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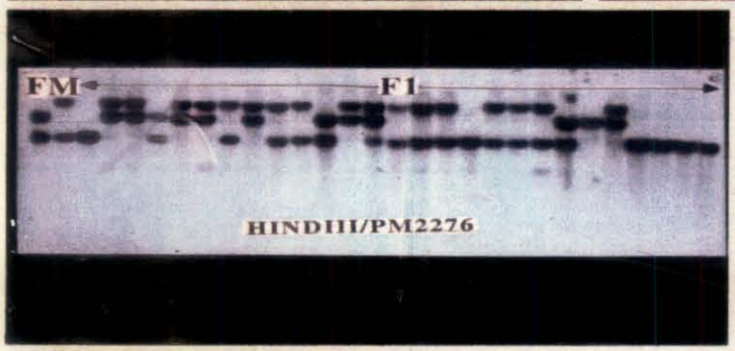
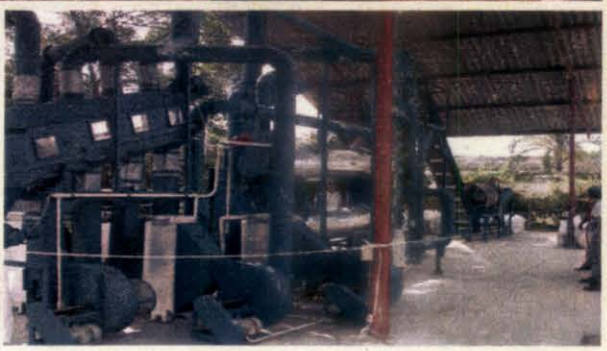
Project IP3:
Improved cassava for the developing world



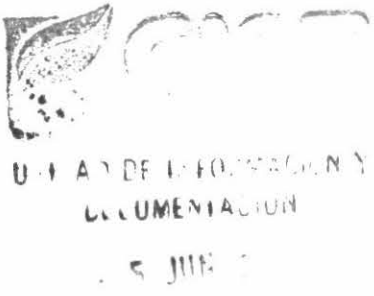


CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Annual Report - 2000

Project IP3

Improved cassava for the developing world



CIAT

Annual Report
Year 2000

Research Highlights

The new economic environment in many developing countries resulting from the globalization of the markets, and the opening of the economies, has had tremendous effects in their agriculture. For instance, the importation of cereals in tropical countries has sky rocketed in recent years, because the agriculture in this region cannot compete with farmers from temperate areas in cereals production. Biological, technological and political factors (including the existence of large subsidies for farmers in temperate regions), explain this lack of competitiveness of cereal production in the tropics. Therefore, economic activities related to the agriculture in tropical regions had to identify activities where it can efficiently compete in world markets. Cassava has benefited by this new situation.

Through the year 2000 some important developments took place in the area of cassava research and development. The increasing interest of the private (processing or end-user) sector, as well as government agencies, have contributed to consolidating the idea of different industrial uses of this crop. Many of the following highlights are a result of this interesting development.

Consolidation of CLAYUCA.

The Latin American Consortium for Cassava Research and Development was created in 1999. One year after its creation, CLAYUCA consolidated and expanded to include new countries. In the process CIAT and the Consortium learned to work together, complementing their activities and supporting each other. Many special projects are currently carried out jointly. The story of CLAYUCA has been so successful that a similar consortium has recently been created in Africa.

The current trend is that IP3 project at CIAT has concentrated in the biological aspects of cassava, and CLAYUCA is targeting its efforts more towards mechanization, processing and utilization of roots and foliage, and the economic aspects related to those issues. There are many areas where CIAT and CLAYUCA are working together such as: the mechanized harvesting of fresh foliage; the organization of an International Course for 30 people from Latin America and de Caribbean on cassava production and utilization; partnership for the creation of a private enterprise for the production of agents for biological control of insect pests, etc.

An important benefit of the existence of CLAYUCA, for IP3 project, is the creation of a ***functional*** network of private and official partners in the country members. This, in fact, will allow CIAT to deploy the technologies and germplasm it develops through the region with a dynamism, not possible before the creation of the Consortium. As an example of the benefit of this association between CIAT and CLAYUCA, a project for

setting up a bank of *vitroplants* from the most important elite varieties is currently underway. About 200-300 *vitroplants,* from each of 20-30 varieties, will be kept constantly in stock so a relatively large number of vitroplants (up to 50) can be shipped any time they are requested by a collaborator (including requests from Asia and Africa). This is a major improvement over the current capacity, which can deliver only 5 *vitroplants* per request. In addition, sometimes 2-3 months were required until the vitroplants were produced.

Furthermore, the diversity of actors in each member country, demonstrate the renewed interest of both private and official sectors in production and utilization of cassava in different agro-industrial pathways.

Finally, the Consortium concept is, itself, a new model for integrating research, industry and government agencies from different countries. For CIAT this concept was not new, because a similar consortium for rice, called FLAR, had been created before CLAYUCA. However, it is very relevant that the first consortium, independent from CIAT, has been created for cassava, and in Africa. CIAT, CLAYUCA and IITA had collaborated and interacted actively to promote the implementation of this idea in the African continent, which materialized during the second semester of the year 2000. CLAYUCA and CIAT received the visit of representatives from IITA, government and private sector from Africa, early in 2000. The visit covered Colombia and Brazil, and its major objective was to learn how CLAYUCA functioned. Later on, in November, two international staff (from CIAT and CLAYUCA) visited IITA mainly to contribute with the initial steps of the new consortium in Africa.

Cassava Breeding.

The changes in the breeding scheme, described in the 1999 Annual Report, have already been implemented in the field. Preliminary evaluations are very satisfactory. The changes imply a shortening of the length of each selection cycle on one hand, and a more efficient selection procedure on the other.

In the previous system, the first selection in the recurrent selection process, carried out in the agro-ecosystem of adaptation, was based on single plant evaluations (stage called F1C1). A very large number of genotypes were eliminated at this stage, even though it was based only on one plant. An additional problem in the evaluation process was that it took three years (three selection stages) to reach replicated trials. In other words, most of the selection was based on single plot trials and, in one case, on single plant observations. It should be emphasized that the evaluations were and still are carried out in marginal environments with several limiting factors. Under these conditions, lack of uniformity through the trials tends to be remarkably high, resulting in large coefficients of variation. The use of replicated trials, therefore, was envisioned as critical to improve the heritabilities of the traits to be improved.

The current evaluation scheme implies that the first selection stage carried out in the target environment will be done on 7-8 plants, non-replicated plots (instead of single plant plots as before). A second major difference is that the second stage of selection will already incorporate the use of replicated trials.

A potential drawback of the new evaluation system was that the first stage of selection was 7-8 times larger that before (because it employs 7-8 plants per genotype rather than one). It was feared that a large proportion of the genotypes included in this initial stage would be agronomically unfit and lack the adaptation for the conditions of the target environment (because no previous selection had been exerted). Preliminary results, however, are very satisfactory. The large trials have an expected and desirable segregation for several traits, with many progenies already showing very poor characteristics, but in general the mean performances of the trials are acceptable.

Another modification to be introduced in the selection process is data gathering. From now on, data will be taken from each and every plot planted in the field. In the previous system, data was taken only on plants or genotypes that survived the first stage of selection. The resulting inherent lack of balance of the data obtained precluded the utilization of these selection trials for generating genetic information (mainly General Combining Ability) about the parents from which the progenies were derived.

Also described in 1999 Annual Report, was the idea of utilizing our breeding plots to generate quantitative genetics information. As proposed, three large diallel experiments were begun and the plantlets are currently in the field. Very valuable basic genetic information will be generated through these experiments. For the year 2001, therefore, the first evaluation in the three major target environments will be following a diallel structure, allowing for the simultaneous selection of the best performing genotypes, but also generating genetic information. For each target environment, up to ten parents were used to generate the respective F1 crosses. Each F1 cross, is represented by 30 *individual genotypes*, In other words each F1 cross is a full sib family made up of 30, genetically unique, individuals. In turn, each *individual genotype*, will be represented by six plants in the evaluation trials. These trials will be carried out at two locations with three replications at each location.

The changes introduced, therefore, will allow not only a more efficient and effective selection process, but also generate valuable information regarding the inheritance of the traits with agronomic relevance. This in turn should make cassava breeding much more efficient in the near future. Some of the F1 crosses from the diallel analysis, can also serve as a starting point for molecular markers studies.

In the case of Colombia, four varieties, specifically adapted to the Northern Coast were released by CORPOICA by mid 2000. The release of two more varieties for the acid soil, eastern savannas was approved. One of them was released on November 10. The remaining one will be released when adequate amount of "seed" has been produced, tentatively by September, 2001. The process to release yet a seventh variety has

begun. This last variety will be the first release of a genotype with genetic resistance or tolerance to white flies.

Two of the varieties released for the Northern Coast were derived from a participatory cassava breeding approach carried out by CORPOICA with genotypes initially developed by CIAT and then transferred to them. The process has benefited from the inputs and know-how offered by participatory research projects from CIAT.

Finally, authorities from Thailand have kindly authorized the release, in Latin American countries, of the variety Rayong 60 (also known as MTAI 8), that was developed in that country jointly by local and CIAT scientists and shows excellent performance in the sub-humid environments.

New kind of data produced.

As a result of the new demands from the processors sectors, CIAT started gathering data that was not routinely taken before. Traditionally, cassava varieties have been harvested at 10-12 months of age. This precluded the identification of early bulking clones. To fill this knowledge vacuum, the elite clones for each agro-ecoregion, were sequentially harvested starting at about seven months of age. Also, fresh foliage production was measured at each sequential harvest. These data is relevant because it will facilitate the combination of varieties / harvest time, so that the industry can maximize the period of time when cassava raw material is available. It also produces information regarding the potential economic benefit for harvesting the fresh foliage at the time the roots are taken. In some instances, higher economic returns for the farmer may be obtained if harvest takes place when leaves still remain in the plant, even if it means not reaching the highest root yield.

Clones, specifically adapted for the agro-industry and adapted to the most important cassava growing regions, were identified. The excellent performance of these genotypes, both from the agronomic and industrial points of view, was confirmed. In different kind of environments, private industrial initiatives based on the use of cassava as raw material, demonstrated their economic feasibility. Examples are the use of cassava for the feed industry in sub-humid environments, and the pre-cooked frozen croquettes made out of cassava grown in the acid-soil savannas.

Interesting results have been found, particularly in relation to carotene content on roots and leaves. A large number of genotypes have been analyzed for vitamins and mineral contents and post-harvest physiological deterioration or PPD. The short shelf life of cassava roots because of PPD remains one of the most limiting characteristics for the large-scale marketing and use of this crop. Within 24 to 72 hours after harvest, cassava roots start to deteriorate, which renders them unpalatable and unmarketable. This phenomenon severely limits the versatility of the crop. Data obtained suggest that high carotene cassava roots tend to delay the onset of PPD. The advantage of developing high yielding cassava varieties with combined higher nutritive value and delayed or

reduced PPD, is obvious (particularly for certain regions of Africa and Asia where chronic Vitamin A deficiencies result in severe negative effects on human populations). Therefore, cassava genotypes with high carotene in their roots have been included in our breeding plots.

Other additional and important efforts have been carried out with the ultimate objective of reducing the problems associated with PPD. A collaborative project with the University of Bath aims at elucidating the biochemical pathway resulting in the physiological deterioration. Simultaneously we have evaluated the segregation in three different populations specifically developed for better understanding the inheritance of the reaction to PPD. The same populations, but with three plants representing each individual genotype were planted by the end of 1999 and will soon be harvested for evaluation of PPD.

The genetic variability for starch quality traits in the germplasm bank collection was started this year. Because of the increased use of cassava by the starch industry and the interest demonstrated by this important processing sector, the search for new, novel starch types was initiated. Several traits affecting physical and chemical properties are currently being measured in starch samples obtained from a large number of genotypes from the germplasm bank collection. A collaborative project with an important starch industry is close to be reached. The agreement will allow CIAT not only to have resources to continue with this initial evaluation process but also to better understanding of the needs from this important cassava processing industry.

In the area of processed cassava for human consumption CIAT has also established important linkages with the private sector. A company, with world-wide operations, has recently entered into the cassava market. The pre-cooked frozen croquettes industry CONGELAGRO was acquired by McCain Food Limited (a company concentrated mainly in frozen french potato products). Therefore, this excellent product (the croquettes) can now be commercialized world wide through McCain, as long as the final price of the product is reduced. With this goal in mind we have established solid relations with CONGELAGRO-McCain for collaborating both to increase the quality of the raw material and reduce the final cost of the croquettes. Few areas or research have already been identified, for instance: use of yellow roots (with reduced PPD and better appearance when used to make croquettes); the use of controlled atmospheres for delayed onset of PPD; and the industrial evaluation of genotypes reaching the final stages of selection. Research on PPD is critical for this industry. Currently, to avoid PPD, cassava roots are locally peeled, selected, cut into pieces and frozen so that they can be transported to the croquette factory (which in some cases is located only two hours away from the area where the roots had been produced). The long-term impact of this activity is enormous: increased demand of cassava, stabilization of the price, generation of rural employment, etc. Similar contacts are currently underway for another important use of cassava in the human food snacks industry: fried cassava chips.

Development of new markets and new partnerships.

Year 2000 has been a turning point for the development of the industrial cassava concept and for CIAT to learn how to interact with the processing industries. It is evident that all the success stories with large and measurable impact about cassava, involve a *"magic triangle"* made up of farmers, the processing industry, and the research community. A fluid communication and interaction among these three actors is fundamental for a successful experience. CIAT is currently learning to actively promote the formation of as many of these *"triangles"* as possible.

In many situations it has been possible to demonstrate that cassava is a competitive crop that can be efficiently used for different industrial processes. The challenge now is to consolidate these emerging enterprises for the benefit of the cassava-growing farmers. An unexpected outcome of this experience has been that a relatively large proportion of the cassava producers benefited by the industrial use of cassava, have been small, resource-limited farmers, from marginal areas.

In other words, these initial experience suggests that the industrial use of cassava (which usually requires higher-inputs agriculture, to be competitive) benefits not only medium or large farmers, as originally expected, but also resource-poor, small farmers. The beneficial effects have been observed as a promotion for the organization of farmers in producing nuclei and by facilitating extension and access to an informal credit system. The industry, has facilitated the access to inputs that otherwise were not available and, therefore, not used in the past (resulting in low yields and non-competitive prices). Furthermore, extension positions linking farmers with the industry, have been created in many cases. This new actor (the extension agent paid by the industry) has greatly facilitated the access to new technologies by the small farmers that, so far, had not been able to benefit from any of the research achievements already available to them. It also allows for a feedback that eventually can help the cassava breeders better focus the objectives of the improvement projects.

Industrial uses of cassava require important efforts to reduce production costs. Several strategies to reduce production costs were mentioned in the 1999 Annual Report. During the year 2000 and jointly with CLAYUCA, two major approaches have been validated through research experiments: the use of organic fertilizers (chicken and swine manure) and the mechanization of planting and harvest. Several machines have been introduced and modified for the efficient mechanical planting of stakes and the harvest of the roots. Research involves not only the technical evaluation and improvement of the machines and operations, but also the economic and environmental impact of their use. A machine for the mechanical harvest of fresh foliage will also be evaluated. A major achievement for this year has been the building of a pilot plant for the artificial drying of cassava roots and foliage. This plant already demonstrated that an artificial drying process can eliminate the cyanide from the roots, even when originally present in high concentrations. It also allows for determining the cost of artificial drying.

Cleaning CIAT experimental station.

In recent years two major biotic problems rendered CIAT Experimental Station at Palmira almost useless: white flies and frog skin disease. These problems are somewhat related because all the available evidence suggests that the frog skin disease (likely to be induced by a virus-like pathogen) can be transmitted by a white fly *(Bemisia tuberculata).* Due to the high incidence of these problems, data from trials conducted at Palmira had large experimental errors; in some cases experiments had to be cancelled (for instance photosynthesis could not be measured in severely infected white-flies cassava); and the self-imposed quarantine measures implied drastic limitations to the movement of germplasm from Palmira to other regions of Colombia.

Drastic measures were taken to reduce to manageable levels the incidence of frog skin disease, particularly in the cassava germplasm bank, the most likely source of inoculum in previous years. All genotype multiplied at Palmira is going through the process of indexation to be certified to be free of from skin disease. This is achieved through a lengthy procedure, involving grafting with a very susceptible clone. Clean materials are then planted in isolated areas (surrounded by sugar cane plantations) outside CIAT. Because the disease is not transmitted through botanical seed, plants from F1 seed obtained in the crossing blocks were also planted in these "disease-free" locations. Multiplication schemes, to produce large amounts of clean propagules of elite germplasm have also been implemented. This "cleaning process" will continue through the year 2001, when most of the germplasm managed is expected to be already certified to be clean of the pathogen.

In addition, to obtain stakes in plantations outside CIAT Experimental Station, the whole plant is first harvested to make sure that they are symptomless. Therefore, all the stakes obtained come from plants that did not show any symptom of the disease (which can only happen when the plants are not infected, they have been infected late in the season and/or because of genetic tolerance to the disease). Previous experience suggests that this measure alone may be enough for quickly reducing the problem of frog skin disease to manageable levels. Furthermore, data has (and will) be taken to measure the epidemiological effect of this cultural practice in the reduction of the incidence of the disease. In addition, CIAT has contributed with the development of several workshops organized jointly with ICA (Insituto Colombiano Agropecuario) to prevent the dissemination of the disease in other regions of Colombia.

In relation to white flies two strategies are currently implemented. A decision was taken to plant and harvest cassava in the experimental station in such a way as to break the breeding cycle of white flies. This means that for about 30 days, every year, there will be no cassava growing at the Experimental Station (this is likely to occur in April, every year). The second strategy lays in a vigorous and early control of the white flies mainly based on the use of biological control agents. Preliminary results, suggest that this strategy is very promising, as long as it is deployed early, and no insecticide is used. Chemical insecticides can be more damaging to the biological control agent than to the white fly it is meant to control.

Progress in exploiting genetic resistance to pests and diseases.

Already a major pest problem in many cassava-growing areas, white flies have became the subject of intense research efforts. CIAT has found resistance to this pest in a landrace from Ecuador (MECU 72), identified the nature of this resistance (antibiosis), and introgressed it into many elite germplasm varieties. Currently we are identifying molecular markers that will facilitate selection for white fly resistance, in the near future. Furthermore, the first cassava variety with resistance to white flies will soon be released.

A new source of resistance to the African Cassava Mosaic Disease (ACMV), or simply CMV, has been discovered by our colleagues at IITA. That source, comes from a cassava landrace, and is more effective against the disease that the previous source, conferring almost immunity to the plants possessing it. Moreover, from the breeding point of view, it is a much better source of resistance because it is dominant in its expression. This source of resistance was recently introduced into CIAT, and will be soon incorporated into our breeding scheme, through the use of molecular markers (the disease is not present in the Americas until now). Incorporating resistance to CMD into Latin American germplasm is strategic because the vector of the disease has been identified in the continent recently. It is feared that the disease, that so far is absent in the region, can suddenly appear and devastate the cassava crop. In general, cassava germplasm in Latin America is very susceptible to the disease. Molecular markers located very close to the physical position of the resistance gene have been identified.

The traditional emphasis that CIAT has placed into breeding cassava genotypes with capacity to withstand major abiotic and biotic limiting factors continues. The new breeding scheme facilitates this integration between breeding, phytopathology and entomology. Information obtained during the last few years has put IP3 project in the position to start using molecular markers in a routine way. MAS will be implemented first for resistance to CMD and white flies.

Strengthening research network through Latin America.

Through several visits, the training of scientists, and the organization of a 15-days international course, CIAT and CLAYUCA have reinforced a debilitated research network for cassava in Latin America. Such a network is fundamental for quickly introducing the technology developed at CIAT into those countries.

Through several visits, the training of scientists, and the organization of a 7-days international workshop (Vietnam), CIAT has also maintained our relations with Asia and Africa. The association between CIAT and IITA continues to be a key issue for both institutions. This year, several scientists from IITA and businessmen and government officials from Nigeria visited Colombia and Brazil, with the collaboration of CIAT and

CLAYUCA personnel. Also several research projects around cassava genetic improvement are currently carried out or being implemented jointly by IITA and CIAT.

Through this highlights section, a strong emphasis in the exploration of new uses, and the intensification of the industrial processes based on cassava has been given. However, it should be mentioned that the efforts at CIAT for the more traditional use of cassava for the fresh market continues undiminished, with the same conviction and understanding of its relevance for many cassava growing farmers and communities, as before. The exploitation of new opportunities does not mean that CIAT is abandoning the traditional emphasis it has given to cassava grown by small, resource-limited farmers, either for self-consumption, or for the local fresh market.

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IMPROVED CASSAVA FOR THE DEVELOPING WORLD

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

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

Activity 1.1. Field evaluation of segregating progenies for studies on post-harvest physiological deterioration (PPD). Activities related to a collaborative project with the University of Bath (United Kingdom).

Specific Objectives:

a) to continue the evaluation of the progenies of contrasting parental lines for PPD;
b) to increase the number of plants representing each individual genotype for a more reliable measurement of PPD;

Rationale: The short post-harvest storage life of cassava is a characteristic that limits the marketability of the root and necessitates either consumption or processing shortly after harvesting. Post-harvest physiological deterioration (PPD) of cassava roots begins within 24 hours of harvest, and results in crop and product quality losses, high marketing margins and risks, and restricted management flexibility for farmers, traders and processors. The reduction of PPD has been identified as a priority target for strategic research because: **a)** A considerable portion of the production is lost when this requirement is not met; and **b)** This feature of cassava roots impose restrictive and expensive constraints for the marketing of the product.

Materials and Methods: Segregating progenies from contrasting genotypes with high and low reaction to PPD were produced the previous year (Table 1.1). The seeds thus produced were germinated and the resulting plants, transferred to the field for an initial evaluation. Each individual genotype was represented by a single plant, therefore, this initial evaluation was considered to be preliminary. PPD is very difficult to measure appropriately, because of the high influence of environmental factors on the expression of the trait. From each plant five stakes were taken to increase the number of plants representing each genotype.

The results of this study are relevant to activities carried out through a collaborative research study with the University of Bath (Project SB2 at CIAT). The study aims to better understand the biochemical basis and pathways that result in the onset of PPD.

Table 1.1. Progenies derived from crosses with contrasting reaction to post-harvest physiological deterioration. Within parenthesis the number of surviving plants evaluated.

Parental Cross	Number of segregating plants
MDOM 5 x CM 523-7	55 (47)
MDOM 5 x SM 625-5	57 (55)
MDOM 5 x SM 985-9	68 (62)

Results : A preliminary evaluation of these three segregating populations was carried out on two plants per genotype (Table 1.2). A second evaluation, with the three remaining plants per genotype will be carried out in November. For this preliminary evaluation total fresh root yield, dry matter content, total dry matter yield and PPD were measured. The original methodology, based on a visual ranking, was employed for evaluating the PPD response. The method consists in leaving the roots in the field for five days, and then using a scale to score the degree of PPD.

Table 1.2. Results of the first evaluation of segregating progenies in three populations developed to elucidate the inheritance and physiological aspects of post-harvest physiological deterioration.

Parameter	MDOM 5 x CM 523-7				MDOM 5 x SM 625-5				MDOM 5 x SM 985-9			
	Yield		Dry	PPD ¶	Yield (t/ha)		Dry	PPD ¶	Yield (t/ha)		Dry	PPD ¶
	Fresh (t/ha)	Dry (t/ha)	mat. (%)	1 → 4	Fresh (t/ha)	Dry (t/ha)	mat. (%)	1 → 4	Fresh (t/ha)	Dry (t/ha)	mat. (%)	1 → 4
Minimum	1.67	0.54	25.87	1.00	3.33	1.12	26.27	1.00	1.00	0.35	30.43	1.00
Maximum	30.00	11.54	41.19	4.00	28.33	9.53	43.92	4.00	32.67	11.95	41.32	4.00
Mean	12.74	4.62	35.63	2.74	13.46	4.73	34.96	2.28	14.37	5.04	35.06	3.26
St. Deviation	7.02	2.66	2.82	1.15	5.56	1.99	2.78	1.06	6.85	2.47	2.73	0.96
Skewness §	0.55	0.57	-0.91	-0.37	0.42	0.24	-0.14	0.05	0.42	0.61	0.12	-1.13
Median	12.00	4.39	36.31	3.00	14.00	4.75	35.17	2.00	13.33	4.87	35.09	4.00

¶ The score for PPD was 1: very low; 2: low; 3: intermediate; 4: high; and 5: very high.

§ Skewness test ranges from negative values (left tales); to 0.0 (perfect symmetry); to positive values (right tales). Larger magnitudes imply larger asymmetry.

There was wide variability in the response to PPD in the three families evaluated, with scores ranging from very low to high (Table 1.2). The other traits also segregated in a

very ample way. It is interesting to note the close and positive association between dry matter content and PPD. The correlations between dry matter content and PPD were, respectively 0.64, 0.49 and 0.40 for the three families evaluated. Combining the three families (162 data points) the correlation was 0.47. This is an undesirable situation because in general the breeding of cassava puts a high emphasis in increasing dry matter content in the roots, which based on this information will result in an increased susceptibility to PPD. The association between the two traits is illustrated in Figure 1.1 below.

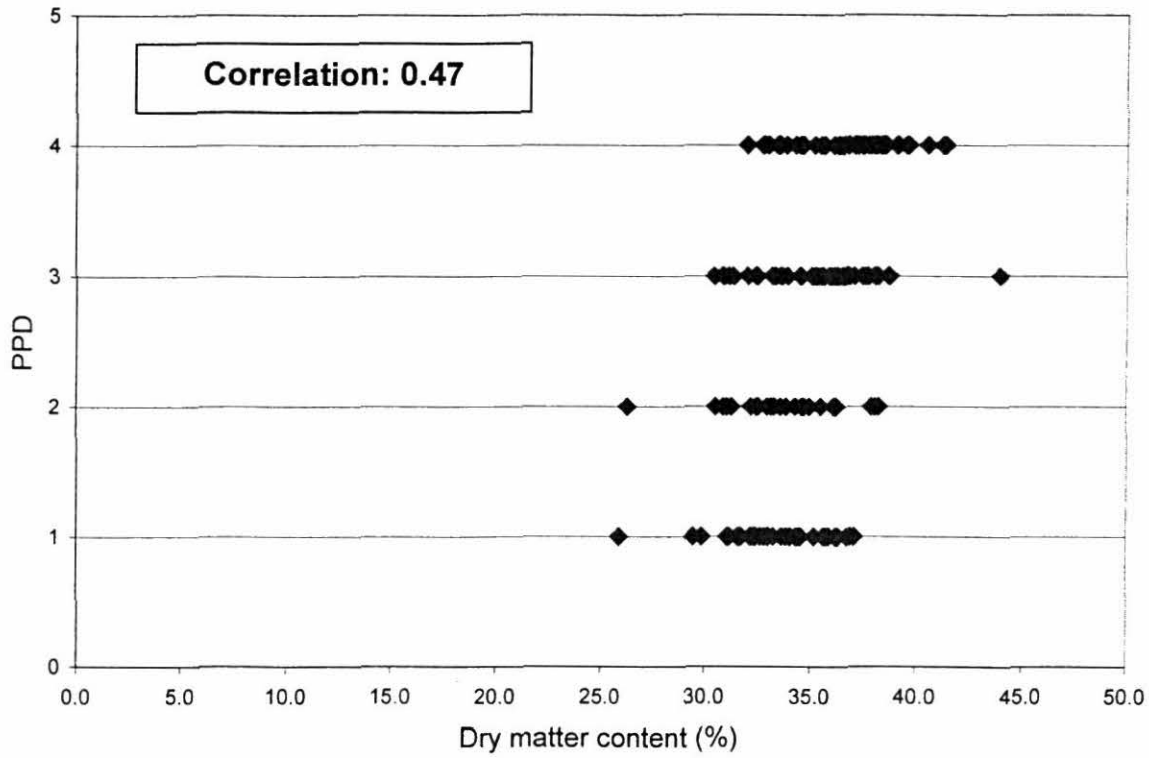


Figure 1.1. Relationship between dry matter content and post-harvest physiological deterioration in cassava roots. Data (166 points) combined from the three families described in Table 1.1.

MDOM 5 had been found to possess good tolerance to PPD in previous evaluations. Because of this characteristic this clone was selected as the tolerant parent in the three crosses described here (Table 1.1). The behavior of this material, however, was far from tolerance. MDOM 5 was included in the evaluation in nine different rows across the experiment. Data from the 18 plants thus harvested show a mean PPD reaction of 3.33 (from intermediate to high), with a range of variation from 3 (intermediate) to 4 (high). Dry matter content was 35.47%. This is a typical situation with PPD. The trait is very difficult to measure satisfactorily, and environmental influence (i.e. variation from year to year) is still high.

Activity 1.2. Evaluation of genetic diversity for vitamins and mineral content in cassava leaves and roots. Collaborative project with IFPRI and the University of Adelaide.

Specific Objectives:

a) *to continue the screening of cassava landraces from CIAT's germplasm collection and a set of elite clones for total carotene and ascorbic acid contents in roots and leaves;*
b) *to correlate total carotene and ascorbic acid contents in both tissues;*
c) *to correlate vitamin contents with physiological post-harvest deterioration.*
d) *to evaluate for the possibility of breaking the linkage between high carotene content and intense yellow pigmentation in the roots.*
e) *to measure mineral contents of a large sample of cassava roots and leaves.*
f) *to search for a group of genotypes with higher protein content in the roots.*

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

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

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

Materials and Methods: Root and leaves samples of plants from several accessions of the cassava germplasm bank, were taken and evaluated for carotene and ascorbic acid contents. In addition, vitamin contents of 100 elite clones were also evaluated. The rationale behind this later evaluation was to identify high yielding clones with high

vitamin concentrations that could be used as donors not only because of their higher nutritive value, but also desirable agronomic performances. Later evaluations concentrated only in carotene content because of the genetic variability observed, the stability of the vitamin content upon processing, and the relevance of vitamin A deficiency in many regions of the world. Samples dry ground leaf or root tissues from about 500 genotypes are currently evaluated for the content of mineral elements, including nitrogen.

Different set of progenies from specific crosses among contrasting genotypes the segregation of other traits were evaluated for post-harvest physiological deterioration, root color pigmentation, and carotene content.

Carotene concentration measurements: the extraction procedure outlined by Safo-Katanga et al. (1984) was adjusted by extracting root parenchyma with petroleum ether. The extraction protocol for leaves had to be modified due to the presence of tannins and chlorophyll. The adjusted protocol included several washing steps with methanol in order to minimize the interference from the other pigments that were present in the leaves. A sample of 5 g was taken out of the root or leaves, taken at random 10 to 11 months after planting. The quantification was done by ultraviolet spectro-photometry using a Simadzu UV-VIS 160A recording spectrophotometer. UV detection was done at λ = 455nm for root extracts and at λ = 490nm for leaves extracts.

Ascorbic acid concentration measurements: the protocol for the determination of ascorbic acid by Fung and Luk (1985) was adjusted for cassava leaf and roots taking as base the procedure outlined and involved the following steps:

a) Homogenization of 1 g of fresh leaves or 6 g of fresh roots in a turrax with 20 ml of extraction buffer (3% phosphoric acid and 8% glacial acetic acid).
b) Centrifugation for 5 minutes at 10 °C and 3000 rpm.
c) Separation of supernatant and vortex of 1 ml of the extracts with 2 ml of 10% hydrochloric acid. Reading was taken immediately with an UV-VIS spectrophotometer. UV detection was done at λ = 245nm. Quantification was done using previously decomposed extract with 1M sodium hydroxide solution as blank. During the whole process, samples were protected from air to avoid oxidation.

PPD measurement: post-harvest physiological deterioration was measured (six days after harvest) on 30 genotypes whose ascorbic acid and carotene root concentrations were known.

Color pigmentation in the roots: a visual scale of nine points was developed for visually scoring the intensity of root pigmentation, and the corresponding cards were printed for the "field study".

Mineral concentration: of leaf and root tissue were prepared by first drying the samples and then grinding them finely. The samples were sent to the University of Adelaide for mineral content determination. **Fe, Mn, B, Cu, Zn, S, Ca, Mg, P, K,** and **Na** are routinely measured using acid digestion and Inductively Coupled Plasma - Atomic Emission

Spectrometry (ICP-AES). Mo, Co, Ni, & Cd are reported but are often below the detection limit of the current instrument. The digestion method used means that Al is reported as an uncertified value. **Total N** is analyzed by Complete Combustion Gas Chromatography.

1.1.a Results of the Evaluation of Genotypes from the Germplasm Bank.

A total of 400 accessions of the cassava world germplasm bank collection held at CIAT, were evaluated for the traits described. These genotypes are do not belong to the core collection which was evaluated the previous year. In a few cases, because plants did not grow adequately or did not develop roots, measurements could not be taken. Those missing data points from previous analyses were revisited during this year. Likewise, missing points from the current evaluations will be completed later. The origin of the accessions evaluated is described in Table 1.3.

Table 1.3. Origin and number of accessions from CIAT's Non-core Collection evaluated for Vitamin C and Carotene in roots and leaves[¶].

Origin	**Number of Accessions Evaluated**
CIAT CM Clones	72
CIAT SM Clones	12
CIAT SG Clones	6
CIAT Thailand Clones	1
CIAT CG Clones	5
Argentina	1
Brazil	137
Colombia	89
Costa Rica	3
Ecuador	2
Guatemala	1
Indonesia	7
Malaysia	2
Mexico	3
Panama	3
Paraguay	10
Peru	33
Philippines	2
Venezuela	11
TOTAL	**400**

[¶] Samples of some accessions could not be obtained or measured because missing plants, small roots and/or damaged roots.

Ascorbic acid concentration in leaf tissue ranged from 6.60 to 487.09 mg/100g FW (Table 1.4), with a mean concentration of 132.22 mg/100g FW and a standard deviation of 71.52 mg/100 g FW. Concentration of ascorbic acid in the roots ranged from 4.88 to 71.19 mg/100 g FW of fresh roots (Table 1.4), showing a mean of 31.37 mg/100 g FW, and a standard deviation of 12.16 mg/100 g FW. Both distributions showed a strong skewness, with values concentrating on the left, and long right tails. In this type of distribution the median is a better measurement of central tendency than the arithmetic mean. The results indicate higher concentration of ascorbic acid for this sample (particularly for roots) than those observed in the core collection, reported last year.

It is obvious that ascorbic acid concentrates on the leaves, rather than in the roots. There was no correlation between the ascorbic acid concentration on leaves and roots (ρ = -0.08, based on 400 data points, Table 1.6).

Table 1.4. Ascorbic acid concentration in leaves and roots of 400 non-core cassava accessions from CIAT's Germplasm Bank Collection.

Data from leaves		**Data from roots**	
Range (mg / 100 g FW)	Frequency	Range (mg / 100 g FW)	Frequency
0.0 – 45.0	35	0.0 – 7.0	4
45.1 – 90.0	86	7.1 – 14.0	18
90.1 – 135.0	109	14.1 – 21.0	53
135.1 – 180.0	85	21.1 – 28.0	101
180.1 – 225.0	46	28.1 – 35.0	82
225.1 – 270.0	26	35.1 – 42.0	70
270.1 – 315.0	7	42.1 – 49.0	33
315.1 – 360.0	3	49.1 – 56.0	26
360.1 – 405.0	0	56.1 – 63.0	7
405.0 – 450.0	2	63.0 – 70.0	5
> 450.0	1	> 70.0	1
Minimum	6.60	Minimum	4.88
Maximum	487.09	Maximum	71.19
Median	123.09	Median	29.83
Skewness†	1.01	Skewness†	0.48
Mean	132.22	Mean	31.37
St.Dev.	71.52	St.Dev.	12.16

† Skewness test ranges from negative values (left tales); to 0.0 (perfect symmetry); to positive values (right tales). Larger magnitudes imply larger asymmetry.

Table 1.5. Carotene concentration in leaves and roots of 400 non-core cassava accessions from CIAT's Germplasm Bank Collection.

Data from leaves		Data from roots	
Range (mg / 100 g FW)	Frequency	Range (mg / 100 g FW)	Frequency
0.0-9.0	0	0.000-0.090	0
9.1-18.0	3	0.091-0.180	121
18.1-27.0	20	0.181-0.270	182
27.1-36.0	89	0.271-0.360	40
36.1-45.0	100	0.361-0.450	23
45.1-54.0	108	0.451-0.540	14
54.1-63.0	58	0.541-0.630	4
63.1-72.0	18	0.631-0.720	4
72.1-81.0	2	0.721-0.810	8
81.1-90.0	0	0.811-0.900	3
> 90.0	2	> 0.900	1
Minimun[§]	12.05	Minimun[§]	0.12
Maximun	96.42	Maximun	0.93
Median	43.99	Median	0.20
Skewness[†]	0.41	Skewness[†]	2.50
Mean	44.47	Mean	0.25
St.Dev.	11.77	St.Dev.	0.14

[†] Skewness test ranges from negative values (left tales); to 0.0 (perfect symmetry); to positive values (right tales). Larger magnitudes imply larger asymmetry

Carotene concentration on leaves ranged from 12.05to 96.42, with a mean of 44.47 and a standard deviation of 11.77 mg / 100 g FW (Table 1.5). As in the case of vitamin C, carotene distribution was also skewed to the left, but to a lesser degree. Carotene concentration on roots showed a strongly skewed distribution to the left, ranging from 0.12 to 0.93, and a mean of 0.25 and a standard deviation of 0.14 mg / 100 g FW. Following the same trend observed with ascorbic acid, carotene concentrated much more on leaves than in roots, illustrating once again the excellent nutritive value of cassava leaves. There was no correlation between carotene concentration (-0.05) on leaves and roots (Table 1.6). Data from Tables 1.4 and 1.5 are illustrated in Figure 1.2.

Correlations among vitamins contents and PPD.

In the evaluation of the core collection (1999 Annual Report) there was no correlation between vitamin contents in different organs. The results from the current report also support the independence of vitamin C and carotene concentrations, as well as the independent accumulation of those vitamins in leaves and roots (Table 1.6).

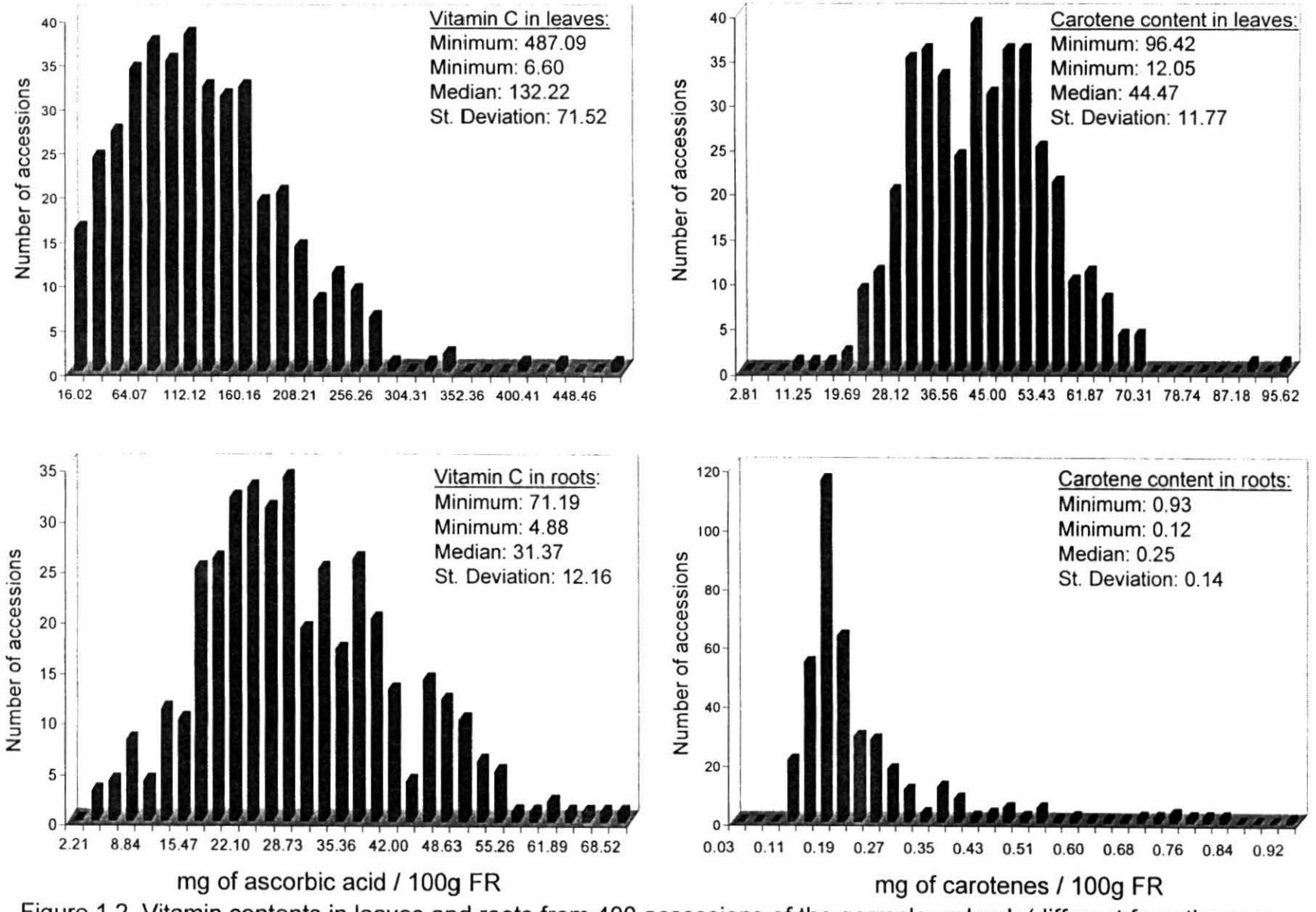


Figure 1.2. Vitamin contents in leaves and roots from 400 accessions of the germplasm bank (different from the core collection.

Table 1.6. Correlations between ascorbic acid and carotene content in leaves and root tissues of 400 genotypes from CIAT's cassava germplasm collection.

	Vitamin C (Roots)	Carotene (Leaves)	Carotene (Roots)
Vitamin C in Leaves	-0.08	0.17	-0.09
Vitamin C in Roots	-.-	-0.20	-0.02
Carotene in Leaves	-.-	-.-	-0.05
Post-harvest Physiological Deterioration	0.47	-.-	-0.16

Correlation between PPD and vitamin C and carotene contents on roots were, respectively, +0.47 and –0.16. It was surprising to find a positive correlation between physiological deterioration and vitamin C content in the roots. This positive correlation between vitamin C and PPD, contrasts drastically with the previous finding of a negative one (-0.30). This contrast may be due to the accumulated errors in measurements for both vitamin C and PPD, or to genetic differences between the genotypes of the core collection and those reported herein.

The relationship between PPD and carotene content seems to be more consistent and was almost identical to the one reported previously (-0.169 vs. –0.160). Therefore, the hypothesis that carotene content can help to reduce PPD (through their antioxidant capacity) is supported again by these results. Furthermore, the graph plotting the relationship between carotene in the roots and PPD (Figure 1.3) suggests that above 50 mg carotene / 100 g FW, PPD does not exceeds 30%. At lower carotene concentrations, the association is lost finding large variation in PPD that cannot be accounted for by carotene content. Although the plot in Figure 1.4 illustrates the relationship suggested by the correlation coefficient = 0.465, the association between vitamin C content and PPD remains unclear.

1.1.b Results of the Evaluation of Elite Genotypes.

A sample of about one hundred elite clones maintained at CIAT because their excellent performance in different agro-ecological zones was also evaluated for their vitamin contents. The reason for this evaluation, as stated above, was to search for a high nutritive value in genotypes that also offer excellent agronomic performance and know adaptation to a particular set of cassava growing environments. Because of their relevance the actual data from each of those elite varieties are presented in Table 1.7.

Figure 1.3. PPD vs. carotene content on the roots

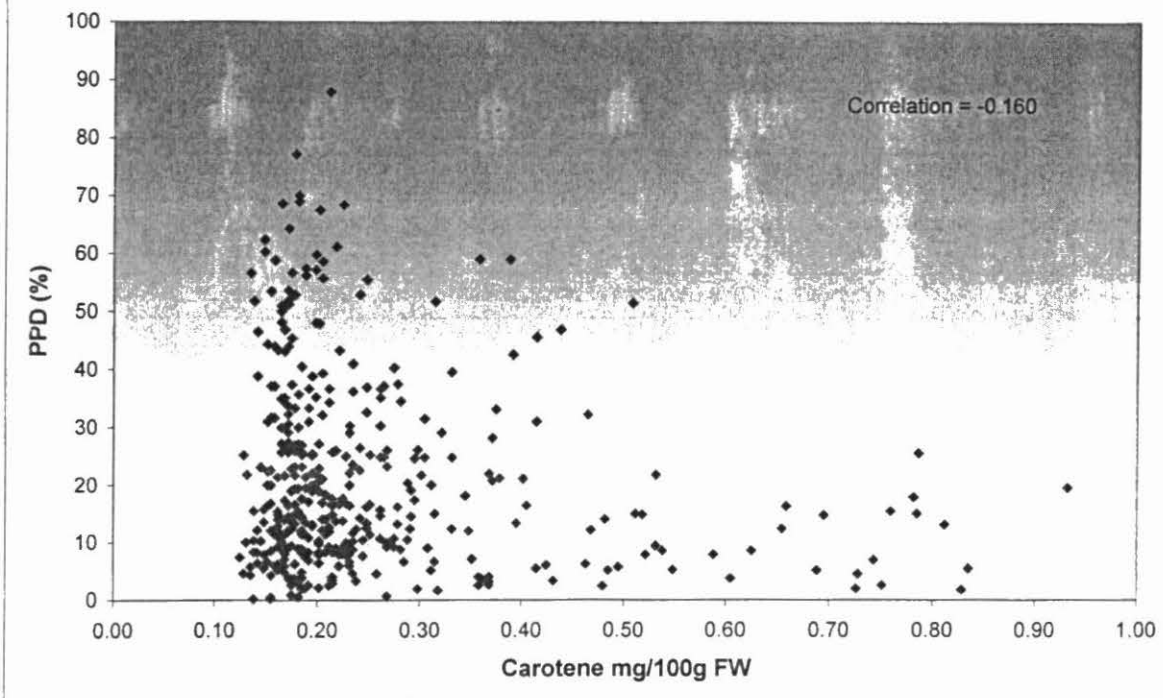


Figure 1.4 PPD vs. viitamin C

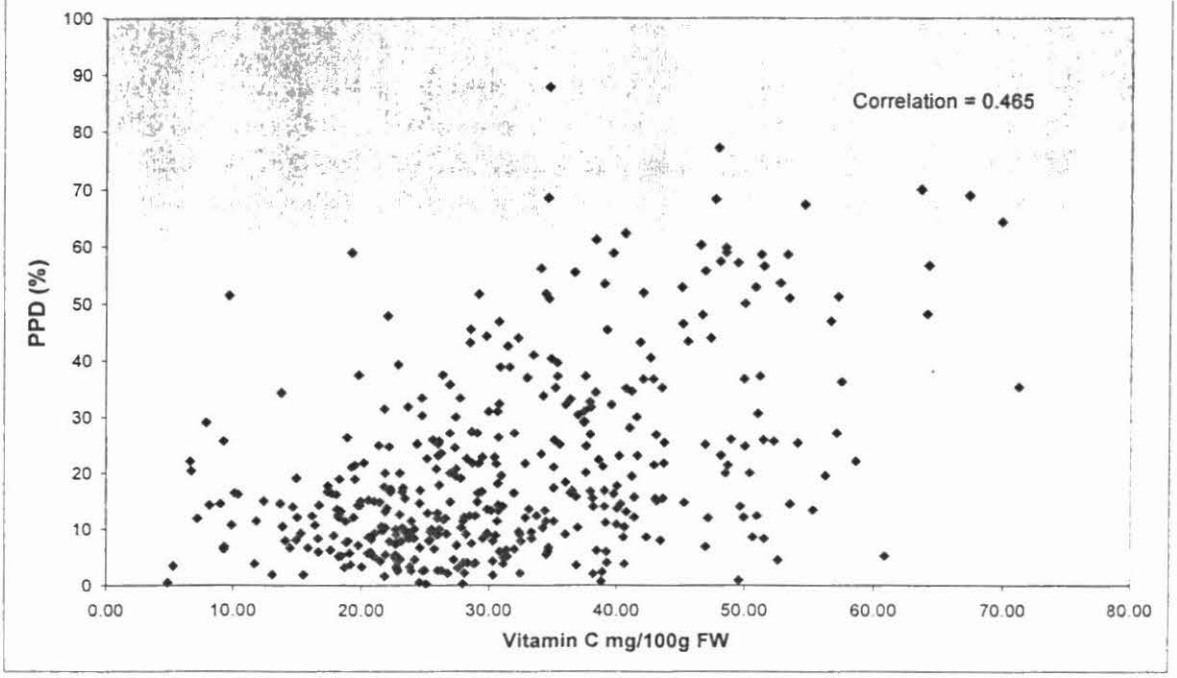


Table 1.7. Carotene and ascorbic acid contents in roots and leaves from 100 elite clones, and their reaction to physiological post-harvest deterioration (PPD).

Genotype	Ascorbic Acid (mg / 100g MF)		Total Carotene (mg/100g MF)		PPD
	In leaves	In roots	In leaves	In roots	(%)
M BRA 12	64.64	11.69	41.21	0.27	57.50
SM 1647- 1	125.91	16.12	33.35	0.29	5.95
M COL 1468	56.18	17.70	32.50	0.21	15.95
M COL 1684	71.50	21.22	37.68	0.46	26.43
M VEN 77	114.22	30.01	38.42	0.25	46.29
HMC 1	42.47	22.29	33.64	0.31	83.10
CM 6787- 4	186.38	14.51	41.40	0.26	46.90
CM 7514- 7	125.11	11.79	38.27	0.28	61.67
CM 7514- 8	97.70	21.56	36.07	0.19	67.62
CM 7686- 5	81.57	14.68	44.96	0.38	48.33
CM 7951- 5	129.95	13.33	47.83	0.25	25.71
CM 8024- 2	230.32	7.09	51.62	0.29	20.71
CM 8151- 1	93.26	19.68	31.10	0.29	24.29
SM 909-25	60.61	9.34	36.54	0.28	37.86
SM 1406- 1	219.03	7.93	33.35	0.25	24.76
SM 1407- 3	50.13	11.93	31.66	0.25	43.81
SM 1479- 8	159.37	14.81	40.62	0.33	14.29
SM 1513- 2	99.71	9.74	39.01	0.23	11.90
SM 1557-17	133.57	12.09	46.14	0.31	20.24
SM 1645- 1	117.85	14.64	57.68	0.27	11.90
SM 1669- 5	105.76	16.76	36.07	0.17	12.62
SM 1690-13	75.93	16.25	29.74	0.15	42.86
SM 1741- 1	158.16	14.71	27.47	0.20	13.81
CM 5438-12	172.67	25.45	24.23	0.19	25.00
CM 6740- 7	74.72	17.26	30.24	0.23	27.38
CM 8224- 2	92.46	18.67	39.63	0.31	4.86
CM 8370-11	97.70	15.01	34.32	0.29	4.76
CM 8370-14	104.95	16.49	42.65	0.21	23.81
CM 8378- 3	93.26	15.45	24.19	0.21	4.76
CM 8383- 7	81.57	14.34	16.86	0.25	7.62
CM 8475- 4	22.72	17.23	27.20	0.29	8.10
SM 1533- 3	42.47	15.31	33.21	0.19	21.43
SM 1543-16	79.56	19.68	32.99	0.20	10.95
SM 1557-27	74.72	14.24	25.53	0.21	25.95
SM 1559-28	92.86	18.44	41.58	0.23	29.71
SM 1583- 8	76.33	24.38	48.05	0.23	22.62
SM 1602-13	97.70	19.95	31.73	0.21	26.19
SM 1636-24	131.96	16.39	58.89	0.23	26.90
SM 1642-20	125.51	12.76	66.39	0.27	20.00
SM 1642-22	51.74	16.42	48.09	0.22	28.81

Table 1.7 cont.

Genotype	Ascorbic Acid (mg / 100g MF)		Total carotene (mg/100g MF)		PPD (%)
	In leaves	In roots	In leaves	In roots	
SM 1645- 6	165.82	18.07	48.16	0.16	17.38
SM 1657-14	215.40	28.00	49.04	0.19	38.33
SM 1660- 4	258.94	22.26	49.52	0.18	32.14
SM 1665- 5	276.27	22.16	53.34	0.19	29.52
SM 1665-13	258.53	16.79	48.75	0.18	38.81
SM 1673-10	226.29	20.68	44.70	0.17	38.10
SM 1772-16	384.70	16.42	44.23	0.26	18.81
SM 1754-46	277.48	21.79	32.94	0.19	4.52
SM 1754-51	253.29	20.95	42.35	0.34	23.81
SM 1775-22	268.61	12.46	59.96	0.17	38.57
SM 1778-44	243.62	22.36	40.48	0.20	6.19
SM 1779- 9	180.74	15.62	36.60	0.19	20.71
SM 1780-27	333.11	25.45	52.75	0.16	63.33
SM 1781-17	339.15	14.48	52.20	0.19	35.00
SM 1781-28	348.83	18.33	41.54	0.21	37.38
SM 1783-31	202.50	15.11	42.02	0.16	23.10
SM 1788-11	248.46	15.25	48.20	0.16	28.10
SM 1812-55	181.14	13.23	45.77	0.22	32.86
SM 1854-17	259.74	11.96	42.87	0.16	31.43
SM 1855-21	230.32	17.66	57.65	0.16	5.71
SM 1868-29	190.01	16.46	27.13	0.57	40.86
SM 1870-24	152.92	13.84	34.96	0.60	11.67
SM 1893-39	151.31	19.14	35.66	0.66	42.38
SM 1896- 3	62.63	16.76	28.72	0.57	36.29
SM 1902- 3	121.88	10.55	52.57	0.59	13.81
SM 1948-29	146.07	15.01	16.89	0.63	16.90
SM 1953-30	184.77	14.44	25.61	0.59	22.62
SM 1955- 6	79.96	14.95	21.14	0.63	25.24
SM 1973-23	125.51	14.98	30.24	0.56	34.76
SM 2069- 2	81.17	13.23	20.53	0.57	22.14
MMEX 108	71.50	10.52	37.13	0.60	8.33
CM 8151- 1	125.91	18.23	28.23	0.56	17.14
CM 8370-10	123.50	13.87	30.68	0.57	31.19
CM 8658-1	219.43	23.64	38.86	0.58	10.24
SB 0216- 9	138.01	20.85	20.24	0.60	46.86
SM 1143-21	90.84	19.74	41.62	0.61	32.14
SM 1144-10	113.42	10.18	37.83	0.55	15.95
SM 1225-15	140.02	10.92	30.13	0.58	23.57
SM 1517- 9	103.74	15.38	30.97	0.56	38.57
SM 1519- 2	140.02	14.78	35.99	0.55	16.43

Table 1.7 cont.

Genotype	Ascorbic Acid (mg / 100g MF)		Total carotene (mg/100g MF)		PPD (%)
	In leaves	In roots	In leaves	In roots	
SM 1657-7	153.73	18.40	66.10	0.17	40.57
SM 1657-12	81.17	24.01	42.54	0.18	13.10
SM 1778-53	155.34	24.91	47.06	0.20	22.38
SM 1788-16	105.36	26.56	51.84	0.16	24.76
SM 1789-20	96.08	23.54	49.63	0.16	10.95
SM 1789-49	178.72	25.62	49.70	0.16	20.00
SM 1822-12	181.94	18.20	44.56	0.18	36.90
CM 2967- 8	158.97	20.62	43.23	0.20	67.71
CM 4365- 3	127.53	30.25	44.26	0.16	38.81
CM 6182- 8	112.21	29.78	38.38	0.16	44.57
CM 6858-3	86.81	21.02	51.62	0.16	39.71
CM 7395- 5	154.13	22.80	48.90	0.16	59.05
CM 7514- 8	54.57	19.58	41.76	0.24	67.43
SM 643-17	100.52	21.15	52.20	0.45	38.57
SM 805-15	137.20	24.34	47.20	0.23	29.43
SM 1201-5	59.81	25.01	25.53	0.20	29.29
SM 1411- 5	50.13	22.70	43.53	0.20	16.57
SM 1433- 4	56.98	22.06	47.46	0.33	30.71
SM 1438- 2	88.02	25.62	49.34	0.41	42.86
SM 1460- 1	23.53	29.95	50.73	0.15	42.29
Maximum	384.70	30.25	66.39	0.66	83.10
Minimum	22.72	7.09	16.86	0.15	4.52
Median	125.31	17.01	40.92	0.23	26.07
Skewness†	1.03	0.38	0.03	1.10	0.84
Mean	139.91	18.00	40.06	0.30	28.70
Standard Dev.	76.22	5.17	10.43	0.16	16.10

† Skewness test ranges from negative values (left tales); to 0.0 (perfect symmetry); to positive values (right tales). Larger magnitudes imply larger asymmetry.

Ascorbic acid in leaves ranged from 22.72 to 384.70 mg / 100 g FW, with a mean of 139.91 and a standard deviation of 76.22 mg / 100 g FW. These values are similar to those presented in Table 1.4. The concentration of this vitamin in roots was also similar to the data presented in Table 1.4, although the highest concentration observed in the germplasm bank (71.19 mg / 100 g FW) could not be matched by the elite varieties whose highest value was 30.25 mg / 100 g FW.

Carotene content in leaves from the elite germplasm ranged from 16.86 to 66.39 mg / 100 g FW, with a mean of 40.06 and a standard deviation of 10.43 mg / 100 g FW. The highest value observed was smaller than that of the germplasm bank sample (96.42 mg / 100 g FW), but the mean and standard deviation values are approximately similar (Table 1.5). In the case of roots, the situation is the same, with mean and standard deviations similar to the values presented in Table 1.5. However, the highest value in the elite clones (0.66 mg / 100 g FW) could not match that of the sample from the bank (0.93 mg / 100 g FW).

The results from the elite clones are as expected. The highest nutrient concentration observed in these varieties could not reach the levels obtained in genotypes from the

germplasm bank. Just the size of the two samples would justify this situation. However, the highest values were already reasonable to the point that they can be considered in the process of introducing the high vitamin cassava trait into commercial varieties for different areas of the world.

1.1.c. Segregation study to brake the linkage between high carotene content and intense yellow coloration in the roots.

Several crosses have been made between yellow and white rooted cassava varieties. In some cases, it is desirable to brake the apparent linkage between high carotene and intense yellow coloration on the roots (Graham et al., 1999). During the period reported herein, 397 progenies from such crosses were evaluated. Table 1.8, summarizes the results from these analyses. Color intensity was estimated using a 1 to 9 visual scale (1= white; 9 = intense yellow –orange roots). As already found, there was a strong correlation between carotene content and color intensity (the correlation coefficient across the 395 genotypes was 0.81). As expected, most of the families exhibit strong correlation coefficients (above 80%). However, families CM 9733–9, and CM 9712–9 each with more than 15 plants had low or negative correlations (–0.202 and 0.673, respectively). Other families had interesting results but their performance were based on few plants (i.e. families CM 9149 – 2, CM 9150 – 1, and CM 9684 – 5).

Based on these preliminary results a few families were selected and, from each plant making up the family, five stakes were obtained and planted for future replicated evaluations to be carried out later in, 2000.

Table 1.8. Families evaluated in the segregation study aiming at breaking or reducing the linkage between high carotene and yellow color in cassava roots.

Family	No. of plants in the family	Carotene Content (mg/100g FW)	Color intensity (1-9)	Carotene/color correlation
CM 7062 - 7	6	0.225	2.167	-0.486
CM 9149 - 2	2	0.296	2.500	-.-
CM 9150 - 1	1	0.296	2.500	-.-
CM 9153 - 9	10	0.291	2.600	0.851
CM 9249 - 9	40	0.486	4.450	0.841
CM 9629 - 9	17	0.330	3.824	0.886
CM 9679 - 9	44	0.387	3.818	0.844
CM 9680 - 9	50	0.307	3.220	0.860
CM 9681 - 9	29	0.304	3.897	0.960
CM 9683 - 9	56	0.257	3.036	0.825
CM 9684 - 5	5	0.251	1.800	-0.559
CM 9712 - 9	50	0.327	3.440	0.673
CM 9714 - 9	50	0.310	3.900	0.923
CM 9731 - 9	20	0.171	2.600	0.237
CM 9733 - 9	17	0.174	2.176	-0.202

1.1.d. Strategies to deploy high carotene cassava varieties.

Finding high vitamin cassava varieties is totally irrelevant if this trait does not benefit the farmers and end-users of the product. At this point it is clear that high-carotene content is a trait that can be exploited to reduce the problems related to its deficiency in human populations. High carotene roots, however, tend to have a strong yellowish-orange coloration. Some efforts are currently underway to get high-carotene cassava varieties closer the people that need it the most:

1. Yellow roots will have a strong preference in some parts of Africa. However, the presence of the viral disease African Cassava Mosaic Virus (ACMV) limits the usefulness of Latin American germplasm in that continent (the disease is not present in Latin America, and the germplasm from the region lacks resistance to it). In a collaborative effort between IITA and CIAT, yellow, low cyanide potential varieties from Latin America, will be crosses with outstanding sources of resistance to ACMV from Africa.

2. In areas where white roots are preferred in the market, there will be a need to break the linkage between high-carotene and intense yellow coloration. The crosses made between white and yellow cassava varieties and the evaluation of their segregating progenies aim at that particular situation, hoping to find some genotypes that show high carotene content with a reduced color intensity.

3. One other strategy to follow is to introduce new products in the market that will prefer the use of yellow cassava roots. Fried cassava chips for the snacks market would be one of those situations. The evaluation of materials for this market with the private sector has been arranged and will be started this year. The high-value added product, however, is likely to be too expensive for the problem of carotene deficiency.

4. Cassava varieties with roots possessing high carotene contents are very important for the feed industry (particularly those products for the poultry industry). In some areas these varieties can be initially adopted to satisfy the need for this industry. But, eventually, they may be spontaneously accepted for direct human consumption. One way or another, however, the carotene initially produced by the plant will reach the human population.

1.1.e. Mineral content of cassava root and leaf tissue.

About 1200 samples of root and leaf tissue were sent to the University of Adelaide in Australia for the determination of their mineral contents. Therefore, information from about 600 genotypes will be available for those minerals measured. At this point, the purpose of the study is merely exploratory to evaluate the genetic variability for those minerals concentrations in a large sample of genotypes. Since data for the corresponding values of vitamin content and PPD will also be available, correlations among these traits will be measured to detect potential linkages that could either facilitate, or make future breeding work more difficult.

It is expected that by the end of the year the results from the entire batch or a large proportion of it will be available.

Activity 1.3. Evaluation of the stability of vitamins and mineral content after processing.

Specific Objectives:

a) *To measure vitamins content after a processing procedure very common in Sub-Saharan Africa (preparation of Gari).*
b) *Upon the availability of a pilot artificial drying plant, to process high carotene cassava roots to measure the actual amount of carotene remaining after the drying process.*

Rationale: cassava is processed before consumption using different treatments that can eventually affect the ascorbic acid and carotene contents. Solar (or oven) drying of cassava flour, and cooking fresh roots for several minutes are common processing procedures used by diverse cultures and for different end uses of the product. The stability of vitamins using these procedures was measured and reported previously (1999 Annual Report). One of the findings was that vitamin C was not as stable as carotene, which in turn motivated this study to concentrate in the latter.

A very common way to consume cassava in Africa is as *gari*. In the preparation of Gari, the roots are washed, peeled and ground. The resulting mass is then put in bags with some pressure exerted on them (by some weight such as a piece of wood or a rock), to extract part of the water originally present on the roots. Since this process takes some days, fermentation occurs. Later the mass is toasted or fried (frequently with oil palm) until it dries. The fermentation gives *gari*, its very peculiar flavor and taste.

Because of the popularity of *gari* in Africa, and the prevalence of carotene deficiency in this continent, the stability of carotene after roots are processed to produce it is relevant to determine the actual potential of high-carotene cassava varieties. The study will be initiated during the second semester of 2000 taking advantage of the presence of native Nigerians who can help in the process to produce *gari*. Roots from few clones, specifically selected for their carotene content on will be processed and the resulting gari evaluated for their carotene content as well as for their taste and presentation.

An additional research, perhaps not directly related to human nutrition, but relevant for the deployment of cassava varieties with high-carotene content in the roots, is the evaluation of carotene stability upon artificial drying. In several tropical countries there has been an increased interest in the use of cassava products for animal feed. An important related activity has been the development of a pilot artificial drying plant. This

plant is fed with cassava roots (or leaves), that are ground and then dried with hot air. It will be used for measuring the amount of carotene remaining after the drying process. It is likely that this product will be very attractive, particularly for the egg producing industries. As mentioned above, the deployment of yellow rooted cassava varieties for the final use by the poultry industry may popularize these kind of varieties, otherwise not preferred in the market.

Activity 1.4. Screening of germplasm bank and elite cassava clones for novel starch forms.

Specific Objectives:

a) To measure other root quality traits in addition to vitamin contents.
b) To increase the interaction with the private sector to explore new avenues for cassava starch and other cassava products utilization.
c) To carry out basic research for better understanding the factors influencing the baking quality of fermented cassava starch.

Rationale: CIAT is currently evaluating germplasm bank and elite cassava clones for vitamins content. Obtaining the samples constitutes a major component for the costs of this particular project. Also the cryopreservation research currently conducted at the Center may allow us in the near future to stop growing the large germplasm bank in the field season after season. It was, therefore, decided to take advantage that tissue samples were being obtained to extract more information from those samples. The kind of data that is or will be taken includes: **1)** dry matter content; **2)** total carbohydrates content; **3)** amylose and amilopectin contents; **4)** starch content; **5)** sugar content; **6)** starch paste clarity; **7)** pasting properties; **8)** resistance to acid media; **9)** resistance to freezing; **10)** syneresis; **11)** swelling power and solubility of granular starches; **12)** starch bound phosphates; and **13)** quantitative measurement of cyanide content.

The food industry saw the need for classifying cassava starch according to the final use of the product. However, a satisfactory quality standard has not been developed. Some early results suggested that fermented starches with higher absorption of water were produced better results in the baking industry.

Materials and Methods: Most of the traits are measured in CIAT's Laboratory at Rural Enterprises. However, for at least one variable (starch bound phosphates), further training and equipment will be required for the proper evaluation. The same germplasm evaluated for vitamins and PPD will also be evaluated for these traits. The methodology to be used for each trait is the standard published in the literature and will not be described herein.

For the quality experiments on fermented starches, CIAT is trying to develop a simple non-expensive, reliable and reproducible method for determining absorption index, solubility, swelling power and their correlation with baking expansion (specific volume) and other functional properties such as gelatinization temperature, viscosity, etc.

Evaluations are carried out at 30, 60 and 90 °C, with a starch suspension of 0.042 % (w/v), maintained for 30 minutes at the corresponding temperature. Samples are then centrifuged at 4900 rpm for 15 minutes. A sample is taken from the supernatant to determine absorption capacity and swelling power. Four replications were used for each source of starch.

Results: a proposal to National Starch & Chemical Company (U.S.A.) for exploring the genetic variability of cassava starch and roots properties is currently under revision. Recently CIAT send a revised proposal for an agreement. It is hoped that this private company will support the evaluation of the entire germplasm collection (≈ 6000 accessions). Additionally the hundred elite genotypes have been evaluated for starch traits as described previously (1999 Annual Report). There are indeed good evidences that ample genetic variability exist for cassava root traits as to justify further evaluations of the germplasm collection.

Novel starch types are strategic for any crop used as a source of starches. The world market for starches, and the proportion of cassava used for starch extraction (particularly in Asia) are growing rapidly. Additionally, chemical modifications of starches are currently avoided as much as possible, because of economic and public concerns matters. Therefore, it is important to evaluate the genetic variability present in the *Manihot* genera for starch quality traits, aiming at eventually taking advantage of it.

Table 1.9. Starch quality data from 558 measurements on 512 genotypes (46 duplicates) from the germplasm bank collection and elite varieties.

Parameter	Leaves		Roots						
	Dry matter (%)	HCN (ppm)	Dry matter (%)	HCN (ppm)	Total sugars (%)	Reduc. sugars (%)	Total starch (%)	Amilose content (%)	PPD (%)
Minimum	16.86	0.00	18.18	13.97	0.23	0.01	72.00	10.29	0.00
Maximum	43.68	3102.9	57.23	2070.3	9.05	7.00	89.00	19.93	95.71
Mean	31.07	696.2	37.28	213.9	2.07	0.61	83.47	14.93	20.83
St. Deviation	4.11	393.79	4.83	274.64	0.90	0.45	2.60	1.43	16.58
Skewness[†]	0.29	1.49	-0.33	3.08	1.53	6.10	-0.54	0.12	1.31
Median	30.50	634.5	37.55	109.9	1.90	0.55	84.00	14.89	15.12

[†] Skewness test ranges from negative values (left tales); to 0.0 (perfect symmetry); to positive values (right tales). Larger magnitudes imply larger asymmetry.

Table 1.9 summarizes the results found in the initial evaluation of 512 genotypes. Dry matter and cyanogenic potential (HCN) were measured both in roots and leaves. Roots were also evaluated for total starch and sugar content. From the observed total of sugars, the fraction corresponding to reducing sugars was measured. Similarly, from the total amount of starches recovered from the roots the amount corresponding to amylose was measured, and by subtraction the amount of amylopectin can be calculated. This

initial evaluation suggests good genetic variability for amilose content (ranging from 10.29 to 19.93 %), total sugars (ranging from 0.23 to 9.05%), and in fact, for all the variables analyzed.

Figure 1.5 illustrates the relationship between dry matter content and post-harvest physiological deterioration. The correlation between the two traits was positive (0.42), that is, roots with lower dry matter content tend to have a slower onset of the deterioration process. An unfortunate association, indeed. Data from PPD varies considerably from one measurement to another, resulting in serious difficulties in achieving satisfactory repeatability. Although this problem is not reflected in the data presented (for each genotype the data from both traits were taken simultaneously), it is an inconvenience for comparing data from different studies.

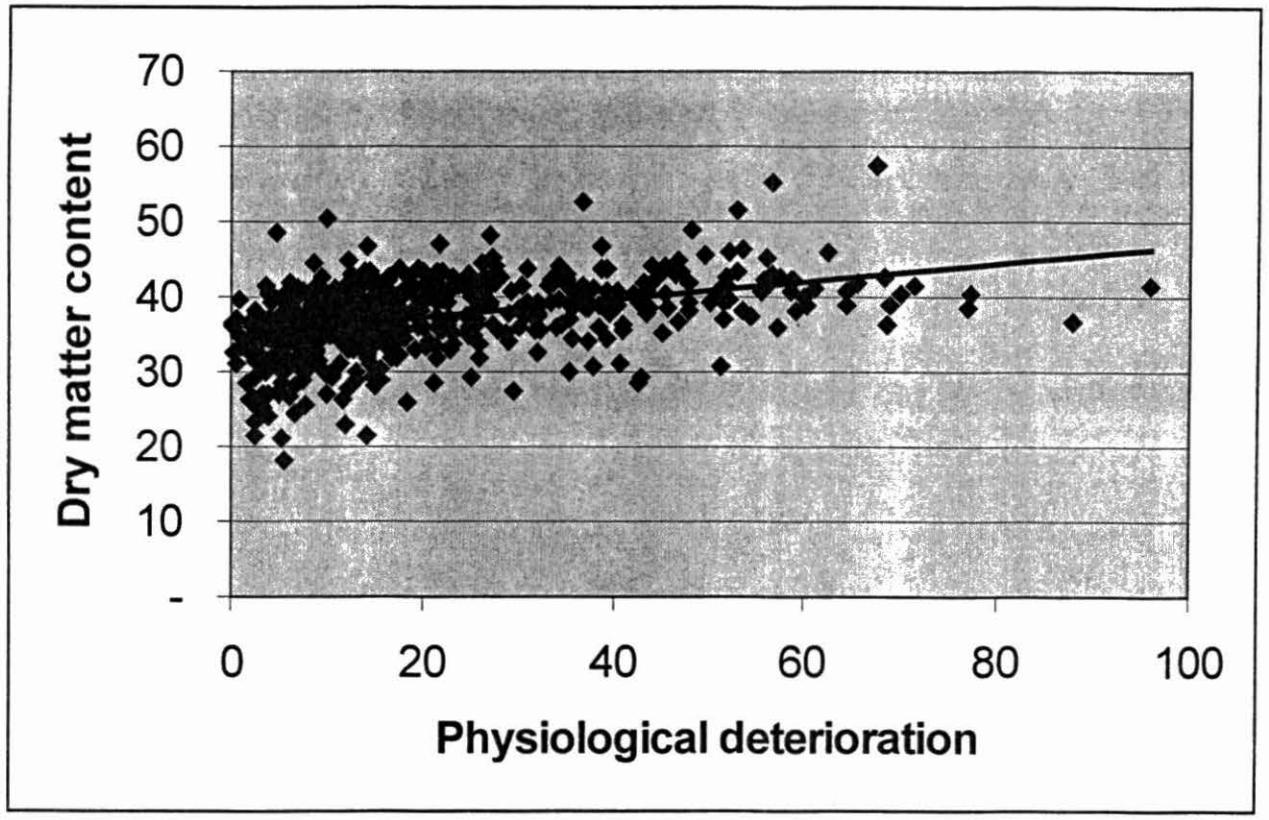


Figure 1.5. Relationship between dry matter content and post-harvest physiological deterioration, measured in more than 500 accessions from the germplasm bank.

In relation to fermented starch quality preliminary results have found clear differences between fermented and native starches. Some of the measurements need adjustments because of unsatisfactory repeatability of the results. Up to now six samples of

fermented starches coming from the non-sophisticated starch plants located North of Cauca Department. Table 1.10).

Table 1.10. Preliminary results of the evaluation of six fermented starches produced in the hillsides, north of Cauca Department. Data based on four replications.

Source of Starch ¶	Calification	WAI	WSI	SP	SV (ml/cm^3)
1	Bad	5.71	7.68	6.04	9.68
2	Bad	6.75	9.47	7.43	9.58
3	Good	13.41	9.45	14.31	13.71
4	Regular	7.95	10.70	8.38	12.94
5	Good	14.28	12.35	15.84	15.08
6	Regular	7.70	10.02	8.16	13.26
Native Starch	-.-	2.51	1.07	2.52	3.00

¶ The source has been identified with a number to protect the interest of the producers.
WAI: Water absorption index; **WSI**: Water solubility index; **SP**: Swelling power; **SV**: Specific volume.

High correlations were found between SV, and WAI (0.86), WSI (0.95), and SP (0.85). However, further confirmation for these results is required. We are currently increasing our database and improving the reliability of the different measurements.

OUTPUT 2: Genetic stocks and improved gene pools developed and transferred to national programs.

The overall objective of this activity 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 do not follow the exact order used to describe them in the respective workplan. This change has been made for more logical and, hopefully, easier to understand description of the research carried out.

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

Specific Objectives:

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

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

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

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

Results: The parents selected for the development of gene pools targeted to specific ecosystems is presented in Table 2.1. The agronomic performance of these materials is described further down in this document. Seed will be harvested from July, 2000 through March, 2001. F1 plants will grow until the planting of the trials early in 2002.

Table 2.1. Parental lines to be used in crosses for three different ecosystems relevant for cassava production in the world. Highlighted in bold italics are the clones possessing yellow roots with high carotene.

#	Sub-humid tropics	Acid-soil savannas	Mid-altitude tropics
1	MTAI 8	MBRA 383	MBRA 383
2	SM 805-15	MCOL 2307	MECU 72
3	SM 1201-5	MCOL 2337	MPER 183
4	***SM 1411-5***	***SM 985-9***	MTAI 8
5	SM 1433-4	SM 1219-9	SM 1210-4
6	SM 1438-2	SM 1565-15	SM 1219-9
7	SM 1565-17	SM 1741-1	SM 1460-1
8	SM 1657-12	SM 1779-8	SM 1557-17
9	SM 1789-20	SM 1820-8	SM 1660-4
10	SM 1871-33	SM 1822-12	***SM 1689-18***
11	CM 3555-6	***SM 1828-11***	SM 1741-1
12	CM 4843-1	SM 1859-26	***CM 2772-3***
13	***CM 6119-5***	SM 1862-25	CM 3306-4
14	CM 6754-8	SM 1871-33	CM 5655-4
15	CM 6758-1	CM 4574-7	CM 6740-7
16	CM 7514-8	CM 6740-7	CM 7514-7
17	CM 7985-24	CM 6921-3	CM 8151-1
18	CM 8027-3	CM 7514-7	CM 8370-11

The criteria for selecting parental lines reflect the new emphasis given to the project, with an increasing interest on industrial uses of cassava. Therefore, many of these genotypes are characterized by the high dry-matter productivity per hectare (i.e. MTAI 8). Other parents have been selected for their excellent characteristics for industrial uses in the food industry (MPER 183), recognized good combining ability (CM 3306-4), or special traits such as resistance whiteflies (MECU 72) or resistance to root rots (CM 4574-7). However, good parental lines for the traditional fresh market have also been included (i.e. MBRA 383, CM 6740-7, CM3306-4)

Many genotypes have been selected for their performance in specific traits and have been part of our controlled crosses during the last 3 years. This group represents a genetically broad population with outstanding performance *per se* and/or through their progenies. Compared with the selection of parental lines made during the 1990s, we have introduced relatively newer clones in the crossing blocks. For instance the excellent variety Catumare (CM 523-7) that had been used for many years as a parental line for the acid-soil savannas, was not included this year. This allowed for the incorporation of more recent materials as parental lines, which will later on be evaluated through the performance of their progenies. Therefore, a more dynamic process of identifying elite materials and including them (only for a few cycles) in the breeding scheme has been initiated. Previously, materials that had been identified as good parental lines were included for many years in the crossing blocks. It took several cycles until new, elite germplasm, began to be utilized as parents.

Other crosses have been planned for incorporating desirable genes into popular varieties in different countries, diversifying the genetic base for resistance to biotic and abiotic stresses, and in few cases, produce adequate genetic materials to study the inheritance of the trait and for the potential identification of molecular markers. For instance new sources of resistance to ACMV have been identified at IITA and shipped to CIAT, to be used in our crossing blocks.

Achievements:

- Introduction of yellow root cassava clones for the incorporation of the high-carotene trait.
- Sources of resistance to pest and diseases as well as specific traits selected for the development of control crosses.
- New criteria have been incorporated to select parental genotypes that reflect the new emphases given to the project.
- In general a higher proportion of new, clones recently identified as superior, has been included as parents.

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

Specific Objectives:

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

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

A key trait where MAS will be applied is for the introgression of resistance to ACMV (partially recessive) into elite Latin American germplasm. A new dominant and better source of resistance to ACMV has been identified and will be utilized for the introgression of resistance to the disease. For this purpose, a molecular marker for the gene has been identified. The vector of this virus has recently been found in the Americas, which makes the apparition of ACMV now a feasible possibility. If ACMV shows up in this continent it would have devastating consequences, because Latin American cassava germplasm lacks resistance to the virus. Traditional breeding for resistance to ACMV in the Americas has been extremely difficult because the virus is not present here yet, the only known resistance was almost completely recessive, and the transfer of materials between Africa and Latin America is expensive and time consuming.

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.

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

For controlled crosses, we plant 10 to 20 plants depending on the flowering capacity of the genotype in question. Each flower has the potential to produce 3 seeds, but in average we obtain no more than 1 seed per cross. This is due to the sensitivity of the stigma to the manipulation during pollination. Seeds from the same cross are mixed together and name as a full-sib family (CM).

Results: A total of 277023 recombinant seeds were produced during the period July, 1999 to September, 2000 (Table 2.2). The number of materials shipped to different regions is presented on Table 2.3. Genetic stocks are being built for traits of high priority in our project. In the case of Africa and ACMV resistance, we are developing "backcross" populations between F1 crosses (African x Latin American), and a different African parent. The strategy is to have progenies with 75% African background. Stocks for root quality traits represent a large proportion of our efforts. For cyanide, white flies, post harvest deterioration and bacterial blight we have crossed genotypes representing the extremes in performance in order to map and tag the genetic factors responsible for the inheritance of those traits.

Table 2.2. Recombinant seed produced within the project (July 1999-September 2000).

Parental population	**Controlled crosses**	**Polycrosses**	**Total**
Wide adaptation	4529	31156	35685
High-P starch	29		29
High yield potential	2765		2765
Resistance to:			
Bacterial Blight	2813		2813
White flies	1655		1655
Post harvest deterioration	1447		1447
Root rot	2269	994	3263
Tetraploid cassava	37		37
Selfings for inbreeding studies	3203		3203
Molecular studies (Family K)	2585		2585
Semi-arid environments	20382	59170	79552
Acid soil savannas	10518	60733	71251
Mid-altitude valleys	12378	53181	65559
Highland tropics	791	6388	7179
Total	65401	211622	277023

Achievements:

- Considerable amount of recombinant seeds produced.
- Large proportion of our work shifted to specific traits, through the development of genetic stocks, and pre-breeding populations.
- More targeted crosses to develop populations for molecular and basic genetic studies.

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

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

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

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

Table 2.3. Shipments of recombinant seed produced within the project from September 1999 to September 2000.

Continents	Genotypes in-vitro	Crosses (families)	Plants (in-vitro)	Seeds in the shipment
Latin America				
In-vitro	43		524	
Hybrid seed		193		10902
Asia				
In-vitro	52		149	
Hybrid seed				
Africa				
In-vitro				
Hybrid seed		15¶		312
Europe + USA				
In-vitro	73		323	
Hybrid seed		3		300
Total				
In-vitro	168		996	
Hybrid seed		211		11514

¶ Hybrid seed from crosses with wild relatives.

Results: Close to 100,000 seeds were produced during the last 4 years of activities combining the work in Thailand and Colombia. About thirty percent of that seed was transferred to four National Programs in the region and to CIAT-HQ. As shown in Table 2.4, close to 30,000 recombinant seeds were produced in 1998 and 1999. The retirement of our cassava breeder stationed in Thailand, implied that since 1998 an increasing proportion of recombinant seed originated in CIAT-HQ. However, it will take longer for Asian National Programs to receive materials from CIAT. In the future, we foresee that the flux of improved germplasm between CIAT-HQ, and the Thai breeding program will continue, and it will be through us that other National Programs will receive progenies involving the latest selections in Thailand. In November, two scientists from Thailand will come to CIAT to receive training in tissue culture (for recovering the shipments of *in vitro* plants) and to be exposed to the breeding scheme we are now following. Through this visit we shall formalize a new avenue for introducing recently

developed genetic materials into Asia. It is recognized that we are through a transition phase in our collaboration with the Asian NARS, which is reflected in the slower transference of genetic materials to and from the region. Additionally, there has been a reduction of shipments of materials *in vitro* because of plant health prevention measures. The visit of the Thai scientists, and a trip to China, Vietnam and Thailand of the cassava breeder from CIAT-HQ early in 2001, aim at re-establishing the strength and dynamism that the relationship between CIAT and the Asian programs has had in the past.

Table 2.4. Cassava F1 hybrid seeds from CIAT (Thailand and/or Colombia) distributed to Asian programs (1998-99).

Country	1998	1999	Total
Indonesia	4621	5893	8611
China	4955	2667	8747
Vietnam	3176	3176	9425
Thailand	4955	3523	
Total	**12752**	**15259**	**29233**

The transfer of recombinant progenies from Latin America to Africa had begun early in the 1990s, with more that 70,000 seeds shipped. Therefore, through this activity, the cassava genetic base for Africa had been greatly increased. A valuable collaboration in this project has been the CNPMF center from EMBRAPA (Bahia Stated, Brazil) and, of course, IITA. CIAT played a key role in crossing the Latin American germplasm with sources of resistance to the ACMV disease.

The utilization in Africa of germplasm from Latin America continued in 1998 and 1999. The germplasm introduced comprised a broad based population of seed, as well as clonal germplasm from IITA, clonal selection of African landraces, and introgressed seed populations containing Latin America germplasm which were distributed in five collaborating countries. Seed populations and clonal materials were derived from crosses, incorporating acceptable levels of cassava mosaic disease (CMD) resistance, between African adapted gene pools and introductions from Latin America.

The new introductions from Latin America with potential adaptation to the semiarid regions of Africa were also introgressed through backcross schemes during 1998 and 1999 into locally adapted germplasm. The process aims at combining high yield potential with tolerance to drought and resistance to CMD. In 1999, about 1,072 families amounting to over 27,000 seeds from controlled biparental crosses between Latin

America and African clones, as well as 227 half-sibs families with about 16,000 seeds from polycrosses were produced in hybridization plots or isolation blocks. They were introduced to collaborating countries for participatory evaluation and selection in 2000.

During the 1998/99 cropping season 54, 425, 550, 495, and 411 genotypes were evaluated (early stage of clonal evaluation) in Brukina Faso, Chad, Ghana, Niger and Nigeria, respectively. Preliminary yield trials in Burkina Faso, Chad, Niger and Nigeria were also carried out with 29, 26, 58, and 139 genotypes respectively. Several materials survived the initial selection process and reached the stage of advanced yield trials in which 17, 24, 15, 17, and 89 genotypes were evaluated in Burkina Faso, Chad, Ghana, Niger, and Nigeria, respectively.

For the 1999/2000 season, 100, 600, 200, and 1067 genotypes in clonal evaluation trials were tested in Burkina Faso, Chad, Ghana, and Nigeria, respectively; 25, 60, 75 and 167 genotypes evaluated in preliminary yield trials in Burkina Faso, Chad, Niger, and Nigeria, respectively. More advanced evaluations (advanced yield trials) involved 120, 15 and 31 genotypes evaluated in Ghana, Niger, and Nigeria, respectively. Finally 25, 32, 10, 28, and 122 genotypes ever evaluated in uniform yield trials in Burkina Faso, Chad, Ghana, Niger, and Nigeria, respectively.

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 have been identified under the local environmental conditions in each of the participating countries.

Achievements:

- Unrestricted support, collaboration and interaction with the Thai breeding program.
- Use of the most elite genotypes in crosses.
- Distribution of segregating progenies of high value for National Programs.
- Valuable collaboration with IITA and EMBRAPA for introgressing cassava germplasm from Latin America into Africa.

Activity 2.4. Use the recombinant seed produced during the 1999-2000 period to start a new selection cycle, but at the same time allow for diallel studies (Collaboration with SB2 *project in areas of cassava biotechnology).*

Specific Objectives:

a) The selection cycle initiated in September 2000 with the planting of F1 seed (see Figure 2.1) has the peculiarity of following a diallel structure.

b) *This procedure will allow for a very extensive genetic study of the inheritance of agronomically relevant traits and simultaneously allow for the identification of superior germplasm.*
c) *The magnitude and relevance of the study will contribute to SB2 project, generating several segregating populations useful for further molecular genetic studies.*

Rationale: There is very little knowledge about the genetics of cassava. Most of the information is related to particular traits, not necessarily of agronomic relevance. Because of the mode of reproduction in cassava, and the very limited work to obtain homozygosity in the crop, it is very difficult to produce segregating materials suitable for relevant genetic studies on agronomically important traits. Diallel studies involve large number of crosses, but allow for the simultaneous analysis of many traits and do not require homozygosity of the parental lines. Therefore, for this particular cycle of selection, seed from controlled crosses targeting the three most important ecosystems, have been planted in the field. Enough seed has been produced among parental lines for a complete diallel analysis. The evaluations will serve to produce valuable data about the genetics on many traits in cassava, but also they will be part of the normal selection process to identify genetically superior germplasm.

Materials and Methods: Three sets of crosses were made for three different target environments representing the most important cassava growing ecosystems in the tropics: semi-arid (Table 2.5); acid soil savannas (Table 2.6) and mid-altitude valleys (Table 2.7). Each set is made of a group of selected parental lines that have been crossed among them to produce sexual recombinant seed, following a diallel arrangement. From each F1 cross, up to hundreds of pollinations were made, to obtain a minimum of 50 viable seeds. The seeds were germinated and transplanted to the field during the last week of September, 2000. At least, 30 adult plants should represent each cross. The plants will be grown in the field for nine months and then, at least, six stakes will be taken from each one of them. This will initiate the clonal multiplication of the plants obtained from the sexual seed.

Once the stakes are obtained they will be shipped to the specific zone of adaptation for an evaluation trial, at two locations, with three replications each. The diallel study, therefore, will be made up of about 45 F1 crosses (when ten parents are included), making up the diallel experiment. Each F1 cross will be represented by 30 *"individual genotypes"*. In turn, each *individual genotype* will be represented by six plants, derived from the six stakes obtained as described above.

Quantitative genetic analysis: From the quantitative point of view, it is a very interesting analysis because it will provide valuable information to be used by cassava breeders. This is the information that, so far, has never been satisfactorily produced. Moreover, it is an unusual study because it offers the possibility of analyzing within-family genetic variation (among the *individual genotype* within the family) and within-family environmental variation (among the six plants representing each *individual genotype*).

This kind of information has very seldom been produced (if at all), so the quantitative model for its analysis requires some theoretical development.

Molecular genetic analysis: Each of the 45 F1 crosses represents a unique genetic population that can be immediately used for molecular analysis. Recognizing that the no less than 30 individuals representing each cross may be a small sized population, the segregation of many important traits among the 45 different F1 crosses will certainly be an excellent starting point for further work. Families showing interesting segregations can be expanded until a satisfactory size is reached.

Cassava breeding: At the same time the evaluation is carried out for the quantitative and molecular genetic analyses, the best performing individuals (based on the performance of the six plants representing each *individual genotype*) will be selected and incorporated into the ongoing breeding project.

Chronogram: F1 plants were transplanted to the field during the last week of September, 2000. Stakes from those plants will be harvested by May, 2001. Evaluation trials will be planted immediately after the stakes are obtained. The most important data will be taken starting in February-March, 2002. Both the quantitative and molecular genetic analysis will be carried out through 2002 and results published at the end of this period. There are late crosses whose seed were put to germinate later and are still being transplanted to the field

Table 2.5. Number of seedlings representing the F1 crosses following a diallel mating design for the semi-arid tropical environment. Within parenthesis additional seedlings being grown but not transplanted to the field by early October.

Genotype	CM 6754-8	CM 8027-3	SM 805-15	SM 1219- 9	SM 1411-5	SM 1565-17	SM 1657-12	SM 1665-2	MTAI-8
CM 523-7	21 (9)	107	105	105	57 (36)	103	54 (9)	101	92
CM 6754-8		71	88	112	42 (15)	101	35 (9)	(94)	59 (6)
CM 8027-3			50 (19)	105	82 (1)	82	93	94	66 (24)
SM 805-15				85	53	75 (42)	81	98	79 (25)
SM 1219-9					38 (61)	97	86	109	104
SM 1411-5						87	31 (3)	(76)	113
SM 1565-17							26 (58)	112	97
SM 1657-12								81 (19)	25 (4)
SM 1665-2									87

Table 2.6. Number of seedlings representing the F1 crosses following a diallel mating design for the tropical environment of acid soil savannas. Within parenthesis *additional seedlings* being grown but not transplanted to the field by early October.

Genotype	CM 6740- 7	CM 7033-3	SM 1219-9	SM 1565-15	SM 2058-2	SM 2219-11	HMC 1	MPER 183	MTAI-8
CM 4574-7	67 (28)	89	95	93	13 (6)	89	39 (47)	104	107
CM 6740-7		104	97	100	60 (2)	103	100	104	97
CM 7033-3			85	81	56	27 (58)	49	78	100
SM 1219-9				104	100	103	101	89	95
SM 1565-15					95	103	87	108	84 (13)
SM 2058-2						95	93	97	95
SM 2219-11							104	84	99
HMC-1								87	115
MPER 183									99

Table 2.7. Number of seedlings representing the F1 crosses following a diallel mating design for the mid-altitude tropical valley environments. Within parenthesis additional seedlings being grown but not transplanted to the field by early October.

	SM 1219-9	SM 1278-2	SM 1636-24	SM 1673-10	SM 1741-1	HMC 1	MECU 72	MPER 183
CM 6740- 7	103	113	93	103	110	104	106	83
SM 1219-9		104	63 (21)	98	96	105	97	103
SM 1278-2			23	95	102	24	85	66
SM 1636-24				31 (16)	100	8 (14)	75	30 (2)
SM 1673-10					81	46 (30)	103	60 (2)
SM 1741-1						90	80	91
HMC 1							86	48 (33)
MECU 72								92

Achievements:

- A breakthrough genetic study was begun.
- Seed was obtained to have a balance, complete diallel mating design.
- The whole experiment could serve for the training of several Ph.D. students.
- Both valuable information and genetic stocks will be produced for the **SB2** project to continue with further molecular studies.

Activity 2.5. Selection of recombinant progenies for broad and specific adaptation within major agro-ecosystems (sub-humid; semi-arid; highland and acid soil savanna).

Specific Objectives:

a) Modify the evaluation procedure to make it more efficient and to adapt it to the new breeding objectives.
b) To develop and evaluate superior germplasm adapted to particular ecosystems.
c) To develop genetic stocks useful for other CIAT projects.

Rationale: Our strategy for cassava germplasm development is centered on the development of improved gene pools for specific edapho-climatic zones with importance for cassava production, as defined in Table 2.8. 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.

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

Traditionally, the progenies generated from the crossing blocks (***F1***) were planted in screen houses and transplanted to the field after 2 months at CIAT. At 6 months after planting, 2 stakes were harvested from each plant and given a consecutive number according to the plant. One of the stakes was planted at CIAT, the other one, was

planted at the main selection site (***F1C1***). Selection was conducted at harvest on individual plants at the main selection site. Planting material taken from the selected genotypes, at CIAT, was used to establish a non-replicated, 6-plant plot, both at CIAT and at the main selection site (***clonal evaluation*** stage). Evaluation was done using the central 3 plants. Selections were transferred to the following stage (***preliminary yield trial)*** and planted in non-replicated, 20-plant plots. Evaluation was done in the central 6 plants, and selections were then passed to the advanced yield trials at 1 or 2 sites, with 3 replications of 25-plant plots. Genotypes selected over 2 consecutive years at the advanced yield trial level were consider as ***"elite genotypes"*** and incorporated in the germplasm collection and the crossing blocks. Since each year a new breeding cycle was initiated, all the stages were simultaneously being conducted in each site.

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

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

2) The *clonal evaluation* will be based on six to eight plants, rather that six as before. An important modification for the sub-humid environment is that the *clonal evaluation* will be carried out in two stages: at the normal harvest time only two plants will be harvested to measure % of dry matter. This trait varies considerably with the time of harvest or age of the plant. Therefore to estimate it correctly, the plants need to be harvested at the proper time. The remaining six plants of each plot will be harvested just prior to normal planting time (one week before). Yield potential will be estimated visually (as had been done traditionally at the F1C1 and clonal evaluations stages) based on the volume of roots produced by the six plants or, if possible, by weighing the total production of roots. Few other traits will also be taken using visual scores: plant architecture, foliar health (for insects and diseases separately), above ground biomass (for a rough estimate of harvest index), and root aspect. A selection index software will be used to make an efficient and fast selection of the approximately 1000-2000 genotypes evaluated at this stage, for each ecosystem.

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

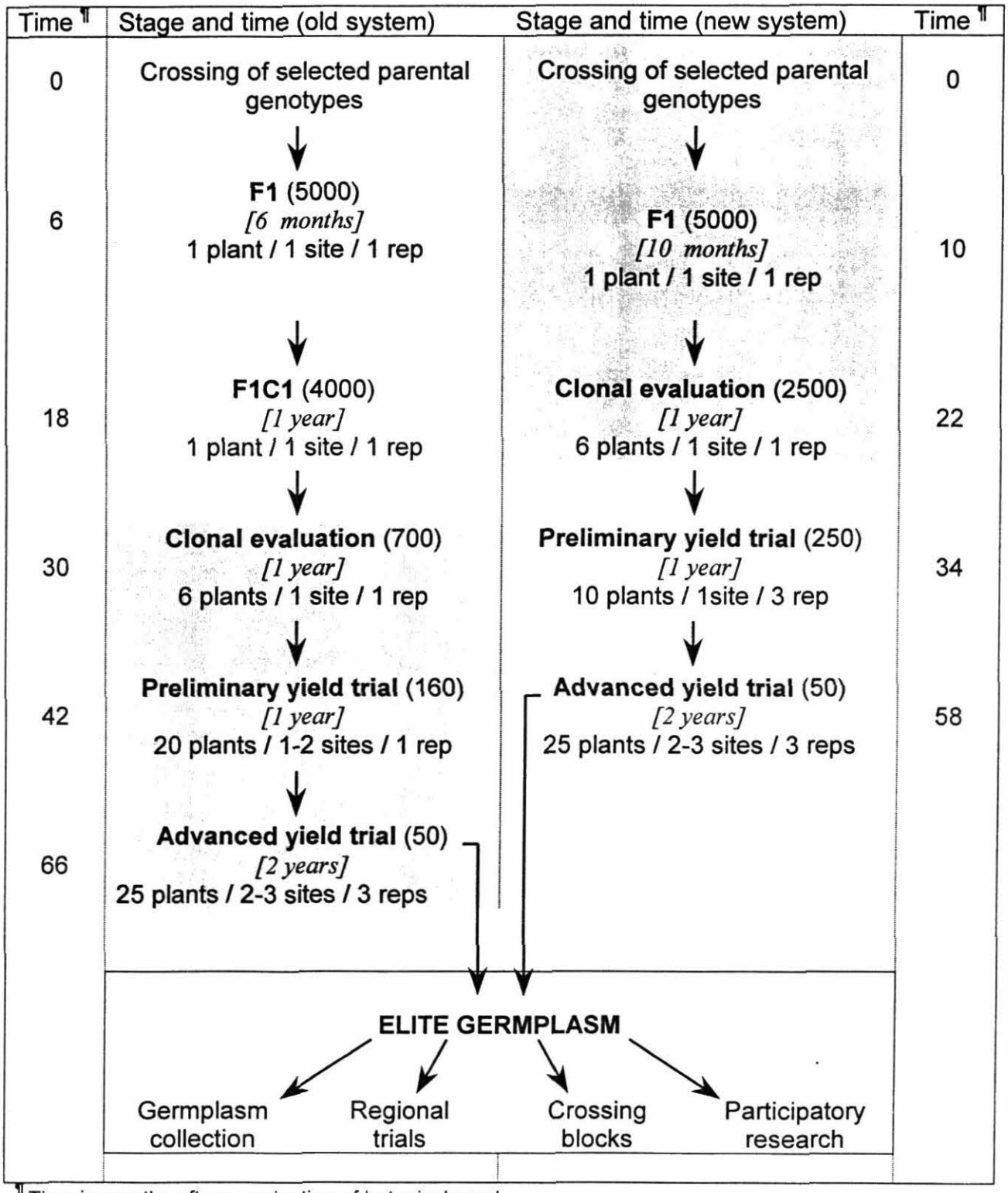
4) A final important modification to the evaluation process is that data will be taken and analyzed for **all** the progenies evaluated. In the past, data was taken only for those families that went beyond the *clonal evaluation* stage. Therefore it was impossible to estimate combining ability of parental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

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

- The duplication of materials maintained at CIAT-HQ is avoided until they reach status of "elite genotype".
- The selection of large number of segregating progenies, at the F1C1 stage, which was based on single plant observations, is avoided.
- The time required to reach the stage of replicated trials is minimized.
- The total length of each cycle of selection is reduced by almost a year.
- Data records will allow for selecting parental material based on combining ability.
- The total cost for each cycle of selection should be reduced.
- Selection will be less subjective by using appropriate software (specifically developed for that purpose).

Table 2.8. Main ecosystems for cassava production, *representative* production regions, and main breeding sites.

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



¶ Time in months after germination of botanical seed.

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

Results: In order to summarize results from the last year of work, we present a description of the main activities at the four most important ecosystems: semi-humid tropics (Tables 2.9 to 2.18); acid-soil savannas (Tables 2.19 to 2.25); mid-altitude tropics (Tables 2.26 to 2.34); and highland tropics (Tables 2.35 to 2.38).

An increasing emphasis has been given to dry matter yield (t/ha) in the last few selection cycles. On the other hand, less weight has been given to HCN. These changes are a response to the demand from the industry. Dry matter yield is the result from a relatively low heritability trait (root yield) and an intermediate to high heritability trait (root dry matter %). In most cases data analysis and selection of progenies is carried out using a selection index (***SI***) that integrates the most important traits from the agronomic point of view. In general the ***SI*** can be described as follows:

SI = (Fresh roots yield * X) + (Dry matter content * X) + (Foliage evaluation * X) + (Harvest index * X)

The "X" value represents a weight given to represent the relative importance desired for the trait in the final value of the SI. Yield and high dry matter content had higher weights than the foliage and harvest index traits. To overcome the problem of scales used to measure each trait, variables were standardized using the following formula:

$$\text{Standardized value for the } i^{th} \text{ observation} = (X_i - \mu) / \sigma$$

where X_i is the actual value for the i^{th} observation, μ and σ are, respectively, the mean and standard deviation for the variable measured across the entire experiment.

Table 2.9. Number of germinated and transplanted sexual seed for the F1 plots planted at CIAT – Palmira.

Purpose of the cross	Germinated seed	Transplanted seedlings
General adaptation	4638	2798
Good cooking quality	1076	690
High yield potential	977	556
Sub-humid tropics	3987	3081
Acid soil savannas	3742	1373
Mid-altitude valleys	2930	1340
Highlands	2068	951
Total	**19418**	**10789**

a. Selections for the sub-humid tropical environments.

A summary of the different trials conducted for the materials adapted to the sub-humid environmental conditions is presented in Table 2.10. These trials were conducted in the northern coast of Colombia (Departments of Atlántico and Sucre). Because of the effects of the *La Niña* phenomenon, there was excessive precipitation, which resulted in the unfortunate flooding of a considerable area of our evaluation plots. Some of the trials (i.e. ***F1C1***) had to be discarded because of unreliable data.

Table 2.10. Trials conducted in the sub-humid ecosystem during the 1999-2000 season.

Trial	Location	N° of Genotypes ¶	N° of Reps	Observations
F1	Cenicaña	See Table 2.9	1	Currently involves the planting of the diallel study as single F1 plants.
F1C1	Caracolí	1011 (1)	1	Severed flooding made this trial useless. Stage eliminated in the new scheme.
Clonal evaluation	Caracolí Caracolí	220 (6) 1319(8) §	1	New selection scheme begun: plants were in the field for 10 months. See Table 2.11 for old system trial.
Preliminary yield trial	Caracolí	81 (20)	1	See Table 2.12
Advanced yield trial	Caracolí	120 (25)	3	See Table 2.13
Regional trials	Caracolí S. Tomás Luruaco Chinú	30 (25) 30 (25) 27 (25) 30 (25) 27 (25)	3 3 2 3 2	See Table 2.14 See Table 2.14 See Table 2.14 See Table 2.15
Seed increases	Several	45	-.-	See Table 2.16

¶ Within parenthesis the number of plants per plot.

§ Clonal evaluation of the new selection scheme.

A summary of the **clonal evaluation** trial can be found in Table 2.11. At the right of the table are listed the values of the selection index for each genotype. The genotype SM 2541-16 was the highest yielding clon (44 t/ha), but its dry matter content was low (31.0 %). Therefore, in spite of its high yield potential (both of fresh roots and dry matter), the selection index has not given the first priority to this, otherwise, excellent genotype. In the high yielding group, materials with good foliage evaluation (rating 1 or 2) and higher dry matter content have been given preference, as was expected, by the selection index.

Table 2.11. Results from the best 20 clones from the **Clonal Evaluation** (Caracolí, Atlántico Department), ranked according to a selection index. In this trial 210 experimental clones and several checks (bottom of the table) were evaluated.

Clon	Yield (t/ha)		Dry Matter (%)	Foliage evaluation (1-5) [§]	Harvest Index (0-1)[¶]	Selection Index
	Fresh Roots	Dry matter				
SM 2546-5	25.67	9.39	36.6	2.0	0.65	13.87
SM 2448-3	24.67	8.09	32.8	1.0	0.58	13.58
SM 2444-5	21.00	7.10	33.8	1.0	0.48	13.13
SM 2547-7	33.33	10.93	32.8	2.0	0.60	12.11
SM 2541-16	44.00	13.64	31.0	3.0	0.55	11.91
SM 2547-10	41.67	12.75	30.6	3.0	0.75	11.55
SM 2545-9	27.00	9.64	35.7	4.0	0.51	10.80
SM 2541-21	21.67	7.87	36.3	3.0	0.53	10.56
SM 2449-5	23.33	8.31	35.6	3.0	0.56	10.46
SM 2547-12	29.67	9.94	33.5	3.0	0.55	10.17
SM 2443-6	19.33	6.88	35.6	3.0	0.77	10.05
SM 2547-14	31.67	10.51	33.2	4.0	0.57	9.87
SM 2547-17	20.67	7.46	36.1	4.0	0.63	9.73
SM 2448-8	18.33	6.69	36.5	3.0	0.47	9.53
SM 2543-1	19.33	7.02	36.3	4.0	0.50	9.08
SM 2546-8	15.33	5.34	34.8	2.0	0.53	8.51
SM 2544-8	23.00	7.91	34.4	4.0	0.56	8.46
SM 2449-4	22.00	7.46	33.9	3.0	0.54	8.24
SM 2547-9	29.33	9.42	32.1	4.0	0.61	8.18
SM 2546-7	14.33	5.15	35.9	3.0	0.58	8.15
CG 1141-1	11.11	3.72	32.8	3.0	0.54	3.82
COL 1505	8.78	2.77	31.4	4.0	0.42	0.88
COL 1684	3.89	1.28	32.6	4.0	0.37	0.16
VEN 77	7.56	2.23	30.1	3.0	0.31	-1.75
BRA 12	3.11	0.87	30.3	4.0	0.19	-3.00
COL 22	2.00	0.59	30.8	5.0	0.28	-3.51
COL 2215	0.00	0.00	31.5	4.0	0.00	-4.37
Minimum [†]	0.00	0.00	23.7	1.0	0.00	
Maximum [†]	44.00	13.64	36.6	5.0	0.77	
Mean [†]	7.27	2.30	31.5	2.98	0.23	
St.Deviation [†]	10.04	3.18	1.96	1.22	0.27	

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = *excellent* ➡ 5 = *very poor*).

[¶] Harvest index = Weight of commercial roots / total biomass. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.12. **Preliminary yield trial** of 72 experimental clones and eight checks at Caracolí (Atlántico Department). Results of the best 20 performing clones (based on their selection index) and checks.

Clon	Foliage evaluation (1-5) [§]	Dry matter (%)	Yield (t/ha)		Harvest index (0-1)[¶]	Selection Index
			Fresh roots	Dry matter		
SM 2438-3	1	39.4	15.17	5.98	0.56	13.08
SM 2443-5	1	33.2	20.17	6.70	0.63	12.15
SM 2448-3	2	35.1	21.67	7.61	0.56	10.12
SM 2448-7	2	32.1	22.83	7.33	0.62	9.17
CM 7985-11	3	33.8	20.67	6.99	0.66	8.2
CM 9020-8	2	35.2	15.83	5.57	0.64	7.68
CM 7985-24	4	36.1	17.83	6.44	0.63	7.46
CM 9067-13	3	32.6	19.83	6.47	0.65	6.96
CM 9020-2	3	34.2	17.33	5.93	0.66	6.80
CM 7985-16	4	34.7	18.00	6.25	0.62	6.58
SM 2439-8	3	36.6	13.50	4.94	0.60	5.94
SM 2352-15	4	33.2	18.50	6.14	0.62	5.90
CM 9067-2	4	34.8	15.67	5.45	0.65	5.67
CM 9067-9	2	33.6	13.83	4.65	0.64	5.59
SM 2446-11	2	31.7	15.83	5.02	0.64	5.39
SM 2440-5	2	34.8	12.67	4.41	0.58	5.34
SM 2441-1	3	32.9	16.00	5.26	0.63	5.01
CM 9067-10	4	33.2	16.33	5.42	0.65	4.94
SM 2441-10	3	34.0	12.00	4.08	0.63	3.63
CM 9024-13	4	33.9	12.50	4.24	0.62	3.19
BRA 12	4	27.0	12.00	3.24	0.61	-1.64
COL 1505	3	28.2	10.50	2.96	0.52	-1.69
COL 22	4	29.3	1.67	0.49	0.67	-5.15
COL 2215	5	35.2	1.17	0.41	0.16	-5.40
CG 1141-1	4	31.2	3.50	1.09	0.45	-4.46
VEN 77	4	26.1	4.17	1.09	0.51	-7.01
COL 1684	5	26.5	3.17	0.84	0.34	-8.81
COL 1468	5	28.0	0.83	0.23	0.10	-10.68
Minimum [†]	1	23.8	0.83	0.23	0.09	
Maximum [†]	5	39.4	22.83	7.61	0.72	
Mean [†]	3.58	31.3	10.03	3.21	0.53	
St.Deviation [†]	0.92	3.06	5.74	1.97	0.15	

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

In Tables 2.12 and 2.13 a summary of the results of the **preliminary and advanced yield trials** are presented. As in the clonal evaluation, the selection index has helped to

identify the best performing genotypes. In the preliminary yield trial all the eight checks had negative selection index, suggesting a performance below the average of the trial. In the advanced yield trial the clon MTAI 8, developed by CIAT/Thailand (Rayong 60) was used as a check, although it is in the process of release as an official variety in Colombia. This "check" as well as other outstanding genotypes, showed an excellent performance compared with local checks, but the results *per se* were disappointing, with very low yields due to the flooding of the plot. The roots that had developed until the sixth month of age all rotted, so the production measured was what the plants could produce during January and February of 2000, after the heavy rains ceased.

Table 2.13. **Advanced yield trial** of 111 experimental clones and nine checks at Caracolí (Atlántico). Results of the best performing clones (including two ckecks identified in bold), according to the ranking of the selection index.

Clon	Yield (t/ha)		Dry matter (%)	Harvest index (0-1) ¶	Foliage evaluation (1-5) §	Selection Index
	Fresh roots	Dry matter				
SM 1619-3	4.30	1.37	31.95	0.48	2.73	15.90
SM 1438-2	2.30	0.77	33.40	0.39	2.50	10.14
MTAI 8	**1.94**	**0.63**	**32.20**	**0.56**	***2.50***	***9.05***
SM 1516-7	2.93	0.83	28.35	0.29	2.50	9.01
SM 1650-7	3.56	1.13	31.90	0.34	3.33	8.52
SM 1669-5	8.85	2.71	30.60	0.57	2.73	8.41
SM 1969-31	2.63	0.85	32.30	0.42	2.50	8.17
SM 1904-4	8.52	2.84	33.30	0.51	3.75	8.17
SM 2182-2	6.30	1.95	30.97	0.50	3.33	8.08
MEX 95	**6.30**	**2.03**	**32.30**	**0.51**	**2.73**	**7.92**
SM 1657-12	7.78	2.65	34.10	0.48	4.29	7.54
CM 8288-43	9.85	3.54	35.95	0.57	4.29	7.43
SM 2081-34	5.89	1.98	33.65	0.50	3.75	7.36
SM 1637-22	4.59	1.35	29.40	0.31	3.75	7.30
SM 1669-7	11.00	3.27	29.75	0.62	3.33	7.15
SM 1785-10	3.59	1.21	33.70	0.44	3.33	7.05
SM 2277-4	1.39	0.00	0.00	0.26	2.86	6.85
SM 1411-5	3.04	0.93	30.70	0.24	2.50	6.64
SM 1521-10	3.59	1.20	33.50	0.39	2.73	6.62
SM 1435-1	8.85	2.56	28.90	0.51	4.29	6.44
Minimum †	0.33	0.00	0.00	0.13	2.14	
Maximum †	12.44	4.55	38.80	0.67	6.00	
Mean †	4.92	1.54	29.64	0.43	3.14	
St.Deviation †	2.64	0.92	7.90	0.11	0.73	

§ Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

¶ Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

† Statistical data based on the complete experiment.

Table 2.14. Regional trials conducted at three locations in the sub-humid environment of Colombia's northern coast.

	LURUACO					SANTO TOMAS						CARACOLI (FLOODED)						
	Yield (t/ha)		Harvest	Dry	Selec.	Yield (t/ha)		Dry	Harvest	Score	Selec.	Yield (t/ha)		Dry	Harvest	Score	Selec.	LU*2
	Fresh	Dry	Index	matter	Index	Fresh	Dry	matter	Index	Foliage	Index	Fresh	Dry	matter	Index	Foliage	Index	ST*2
Genotype	roots	matter	(%)	(%)	[1]	roots	matter	(%)	(%)	(1-5)	[2]	roots	matter	(%)	(%)	(1-5)	[3]	CA*1
TAI 8	44.50	14.95	0.56	33.6	1	38.89	13.73	35.3	0.72	4	2	5.74	1.65	28.90	0.45	2.33	14	4.0
CM 7514-8	30.17	9.92	0.51	32.9	10	37.56	13.33	35.5	0.57	3	3	5.81	1.83	31.60	0.54	2.33	8	6.8
SM 1433-4	40.33	13.07	0.52	32.4	3	36.11	11.92	33	0.55	3	13	7.63	2.32	30.35	0.55	2.67	10	8.4
BRA 384	36.17	11.18	0.49	30.9	4	41.33	13.27	32.1	0.64	2	6	5.04	1.40	27.80	0.50	3.67	25	9.0
SM 1431-2	32.33	10.35	0.45	32.0	8	35.89	12.09	33.7	0.50	4	15	9.74	3.06	31.45	0.47	2.67	2	9.6
SM 1427-1	26.17	8.24	0.49	31.5	15	46.11	14.25	30.9	0.60	2	5	6.04	1.85	30.70	0.54	2.67	12	10.4
VEN 25	19.17	6.15	0.35	32.1	24	46.00	15.41	33.5	0.55	2	1	7.59	2.50	32.90	0.54	3.67	7	11.4
CM 6119-5	31.83	11.11	0.54	34.9	5	27.33	9.38	34.3	0.62	3	21	4.26	1.42	33.25	0.43	2.67	6	11.6
CM 4843-1	28.83	9.23	0.49	32.0	12	34.67	11.75	33.9	0.68	5	10	5.70	1.80	31.60	0.58	4.33	17	12.2
SM 1411-5	22.67	6.98	0.41	30.8	19	38.89	12.87	33.1	0.64	3	7	11.70	3.44	29.35	0.58	3.00	9	12.2
CM 6182-8	22.83	7.69	0.54	33.7	16	26.67	10.03	37.6	0.66	5	11	5.30	1.71	32.20	0.48	3.67	11	13.0
SM 643-17	32.83	10.21	0.40	31.1	9	25.56	9.10	35.6	0.36	3	23	10.44	3.85	36.85	0.62	2.67	1	13.0
CM 4919-1	30.00	9.36	0.50	31.2	11	33.78	11.38	33.7	0.67	4	14	5.63	1.74	30.85	0.53	3.33	16	13.2
CM 8027-3	33.83	10.69	0.41	31.6	6	34.22	11.53	33.7	0.58	4	16	2.44	0.73	29.85	0.40	5.00	26	14.0
CG 1141-1	15.67	4.98	0.38	31.8	27	31.67	11.31	35.7	0.68	3	8	6.52	2.06	31.55	0.45	2.67	4	14.8
CM 6758-3	24.50	6.86	0.44	28.0	17	34.00	11.76	34.6	0.60	3	9	1.67	0.54	32.40	0.33	4.00	23	15.0
CM 6758-1	41.33	13.27	0.46	32.1	2	23.33	7.96	34.1	0.45	4	28	3.52	1.13	32.10	0.51	4.00	19	15.8
CM 3555-6	20.17	6.07	0.42	30.1	22	52.22	16.19	31	0.51	4	4	3.30	0.89	27.05	0.41	3.33	28	16.0
CM 3306-19	29.50	9.03	0.49	30.6	13	43.89	13.04	29.7	0.62	4	17	5.41	1.61	29.90	0.55	3.67	21	16.2
SM 1201-5	21.67	7.00	0.45	32.3	20	31.67	10.74	33.9	0.58	5	20	9.63	3.16	32.80	0.59	4.00	5	17.0
COL 2215	30.00	10.89	0.55	36.3	7	25.56	8.84	34.6	0.54	5	26	1.33	0.42	31.70	0.19	4.33	22	17.6
SM 805-15	28.00	8.01	0.47	28.6	14	30.44	10.38	34.1	0.62	3	18	2.11	0.60	28.60	0.33	3.67	27	18.2
CM 3306-4	22.83	6.67	0.46	29.2	21	26.33	10.14	38.5	0.50	4	12	0.41	0.12	30.57	0.14	4.00	29	19.0
IND 39	21.83	7.23	0.44	33.1	18	25.00	8.60	34.4	0.52	3	24	6.26	1.66	26.45	0.50	2.00	18	20.4
CM 4365-3	15.50	5.46	0.41	35.2	25	22.22	7.87	35.4	0.52	4	27	8.22	2.60	31.60	0.49	2.67	3	21.4
SM 1438-2	13.17	4.33	0.33	32.9	28	25.89	9.45	36.5	0.62	4	19	1.59	0.45	28.35	0.22	2.67	24	23.6
COL 1505	13.50	4.29	0.35	31.8	29	27.22	9.20	33.8	0.60	4	22	4.85	1.35	27.85	0.41	2.67	20	24.4
CT 20-2	19.17	5.50	0.31	28.7	26	25.00	8.43	33.7	0.41	5	29	7.37	2.20	29.90	0.49	3.67	13	24.6
SM 1539-2	19.67	6.12	0.40	31.1	23	25.56	8.41	32.9	0.64	3	25	1.11	0.31	27.90	0.23	4.00	30	25.2
CM 6754-8	12.83	3.77	0.40	29.4	30	24.44	7.24	29.6	0.46	4	30	10.07	3.10	30.80	0.68	3.67	15	27.0
Promedio	26.03	8.29	0.45	31.73		32.58	10.99	33.95	0.57		15.50	5.55	1.72	30.57	0.46	3.32		

[1] =Selection Index = (Fresh Root Yield * 10)+(Dry matter content * 5)+(Iharvest Index * 50)

[2] = Selection Index = [(Dry matter yield * 5) + (Dry matter content * 2) + (Harvest Index) + (Inverse Foliage Score)

[3] = Selection Index = (Dry matter yield *2) + (Dry matter content * 2) + (Harvest Index) + (Inverse Foliage Score)

In Table 2.14 and 2.15, the complete results of regional trials are presented. There is a clear contrast between the drier environment of the Atlántico Department (Table 2.14) and the more humid conditions of the Sucre Department (Table 2.15). General performance (according to the selection index criteria) indicated a very strong genotype by environment interaction, which motivated the presentation of the data separated in two different tables.

Table 2.14 includes the results of three different regional trials in the Atlántico Department (Luruaco, Santo Tomás and Caracolí). Fortunately the first two sites suffered intense rains by the end of 1999, but were not flooded as Caracolí. Yields were, therefore, much higher in these two locations. Selection indices for each location were slightly different to adjust the methodology to the peculiarities of the results from each site. The ranking of the genotypes at each location, rather than the actual selection index value, is presented. At the right of Table 2.14 a weighted average of these rankings is presented. Data from Luruaco and Santo Tomás had twice as much weight in this average, as the information coming from Caracolí.

As expected, MTAI8 was the best performing variety. This confirms the previous finding that this genotype is genetically superior and worth releasing as a variety for Colombia and other Latin American countries. CIAT has requested Thailand authorities their consent to proceed, and we have received a kind and positive answer.

At the bottom of Table 2.14 is the genotype CM 6754-8. This is an interesting material with a short, bushy type of plant. This kind of architecture has been identified as the most nutrient efficient type by cassava physiologists at CIAT. It does not perform well in these locations included in Table 2.14. However, it was (as in previous years) the best performing variety at Chinú (Table 2.15). The differential performance of CM6754-8, as well as that of other varieties such as MTAI8, in the Atlántico and Sucre Departments illustrates the strong genotype by environment interactions that can be observed when cassava is grown in key locations. In similar evaluations carried out during the previous year, the genotypes MTAI8, CM 6754-8, SM1411-5, CM4919-1, CM 3555-6, and MBRA384 have shown excellent results in regional trials (1999 Annual Report). Through these evaluations it is possible, therefore, to identify a group of genotypes with outstanding yield potential and reliable stability.

Tables 2.16 through 2.19 present the results of sequential samplings and data for agronomically important traits. The first sampling (December 13) was taken when the plants were about seven months old. This evaluation aimed at detecting early bulking on one hand, and the capacity to maintain dry matter content after the initiation of the rainy season, on the other. As can be seen (Table 2.18) dry matter content drops drastically when the growth of the plant begins upon the arrival of the rains, because part of the accumulated energy is used for producing the new tissue. A lower dry matter content in the roots reduces its industrial quality. Both traits (early bulking and sustained dry matter content) are highly desirable for areas with a long dry season, because it would contribute to a continuous availability of raw materials for the industry.

Table 2.15. **Regional trial** at Chinú (Sucre Department). Varietal performance at this location contrasts drastically with their behavior in drier environment (See Table 2.14).

CLON	Commercial roots (No.)	Dry matter (%)	Yield (t/ha)		Harvest index (0-1)	Selection index
			Fresh roots	Dry matter		
CM 6754-8	28	32.0	32.11	10.28	0.64	8.01
SM 1539-2	37	33.4	27.33	9.13	0.68	7.71
MBRA 384	35	33.0	22.33	7.37	0.75	5.82
SM 805-15	22	31.5	23.89	7.53	0.67	3.63
CM 4843-1	24	33.3	16.33	5.44	0.74	2.70
CM 7514-8	18	33.3	15.11	5.03	0.75	2.32
SM 1438-2	20	34.7	15.22	5.28	0.63	2.02
CM 3306-4	12	34.3	15.89	5.45	0.63	1.90
CM 6758-1	20	33.7	18.78	6.33	0.55	1.54
CM 4919-1	28	33.2	18.78	6.23	0.57	1.19
M VEN 25	20	34.2	14.22	4.86	0.63	0.91
M TAI 8	15	32.9	14.00	4.61	0.72	0.71
M IND 39	33	32.2	18.33	5.90	0.60	0.43
SM 1431-2	39	33.1	18.22	6.03	0.54	0.31
CM 6182-8	18	35.0	10.44	3.66	0.64	0.02
CT 20- 2	31	32.5	18.67	6.07	0.54	-0.06
SM 1433-4	26	32.7	16.00	5.23	0.62	-0.07
MCOL 2215	23	33.9	12.56	4.26	0.63	-0.35
SM 1427-1	37	30.9	18.67	5.77	0.63	-0.42
SM 1201-5	12	33.2	12.89	4.28	0.63	-0.97
SM 1411-5	37	31.9	15.44	4.93	0.63	-1.00
CM 3306-19	15	30.7	14.00	4.30	0.73	-1.56
CM 6119-5	20	30.2	18.78	5.67	0.57	-2.18
CM 4365-3	15	34.3	8.11	2.78	0.59	-2.88
MCOL 1505	45	31.2	15.11	4.71	0.54	-3.47
SM 643-17	10	33.6	7.00	2.35	0.56	-4.67
CM 6758-3	17	30.2	11.56	3.49	0.63	-4.98
CM 8027-3	16	30.7	6.11	1.88	0.66	-6.79
CM 3555-6	22	26.4	10.11	2.67	0.64	-9.84
Minimum	10.00	26.40	6.11	1.88	0.54	-9.84
Maximum	45.00	35.00	32.11	10.28	0.75	8.01
Mean	23.97	32.49	16.07	5.22	0.63	0.00
St.Deviation	9.34	1.78	5.67	1.85	0.06	3.85

¶ Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

Results indicate that at 7-8 months of age adequate yields (about 10 t/ha of dry matter or 30 t/ha of fresh roots) can already been obtained (Tables 2.16 and 2.17). This early harvest may not maximize the yields of roots (which is attained one or two months later), but allows for the harvest of a valuable volume (about 7 t/ha) of fresh foliage. Later on, the dry season induces the loss of almost all the leaves (Table 2.19). However, the data comes from a few plants from each sampling date, so conclusions at

this point are preliminary and needs further confirmation from ongoing trails currently in the field.

The genotypes 4919-1, SM1433-4, and SM 1411-5 seem to be early bulking, reaching its highest recorded production between the middle of January and February. CM 3306-19, MVEN25, and CM 3555-6, on the other hand seem to be late bulking genotypes (Table 2.16). Regarding the capacity to maintain dry matter content after the initiation of the rains (which occur in April), some genotypes were very undesirable (CM 3555-6) but unfortunately several plots had been harvested earlier by passers by, and the data are missing. It is interesting to note that CM3555-6 seems to be early bulking. Perhaps early bulking is somewhat associated with a susceptibility to loose dry matter content drastically upon the arrival of the rains. Varieties CM 6119-5 and CG 1141-1, on the other hand showed some "tolerance to rains", and tended to maintain higher dry matter content than the remaining varieties.

Fresh foliage production is a new trait included in the evaluations. Cassava leaves have high protein concentration (about 20-24 % fresh weight basis) as well as vitamins (see Tables 1.2 and 1.3). There is a growing interest in utilizing cassava directly for feeding swine and cattle, because at this. A combination of foliage and roots (either dried or as a silage) offers and ideal solution to the problem. The short plant type of CM6754-8 reveals a high production of fresh foliage, even well after the dry season begun, with 12.8 t/ha of fresh foliage in the January measurement (Table 2.18).

Table 2.16. Dry matter production (t/ha) in sequential samplings taken at Santo Tomás (Atlántico). Planting date was May 10.

Clon	Dec. 13	Jan. 24	Feb. 21	Mar. 15	***Mean*** [§]	Apr. 24	May 23	***Overall Mean***
CM3306-19	10.57	9.96	14.98	13.04	***12.14***	[¶]	[¶]	***-.-***
MVEN 25	11.31	9.61	15.18	15.41	***12.88***	[¶]	[¶]	***-.-***
CM4919-1	11.28	8.35	10.69	11.39	***10.43***	9.93	10.28	***10.34***
MCOL 2215	6.61	9.00	8.18	8.86	***8.16***	9.09	7.29	***8.17***
SM8027-3	9.70	10.77	12.80	11.53	***11.20***	11.09	9.32	***10.92***
SM1433-4	11.31	11.01	13.89	11.91	***12.03***	16.22	9.25	***12.23***
CM3555-6	5.66	10.79	13.96	16.18	***11.65***	11.80	13.26	***11.90***
CM6119-5	9.34	6.98	9.98	9.36	***8.92***	10.73	10.57	***9.41***
CM6754-8	8.67	7.15	8.23	7.22	***7.82***	9.94	7.53	***8.08***
SM1411-5	15.29	16.94	18.25	12.88	***15.84***	17.12	[¶]	***-.-***
SM1438-2	10.65	11.34	14.19	9.45	***11.41***	17.66	[¶]	***-.-***
MTAI-8	14.03	18.16	17.78	13.73	***15.93***	13.26	11.47	***14.91***
CM4843-1	10.52	12.44	12.60	11.76	***11.83***	12.59	11.18	***11.85***
MCOL1505	10.05	6.55	11.01	9.19	***9.20***	8.73	10.13	***9.26***
CG 1141-1	7.92	9.49	9.24	11.32	***9.49***	12.08	12.87	***10.34***
CM3306-4	2.62	8.70	7.90	10.13	***7.34***	8.24	4.57	***7.07***
Mean	***9.72***	***10.45***	***12.43***	***11.46***	***11.02***	***12.03***	***9.81***	***10.37***

[§] Average performance until normal harvest date (March 15).

[¶] Plots where roots were stolen before harvest.

Table 2.17. Fresh roots production (t/ha) in sequential samplings taken at Santo Tomás (Atlántico). Planting date was May 10.

Clon	Dec. 13	Jan. 24	Feb. 21	Mar. 15	***Mean*** [§]	Apr. 24	May 23	***Overall Mean***
CM3306-19	31.00	32.20	42.80	43.90	***37.48***	[¶]	[¶]	***-.-***
MVEN 25	30.40	27.40	42.00	46.00	***36.45***	[¶]	[¶]	***-.-***
CM4919-1	29.20	22.80	29.80	33.80	***28.90***	32.20	36.60	***30.47***
MCOL 2215	17.20	22.80	21.40	25.60	***21.75***	30.60	23.60	***23.28***
SM8027-3	26.40	30.60	37.20	34.20	***32.10***	36.00	32.60	***32.73***
SM1433-4	30.40	30.20	40.60	36.10	***34.33***	52.00	33.40	***36.72***
CM3555-6	15.80	32.80	42.00	52.20	***35.70***	41.60	51.40	***38.79***
CM6119-5	23.80	19.20	26.20	27.30	***24.13***	31.60	34.20	***26.63***
CM6754-8	24.40	21.40	25.00	24.40	***23.80***	30.20	30.00	***25.60***
SM1411-5	35.20	42.40	50.60	38.90	***41.78***	59.00	[¶]	***-.-***
SM1438-2	27.80	30.80	38.20	25.90	***30.68***	53.40	[¶]	***-.-***
MTAI-8	38.40	49.80	51.00	38.90	***44.53***	38.00	42.00	***43.23***
CM4843-1	29.00	35.20	35.80	34.70	***33.68***	38.40	40.20	***35.28***
MCOL1505	26.00	17.60	29.80	27.20	***25.15***	28.80	39.40	***27.71***
CG 1141-1	21.40	27.20	25.20	31.70	***26.38***	35.60	42.60	***30.01***
CM3306-4	8.20	24.20	19.60	26.30	***19.58***	22.60	15.60	***19.44***
Mean	***25.91***	***29.16***	***34.83***	***34.19***	***31.02***	***37.86***	***35.13***	***30.82***

[§] Average performance until normal harvest date (March 15).
[¶] Plots where roots were stolen before harvest.

Table 2.18. Dry matter content (%) in sequential samplings taken at Santo Tomás (Atlántico). Planting date was May 10.

Clon	Dec. 13	Jan. 24	Feb. 21	Mar. 15	***Mean*** [§]	Apr. 24	May 23	***Overall Mean***
CM3306-19	34.08	30.94	35.00	29.70	***32.43***	[¶]	[¶]	***-.-***
MVEN 25	37.21	35.06	36.13	33.50	***35.47***	[¶]	[¶]	***-.-***
CM4919-1	38.64	36.63	35.87	33.70	***36.21***	30.83	28.10	***34.28***
MCOL 2215	38.43	39.48	38.23	34.60	***37.69***	29.69	30.90	***35.57***
SM8027-3	36.74	35.20	34.41	33.70	***35.01***	30.81	28.60	***33.50***
SM1433-4	37.21	36.47	34.22	33.00	***35.22***	31.20	27.70	***33.57***
CM3555-6	35.80	32.90	33.24	31.00	***33.24***	28.36	25.80	***31.48***
CM6119-5	39.26	36.38	38.09	34.30	***37.01***	33.94	30.90	***35.70***
CM6754-8	35.53	33.40	32.92	29.60	***32.86***	32.90	25.10	***31.76***
SM1411-5	43.44	39.95	36.07	33.10	***38.14***	29.01	[¶]	***-.-***
SM1438-2	38.30	36.80	37.14	36.50	***37.18***	33.07	[¶]	***-.-***
MTAI-8	36.53	36.47	34.87	35.30	***35.79***	34.89	27.30	***34.45***
CM4843-1	36.27	35.34	35.20	33.90	***35.18***	32.78	27.80	***33.78***
MCOL1505	38.64	37.21	36.94	33.80	***36.65***	30.30	25.70	***34.18***
CG 1141-1	37.00	34.88	36.67	35.70	***36.06***	33.94	30.20	***34.92***
CM3306-4	31.96	35.96	40.30	38.50	***36.68***	36.45	29.30	***35.59***
Mean	***37.19***	***35.82***	***35.96***	***33.74***	***35.68***	***32.01***	***28.12***	***34.07***

[§] Average performance until normal harvest date (March 15).
[¶] Plots where roots were stolen before harvest.

Table 2.19. Fresh foliage production (t/ha) in sequential samplings taken at Santo Tomás (Atlántico). Planting date was May 10.

Clon	Dec. 13	Jan. 24	Feb. 21	Mar. 15	***Mean*** [§]	Apr. 24	May 23	***Overall Mean***
CM3306-19	8.20	1.60	0.80	-.-	***3.53***	[¶]	[¶]	**-.-**
MVEN 25	7.60	4.20	1.00	-.-	***4.27***	[¶]	[¶]	**-.-**
CM4919-1	2.00	1.40	0.20	-.-	***1.20***	2.00	2.80	**1.60**
MCOL 2215	3.60	3.40	0.20	-.-	***2.40***	1.40	5.60	**2.77**
SM8027-3	5.60	8.20	0.80	-.-	***4.87***	3.20	7.00	**4.94**
SM1433-4	5.60	6.00	1.00	-.-	***4.20***	2.80	5.60	**4.20**
CM3555-6	4.80	6.60	0.40	-.-	***3.93***	3.60	10.00	**4.89**
CM6119-5	2.80	2.20	0.60	-.-	***1.87***	2.40	4.00	**2.31**
CM6754-8	9.00	12.80	0.80	-.-	***7.53***	3.20	4.00	**6.22**
SM1411-5	9.00	9.20	1.20	-.-	***6.47***	[¶]	[¶]	**-.-**
SM1438-2	9.40	10.20	0.80	-.-	***6.80***	[¶]	[¶]	**-.-**
MTAI-8	9.00	6.40	2.00	-.-	***5.80***	1.20	6.60	**5.17**
CM4843-1	7.20	10.00	1.00	-.-	***6.07***	1.80	4.40	**5.08**
MCOL1505	15.20	12.80	1.20	-.-	***9.73***	2.80	6.20	**7.99**
CG 1141-1	9.00	11.80	0.80	-.-	***7.20***	3.40	7.00	**6.53**
CM3306-4	9.20	7.20	1.80	-.-	***12.73***	2.40	4.20	**9.59**
Mean	***7.33***	***7.13***	***0.91***	***-.-***	***5.54***	***2.52***	***5.62***	***5.11***

[§] Average performance until normal harvest date (March 15).

[¶] Plots where roots were stolen before harvest.

b. Selections for the acid-soil savanna environments.

The same scheme of selection illustrated in Figure 2.1 was followed in the acid soil savannas environment. The different trials conducted in the region are described in Table 2.20. There are additional trials planted in the second semester of 1999, whose results are not described in this Table. The major abiotic constraint for cassava production in this environment is the poor fertility of the soil, which are acidic, poor in calcium and phosphorous, and high in aluminum. Water availability is adequate through the year although there is a dry spell in December and January. Two diseases are very predominant in the area the bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*) and fungal super elongation disease (*Spacheloma manihoticola*). Breeding efforts, therefore are directed at increasing yield potential under these abiotic and biotic limiting factors, which are also relevant in many other cassava-growing regions of the world.

The new breeding scheme (Figure 2.1) involves the planting of clonal evaluation directly from the F1 plants. In the acid-soil environments, 1525 families (represented by seven plants each) are currently evaluated. The segregation for their reaction to the diseases mentioned above is very dramatic. It is a new experience for the cassava-breeding project, repeated in the sub-humid environment (1350 families) and the mid-altitude valleys (1100 families). A clear advantage of this evaluation already apparent is that selection for disease resistance can be done more reliably when several plants (rather than one as in the previous system) are representing each family.

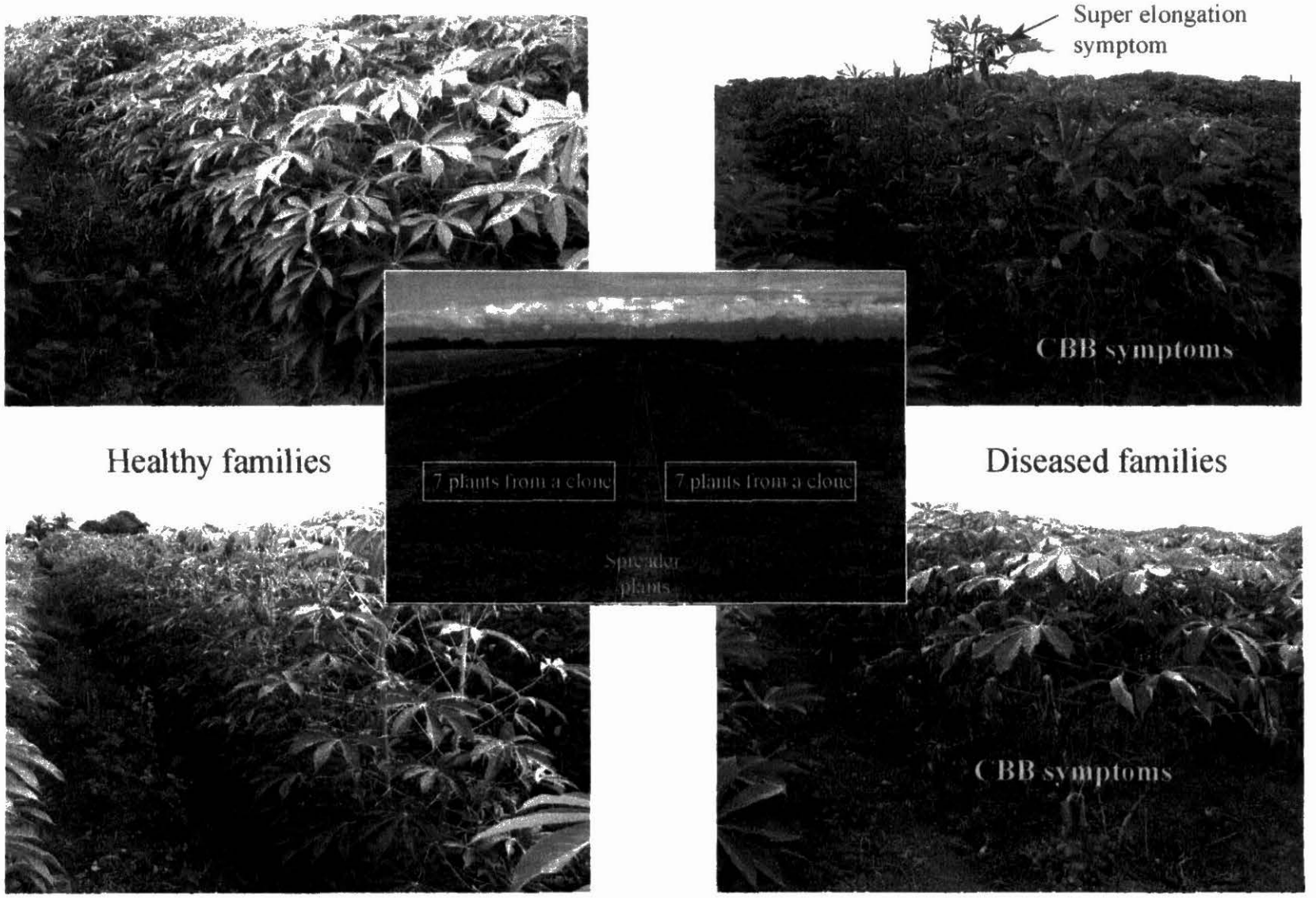


Figure 2.2. Clonal evaluation at CORPOICA La Libertad (Meta). Many spreader plants were dead one months after planting. At three months excellent segregation for CBB and super elongation disease could be observed.

Figure 2.2 illustrates a general view of the clonal evaluation plot at CORPOICA La Libertad (Villavicencio, Meta), as well as some pictures depicting the wide segregation for disease resistance found among the genotypes included in the trial. The families that were planted one after the other in the same row, were separated by one plant of a mixture of materials. This mixture was made up of susceptible genotypes and was included in the trial to serve as spreader plants for different diseases. The susceptibility of these plants was already apparent very early in the season (Figure 2.2). Since this trial is very large the border rows, as well as a central one was uniformly planted with the variety CM 6740-7 (Reina). The performance of this variety through the whole experiment will be used as a check to adjust results to the unavoidable lack of soil uniformity, in such a large plot.

Table 2.20. Trials conducted in the acid-soils ecosystem during the 1999-2000 season.

Trial	Location	N° of Genotypes [¶]	N° of Reps	Observations
F1	Cenicaña	See Table 2.9	1	Currently involves the planting of the diallel study as single F1 plants.
F1C1	La Libertad	1426 (1)	1	Stage eliminated in the new scheme.
Clonal evaluation	La Libertad La Libertad	116 (6) 1525 (7) [§]	1	New selection scheme begun: plants were in the field for 10 months. Data for this trial not presented.
Preliminary yield trial	La Libertad	150 (20)	1	See Table 2.21
Advanced yield trial	La Libertad Matazul Matazul	103 (25) 52 (25) 25 (25)	3 3 3	See Table 2.22
Regional trials	San Martín Matazul Granada La Libertad	20 (25) 20 (25) 22 (25) 20 (25)	3 3 3 3	See Table 2.23 See Table 2.23 See Table 2.23 See Table 2.23
Seed increases	Several	35	-.-	-.-

[¶] Within parenthesis the number of plants per plot.

[§] Clonal evaluation of the new selection scheme.

Table 2.21 presents the results of the **preliminary yield trials** conducted at La Libertad. A total of 150 genotypes were evaluated but only the best 20 performing clones and nine representative checks are listed here. With the exception of Brasilera and MVEN77, all the checks had negative selection indices, suggesting that they had a performance below the average of the experiment. Among the best 13 progenies, four were of sister lines from the family SM 2361. These materials are derived from an open pollination using the CM 4574-7, as female progenitor.

Table 2.21. **Preliminary yield trial** at en Villavicencio (Meta). The results from the best 20 performing genotypes are presented along with nine checks. The order of the materials follows the ranking based on the selection index criteria.

Genotype	Foliage Evaluation (1-5) [§]	Dry Matter (%)	Yield (t/ha)		Harvest Index [¶]	Selection Index
			Fresh roots	Dry matter		
SM 2371-6	1	31.7	31.00	9.83	0.74	12.38
SM 2361-31	1	36.3	23.33	8.47	0.64	9.55
SM 2361-29	1	36.0	23.33	8.40	0.55	8.73
SM 2382-5	2	33.7	27.67	9.32	0.52	7.31
SM 2370-1	2	33.9	26.67	9.04	0.57	7.25
SM 2361-30	1	35.1	21.00	7.37	0.54	7.18
SM 2382-8	3	35.9	25.67	9.21	0.63	7.12
SM 2425-3	3	29.7	28.67	8.51	0.66	6.36
SM 2365-18	1	33.2	19.00	6.31	0.62	6.22
SM 2375-16	2	33.1	24.33	8.05	0.62	6.20
SM 2365-7	1	31.7	20.00	6.34	0.61	6.15
SM 2458-9	3	38.1	23.33	8.89	0.53	5.90
SM 2361-10	1	34.5	18.67	6.44	0.55	5.89
SM 2458-10	1	35.9	17.67	6.34	0.54	5.59
SM 2454-6	2	36.3	22.00	7.99	0.55	5.55
SM 2367-16	4	32.1	26.33	8.45	0.61	5.45
SM 2371-1	3	31.7	25.67	8.14	0.60	5.29
SM 2366-17	1	33.9	18.33	6.22	0.49	5.01
CM 8288-46	4	34.3	22.67	7.77	0.67	4.88
SM 2375-17	2	32.7	21.00	6.87	0.64	4.64
BRA 12	3.5	32.7	7.67	2.53	0.36	-5.15
COL 22	5	12.7	1.33	0.38	0.38	-8.68
COL 1468	4.5	25.9	9.75	2.57	0.32	-5.78
COL 1684	3.5	30.6	10.67	3.27	0.58	-2.30
VEN 77	3	32.0	19.17	6.13	0.58	2.21
CM 523-7	3.5	36.3	12.00	4.28	0.46	-1.71
CM 2177-2	3.5	31.3	9.33	2.80	0.36	-4.72
BRASILERA	3.5	35.5	22.17	7.88	0.65	5.10
CHIROZA	4	28.7	13.17	3.76	0.48	-2.58
Minimum [†]	1	0.0	0.67	0.22	0.04	-10.94
Maximum [†]	5	38.3	31.00	9.83	0.76	12.38
Mean [†]	3.25	25.9	14.92	4.95	0.51	0.00
St. Deviation [†]	1.09	13.8	5.87	2.03	0.12	4.17

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.22. **Advanced yield trial** at Villavicencio (Meta). Results from the 20 best performing experimental clones (out of 103) are presented along with nine relevant checks.

Genotype	Foliage Evaluation (1-5) [§]	Yield (t/ha)		Dry matter (%)	Harvest Index [¶]	Selection Index
		Fresh roots	Dry matter			
SM 1807-1	1.7	27.4	10.1	36.85	0.57	11.16
SM 1363-11	2.7	24.4	9.5	39.15	0.59	9.83
SM 1807-2	1.0	22.0	8.0	36.3	0.45	9.28
SM 2219-11	2.3	22.9	8.4	36.6	0.58	7.18
SM 2069-49	1.3	20.7	7.4	35.7	0.63	7.06
CM 7073-7	1.3	20.7	7.4	35.65	0.45	6.44
SM 1152-13	3.3	22.7	8.4	37.05	0.55	6.40
SM 1812-69	2.7	25.5	8.5	33.35	0.64	6.01
CM 8748-2	2.3	25.6	8.4	32.95	0.62	5.94
SM 2182-3	3.3	23.2	8.0	34.4	0.68	5.78
SM 1143-18	2.7	21.9	7.9	35.9	0.67	5.58
SM 2288-3	3.0	21.6	8.0	36.85	0.54	5.49
SM 1245-29	3.0	23.6	8.2	35	0.52	5.37
SM 1363-12	2.7	21.9	8.0	36.3	0.59	5.25
SM 1861-18	3.3	21.8	7.8	35.65	0.60	5.02
CM 8779-4	2.3	22.2	7.7	34.85	0.51	4.85
SM 2291-7	3.7	25.3	8.6	34.1	0.63	4.83
SM 1859-26	1.0	18.2	6.0	33.15	0.64	4.68
SM 2261-3	4.7	25.0	8.3	33.05	0.49	4.58
SM 1245-25	4.0	25.4	8.6	33.85	0.63	4.51
CM 8785-1	3.0	22.4	7.7	34.35	0.53	4.22
SM 1920-1	2.0	19.6	7.2	36.9	0.56	4.17
BRA 12	4.3	5.9	1.7	28.5	0.43	-14.05
COL 22	4.7	7.0	2.1	30.1	0.61	-11.52
COL 1468	4.7	14.4	4.0	27.9	0.47	-9.68
COL 1684	4.7	12.5	4.1	32.4	0.46	-3.57
VEN 77	3.0	19.9	6.0	30.15	0.60	-1.22
CM 523-7	3.7	7.7	2.7	34.5	0.42	-7.47
CM 2177-2	2.3	13.9	4.5	32.25	0.48	-3.56
BRASILERA	3.3	21.5	7.4	34.5	0.54	3.30
Chiroza Morada	2.7	17.9	5.7	32.2	0.53	-2.74
Minimum [†]	1	5.04	1.67	278.5	3	-14.04723
Maximum [†]	5	27.41	10.1	397.5	9	11.16363
Mean [†]	3.318452	17.35	5.93	341.57	6.63	0.00
St. Deviation [†]	0.901992	4.76	1.69	24.70	1.70	4.67

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.23. **Advanced yield trial** at Matazul (Meta). Results from the 20 best performing clones (out of 52) are presented along with eight relevant checks.

Genotype	Foliage evaluation (1-5) [§]	Yield (t/ha)		Dry matter	Harvest Index [¶]	Selection Index
		Fresh roots	Dry matter			
SM 1565-15	1.67	17.04	5.97	35.1	0.5	7.4
SM 1812-29	2.00	15.63	5.76	36.9	0.5	7.2
SM 1794-18	3.67	15.11	5.85	38.7	0.6	6.6
SM 1807-2	1.67	16.44	5.67	34.5	0.5	6.3
SM 2110-2	3.67	18.59	6.32	34.0	0.7	6.3
SM 1807-1	3.00	18.74	6.36	34.0	0.6	6.2
SM 1871-32	3.00	18.89	6.44	34.1	0.6	5.7
SM 1773-2	1.67	13.63	4.99	36.6	0.5	5.7
SM 1861-18	3.67	18.07	6.16	34.1	0.7	5.6
SM 1583-8	4.00	16.00	5.71	35.7	0.7	5.0
SM 1483-1	3.67	17.78	6.17	34.7	0.6	4.7
SM 1960-1	3.67	16.74	5.78	34.6	0.7	4.7
SM 1152-13	3.33	15.19	5.47	36.0	0.6	4.6
SM 1872-9	4.00	18.81	6.15	32.7	0.7	4.6
SM 1674-1	3.00	15.19	5.42	35.7	0.6	4.3
SM 1859-15	2.67	15.48	5.39	34.8	0.6	4.1
CM 6975-14	2.67	14.89	5.28	35.5	0.5	3.9
SM 1859-26	3.33	17.33	5.72	33.0	0.6	3.7
SM 1241-12	3.33	17.11	5.60	32.7	0.7	3.5
SM 2104-1	3.67	15.78	5.28	33.5	0.7	3.2
SM 2069-4	4.00	14.30	5.11	35.8	0.6	2.9
SM 1883-17	3.67	12.44	4.65	37.4	0.5	2.5
BRA 12	4.33	6.00	1.94	32.3	0.4	-10.9
COL 1468	5.00	4.22	1.34	31.7	0.4	-12.9
COL 1684	5.00	8.67	2.81	32.5	0.7	-5.0
VEN 77	4.00	9.70	2.89	29.8	0.4	-9.1
CM 523-7	4.00	10.22	3.76	36.8	0.5	-0.3
CM 2177-2	3.00	13.93	4.35	31.3	0.6	-1.5
BRASILERA	4.00	11.11	3.92	35.3	0.6	-0.6
CHIR MORADA	4.00	4.52	1.32	29.2	0.3	-16.2
Minimum [†]	1.67	4.22	1.32	29.20	0.26	-16.18
Maximum [†]	5.00	18.89	6.44	38.70	0.70	7.40
Mean [†]	3.57	13.38	4.53	33.76	0.55	0.00
St. Deviation [†]	0.72	3.46	1.23	1.93	0.09	5.15

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.24. Results from regional trials carried out at three locations in the acid soil savannas.

Genotype	San Martín				Matazul					La Libertad					Ranking based Selection Index		
	Yield (t/ha)		Harv.	Dry	Yield (t/ha)		Harv.	Dry	Fol.	Yield (t/ha)		Harv.	Dry	Fol.			
	Fresh	Dry	Ind.	Matter	Fresh	Dry	Ind.	Matter	Eval.	Fresh	Dry	Ind.	Matter	Eval.	S.M.	M.A.	L.L.
BRA 502	24.52	7.89	0.53	32.2	8.15	2.61	0.41	32.1	2.3	16.30	5.81	0.49	35.7	3.8	17	28	19
Brasílera	19.48	6.52	0.60	33.5	12.96	4.32	0.52	33.4	2.7	20.74	7.28	0.58	35.1	3.0	16	19	10
Chir.Morada	14.59	4.36	0.53	29.9	4.74	1.32	0.30	27.8	2.5	13.41	4.35	0.43	32.4	2.5	27	32	32
CM 523- 7	13.78	4.52	0.64	32.8	8.89	3.02	0.50	34.0	2.5	14.52	5.40	0.54	37.2	2.7	22	22	21
CM 2177- 2	21.04	7.50	0.51	35.7	10.59	3.20	0.47	30.2	3.3	18.44	6.23	0.55	33.8	3.8	10	26	18
CM 4574- 7	***25.63***	***8.64***	***0.52***	***33.7***	***17.04***	***5.94***	***0.48***	***34.9***	***5.0***	***19.19***	***6.71***	***0.57***	***35.0***	***4.3***	***8***	***6***	***5***
CM 5306- 8	20.44	6.47	0.51	31.7	10.15	3.43	0.43	33.8	2.5	15.33	5.25	0.52	34.2	4.3	23	24	26
CM 6438- 14	***19.48***	***5.99***	***0.57***	***30.8***	***19.93***	***7.22***	***0.52***	***36.3***	***3.0***	***16.74***	***6.17***	***0.49***	***36.9***	***3.8***	***24***	***3***	***12***
CM 6740- 7	***24.52***	***8.02***	***0.54***	***32.7***	***10.52***	***3.55***	***0.55***	***33.8***	***2.3***	***25.19***	***9.45***	***0.64***	***37.5***	***3.0***	***14***	***21***	***1***
CM 6921- 3	26.59	8.39	0.57	31.6	15.33	4.95	0.43	32.3	4.3	15.56	5.60	0.59	36.0	2.5	13	14	23
CM 6975- 14	16.22	4.94	0.55	30.5	15.19	5.17	0.48	34.1	3.0	19.11	7.05	0.52	36.9	2.7	26	11	9
CM 7052- 3	22.07	7.20	0.56	32.6	8.67	2.78	0.56	32.1	2.3	20.22	7.03	0.55	34.8	3.3	19	27	13
CM 7073- 7	26.56	8.82	0.58	33.2	6.07	1.93	0.48	31.8	2.3	18.00	6.44	0.56	35.8	4.3	5	30	7
SM 1143- 18	-.-	-.-	-.-	-.-	***21.56***	***7.02***	***0.57***	***32.6***	***3.8***	***22.67***	***8.33***	***0.57***	***36.7***	***3.0***	-.-	***2***	***3***
SM 1152- 13	5.85	1.81	0.50	31.0	13.56	4.85	0.57	35.8	3.0	21.11	7.56	0.63	35.8	2.7	29	9	6
SM 1225- 15	20.89	6.91	0.56	33.1	14.15	4.75	0.61	33.6	2.3	12.00	4.19	0.55	34.9	2.1	18	15	31
SM 1241- 12	26.26	8.36	0.58	31.9	15.63	4.99	0.64	31.9	2.7	14.07	4.68	0.61	33.3	2.3	11	12	30
SM 1363- 11	***30.00***	***9.65***	***0.55***	***32.2***	***15.56***	***5.87***	***0.55***	***37.8***	***3.3***	***17.78***	***6.49***	***0.62***	***36.5***	***2.5***	***6***	***4***	***14***
SM 1483- 1	25.78	8.38	0.59	32.5	16.07	5.55	0.54	34.6	2.7	19.48	6.77	0.50	34.8	2.5	9	10	24
SM 1565- 15	24.07	7.75	0.59	32.2	15.19	5.25	0.46	34.6	5.0	11.33	4.06	0.48	35.8	3.8	12	7	28
SM 1674- 1	24.89	8.03	0.64	32.3	14.52	4.78	0.52	33.0	3.0	14.52	4.80	0.52	33.1	3.3	7	17	29
SM 1694- 2	14.74	4.15	0.45	28.2	9.70	3.25	0.39	33.5	3.0	16.44	6.01	0.48	36.6	3.8	28	25	15
SM 1697- 1	5.11	1.47	0.36	28.8	13.26	4.12	0.49	31.1	2.1	20.74	7.04	0.56	33.9	2.7	30	23	17
SM 1794- 18	13.85	4.70	0.57	33.9	18.59	7.14	0.58	38.4	3.3	15.78	6.00	0.54	38.0	2.5	21	1	16
SM 1812- 69	23.78	7.45	0.64	31.4	15.48	4.77	0.61	30.8	2.5	23.26	7.96	0.66	34.2	2.3	15	18	8
SM 1821- 7	***23.78***	***8.22***	***0.58***	***34.6***	***18.96***	***6.57***	***0.68***	***34.7***	***2.7***	***15.70***	***5.62***	***0.63***	***35.8***	***2.5***	***2***	***5***	***22***
SM 1854- 23	-.-	-.-	-.-	-.-	20.22	6.32	0.65	31.3	2.5	18.89	6.48	0.64	34.3	2.7	-.-	8	20
SM 1859- 26	25.26	7.74	0.59	30.7	13.48	4.26	0.55	31.6	2.5	20.44	7.03	0.59	34.4	5.0	20	20	4
SM 1862- 25	24.30	8.59	0.51	35.4	14.96	5.09	0.55	34.1	2.7	17.04	5.87	0.60	34.4	2.5	3	13	27
SM 1881- 17	30.67	10.73	0.62	35.0	7.11	2.00	0.36	28.1	2.3	19.11	6.50	0.63	34.0	2.3	1	31	25
SM 2068- 3	28.67	9.16	0.61	32.0	7.56	2.46	0.39	32.5	2.3	18.96	6.79	0.53	35.8	3.3	4	29	11
SM 2219- 11	11.67	3.78	0.59	32.4	12.52	4.17	0.64	33.3	2.7	24.15	8.81	0.66	36.5	2.7	25	16	2
Minimum	5.11	1.47	0.36	28.2	4.74	1.32	0.30	27.8	2.1	11.33	4.06	0.43	32.4	2.1			
Maximum	30.67	10.73	0.64	35.7	21.56	7.22	0.68	38.4	5.0	25.19	9.45	0.66	38.0	5.0			
Mean	21.15	6.87	0.56	32.3	13.32	4.46	0.51	33.1	2.9	18.01	6.37	0.56	35.3	3.1			
St. Deviation	6.54	2.24	0.06	1.7	4.31	1.58	0.09	2.3	0.7	3.38	1.27	0.06	1.4	0.7			

Tables 2.22 and 2.23 summarize the results of two different **advanced yield trials** conducted respectively at La Libertad and Matazul, both in the Meta Department. The trial at La Libertad had 103 entries, the one in Matazul only 52.

It is remarkable that two sister clones, from the family **SM 1807**, occupied the first and third place at La Libertad, based on their selection index performance (Table 2.22). The same related materials, also had an outstanding behavior in the advanced yield trial at Matazul, occupying the sixth and fourth places, respectively (Table 2.23). The female progenitor of this family was, again, **CM 4574-7**. This parental line has excellent adaptation for the conditions of the acid-soil savannas, with a reported tolerance to root rots (1999 Annual Report). It was included as one of the parents in the diallel established for this ecosystem (Table 2.6), and was included as a parental line again this year (Table 2.1). Except for Brasilera at La Libertad, all the checks in both trials had negative selection indexes. Notice that the mean selection index is always 0.00. This is so because the values that participate in their definition have been standardized, each with a mean of zero. Negative selection index, therefore, imply a performance poorer than the mean of the general population.

The results of three **regional trials** conducted in three different locations are summarized in Table 2.24. Based on the ranking from the selection indices across the three sites, genotypes CM 4574-7, CM 6438-14, CM 6740-7, SM 1143-18, SM 1363-11 and SM 1821-7 showed a good performance (highlighted in bold). In fact, the release of two of those varieties was officially approved. Cultivar CM 6740-7 or ***"Reina"*** was released on November 10, and CM 6438-14 will be released next year when enough seed is available. Reina is a variety with wide adaptation to different environments, but does not posses enough tolerance to do well in the acid soil savannas, but rather in the intermediate environments (called *"piedemonte"*) between the savannas and the hills. CM 6438-14, on the other hand, does not perform very well in the *piedemonte*, but it is an outstanding material for the more limiting environment of the true acid soil savannas.

As was the case in the sub-humid environment several sequential samplings were taken from different genotypes to evaluate the evolution of productivity for roots and foliage. The results of this evaluation are described in Table 2.25. As early as the eight month, the recently released variety CM 6740-7 already produced more than 10 t/ha of dry matter. The oscillations along consecutive harvest times were natural, because just a few plants were taken each time. So, in general, only the trends and final mean should be more carefully analyzed.

In contrast with the observed results at the sub-humid environment, there was not a drastic drop in dry matter content from the 8^{th} to the 13^{th} month of age. Likewise, fresh foliage production remained more or less constant through the different ages of the plant, although it tended to be lower at the 10^{th} month.

Table 2.25. Results from sequential samplings taken at La Libertad (Meta) from five relevant genotypes. Within parenthesis the age of the plants at the time of each sampling.

Genotype	Jan. 18 (8)	Feb. 18 (8.5)	Feb. 1 (9)	Mar. 3 (9.5)	Mar. 17 (10)	Apr. 3 (10.5)	Apr. 30 (11.5)	May 15 (12)	May 31 (12.5)	Jun. 15 (13)	**Mean**
					Fresh roots production (t/ha)						
CM 523-7	20.6	26.3	19.4	28.1	21.6	21.6	16.9	26.6	23.8	23.4	**22.8**
CM 2177-2	20.3	20.6	25.9	18.8	25.9	36.3	26.3	35.6	25.9	29.1	**26.5**
SM 1565-15	15.6	16.3	21.9	23.4	17.5	27.8	29.4	30.3	31.9	32.5	**24.7**
CM 6740-7	36.9	30.0	31.3	30.0	29.1	30.6	46.3	39.1	42.5	32.2	**34.8**
Brasilera	25.3	19.7	20.6	25.6	25.6	24.7	17.5	19.4	29.7	27.5	**23.6**
Mean	**23.74**	**22.58**	**23.82**	**25.18**	**23.94**	**28.20**	**27.28**	**30.19**	**30.75**	**28.94**	**26.46**
					Dry matter production (t/ha)						
CM 523-7	7.9	10.3	7.5	11.2	7.8	7.4	6.3	9.9	8.7	8.5	**8.5**
CM 2177-2	6.7	7.2	8.8	6.3	9.2	12.6	8.9	12.3	9.0	10.1	**9.1**
SM 1565-15	5.4	6.1	8.4	8.4	6.2	9.9	10.9	11.7	12.3	12.5	**9.2**
CM 6740-7	14.4	12.1	12.0	11.6	10.8	11.5	17.2	13.6	15.7	11.8	**13.1**
Brasilera	9.3	7.3	7.4	9.5	8.9	8.9	6.0	6.9	10.7	9.9	**8.5**
Mean	**8.72**	**8.60**	**8.81**	**9.42**	**8.57**	**10.08**	**9.87**	**10.87**	**11.29**	**10.56**	**9.68**
					Dry matter content (%)						
CM 523-7	38.3	39.1	38.6	39.8	35.9	34.1	37.5	37.1	36.8	36.1	**37.3**
CM 2177-2	33.1	34.9	34.1	33.6	35.5	34.8	34.0	34.6	34.6	34.7	**34.4**
SM 1565-15	34.6	37.5	38.4	36.0	35.3	35.7	37.0	38.5	38.6	38.6	**37.0**
CM 6740-7	38.9	40.3	38.2	38.7	37.0	37.7	37.1	34.9	36.9	36.8	**37.7**
Brasilera	36.6	37.1	35.8	37.3	34.9	36.2	34.4	35.5	36.2	35.9	**36.0**
Mean	**36.30**	**37.78**	**37.02**	**37.08**	**35.72**	**35.70**	**36.00**	**36.12**	**36.62**	**36.42**	**36.48**
					Fresh foliage production (t/ha)						
CM 523-7	4.7	5.0	3.1	4.4	3.4	4.1	4.1	6.9	4.7	4.7	**4.5**
CM 2177-2	4.7	5.9	3.8	5.0	4.7	5.3	7.8	8.4	5.0	4.4	**5.5**
SM 1565-15	4.4	4.4	3.1	5.0	2.5	4.7	7.2	8.4	7.2	5.3	**5.2**
CM 6740-7	3.8	3.8	3.1	3.1	2.8	2.2	7.2	5.0	3.1	5.3	**3.9**
Brasilera	3.8	3.1	3.1	4.4	4.1	2.2	3.4	5.3	5.3	3.4	**3.8**
Mean	**4.28**	**4.44**	**3.24**	**4.38**	**3.50**	**3.70**	**5.94**	**6.81**	**5.06**	**4.63**	**4.59**

c. Selections for the mid-altitude valleys.

The trials conducted for this ecosystem are described in Table 2.26. Because of a conjunction of factors, there has been an increased problem with whiteflies in our experimental station. Along with the increase in whiteflies population there has been an increased problem of the *frog skin* disease transmitted by them. There is ample evidence that the frog skin disease is induced by a virus. Because of this problem our operations at the experimental station have been modified and restricted. Results from these trials have been affected by this situation. Further information in this regard can be found below in Activity 2.6.

Table 2.26. Trials conducted in mid-altitude valleys ecosystem during the 1999-2000 season.

Trial	Location	N° of Genotypes [¶]	N° of Reps	Observations
F1	Cenicaña	See Table 2.9	1	Currently involves the planting of the diallel study as single F1 plants
F1C1	Palmira	1426 (1)	1	Stage eliminated in the new scheme.
Clonal evaluation	Palmira CEUN Quilichao	475 (6) 653 (6)[§] 653 (6)[§]	1	New selection scheme begun: plants were in the field for 10 months. Data for this trial not presented.
Preliminary yield trial	Palmira	197 (20)	1	See Table 2.27
Advanced yield trial	Palmira Quilichao	171 (25) 48 (25)	3 3	See Table 2.28 See Table 2.29
Regional trial	Palmira	48 (25)	3	See Table 2.30
Seed increase	Several	Varios	-.-	-.-

[¶] Within parenthesis the number of plants per plot.
[§] Clonal evaluation of the new selection scheme.

Table 2.27 presents the results of the **preliminary yield trial**. The performance of the best 20 progenies included in the table was outstanding and much superior than the set of checks presented. The best genotype (according to the selection index criteria) did not reach the maximum dry matter yield, but had better foliage evaluation and an acceptable harvest index, and therefore, it was shown first in the list. Both dry matter yield and dry matter content were excellent in this trial. In Tables 2.28 and 2.29 the results of **advanced yield trials** conducted at Palmira (Valle del Cauca Deparment) and Santander de Quilichao (Cauca Department) are presented, with 171 and 48 clones, respectively. Most of the checks have negative selection indices, whereas the best performing clones had large positive values, indicating their genetic superiority over the checks.

The results of a **regional trial** conducted at CIAT-Palmira are presented in Table 2.30. Many of the genotypes included have survived several years of intense selection and constitute the best performing materials that CIAT has to offer at this point in time. Clones CM 8370-11 (included as a parent in the crosses of this year, Table 2.1), SM 1855-15, SM 1636-24 and SM 1602-13, showed excellent performance.

An interesting evaluation was carried out in the fields of a poultry producer at Jamundí (Valle del Cauca Department). About 10 hectares of commercial plantings accompanied the experimental trials and confirmed their results. Fifteen clones were planted in plots fertilized with chemical fertilizers and chicken manure. As was the case for the other two ecosystems, sequential samplings were taken starting in November 9, when the plants were about seven months old. Samples consisted of four plants from each treatment

(type of fertilizer) for a total of eight plants per genotype per sampling date. There was no significant difference for the type of fertilizers, so the average of the eight plants is presented herein. Tables 2.31 through 2.34 summarize the results for dry matter production, fresh root production, fresh foliage production, and dry matter content. Chicken manure proved to be a good alternative for fertilizing cassava, an appealing feature for poultry producers interested in also producing cassava.

Table 2.27. **Preliminary yield trial** at CIAT – Palmira. The results of the best 20 performing clones out of 191 (based on the selection index criteria) and six checks are presented.

Genotype	Yield (t/ha)		Dry matter (%)	Foliage evaluation (1-5) [§]	Harvest Index [¶]	Selection Index
	Fresh roots	Dry matter				
SM 2058-2	39.17	15.47	39.5	2.0	0.57	13.90
SM 2371-6	40.00	15.72	39.3	3.0	0.65	11.73
SM 2373-2	33.00	13.73	41.6	3.0	0.55	8.37
SM 2363-8	31.67	12.79	40.4	3.0	0.61	8.33
SM 2375-13	32.50	12.51	38.5	3.0	0.58	8.13
SM 2372-3	30.33	11.77	38.8	3.0	0.63	7.79
SM 2376-5	33.33	12.63	37.9	3.0	0.50	7.55
SM 2371-1	31.67	11.62	36.7	3.0	0.57	7.50
SM 2382-8	29.00	11.25	38.8	3.0	0.60	7.04
SM 2349-19	30.00	11.16	37.2	3.0	0.57	6.88
CM 9021-1	20.00	8.12	40.6	2.0	0.58	6.85
SM 2375- 3	30.00	11.79	39.3	3.0	0.52	6.69
SM 1789-52	28.00	12.12	43.3	3.0	0.54	6.58
SM 2361-9	30.50	11.93	39.1	3.0	0.49	6.57
SM 2368-2	30.83	12.15	39.4	3.0	0.46	6.42
SM 2363-3	23.00	9.11	39.6	2.0	0.43	6.40
CM 8907-1	27.17	11.14	41.0	3.0	0.58	6.37
SM 2455-3	28.83	11.50	39.9	3.0	0.51	6.20
SM 2465-3	31.00	11.35	36.6	4.0	0.61	6.00
SM 2369-3	28.83	10.58	36.7	3.0	0.52	5.90
BRA 12	22.06	8.31	37.57	3.3	0.52	2.94
COL 22	3.11	1.26	40.60	4.3	0.49	-5.58
COL 1468	9.22	3.42	37.70	4.0	0.47	-3.52
COL 1684	8.57	3.01	35.07	3.6	0.43	-3.92
VEN 77	14.80	5.29	35.36	3.5	0.47	-0.93
HMC 1	18.16	6.90	37.65	3.4	0.47	0.68
Minimum [†]	1.83	0.50	0.00	5.0	0.17	
Maximum [†]	40.00	15.72	45.50	2.0	0.65	
Mean [†]	18.64	6.87	27.44	3.2	0.46	
St. Deviation [†]	7.94	3.47	17.99	19.4	0.10	

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.28. **Advanced yield trial** at CIAT – Palmira. Results of the best 20 genotypes (out of 162) and six checks are presented.

Genotype	Foliage evaluation (1-5) [§]	Yield (t/ha)		Dry matter (%)	Harvest Index [¶]	Selection Index
		Fresh roots	Dry matter			
CM 8224-2	2.00	39.11	15.92	40.70	0.66	17.64
SM 1812-72	2.00	31.56	12.18	38.60	0.60	11.49
SM 1960-28	3.00	28.33	11.84	41.80	0.62	10.14
SM 1783-31	3.00	27.78	11.76	42.35	0.55	9.49
SM 1955-6	3.00	26.67	10.71	40.15	0.63	8.11
SM 1665-13	2.00	22.78	8.67	38.05	0.64	7.18
CM 8785-2	4.00	29.78	11.60	38.95	0.59	6.79
SM 1855-15	2.00	22.44	8.75	39.00	0.55	6.77
SM 1871-39	2.00	28.44	10.04	35.30	0.54	6.54
SM 2186-5	3.00	23.56	9.45	40.10	0.58	5.94
SM 1517-9	3.00	23.67	9.47	40.00	0.58	5.87
SM 2194-1	2.00	26.33	9.32	35.40	0.56	5.81
CM 6979-3	2.00	16.11	6.57	40.75	0.61	5.79
SM 1636-24	3.00	22.22	8.94	40.25	0.59	5.48
SM 2073-1	3.00	23.33	9.21	39.45	0.60	5.46
SM 1225-15	2.00	21.67	8.48	39.15	0.46	5.44
SB 216-9	2.00	21.11	7.99	37.85	0.57	5.40
SM 1778-44	3.00	24.89	9.53	38.30	0.58	5.12
SM 2081-34	4.00	28.22	11.27	39.95	0.44	5.07
SM 1533-3	4.00	22.00	9.06	41.20	0.59	4.84
MEX 108	3.00	26.78	9.49	35.45	0.55	3.28
BRA 12	4.00	4.44	1.48	33.25	0.44	-12.12
COL 1468	4.00	2.78	0.92	33.10	0.37	-13.90
COL 1505	4.00	5.22	2.00	38.35	0.51	-6.68
HMC 1	3.00	11.67	4.31	36.95	0.62	-2.05
CM 3306-4	3.00	14.07	6.16	43.80	0.54	3.84
Minimum [†]	2.00	2.78	0.92	30.40	0.26	
Maximum [†]	4.00	39.11	15.92	44.15	0.70	
Mean [†]	3.04	16.80	6.34	37.68	0.52	
St. Deviation [†]	0.50	6.10	2.36	2.42	0.09	

[§] Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

[¶] Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

[†] Statistical data based on the complete experiment.

Table 2.29. **Advanced yield trial** at Santander de Quilichao (Cauca). Results of the best 10 genotypes (out of 25) and nine checks are presented.

Genotype	Foliage evaluation	Yields (t/ha)		Dry matter (%)	Harvest Index ¶	Selection Index
		Fresh roots	Dry matter			
SM 2069-55	2.67	10.07	3.58	35.50	0.57	11.11
SM 1855-25	3.33	7.33	3.03	41.35	0.61	9.53
SM 2219-11	3.67	9.89	3.44	34.80	0.60	8.14
SM 1557-26	2.67	8.78	3.05	34.80	0.41	7.06
SM 593-5	3.00	6.37	2.42	38.00	0.48	5.38
CM 6740-7	3.33	6.96	2.56	36.75	0.54	5.28
SM 1881-9	2.67	7.07	2.34	33.10	0.51	4.76
SM 1870-24	4.00	7.52	2.76	36.70	0.53	4.60
CM 6370-2	3.00	6.48	2.16	33.30	0.63	4.28
SM 2065-4	3.33	8.33	2.57	30.85	0.61	4.27
BRA 489	3.00	5.26	1.85	35.15	0.45	1.73
BRA 12	3.67	2.48	0.86	34.70	0.28	-5.95
COL 1468	3.67	5.59	1.68	30.05	0.48	-2.30
COL 1684	3.67	2.11	0.71	33.50	0.44	-5.47
VEN 77	3.33	5.78	1.78	30.80	0.53	-0.32
CM 507-37	3.33	7.89	2.46	31.20	0.53	2.96
CM 523-7	3.67	2.00	0.76	37.90	0.32	-4.19
CM 2177-2	3.67	3.48	1.05	30.05	0.42	-5.93
CM 3306-4	3.67	3.26	1.25	38.25	0.45	-0.71
Minimum †	2.67	0.63	0.18	27.45	0.23	
Maximum †	4.67	10.07	3.58	41.35	0.63	
Mean †	3.45	5.20	1.74	33.28	0.50	
St. Deviation †	0.44	2.13	0.77	3.14	0.09	

§ Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

¶ Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

† Statistical data based on the complete experiment.

Table 2.30. Results from the **regional trial** conducted at CIAT – Palmira during the period 1999-2000.

Genotype	Yield (t/ha)		Dry matter (%)	Foliage Evaluation (1-5)	Harvest Index ¶	Selection Index
	Fresh roots	Dry matter				
CM 8370-11	31.89	13.07	41.00	2.00	0.63	13.60
SM 1855-15	23.67	10.00	42.25	2.00	0.65	9.70
SM 1602-13	32.19	12.10	37.60	2.67	0.61	9.01
SM 1636-24	27.93	10.95	39.20	3.00	0.59	6.38
SM 1741-1	19.30	8.01	41.50	2.00	0.60	5.84
SM 2141-1	19.59	8.62	44.00	2.33	0.56	5.71
SM 1557-17	21.52	8.70	40.45	2.33	0.61	5.33
SM 1871-33	23.56	9.54	40.50	3.00	0.58	4.42
CM 3306-4	18.07	7.94	43.95	3.00	0.62	3.95
CM 8370-10	20.81	8.72	41.90	2.67	0.54	3.95
SM 1513-2	20.41	8.81	43.15	3.00	0.55	3.89
SM 1210-4	18.78	7.91	42.15	2.67	0.59	3.56
SM 1645-6	23.52	9.05	38.50	2.67	0.54	3.39
CM 7514-7	14.15	6.73	47.60	3.00	0.58	3.26
CM 5655-4	22.26	8.00	35.95	3.00	0.64	1.63
SM 1521-7	17.81	7.36	41.30	3.00	0.59	1.62
SM 2074-1	20.93	7.46	35.65	3.00	0.71	1.55
CM 523-7	22.41	9.36	41.75	3.00	0.63	5.27
BRA 12	18.19	6.66	36.65	3.00	0.58	-1.35
PER 183	19.78	6.78	34.30	3.00	0.60	-1.49
COL 1505	14.19	5.45	38.45	3.00	0.53	-3.22
COL 1468	9.89	3.48	35.20	4.00	0.51	-9.75
Minimum †	9.07	3.21	32.45	2.00	0.37	
Maximum †	32.19	13.07	47.60	4.00	0.71	
Mean †	18.17	7.03	38.50	2.91	0.56	
St. Deviation †	4.93	2.10	3.10	0.41	0.07	

§ Foliage evaluation integrates plant architecture, foliage health and capacity of stakes production (1 = excellent ➡ 5 = very poor).

¶ Harvest index = Total weight of commercial roots / total biomass. Total biomass is the summatory of the total weight of roots and the above ground tissues. A harvest index larger than 0.50 is desirable

† Statistical data based on the complete experiment.

As early as at seven months of age an average of more than 11 t/ha of dry matter were harvested across the fifteen clones (Table 2.31). Some genotypes showed early bulking capacity (MBRA 383, CM 7514-7, and CM 7951-5), whereas other tended to be late (CM 6740-7, SM 1741-1, and MPER 183). The production of fresh roots was excellent with more than 40 t/ha at the optimum harvest time (Table 2.32). Because of the more or less continuos occurrence of rains, fresh foliage production remains approximately constant, around eight t/ha (Table 2.33), as well as the content of dry matter (Table 2.34). The high root production plus the excellent amount of foliage that can be harvested highlights the enormous potential of the crop for the industry (particularly for feed but also for starch production).

Table 2.31. Dry matter production (t/ha) of 15 elite clones evaluated at Jamundí. The age of the crop at each sequential sampling is specified at the top of the table.

Genotype	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	Mean
CM849-1	11.64	11.22	8.91	12.86	15.89	13.27	17.12	16.10	**13.38**
CM6740-7	9.10	12.40	13.02	17.69	12.17	18.50	18.64	18.29	**14.98**
CM7514-7	13.25	15.17	13.34	13.26	14.91	16.04	21.83	18.87	**15.84**
CM7951-5	13.74	12.45	20.44	19.45	19.77	14.89	18.87	21.98	**17.70**
SM653-14	7.56	9.47	10.40	12.21	10.37	8.72	12.66	14.89	**10.79**
SM909-25	10.81	13.43	16.63	18.28	17.52	22.35	19.41	17.03	**16.93**
SM1219-9	11.58	12.42	12.78	11.66	18.81	17.45	22.95	20.51	**16.02**
SM1460-1	11.66	12.25	15.30	13.58	16.36	14.29	26.60	18.95	**16.12**
SM1543-16	13.77	10.09	12.53	16.37	14.54	13.59	24.59	15.25	**15.09**
SM1557-17	5.92	10.84	12.31	13.88	17.65	17.54	21.02	15.89	**14.38**
SM1741-1	9.17	10.94	14.44	17.46	16.40	19.62	20.62	20.74	**16.17**
MBRA 383	15.77	16.47	15.54	18.78	17.60	17.51	26.10	18.60	**18.29**
MPER 183	7.84	9.04	13.71	13.70	13.12	15.99	18.50	16.74	**13.58**
CM523-7	12.13	10.13	13.42	8.37	13.06	15.89	17.19	16.78	**13.37**
CM3306-4	15.28	15.73	15.28	13.04	14.57	16.21	15.45	11.25	**14.60**
Mean	**11.28**	**12.14**	**13.87**	**14.71**	**15.51**	**16.12**	**20.10**	**17.46**	**15.15**

Table 2.32. Fresh root production (t/ha) of 15 elite clones evaluated at Jamundí. The age of the crop at each sequential sampling is specified at the top of the table.

Genotype	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	Mean
CM849-1	32.27	32.50	24.66	35.00	42.95	35.91	44.66	41.79	**36.22**
CM6740-7	26.25	35.23	35.91	47.73	33.75	50.34	50.68	48.38	**41.03**
CM7514-7	32.86	38.41	32.61	31.48	35.91	38.86	51.71	45.37	**38.40**
CM7951-5	39.43	37.39	54.21	52.73	53.41	44.66	52.39	56.31	**48.81**
SM653-14	23.07	27.27	28.18	32.73	27.39	23.52	33.64	37.36	**29.14**
SM909-25	30.57	37.27	46.14	47.16	45.80	58.64	50.11	44.97	**45.08**
SM1219-9	31.82	35.23	35.91	31.25	50.80	46.71	59.09	54.83	**43.20**
SM1460-1	32.39	33.98	42.61	39.32	44.66	38.07	71.36	49.35	**43.97**
SM1543-16	38.18	28.75	34.55	42.50	38.18	36.36	65.11	40.80	**40.55**
SM1557-17	17.39	30.68	33.98	36.70	45.46	44.09	52.16	41.88	**37.79**
SM1741-1	26.25	30.57	39.66	47.84	43.98	52.05	55.57	49.97	**43.24**
MBRA 383	43.52	45.34	51.02	58.64	55.34	56.36	77.73	46.56	**54.32**
MPER 183	26.48	29.55	35.91	35.91	34.43	40.23	46.14	48.18	**37.10**
CM523-7	31.93	26.02	37.39	23.52	37.39	42.95	45.34	40.85	**35.68**
CM3306-4	37.95	37.73	35.68	30.91	35.45	38.86	35.68	26.59	**34.86**
Mean	**31.36**	**33.73**	**37.89**	**39.56**	**41.66**	**43.17**	**52.76**	**44.88**	**40.63**

Table 2.33. Fresh foliage production (t/ha) of 15 elite clones evaluated at Jamundí. The age of the crop at each sequential sampling is specified at the top of the table.

Genotype	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	Mean
CM849-1	n.a.	14.77	6.93	9.32	7.50	13.07	13.30	7.39	**10.32**
CM6740-7	n.a.	11.59	11.70	19.20	7.73	12.84	5.57	6.59	**10.75**
CM7514-7	n.a.	8.18	6.02	5.68	7.05	7.95	9.09	4.35	**6.90**
CM7951-5	n.a.	7.05	8.41	8.75	6.93	4.66	5.80	3.21	**6.40**
SM653-14	n.a.	18.52	7.73	15.68	6.25	6.82	10.91	4.12	**10.00**
SM909-25	n.a.	9.09	9.89	12.27	6.25	8.41	8.98	6.14	**8.72**
SM1219-9	n.a.	6.70	5.34	4.89	5.11	5.00	4.43	3.81	**5.04**
SM1460-1	n.a.	6.93	7.61	6.02	5.11	4.89	6.82	5.48	**6.12**
SM1543-16	n.a.	5.80	5.57	10.34	4.66	5.23	6.48	4.83	**6.13**
SM1557-17	n.a.	8.30	10.45	8.86	9.55	6.36	8.64	4.74	**8.13**
SM1741-1	n.a.	7.50	7.84	9.66	9.43	8.52	9.20	4.35	**8.07**
MBRA 383	n.a.	9.09	6.82	8.64	6.14	6.93	11.36	4.43	**7.63**
MPER 183	n.a.	8.18	16.14	11.48	8.52	14.09	8.64	5.65	**10.39**
CM523-7	n.a.	9.77	15.80	11.48	8.98	8.07	14.20	4.49	**10.40**
CM3306-4	n.a.	14.09	8.41	12.05	7.50	8.64	7.27	7.95	**9.42**
Mean	**n.a.**	**9.70**	**8.98**	**10.29**	**7.11**	**8.10**	**8.71**	**5.17**	**8.29**

n.a. (not available)

Table 2.34. Dry matter content (%) of 15 elite clones evaluated at Jamundí. The age of the crop at each sequential sampling is specified at the top of the table.

Genotype	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	Mean
CM849-1	36.09	34.65	35.85	36.70	37.00	36.70	38.25	38.50	**36.72**
CM6740-7	34.65	35.20	36.25	37.20	36.10	36.75	36.80	37.85	**36.35**
CM7514-7	40.26	39.50	40.90	42.30	41.55	41.30	42.35	41.60	**41.22**
CM7951-5	34.88	33.30	35.40	37.20	37.40	37.60	38.85	39.05	**36.71**
SM653-14	32.71	34.80	37.70	36.80	37.00	33.20	36.45	39.85	**36.06**
SM909-25	35.38	36.00	37.05	37.25	37.85	37.05	37.65	37.90	**37.02**
SM1219-9	36.34	35.45	35.70	37.00	36.95	36.50	36.75	37.40	**36.51**
SM1460-1	35.99	36.05	35.60	37.30	37.00	37.35	38.80	38.40	**37.06**
SM1543-16	36.07	34.70	35.90	34.55	36.65	37.50	37.25	37.40	**36.25**
SM1557-17	33.94	35.30	36.30	38.45	38.05	37.15	37.85	37.90	**36.87**
SM1741-1	34.85	35.65	36.25	37.80	38.80	39.85	40.30	41.55	**38.13**
MBRA 383	36.25	36.35	36.45	36.50	37.30	37.70	37.10	39.90	**37.19**
MPER 183	29.40	30.60	30.40	32.15	31.80	31.10	33.50	34.75	**31.71**
CM523-7	37.98	38.70	38.20	38.10	38.10	39.75	40.10	41.05	**39.00**
CM3306-4	40.3	41.7	41.0	42.20	41.10	41.70	43.30	42.30	**41.70**
Mean	**35.67**	**35.86**	**36.60**	**37.43**	**37.51**	**37.41**	**38.35**	**39.03**	**37.23**

d. Selections for the highlands.

Cassava is grown up to 1800 meters above sea level. There is relatively less cassava growing in highland environments, and therefore, the breeding and other related research activities for this kind of ecosystem is smaller than for the other three environments

The higher locations (1600 to 1800 m.a.s.l.) are frequently dedicated to cassava used for the production of fermented starches. Cassava is generally harvested at 18 months of age. Because of low radiation and lower temperatures, yields tend to be lower than for lower altitude environments. In other regions, such as the coffee growing area of Colombia, cassava can be associated with coffee, or used in rotations. In these conditions and at about 1400 m.a.s.l.,the crop can produce a high quality product with excellent acceptance in the traditional markets for fresh table consumption. The area around Armenia (Quindío Department) produces the most valued cassava variety (Chiroza), which is sold in the Bogotá market at top prices. A considerable portion of the cassava grown in this area is also used for the high value added products such as the precooked frozen croquettes. CIAT is conducting several trials to satisfy the needs for farmers growing cassava between 1400 and 1800 meters. It has to be emphasized that much of these areas are generally characterized by poverty and by few alternatives for the farmers, especially in Colombia.

Table 2.35. Performance of established and experimental cassava clones in the coffee-growing region. Data across five regional trials (except for Chiroza which produced data in four of the five trials).

Genotype	Fresh root yield (t/ha)	Dry matter content (%)	Dry matter yield (t/ha)	Cooking quality (1 a 5) ¶
Chiroza (MCOL 2066)	29.49	37.62	12.25	5.0
Catumare (CM 523-7)	45.61	40.51	18.98	4.0
P12 (MCOL 1505)	34.34	38.87	14.91	4.0
SM 1406-1	37.70	39.61	15.31	4.3
Reina (CM 6740-7)	40.07	38.44	16.07	5.0
Parrita (MCOL 2758)	33.66	34.62	11.60	4.0
ICA (HMC 1)	43.04	37.90	16.44	4.7
Mean	37.70	38.22	15.08	4.4

¶ Cooking quality test 5= excellent; 4= good; 3=regular; 2= poor; and 1= very poor.

Table 2.35 shows the results of five regional trials conducted through the coffee growing area. Chiroza is a standard of quality for cassava in Colombia. The cooking quality and presentation of this landrace are excellent. However, its yield, is not high for the mean productivity in the area, which is one of the highest in the country. CIAT has developed

other varieties that are very promising. Catumare (released about 10 years ago) has been adopted in the area, but its quality is not as good as Chiroza. The clone CM6740-7 (Reina), on the other hand, shows high yield potential (16.1 t/ha of dry matter) combined with excellent cooking quality (the mean rating of five, means that in every of the five locations, the cooking quality test gave a score of excellent).

Table 2.36. Regional trial of promising clones in the coffee- growing region. In bold are the results of regional checks.

Genotype	Yield (t/ha)		Dry matter	Harvest	Potential	Cooking
	Fresh roots	Dry matter	Content (%)	Index		Quality[¶]
Chiroza (T)	**37.4**	**12.5**	**33.5**	**0.6**	**Good aspect**	**4**
M PER 183	35.2	11.3	32.1	0.6	Good aspect	4
SM 1583-8	33.8	12.3	36.3	0.6	Good aspect	4
SM 1673-10	32.6	12.3	37.8	0.5	Good aspect	4
HMC 1	31.9	11.4	35.9	0.6	Good aspect	4
SM 1788-13	29.4	9.8	33.3	0.5	Good aspect	3
ICA (T)	**26.7**	**9.3**	**34.7**	**0.5**	**Good aspect**	**4**
SM 1781-17	26.5	9.7	36.7	0.6	Good aspect	4
M COL 1468	25.2	8.4	33.3	0.5	Good aspect	3
SM 1871-33	25.1	9.9	39.6	0.5	Good aspect	4
CM 523-7	21.7	7.9	36.3	0.4	Good aspect	4
SM 1225-15	17.7	6.6	37.1	0.4	Good aspect	3
SM 1557-27	11.2	3.9	35.1	0.4	Good aspect	4
SM 1778-44	30.1	12.1	0.3	0.5	Industrial	4
CM 8378-3	29.9	10.9	36.3	0.5	Industrial	4
CM 3306-4	29.2	11.6	39.6	0.6	Industrial	3
SM 21559-28	29.1	10.5	36.0	0.5	Industrial	4
SM 2201-4	28.4	10.3	36.3	0.6	Industrial	4
M COL 1505	24.6	8.8	35.6	0.4	Industrial	4
SM 1781-28	23.7	9.4	39.6	0.5	Industrial	1
SM 2205-5	21.7	7.6	34.9	0.5	Industrial	2
SM 2211-3	20.4	6.8	33.2	0.5	Industrial	2
SM 2214-1	17.5	6.5	37.1	0.5	Industrial	2
SM 2098-4	11.9	4.7	39.8	0.3	Industrial	4
M CUB 74	10.6	3.6	34.3	0.3	Industrial	4

[¶] Cooking quality test 5= excellent; 4= good; 3=regular; 2= poor; and 1= very poor.

The generalized use of Chiroza in the area can be understood looking at Table 2.36, where this clone shows the highest dry matter yield (12.5 t/ha). None of the experimental clones had higher yields. Reina (CM6740-7), unfortunately, was not included in this trial. However, the genotypes SM 1583-8 and SM 1673-10 had similar dry matter yields (12.3 t/ha), the same cooking quality score as Chiroza (4 = good), but higher dry matter content (36.3 and 37.8 % versus 33.5% of Chiroza). High dry matter content is a very desirable trait for the croquettes industry.

For many years CIAT conducted evaluations in Cajibío (Cauca Department) for the highland environment (1800 m.a.s.l.). A major emphasis of the research was to evaluate the possibility of shortening the cycle from 18 to 12 months. The accumulated information from 10 years will be evaluated and an economic analysis made. We shifted our operations from Cajibío to Morales, which still offers good environmental conditions to evaluate the reaction to foliar diseases (CBB, Phoma, and Cercospora), low fertility, and acidic soils.

Tables 2.37 and 2.38 summarize the results of the two regional trials conducted at Cajibío and Morales, respectively. In general, Morales showed higher yields than Cajibío. The mean yield of the five best clones at Cajibío was almost twice as large as that of the checks from the experiment. At Morales, the yield superiority of experimental clones, in some cases, was close to three times the average of the checks. One clear trend for the experimental clones at both locations was their higher dry matter content. Cooking quality for this environment is important but not as much as for the coffee growing area, because a considerable portion of the cassava goes to the fermented starch factories that are very common in the area.

Table 2.37. Five best performing genotypes in the highland environment of Cajibío (Cauca Department). The trial was planted by mid 1998 and harvested at the end of 1999.

Genotype	Harvest Index	Dry Matter Content (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Cooking Quality ¶
SM 526-3	0.44	35.8	25.9	9.2	1.0
SM 1716-1	0.57	37.1	25.7	9.6	5.0
SM 1944-17	0.36	35.4	24.4	8.6	1.0
SM 856-11	0.57	38.4	23.7	9.1	3.0
SM 1712-15	0.48	39.1	23.5	9.2	3.0
Mean (trial)	0.49	35.8	18.2	6.5	2.9
Mean (checks)	0.50	34.2	15.4	5.3	2.2

¶ 1: Excellent; 2: Good; 3: Regular; 4: Poor; 5: Very poor.

Table 2.38. Five best performing genotypes in the highland environment of Morales (Cauca Department). The trial was planted by early 1999 and harvested by mid 2000.

Genotype	Harvest Index	Dry Matter Content (%)	Fresh root yield (t/ha)	Dry matter yield (t/ha)	Cooking Quality ¶
SM 1713-25	0.54	37.7	42.0	15.8	3.0
SM 7138-7	0.59	36.9	34.0	12.5	1.6
SM 1495-5	0.57	33.9	30.4	10.3	2.3
SM 1713-26	0.59	39.0	27.3	10.6	5.0
CM 7436-7	0.54	34.9	27.0	9.4	2.3
Mean (trial)	0.49	34.6	18.4	8.4	3.8
Mean (checks)	0.52	32.6	17.0	5.6	4.3

¶ 1: Excellent; 2: Good; 3: Regular; 4: Poor; 5: Very poor.

e. Other research activities.

There have been additional research carried out with elite germplasm of the cassava breeding project. A collaborative research with CLAYUCA, two varieties: Catumare (CM 523-7) and Reina (CM6740-7) were planted at CORPOICA La Libertad in large plots. Each variety was fertilized with six different alternatives, including varying doses and combinations of chemical fertilizers and pig manure (Table 2.35). Fresh root yields were around 20 t/ha, with a slight superiority of Reina over Catumare. Dry matter content, however, was lower for Reina, resulting in a similar dry matter yield. Total foliage production was around 14 t/ha. The two cassava clones had an excellent response to fertility. Dry matter yields almost doubled with treatments four and six. Pig manure provided enough nutriments for the plants to show a clear trend, but the best performance was achieved by combining pig manure and chemical fertilizers.

From the three main research activities there has been an expansion to new areas. In the Huila Department a join research was carried out with the collaboration of a feed industry (PROCEAL) of the region. Eight genotypes were evaluated as a first step in the introduction of cassava as an industrial crop. This Department is well known for its intense fish growing activities. Feed made with cassava flour tends to precipitate slower than other feed, therefore providing an additional advantage for cassava as a raw material. Fresh root yields ranged from 28.5 to 47.0, with a mean of 36.3 t/ha (Table 2.36). The highest yielding clone was CM6740-7 (Reina) with 47 t/ha. This clone was officially released by CORPOICA on November 10, and shows once again its genetic superiority and wide adaptation to varying environmental conditions. It shows an

outstanding performance in the eastern savannas (Meta Department), in mid-altitude valleys with acidic soils (Cauca Department) and mid-altitude valleys with fertile soils (Huila Department), and in the coffee growing area. In other evaluations carried out in other Departments (Antioquia, Magdalena, Casanare) this genotypes has performed equally well. A new clone that is performing consistently well in mid-altitude environments is SM 1219-9. In the Huila Department was the highest yielding genotype, with 15.55 t/ha of dry matter. This value is similar to the one reported for Jamundí (Valle del Cauca Department) in Table 2.31.

Table 2.35. Yield response to different fertilization alternatives including varying doses and combinations of chemical fertilizers and pig manure. Data from seven samples of 25 plants each, taken at La Libertad (Villavicencio).

Tmt ¶	Fresh root yield (t/ha)			Foliage production (t/ha)			Dry matter content (%)			Dry matter yield (t/ha)		
	Catumare	Reina	**Mean**	Catumare	Reina	**Mean**	Catumare	Reina	**Mean**	Catumare	Reina	**Mean**
1	13.25	15.02	**14.13**	11.67	10.31	**10.99**	36.5	33.49	**35.00**	4.831	5.028	**4.93**
2	17.31	21.01	**19.16**	15.45	14.04	**14.74**	38.57	34.7	**36.64**	6.665	7.289	**6.98**
3	19.53	21.38	**20.46**	16.59	14.98	**15.79**	37.95	34.14	**36.04**	7.417	7.291	**7.35**
4	21.46	24.27	**22.87**	18.32	15.32	**16.82**	37.67	34.26	**35.96**	8.098	8.311	**8.20**
5	19.07	22.88	**20.98**	14.97	13.04	**14.00**	38.06	34.59	**36.32**	7.238	7.914	**7.58**
6	21.65	27.13	**24.39**	15.73	15.95	**15.84**	37.85	35.15	**36.50**	8.169	9.536	**8.85**
Mean	**18.71**	**21.95**	**20.33**	**15.46**	**13.94**	**14.70**	**37.77**	**34.39**	**36.08**	**7.07**	**7.56**	**7.32**

(1) Check (no fertilizer, no pig manure).
(2) 1.5 t/ha pig manure.
(3) 3.0 t/ha pig manure.
(4) 500 kg/ha 10-20-20 NPK + 87 kg/ha $ZnSO_4$.
(5) 0.75 t/ha pig manure + 250 kg/ha 10-20-20 NPK + 43.5 kg/ha $ZnSO_4$.
(6) 1.50 t/ha pig manure + 250 kg/ha 10-20-20 NPK + 43.5 kg/ha $ZnSO_4$.

Table 2.36. Yield performance of eight clones introduced to the Huila Department (Neiva) for their evaluation as raw material for the feed industry (PROCEAL). Results from two replications of five plants each, taken at ten and a half months of age.

	Fresh root Yield (t/ha)	Dry matter content (%)	Dry matter yield (t/ha)
M.COL 2737	29.5	28.8	8.49
CM 849-1	35.6	35.2	12.53
CM 3306-4	38.5	34.2	13.16
CM 6740-7	47.0	31.1	14.61
CM 7514 –7	28.5	38.1	10.85
SM 909-25	37.6	30.1	11.31
SM 1219-9	44.7	34.8	15.55
MPER183	28.8	25.9	7.45
Mean	**36.3**	**32.3**	**11.74**

Achievements:

- Several outstanding, genetically superior varieties have been identified for each ecosystem.
- Selected genotypes show excellent stability, by performing above the population mean in most (or all) the trials where they were evaluated.
- The new emphasis for clones specially designed for industrial uses, has been implemented, and the first *industrial genotypes* identified.
- The new selection scheme was initiated and has reached the stage of clonal evaluation. Preliminary results are very promising.
- Good segregation progenies for studies on CBB and super elongation disease.
- A selection index has been implemented to facilitate the selection process. Although the first results are already satisfactory, there is a need for further refinement.
- On going research for the integration of cassava production to swine or poultry activities are producing the first results (i.e. the use of pig or chicken manure)

Activity 2.6. Develop and implement a vigorous strategy for reducing the levels of incidence of frog skin disease in breeding and elite germplasm at CIAT.

Specific Objectives:

a) *To reduce the level of incidence of frog skin disease in cassava germplasm under CIAT management.*
b) *To recover CIAT-Palmira as a location where valuable and reliable data could be obtained*
c) *To test the value of cultural practices aimed at the solution of the frog skin and white flies problems, currently affecting the cassava research at CIAT-Palmira.*
d) *To produce data for better knowledge of the epidemiological aspects of the frog skin disease.*
e) *To set up a rapid propagation scheme for the production of disease-free propagules.*

Rationale: For a few years, and for a diversity of reasons, the problems of frog skin disease and white flies have gradually increased in the cassava plots at CIAT – Palmira. Urgent and drastic measures needed to be taken to invert the trend, and gradually but consistently reduce the incidence of both problems. Frog skin disease is most likely induced by a viral agent, which is transmitted inefficiently by the whitefly *Bemisia tuberculata*.

The measures taken to reduce the problem of frog skin involve the indexation of all the germplasm bank material (our most likely source of inoculum). The indexation is a lengthy and costly process that involves the grafting of each accession on a genotype very susceptible to the disease ("*Secundina*"). Accessions scoring positive for the

presence of the disease, are cleaned by a combination of thermoterapy and meristem culture. Materials that are certified to be clean of the viral agent have been planted in two isolated plots outside and far away from CIAT (at CENICAÑA and CEUN). During the year these two locations have grown in size as more and more materials were declared free of the disease. Simultaneously the plantings of cassava at CIAT were reduced. F1 plats derived from sexual seed, are free of the disease, so they have been planted outside CIAT, at CENICAÑA.

To overcome the increasing problem of whiteflies at CIAT, which to a large extent is due to the sequential planting of cassava year around, starting in 2001, there will be a time period during the year when there will be no cassava in the field. Plantings will be made so that harvest of all the cassava in the field will be completed by the end of March 2002. During April, there will be no cassava plants in the field (with one exception) to break the breeding cycle of the whitefly. The exception will be approximately one hectare of cassava occupied by the crossing blocks. This cassava will be planted isolated in one extreme of the experimental station. All the other plantings will be at the opposite side of CIAT. Whiteflies management in the crossing blocks will be made in a very intensive way.

During the harvest of plots at Jamundí (see Tables 2.31 to 2.34) neighboring commercial plots were also harvested. Three genotypes (Table 2.35) from these commercial plots were chosen for a follow-up study, to produce information related to epidemiological aspects of the frog skin disease. An initial restriction in the way harvest took place, was **not** to obtain stakes from plants without first inspecting their roots to detect the eventual presence of symptoms of the disease. Frog skin symptoms, in most instances, can only be detected in the roots of the plants. In the entire area to be harvested, therefore, the plants were pulled to expose the roots. The spatial location of plants showing symptoms of the disease was recorded to obtain a map of the distribution of the disease in the field and a precise estimate of the frequency of plants showing symptoms.

This information will be very useful in many ways (see "Sampling for Decision Making in Crop Loss Assessment and Pest Management: Sampling for Plant Disease Incidence" 1999. L.V. Madden and G. Hughes, Phytopathology 89:1088-1103). First, information was needed to determine the size and kind of sampling required for adequately assessing disease incidence in the field. Secondly, spatial variation may provide some evidence of the relative frequency of disease transmission from infected plants to healthy ones. Furthermore, we applied the most recommended measure for controlling frog skin disease: discarding the stakes coming from plants suspected to be diseased. The stakes from asymptomatic plants were planted in plots properly identified. During the harvest by the end of 2000 or beginning of 2001, the roots of every plant will be inspected and the frequency and spatial distribution will be again determined. This information will allow further understanding of the epidemiology of the disease, and indicate how efficient is the practice of inspecting the roots before taking stakes from them, to reduce the incidence of the disease in commercial plots.

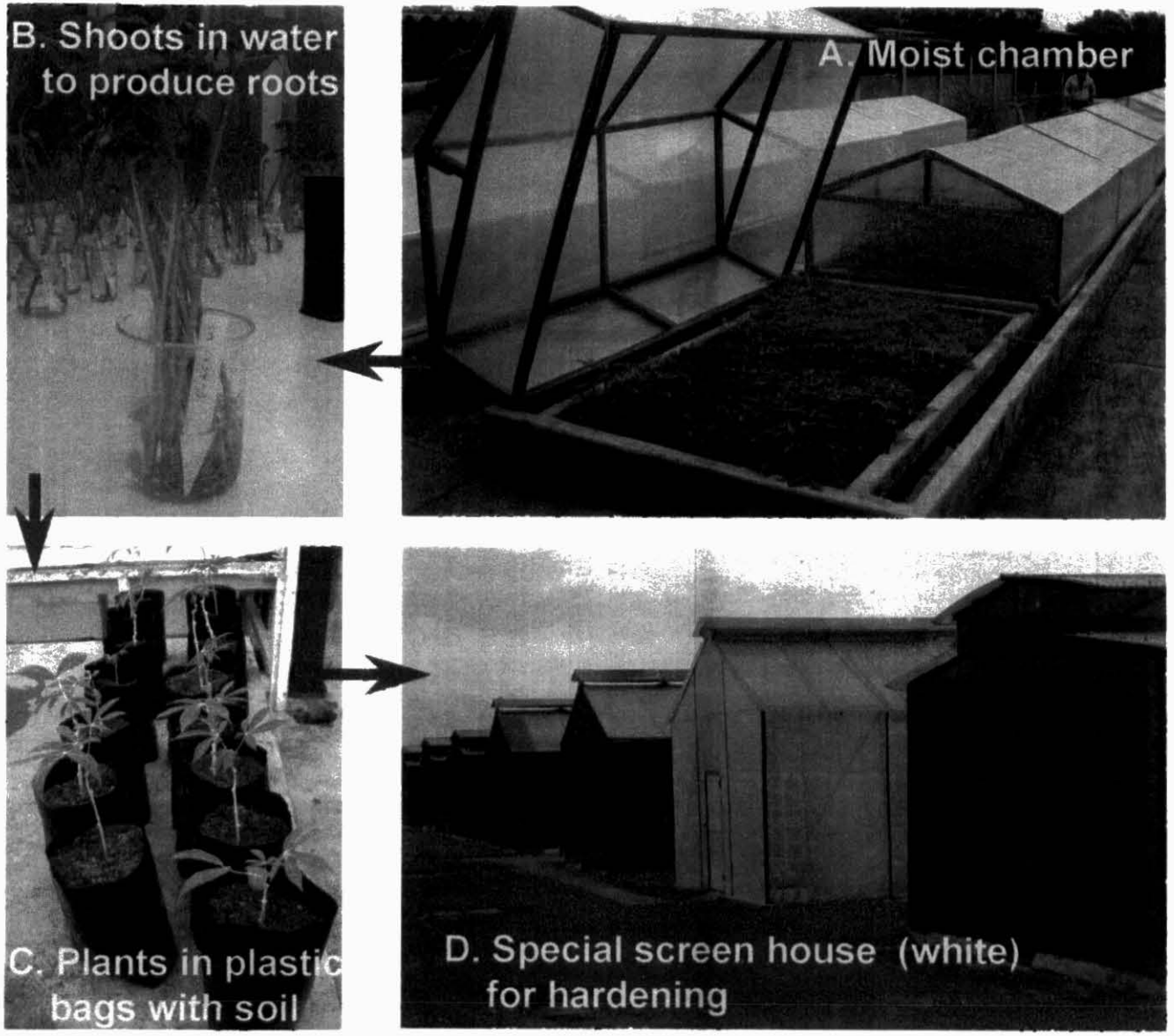


Figure 2.3. Rapid multiplication system based on the use of micro stakes.

Table 2.35. Data from frog skin disease incidence in commercial fields planted with three different cassava genotypes.

Genotype	Plants harvested (number)	Plants with symptoms (number)	Incidence (%)
MBRA 383	6800	68	1.00
SM 1219-9	4830	135	2.79
SM 1543-16	3625	159	4.39

Because of the growing interest in cassava as an industrial crop, and frequent requests of stakes for large plantations (which cannot possibly be met), a special project was initiated for setting up a rapid multiplication scheme based on the use of micro-stakes. Micro-stakes (two buds only, and about 8 cm long) are obtained from plants that are certified to be free of frog skin. Because of their reduced length, up to 50 stakes can be taken from a single plant. Micro-stakes are then put to sprout in high moisture chambers. Every other week, up to two shoots can be harvested. The shoots collected are then put in flasks with water to produce roots, and then, into plastic bags with soil for hardening in shaded conditions. Until this point, the entire process has been free from whiteflies that could eventually introduce the frog skin disease into the rapid propagation scheme. The hardening of the plants takes place in screen houses made of an special mesh that does not allow the passage of small insects such as whiteflies or aphids, therefore guaranteeing plant health until they are transplanted into the field.

The process is useful in many ways: **a)** produces large amount of disease free plants in a relatively short period of time; **b)** produces information about multiplication rate and best approaches to maximize it; and **c)** produces information regarding the actual costs for each plant produced through this system. All these aspects are relevant for the eventual development of a private initiative for the production of certified cassava "seed" (either as plants or stakes). The final stages of the process can also be used for plants regenerated from the RITA system (temporal immersion) implemented by the biotechnology project (SB2).

Activity 2.7. Recovery of elite Asian materials to be included in crossing blocks for breeding purposes for Asia and Latin America.

Rationale: CIAT has carried out intense breeding activities for the Asian cassava growing countries. The work was carried out in close association with the Thai cassava research programs (mainly at Rayong Farm and Kasetsart University). This work has produced many successful varieties for the region (some of them had been derived from crosses made at CIAT-Palmira, whereas others came directly from the Thai-CIAT program). It is important that the varieties are brought back to CIAT headquarters because they can: **a)** be used as parental lines for new crosses to be shipped to Asia;

b) evaluated for their potential use in Latin America, both as parental lines or as elite germplasm to be exploited by farmers; and **c)** eventually to be subjected to genetic transformation for the introduction of whatever gene was desirable to our Asian collaborators, upon their request..

Most of the Asian elite germplasm has been introduced back to CIAT-Palmira, and have been, or are currently in the process of evaluation, in different environments. It has already been mentioned the excellent performance of MTAI8 (Rayong 60) in the sub-humid environments. We are in the process of obtaining official authorization from the Thai government for the release of this material as a variety in Colombia. It has also been extensively used in crosses whose progenies are already being evaluated with promising results. Seed produced from crosses between Asian and Latin American germplasm will also be shipped to Asia, for their evaluation by the National Programs in that continent.

Activity 2.8. Introduce into cassava resistance to ACMV and collaborate in the process of MAS using the molecular marker identified at CIAT.

Rationale: Cassava mosaic disease (CMD) remains the most economically damaging disease of the crop in Africa, and it is a threat to cassava production in the Americas. The whitefly vector biotype of CMD has been recently found the New World increasing the possibility that the disease may eventually cross over to cassava. Breeding efforts in Latin America have not placed an important value on resistance to the disease for two reasons: **a)** the disease is not present in the Americas and, therefore, there was no need for resistance against it and **b)** since the disease is not present, we cannot select for it. Latin American varieties, consequently, are very susceptible to the disease. Resistance to CMD is the only reliable method for disease control. The first source of resistance was probably derived from *M. glaziovii* and its mode of action was partially recessive. A better source of resistance, controlled by a single dominant gene was recently identified in Nigeria.

Materials and Methods: Until recently several thousand crosses between the *M. glaziovii* source of resistance and elite Latin American germplasm have been produced. As a result, 88541 F1 seeds are currently stored in our cold room. However, given the excellent level of resistance conferred by the new gene and its dominant mode of action, we will start using this new source. The advantage is that being dominant, the F1 thus produced will also show high levels of resistance. The old source of resistance required a back-cross to the donor which complicated greatly the breeding process and made considerably more difficult the recovery of the desirable traits of the Latin American elite lines.

To bypass the restriction for introducing vegetative materials from Africa into Latin America we introduced segregating materials from the cross between **TME3** (source of

the new resistance) and **TMS 30555** (and improved variety from IITA). Many progeny were obtained and the seed thus produced were germinated in confined conditions to avoid any risk of infection by the virus. From the plants thus grown, a replicate was obtained vegetatively for evaluation in the field. Only in vitro plants whose field replicate did show clear and adequate levels of resistance where then shipped from Nigeria to CIAT. The *in vitro* plantlets were tested on arrival by ELISA and PCR methods for the presence of ACMV before sub-culturing and transfer to the green house. Permission to transfer the plants to the field will be obtained from the Colombian quarantine authorities, and the plants will be transferred to a healthy site for hybridization

Once we have plants in the field we will start the crosses between these sources of resistance and about 20 elite varieties from tropical (low and highlands) and subtropical adaptation. In addition, we will also use these materials for crosses to be shipped to Africa. CIAT and IITA are interested in continuing the introgression of germplasm from Latin America into Africa. Particular goals here will be to combine resistance to ACMV with: **a)** high carotene levels in the roots, a trait present in several Latin American materials with yellow/orange coloration; and **b)** drought tolerance, also present in Latin American genotypes (particularly from North East Brazil).

OUTPUT 3: Collaboration with other institutions.

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

Specific Objectives:

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

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

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

➢ *Photosynthesis enzymatic activity measurements in 30 germplasm bank accessions.*

Rationale: Photosynthesis occurs in two phases: **a)** the luminous stage when light energy is captured by the plant pigments and chemically stored for the following step. **b)** the biochemical phase when different carboxilation enzymes reduce the atmospheric CO_2 and use it to produce sugars. The

efficiency of the process is determined by several environmental (CO_2 concentration, light intensity, water potential and temperature) and genetic factors. Biomasss and yield heavily depend on the efficiency of photosynthesis. Some basic research carried out at CIAT suggested some atypical behavior of enzymes related to the photosynthetic carboxilation, particularly for phosphoenolpiruvate carboxilase (PEPC). It has been postulated that this unusual biochemical situation could explain the relatively high cassava productivity under temperature and water stress conditions that lead other crops to reduced production and even death.

Materials and methods: the enzymatic activity for phosphoenol pyruvate carboxylase (PEPC), D-ribulose, 1-5 Diphosphate carboxylase (RUBISCO), pyruvate Pi-dikinase (PPDK) and NAD-malate dehydrogenase will be determined in foliar tissue of thirty different accessions from the cassava germplasm bank, with additional measurements in *M grahami*, *M cartaginensis* and *M. rubricaulis*. On the same materials the photosynthesis (CO_2 absorption) will be measured ten times through a period of two months.

➢ *Monitoring root growth using a capacitance meter for a non-destructive evaluation of productivity through time.*

Rationale: It is very difficult for cassava to determine the optimum age for harvesting. Since the crop is perennial, it can be harvested at any time and there is no morphological trait that can help to decide the best time for harvest. As a result the breeding projects generally harvest the segregating progenies at about 10-11 months of age for tropical environments. For highlands and subtropical areas the harvest can be postponed until 18 months. This procedure, in fact, prevents the detection of those genotypes that have an early bulking capacity, a trait that could benefit cassava farmers greatly. An alternative would be to harvest all segregating population at two ages (for instance at seven and ten months after planting) but this would complicate field operations and increase the costs of the research.

Materials and methods: An article (van Beem et al. 1998. Agron. J. 90:566-570) for monitoring root growth, using a capacitance meter, gave some hopes for developing a non-destructive method for following up root production. The method is relatively simple (Figure 3.1) and the equipment is not expensive. We are currently measuring the capacitance of four plants for each of four genotypes. Measurements are first taken under field water capacity, and then, the following day, harvested for weighting root production. Measurements were started at the fourth month of age and taken every month thereafter.

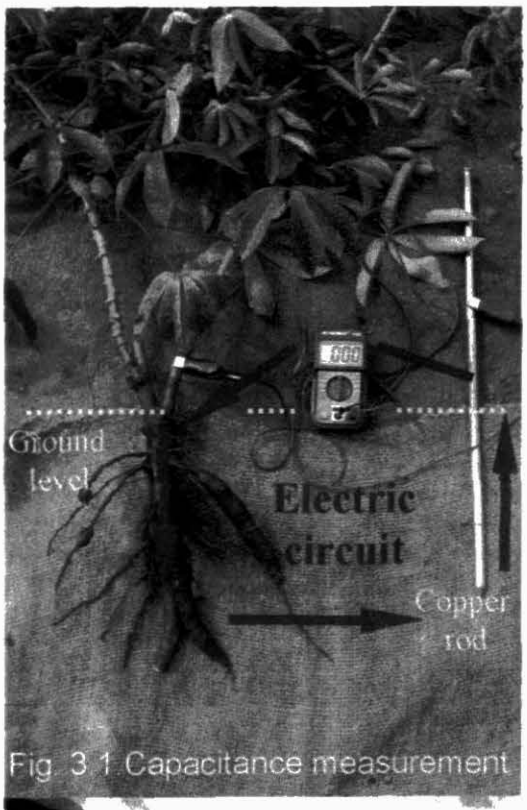


Fig. 3.1 Capacitance measurement


Fig. 3.2 Mechanical planting of cassava


Fig. 3.4 Artificial drying plant


Fig. 3.3 Mechanical harvester


Fig. 3.5 Commercial field fertilized with chicken manure

➢ *Induction or inhibition of flowering of cassava through hormonal treatments.*

Rationale: One important aspect of cassava breeding has been for plant architecture. This aspect influences the facility for field operations, amount of stakes produced, risk of soil erosion, and productivity. Short, branching types were found to be more efficient in nutrient utilization. Their canopy covers the grown quickly and, therefore, they are less prone to soil erosion. Stakes production for these varieties, however, is low, and the field operations after the plants have developed are somewhat cumbersome. Branching in cassava is directly correlated to flowering. On the opposite side there are varieties that never flower and, therefore, they do not branch. To develop a methodology for modifying the flowering habit in some cassava genotypes, through exogenous application of phytohormones, is very convenient. For the non-flowering types, inducing flowering will facilitate their use in our crossing blocks. This would be the case for MTAI-8, a clone that show excellent agronomic characteristics, but from whom it is very difficult to obtain seeds, due to its reluctance to flower in the Palmira environment. Inhibiting flowering in the short, branching types would facilitate stakes production.

Materials and methods: Four varieties (two short and branching and the other two erect with little or no flowering habit) will be used in this experiment. A large number of plants will be planted in the field and different hormones and doses will be employed for inducing or inhibiting flowering trends. Because of the changes in field operations at CIAT (no cassava plant will be left on the field for a month in April next year), the actual experiment will began in May, 2001.

☞ ***Official release of two cassava varieties for the acid-soil savannas.*** The release of two varieties for the acid soil savannas has been initiated by CORPOICA. One of the varieties (**CM 6740-7**) is specifically adapted for the transition environment between non-acidic fertile soils and the severely acidic infertile soils of the savannas *("Piedemonte")* . The identification of this variety should be familiar to the reader, because it is ***Reina***, a genotype that has shown excellent performances in the mid-altitude valleys, as well as in the coffee growing area. The other genotype (**CM 6438-14**) is more rustic and outperforms genotypes when tested in the marginal soils of the acid soil savannas. The official name of this clone will be ***Vergara***, to honor the late Juan Vergara a good friend and innovative collaborator, that greatly contributed to the development of cassava production in the eastern savannas of Colombia.

Tables 3.1 and 3.2 illustrate the performance of **CM 6740-7** (***Reina***) respectively in the transition zone (*Piedemonte*) and true savanna conditions. Dry matter yields are higher in the new variety than in the most commonly grown regional checks (Table 3.1). Furthermore, Reina shows higher dry matter content, an important feature because part of the production in the area goes to the croquette end use in Bogotá. In the true savanna conditions, however, *Reina* does not show the superiority observed in the

Piedemonte conditions (Table 3.2). In this environment soils are more acidic, less fertile and the presence of foliar diseases much more prevalent (probably because the plants are subjected to the edaphic stress).

Table 3.1. Comparison (in *Piedemonte*) of cultivar **CM 6740-7** with relevant checks in different regional trials where the genotypes compared had been grown together.

Genotype	Yield (t/ha)		Dry matter	HCN
	Fresh roots	Dry matter	content (%)	(1 → 9)[¶]
Comparison with CM523-7 (or *Catumare*) based on 9 trials.				
CM 6740-7	23.72	8.48	35.3	6.1
CM 523-7	16.34	5.95	34.2	6.8
Comparison with *Brasilera* based on 6 trials.				
CM 6740-7	27.43	9.86	35.9	6.0
Brasilera	24.52	8.58	35.0	7.3

[¶] 1: low cyanogenic potential; 9: very high cyanogenic potential.

Table 3.2. Comparison (in true savanna conditions) of cultivar **CM 6740-7** with relevant checks. Results from four regional trials.

Genotype	Yield (t/ha)		Dry matter	HCN
	Fresh roots	Dry matter	content (%)	(1 → 9)
CM 6740-7	12.28	4.00	32.4	4.8
CM 523-7	15.83	5.63	35.3	6.5
Brasilera	12.13	3.98	32.3	6.0

[¶] 1: low cyanogenic potential; 9: very high cyanogenic potential.

Table 3.3. Comparison (in *Piedemonte*) of cultivar **CM 6438-14** with relevant checks in different regional trials where the genotypes compared had been grown together.

Genotype	Yield (t/ha)		Dry matter	HCN
	Fresh roots	Dry matter	content (%)	(1 → 9)
Comparison with CM523-7 (or *Catumare*) based on 20 trials.				
CM 6438-14	17.37	6.38	36.0	5.8
CM 523-7	13.89	5.10	35.9	6.8
Comparison with *Brasilera* based on 10 trials.				
CM 6438-14	18.80	6.80	36.0	5.9
Brasilera	20.55	7.08	34.5	6.3

[¶] 1: low cyanogenic potential; 9: very high cyanogenic potential.

Table 3.4. Table 3.2. Comparison (in true savanna conditions) of cultivar **CM 6438-14** *with relevant checks. Results from four regional trials.*

Genotype	Yield (t/ha)		Dry matter	HCN
	Fresh roots	Dry matter	content (%)	(1 → 9)
CM 6438-14	15.30	5.43	34.9	5.3
CM 523-7	11.43	4.01	34.6	6.8
Brasilera	11.95	3.89	32.4	5.0

¶ 1: low cyanogenic potential; 9: very high cyanogenic potential.

Tables 3.3 and 3.4 illustrate the performance of **CM 6438-14** (***Vergara***) respectively in the *Piedemonte* and true savanna conditions. It should be explained that data is different for each set of comparisons. The database was screened to identify all the trials where the two varieties to compare had been included. For example, the trials that were used to estimate the mean performance of CM523-7 in Table 3.1 are different than those used for Table 3.3, and therefore, the mean performance of that variety will also be different.

In *Piedemonte* conditions (Table 3.3), *Vergara* was not superior to *Brasilera* (6.80 vs. 7.08 t/ha of dry matter), although the dry matter content was indeed higher (36.0 versus 34.5 %). However, in the stressful conditions of the true savanna (Table 3.4), *Vergara* shows a clear superiority over the two relevant checks.

☞ ***Official release of four cassava varieties for the sub-humid environments.*** After a period of ten years without a release of new varieties in Colombia, in July CORPOICA released four varieties originated in the crossing blocks of CIAT adapted for the sub-humid environments. Two of those varieties are specifically suited for industrial uses: ***Sucreña*** (CM3555-6) and ***Colombiana*** (CM 3306-19). These materials are mentioned in the results of several trials described in Output 2. They have had a satisfactory performance through the years, and show reliable and stable yields. It will be noted that these varieties, perhaps, are not at the top of the lists in the different trials were they have been included (see Tables 2.14 through 2.19), but this is because there are newer clones that outperform them, but still have not been so extensively tested.

The remaining two varieties are double purpose (fresh consumption and industry). They were produced at CIAT crossing blocks, and then transferred to CORPOICA after the F1C1 stage (See Figure 2.1). These varieties were selected using the participative breeding methodology described in more detail later in this document. The name of the varieties are ***Caribeña*** (SGB 765-2), and ***Rojita*** (SGB 765-4). These varieties have good acceptance by the farmers, but the yields (as is frequently the case for double purpose materials) are not necessarily the highest.

☞ ***Evaluation of segregating progenies in Ecuador and training course.*** A workshop lasting a week took place in Santo Domingo de los Colorados (Manabí Province), Ecuador. The event, a join effort by CLAYUCA and CIAT, covered from genetics to agronomy and processing cassava. In addition, 1000 genotypes (50 seeds from 20 different crosses) from CIAT were shipped and planted in two locations in Ecuador. The harvest of the F1C1 progenies (See Figure 2.1) was carried out by the end of October with the presence of a CIAT staff. An interesting detail is that all these activities were carried out in collaboration with PRONACA (Procesadora Nacional de Aves) and ETAX S.A., two private companies associated with CLAYUCA.

☞ ***Seed shipment to Haiti and training of a Haitian scientist.*** As part of the international efforts aiming at improving the conditions in Haiti, CIAT is actively promoting the introduction in that country of new germplasm from several crops, including cassava. A Haitian scientist was also trained in cassava breeding and agronomy during a week at CIAT headquarters. Two visits to the country by CIAT senior staff were also paid, for better determining the needs for improving cassava production in the country.

☞ ***Training of two Thai scientists in breeding and tissue culture.*** Thailand has always been a key collaborator for CIAT in the area of cassava research. CIAT is maintaining as close a relationship as possible after the departure of the cassava breeder based in Bangkok. As part of this collaboration, two scientists were invited for a two-week training, at CIAT headquarters, in tissue culture and cassava breeding. This activity is important to facilitate and maximize the flow of germplasm and information from Colombia to Thailand, and vice versa.

☞ ***International course on cassava breeding, agronomy and processing.*** In October 20, a two-week training course was initiated in CIAT headquarters. The course included genetics, agronomy, pest and disease management, economics, and processing of cassava roots and leaves. Scientists from Colombia, Ecuador, Peru, Venezuela, Haiti, Paraguay, Brazil, Cuba, and Bolivia attended to the meeting, which was a joint effort between CLAYUCA and CIAT.

☞ ***VI Asian Cassava Research Workshop in Vietnam.*** In February, 2000 the VI Asian Cassava Research Workshop took place in Ho Chi Minh city, Vietnam. Two scientists from CIAT Headquarters and the Director of CLAYUCA attended to the meeting and gave presentations. The meeting offered and ideal opportunity to interact with cassava researchers from Asian countries. The trip also involved visiting and travelling around the most important cassava growing regions of Thailand. In addition CIAT is contributing with the maintenance of six key cassava research projects in Vietnam and China. Shipment of vegetative materials in vitro, was initiated this year

after a long period of self imposed restriction until the accessions were satisfactorily screened for a new viral agent (Vein Mosaic Virus).

☞ ***Visit of Nigerian businessmen and cassava scientists from IITA***. In May a group of businessmen and government officials (including His Excellency the Minister of Agriculture) from Nigeria visited the region. The group included the presence of two cassava scientists from IITA. The purpose of the trip was to get exposed to different cassava growing and processing methodologies in Latin America and to learn about the functioning of CLAYUCA. The tour included a visit to Bogotá, Armenia (coffee growing region) and Valle del Cauca and Cauca Departments. The group, including a CIAT/CLAYUCA scientist, then went to Brazil for an additional week.

☞ ***Training workshops on the prevention of frog skin disease***. Six two-day workshops took place during the year for the prevention of frog skin disease. These events were organized by ICA and took place in different regions of Colombia. The content, lectures and field visits were carried out mainly by CIAT staff.

Achievements:
- ☞ Collaboration with partners in Asia, Africa and Latin America and the Caribbean continues.
- ☞ New partners identified (i.e. PRONACA and ETAX).
- ☞ Shipment of plants *in vitro* re-established.
- ☞ New research areas possible through the collaboration with partners.
- ☞ Training was an important issue in these collaborative initiatives.
- ☞ Six new cassava cultivars officially released in Colombia.

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

Specific Objectives:

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

Rationale: As a result of the changes that took place during the last decade in most of the Latin American countries, the traditional partners with whom CIAT interacted in relation to cassava changed considerably. CLAYUCA came into existence, in part, to fill

up some of the spaces that have been left empty and contributes considerably to the transference of technology and germplasm developed at CIAT into the affiliated countries. As a new institution CLAYUCA is already playing a key role in cassava research and development in the region. CIAT and CLAYUCA have developed a close and complementary research agenda that strengthens both institution on one hand, and avoids duplication of efforts on the other.

Results: Upon its creation the list of founding countries (Colombia, Cuba, Ecuador, and Venezuela) increased to include Bolivia, Paraguay, and Brazil. Other countries are likely to join the consortium (Haiti and Mexico). In relation to the collaboration in several research activities, the summary of these results is presented below (CLAYUCA, Informe Annual. Abril 1999 – Julio 2000).

☞ *Mechanization*. It has been interesting to see the evolution of this concept, first mentioned in the 1999 Annual Report. Since then, planting and harvesting implements for the mechanization of cassava cultural practices, have been introduced and successfully used in several regions of Colombia. CIAT collaborated with CLAYUCA in demonstration field days to promote the use of the planting machine (see Figure 3.2) in Palmira and Jamundí (Valle del Cauca), Luruaco (Atlántico), Neiva (Huila) and Villavicencio (Meta).

Harvesting equipment (see Figure 3.3) has also be bought and is currently being evaluated to determine their efficiency, operational costs, and amount and quality of their output. It has been mentioned that mechanical harvesting of cassava can reduce the percentage of roots that remain in the soil from 20% down to 5%. These statements need verification under a range of cassava growing conditions (particularly for specific type of soils), and for different shapes of the root system (which is genotype dependent).

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

☞ *Artificial drying of cassava*. A project was presented by CLAYUCA and funded by the Colombian Ministry of Agriculture to build a pilot plant to conduct research in the area of artificial drying of cassava tissue (both roots and leaves). The plant (see Figure

3.4) is already functioning. Project IP3 contributed with the evaluation process of the products of the plant. As a result of this research we can now conclude that artificial drying of cassava tissue is feasible from the economic point of view and that the product has cyanide levels below the maximum tolerated. All the adjustments of the pilot plant so far made (or to be made in the future) were based, in part, on the close monitoring of the quality of the cassava flour produced. A major achievement of this process is that it produces no water effluent.

☞ *Fertilization of cassava*. Some of the results of this collaborative effort were already presented (See Table 2.35) for the inclusion of swine manure in cassava grown in the acid soil savannas. The use and effect of poultry manure in cassava productivity was also evaluated (See the explanation for Tables 2.31 to 2.34), this time at Jamundí (Valle del Cauca). Figure 3.5 depict a nice cassava field, well taken care of, and fertilize with chicken manure. This project has also contributed to the following up of fertility trials conducted in the coffee growing areas of the Quindío Department.

☞ *Integrated pest and disease management*. This is one of the six priority areas of activity for CLAYUCA. Some of the activities where CIAT has contributed are the evaluation of different isolates of Phytophthora root rot organisms from cassava grown in different regions. Research was carried out to determine pathogenicity and the effect of thermotherapy. In the area of insect (and arthropods) resistance and control CIAT is contributing to the development of a commercial enterprise for the production of the baculovirus that has been successfully used in the control of the horn worm (*Erinnyis ello*). The use of *Metarhizium anisopliae* and *Beaveria sp.* for the control of the burrower bug *Cyrtomenus bergi*.

Achievements:
- ☞ CLAYUCA consolidated and expanded.
- ☞ Very important complementary research activity between CIAT and CLAYUCA resulted in increased impact for the cassava sector.
- ☞ It demonstrates, once again, the feasibility and benefits of this type of consortium.

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

Specific Objectives:

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

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

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

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

- *Processed cassava for human consumption*. These high value-added products (frozen croquettes or fried cassava chips), require very specific products. The late Juan Vergara (see: "Official release of two cassava varieties for the acid-soil savannas" above) produced cassava roots for CONGELAGRO (the largest producer of croquettes in Colombia). He initially cultivated the variety "Brasilera", but upon sending a 50 Kg sample of roots from Reina (CM 6740-7) he switched his emphasis to this cultivar (part of the change is due to its higher dry matter content). We are also developing a collaborative work to determine the best characteristics in cassava roots for their use in the production of fried cassava chips for the snacks market. This work will include the use of yellow (high carotene) cassava roots (Figure 3.6).

  It should be emphasized that this emerging market has a huge potential. CONGELAGRO started exporting their frozen products to Japan. The fried cassava chips are currently sold in the U.S. snacks market (although restricted to areas with strong presence of Latin American people). The advantage of this activity lies on its potential for growth, its need for a constant supply of raw material which would help stabilizing the price of cassava, the demand of labor for processing the products in rural areas (before the roots are sent to the final processing plant), the creation of a relatively large alternative market for cassava, and the increase of demand for a high quality product. The technology for croquettes was very appealing to the Nigerian group of businessmen that visited the region. Similar products are also produced in other countries such as Brazil, with an enormous market.

➢ *Starch industry*. One of the challenges for industrial uses of cassava is the constant supply of roots. This may be a difficult task in areas with long dry periods when planting is not possible for a period of 3—4 months. That is the case of the northern coast of Colombia. An important starch factory is based nearby Barranquilla (Figure 3.6). We have worked together with this enterprise which has been fundamental in the promotion of new industrial clones in the region.

In addition to providing seed for their operations, we have contributed with them in training of their personnel in the agronomy of cassava, and recently, contributed in setting up a tissue culture lab for a rapid multiplication scheme of elite germplasm developed by CIAT. Such an initiative is very relevant because it demonstrates the renewed interest of the industry in this crop on one hand, and because it will eventually facilitate the availability of clean stakes of new cassava clones in the region. Initially the vegetative material produced will be for their own fields, but then for general use by the farmers in the long term.

➢ *Feed industry*. We have received strong support by poultry associations in different countries of the region. Their interest is in the use of dried cassava roots in up to 20-30 % of the diets. It has been a very productive and intense collaboration. A representative of the Colombian Poultry Association visited Asia along with the CIAT staff. During the year, every single issue of their monthly publication ("Avicultores") has had an article on the use of cassava in the poultry business. Several of them originated in CIAT. Again, the creation of CLAYUCA has been fundamental to facilitate this interaction, also resulting in several research projects approved, and partially funded, by the Poultry Associations (see CLAYUCA: Informe Annual (Abril 1999 – Julio 2000).

A very good example of the synergism that can be achieved through this collaboration is the activity currently carried out in Jamundí with a farmer and poultry grower (Juan Pablo Velez – AVICAUCA). This model farmer has obtained excellent yields using CIAT germplasm and technology (see Tables 2.31 to 2.34, and Figure 3.6). He has also been the first poultry grower that has fed his chickens with cassava produced by him. This activity, in turn, has helped to reduce the reluctance and concerns of other poultry growers to change the composition of the diets that they have traditionally used for many years. Again all this activity is not restricted to Colombia alone. PRONACA from Ecuador is an important chicken growing industry and is actively following up all these developments.

Achievements:

- Collaboration with the private sector promotes the use of technologies and germplasm developed by CIAT.
- It has helped CIAT to adjust its breeding objectives and other research goals to the needs of the industry.
- It has contributed financially to carrying out some specific research projects.

A 40 ha field with MTAI-8 grown by the feed industry at Molinero (Atlántico)

A 10 ha field with MTAI-8 at Malambo (Atlántico). Roots are for the starch industry.

10 ha field with "Reina" (CM 6740-7) (Restrepo, Meta), using minimum tillage practice. Cassava production for the frozen croquette industry.

Commercial cassava (60 ha) field in Jamundí (Valle del Cauca) from a poultry producer.

Figure 3.6. Commercial cassava field for the industry in different agro-ecological regions of Colombia.

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

Specific Objectives:

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

Rationale: In Northern Colombia, cassava is a major staple crop and provides an important linkage for small farmers to urban and processing markets. Close to 70% of the root production is used for direct human consumption. Therefore, cassava varieties need to produce well and meet stringent consumer preferences.

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

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

Results: Farmer, middlemen, table consumer, starch producer, chip producer, and animal feed industry were the general users considered.

Farmers: Based on the statistics pseudo t^2 and pseudo F, and a $R^2 = 0.86$, five farmer types were identified according to preferences in six stages of cassava cycle growth. Characteristics for each type were described using frequency tables.
Middlemen: In this case, three types of middlemen, allowed to explain 92% of the variability among them. This means that they have different criteria of preferences to buy cassava.

Consumers: With a R^2 of 0.71, four types of consumers were identified in the region. There was a strong difference between consumers depending whether they came from a village, a small town or a city.

For the cassava chip and starch producers and animal feed industry, it was impossible to run a cluster analysis because the sample was smaller than 10. However, a comparative description about preferences in cassava roots was obtained.

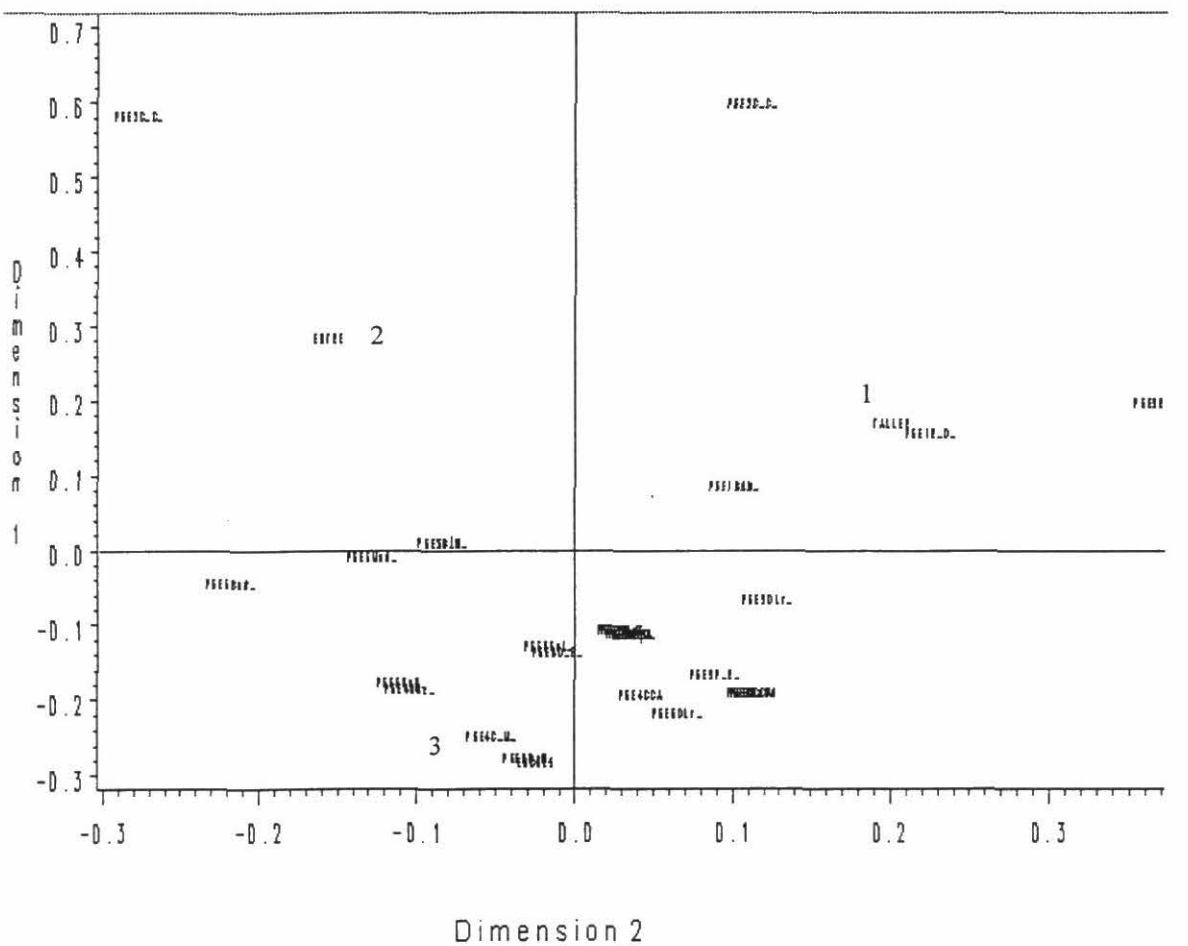


Figure 4.1. Multiple correspondence for the interview, workshop and survey Methods (1 = Worshop; 2 = Interview; 3 = Survey)

Comparison of Methods.

In the case of farmers, there was a preference's criteria group by growth's phase of the crop, which was common to the interview, workshop and survey. However, there were

criteria, which were specifically associated with the workshop and the interview processes. The survey did not have a discriminatory capacity (Figure 4.1. This means that the interview and the workshop provide additional and specific information complementing whatever was obtained through the survey. Comparing costs, the survey provided the less expensive information by farmers, but the additional information provided by the others tools contributed to get a clearer explanation of farmer's preferences.

The criterion associated with the interview was canopy diameter in the phase from 60 to 120 days after planting. Meanwhile, for the workshop, the criteria associated were different. For the phase from cutting selection to 60 days after planting, healthy, disease-free cuttings, with high latex content, and resistance to drought were prevalent traits. From 120 to 180 after planting the most important criteria were stem-borer (*Chilomima clarkeii*) resistance, thick roots, high starch content and few branching.

It was possible to do a comparative analysis of farmers', industry's, middle men's and end users' preferences at one site (Barranquilla, on the Atlantic Coast). In total 15 interviews were conducted in the different categories (women = 4, middle men = 3, farmers = 5, industry 3), for of the general users reported above.

The feed industry "Concentrados del Norte," also located in Barranquilla, has been grwoing the cassava varieties MTAI 8, CM 4843-1, CM 4919-1, ICA Costeña and ICA-Negrita. They have identified specific requirements for these varieties, which are to be used in dehydrated feed. These clones, transferred by the CIAT Cassava Program, have characteristics such as a minimum of 34% dry matter, low cyanogen content and an average root yield of 25 t/ha, fresh root (Table 4.1). However, the some characteristics relevant to the industry, such as low cyanogenic and root color, should be further analyzed to clarify or explain in detail each one of them.

The challenge was to determine whether the participatory methodology incorporated into cassava breeding (developed from 1986-1992) identified, analyzed, and incorporated end users' criteria. It should be emphasized that new possibilities of transforming the crop from a subsistence to an income-generating alternative arose as a result of the release of ICA-Negrita and ICA-Costeña. These cassava varieties were identified through participatory breeding processes.

This evaluation revealed that women have their own selection criteria, related to their particular industry of making *bollos* (home-made " cassava dumplings), a typical breakfast food, prepared with cassava and cheese. It was evident that cassava varieties should meet certain cooking requirements such as high starch content for good cooking quality, uniformity of roots, and flesh color (yellow or white) (Table 1). These characteristics result in possibilities for new cassava germplasm in relation to this small-industry activity. The cassava-producing families also contributed information on quality-related traits to the selection process. There are areas where cassava plays an important role in more than one particular market. The broadening of the user base should be an objective in order to improve the effectiveness of the participative breeding

methodology in the future. This project, therefore, may provide insight into a new perspective about cassava-related women's and family earnings on the Atlantic Coast.

Table 4.1. Comparison of selection criteria used by different cassava users in the northern coast of Colombia.

INDUSTRY	WOMEN (qualitative evaluation)	MIDDLE MEN (qualitative evaluation)	FARMERS - FRESH MARKET (qualitative evaluation)
Root dry matter ≥ 34%	High root dry matter (≈ high starch content) for good cooking quality	High root dry matter (≈ high starch content)	High root dry matter (≈ high starch content)
Low cyanogenic content	Not mentioned	Not mentioned	Not mentioned
Good production of stakes	Not mentioned	Not mentioned	Not mentioned
Not mentioned	Uniformity of roots	Uniformity of roots	Uniformity of roots
Fresh root yield ≥ 25 t/ha	Root yield = one "carga" should produce 280, each selling at Ps$250	High root yield	High root yield
Not mentioned	Root thickness	Root thickness	Root thickness
White flesh color	Yellow or white flesh	White flesh color	White flesh color
Not mentioned	Root length	Root length	Root length
Not mentioned	Not mentioned	Health (without diseases and insects	Health (without diseases and insects

The most important selection criteria for middlemen were high root dry matter, root morphological traits, external root (dark brown) and flesh color (white), root thickness and length, health (without diseases and insects) and no constrictions. In addition to having all the previously described characteristics, end users prefer selecting high-yielding varieties with an average of 5 roots/plant (Table 4.1).

Table 4.2 shows an interesting difference among women, middle-men and end user criteria. Yellow-fleshed clone CM 6119-5 has high acceptance by women (for the home-made *"bollos"*), in contrast to middle-men and the end user. It can be observed that yellow flesh is of intermediate acceptance. This means that for women the criterion root

color could have two acceptable possibilities: white and yellow. However, none of the groups accepted light brown color in the external root, as in CM 3555-6 with an intermediate to low acceptance (Table 4.2). It was evident that some cassava varieties (e.g. Venezolana, ICA Costeña and SM 1411-5) play an important role in more than one particular market (Table 4.2).

Table 4.2. Comparison of preference ranking among women's, middle men's and end users' (fresh market) criteria. Barranquilla, Northern Colombia.

Women			Middle-men			Farmers		
Genotype	PR	RS	**Genotype**	PR	RS	**Genotype**	PR	RS
Venezolana*	1	H	Venezolana	1	H	Venezolana	1	H
SM-1411-5	2	H	Costeña	2	H	Negrita	2	H
CM 6759-8	3	H	SM-1411-5	3	H	SM-1411-5	3	H
CM 6119-5	4	H	CM 3306-19	4	H	CM 6759-8	4	H
Costeña*	5	H	Negrita	5	I	Costeña	5	H
Negrita*	6	H	CM 6759-8	6	I	CM 6119-5	6	I
SM 805-17	7	I	CM 3555-6	7	I	SM 805-17	7	I
CM 1433-4	8	I	CM 6119-5	8	I	CM 3306-19	8	L
CM 3555-6	9	L	CM 1433-4	9	L	CM 3555-6	9	L
CM 3306-19	10	L	SM 805-17	10	L	CM 1433-4	10	L

* Varieties Released
RS: Rating scale: H = High acceptance, I = Intermediate acceptance, L= Low acceptance.
PR= Preference Ranking

The evaluation process in this project has resulted in specific benefits: better knowledge of the market system in a given area and the refinement of selection criteria based on cassava farmers, women, middle men and end users.

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

CIAT has been trusted with the maintenance of the cassava world germplasm bank, which includes more than 6000 accessions of *Manihot esculenta* and other *Manihot* species. Research progress on the micro-nutrient and starch properties of hundreds of genotypes from the germplasm bank was reported as Output 1. In the following pages a summary of other activities related to the germplasm bank will be described.

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

Specific Objectives:

a) *To contribute with the maintenance the cassava and relates wild species germplasm bank in the field.*
b) *Implementation of scheme for reducing incidence of frog skin disease in germplasm bank and elite clones (also see Activity 2.6).*

Rationale: The Genetic Resources Unit is officially in charge of the maintenance of the cassava germplasm bank, both *in vitro* and in the field. However, for practical reasons, the field operations are coordinated by IP3 project. During the past year and until 2001 there will be an extensive activity to clean up from frog skin disease, the germplasm bank (see Activity 2.6). Plots from the germplasm bank maintained in the field, because of its very nature, could not be eliminated even if frog skin disease appeared in some of the plants. Eventually the incidence of the disease increased to unacceptable levels. Therefore, since this year drastic measures were taken to reduce the level of incidence to acceptable levels. The strategy implies four main elements:

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

Planting of disease free plantlets outside CIAT, in isolated fields. Because of the higher incidence of frog skin disease at CIAT (mainly at the germplasm bank collection in the field), plants that are certified to be disease free, or those developed from botanical seeds (which do not transmit viral agents to the plants germinating from them), were planted outside CIAT in isolated plots. Only virus-free plants will be planted in those isolated plots. In the meantime, plantings at CIAT are reduced as a higher proportion of the cassava germplasm was certified to be virus-free. In short the outside plantings are certified to be *"clean"*, whereas the plantings at CIAT are not. This situation will be maintained until the middle of 2001, when materials not certified to be disease free will move out of CIAT, and those that are *clean*, will come back.

Breaking the life cycle of the white flies at CIAT. In addition of maintaining an ideal reservoir for the agent of the frog skin disease in the germplasm bank, there is a second factor that facilitated the spread of the disease. In effect, the white flies problem has increased considerably during the last few years. A major factor for this increment has been the continuous planting of cassava year round. The insects, therefore, had an ideal condition for maintaining high population densities. Starting in 2001, there will be a period of approximately one month, when no cassava will be maintained in the field (with an exception already described in Activity 2.6). This will greatly reduce population densities for the insect, and in turn, will reduce to a minimum the already inefficient transmission of the frog skin disease agent to healthy plants.

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

It is expected that by mid 2001, all the accessions from the germplasm bank will be already indexed and certified to be disease free. Until then they are maintained in the isolated plots outside CIAT, but then they will be brought back to the normal plantings at the Experimental Station.

Achievements:

- By the end of 2001 the entire germplasm bank collection will have been passed through the process of indexation to certify that each accession is free from frog skin.
- Procedures have been established and agreed upon for reducing population densities of white flies.
- Procedures have been established and agreed upon for minimizing the transmission of frog skin through the stakes.

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

Specific Objectives:

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

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

So far the process has yielded no positive results, but only a fraction of the entire germplasm bank collection has been screened, because of the transitional stage most of the accessions are currently on (to be cleaned from frog skin). This activity which has low probability of success will be maintained until the entire collection has been properly tested.

We are currently trying to obtain financial support for a more aggressive project on apomixis. This project would be a collaborative effort with Professor Nagib Nasser from the Universidade de Brasilia (Brazil). As a kind gesture professor Nasser already sent to us F1 seed from inter specific crosses with wild relatives, a material that according to his publications has shown the occasional occurrence of apomixis.

Achievements:
- We continue to look into the germplasm collection for the natural occurrence of apomixis.
- Potential collaborative effort with the Universidade de Brasilia.

OUTPUT 6: Breeding for insect (and other arthropods) resistance and development of alternatives methods for their control.

An important feature of the IP3 project relates to the integration of breeding, entomology plant pathology and the development and use of tools from biotechnology. In spite of the "divisions" created by the project structure, the four areas of science have maintained as much a close relationship as possible. In Output 6 the progress related to insect and other arthropods is summarized. In Output 7, the same is done but for disease resistance.

Activity 6.1. Evaluation of M. esculenta *and related species from the germplasm collection for useful traits, particularly for the natural occurrence of apomixis.*

Specific Objectives:

1) *To evaluate cassava germplasm for resistance to* Bemisia tabaci.
2) *To establish working colonies of* B. tabaci.
3) *To evaluate cassava germplasm for resistance to* Aleurotrachelus socialis.

Rationale: Whitefly populations on cassava during the 1999-2000 growing season continued to remain high at CIAT. As studies during 1999 (See IP3 Annual Report, 1999) indicated, the predominant species is *Aleurotrachelus socialis*, which accounts for 98.5% of the population. *Bemisia tuberculata* and *Trialeurodes variabilis* account for the remaining 1.5%. Whitefly populations were so high and extensive (all cassava plots/fields were heavily infested) throughout the CIAT farm that it was impossible to carry out experiments on other pests or almost any other type of cassava experiments (i.e. agronomic or physiological). In addition, the species *B. tuberculata* is reported as a vector of cassava frog skin virus disease. This disease is also endemic at CIAT, with high incidence and most varieties/fields being infested.

The combination of high whitefly populations and frog skin disease has rendered cassava field research at CIAT impractical. Land outside of CIAT, in areas where there is scarce cassava plantings and low whitefly populations has been obtained to produce pest and disease free cassava for entomological experimentation. Clean cassava plants are required for host-plant resistance mechanism studies with *A. socialis* and *B. tabaci* and for the maintenance of pest and parasitoid studies with mealybugs and whiteflies and predator research with mites.

Whiteflies are one of the most difficult pests to control, especially using chemical pesticides. They rapidly acquire resistance to pesticides and their short lifecycles (30-35 days) make chemical control economically difficult for resource scarce small farmers. Also, pesticide use, in addition to human health hazards, can destroy any balance that might exist between the pest and its natural enemies. Pesticide control, if it is to be successful, must be initiated early in the crop cycle, at the first sign of whitefly infestation. Studies carried out during the early 1980's with *A. socialis* showed that 3 to 4 pesticides applications, initiated early in the crop cycle and repeated every 3 to 4 months reduce whitefly populations and maintained then at economically feasible levels (i.e. cassava farmers could still turn a profit).

Whitefly population eruptions and epidemiology is not well documented and understood. *A. socialis* has been observed and collected on the CIAT farm for more than 25 years. However it is only in the past 5 years that populations have reached epidemic proportions. This could be due to several factors, some not well understood:

- Most cassava germplasm planted at CIAT is susceptible.
- Cassava has been continually grown at CIAT for more than 30 years.
- Environmental conditions, especially adequate to high rainfall, are favorable for whitefly population increases.
- The staggered planting pattern followed at CIAT, where cassava plantings are programmed almost continually throughout the year, provides a continuum of young, vigorous foliage that is preferred for oviposition and feeding.
- Pesticides use in the Germplasm Bank and on other experimental fields may have caused a disequilibrium in the pest-natural enemy relationship.
- A new "biotype" of *A. socialis*, that is particularly aggressive and with a high reproductive capacity may have been inadvertently introduced into the region.

The third factor; the high rainfall pattern of recent years is considered to be the major factor for increased populations. Number 6, the introduction of a new biotype is least favored of the reasons indicated.

Evaluation of cassava germplasm for resistance to Bemisia tabaci

In recent years, *B. tabaci*, the vector of African Cassava Mosaic Disease (ACMD), has been collected feeding on cassava in the neotropics. ACMD has not been observed in the Americas, and it has been speculated that its absence may have been related to the inability of its vector, *B. tabaci* to feed on cassava. Since the early 1990's a new biotype (B) of *B. tabaci* (considered by some investigators to be a separate species, *B. argentifolii*) has been found feeding on cassava in several areas of the neotropics,

including Brazil, Ecuador, Colombia and several countries in the Caribbean region. ACMD, therefore, now poses a more serious threat, as most traditional varieties in the neotropics are susceptible to the disease.

Several cassava varieties and hybrids have been identified for resistance to *A. socialis* (see previous and this Annual Report). A research project, involving a student MS thesis, has been initiated to evaluate *B. tabaci* feeding, oviposition and development on whitefly (*A. socialis*) resistant varieties. The hypothesis is that whitefly (vector) resistant varieties, combined with ACMD resistant varieties could provide an effective option for controlling or reducing ACMD levels and incidence.

It has been difficult to rear *B. tabaci* (See IP-3 Annual Report, 1999) on Cassava. Therefore *B. tabaci* colonies are presently being established on alternate hosts beans (*Phaseolus vulgaris*) and poinsettia (*Euphorbia palcherrima*). Poinsettia was chosen for the obvious reason that being an Euphorbiaceae, it shares commonalties with cassava. The *B. tabaci* that we are using originated on beans and a working colony has now been established on beans.

By placing pupae from the bean colony onto poinsettia leaves in 1m x 1m nylon mesh cages in the greenhouse, it was possible to establish a now flourishing colony on poinsettia. In collaboration with the virology unit, and using the RAPD's-PCR technique, it has been possible to generate molecular markers for whitefly biotype identification. The amplified products indicate a polymorphism between biotypes A & B of *B. tabaci*. Fragments of amplified DNA observed in biotype B were absent in A. It was therefore possible to confirm the establishment of biotype B on both beans and poinsettia.

Cassava plants were then infested with the (confirmed) B-biotype, using a leafcage technique previously developed at CIAT for establishment of whitefly colonies. Ten whitefly adults were introduced into each leafcage and allowed to feed and oviposit. Oviposition and life cycle of *B. tabaci* biotype B will be evaluated on selected cassava varieties and hybrids.

Evaluation of cassava cultivars for resistance to Aleurotrachelus socialis.

Cassava germplasm from several sources was evaluated for whitefly resistance during the 1999-2000 crop cycle at CIAT. Due to the heavy infestation of frog skin disease in CIAT germplasm, it was not possible to introduce this germplasm to the CORPOICA, Nataima station for evaluation at that site. Germplasm evaluated included core collection, crossing blocks, multiplication and yield trial materials. All were planted by the cassava germplasm improvement project and sown at CIAT and subjected to high field populations of *A. socialis*. Emphasis was given to those varieties/cultivars that had not been previously evaluated, had few evaluations or had received very low damage ratings in previous evaluations. Approximately 450 materials were evaluated.

A considerable portion of the germplasm evaluated were hybrids (CM & SM) while others were from the core collection, mostly MBRA, MCOL, MECU, MGUA, and MMEX. The hybrids evaluated were developed for several ecosystems, such as the lowland tropics, acid-soil savannas and inter-andean valleys and also include materials for genetic mapping, whitefly resistance mapping, post harvest root deterioration and phytophthora and bacteriosis mapping.

Periodic evaluations for plant damage and whitefly populations were made throughout the crop cycle. A 1 to 6 damage scale (1 = no damage, 6 = severe damage) is used to measure whitefly damage, and a 1 to 6 scale is also used to measure whitefly populations (Table 6.1).

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

Population scale (nymphs and pupae)
1 = no whitefly stages present
2 = 1-200 individuals per cassava leaf
3 = 201-500 per leaf
4 = 501-2000 per leaf
5 = 2001-4000 per leaf
6 = > 4000 per leaf
Damage scale
1 = no leaf damage
2 = young leaves still green but slightly flaccid
3 = some twisting of young leaves, slight leaf curling
4 = apical leaves curled and twisted; yellow-green mottled appearance
5 = same as 4, but with "sooty mold" and yellowing of leaves
6 = considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems.

Results show that whitefly populations at CIAT were extremely high and caused considerable selection pressure on the cassava clones. Of the 449 clones evaluated, more than 50% had a damage rating above 4.0, and 86% above 3.0 (Figure 6.1). However, in spite of the high selection pressure, 30 cultivars (6.6%) presented no damage symptoms, and 35 cultivars had low damage ratings (between 2.0 and 3.0) on the 1 to 6 damage scale. 91 cultivars had a damage rating below 3.5, indicating that they will be re-evaluated in subsequent planting cycles.

Table 6.2. Cassava cultivars evaluated for whitefly (*Aleurotrachelus socialis*) damage during 1999-2000 crop cycle, at CIAT, with damage ratings below 3.5 (1=no damage, 6=severe damage).

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupa[1]	Pupa[2]
CM 6320 - 2	MULTN3	1.0	2.0	2.0	1.5	2.0	1.0
CM 8024 - 2	MULTN3	1.0	2.0	2.0	2.0	2.5	2.0
SM 1143 - 21	MULTN3	1.0	2.0	2.0	2.0	2.0	2.0
SM 1517 - 9	MULTN3	1.0	2.0	2.0	1.0	1.0	1.0
SM 1519 - 2	MULTN3	1.0	1.0	1.0	2.0	2.0	2.0
SM 1624 - 2	MULTN3	1.0	1.0	1.0	2.0	1.0	1.0
SM 1657 - 7	MULTN3	1.0	1.0	1.0	1.0	2.0	2.5
SM 1673 - 11	MULTN3	1.0	1.5	1.0	1.0	2.0	2.0
SM 1673 - 5	MULTN3	1.0	1.0	1.0	1.0	1.0	1.0
SM 1684 - 13	MULTN3	1.0	1.5	1.5	1.0	1.5	2.0
SM 1694 - 2	MULTN3	1.0	2.0	1.0	1.0	2.0	2.0
SM 1778 - 53	MULTN3	1.0	1.0	1.0	1.0	2.0	2.0
SM 1896 - 3	MULTN3	1.0	2.0	2.0	1.5	1.5	2.0
SM 2065 - 8	MULTN3	1.0	2.0	2.0	1.5	1.5	1.0
SM 985 - 9	MULTN3	1.0	1.5	1.0	2.0	2.0	1.0
MPER 385		1.5	2.0	2.0	2.5	2.5	2.0
SM 1788 - 16	MULTN3	1.5	2.0	2.0	1.0	1.0	1.5
SM 1955 - 6	MULTN3	1.5	2.0	2.0	2.0	2.5	1.5
SM 2069 - 2	MULTN3	1.5	2.0	2.0	2.0	2.0	2.0
CM 7951 - 5	MULTN3	2.0	2.0	2.0	2.5	2.5	2.0
CM 909 - 25	MULTN3	2.0	2.0	2.0	2.5	2.5	2.5
MBRA 489	MULTN3	2.0	1.0	1.0	1.0	2.0	2.0
MCOL 2019		2.0	2.0	2.0	4.0	4.0	3.0
MCOL 297		2.0	1.0	1.0	2.0	2.0	2.0
MECU 82		2.0	2.0	2.0	3.0	3.0	2.0
MECU 72	CruzMap MoscaBca	2.0	1.0	1.0	0.0	0.0	1.0
SM 1778 - 44	MULTN3	2.0	2.0	1.5	1.5	2.0	1.5
SM 1794 - 18	MULTN3	2.0	1.0	1.0	1.0	2.0	3.0
SM 1828 - 11	MULTN3	2.0	1.0	1.0	1.0	2.0	2.0
SM 1673 - 10		2.0	2.0	2.0	3.0	4.0	3.0
MCOL 317		2.5	3.0	3.5	4.0	4.0	2.0
MPER 368		2.5	2.5	2.5	4.0	4.0	2.5
MVEN 67 B		2.5	3.0	3.0	3.0	3.0	2.0
SM 1682 - 2	MULTN3	2.5	1.5	1.5	1.5	2.5	3.0
SM 1948 - 29	MULTN3	2.5	2.0	2.0	3.0	2.5	3.0
SM 2141 - 1		2.5	2.0	2.0	2.0	2.0	3.0
CM 1111 - 8	ERCIAT00	3.0	2.0	2.0	3.5	3.5	2.5

[1] Pupae on leaves of mid 1/3 of plant. [2] Pupae on leaves of lower ½ of plant.

Table 6.2 (cont.)

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupa[1]	Pupa[2]
CM 5438 - 12	MULTN3	3.0	2.0	2.0	2.5	2.5	2.0
CM 7593 - 15	ERCIAT00	3.0	2.0	2.0	3.0	3.0	3.5
CM 8378 - 3	MULTN3	3.0	2.0	2.0	2.5	3.0	2.0
MCOL 113	Macho esteril	3.0	3.0	4.0	3.0	3.0	4.0
MCOL 1522		3.0	4.0	4.0	4.0	4.0	2.0
MCOL 1795		3.0	3.5	5.0	5.0	5.0	2.5
MCOL 2131		3.0	4.0	4.0	3.5	3.5	2.0
MCOL 346		3.0	2.0	2.0	3.0	2.5	2.0
MCOL 725		3.0	4.0	4.0	4.0	4.0	3.5
MECU 151		3.0	3.0	3.0	4.5	4.5	2.5
MECU 41		3.0	4.0	3.5	5.0	5.0	2.5
MECU 43		3.0	3.0	3.5	3.0	3.0	3.5
MEX 71		3.0	3.0	3.0	3.5	3.0	3.0
MMEX 108	MULTN3	3.0	2.0	2.0	2.5	3.0	2.5
MPER 183		3.0	2.0	2.0	2.0	3.0	3.5
MPHI 3		3.0	2.0	2.0	2.0	3.0	3.0
SM 1688 - 20	ERCIAT00	3.0	2.0	2.0	2.0	3.0	3.0
SM 1689 - 18	MULTN3	3.0	2.0	2.0	2.0	2.0	3.0
SM 1799 - 18	MULTN3	3.0	2.0	2.0	1.0	1.0	3.0
SM 1812 - 55	MULTN3	3.0	2.0	2.0	3.5	3.5	3.0
SM 1861 - 18		3.0	3.0	3.0	3.0	3.0	3.0
SM 1862 - 25		3.0	2.0	2.0	2.0	2.0	3.0
SM 1870 - 31		3.0	2.0	2.0	3.0	3.0	3.0
SM 1920 - 1	MULTN3	3.0	2.0	2.0	2.0	3.0	3.0
SM 1927 - 9	MULTN3	3.0	1.0	1.0	1.0	2.0	3.0
SM 1953 - 30	MULTN3	3.0	1.5	1.5	2.0	2.5	2.5
SM 1965 - 1	ERCIAT00	3.0	2.0	2.0	2.0	2.0	3.0
SM 909 - 25	MULTN3	3.0	2.0	2.0	3.0	3.5	2.0
CM 5655 - 4	Z4	3.5	2.0	2.0	3.0	3.0	3.5
CM 6370 - 2	MULTN3	3.5	2.0	2.0	2.5	2.5	3.0
CM 6787 - 4	MULTN3	3.5	2.0	2.0	3.5	3.5	2.5
*Brasilera		3.5	2.0	3.0	3.0	3.5	3.5
MCOL 1722		3.5	4.0	5.0	4.5	4.5	3.5
MCOL 1780		3.5	4.0	5.0	5.0	5.0	3.0
MCOL 191		3.5	3.0	3.0	3.5	4.5	3.0
MCOL 1964		3.5	4.5	5.0	5.0	5.5	3.0
MCOL 2493		3.5	3.5	4.0	4.5	5.5	2.0
MCUB 42		3.5	3.0	3.5	4.0	5.0	4.0
MDOM 5		3.5	3.0	3.5	5.0	5.0	2.0
MECU 171		3.5	2.5	3.0	4.0	4.0	3.0

[1] Pupae on leaves of mid 1/3 of plant. [2] Pupae on leaves of lower ½ of plant.

Table 6.2 (cont.)

Clone	Code	Damage Grade	Population Levels/Leaf				
			Adult	Eggs	Nymph	Pupa[1]	Pupa[2]
MECU 47		3.5	4.5	5.0	5.0	4.5	4.0
MFJI 6		3.5	6.0	4.5	5.0	5.0	2.5
MPER 209		3.5	4.0	4.0	3.5	3.5	3.0
MPER 372		3.5	2.0	2.0	2.5	4.0	3.5
MPER 488		3.5	4.0	3.5	4.0	3.5	3.5
SM 1468 - 9	MULTN3	3.5	2.0	2.0	2.5	3.0	2.5
SM 1543 -16		3.5	2.0	3.0	4.0	3.0	3.0
SM 1602 - 13	MULTN3	3.5	2.0	2.0	3.0	3.5	3.0
SM 1642 - 20	MULTN3	3.5	2.0	2.0	3.0	3.0	3.0
SM 1754 - 21		3.5	2.0	3.5	2.5	3.5	3.0
SM 1754 - 46	MULTN3	3.5	2.0	2.0	3.0	3.0	3.0
SM 1779 - 8		3.5	3.0	3.0	3.0	4.0	3.0
SM 1780 - 27	MULTN3	3.5	2.0	2.0	3.5	3.0	3.0
SM 1868 - 29	MULTN3	3.5	1.0	1.0	2.0	2.0	2.0
SM 2160 - 2		3.5	2.0	3.0	3.5	3.0	3.5
SM 2216 - 12	ERCIAT00	3.5	2.0	2.0	2.0	2.0	2.0
SM 653 - 14	MULTN3	3.5	2.5	2.0	3.0	3.5	2.0

[1] Pupae on leaves of mid 1/3 of plant. [2] Pupae on leaves of lower ½ of plant.

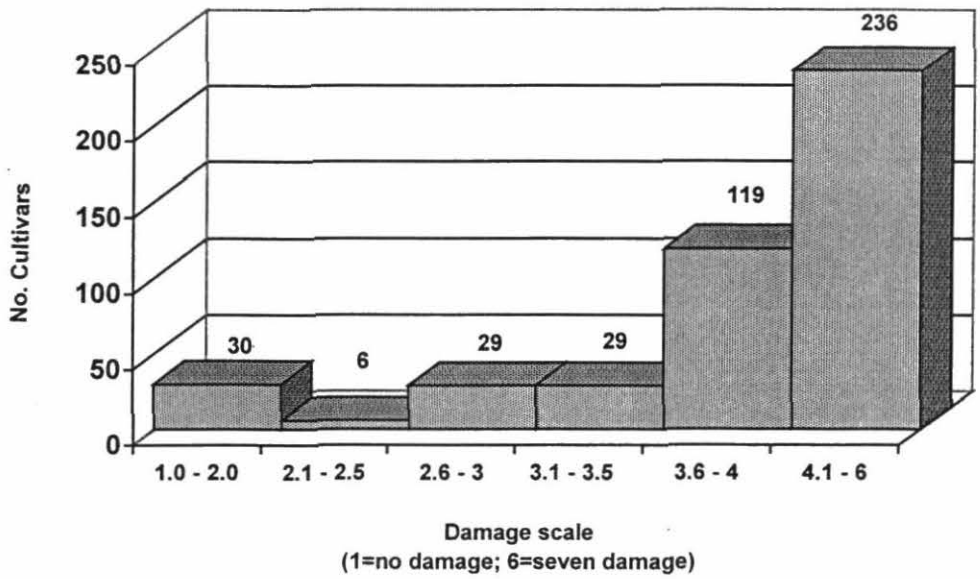


Figure 6.1. Evaluation of 449 cassava cultivars (hybrids and core collection varieties) for whitefly (*Aleurotrachelus socialis)* damage at CIAT/Palmira during 1999-2000 crop cycle.

In general the hybrid clones presented very low damage ratings (Table 6.2). This table includes those clones and cultivars that received a damage rating below 3.5 and will be re-evaluated. Approximately 64% of these are represented by hybrids (SM = 47, 51.6% and CM = 11, 12%) of the germplasm accessions, MCOL represents 15% and MECU and MPER 6% each. These results indicate that there is a good basis for resistance to whiteflies in Andean germplasm, a phenomenon that has been noted in previous evaluations. These results also reinforce previous observations that the cultivar MECU-72 continues to display high levels of resistance, even under high levels of whitefly selection pressure.

Whitefly Resistance in Cassava Germplasm Collection

The research for resistance to cassava whiteflies (especially the species *Aleurotrachelus socialis)* has been an important segment of the cassava research program for several years. This research has been successful in identifying numerous cultivars with resistance to whiteflies (whitefly resistance in agricultural crops is rare) and in developing commercial hybrids with resistance.

From 1992 to present, 5363 genotypes have been field evaluated for whitefly damage/resistance at four different sites in Colombia. The bulk of these screening have been at two sites, CIAT and the CORPOICA station in Nataima, Tolima. The results of these evaluations, which include both plant damage and whitefly population ratings, are contained in a database and available to researchers. In the case of numerous (most) clones, more than one evaluation has been made; the highest score received is considered the most important and first that appears in the data bank.

Of the 5363 genotypes screened, 3897 (73%) have received a damage rating above 3.5 (1 = no damage, 6 = severe damage) and are considered susceptible (Figure 6.2). No further evaluations are planned for these materials. The remaining 1466 genotypes (27%) with damage ratings below 3.5 are considered "promising" and will continue to be evaluated. Emphasis will be given first to those materials with damage rating below 2.0 (479 or 8.9%). Most of these are probably escapes, i.e. selection pressure was not sufficiently high enough to get an accurate evaluation.

Approximately 44% of the materials evaluated are hybrids (Figure 6.4). This figure has increased considerably in recent years as more crosses and subsequent hybrids are being produced by the germplasm development project and many of these are screened for arthropod resistance, especially whiteflies, mites and thrips.

Germplasm (landrace varieties) from several other countries have also been screened and can be appreciated in Figures 6.3 and 6.4. Based on present results, since much of the whitefly resistant germplasm has originated in the Andean zone, increased emphasis will be given to accessions from Ecuador and Peru.

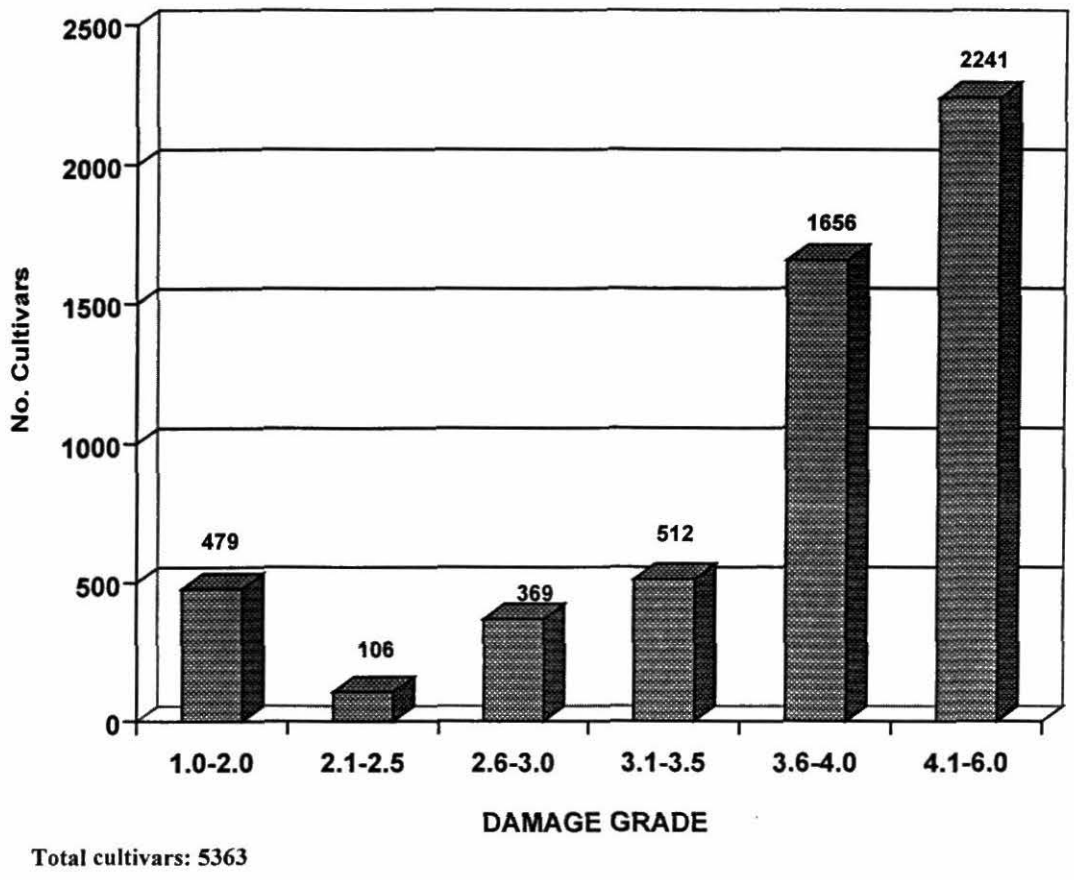


Figure 6.2. CIAT cassava germplasm evaluated in Colombia for resistance to whiteflies (*A. socialis*) from 1992 to 2000; damage scores are based on a 1 (no damage) to 6 (severe damage) rating scale.

Landrace varieties have been collected from numerous countries, especially in the neotropics and these have been included in the CIAT germplasm bank. These accessions have also been systematically screened. The highest number are from Colombia 1030 (33.6%) of the 3038 accessions screened (Figure 6.3). Colombia is followed by Brazil (167 or 5.5%), Venezuela (118 or 3.9%) and, finally, Ecuador (115 or 3.8%).

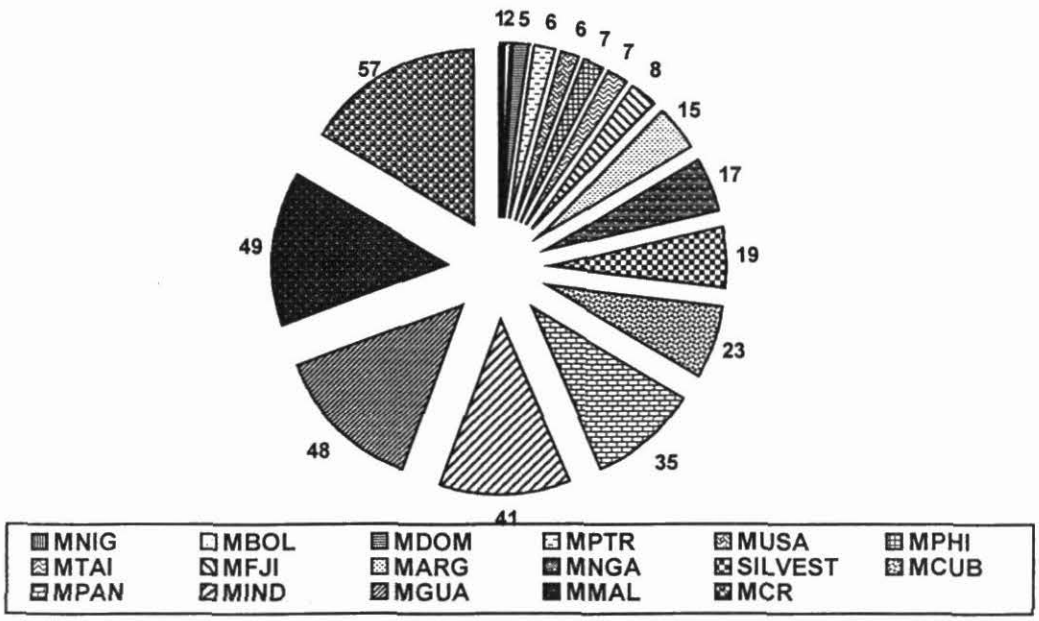


Figure 6.3. Cassava landrace cultivars collected from several countries, and CIAT-produced hybrids, evaluated for whitefly (*A. socialis*) resistance from 1999 to 2000.

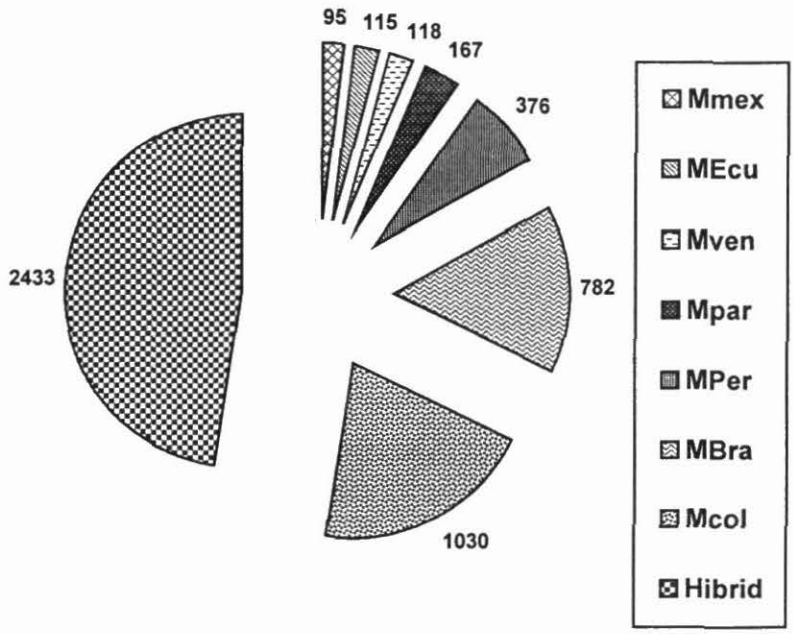


Figure 6.4. Cassava landrace cultivars collected from numerous countries, evaluated for whitefly (*A. socialis*) resistance from 1999 to 2000.

Achievements:
- Thousand of genotypes have been screened for different species of whiteflies.
- Several potential sources of resistance have been identified.

Activity 6.2. Gene tagging of resistance to whiteflies in cassava.

Specific Objectives:

1) To identify molecular marker(s) associated with the gene(s) for resistance to whiteflies.

Rationale: The whitefly is one of the most serious pest and disease vectors affecting agricultural production around the world. In cassava (*Manihot esculenta* Crantz), the whitefly causes from 70-80 percent economic losses. The principal symptoms in the plant are: total chlorosis of the leaves, curling of the apical leaves, yellowing and drying of the basal leaves and retard plant development.

Different sources of resistance to whitefly have been reported (CIAT, 1995). The most important source of resistance genes was genotype MECU-72. Due to the importance of the whitefly as a pest and virus vector, it is necessary to know about the nature of genes that confer resistance to whitefly in genotypes like MECU -72. For this purpose it would be useful to know the F1 segregation of the cross MECU -72 (resistant genotype) x any highly susceptible genotype, using molecular markers. This would help accelerate selection of materials resistant to whitefly and the isolation of the resistance genes.

Materials and Methods: For this study, the cross MECU -72 (as the resistant parent) x MCOL 2246 (as the susceptible parent) was used. Although the latter has advantages such as tolerance to other pests including mites and thrips, and good flowering, it seems to be very susceptible to the whitefly. An F1 offspring of 282 individuals was produced by this cross.

Botanical seeds of this cross were cultured in sterile soil in plastic dishes with 67 sprouts under greenhouse conditions for 6-8 weeks. The temperature was ±30°C.The seedlings were transferred to the field for multiplication.

For the greenhouse evaluation, the seeds were multiplied *in vitro* to obtain enough material in a short period of time (approximately 3 months). This period is brief compared to the normal 6-month period that cassava requires to produce stakes. Optimal health conditions were also achieved.

In vitro propagation methodology, developed by Escobar (1991), will be used in this work. This methodology is based on cutting plant tips, which are transferred to the lab, disinfected first by washing them with sterile deionized water, ethanol 70%, hypochlorite 0.25% and finally washed three times with sterile deionized water. The tips are cultured in 4E medium (Roca, 1984) in 16-ml assay tubes. The calculated growing period is from 60-80 days. Following this period a second in vitro propagation in 4E medium in small, 100-ml flasks will be performed to increase the amount of material per clone. After this the tips of each clone will be cut for culturing in 17N rooting medium (Roca, 1984) for 30-40 days. Finally the plants will be transferred to the greenhouse. This methodology will allow the conservation of material under optimal health conditions, and it will supply sufficient material in a reduced space.

The parents MECU-72, MCOL 2246 and their offspring will be evaluated in the greenhouse, using the "clip cage" methodology, which consists of two polyethylene cylinders of different height joined by forceps. Both cylinder bases are covered by muslin, and the higher cylinder has a small hole through which flies are introduced. With this evaluation it is expected to be able to identify the gene segregation in the offspring and identify the resistant and susceptible materials.

Simple sequence repeats (SSRs) are being used to find markers associated with resistance for mapping and ultimately cloning the resistant genes. The SSRs are random repeat sequences across all eukaryotic genome. These simple repeats can range from 2-6 base pairs (bp). SSRs show high polymorphism, are locus-specific and multiallelic, they have a mendelian inheritance and also are codominant. Silver staining is being used to visualize the allelic segregation of the markers.

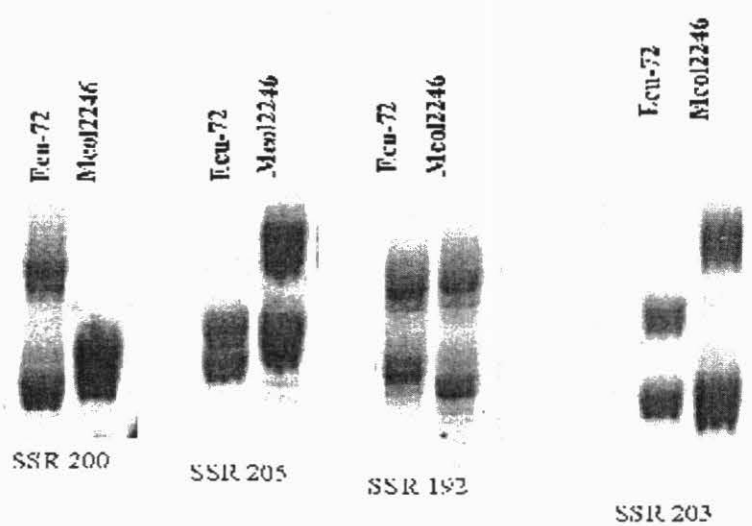


Figure 6.5. Silver-stained polyacrylamide gel showing SSRs of cDNA in both parents MECU-72 (female) and MCOL 2246 (male).

Results: Both parents, MECU-72 and MCOL 2246, were evaluated with 218 cassava SSRs including 32 recently developed cDNA SSRs (Mba et al., submitted). Approximately 60% of the SSRs were polymorphic (Figure 6.5 and Table 6.3).

Table 6.3. SSRs in the parents MECU-72 x MCOL 2246.

SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism	SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism
SSRY1	197	45	X	SSRY51	298	50	X
SSRY2	225	55	X	SSRY52	266	55	X
SSRY3	247	45	X	SSRY53	138	55	Monomorphic
SSRY4	287	45	X	SSRY54	151	55	X
SSRY5	173	55	X	SSRY55	145	50	X
SSRY6	298	45	X	SSRY56	137	50	Monomorphic
SSRY7	250	45	X	SSRY57	293	55	X
SSRY8	288	45	X	SSRY58	217	55	X
SSRY9	278	55	Monomorphic	SSRY59	158	55	X
SSRY10	153	55	X	SSRY60	137	55	X
SSRY11	265	55	X	SSRY61	233	55	Monomorphic
SSRY12	266	55	Monomorphic	SSRY62	250	55	Monomorphic
SSRY13	234	50	X	SSRY63	290	55	Monomorphic
SSRY14	300	55	Monomorphic	SSRY64	194	55	X
SSRY15	215	50	Monomorphic	SSRY65	299	55	X
SSRY16	218	55	X	SSRY66	261	55	Monomorphic
SSRY17	277	50	X	SSRY67	278	55	Monomorphic
SSRY18	198	44	Monomorphic	SSRY68	287	55	X
SSRY19	214	50	X	SSRY69	239	55	X
SSRY20	143	55	X	SSRY70	249	55	X
SSRY21	192	55	X	SSRY71	217	55	X
SSRY22	299	43	Monomorphic	SSRY72	141	55	X
SSRY23	247	45	X	SSRY73	265	50	Monomorphic
SSRY24	100	45	Monomorphic	SSRY74	114	55	X
SSRY25	296	45	Monomorphic	SSRY75	284	55	X
SSRY26	121	55	X	SSRY76	273	55	X
SSRY27	277	50	X	SSRY77	275	55	X
SSRY28	180	55	Monomorphic	SSRY78	248	55	X
SSRY29	281	55	Monomorphic	SSRY79	210	55	X
SSRY30	220	50	X	SSRY80	299	55	X
SSRY31	188	50	X	SSRY81	204	55	Monomorphic
SSRY32	298	50	Monomorphic	SSRY82	211	55	X
SSRY33	273	50	Monomorphic	SSRY83	239	55	Monomorphic
SSRY34	279	55	X	SSRY84	203	55	X
SSRY35	282	55	Monomorphic	SSRY85	292	50	X
SSRY36	134	55	X	SSRY86	296	50	X
SSRY37	187	50	Monomorphic	SSRY87	102	55	X
SSRY38	122	55	X	SSRY88	243	55	X
SSRY39	293	50	X	SSRY89	120	55	X
SSRY40	231	50	X	SSRY90	193	55	Monomorphic

Table 6.3 (cont.)

SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism	SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism
SSRY41	271		X	SSRY91	300	55	Monomorphic
SSRY42	221	50	X	SSRY92	171	55	Monomorphic
SSRY43	255	43	Monomorphic	SSRY93	289	55	X
SSRY44	194	50	Monomorphic	SSRY94	268	55	X
SSRY45	228	50	X	SSRY95	282	55	X
SSRY46	268	50	Monomorphic	SSRY96	149	55	X
SSRY47	244	55	X	SSRY97	194	55	X
SSRY48	178	50	Monomorphic	SSRY98	209	55	Monomorphic
SSRY49	300	50	Monomorphic	SSRY99	192	55	X
SSRY50	271	50	X	SSRY100	210	55	X
SSRY101	213	55	X	SSRY153	117	45	X
SSRY102	179	55	Monomorphic	SSRY154	318	55	X
SSRY103	272	55	X	SSRY155	158	55	X
SSRY104	258	52	Monomorphic	SSRY156	160	44	Monomorphic
SSRY105	225	55	Monomorphic	SSRY157	500	45	Monomorphic
SSRY106	270	55	X	SSRY158	224	45	Monomorphic
SSRY107	120	45	X	SSRY159	159	45	Monomorphic
SSRY108	203	55	X	SSRY160	151	50	X
SSRY109	125	55	X	SSRY161	220	55	X
SSRY110	247	55	Monomorphic	SSRY162	126	43	X
SSRY111	235	55	Monomorphic	SSRY163	231	44	Monomorphic
SSRY112	117	55	X	SSRY164	187	55	X
SSRY113	187	45	X	SSRY165	243	55	X
SSRY114	167	55	X	SSRY166	244	55	X
SSRY115	296	.	No amplified	SSRY167	183	45	X
SSRY116	167		No amplified	SSRY168	277	55	Monomorphic
SSRY117	142	55	X	SSRY169	100	55	X
SSRY118	169	55	Monomorphic	SSRY170	299	55	X
SSRY119	155	55	X	SSRY171	291	55	X
SSRY120	139	55	X	SSRY172	201	55	X
SSRY121	168	43	X	SSRY173	281		NO
SSRY122	273	45	X	SSRY174	136	43	X
SSRY123	136	55	X	SSRY175	136	55	X
SSRY124	146	55	Monomorphic	SSRY176	112	45	Monomorphic
SSRY125	247	55	Monomorphic	SSRY177	268	55	X
SSRY126	245	55	Monomorphic	SSRY178	104	55	Monomorphic
SSRY127	130	44	Monomorphic	SSRY179	226	55	X
SSRY128	243	45	X	SSRY180	163	55	X
SSRY129	205	55	Monomorphic	SSRY181	199	55	X
SSRY130	223	55	X	SSRY182	253	50	Monomorphic
SSRY131	111	45	Monomorphic	SSRY183	221	50	X
SSRY132	196	45	Monomorphic	SSRY184	163	50	X
SSRY133	295	55	Monomorphic	SSRY185	243	50	X
SSRY134	213	55	Monomorphic	SSRY186	297	55	
SSRY135	253	55	X	SSRY187	160	55	
SSRY136	296	55	Monomorphic	SSRY188	198	55	Monomorphic
SSRY137	157	55	Monomorphic	SSRY189	185	55	X

Table 6.3 (cont.)

SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism	SSR #	Size (bp)	Anneal.T. (°C)	Polymorphism
SSRY138	129	50	Monomorphic	SSRY190	164	55	
SSRY139	129	44	Monomorphic	SSRY191	186	55	Monomorphic
SSRY140	212	43	Monomorphic	SSRY192	183	55	X
SSRY141	262	55	X	SSRY193	218	55	X
SSRY142	206	55	X	SSRY194	196	55	
SSRY143	153	55	Monomorphic	SSRY195	186	55	X
SSRY144	117	55	X	SSRY196	188	55	
SSRY145	143	45	X	SSRY197	209	55	X
SSRY146	139	45	X	SSRY198	219	55	
SSRY147	113	45	Monomorphic	SSRY199	205	55	
SSRY148	114	55	Monomorphic	SSRY200	205	55	X
SSRY149	500	45	X	SSRY201	197	55	X
SSRY150	175	45	Monomorphic	SSRY202	191	55	
SSRY151	182	55	X	SSRY203	246	55	X
SSRY152	233	45	X	SSRY204	182	55	X
SSRY205	201	55	X	SSRY212	238	55	
SSRY206	219	55		SSRY213	199	55	
SSRY207	199	55		SSRY214	234	55	
SSRY208	198	55		SSRY215	204	55	X
SSRY209	195	55		SSRY216	210	55	
SSRY210	219	55	Monomorphic	SSRY217	181	55	X
SSRY211	202	55	Monomorphic	SSRY218	203	55	X

At present 130 polymorphic SSRs have been obtained for the parents. After isolating the DNA in 282 individual offsprings, the polymorphic SSRs will be evaluated.

Achievements:

- A high percentage (>60%) polymorphism was found between parents Ecu-72 and M Col-2246.
- Segregation data from the SSR and greenhouse evaluation of the 282 F1 individuals will allow the construction of a linkage map for whitefly resistance.

Activity 6.3. Evaluation of cassava germplasm for resistance to mites.

Specific Objectives:

1) *To screen different cassava genotypes for their reaction to mites.*
2) *To identify phytophagous mite species collected in different countries.*

Rationale: Mites are a universal pest of cassava, causing serious yield losses in the Americas and Africa. Of the 40 species reported feeding on cassava, the most frequent are *Mononychellus tanajoa* (syn = *M. progresivus*), *M. caribbeanae, Tetranychus cinnabarinus* and *T. urticae* (also reported as *T. bimaculatus* and *T. telarius*). Cassava is the main host for the *Mononychellus* species while the *Tetranychus* species have a wide host range. Other mite species (e.g. *Oligonychus peruvianus, O. biharensis, Eutetranychus banksi or M. mcgregori* are not economically important, feeding on cassava only sporadically. Cassava green mite (***CGM***), *M. tanajoa*, the most important species, is reported causing crop losses in the Americas and Africa, especially in seasonally dry regions of the lowland tropics. Native to the Neotropics, *M. tanajoa* was originally reported from NE Brazil in 1938 and first appeared in Africa (Uganda) in 1971. Molecular studies on *M. tanajoa* populations indicate that the origin of the population introduced into Africa was probably Northern South America. It is therefore proposed that the identification of host plant resistance in Cassava germplasm would serve cassava producers in Africa as well as the Americas (especially in NE Brazil where *M. tanajoa* are high and causing yield reductions.

CGM populations feed preferentially on the undersides of young emerging leaves, which develop a mottled whitish-to-yellow appearance; and they may become deformed or reduced in size. The CGM is a serious problem only in dry regions, where heavy infestations cause defoliation beginning at the top of the plant, often killing apical buds and shoots. Regrowth may occur; but if the rains are scarce, this new flush of leaves will also be attacked.

Research into the control of *M. tanajoa* has had two main thrusts, host plant resistance and biological control (See PEI Annual Reports). The continued use of pesticides is not an economical option for low-income farmers; moreover, their use is not recommended because of their adverse effect on natural enemies, which play an important role on CGM IPM.

Substantial efforts have been made at CIAT (and also IITA), and national research programs to identify cassava cultivars with resistance to CGM. Of the approximately 5000 landrace cultivars in the CIAT germplasm bank evaluated for CGM resistance, only about 6% (300 cultivars have been identified as having low to moderate levels of resistance (Table 6.4).

Field evaluations of cassava germplasm for resistance to CGM has traditionally been done on the Colombian North Coast (Caribbean Region), where, because of prolonged seasonally dry periods, CGM populations are high enough to provide adequate selection pressure. In recent years, due to the political insecurity in that region, field evaluations have been suspended. Meanwhile, high whitefly populations at CIAT, Palmira, have rendered this site inadequate for CGM field evaluations.

The continued field evaluation of selected germplasm until 1999 has now identified 389 genotypes as promising for resistance to CGM (Table 6.5). These materials have consistently been recorded with a damage evaluation of 3 or less, on a 1.0 to 5.0 damage scale at CIAT. In total, nearly 600 genotypes have received a 3.0 or below damage rating at CIAT, but many of these show a higher (3.5) rating under the more severe selection pressure of the Atlantic Coast (Pivijay). In this group of 600, 72 genotypes, that have consistently received a 3.0 or lower damage rating at both CIAT and Pivijay, were selected for further evaluation, including resistance mechanism studies.

Antixenosis (preference vs. non-preference) for *M. tanajoa* oviposition were carried out on several resistant cultivars. The susceptible check was CMC-40 (MCOL 1468). The experimental arena used was a petri dish with a layer of moisturized cotton place on the bottom. In a non-choice test, *M. tanajoa* mite females were allowed to oviposit on 72 selected cassava cultivars. One female was placed on a 3 cm^2 leaf disc and oviposition was recorded daily. There were 30 repetitions of each cultivar and oviposition was recorded for 3 days.

Table 6.4. Cassava cultivars field evaluated at CIAT for resistance to the green mite, *Mononychellus tanajoa.*

Year	No. Genotypes Evaluated*
1977	2261
1978	2298
1995	4664
1996	1400

* Some cultivars evaluated several times.

Table 6.5. Cassava cultivars from the CIAT germplasm Bank selected as promising for resistance to the green mite, *Mononychellus tanajoa* at Pivijay, Magdalena.

Year	No. Genotypes Evaluated
1993	575
1994	406
1995	546
1996	808
1997	387
1998	474
1999	389

Results show high oviposition levels on CMC-40 (MCOL-1468), the susceptible check; an average of 5.6 eggs per day. The results of the selected genotypes are varied. Several of them had oviposition levels similar or higher than CMC, while others showed much lower oviposition (Table 6.6). Thirty of the cultivars averaged 5.0 or more eggs oviposited per female, 11 of these were above 6.0 eggs/female. These results indicate that these cultivars are as susceptible to *M. tanajoa* oviposition as the susceptible check, CMC-40. However 29 cultivars has less than 4.0 eggs per female oviposited, and 15 of these less than 3.0 eggs per female. In addition, 2 cultivars MPeru 335 and CM 8424-5, showed less than 2.0.

Several cultivars showing low levels of oviposition are hybrids that resulted from two crosses; the MECU-72 x MBRA-12 cross produced the CG489 progeny and the CM8424 hybrids are the result of a CG489-4 x CG 489-34 cross. In other words all of these hybrids are progeny that originated from the MECU 72 x MBRA 12 cross. MECU 72 was originally selected for resistance to whiteflies (*A. socialis*) and is actually our most whitefly resistant variety. MBRA-12 was originally selected as "field resistant" to both mites and whiteflies. MECU-72, as in previous trials, has very low mite oviposition (2.1 eggs per female) and CG489-31, CG489-4 and CG489-34 had 2.1, 2.4 and 2.7 eggs oviposited per female, respectively.

CM 8424-5, CM 8424-25, CM 8424-10, CM 8424-33 and CM 8424-6 had 1.9, 2.2, 2.5 and 2.6 eggs/female oviposited respectively. These results indicate a strong ovipositional antixenosis associated with MECU-72. Several additional cultivars also had low oviposition numbers (Table 6.6). These include MPER-335, MPER-273, MPER-320, MPER-5648, MPER-608; MPER-335 has also been selected for whitefly resistance and adds interest to the phenomenon that high, levels of arthropod resistance exists in Peruvian and Ecuadorian germplasm.

Reduced oviposition on the cultivars MECU-72 and its progeny from the MBRA-12 cross is supported by similar data from experiments with these same cultivars and whitefly oviposition. *A. socialis* oviposition was reduced by 56% on MECU-72 and by nearly 80% on CG489-34 and CG489-31 when compared to susceptible cultivars. These results reinforce 1999 observations and indicate that there may be two or three mechanisms for resistance to mites (and whiteflies) involved in selected cassava clones.

As figures 6.6 and 6.7 show, there is a reduction larger than 50% in mite oviposition on the clone MECU-72, when compared to CMC-40, the susceptible check. In MECU-72, daily oviposition did not get higher than 3 eggs, while on CMC-40 it seldom fell below 3 and reached peaks of 7 eggs per day (Figure 6.7).

Table 6.6. Average daily oviposition of *Mononychellus tanajoa* on cassava cultivars selected as promising for resistance to CGM.

Cultivar	Eggs/day	Maximum damage rating		Cultivar	Eggs/day	Maximum damage rating	
		CIAT	Pivijai			CIAT	Pivijai
Above 6.0 eggs/day							
MCol 1439	7.7	3.0 (2)	3.0 (2)	CG 959-1	6.2	3.5 (1)	3.0 (1)
Mper 324	6.9	3.0 (3)	3.0 (2)	MVen 216	6.2	3.0 (1)	3.0 (3)
MPer 415	6.6	2.0 (3)	3.0 (3)	MBra 292	6.1	3.0 (2)	3.0 (2)
MBra 420	6.5	4.0 (1)		MPer 221	6.1	3.0 (3)	3.0 (3)
MCol 2179	6.4	3.0 (3)	3.0 (5)	MBra 276	6.0	3.0 (1)	2.5 (4)
MBra 124	6.4	3.5 (1)	1.0 (1)				
5.0 to 5.9 eggs/day							
SG 106-59	5.9	3.5 (2)	4.0 (4)	CM 8424-24	5.2	*2.5 (1)	
MBra 69	5.9	3.0 (2)	2.0 (4)	MCol 2025	5.2	3.5 (2)	3.0 (3)
MCol 1254	5.8	3.0 (3)	3.0 (4)	MPer 372	5.2	3.0 (3)	
MMex 71	5.8	3.0 (3)	3.5 (4)	MEcu 10	5.2	3.5 (3)	4.5 (5)
CM 5286-3	5.8	3.5 (3)	3.0 (4)	MGua 7	5.1	3.0 (3)	3.5 (3)
MVen 321	5.6	5.0 (1)	3.5 (1)	CM 849-1	5.1	3.5 (1)	3.0 (3)
MBra 878	5.5	3.5 (1)	3.0 (2)	MVen 276	5.1	3.0 (3)	3.0 (6)
MBra 826	5.4	3.0 (1)	3.0 (2)	MUSA 8	5.0	3.5 (1)	3.0 (3)
MCol 2550	5.4	3.0 (2)	2.0 (2)	MMex 17	5.0	3.0 (3)	3.0 (6)
MCR 32	5.3	3.5 (1)	2.0 (1)	***Mcol 1468***	***5.6***	***4.5 (1)***	***4.5 (7)***
4.0 to 4.9 eggs/day							
CM 4574-7	4.9	3.0 (3)	3.0 (5)	MPer 317	4.5	3.0 (3)	3.5 (3)
MPan 51	4.8	3.5 (1)	3.5 (3)	MPer 178	4.5	3.0 (1)	4.0 (2)
MPan 70	4.7	3.5 (2)	3.5 (4)	MBra 64	4.4	2.5 (3)	3.0 (5)
MPan 38	4.7	3.0 (2)	3.0 (1)	MBra 191	4.4	3.0 (3)	3.0 (6)
MPer 315	4.7	3.0 (2)	3.0 (3)	MPer 465	4.3	3.0 (3)	4.0 (7)
MBra 165	4.7	3.5 (1)	3.0 (3)	MBra 89	4.1	3.0 (3)	3.5 (4)
MVen 174	4.6	3.0 (1)	3.0 (6)	MVen 117-B	4.1	3.5 (2)	3.0 (1)
MPer 368	4.6	3.0 (2)	3.0 (4)	MVen 210	4.1	3.5 (2)	3.0 (2)
3.0 to 3.9 eggs/day							
MCR 52	3.9	3.0 (3)	2.0 (2)	CG 1141-1	3.7	3.0 (3)	3.0(7)
CG 402-11	3.9	3.5 (1)	2.0 (2)	MBra 12	3.4	3.5 (2)	4.0(5)
CG 489-23	3.8	3.0 (3)	3.5 (5)	MPer 273	3.1	3.0 (3)	3.0(1)
CM 8424-9	3.7	*2.5(1)		CG502-1	3.0	3.0(2)	2.5(5)
2.0 to 2.9 eggs/day							
MEcu 72	2,8	2.5(3)	3.0(7)	MPer 273	2.5	3.0 (3)	3.0 (1)
CG 489-34	2.7	3.5 (3)	3.5(6)	CM 8424-10	2.4	*2.0 (1)	
MPer 320	2.7	3.0 (1)		CG 489-4	2.4	3.5 (2)	3.5 (4)
MPer 564	2.7	2.5 (3)	3.0 (5)	MPer 608	2.3	3.0 (3)	2.0 (3)
CM 8424-6	2.6	*2.5 (1)		CM 8424-25	2.2	*3.0 (1)	
CM 8424-33	2.5	*2.0 (1)		CG 489-31	2.1	2.5 (3)	2.5 (6)
1.0 to 1.9 eggs/day							
CM 8424-5	1.9	*3.0 (1)		MPer 335	1.5	2.5 (3)	3.5 (3)

* Evaluations only in screenhouse conditions. ( ) = number of years evaluated.

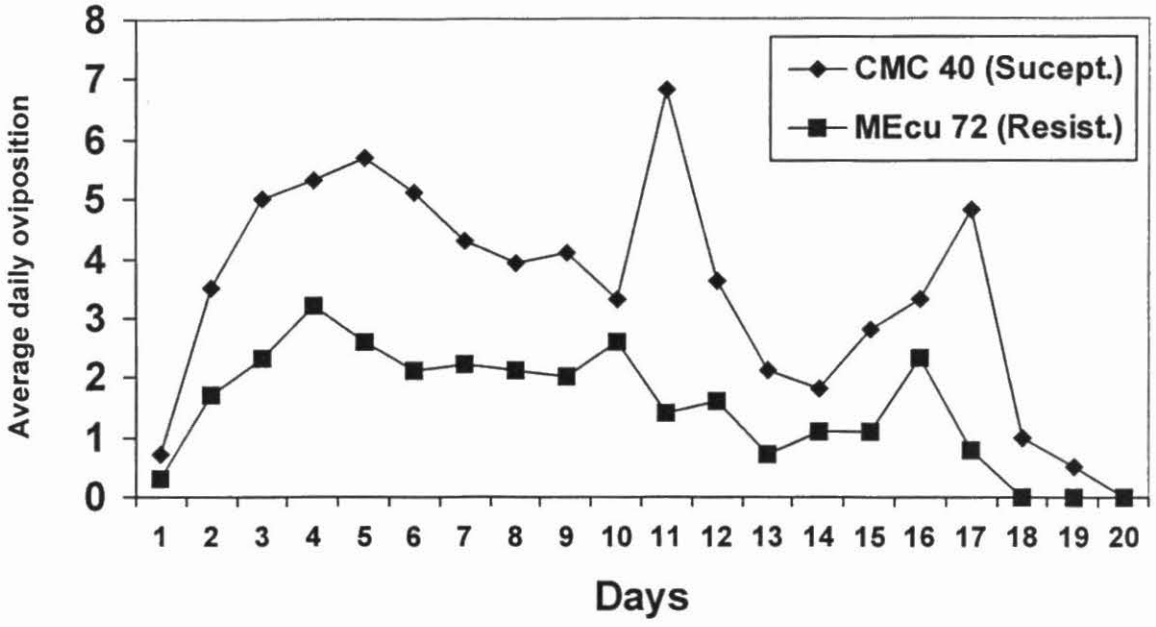


Figure 6.6. Average total oviposition of female *Mononychellus tanajoa* mites feeding on two cassava varieties, MECU-72 (Resistant) and CMC-40 (Susceptible).

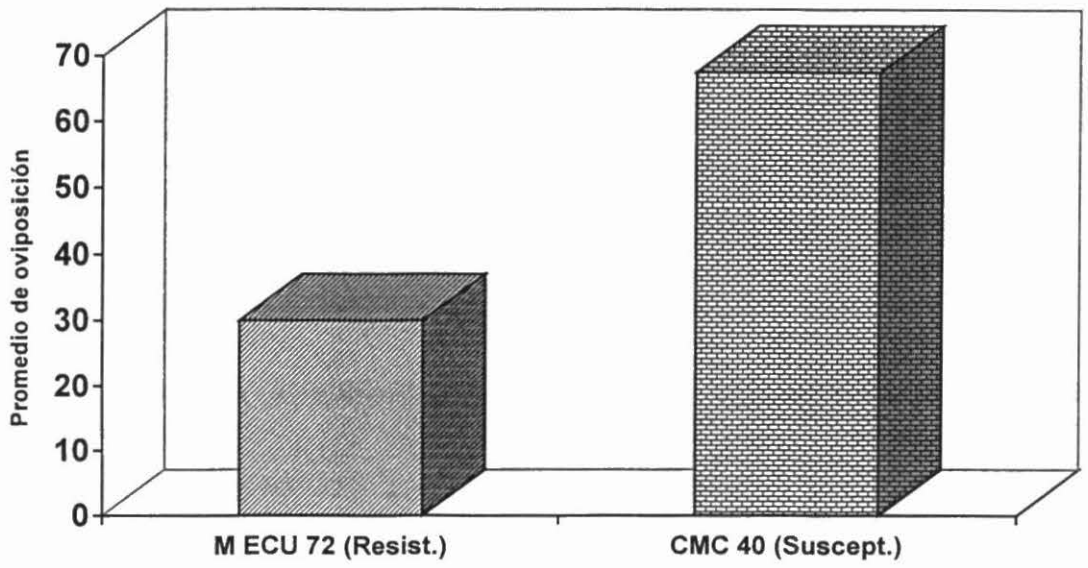


Figure 7. Average daily oviposition of female *Mononychellus tanajoa* mites feeding on two cassava varieties MECU-72 (Resistant) and CMC-40 (susceptible).

Phytophagous Mite Identification

The CIAT Acarology Laboratory maintains a collection of phytophagous mites collected mostly from cassava, but also includes other crops. The collection contains more than 20,000 specimens, mounted on slides and collected from numerous countries in the Americas (North, South & Central America and the Caribbean) Africa and Asia.

During the past few years, while involved in explorations for whiteflies and their natural enemies, phytophagous mites have also been collected and added to the CIAT collection. Specimens were collected from several crops but only those found on cassava are reported in Table 6.7. Thirty-four records were added to the collection, 12 were from cassava and 24 from other crops. Collections were made from Colombia, Ecuador, Haiti and Venezuela. It is interesting to note that the species *M. caribbeanae*, found primarily on the Colombian Atlantic coast in very dry areas, is quite wide-spread and also collected from Ecuador, Venezuela and Haiti. Collection in Venezuela (Register No. 2525, Table 6.7) revealed that 66% of the population was *M. caribbeanae* and 34% *M. tanajoa*.

Table 6.7. Phytophagous mite species collected from cassava in Colombia, Ecuador, Haiti, and Venezuela during 1999-2000.

Record #	Country	Department	Site	Host	Species
2495	Colombia	Valle	CIAT- Palmira	M. tristis	Oligonychus sp
2502	Ecuador	Manabí	Olina, Jipijapa	Cassava	*Allonychus*
2504	Ecuador	Guayas	Km.17, Boliche	Cassava	*M. Caribbeanae*
2505	Ecuador	Guayas	Km.18, Sta. Elena	Cassava	*M. Caribbeanae*
2506	Ecuador	Imbabura	Chota	Cassava	*Eotetranychus lewisi*
2510	Haití	Le Matinere	Les Caves	Cassava	*M. caribbeanae*
2512	Ecuador	Manabí	San Clemente, Rocafuerte	Cassava	*M. caribbeanae*
2513	Colombia	Valle	CIAT, Palmira	Cassava	*M. mcgregori*
2517	Colombia	Valle	CIAT, Palmira	Cassava	*Oligonychus sp*
2518	Colombia	Valle	CIAT, Palmira	Cassava	*Oligonychus gossypii*
2519	Colombia	Valle	CIAT, Palmira	Cassava	*Tetranychus pos. desertorum*
2525	Venezuela	Anzoategui	Agroselect, Cantaura	Cassava	*M. Caribbeanae, M. tanajoa*

OUTPUT 7: Disease resistance in cassava.

An important feature of project IP3 relates to the integration of breeding, entomology plant pathology and the development and use of biotechnolgy tools. The four areas of science have maintained as much a close relationship as possible. Output 7 summarizes the progress related to disease resistance in cassava.

Activity 7.1. Fifty genotypes characterized for their reaction to CBB under greenhouse conditions, using 12 isolates.

Specific Objectives:

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

Twenty-five genotypes were characterized under greenhouse conditions for their reaction to 12 isolates of *Xanthomonas axonopodis* pv. *manihotis*, causal agent of cassava bacterial blight (CBB). The isolates were selected from a group of 30 that were collected from different genotypes and edaphoclimatic zones in Colombia. The zones were Santander de Quilichao (Cauca Department); Jamundí (Valle del Cauca Department); La Tebaida and Montenegro (Quindío Department); and Villavicencio and Puerto López (Meta Department) (Table 7.1). Under greenhouse conditions, 30-day-old cassava plants of each genotype were inoculated with 12 isolates, using stem injection and a bacterial suspension of 1×10^6 cfu/mL. Disease severity was recorded 10, 17, and 24 days after inoculation.

Table 7.1. Origin, source, and pathogenicity of 42 isolates of *Xanthomonas axonopodis* pv. manihotis (causal agent of cassava bacterial blight) obtained from cassava.

Isolate	Origin (Department)	Source	Pathogenicity[a]
SQ 1	Santander de Quilichao (Cauca)	K 7	5.0
SQ 2	Santander de Quilichao	ICA Catumare	4.0
SQ 3	Santander de Quilichao	M Col 1505	4.5
SQ 4	Santander de Quilichao	ICA Catumare	3.5
SQ 1A	Santander de Quilichao	K 15	3.0
SQ 1B	Santander de Quilichao	M Col 1468	2.0
SQ 1C	Santander de Quilichao	K 16	2.0
SQ 1D	Santander de Quilichao (Cauca)	ICA Catumare	1.5
SQ 1E	Santander de Quilichao	M Col 1505	3.0
M1	Montenegro (Quindío)	Manzana	2.5
M2	Montenegro	HMC-1	2.0
M 1F	Montenegro	Manzana	2.0

Table 7.1 (cont.)

Isolate	Origin (Department)	Source	Pathogenicity[a]
M 1G	Montenegro	HMC-1	2.5
M 1H	Montenegro	HMC-1	2.5
M 1I	Montenegro	Manzana	5.0
V 1J	Villavicencio (Meta)	ICA Catumare	1.5
V 1K	Villavicencio	ICA Catumare	1.5
V 1L	Villavicencio	CM 6787-9	2.0
V 1	Villavicencio	CM 2177-2	4.0
V 1N	Villavicencio	SM 1545-19	1.5
V 4	Villavicencio	SM 1855-9	4.0
V 1P	Villavicencio	SM 1642-13	1.5
V 1Q	Villavicencio	SM 1788-16	1.5
V 1R	Villavicencio	SM 2061-1	2.0
V 1S	Villavicencio	SM 1468-9	1.5
V 1T	Villavicencio	SM 1812-72	2.0
V 3	Villavicencio	M Cub 74	5.0
V 1V	Villavicencio	M Col 2409	1.5
V 1W	Villavicencio	SM 1665-5	1.5
V 1X	Villavicencio	SM 1583-8	2.0
V 2	Villavicencio	SM 1642-13	4.5
TQ 3	La Tebaida (Quindío)	HMC-1	4.0
TQ 4	La Tebaida	M Per 183	4.0
TQ 5	La Tebaida	M Col 2066	5.0
JV 6	Jamundí (Valle)	CM 849-1	2.0
JV 7	Jamundí	CM 6740-7	5.0
JV 8	Jamundí	M Bra 383	5.0
CIO 616 (Control)	Puerto López (Meta)	CM 2177-2	4.0
CIO 277 (Control)	Monagas (Venezuela)	Tres Brincos	4.5
CIO 285 (Control)	Monagas	Paiguana Negra	4.5
CIO 421 (Control)	Mondomo (Cauca)	Algodona	3.5
CIO 763 (Control)	Mitú (Vaupés)	Amarilla	5.0

a. Score for damage, rated on a scale where 1-2 = resistant; 2.5-3.5 = intermediate; 4-5 = susceptible, when inoculated on cassava genotype M Col 1505.

The most aggressive isolates were SQ1, M1, and V2, with a virulence percentage of more than 60%. Ten varieties, equivalent to 40%, were either intermediately resistant or resistant to 66.7%–83.3% of the isolates. Genotypes M Esc Fla 021 and MCOL-2066 ('Chiroza', local variety from Quindío) were resistant to 66.7% of the isolates, while M Esc Fla 039 was either intermediately resistant or resistant to 83.3% of the isolates (Table 7.2).

Table 7.2. Disease reaction[a] to 12 *Xanthomonas axonopodis* pv. *manihotis* isolates, causal agent of cassava bacterial blight of cassava genotypes.

	Isolate[b]												Total			R + I
Genotype	SQ 1	SQ 3	J 7	J 8	M 1	TQ 3	TQ 4	TQ 5	V1	V2	V3	V4	R	I	S	(%)[d]
M Esc Fla 039	I	I	R	I	I	S	R	R	S	I	I	R	4	6	2	83.3
M Esc Fla 021	I	R	R	R	S	R	R	R	R	S	R	S	8	1	3	75.0
M Col 2066	R	R	S	R	S	R	R	R	I	S	R	R	8	1	3	75.0
SM 1225-12	S	R	I	R	S	R	R	R	R	S	R	I	7	2	3	75.0
MCOL-2737	I	I	S	R	I	R	I	R	S	S	R	R	5	4	3	75.0
SM 1828-11	I	R	I	S	S	R	R	I	R	I	R	S	5	4	3	75.0
CM 523-7	S	S	S	R	R	R	I	R	R	S	R	R	7	1	4	66.7
SM 1812-72	I	R	I	S	S	R	R	R	R	S	R	S	6	2	4	66.7
CM 6055-3	I	I	I	S	S	R	R	R	R	S	R	S	5	3	4	66.7
SM 2061-1	S	R	I	I	S	R	R	I	R	S	R	S	5	3	4	66.7
CM 8370-14	S	S	R	R	S	I	R	R	S	S	R	R	6	1	5	58.3
HMC-1	S	R	S	R	S	R	I	R	S	S	R	R	6	1	5	58.3
MCUB- 74	S	S	S	R	I	R	R	R	S	S	R	R	6	1	5	58.3
Manzana[e]	S	I	S	R	S	R	I	R	S	S	R	R	5	2	5	58.3
SM 1555-17	I	I	I	S	S	R	R	S	R	S	R	S	4	3	5	58.3
SM 1673-11	S	R	R	S	S	I	R	R	R	S	S	S	5	1	6	50.0
SM 1868-34	S	I	I	I	S	S	R	I	S	S	R	S	2	4	6	50.0
SM 2219-9	I	S	S	I	S	S	I	I	S	I	S	I	0	6	6	50.0
SM 1665-5	S	S	S	S	S	S	R	R	I	S	I	R	3	2	7	41.7
SM 2069-2	I	S	S	S	S	S	R	R	S	S	I	R	3	2	7	41.7
MBRA-489	S	S	S	S	S	S	R	R	I	S	S	R	3	1	8	33.3
MCOL- 2329	S	S	S	S	S	S	I	R	R	S	S	R	3	1	8	33.3
SM 2069-57	S	S	S	S	S	S	R	R	S	S	I	R	3	1	8	33.3
MCOl-2387	S	S	S	S	S	S	I	I	S	S	S	R	1	2	9	25.0
MCOL-1505 (control)	S	S	S	S	S	S	S	S	S	S	S	S	0	0	12	0.0
Resistant	1	8	4	9	1	13	17	18	10	0	15	14				
Intermediate	9	6	7	4	3	2	7	5	3	3	4	2				
Susceptible	15	11	14	12	21	10	1	2	12	22	6	9				
Virulence (%)[f]	60	44	56	48	84	40	4	8	48	88	24	36				
Correlation[g]									0.3	0.3	-0.5	-0.3				

a. Disease reaction: R = resistant; I = intermediate; S = susceptible. **b.** Isolates SQ1 and SQ3 = S. de Quilichao (Cauca); J7 and J8 = Jamundí (Valle); M1 = Montenegro; TQ3, TQ4, and TQ5 = La Tebaida (Quindío); V1, V2, V3, and V4 = Villavicencio (Meta). **c.** Total of isolates to which each genotype shows either resistance (R), intermediate resistance (I), or susceptibility (S). **d.** % of isolates to which each genotype shows either resistance or intermediate resistance. **e.** Local variety from Montenegro (Quindío). **f.** % of genotypes susceptible to each isolate. **g.** Correlation between field and greenhouse disease reaction, according to the origin of the isolates. Correlation was done with the genotypes evaluated in the field in each zone.

Activity 7.2. One hundred cassava genotypes evaluated for resistance to Cassava Bacterial Blight (CBB) and Superelongation Disease (SED) at two locations in the eastern savannas of Colombia.

Specific Objectives:

1) *To evaluate reaction of 100 genotypes to two different diseases and measure their root yield potential.*
2) *To obtain the correlation between field and greenhouse disease reactions.*

At Villavicencio and Matazul (Meta), cassava genotypes were characterized for their reactions to CBB (88 genotypes) and SED (80) under natural disease pressure from several pathotypes of each causal agent.

In Villavicencio, 55 genotypes were intermediately resistant to CBB and resistant to SED, 7 varieties were intermediately resistant to both diseases, and 80 genotypes were resistant to SED. In Matazul, 51 genotypes were resistant to CBB and SED, indicating that the disease pressure was less in this location. That is, across the two sites, 31 genotypes were intermediately resistant to CBB and resistant to SED in Villavicencio and resistant to both diseases in Matazul. Table 7.3 shows the results from 73 genotypes evaluated in both localities. The elite clone CM 6740-7 (not shown in table) was intermediately resistant to CBB and SED in Villavicencio, but was not evaluated in Matazul.

The genotype MBRA-489 had the highest yield at Villavicencio (35.6 t/ha) and was intermediately resistant and resistant to CBB and SED, respectively. *'Chiroza Morada'* was tolerant to diseases, producing 35 t/ha, despite presenting strong disease symptoms. Other genotypes with high yield were SM 1871-39, MBRA-466, and MCOL-2329.

Correlation between field and greenhouse disease reaction, according to isolate's origin, is shown in Table 7.2 (last row). Genotypes evaluated at Villavicencio had a correlation of 0.3 with those inoculated in greenhouse with isolates V1 and V2.

Table 7. 3. Disease reaction[a] to CBB and SED and yield performance of cassava genotypes at Matazul and Villavicencio (Meta Department).

Genotype	Villavicencio			Matazul		Genotype	Villavicencio			Matazul	
	CBB	SED	T/ha	CBB	SED		CBB	SED	T/ha	CBB	SED
Chiroza Morada	S	I	35.0	I	R	SM 1363- 3	S	R	10.0	R	R
CM 523- 7	S	R	5.0	I	R	SM 1411- 5	S	R	13.8	R	R
CM 2177- 2	I	R	12.0	R	R	SM 1460- 1	I	R	20.8	S	R
CM 6055- 3	I	R	3.8	I	R	SM 1468- 9	I	R	17.1	R	R
CM 6697- 2	I	R	14.6	R	R	SM 1545-19	I	R	8.8	R	R
CM 6787- 9	S	R	10.0	I	R	SM 1545-22	I	R	6.3	R	R
CM 6975-14	I	R	8.3	R	R	SM 1545-25	S	R	7.5	S	R
CM 7086-17	I	R	11.3	R	R	SM 1553-23	I	R	6.3	R	R
CM 7389- 9	S	R	1.9	R	R	SM 1555-17	I	R	22.1	I	R
CM 7747- 7	S	I	21.3	R	R	SM 1583- 8	I	R	15.4	R	R
MBRA-461	I	R	10.8	S	R	SM 1588- 1	S	R	15.0	R	R
MBRA-466	I	I	27.9	S	R	SM 1642-13	S	R	0.0	R	R
MBRA-489	I	R	35.6	R	R	SM 1673-11	I	R	12.9	R	R
MCOL-707	I	R	15.0	R	R	SM 1682- 2	I	R	14.4	R	R
MCOL-2307	I	R	21.7	S	R	SM 1788-16	S	R	7.5	R	R
MCOL-2329	I	R	27.5	R	R	SM 1811-36	I	I	7.5	I	R
M Col 2737	S	R	16.4	I	R	SM 1812-56	S	R	5.4	I	R
MCR-32	I	R	12.9	R	R	SM 1812-72	I	R	10.0	S	R
MECU-72	S	R	3.8	R	R	SM 1820- 8	I	R	11.7	I	R
M Esc Fla 007	I	R	8.8	R	R	SM 1821- 7	S	R	12.2	I	R
M Esc Fla 039	I	R	15.0	R	R	SM 1822-12	I	R	7.9	I	R
M Esc Fla 075	I	I	9.4	R	R	SM 1828-11	I	R	15.0	R	R
MVEN-77	I	R	17.1	R	R	SM 1855- 9	I	R	18.3	R	R
SB 0240- 8	S	-	-	I	R	SM 1855-21	I	R	12.1	R	R
SG 104- 74	I	R	21.3	I	I	SM 1859-26	I	R	12.9	R	R
SM 1080- 1	I	R	2.5	R	R	SM 1860-19	I	R	10.0	I	R
SM 1143-22	I	R	16.3	R	R	SM 1862-25	S	I	3.3	I	R
SM 1144- 4	I	R	3.8	I	R	SM 1871-29	I	R	16.3	I	R
SM 1152-16	I	I	11.7	S	R	SM 1871-32	I	R	12.9	R	R
SM 1152-19	I	I	21.9	I	R	SM 1871-38	S	R	-	R	R
SM 1215- 1	I	R	3.3	R	R	SM 1871-39	I	I	33.3	R	R
SM 1223-20	I	R	2.5	R	R	SM 1912- 4	S	R	7.1	I	R
SM 1225-11	I	R	4.4	R	R	SM 2061- 1	S	I	3.8	R	R
SM 1225-12	I	R	19.6	I	R	SM 2062- 2	S	R	2.5	R	R
SM 1225-13	I	R	6.3	I	R	SM 2069- 4	I	R	5.4	R	R
SM 1345-10	I	R	20.0	I	I	SM 2219- 9	I	R	24.6	R	R
SM 1361- 8	I	R	10.6	R	R						

[a] R= resistant; I= intermediate; S= susceptible; - = not determined.

Activity 7.3. Fifteen cassava genotypes evaluated in the greenhouse for resistance to 19 isolates of Sphaceloma manihoticola, causal agent of superelongation disease.

Specific Objectives:

1) To identify and determine the virulence phenotypes of 6 isolates from different regions in Brazil.

Virulence variation was determined by inoculating through wounding sprouts of 15 cassava variety differentials. The inocula were six selected isolates from different regions of Brazil. The inoculated sprouts were incubated for 5 days at 95% relative humidity and 27°C, then transferred to the greenhouse and observed for symptom development for 7, 14, 21, and 28 days after inoculation.

Table 7.4. Identification and virulence phenotypes of 6 isolates, of *Sphaceloma manihoticola* inoculated on 15 cassava genotypes in the greenhouse.

	Genotypes[a]															
Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Severity Group[b]
Bh	MR[d]	MR	MR	MR	MR	MR	HS	HS	HS	MR	HS	MR	MR	I	R	3
1	MR	MR	R	S	I	MR	I	S	HS	MR	HS	MR	R	MR	R	2
4p	HS	HS	HS	HS	HS	MR	HS	HS	HS	S	HS	MR	I	I	R	4
5	MR	MR	R	MR	MR	S	S	HS	HS	I	S	HS	S	HS	S	1
7h	MR	MR	R	MR	MR	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	4
25h	MR	S	HS	HS	S	I	HS	S	S	MR	HS	HS	HS	HS	MR	4
AUDPC[c]	42.6	41.5	38.8	49.1	43.3	44.2	50.9	54.6	56.2	43.9	54.9	45.8	48.2	51.9	38.8	
Resistance Group[d]	MR	MR	R	I	MR	MR	S	HS	HS	MR	HS	I	I	S	R	

[a] Cassava genotypes inoculated: 1= MBRA-703, 2= MBRA-97, 3= MBRA-12, 4= MBRA-917, 5= MBRA-886, 6= MCOL-2737, 7= MNGA-2, 8= MBRA-1044, 9= MBRA-237, 10= MBRA-1045, 11= MCOL-2215, 12= CG 6915-1, 13= CM 3306-9, 14= CM 3306-4, 15= CM 2177-2.

[b] Severity of the isolates by Ward's minimum variance analysis (R^2=99%): 1: low severity; 2: intermediate; 3: moderately severe; 4: severe

[c] AUDPC Area under disease progress curve

[d] Genotype resistance: R= resistant; MR= moderately resistant; I= Intermediate; S= susceptible; HS= highly susceptible.

Based on the 15 cassava variety differentials, 6 isolates were grouped into 5 pathotypes. Isolate **4p** was found to infect 12 of the 15 genotypes and was considered as the most virulent. Isolate **Bh** was the least virulent, infecting only five differentials. Of the cassava genotypes, MBRA-1044, MBRA-237, and MCOL-2215 are in highly susceptible groups and the most susceptible is MBRA-237. The most tolerant genotype was CM 2177-2. (Table 7.4). Some correlation between sample source, geographical origin, virulence variation, and DNA polymorphism was also observed.

Activity 7.4. Alternative methods for the control of Phytophthora *root rot in the field at four Colombian locations.*

Specific Objectives:

1) *To evaluate reaction of six different cassava genotypes to root rots.*
2) *To evaluate three alternative methodologies for root rot control.*

Three alternative methods of controlling *Phytophthora* root rots in cassava (with two treatments each) were evaluated at Caicedonia (Valle del Cauca). The methods were:

- Two potassium sources: KCl and KNO_3.
- Two thermal treatments of cuttings in water: at 49°C for 49 min (thermotherapy).
- Two biological root rot control with: *Trichoderma* spp. and Micobiol®.

Micobiol® is a mixture of *Trichoderma* spp., *Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisople, Paecilomyces lilacinus, Paecilomyces fumosoroseus, Nomureae rileyi, Entomophthora muscae, Hirsutella thompsonii*, and *Verticillium lecanii.* The cassava genotypes evaluated in this trial were ICA Catumare, MCOL-1505, and MPER-183.

Evaluations carried out in Valle del Cauca.

The variety MCOL-1505 did not show necrosis in stems, but plant establishment was only 65%. Plant height increased with the application of *Trichoderma* spp. and KNO_3. Applications of KCl increased plant drying, whereas *Trichoderma* spp. and KNO_3 decreased damage. Thermotherapy of cuttings, *Trichoderma*, KNO_3, and Micobiol® significantly reduced plant drying (Table 7.5). Local variety HMC-1 was more tolerant than the other genotypes evaluated.

Evaluations carried out in Quindío.

Different control practices were evaluated for their effect on crop yield, bacterial blight, and root rot at Montenegro (Quindío). Main results are presented at Table 7.6. Yields were higher for the practices proposed by CIAT, like immersion of stakes in a suspension of the biocontrol agent *Trichoderma* spp. (isolate 14PDA-4) and its frequent application to the plants. Applications of potassium sulfate and chloride were also beneficial to crop yield, compared with local practices. No significant differences were observed between the integrated pest management (IPM) package proposed by CIAT and farmers' crop management.

Table 7.5. Effect of different treatments on cassava performance and stem rot disease in a field trial established in the Valle del Cauca Department.

Treatment[a]	Performance (%)	Height (m)[b]	Severity of stem rot	Incidence of stem rot (% plants)[c]		
				1	2	3
Treatment for cuttings						
Water bath (49°C for 49 min)	94	1.23	1.5	55	36	9
Water heated on firewood (49°C for 49 min)	92	1.28	1.8	42	39	19
Traditional management +						
Micobiol®[d]	96	1.24	1.8	37	45	18
Trichoderma spp. (1×10^4 conidia/mL)	96	1.39	1.5	58	31	11
Traditional management +						
KCl (20 g/plant)	96	1.16	2.0	31	35	34
KNO_3 (20 g/plant)	88	1.51	1.4	73	17	9
Traditional management +						
ICA Catumare	98	1.28	1.8	52	21	27
MCOL-1505	65	1.14	1.0	100	0	0
MPER-183	88	1.29	1.7	40	48	12
Traditional (farmer) management	100	1.28	1.7	46	35	19

a. Ten months after planting. **b.** Average plant height. **c.** Evaluation scale: 1 = plant with no sprout rot; 2 = plant with some sprout rot; 3 = plant with 100% sprout rot. **d.** Biological product based on entomopathogenic fungi for disease and pest control (immersion for 30 min in 6 kg/L suspension and application at 50 mL per plant).

Table 7.6. Effect of different practices on cassava performance, and root rot (RR) and cassava bacterial blight (CBB) control at Montenegro, Quindío Department [v].

Control practice	Plant height (m)	Root yield (t/ha)	Stakes per plant (#)	CBB		RR	
				Incidence [¶]	Severity (%)	Incidence [w]	Severity [§]
Hot water treatment[x]	1.73	62 a	36 a	21 a	89	2.0 a	3.7 a
Biocontrol agent *Trichoderma* spp.[y]	1.89	63 a	36 a	16 a	89	2.0 a	1.8 a
Micobiol®[z]	2.31	60 a	37 a	12 a	56	1.3 a	0.2 a
Ridomil (metalaxyl)	1.91	70 a	39 a	16 a	89	1.7 a	1.0 a
Potassium chloride	1.90	70 a	37 a	18 a	100	2.0 a	0.3 a
Potassium sulfate	1.90	80 a	38 a	24 a	100	2.0 a	1.1 a
Local commercial varieties							
Manzana	1.93	41 a	36 a	21 a	100	2.0 a	7.1 a
HMC-1	1.86	51 a	37 a	22 a	100	1.8 a	6.1 a

[¶] % of plants affected. [§] tons of affected roots/ha **v.** Duncan's multiple range test, alpha = 0.05. **w.** Values on a scale of 0 to 3, where 0 = no symptoms and 3 = highly infected. **x.** Oil drum and firewood, water temperature at 49°C for 49 min. **y.** Isolate 14PDA-4. **z.** *Trichoderma* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Bacillus thurigiensis*

Evaluations carried out in Cauca.

Two experiments were established at San Jerónimo and Mondomito (Santander de Quilichao, Cauca) to evaluate the control some crop management practices had over *Phytophthora* spp. The control methods evaluated were the fertilizer Agropremix® (nutrient content 20g/plant) potassium chloride (200 kg/ha K_2O), potassium sulfate (200 kg/ha K_2O), thermotherapy of cuttings in water heated on firewood, *Trichoderma* isolates 14 PDA-4 and 19 TSM-3A (1×10^4 conidia/mL), and cutting selection. The performance of the elite genotype CM 6740-7 was also evaluated.

Results of the different control methods evaluated were then compared with farmer management practices, which incorporated chicken manure (250 g/plant) into the soil. In San Jerónimo, farmers also incorporated 500 kg/ha of calcium and magnesium lime. Fertilizer treatments were applied 35 days after planting in San Jerónimo and at planting in Mondomito. The variety planted was Verdecita (MCOL-1505), whose cuttings were obtained from San Jerónimo, where the disease was present in a previous crop. Germination was not affected by thermotherapy (Table 7.7).

Table 7.7. Germination of cassava stakes after different treatments in two farms at Santander de Quilichao, Cauca Department.

Treatment	Stake germination (%)	
	San Jerónimo	Mondomito
Fertilizers		
Agropremix® (30 g/plant)	96.7	98.3
K_2SO_4 (36 g/plant)[b]	85.0	100.0
KCl (30 g/plant)[b]	80.0	98.3
Thermotherapy	92.5	98.3
Biological control		
Trichoderma 14 PDA-4	83.3	93.3
Trichoderma 19 TSM-3A	92.5	-[a]
Cutting selection	75.0	96.7
Genotype CM 6740-7	91.7	98.3
Farmer control (chicken manure)	85.0	98.3

a. Not evaluated. **b.** Equivalent to 18 g/plant of K_2O.

Table 7.8. Percentage of germination in plants established at three farms in the Quindío Department.

Treatment	Farm		
	El Jardín	Las Mercedes	Guayaquil
Fertilization			
KCl (30 g/plant)	88.3	66.6	96.7
K_2SO_4 (36 g/plant)	91.7	95.0	95.0
Farmer control[a]	91.7	85.0	88.3
Control: no fertilization	93.3	93.3	86.7
Treatment of cuttings			
Thermotherapy (49°C for 49 min)	40.0	26.7	68.3
Orthocide® (4 g/L) + Ridomil® (3 g/L)	100.0	96.7	73.3
Lonlife® 4% (ascorbic acid)	-	-	96.7
Biological control			
Trichoderma (19 TSM-3A)	88.3	98.3	95.0
Trichoderma (41 PDA-3A)	96.7	71.7	90.0
Varietal resistance			
HMC-1	100.0	100.0	91.7
ICA Catumare	88.3	86.7	89.8
M Per 183	93.3	95.0	-
Chiroza	93.3	83.3	70.0

a. Ammonium sulfate + borax (50:1.5) at 35 g/plant at "El Jardín" and "Las Mercedes"; N-P-K (10-30-10) at 50 g/plant at "Guayaquil".

Three experiments were conducted at the farms "El Jardín" (La Tebaida, Quindío), "Las Mercedes" and "Guayaquil" (Montenegro, Quindío) to evaluate the effect of some crop management practices on *Phytophthora* spp. control. Treatments evaluated were:

1. Fertilization with KCl (30 g/plant) and K_2SO_4 (36 g/plant) was compared with farmer fertilization: "El Jardín" and "Las Mercedes" used 35 g/plant of ammonium sulfate and a borax mixture at a 50:1.5 rate; "Guayaquil" used 50 g/plant of N-P-K (10-30-10). Fertilizer treatments were applied 45 days after planting.

2. Treatments for cuttings were thermotherapy (49°C for 49 min), chemical immersion for 5 min in Orthocide® (captan, 4 g/L of commercial product) and Ridomil® (metalaxyl, 3 g/L of commercial product), and immersion in Lonlife® (ascorbic acid) at 4%.

3. Biological control by immersion of stakes for 10 min in a *Trichoderma* suspension (1×10^4 conidia/mL), containing isolates 19 TSM-3A and 41 PDA-3A. The area close to stakes was watered with 100 mL/plant of fungal suspension.
4. Varietal resistance, using the genotypes HMC-1, ICA Catumare (CM 523-7), MPER-183, and the local variety Chiroza (MCOL-2066).

Plant germination was too low (Table 7.8) with thermotherapy (26.7%-68.3%), compared with the same treatment at Cauca (92.5 to 98.3%, Table 7.7). Possibly the temperature was inappropriate because of the recipient used, which was different to that used at Cauca experiments. At "Las Mercedes" and "El Jardín", where there was CBB incidence, some 35-day-old plants were observed as being affected by *Xanthomonas axonopodis* pv. *manihotis* in treatments with KCl, farmer control, *Trichoderma* spp., chemical treatment, and in the genotypes MPER-183 and HMC-1.

Activity 7.5. F_1 progeny and parents of K family (M Nga 2 × CM 2177-2) characterized for resistance to Phytophthora root rot.

Specific Objectives:

1) To evaluate individuals from family K for their reaction to root rot.
2) To determine disease reaction of key crosses to six Phytophthora *isolates.*
3) To study the genetic basis of inheritance to resistance to root rots.

The genotypes MBRA-1045, MCR-81, MCOL-22, CM 523-7, MNGA-2, and CM 2177-2, which are parents of three crosses, were inoculated with six isolates of *Phytophthora* spp. (P4, P12, 44, 69, MTR4, and MTR6), to search for contrasting disease reactions. Inoculations were performed on young stem fragments with an average length of 30 mm. Paper discs, carrying a fungal suspension, were placed on the fragments' edge.

Family K parents, CM 2177-2 and MNGA-2, were inoculated on root cylinders, 30 mm in diameter and 15 mm high, by placing discs carrying medium with a fungal growth. Evaluations were conducted 2, 4, and 6 days after inoculation.

Genotypes were grouped into five categories by Ward's minimum variance cluster analyses, with R^2 up to 90%: resistant (R), intermediately resistant (MR), intermediate (I), susceptible (S), and highly susceptible (HS). Segregation for disease resistance was observed in the progeny when inoculated with isolates 69, 44, and MTR6 (Figure 7.1).

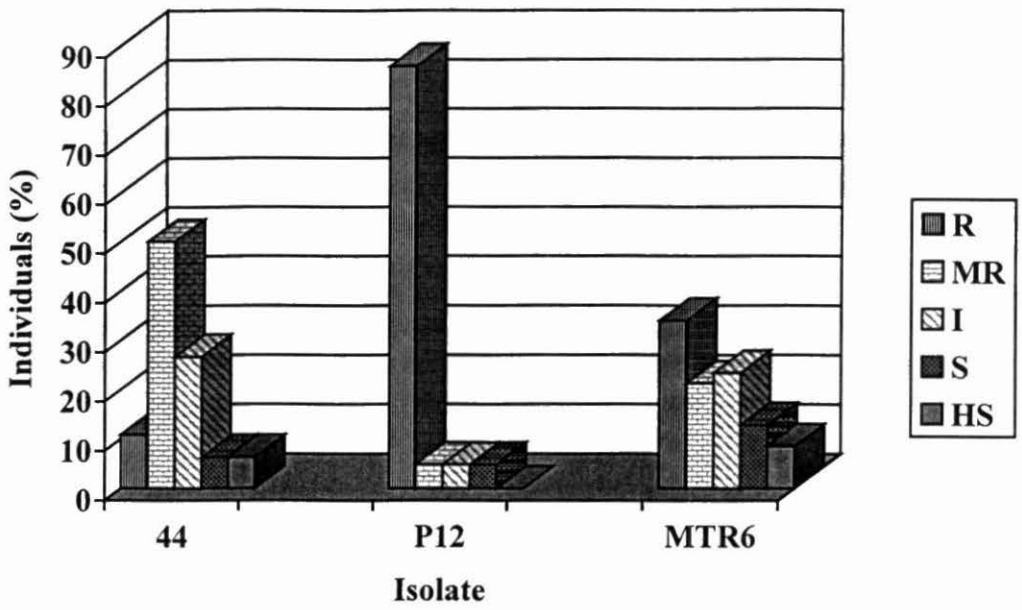


Figure 7.1. Distribution of individuals from family K according to their resistance to *Phytophthora* spp. isolates 44, P12, and MTR6, inoculated on root cylinders (R = resistant; MR = intermediately resistant; I = intermediate; S = susceptible; HS = highly susceptible).

Table 7.9 shows each parent's resistance reaction. Family K parents showed no contrasts, except for the isolate MTR6, to which CM 2177-2 was resistant and MNGA-2 was highly susceptible. Figure 7.1 shows the distribution of the progeny from family K, according to resistance cluster, for three of the isolates inoculated on roots. Although parents were intermediate or susceptible, progeny segregated in different resistance categories for each fungus isolate, because of cassava heterozygocity and pathogen diversity.

Host resistance is the most effective solution for root rot management. An F_1 population of 132 to 138 genotypes obtained by a cross between MNGA-2 and CM 2177-2 was used to analyze distribution of resistance to different fungal pathogens. Roots of each evaluated genotype were inoculated by perforation with fungal discs of *Phytophthora capsici* (isolate 44) *Pythium* spp. (isolate 69), and *Phytophthora palmivora* isolate P4. After 7 days, roots were evaluated and the diameter of the lesion in the affected area was recorded.

All the pathogens studied caused severe root rot. Damaged areas caused by isolate 69 were bigger and the affected tissue softer than those caused by P4 and 44. Table 7.9 presents the main results. Most genotypes of the analyzed cassava population are highly susceptible to all pathogens.

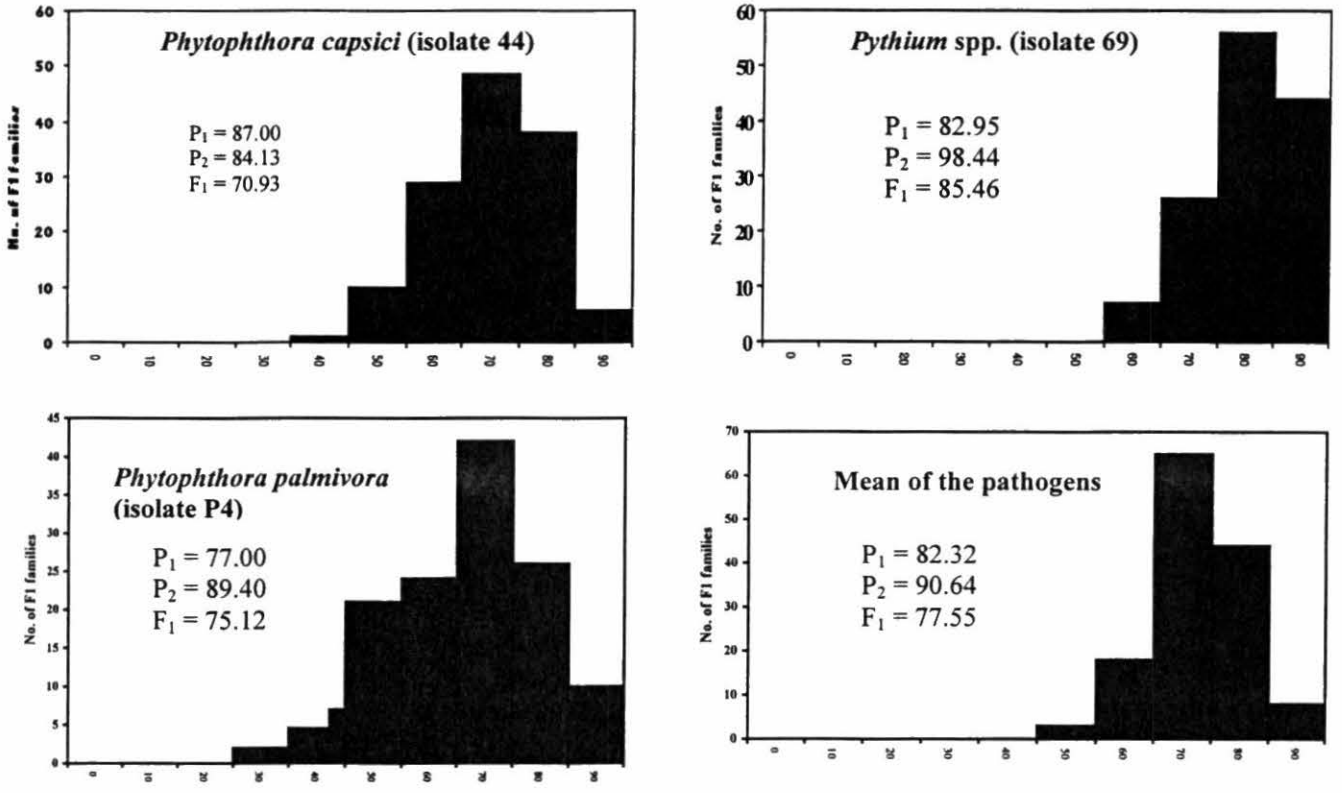


Figure 7.2.Frequency distributions of root rot means of F_1 genotypes for three different causal agents.

Variety MNGA-2 is less susceptible to isolate 69 than is CM 2177-2 at, respectively, 82.95% and 98.44% of the affected root area. For the other pathogens, no major differences in susceptibility were observed between the parents. Cassava varieties MBRA-1045 and MCOL-2066 were included in each trial as field resistant and susceptible controls. These two varieties reacted to the pathogens *in vitro* in the same manner as in the field, which shows that the inoculation technique used is appropriate for evaluating resistance to root rot. Genotype K110 has high levels of resistance to all evaluated pathogens: 44.55% of affected root area for isolate 44, 70.26% for isolate 69, and 50.00% for isolate P4. Variation of resistance in the population is high for P4 and low for 69 (Figure 7.1).

Table 7.9. Simple statistics of genotype/pathogen combinations for the P_1[a], P_2[b], and F_1 cassava families.

Genotype	Root area affected (%)			
	Pathogen			Average
	44[c]	69[d]	P4[e]	
F_1	70.93	85.46	75.12	77.55
N[c]	132	133	133	138
Standard deviation of the mean	13.69	8.75	10.03	8.13
Minimum mean of affected area observed	30.95	64.68	44.63	46.75
Maximum mean of affected area observed	100	100	100	100
Parents:				
M Nga 2 (less susceptible)	87.00	82.95	77.00	82.32
CM 2177-2 (more susceptible)	84.13	98.44	89.40	90.64
Controls:				
MBRA-1045 (resistant)	20.43	79.01	56.20	51.88
MCOL-2066 (susceptible)	84.60	84.71	90.00	86.43

a. P_1 = Parent one = M Nga 2. **b.** P_2 = Parent two = CM 2177-2. **c.** *Phytophthora capsici* **d.** *Pythium* spp. **e.** *Phytophthora palmivora.*

Activity 7.6. Evaluation of ten probes of resistance gene analogs in 140 individuals of family K for resistance to Phytophthora root rot.

Specific Objectives:

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

Rationale: In many countries, root rot symptoms include a sudden wilting that kills or severely reduces the quality of cassava planting materials. Cassava root rots are widespread and destructive, causing losses of 20% in world production. Locally, they can cause losses as high as 70% (Lozano 1992; Sánchez 1998). *Phytophthora drechsleri* Tucker, *P. nicotianae* Breda de Haan var. *parasitica* (Dastur), *P. cryptogea* Pethybr & Lafferty, and *P. erythroseptica* Pethybr have been reported as causal agents (Fassi 1957). Other species isolated from cassava and identified by David Cook of the Scottish Crop

Research Institute are *P. sinensis, P. meadii*, and *P. arecae* (Alvarez et al. 1997a, 1997b).

Automated dideoxy sequencing (Sanger and Coulson 1975) of the ribosomal DNA internal transcribed spacer (ITS) region of different Phytophthora isolates were performed for identification. *Phytophthora vignae* and *P. citricola* were also identified as infecting species other than cassava.

Colombian studies of 80 Phytophthora isolates from roots, shoots, and soil have established this fungus complex's high genetic diversity in terms of pathogenicity, virulence, morphology, and molecular characteristics of the ITS region of the ribosomal DNA (Alvarez et al. 1997a, 1997b). Isolates from different genetic groups were also found in the same geographic regions (Sánchez 1998).

Resistance to Phytophthora root rot and identifying resistant materials. Because of its efficiency, economy, and low social costs, integrated pest management, based on resistant materials, has always stimulated the search for resistant genotypes. Major constraints to rapid progress in the genetic improvement of cassava are the long vegetative period, difficulty in obtaining swollen cassava roots, and evaluating disease resistance *in situ*.

To overcome these problems, methods for early evaluation have been developed to detect resistance by inoculating roots and different parts of young plants like rootlets, leaves, shoots, and stems (Loke and Alvarez 1999). To rapidly evaluate the large germplasm collection a methodology has been developed for inoculating 20 to 40-day-old plantlets. 430 cultivars have been evaluated and 14 cultivars resistant to *P. drechsleri*, have been identified with wide differences in resistance levels to other *Phytophthora* species (Alvarez et al. 1998).

Resistance gene analogs (RGAs). Several disease-resistance genes have been cloned from different plant species (Christensen et al. 1998; Hahn et al. 1993; Martin et al. 1993; Mindrinos et al. 1994; Yu et al. 1996). Although these genes confer resistance to different bacterial, fungal, viral, and nematode pathogens, their products share structural similarities, suggesting a high degree of mechanistic conservation among the pathways that plants use to trigger defense responses (Baker et al. 1997; Bent 1996).

On comparing disease-resistance gene sequences with genes from different organisms, structurally conserved motifs of protein coding are observed. Resistance genes to *P. infestans* in potato are effective against pathogens as different as viruses, bacteria, and fungi, and have been cloned from unrelated plants such as tobacco, *Arabidopsis thaliana,* flax, and rice (Leister et al. 1996). Homologs of those genes can confer resistance to fungi in one species and nematodes in another.

Kanazin et al. (1996), working with soybean, demonstrated that conservative disease-resistance gene sequences, cloned from a large range of species, can be used to identify related genes in other species. Those related sequences are distributed

throughout the genome, occurring in gene class microclusters, and are associated with known resistance genes. Mapping RGA sequences can place genetic markers in close proximity of known resistance genes.

Leister et al. (1998) used conserved domains in the major class (nucleotide-binding site + leucine-rich repeat) of dicotyledonous resistance genes to isolate related gene fragments via PCR. They used degenerated primers from rice and barley (monocotyledonous species). Comparisons of peptide sequences of dicotyledonous R genes and monocotyledonous R-like genes revealed shared motifs, but provided no evidence for a monocotyledon-specific signature.

Materials and Methods: DNA was extracted by the Gilbeston-Dellaporta protocol, from leaf tissues of seven cassava parental genotypes, CM 2177-2, M Nga 2, M Col 22, CM 523-7, M Bra 1045, M CR 81, and M CR 54, which show different resistance levels to *Phytophthora* spp. Genomic restriction with the enzymes *Eco* RI, *Eco* RV, *Hae* III, *Hind* III, *Dra* I, and *Taq* I was done after gel depuration and denaturation. The digested DNA was transferred overnight to a Hybond N+ membrane, using 10× SSC (NaCl and trisodic citric acid) as transferring solution. The DNA was fixed on the membrane by ultraviolet light in a Stratalinker.

Escherichia coli DH5α cells were transformed by electroporation, introducing pGEM-T Plasmid Vector System (Promega), containing ten disease resistance genes analogs isolated from maize and rice. Transformed cells were kept at -80°C in glycerol 30%. Minipreps were prepared with Concert Rapid Plasmid Purification Systems (Gibco-BRL) from transformed cells. A PCR, using primers T7 and SP6, was done to amplify the insert, which was then used as a probe by marking with ^{32}P[dATP] to hybridize with restricted cassava genome of the seven parents described above.

Results: A clear and concentrated plant DNA was obtained from seven cassava parents. The complete digestion of genomic DNA was observed using six enzymes. Southern analysis for each enzyme and variety was carried out. Afterwards all filters were hybridized with three different probes from rice and maize. Another probe from cassava was included as a control. Using a non-radioactive labeling system a weak signal was observed with a probe isolated from maize and two cassava varieties. DNA labeled with ^{32}P is currently used to confirm the obtained results. Ten RGAs were successfully multiplied in *Escherichia coli* by Cell-Porator® Voltage Booster from Gibco BRL. All RGAs will be evaluated using the established methodology.

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Activity 7.7. Six resistant and susceptible parents identified from established populations for further identification of molecular markers associated with resistance to Phytophthora root rot

Specific Objectives:

1) To evaluate reaction to root rot in three populations segregating for their reaction to the disease.

Young stem fragments of three cassava populations were inoculated with six Phytophthora isolates. Populations inoculated were CM 7857—K family—conformed by 79 individuals; CM 9582 (MBRA-1045 × MCR-81) with 22 individuals; and CM 9600 (M CR 54 × M CR 81), with 10 individuals. Table 7.10 shows the percentage of individuals in each resistance category. Populations segregated as resistant, intermediate, and susceptible in variable amounts, depending on the isolate inoculated. Figure 7.3 shows the progeny distribution of populations CM 9582 and family K, according to resistance to P4 and 44 isolates. Probably, major genes control the resistance to these isolates, whereas, in family K, minor genes control resistance to the isolate 44 (*P. capsici*). Correlation between disease in stems and roots was not observed, suggesting different resistance mechanisms, depending on tissue.

Table 7.10. Percentage of segregating cassava genotypes from three crosses (CM 7857, CM 9582, and CM 9600) with different levels of resistance to six Phytophthora isolates.

	Isolate [a]						
Progeny	69	44	MTR6	P12	MTR4	P4	Mean
CM 7857–K family (M Nga 2 × CM 2177-2)							
Resistant	16.5	17.7	23.2	20.0	16.3	8.0	16.95
Moderately resistant	29.1	24.1	30.4	36.4	26.5	14.0	26.75
Intermediate	22.8	22.8	21.7	36.4	30.6	30.0	27.38
Susceptible	24.1	17.7	20.3	5.5	22.4	26.0	19.33
Highly susceptible	7.6	17.7	4.3	1.8	4.1	22.0	9.58
CM 9582 (M Bra 1045 × M CR 81)							
Resistant	13.6	4.5	4.5	36.4	14.3	9.5	13.80
Moderately resistant	36.4	54.5	36.4	31.8	23.8	52.4	39.22
Intermediate	40.9	31.8	36.4	22.7	42.9	23.8	33.08
Susceptible	4.5	9.1	18.2	9.1	19.0	9.5	11.57
Highly susceptible	4.5	0.0	4.5	0.0	0.0	4.8	2.30
CM 9600 (M CR 54 × M CR 81)							
Resistant	60.0	30.0	30.0	70.0	20.0	44.4	42.40
Moderately resistant	10.0	20.0	50.0	10.0	50.0	22.2	27.03
Intermediate	20.0	40.0	0.0	0.0	30.0	33.3	20.55
Susceptible	0.0	10.0	10.0	20.0	0.0	0.0	6.67
Highly susceptible	10.0	0.0	10.0	0.0	0.0	0.0	3.33

a. Isolate 44 = *Phytophthora capsici,* P12 = *P. vignae*; P4 = *P. palmivora*..

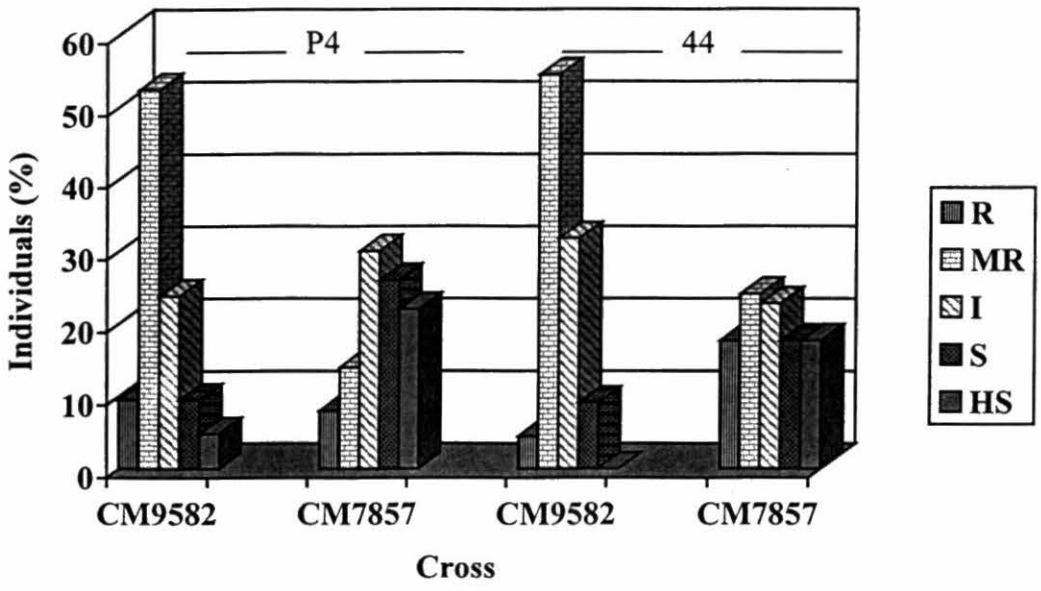

Figure 7.3. Distribution of genotypes from crosses CM 9582 and CM 7857 according to their resistance to Phytophthora isolates P4 and 44, inoculated on young stem fragments.R = resistant; MR = moderately resistant; I = intermediate; S = susceptible; HS = highly susceptible.

Activity 7.8. Genetic interaction between cassava varieties and Phytophthora isolates in the expression of resistance to root rot disease

Specific Objectives:

1) To determine the genetic interaction between 12 cassava varieties and five isolates of Phytophthora spp. in the expression of resistance to root rot disease.

To carry out this study, we used *in vitro* systems, based on sprouts and detached leaves and applying different inoculum levels. The main goals were to determine **a)** the location of resistance mechanisms; **b)** how they differ in a group of 12 cassava genotypes; and **c)** identify the pathogen's variability of virulence and the implications such variability would hold for developing host resistance to manage the disease.

Table 7.11. Interaction between resistance genes of cassava and virulence groups of *Phytophthora* and *Pythium* spp. by inoculating sprout fragments and detached leaflets.

Sprouts							Leaflets						
Resistance group	Genotype	Resistance genes or factors	Virulence group (and isolate)[a] 1 (43)	2 (P12)	3 (120)	4 (71)	Resistance group	Genotype	Resistance genes or factors	Virulence group (and isolate)[a] 1 (P12)	2 (120)	3 (43)	4 (71)
0	HMC-1	R	S	S	S	S	1	MNGA- 2	R1R2	R	S	R	S
0	CM 2177-2	R	S	S	S	S	2	MBRA-532	R1R3	R	R	S	S
0	MCOL-2066	R	S	S	S	S	3	HMC-1	R1R2R3	R	R	R	S
1	MBRA-12	R1	S	R	S	S	3	MBRA-12	R1R2R3	R	R	R	S
2	MBRA-1044	R1R2	S	R	R	S	3	MBRA-222	R1R2R3	R	R	R	S
3	MBRA-532	R3	R	S	S	S	3	CM 2177-2	R1R2R3	R	R	R	S
3	MBRA-311	R3	R	S	S	S	3	MCOL- 2066	R1R2R3	R	R	R	S
4	MCR-81	R2R3	R	S	R	S	3	MBRA-1044	R1R2R3	R	R	R	S
5	MBRA-1045	R1R2R3	R	R	R	S	4	MCOL- 1522	R1R2R3R4	R	R	R	R
5	MBRA-222	R1R2R3	R	R	R	S	4	M Tai 8	R1R2R3R4	R	R	R	R
5	MARG-6	R1R2R3	R	R	R	S	4	MBRA-1045	R1R2R3R4	R	R	R	R
Mean ABCPE			8	10	13	24	**Mean ABCPE**			6	31	30	128

Materials and methods: The cassava varieties used were those that had demonstrated different reactions to the disease in the field. *Phytophthora* isolates were collected from regions that differ in disease pressure. Inoculations were carried out on fragments of sprouts cut from 20 to 40-day-old cassava plants propagated by stem cuttings. The fragments were obtained from 20 to 40-mm lengths of young stems carrying two or three buds. On the upper surface of each fragment, a disc of filter paper, with a 5-mm diameter, was fixed with a needle. The disc was inoculated with a drop of fungal suspension + soil extract + a given concentration of mainly zoospores.

Each leaflet of detached leaves of 20 to 40-day-old cassava plants propagated by stem cuttings was perforated with a needle having a 2-mm diameter. Each perforation was also inoculated but with a suspension of mainly zoospores in soil extract.

The sprout fragments and detached leaflets were incubated on the laboratory bench. In both inoculation systems, the evaluation parameter was the extension of area attacked by the pathogen after 2, 4, and 6 days after inoculation. Areas under the disease progress curve (AUDPC) was calculated. Isolates were clustered into virulence groups by means of AUDPC/day and the sum of binary values of the compatible combinations variety * isolate. An infection level of more than 3% AUDPC/day was considered as a compatible reaction. The point of separation between incompatible and compatible was 3% AUDPC/day, which corresponds to a very small lesion of about 4 mm^2 in a leaf and 2 mm on a sprout. Genotype groups, within each virulence group, were determined by Ward's method, based on variance between replications.

Results: For each different plant tissue, different virulence groups were found (Table 7.11). According to the results obtained for sprout inoculation, each of the isolates 43, 44, 69, and P12 belong to a different virulence group. Isolate 44 (*P. capsici*) was the most pathogenic, considering its virulence and severity under both inoculation systems. These results demonstrate the pathogenic variability of Phytophthora isolates obtained from Colombian cassava.

Through sprout inoculation, we could demonstrate that five resistance groups exist, to which one or more cassava varieties belong to (Table 7.11). In sprout fragments, the variety MARG-6 is resistant to the four virulence groups. The varieties MARG-6 and MBRA-1045 possess the highest levels of resistance for sprouts. This resistance may be durable because it is not caused by hypersensitive reactions. We conclude, then, that adequate levels of horizontal resistance exist.

Leaf reaction was different to sprout reaction: four instead of five resistance groups were found. A plant's hypersensitive reaction indicates that vertical resistance occurs. Hypersensitivity was observed with isolates 43 (MARG-6, MBRA-1044, MBRA-1045, and MBRA-222); 69 (MBRA-12, MBRA-222, and M TAI 8); and P12 (MBRA-532, MBRA-1044, MBRA-1045, MCOL-2066, and MBRA-222). These reactions were not observed under sprout inoculation conditions. Probably, one or a few genes dominate this reaction. The

absence of visible infection by P12 (*P. vignae*) in leaves indicates that the resistance mechanism in this tissue is different to that in sprouts.

The varieties MARG-6 and MBRA-1045 showed the highest levels of resistance across the different plant tissues. In contrast, varieties HMC-1 and CM 2177-2 were highly susceptible. A similar number of resistance groups (six) was observed for each plant tissue, suggesting that, in the studied population, a similar level of genetic diversity exists for resistance in sprouts and leaves. The correlation between lesion size of sprouts and leaves, based on four isolates and 12 varieties, is +0.54. HCN does not affect disease development in sprouts or leaves.

Both inoculation systems indicated a high genetic interaction between varieties and the *Phytophthora* virulence groups.

For the inoculation of fragments of sprouts and detached leaflets, a concentration of 1×10^5 and 1×10^5 zoospores per mL were the most effective in causing disease. Nevertheless, to determine different resistance mechanisms, the three most effective concentrations for causing disease were used.

The results suggest the existence of different resistance mechanisms operating in different plant tissues against the pathogen's specific virulence mechanisms. The methodology described seems effective in detecting different horizontal and vertical resistance mechanisms against this disease. No varieties were observed to possess high levels of resistance in both sprouts and leaves, which would be desirable for managing the disease by durable resistance. The techniques developed so far can also be applied to genetic studies and the selection of biocontrol agents and resistance inductors. HCN content was proved not to be a resistance mechanism.

Activity 7.9. Resistance of native varieties from Mitú (Colombia) to Phytophthora isolates.

Cassava roots from 20 native cassava varieties collected from indigenous settlements at Mitú (Vaupés Department) were inoculated with fungal discs of the *Phytophthora* isolates MTR4, MTR6, MTR7, and 2X Mitú, all from Mitú; and 44 (*P. capsici*) from the Quindío Department, which was used as a known control. Root damage was determined by measuring width and length of lesions at 7 days after inoculation.

Ward's minimum variance cluster analysis at 96.5% of reliability formed five variety groups according to disease resistance.

Table 7.12. Native cassava genotypes from Mitú (Vaupés), evaluated for resistance[a] to different Phytophthora isolates by inoculating roots under laboratory conditions.

Genotype	Isolate[b]					Average lesion size (cm^2)
	44	2X Mitú	MTR4	MTR6	MTR7	
Abiyú	3.30	-	-	3.62	-	3.46
Brava Blanca	4.97	1.62	2.61	4.37	5.70	3.86
Busá	13.30	-	-	2.29	-	7.80
Butisé	16.62	2.06	5.37	0.69	1.73	5.29
Carayurú	5.48	-	9.29	2.69	3.89	5.34
Flores	10.06	-	-	2.48	-	6.27
Garza	10.96	-	-	1.10	-	6.03
Guaracú	8.06	-	1.91	1.04	-	3.67
Hoja de Plátano	5.19		1.09	0.73	-	2.34
Inayá	5.86	1.56	-	1.01	1.25	2.42
Mico (Roja)	6.68	-	9.62	1.41	-	5.90
Piña	8.36	-	-	1.40	-	4.88
Pintadillo	8.68	9.88	13.25	5.34	3.23	8.08
Rana	4.40			1.65	1.07	2.37
Santa Catalina	13.85	3.00	2.60	2.74	12.43	6.92
Siringa	4.98	-		10.59	-	7.79
Totuma	6.20	-	4.00	5.06	2.03	4.32
Wasoco	4.12	-	-	0.69	-	2.40
Yuca de Uva	7.50	-	-	2.10	-	4.80
Zancudo	-	-	12.98	1.51	-	7.24
M Bra 12 (control)	13.84	1.99	3.48	0.13	4.95	4.88
Average	8.12	3.35	6.02	2.51	4.03	-
LSD α 5%	6.09	6.09	6.09	6.09	6.09	2.49

a. Resistance groups: resistant = 0–2.2 cm^2; intermediately resistant = 2.3–4.7 cm^2; intermediate = 4.71–7.0 cm^2; susceptible = 7.1–12.0 cm^2; highly susceptible = 13.0–17.0 cm^2; - = not determined.
b. Origin of isolates: 44 = Quindío, Colombia; 2X Mitú, MTR4, MTR6, and MTR7 = Mitú, Colombia.

Isolate 44 was observed as the most aggressive, followed by MTR4. Least significant difference at α = 5% between isolates was 2.49 cm^2 and between isolates for each variety was 6.09. 'Hoja de Plátano', 'Rana', 'Wasoco', and 'Inayá' were the most resistant varieties, while 'Pintadillo' was the most susceptible. 'Abiyú' showed high resistance to isolate 44, while 'Santa Catalina' was the native variety most attacked by isolate 44. The control variety MBRA-12, was highly susceptible to isolate 44, but showed high or intermediate resistance to isolates from Mitú.

Activity 7.10. Evaluation of frogskin disease in cassava varieties at Palmira and in the Meta Department.

In experiments in the Llanos Orientales, sources of resistance to frogskin disease (FSD) were identified.

From 1997, the incidence of this disease was 15%, and was controlled by eliminating affected plants and disinfecting the machete with 1% hypochlorite or soap solution each time it was used. With these practices, disease incidence dropped from 4.4% between 1996 and 1997 to 3.9% at 1998 (Table 7.13).

Table 7.13. Evaluation of resistance to cassava bacterial blight (CBB) and super elongation disease (SED) at Villavicencio, Carimagua, and Matazul between 1996 and 1999, with information on incidence of FSD.

Description	1996-1997		1997-1998		1998-1999	
	V/cencio	Carimagua	V/cencio	Carimagua	V/cencio	Matazul
Genotypes evaluated (no.)	345	387	158	219	198	246
% of genotypes resistant or intermediately resistant to CBB and SED	30.4	26.8	3.2	10	50.5[a]	12.2[a]
% of genotypes with FSD	15.1	9.7	4.4	0[b]	8.1	3.7

a. All selected genotypes demonstrated an intermediate reaction to CBB.
b. None of the 23 genotypes selected for planting at Matazul were affected by FSD.

Table 7.14. Evaluating the incidence of frogskin disease (FSD) in a field at CORPOICA, Palmira.

Description	Number	Percentage
Evaluated genotypes	140	100.0
Genotypes affected by FSD	132	94.3
Genotypes severely affected	15	10.7
Genotypes without visible symptoms[a]	8	5.7

a. CG 1367-1, CM 3311-3, CM 6438-14, CM 7052-2, MBRA-532, MBRA-1045, MCOL-2280, and MVEN-77.

During the 1999 harvest at Villavicencio and Matazul, 8.1% and 3.7%, respectively, of the genotypes were affected by FSD. Most genotypes had come from Palmira. None of the 23 genotypes from Carimagua and planted at Matazul showed the disease.

During an evaluation of a stake-multiplication field, established at CORPOICA (Palmira), 94.3% of the genotypes were affected by FSD. About 10% of the genotypes presented severe symptoms of the disease. Some of the stakes of the affected genotypes came from the germplasm collection held at CIAT (Table 7.14).

Table 7.15 shows the incidence of FSD among cassava genotypes selected for resistance to CBB and SED in experiments at Villavicencio and Carimagua, during 1997 and 1998. These genotypes were also evaluated for FSD at CORPOICA (Palmira).

Table 7.15. Evaluation for reaction to frogskin disease (FSD) among cassava genotypes selected for resistance to cassava bacterial blight (CBB) and superelongation disease (SED) at Carimagua (C) and Villavicencio (V), after several cycles of evaluation per genotype.

Genotype	Evaluation				FSD[b]	
	Location	cycles (no.)	CBB[a]	SED[a]	Eastern Savannas	Palmira
CG 1367-1	C	5	I	I	NS	NS
M Bra 703	C	4	I	I	NS	S
M Bra 903	C	5	I	I	NS	
M Bra 917	C	4	I	I	NS	S
M Ecu 82	C	5	I	I	NS	S
SG 104-74	C	5	I	I	NS	S
SM 1152-13	C	4	I	I	NS	
SM 1821-7	C	2	I	I	NS	S
CM 2772-3	V	3	I	R	NS	S
M Col 707	V	3	I	R	NS	S
M Col 2387	V	3	I	I	NS	
M Col 2409	V	3	I	I	NS	S
M Col 2538	V	3	I	R	NS	S

a. I = intermediate; R = resistant.
b. NS = no symptoms: S = symptoms.

Activity 7.11. Other various activities related to disease resistance in cassava.

Multiplication of promising cassava genotypes: We multiplied 280 promising cassava genotypes at CORPOICA, Palmira, for greenhouse experiments on varietal resistance and disease management.

Detecting cassava bacterial blight in Quindío and Valle del Cauca: A cassava crop suffering severe incidence, and with severe symptoms, of CBB was detected on a farm located at La Tebaida (Quindío). The 45 ha were planted to the cassava varieties MPER-183, HMC-1, and the local Chiroza (MCOL-2066). Field evaluations showed that 'Chiroza' suffered least, showing fewest symptoms. On another farm near Montenegro (Quindío), varieties HMC-1 and the local Manzana were seen with intermediately severe symptoms of CBB.

The blight was also detected in M Bra 383, CM 6740-7, and CM 849-1 on a farm at Jamundí (Valle del Cauca) and in the genotypes MCOL-1505, MCOL-1468, and CM 523-7 (ICA Catumare) at the CIAT Experiment Station in Santander de Quilichao (Cauca). Isolates were obtained from all three sites and 25 cassava genotypes inoculated to evaluate for varietal resistance.

Identifying a mycoplasm that causes witch's broom disease in cassava: A mycoplasm was identified as causing witch's broom disease in the cassava varieties Mirití and Flores in young plants grown in the greenhouse at CIAT. These plants were obtained from stem cuttings at Vaupés. The diagnosis was confirmed through PCR, using specific primers for detection. The CIAT Virology Unit then standardized the PCR technique.

Detecting a Scytalidium species in cassava stems at Mondomo, Cauca: An important cassava root rot pathogen, reported previously in Brazil and the Colombian North Coast, was detected in the Cauca Department this year. The fungus *Scytalidium* sp. was isolated from a 5-month-old cassava plant, cv. Algodona (M Col 1522), growing on a farm at Mondomo, Santander de Quilichao, Cauca. The symptoms observed on cassava plants are wilting, constricted and necrotic stems, and canker development at stem bases.

Isolates of the fungus were obtained by washing stem pieces under running tap water for 20 min, followed by sterilization in 70% alcohol for 1 min, and finally rinsing with sterilized distilled water. These fragments were placed on PDA medium and incubated for 4 days at 28°C under alternating light.

Activity 7.12. Information Dissemination during 2000.

Presentations for conferences and other meetings:

Alvarez E; Claroz JL; Loke JB; Echeverri C. Pathogenicity and genetic diversity of *Sphaerotheca pannosa* var. *rosae*, causal agent of powdery mildew of roses in Colombia. American Phytopathological Society, New Orleans, August 13 and 14.

Alvarez E; Mejía JF; Losada T. Pathogenic and molecular characterization of Brazilian isolates of *Sphaceloma manihoticola*. American Phytopathological Society, New Orleans, August 13 and 14.

Alvarez E; Mejía JF; Lozada T. Caracterización molecular y patogénica de aislamientos de *Sphaceloma manihoticola* de Brasil. XXI Congreso Nacional de Fitopatología y Ciencias Afines, held at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, September.

Alvarez E; Claroz JL; Loke JB; Echeverri C. Diversidad genética y patogénica de *Sphaerotheca pannosa* var. *rosae*, causante del mildeo polvoso en la rosa en Colombia. XXI Congreso Nacional de Fitopatología y Ciencias Afines, held at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, September 1.

Alvarez E; Cuero SP; Castellanos G; Claroz JL; Llano GA; Loke JB. Potencial de un extracto vegetal y de un fertilizante foliar para el control del mildeo polvoso de la rosa causado por *Sphaerotheca pannosa* var. *rosae*, en Colombia. XXI Congreso Nacional de Fitopatología y Ciencias Afines, held at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, September 1.

Llano GA. Manejo integrado de pudriciones radicales causadas por *Phytophthora* spp. Presentación de avances de actividades para el Informe Annual de CLAYUCA.

Alvarez E; Llano GA. Investigación participativa para el control de pudriciones de yuca en comunidades indígenas de Mitú (Colombia), II: Seminario Regional de Agrociencia y Tecnología, Siglo XXI, Orinoquía Colombiana, August 23—25, Villavicencio.

Presentations to university groups:

Llano GA; Claroz JL; Mejía JF. Diagnóstico, caracterización y control de enfermedades de yuca y flores. To biology students from the Universidad Pedagógica de Bogotá on May 27.

Claroz JL; Llano GA; Loke JB. Técnicas moleculares aplicadas a fitopatología; aislamiento de *Phytophthora* spp.: Investigación participativa para el control de pudriciones de yuca en comunidades indígenas de Mitú (Colombia). To agroecological engineering students from the Universidad de la Amazonia de Florencia, Caquetá on September 22.

The training offered:

At CIAT, Palmira, December 9—18. Técnicas moleculares aplicadas a la identificación de la resistencia a enfermedades de diferentes cultivos. Course to researchers from different Colombian universities and institutes. *Funding provided by the Colombian* Ministry of Agriculture.

Pereira, Risaralda, April 3—6. Training given to multipliers of technology transfer: technicians from SENA, Armenia; *Comité de Cafeteros de Risaralda; Secretaría de* Agricultura de Risaralda; UMATAs of Risaralda, Quindío, and Norte del Valle. Farmers from Quindío, Risaralda, and northern Valle del Cauca were also given training.

Aguazul, Casanare, May 30—June 2. *Technicians from the Fundación* CEMILLA, Secretaría de Agricultura y Desarrollo Rural de Agua Azul, and UMATAs of Casanare. Training given in cassava disease integrated management.

CIAT, September 2000. Dr. Hanne Nielsen of the collaborative project with the Danish Government Institute of Seed Pathology (DGISP), Copenhagen, Denmark. Training in Molecular characterization of bacterial and fungal pathogens.

CIAT, October 2000. María del Socorro Balcázar, Project SB-2, CIAT. Training in molecular characterization of *Xanthomonas campestris*.

The training received:

Participation in training courses of research assistants: José Luis Claroz, Michigan State University (USA), and John B. Loke, Plant Protection Service, Wageningen, (Netherlands), Danish Government Institute of Seed Pathology (Denmark), and Scottish Crop Research Institute (Scotland).

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Ramírez AM. Caracterización patogénica y molecular de aislamientos de *Phytophthora* spp., obtenidos de los departamentos de Cauca, Valle del Cauca y Quindío.

Awards:

Award in Mutual Extension Efforts given by the Universidad Nacional de Colombia for "Desarrollo agrícola de la población indígena de la zona de influencia Mitú—Monfort (Vaupés, Colombia): *Control de pudriciones en yuca mediante investigación participativa*" as being the best research work on technology transference in Colombia,.

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Instituto Agronómico de Campinas (IAC)
Instituto de Investigaciones de Viandas Tropicales (INIVIT), Cuba
Universidad Nacional de Colombia—Palmira (Valle del Cauca, Colombia)
Secretaría de Agricultura del Vaupés (Mitú, Vaupés, Colombia)
UMATAs from Mitú, Santander de Quilichao, Buenos Aires, Caicedonia, La Tebaida, and Montenegro (Colombia)

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Highlights:

- Promising varieties with resistance to cassava bacterial blight (CBB) and superelongation disease (SED) were identified at Villavicencio and Matazul (Meta Department).
- Six cassava genotypes (M Esc Fla 039, M Esc Fla 021, MCOL-2066, SM 1225-12, MCOL-2737, and SM 1828-11), highly resistant to CBB, were selected in the greenhouse at CIAT.
- Two cassava genotypes (MBRA-12 and CM 2177-2), highly resistant to SED, were selected in the greenhouse at CIAT.
- F_1 progeny and parents of K family (MNGA-2 × CM 2177-2) were characterized for resistance to Phytophthora root rot.
- Cassava variety, HMC-1, was identified as highly resistant to Phytophthora root rot in the field.
- The native varieties, 'Hoja de Plátano', 'Rana', 'Wasoco', and 'Inayá', were found to be highly resistant to Phytophthora root rot.
- From 132 genotypes evaluated in the field at the Instituto Colombiano Agropecuario (ICA), Palmira, eight cassava genotypes (CG 1367-1, CM 3311-3, CM 6438-14, CM 7052-2, MBRA-532, MBRA-1045, MCOL-2280, and MVEN-77) were unaffected by frogskin disease (FSD).
- Cassava variety MBRA-1045 was free of FSD by indexing with 'Secundina'. Progeny of this variety segregated with resistance to FSD.

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

Cassava is a remarkable crop with many advantages over other crops, particularly in relation to stability of performance, capacity to achieve acceptable productions in low fertility soils, and general tolerance to biotic and abiotic stresses. However, the crop also has some clear disadvantages. Among some of the limitations, the low reproductive rate and the length of each cycle of selection, slows down the genetic progress achieved and limits the amount of genetic information available for the species. New biotechnology tools can help overcoming or reducing some of these problems particular for cassava.

Activity 8.1. Molecular genetic markers for the new source of resistance to the Cassava Mosaic Disease (CMD).

Specific Objectives:

a) to identify molecular markers that flanks the new source of resistance to ACMD.
b) to confirm that the new source of resistance is controlled by a single dominant gene.
c) to develop tools for marker-assisted breeding of CMD resistance at CIAT.

Rationale: Cassava mosaic disease (CMD) remains the most economically damaging disease of the crop in Africa, and it is a threat to cassava production in the Americas. The whitefly vector biotype of CMD has recently invaded the New World increasing the possibility that the cassava mosaic virus (CMV) or a native geminivirus will cross over to cassava in the near future. CMD also prevents the transfer of germplasm from the crop's center of diversity in Latin America to Africa; resistance to CMD has not, until recently, been emphasized in gene pool development goals in LA. Host-plant resistance to CMD is the best method of containing the disease and currently deployed resistance was originally obtained from a wild relative of cassava, *M. glaziovii*. A more extreme source of resistance controlled by a single dominant gene (Akano et al. 2000) was identified in closely related Nigerian cassava land races in IITA. Given CIAT's global cassava mandate it is necessary to develop gene pools adapted to CMD for Latin America should the CMD disease makes its debut in the region, and for the transfer of useful variability to Africa, such as high carotene content, and tolerance to drought. CMD breeding at CIAT requires a method to select in absence of the disease agent a task for which molecular makers are best suited.

To develop markers for marker-assisted breeding of CMD resistance, populations segregating for genes controlling both sources of resistance were developed and evaluated under high disease pressure in Nigeria over a period of two years under the auspices of a Rockefeller Foundation funded CMD resistance gene mapping project. Last year a SSR marker was found for the currently deployed CMD resistance

introgressed from *M. glaziovii*, which is recessive in nature. We report here identification of a simple sequence repeat marker and an RFLP marker that are tightly linked and flank the dominant R gene controlling the new source of resistance to CMD. This result opens up the possibility of marker-assisted selection for breeding resistance to CMD in the absence of the pathogen as in CIAT and for rapidly introducing the gene into African cassava gene pools.

Materials and Methods: The mapping population was a F_1 progeny from a cross between a Nigerian land race (TME3) that represent the new source of ACMD resistance and a susceptible improved line (TMS 30555). The F_1 progeny, of 240 individuals, was established from embryo axes, multiplied and six copies per genotype were transferred to the field in a low CMD pressure site in Nigeria and two copies kept *in-vitro*. For field evaluation of CMD resistance, the mapping population was established from woody cuttings 15-20cm long, in two CMD high-pressure sites, Onne, a high humid forest agro-ecology and Ikenne, a low humid forest agro-ecology, both in Nigeria. The experimental design was an augmented randomized complete block design with 10 plant rows per genotype per block. CMD disease resistance was evaluated at 3 and 6 months after planting (MAP). Visual assessment of symptom intensity was conducted according to the IITA scale, ranged from 0 for no observable symptom to 5 for very severe chlorosis and reduction in leaf area, for each leaf on each plant and averaged by genotype. Genotypes were observed to fall into two broad classes of no symptom score of 1, and very severe symptom score of 4, in both sites, suggesting a qualitative inheritance. Genotypes were therefore scored as resistant and susceptible and a chi square test was performed to test a single heterozygous gene model of resistance.

A bulk segregant analysis (BSA) using two pools of 40 susceptible and 40 resistant genotypes from the mapping progeny was used as a quick method to identify markers associated with the resistance gene. The two bulks and two parents were screened for with the 186 Cassava MapPairs SSR markers using PCR conditions as described by Mba et al. (2000). Any marker found to be polymorphic in the two parents and the two bulks was employed to evaluate members of the bulks individually and markers that were still polymorphic in the resistant and susceptible progeny were used to genotype a subset of 162 genotypes from the progeny. Single point marker analysis using a simple linear regression of CMD resistant data on the SSR marker genotypic classes as independent variable was conducted to determine how much of the phenotypic variation was explained by the marker.

Results: The ratio of resistant to susceptible genotypes in both sites was not significantly different from a ratio of 1:1 in the mapping progeny by a Chi square test at a probability level of 0.01. This suggests a single dominant gene heterozygous in the ACMD resistance parent. In the bulk segregant analysis, only one SSR primer SSRY 28 showed an allele that was present in the resistant parent and in the resistant bulk but absent in the susceptible bulk and the susceptible parent. The polymorphism was confirmed when members of the bulks were individually screened although there were 6 recombinants (Figure 8.1). Analysis of a total of 162 progeny revealed 16

recombinants, suggesting the marker is at least 10cM from the CMD resistance gene. A simple regression analysis also revealed that the marker explains 70 % of the phenotypic variance of CMD resistance. SSRY 28 was found to be located on linkage group R of the male-derived map of cassava linked in coupling to marker GY1 (RFLP), and in repulsion to marker Ai19 (RAPD). See Figure 8.2. RFLP analysis of the mapping progeny with GY1 revealed the CMD R gene is located between SSRY 28 and GY 1 at a distance of 10cM, and 6cM respectively.

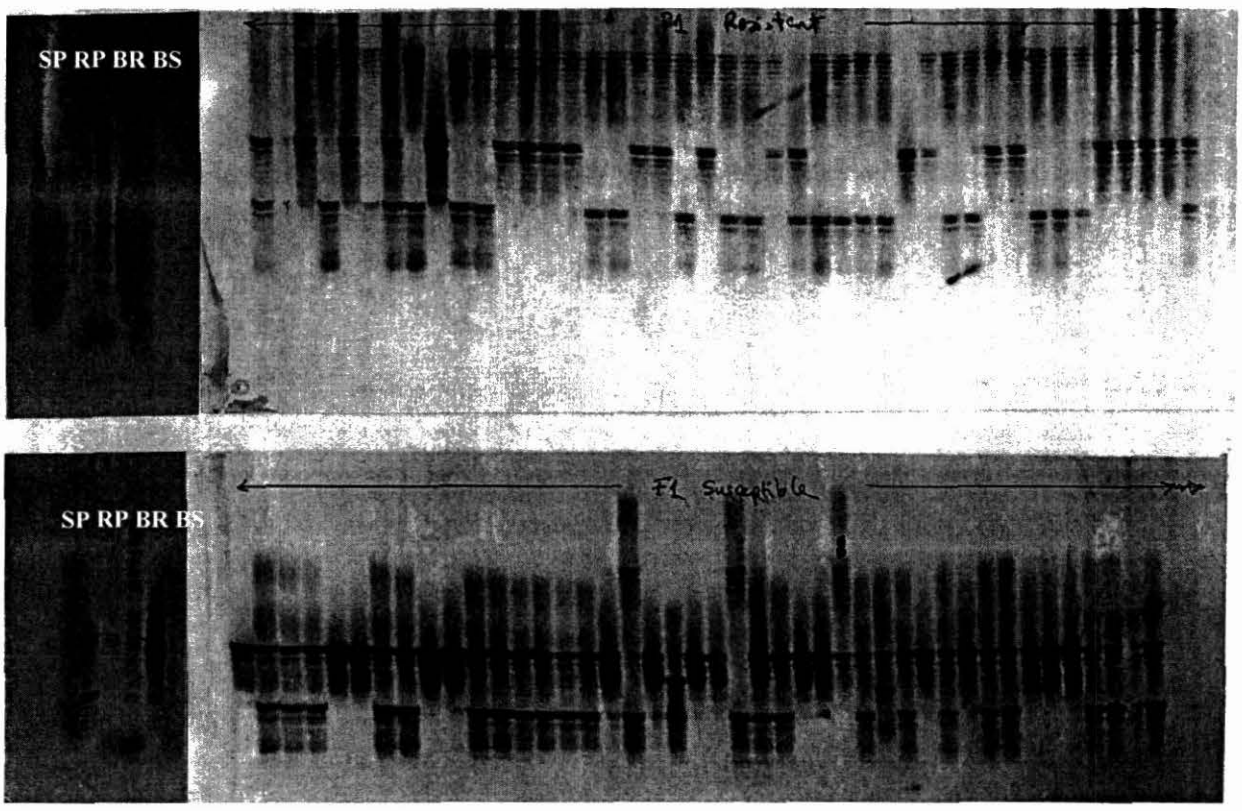

Figure 8.1. Bulk segregant analysis (BSA) of CMD susceptible and resistant progeny with SSR Marker SSRY28 (SP, SB: susceptible parent, susceptible bulk; RP, RB: resistant parent, resistant bulk).

The two markers that flank the CMD resistance gene are linked in coupling and can be used together to determine genotypes which bear the CMD resistance gene. However, the RFLP marker does not readily lend itself to high-throughput genotyping required for MAS compared to the SSR marker. It is therefore planned for next year, to convert the RFLP marker to an SSR by identification of BAC clones that contain GY1, and an SSR sequence.

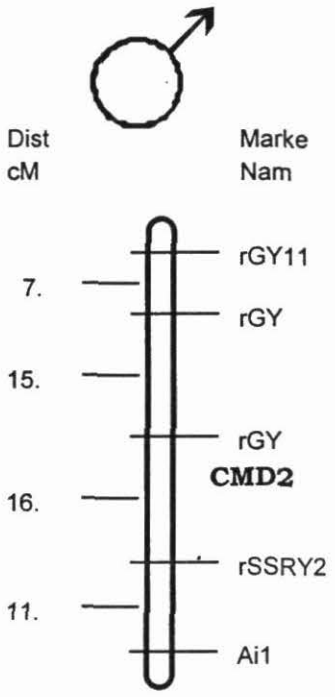

Figure 8. 2. Linkage group R of the male-derived Map showing the location of the dominant gene, **CMD 2**, controlling the new source of resistance to the African Cassava Mosaic Disease.

Achievements:

- Identification of a SSR and RFLP marker that flank, at a distance of 10cm and 6cM respectively, the single dominant gene controlling resistance to CMD.
- Tools for marker-assisted breeding of CMD resistance at CIAT.

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Activity 8.2. Serial analysis of gene expression of resistance to CMD: candidate genes for the positional cloning of the CMD resistance gene.

Specific Objectives:

a) to identify molecular markers tightly linked to the new sources of CMD resistance.
b) to clone the resistance gene(s) for faster deployment of resistance genotypes

Rationale: The objectives of the second phase of the RF funded CIAT-IITA CMD resistance mapping project is to identify molecular markers tightly linked to different sources of CMD resistance and to clone the resistance gene(s) for faster deployment of resistance genotypes and as tightly linked (zero cM) markers for MAS. Having achieved the first objective a strategy to identify candidate R genes, differentially expressed in CMD resistant genotypes, for map-based cloning was outlined based upon the serial analysis of gene expression (SAGE). The SAGE technique is a powerful sequence-based method that is routinely used in human genetics research, especially cancer research, to identify novel genes differentially expressed in cancerous cells as target for new medicines. The SAGE profiling of genes expressed in plants was first described by Matsumura et. al., of the paddy-field rice research project, Iwate Biotech Research Center (IBRC) to reveal the gene expression pattern of rice plants under different germination and disease conditions. At CIAT, the bulked segregant analysis (BSA) approach had earlier been used to identify a simple sequence repeat (SSR) marker linked to a dominant gene that confers resistance to the devastating cassava mosaic disease (CMD). The SAGE and BSA techniques were combined to obtain the gene expression pattern in CMD resistant and susceptible plants as a first step to identifying candidate genes for map-based cloning of the CMD resistance gene.

Materials and Methods: Leaf tissue for SAGE analysis came from progeny of the TME3 X TMS30555 CMD resistance mapping progeny. About 1g of young leaf shoots were harvested from each of the 40 resistant plants and 40 susceptible plants used earlier to identify a CMD resistance marker by BSA. The plants have been kept in the field under very high disease pressure at the IITA high rain forest Station, Onne, Nigeria for more than two years and were pruned at 12 months after planting. Pruning cassava plants greatly increases virus disease pressure. The leaf samples were bulked for resistant and susceptible plants, ground to a fine powder in liquid nitrogen and dissolved in 5 volumes of "RNA later" (Ambion Inc.) and shipped directly to Japan.

Leaf tissue in "RNA later" was recovered by centrifugation in microfuge tubes, 2min room temp, and total RNA isolated using the Qiagen (Gmbh) RNA mini or midi prep kit. Typical yields were about 800ug total RNA/g of leaf tissue. Messenger RNA isolation was by the Amersham-Pharmacia Biotech (PLC) mRNA isolation kit. Construction of the SAGE library was with 4.5ug and 6ug mRNA for the resistant and susceptible

samples respectively, according to the SAGE protocol, as modified by Dr H. Matsumura. Additional modifications made include:

- Optimization of ditag PCR, by performing a $MgCl_2$ concentration curve of 1.5mM, 2mM, 2.5mM, 3mM, 3.5mM, 4mM, 6mM, and 8mM using ditag template dilutions of 1:5, 1:10, and 1:20, maximizes efficiency of ditag generation. To prevent contamination of ditag PCR with previously amplified ditag, the required PCR reactions, 600 in all, were performed the same day prior to running ditag PCR PAGE gels.

- Treatment of NlaIII digested ditag with 400ul of streptavidin beads before and after PAGE electrophoresis, to reduce the risk of contamination by linker molecule, and thus increase the purity of ditag molecules which greatly enhances the efficiency of concatemerization of ditags

- Replacing the pZERO plasmid vector of the SAGE protocol with another vector that permits the blue/white screening of recombinant plasmids; in this case phosphorylated (RT Inc.) pGEM-3Z (Promega inc.) plasmid was used. Nine nanograms of vector is the optimum amount for the ligation reaction with a 3:1 insert:vector ratio in a 10ul reaction volume, and 4 hours incubation time at 16^0C. Knowing the exact the concentration of inserts, for example by ethidium bromide dot quantitation, is therefore required.

- Electroporation into 40ul of ElectroMax DH10B cells (GibcoBRL) of the ethanol precipitated ligation, using the BIO-RAD Electro-pulser and the following conditions: 2.5kV, 25uF, 100ohms, 0.1cm cuvette. Electroporated cells are recovered in 1ml SOC and 100ul plated on 10cm ampicillin (100ug/ul), IPTG/X-gal LB plates.

White colonies were picked into 10ul of sterile water and 10ul of a PCR-mix added; PCR thermal cycling was according to the SAGE protocol. About 3ul of the PCR reaction were run on a 1.2% agarose gel, stained with ethidium bromide, and visualized on an UV trans-illuminator. Inserts above 586bp, approximately 29 tags, were selected for PCR clean up, with the QIAGEN kit, for sequencing. Sequencing was done with the T7 primer on a Perkin Elmer 377 Automated sequencer, using 1.5ul from the approximately 50ul volume obtained after PCR product purification. Sequence data was edited and saved as several text files, no larger than 9kb, then inputed into the SAGE bioinformatics program.

Results: Two SAGE libraries, of more than 100,000 clones with an average insert size of 620bp, were constructed from mRNA isolated from the resistant and susceptible progeny bulks. About 288 clones were sequenced from each library and a total of 5733 and 7053 tags, total of 12,786 tags, were obtained from the resistant and susceptible SAGE libraries. About 1,700 unique genes were expressed at equal levels in both samples. Of these, 10 abundantly expressed genes accounted for about 5% of all expressed transcripts. One hundred and eight transcripts were differentially expressed

in the resistant bulk, at >5% probability level, including several transcripts that were completely absent in the susceptible bulk; twenty eight transcripts were at least 5 times more abundant in the resistant bulk (Figure 8.3). Primers were synthesized from these 28 tags for further analysis, namely tag annotation and genetic mapping. Transcripts that are found to map to the same region as the CMD resistant gene, in other words, that are strongly associated with the CMD resistance phenotype, will be used for fine-mapping, to screen BAC libraries for contig mapping and finally complementation. Partial length cDNAs for more than10 of the 28 tags have been recovered by PCR, using the tags as sense primers and an anti-sense primer from the plasmid vector, from a cDNA library constructed from mRNA of the resistant bulk in the vector pYES (Pharmacia biotech). Sequencing of the partial cDNA revealed down stream, known to be expressed in response to pathogen attack in plants, including a peroxidase gene. Annotation of other tags is still ongoing.

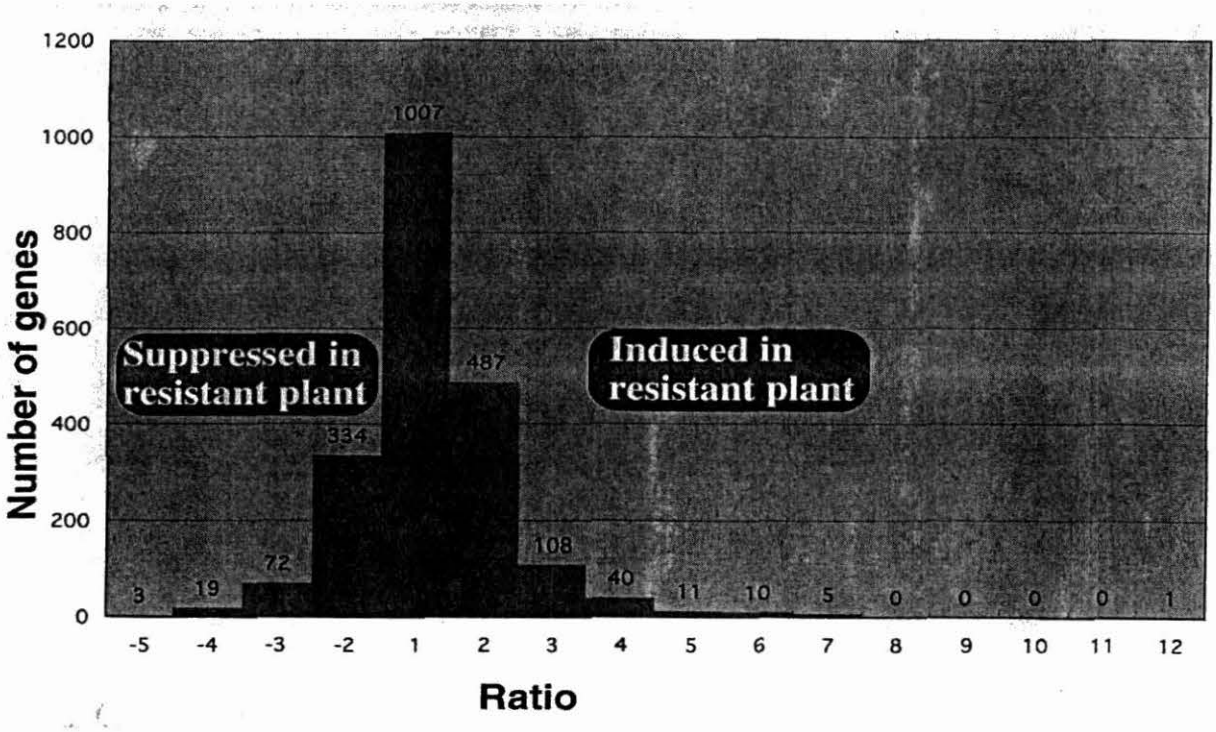


Figure 8.3. Transcripts differentially expressed in bulks of CMD resistant and susceptible genotypes.

The lack of ESTs in cassava, especially in CMD resistant genotypes has considerable slowed down the annotation of the tags. This work will continue during year 2001. The main objectives for next year are the genetic mapping of the 28 tags, and the generation of ESTs from the resistant bulk project to hasten tag annotation, in collaboration with the Iwate Biotech Center, Kitakami (Japan).

Achievements:
- Implementation of the SAGE protocol for cassava.
- Generation of 5,733 and 7,053 tags respectively for 2 mRNA samples obtained from CMD resistant and susceptible bulks, a total of 12,786 tags.
- Annotation of 10 of the 28 differentially expressed tags in the two bulks.

Activity 8.3. Positional cloning of a CMD resistance gene.

Specific Objectives:

a) to construct a BAC library from the TM3 source of resistance against CMD.
b) to test in cassava BIBAC agro-bacterium based transformation.

Rationale: The heterozygous nature of cassava implies that any attempt to introduce any trait, even those controlled by a single gene leads to the loss of a favorite variety. A more efficient way to introduce single gene controlled traits, such as CMD resistance, is through genetic engineering. Following the discovery of genetic markers linked to the gene controlling the new source of resistance to CMD and current efforts to identify candidate genes by the serial analysis of gene expression of CMD resistance, the stage is set for positional cloning of the CMD resistance gene. Three important criteria for positional cloning are a fine map of the appropriate genome region, based on a large mapping population, a bacterial artificial chromosome (BAC) library, and an efficient transformation protocol for complementation analysis. An appropriate cross for fine mapping was identified at IITA and permission was sought and obtained to isolate DNA from the cross. A cassava BAC library has been constructed by CIAT in collaboration with the Clemson University Genome Institute (CUGI), however the BAC library was in a cassava genotype other than TME3, donor parent of the new source of CMD resistance, plans are underway to construct another library from TME3. Finally the BIBAC agro-bacterium based transformation kit for the introduction of large DNA fragment into plants was obtained from Cornell University (Dr. Carol Hamilton) and is being tested out on cassava at CIAT.

Materials and Methods: The fine mapping population consists of two crosses between TME 3, the new source of CMD resistance, and TME117 or TMS30555, a susceptible land race and a susceptible improved line with a total of 789 progeny. The population was phenotyped for resistance to CMD under high, natural field infection pressure in June 2000. DNA was isolated from very young fresh leaves according to Dellaporta et al. (1983). Total DNA obtained was dissolved gently overnight at 4 °C in TE (10mM Tris-HCL 1mM EDTA) and quantified by fluorimetry (TKO 100 Hoefer). The CMD

phenotyping and DNA isolation of the mapping population was done at IITA during a visit by the post-doc CIAT scientist, and the DNA was shipped to CIAT for genotyping with candidate genes that will soon be identified.

In preparation for transformation of candidate BAC clones with the BIBAC vector, friable embryogenic callus (FEC) were induced in a CMD susceptible lines CM2177-2 using a protocol described by Taylor et al. (1996. Embryos were obtained by direct embryogenesis, culturing somatic embryos on Greshoff and Doy salts supplemented with vitamins and 10 mg/l picloram. Several selection rounds were carried out in this medium to obtain pure friable embryogenic cultures. The FECs are intended for use in the BIBAC agrobacterium-mediated transformation system; in preparation for this four different *A. tumefaciens* strains containing BIBAC2.H150 have been cultivated in medium AB and the bacteria concentration measured.

Results: About 200-500microgram of good quality DNA was obtained for each of the 537 genotypes, sufficient for both RFLP and PCR-based analysis of the population. CMD resistance valuation revealed the expected ratio 1:1. FECs were successfully induced in CM 2177-2 although the frequency was very low, they are currently being proliferated in liquid medium supplemented with Schenk and Hildebrand salts and vitamins and 10mg/l Picloram. Also the cultivation of BIBAC constructs in medium AB was successful.

Plans for the next year include: **a)** genotyping the 789 progeny with candidate genes identified in the cassava SAGE experiment; **b)** making a BAC library from TME 3 and build a contig around genome region carrying the CMD resistant gene; and **c)** transforming the FECs with candidate BAC clones carrying the CMD gene using the BIBAC system.

Achievements:

- DNA isolation of 537 genotypes from population derived from a cross between CMD resistant and susceptible African landraces.
- Evaluation of CMD resistance under high disease pressure of the population.
- FECs developed in cultivars CM 2217-2.
- BIBAC transformation system received from Connell University confirmed to be still viable.

References:

Taylor N.J., Edwards M., Kiernan R.J., Davey C., Blakesley D. and Henshaw G.C. 1996. Development of friable embryogenic calli and suspension cultures in cassava (*Manihot esculenta Crantz*). Nature Biotechnology 14: 726-730

Activity 8.4. Marker-assisted breeding of resistance to CMD in Latin American cassava gene pools.

Specific Objectives:

a) to introduce into CIAT the new source of resistance to CMD.
b) to initiate crosses between CMD-resistant genotypes and elite Latin American cassava germplasm.

Rationale: The availability of molecular markers for CMD resistance has made possible for the first time its breeding at CIAT. As a first step to CMD resistance breeding at CIAT, the new source of resistance to CMD, found in a group of closely related Nigerian cassava land races were imported from IITA to CIAT. Due to quarantine restrictions, the land races themselves could not be imported into Colombia, rather F_1 progeny obtained by crossing the resistant parent to an improved line and resistant to CMD maintained as *in vitro* culture of embryo axes were imported as the source of CMD resistance. The CMD donor lines were imported with the approval of the Colombian quarantine authorities. The *in vitro* plantlets were tested on arrival by ELISA and PCR methods for the presence of ACMV or EACMV before sub-culturing and transfer to the green house. Permission to transfer the plants to the field will be obtained from the Colombian quarantine authorities, and the plants will be transferred to a healthy site for hybridization with the elite parents of Latin American cassava gene pools at CIAT.

Materials and Methods: Twenty cassava lines from an F_1 mapping population having TME3, one of the land races with the new source of CMD resistance as parent, and resistant to CMD were shipped to CIAT from IITA as *in vitro* plantlets (Table 8.1). The plantlets were obtained from embryo axes cultures that have always been kept *in vitro* and have never been in the field, neither have they been in contact with the virus or its vector. The plantlets were sub-cloned using 2 nodal cuttings cultured in Murashige and Skoog's (1962) basal medium supplemented with 2% sucrose, $1mgl^{-1}$ GA3 (growth hormone) and 0.7% agar, pH adjusted to 5.6± 0.1 before sterilization. All plant cultures were maintained at 25-29oC with 12 hours photoperiod and 5,000 lux in culture rooms. The plantlets were tested for presence/absence of cassava mosaic virus (CMV) using enzyme linked immunosorbent assay (ELISA) and PCR-based diagnostic methods.

Plantlets were transplanted to the screen house after 5-6 weeks of culturing. Plantlets were carefully shaken out of culture tubes into a plastic bucket containing clean water and culture medium was washed off from the roots and then placed in jiffy peat pots three-quarters full of peat moss mixture. They were kept for hardening under a humidity chamber with an initial relative humidity (RH) of 100% for the first 3 days at 25-35oC in a shaded screen house. The RH in the chamber was gradually reduced, by perforating the chamber increasingly until it was equal to atmospheric humidity, by the 6th-8^{th} day after transplanting. After 3 weeks of hardening, the plants were transplanted into polythene plastic bags filled with sterile topsoil and kept in the screen house.

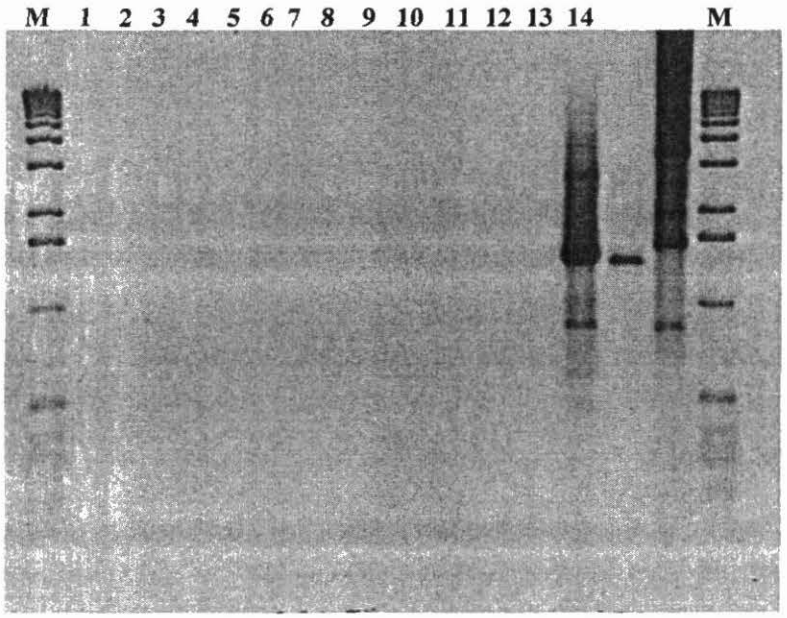
Table 8.1: List of resistant cassava lines sub-cloned *in-vitro* and currently available in the green house and the tissue culture room of the BRU.

Genotypes	Numbers sub-cloned *in-vitro*
C54	6
C35	4
C39	7
C22	3
C41	3
C33	6
C413	5
C24	5
C6	8
C243	7
C18	7
C127	8
C43	5
C373	9
C227	6
C101	8
C400	5
C377	2
C151	3
C19	8

Table 8.2. Result of the ELISA test to detect ACMV in cassava plantlets introduced from IITA (Nigeria), using Monoclonal antibibody 4C1-3F7.

Sample numbers	Genotypes	Absorbance (405nm)*	ELISA reaction (±)
1	C33	-0.016	-
2	C41	-0.016	-
3	C43	-0.018	-
4	C54A	-0.017	-
5	C54B	-0.016	-
6	C71	-0.016	-
7	C227	-0.017	-
8	C243	-0.017	-
9	C373	-0.015	-
10	C401	-0.003	-
11	Blanco	-0.096	-
12	Negative control (healthy)	-0.011	-
13	Positive control	1.488	+

*Any absorbance value more than twice that of the healthy control, was considered positive.



1. C33
2. C41
3. C43
4. C54A
5. C54B
6. C71
7. C227
8. C243
9. C373
10. C401\
11. Healthy check (-)
12. Positive check (BGMV)
13. Positive check (potato)
14. Positive check (Q3 plasmid)

M. 1 Kb molecular marker

Figure 8.4. PCR for the detection of the African Cassava Mosaic Virus (ACMV).

Results: An average of 5 plants each, per genotype, were successfully sub-cloned *in-vitro* (Table 8.2). A copy each from the 20 genotypes is maintained *in-vitro* in the tissue culture lab, while the remainder have been moved to the screen house. The results from ELISA and PCR diagnostics tests carried out on samples showed absence of the cassava mosaic virus and are shown below (Table 8.2 and Figure 8.4).

The CMD resistance donor plants are currently in the green house and will be moved to the field for hybridizations with elite parents of Latin American cassava gene pools once approval for this is obtained from the Colombian quarantine authorities. Next year, cassava plants from the 20 resistant lines will be planted on the field and hybridized with elite Latin American elite germplasm. Hybridized lines will be used in marker-assisted selections in collaboration with IITA, Nigeria in Africa.

Achievements:

- Cassava plantlets from 20 resistant lines to CMD successfully transferred *in-vitro* from IITA, Nigeria to CIAT.
- Cassava plantlets free from CMD from the 20 lines subcloned and copies maintained *in-vitro* in the tissue culture room of CIAT's BRU.
- Cassava plants from 20 resistant lines successfully established in the green house in CIAT.

Activity 8.5. A comparison of MAS and conventional approaches to test the utility of molecular genetic markers for the rapid conversion of African cassava gene pools to CMD resistant lines.

Specific Objectives:

a) to test marker-assisted selection of CMD resistance in African cassava gene pools.

Rationale: One of the primary objectives of genetic mapping and gene tagging efforts in cassava is to provide tools that can increase the efficiency and cost-effectiveness of cassava breeding. Markers increase the efficiency of breeding by the elimination of inferior genotypes at the seedling stage allowing the breeder concentrate on fewer genotypes for the crucial single row trial stage. Fifteen years of cassava breeding at the CIAT-Asia program has shown that that negative selection at the seedling stage, combined with indirect selection for yield, using harvest index, is most the efficient breeding scheme for yield in cassava. The availability of genetic markers for the new and more durable source of CMD resistance provides a unique opportunity to test the cost effectiveness and efficiency of marker-assisted breeding compared to conventional breeding for the introduction of CMD resistance gene into African cassava gene pools. The advantage of genetic markers for CMD resistance is that it enables the breeder to eliminate, at an early stage inferior genotypes and thus considerably reduce, by 50%, the materials to be established in the field, thus cutting his task and costs by half. Provided a cheap, slab gel-free method of genotyping can be developed, the cost-savings and increase in efficiency can be considerable. A joint project to test marker-assisted selection of CMD resistance was initiated with IITA this year.

Materials and Methods: The Nigerian cassava land races TME 3, TME4 and TME28, that carry the single dominant CMD resistance gene, were crossed to TMS 53808, TMS 30572, and TMS71173, and TMS91934, important parental lines at IITA. A target of about 500 seeds for each of the 12 crosses, making a total of 6000 seeds, has been set for the experiment. Seeds from all 6 crosses will be divided equally into two and designated as population A and population B. Seeds from populations A will be planted in the seedling nursery and evaluated with markers associated with the CMD resistance gene at 3 weeks after planting, while seeds from B will be planted but not evaluated. Plants bearing the CMD resistance gene from population A and all plants from population B will be introduced into the regular IITA cassava breeding scheme of seedling trial (ST), and single row trial (SRT). At the end of SRT the cost of maintaining populations A and B, and of genotyping population A will be estimated. The selection efficiency of markers against no markers will be also estimated for population A and B by determining the mean of both populations for traits of agronomic interest such as resistance to CMD, CBB, dry matter content and dry matter yield. The above activities

are the objectives of a Ph.D dissertation to be conducted by a student at IITA with technical and partial financial support from CIAT.

Results: More than 1500 pollinations each were performed for all possible crosses between the CMD donor parentsTME 3, TME9, TME28, and the IITA breeding parents TMS 53808, TMS 30572, TMS71173 TMS91934 at the IITA crossing block in Ubiaja, Nigeria. The total number pollinations are more than 18,000. A minimum of about 500 seeds is expected per cross. The seeds will be harvested beginning late October and will be established in the seedling nursery by early January 2000.

For next year the 6000 progeny, divided into population A and B, will be established. Seedlings from population A will be marker evaluated. Plants having the CMD resistance gene in population A and all plants from population B will then be transplanted to the field and evaluation of both populations will be carried out at the seedling stage.

Achievements:
- Generation of 12 crosses of about 6000 progeny in total for the comparison of MAS and conventional approach to cassava breeding.

Activity 8.6. SSR Marker assessment of the genetic structure of cassava landraces from principal growing regions in Sub-Saharan Africa and Latin America.

Specific Objectives:

a) *to evaluate genetic variability in cassava populations from Tanzani, as a model for the study of genetic structure of cassava in principal production regions.*

Rationale: The principal objectives of the cassava molecular genetic diversity study of cassava in major growing countries in Sub-Saharan Africa and Latin America is to elucidate the genetic structure of cassava land races, determine the salient factors responsible for the structure and use the information to rationalize cassava improvement strategies. A pilot genetic diversity assessment study was conducted in an important cassava growing area of Southern Tanzania, a region that has one of the highest per capita consumption of cassava in the entire African continent. A sub-set of 92 SSR markers from the Cassava MapPairs of SSR markers was multiplexed in the Tanzanian collections and 110 genotypes from CIAT. To graphically represent relationships between the genotypes, the raw SSR data was converted into genetic distance matrices and analyzed by a principal coordinate analysis (PCA. The

experienced gained in the pilot study will be extended to an assessment of genetic diversity of cassava land races in subsequent studies which includes studies of cassava collections from Nigeria, Uganda and the Amazonian region of Colombia.

Materials and Methods: A survey of genetic diversity in cassava was carried out in the Mtwara, Newala, Masasi, and Nachingwea districts of Southern Tanzania, a region between latitude 10° and 20° and longitude 30° and 40°. A total of 10 villages were visited. At each village farmers were invited to share their knowledge on the cassava land races grown by them and a few stakes, usually 4 to 5 500cm stakes, were then requested for each land race from the farmer. A total of 96 genotypes were collected. Another 100 land races were collected from the cassava germplasm banks at the Agricultural Research Institute (ARI) at Naliendele, and at Kibaha, where land races from all over Tanzania are kept for breeding purposes. Woody stakes of all genotypes were planted in the green house in 20litre pots at the Kibaha agriculture research station and young fresh shoots were harvested after 3-4 weeks for DNA isolation. DNA extraction, according to Dellaporta et. al (1983), was carried out at the biotech laboratories of ARI Mikocheni (Dr Allois Kulaya). Quarantine restrictions due to the African cassava mosaic disease (ACMD), prohibits the shipment of cassava tissue from Africa to Latin America. Leaf tissue was from 3-4 week old plants of the collection established in Kibaha, and DNA extraction was. The DNA samples were shipped to CIAT, Cali, Colombia, quantified and diluted in preparation for the SSR Marker analysis. DNA from the core collection genotypes was isolated from the CIAT and IITA accessions by the same method.

A subset of 92 SSR markers, with broad coverage of the genome, from the 186 SSR markers developed at CIAT (Mba et. al. 2000), were organized into 23 quadriplexes (multiplexes of 4 markers). The quadriplexes were designed by searching for sets of 4 markers, from the pool of 186 SSR markers, with the condition that their primers do not form heteroduplexes at the 3' end. The primers were multiplexed on all the 315 genotypes using flourescent primer pairs. PCR product was denatured and electrophoresed on 4% polyacrylamide gels using an automated DNA sequencer ABI model 377 (Perkin Elmer Inc.). Extraction of the raw gel data was done using the ABI PRISM Gene Scan analysis software (Perkin Elmer Inc), and the Genotyper software.

The SSR raw allele data obtained from genotyper was transformed into genetic similarity matrices by the Nei's standard distance (Gst), and proportion of shared alleles (PSA) using the software microsat (http:www.lotka.stanford.edu/microsat.html). Prinicipal Component Analysis (PCA) (Sneath and Sokal 1973) to test the degree of clustering among land races was performed on the similarity matrix using the JMP computer software (SAS Institute 1995).

Results: Raw SSR allele data could be extracted for 68 SSR markers; 24 markers yielded complex non-disomic marker genotypes or had too many missing data points. The PCA analysis of relationships from similarity indices of the Tanzanian genotypes is shown in Figure 8.5. The PCA reveals genetic differentiation amongst the land races that is not strictly according to taste or location. The other basis for the clusterings may

be the source of the land races; attempts are therefore being made to trace the sources of the land races. The clusters may also represent heterotic groups which is of benefit to cassava breeding. An effort is being made with collaborators in Africa to perform crosses between representatives of the clusters.

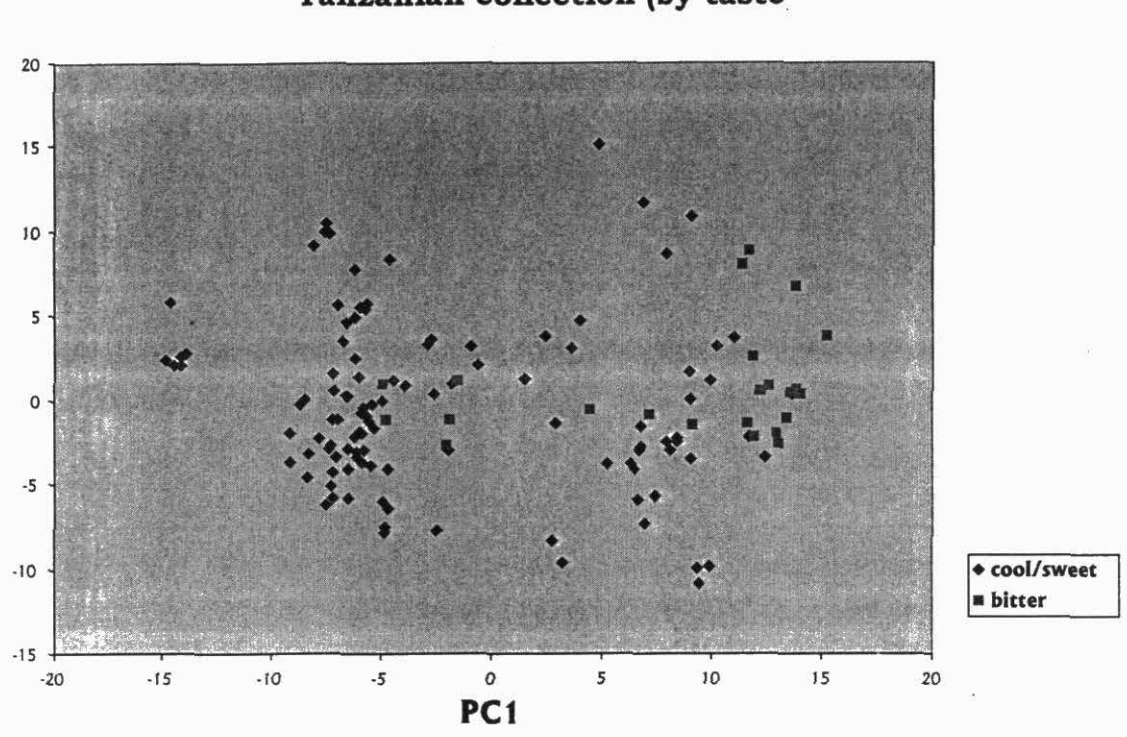


Figure 8.5. Principal Component analysis of similarity indices derived from allele sizes from 68 SSR markers of 173 cassava land races from Tanzania.

For the following year a completion of the comparison of the Tanzanian materials and core collections from CIAT using an analysis of molecular variation (AMOVA) will be carried out. Then a search for parent-offspring relationships in the Tanzanian genotypes using SSR allele data will be initiated. An analysis of similar collections from Nigeria, Uganda and the Amazonian region of Colombia with SSR markers, is also planned.

Achievements:

- Elucidation of the genetic structure of cassava land races from a major growing region of Southern Tanzania.
- Development of a set of 68 SSR markers, with broad coverage of the cassava genome, for molecular characterization of cassava.
- Establishment of a procedure for the collection and SSR marker analysis of cassava germplasm.

Activity 8.7. First Workshop on the Molecular Genetic Diversity of Cassava Network (MOLCAS), held at CIAT August 22-24, 2000.

Specific Objectives:

a) to organize a workshop on assessing molecular genetic diversity of cassava landraces.
b) to strengthen collaboration among cassava scientists from different continents.

The MOLCAS Network: The molecular genetic diversity network of cassava (MOLCAS) comprises of scientists drawn from institutes in Malawi, Uganda, Tanzania, Nigeria, Brazil, Sweden, Colombia, France, and the USA, and it is funded by the International Chemical Sciences Program (IPICs), University of Uppsala. The goal of the network is to enhance the enduring and emerging roles of cassava as a food security crop and industrial crop by the assessment and exploitation of genetic variation. The network assists members, with tools, information, and in some instances funds, with the collection molecular and agronomic characterization of cassava land races in Africa and Latin America.

Studies concluded to date by members of the network include:

- ✓ A SSR study of cassava land races in Northern Malawi (Bvumbwe Agricultural Research Station Malawi, SLU, Uppsala, Sweden).
- ✓ A SSR study of cassava land races in Southern Tanzania (CIAT, SLU, ARI-Mwanza, ARI-Mikocheni Tanzania).
- ✓ A molecular marker SSR study of cassava land races from the Amazonian basin and North Eastern coast of Brazil (ICA-Campinas, Brazil).

Ongoing studies include:

- ✓ SSR study of a cassava collection from the Amazonian region of Colombia (CIAT, Uni Valle Colombia).
- ✓ SSR study of land races in Nigeria (IITA, NRCRI Nigeria, CIAT).
- ✓ Test for heterotic (hybrid vigor) patterns in clusters of previous collections (CIAT).

Summary of Workshop Report: After 2 years of the existence of the network, it was decided to hold a workshop to review progresses, and discuss future perspectives. The first MOLCAS workshop was therefore planned for August 22-24, 2000 at CIAT headquarters, Cali, Colombia. Invitations were sent out in May to members and interested CIAT participants. Two days were spent reviewing the results of cassava collections and their analysis with molecular markers in Brazil, Malawi, Tanzania, CIAT, and IITA collections, the third day was spent discussing future projects and deciding on priorities.

A total of 8 presentations were made during the workshop. Three talks dwelled on new methodologies, namely: recent advances in plant genomics and the implications for study and use of genetic diversity; GIS tools for guiding collection of genetic diversity; and the need to involve farmers in the study of genetic resources. There were 3 genetic diversity assessment reports, conducted in Brazil, Malawi and Tanzania respectively; the other 2 presentations were from CIAT on the study of genetic diversity in wild relatives and the CIAT cassava collection. The presentation on advances in plant genomics, given by Dr Matthew Blair, CIAT bean geneticist, on behalf of Professor Steve Kresovich, was a good introduction to advances and powerful new tools of plant genomics that are redefining traditional thinking on genetic diversity. In particular, the new methods of uncovering bi-allelic, highly abundant single nucleotide polymorphisms (SNPs) promises to provide unprecedented resolution for genetic studies.

The studies of genetic diversity assessment of cassava land races in two African and one Latin American country provided new evidence on the elucidation of the genetic structure of cassava in its primary and a secondary center of diversity. The studies, conducted with SSR, AFLP, and RAPD markers revealed genetic differentiation in the land races along lines that are not fully understood at the moment, although some evidence points to bitter and sweetness of roots, and multiple introductions. Questions raised about the underlying reasons for observed organization of genetic variation and the preservation of the genetic structure in the primary and secondary centers of diversity will be the objective of further studies. Finally, we were reminded about the wisdom of properly marrying farmers' knowledge of cassava with molecular tools to make the best interpretation of results.

Future Activities: IPICs, the principal donor of MOLCAS has invited the network to apply for funding during the period 2001-2003, the last session of the workshop was therefore spent brainstorming projects the network considers priority for the future. Anke Van de Hurk, IPGRI scientist based at CIAT, served as a very able facilitator in this process. In the period 2001-2003, the network has prioritized the following studies:

- SSR-marker study on the role that seedlings play in preserving genetic diversity during the CMD disease epidemic (Uganda).
- A study on broad adaptation of cassava genotypes to Southern Africa (Malawi, Tanzania, Mozambique, and Uganda).
- Farmer keys for identification of their varieties (Malawi).
- A study of broad adaptation of cassava genotypes in Brazil (Brazil).
- Continuation of the country study of genetic diversity structure (Democratic Republic of Congo, Ghana and Mozambique).
- Development of a set of highly polymorphic markers for studying genetic diversity in cassava.

Activity 8.8. Root quality genes from wild relatives of cassava for broadening the crop genetic base.

Specific Objectives:

a) to produce interspecific hybrids and establish the seedlings in the field.
b) to evaluate root quality traits as a start point for the advanced backcross QTL mapping scheme.
c) to search for the natural occurrence of apomixis.

Rationale: For several years now, it has been shown that the "tremendous genetic potential locked up in germplasm banks can be released by shifting the paradigm from searching for phenotypes to searching for superior genes with the aid of new tools of "genomics" (Tanksley and McCouch, 1997). The value of exotic species as a source of useful alleles, while predicted by Vavilov, the founder of modern gene banks, more than 50 years ago, is only now being recognized and exploited using molecular genetic maps and the advanced back cross mapping scheme (Tanksley and McCouch, 1997). The tools, in particular a molecular genetic map of cassava, and easily assayed PCR-based molecular markers are now available in cassava (Fregene et. al. 1997, Mba et al., 2000), opening new horizons for the efficient use of exotic germplasm. Root quality traits known to exist in the wild and not in cassava include higher levels of protein content from *M.tristis* and *M. carthaginensis* and waxy starches from *M. crasisepala*. Resistance to post-harvest deterioration have also been reported to occur in *M. walkerae*

Materials and Methods: An inventory was made of *Manihot* species and their hybrids with cassava available as seeds and plants at CIAT in collaboration with the genetic resources unit. A representative sub-set of germplasm available at CIAT was germinated from seeds and stakes in the green house and transferred to the field at the Universidad Nacional (Palmira Campus) experimental farm in August. At 9 months after planting, two roots will be "milked" from the wild *Manihot* accessions and interspecific hybrids and evaluated for the following root quality traits according to standard procedure established at CIAT:

- Protein content.
- Dry matter percentage.
- Amylose / amylopectin content.
- Percent post-harvest deterioration; evaluated at 3 days after harvest.

Genotypes that show high root protein or dry matter, or amylopectin content or resistance to PHD will be crossed or backcrossed (for inter-specific hybrids) to elite parents of the cassava gene pools, these parents have also been established nearby for this purpose. Evaluation of root quality traits on roots of the *Manihot* species and inter-specific hybrids will begin next year. Crosses and backcrosses to initiate the advanced backcross scheme. Since plants will be in the field, inflorescences from each plant will be covered with a cloth bag (prevents outcrossing) in order to identify the eventual occurrence of apomixis.

Achievements:

- Inventory of available seeds and plants of *Manihot* species and inter-specific hybrids available at CIAT.
- Field establishment of a representative sub-set of seeds of *Manihot* species and inter-specific hybrids for evaluation of root quality traits.

References

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Tanksley S D, and McCouch S 1997. Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. Science, vol 277: 1063-1066.

Activity 8.9. Progress towards a PCR-marker based map of cassava and its utilization in cassava breeding.

Specific Objectives:

a) to develop a large-scale high throughput genotyping system useful for breeding purposes.
b) to identify many SSR markers to provide wider coverage of the genetic map of cassava.
c) to convert existing mapped RFLP markers to PCR-based markers.

Rationale: The application of the molecular genetic map of cassava in crop improvement is becoming more and more of a reality in light of successes in discovering

associations between mapped markers and genes controlling traits of agronomic traits. However one draw back remains and that is the majority of the markers on the map do not lend themselves easily to the large-scale high throughput genotyping required for MAS. An effort to remedy the situation was initiated two years ago and has currently yielded more than 200 SSR markers of which at least 90 have been placed on the male- and female-derived maps. However many more SSR markers are required to provide wider coverage, and there is a need to convert existing mapped RFLP markers to PCR-based markers. The need for a PCR-based map of cassava is all the more urgent considering that an advanced back cross QTL (ABC-QTL) mapping project has been initiated to introgress enhanced root quality traits from wild relatives of cassava. The success of any ABC-QTL relies heavily on a reliable frame-work map amongst other criteria.

Materials and Methods: The SSR enriched library from variety CMC40 enriched for $(CT)_8$, $(GT)_8$, developed by Keith Edwards, Bristol University, UK, and cloned in pJV1, has been used to identify more than 150 SSR markers (Mba et al., 2000). It was decided to plate and screen many more clones from the library again with the oligos $(CT)_{20}$, and $(GT)_{20}$. The dot blot screening of the library is as described earlier (Mba et. al. 2000). Plasmid DNA was isolated from a total of 1000 positive clones by the QIAprep plasmid isolation kit, and 1-3ul of plasmid preparation was sequenced on an ABI377 automated sequencer, using the Universal M13 primers at the Cornell University Biotechnology Resource Center. Primer design was by the web based software Primer 3.0 found at http://waldo.wi.mit.edu/cgi-bin/primer/primer3.cgi.

The genetic map of cassava comprises of at least 250 RFLP markers on the male- and female-derived maps. It is an important resource and if they can be converted into co-dominant PCR-based markers sequencing and primer construction they will go a long way to ensure a quick completion of a PCR-based map of cassava. Plasmid DNA was prepared from overnight mini-prep cultures of *E.coli* stocks containing the appropriate RFLP probe using the QIAprep plasmid isolation kit. The clones are ready and will be sequenced shortly from both ends using the universal and reverse M13 primers (for PUC18 plasmids), and T3 and T7 primers (for pBlueScript plasmids).

Results: A total of 2400 putative clones were identified from screening 10,000 clones. A sub-set of 1000 clones was selected for sequencing. Of this about 450 clones contained unique SSR markers and primers, 20-mers long, could be designed for 300 clones from regions flanking the repeats. Other clones had the SSR too close to the end of the fragment, or had very short, <4 di- or tri-nucleotide repeats. The primers will be synthesized and tested in the parents of the mapping population and polymorphic ones scored in the progeny. Plans for next year include:

a) Survey of 300 new SSR primer pairs in the parents of the cassava map population and evaluation of polymorphic markers in the progeny.

b) Primer design for 250 RFLP and evaluation of their ability to detect polymorphisms as PCR products (STSs) or after digestion with restriction enzymes (CAPs)

Achievements:

- Sequencing of 1000 positive SSR clones and the design of primer pairs for 300 new SSR markers.
- Initiation of the conversion of RFLP markers on the genetic map of cassava to PCR based markers such as sequence tagged sites (STS) of cleaved amplified polymorhic sites (CAPs).

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Mba REC, Stephenson P, Edwards K, Melzer S, Nkumbira J, Gullberg U, Apel K, Gale M, Tohme J, Fregene M 2000. Simple Sequence Repeat (SSR) Markers Survey of the Cassava (Manihot esculenta Crantz) Genome: Towards a SSR-Based Molecular Genetic Map of Cassava. (Theor and Appl Genet in press)

Activity 8.10. QTL mapping of early bulking in cassava.

Specific Objectives:

a) to identify genotypes contrasting for early bulking.
b) to identify traits strongly associated with early bulking.
c) to find markers linked to genes or QTLs controlling such traits for marker-assisted breeding of earliness.

Rationale: Based upon the results of a preliminary evaluation of early bulking in the F_1 cassava map progeny, a second study, to study the genetics of early bulking was initiated January 1999 and concluded late last year. Early bulking is one of the most important traits in the acceptance or rejection of cassava varieties in Sub-Saharan Africa based on the findings of the collaborative study of cassava in Africa (COSCA) (Nweke et al, 1994). It is also a much sought after trait in Latin America due to the increased flexibility of supply of raw material it offers in the industrial production of cassava for animal feed and starch. The objectives of the early bulking study were to identify traits strongly associated with early bulking, and subsequently find markers linked to genes or QTLs controlling such traits for marker-assisted breeding of earliness. Results obtained from the early bulking study are presented in this report. To verify

QTLs identified in the early bulking study, a new F_1 S_1 mapping population of 240 individuals was developed and established *in vitro* from embryo axes and transferred to the field. The population will be genotyped on a genome-wide basis with SSR markers and evaluated in replicated trials for traits found to be most strongly associated with early bulking next year.

Materials and Methods: A preliminary assessment of early bulking was conducted in 1998 by harvesting the F_1 mapping population at 7 MAP in CIAT Palmira location (Fregene et. al. 2000). Dry matter yield was determined on three plants per genotype. Based on results from this evaluation, 40 early bulking genotypes and 40 late bulking groups were selected. Carefully picked healthy cuttings of the 80 selected genotypes were planted in a new experiment in December 1998 in CIAT Palmira. A early bulking cassava land race (Mandioca de tres meses) introduced from Brazil was used as control. Field layout was a randomized complete block design of two replications. Each plot had 60 plants of each genotype in a 6 x 10 (column by row) arrangement; the central 32 plants, arranged as eight rows of four plants, were used in the sequential harvest with an interval of three weeks, beginning at 6WAP through to 30 WAP. A total of nine harvests were done within a seven month duration after which the experiment was terminated (July 1999).

At each harvest, four plants in a row within a plot, per genotype, were evaluated for root yield and other traits assumed related to bulking. The traits evaluated were: plant height, plant vigor, leaf area index, fresh root yield, fresh foliage, number of roots per plant, root diameter of the biggest five storage roots. Others were harvest index, measured as the ratio of root yield to total harvested biomass, root dry matter and dry foliage. Plant vigor was evaluated on a visual rating scale of 1 – 5 (1 = poor; 5 = best). Dry matter assessment for root and foliage were carried out by taking samples of each plant and oven-drying to a constant weight to determine dry matter content. Starch initiation (or commencement in bulking) was also evaluated. Samples from roots were randomly picked, sectioned and then stained with iodine for blue black coloration test for starch presence.

Multiple regression analyses were performed to determine the linear relationships between evaluated traits (independent variable) and dry matter root yield (dependent variable) for each harvest time based on the linear equation:

$$Y = a + b_1X_1 + b_2X_2 + \ldots b_kX_k + e$$

Where Y is the dependent variable (dry matter root yield); a is the intercept and b (1,2...k) is the partial regression coefficient of the corresponding independent variable and X (1,2,...k).refer to the independent variables, with e as the error term.

Regression coefficient of each trait variable was accepted as significantly associated to early bulking at $P < 0.05$. Single linear regression of dry matter root yield with time (i.e. progressive increase in dry matter root yield (bulking) over the nine harvest periods) per

genotype was done to determine rate of bulking given by the regression coefficient. QTL analysis was done for traits significantly linked to early bulking using QGENE (Nelson, 1997) and markers linked to the traits associated with early bulking were declared significant at $P< 0.005$. The PGRI computer package (Liu, 1995) was also used for QTL analysis.

Table 8.3. *P* levels of traits evaluated at each harvesting stage of early bulking assessment at Palmira over a 30-week period.

Variables	6 WAP	12 WAP	12 WAP	15 WAP	18 WAP	21 WAP	24 WAP	27 WAP	30 WAP
Starch initiation	0.0040*	0.9275	0.5489	0.5575	0.0463	0.7878	0.1399	0.7663	0.3926
Size dif/tion	0.0857	0.0192*	0.1056	0.5250	0.4214	0.5839	0.8279	0.0995	0.2152
Root diameter	0.0001*	0.0001*	0.0002*	0.2195	0.0788	0.3129	0.2702	05153	0.3672
Dry foliage	-	-	0.0254*	0.0701	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Harvest index	-	-	*0.0018**	*0.1418*	*0.0001**	0.0001*	0.0001*	0.0096*	0.0001*
Number roots	-	-	0.0638	0.0254*	0.4147	0.9160	0.0100*	0.4294	0.1197
Plant height	-	-	0.4876	0.7258	0.2932	0.5566	0.1149	0.2296	0.0980
Plant vigor	-	-	0.1393	0.9957	0.4120	0.3618	0.7240	0.2124	0.1332
Adj. R^2	**0.51**	**0.83**	**0.82**	**0.72**	**0.84**	**0.82**	**0.86**	**0.67**	**0.82**

*Significant variables at each evaluation stage

Results: At 6WAP when sequential harvesting commenced, it was observed that 79% of the early bulking population had already started synthesizing starch implying little genetic variation in starch initiation within the population but only 16% of the population had shown development of storage roots based on visual observation. At the next evaluation, 9WAP, over 75% of the population had storage roots. Results showed that yield was significantly correlated to all other traits evaluated. The multiple linear regression model using all other traits as independent variable adequately explained variation observed for early bulking. The proportion of variation accounted by the independent variables, given by the multiple coefficient of determination (R^2), was high and the adjusted R^2 varied from 0.67 – 0.86 between 12 WAP and 30WAP. Our analysis reveal that foliage and harvest index are the two most important factors influencing early bulking. These traits were evaluated between 12WAP and 30 WAP and were found to be significantly associated to early bulking for most of the times (Table 8.3). Within the evaluation period of 12WAP and 30 WAP, dry foliage and harvest index were highly significant in their partial regression coefficients for early bulking in five of the six evaluation times or 83% of the evaluation period. All other traits were most of the times non-significant in their partial regression coefficients and thus weakly related to early bulking (Table 8.3). Based on our result, root dry mater yield is a

function of total plant biomass, harvest index and dry matter percentage of fresh root yield.

QTL analysis for harvest index and dry foliage showed genomic regions with significant effects for these traits associated with early bulking. Three QTLs each were found for foliage dry weight and harvest index (Table 8.4). An RFLP marker CDY76 on linkage group J alone explains about 33% of the phenotypic variance for foliage at 24 WAP and hold great potential for marker assisted breeding given its major effect for foliage/shoot development. For harvest index, a major QTL (rBEST-2) chromosome A in the male map accounted for 32% of the phenotypic variance at 18WAP (Table 8.4). Kawano et. al. (1998) showed that selection of harvest index in breeding scheme is an efficient indirect selection parameter for root yield. Based on the analysis and information from the early bulking study, it is apparent that early bulking (and by extension yield), can be increased more effectively by using a selection criteria based on foliage (or total plant biomass), and harvest index in addition to yield potential of the genotype.

Table 8.4. Markers identified to dry foliage, harvest index and bulking rate in the early bulking trial.

Trait	Marker	Chromosome	WAP	R^2	P
Dry foliage	CDY 76	NgJ	24	0.33	0.0002
	CDY131	NgL	24	0.25	0.0018
Harvest index	GY142		15	0.29	0.0009
	GY212		27		
	rCDY106	NgK	15, 27	0.23	0.0026
	rGY55	CmF	15	0.19	0.0044
	rBEST-2	CmA	18	0.32	0.0002
	GY68	CmA	18	0.19	0.0050
	nGY162	CmE	18	0.19	0.0047
Bulking rate	GY202			0.14	0.0012
	GY142			0.19	0.0038
	rP3	NgQ		0.23	0.0032

*Chromosomes with prefix "Ng" are for the female derived map while chromosomes with prefix "Cm" are for the male derived map.

Identification of putative QTLs for traits associated with early bulking provides a first step in the understanding of the genetics of these complex traits. Deploying these marker tools in crop improvement requires further testing. To confirm putative QTLs identified for early bulking and to propose a model of genetic control for these traits, a F_1 S_1 population was developed by selfing a genotype from the F_1 mapping population possessing high foliage and high harvest index, and favorable QTLs alleles for these

traits. Self-pollination resulted in 725 seeds, which were then tested for viability by soaking seeds in water. After the viability test, embyro culture was carried out for 473 seeds in the 17N culture medium (1/3 medium, supplemented with 0.01 mg l^{-1}NAA, 0.01 mg l^{-1} GA_3, 1.0 mg l^{-1} thiamine-HCL, 100 mg l^{-1} inositol, 2% sucrose, 0.7% agar (Sigma Co.) and 25 mg l^{-1} of a commercial fertilizer containing: N 10, P 52, K 10, pH 5.7-5.8 (Roca 1984) as follows. Mature seeds were treated with sulfuric acid for 50 minutes, then thoroughly washed and rinsed before soaking in water for 30 minutes. Seeds were surface-sterilized by immersion in 70% alcohol for 5 minutes followed by immersion in 5% sodium hypochlorite and tween for 20 minutes, and then rinsed thrice. Under aseptic condition, the seeds were split along the longitudinal axis and embryonic axes removed by means of sterile forceps and scapel germinated. Excised embryonic axes were placed radicle down on 17N medium. The embryo cultures were incubated in darkness for three days to promote radicle growth and then transferred to growth chambers with a 12hr photoperiod. A total of 240 progeny were hardened and established in the field.

Next year the F_1 S_1 population will be genotyped on a genome-wide basis with SSR markers and evaluated in replicated trials for traits found to be most strongly associated with early bulking.

Achievements:

- Identification of traits (harvest index and dry foliage weight) with strong influence on early bulking.
- QTLs associated with harvest index and dry foliage weight.
- Development, in vitro and field establishment of F_1 S_1 population for marker inheritance and fidelity studies

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