CHAPTER 17

Manihot Genetic Resources at CIAT (Centro Internacional de Agricultura Tropical)

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Introduction

Among the dozens of *Manihot* species, cassava (*M. esculenta* Crantz) is unique in being cultivated. Its allogamous reproductive mode and its highly heterozygous genetic constitution are the main reasons for propagating the crop by cuttings (or stakes) instead of by sexual seed. To preserve the visible phenotypic characters, the species has been cultivated and maintained over the years by continuous vegetative propagation.

The primary center of origin and diversity is the western Amazon Region. In pre-Columbian times, cassava migrated westwards to Peru, and then northwards to Colombia, and from there entered Central America. It also migrated southwards to Paraguay and Argentina, although when this migration occurred is not precisely known (D Debouck 2001, pers. comm.). In the 1500s, cassava was taken by the Spanish and Portuguese to Africa and Asia, which then became secondary centers of diversity (Hershey and Amaya 1979).

Within the system of the Consultative Group on International Agricultural Research (CGIAR)[2], CIAT has the global responsibility to conserve the genetic resources of *M. esculenta*. Currently, the collections held at the CGIAR centers are under the auspices of the Food and Agriculture Organization of the United Nations (FAO), as patrimony for humanity. As with other crops, the conservation of cassava germplasm is justified by the following points:

1. To prevent the loss of wild and cultivated species to *genetic erosion*, caused by pressure factors such as the adoption of modern varieties, land clearing for urbanization, and alteration of natural habitats.

2. To maintain a high degree of *genetic variation* for use in crop improvement programs.

Although cryopreservation techniques are currently being enhanced, conservation in the germplasm bank at CIAT is based mainly on two systems: field and *in vitro*. These two modalities of *ex situ* conservation successfully maintain the status of gene combinations, that is, without change, as verified by the clones' genetic stability. They also contribute important elements for the conservation, characterization, and use of germplasm (Debouck and Guevara 1995).

This chapter compiles information from several scientific articles and discusses collection, conservation, characterization, documentation, and distribution—all activities for managing a cassava germplasm bank.

Clone Codification and Nomenclature

According to Jaramillo (1993), the germplasm bank held at CIAT uses the following scheme:

Manihot esculenta varieties

For landraces collected inside and outside Colombia, CIAT assigns a three-part code:

M + country + consecutive number

- The letter "M" corresponds to the first letter of the genus name (*Manihot*).

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2. For an explanation of this and other acronyms and abbreviations, see *Appendix 1: Acronyms, Abbreviations, and Technical Terminology*, this volume.

- "Country" refers to the country of origin, and is expressed as the first two or three letters of the country's name, following the FAO code.

- The consecutive number is in Arabic numerals, and indicates the material's order of entry at CIAT.

For example, M Bra 383 indicates a cassava clone of Brazilian origin that had entered the collection at CIAT as number 383.

Improved hybrids

The original identification of hybrids from the CIAT Cassava Improvement Project is conserved by assigning a four-part code:

> Type of cross + record of the cross + hyphen + selected genotype

For example, CM 340-55 indicates a hybrid of controlled pollination (CM). The parents crossed were M Col 22 and M Col 645, with the cross being recorded as 340. The plant selected was number 55. We point out that a clone's code never changes. Even in the event that one disappears or dies, its code is never assigned to another clone.

Vulgar, regional, or common names for cassava clones are also important. Usually, farmers give varieties simple names that relate to some characteristic of the plant or to its place of origin, for example:

Algodonas:	Varieties that are easy to cook
Rojitas:	Varieties with red petioles
Llaneras:	Varieties from the *Llanos* (i.e., Eastern Plains of Colombia)
Negritas:	Varieties with dark stems or crown

The use of common names has many limitations and can be confusing, particularly as a common name may be used for two or more very different or contrasting genotypes.

Released materials are also given common names, usually by the institutes or agencies who do the releasing. These names relate to details specific to the clone or release site such as Catumare, Costeña, Caribeña, and Rojita. Table 17-1 details the most common regional cassava varieties in Colombia and the materials released in the country to date.

Wild *Manihot* species

The species collected are introduced into CIAT. Seeds are used to obtain varieties, for which the following five-part code is proposed:

> M + abbreviation + seed population + hyphen + selected genotype

Examples include M alt 003-004, and M fmt 001-001, where:

- The letter "M" corresponds to the first letter of the genus name (*Manihot*).

- The "abbreviation" consists of three letters that refer to the species, as according to the list proposed by Chávez et al. (1987; Table 17-2).

- "Seed population" refers to a consecutive number given in the order in which the wild species was introduced into CIAT.

- "Selected genotype" refers to the code number of the selected plant. This number is then used consecutively.

Cultivated cassava × wild relative hybrids

A four-part code has been proposed, as follows:

> Type of cross + record of cross + hyphen + selected genotype

- Type of cross:
  Open pollination = OW (open wild)
  Polycross = SW
  Self-pollination = AW
  Controlled pollination = CW

- Record of cross:
  This consecutive number refers to the parents used in the cross. A fictitious example would be:

Type	Record, which refers to cross:	Mother	×	Father
CW	1	M Col 1505		M aes 001-002

- Selected genotype:
  This selection number is assigned consecutively, starting at 1. Using the previous example, this would be CW 1–001, CW 1–002, and so forth.

Table 17-1. Examples of important cassava clones, their assigned codes, regional names, year of release in Colombia, and planting site.

CIAT code	Regional name	Other codes assigned by ICA or CIAT	Year of release in Colombia	Planting site: country or locality in Colombia
M Bra 356	Ornamental	M Col 2264		Brazil
M Col 113	Valluna			Hillsides of Valle and Cauca
M Col 1438	Llanera	CMC 9		Eastern Plains
M Col 1468	Mantiqueira	Manihot ICA P-11; CMC 40	1984	Inter-Andean valleys
M Col 1505	Verdecita	Manihot ICA P-12; CMC 76	1984	Inter-Andean valleys
M Col 1522	Algodona			Caucan hillsides
M Col 1684	Matasuegra			Quilichao
M Col 2058	Popayán			Caucan hillsides
M Col 2059	Sata Dovio			Caucan hillsides
M Col 2060	Regional Amarilla			Caucan hillsides
M Col 2061	Regional Morada			Caucan hillsides
M Col 2063	Secundina			North Coast
M Col 2066	Chiroza Gallinaza			Quindío
M Col 2215	Venezolana 1; Coñito			North Coast
M Col 2216	Venezolana 2; Ven. Negra			North Coast
M Col 2253	Blanca Mona			North Coast
M Col 2257	Americana			Mondomo
M Col 2258	Batata			Mondomo
M Col 2259	Selección 40			Mondomo
M Col 2260	Negrita			Mondomo
M Col 2261	Panameña			La Cumbre/Cajibío
M Col 2478	Chiroza Llanera			San Martín, Meta
M Col 2479	Vajuna Negra			Caucan hillsides
M Col 2625	Vivas			Cajibío
M Col 2627	Chiroza Morada			La Libertad, Meta
M Col 2628	Chiroza Blanca			La Libertad, Meta
M Col 2733	Chiroza Falsa			Mondomo
M Col 2737	Brasilera			Meta
M Col 2740	Sata			Caucan hillsides
M Col 2752	Cogolliroja			Flandes, Tolima
M Col 2753	Aroma			Flandes, Tolima
M Col 2756	Costeña			Supatá, Cundinamarca
M Col 2758	Parrita			Quilichao/Jamundí
M Col 2759	Chiroza Manzana			Alcalá, Valle
M Cub 74	Señorita Falsa			Cuba
M Pan 139	Dayana			Panama
M Tai 1	Rayong 1			Thailand
HMC 1	ICA Armenia	Manihotica P-13	1986	Inter-Andean valleys
CG 1141-1	ICA Costeña		1991	North Coast
CM 523-7	ICA Catumare (Raya 7)		1990	Eastern Plains
CM 2177-2	ICA Cebucán		1990	Eastern Plains
CM 3306-4	ICA Negrita		1993	North Coast
CM 3306-19	CORPOICA Colombia		2000-B	North Coast
CM 3555-6	CORPOICA Sucreña		2000-B	North Coast
SGB 765-2	CORPOICA Caribeña		2000-B	North Coast
SGB 765-4	CORPOICA Rojita		2000-B	North Coast
CM 6740-7	Reina		2000-B	Eastern Plains
CM 6438-14	Juan V			Eastern Plains

Table 17-2. Manihot species, in alphabetical order and with their respective abbreviations.

No. in series	Species	Abbr.	No. in series	Species	Abbr.
1	*M. acuminatissima* Müller von Argau	acu	51	*M. michaelis* McVaugh	mic
2	*M. aesculifolia* (Kunth) Pohl	aes	52	*M. mirabilis* Pax	mbl
3	*M. affinis* Pax & K. Hoffmann	alf	53	*M. mossamedensis* Taubert	mos
4	*M. alutacea* Rogers & Appan	alt	54	*M. nana* Müller von Argau	nan
5	*M. angustiloba* Müller von Argau	ang	55	*M. neusana* Nassar	neu
6	*M. anisophylla* Müller von Argau	aph	56	*M. oaxacana* Rogers & Appan	oax
7	*M. anomala* Pohl	anm	57	*M. oligantha* Pax & K. Hoffmann	oli
8	*M. attenuata* Müller von Argau	att	58	*M. orbicularis* Pohl	orb
9	*M. auriculata* McVaugh	aur	59	*M. paviifolia* Pohl	pav
10	*M. brachyandra* Pax & K. Hoffmann	bnd	60	*M. peltata* Pohl	pel
11	*M. brachyloba* Müller von Argau	blo	61	*M. pentaphylla* Pohl	pnt
12	*M. caerulescens* Pohl	cae	62	*M. peruviana* Müller von Argau	per
13	*M. carthaginensis* (Jacq.) Müller von Argau	cth	63	*M. pilosa* Pohl	pil
14	*M. catingae* Ule	cng	64	*M. pohlii* Wawra	poh
15	*M. caudata* Greenman	cdt	65	*M. populifolia* Pax	plf
16	*M. cecropiifolia* Pohl	cec	66	*M. pringlei* S. Watson	pri
17	*M. chlorosticta* Standley & Goldman	chl	67	*M. procumbens* Müller von Argau	pcb
18	*M. condensata* Rogers & Appan	con	68	*M. pruinosa* Pohl	pru
19	*M. corymbiflora* Pax	cmf	69	*M. pseudoglaziovii* Pax & K. Hoffmann	pse
20	*M. crassisepala* Pax & K. Hoffmann	cra	70	*M. purpureocostata* Pohl	pur
21	*M. crotalariiformis* Pohl	ctl	71	*M. pusilla* Pohl	psa
22	*M. davisiae* Croizat	dav	72	*M. quinquefolia* Pohl	qfl
23	*M. dichotoma* Ule	dch	73	*M. quinqueloba* Pohl	qba
24	*M. divergens* Pohl	dve	74	*M. quinquepartita* Huber ex Rogers & Appan	qpt
25	*M. epruinosa* Pax & K. Hoffmann	epr	75	*M. reniformis* Pohl	ren
26	*M. esculenta* Crantz	esc	76	*M. reptans* Pax	rpt
27	*M. falcata* Rogers & Appan	fal	77	*M. rhomboidea* Müller von Argau	rho
28	*M. filamentosa* Pittier	fmt	78	*M. rubricaulis* I.M. Johnston	rub
29	*M. flemingiana* Rogers & Appan	fgn	79	*M. sagittatopartita* Pohl	sag
30	*M. foetida* (Kunth) Pohl	foe	80	*M. salicifolia* Pohl	slc
31	*M. fruticulosa* (Pax) Rogers & Appan	fru	81	*M. sparsifolia* Pohl	spr
32	*M. glaziovii* Müller von Argau	gla	82	*M. stipularis* Pax	sti
33	*M. gracilis* Pohl	gcl	83	*M. stricta* Baillon	str
34	*M. grahamii* Hooker	grh	84	*M. subspicata* Rogers & Appan	sub
35	*M. guaranitica* Chodat & Hassler	gut	85	*M. surinamensis* Rogers & Appan	sur
36	*M. handroana* Cruz	han	86	*M. tenella* Müller von Argau	ten
37	*M. hassleriana* Chodat	hsl	87	*M. tomatophylla* Standley	tll
38	*M. heptaphylla* Ule	hph	88	*M. tomentosa* Pohl	tsa
39	*M. hunzikeriana* Martinez-Crovetto	huk	89	*M. tripartita* (Sprengel) Müller von Argau	tpa
40	*M. irwinii* Rogers & Appan	irw	90	*M. triphylla* Pohl	tph
41	*M. inflata* Müller von Argau	inf	91	*M. tristis* Müller von Argau	tst
42	*M. jacobinensis* Müller von Argau	jac	92	*M. variifolia* Pax & K. Hoffmann	var
43	*M. janiphoides* Müller von Argau	jnp	93	*M. violacea* Pohl	vio
44	*M. jolyana* Cruz	jol	94	*M. walkerae* Croizat	wlk
45	*M. leptophylla* Pax & K. Hoffmann	lph	95	*M. warmingii* Müller von Argau	wrm
46	*M. leptopoda* (Müll. Arg.) Rogers & Appan	da	96	*M. websteri* Rogers & Appan	web
47	*M. longipetiolata* Pohl	lon	97	*M. weddelliana* Baillon	wdd
48	*M. maguireana* Rogers & Appan	mag	98	*M. xavantinensis* Rogers & Appan	xav
49	*M. maracasensis* Ule	mcn	99	*M. zehntneri* Ule	zeh
50	*M. marajoara* Huber	mjr			

SOURCE: Chávez et al. (1987).

Collection or Acquisition

Accessions of germplasm banks are usually landraces or traditional varieties selected by farmers over the years. Many germplasm banks also hold modern varieties, including those in disuse, and wild species. For JG Hawkes, University of Birmingham (pers. comm.), collection is the first and fundamental stage on which to develop an appropriate set of holdings. With it, the following can be guaranteed:

a. An optimal collection size that is reasonable in terms of costs and management, and possessing broad genetic diversity.

b. The exploration of high-priority areas.

c. The exploration of areas at high risk of genetic erosion.

d. The introduction of the smallest possible number of duplicates.

e. Reduced risk of introducing pests and diseases. To achieve this, full knowledge of the species to be cultivated should be available.

To attain these objectives, as much scientific preparation is needed as for logistics.

Passport data

This basic information is taken from both the sample and collection site. Hence, for each sample collected or introduced, the respective passport information must be completed. For this purpose, the standard form (Appendix 1, page 340), established by Gulick et al. (1983), should be used. The minimum data for morphological descriptors should also be recorded on this format.

Passport information is of vital importance. It not only identifies each sample, but it also reduces the risk of collecting or introducing duplicates, and permits the recovery of materials missing in the collection. Indeed, a sample with no passport data has no value.

Status of the collection at CIAT

Table 17-3 presents the *Manihot* accessions that CIAT conserves in the *in vitro* germplasm bank. This bank represents the world's largest *Manihot* collection. To date, it holds 6739 accessions. Of these, 5301 (i.e., about 87%) are *M. esculenta* clones and the other 883 accessions (13%) correspond to 33 wild species. Of the cassava clones themselves, about 91% are landraces, while the rest comprise advanced (improved) cultivars, hybrids, and genetic stock (K family for genetic mapping).

Table 17-3. Number of *Manihot* accessions conserved in the *in vitro* germplasm bank held at CIAT.

Source of accession	ISO code	Number of *in vitro* accessions
Argentina	ARG	122
Bolivia	BOL	7
Brazil	BRA	1281
Colombia	COL	2000
China	CHN	2
Costa Rica	CR	102
Cuba	CUB	84
Dominican Republic	DOM	5
Ecuador	ECU	116
Fiji	FJI	6
Guatemala	GUA	92
Honduras	HND	36
Indonesia	IND	253
Jamaica	JAM	22
Malaysia	MAL	61
Mexico	MEX	106
Nigeria	NGA	19
Nicaragua	NIC	4
Panama	PAN	51
Paraguay	PAR	208
Peru	PER	421
Philippines	PHI	6
Puerto Rico	PTR	17
Salvador	SLV	11
Thailand	TAI	37
USA	USA	10
Venezuela	VEN	253
Vietnam	VTM	9
ICA-CIAT hybrids	CG; CM[a]	
	SG; SM[b]	408
Subtotal		**5749**
Genetic stock[c]		**146**
Wild species	33 species *in vitro*	**883**
Total		**6778**

a. CG and CM = hybrids obtained through controlled pollination.
b. SG and SM = hybrids obtained through open pollination.
C. Genetic stock = K family for the study of genetic mapping.

SOURCE: www.ciat.cgiar.org/urg

According to Debouck and Guevara (1995), 94% of the cultivated cassava germplasm collection at CIAT consists of Latin American accessions, that is, from the region recognized as the primary center of diversity. The introduction of about 800 accessions from the National Cassava & Fruits Research Center (CNPMF, its Portuguese acronym), Brazil, has broadened the holdings at CIAT with a highly representative sample of genetic diversity, especially from Northeast Brazil.

Of the 61 countries where *M. esculenta* is important, 24 (39%) have contributed to the collection. High-priority areas for acquiring germplasm are Central America (Nicaragua, Honduras, and El Salvador); Amazon Region (central and western Brazil); Chaco Region (Paraguay and Bolivia); Venezuela and eastern Colombia; the Guianas and the Ecuadorian mountains; and, lastly, the Caribbean Region (Dominican Republic and Haiti, which are regarded as of moderate priority for collection).

With respect to Asia, important elite genotypes were introduced into CIAT from national improvement programs, especially that of Thailand. This country, together with China, Vietnam, and the Philippines, has recently become a priority area for acquiring germplasm that would effectively represent this secondary center of diversity.

The absence of a centralized collection of African germplasm has restricted representation of this continent. However, this situation will improve in the near future because the International Institute of Tropical Agriculture (IITA) is compiling information on national collections and consolidating regional collections of African germplasm. Advances in virus indexing techniques will also help.

More important than country representation is that of existing diversity, for which strategies of acquisition and collection have been established.

Germplasm Conservation Methods

CIAT conserves the international germplasm collection by using two systems: active conservation in the field, and active conservation *in vitro*. Replication of germplasm through these two systems guarantees its safety against unforeseen contingencies or natural disasters. Future plans are directed towards enhancing cryogenic conservation techniques to thereby guarantee long-term conservation that is safe and economic.

In-Field Active Genebank

For the CIAT Cassava Improvement Project, this traditional form of conservation has advantages and disadvantages. The advantage is one of immediate and almost permanent availability of stakes and leaves to make evaluations, either locally or at the experiment station, on, for example, morphology, physiology, resistance to pests and diseases, and nutrient contents.

The disadvantages include the heavy need for space; higher risk of losing materials to problems such as pests, diseases, poor soils, and lack of adaptation; and higher costs of maintenance and conservation.

Planting methods

Showcase collection of existing variability. A showcase collection, highly visible to visitors from the road, is planted at the beginning of the genebank. One or two furrows of five plants each are planted at 1×1 m, leaving 2 to 3 m between plots. Each plot receives a placard with the clone's name and a description of its main characteristics. The clones and their characteristics are studied before being chosen for this planting.

The genebank proper. All the bank's accessions are planted in plots, with the number of plants depending on the size of the plot and maintenance costs. A plot may be planted like a trial, with rows, or like an observation field, with rows planted at 5 to 6 plants each and separated by a row in between to prevent competition between genotypes. They may also be planted on plots of two furrows, with 5 to 10 plants per plot, leaving a space of at least 2 or 3 m between plots. To reduce the risk of losing materials, the planting should be replicated.

Furthermore, major collections such as that at CIAT, where the entire collection would occupy more than 6 hectares, the accessions are best classified according to vigor or architecture. The scale used ranges from 1 to 3, where 1 refers to non-branching, 2 to medium branching, and 3 to highly branching. Planting distances are therefore set according to vigor, thus saving area and costs.

Planting for the field genebank is carried out in alphabetical order of the accessions, according to countries of origin (e.g., M Arg, M Bol, M Bra, and M Cub) and then by number of accession within each country (e.g., M Arg 1, M Arg 2, M Arg 3, and so forth).

Hybrids are best planted by group, starting with vigor, and following the order: CG, CM, SG, and SM.

Renewal period

To reduce the risk of losing materials to biotic and abiotic factors, the field bank should be renewed every year at the beginning of the rainy season. Once renewal is accomplished, the old bank must be kept for at least another 6 to 8 months; it should not be immediately eliminated from the field. This way, stakes for replanting the new bank are guaranteed and always available. Thus, every field bank remains standing for 16 to 18 months.

Maintenance

A germplasm collection in the field is more complicated to maintain than cassava trials or other types of plots, because of, for example, wide variability in plant size and adaptation to soil conditions, and the different degrees of resistance and susceptibility to pests and diseases. Hence, to be on top of any problem that may occur, the bank should be monitored every 2 or 3 weeks. Pests and diseases must be controlled by applying integrated pest-and-disease management (IPDM), as follows:

Weed control. Weeds constitute the factor that most increases field management costs. Consequently, herbicide use is recommended.

Timely replanting. For a field bank, the minimum number of plants per plot must be determined. If a plot has fewer plants than this number, then a replanting must be planned. The replanting must be done no later than 2 months after the original planting, using stakes 40 cm long.

Controlling thrips and other pests. Pests that delay growth such as thrips must be controlled until the plants are at least 6 months old to ensure that these produce seed (stakes) that guarantee renewal. Thrips can be controlled with Sistemin® (a dimethoate) at a dosage of 2 cc/L (commercial product).

Controlling bacterial blight and superelongation disease. Bacterial blight (*Xanthomonas axonopodis*) is a disease that must not exist in a cassava field bank. If, for whatever reason, this disease appears, commercial formalin at 5% must be applied to both the infected plants and their neighbors. The diseased plants are then immediately eradicated.

Control measures for superelongation disease (*Sphaceloma manihoticola*) include the eradication of infected plants and later fumigation with cupric fungicides such as Kocide® and copper oxychloride.

Soil problems. The field bank occupies a large area. Some plots are therefore often affected by soil problems such as waterlogging and salinity. In such cases, planting may take place elsewhere in the field or the plants may be moved to bags that are provisionally placed in an appropriate screenhouse or greenhouse.

Excessive branching. The most vigorous clones may close alleys with their branches and foliage, hampering personnel movement and data collection. Unfortunately, cutting the foliage increases the possibility of attack from stemborers. Thus, pruning the foliage of highly vigorous clones must be as minimal as possible.

Quarantine measures

The use of quarantine measures in the field bank aims to prevent the introduction or dissemination of diseases and pests. Recommendations include:

- Whenever seed (stakes) or foliage must be cut, workers should each carry a recipient containing soap or commercial formalin to disinfect the machetes.
- Workers who come from other plots must shake out and clean their work clothes before entering the bank.
- All tools and machinery to be used in the bank must be disinfected.
- New clones entering the bank, even if they are from the same region, state, or country, should never be directly introduced. Instead, they should be first planted within a greenhouse or screenhouse. Later, they may be planted in an isolated plot and then, once their health is verified, they may enter the bank.

Morphological and agronomic descriptors. "Morphological descriptor" is understood as that set of characteristics that easily identify and differentiate a genotype, including heritability and stability before environmental changes. Descriptors are mainly used to characterize the accessions of any given collection.

For the morphological characterization of *M. esculenta*, an up-to-date list of the morphological and

agronomic descriptors is used. This list was standardized for cassava by the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) at a 1995 meeting held in Cruz das Almas (Bahia), Brazil (Gonçalvez et al. 1996).

As Gonçalves and Guevara noted (1998), the list comprises 13 minimal descriptors that are necessarily included in passport data, another 13 principal and 11 secondary descriptors, a further 21 for preliminary agronomic evaluation, and 17 for complementary evaluation (e.g., flowers, fruits, and seeds), totaling 75 descriptors. Wild *Manihot* species require a second table with different descriptors.

To manage a given collection, all accessions must be duly characterized morphologically. As Iglesias et al. (1995) point out, the integration of morphological with biochemical and molecular descriptors, and accompanied by passport data, form a valuable tool for identifying duplicate accessions in that collection.

Biochemical descriptors, using isoenzymes. Ocampo et al. (1993) indicated that, because of the limitations presented by morphological markers for evaluating a collection's materials, techniques for the electrophoresis of total seed protein must be used. However, these are limited to differentiating among genetically close materials. The later development of isoenzyme electrophoresis techniques largely resolved this limitation. These techniques use an electrical field to separate enzymes present in a raw extract of a tissue. Because of their electrical charges and sizes, the enzymes migrate to different positions within a gel matrix of either starch or polyacrylamide.

Because enzymes catalyze specific biochemical reactions, an enzyme's location in the gel can be seen. Through adding a substratum and appropriate cofactors, reaction products can be detected through color reactions. Thus, a visible band is formed at the site where a given enzyme is located. When different molecular forms of an enzyme have affinity for a single substratum, these forms make up an isoenzyme family.

The pattern of isoenzyme bands is analyzed quantitatively, using a laser densitometer. It is also qualitatively codified according to the presence or absence of each of the 22 bands (Figure 17-1).

Molecular characterization. According to Ocampo et al. (1995), given the limitations of morphological and biochemical descriptors, materials should be evaluated directly from their genomes. This

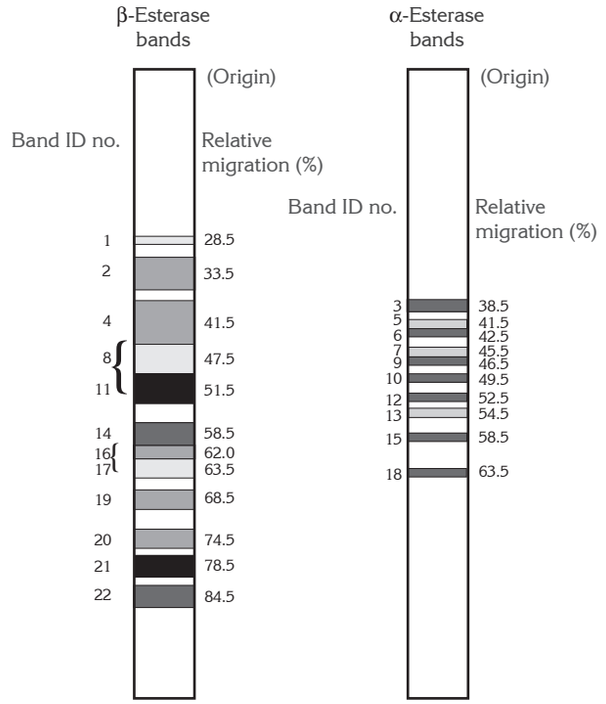

Figure 17-1. Zymogram showing patterns of isoenzyme bands of α- and β-esterases obtained from cassava tissues.

was initially done, using the "restriction fragment length polymorphism" (RFLP) but several markers are now available such as amplified fragment length polymorphism" (AFLP), microsatellites, simple sequence repeats (SSR), or single nucleotide polymorphism (SNP).

The evaluation of major germplasm collections, using only different types of molecular markers or isoenzymes, would be laborious and expensive, although the costs and the efficiency has improved astonishingly fast in recent years. The importance of morphological and agronomic characterization should not be ignored, but used to complete the first stages of characterization. Thus, a large collection would be reduced to small groups, which can then be more efficiently and economically evaluated, using the isoenzyme or different molecular markers techniques.

Duplicate identification and elimination. In a germplasm collection that is maintained vegetatively, accessions are often duplicated. Preliminary observations of the collection at CIAT estimated that the level of duplication is between 20% and 25%. Hershey, cited by Iglesias et al. (1995), noted that the presence of a large number of duplicates in a germplasm collection has negative implications for their management and use in improvement programs, such as:

- Significant increase in the costs of conservation and evaluation
- Skewing of genetic variability
- Narrowing of the genetic base
- Undesirable homozygosis in crosses

Iglesias et al. (1995) pointed out that, over the years, the *Manihot* collection at CIAT has been classified by basic morphological descriptors, which had first been defined by the International Board for Plant Genetic Resources (IBPGR, now Bioversity International). In themselves, they do not reliably identify duplicates. However, if biochemical characterization is included, based on codifying the presence or absence of 22 isoenzyme bands of α- and ß-esterases in STET gels, confidence levels increase greatly.

Given the above considerations and to eliminate duplicates from the international cassava collection, CIAT developed and applied a model based on the following criteria:

- *Preliminary grouping of clones.* Grouping is based on within-group identification, the selection of four primary morphological characteristics, and 12 electrophoretic bands of high-level confidence:
  - Morphological characteristics: Stem colenchyma, stem epidermis, stem growth habit, and root external color
  - Presence or absence of electrophoretic bands coded 3, 4, 9, 10, 12, 13, 14, 15, 19, 20, 21, and 22

- *Secondary grouping of clones.* In groups larger than 10 clones, a second level of grouping is made by cluster analysis, using the following group of morphological characteristics and electrophoretic bands of secondary level of confidence:
  - Morphological characteristics: Height of first branching, color of apical leaf, pubescence, vein color, lobe shape, lobe width, petiole color, cortex color, and root pulp color
  - Presence or absence of electrophoretic bands coded 1, 6, 8, 18, 2, 5, 7, and 17

- *Confirmation in the field.* Clones grouped with possible duplicates are planted in the field and the morphological descriptors are reevaluated. Clones with identical descriptors are checked for their passport data. If these are the same, the duplicates are eliminated from the field collection, but they remain in the *in vitro* collection for later confirmation with RFLP molecular markers.

Preliminary agronomic evaluation

The CIAT Cassava Improvement Project uses the following strategy for the preliminary agronomic evaluation of the cassava germplasm bank:

a. Define and select edaphoclimatic areas that contrast and represent cassava-producing areas.

b. Select the group of accessions to be evaluated.

c. Plant according to the system "bank's observation field", which consists of a row of six plants per accession, separated by one furrow in between.

d. Select the best materials and later evaluate these in a preliminary yield trial, followed by conventional yield trials.

e. Select the materials that best show integration of adaptation, yield potential, resistance to pests and diseases, and root quality.

f. After several cycles, followed by advanced stages, classify the selected materials as "elite" and recommend them to national programs or use them as parental materials in hybridization schemes.

Documentation and exchange

According to Debouck and Guevara (1995), this stage encompasses the following genebank activities to provide information for entering institutional documentation system (Oracle):

- Passport data
- Morphological and isoenzymatic characterization
- Preliminary agronomic evaluation
- Conservation methods and techniques
- Indexing tests
- Germplasm exchange

The mandate assigned to CIAT by the CGIAR includes not only germplasm conservation, but also its *distribution or exchange*. Given these goals, the following protocol (Figure 17-2) was established for field conservation to minimize the distribution of pests and diseases:

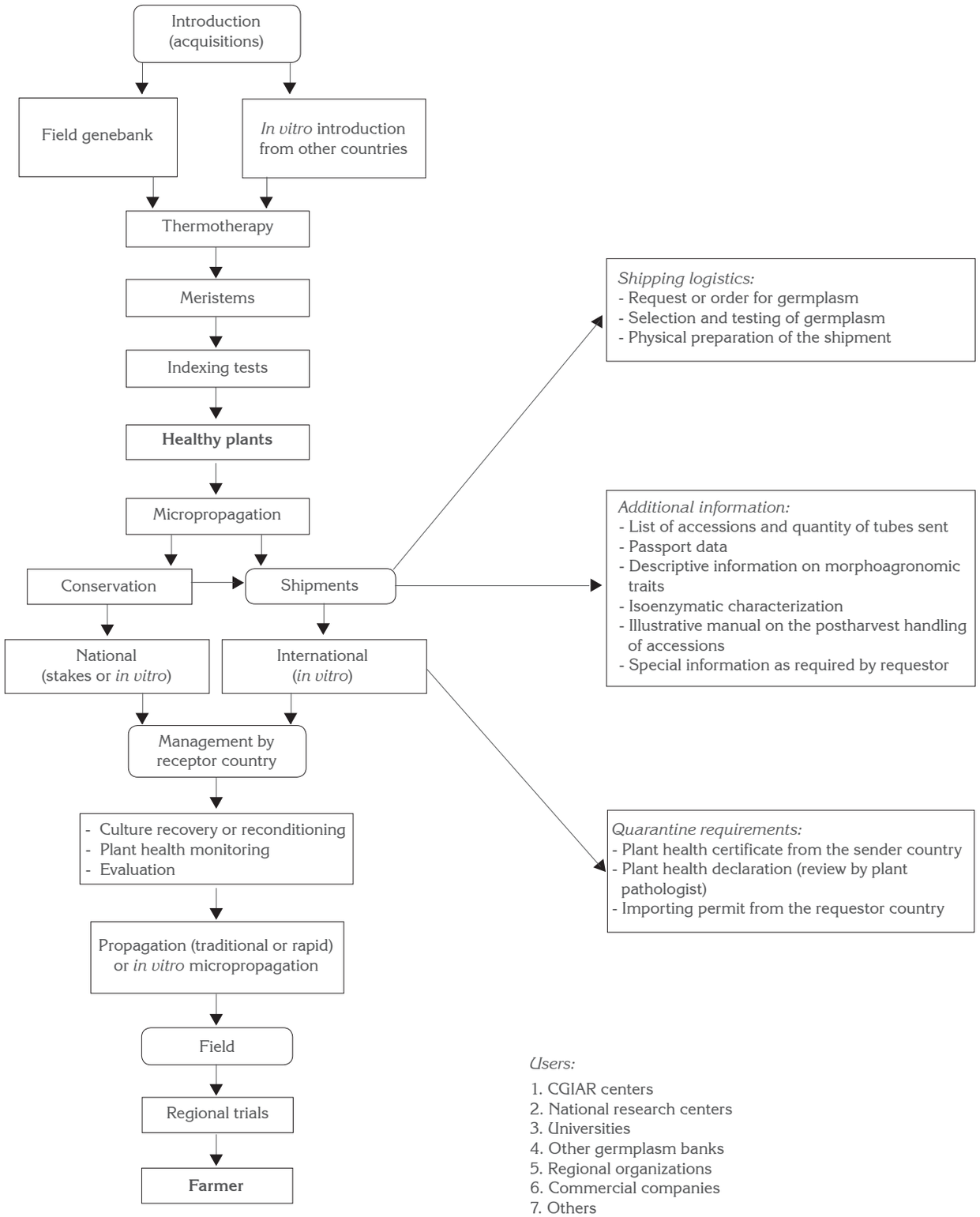

Figure 17-2. Exchange of *Manihot* germplasm (from Debouck and Guevara 1995).

- Prohibit shipments to the exterior of all materials in the form of stakes.

- Only indexed stakes may be sent when the materials are for purely experimental or very specific purposes, and will be planted in the greenhouses of non-cassava-producing countries of temperate areas. Furthermore, the stakes must be accompanied by the exporting country's plant health certificate and the importing country's previously obtained importing permit.

- Cassava plant materials may be distributed to other countries only as meristem culture from plants that underwent thermotherapy and indexing. Sexual seed may also be distributed, provided that plant health certificates and importing permits have been issued.

In Vitro Active Genebank

Debouck and Guevara (1995) noted that the cassava *in vitro* active genebank (IVAG) consists of maintaining the plants under slow-growth conditions by providing physical and chemical conditions that extend, as far as possible, the interval before transfer to fresh media is needed. In *in vitro* conservation, the growth rate of cultures can be controlled by managing the following factors:

- Temperature
- Inorganic and organic substances
- Growth regulators
- Osmotic regulators
- Ethylene inhibitors and capturers

Conditions for conservation and renewal

The findings of several years of research by CIAT scientists indicated the following growth conditions for *in vitro* cassava conservation:

- A constant temperature between 23 and 24 °C
- A photoperiod of 12 h of light
- Light intensity at 1000 lux
- Modified culture media (MS) (Table 17-4)
- Test tubes of 25 × 150 mm, covered with aluminum foil and sealed with plastic
- Conservation of five tubes per clone

Under these conditions, the *in vitro* collection presents an average period of conservation of 12.8 months, ranging from 10.3 to 18.5 months, according to country of origin.

Procedures for *in vitro* conservation

Debouck and Guevara (1995) suggest the following procedures:

- Enter the materials
- Establish the *in vitro* culture
- Evaluate and monitor the cultures' aseptic state
- Maintain and renew the materials
- Monitor viability and genetic stability
- Document and systematize the bank

Table 17-4. Culture media used for the operations of introduction, conservation, transfer to greenhouse, and exchange of *in vitro* cassava clones.

Constituents of the medium	Concentration in medium:		
	4E (for meristem initiation, micropropagation, and exchange)	8S (for conservation)	17N (for transfer to greenhouse)
Inorganic salts	MS	MS	1/3 MS
m-Inositol	100 mg/L	100 mg/L	100 mg/L
Thiamine HCl	1 mg/L	1 mg/L	1 mg/L
Sucrose	2%	2%	2%
BAP	0.04 mg/L	0.02 mg/L	—
GA	0.05 mg/L	0.10 mg/L	0.01 mg/L
ANA	0.02 mg/L	0.01 mg/L	0.01 mg/L
Agar	0.7 g	0.7 g	0.7 g
pH	5.7–5.8	5.7–5.8	5.7–5.8

SOURCE: Debouck and Guevara (1995).

With regard to "entering the materials", Colombian materials may be introduced as plant materials (or stakes), whereas introductions from other countries are made only *in vitro*. The cultures are then multiplied or micropropagated. After micropropagation, the cultures are planted in 8S culture medium to conserve them and then placed under conditions specific to their establishment.

Under these conditions, the cultures are left for more than 2 weeks and then evaluated for plant development and health, taking into account the following basic aspects: state of the medium, state of the tube's cover and seal, seedling development, plant health, and each tube's nomenclature and identification. Once the evaluation is completed, each material is registered in the database, identified by its varietal name, date of entry, culture medium, and location in the conservation room.

The materials are stored within this room at five tubes per variety. These are located on shelving and are ordered according to code. Arrangement by stand, row, shelf, and test-tube rack facilitates searching.

Maintenance and renewal

In vitro conservation requires that conditions in the conservation room be maintained constant, using equipment for regulating temperatures, relative humidity, and light. Tasks for renewing materials are also carried out here.

The materials coming from conservation are micropropagated and then placed in 4E growth medium for recovery and strengthening. When these materials are established, they are propagated again and moved to 8S medium for conservation. The following information is recorded in the database: date of exit for subculturing and cause of exit, whether contamination, subculturing, elimination, or exchange. Finally, genetic stability is monitored, using morphoagronomic and biochemical criteria.

Cleaning clones

According to Guevara and Valderrama (1995), the literature reports more than 50 cassava diseases produced by viruses, bacteria, fungi, and phytoplasmas. Among the most important viral diseases are:

- African mosaic virus (ACMV), caused by viruses from the Geminivirus group
- Vein mosaic virus (CVMV), caused by the Caulimovirus group
- Common mosaic virus (CsCMV), belonging to the Potexvirus group
- Frogskin disease (CFSD) complex, including Caribbean mosaic (CMD)
- Colombian symptomless virus (CCSpV), belonging to the Potexvirus group

All these viruses can be eliminated by using thermotherapy techniques associated with meristem culture. Figure 17-3 illustrates the general scheme for eliminating viruses from cassava. The steps are:

- Applying thermotherapy to meristem culture (i.e., to *in vitro* seedlings) or to shoots that have germinated from stakes originating in the field
- Thermally treated materials undergo indexing tests
- Micropropagation of healthy clones, using *in vitro* culture techniques
- Virus detection

Indexing tests

Indexing tests for cassava viruses can be applied to both *in vitro* seedlings and greenhouse plants. The general methodology used to eliminate cassava viruses includes the following techniques:

- *Grafting with the highly susceptible clone M Col 2063 or 'Secundina'*. This test is used mainly to detect frogskin disease. To graft, the material being evaluated is the main plant (stock), while Secundina is the graft (i.e., grafted onto the main plant). Should the stock be infected, symptoms will be expressed in the graft (Figure 17-4). With this hypersensitive material, readings are normally made at 30 days. The graft must be absolutely clean to prevent the reading of false positives.
- *The ELISA test* is used for the following viruses: African mosaic virus (ACMV), vein mosaic virus (CVMV), Colombian symptomless virus (CCSpV), and Caribbean mosaic (CMD).
- *Double-stranded RNA* (dsRNA) is used to test for the RNA of the following cassava viruses: frogskin disease (CFSD); common mosaic virus (CsCMV); and latent viruses.

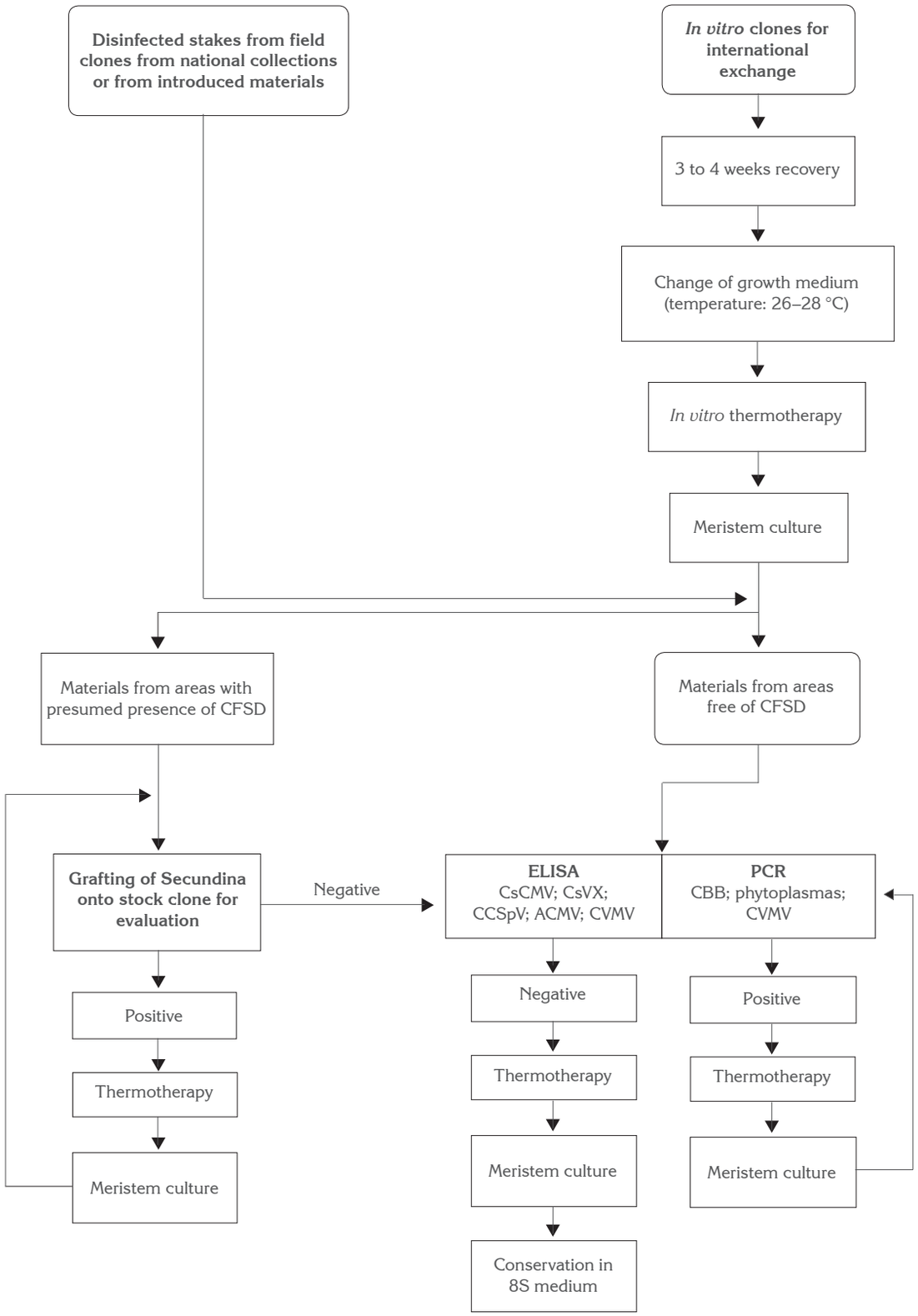

Figure 17-3. Procedures for detecting and eliminating cassava pathogens (from Debouck and Guevara 1995). For an explanation of abbreviations and acronyms, see *Appendix 1: Acronyms, Abbreviations, and Technical Terminology*, this volume.

Figure 17-4. The highly susceptible cassava variety Secundina (with leaves) is grafted onto the stock (stake of the clone being evaluated). (Photo by Norma Flor, Genetic Resources Unit, CIAT.)

- *Polymerase chain reaction* (PCR) is used to detect bacterial blight (CBB), phytoplasmas, and vein mosaic virus (CVMV).

Cryogenic Conservation

According to Escobar et al. (1998), the *in vitro* base genebank (IVBG) is founded on the cryoconservation of clones, that is, on the total suppression of their growth, metabolism, and other biological processes by applying very low temperatures. Mutations are also prevented. Conservation thus becomes indefinite.

The method consists of isolating precultured meristems by using a cryoprotectant agent, completing a stage of controlled cooling, and transferring to liquid nitrogen at 196 °C. The protocol established for cassava cryopreservation at CIAT is as follows:

- Isolation of meristems that measure 2 to 3 mm long and had been taken from a 3 to 4-month-old *in vitro* culture
- Pretreatment in 4E medium for 3 days
- Preculture on solid C4 medium for 3 days, in the dark, and at temperatures between 26 and 28 °C
- Cryoprotection in liquid medium for 2 h on ice
- Dehydration or drying for 1 h, using filter paper, at room temperature
- Programmed slow cooling, using a CryoMed freezer at -40 °C
- Immersion and storage in liquid nitrogen for at least 3 h
- Heating to 37 °C for 45 s
- Re-culturing:
  - R1 and R2 balance media for 2 days each
  - Transfer to CIAT 4E semisolid medium
- Evaluation:
  - Tissue survival or viability
  - Shoot formation after 1 month

Studies on cryogenic conservation currently conducted at CIAT have led to the development of two main methodologies:

- For *botanical seed*, working with *M. esculenta* and *M. carthaginensis*. This method permits total recovery of viable plants.
- For *planting materials*, which itself has two methodologies:
  - Classical, where the varietal response is modified.
  - New, which has a practical sense in that active work is done, especially on the "core'" collection. The technique is called encapsulation/dehydration.

The IVBG constitutes a basic working, but inactive collection that is conserved for the long term. Once the technique is completely developed, this bank will be able to totally maintain the germplasm's genetic stability. This collection is expected to become an efficient and economic alternative for conserving cassava clones. Thus, cryopreservation would be a safe method for long-term storage in a reduced space. It would also be free of changes and relatively low-cost.

Debouck pointed out that cryoconservation is not envisioned as a distribution method, for which the

in vitro active genebank is more suitable. The main activities involved in cassava germplasm exchange by the CIAT *in vitro* bank are presented in Figure 17-2.

Nucleus or Core Collection

The concept

The concept of a "nucleus" or "core" collection was proposed by Frankel in 1984 (cited in Iglesias et al. 1992) to define a set of accessions that, with a minimum of repetition, would represent the genetic diversity of a given species. The accessions that become part of a core collection are selected for their representativeness and ecological or genetic differences. Iglesias et al. (1992) also discuss the following points:

- A core collection must be constituted in such a way that its genetic diversity is maximized. This means that duplicated or closely related accessions must be excluded. Normally, core collections for cultivated species are separated from those of their wild relatives.

- For a given species, other groups of accessions may exist for specific purposes. These can also be core collections. An example is the group of elite clones within the germplasm collection held at CIAT.

Advantages of a core collection

As a representative sample, a core collection has the following advantages:

- It increases efficiency in the use of genetic resources by facilitating evaluation and access to existing genetic variability.

- It enables the use of methodologies that can later be extended to the entire collection.

- Facilitates the possibility of duplicating accessions for other institutions.

Requisites

Ideally, a core collection for a cultivated species has the following characteristics:

- It covers the total range of genetic variability existing in that species

- It consists mainly of landraces, for which the passport data are complete.

- It does not include duplicated accessions.

- It has been well characterized, using morphological and molecular descriptors.

- Traits such as agronomic and physiological characteristics, root quality, and resistance to diseases and pests have been evaluated.

- Information on the crop's evolution and different centers of genetic diversity is adequate.

Collection size

Brown (cited in Iglesias et al. 1992) recommends selecting 5% of all accessions in large collections such as for maize, and 10% for small collections such as for cassava. Also taken into account are factors for conservation and the limits imposed by sample size on the evaluation of certain characteristics. Hence, a core collection of 600 to 650 accessions was first proposed as an objective for the cassava collection at CIAT.

Parameters for definition

The general criteria for defining the cassava core collection were classified into four groups:

- Geographic origin
- Diversity of morphological characteristics
- Diversity in the band patterns of α- and ß-esterases
- A priori selection of accessions based on the following requisites:
  - Clones included in studies by the Cassava Biotechnology Network (CBN)
  - The most frequently planted local varieties
  - Elite clones from the cassava improvement program; these genotypes represent, with high frequency, those genes that favor a large number of characteristics

To sample the main collection's genetic diversity, emphasis was given to geographic origin. About two-thirds of accessions in the core collection were selected this way (Table 17-5).

Table 17-5. Parameters, including country of origin, for determining the number of accessions to be selected for the cassava core collection held at CIAT[a].

Origin	No. of access.	Local cultivars (%)	Level of duplic. (%)	Base number of local cultivars	Importance as diversity center		Total diversity of country in collection at CIAT		Diversity of ecosystems		Factor of correction by size[b]	Sum of weights[c]	Geographic origin[d]	Morphological diversity[e]	Divers. of esterase	A priori selection[f]	Final no. of access.[g]
					Score	Weight 1	Score	Weight 2	Score	Weight 3							
Argentina	16	40	10	6	1	1.00	25	0.75	2	0.40	1.00	2.15	2	4	0	3	8
Bolivia	3	100	0	3	1	1.00	5	0.95	2	0.40	1.00	2.35	1	2	0	3	3
Brazil[h]	1637	95	20	1244	1	1.00	40	0.60	5	1.00	0.20	0.52	110[i]	13	15	20	101
China	2	100	0	2	3	0.50	25	0.75	3	0.60	1.00	1.85	1	0	0	2	2
Colombia	1907	95	20	1449	1	1.00	75	0.25	5	1.00	0.20	0.45	111	15	13	14	146
Costa Rica	147	40	20	47	2	0.75	80	0.20	2	0.40	0.80	1.08	9	7	5	4	23
Cuba	74	90	20	53	2	0.75	80	0.20	2	0.40	0.80	1.08	10	5	1	2	18
Domin. Rep.	5	100	10	5	2	0.75	10	0.90	3	0.60	1.00	2.25	2	2	0	4	5
Ecuador	117	100	25	88	1	1.00	50	0.50	3	0.60	0.80	1.68	25	6	0	4	32
Fiji	6	100	10	5	3	0.50	50	0.50	1	0.20	1.00	1.20	1	0	0	2	2
Guatemala	91	100	50	46	2	0.75	80	0.20	2	0.40	0.80	1.08	8	6	0	2	15
Indonesia	51	10	15	4	3	0.50	10	0.90	3	0.60	0.80	1.60	1	0	2	5	7
Malaysia	68	70	15	40	3	0.50	50	0.50	2	0.40	0.80	1.12	8	0	1	6	15
Mexico	100	95	30	67	2	0.75	75	0.25	3	0.60	0.80	1.28	14	6	0	2	20
Panama	42	100	20	34	2	0.75	75	0.25	2	0.40	0.80	1.12	6	2	0	2	9
Paraguay	192	100	20	154	1	1.00	80	0.20	2	0.40	0.60	0.96	25	8	3	7	40
Peru	405	95	20	308	1	1.00	60	0.40	2	0.60	0.60	1.20	63	10	3	2	76
Philippines	6	30	0	2	3	0.50	5	0.95	2	0.40	1.00	1.85	1	0	0	2	2
Puerto Rico	15	40	15	5	2	0.75	60	0.40	2	0.40	1.00	1.55	1	2	0	4	7
Thailand	8	10	0	1	3	0.50	75	0.25	2	0.40	1.00	1.15	0	0	0	4	4
USA	9	0	0	0	3	0.50	100	0	1	0.20	1.00	0.70	0	0	0	4	4
Venezuela	240	95	20	182	1	1.00	60	0.40	4	0.80	0.60	1.32	41	9	3	3	55
CIAT clones	317	0	0	0									0	3	5	27	33
IITA clones	19	0	0	0									0	0	0	3	1
Total	**5477**			**3744**									**440**	**100**	**51**	**121**	**630[j]**

a. Access. = accessions; duplic. = duplication; Score of a scale; divers. = diversity.
b. Factor of correction according to the size of the collection, where >1000 = 0.2; 400–1000 = 0.4; 100–400 = 0.6; 20–100 = 0.8; 1–20 = 1.0.
c. Sum of weights (1, 2, and 3) × factor of correction according to size of collection.
d. Number of accessions for core collection = (sum of weights × base number of local cultivars × constant), where the constant = 0.17.
e. Clones included in the pilot *in vitro* active genebank (IVAG) at CIAT/IBPGR (now Bioversity International).
f. Selected by three criteria:
   - Included in studies conducted by the Cassava Biotechnology Network (CBN), based on the diversity of geographic origin and agronomic value
   - Most widespread cultivars
   - Elite clones held at CIAT and the International Institute of Tropical Agriculture (IITA)
g. The final number may be less than the sum of the columns, given that the same clone may have been selected for different parameters.
h. Includes 800 accessions introduced in 1991/92.
i. Sixty accessions will be introduced, followed by another 800 new accessions, totaling 970 in all.
j. The final number may be smaller after detecting and eliminating duplicates.

SOURCE: Iglesias et al. (1992).

Clones included in the core collection

The application of all the parameters mentioned above enabled the definition of a first list of clones to include in the core collection at CIAT (Table 17-6).

Iglesias et al. (1992) also noted that, when defining a core collection, the question arises of how flexible its structure should be in accepting changes. Presumably, excessive dynamism would not be good if what is desired is a reference sample for the systematic evaluation of different characteristics. However, such a structure should allow the incorporation of new accessions that will increase even more the selected sample's representativeness of the genetic diversity existing in the field.

In practical terms, 70% to 80% of the core collection, as initially defined, could reasonably be expected to remain unmodifiable. The remainder may be subjected to change, in accordance with new information obtained over the short and medium term.

Wild *Manihot* Species

Few crops have such a high number of related or wild species as *M. esculenta*. According to Chávez (1990), wild *Manihot* species constitute a valuable resource for improvement programs, because of their:

- High potential as sources of genes for resistance to pests and diseases

Table 17-6. Clones included in the core collection at CIAT, using different parameters.

Origin	Number of clones included according to parameter:				Final number in core
	Geographic origin	Morphological diversity	Diversity of esterases	A priori selection	
Argentina	2	4	0	3	8
Bolivia	1	2	0	3	3
Brazil	110	13	15	20	101
China	1	0	0	2	2
Colombia	111	15	13	14	146
Costa Rica	9	7	5	4	23
Cuba	10	5	1	2	18
Dominican Republic	2	2	0	4	5
Ecuador	25	6	0	4	32
Fiji	1	0	0	2	2
Guatemala	8	6	0	2	15
Indonesia	1	0	2	5	7
Malaysia	8	0	1	6	15
Mexico	14	6	0	2	20
Nigeria	0	0	0	3	3
Panama	6	2	0	2	9
Paraguay	25	8	3	7	40
Peru	63	10	3	2	76
Philippines	1	0	0	2	2
Puerto Rico	1	2	0	4	7
Thailand	0	0	0	4	4
USA	0	0	0	4	4
Venezuela	41	9	3	3	15
Hybrids	0	3	5	27	33
Total	**440**	**100**	**51**	**131**	**590**

SOURCE: Iglesias et al. (1992).

- Tolerance of most of the common abiotic stresses
- Broad genetic variability for important agronomic and biochemical characteristics such as low hydrocyanic acid content and high protein content
- Highly desirable C4 photosynthetic route

Because of the importance of these species and the considerable genetic erosion they suffer, one conservation option is to establish an *ex situ* germplasm bank with these valuable materials.

Coding and abbreviations

Within the *Manihot* genus, all species studied have the same number of chromosomes: 2n = 36. To date, 98 wild species plus cassava have been recognized, with five more being described. Taxonomically, the *Manihot* genus is separated into 18 sections.

Chávez et al. (1987) indicated that, for coding, CIAT has developed and set up a standardized system of nomenclature for *Manihot* species and sections. Tables 17-2 and 17-7 list the abbreviations of all 99 species and 18 sections. In this system, an abbreviation is made up of three lowercase letters to represent the species, with the first letter being taken from the first letter of the species's name. No abbreviation is repeated. For the sections, the abbreviations used each consists of three uppercase letters, thus differing from the lowercase abbreviations for species.

Table 17-7. *Manihot* sections in alphabetical order, with their respective abbreviations.

Serial number	Section	Abbreviation
1	Anisophyllae Rogers & Appan	ANY
2	Brevipetiolatae Pax	BRE
3	Caerulescentes Rogers & Appan	CAE
4	Carthaginenses Rogers & Appan	CAR
5	Crotalariaeformes Rogers & Appan	CRO
6	Foetidae Rogers and Appan	FOE
7	Glaziovianae Pax	GLA
8	Graciles Rogers & Appan	GCL
9	Grandibracteatae Pax	GND
10	Heterophyllae Pax	HET
11	Manihot P. Miller	MAN
12	Parvibracteatae Pax	PAR
13	Peltatae Pax	PEL
14	Peruvianae Rogers & Appan	PER
15	Quinquelobae Pax	QUI
16	Sinuatae Pax	SIN
17	Tripartitae Rogers & Appan	TRI
18	Variifoliae Rogers & Appan	VAR

The list contains all the taxonomically critical wild species of the *Manihot* genus as published by Rogers and Appan (1973). It also includes the new species recently described by Nassar (2000). Synonyms are excluded. Allem (2002) provided an update of the origins and taxonomy of cassava.

Possible contributions

For Chávez (1990), current studies have demonstrated that many of the wild species have potential in improvement programs as sources of genes for beneficial characteristics, including resistance to pests and diseases, adaptation, and tolerance of abiotic stresses. Table 17-8 details the possible contributions that some wild species may make.

Table 17-8. Outstanding characteristics and possible benefits from wild *Manihot* species.

Species	Characteristic and/or benefit
M. pringlei	Low cyanide content
M. glaziovii	Resistance to African mosaic virus
M. pseudoglaziovii	Resistance to bacterial blight; resistance to drought; tolerance of cold
M. reptans	Resistance to bacterial blight
M. tristis	High starch content
M. angustiloba	High starch content
M. neusana	Resistance to stemborer
M. pohlii	Resistance to stemborer
M. grahamii	Resistance to stemborer; tolerance of cold
M. chlorosticta	Adaptation to saline soils
M. carthaginensis	Resistance to drought
M. dichotoma	Resistance to drought
M. irwinii	Excellent adaptation to lateritic acid soils
M. tripartita	Excellent adaptation to lateritic acid soils
M. orbicularis	Excellent adaptation to lateritic acid soils
M. peltata	Tolerance of acid soils
M. attenuata	Tolerance of cold
M. rubricaulis	Tolerance of cold
M. gracilis	Dwarf type

SOURCE: Chávez (1990).

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To save space, the acronym "CIAT" is used instead of "Centro Internacional de Agricultura Tropical".

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Appendix 1:

Form for Collecting Cassava Materials

Note: The section on descriptors must be answered

Genus: _____ Species: _____ Subspecies: _____

Name of collectors (initials): _____ Provisional code (collected sample) _____

Institution responsible: _____

Collection date (year/month/day): _____ International code (collected sample) _____

Country of collection: _____ Province/State: _____

Site: Closest municipality or town: _____

Distance (km): _____ Address: _____

Latitude: degrees: _____ minutes: _____ North _____ South _____

Longitude: degrees: _____ minutes: _____ East _____ West _____

Altitude: meters above sea level: _____

Sample's immediate origin (encircle):

Wild	1	Local market	5
Farm field	2	Commercial market	6
Local store	3	Institute	7
Household plot or garden	4	Other: _____	8

Sample's status (encircle):

Wild	1	Landrace	4
Weedy	2	Improved cultivar	5
Improved line	3	Other: _____	6

Local name: _____

Photo (encircle): Yes No Photo code: _____

Sample type (encircle): Plant 1 Seed 2 Both 3

Herbarium sample from the site (encircle): Yes No

Amount of material (number of seeds or stakes): _____

Primary morphological **descriptors** (encircle):

Color of apical leaf		3	5	7	9				
Color of adult leaf		3	5	7	9				
Color of petiole		1	2	3	4	5	7	9	
Lobe shape	1	2	3	4	5	6	7	8	9
External stem color	3	4	5	6	7	8	9		
External root color		1	2	3	4				
Color of root cortex	1	2	3	4					
Color of root pulp		1	2	3					

Growth habit (encircle):

Tree 1 Shrub 2 Creeper 3 Other 4 _____

Part of plant used (encircle): Roots 1 Foliage 2

Principal use (encircle):

Human consumption (fresh)	1	Animal consumption (dried or processed)	4
Human consumption (dry or processed)	2	Starch extraction	5
Animal consumption (fresh)	3	Other: _____	6

Special qualities according to farmers (encircle):

Yield	1	Resistance to diseases	5
Starch content	2	Resistance to pests	6
Culinary quality	3	Edaphic adaptation	7
Roots tolerant of PPD	4	Other: _____	8

Notable defects according to the farmer: _____

Diseases or pests and their severity:

(Scale of severity, where 1 = little damage; 2 = moderate damage; 3 = severe damage)

Disease or pest	Severity	Disease or pest	Severity

Crops in association: Yes 1 None 2 Details: _____

Information on wild species sample: _____

Natural vegetation (encircle):

Wet rainforest	1	Spiny forest	6
Humid rainforest	2	Desert thicket	7
Semi-humid rainforest	3	Desert	8
Dry forest	4	Other: _____	9
Very dry forest	5		

Topography (encircle):

Swampy	1	Undulating	5
Flood-prone plains	2	Hills	6
Vega	3	Mountainous	7
Plains	4	Other: _____	8

Soil texture (encircle):

Sandy	1	Clayey	5
Loamy-sandy	2	Stony	6
Loam	3	Organic	7
Loamy-clayey	4	Other: _____	8

Drainage (encircle):

Poor 1 Moderate 2 Good 3 Excessive 4

Slope (encircle):

Flat or almost flat (<4°) 1 Moderate slope (4°–14°) 2 Steep slope (>14°) 3

Brightness (encircle):

With sun 1 With shade 2

Comments: _____