and present long setae; **P)** Papillae present in the submargin. Lobulated lingula and coxae with setae.
Aleuronudus sp pupae. **Q)** Pupae are ovoid and present long and short waxy filaments; **R)** Six pairs composed pores, varying in size..
Aleurodicus dispersus pupae. **S)** Abundant secretion of cottony, long, crystalline waxed filaments; **T)** Four pairs of composed pores in the abdomen.
Aleurodicus flavus pupae. **U)** Covered with a cottony, waxy secretion irradiating in all directions.

Paraleyrodes sp pupae. **V)** Ovoid with long, crystalline, filamentous setae; **W)** Six pairs of composed pores on segments III-VIII.



These parasitoids were grouped in seven genera from families Aphelinidae:(*Encarsia* y *Eretmocerus*), Eulophidae (*Aleuroctonus* y *Euderomphale*), Platygasteridae (*Amitus*), Encyrtidae (*Metaphycus*) and a possible Signiphoridae hyper-parasite. **Table 7.2** presents the list of parasitoids found in different hosts collected in four countries: Colombia with 19 species, Venezuela with ten, Ecuador with nine and Brazil with four parasitoid species. Of the parasitoid species *E .hispida* was the commonest and proved to be the most generalist as well. Five new *Eretmocerus* species were found. This genus shows great potential for the

control of whiteflies worldwide. However, the generalized lack of knowledge on the native species makes their analysis, assessment of their potential and actual utilization difficult.

Table 7.1. Distribution of whiteflies species associated with cassava in different South American countries.

Species	**Brasil**	**Colombia**	**Ecuador**	**Venezuela**
Aleuroglandulus malangae Russell		x		
Aleurodicus dispersus Russell	x	x	x	x
Aleurodicus flavus Hampel			x	
Aleuronudus sp		x	x	
Aleurotrachelus socialis Bondar	x	x	x	x
Aleurothrixus aepim (Goldi)	x		x	
Bemisia tabaci (Gennadius)		x	x	
Bemisia tuberculata Bondar	x	x	x	x
Paraleyrodes sp	x	x		
*Tetraleurodes ursorum C*ockerell				x
Tetraleurodes sp		x	x	
Trialeurodes vaporariorum (Westwood)			x	
Trialeurodes variabilis (Quaintance)	x	x	x	x

Acknowledgement:

This work was possible only because the valuable contributions of many people collecting the samples in different countries. Contributions by Research Assistants and field workers from CIAT, INIA, EMBRAPA and CENIAP are hereby acknowledged:.

José María Guerrero, Harold Trujillo, Adriano Muñoz, Carlos Ñañez, Gerardino Perez, Oswaldo Valarezo ,Alba R. Farias, Vanda Piestroswky.

References:

Bellotti, A., J. Peña, B. Arias, J.M. Guerrero, H. Trujillo, C.M. Holguin and A. Ortega. 2005. Biological control of whiteflies by indigenous natural enemies for major food crops in the

neotropics. In. Whitefly and whitefly-borne viruses in the tropics: Building and Knowledge base for global action. Edited by: P.K.Anderson and F.J. Morales. 313-323 pp.

Table 7.2. Distribution of parasitoids associated with different whitefly species feeding on cassava.

Parasitoides	**Brasil**	**Colombia**	**Ecuador**	**Venezuela**
Amitus macgowni Evans & Castillo		X [5, 13]	X [5]	
Amitus sp.				X [5]
Aleuroctonus vittatus (Dozier)		X [2]		
Encarsia americana (Debach & Rose)		X [5, 10]		
Encarsia. aleurothrixi Evans & Polaszek	X [6]			
Encarsia. Bellotti Evans & Castillo		X [5, 13]		X [5]
Encarsia cubensis Gahan				X [5]
Encarsia desantisi Viggianni		X [1]		
Encarsia guadeloupae Viggianni		X [1]		
Encarsia hispida DeSantis	X [6]	X [5, 8, 10, 13]	X [5, 10]	X [5, 8]
Encarsia luteola group		X [5, 13]		
Encarsia nigricephala Dozier		X [13]		
Encarsia pergandiella Howard		X [8, 13]	X [13]	
Encarsia sophia (Girault & Dodd)		X [5, 8, 13]	X [13]	X [5, 8]
Encarsia strenua group		X [13]		
Encarsia sp.	X [8]	X [1, 2, 5, 10, 13]	X [5, 7, 10]	
Encarsia tabacivora Viggiani		X [8, 13]	X [5, 12]	X [5, 8]
Encarsia sp. prob. *variegata*		X [5]		
Euderomphale sp.		X [5, 8]	X [2]	X [5, 8]
Eretmocerus spp (5 especies)	X [6]	X [2, 5, 8, 10, 13]	X [5, 10, 13]	X [5, 8]
Metaphycus sp.		X [5, 8]		X [8]
Signiphora aleyrodis Ashmead		X [5, 13]	X [10]	X [5]

A. malangae [1] *, A. dispersus* [2]*, A. flavus* [3] *, Aleuronudus* [4] *, A. sociales* [5] *, A. aepim* [6] *, B. tabaci* [7]*, B. tuberculata* [8]*, Aaleyrodes sp* [9]*, Tetraleurodes sp* [10]*, T. ursorum* [11]*, T. vaporariorum* [12] *and T. variabilis* [13]

7.2 SCREENING FOR REACTION TO DIFFERENT PEST IN SEGREGATING PROGENIES

During the planting of the clonal evaluation trial (CET) in Palmira (2207-2008 cycle), a complete screening and rating of reaction to the green mite (*Mononychellus tanajoa*); and other mite species (*Scyrtotrips manihoti* and *Olygonichus peruvianus*) was conducted. This activity was coordinated by Bernardo Arias and benefited from the collaboration of Gerardino Pérez, Carlos Náñez and Adriano Muñoz.

This trial included 1400 genotypes planted in rows with 8 plants. This is a standard CET trial conducted for general agronomic performance for adaptation to the mid-altitude valleys environment. However, screening for resistance to the most common pests is a key activity in search of new sources of resistance/tolerance. Because of the success in the management of the whiteflies problem at the Experimental Station we can no longer select for resistance to whiteflies in standard trials in the field. There is just too little pressure from the insect. As whiteflies became a no-problem, however, mites pressure increase. In a way, this is a measure of the success of the approached used to overcome the problem of white flies. The specific pests that managed to exert some pressure on the crop were mainly *Mononychellus tanajoa (cassava green mite)* and *Scyrtotrips manihoti* (a thrip pest). The flat mite Q*lygonichus peruvianus* was observed but the population density and pressure on the crop was not high enough to allow for reliable assessment of the reaction of cassava to it.

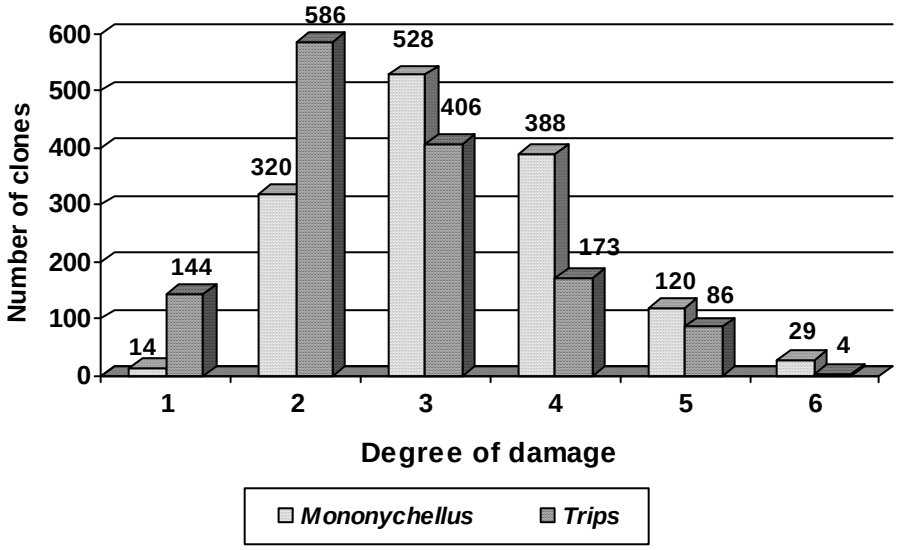


Figure7.3. Evaluation of pests in the Clonal Evaluation Trial planted in Palmira, Valle del Cauca, Colombia during the 2007-2008 cycle.

The evaluation was conducted using a 1-6 score system where 1= clean, healthy plants and 6=maximum damage for each of the plants analyzed from each genotype. According to this scale materials are susceptible when the score is above 4. Score below 3 would represent materials that are highly resistant. A score of 3 would describe a genotype that has an intermediate reaction. Materials with a rating below 4, need to be assessed again next cycle to confirm their resistance. Evaluation and selection for pest resistance can be made after final selection based on agronomic performance has been completed.

Figure 7.3 summarizes the results observed for the two most important pest based on the natural pressure observed in the field. Most genotypes were affected simultaneously by the two pests. Only 14 out of the 1399 genotypes (1%) did not have damage induced by the green mite. On the other hand about 10% of the clones (144 genotypes) did not have damage by the thrips. It is clear that population wise mites were more important than thrips.

In the case of green mite about 23% of the genotypes (320 clones) had very low damage levels (score of 2); 38% had an intermediate reaction (528 clones with a score of 3), and the remaining 38% of the materials had a susceptible reaction (scores > 4). For thrips, about 42% of the segregating progenies had a rating of 2. Therefore, more than 50% of the germplasm shows high to intermediate levels of resistance to thrips. A total of 406 clones (29%) showed intermediate reaction to thrips and the rest (19% had a susceptible reaction with scores > 4).

The 14 clones that had very little or no damage caused by the green mite are listed in **Table 7.3.** It is obvious that many of these genotypes were also highly or moderately resistant to the thrips, with the expection of clone SM 3340-20. In general there is an interesting finding that implies that resistance to these two pests in the same genotype is feasible. Over the whole trial correlation coefficient for the reaction to the two pests was 0.46 and is illustrated in **Figure 7.4**.

Table 7.3. List of the best 14 clones based on the reaction to *Mononychellus tanajoa*. Their reaction to thrips and the flat mite is also presented

Clone	*Mononychellus tanajoa*	*Scyrtotrips manihoti*	*Olygonichus peruvianus*
GM 1443-4	1	1	1
GM 1483-2	1	2	1
GM 1483-3	1	1	1
GM 1491-6	1	2	1
SM 3223-15	1	2	1
SM 3225-21	1	2	1
SM 3339-9	1	1	1
SM 3339-12	1	1	1
SM 3340-20	1	4	1
SM 3343-22	1	2	1
SM 3347-2	1	2	1
SM 3351-14	1	2	1
GM 1442-13	1	1	1
SM 3339-13	1	1	1

7.3 SCREENING FOR REACTION TO GREEN MITE IN ACCESSIONS FROM THE GERMPLASM COLLECTION

As part of the collaboration between the genetic resources unit with field breeding and cassava entomology personnel a systematic screening of the reaction of accessions from the

germplasm collection has been made over the years. During the 2007-2008 growing cycle reaction to green mite in 542 clones was added to the data base of the germplasm collection. The origin of these accessions is described in **Table 7.4**. The frequency distributions of these accessions based on their scores are presented in **Figure 7.5**.

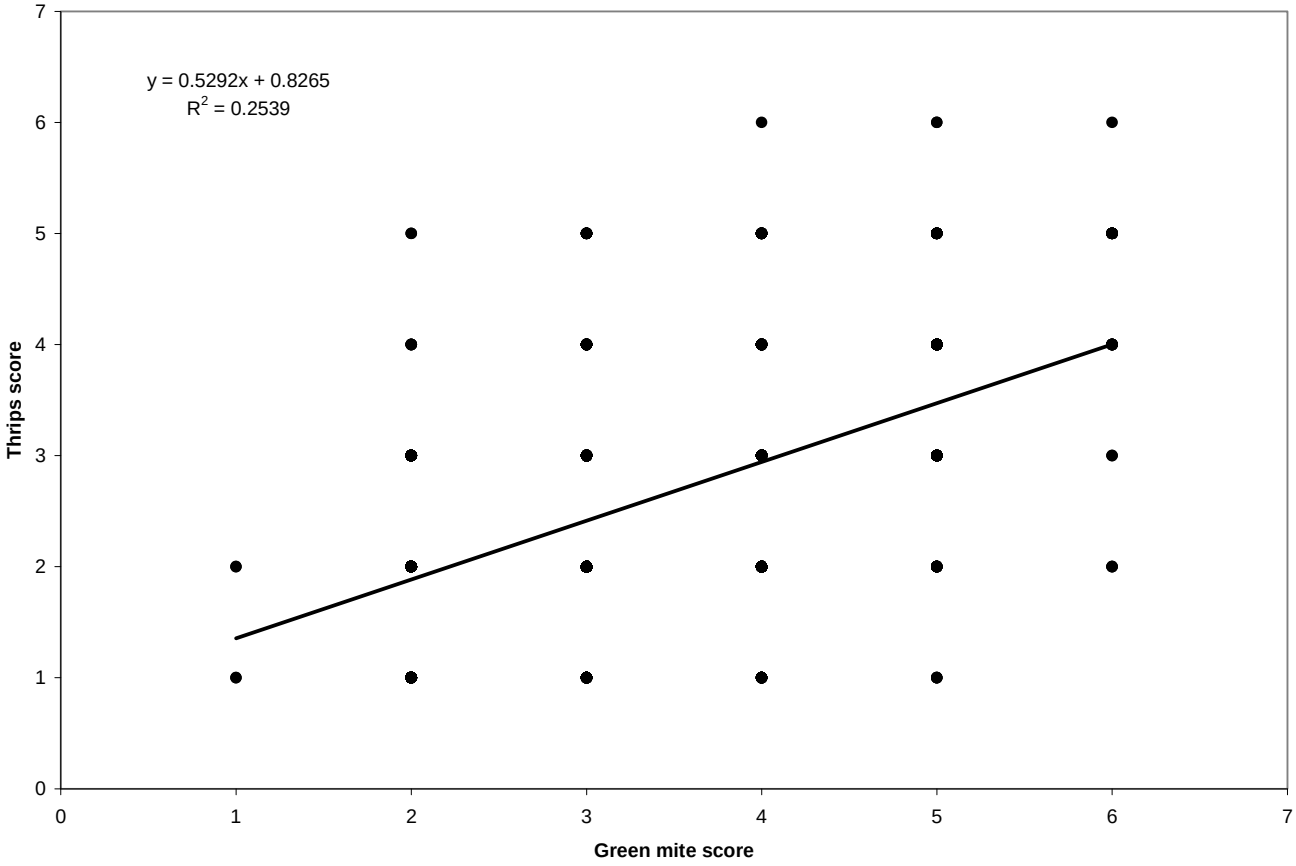


Figure 7.4. Relationship between the reaction to green mite and thrips in the 1399 clones evaluated in the Clonal Evaluation Trial.

This systematic screening of the germplasm collection has lead to knowledge of the reaction to green mites in about 90% of the collection (**Figure 7.6**). However, as explained above, if possible the assessment of the reaction to different pest should be based in more than one observation. About 50% of the accessions have been scored once, about 1500 clones (28%)

have been evaluated twice, 600 clones (11%) three times and in slightly more than 1% (67 clones) up to four times for reaction to the green mite.

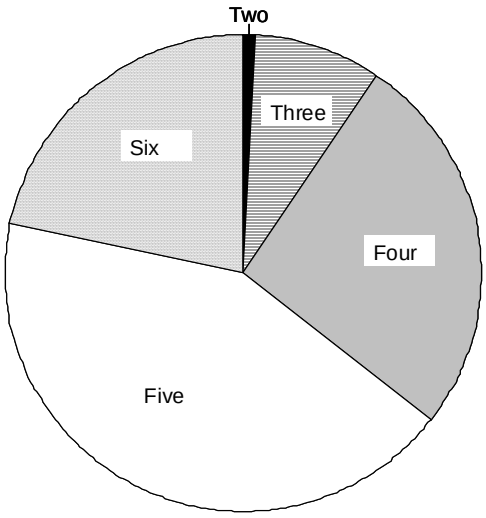


Figure 7.5. Relative frequencies of the different scores assigned to accessions from the germplasm collection for their reaction to the cassava green mite.

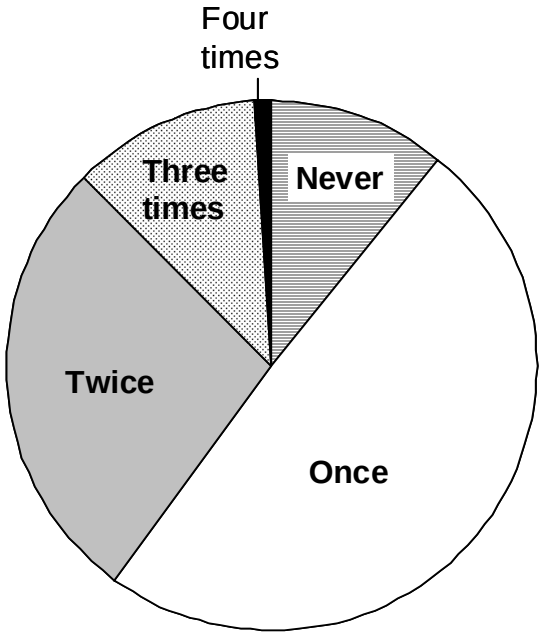


Figure 7.6. Relative frequencies of the number of times the accessions of the germplasm collection have been evaluated for their reaction to the cassava green mite. .

Table 7.4. Country of origin of the 532 accessions from the germplasm collection that have been evaluated for their reaction to the cassava green mite during the 2007-2008 growing cycle.

Country	# accessions	Country	# accessions
Argentina	8	Mexico	16
Bolivia	3	Nigeria	3
Brasil	89	Panama	9
Colombia	117	Paraguay	30
Costa Rica	21	Peru	58
Cuba	17	Philippines	2
Dom. Rep.	4	Puerto Rico	5
Ecuador	26	Thailand	4
Fiji	2	USA	4
Guatemala	15	Venezuela	49
Indonesia	7	Improved	29
Malaysia	14	**TOTAL**	**532**

7.4 ASSESSMENT OF EMERGING PESTS OF CASSAVA IN THAILAND

Perhaps as a result of the climate changes or the extensive and continuous growth of cassava for many years in Thailand, there is a growing concern of pest problems in that country. There has been a particular rise in the populations of a mealybug species whose true identity is still waiting for confirmation. Tentatively we have identified it as *Pseudococcus jackbeardsleyi.* This is important because depending on the species the strategies for their control based on the introduction of efficient agents for their biological control. It has been the experience of the cassava entomology team that there is a great deal of specificity in the efficiency of different agents for the biological control. Dr. Tony Bellotti spent two weeks in Thailand during the second semester of 2008. He took the opportunity to visit several fields in different regions of Thailand collecting different species of insects and mites. During 2008 there has been many reported cases of severe damage on cassava fields caused by a mealybug. This is a new phenomenon in cassava for Thailand.

In addition to the problem of the mealybug there has also been a growing concern with the spiraling whitefly (*Aleurodicus dispersus*) which is a species that seldom causes serious problems in cassava grown in Latin America. Since none of these two insects (whitefly and the tentative mealybug species) cause serious problem in Latin America but are increasingly present in Asia, it is suspected that there is a lack of agents for the biological control of these insects in Asia.

Figure 7.7 presents photographs illustrating the two insect pests mentioned in this section. In addition photographs of a red mite (it can be either *Tetranychus truncatus* or *Oligonychus biharensis)* which completes the list of the most generalized and serious arthropod problems for cassava grown in Asia.

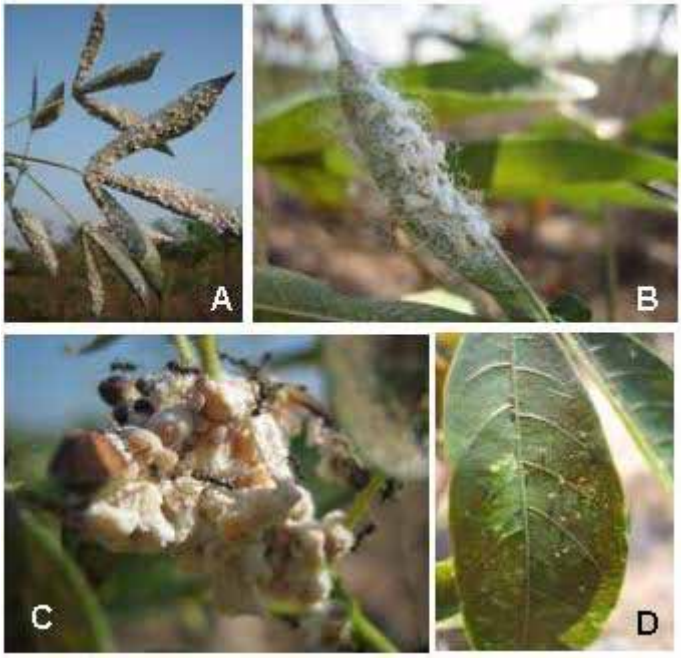


Figure 7.7. Illustrations of the insects that can feed in Thailand. A and B. Spiraling whitefly (*Aleurodicus dispersus*); C) Mealy bug (tentatively *Pseudococcus jackbeardsleyi)*; D. Red mite (this particular photograph may be of *Tetranychus truncatus* or *Oligonychus biharensis)*

CHAPTER 8

BREEDING FOR DISEASE RESISTANCE AND DEVELOPMENT OF ALTERNATIVE METHODS FOR THEIR CONTROL

INTEGRATED MANAGEMENT OF CASSAVA FROGSKIN DISEASE

Of the diseases attacking cassava, CFSD is currently considered as one of the most limits cassava cultivation, causing losses of more than 90%. It affects not only farmers but also researchers—breeders, entomologists, physiologists, and others—whose research focuses on improving varietal yields and ensuring production stability[1]. These problems occur because little is known about the causal agent. Hence, evaluations under controlled conditions to readily select undesirable materials are impossible. Without these methodologies, conducting yield trials and regional evaluations that are free of susceptible materials becomes increasingly difficult. Nor can susceptible materials be identified to monitor them and thus prevent the continual presence of disease-bearing inocula.

Compounding these problems is the factor of continuity in cassava breeding programs. Continuity is particularly important because of the length of each selection cycle, which may be as long as 5 years. Hence, a constant risk exists of losing materials, with significant traits such as yield, resistance to pests, carotenes, dry matter, and starch, to severe attacks from the disease, thereby cutting the cycle and putting back experiments. Quarantines and closed seasons must be established, and materials cleaned through tissue culture. These processes are costly and time-consuming.

Pathologists are seeking the most efficient and lasting tools and methodologies that would speed up and improve the management of this disease. Increasingly, they are focusing more on using genetic resistance as a major component of the integrated management of frogskin disease. To do so, they work with classical and molecular breeders.

To discover, within plant genomes, genes associated with agricultural traits of interest, molecular breeders develop highly saturated, molecular, genetic maps. Saturation depends on the speed with which markers can be found for saturating a map. This process can be slow and expensive. Molecular markers that can be obtained quickly at low cost are very helpful, especially if little molecular information exists on the plant of interest, as is the case for most Neotropical plants, including cassava (Duque 2003).

DArT is one of the latest techniques employing microarrays. It is based on the generation of an array or panel of DNA segments with unknown sequences of two or more genomic DNA individuals. These segments are hybridized with fluorescent-labeled DNA probes of the two individuals used for creating the panel. The technique has been tried for cassava to (1) find differences in genomic DNA sequences between parents of the family of the genetic map (K

1. Production stability is important for food security and is achieved when the material developed has genetic tolerance or resistance to the principal biotic and abiotic factors that limit production (CIAT 2002).

Family, M Nga 2 × CM 2177-2) (Duque 2003, and (2) generate a microarray on the diversity of cultivated and wild species (Xia et al., 2005). So far, it has not been used to search for markers associated with resistance to diseases in cassava or other cultivated species. This technique is a suitable option for generating genetic map saturation in Neotropical plants such as cassava because (1) no previous sequencing is needed and (2) throughput is high and rapid, making the technique inexpensive (CIAT 2002).

8.1. MATERIALS AND METHODS

Collection of materials, extraction of genomic DNA, data analyses, and evaluations of candidate markers were carried out at CIAT. The reading and running of DArTs were conducted at DArT P/L, based at the Centre for the Application of Molecular Biology to International Agriculture (CAMBIA) in Canberra, ACT, Australia.

We worked with the entire K Family (147 individuals) conserved at the Genetic Resources Unit at CIAT. We also established a contrasting population for CFSD with 100 individuals of commercial varieties and genotypes, where 50 were tolerant and 50 susceptible. They were evaluated by CIAT's plant breeding programs and the Virology Unit in the field under various conditions such as selection cycles in regional trials, and experimental and F_1 fields (CIAT 2004). Sites were at CIAT stations: Palmira (Valle del Cauca), Santander de Quilichao (Cauca), North Coast (Sincelejo), and the Eastern Plains.

Table 8.1. Scale for evaluating the severity of symptoms of cassava frogskin disease, as modified by the cassava plant pathology and breeding programs at CIAT.

Score	Category of infection	**Symptoms observed**
0	Healthy plant	Roots are filled; no symptoms; peel is thin and flexible
1	Very light	Root are filled; **a few roots** with **some fissures** or splits with lip form
2	Light	Roots are filled, **many roots** with **some fissures** or splits with lip form
3	Moderate	**Large number of fissures** or splits with lip form in **any part of the root** (basal, intermediate, and distal zones), with some reduction of root filling
4	Severe	Presence of a network or honeycomb in some or many roots, with a **moderate reduction** of root filling; the skin is thick, cork-like, and brittle
5	Very severe	Presence of a network or honeycomb in some or many roots, with a **severe reduction** of root filling; roots appear woody or fibrous; the skin is thick, cork-like, and brittle

Another population was also evaluated. This included 65 individuals of the cassava family GM 306 (M Ecu 72 [maternal, highly susceptible] × M Per 183 [paternal, tolerant]), encompassing genotypes GM 306-88 to GM 306-153. Individuals came from stakes planted

in the field at CIAT–Palmira, Valle del Cauca, where the disease is endemic. Specifically, the plants came from observation field Zone 4–ZEC 04 of F_1 selections and preliminary yield trials.

To confirm and select the best population, we took data from phenotypic evaluations to form two contrasting groups according to the level of disease observed in field evaluations. Based on a scale of severity of attack, one group comprised 50 individuals that were highly tolerant, showing a symptomatological score between 0 and 1.5, whereas the other group included susceptible individuals that scored from 1.51 to 5 (**Table 8.1**).

To select for the groups, the frequency distribution of CFSD was graphed for 100 and 65 individuals. The percentages of individuals that were resistant, moderately resistant (intermediate), and susceptible were also graphed. Individuals considered as resistant were those that scored 0 to 1.5 on the scale of severity for CFSD; intermediate, between 1.5 and 3.0; and susceptible, 3.5 to 5.0.

We used leaves from *in vitro* materials and shoots found in the field. We used the total genomic DNA of all the materials being evaluated, extracting it according to the extraction protocol described by DArT P/L, with some modifications for volumes.

We added 0.6-mL aliquots of preheated (65 °C), well-mixed, freshly prepared, buffer solution to 1.5-mL tubes. The tubes were then placed in an incubator or water bath at 65 °C. The requisite amount of plant material was ground in liquid nitrogen with a mortar and pestle to a fine powder, checked for lumps and, if necessary, vortexed. The plant material was then suspended in 0.6 mL of the hot buffer solution and the whole incubated at 65 °C for 1.0–1.5 h. The tubes were either inverted every 20 min or incubated on a gently agitating shaker. The tubes were left to cool for 5 min and 0.6 mL were added of a mixture of chloroform and isoamyl alcohol at 24:1. The whole was mixed well for 30 min, centrifuged for 20 min at 3000 *g*, and the water phase transferred to a fresh tube.

Ice-cold isopropanol was then added and the tube inverted about 10 times, after which nucleic acids became visible. The whole was centrifuged at 3000 *g* for 30 min at 10 °C. The supernatant was discarded and the pellet washed with 0.2 mL of ethanol at 70%. The ethanol was then discarded, and the pellet dried and dissolved in 200–500 µL 1X TE (10 mM Tris-HCl, pH 8.0; and 1 mM EDTA, pH 8.0).

The resulting DNA was checked for quality and quantity on 0.8% agarose gel. Because we were using DArT technology, we did not treat the product with RNase, as the RNA quantity was a lot less than the DNA.

Segregation data from each parental genotype were analyzed independently. The presence or absence of each polymorphic fragment was visually determined and recorded for each progeny. A x^2 test was used to determine the goodness of fit between the observed and expected number of genotypes for each class of segregation ratio, using the MapDisto program (http://www.mpl.ird.fr/~lorieux/mapdisto) with a significance level of 5%.

For single-dose amplification fragments (SDAFs) that were polymorphic between parents (i.e., present in CM 2177-2 and absent in TMS 30572, or vice versa), the expected segregation ratio was 1:1. The fragments represent the segregation equivalent of an allele at a heterozygous locus in a diploid or allopolyploid genome, or a simplex allele in an

autopolyploid. They are suitable for linkage analysis in an F_1 population when several unique segregating polymorphisms (heterozygosity) and normal meiosis in either or both parents of the mapping polyploid genomes are present (Fregene et al. 1997).

For simplex markers that are homomorphic for parents but segregating in the population (biparental single-dose fragments or BSDF), the expected ratio was 3:1 for either disomic or polysomic inheritance. The linkage map was based on the use of undistorted SDAFs to develop two separate linkage maps, based on male and female parents. Map units in centimorgans (cM) were derived from the Kosambi mapping function. An LOD score of 2.0 or more was used to place markers within the existing linkage groups, using the JoinMap v.3 software.

Marker fragments were scored as present (1) or absent (0). Fragments that could not be scored reliably were declared as missing values (2). Similarity of marker distribution in the population was calculated by distance analysis between binary variables (simple similarity) using NTSYSpc software v.2.02. Marker genotype classes were tested for association with phenotypic scores with the non-parametric Mann–Whitney–U test, using the StatsDirect® software (Cheshire, UK). The null hypothesis of no association was rejected at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and $P \leq 0.0001$.

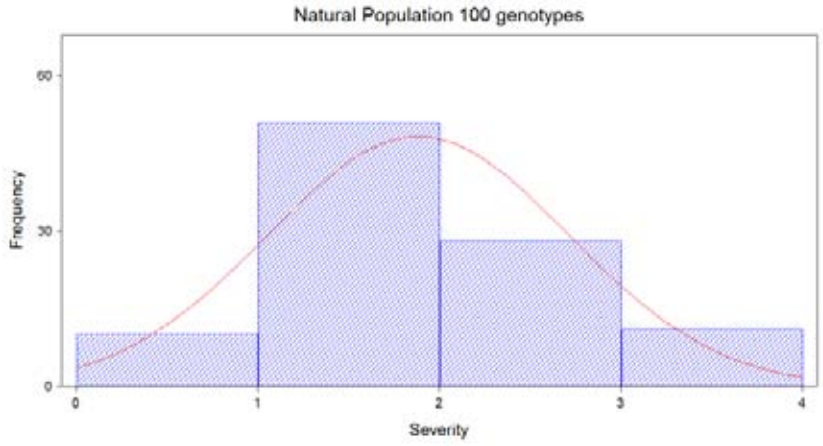
After analyzing the results obtained with the DArT technology, we selected clones located in the diversity microarray that presented polymorphisms or association with resistance to the disease. Sequencing was carried out and homologies with genes reported for resistance to diseases in diverse crops searched in the database of the National Center for Biotechnology Information (NCBI), using an application of the tool Basic Local Alignment Search Tool (BLASTx at www.ncbi.nlm.nih.gov). Homologies were found with sequences of nucleotides or amino acids (of known proteins) registered in the database. We aligned our sequences with each other through the "alignment" option of the MEGA program v.4.1 and constructed a phylogenetic tree, using Parsimony and bootstrap analysis with 10,000 replicas.

8.2 RESULTS

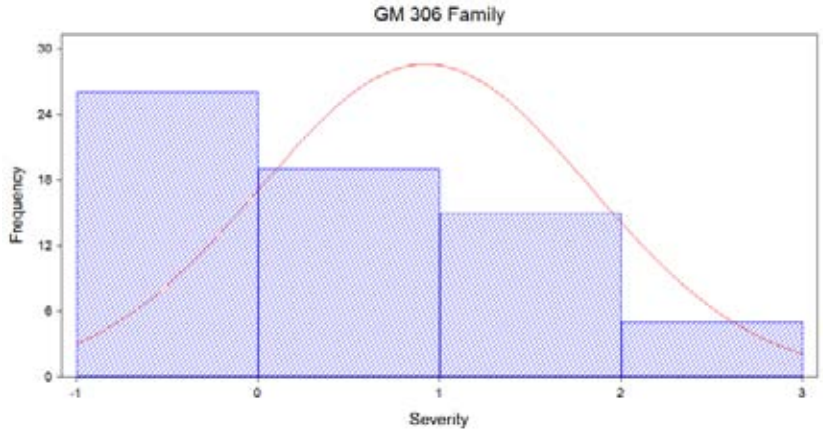
A frequency distribution graph was drawn for 100 and 65 genotypes on the basis of their resistance or susceptibility as according to the scale described above (**Figure 8.1**).

We had chosen 165 cassava varieties and genotypes from four CIAT stations in Colombia. From these materials, 100 individuals were selected to create two contrasting populations (50 susceptible and 50 tolerant) for use in identifying a molecular marker associated with resistance to CFSD.

The extraction methodology was reproducible for *in vitro* leaf tissues and shoots from the field. So far, we have obtained DNA from 247 genotypes, both resistant and susceptible to CFSD, from observation fields. Of the genotypes, 147 were from the K Family and 100 were elite (**Figure 8.2**). All the DNA was sent to DArT P/L for hybridization with the microarray designed by Xia and co-workers (2005).



A.



B.

Figure 8.1. Frequency distribution of severity of disease in **(A)** a natural population and **(B)** the GM 306 family, which had 100 and 65 varieties or genotypes, respectively. Severity was scored in terms of resistance, intermediate resistance, or susceptibility to cassava frogskin disease (CFSD).

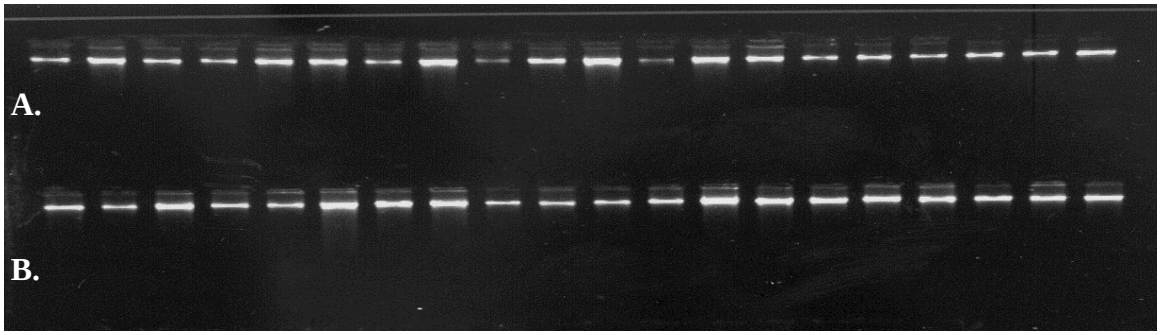


Figure 8.2. Gel for testing the quality of DNA obtained from **(A)** leaves of *in vitro* seedlings and **(B)** shoots from the field, according to the protocol recommended by DArT P/L, based at

the Centre for the Application of Molecular Biology to International Agriculture (CAMBIA), Australia.

Association between markers and disease resistance.

The 100 individuals selected were hybridized with 804 clones from the diversity array designed by Xia and co-workers (2005). The analysis of the nonparametric Mann–Whitney–U test showed four levels of probability that several DArT markers were significantly associated with CFSD: $P \leq 0.05$ (108 markers), $P \leq 0.01$ (28 markers), $P \leq 0.001$ (6 markers), and $P \leq 0.0001$ (9 markers). To continue with the analyses, we took into account 15 markers that showed the highest degree of significance ($P \leq 0.001$ and $P \leq 0.0001$; **Table 8.2**).

Table 8.2. Average of values of resistance to cassava frogskin disease (CFSD) for clones with or without a marker, significant at $P \leq 0.001$ and $P \leq 0.0001$, according to the nonparametric Mann–Whitney–U test.

Marker	**Location on the map**	**Marker class**	**Number of genotypes**	***Resistance to CFSD***	
				Average (d.s.)	***P***
247586	CM 2177-2	1	65	2,1392 (0,7403)	< 0,0001
		0	32	1,3612 (1,0173)	
80664	-	1	24	1,2922 (0,4348)	< 0,0001
		0	71	2,1365 (0,8147)	
255287	CM 2177-2	1	65	2,1392 (0,8392)	< 0,0001
		0	34	1,3893 (0,5151)	
255179	CM 2177-2	1	65	2,1392 (0,8392)	< 0,0001
		0	34	1,3893 (0,5151)	
250307	CM 2177-2	1	59	2,1112 (0,8522)	< 0,0001
		0	33	1,4369 (0,5363)	
250272	CM 2177-2	1	65	2,1392 (0,8392)	< 0,0001
		0	28	1,4128 (0,5088)	
248286	CM 2177-2	1	65	2,1392 (0,8392)	< 0,0001
		0	35	1,4124 (0,5254)	
255773	TMS 30572	1	29	1,3825 (0,4112)	0,0001
		0	69	2,1107 (0,861)	
81019	-	1	20	1,2575 (0,4521)	0,0001
		0	74	2,0694 (0,8302)	
248137	TMS 30572	1	29	1,3825 (0,4112)	0,0002
		0	60	2,1153 (0,8754)	
82210	TMS 30572	1	58	2,0971 (0,8304)	0,0002
		0	34	1,4731 (0,5537)	
249344	CM 2177-2	1	74	1,9692 (0,8095)	0,0003
		0	12	1,2503 (0,5256)	
256481	-	1	53	2,1774 (0,8626)	0,0003
		0	35	1,475 (0,5953)	
81251	-	1	59	1,6247 (0,7225)	0,0003
		0	38	2,2218 (0,8182)	
248961	-	1	68	1,7463 (0,8104	0,0008
		0	24	2,3624 (0,7521)	

Identifying polymorphic clones and comparing sequences of tolerant and susceptible individuals.

At DArT P/L, 15 clones were selected and sequenced for their resistance to CFSD. Of the 15 clones, 12 provided good sequences. Fasta files with vector and adaptor sequences were removed, and sequence length and redundancy were obtained. Four clones were found redundant, forming one cluster, while the remaining eight were unique sequences (**Table 8.3**). The 12 sequences were analyzed, using BLAST tools, and similarities identified, together with description and significance. BLASTN, BLASTX, and TBLASTX were performed on the range of publicly available databases (**Table 8.4**) and restriction enzymes sites identified within the sequences (**Table 8.5**).

Table 8.3. Sequence length and cluster information for 12 cassava clones.

CloneID	Sequence Length	Cluster No.	Cluster name	No. clones per cluster
250272	341	1	250272	4
255179	341	1	250272	4
250307	325	1	250272	4
255287	256	1	250272	4
248286	116	2	248286	1
82210	808	3	82210	1
81251	594	4	81251	1
249344	405	5	249344	1
248961	372	6	248961	1
256481	302	7	256481	1
255773	264	8	255773	1
80664	102	9	80664	1

Data analysis and linkage map construction.
As a result of using a tetraploid F_1 mapping population derived from noninbred parents, different allelic configurations per locus were expected. Segregation data were studied for the presence of (1) paternal markers (fragments segregating from CM 2177-2), (2) maternal markers (fragments segregating from TMS 30572), or (3) biparental markers (fragments present in both parents and segregating in the progeny). The fragments were then used to compare maternal and paternal linkage maps (**Table 8.5, Figure 8.3**). The number of markers scored in each class of segregation ratio is shown in Table 6. In both genotypes, most polymorphic fragments segregated as SDAFs. Table 6 shows the allelic configuration of

the detected biparental makers, of which 89 fitted the segregation ratio expected for BSDFs (3:1).

Table 8.4. BLAST-identified similarities among sequences for 12 cassava clones.

CloneID	Best hit for:	Description	Significance
	est_others_Manihot_blastn		
250272	gb|FF381943.1|	CASLS06TF CASL *M.esculenta* cDNA 5'; mRNA sequence.	8,00E-53
255179	gb|FF381943.1|	CASLS06TF CASL *M. esculenta* cDNA 5'; mRNA sequence.	5,00E-51
250307	gb|FF381943.1|	CASLS06TF CASL *M. esculenta* cDNA 5'; mRNA sequence.	3,00E-43
248286	gb|FF381943.1|	CASLS06TF CASL *M. esculenta* cDNA 5'; mRNA sequence.	9,00E-10
248961	gb|DV441158.1|	CV01003B1A12.f1 CV01-normalized library *M. esculenta* cDNA clone CV01003B1A12.f1; mRNA sequence.	1,00E-11
	plant_genomic_blastn		
81251	ref|NC_008471.1|	*Populus trichocarpa* linkage group V	4,00E-24
249344	ref|NC_008474.1|	*P. trichocarpa* linkage group VIII	7,00E-28
256481	ref|NC_008474.1|	*P. trichocarpa* linkage group VIII	6,00E-09
	plant_protein_blastx		
249344	ref|NP_187272.3|	Binding [*Arabidopsis thaliana*]	4,00E-20
	plant_rna_tblastx		
81251	Ref|NM_106398.2|	*A. thaliana* TAPX; L-ascorbate peroxidase (TAPX) mRNA; complete cds	4,00E-08
249344	Ref|NM_121903.3|	*A. thaliana* binding (AT5G18980) mRNA; complete cds	7,00E-29
256481	Ref|NM_113503.3|	*A. thaliana* DNA-binding protein GT-1-related (AT3G25990) mRNA; complete cds	4,00E-14
	Arabidopsis_thaliana_blastn		
81251	gb|AE005173.1|	*A. thaliana* chromosome 1 bottom arm; complete sequence	1,00E-14
256481	dbj|BA000014.8|	A. *thaliana DNA; chromosome 3;* complete sequence	3,00E-07

Table 8.5. Total DArT molecular markers scored on tetraploid cassava mapping population.

Type of marker	***DArT***	***%***
Paternal	127	29.5
Maternal	110	
Biparental	326	40.5
Absence in both parents	98	12.2

Marker with missing values >5%	143	17.8
Total	**804**	**100.0**

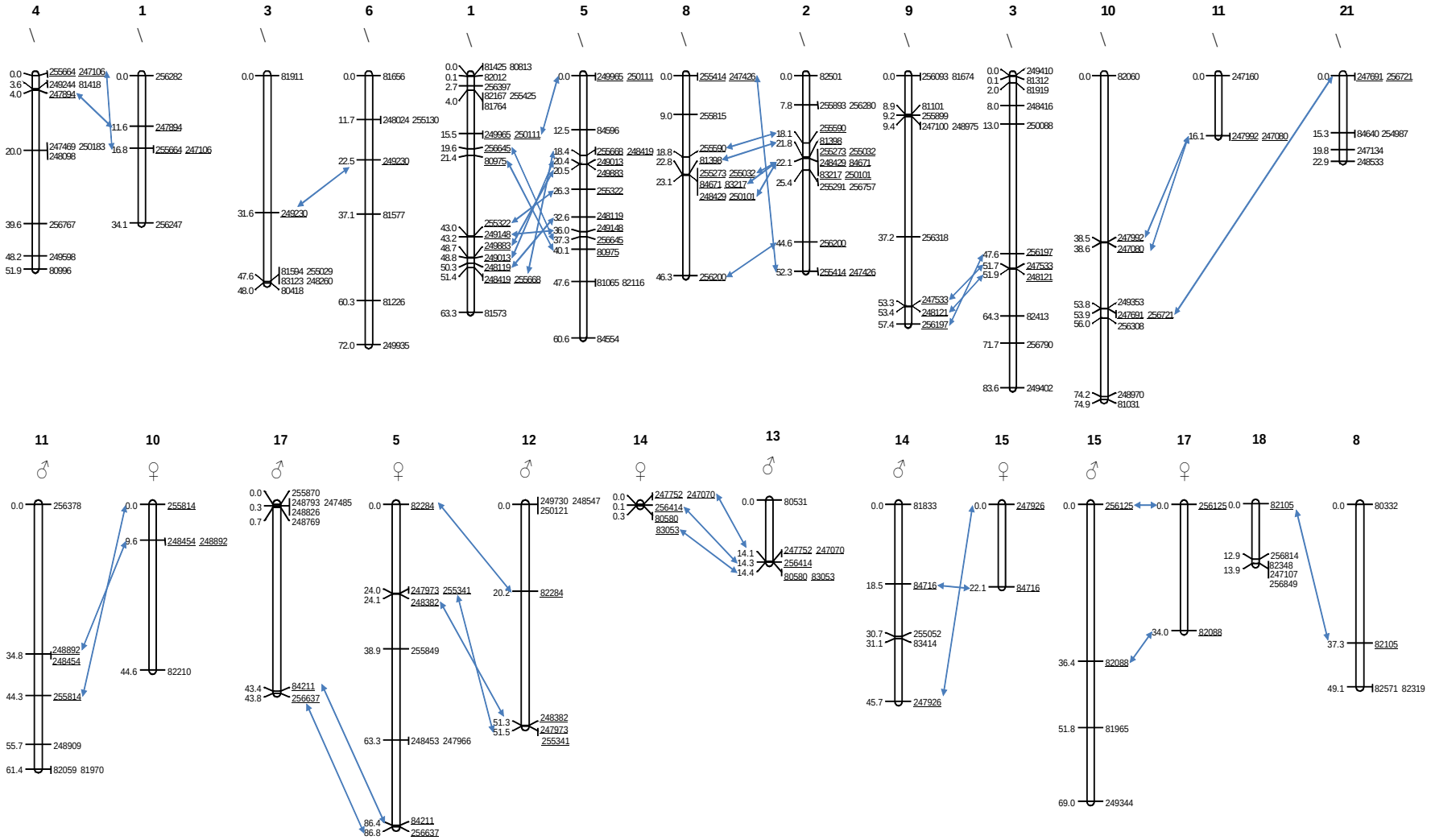


Figure 8.3. Linkage map of cassava DArTs. Markers' names and distances in cM (Kosambi) are indicated on the right and left. SDAFs groups were mapped at LOD values 10.0–2.0. BSDF markers (underlined) were allocated and used to compare paternal and maternal maps. Asterisks indicate markers showing association of resistance to cassava frogskin disease.

Table 8.6. Number of markers scored in each class of segregation ratio.

Type of marker	Allelic configuration and expected segregation ratio[a]
	SDAF
	1:1
Paternal	85
Maternal	75
	BSDF
	3:1
Biparental	89
Total	**249**

a. SDAF refers to single-dose amplification fragments;
BSDF refers to biparental single-dose amplification fragments.

The linkage maps of CM2177-2 and TMS 30572 were based on 174 and 164 paternal SDAF and BSDF loci, respectively (**Tables 8.5 and 8.6**). Twenty-one linkage groups were defined at LOD > 2.0 in both maps. The two maps shared 13 linkage groups, identified according to the markers segregating in the progeny at 3:1 (**Figure 8.3**).

The similarity between the markers and between the genotypes was calculated. In this case, like genotypes were not excluded but in later analyses they were excluded to prevent redundancy. Of the 174 markers selected in the parent CM 2177-2, 130 DArTs could be included on the map and 34 were unsuccessfully located in any linkage group or they represented a region saturated with markers of a similarity of more than 0.98 and which really did not represent linkage groups. For the parent TMS 30572, of 164 markers, 127 DArTs could be included on the map and 37 were unsuccessfully located in any linkage group or they represented a region saturated by markers with high similarity.

Several markers had similarity values that equaled 1, which indicated that they were the same marker and, as such, were located in the same position on the map. We defined 18 linkage groups and 3 groups of markers located independently in small “pseudogroups”. These three “linkage groups” really could not be considered as groups because they were only markers with high similarity located in the same position and no other markers linked with them. Possibly, they are part of other groups already defined but confirmation is needed with more markers on complete map. Likewise, markers that could not be located on the map (34 for the paternal and 37 for the maternal parent) must be confirmed with more markers on the complete map.

So far, of the 15 markers selected for their high significance or close association with resistance to CFSD, 12 were polymorphic (7 for the paternal and 5 for the maternal parent), 3 were monomorphic, 11 complied with the expected segregation of 1:1, and 10 could be mapped (**Table 8.7; Figure 8.3**).

Table 8.7.Results of the linkage analysis of 15 cassava clones selected for their association with resistance to cassava frogskin disease.

CloneID	*Poly (P)- or mono (M)-morphic in the parents*	*Location on the map*	*Expected segregation*
247586	P	CM	1:1
248137	P	TM	1:1
248286	P	CM	1:1
248961[a]	P	-	-
249344	P	CM	1:1
250272	P	CM	1:1
250307	P	CM	1:1
255179	P	CM	1:1
255287	P	CM	1:1
255773	P	TM	1:1
256481[b]	P	-	1:1
80664	M	-	-
81019	M	-	-
81251	M	-	-
82210	P	TM	1:1

[a] No segregation expected

8.3 CONCLUSIONS

Because of the crop's cycle and the particular characteristics of CFSD, the selection of resistant materials in the field was slow and affected breeding procedures. So far, no studies have been done on the association of any molecular marker that would enable rapid selection of genotypes resistant to the disease.

The use of a DArT technique for cassava would optimize rapid selection of genotypes resistant to CFSD. Thus, time and money spent on procedures would be economized, enabling us to give farmers quicker and more viable options for managing the disease. Obtaining resistant materials would also help us support other improvement programs in Central and South America where the disease has caused losses as high as 90% in commercial crops.

We obtained a sufficient number of polymorphic markers that can be evaluated across the contrasting populations. High correlations among field evaluations and results obtained with molecular markers will allow us to establish a molecular marker that will identify genotypes with resistance or tolerance of CFSD.

Contributors:
Juan Fernando Mejía, Elizabeth Álvarez, Martin Fregene, Paula Hurtado, and Andrzej Kilian

Highlights

- Identified diversity array technology (DArT) as an alternative to marker-assisted selection for resistance to frogskin disease.

- Identified candidate markers from DArT technology for saturating the cassava genetic map (K Family), that is, 110 markers for the maternal parent TMS 30572 and 127 for the paternal parent CM 2177-2.

8.4 REFERENCES

Center for the Application of the Molecular Biology to International Agriculture DArT P/L (CAMBIA) Canberra, ACT, Australia

CIAT (2002). La yuca en el Tercer Milenio: sistemas modernos de producción, procesamiento, utilización y comercialización. Cali-Colombia, Publicación CIAT; no. 327. 586p.

CIAT (2004). Centro Internacional de Agricultura Tropical. Virology. Activity 1. Resistance for cassava frogskin disease is widespread in cassava germplasm Annual Report. Cali-Colombia**:** p 199-200.

Duque, D. P. (2003). Identificación de secuencias de ADN genómico polimórficas entre las variedades de yuca (*Manihot esculenta* Crantz) CM2177-2 y NGA-2 asi como de MECU 72 y MCOL 2246 mediante la tecnología DArT (Diversity Arrays Technology) basada en microarreglos. Facultad de Ciencias, Programa academico de Biología. Santiago de Cali, Universidad del Valle**:** 97.

Jaccoud, D., K. Peng., D. Feinstein and A. Kilian (2001). "Diversity Arrays: a solid state technology for sequence information independent genotyping." Nucleic Acids Research **29**(4): 7.

Xia, L., K. Peng., S. Yang., P. Wenzl., M. C. de Vicente., M. Fregene and A. Kilian (2005). "DArT for high-throughput genotyping of Cassava (*Manihot esculenta*) and its wild relatives." Theor Appl Genet **110**: 1092-1098.

CHAPTER 9

ACTIVITIES IN ASIA

9.1. CASSAVA TRAINING COURSE

A 2-week Regional Cassava Training Course was organized in October 2008. The course was taught in English by experienced cassava researchers from CIAT-Colombia and CIAT-Asia covering their specialized disciplines, such as cassava breeding, biotechnology, physiology, insects and diseases, cassava nutrition and fertilization, soil management and agronomic practices, as well as the use of cassava in food preparations, processing into starch and fuel-ethanol, and for animal feeding. Several Thai cassava researchers made additional presentations in their specialized fields (**Table 9.1**). The participants were mainly young cassava researchers from the various national programs who had not had previous opportunities for intensive training in their disciplines of cassava research (**Table 9.2**). Activities were held during the first week at Rayong Field Crops Research Center in Huay Pong, Rayong, and the second week at the Thai Tapioca Development Institute (TTDI)'s Research and Training Center in Huay Bong, Nakhon Ratchasima. The course also included field trips to cassava chip drying yards and starch factories. Each resource person is asked to write one or more chapters covering the material presented during the course. These chapters will be assembled and printed as a "Handbook of Cassava Research and Development" to be used as a reference, summarizing over 30 years of cassava research by scientists from CIAT and from various national programs.

Table 9.1. List of lecturers in the Cassava Training Course held in Thailand (October 6-19) and their respective areas of research

Lecturer	Area of Research
Dr. Reinhardt Howeler	Agronomy
Dr. Tin Maung Aye	Agronomy
Dr. Elizabeth Alvarez	Pathology
Dr. Henarn Cebalos	Breeding
Dr. Bernardo Ospina	Processing
Dr. Julian Buitrago	Animal feeding
Dr. Anthony Bellotti	Entomology
Dr. James Cook	Physiology
Dr. Emmanuel Okogbenin	Molecular markers
Dr. Kaival Klakhaeng	Agronomy
Mr. Watana Watananonta	Agronomy/breeding
Mr. Banyat Vankaew	Agronomy/breeding
Ms Amporn Youngmod	Genetic Resources
Mrs. Wilawan Vongkasem	Agronomy
Mr. Danai Suparhan	Breeding
Ms. Kuakoon Ph.D	Starch
Prof. Dr. Charoensak Rojanaridpiched	Breeding
Dr. Atchara Limsila	Breeding

Table 9.2. List of 59 participants in the Cassava Training Course held in Thailand (October 6-19) grouped by country of origin.

Cambodia
Mr. Ung Sopheap
Mr. Orn Chhourn
Mr. Sok Sothearith
Mr. Sok Sunnara
China - Hainan
Mr. Huang Jie
Mr. Ye Jianqiu
Mr. Yan Qingxiang
Mr. Zhang Zhenwen
Mr. Jiang Shengjun
Mr. Ou Wenjun
Mr. Wu Chuanyi
Mr. Xue Maofu
Mr. Zheng Yongqing
Mr. Song Fuping
Mr. Lu Cheng
Ms. Song Hongyan
Ms. Qi Lan
China - Guangxi
Mr. Ma Chongxi
Mr. Fu Haitian
Mr. Huang Jianqi
Mr. Wei Zusheng
Ms. Chen Xianshuan
Ms. Wen Feng
Ms. Pan Huan
Ms.Lu Saiqing
Ms. Yang Qin
Ms. Luo Yanchun
East Timor
Mr. Telio Moniz
Mr. Abril Fatima Soares
India
Dr. G. Byju
Dr. M. Nedunchezhiyan
Dr. G. Ramanandam
Myanmar
Mr. Thant Lwin Oo
Indonesia
Dr. Sholihin
Dr. Marjuki
Mr. Aldon Sinaga
Mr. Budi Waluyo
Ms. E. Retnaningtyas
Laos
Mr. V.Phimphachanhvongsod
Mr. P. Khanthavong
Ms. P.Soisouvanh
Mr.Lao Thao
Malaysia
Mr. NurulNahar bin Esa
Philippines
Mr. Dioscoro M. Bolatete Jr
Thailand
Dr. Chalermpol Phumichai
Mr. Jumnong Chanthaworn
Mr. Preecha Phetprapai
Ms. Supatra Chaokongjugr
Mr. Wasan Wannajuk
Ms. Supawadee Boonma
Ms. Methapond Putkhao
Ms. Chansawang Srihata
Ms. Siwilai Lapbanjob
Ms. Rungravee Boontung
Vietnam
Ms. Pham Thi Nhan
Ms. Vu Thi Nguyen
Mr. Nguyen Phuong

9.2. 8TH REGIONAL CASSAVA WORKSHOP.

As mentioned above, after the training course in Thailand the 8th Regional Cassava Workshop took place at the end of October, 2008 in Vientiane, Lao. This has been an important event that groups together a large number of cassava researchers from the region. **Table 9.3** provides a list of the participants in the workshop, grouped by country of origin. **Table 9.4** provides a summary of the topics covered during the workshop. CIAT is grateful to the Nippon Foundation for the many years it has supported cassava research and training activities in Asia. A total of 118 people from fifteen different countries participated in the workshop. The Nippon Foundation also financed the organization of the Training Course which benefited from the logistic and valuable support of the Department of Agriculture and Thai Tapioca Development Institute.

Table 9.3. List of participants in the 8th Cassava Workshop, held in Vientiane (LAO) (October 20-24) grouped by country of origin.

Bangladesh
Borhan Ahmed
Mohammad S. H. Bhouiyea
Major Md Abdullah O. Sharif
Cambodia
Ung Sopheap
Thun Vathany
El Sotheary
Sok Sunnara
Chhay Ty
Hideo Okuda
China
Tian Yinong
Li Jun
Fu Haitian
Lu Saiqing
Luo Yanchun
Li Kaimian
Huang Jie
Ye Jianqiu
Jiang Shengjun
Zhang Zhenwen
Wang Wenquang
Chen Xin
Lu Cheng
Liang Guo Tao
Colombia
Hernan Ceballos
Bernardo Ospina
Elizabeth Alvarez
Emmanuel Okogbenin
Anthony Bellotti
Julian Buitrago
E. Timor
Brian Monaghan
India
K. Abraham
Vinayaka Hegde
Jayaprakas
G. Byju
M. Nedunchezhiyan
G. Ramanandam
Indonesia
Wani Hadi Utomo
Marjuki Marjuki
Sholihin
Yudi Widodo
Endah Retnaningtyas
J. Wargiono
Augustinus Omar Rahmanadi
Japan
Shuichi Ohno
Kenya
Jonathan Schofield
Laos
Rod Lefroy
Tin Maung Aye
Keith Fahrney
Lao Thao
Thiphavong Boupha
Hongthong Phimmasan
V. Phimphachanhvongsod
Bounthong Bouahom
Monthathip Chanphengxay
Soukanh Keonouchanh
Chay Bounphanousay
Phoumi Inthapanya
Phanthasin Khanthavong
Paulina Naranjo Taco
Eliana Nunez Ponce
Rob Kelly
Silinthone Sacklokham
Khonesavanh
Mr. Siphone Dalaphone
Keutkhuanchai Malychansy
Somsack Pongkhao
Malaysia
Engku Ismail Engku Ahmad
NurulNahar Esa
Philippines
Algerico Mariscal
Dioscoro Bolatete Jr.
Thailand
Morakot Tanticharoen
Chareinsak Rojanaridpiched
Klanarong Sriroth
Kuakoon Piyachomkwan
Sutkhet Nakasathien
Yupa Pankaew
Metha Wanapat
Anan Petlum
Vongpasith Chanthakhoun
Methapond Putkhao
Somsak Thongsri
Surapong Charoenrath
Danai Suparhan
Somlak Jutunka
Jinnajar Hansethasuk
Suchirat Sakuanrungsirikul
Peaingpen Sarawat
Theerawut Wongwarat
Nilubon Taweekul
Kobkiet Paisancharoen
Wasan Wannajuk
Chansawang Srihata
Banyat Vankaew
Pitsanu Detyothin
Wilawan Vongkasem
Kaival Klakhaeng
Chumpol Nakaviroj
Uthai Cenpukdee
Watana Watananonta
Rungravee Boontung
Suthasinee Sontirat
Boonmee Wattanaruangrong
Taweesak Chodchoi
Somruedee Ridthaisong
Chantasing Duangbansao
Reinhardt Howeler
USA
Francisco Hoyos
Vietnam
Tran Ngoc Ngoan
Nguyen Vu Thi
Nguyen Thi Hoa Ly
Hoang Kim
Phuong Nguyen
Nguyen Huu Hy
Pham Thi Nhan
Nam Ho Dai
Nhat Le Quang
The Le Van
Khanh Ton That Minh

Table 9.4. General agenda of presentations at the 8th Cassava Workshop, held in Vientiane (LAO) (October 20-24).

Sunday – Oct 19	
Arrival of participants in Vientiane	
Monday – Oct 20	
Morning	**Registration and opening ceremony and keynote address** Chairperson: Rod Lefroy
Afternoon	**Cassava Germplasm Conservation and Breeding** Chairperson: Hernan Ceballos
Tuesday – Oct 21	
Morning	**Cassava in Asia** Chairperson: Morakot Tanticharoen
Afternoon	**Old and New Cassava Initiatives in Asia** Chairperson:Wani Hadi Utomo
Wednesday – Oct 22	
Morning	**Old and New Cassava Initiatives and Cassava Agronomy** Chairperson: Chareinsak Rojanaridpiched
Afternoon	**Field trip** Naphok Rice and Cash Crop Research Center – Phoumi Inthapanya/Tin Aye
Thursday – Oct 23	
Morning	**Cassava Disease and Pest Management** Chairperson: Anthony Bellotti
Afternoon	**Cassava Processing and its Use in Animal Feeding** Chairperson: Keith Fahrney
Friday – Oct 24	
Morning	**The role of cassava in rural development and poverty alleviation** Chairperson: Reinhardt Howeler
Afternoon	Departure of participants by bus or airplane

9.3 INSTITUTIONAL COLLABORATION

Table 9.5 shows the institutions and individuals at the national, provincial and district levels that collaborated in the execution of the project in 2008. In Lao PDR the project is being coordinated by the National Agric. and Forestry Research Institute (NAFRI) with headquarters and two research centers near Vientiane, while the on-farm trials are conducted mainly by provincial (PAFO) and district (DAFO) staff in several provinces. In 2008 the project has expanded to new districts and new provinces, such a Houa Phan and Saravanh.

The main partners in Cambodia are the Cambodian Agricultural Research and Development Institute (CARDI) with headquarters just outside Phnom Penh, and with substations in Preah Vihear province and Pailin Town. CARDI collaborates in the project mainly for the conducting of experiments on varietal selection and agronomic practices, and coordinates the conducting of on-farm and farmer participatory research (FPR) trials in target provinces nation-wide through the provincial extension offices of the Department of Agriculture under the Ministry of Agriculture and Forestry.

Table 9.5. Institutions and principal individuals that are collaborating in the Nippon Foundation Cassava Project in Lao PDR and Cambodia in 2008.

Institution	Location	Person	Specialty
Lao PDR			
1. National Agric. and Forestry Research Institute (NAFRI)	Vientiane	Mr. Viengsavanh Phimphachanhvongsod	Animal Nutrition
		Mr. Soukanh Keonouchanh	Director Nam Xuang
		Mr. Sopha Xaipha	Animal Nutrition
		Mr. Sitone Kongvongxay	Cassava
		Mr. Phoumi Inthapanya	Director Naphok
		Mrs. Sengkham Lakmaitry	Cassava
		Mr. Phanthasin Khanthavong	Cassava
		Mr. Saythong Udthachit	Cassava
2. Provincial Agric. and Forestry Offices	Oudomxay	Mr. Somsamouth Phongsavath	PAFO Crops
	Oudomxay	Mr. Moua Yang	PAFO Livestock
-in Oudomxay	La	Mr. Odon	DAFO Director
	La	Mr. Bounpheng Thanthonbai	DAFO Crops
	Namor	Mr. Bounson Duangphasith	DAFO Director
	Phonesavan	Mr. Amphone Phommavong	PAFO Crops
	Phonesavan	Mr. Sonthavath Vanthala	PAFO Livestock
	Phonesavan	Mr. Khamphai Phommavong	PAFO Livestock
-in Xieng Khouang "	Phonesavan	Mr. Hongthong Phimmasau	Director Cattle Bank
	Phonesavan	Mr. Khammanh Chansingbang	Crops
	Nong Het	Mr. Vong Philavong	DAFO Livestock
	Nong Het	Mr. Neuakhom Thepphanit	DAFO Crops
-in Luang Prabang	Luang Prabang	Mr Sengpasith Thongsavath	PAFO Livestock
	Luang Prabang	Mr. Soulideth Phaphonxay	PAFO Livestock
	Xamneua	Mr. Satian Vannasouk	PAFO Livestock
-in Houa Phan		Mr. Mayphuot Ban Vi Done	PAFO Crops
		Mr. Lee Cha	PAFO Livestock
		Mr. Siviengxam Phengphomma	PAFO Agric. Extension
	Saravanh	Mr. Somkit Senthay	PAFO Livestock
-in Saravanh		Mr. Sysomphone Fangkham	PAFO Crops
		Mr. Thongdy Chanthavong	PAFO Agriculture
-in Vientiane Municipality	Xaythany	Mr. Banelom Siakkhasone	DAFO Director
3. National University of Laos	Vientiane	Dr. Silinthone Sacklokham	Agric. Ecom
Faculty of Agriculture	Vientiane	Mr. Sitthisack Phoulivonk	Agric. Ecom
	Luang Prabang	Mr. Khamphoul Phonexay	Director
	Luang Prabang	Mr. Chanphone Keoboualapheth	Dep. Dir
	Luang Prabang	Mr. Aphaivanh Souksanti	Livestock
4. Luang Prabang Agric. Forestry College	Luang Prabang	Mr. Outhai Soukkhy	Agriculture
	Luang Prabang	Mr. Thonsamouth Phoummasone	Dep. Dir. Education
	Luang Prabang	Mr. Bounxou Xaysana	Agriculture
	Luang Prabang	Mr. Somkit Chaleinphan	Agriculture
	Luang Prabang	Mrs. Chamanny Souphanavong	Agriculture
Cambodia			
1. Cambodia Agric. Research and Developm. Inst. (CARDI)	Phnom Penh	Mr. Ung Sopheap	Agronomy
		Mr. Pith Khon Hel	Varietal Impr.
		Mr. Orn Chhourn	Varietal Impr.
		Dr. Nget Sivutha	Soil/Water Cons.
		Mr. Sok Sophearith	Soil/Water Cons.
2. Prov. Dept. Agric., For.& Fish.	Kampong Cham	Mr. Seung Bunny	Agronomy
-in Kampong Cham	Kampong Cham	Mr. Kou Nakry	Agronomy
	Kampong Cham	Mr. Mean Samay	Agronomy
-in Battambang	Battambang	Mr. Nou Praneth	Agronomy
	Battambang	Mr. Sarhai Rachana	Livestock
3. CelAgrid	Kandal Stung	Dr. Khieu Borin	Animal Nutrition
		Mr. Chhay Ty	Animal Nutrition

Research and development of the use of cassava for animal feeding is done mainly by the Center for Livestock and Agriculture Development (CelAgrid) located in Kandal province, just south of Phnom Penh. This NGO conducts research at their own research farm, mainly through research projects of university students; it also has offices and personnel in several provinces to work with small-scale farmers to increase their income through improvements in the management of livestock, mainly by the use of locally available feed resources, such as cassava roots and leaves.

In Cambodia the activities in 2008 were mostly concentrated in Chamcar Leu and Tbong Khmon districts of Kampong Cham province and in Rattanak Mondul district of Battambang province. Since the headquarters of the Cambodian Agric. Research and Development Institute (CARDI) is located on typical paddy soils, which are too wet in the rainy season for growing cassava, all replicated cassava experiments in 2008/09 were conducted on red upland soils at the Chamcar Leu Seed Farm of the Dept. of Agronomy and Agric. Land Improvement (DAALI) in Kampong Cham province, about a 2-3 hour drive northeast of Phnom Penh. Although the soils at this station are possibly too fertile to be representative of typical cassava growing areas, the type of soil is quite representative of major cassava growing areas in the province, and this province produces about 50% of all cassava in Cambodia.

9.4 Collaborative On-Station Research

Cassava germplasm collections

Since the start of the Nippon Foundation-funded Cassava Project in Laos in 2004, CIAT has introduced new varieties from Thailand, Vietnam, China and Indonesia. With the introduction of these new varieties as well as the collection of local landraces, the cassava germplasm collection in Laos has been growing rapidly and now contains 43 accessions, of which 10 from Laos, 10 from Thailand, 7 from Vietnam, 15 from China and 1 from Indonesia. The newly introduced varieties are being characterized and evaluated, and then multiplied for further testing in comparison with the existing improved varieties. The whole cassava germplasm collection was replanted in a new field at the Rice and Cash Crop Research Center near Vientiane in August 2008.

Samples of young leaves of 37 germplasm accessions were sent in 2007 to the Field Crops Research Center of DOA in Khon Kaen, Thailand, for DNA finger printing, to see whether these varieties are genetically related or duplicates. The results from the dendrogram indicate that some of the varieties are very closely related and some of them are genetically very different. But some results from the dendrogram were rather unexpected. Therefore, in 2008 another batch of 20 samples were sent to the Field Crops Research Center in Khon Kaen for a repeat analysis to confirm the results. The new dendrogram is shown in **Figure 9.1**. It confirms that several local eating varieties are very closely related to the Thai variety Hanatee, and that the Vietnamese-improved variety KM 98-1 is very closely related to the Thai variety Rayong 72.

Since 2004 CIAT has also introduced small amounts of planting material of Thai, Vietnamese and Chinese varieties into Cambodia, which were planted at CARDI for characterization and multiplication. Since the soil at the institute is too wet during much of the rainy season and

very dry during the 6-month dry season, plants did not grow well, but at least most accessions survived. Some of these have now been replanted in a new cassava germplasm bank at the Cham Car Leu Seed Farm in Kampong Cham. The collection now has 31 accessions of which three local varieties, 13 from Thailand, 8 from Vietnam and 7 from China (**Table 9.6**).

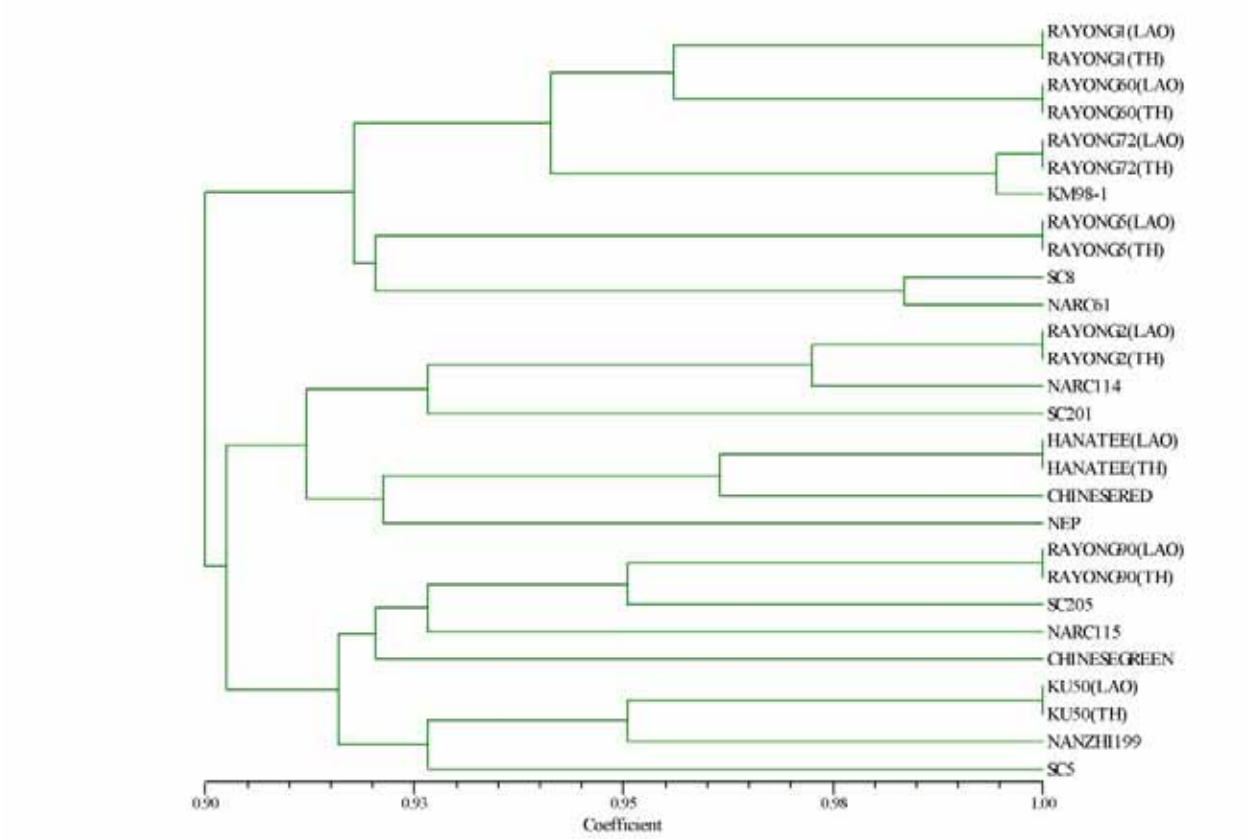


Figure 9.1. Dendrogram of cassava varieties in the germplasm collection at Naphok Agricultural Research Center, Vientiane, Lao PDR. March 2008.
Source: Suchirat Sakuanrungsirikul, Khon Kaen Field Crops Research Institute, DOA, Thailand.

Variety evaluation experiments
A varietal evaluation trial was planted at the Naphok Agric. Research Center (NARC) near Vientiane, Lao PDR, in June 2007 and harvested in April 2008. Twenty varieties were evaluated in this experiment: two local varieties, eight Thai, six Vietnamese, three Chinese and one Indonesian variety. All varieties had good germination and were reasonably vigorous. The results of this experiment are shown in **Table 9.7**.

Fresh root yields and root starch contents were low this year as compared to last year due to excessive rain. Highest root yields were obtained with HL 23, KU 50, Rayong 72, KM 98-1 and Rayong 90, and lowest yields with two eating varieties, Mentega from Indonesia and Vin Phu from Vietnam. The root yields of OMR 36-31-1 and SC 5, both from China, were not high in contrast with the high yields obtained with these varieties in Luang Prabang. In May

2008, this trial was replanted in NARC to determine whether these Chinese varieties are actually suitable for low elevation areas in Laos.

Table 9.6. Local and introduced cassava varieties grown in Cambodia. The year they were introduced, their origin and main characteristics are provided.

Variety	Year	Origin	Characteristics
Damlong Kor	-	-	Local eating variety similar to Hanatee; low yields
Damlong Mi	-	-	Local eating variety; early maturing
Damlong Slek Toch	-	-	Local industrial variety; high yield, low starch content
Malaysia	~2000	Vietnam	Same as KM 94 = KU 50; high yield, high starch
KU 50	~2000	Thailand	Industrial variety; high yield, high starch
Hanatee	2004	Thailand	Eating variety; low yield, low starch
Rayong 1	2004	Thailand	Industrial variety; relatively high yield and starch
Rayong 2	2004	Thailand	Excellent eating quality; yellow flesh, low starch
Rayong 3	2004	Thailand	Industrial variety; high yield and starch on good soils
Rayong 5	2004	Thailand	Industrial variety; high yield and starch
Rayong 60	2004	Thailand	Industrial variety; high yield, early maturing
Rayong 72	2004	Thailand	Dual purpose variety; high yield, high starch, drought tolerant
Rayong 90	2004	Thailand	Industrial variety; high yield and very high starch
Kasetsart 50	2004	Thailand	Industrial variety; high yield, high starch, good germination
Huay Bong 60	2004	Thailand	Industrial variety; high yield, high starch on good soils
Rayong 7	2006	Thailand	Industrial variety; high yield, high starch on good soils
Rayong 9	2006	Thailand	Industrial variety; high yield and starch, high ethanol yield
Vinh Phu	2005	Vietnam	Dual purpose variety; relatively high yield and starch
Ba Trang	2005	Vietnam	Eating variety; low yield, early maturing
Nep	2005	Vietnam	Eating variety; low yield, high starch, sticky
KM 95-1	2006	Vietnam	Dual purpose variety; high yield
KM 98-1	2006	Vietnam	Dual purpose variety; high yield
KM 98-5	2006	Vietnam	Industrial variety; high yield
KM 140	2006	Vietnam	Dual purpose variety; high yield
SM 937-26	2006	Vietnam	Industrial variety; high yield, high starch
SC 5	2006	China	Industrial variety; high yield on poor soil
SC 8	2006	China	Industrial variety; high yield
SC 9 (Yolk)	2006	China	Eating variety; low yield, yellow flesh
SC 201	2006	China	Eating variety; relatively high yield on poor soils, low starch
GR 891	2006	China	Industrial variety; high yield on good soil, high starch
Nanzhi 199	2006	China	Industrial variety; high yield, high starch
OMR 36-31-1	2006	China	Industrial variety; high yield, high starch

Another cassava variety trial was planted at the College of Agriculture and Forestry in Luang Prabang province by the second group of Zamorano interns in early June 2007. The cassava plants had grown very tall and vigorous during the wet season. They were harvested at the end of March 2008 with the help of two new Zamorano interns and College students. In this trial, Vietnamese, Chinese, Thai and local varieties were included, both those for eating and for industrial use. Many farmers still want to grow some of their local varieties for their own consumption. **Table 9.8** shows that root yields varied from 11 to 37 t/ha, and starch contents varied from 19 to 34%. Rayong 72 from Thailand had the highest fresh root yield (37 t/ha) as well as starch yield (9.3 t/ha) among tested varieties even though its starch content

was only 25%. The local varieties, NARC 48 and LAFC, had low yields (13 and 11 t/ha) and rather low starch contents (20 and 19%). KU 50 had a rather low yield but had the highest starch content (34%), while Rayong 90 produced a high starch yield (8 t/ha). Farmers in the various pilot sites have already selected KU 50, Rayong 72 and Rayong 90 as the most suitable varieties for feeding their animals and for sale. The two Chinese varieties, OMR-36-31-1 and SC 5, and the Vietnamese variety KM140 produced higher fresh roots yields and starch yields than most other varieties tested. The HCN contents of the roots were also determined. Although the methodology used was not very quantitative, the obtained results clearly separated the low-HCN eating varieties from the high-HCN industrial varieties. However, the HCN content of Rayong 90 determined this year was much lower than that of last year; environmental conditions may have affected the HCN content of the roots.

Table 9.7. Results of the varietal evaluation experiment conducted at Naphok Agriculture Research Center in 2007/08. (10 months after planting).

No.	Variety	Root yield (t/ha)	Starch content (%)	Starch yield (t/ha)
1	HL 23	22.2	20.6	4.6
2	KU 50	20.4	24.8	5.1
3	Rayong 72	20.3	23.5	4.8
4	KM 98-1	19.8	26.4	5.2
5	Rayong 90	19.2	24.8	4.8
6	Rayong 60	18.9	23.5	4.4
7	Rayong 5	17.2	22.7	3.9
8	KM 140	15.9	22.9	3.6
9	NARC 61	15.6	24.5	3.8
10	Rayong 1	15.3	22.9	3.5
11	NARC 48	15.1	19.3	2.9
12	SC 205	14.7	20.0	3.0
13	Ba Trang	14.3	20.6	2.9
14	Rayong 2	13.1	17.2	2.3
15	Hanatee	12.8	19.5	2.5
16	NEP	12.6	25.1	3.2
17	OMR 36-31-1	11.5	22.4	2.6
18	SC 5	11.5	20.8	2.4
19	Mantega	10.4	22.9	2.4
20	Vin Phu	10.1	20.6	2.1

Another cassava variety trial was planted at the Cattle Bank Station in Paek district of Xieng Khouang province in early 2007. The station is located at about 1,100 meters above sea level (masl) and has an extremely acid and infertile soil. Because of its high elevation it has year-round low temperatures, especially in winter (Nov-Feb) when night temperatures can be below 0° C. For these reasons, cassava growth is usually very slow, and the trial was therefore harvested only after two years. **Table 9.9** shows that after two years yields of some varieties were exceptionally high, reaching nearly 100 t/ha for the Vietnamese variety KM 140 and 66 t/ha for the Thai variety Rayong 1. The same varieties were replanted to confirm these unexpected results.

Table 9.8. Results of the cassava varietal evaluation experiment conducted at the Luang Prabang College of Agriculture and Forestry in 2007/08 by the Zamorano graduates. (10 months after planting)

No	Variety	No. of harvested plants	Root yield (t/ha) [1)]	Starch content (%)	Starch yield (t/ha)	HCN content (ppm)[2)]
1	Rayong 72	14	36.88	25	9.3	50
2	OMR 36-31-1	14	33.75	26	8.7	30
3	SC 5	15	31.88	25	7.9	400
4	HL 23	13	31.25	21	6.4	30
5	Vinh Phu	14	30.00	23	6.9	50
6	KM 140	15	29.39	27	7.8	30
7	Rayong 90	15	29.38	27	8.0	50
8	KM 98-1	13	27.50	28	7.7	100
9	Ba Trang	12	25.62	22	5.7	30
10	Rayong 1	11	25.00	20	5.0	30
11	NARC 114	12	25.00	23	5.7	200
12	Hanatee	13	22.50	22	5.0	30
13	Local Ban Bo Hee	14	21.88	23	5.0	30
14	KU 50	12	21.88	34	7.5	400
15	Nep	11	21.25	27	5.8	30
16	SC 205	11	20.62	22	4.5	50
17	NARC 61	11	20.62	29	5.9	30
18	Rayong 5	16	20.62	28	5.6	30
19	Rayong 2	10	18.75	20	3.8	20
20	NARC 48	9	13.12	20	2.7	30
21	Local LAFC	10	11.25	19	2.1	10

[1)] based on 16 m^2 of harvested area
[2)] on fresh weight basis

Table 9.9. Results of the cassava varietal evaluation experiment conducted at the Cattle Bank Stationin Xieng Khouang province in 2006/08. (two-year cassava)

No	Variety	Fresh root yield (t/ha)	Starch content (%)	Starch yield (t/ha)
1	KM 140	98.8	26.1	25.7
2	Rayong 1	65.6	26.1	17.1
3	KM 98-1	56.9	26.1	14.8
4	Rayong 60	55.0	26.9	14.8
5	KM 98-7	62.1	23.3	14.5
6	Nep	43.3	31.5	13.7
7	KU 50	41.0	29.9	12.3
8	HL 23	46.5	26.1	12.1
9	Rayong 90	42.7	26.1	11.1
10	Rayong 72	46.7	22.3	10.4
11	Ba Trang	22.3	29.0	6.5

In Cambodia a set of 15 varieties was planted in a replicated trial at the Chamcar Leu Seed Farm in Kampong Cham in May 2008 and harvested in early March 2009 at 9 MAP. **Table 9.10** shows that root yields were quite high, ranging from 29.0 t/ha for the local eating variety, Damlong Kor, to 53.9 t/ha for the Thai variety KU 50; the latter is the most widely grown variety in Thailand, Indonesia, Vietnam and Cambodia, because of its high yield and starch content. In this experiment KU 50 produced a starch yield of 15.4 t/ha in only 9 months.

Table 9.10. Results of a cassava varietal evaluation experiment conducted in Chamcar Leu Seed Farm in Kampong Cham province of Cambodia in 2008/09.

Varieties	Plant stand (%)	Root yield (t/ha)	Starch content (%)	Starch yield (t/ha)	Ranking
1. Damlong Kor	89	28.97	24.2	7.01	15
2. Damlong Slek Toch	100	52.64	29.0	15.27	2
3. KU 50	73	53.92	28.6	15.42	1
4. Rayong 1	97	37.85	28.4	10.75	10
5. Rayong 2	54	39.47	25.3	9.99	12
6. Rayong 5 (from Laos)?	83	38.65	27.5	10.63	11
7. Rayong 7	94	50.13	29.6	14.84	5
8. Rayong 9	94	50.80	29.3	14.88	4
9. Rayong 60	82	50.31	29.6	14.89	3
10. Rayong 72	87	51.15	28.3	14.48	6
11. Rayong 90	90	40.28	27.6	11.12	9
12. V_2 (from Vietnam) ?	83	43.08	29.7	12.79	7
13. Vinh Phu	92	35.00	24.2	8.47	14
14. Nep	92	38.79	29.2	11.33	8
15. Ba Trang	94	34.70	25.9	8.99	13

Long-term NPK experiments

Three long-term NPK experiments were planted in 2008, one in south Vietnam, one in Hainan province of China, and a new one in Kampong Cham province of Cambodia, while a fourth one was planted in Xieng Khouang province of Lao PDR in 2007 and will be harvested in early 2009.

In Hung Loc Agric. Research Center in Dong Nai province of South Vietnam the long-term NPK trial was planted for the 19th consecutive year and harvested in late Feb 2009. **Table 9.11** shows the soil chemical characteristics before the first planting in 1990 and before the 18th planting in 2007. It is clear that even in the unfertilized check plots the soil pH had declined considerably while the exchangeable Al concentration had increased and the Ca, Mg and K concentrations had all decreased, resulting in a marked increase in the Al-saturation to levels that would seriously affect the growth of most other crops, and may have also affected the yield of cassava. Application of high levels of N did not increase the soil OM content, but application of P markedly increased the available P content, while only the high rate of application of K increased the exchangeable K content of the soil. **Table 9.12** shows the effect of N, P and K applications on the yields and starch contents of two cassava varieties, as well as the gross and net income during the 18th cropping cycle. In spite of

relatively high levels of soil organic matter (OM) and available P there were significant responses to medium levels of N and P applications, while there was a very marked response to the application of K. Without K or without NPK, yields were only 7-10 t/ha, while application of medium levels of N, P and K increased yields to 25-30 t/ha. **Figure 9.2** shows the change in yields, in relative yields, and in the exchangeable K and available P contents of the soil as affected by annual N, P and K applications during the course of 18 years of consecutive cropping. It is clear that, like in most other long-term fertilizer trials with cassava, K became the first and most limiting nutrient, followed by N and P. Lack of K application also resulted in a steady decline in the exchangeable K content of the soil, while lack of P application had no significant effect on the available P content.

Table 9.11. Effect of annual applications of various levels of N, P and K fertilizers on soil fertility characteristics mafter 17 years of continuous cassava cultivation at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007. (before 18th year planting)

		(%)	(ppm)	(me/100g)				(%)	(ppm)					(%)			
Treatments[1)]	pH	OM	P	Al	Ca	Mg	K	Al	B	Zn	Mn	Cu	Fe	sand	silt	clay	texture
1st year (1990)	4.72	2.95	18.4	2.01	1.14	0.40	0.19	53						6.7	19.8	73.5	clay
18th year (2007)																	
1. $N_0P_0K_0$	4.06	2.40	26.74	3.07	0.55	0.15	0.09	80	0.54	2.03	86.6	0.7717.4		2.9	17.9	79.2	clay
2. $N_0P_2K_2$	4.20	2.45	45.26	3.02	0.58	0.10	0.14	79									
3. $N_1P_2K_2$	4.19	2.30	44.30	3.12	0.57	0.11	0.11	80									
4. $N_2P_2K_2$	4.26	2.29	47.00	3.07	0.79	0.13	0.15	74									
5. $N_3P_2K_2$	4.11	2.34	35.85	3.12	0.61	0.09	0.15	79									
6. $N_2P_0K_2$	4.19	2.34	23.10	2.91	0.53	0.13	0.17	78									
7. $N_2P_1K_2$	4.16	2.53	27.88	2.96	0.62	0.11	0.14	77									
8. $N_2P_3K_2$	4.31	2.43	92.65	2.76	1.20	0.13	0.13	65									
9. $N_2P_2K_0$	4.14	2.33	46.52	3.28	0.51	0.10	0.07	83									
10. $N_2P_2K_1$	4.28	2.23	47.73	3.17	0.46	0.06	0.09	84									
11. $N_2P_2K_3$	4.32	2.47	53.07	2.70	0.79	0.11	0.28	70									
12. $N_3P_3K_3$	4.33	2.56	81.84	2.55	1.16	0.13	0.22	63	0.70	1.68	88.4	0.6218.6		4.4	21.6	74.0	clay

1) N_0 = 0N; P_0 = 0P; K_0 = 0K
N_1 = 40 kg N/ha; P_1 = 20 kg P_2O_5/ha; K_1 = 40 kg K_2O/ha
N_2 = 80 kg N/ha; P_2 = 40 kg P_2O_5/ha; K_2 = 80 kg K_2O/ha
N_3 = 160 kg N/ha; P_3 = 80 kg P_2O_5/ha; K_3 = 160 kg K_2O/ha

Table 9.12 also shows that fertilizer application was highly economic, with the highest net income being obtained with the highest rates of N, P and K applied. This is partly due to the exceptionally high price of cassava roots in early 2008. In most years the most economic rate of application is the intermediate level of 80 kg N, 40 P_2O_5 and 80 K_2O/ha.

A similar long-term NPK experiment was initiated in Chamcar Leu district of Kampong Cham province of Cambodia. **Figure 9.3** shows the response in terms of root yield and starch content of two varieties to the application of various levels of N, P and K, as well as the combined application of these three nutrients. The yields and starch contents of the Thai industrial variety KU 50 were significantly higher than those of the local eating variety Damlong Kor. KU 50 responded markedly to N application while Damlong Kor showed only a minor response to N and actually a negative response to the combined application of N, P and K. Neither variety showed a consistent response in terms of starch content. Since plant growth was quite vigorous and yields of the industrial varieties were high, it is clear that the

soil is still quite fertile. It is expected, however, that continuous production of cassava (or any other crop) without fertilizer application will eventually lead to nutrient depletion and declining yields, making fertilizer application not only essential but also highly economic in the long-term.

Table 9.12. Effect of annual applications of various levels of N, P and K fertilizers on the root yield and starch content as well as the gross and net income obtained when two cassava varieties were grown at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08. (18th year)

Treatments[1)]	Root yield (t/ha)		Starch content (%)		Av. root yield (t/ha)	Av. starch content (t/ha)	Gross income[2)]	Fertilizer costs[3)]	Production costs[3)]	Net income
	KM 60	SM 937-26	KM 60	SM 937-26			('000 dong/ha)			
1. $N_0P_0K_0$	6.44	9.90	22.2	24.6	8.17	23.4	9,559	0	4,800	4,759
2. $N_0P_2K_2$	15.08	16.17	24.8	26.5	15.62	25.6	18,275	1,027	6,127	12,148
3. $N_1P_2K_2$	19.61	21.24	23.9	25.6	20.42	24.8	23,891	1,505	6,605	17,286
4. $N_2P_2K_2$	24.58	31.31	23.4	25.6	27.94	24.5	32,690	1,983	7,083	25,607
5. $N_3P_2K_2$	26.13	31.10	24.8	25.7	28.62	25.2	33,485	2,940	8,040	25,445
6. $N_2P_0K_2$	12.79	21.61	23.3	23.7	17.20	23.5	20,124	1,583	6,683	13,441
7. $N_2P_1K_2$	23.82	25.78	23.4	24.6	24.80	24.0	29,016	1,783	6,883	22,133
8. $N_2P_3K_2$	28.59	32.12	24.5	25.8	30.36	25.2	35,521	2,383	7,483	28,038
9. $N_2P_2K_0$	6.67	10.98	22.4	24.0	8.82	23.2	10,319	1,356	6,456	3,863
10. $N_2P_2K_1$	21.14	23.99	23.8	25.3	22.56	24.6	26,395	1,669	6,769	19,626
11. $N_2P_2K_3$	25.03	28.21	25.2	26.7	26.62	26.0	31,145	2,609	7,709	23,436
12. $N_3P_3K_3$	31.68	34.29	24.8	25.6	32.98	25.2	38,587	3,966	9,066	29,521

1) N_0 = 0N P_0 = 0P K_0 = 0K
N_1 = 40 kg N/ha P_1 = 20 kg P_2O_5/ha K_1 = 40 kg K_2O/ha
N_2 = 80 kg N/ha P_2 = 40 kg P_2O_5/ha K_2 = 80 kg K_2O/ha
N_3 = 160 kg N/ha P_3 = 80 kg P_2O_5/ha K_3 = 160 kg K_2O/ha

2) Prices: cassava: VND 1,170/kg

3) *Costs:urea (46% N)* *5,500/kg*
SSP (17% P_2O_5) *1,700/kg*
KCl (60% K_2O) *4,700/kg*
land preparation *900,000/ha*
planting material *1,000,000/ha*
planting *700,000/ha*
weeding *2,200,000/ha*
fertilizer application 300,000/ha
labor 45,000/manday

Long-term soil improvement experiments

An experiment was started at Hung Loc Agric. Center in 1992 to study the effect of fertilizers and various green manures on the long-term fertility maintenance and yields as a result of continuous cassava cropping. In 2008 the 16th crop was harvested. Chemical fertilizers were applied to all plots during the first 7 years, were not applied during the 8th to 10th year and plots were split, with and without fertilizers, from the 11th year onward. Main plots had peanut or cowpea intercrops; *Mucuna, Crotalaria juncea* and pigeon pea intercropped green manures; and *Leucaena leucocephala* and *Gliricidia sepium* leguminous tree hedgerows in alley cropping systems.

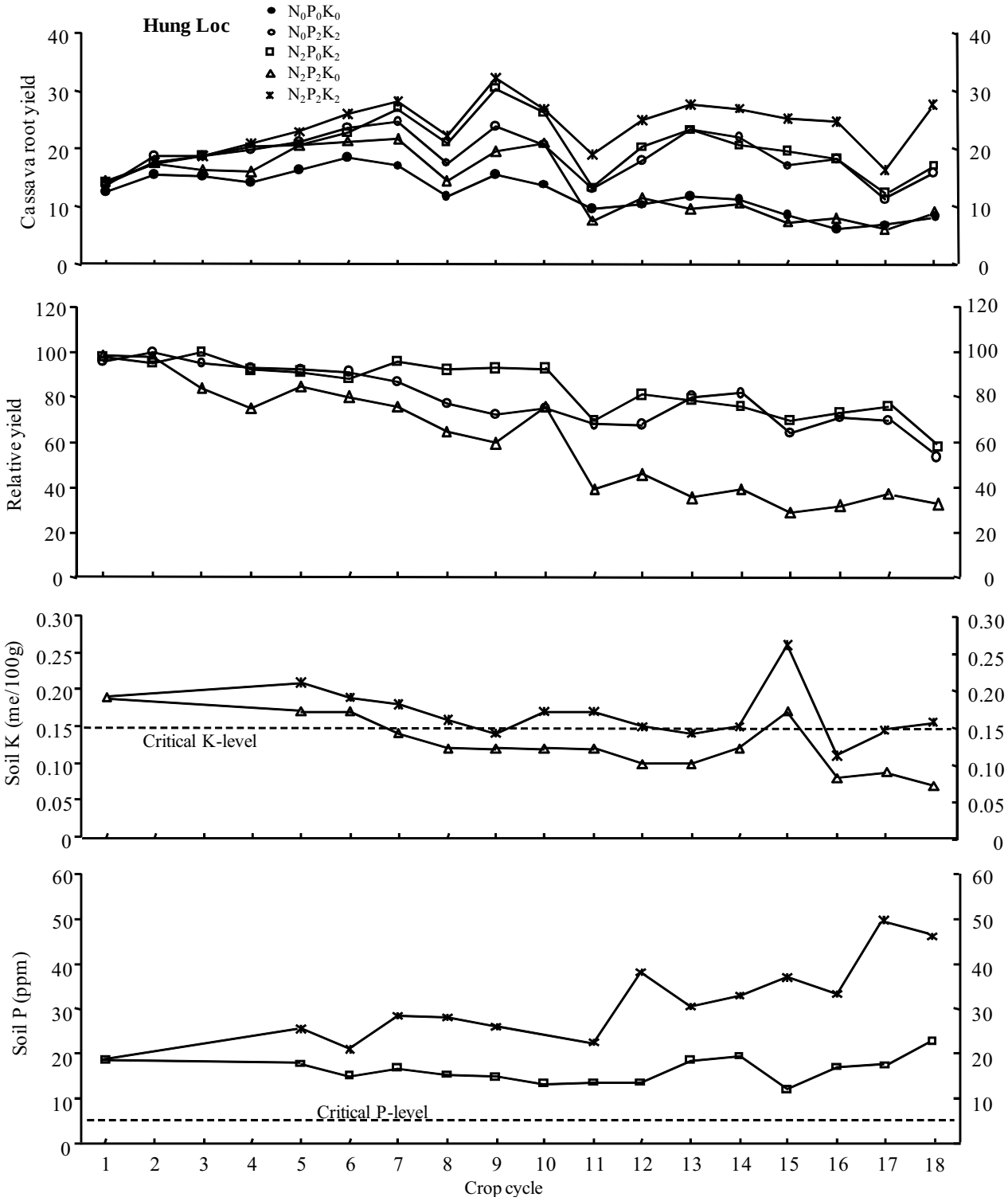


Figure 9.2. Effect of annual applications of N, P and K on cassava root yield, relative yield (yield without the nutrientover the highest yield with the nutrient) and the exchangeable K and available P (Bray 2) content of the soil during eighteen years of continuous cropping in Hung Loc Agriculture Research Center in South Vietnam.

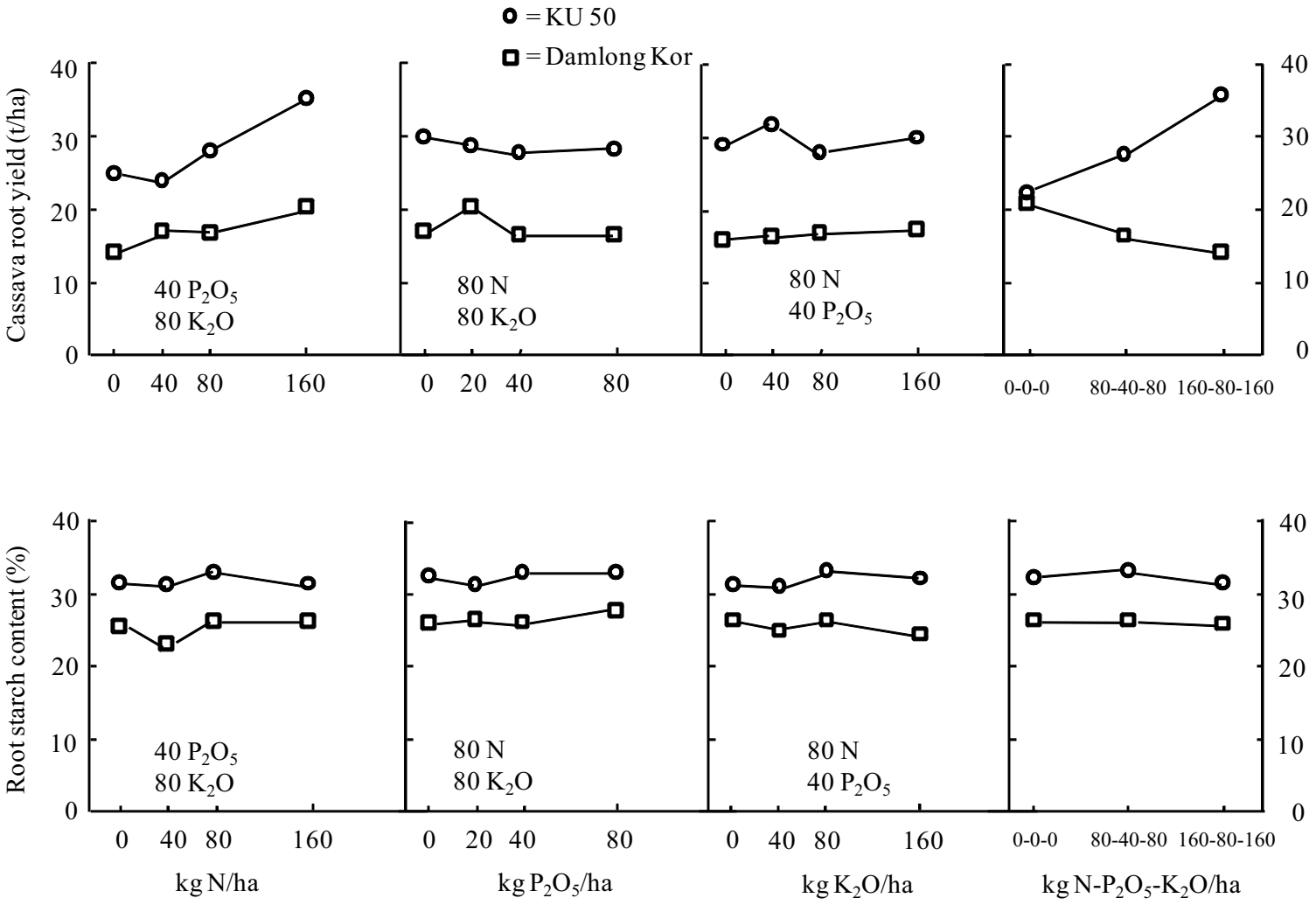


Figure 9.3. Effect of the application of various levels fo N, P and K on the root yield and starch content of two cassava varieties grown at the Chamcar Leu Seed Farm in Kampong Cham province of Cambodia in 2008/09 (1st year).

Table 9.13 shows the results of soil analyses before the 3rd year as well as before the 16th year of planting. Cassava cultivation without fertilizers reduced soil pH, soil OM, exchangeable Ca, Mg and K, while it markedly increased the soil P content, exchangeable Al, and the Al-saturation. NPK fertilizer application generally increased the soil P and Ca contents, but had little effect on the Mg and K contents. Intercropping with grain legumes or green manures had little effect on the soil chemical characteristics, but mulching the prunings of the tree legumes, especially those of *Leucaena,* increased the OM, P, Ca, Mg and especially the K content of the soil, probably by recycling those nutrients from deeper soil layers to the top soil. **Table 9.14** shows that these two alley cropping treatments also resulted in significantly higher cassava yields and starch contents, both with and without fertilizers, and produced the highest net incomes. Intercropping with peanut also resulted in a high cassava yield and net income, but only in plots that received fertilizers.

Table 9.13. Effect of planting intercrops, green manures and alley crops, with or without fertilizers, on soilfertility characteristics after 15 years of continuous cassava cultivation at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08. (before 16th year planting)

Treatments[1)]	pH		OM(%)		P(ppm)		Al(me/100g)		Ca(me/100g)		Mg(me/100g)		K(me/100g)		Al(%)	
	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert
3rd year (1994)	4.4		3.1		9.4		1.00		1.66		0.57		0.32		28	
16th year (2007)																
1. C monoculture[1)]	4.11	4.38	2.24	2.35	15.73	13.96	2.80	2.91	0.63	0.64	0.16	0.15	0.16	0.13	63	76
2. C+pigeon pea GM	4.09	4.43	2.46	2.52	14.53	16.31	2.96	2.81	0.58	0.63	0.15	0.14	0.13	0.15	77	75
3. C+*Mucuna* GM	4.12	4.34	2.35	2.46	14.33	13.08	2.81	2.76	0.72	0.66	0.22	0.17	0.14	0.10	72	75
4. C+peanut IC[4)]	4.06	4.35	2.48	2.59	18.86	26.39	3.07	2.86	0.55	0.71	0.16	0.14	0.13	0.12	79	75
5. C+cowpea IC	4.11	4.28	2.36	2.07	17.70	19.23	2.91	2.70	0.49	0.67	0.17	0.14	0.22	0.13	77	74
6. C+*Crotalaria* GM	4.14	4.30	2.44	2.56	15.00	16.26	2.81	2.76	0.66	0.62	0.15	0.16	0.16	0.13	74	75
7. C+*Leucaena* AC	3.97	4.21	2.82	3.08	18.26	28.82	2.86	2.55	0.76	0.82	0.25	0.26	0.23	0.18	70	67
8. C+*Gliricidia* AC	3.98	4.20	2.51	2.62	15.33	21.77	2.86	2.76	0.78	0.63	0.25	0.17	0.22	0.14	70	75
Average	**4.07**	**4.31**	**2.46**	**2.53**	**16.22**	**19.47**	**2.74**	**2.76**	**0.65**	**0.67**	**0.19**	**0.17**	**0.17**	**0.14**	**73**	**74**

1) Cassava variety is KM 60; -F = without fertilizers; +F = with 80 kg N, 40 P_2O_5, 80 K_2O/ha
GM = GREEN MANURE, IC = INTERCROP, AC = ALLEY CROP

Erosion control experiments

In 1997 a long-term erosion control experiment was planted on about 12% slope at Hung Loc Agric. Research Center, comparing the effectiveness of intercropping with peanut and mung bean with contour hedgerows of vetiver grass or the tree legumes *Leucaena leucocephala* or *Gliricidia sepium* in reducing soil loss by erosion and maintaining soil fertility. **Table 9.15** shows the soil fertility status of the soil before the first planting in 1997 and before the 11st planting in 2007. Annual application of fertilizers (80 kg N, 40 P_2O_5 and 80 K_2O/ha) did not change the soil pH, OM and K content, but markedly increased the P content and exchangeable Al, while decreasing the Ca and Mg contents. Soil erosion, especially in the check plot of monoculture cassava, markedly decreased the sand and increased the silt and clay contents of the soil. **Table 9.16** shows the results of the 11th consecutive year of

cropping. As in previous years, hedgerows of vetiver grass were the most effective in reducing erosion, followed by those of tree legumes, while intercropping with mungbean or peanut was considerably less effective. Vetiver grass hedgerows reduced erosion to about 30% of the check plot. **Figure 9.4** shows that all contour hedgerows became more effective over time and that vetiver grass was consistently more effective in reducing erosion than *Leucaena* or *Gliricidia*. Vetiver grass also tended to increase yields slightly more than the two tree legumes. Intercropping with peanut, however, resulted in the highest cassava yields and net income (**Table 9.16**).

Table 9.14. Effect of planting intercrops, green manures and alley crops, with or without fertilizers, on cassavaand intercrop yields, as well as the gross and net income obtained when cassava, KM 60, was grown for the 16th consecutive year at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08.

Treatments[1]	Root yield (t/ha)		Starch content (%)		Gross income[2] ('000 d/ha)		Product. costs[3] ('000 d/ha)		Net income ('000 d/ha)	
	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert
1. C monoculture	17.44	4.81	23.28	21.28	0,405	5,628	6,008	3,800	14,397	1,828
2. C+pigeon pea GM	15.62	6.75	23.60	21.70	3,275	7,898	8,108	5,900	10,167	1,998
3. C+*Mucuna* GM	17.82	8.56	24.45	22.35	0,849	10,015	8,108	5,900	12,741	4,115
4. C+peanut IC[4]	20.41	8.62	25.35	24.08	4,824	10,085	8,108	5,900	16,716	4,185
5. C+cowpea IC	19.44	7.44	24.92	22.65	2,745	8,705	8,108	5,900	14,637	2,805
6. C+*Crotalaria* GM	18.75	8.50	24.95	21.72	1,938	9,945	8,108	5,900	13,830	4,045
7. C+*Leucaena* AC	20.68	13.39	25.52	24.40	4,196	15,666	7,708	5,500	16,488	10,166
8. C+*Gliricidia* AC	19.30	16.75	26.32	24.95	2,581	19,597	7,708	5,500	14,873	14,097
Average	**18.68**	**9.35**	**24.80**	**22.89**	**1,977**	**10,942**	**7,745**	**5,538**	**14,231**	**5,404**

[1] C = cassava, GM = green manure, IC = intercrop, AC = alley crop

[2] Prices: cassava dong 1,170/kg fresh roots
Peanut: 8,000/kg dry pods

[3] Costs:

land preparation	900,000/ha
cassava planting	700,000/ha
weeding	2,200,000/ha
fertilizers (80:40:80 kg/ha)	1,983,000/ha
- urea (46% N)	5,500/kg
- SSP (17% P_2O_5)	1,700/kg
- KCl (60% K_2O)	4,700/kg
intercrop planting	500,000/ha
cost of labor	45,000/manday
fertilizer application (5 mdays/ha)	225,000/ha
intercrop harvest and cutting	*1,200,000/ha*
seed of intercrops or GM	*400,000/ha*

[4] peanut yield +fertilizers 118 kg dry peanut pods/ha; -fertilizers 0 peanut yield

[5] 1 US$ = 17,000 dong in 2008

Table 9.15. Effect of cropping systems and the planting of contour hedgerows on soil fertility characteristics after 10 years of continuous cassava cultivation on 12% slope at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08. (before 11th year planting)

Treatments[1)]		(%)	(ppm)	—— (me/100g)——				(%)	————(ppm————					——(%)——			
	pH	OM	P	Al	Ca	Mg	K	Al	B	Zn	Mn	Cu	Fe	sand	silt	clay	texture
1st year (1997)	4.80	3.50	8.03	0.62	2.70	1.20	0.14	13	0.34	1.30	159.7	0.74	17.8	24.3	12.7	63.0	clay
11th year (2007)																	
1. C monoculture, no hedg.	4.71	3.14	26.06	1.77	1.87	0.50	0.15	41	0.72	2.77	151.1	0.89	20.7	7.2	22.2	70.6	clay
2. C+mungbean intercrop	4.50	3.22	35.37	2.34	1.49	0.31	0.22	54									
3. C+peanut intercrop	4.60	3.16	30.63	2.18	1.40	0.38	0.17	53									
4. C+vetiver hedgerows	4.70	3.34	27.51	1.98	1.89	0.47	0.15	44									
5. C+*Leucaena* hedgerows	4.72	3.49	36.27	1.72	2.28	0.54	0.17	37									
6. C+*Gliricidia* hedgerows	4.63	3.34	33.49	1.77	2.18	0.53	0.18	38	0.89	2.83	136.1	0.86	20.8	5.7	18.4	75.9	clay

Table 9.16. Effect of cropping systems and the planting of contour hedgerows on the yield of cassava andintercrops, on dry soil loss by erosion, and on gross and net income during the 11th consecutive year ofcropping on 12% slope at Hung Loc Agric. Research Center in Thong Nhat district, Dongnai, Vietnamin 2007/08.

Treatments[1)]	Dry soil loss (t/ha)	Root yield (t/ha)	Starch content (t/ha)	Hedgerow yield (%)	Gross income[2)]	Product. cost[3)]	Net income
					————('000 d/ha)———		
1. C monoculture, no hedgerows	33.56	27.06	27.90	-	31,660	6,008	25,652
2. C+mungbean IC	28.84	32.60	28.03	2.19	38,142	8,108	30,034
3. C+peanut IC[4)]	22.46	34.58	29.43	3.76	41,595	8,108	33,487
4. C+vetiver hedgerows	10.03	30.45	28.73	10.10	35,626	7,008	28,618
5. C+*Leucaena* AC	16.50	30.09	30.00	10.21	35,205	7,008	28,197
6. C+*Gliricidia* AC	18.11	29.58	28.18	8.45	34,609	7,008	27,601

1) C = cassava; IC = intercrop; AC = alley crop

2) Prices: cassava dong 1,170/kg fresh roots
Peanut 8,000/kg dry pods

3) Costs:

land preparation	900,000/ha
planting cassava	700,000/ha
planting intercrops	500,000/ha
seed intercrops	400,000/ha
weeding	2,200,000/ha
harvest or cutting of intercrops	1,200,000/ha
fertilizers (90:40:80 kg/ha)	1,983,000/ha
fertilizer application	225,000/ha

4) peanut yield: 142 kg dry pods/ha = dong 1,136,000

Another erosion control experiment was planted on about 10-12% slope at the Cattle Bank in Xieng Khouang province of Lao PDR in 2007. **Table 9.17** shows the treatments and the dry soil loss by erosion during the first year of cropping. Erosion could be markedly reduced by planting contour hedgerows of *Paspalum atratum, Tephrosia candida* or vetiver grass, while contour ridging, closer plant spacing and intercropping with peanut were also highly effective. Up-and-down ridging, however, greatly increased soil losses by erosion. The trial will be harvested in March 2009, so no cassava yield data are yet available.

Method of planting and plant spacing experiment

A plant spacing times method-of-planting experiment was planted at the Chamcar Leu Seed Farm in Kampong Cham province of Cambodia in May 2008 and harvested in March 2009 at 9 MAP. Growth of KU 50 was extremely vigorous and yields ranged from 42 to 58 t/ha (**Table 9.18**). Planting at the closer spacing of 0.8 x 0.8 m produced higher yields than the wider spacing of 1.0 x 1.0 m, even though the latter is usually recommended for high-fertility soils. Planting one stake per hill, there were no significant differences between planting vertically, inclined or horizontally. Vertical planting, however, tends to result in more rapid germination and canopy closure, thus reducing weed competition and erosion, but this method also leads to roots going deeper, making the root harvest more difficult. But if farmers were to use some simple but very effective harvesting tools, developed by Thai farmers, this may not be a problem. Planting two stakes per hill, as many farmers in Cambodia do, resulted in significantly lower yields due to interplant competition.

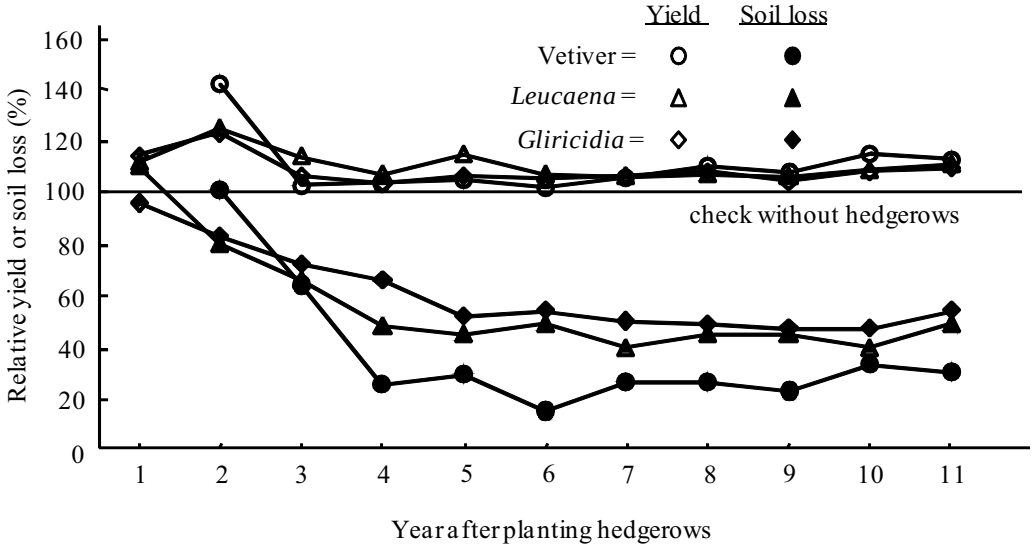


Figure 9.4 Trend in relative yield and relative soil loss by erosion when cassava was planted with contour hedgerows of vetiver grass, *Leucaena leucocephala* or *Gliricidia sepium* in comparison with the check without hedgerows during then consecutive years in Hung Loc Agric. Research Center in South Vietnam from 1997/98 to 2008/09

Table 9.17. Effect of various soil erosion control practices on dry soil loss by erosion in the erosion control trial at the LESC in Xieng Khouang province in 2007/08.

Treatments	Dry soil loss (t/ha)
Traditional practice: no fertilizer or lime; no hedgerows; 2 stakes/hill; no ridging; 0.9 m x 0.9 m plant spacing	16.8
No ridging; with fertilizers and lime; no hedgerows; 1 stake/hill; 0.9 x 0.9 m plant spacing	11.0
Intercropped with 2 rows of peanut; with fertilizers and lime; no hedgerows; 1 stake/hill; 0.9 x 0.9 m plant spacing	8.5
Hedgerows of pineapple; with fertilizers and lime; 1 stake/hill; 0.9 x 0.9 m plant spacing	10.0
Hedgerows of *Paspalum atratum*; with fertilizers and lime; 1 stake/hill; 0.9 x 0.9 m plant spacing	6.6
Hedgerows of *Tephrosia candida*; with fertilizers and lime; 1 stake/hill; 0.9 x 0.9 m plant spacing	7.4
Hedgerows of vetiver grass (Vietnam); with fertilizers and lime; 1 stake/hill; 0.9 x 0.9 m plant spacing	8.0
Closer plant spacing (0.7 x 0.7 m); with fertilizers and lime; 1 stake/hill; no hedgerows	8.4
Contour ridging; with fertilizers and lime; 1 stake/hill; no hedgerows; 0.9 x 0.9 m plant spacing	8.1
Up-down ridging; with fertilizers and lime; 1 stake/hill; no hedgerows; 0.9 x 0.9 m plant spacing	30.0

9.5 On-farm and Farmer Participatory Research (FPR)

The objectives and methodologies used in these trials have been described in the 2004 and 2005 Annual Reports of IP-3. Both on-farm and FPR trials are conducted on farmers' fields, but in on-farm trials the experiment is designed and managed mainly by researchers, while in FPR trials farmers decide on the types of trials to be conducted and the treatments to be tested; in this case the farmers are the "owners" of the trials and they discuss and select the best treatments. In both cases researchers or extension workers help the farmers set out and plant the trials and measure the yields obtained in each treatment.

Table 9.18. The effect of method-of-planting cassava stakes and plant spacing on the root yield of KM 94 planted at the Chamcar Leu Seed Farm in Kampong Cham province of Cambodia in 2008/09.

Method of planting	Cassava root yield (t/ha)		
	A[1)]	B[1)]	**Average**
1. Vertical, 1 stake per hill	49.92	57.73	**53.82**
2. Horizontal, 1 stake per hill	49.58	58.16	**53.87**
3. Inclined, 1 stake per hill	53.65	54.92	**54.28**
4. Inclined, 2 stakes per hill	43.03	42.83	**42.92**
Average	**49.04**	**53.41**	**51.22**

[1)] A = plant spacing of 1.0 x 1.0 m
B = plant spacing of 0.8 x 0.8 m

On-farm and FPR trials in Lao PDR

On-farm and FPR trials were initially conducted in 1-2 districts of Oudomxay, Luang Prabang and Xieng Khouang provinces, but have now expanded to additional districts and to two new provinces, Houa Phanh province and Vientiane municipality.

Oudomxay province

In Houn, La and Nga districts of Oudomxay province, several FPR trials were planted in May and June 2008. These FPR trials were mostly located between 600 and 1,000 masl. Most cassava varieties grow vigorously when planted in fertile soil at that elevation and at the relatively high latitude of about 20° north. Soil fertility was quite variable but nearly all soils had a clay texture, a low-medium pH, were high in Ca, Mg, and K (with some exceptions) and were low-medium in P.

In June 2007, five FPR trials were planted in Phou Lath village and two trials in Chanh Vang village, both in Houn district; and four trials in Houay Xay village in La district. Seven cassava varieties, i.e. Nep, Ba Trang, Vinh Phu, NARC 61, Rayong 2, Hanatee and the local variety, were planted in FPR trials for evaluation of those varieties for eating purposes. In the strip-cropping trials, farmers planted cassava (KU 50) with contour strips of pineapple, *Stylosanthes* and Thai ginger. In the intercropping trials farmers planted Rayong 72 or KU 50 intercropped with peanut, yard-long bean and cowpea. In these trials cassava was not harvested in March 2008 because the farmers preferred to harvest these trials in early 2009. One of the reasons is that farmers saw the very high yields and starch content of 2-year old cassava plants that were harvested last year.

Phou Lath village and Chanh Vang village in Houn district

Phou Lath village is located at 630 masl in the mountains about 30 km from Houn town, capital of Houn district. Chanh Vang village is located at about 900 masl and on the way from Houn to Phou Lath village.

In Phou Lath village several cassava variety trials have been planted by farmers in 2005 and 2006. In addition, an FPR variety trial was planted in May, 2007 and will be harvested in March 2009 when plants will be nearly two years old. The trials have two eating varieties (Rayong 2 and Hanatee) and five industrial varieties (Rayong 5, Rayong 60, Rayong 72, Rayong 90 and KU 50) from Thailand. Farmers are now planning to expand the planting of these varieties on their larger fields.

In the FPR trials in both villages cassava was growing very vigorously, especially the varieties Rayong 72 and KU 50. Farmers are expecting that the yields of the introduced varieties will be significantly higher than their existing local varieties. These varieties can be fed to their animals or sold to traders after making dry chips. Because of the farmer training and field days, many farmers now understand that all introduced varieties can be fed to the animals, but only after sun-drying or ensiling, or can be fed fresh in small amounts. Farmers are now starting to use these feed preparation techniques.

The grain legumes in the intercropping trials have been harvested in Houn district and the yields in Phou Lath village are shown in **Table 9.19**. Many farmers in this district are interested in growing intercrops with cassava rather than grow cassava alone. These farmers like to intercrop cassava with cowpea and yard-long bean rather than peanut.

Table 9.19. Results of an FPR cassava and legume intercropping trial conducted by a farmer in Phou Lath village, Houn district of Oudomxay province in Lao PDR in 2007/09 (legume yields only).

Varieties	Legume yield (kg/ha)	Harvest date	Farmers' preference ranking
Cowpea	83,900[1)]	1 Aug, 9 Aug, 14 Aug	1
Peanut	1,360	15 Sept	3
Yard-long bean	9,660[1)]	1 Aug, 9 Aug, 14 Aug	2

[1)] fresh weight

Houay Xay village in La district

The village is located at 869 masl in the mountains at 20º northern latitude. In early 2007, an FPR cassava variety trial was planted for eating purposes with seven varieties: Nep, Ba Trang, Vinh Phu, NARC 61, Rayong 2, Hanatee, and the local variety. All cassava varieties are growing very well because of high soil fertility and good crop management. These trials will be harvested in March 2009, and a farmers' field day will be organized to evaluate these eating varieties.

In May 2007 cassava intercropping trials with grain legumes were planted. Farmers selected Rayong 72 and KU 50 for cassava, and peanut, yard-long bean and cowpea for the intercrops. The grain legumes in these FPR trials have been harvested and the yields are shown in **Table 9.20**. At harvest of the legume intercrops a field day was held for farmers to evaluate these crops. Farmers from nearby villages participating in the field day were surprised to see the high yields of yard-long bean and cowpea. The local farmers are now

interested in growing cassava with legume intercrops. It is clear that many minority people would like to eat cowpea and yard-long beans as a vegetable.

Table 9.20. Results of an FPR cassava and legume intercropping trial conducted by a farmer[1)] in Houay Xa village, La district of Oudomxay province in Lao PDR in 2007/09 (legume yields only).

Intercrops	Legume yield (kg/ha)	Harvest date[2)]	Farmers' preference ranking
Cowpea	99,100[2)]	30 July, 2 Aug, 7 Aug	1
Peanut	895	15 Sept	3
Yard-long bean	93,100[2)]	30 July, 2 Aug, 7 Aug	2

[1)] farmer: Mr Loy; [2)] fresh weight

Luang Prabang province
Many farmers in the three target districts of Luang Prabang province have been planting new cassava varieties, particularly KU 50, Rayong 72 and Rayong 90, since 2006. Some of the district agriculture and forestry office (DAFO) staff in these three districts have grown the three preferred Thai varieties on their own farms, and are now selling the planting material and are helping in the dissemination of these high-yielding varieties. In 2008, six progressive farmers in these districts have planted KU 50 and Rayong 72 in large areas to get large quantities of roots, and to speed up the multiplication of planting material. But farmers generally grow small amounts of their local eating variety for their own consumption.

Next year, planting material of the promising eating varieties from the on-station trials and FPR trials from Oudomxay province will be provided to farmers to test in Luang Prabang province. These eating varieties may be included in their on-farm FPR trials.

Xieng Khouang province
First clonal evaluation of highland varieties and planting of Preliminary Yield Trial in Nonghet district of Xieng Khouang

The highland breeding lines (grown from sexual seeds) that had been planted at Naphok Agric. Research Center were harvested and stems were collected to conduct the first clonal evaluation in March 2007. Planting materials of these 290 highland lines were transported to Nonghet district in Xieng Khouang province. With the help of the second group of three Zamorano interns and DAFO staff of Nonghet, these had been planted in March 2007 on a farmer's field in Thumphong village. The area is located at the latitude of 19º north and at 1,318 m above sea level.

This Single-Row Trial had been well maintained by the farmer, and by two DAFO staff who had previously attended the cassava production and utilization training course in Xieng Khouang province in 2006. The plants were harvested and evaluated with the third group of

two Zamorano interns in March 2008. The 34 best lines were selected from this Single-Row Trial. These lines, plus KU 50, Rayong 72 and the local variety, were replanted in a Preliminary Yield Trial in a similar high altitude location in the same village in March 2008. From field observation it seems that some lines are better than KU 50 and Rayong 72 in terms of canopy formation, plant growth and plant type. This trial has three replications and will be harvested and evaluated in March 2009. The selected varieties will be further evaluated in 2009 in three or four high altitude locations in Regional Evaluation Trials before further testing in on-station and FPR trials. The best varieties considered for release, will be selected from on-station, and on-farm and FPR trials.

FPR trials in Khoune and Paek districts of Xieng Khouang
At the request of farmers and DAFO staff from Khoune and Paek districts, new planting material of KU 50, Rayong 72, Rayong 90, Rayong 5 and Hanatee were distributed to Namlane and Sone Khing villages in Khoune district, and in Hat Gne, Khang Done, Phonethong and Phone villages in Paek district.

Khoune district
Cassava plants in Khoune district were growing well and much better than those planted in Phou Khout district in previous years. One of the main reasons is that soils in Khoune district are more fertile than soils in Phou Khout district which is located on very acid and infertile soils in the Plain of Jars. Also day and night temperatures and humidity in Koune district, especially in winter season, seem to be higher than in Phou Khout district. Farmers decided that their trials should be harvested in early 2009.

Paek district
In Paek district soils are also generally better than in Phou Khout district. Farmers in Paek district planted the Thai cassava varieties Hanatee, Rayong 5, Rayong 60, Rayong 72, Rayong 90 and KU 50. Most of the cassava plants showed severe P, Mg and Zn deficiencies in the early stages of plant growth, but at 4-5 months after planting these nutrient deficiency symptoms had disappeared.

Since 2006 the cassava project has also introduced some Thai and Vietnamese varieties to Na Larm village in Paek district. Farmers were very impressed with the introduced varieties. Some farmers have already adopted KU 50 and Rayong 72 from Thailand and KM 140 and KM 98-1 varieties from Vietnam. Farmers have expanded these varieties themselves within a short period. Farmers in Na Larm have also provided planting materials of KU 50, Rayong 5 and Rayong 72 for testing in FPR trials in Houaphan province. In addition, the cassava project has distributed cassava harvesting tools to farmers. Farmers are now using the cassava harvesting tools which can save 2 to 4 times the farmers' labor as compared to harvesting by hoe.

Vientiane municipality

On-farm trials in Xaythany district of Vientiane municipality

At the request of the local DAFO director and farmers, three on-farm FPR trials were planted in Xaythany district, Vientiane municipality in August and September 2007. Farmers wanted to test high yielding and high starch cassava varieties for sale to the Indo-China

cassava starch factory in this district. The trial was planted at an unusual time, towards the end of the wet season. But, cassava is generally considered an easy crop to grow as long as the planting material is good and enough soil moisture is present at time of planting. Two variety trails were conducted at Thading Deng village, and one trial at Ban Bor Lex village. Cassava plants were growing well in these three trials at the beginning of the growth cycle. Unfortunately, the two trials at Thading Deng village could not be harvested due to animal damage in April. The FPR trial located at Ban Bor Lex village was harvested and the results are shown in **Table 9.21**. The trial was located at 18° north latitude and at 162 meters above sea level; the soil texture was sandy clay loam.

Among five tested cassava varieties, the Thai variety KU 50 had both the highest root yield and starch content, while the eating varieties (NARC 61 and Nep) had low yields and intermediate starch contents. KU 50 produced a high root yield of 26.88 t/ha and a starch yield of 7.17 t/ha within 11 months. Famers were very pleased with these results and more farmers are now interested in planting cassava on their fields.

Table 9.21. Results of the FPR variety trial conducted at Ban Bor Lex village, Xaythany district of Vientiane municipality in 2007/08.

Variety	Origin	No of plants harvested in 48 m^2	Root yield (t/ha)	Starch content (%)	Starch yield (t/ha)
KU 50	Thailand	46	26.88	26.69	7.17
KM 140	Vietnam	45	17.92	23.98	4.30
Rayong 72	Thailand	38	16.67	23.98	4.00
NARC 61	Laos	40	9.13	25.06	2.29
Nep	Vietnam	39	8.13	25.60	2.08

Farmer name: Mr Vi; planted Aug 24, 2007; harvested July 31, 2008.

On-farm and FPR trials in Cambodia

On-farm and FPR trials by CARDI in 2007/08

Twenty eight on-farm trials were planted in May-July of 2007 and harvested in March-April of 2008. These trials were designed and planted by CARDI staff, but were managed mainly by collaborating farmers under supervision of provincial or district agricultural extension staff. They were conducted in ten villages in six districts of four provinces, mainly in Kampong Cham and Battambang. The trials studied the effect of varieties, fertilization with chemical fertilizers and manures, and methods of planting. Most trials had no replications, but each farmer in the district was considered a replication as most trials had the same treatments throughout the district.

Table 9.22 shows the overall results of the 11 on-farm variety trials conducted in three provinces in 2007/08. Unfortunately, the germination of many varieties was low or very low, especially in Kampong Cham. This is probably because the planting stems, stored at CARDI after the previous harvests, had been cut into short stakes before transport to the provinces. While this makes the transport of the planting material more efficient and the planting of the trials quicker, it also subjects those short stakes to severe dehydration during transport in an open truck, which in turn effects germination. With the exception of a local narrow-leafed variety (called Damlong Slek Toch or "narrow leaved" Damlong) and KU-50, the germination of most other varieties was greatly reduced in all but one trial in Kampong Cham, and somewhat reduced in most trials in Battambang, resulting in low or no yield. The clearly superior variety was KU 50, which produced high yields in five out of seven trials, with an average yield of 30.6 t/ha; this variety also had the highest starch content of all varieties tested. The ability of this variety to germinate well under sub-optimal conditions or after long stem storage, combined with its high yield and starch content, has made this the most popular cassava variety in Asia, including Cambodia, where it is generally known as "Malaysia" or KM 94.

The agronomic trials on fertilization and planting method were much less affected by poor germination because of the use of KU 50 as the test variety.

Table 9.23 shows the results of six fertilization trials, four conducted in Kampong Cham, and one each in Battambang and Preah Vihear provinces. Yields were reasonably good in Kampong Cham province and very high in Battambang and Preah Vihear provinces. There was no positive response to the application of 10 t/ha of cow manure and only a minor response to the application of a low level of 15 kg/ha each of N, P_2O_5 and K_2O in combination with cow manure. Highest yields were generally obtained with the intermediate level of 45 kg/ha each of N, P_2O_5 and K_2O, while the higher application of 80 kg N, 40 P_2O_5 and 80 kg K_2O/ha was much less effective in increasing yields. Since most soils on which cassava is currently grown are rather fertile, the response to fertilizer application is not very dramatic. However, considering the relatively high yields obtained, and consequently, the high nutrient removal in the harvested roots, yields could well decline in the coming years, unless the right balance and adequate amounts of N, P and K are applied. For that reason it will be useful to start conducting some long-term NPK trials in Kampong Cham and Battambang provinces in order to monitor the effect of continuous cassava production on soil fertility, and to determine the best rates and balance of N, P and K to maintain soil fertility and high cassava yields.

Table 9.24 shows the results of the planting method trials conducted on seven farms in four provinces. Results varied but vertical planting produced the highest yields in Kampong Cham, while inclined planting produced highest yields in Battambang and Preah Vihear provinces. The inclined planting of two stakes per hill consistently produced the lowest yield due to interplant competition. Yields were very low in Kampong Speu due to water logging of the soil.

On-farm and FPR trials conducted by CARDI in 2008/09

In May-June of 2008 CARDI planted eight on-farm trials in Rattanak Mondul district of Battambang province and ten on-farm trials in Tbong Khmon district of Kampong Cham province. The trials were planted in collaboration with the district agricultural extension staff in each location and had basically the same treatments as in 2007/08.

Table 9.22. Results of on-farm cassava variety evaluations conducted in four farmers' fields in Kampong Cham (KC), three in Battambang (BB), and one in Kampong Speu (KS) provinces of Cambodia in 2007/08.

Cassava root yield (t/ha)[1]											
Varieties	KC-1[2]	KC-2	KC-3	KC-4	**Av. KC**	BB-1	BB-2	BB-3	**Av. BB**	KS-1	**Av.**
Damlong Kor		12.50								2.92 [3]	
Damlong Slek Toch	26.66	9.60	7.08	5.30	**12.16**	17.92 [3]	9.17 [3]	40.42	**22.50**		**16.59**
Rayong 5	-		-	-				8.58 [3]		3.33 [3]	
Rayong 60		20.83				18.75 [3]	35.67 [3]	45.25	**33.22**	28.92	**29.88**
Rayong 72							27.17 [3]	15.58 [3]	**21.37**		
Rayong 90		11.66								10.42 [3]	
KU 50	29.16	31.66	7.90	5.83	**18.64**	23.92	63.42	52.50	**46.61**		**30.63**

Cassava starch content (%)											
Varieties	KC-1	KC-2	KC-3	KC-4	**Av. KC**	BB-1	BB-2	BB-3	**Av. BB**	KS-1	**Av.**
Damlong Kor										26.93	
Damlong Slek Toch						19.79	23.45	22.92	**22.05**		**22.05**
Rayong 5								22.39		28.90	
Rayong 60						22.39	22.92	22.39	**22.57**	21.34	**22.26**
Rayong 72							23.45	23.99	**23.72**		**23.72**
Rayong 90										23.45	
KU 50						23.99	26.15	26.69	**25.61**		**25.61**

[1] Root yields based on 12 m^2 effective plot size

[2] KC-1= farmer Mung Heng, Srork village, Srork commune, Kampong Seam district, Kampong Cham

KC-2= farmer Chor Choeung, Romoul village, Roang commune, Kampong Seam district, Kampong Cham

KC-3 = farmer Chon Sren, Chroy Changha village, Mong Reav commune, Tbong Khmom district, Kampong Cham

KC-4 = farmer Kong Thon, Chroy Changva village, Mong Reav commune, Tbong Khmom district, Kampong Cham

BB-1 = farmer Rean Kong, Daun Meay village, Sdao commune, Rattanak Mondul district, Battambang

BB-2 = farmer Rath Chamreoun, Daun Meay village, Sdao commune, Rattanak Mondul district, Battambang

BB-3 = farmer Chheng Rith, Prey Ampor village, Andeuk Hep commune, Rattanak Mondul district, Battambang

KS-1 = farmer Lim Sokhom, Trapieng Saray village, Treng Taying commune, Phnom Srouch district, Kampong Speu

[3] Plant population less than 75% in effective plot

Table 9.23. Effect of five six fertilizer treatments on the yield of cassava, KU 50, planted in four on-farm trials in Kampong Cham, and one each in Battambang and Preah Vihear provinces of Cambodia in 2007/08.

Treatments	Cassava root yield (t/ha)							
	KC-1[1)]	KC-2	KC-3	KC-4	**Av. KC**	BB-1	PV-1	**Av.**
1. $N_0P_0K_0$	30.00	28.33	12.50	16.67	**21.88**	40.42	41.25	**28.20**
2. $N_{80}P_{40}K_{80}$	31.67	30.00	14.17	20.42	**24.06**	33.75	65.83	**32.64**
3. $N_{15}P_{15}K_{15}$	30.83	31.67	16.67	21.67	**25.21**	58.33	49.17	**34.72**
4. $N_{45}P_{45}K_{45}$	31.67	31.67	17.92	22.92	**26.04**	56.67	75.42	**39.38**
5. T_3 + cow manure	35.00	33.33	19.17	24.17	**27.92**	-	46.67	**31.67**
6. 10 t/ha cow manure	29.17	20.83	13.75	19.17	**20.73**	-	42.50	**25.08**

[1)] KC-1 = farmer Mung Heng, Srork village, Srork commune, Kampong Seam district, Kampong Cham

KC-2= farmer Chor Choeung, Romoul village, Roang commune, Kampong Seam district, Kampong Cham

KC-3=farmer Chon Sren, Chroy Changha village, Mong Reav commune, Tbong Khmom, Kampong Cham

KC-4=farmer Kong Thon, Chroy Changva village, Mong Reav commune, Tbong Khmom, Kampong Cham

BB-1 = farmer Rean Kong, Daun Meay village, Sdao commune, Rattanak Mondul district, Battambang

BB-2 = farmer Rath Chamreoun, Daun Meay village, Sdao commune, Rattanak Mondul district, Battambang

BB-3= farmer Chheng Rith, Prey Ampor village, Andeuk Hep commune, Rattanak Mondul, Battambang

BB-4=farmer Sok Rem, Prey Ampor village, Andeuk Hep commune, Rattanak Mondul, Battambang

KS-1= farmer Lim Sokhom, Trapieng Saray village, Treng Taying commune, Phnom Srouch, Kampong Speu

PV-1= farmer Chea Cheu, Svay Po village, Romdos commune, Ror Veang district, Preah Vihear

Table 9.24. Results of on-farm trials on planting method of cassava, KU 50, conducted in three farmers fields in Kampong Cham (KC), two in Battambang (BB), and one each in Kampong Speu (KS) and Preah Vihear (PV) provinces of Cambodia in 2007/08.

Treatments	Cassava root yield (t/ha)									
	KC-1[1)]	KC-3	KC-4	**Av. KC**	BB-1	BB-4	**Av. BB**	KS-1	PV-1	**Av.**
1. horizontal	33.33	20.00	21.25	**24.86**	10.00	41.25	**25.62**	6.67	19.58	**21.73**
2. vertical	36.67	19.58	22.50	**26.25**	10.83	38.33	**24.58**	4.58	23.75	**22.32**
3. inclined (1 stake/hill)	31.67	18.83	22.08	**24.19**	20.83	52.50	**36.66**	7.50	32.50	**26.56**
4. inclined (2 stakes/hill)	30.83	16.67	20.67	**22.72**	7.50	42.08	**24.79**	5.00	12.08	**19.26**

[1)] See footnote under **Tables 9.23** and **9.24**.

Table 9.25 shows the results of the six on-farm variety trials conducted in Kampong Cham and Battambang provinces. Unlike in 2007/08, this year most varieties germinated quite well. In both provinces Rayong 60 produced the highest yields, followed by KU 50. Rayong 60 is mainly suitable for chipping and drying for the animal feed industry because of the light yellow color of its root parenchyma, indicating high carotene content.

Table 9.26 shows the results of five on-farm NPK/manure trials conducted by farmers in Kampong Cham and Battambang provinces. Soil fertility and thus the fertilizer response varied markedly among farms, depending mainly on the number of years of previous cropping with cassava without fertilizer application. Some farmers in Kampong Cham have grown cassava in the same fields for up to 20 years without fertilizer application or with application of only urea. They commented that yields had been declining during the past few years. Farmers helping with the harvest of the trials could clearly see the need for fertilizer application even in a soil that used to be quite fertile. Among the various fertilizer treatment, the use of 80 kg N, 40 kg P_2O_5 and 80 kg K_2O/ha produced the highest yields, as it does in many similar soils in Vietnam. It usually also produces the highest net income. The use of 10 t/ha of cow manure alone was clearly not as effective as the chemical fertilizers, but its use in combination with 15-15-15 fertilizers did increase yields on average about 3 t/ha (treatment 5 vs 3)

Table 9.25. Results of on-farm variety trials conducted by three farmers in Tbong Khmom district of Kampong Cham province and by three farmers in Rattanak Mondul district of Battambang province of Cambodia in 2008/09.

Varieties	Cassava root yield (t/ha)								
	KC-1[1]	KC-2	KC-3	**Av. KC**	BB-1[2]	BB-2	BB-3	**Av. BB**	**Av.**
1. KU 50	40.0	21.7	27.5	**29.7**	26.5	15.8	28.7	**23.7**	**26.7**
2. Damlong Slek Toch	35.8	17.5	25.0	**26.1**	14.2	18.3	20.2[5]	**17.6**	**21.8**
3. Rayong 5 (from Laos)	20.8	18.3	32.5	**23.9**	33.2	28.2	21.7	**27.7**	**25.8**
4. Rayong 60	60.0	25.8	39.2	**41.7**	56.6	7.0[3]	28.2	**30.6**	**36.1**
5. Rayong 72	-	17.5	31.7	**24.6**	32.8	11.7[4]	20.8[5]	**21.8**	**22.9**

[1] KC-1 = Mr. Matt Ya, Chrouy Kor village, Chi Rour 2 commune, Tbong Khmom, Kampong Cham
KC-2 = Mr. Yeay That, Kean Roung village, Vihaer Loung commune, Tbong Khmom, Kampong Cham
KC-3 = Mr. Ta Chhun, Kean Roung village, Vihaer Loung commune, Tbong Khmom, Kampong Cham

[2] BB-1 = Mr. Heun Chek, Doun Meay village, Sdao commune, Rattanak Mondul, Battambang
BB-2 = Mr. Heun Chek, Doun Meay village, Sdao commune, Rattanak Mondul, Battambang
BB-3 = Mr. Tep Outdom, Prey Arm Por village, Andeuk Heap commune, Rattanak Mondul, Battambang

[3] very low yield due to water logging and root rots

[4] low yield due to water logging

[5] low yield due to competition from *Imperata* grass

Table 9.26. Results of on-farm fertilizer/manure trials conducted by three farmers in Tbong Khmomdistrict of Kampong Cham province and by two farmers in Rattanak Mondul district ofBattambang province of Cambodia in 2008/09.

Fertilizer treatments	Cassava root yield (t/ha)							
N-P_2O_5-K_2O (kg/ha)	KC-4[1)]	KC-5	KC-6	**Av. KC**	BB-4[1)]	BB-5	**Av. BB**	**Av.**
1. 0-0-0	11.2	8.9	22.5	**14.2**	31.8	15.0	**23.4**	**17.9**
2. 80-40-80	35.4	34.4	28.3	**32.7**	35.0	30.6	**32.8**	**32.7**
3. 15-15-15	20.0	31.7	27.5	**26.4**	28.0	24.9	**26.4**	**26.4**
4. 45-45-45	24.2	35.2	28.3	**29.2**	31.6	17.9	**24.8**	**27.4**
5. 15-15-15 + 10 t/ha CM	17.5	39.0	34.2	**30.2**	31.2	25.7	**28.4**	**29.5**
6. 10 t/ha cow manure (CM)	17.3	23.0	26.7	**22.3**	26.0	23.2	**24.6**	**23.2**

[1)] KC-4 = Mr. Chea Chrock, Chrouy Kor village, Chi Rour 2 commune, Tbong Khmom, Kampong Cham
KC-5 = Mr. Yen Sengsor, Chrouy Kor village, Chi Rour 2 commune, Tbong Khmom, Kampong Cham
KC-6 = Mr. Leng Sokchea, Kean Roung village, Vihaer Loung commune, Tbong Khmom, Kampong Cham
BB-4 = Mr. Tep Somnith, Prey Arm Por village, Andeuk Heap commune, Rattanak Mondul, Battambang
BB-5 = Mr. Heun Chek, Doun Meay village, Sdao commune, Rattanak Mondul, Battambang

Table 9.27 shows the results of seven on-farm methods-of-planting trials conducted by farmers in Kampong Cham and Battambang provinces. In both provinces planting the stakes vertically produced the highest yields, but unlike the experiment in Chamcar Leu (**Table 9.18**) the traditional method of planting two stakes per hill did not reduce yields as compared to planting inclined with only one stake per hill. Nearly all farmers in Cambodia, China and Vietnam plant cassava stakes horizontally, as this would result in shallow root formation which facilitates the harvest. But if they were to use the simple harvesting tools developed in Thailand, this may not be a problem and yields could increase substantially if they were to change to vertical planting, as is being practiced by farmers in Indonesia, Thailand and India. Conducting simple on-farm or FPR trials with farmers is an effective way of showing farmers the potential benefits of better agronomic practices, and thus enhances adoption and increase yields and farmers' income.

Pig feeding trials by CelAgrid

In 2006 CelAgrid had conducted an FPR pig feeding trial in two villages of Takeo province to test diets based on either ensiled cassava leaves or ensiled cassava leaves and water spinach. In that experiment each farmer was given two piglets and both were fed with either one of the two diets. Since pig management varies considerably among farmers, the effect of differences in diets was partially obscured due to differences in management.

Table 9.27. Results of on-farm planting method trials conducted by four farmers in Tbong Khmom district of Kampong Cham province and by three farmers in Rattanak Mondul district of Battambang province of Cambodia in 2008/09.

Planting methods	Cassava root yield (t/ha)									
	KC-7[1)]	KC-8	KC-9	KC-10	**Av. KC**	BB-6[1)]	BB-7	BB-8	**Av. BB**	**Av.**
1. Vertical, 1 stake per hill	34.2	63.3	26.7	25.0	**37.3**	29.8	29.3	41.7	**33.6**	**35.7**
2. Horizontal, 1 stake per hill	30.8	35.8	25.0	16.7	**27.1**	26.6	10.5[2)]	34.5	**23.9**	**25.7**
3. Inclined, 1 stake per hill	27.1	39.2	20.8	20.8	**27.0**	29.4	24.0	32.3	**28.6**	**27.7**
4. Inclined, 2 stakes per hill	34.0	30.0	22.5	19.2	**26.4**	30.4	29.5	30.3	**30.1**	**28.0**

[1)] KC-7 = Mr. Ya Phalkun, Chrouy Kor village, Chi Rour 2 commune, Tbong Khmom, Kampong Cham
KC-8 = Mr. Matt Ya, Chrouy Kor village, Chi Rour 2 commune, Tbong Khmom, Kampong Cham
KC-9 = Mr. Kan Nang, Kean Roung village, Vihaer Loung commune, Tbong Khmom, Kampong Cham
KC 10 = Mr. Ta Chhun, Kean Roung village, Vihaer Loung commune, Tbong Khmom, Kampong Cham
BB-6 = Mr. Tep Somnith, Prey Arm Por village, Andeuk Heap commune, Rattanak Mondul, Battambang
BB-7 = Mr. Heun Chek, Doun Meay village, Sdao commune, Rattanak Mondul, Battambang
BB-8 = Mr. Tep Outdom, Prey Arm Por village, Andeuk Heap commune, Rattanak Mondul, Battambang
[2)] low yield due to water logging

In 2007 a similar trial was conducted in Chung Ruk village of Kravanh district in Pursat province of central Cambodia. While farmers in this area grow mostly rice, they also raise pigs and grow cassava on slightly higher ground. This time nine farmers each received two piglets. The two piglets of each farmer were fed a different diet, i.e. one of three different diets being tested; in total, six pigs were fed with diet 1, six with diet 2 and six with diet 3. Diet 1 consisted of a traditional diet that farmers normally use to feed pigs; diet 2 consisted of rice bran and ensiled cassava leaf silage, while diet 3 used rice bran with both cassava leaf and root silage. **Table 9.28** shows that the average daily weight gain was highest for diet 3, followed by diet 2, but that the feed conversion ratio was better for diet 2 than 3, due to the much greater feed intake of diet 3. The daily weight gain of pigs fed with diet 3 was more than twice that of pigs fed the traditional diet. An economic analysis of feed costs per kg live weight gain will need to be made to determine which diet results in the highest economic benefit for the farmer.

9.9 TRAINING COURSES AND WORKSHOPS

Two FPR training courses were held in Lao PDR and Cambodia to prepare the provincial and district staff collaborating in the project for the greater use of FPR methodologies in order to increase farmers' involvement and ownership of the trials during the second phase of the project. This will also allow for the rapid increase in the number of FPR trials to be conducted by local staff and farmers at an ever increasing number of pilot sites in order to achieve adoption and significant impact on cassava yields and farmers' income

The training course in Laos was held from March 24 to 29, 2008 at Naphok Agric. Research Center near Vientiane, with participation of 26 persons from various institutions collaborating in the project (**Table 9.29).** The course was taught mainly by two FPR specialists from the Thai Dept. of Agric. Extension, with additional lectures by Lao and CIAT staff.

Table 9.28. Results of an FPR pig feeding trial conducted by nine farmers in Chung Ruk village, Ptas Rong commune, Kravanh district of Pursat province in Cambodia in 2007.

Parameter	Treatment 1 Traditional diet	Treatment 2 RB + CLS[1)]	Treatment 3 RB + CLS + CRS[1)]
No. of pigs	6	6	6
Live weight (kg)			
-initial	14.8	17.9	29.0
-final	27.3	40.3	57.4
Weight gain in 113 days (kg)	12.5	22.4	28.4
Daily weight gain (g/day)	110.6	198.2	251.3
Feed intake (g DM/day)	-	1,701.1	2,281.2
Feed conversion ratio (kg DM/kg weight gain)	-	8.58	9.08

[1)] RP = rice bran; CLS = cassava leaf silage; CRS = cassava root silage

The FPR course in Cambodia was held from June 30 to July 5, 2008 at CARDI headquarters near Phnom Penh, with participation of 32 persons from various Cambodian institutions collaborating in the project. The course was again taught mainly by the (**Table 9.30**). Both courses consisted of 4½ days of lectures and class-room practice, and one day of field practice with farmers in a nearby village.

Collaborators

Within CIAT:

Rod Lefroy, Coordinator of CIAT-Asia, stationed in Vientiane, Lao PDR
Tin Maung Aye, Cassava Project in Asia, stationed in Vientiane, Lao PDR
Keith Fahrney, PRDU Project in Asia, stationed in Vientiane, Lao PDR
Lao Thao, PRDU Project in Asia, stationed in Vientiane, Lao PDR
Hernan Ceballos, Project Manager IP-3, stationed in Cali, Colombia

The institutions and principal individuals collaborating in the Nippon Foundation-funded cassava projects in Lao PDR and Cambodia are shown in **Table 9.5**.

Table 9.29. Participants and resource persons of the FPR Training Course at the Rice and Cash CropsResearch Center in Vientiane, Lao PDR, March 24-29, 2008.

No.	Participants	Office/Institute	Province/Country
1	Mr. Phanthasin Khanthavong	Rice and Cash Crop Research Center	Vientiane Munic.
2	Mr. Saythong Oudthachith	Rice and Cash Crop Research Center	Vientiane Munic.
3	Mr. Soulyphonh Intaphone	Agriculture & Forestry (Koun district)	Vientiane Munic.
4	Mr. Kongvang Nhia Veu	Livestock Research Center	Vientiane Munic.
5	Mr. Khammand Chansingbang	Livestock Extension and System Center	Vientiane Munic.
6	Mr. Sayvisene Boulom	Faculty of Agriculture	Vientiane Munic.
7	Mr. Khamphet Kongthavixay	Agriculture & Forestry Office (Xaythany district)	Vientiane Munic.
8	Ms. Keo Sakhone	Agriculture & Forestry Office (Xieng Ngeun district)	Luang Prabang
9	Ms. Chansouk Chathanon	Agriculture & Forestry Office (Luang Prabang district)	Luang Prabang
10	Ms. Chansamone Beer Lao	Agriculture & Forestry Office (Luang Prabang district)	Luang Prabang
11	Mr. Bouathong Sivongxay	Luang Prabang Agriculture & Forestry College	Luang Prabang
12	Mr. Soulideth Phaphonxay	Agriculture & Forestry	Luang Prabang
13	Mr. Bounxou Xayxana	Luang Prabang Agriculture & Forestry College	Luang Prabang
14	Mr. Maiphout Bavidone	Provincial Agriculture & Forestry Office	Houa Phanh
15	Mr. Siviengxam Phengphomma	Provincial Agriculture & Forestry Office	Houa Phanh
16	Mr. Pisa Keoned	Agriculture & Forestry Office (Long Ngam district)	Saravanh
17	Ms. Songsone	Agriculture Section, PAFO	Saravanh
18	Mr. Keo Anong Siphaseuth	Agriculture & Forestry Office (Pek district)	Xieng Khouang
19	Mr. Amphone Phommavong	Agriculture & Forestry Office (PhaXay district)	Xieng Khouang
20	Mr. Bounpheng Thavsai	Agriculture & Forestry Office (Nonghed district)	Xieng Khouang
21	Mr. Vong Philavong	Agriculture & Forestry Office (Nonghed district)	Xieng Khouang
22	Mr. Aenoy Keophilavanh	Livestock & fisheries section	Luang Namtha
23	Mr. Phouvanh Phontavong	Agriculture and forestry Office (Xay district)	Oudomxay
24	Mr. Sivone Souksitthi	Agriculture and forestry Office (Houn district)	Oudomxay
25	Mr. Bounpheng Thanthongbai	Agriculture and forestry Office (La district)	Oudomxay
26	Ms. Eliana Nuncz Ponce	Luang Prabang Agriculture & Forestry College (from Zamorano University)	Luang Prabang
27	Ms. Paulina Narajo Taco	Luang Prabang Agriculture & Forestry College (from Zamorano University)	Luang Prabang
	Resource persons		
1	Mr. Viengsavanh Phimphachanhvongsod	NAFRI	Vientiane, Lao PDR
2	Mr. Vanthong Phengvichit	NAFRI	Vientiane, Lao PDR
3	Ms. Wilawan Vongkasem	DOAE – Thailand	Bangkok, Thailand
4	Mr. Kaival Klakhaeng	DOAE – Thailand	Bangkok, Thailand
5	Dr. Tin Maung Aye	CIAT	Vientiane, Lao PDR
6	Mr. Lao Thao	CIAT	Vientiane, Lao PDR
7	Dr. Reinhardt Howeler	CIAT	Bangkok, Thailand

Table 9.30. Participants and resource persons of the FPR Training Course in CARDI, Phnom Penh, Cambodia, June 30-July 5, 2008.

	Participants Name	Office/Institute	Province/Country
1.	Mr. Sim Socheat	Farming System, CARDI	Phnom Penh
2.	Ms. Tith Jannin	Farming System, CARDI	Phnom Penh
3.	Mr. Sok Sopheaprith	Soil and water, CARDI	Phnom Penh
4.	Mr. Orn Chhourn	Blant Breeding, CARDI	Phnom Penh
5.	Mr. Phin Phal	Blant Breeding, CARDI	Phnom Penh
6.	Mr. Huon Serey Vuth	Agricultural Engeering, CARDI	Phnom Penh
7.	Mrs. El Sotheary	Socio-Economic, CARDI	Phnom Penh
8.	Ms. Srey Sinath	Socio-Economic, CARDI	Phnom Penh
9.	Mr. Lim Saluot	Koh Ke Station, CARDI	Preah Vihear
10.	Mr. Khim Sam El	Koh Ke Station, CARDI	Preah Vihear
11.	Mr. Pen Pichpounnareay	Agricultural Department	Preah Vihear
12.	Mr. Leang Vuthy	Chamcar Leu Agricultural District	Kampong Cham
13.	Mr. Soth Chamreun	Tbong Khmom Agricultural District	Kampong Cham
14.	Mr. Mean Samay	Agricultural Department	Kampong Cham
15.	Mr. Kou Nakry	DAALI	Kampong Cham
16.	Mr. Choun Sovann	Ratanak Mondul Agricultural District	Battambang
17.	Mr. Nou Pra Neth	Ratanak Mondul Agricultural District	Battambang
18.	Mr. Chea Chheang Ly	Centre for Livestock & Agriculture Dev.	Kandal
19.	Mr. Pann Khemrin	Agricultural Department	Kandal
20.	Mr. Nauv Enghay	Agricultural Department	Kandal
21.	Mr. Mam Somony	Department of Animal and Health	Phnom Penh
22.	Mr. Sem Keovirak	Agricultural Department	Prey Veng
23.	Mr. Prak Sokha	Agricultural Department	Svau Rieng
24.	Mr. Vath Kim Cheang	Agricultural Department	Kampong Thom
25.	Mr. Yong Kady	Agricultural Department	Takeo
26.	Mr. Thiv Vanthy	Agricultural Department	Pursat
27.	Mr. Chhim Neat	Agricultural Department	Kampong Chooang
28.	Mr. Hien Ngon	Agricultural Department	Kampot
29.	Mr. Sem Phay	Agricultural Department	Pailin
30.	Mr. Hideo Okuda	Cassava Company	Bantey Mean Chey
31.	Ms. Trinh Thi Niem	Cassava Company	Bantey Mean Chey
32.	Ms. Majem	Cassava Company	Bantey Mean Chey
	Resource Persons		
1.	Mrs. Wilawan Vongkasem	DOAE	Bangkok, Thailand
2.	Mr. Kaival Klakhaeng	DOAE	Bangkok, Thailand
3.	Mr. Chhay Ty	Cel Agrid	Phnom Penh ,Cambodia
4.	Mr. Orn Chhourn	CARDI	Phnom Penh, Cambodia
5.	Mr. Ung Sopheap	CARDI	Phnom Penh, Cambodia
6.	Dr. Tin Maung Aye	CIAT	Vientiane, Lao PDR
7.	Dr. Reinhardt Howeler	CIAT	Bangkok, Thailand

CHAPTER 10

DEVELOPMENT AND USE OF BIOTECHNOLOGY TOOLS FOR CASSAVA IMPROVEMENT

10.1. JUSTIFICATION AND ADVANTAGES OF INBREEDING IN CASSAVA

This activity has also been reported as related to the outcome line in biotechnology. As already explained above, cassava is a highly heterozygous species and as such shows strong inbreeding depression for several traits, but particularly fresh root yield which showed a depression of around 64%. However, inbreeding depression for plant height (a trait related to plant vigor) was considerably lower, around 10%. This is important because it would suggest that there should not be major biological limitations for homozygous cassava plants to produce viable plants that can flower, and bear at least a few viable botanical seeds. These findings are also important because for fresh root yield to show such strong levels of ID, would also suggest a huge opportunity for exploiting heterosis, which is the opposite phenomenon to inbreeding depression. The introduction of inbreeding in the process of cassava breeding offers several advantages that are summarized below (Ceballos et al. 2007a).

10.1.1 REDUCTION OF GENETIC LOAD

The heterozygous nature of cassava allows for a large frequency of undesirable alleles to be maintained in breeding populations taking advantage of their frequent recessive nature. All the undesirable and deleterious alleles present in a given individual is known as genetic load. Inbreeding exposes these undesirable alleles, allowing for the elimination of genotypes exposing them. The ultimate consequence of this action is that there is a gradual reduction of the frequencies of these undesirable alleles. The evolution of productivity in maize inbred lines in the past century is a perfect example of the impact that a reduction of genetic load can have in a given crop (Duvick, 1999). The introduction of inbreeding, to produce fully homozygous or partially inbred progenitors, would allow for a rapid reduction in the levels of genetic load in elite cassava germplasm and should lead, by default to improved performance of the hybrids they produce.

10.1.2 DISCOVERY OF USEFUL RECESSIVE TRAITS

This is opposite to the argument presented above. It is recognized that some recessive traits may be desirable. Examples have already been reported for many crops in the literature and are gradually emerging for cassava as well. Ceballos et al. 2007b; and Ceballos et al., 2008 reported on two recessive starch mutations that were fond from through self-pollinating accessions of the germplasm bank and mutagenized populations, respectively. Routine production of inbred germplasm would allow for the identification and subsequent exploitation of these useful recessive traits. As another example **Figure 2.1** illustrates a peculiar plant type that was recently identified in an S1 family (obtained after self-pollinating an accession from the germplasm collection at CIAT). A total of 12 plants were grown and half of them showed the phenotype depicted in **Figure 2.1**. Leaves lack petioles and in several

cases there was no flowering or branching. This particular phenotype could be interesting because the foliage, if harvested, would be of much better quality (petioles contribute considerably to fiber of dried foliage flour limiting its uses in animal feeding). More importantly this particular phenotype could allow for higher plant densities (perhaps as high as 30,000 or 40,000 plants per hectare). As explained above one of the reasons for the increase productivity in maize has been higher plant densities (Duvick, 1999). Although there is a lot to learn about this recessive trait and perhaps its usefulness will never materialize, it serves as an example of the potential benefits of inbreeding.

10.1.3 POSSIBILITY OF IMPLEMENTING THE BACK-CROSS SCHEME

The research conducted in cassava during the past 20-30 years is finally producing large volume of useful information and the identification of useful germplasm. Many examples of sources of resistance to diseases (Hahn et al. 1980a:b) and pests (Bellotti et al., 2002) or desirable root quality traits (Ceballos et al. 2007b; Ceballos et al., 2008) have been identified. However the impact of these high-value characteristics is limited because their introgression requires breeding a new variety *de novo*. For example, Thailand was the first country to invest in the development of a commercial waxy (amylose-free) starch cassava variety. The process implies making crosses between the source of waxy starch with elite germplasm to produce F1 genotypes which will not produce waxy starch given the recessive nature of the waxy mutation. Several unrelated F1 genotypes will then be crossed to produce an F2 generation that will show a certain proportion of progenies with waxy starch. However, if the parents of an elite clone (such as the widely grown cultivar KU50) were homozygous, the introgression of the waxy starch trait would be straight through a back cross scheme. In few cycles a waxy version of the two parents could be available and when crossed they would produce exactly the same outstanding KU50 hybrid, but with the waxy starch trait expressing. In other words there would be no need to develop again such an outstanding hybrid. Unfortunately all cassava breeding projects use heterozygous progenitors preventing the application of the back-cross scheme which is one of the most widely used and successful breeding approaches used both in self and cross-pollinated crops (Allard, 1960). Similarly, assuming that whiteflies become an unmanageable problem in a given country, the dominant source of resistance found in MECU72 (Bellotti, 2002) could be crossed with one (or two) of the parents of the most outstanding hybrid grown in that country. Provided they were homozygous they could be recovered completely (with the exception of the introgressed source of resistance to whiteflies) through the backcross scheme. These progenitors would have been '*converted*' resistant and when crossed the outstanding hybrid they produce would carry now the source of resistance to the insect. So, by a very controlled and predictable way the progenitors and the outstanding hybrid they produce could be turned into resistant to the white flies. The value of these sources of resistance or high-quality roots increases considerably because their exploitation becomes much more efficient. **Figure 10.1** illustrates the typical scheme used to introduce a dominant gene into a homozygous progenitor.

10.1.4 FACILITATED GERMPLASM EXCHANGE AND CONSERVATION

The imposition to exchange germplasm in vitro (for phytosanitary reasons) restricts considerably the exchange of germplasm between the few cassava-breeding projects of the world. Maintenance of germplasm can only be made through expensive in vitro operations or else growing it in the field. Both alternatives are expensive and prone to problems that ultimately lead to the risk of loosing germplasm. Since our current effort is directed to identify (by chance) outstanding hybrids the key germplasm to exchange is the finished product (the outstanding hybrid), which generally has only limited application outside the environment where it was developed.

If cassava breeding were based on the development of good homozygous progenitors that produce outstanding hybrids, the key research product would not be the hybrid but the homozygous progenitors. Progenitors that are selected because of their adequate general combining ability and breeding value could be shared among breeding projects and crossed with local (homozygous) progenitors in search of outstanding hybrids. That is basically the way the maize breeding industry developed based on few university projects, which then opened the possibility for the private sector investments. For decades, public and private breeding project worked and collaborated, exchanging germplasm and information. This process leaded to the identification of '*venerable*' maize homozygous lines, such as Mo17 and B73. It is impossible to quantify the significance and impact of this synergism, but most likely has played a very significant role in the development of conventional breeding projects in different crops.

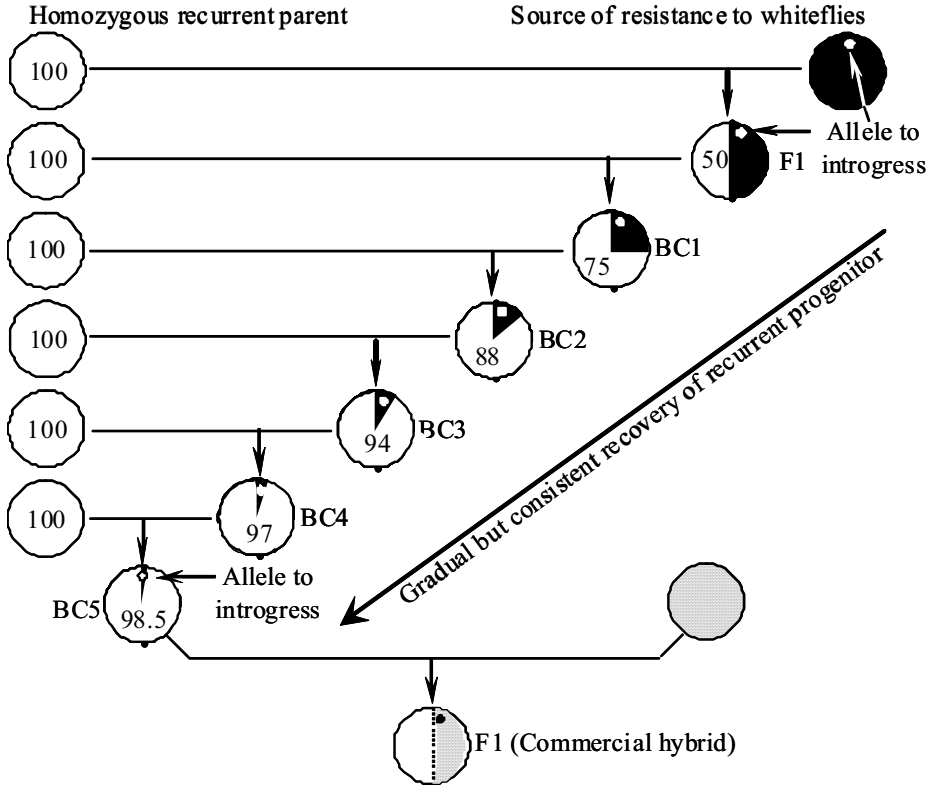


Figure 10.1. Illustration of the back-cross scheme to introgress a desirable gene into the homozygous recurrent parent through successive back-crosses. There is a gradual recovery of the 'blood' of the recurrent parent. In different stages of the process many progenies are produced but only a genotype that carries the desirable gene is back-crossed to the recurrent parent. Since the progenitor is homozygous each back-cross contributes with gametes that are genetically identical and this allows a gradual and consistent recovery of the recurrent parent. The ultimate objective is to use the 'converted' recurrent progenitor in a cross to produce the same outstanding hybrid but with the addition of the allele that has been

introgressed. When the allele to introgress is recessive, self-pollinations need to be made at each back-cross stage to identify which genotypes carry the allele.

10.1.5 Facilitated phytosanitary maintenance of superior clones.

When an outstanding hybrid is identified, it is multiplied and maintained vegetatively. However, the continuous grow of a clone in the field, year after year, eventually results in decreased performance due to the 'contamination' with organisms that can be pathogenic or just epiphytes that do not induce disease proper but ultimately affect the performance of cassava. When disease problems are chronic and severe (i.e. bacterial blight or cassava mosaic disease), the only way to recover the productivity of the clone is by meristem culture to clean the planting material from undesirable micro-organisms. This is an expensive process that could be avoided if the outstanding clones were produced from inbred progenitors. They could be crossed again and the same hybrid would be produced through botanical seed. A few crosses (for example to produce 100-200 botanical seeds) every now and then could provide a new generation of the same hybrid but clean of diseases.

10.1.6 Facilitated conventional and molecular genetic studies.

The availability of homozygous progenitors would facilitate greatly the logistics of genetic studies (both conventional and molecular genetics). Segregating progenies could be selected for a higher contrast and 'cleaner' segregations. This in addition to the obvious fact that a larger number of recessive traits (desirable and undesirable) would be properly identified allowing for the analysis of genetic segregations that we are not aware they are actually happening but remain masked behind the heterozygous nature of the crop.

10.1.7 Development of superior hybrids by design, not by trial and error.

Dominance and epistatic effects can be systematically exploited for enhanced heterosis (hybrid vigor). These sources of genetic variation can also be exploited through reciprocal recurrent selection methods (Hallauer and Miranda, 1988) without the use of inbreeding, but genetic gains would be slow. In the case of cassava, as for other crops, the shaping of two reciprocal populations for enhanced heterosis would be very slow if no inbreeding were employed. The development of hybrids in the maize industry can be used as an example of the power that inbred progenitors have in the development of a crop. **Figure 10.2** presents the evolution of maize yields in the last 150 years (Troyer, 2006).

About 50 to 60 % of the gains depicted in **Figure 10.2** have been demonstrated to be due to genetic causes. The remaining 40 to 50% of the gains are due to management practices such as increases in nitrogen fertilizers and higher plant densities. It has been estimated that 15% of the gains in productivity are due to heterosis (Duvick, 1999).

The sharp increase in maize productivity observed after the year 1935 is due to the shift from farmers planting seed from open-pollinated varieties to planting seed from hybrids produced by crossing selected inbred progenitors. Because the inbred parents available early on still had considerable amount of genetic load their productivity was low. As tolerance to inbreeding was built, the productivity of inbred lines increased from 2 to 4 t/ha (average of selected elite inbred lines from different eras of maize breeding) allowing the commercial exploitation of single-cross hybrids which further increased (at a higher rate) grain yields (Duvick, 1999; Troyer, 2006). All data from **Figure 10.2** depict yields of hybrid maize. Open pollinated varieties were obtained from heterozygous progenitors and were a mixture of hybrids. Double and single-cross hybrids, on the other hand, were derived from inbred parents and only the best performing hybrid (not a mixture) was planted by farmers.

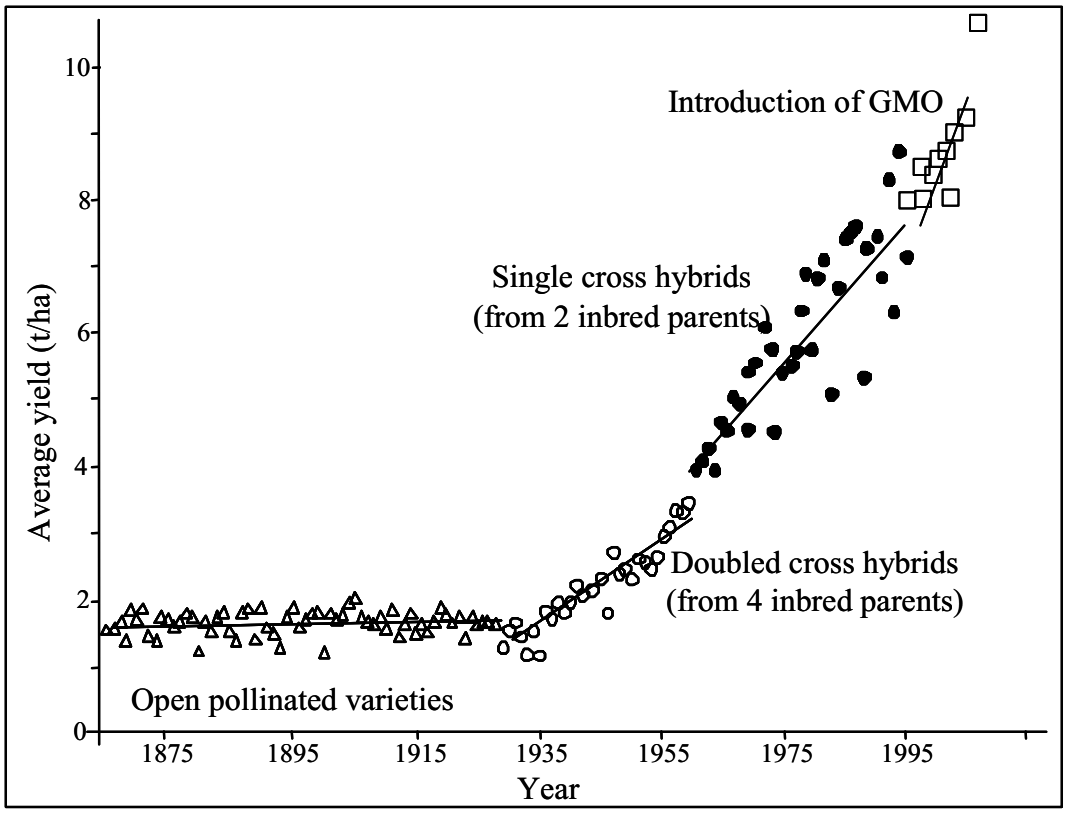


Figure 10.2. Evolution of maize productivity in the USA during the last 150 years. The introduction of double-cross and single-cross hybrids from inbred progenitors significantly increased maize productivity (Adapted from Troyer 2006).

10.1.8 Shortening the length of evaluation and selection cycles.

In the current selection approach crosses among elite (heterozygous) progenitors are made to produce full-sib families. Each seed will represent a unique genotype. The seeds are germinated to produce an *F1* plant. Selection can or cannot be made at this single-plant stage. The main function of these F1 plants, however, is to produce vegetative cuttings (7-10 cuttings per genotype) to plant the clonal evaluation trial (*CET*), which for some breeding projects is the first stage of selection. The seven to ten plants used in the *CET*, in turn are source of cuttings for the third stage in the selection process: the preliminary yield trial (*PYT*), which in the case of CIAT would be based on three replications of 10-plant plots, for a total of 30 plants per genotype. If inbred lines were used to make the F1 crosses several pollinations between the same inbred parents would yield the same F1 hybrid (as it does in maize). Therefore, by making several crosses among two inbred parents as many as 30 botanical seeds could be produced, in such a way that the first stage of selection would be the *PYT*. This is very important because: a) it makes the breeding cycle two years shorter by eliminating the *F1* and *CET* stages of selection; and b) it allows avoiding these early selection

stages which are based on unreplicated observations and, therefore, prone to large experimental errors. A better understanding of this advantage can be obtained by reviewing the chapter on Cassava Breeding (Ceballos et al., 2007a).

10.2. PROBLEMS AND BOTTLENECKS FOR INBREEDING CASSAVA.

There are few technical approaches to develop homozygous genotypes. The most common approach has been through successive self-pollinations, which is the fastest method of inbreeding through sexual reproduction. However, in practice this would be impractical because of the time required to produce 5-6 successive self-pollinations (which is the standard approach for breeders to consider germplasm as 'fixed'). It is estimated that up to 12 years may be required to produce inbred lines through this approach. Moreover, in the process of inbred line development breeders generally take the opportunity to make selections in the successive segregating generations. In the case of cassava, unfortunately the breeder ends up selecting mostly for plants that flower, and not for plants that show adequate plant architecture, disease or pest resistance, and other traits related to good agronomic performance and productivity (particularly tolerance to inbreeding). It should be emphasized that in the case of cassava, the capacity to flower is not necessarily related to vigor. Many full-vigor and productive genotypes fail to flower. As a matter of fact, these non-flowering types offer the erect plant-architecture that farmers prefer. So, ultimately the pressure to produce lineages that have the capacity to flower would result in producing germplasm with undesirable plant architecture characteristics. Therefore, other approaches for the production of inbred germplasm must be considered.

10.2.1 PRODUCTION OF DOUBLED-HAPLOIDS THROUGH TISSUE CULTURE

A very common approach to produce instant homozygosity is through anther (or less commonly ovule) culture. Immature pollen is harvested, subjected to some sort of stress and cultured in vitro in such a way that its biological pathway is changed. Eventually cell division in induced in the microspore to produce a micro-callus, and from there embryos that can be induced to develop a plantlet. Because the explant is an immature microspore with half (N) the somatic number of chromosomes (2N) the tissue developed is haploid in nature. There is a frequent doubling of chromosomes that occurs spontaneously. The process, however, may have to be induced through the use of colchicine. This is the reason why this technology and products it develops are known as doubled-haploids. The double- haploids technology is used extensively even for species that can produce 'fixed' lines in a matter of three years (maize and rice for example). Considering the reasons given above, the technology is more appealing for the case of cassava.

Work to develop the protocol for the production of doubled-haploids in cassava started in 2003 with a project supported by The Rockefeller Foundation. Early activities were directed for the understanding of microspore development in cassava so uniform suspension of large number of microspores at the right developmental stage could be obtained. Then the research faced an unexpected problem: the thick and autofluorescent exine wall of cassava microspore. The thickness of the wall prevented the observation inside the cells to see which treatment favored cell-division. The thick exine wall may have even prevented, by its pure physical strength, those cases where cell division had been initially induced to prosper and form a micro-callus. The autofluorescence prevented the use of special dyes to identify living from death tissue. By the end of 2007 a methodology for degrading the exine wall was finally developed and this allowed for the routine development of multicellular structures.

The current work turns now around the final stage for the development of doubled-haploids: produce an embryo from the micro-callus, regenerate a viable plant and harden it so it can be transplanted to the field and be used to make crosses. Further details of the progress in the development of the protocol are described below, in Section 10.3.

10.2.2 PRODUCTION OF DOUBLED-HAPLOIDS *IN VIVO*

There are examples reported in the literature where homozygous plants have been produced through sexual reproduction using wide crosses. The modes of origin vary, but in any case haploids arise following abnormal events during or soon after fertilization (frequently as a result of wide crosses between different species). The frequency of haploids is controlled by the genotype of the progenitors and there is an important influence of the environment, chemicals, timing of pollination and the effect of alien cytoplasm. Hermsen (1984) listed four different pathways for the production of haploid seed after sexual reproduction in different plant species: a) Pseudogamy; b) Preferential elimination of chromosomes; c) Semigamy; and d) Androgenesis.

With the exception of the exploitation of a gene in maize that allows for a predictable production of haploids or doubled-haploids *in vivo*, these systems are not as common as the *in vitro* approach. In the case of maize, 'inducer' lines have been developed to produce doubled-haploids in vivo (Röber et al., 2005). . The use of an inducer line is a simple, fast, and inexpensive method of haploid production and is referred to as in-vivo haploid induction.

10.2.3 INDUCTION OF FLOWERING THROUGH EXOGENOUS APPLICATIONS OF PHYTO-HORMONES.

Flowering in cassava, as in every other crop, is controlled genetically. However, cassava is a perennial crop that does not need sexual reproduction for survival. The plant does not follow a pre-established phonological development (seed germination, vegetative growth, flowering, grain filling period, senescence and death) typical of many grain crops. Cassava shows marked genetic differences for flowering habit. Some genotypes will flower early and frequently leading to branching type architecture. Other genotypes flower late and scarcely (or not at all), leading to non-branching, erect types. In several crops flowering can be stimulated by exogenous application of hormones (Botha et al., 1998; Wilson et al., 1990). The induction of flowering in pineapple has been known for a long time (Rodrigues, 1932) and the crop fits well for this kind of technology (Pinto da Cunha, 2005). Smoking was the first procedure used for artificial induction of pineapple flowering, after what may have been an accidental observation in the Azores Island. Later on it was discovered that the smoke agent that initiated the flowering was the gas ethylene. Ethylene is now applied commercially in pineapple production and has even allowed for some patents being granted in the way it is applied (U.S. patent 3819359).

The induction of flowering in cassava would offer very interesting alternatives not only to facilitate (actually allow) the production of inbred lines through successive self-pollinations but also in the general operations of cassava breeding. When crosses between a set of progenitors are planned, it takes basically two years to obtain the botanical seed from these crosses. If a method to induce flowering in cassava breeding nurseries were available, then the time required to obtain the seed could be reduced considerably, perhaps to just six months.

10.3 Development of an *In Vitro* Protocol for the Production of Cassava Doubled-Haploids and its Use in Breeding

Cassava is a staple food in many parts of the world, where subsistence farmers in developing countries often grow it as a carbohydrate source. Cassava is often grown in marginal areas because of its ability to tolerate adverse environmental conditions. Traditional breeding of cassava is difficult due to its allopolyploid nature, heterozygosis and its low natural fertility. The true breeding, homogenous line, offers several advantages for an efficient breeding program which have been summarized above.

As also explained above, however, to develop true breeding lines in cassava through successive self-pollinations is a time-consuming work that would take about 10-15 years for completion. Moreover, the process leads to the unavoidable selection in favor of genotypes that flower profusely. As reported in the literature (Ceballos et al., 2007a) flowering results in branching and, in general, farmers prefer non-branching plant types. It is obvious that self-pollination particulary when it is based on single plant per genotype would lead to the production if inbred lines that are highly undesirable. *In vitro* production of doubled haploid (DH) plants by androgenesis is a highly valuable tool for obtaining completely homozygous or inbred lines from F1 crosses in a single generation. Nevertheless, the production of DH lines is subject to the constraints from many factors, including the physiological status of donor plants, special developmental stage of microspore, proper pretreatment on the microspores (generally based on the induction of some sort of stress), and microspore culture conditions (the medium species and its composition, the cell density, the temperature regime, the application of plant growth regulators, and the conditioned media).

Our work combined androgenesis protocol established in the last few years with the right developmental stage of microspores resulted in switching the microspores from the gametophytic pathway to a sporophytic development confirmed by confocal laser scanning microscope (CLSM). A first stage of microspore response to *in vitro* culture is the production of multicellular structures (MCS) and advanced structures commonly names as embryo-like structure (ELS) are now obtained in the cultures reproducibly. Current work is aiming at increasing further the frequency of success. These responses have been considered as evidence of the initiation of an embryogenic potential in several model systems for androgenesis. Cassava microspores have a strong and thick exine wall that constraint the MCS development, prevented the observation inside the microspore (therefore no assessment of pretreatments and/or treatments that favored the induction of cell division could be done), and its autoflourescence prevented distinguishing living from dead cells or tissue. The exine wall is resistant to enzyme digestion that commonly used for protoplast release [Szabados et al. 1987] as other plant species due to the sporopollenin it contains [Southworth, 1974]. Physical and chemicals treatments were used hoping to degrade the exine wall with no avail. Finally only with the treatment sodium hypochlorite (NaClO) was possible to digest the exine wall without killing the microspore (Wang *et al.*, 2008). However ions as Cl^- and Na^+, present in the NaOCl affected the development of the plants when they are in a relatively high concentration (Kronzucker et al., 2008), the pre-culture and the carefully digestion of microspore's exine, and different changes in the medium composition have offered results such as major and reproducible production of advanced structures of androgenesis, embryo-like structure.

Based on the work in 2007 which allowed the digestion of the exine wall through the treatment with sodium hypochlorite, different changes to the medium composition has been included to the protocol. This new protocol allows obtaining high reproducibly and better

quantity of ELS. Another breakthrough of during 2008 was, a reliable and easy method development in CIAT to determine the developmental stage of the microspore for culture, as well as to monitor the in vitro microspore division using the light microscope.

10.3.1 Materials and Methods

Cultivar SM1219-9 was used for these early experiments but results were confirmed with two other genotypes. The donor plants of buds were planted in the fields of ICA experimental station at Palmira, Colombia, under natural conditions using stem cuttings from 2006-2007.

Microspore isolation:

Male inflorescences were harvest and immediately stored in a polystyrene box with refrigerant gel (Glacier Ice Co., USA) to maintain as mush as the viability of the microspores before being brought back to laboratory and after stored at 10ºC for 3 days in the cold room. After pre-treatment, the floral buds were precisely selected for its size (2.3-2.6mm), color and the size of female flowers (≤5mm). All the buds are selected on ice. Once measured, 500-1000 buds were placed in a baby food jar containing 5.25% NaClO solution, with 2 drops of Tween 20 added, for 15 min. The NaClO solution was then descanted and the buds were rinsed with sterile water for three times, 5 min each. After washing, the buds were transferred into a blender cup containing 40 ml of pre-cooled 0.4 M mannitol solution and blended for 20s with low speed (Warings, USA). The slurry was filtered through a series of nylon meshes (218,150 and 102 μm pore size in turn). The cells blocked on the filters of 102 were recovered by mannitol washing and pellet by centrifugation at 100g for 1min. The pellet was re-suspended in mannitol and layered onto a 50% of percoll solution followed by a centrifugation at 150g for 3min. The microspores were collected on the percoll and then washed with medium for 3 times. The cell yield was determined by means of series dilution. The suspension, with the concentration of 10^4cells/ml, was pre-cultured in 60×15mm Petri dishes and incubated at 26 ºC in the dark and after 4 days the microspore were recovered and subjected to sodium hypochlorite (NaClO) digestion for 30-40 second at 5.25% of concentrations. The partially digested microspores were culture in the same o different pre-culture medium and incubated at 26 ºC in the dark. All the media were refreshed every 2 or 4 weeks depending on different experiments and cultured for at least two months. A minimum of 3 replicates culture dishes were prepared for each treatment.

Ovary dissection:

Female flowers, prior to opening for pollination, were excised from the donor plants of TMS60444, which is available for a long time and shows good effect on androgenesis induction, and place in a cold box immediately as described above for male flower collection. After washing with running water, these female flowers were disinfected in 5.25% NaClO solutions with 2 drops of Tween 20 for 15 min, followed by sterile water washing for 3 times. The ovaries were released from the flowers by cutting the flower at the end of the calyx close to the receptacle and then transferred to a Petri dish containing fresh medium. The Petri dish was put at 28ºC over night to eliminate the contaminated ovary preparation. The density for co-culture of ovary is 2 ovaries per milliliter of medium and refreshed every one-week with the medium. The cases, the addition of ovaries in the culture medium induced more MCS. It seems that the ovary may provide a “buffer” environment for MCS induction, most likely though balancing the hormone level or other signal molecules in the medium. Although this nourishment effect of the ovary has been know for years in wheat androgenesis (Zheng et al. 2003).

Microspore culture
The effects of KNO_3, $Ca(NO_3)$ and NH_4NO_3 have been tested on enlarged microspore induction. NLN medium supplemented with 100 mg/L Larcoll and 30 mg/L Glutation and the ovary co-culture was used. The treatments of different concentrations of 125, 250, 500, 1000, 2000 and 3000mg/L KNO_3, and 500, 750, 1000, 1250, 1500 and 1750mg/L $Ca(NO_3)$ and 82.5, 165, 330, 660 and 1320mg/L NH_4NO_3 were tested. In preliminary studies for the ELS yield improvement and examination its effect on MCM, different chemical as auxines (IAA, ANA, 2,4-D, Dicamba, Picloram and PAA) citoquinines (Kinetin, Zeatine, BAP, IBA and 2i-P) polyamines (PVP, SPD and PUT) and others chemicals inductors (GA_3, ABA, 2-HNA and Colchicine) were tested. We grouped the cells by their physical characteristics (**Figure 10.3**) to evaluate the production of advanced structures of androgenesis (ELS).

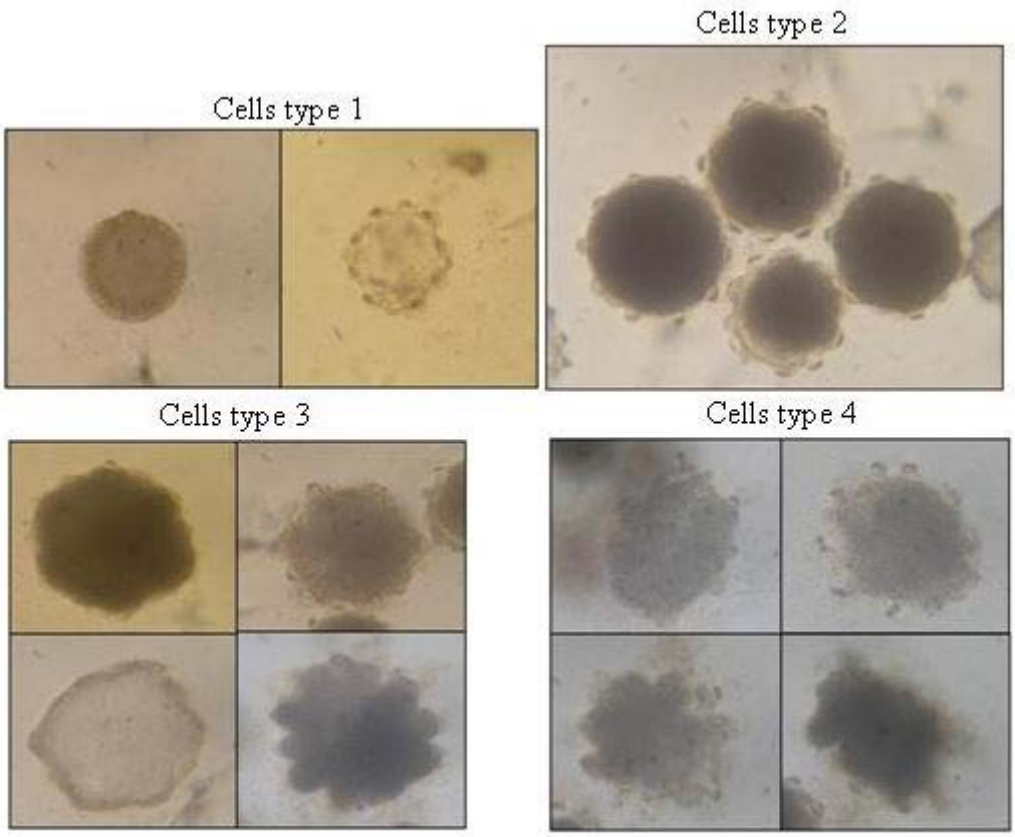


Figure 10.3. Cells type 1. Microspores with exine partially or completely digested, small sizes and dark or semi-transparent color. Type 2. Microspores with exine digested, small or big sizes and dark color. Type 3. Bigger microspores with semi-transparent color, the spherical aspect, and presence of small peaks in the periphery and small circles are observed within these. Type 4. Elongated or deformed microspores, small circles are observed within these.

Exine digestion and microspore culture:
The exine wall of cassava microspore is very thick, which probably protects the cell preventing moisture loss in dry environments. This thick cell wall appears to limit the penetration of any components of medium that allow the development of ELS (Wang *et al.*,

2008). Partially or completely exine wall digestion with 10, 5.26, 2.63, and 1.32% NaOCl was evaluated. Other chemicals for this propose as Calcium hypochlorite (CaOCl) and Potassium permanganate ($KMnO_3$) in different concentrations were tested too.

Light microscopic analysis:
Immature flower buds were collected when plants were about 8-12 month-old when they were between 0.8 mm and 3.1 mm in diameter. Anthers of 10 buds were dissected and then squashed in ddH_2O and collected in eppedorf tube, the supernatant was eliminated and the pellet was resuspended in 5.25% sodium hypochlorite for 1 min. Then partially digested microspores were washed in ddH_2O for three times and stained in 1 $mg.ml^{-1}$ DAPI (4',6-diamidino-2-phenylindole) or 5µl Picogreen (Quant-iT™ PicoGreen® dsDNA reagent) for 15 minutes and was observed under Leitz epifluorescence microscope (Wild Leitz GmbH, Wetzlar, Germany).

10.3.2 Results and Discussion

Various culture media were tested including NLN (Lichter, 1982), NN (Nithch and Nitch, 1969), B5 (Gamborg *et al.*, 1968), B5-P (Szabados *et al*, 1987), M1 (Quintero *et al*, 2003), GD (Gresshoff and Doy, 1972), and NPB (Liu *et al.*, 2002) with or without plant growth regulator, before and/or after digestion exine wall (data not shown). Of the media tested, NLN is the optimal medium for MCS induction according with Wang *et al.* (2008) followed by NN and M1, in the other medium the MCM were not seen. On the other hand, went the microspores were treatments with NaOCl (5.25%) for exine wall digestion and culture in the same medium, alone in NLN medium ELS were observed although in very low quantity and frequency (0.16 ELS per $2x10^4$cells in average) and these did not continue their development. An increase ten at four fold in the concentrations of KNO_3 and $Ca(NO_3)$ respectively, in the pre-culture medium, independently, increased significantly MCS quantity, in 37.3% and 18% respectively in relation with control (NLN). When these macro-nutrients were combined in the same medium (CM_1) an increase significant of 40.2% was observed. Nevertheless, addition of 165mg/L NH_4NO_3 in the CM_2 medium, the response enhanced significantly in 12% in relation with control (CM_1) (**Figure 10.4**). Additionally all changes improvement in quantity and frequency of ELS (1 - 3 ELS per $2x10^4$cells in average) induced. Ions as Cl^- and Na^+, present in the NaOCl may be affect the development of the cells when it is accumulated (Kronzucker et al., 2008), the pre-culture in CM_2 (NLN modified) and the carefully digestion of microspore's exine have offered results such as major and reproducible production of the first (MCS) and advanced structures of androgenesis, (ELS). According with Kronzucker et al. (2008), K^+ and $N\text{-}NO_3^-$ can to work against the adverse effects of Na^+ and Cl^- on the metabolism of cells, the Ca_2^+ is very important in the structure membranes and restricts the influx of Na^+. Inorganic nitrogen sources such as N-NO3 and N-NH in a ratio of 10:1 was used taking account the demand for cassava demonstrated by Howeler, R. (1981).

Nowadays, the main obstacle in the process has been that ELS are not competent to become embryos. Other types of stresses applied within the response to induce embryos such as auxins (Reynolds, 1997) and other chemicals (Zheng et al., 2001) were used. The principal target with these preliminary tests was to evaluate the effect of the different hormones in the MCS by then make combinations to obtain embryos. The hormones with better results in the induction of ELS were PAA (8mg/L), IAA (1mg/L), 2-HNA (25mg/L), KT (1mg/L), IBA (1mg/L), 2i-P (1mg/L), Put (129mg/L), GA_3 (2mg/L) and Colchicine (100mg/L) (**Table 10.1**). Taking

account that mainly auxin and other chemical inducers can trigger androgenesis, we have considered different hormone combinations, in order to realize in the future.

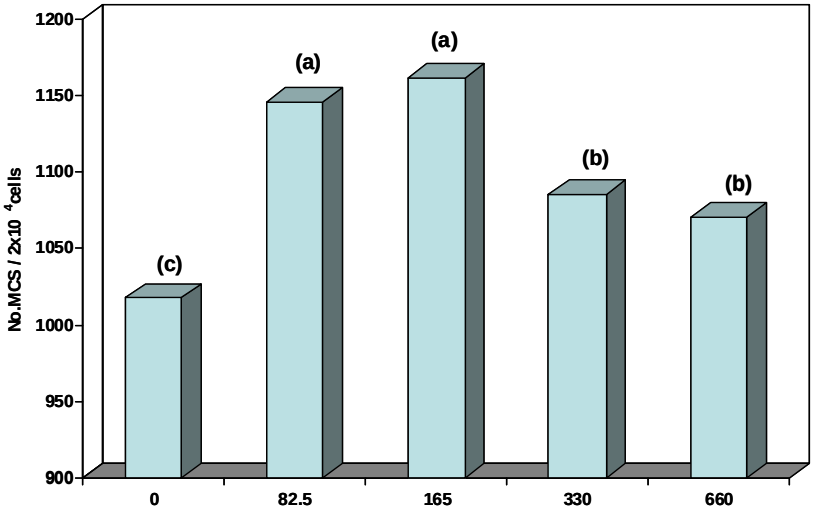


Figure 10.4. Effect of the ammonium nitrate **(**NH_4NO_3) in the MCS induction in different concentration using CM_1 medium (NLN modified) as control.

Table 10.1. Amount of induced cell by type (Type 3 or Type 4) grown in CM_2 culture media supplemented with different type of hormones and based on a base cell population of de $2x10^4$cells observed eight days after exine degradation.

Hormonal Supplement	Type 3	Type 4
CM2 (Free-hormones)	97.0	1.0
2,4-D	104.3	0.0
Picloram	71.3	0.0
Dicamba	92.0	1.0
ANA	133.0	1.7
IAA	124.3	3.3
AFA	155.0	2.8
TDZ	122.5	0.0
Zeatine	124.3	1.7
Kin	158.0	2.7
BAP	102.0	1.0
IBA	153.7	4.3
2iP	152.5	2.5
PVP	198.5	0.5
Put	225.7	4.3
2HNA	82.7	2.0
GA3	210.7	5.3
Colchicine	148.3	3.0

During the course of this research, an advanced MCS (**Figure 10.5A)** and ELS (**Figure 10.3**, cells type 4) were observed in certain treatments in a reproducible manner (98 - 250 MCS and 3 – 6 ELS per each 30 x 60mm cases) after exine wall digestion, these structures are signs of a possible continuous development of cassava microspore. Observation led to the conclusions that these signs of development were the results of factors such as medium composition (combination of macro-elements and hormones) that offer to cells during the pre-culture and isolation process that weaken the exine, resistance to adverse conditions. According to Wang *et al.* (2007), more hormones should not be used in the MCS induction medium (pre-culture medium), these have a better effect in adding to the culture medium after treatment of exine digestion (**Table 10.1**). Notably, those plant hormones commonly used for androgenesis induction do not work in our study (data not shown). This is consistent with the rapeseed microspore culture in which a hormone-free medium is used (Custer *et al.*, 2003).

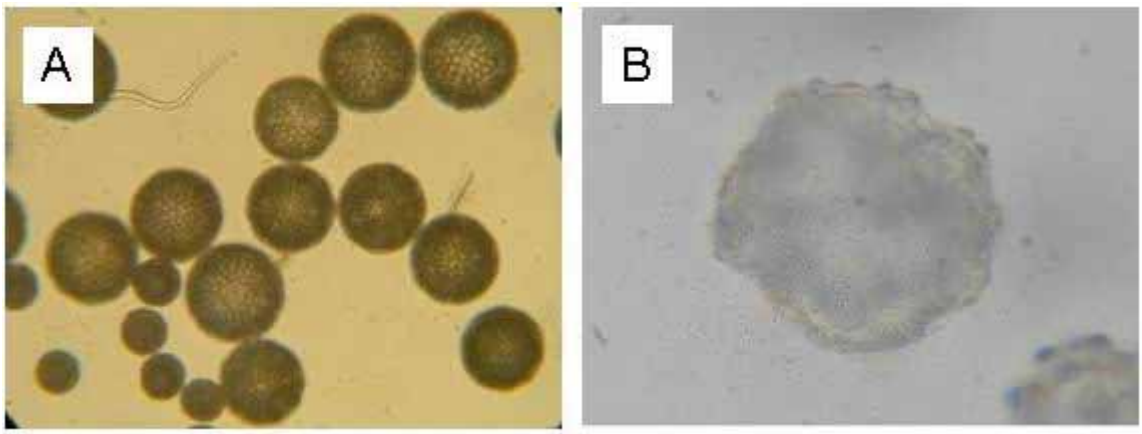


Figure 10.5. Multi-cellular structure induced in CM_2 medium (A) four days of pre-culture, and (B) after treatment of exine wall digestion.

Exine digestion and microspore culture:
In the last year, various physical treatments (i.e. ultrasonic shocks, blending, etc.), chemicals products (i.e. acetic acid, HCl, KOH, NaOH) and enzyme digestion, were tested to remove or weaken the exine of isolated microspore prior to culture unsuccessfully (Wang *et al.*, 2008). In this year partial or complete exine wall digestion was achieved when different NaOCl concentrations were used. Other chemicals tested are not competent for this purpose. Deleterious and detrimental effects were caused on cells with CaOCl and $KMnO_3$ after 15 minutes and 24 hours respectively.

The speed of the digestion was related at high concentrations, this processes began about 20 seconds to MCS and reaches 50 to 90% of cells with degraded exine in just 30 to 60 seconds in treatments 5.26 and 10% NaOCl. At lower concentrations of NaOCl the process began at 40 seconds and these percentages were obtained at 60 to 150 seconds (**Figure 10.6**). We selected 5.25% NaOCl for 50 to 60 seconds as the best treatment because the combination of

reduced exposure time and the dissolving the exine in a short time, that could have minor effects on the cells viability.

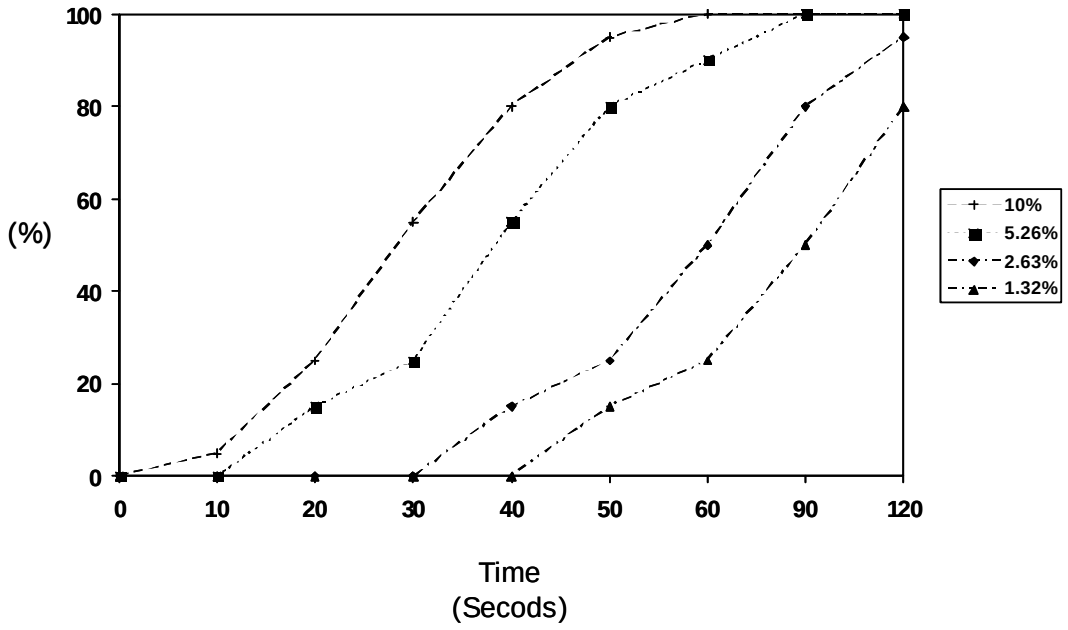


Figure 10.6. Characterization of exine wall digestion on cassava microspores with different concentrations of NaOCl (10, 5.26, 2.63 and 1.32%) in determinate in different times.

Light microscopic analysis:
The exine wall emits intensive auto-fluorescence excited by both UV-light (λ 360-380 nm) and visible lasers (λ 476 and 488 nm), hindering the attempt to examine the developmental stage and to witness the cell division by fluorescence staining, such as Acridine Orange or DAPI (4',6-Diamidino-2-phenylindole), which has been widely used in other plant androgenic systems (Wang *et al.*, 2007). In the last year, the whole process of in vivo microsporogenesis had been deciphered by combining of light- and electron microscopic techniques, together with a CLSM analysis. In this year an easy protocol to identify the relationship between microspore development stage and the bud size were established in cassava. This methodology allowed re-establishing that Mother cells (MC) can be found in buds of 1.8-2.2mm in diameter and other stages as Tetrads (T), Early-uninucleate, Late-uninucleate, Early-bynucleate and Late-bynucleate were observed in ranges of 2.0-2.3, 2.2-2.5, 2.3-2.6, 2.5-2.8, and ≥2.9mm respectively. This association allows a rapid selection of floral buds with similar microspore developmental stages, useful when a large number of homogeneous cells are needed for analysis and for in vitro induction of androgenesis. The nuclear stage progression during microspore developmental stages is illustrated in the **Figure 10.7**, where is possible to see the asymmetrical division that produced two different sized nuclei (presumably vegetative and generative nuclei) at opposite of the cell (Bi-nucleated stage). These results can be used in a cytological study of androgenesis in cassava to allow improve isolated microspore cultures, and can be used in those species when the monitoring of the androgenesis has been constrained by the thick exine wall, such as lupin (Bayliss et al., 2004).

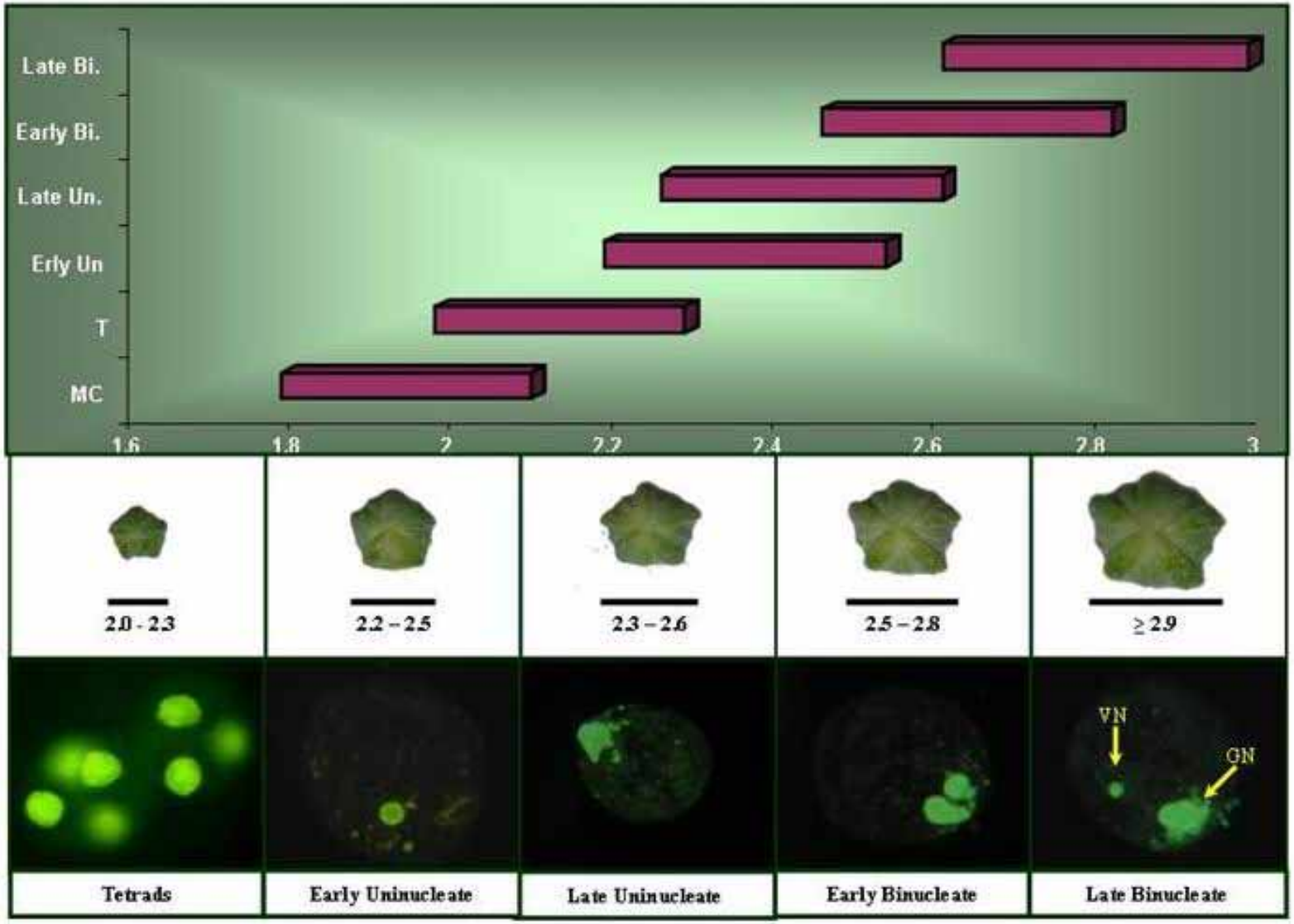


Figure 10.7. Relationship between microspore developmental stage and the bud size from which it is harvested.

10.3.3 FUTURE ACTIVITIES

Next year our efforts will concentrate in continuing the development of the protocol perhaps based on a single genotype to evaluate new avenues to advance in the protocol but always confirming that what works with one genotype also works with the other two.Based on the reliable system to induce cell division and produce MCS the focus will center around the production of embryos. Different trials will be conducted to combine different types of hormones or chemical inducers and as this trial and error process advances the direction of the research will evolve. In this process we hope to fine tune a system that will allow us to check on cell viability at each stage so point changes in the protocol as specific times can be suggested by the results obtained.

10.4. REFERENCES.

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons, New York.

Bayliss K.L., Wroth J.M., Cowling W.A. 2004. Pro-embryos of Lupinus spp. produced from isolated microspore culture. Austrilian Journal of Agricultural Research 55: 589-593.

Botha, M.L., C.S. Whitehead and A.H. Halevy. 1998. Effect of octanoic acid on ethylene-mediated flower induction in Dutch iris. Plant Growth Regulation 25(1):47-51.

Ceballos, H., M. Fregene, J.C. Pérez, N. Morante and F. Calle. 2007a. Cassava Genetic Improvement. Breeding Major Food Staple. Chapter 12. p:965-991.

Ceballos, H., T. Sánchez, N. Morante, M. Fregene, D. Dufour, A. M. Smith, K. Denyer, J.C. Pérez, F. Calle, and C. Mestres. 2007b. Discovery of an Amylose-free Starch mutant in cassava (*Manihot esculenta* Crantz). Journal of Agric. Food Chem. 55(18): 7469-7476.

Ceballos, H., T. Sánchez, K. Denyer, A. P. Tofiño, E. A. Rosero, D. Dufour, A. Smith, N. Morante, J. C. Pérez, and B. Fahy. 2008. Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). Journal of Agricultural and Food Chemistry 56(16): 7215-7222.

Custer J.B.M. 2003. Microspore culture in rapeseed (Brassica napus L.). In: Maluszynski, M., K.J. Kasha, B.P. Forster, and I. Szarejko, 2003. Doubled haploid production in crop plants- A Manual. Kluwer Academic Publisher, The Netherlands. ISBN 1-4020-1544-5 . pp .185-193

Duvick, D.N. 1999. Heterosis: feeding people and protecting natural resources. In: The genetic exploitation of heterosis in Crops. (J. G. Coors and S. Pandey Eds.) American Society of Agronomy, Madison WI. USA p. 19-29.

Gamborg, D.L., Miller R.A., Ojima K. 1968. Nutrient requirements of suspension culture of soybean root cells. Exp. Cell Res. 50:151-158

Gresshoff P.M., Doy C.H. 1972. Haploid Arabidopsis thaliana callus and plants from anther culture. Aust. J. Biol. Sci. 25: 259-264.

Hallauer A.R., and J.B. Miranda Fo. 1988. Quantitative Genetics in Maize Breeding. Second Edition. Iowa State University Press. USA., pp 45-114.

Hermsen, J.G. T. 1984. Haploids as tool in breeding polyploids. Iowa State Journal of Research 58(4):449-460.

Howeler, R. 1981. Nutrición mineral y fertilización de la yuca. Cali, Colombia, Centro Internacional de Agricultura tropical. 55p

Kronzucker H.J., Szczerba M.W., Schulze L.M. and Dev T. Britto, 2008. Non-reciprocal interactions between K+ and Na+ ions in barley (Hordeum vulgare L.). Journal of Experimental Botany, 2008. pp 1 – 9

Lichter R. 1982. Induction of haploid plants from isolated pollen of Brassica napus, Z Pflanzenphysiol 105: 427–434

Liu W., Zheng, M.Y., Polle, E. and Konzak, C.F. 2002. Highly efficient doubled-haploid production in wheat (*Triticum aestivum* L.) via induced microspore embryogenesis. Crop Sci 42: 686-692

Pinto da Cunha, G. A. 2005. Applied aspects of pineapple floweing. Bragantia 64(4):499-516.

Nithch J.P., Nitch C. 1969. Haploid plants from pollen grains. Science 163:85-87

Quintero M.A., Tabares E., Delgado G., Lentini Z. 2005. Improvement of callus induction and dobled haploid plant regeneration from rice anther cultures. Annual Report 2005. CIAT, Cali, Colombia pp 29-35.

Reynolds T.L. 1997. Pollen embryogenesis. Plant Mol. Biol. 33:1-10

Röber. F.K., G.A. Gordillo, and H.H. Geiger. 2005. In vivo haploid induction in maize – performance of new inducers and significance of doubled haploids lines in hybrid breeding. Maydica 50(3-4):275-283.

Rodrigues, A. G. 1932. Smoke and ethilene and pineapple flowering. Journal of Agriculture Univ. Puerto Rico 16:5-6.

Southworth] D (1974). Solubility of pollen exines. Amer.J. Bot. 61: 39-44.

Szabados L., Hoyos R., Roca W. 1987. In vitro somatic embryogenesis and plant regeneration of cassava. Plant Cell Rep. 6 (1987) 248-251.

Troyer, A. F. 2006. Adaptedness and heterosis in corn and mule hybrids. Crop Sci. 46:528-543.

Wang, Changhu, E. Tabares, M. Quintero, H. Ceballos, C. Sautter, Z. Peng and Z. Lentini (2008). Development of the microspore in cassava (*Manihot esculenta*, Crantz) and androgenesis. First Scientific Meeting of the Global Cassava Partnership GCP-1. Ghent, Belgium.

Wilson, J. E., S. Tjendana Tedeschi and K. Wong. 1990. Taro Breeding. Agro-Farcts. Ireta Publication No. 3/89. USP Alafua Campus, Apia, Western Samoa. P. 9-20.

Zheng M.Y. 2003. Microspore culture in wheat (*Triticum aestivum* L.) doubled haploid production via induced embryogenesis. Plant Cell Tissue and Organ Culture 73: 213-230

Zheng M.Y., Liu W., Weng Y., Polle E., Konzak C.F. 2001. Culture of freshly isolated wheat (*Triticum aestivum* L.) microspores with inducer chemicals. Plant Cell Reports 20, 685-690.