OUTPUT 3
Development of new genetic stocks and improved gene pools for their evaluation in key target environments.

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

Activity 3.1 Selection of progenitors based on previous cycle results and information from other outputs (i.e., resistance/tolerance, root quality traits, etc.).

Rationale

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

Specific Objectives

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

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, clones with outstanding performance for the most important agronomic traits are selected. After the analysis of results is conducted with data across two years, those genotypes exceeding at least one standard deviation from the overall mean are considered as parents for the next generation. Sometimes, landraces or already released cultivars that can contribute special features to the progenies generated are also included. Lately, thanks to the modifications introduced to the evaluation process selection of parents is greatly affected by data of the progenies they produce (≈ general combining ability). It is envisioned that about 15-20% of the parental lines will be changed, eliminating those with poor general combining ability and introducing new clones that have had outstanding performance *per se* in

Advanced Yield Trials to assess their breeding value.

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

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

Results

The parents selected for the development of gene pools targeted to specific ecosystems is presented in Table 3.1. The agronomic performance of these materials is described further down in this document. Seed will be harvested from July, 2005 through December, 2005. F1 plants will grow until the planting of the trials early in 2006. A major decision to take in the genetic improvement of crops is how to choose materials for use as parents that will produce new varieties with increased production potential and adequate adaptation to the environmental conditions under which they will be cultivated.

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

During this year, the genotypes listed in Table 3.1 were selected to produce a new generation of crosses. These materials had stood out for their excellent performance *per se*, and for demonstrating good levels of *general combining ability* in relation to the results observed in the respective *Clonal Evaluation Trials*. The agronomic performance of some of these materials *per se* is also described below. At the bottom of the table parental lines for special purpose crosses have also been listed. The seed produced from the current crossings will be harvested until December 2005.

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

General purpose crosses			
Sub-humid tropics	**Acid-soil savannas**	**Mid-altitude valleys**	**High-altitude environments**
CM 4365-3	CM 523-7	CG 489-31	CM 7138-7
CM 6119-5	CM 2177-2	CM 2772-3	CM 7138-12
CM 6754-8	CM 6921-3	CM 7514-7	CM 7190-2
CM 6758-1	CM 6975-14	CM 7951-5	CM 7438-14
CM 7514-8	CM 7052-3	CM 8370-10	CM 7595-1
CM 8027-3	CM 7951-5	CM 8370-11	CM 8106-4
CM 8475-4	CM 9460-9	SM 1642-22	SM 1053-23
CM 9067-2	CM 9460-12	SM 1660-4	SM 1958-13
SGB 765-2	CM 9460-15	SM 1779-7	SM 1495-22
SGB 765-4	CM 9460-41	SM 1855-15	SM 1703-17
SM 1427-1	CM 9461-1	SM 1871-33	SM 1937-1
SM 1433-3	CM 9461-15	SM 1965-1	SM 1946-2
SM 1511-6	CM 9463-19	SM 2052-4	SM 2227-21
SM 1521-10	CM 9464-33	SM 2058-2	SM 2229-36
SM 1565-17	CM 9464-36	SM 2985-7	SM 2233-11
SM 1637-22	SM 1363-11	SM 2211-3	MCOL 2261
SM 1650-7	SM 1565-15	HMC 1	
SM 1656-7	SM 1812-69	INIVIT-Cuba	
SM 1669-5	SM 1821-7	**Special purpose crosses**	
SM 1669-7	SM 1859-26	**Yellow roots**	**ACMD resistance**
SM 1759-29	SM 1864-10	AM 320-52	C-4
SM 1778-45	SM 2219-11	AM 320-80	C-6
SM 1973-25	SM 2452-6	AM 320-120	C-18
SM 2081-34	SM 2201-44	AM 320-145	C-19
SM 2546-32	SM 2632-4	AM 262-8	C-24
SM 1629-4	SM 2636-6	CM 2772-3	C-33
SM 2629-36	SM 2638-13	CM 4919-1	C-39
SM 2772-5	SM 2640-6	CM 6119-5	C-41
SM 2782-4	SM 2727-12	SM 1859-26	C-43
MTAI 16	SM 2730-1	MBRA 337	C-54
MVEN 25	SM 2739-4	MBRA 463	C-101
	SM 2786-10	MCR 87	C-127
	SM 2792-31	MBRA 502	C-227
	SM 2792-32	MBRA 1107	C-243
	MBRA 502	MBRA 1251	C-373
	MCOL 638	MBRA 1400	C-377
	MCOL 2737	MCOL 1734	C-400
		MCOL 2141	C-413
		MCOL 2199	
		MCOL 2279	
		MCOL 2318	

Planting materials were also selected from these parents to seed the *F1* in July 2004. In addition to crossing, these lines were also self-pollinated to begin an S_2 recurrent selection scheme to improve each of them for tolerance to inbreeding. The justification for this approach is given later when the description of a cassava-breeding scheme based on the production of doubled-haploids described in Output 2.

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

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

Activity 3.2 Establishment of crossing blocks and production of recombinant seed from previously established blocks.

Rationale

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

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

Specific Objectives

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

Materials and Methods

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

Table 3.2. Production of recombinant cassava seed at CIAT, Palmira, Valle del Cauca, Colombia, between July 2003 and October 2004.

Objective of the cross	**Controlled crosses**	**Polycrosses**	**Total**
Interespecific crosses			
Waxy starch	25	-.-	25
Posharvest physiological deterioration	23	-.-	23
High protein content n the roots	110	-.-	110
Protein content & resistanse to ACMD	2754	-.-	2754
Resistance to mites & ACMD	2721	-.-	2721
Self-pollinations			
S_1 plants	8436	-.-	8436
S_2 plants	3645	-.-	3645
S_3 plants	391	-.-	391
Yellow roots	3148	-.-	3148
Foliar retention	-.-	19403	19403
Herbicide resistance	-.-	2663	2663
Resistanse to CBB	2818	-.-	2818
Resistanse to ACMD	6771	-.-	6771
Specific adaptation to			
Sub-humid environment	3814	14351	18165
Acid-soil savannas	1451	10398	11849
Mid-altitude valleys	2689	12521	15210
Hillsides	502	12365	12867
Total	**39298**	**71701**	**110999**

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

Results

More than 110,000 recombinant cassava seeds were produced at CIAT's Experiment Station, Palmira, during July 2003 to October 2004 period (Table 3.2). Although the recombinant seed was produced at CIAT, the generated seedlings used to be transplanted to fields outside the Experiment Station and under conditions of isolation from other cassava crops. Thus, the generated *F1* plants grew and were maintained under conditions where possibilities of contamination from frogskin disease were minimized. This strategy, as can be seen in the description of results from different *Clonal Evaluation Trials*, has been highly successful in virtually eliminating the incidence of this disease from the nurseries for cassava improvement at CIAT. The production of botanical seed within the CIAT Experiment Station did not represent high risk because this disease, which is probably induced by a virus, viroid or phytoplasm, is not likely to be transmitted through botanical seed.

Activity 3.3 Generation and distribution of advanced breeding materials for National Programs.

Rationale

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

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

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

Results
A considerable fraction of the seed produced by the project has been transferred to National Programs in different regions of the world. As shown in Table 3.2, more than 110,000 recombinant seeds were produced between June 2003 and October 2004 and about 30% of that seed (34366) has been shipped to our collaborators (Table 3.3). Since the retirement of our cassava breeder stationed in Thailand in 1998 an increasing number of recombinant seed originated in CIAT-HQ has been shipped to Asia. In the future, we foresee that the flux of improved germplasm between CIAT-HQ, and the Thai and other Asian breeding programs will continue, and it will be through CIAT that other National Programs will receive progenies involving the latest selections of elite germplasm from Asia.

Table 3.3. Shipments of recombinant seed produced within the project from September 2003 through September 2004.

Continents	Genotypes in-vitro	Crosses (families)	Plants (in-vitro)	Seeds in the shipment
Latin America				
In-vitro	52		476	
Hybrid seed		75		17043
Asia				
In-vitro	275		649	
Hybrid seed		100		15323
Europe + USA				
In-vitro	242		266	
Hybrid seed		10		2000
Total				
In-vitro	569		1391	
Hybrid seed		185		34366

Because of a self-imposed restriction for in-vitro shipments of cassava germplasm CIAT shipped a limited number of vitro-plants in the last two years. This restriction, however, has been gradually eliminated and therefore CIAT will increase the shipment of vitro-plants. To recover the lost time, the project has set up a tissue culture laboratory that produces large

quantities of vitroplants for our colleagues. The Genetic Resources Unit previously carried out this activity but the number of clones to be produced and shipped far exceeds the capactity and function of that Unit. Several plants from each clone have been or will be sent before the end of the year to countries in Asia, Latin America and the Caribbean and to IITA. As a result of this comprehensive on-station participatory evaluation and selection with the farmers, and NARS partners of the various countries, promising improved genotypes with desirable characteristics for end users will be identified (as has been the case in the past) under the local environmental conditions in each of the participating countries. A total of 1391 vitroplants, representing 569 genotypes were shipped during the past year.

Activity 3.4. Selection of recombinant progenies for broad and specific adaptation within major agro-ecosystems.

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 3.4. The most relevant ecosystems are the semi-arid and sub-humid tropics, for which we devote the majority of our efforts. The main selection activity is conducted in sites selected to represent the conditions of the target ecosystem. For every genotype that was tested in those sites, a copy was maintained at CIAT-HQ. This location is considered to be free of bacterial blight and some important viruses, and to maintain that condition, the introduction of vegetative material from other areas is restricted. In case vegetative material has to be brought to HQ, then it has to pass through quarantine, which usually takes more than a year.

Specific Objectives

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

Materials and Methods

For each of the zones we conduct a recurrent selection program, with a progressive set of stages as described in Figure 3.1**.** As the stages progress, we give more emphasis to traits of lower heritability, because we have more planting material for each genotype, and the evaluation can be conducted in bigger plots with replications. Certain selection criteria are of general importance across ecosystem (i.e. yield potential, dry matter content), while others are specific for each ecosystem (i.e. pest and diseases).

Traditionally, the progenies generated from the crossing blocks (***F1***) were planted in screen houses and transplanted to the field after 2 months at CIAT. At 6 months after planting, 2 stakes were harvested from each plant and given a consecutive number according to the plant. One of the stakes was planted at CIAT, the other one, was planted at the main selection site (***F1C1***). Selection was conducted at harvest on individual plants at the main selection site. Planting material taken from the selected genotypes, at CIAT, was used subsequently to establish a non-replicated, 6-plant plot, both at CIAT and at the main selection site (***Clonal Evaluation*** stage). Evaluation was done using the central 3 plants. Selections were transferred to the following stage (***Preliminary Yield Trial)*** and planted in non-replicated, 20-plant plots. Evaluation was done in the central 6 plants, and selections

were then passed to the ***Advanced Yield Trials*** at 1 or 2 sites, with 3 replications of 25-plant plots. Genotypes selected over 2 consecutive years at the *Advanced Yield Trial* level were considered as **"*elite genotypes*"** and incorporated in the germplasm collection and the crossing blocks. Since each year a new breeding cycle was initiated, all the stages were simultaneously being conducted in each site.

Table 3.4. Main ecosystems for cassava production, representative production regions, and main breeding sites.

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

masl; meters above sea level

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

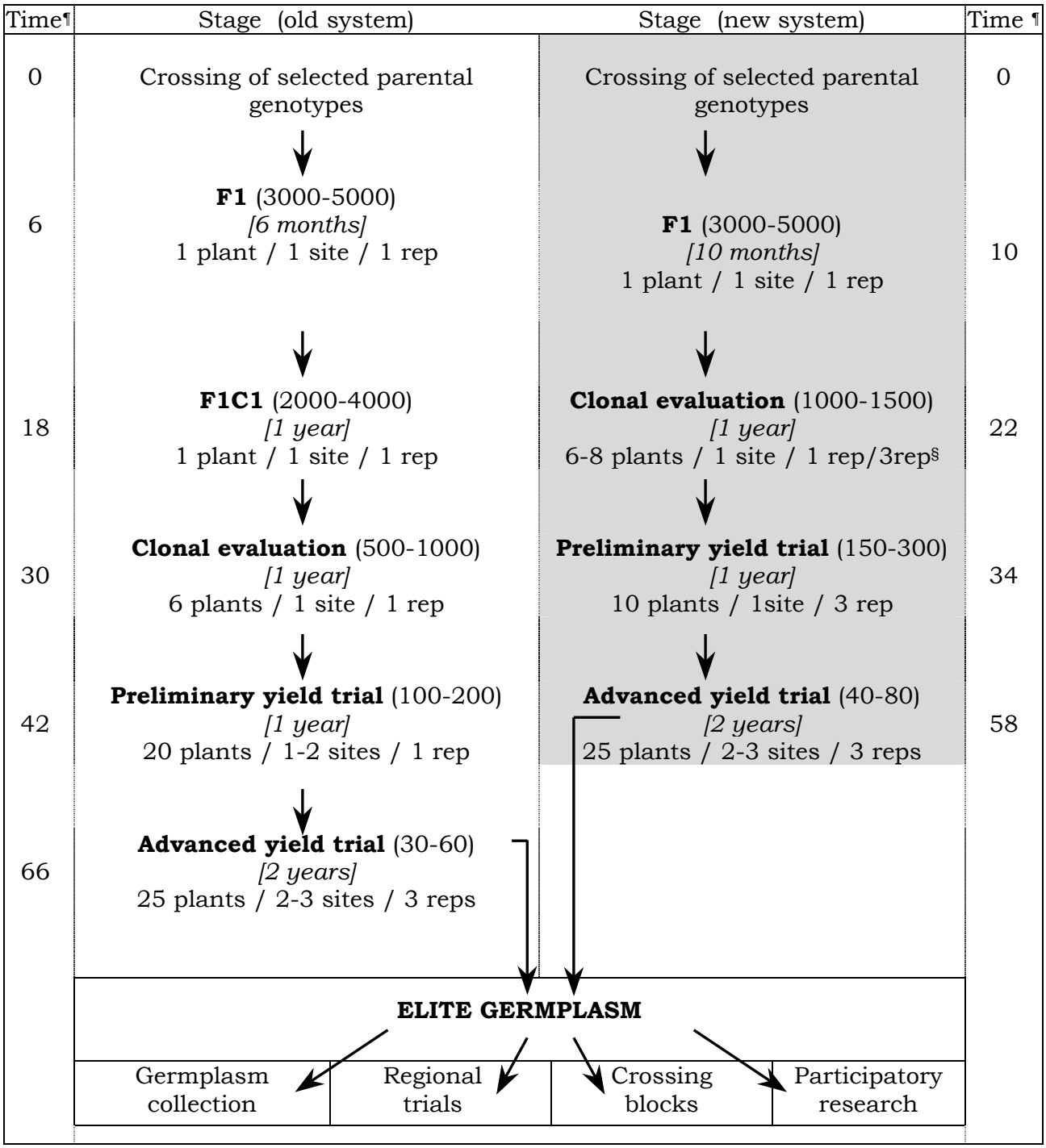
1) The *F1* plants were grown for 10 months rather than 6. At that age they can produce up to 8-10 stakes. The stakes will be sent to the proper evaluation site for the *Clonal Evaluation*. This implies that the *F1C1* stage is eliminated and that no duplicate of each genotype is necessarily maintained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

2) The *Clonal Evaluation Trials* were based on up to eight plants, rather that six as before.. Few other traits will also be taken using visual scores: plant architecture, foliar health (for insects and diseases separately), above ground biomass (for an estimate of harvest index), and root aspect. A selection index was 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 eight plants (except for those cases were stakes did not germinate or plants died), rather than three, as in the past. These eight plants will produce more than 30 cuttings, which will be used for the first replicated trial based on three replications and two row plots with ten plants per plot. It is recognized that this evaluation will result in some competition effect among neighboring plots. However, it is hoped that the number of replications will neutralize most of these effects. Also, row spacing between plots can be increased and the plant-to-plant distance within the plot reduced. This will maintain the density unchanged, while favoring competition among plants from the same genotype.

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

5) One final modification introduced this year was the field planting arrangement for the *Clonal Evaluation Trials*. In previous years, all the clones belonging to the same family were planted together one after the other. Because of the large size of theses trials environmental factors had a relatively large effect on the performance of the genotypes evaluated. Therefore, each family into three groups of clones each group having approximately the same size. The experimental plot was also divided into three "blocks". Each group of clones from a given family was randomly allocated to one of these "blocks". This modification allowed for a replicated presence for each family. The individual clones, of course, could not be replicated. On the other hand, the family means are based on three replications and therefore, more precisely estimated. Selection of individual clones was done within each "block", following the ideas behind stratified mass selection proposed by Gardner in the 1960s.



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

Figure 3.1. Basic cassava breeding schemes applied for each of the priority ecosystems. On the right is the new scheme currently under implementation (shaded area). Later stages of selection are made following the old system (shaded area on left).

Preparing new F1 field
About 17,000 recombinant, botanical seeds were germinated early in 2003, and approximately 11,764 of the resulting plantlets were transplanted at CIAT Experimental Station in Palmira for the first time in four years (Table 3.5). This material represents the *F1* stage described in Figure 3.1.

Table 3.5. Cassava seed processed for producing F1 plants for various purposes at CIAT, Palmira, Valle del Cauca, Colombia. F1 nursery was transplanted in July 2004.

Purpose of cross	**Germinated seed**	**Transplanted Seed**
Sub-humid environment	4452	3091
Acid-soil savannas	4365	2938
Mid-altitude valleys	4302	3144
Yellow Roots	2306	1610
Foliar retention	2671	1649
Self-pollinations	2793	1995
Total of crosses	**20889**	**14427**

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

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

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

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

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

Plant type integrates several important aspects for cassava: (1) plant health, inasmuch as a

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

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

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

The most relevant results obtained in six major cassava-producing regions of Colombia during the cycle that finished with harvests during March to June, 2004 are summarized in the following Outputs.

Activity 3.5. Precision of selection in early stages of cassava genetic improvement. (Manuscript submitted to Crop Science)

Rationale

Cassava breeding is difficult, expensive and to certain degree inefficient. Kawano et al. (1998) mention that during a 14-years period about 372,000 genotypes, derived from 4130 crosses, were evaluated at CIAT-Rayong Field Crop Research Center. Only three genotypes emerged the selection process to be released as official varieties. Similar experiences have been observed at IITA, CIAT- Colombia and Brazil. In spite of the difficulties, significant progress has been achieved in the past few decades (Johnson et al. 2003). Modifications to overcome some of the limitations of the original evaluation scheme and to take advantage of the opportunities that cassava faces were introduced for cassava breeding at CIAT starting in 2000.

The objective of this study was to learn how well measurements taken early in the selection process correlate with the same traits in the last stages of selection and to what extent these traits are useful for identifying the best germplasm.

Materials and methods

Multiplication rate in cassava (based on vegetative cuttings) is low. Under good environmental conditions a cassava plant from a modern clone can easily yield up to 20 cuttings. However, when thousands of clones are handled under non-optimal conditions, which are the typical target environments for most cassava-breeding projects, a realistic multiplication rate will range only from 5 to 10. This imposes a critical limitation because it takes several years until enough planting material is available for the multi-location trials.

Cassava genetic improvement starts with the production of new recombinant genotypes derived from selected elite clones. Parental lines are selected based mainly on their *per se* performance and little progress has been made to use general combining ability performance as a criteria for parental selection. Pollinations can be done manually, in a controlled way, to produce full sib families or else in polycross nurseries where open pollination takes place and half-sib families are produced (Kawano, 1980).

Recombinant seed is germinated and the seedling transplanted to the field. The resulting plant is used as source of vegetative cuttings to start the evaluation and selection process. Because of the low multiplication rate as many as six years are needed to complete a selection cycle. Typically, a large number of segregating clones are evaluated in the first year. Drastic selection is used to reduce the number of clones in the early stages of selection (first 1-2 years). Selected clones are then planted in successive evaluation trials that gradually reduce the number of genotypes and increase the size of the plots, introduce replications and then add locations. A critical issue in this scheme is the effectiveness of selection early in the process, when segregating clones are evaluated in non-replicated trials with plots ranging from one to ten plants in size.

The cassava-breeding project at CIAT-Colombia uses three main target environments, which represent the main cassava growing conditions in the tropics and allow for the selection against the main biotic or abiotic limiting factors (except the viral diseases only present in Africa and India). Results from the following environments are reported in this article (disease problems seriously affected the reliability of data from the third environment).

Acid-soil savannas, where the bacterial blight induced by *Xanthomonas axonopodis* pv. *Manihotis* (also known as *X. campestris* pv. *manihotis*) and super elongation disease induced by *Elsinoe brasiliensis* (also known as *Sphaceloma manihoticola*) are the major constraints, in addition to the edaphic conditions related to soil acidity.

Sub-humid conditions, where lack of rains can last as many as five months and pests become the most serious limiting factor for cassava growth (Bellotti et al., 2002). The green mite (*Mononychellus tanajoa*) and other mites (*Tetranychus urticae, T. cinnabarinus, Mononychellus caribbeanae* and *Oligonychus peruvianus),* mealybugs (*Phenacoccus manihotis* and *P. herreri)* and thrips (particularly *Frankliniella williamsi* and *Scyrtotrips manihoti*) are common in these environments.

<u>*Original evaluation and selection system*</u>

Originally plants germinated from botanical seed (F1 in Figure 3.1) were grown for only six months. Two stakes were taken from each plant. One stake was sent to the target environment for evaluation in the F1-C1 trial and the sister stake was planted at CIAT-Palmira. The first stage of selection, therefore, was based on the single plant evaluations at the F1-C1 trial. Selection was based on a visual assessment of each plant. High heritability traits such as plant architecture, root and pulp color and resistance to diseases and pests were the criteria of the selection at this stage. A drastic reduction of the number of genotypes (for example from 4000 to 1500 genotypes) was based on these single plant evaluations.

Planting material (six stakes) from selected genotypes at the F1-C1 was obtained from the sister plants grown for ten months at Palmira. The second stage of selection (Figure 3.1) was the *Clonal Evaluation Trials* (CET). These trials were grown in the proper target environment

and were based on six plants. No data was recorded in the first two stages of selection. The next evaluation and selection stage, the *Preliminary Yield Trials* or PYT, grown in one representative location for the target environment. One replication of a 20-plant plot (four rows and five plant per row) was used to represent each clone at this stage. The six central plants were harvested for evaluation purposes and the 14 border plants of each plot were used as source of planting material.

The *Advanced Yield Trial* (AYT) was the first selection stage based on replicated evaluations. In addition, plots had 25 plants (5 rows and five plants per row) and the nine central plants were harvested and the data generated used for selection purposes. The remaining 16 plants were left as source of planting material. This basic planting scheme was used frequently for a second year of AYTs and the selected clones joined the Regional Trials (RT), which included several of the best genotypes grown in the target environment (Figure 3.1).

The previous evaluation scheme had two major problems: i) the selection during the first three stages was based on non-replicated trials (Figure 3.1). Because of the large size, particularly for CETs, experimental error and the impact of the environment is expected to be very large (a typical CET would require about one hectare); ii) No data was taken in these early stages of selection and, therefore, little could be learned about the breeding value of the parental clones, nor about the efficiency of the breeding system and alternative ways to improve it.

New evaluation and selection system

The main modifications introduced to the evaluation and selection system occur in the early stages. The F1 plants are grown for 10 months (not six) and, therefore, eight stakes (instead of two) can be obtained. This change allows the elimination of the F1-C1 stage of selection, which was based on single plant evaluations.

The CETs also went through important changes. The most relevant modifications can be summarized as follows: 1) Number of plants representing each clone has been increased to eight; 2) Every plant in the CET is harvested. Data from each clone is therefore based on eight plants and not three as in the previous scheme. In addition the eight plants are used as source of planting material (instead of the three plants used in the previous system). This change allows a larger number of stakes for the next stage of selection (the PYT), as well as more reliable data; 3) To avoid the competition between neighboring clones rows are separated 1.2 m (not 1.0 m as usually done) and plants within the row are planted closer to each other (0.8 m instead of 1.0 m). Overall plant density is not changed drastically, but within family plant competition is favored over the between family competition; 4) The whole area where the CET is planted is divided in three blocks of equal size. Clones from each same full- or half-sib family are randomly assigned to each of three blocks in the CET. This approach allows a replication effect for each family. In addition, selection is conducted independently within each block, in a way that does not differ much from that suggested by Gardner (1961) for the stratified mass selection; and 5) An additional change in the CETs is that data, including dry matter content in the roots, is collected from every row.

Finally, at the PYTs some important changes have also been introduced (Figure 3.1). In the previous scheme this stage of selection was based on single replications of 20-plant plots. In the current system three replications of 10-plant plots (two rows of five plants each) are used. Since each clone is planted in two-row plots, half of the between-row competition is against sister plants from the same clone. Further reduction of undesirable between-family

competition originating in differences in plant vigor can be achieved because the planting distances between-row is reduced to favor of within-row competition (as done for CETs).

Significant consequences of the modifications introduced are: i) the second (not the fourth) stage of selection is based on replicated evaluations. Only one stage of selection (CET) is based on non-replicated evaluations, not three as in the previous scheme (F1-C1, CET and PYT). ii) Data was taken at every stage of selection (except at the F1 which is carried out in Palmira, not in the target environment and where the only selection criterion is capacity to produce the eight stakes required for the CET).

Data is used for a selection index integrating the most relevant variables as described in Activity 3.4. Harvest index has been consistently favored as one relevant variable to be included in early stages of selection such as at CETs (Kawano et al. 1998; Kawano, 2003) and is estimated as the ratio between root-weight over the total biomass of the plant. Plant type has been reported to play an important role in early stages of selection (Hahn et al, 1979). In our case we use a 1 to 5 scale, where 1 represents an excellent plant type and 5 a poor one. The plant type score integrates plant architecture (erect, non branching types), plant height (intermediate types 2-3 m tall), plant health, and foliage retention. Because the lower scores represent the desired phenotype, a negative sign is assigned to plant type in the selection index above. In areas where disease resistance is very important (such as the acid-soil savannas) the weight for plant type may be as high as five. In other areas where the main trait affecting plant type is its architecture the weight may be reduced down to three. Since SI is estimated using the standardized values, a positive SI means a performance better than the average, while a negative one means a poor performance. The more negative the worse performance.

Results from two different types of evaluations were obtained and analyzed: the normal five-year recurrent selection cycle, and data from a diallel study.

Normal five-year recurrent selection cycle.
Results from different trials in these three agro-ecosystems are analyzed in this article. Because of the unexpected problems faced during its implementation, occasionally the scheme is modified and does not follow the normal step-by-step scheme described in Figure 3.1. In the sub-humid conditions, the lack of rains at planting time may affect the sprouting of the stakes, resulting in poor plant stands. When this happens the selection process is interrupted and a multiplication of the surviving planting material is conducted instead. This causes a one-year delay in the selection process but guarantees good and uniform planting densities and vigor for later stages of selection.

Data from diallel studies
During the July 2001- April 2002 evaluation cycle in addition to the standard evaluations, a group of clones representing a diallel set of crosses was planted in the sub-humid and acid soil savannas. In a way this evaluation could be envisioned as a CET trial, except that the six plants representing each clone were distributed in two locations (Pitalito and Stanto Tomás in the sub-humid environment and at two contrasting soil types in the acid-soil savannas) with three replications at each location. Mean performance of each clone, therefore, was much less affected by environmental error and genotype by environment interaction. Data from the diallel set was analyzed to learn about the inheritance of the most important variables, but the best performing clones, across the two locations for each agro-ecological

zone, were selected and planted first in a standard CET and then the subsequent year as a PYT.

The most important variables analyzed are Plant Type score (PT) ranging from one to five, Fresh Root Yield (FRY) and Fresh Foliage Yield (FFY) both measured in t ha^{-1}, Harvest Index (HI) ranging from zero to one, percentage of Root Dry Matter Content (DMC), Root Dry Matter Yield (DMY) measured in t ha^{-1}, and Selection Index (SI).

The main purpose of this article is to describe the relationship between the evaluations of the same genetic materials during the successive selection stages. For that purpose, phenotypic correlations (Steel and Torrie, 1960) were estimated using the Microsoft EXCEL spread sheet (Lirola Terrez, 1997).

Results

Normal five-year recurrent selection cycle.

Table 3.6 describes the size of the trials, from the five-year recurrent selection cycles whose results are described in the present article. The same clones are evaluated during CET and then, if selected, later on at PYT and AYT (Figure 3.1). The clones evaluated in a CET in any given year represent the "class" for that year and for that particular agro-ecosystem.

Table 3.6. Number of genotypes evaluated in trials at different stages of the selection process in sub-humid and acid-soil savannas environments.

Environment	Class	CET¶	PYT¶	AYT¶	AYT1¶
Sub-humid conditions	2000	1350	218	60	31
	2001	1952	198	n.a.	n.a.
	2002	1967	310	n.a.	n.a.
Acid-soil savannas	2000	1525	269	60	n.a.
	2001	1170	180	60	n.a.
	2002	1235	178	n.a.	n.a.

¶CET (clonal evaluation trial); PYT (preliminary yield trial); AYT (advanced yield trial).

Results from the different five-year recurrent selection cycles trials conducted in the sub-humid environment in Colombia's Caribbean coast are presented in Table 3.7. Two types of correlation coefficients were estimated: the correlation for the same variable measured in different trials and the correlations of different variables with root dry matter yield in the latest evaluation trial for a given class. Ultimately, root dry matter yield is the most important variable when cassava is used for starch or animal feed production (Kawano et al. 1998). Only the 2000 class could complete the normal recurrent cycle. AYT1 was conducted at four different locations. PYT trial from class 2001 failed to sprout uniformly due to lack of adequate rains and was replanted the following year.

Correlations among the same variables taken in CET and AYT1 for the class 2000 are the most relevant ones: the quality of AYT1 trials evaluated in four different locations with three replications at each location and 25-plant plots provide a reliable assessment of the genotypic value of the clones involved. Root dry matter content showed the highest correlation coefficient (0.79) indicating that measurements for this variable early at CET is reliable and selection likely to be effective (Table 3.7). Plant type score was the second highest

correlation (0.61) but, lower in few other trials combinations. Harvest index, fresh foliage and fresh root yields all showed correlation coefficients larger than 0.30. Our results supports those reported by Kawano et al. 1998 showing the consistency of harvest index measurements at different phases of the evaluation process. Fresh root yield showed a much better correlation (0.39) than expected and reported by Kawano et al. in 1998 and was very similar to that of harvest index (0.41). The low correlation for selection index is probably due to the fact that it combines four different variables, some of them with a tendency for a negative correlation themselves (i.e., fresh root yield and dry matter content).

Table 3.7. Correlations for several variables in the different trials conducted in the sub-humid conditions during the 2000-2004 period.

Class	**2000**						**2001**	**2002**	**Mean**
Trials compared	AYT1 AYT	AYT1 PYT	AYT1 CET	AYT PYT	AYT CET	PYT CET	PYT CET	PYT CET	PYT CET
Sample size	31	31	31	60	60	218	198	310	242
Variable§									
Correlations between the same variable measured at different stages									
PT	0.74	0.65	0.61	0.51	0.25	0.31	0.31	0.07	0.23
FRY	0.67	0.23	0.39	0.25	0.29	0.13	0.13	0.26	0.17
FFY	0.46	0.48	0.36	0.57	0.28	0.30	0.30	0.27	0.29
HI	0.62	0.49	0.41	0.58	0.35	0.51	0.51	0.29	0.43
DMC	0.83	0.72	0.79	0.64	0.68	0.64	0.68	0.59	0.64
DMY	0.48	0.07	0.32	0.14	0.04	0.11	0.05	0.28	0.14
SI	0.52	0.09	0.24	-0.06	0.27	-0.10	0.02	0.08	0.00
Correlations of dry matter yield (t ha^{-1}) at later stages versus different traits measured early									
PT	0.04	0.04	-0.03	-0.10	-0.07	0.00	-0.08	0.11	0.01
FRY	0.48	0.10	0.31	0.18	0.25	0.03	0.01	0.16	0.08
FFY	0.20	-0.03	-0.16	-0.28	-0.14	-0.09	0.05	0.10	0.07
HI	0.20	0.07	0.42	0.47	0.31	0.11	-0.07	0.01	-0.03
DMC	-0.07	-0.07	0.05	-0.05	-0.08	0.18	0.08	0.25	0.16
DMY	0.48	0.07	0.32	0.14	0.21	0.11	0.05	0.28	0.16
SI	0.35	0.04	0.40	-0.05	0.28	0.15	0.07	0.15	0.11

¶CET (clonal evaluation trial); PYT (preliminary yield trial); AYT (advanced yield trial).

§ PT (plant type); FRY (fresh root yield); FFY (fresh foliage yield); HI (harvest index); DMC (dry matter content); DMY (dry matter yield); SI (selection index).

The correlations between measurements at CET and the same variables evaluated at PYTs and AYTs showed similar trends but were generally lower in magnitude compared with those observed for AYT1.

The comparisons between PYTs and CETs from classes 2001 and 2002 are also presented in Table 3.7. Root dry matter content showed again the highest correlations (0.68 and 0.59, respectively for 2001 and 2002). Harvest index showed a good correlation for 2001 (0.51), but was much lower for 2002 (0.29). The right column in Table 3.7 shows the average correlation between CET and PYT across classes 2000, 2001, and 2002. Root dry matter content stands alone as thee highest correlation (0.64), followed by harvest index (0.43), fresh foliage yield (0.29), plant type score (0.23), fresh root yield (0.17), dry matter yield (0.14 and selection index (0.00). In general, these results are similar to those presented by Kawano et al. in

1998, but have the additional information on root dry matter content which was not measured by these authors at CET trials. Overall, correlations between fresh root yield at different stages of the selection cycle tended to be higher than those reported by Kawano et al. (1998).

As expected, correlations among measurements of the same variable at later stages of the evaluation cycle (for instance AYT vs. AYT1), were generally higher than those among earlier trials (Table 3.7). As in the previous comparisons, root dry matter content was the most consistent variable (correlation of 0.83), followed by plant type score (0.74), fresh root yield (0.67), harvest index (0.62), selection index (0.52), dry matter yield (0.48), and fresh foliage yield (0.46). The most noticeable change in this comparison is the excellent correlations observed for selection index and fresh root yield. These results also agree with those presented by Kawano et al. in 1998.

The most important information in Table 3.7, however, are those in the lower half of the table, where the correlations among different variables in early stages of selection and dry root yield at the latest available stage of evaluation are shown. For class 2000, harvest index (measured at CET) showed the highest correlation with dry matter yield (measured at AYT1). Selection index showed the second highest correlation (0.40), followed by dry matter yield and fresh root yield (0.32 and 0.31, respectively). As expected plant type score showed a negative correlation, since the lower score indicates the best plant type.

It is worth emphasizing the good correlation of selection index at CET and dry root yield at AYT1 (0.40). The selection index incorporates the plant type score that is not always correlated with high root productivity as illustrated by the small negative correlation (-0.03) shown in Table 3.7. Plant type score includes plant architecture, which is an important criteria for cassava farmers, but does not necessarily contributes to higher productivity. It is also interesting to note that the correlation between selection index at early stages of evaluation and dry matter productivity at later stages of selection increased from CET vs. PYT (0.15), to CET vs. AYT (0.28), to CET vs. AYT1 (0.40).

The bottom of the right column in Table 3.7 shows the average correlation between different variables (measured at CET) and dry matter productivity (measured at PYT) across classes 2000, 2001, and 2002. Results are very contrasting with the correlations between CET and AYT1, with coefficients generally much lower in magnitude. The highest correlations were for dry matter content and dry matter yield (both at 0.16), followed by selection index (0.11). Correlation coefficients for fresh root yield and fresh foliage yield at CET and dry matter yield at PYT where higher (0.08 and 0.07, respectively), than for harvest index (-0.03).

Results from the different five-year recurrent selection cycles trials conducted in the acid-soils environment are presented in Table 3.8. Because of lack of enough planting material, the AYT1 trial from class 2000 was delayed for a year.

As it was the case for the sub-humid environment, root dry matter content showed excellent correlations between early and late measurements. Across years the correlation for root dry matter between measurements at CET and then at the AYT was 0.61, followed by fresh foliage yield (0.46), harvest index (0.44), plant type score (0.43), fresh root yield (0.34), dry matter yield (0.28) and selection index (0.21).

Table 3.8. Correlations for several variables in the different trials conducted in the acid-soil savannas environment during the 2000-2004 period.

Class	**2000**			**2001**			**2002**	**Averages**		
Trials compared	AYT PYT	AYT CET	PYT CET	AYT PYT	AYT CET	PYT CET	PYT CET	AYT PYT	AYT CET	PYT CET
Sample size	60	60	259	60	60	180	178	60	60	205
Variable§										
Correlations between the same variable measured at different stages										
PT	0.54	0.48	0.41	0.43	0.38	0.20	0.43	0.49	0.43	0.35
FRY	-0.03	0.48	0.15	0.27	0.19	0.13	0.04	0.12	0.34	0.11
FFY	0.52	0.49	0.47	0.50	0.43	0.35	0.16	0.51	0.46	0.32
HI	0.56	0.62	0.41	0.66	0.26	0.45	0.46	0.61	0.44	0.44
DMC	0.72	0.67	0.64	0.66	0.55	0.58	0.49	0.69	0.61	0.57
DMY	-0.12	0.35	0.18	0.25	0.22	0.18	0.00	0.06	0.28	0.12
SI	0.05	0.26	0.18	0.33	0.17	0.15	0.13	0.19	0.21	0.15
Correlations of dry matter yield (t ha^{-1}) at later stages versus different traits measured early										
PT	-0.01	-0.04	-0.04	-0.08	0.08	0.00	-0.07	-0.04	0.02	-0.04
FRY	-0.09	0.35	0.07	0.20	0.21	0.10	-0.04	0.06	0.28	0.04
FFY	0.03	0.22	0.14	0.10	0.15	0.10	-0.03	0.06	0.18	0.07
HI	-0.15	0.03	-0.15	0.06	-0.16	-0.04	-0.01	-0.04	-0.06	-0.06
DMC	-0.09	-0.10	0.17	0.13	0.11	0.26	0.08	0.02	0.00	0.17
DMY	-0.12	0.35	0.18	0.25	0.22	0.18	0.00	0.06	0.28	0.12
SI	-0.11	0.21	0.19	0.30	0.04	0.17	0.09	0.10	0.13	0.15

¶CET (clonal evaluation trial); PYT (preliminary yield trial); AYT (advanced yield trial).
§ PT (plant type); FRY (fresh root yield); FFY (fresh foliage yield); HI (harvest index); DMC (dry matter content); DMY (dry matter yield); SI (selection index).

Correlations between root dry matter yield at AYT and other variables measured in earlier trials are of prime interest. The highest correlations between root dry matter yield at AYT and variables measured at CETs were observed for fresh root yield and root dry matter yield (0.28 in both cases), followed by fresh foliage yield (0.18), selection index (0.13). Other correlations, including that for harvest index, were negligible. Coefficients for plant type score at different trials, which averaged 0.43 in the AYT vs. CET trials across the acid-soils savannas trials were in general higher than those observed in the sub-humid environment. This is probably because of the importance of the reaction to foliar diseases, which are prevalent and, therefore, constitute a major objective for the acid-soils environment.

Data from diallel studies

Table 3.9 shows the results from evaluation of clones within a diallel mating design at two locations with three replications, the same clones in a standard CET and then in a PYT.

In the sub-humid environment correlations for the same variable measured at PYT and in the diallel or the standard CET were generally higher for the former, except for root dry matter content and selection index (Table 3.9). As it was the case in the trials described above, dry matter content and harvest index showed the highest correlations, both in the PYT vs. CET and PYT vs. diallel comparisons. Correlations involving different variables in the diallel or CET trials with dry matter productivity at the PYT were generally low.

Table 3.9. Phenotypic correlations measured in clones evaluated in a diallel mating design (each clone evaluated at two locations and three replications per location) and the same clones in a CET and PYT trial. Two independent sets of trials conducted for the sub-humid environment and the acid-soil savannas.

	Sub-humid environment			Acid-soil savannas		
Variable§	PYT CET¶	PYT Diallel¶	CET Diallel¶	PYT CET¶	PYT Diallel¶	CET Diallel
Sample size	49	49	216	46	46	261
Correlations between the same variable measured at different stages						
PT	0.07	0.26	0.09	0.38	0.19	0.25
FRY	0.04	0.11	0.05	-0.00	-0.12	-0.11
FFY	-0.05	0.18	0.18	0.22	-0.11	-0.04
HI	0.41	0.42	0.44	0.49	0.48	0.29
DMC	0.67	0.61	0.65	0.50	0.48	0.21
DMY	0.04	0.06	0.07	-0.02	-0.09	-0.10
SI	0.25	0.08	0.22	0.23	-0.19	-0.11
Correlations of dry matter yield (t ha^{-1}) at later stages versus different traits measured early						
PT	0.00	0.10	0.00	-0.40	-0.37	-0.11
FRY	-0.00	0.08	-0.01	-0.03	-0.12	-0.11
FFY	-0.11	0.00	-0.12	0.02	-0.33	-0.11
HI	0.18	0.04	0.16	-0.02	0.32	-0.01
DMC	0.09	-0.12	0.20	-0.01	0.31	0.03
DMY	0.04	0.06	0.07	-0.02	-0.09	-0.10
SI	0.12	-0.06	0.21	0.19	-0.15	-0.03

¶CET (clonal evaluation trial); PYT (preliminary yield trial); AYT (advanced yield trial).

§ PT (plant type); FRY (fresh root yield); FFY (fresh foliage yield); HI (harvest index); DMC (dry matter content); DMY (dry matter yield); SI (selection index).

In the acid-soil savannas dry matter content and harvest index showed the highest correlations when measured at different trials (ranging from 0.48 to 0.50). Plant type scores showed a good correlation between PYT vs. CET (0.38), which was not as high in the PYT vs. diallel comparison (0.19). Correlations between different variables at CET or diallel with dry matter yield at PYT were generally low. The best correlation was found for plant type (-0.40 for PYT vs. CET and -0.37 for PYT vs. diallel). Negative correlations in this case are to be expected because lower plant type score identifies the best phenotypes. This was an interesting finding because it highlights the importance of resistance to foliar diseases in this environment and its ultimate effect in root dry matter productivity. Harvest index and dry matter content measured in the diallel trial correlated well with dry matter productivity at PYT (0.32 and 0.31, respectively). However, when these two variables were measured at CET they correlated poorly with root dry matter productivity at PYT (-0.02 and -0.01).

An additional change in the way the CET trials were conducted was their blocking. As explained in the Materials and Methods section, clones from each family were randomly split into three groups, which were then planted in one of the three blocks CET trials were divided into. Table 3.10 provides and illustration of the changes in the mean performance of clones at different blocks. Since clones from each family were randomly assigned to different blocks the mean performance for each block depended much more on environmental differences than in genetic ones. Stratifying the selection within each block reduced the influence of

environment at the CETs single-plot evaluations. The reduction of that influence is proportional to the difference in the averages of each block (Gardner, 1961). The variations in the mean performance of each block shown in Table 3.10 fully justify the little additional trouble of dividing each family into three groups rather than planting all the clones from a given family together in one group. Moreover, if possible CET trials should be divided in larger number of blocks so that the within-block environmental variation can be further reduced.

Table 3.10. Results of the *Clonal Evaluation Trials* for the three main target environments harvested in May 2003. Data present the variation between the three blocks in which each CET was divided.

Block	Yield (t/ha)		Harvest Index (0 to 1) ¶	Plant type (1 to 5) §	Dry matter content (%)	Selection Index
	Fresh roots	Dry matter				
Averages of the412, 412 and 411 clones in Blocks 1, 2 and 3, respectively from the CET targeting the acid-soil savannas.						
Block 1	20.88	6.66	0.50	3.33	31.59	0.00†
Block 2	21.73	6.88	0.49	3.35	31.24	0.00†
Block 3	22.30	7.28	0.50	3.48	32.44	0.00†
Averages of the 749, 746 and 705 clones in Blocks 1, 2 and 3, respectively from the CET targeting the sub-humid conditions.						
Block 1	14.19	3.70	0.50	2.87	26.09	0.00†
Block 2	14.37	3.91	0.46	2.88	27.21	0.00†
Block 3	12.89	3.38	0.44	2.87	26.26	0.00†
Averages of the605, 588 and 568 clones in Blocks 1, 2 and 3, respectively from the CET targeting the mid-altitude valleys.						
Block 1	24.05	8.86	0.63	2.68	36.61	0.00†
Block 2	28.08	10.21	0.57	2.63	36.02	0.00†
Block 3	27.51	9.76	0.54	2.97	35.09	0.00†

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

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

† Average election index within blocks must be zero, because it is based on a combination of standardized variables.

Discussion

The analysis of the results presented in this article should take into account that year to year comparisons are affected by the changes in growing conditions from year to year, as well as changes in the specific locations were trials were conducted. They involve data taken from large experiments conducted in marginal growing conditions and, therefore, prone to relatively large coefficients of variability. The relatively low values for some of the correlation coefficients observed or the lack of consistency from trial to trial, therefore, should be envisioned with these facts in mind.

Our results support the findings reported by Kawano et al. in 1998 regarding the reliability of harvest index measurements at different stages of the evaluation process. They also support its usefulness as an indirect selection criterion for increased dry matter productivity. Overall,

however, the importance of this variable in our work is lower that that reported previously. There are two feasible explanations for this situation: i) harvest index has been improved during the last twenty years and cassava breeding populations are now nearing the ideal harvest index level, compared with the populations reported in Kawano and co-workers' article; ii) changes in planting distances increased the within clone competition and reduced the between clone competition, therefore reducing one of the problems that harvest index could help overcoming.

Changes introduced in the planting distances may also explain the better relationship between fresh root yield at CETs and dry root yield at later stages of the evaluation process, compared with previous reports. This is the case, for instance in the acid soil environment were fresh root yield at CET had a much better average correlation with dry root yield at AYT (0.28) than harvest index (-0.06).

Dry matter content was consistently the most reliable trait measured at different stages of the evaluation process. Our results indicate that high dry matter content can be properly identified and selected for at the CET trials. Dry matter content is important because of its direct effect of dry matter productivity. It is also important because cassava-processing industries (starch and root drying for animal feed) would frequently penalize or reject roots with lower than optimum dry matter contents. These results justify the additional costs involved in measuring dry matter content in large trials such as CETs. A major consequence of this finding is the future increase in the relative value of dry matter content in the selection index used by the cassava-breeding project at CIAT at CETs

The comparison of data from the diallels and the normal recurrent selections suggests that there is not much advantage in splitting the CET trials so that the plants of each clone can be planted in replicated trials in more than one location. It is clear that results such as those from the diallels are less affected by genotype by environment interactions. However, the amount of data taken and handled is six-fold larger (individual data from each of six plants rather than totals of a six-plant row) and thus prone to larger experimental errors and more frequent mistakes. There was no clear evidence that the additional complications of planting CETs in ways similar to the diallels approach would result in significant gains in the precision of the information.

The analysis of the relationship between variables at different stages of the selection process through their correlation coefficients should be taken with caution. One major change throughout the selection process is, precisely, the selection of genotypes, which unavoidably will have an effect on the performances of the successive trials and may eventually affect the values of the correlations obtained. The number of genotypes in later stages of selection is considerably smaller than in early stages of selection, so there are fewer degrees of freedom for their respective correlations. The magnitude and nature of this influence, however, is difficult to assess.

The stratification of CETs into three blocks was useful for highlighting the large environmental variation within these large trials. The little additional effort to split clones from a given family into three groups to be randomly allocated to each block is justified based on the large differences in the averages measured at each block. Furthermore, this approach allows for a replication effect for family means and a more precise estimation of their mean performance, which in turn results in more reliable data on the general combining ability of parental lines used to generate each CET.

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