ANNUAL REPORT 1999

CIAT Project on Saving Agrobiodiversity SB-01

Genetic Resources Unit
Report on Achievements and Progresses

CIAT
SEPTEMBER, 1999

Executive Summary

This report presents achievements against planned targets for the delivery of outputs as resented in the logical framework of the Project SB-01. The upgrading plan to put the FAO Designate collections in line with the FAO/IPGRO international standards has continued in 1999. As part of a systemwide initiative in view of the FAO Commission meeting of November 1999, we have re-checked and updated the lists of Designate Germplasm for the three groups of commodities. The GRU is responsible for a total of 53,947 accessions, and continues to include into the Designate Collections valuable materials received in the past at CIAT. A total of 1,021 materials were introduced into the whole process in 1999, and 4,908 bean accessions are currently under 1st multiplication. Regeneration is becoming an urgent task, as many seed stocks are aging (first multiplications done in the period 1974-1980!). Regeneration was undertaken/ completed for a total of 20,516 accessions of beans and forages. A total of 3,439 accessions of forages and beans were packed for short- and long -term conservation. A set of 3,000 accessions of bean germplasm is ready for security backup at CENARGEN. To date 70% of the cassava collection has been processed through thermotherapy as a step towards the cleaning against viruses and viroids of quarantine importance and thus effective availability. GRU has distributed 10,585 samples from the Designate collections to both internal and external users. As part of the upgrading effort, thanks to CIAT Capital fund, the GRU also upgrades the cleaning room and the field area, and put another cold store into service. Vacuum counting devices were also installed into five different sections of GRU.

Research in SB-01 focuses on targets for conservation and on methods for safer and more reliable conservation and distribution of germplasm. The definition of targets for conservation includes a molecular study of *Brachiaria* and *Urochloa*. Information about the early evolution and species relationships was generated for *Phaseolus* beans, with the help of multiple molecular markers and pollen data. Several of these markers were successfully applied to investigate species relationships in *Capsicum* peppers. Reproductive mode of *Brachiaria* grass germplasm has been characterized for the entire collection, limiting special multiplication procedures to the sole allogamous accessions. A study has been conducted to define the best protocols to limit drift and genetic erosion in wild bean accessions. Detection of bacterial diseases in bean seeds was investigated with immunofluorescence staining. Micrografting techniques in vitro and in vivo offer hope to speed up the indexing of the entire cassava collection against Frog Skin Disease in cassava. Research to find a PCR diagnostic tool for CVMV continued during 1999. As a step towards security backup of the cassava collection slow-growth protocols allowing subculturing intervals of 20 months are now being tested for genetic stability. Protocols for in vitro conservation of tropical fruit genetic resources are being investigated. Seven publications by SB-01 Staff were published in 1999. Ing. Agr. Juan Manuel Osorno G.. won the "Otto de Greiff" award for the Best Thesis (carried out at GRU in 1997) at graduate level in Colombia.

CIAT Project on Saving Agrobiodiversity SB-01
Report on Achievements and Progresses for 1999

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1. Introduction

This report presents achievements , progresses and activities carried out for the maintenance and service to the FAO Designate Collections held in trust in CIAT during the period October 1998 through September 1999. It also presents progresses made for the filling of objectives of SB-01 CIAT Project on Saving Agrobiodiversity.

2. Project work breadown structure and Objectives

The objectives of Project SB-01 as per the Mid-term Strategic Plan of CIAT are to:
+ make the FAO Designate collections fully meeting the international FAO/IPGRI standards for genebanks
+ make the FAO Designate collections and their pertinent information fully available
+ make the FAO Designate collections genetically and socially relevant
+ contribute to the formation of human resources in conservation methodologies in the region
+ provide scientific input in *in situ* conservation of farmers' landraces and wild relatives

Each objective area can be better visualized as an inter-related sub-project; there is thus a log-frame for each sub-project that is presented separately in Annex 1. There are thus specific outputs for each area.

3. Project Logical Framework

The logical framework developed in 1996-7 and in use in 1998 is presented in Annex 1. Research outputs shall be referred to using the same numbering.

4. Highlights

+ A total of 53,947 accessions (31,880 beans, 5,728 cassava, 16,339 tropical forages) has been reconfirmed/ designated to FAO in September as a systemwide activity

+ The encapsulation/ dehydration technique is extended to a subste of the cassava core collection

+ Micrografting techniques in vitro allow safe and fast indexing of cassav clones against FSDA

+ Development and use of biochemical markers for studies on inter- and intraspecific relationships in *Capsicum*

+ Simulations and genetic diversity markers help to control drift and erosion in wild bean accessions

+ Phylums and interspecific relationships are established in *Phaseolus* with the help of multiple markers and palynological analysis

5. Progress Report

5.1. Sub-Project # 1: The International Standards

The activities include:
+ the processing of backlogs of original materials, obtained either from explorations or from donations
+ the multiplication/ regeneration of materials already introduced in CIAT collections in the past (and declared to FAO as Designate Collections)
+ research on protocols to limit risks of genetic contamination, drift and genetic erosion
+ research on reliable and cost-effective conservation technologies
+ upgrading of GRU facilities

Outputs 1.1 and 1.2. : germplasm received at CIAT processed and multiplied

Activities 1.1. and 1.2.: Processing backlogs through quarantine and first multiplication

This group of activities refers to the introduction and first multiplication of materials, obtained either from germplasm explorations or donations by NARS.

Table 1. Status of materials introduced into process.

	Beans	Forages	Total
Awaiting processing	14,542	2,227	16,769
Processed in 1999	211	810	1,021

During this process, 350 bean accessions and 183 forage accessions did not germinate and could be considered as 'lost accessions' due to poor viability at collecting and/ or poor drying and storage during the first two decades of GRU work. Apart from this, a total of 4,908 bean materials (we doubled the figure of 1998) are under 1st multiplication as follows:

Table 2. *Phaseolus* beans accessions processed for multiplication under greenhouse/ meshouse and field conditions.

	Greenhouse	Field[2]	Total
Palmira	561	1,084	1,645
Popayán[1]	558	-	558
Tenerife	146	2,559	2,705
Total	1,265	3,643	4,908

[1]Including 206 accessions of the complex of *P. coccineus*.
[2]Including material designated to FAO in 1999 and in process of regeneration.

Output 1.3: FAO Designate Collections regenerated (thus satisfying criteria of amounts and viability)

Activity 1.3: Regeneration of FAO Designate Collections

Table 3. Regeneration of FAO Designate Collections

	Beans				Forages	Total
	Acutif	*Lunat*	*Cocc/polya*	*Vulg*		
Awaiting regeneration	1	648	235	10,884	5,884	17,652
Processed in 1999	318	1,475	834	17,259	630	20,516

The multiplication site of Tenerife has been re-activated and a special multiplication plan has been put in place to quickly regenerate ageing bean accessions. During this process, 145 forage accessions could be considered as 'lost accessions' as they did not germinate due to poor drying/ storage in the past or failed to give fully developed plants due to poor adaptation.

The regeneration process also includes two other groups of activities: viability testing and final packing (**Output 1.4.** and Activity 1.4). The following tables indicate flows of materials during 1999.

Table 4. Viability testing for *Phaseolus* beans and tropical forages during 1999

	PHASEOLUS					FORAGES		
	Germination %	*P.vulg.* # acces	*P. lun.* # acces	*P.Acut.* # acces.	*P. cocc.* # acces.	Germination %	LEGUMINOSAE # acces.	POACEAE # acces.
Already stored materials	1-50	20	5			1-50	-	-
	51-84	157	32	1	9	51-84	-	-
	85-100	1,195	88	4	1	85-100	-	-
TOTAL		1,372	125	5	10		0	0
Recently multiplied materials	1-50	17				85-100	-	-
	51-84	194				51-84	-	-
	85-100	3,616				85-100	215	5
TOTAL		3,827					215	5

The milestone for 1999 was 8,000 accessions (6,000, for those processed through regeneration, and 2,000 for those of already stored materials). The shortfall to target is partly explained by the lack of seed to perform testing. We preferred to regenerate larger numbers of accessions now, although with fewer seed produced, with the need to come back for larger amounts of seed in the future.

Table 5. Final storage and packing of accessions of tropical forages processed during 1999

	Accessions previously multiplied with enough seed for packing		Accessions recently multiplied with enough seed for packing	
	LEGUMES	POACEAE	LEGUMES	POACEAE
LONG TERM (Base + Duplicates + Repatriation) + SHORT TERM (Distribution-Monitoring)	0	0	215	5
SHORT TERM only (Distribution-Monitoring)	0	0	215	5
TOTAL	0	0	215	5

Accessions multiplied in the past do not comply with the criteria of amounts/ high viability to allow packing for long-term.

Table 6. Final storage and packing of accessions of *Phaseolus* beans processed during 1999

	P. vulgaris		
	TEN 1996B	TEN 1997A	TEN 1997B
LONG TERM (Base + Duplicates + Repatriation) + SHORT TERM (Distribution-Monitoring)	875	672	865
SHORT TERM only (Distribution-Monitoring)	218	310	202
TOTAL	1,119*1	1,015*2	1,085*3

*1 (26 plots did not yield any seed)
*2 (33 plots did not yield any seed)
*3 (18 plots did not yield any seed)

As part of a systemwide activity throughout the 1st semester of 1999, the GRU revised the list of designated materials to FAO. A total of 16,339 accessions of tropical forages (including 1,491 recently multiplied materials) have been designated to FAO in September 1999. Here follows the detail for beans and cassava.

Table 7. Status of the *Phaseolus* beans germplasm collections conserved at GRU-CIAT (by September 1999)

Germplas collection	Genera	Species	Source Countries	Total No. accessions
Phaseolus beans	1			28,182
P.vulgaris cultivated and wild		1	91	3,509
Other cultivated and wild spp.		4	53	189
Other wild species		22	12	189
Total	1	27	97	31,880[(1)]

[1]Subdivisions is made when genetic mixtures appeared.

Table 8. Status of the *Manihot* FAO Designate Collections (by September 1999)

CULTIVATED	FAO Designated	Available for distribution to 1999	Termotherapy in vitro	Grafting FSD
Argentina	65	8	55	-
Bolivia	3	2	2	-
Brazil	1,317	242	1,132	36
Colombia	1,995	380	1,701	29
China	2	2	-	-
Costa Rica	148	23	39	86
Cuba	77	72	8	-
Dominican Republic	5	2	-	1
Ecuador	116	45	86	4
Fiji	6	2	2	-
Guatemala	91	17	67	1
Indonesia	51	10	39	-
Malaysia	67	16	49	-
Mexico	102	30	79	1
Nigeria	19	3	16	-
Panama	43	11	26	1
Paraguay	208	56	136	1
Peru	405	90	314	11
Philippines	6	3	2	-
Puerto Rico	15	5	9	-
Thailand	31	4	25	-
United States	9	4	5	-
Venezuela	241	62	165	7
CIAT/ICA Hybrids	384	219	97	43
CROSSING FOR G. MAPPING				
SUBTOTAL	5,406	1,308	4,054	221
WILD SPECIES				
30 spp in vitro	322	-	-	-
3 Undefined spp				
TOTAL	5,728	1,308	4,054	221

Output 1.5: Improved conservation techniques

Activity 1.5.1: Research on protocols to limit risks of genetic contamination, drift and genetic erosion

Management practices to limit genetic drift and erosion in wild bean accessions

F. A. Guzmán (Universidad del Valle, Colombia), O. Toro, C. H. Ocampo, I. Sánchez (Corpoica) and D. G. Debouck

Introduction

Once a new accession arrives in CIAT 's genebank, its original seed is multiplied in a quarantine glass-house, and the next generation in a field. During this process, a serious genetic drift (i.e., change of gene frecuency) could occur. The main objective of this project is to develop optimal management strategies of a genebank to limit genetic drift in conserved accessions. In order to develop this strategy is necessary understand the genetic structure of the original accession and the effects of different initial seed increase methods on the genetic contents of that accession. We use the wild common bean model, since it has proven to be variable between and wihtin accessions. This variation has proven to be useful (e.g. arcelin for resistance to bruchids, photosynthesis parameters).

Results

Four wild populations of bean common (*Phaseolus vulgaris* L.) from different geographic origins (Guatemala, Colombia, Perú y Argentina) were chosen. A total of 80 plants of each population were planted in three different environments (Palmira, Popayan and modified Popayan). Eleven enzymes (18 loci) and phaseolin marker (seed storage protein) were chosen for analyzing the genetic structure of the four populations, and for monitoring gene frecuency changes during initial seed increase (Table 9). Enzymes were found to display clear bands, repeatability and polymorphism at both inter - and intrapopulational levels. The control genetic of the polymorphism for these enzyme systems in wild bean has been determined previously (Koenig and Gepts, 1989a).

The phaseolin analysis of wild beans revealed the typical patterns established for cultivated bean in each area, except the CH type found in Colombia which is similar to M16 in one di-SDS-PAGE, by different in two di-IEF-SDS-PAGE (Table 9). Enzymes display that Guatemala and Peru were the most variable populations while Colombia and Argentina presented the lowest intrapopulational polymorphism (Table 10). The enzyme loci MDH-1, PGM-2 revealed high intrapopulational variation. The enzyme loci PRX, DIAP-1, SKDH, MDH-1 and MDH-2 were found as the major contributors to interpopulational differentiation.

The cluster analysis based on Nei's distances corroborated the existence of three major groups at 80% similarity level. The first group from Mesoamerica (Guatemala) also included a Colombian population; the second group included Peru and the third group included Argentina (Figure 1). Some rare alelles were observed in the selected populations; MDH-1 (102), MDH-2 (102), IDH (98), PGM-2 (105, 103), PGM-1 (103) in Guatemala, as well as PGM-2 (102) in Peru (Table 11). It is necessary to preserve them across the germplasm handling procedures for monitoring changes that can cause genetic drift during this process.

Prospects

We have gained evidence to understand the genetic structure of these populations wich help to develop a simulation study. This information helps to support decisions for the management and maintenance of the genetic diversity present in the CIAT and other genebanks.

References

Koenig, R. and Gepts, P. 1989. Allozyme diversity in wild *Phaseolus vulgaris*: Further evidence for two major centers of genetic diversity. *Theor. Appl. Genet.* 78: 809-817.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* USA 70: 3321-3323.

Table 9. Enzymes systems assayed, loci and alleles in wild populations.

Enzyme	Tissue	Gel	Buffer	Loci	Alleles
Esterases	Root	PAA[1]	TB[2]	α-Est-1 α-Est-2 β-Est	96, 98, 100 98, 100 93, 95, 98, 100, 102
Diaphorase	Root	STARCH	HC[3]	Dia-1 Dia-2	95,100 100
Peroxidase	Root	PAA	TB	Prx	98, 100
GOT (AAT)	Root	PAA	TB	Got	13, 20, 30
Isocitric dehydorgenase	Root	STARCH	HC	Idh	98, 100
Malate dehydrogenase	Root	STARCH	HC	Mdh-1	100, 102 95, 98, 100, 102
Malic enzyme	Leaf	STARCH	HC	Me	98, 100, 102
Shikimate dehydrogenase	Root	STARCH	HC	Skdh	98, 103, 105
6-phosphogluconate dehydrogenase	Root	STARCH	HC	6-Pgdh	98, 100, 102
Phosphoglucomutase	Root	STARCH	HC	Pgm-1 Pgm-2	100, 103 95, 100, 102, 103, 105
Acid phosphatase	Root	PAA	TB	Acp-1 Acp-2	95, 100, 102 100, 104

[1] Polyacrylamide gel
[2] Tris-borate buffer system
[3] Histidine-citrate buffer system

Table 10. Selected populations for drift genetic studies: phaseolin data

Identification	Origin of population	No.of sampled seeds	Phaseolin
DGD – 621	Argentina (Jujuy)	138	C, H_1, J_4, T
DGD – 1956	Perú (Cajamarca)	138	I
DGD – 2423	GTM (Chiquimula)	173	M16
OT – 491	CLB (Boyacá)	87	CH

Table 11. Selected populations for drift genetic studies: Enzyme data (frecuencies of alleles).

LOCUS	Mdh-1		Mdh-2				Pgi-1	Pgi-2	Pgm-1		Pgm-2					Skdh		
ORIGIN	100	102	95	98	100	102	100	100	100	103	95	100	102	103	105	98	103	105
GTM	0.9867	0.0133	0	0	0.9867	0.0133	1	1	0.9867	0.0133	0.0133	0.9733	0	0.0067	0.0067	0	0.9733	0.0267
CLB	1	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	1	0
PER	1	0	0.038	0.962	0	0	1	1	1	0	0	0.9873	0.0127	0	0	0	1	0
ARG	1	0	0	0.0135	0.9865	0	1	1	1	0	0.0068	0.9932	0	0	0	1	0	0

LOCUS	Acp-1			Acp-2		Prx		A-Est-1			Dia-1		Dia-2	Idh		Me		
ORIGIN	95	100	102	100	104	98	100	96	98	100	95	100	100	98	100	98	100	102
GTM	0.2267	0.7733	0	1	0	0.0333	0.9667	0	0.12	0.88	0	1	1	0.0133	0.9867	0	0.2	0.8
CLB	0.954	0.046	0	1	0	0	1	0	0.069	0.931	0.069	0.931	1	0	1	0.3103	0.6897	0
PER	0.9494	0	0.0506	0	1	0.9367	0.0633	0.0633	0.9367	0	0.3924	0.6076	1	0.0127	0.9873	0	0.0759	0.9241
ARG	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	0.973	0.027

LOCUS	6-Pgdh			Got		
ORIGIN	98	100	102	1	2	3
GTM	0.9467	0	0.0533	0.9867	0.0133	0
CLB	0.046	0	0.954	1	0	0
PER	0	0.9873	0.0127	0.9873	0	0.0127
ARG	0	0	1	1	0	0

```
**********************************************************************************
   **         Nei's Original Measures of Genetic Identity and Genetic distance      **
   **                       [See Nei (1972) Am. Nat. 106:283-292)]                  **
   **********************************************************************************

================================================
pop ID    1         2         3         4
================================================
1           ****    0.8730    0.7543    0.6454
2         0.1358      ****    0.8846    0.6655
3         0.2819    0.1226      ****    0.6735
4         0.4379    0.4072    0.3953      ****
================================================
Nei's genetic identity (above diagonal) and genetic distance (below diagonal).
```

Figure 1. Dendrogram of allozyme diversity in wild populations.

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   **********************************************************************************
   **         Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA       **
   **            --Modified from NEIGHBOR procedure of PHYLIP Version 3.5          **
   **********************************************************************************

                              +-----------------------------pop1 (Argentina)
  +---------------------------2
  !                           !           +-----------------pop2 (Colombia)
--3                           +-----------1
  !                                       +-----------------pop3 (Guatemala)
  !
  +-----------------------------------------------------------pop4 (Perú)


Between        And             Length
-------        ---             ------
   3             2            10.22962
   2          pop1            10.44437
   2             1             4.31509
   1          pop2             6.12928
   1          pop3             6.12928
   3          pop4            20.67400
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Activity 1.5.2: Research on protocols to assess viability of forage seeds

E. Velasquez

The assessment of viability in forages was done with 51 genera and 158 species through germination tests with determinations of vigor and moisture content (Table 12). Scarification tests were performed either through mechanical means or acid action. Different germination media (sand, germination papers, vials) and germination conditions for the different species (temperature and photoperiod in growth chambers) were also tested. Grasses usually need a special test using TZC tetrazolium staining due to dormancy.

Table 12. Research on protocols for viability testing for Forages during 1999

	# Genera	# Species
Legumes	47	152
Grasses	4	6
Total	51	158

Activity 1.5.3. Micrografting: a diagnostic method for the detection of Frog Skin Disease in Cassava

Roa, J.C. and G. Mafla

Problem

Cassava is severely affected by frog skin disease (FSD), which can cause losses up to 90% or more in certain areas (Lozano J.C. et al. 1983a). In addition, FSD is a disease of quarantine importance (Frison and Feliu, 1991).

At present, a *detection* method using serology such as ELISA is not available because the purification of the virus has not been possible. The only reliable diagnostic method is grafting a hypersensitive clone 'Secundina' on the *clone to be checked in* greenhouse (CIAT, 1998). Time delays (up to six months) and greenhouse space to produce appropriate material for grafting and symptom expression are the major constraints.

Micrografting has been used for the production of plants free of diseases, as for *Citrus* (Navarro L., 1977), grapes, apples, as well as in the vegetative propagation of woody plants. We have been interested in developing an *in vitro* micrografting-like technique for fast diagnostic of FSD for *in vitro* material. We also develop the *in vivo* micrografting to evaluate plants coming from the field.

Objectives

To develop simpler techniques that allow i) high levels of successful grafting, ii) high grade of virus transmission, iii) great sensibility of the test to low concentrations of the virus, and iv) decrease of the periods required to obtain plants ready for indexing.

Materials and Methods

In vitro micrografing

Fifty-six varieties were selected from the cassava in vitro bank (more than 3 old months), they were used like rootstock and the hypersensitive variety 'Secundina' (MCOL 2063) served as scion. The positive control was CM 5460-10 clone.

The plants that were used like rootstock were extracted of the test tubes (culture mediun, 8S or 4E) (Roca et al, 1984), and were placed in petri dishes, ensuring continuous growth. All axillary buds were removed. A cut above the node was made vertically with a surgical blade (No. 11) (size of the rootstock: 2 cm). The plant was placed again in the test tube, and with a large forceps the secundina shoot (2 cm, with 1 or 2 leaves) was inserted into vertical splits made in the rootstock. The micrografted seedlings were then cultured in the same medium, sealed and were

kept in a conservation room at 23-24 °C, 12 hour photoperiod, and 1,000 lux. After 4 weeks, the micrografting were evaluated.

In vivo micrografting

Stem cuttings of the Cassava Field Gene Bank with frog-sking disease were utilized and secundina shoots of the Cassava in vitro Gene Bank were used as scion. The scion was inserted in the vertical cut of the rootstock and held in place with parafilm. This set was covered with a transparent plastic bag for a period of a week in order to avoid deshydratation of the micrografting. They were kept in a growth chamber with temperatures of 23-24 °C, 2,000 lux and under 12 hour-photoperiod. After 3 weeks the plants were evaluated.

Table 13. Success rate and growth of in vitro micrografting in cassava after four weeks of culture.

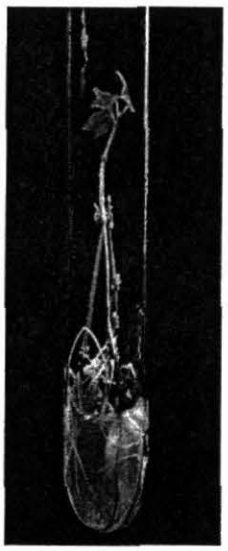
Variety	Leave(no.)	Roots (no.)	Successful	Symptom
COL 1655	3	1	YES	NO
COL 1786	3	1	YES	NO
COL 1633	3	2	YES	NO
COL 1658	2	2	YES	NO
COL 1669	3	1	YES	NO
COL 1673	3	1	YES	NO
COL 1676	2	1	YES	NO
COL 1683			NO	
COL 499A	3	3	YES	NO
COL 595	4	1	YES	NO
COL 963	2	1	YES	NO
COL 1539	3	2	YES	NO
COL 1080	2	2	YES	NO
COL 1112B	5	1	YES	NO
COL 1109	4	2	YES	NO
COL 452A	2	1	YES	NO
COL 1994	3	1	YES	NO
COL 850	4	1	YES	NO
COL 1559			NO	
COL 868	2	2	YES	NO
BRA 60	5	3	YES	NO
BRA 460	6	1	YES	NO
BRA 550	3	1	YES	NO
BRA 1129	3	1	YES	NO
BRA 1366	5	2	YES	NO
COL 1940	7	2	YES	NO
COL 2632			YES	NO
MEX 33	4	3	YES	NO
CM 4867- 1	5	5	YES	NO
CM 5460- 5			NO	
CM 7073- 7			NO	
CM 7463- 2	7	3	YES	NO
CM 6119- 5	4	3	YES	NO
CM 6082- 1			NO	
BRA 295	2	1	YES	NO
BRA 550	4	1	YES	NO
BRA 55	5	2	YES	NO
BRA 246	2	4	YES	NO
CM 7857-8	2	0	YES	NO
CM 7857- 10	2	1	YES	NO
CM 7857- 15	2	0	YES	NO
CM 7857- 27	2	2	YES	NO
CM 7857- 29	5	0	YES	NO
CM 7857- 32	2	2	YES	NO
CM 7857- 45	2	0	YES	NO
CM 7857- 49	2	1	YES	NO
CM 7857- 53	2	0	YES	NO
CM 7857- 64	2	1	YES	NO
CM 7857- 34	2	1	YES	NO
CM 7857-128	3	1	YES	NO
CM 7857- 94	2	1	YES	NO
CM 7857- 78	2	1	YES	NO
CM 7857-126	2	1	YES	NO
CM 7857-122	2	2	YES	NO
CM 7857- 68	2	1	YES	NO
CM 7857-36	2	0	YES	NO
CM 5460- 10	3	3	YES	YES

Results

Ninety-two percent of the micrografting were successful with the developed methodology, and one could observe the presence of a callus in the union of the scion with the rootstock. New leaves and roots were produced, indicating success in the micrografting (Table 13). The death of some micrografting could be more related with the size of the cut than an effect of genotype. Deep cuts make that the tissue is opened and could not have a good contact with the vascular cambium of the rootstock producing death of the micrografting (Figure 2a).

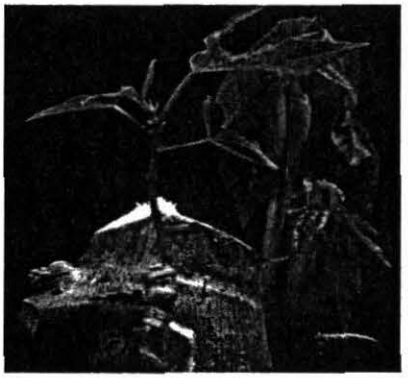
Twenty-five days after grafting typical symptoms developed on CM 5460-10 that was used as positive control. This would indicate that virus translocation is occurring across micrografting. This would mean a gain in time (six weeks) and the security of not reinfecting because all process is developed in sterile conditions (Figure 2b).

The in vivo thermoterapy technique is being adjusted, but has been able to observe a quick expression of symptoms like one can see in the Figure 3.



b

Figure 2. a) In vitro micrografting. b) Grafts showing symptoms



b

a

Figure 3. a) In vivo micrografting. b) Graft showing symptoms.

Prospects
With these preliminary outputs, we could affirm that in vitro and in vivo micrografting could become an excellent tool for the prompt diagnostic of one of the most important pathological problems in the cassava crop. We propose in the next step to compare the three existent methodologies: minigrafting, in vitro and in vivo micrografting. Taling into acount the severity of viral infection, we would like to test the sensibility of each of these techniques.

References
Frison, E.A. and Feliu, E. (eds.). 1991. FAO/IPGRI Tecnichal guidelines for the safe movement of cassava germplasm. Food and Agriculture Organization of the United Nations, and International Board for Plant Genetic Resources, Rome.

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Activity 1.5.4. Use of in vitro thermoterapy for the safe movement of cassava germplasm

G. Mafla and J.C. Roa

Introduction
There is an urgent need to duplicate the existing Cassava collection in order to reduce the risk of losing valuable genetic material. To achieve that and to facilitate the safe movement of cassava germplasm, protocols have been established by FAO (Frison, E.A. and Feliu, E. 1991). A cleaning program of the cassava designated collection has been initiated three years ago and is being strengthened with certification of all materials. It should be massive so that the widest diversity is made available in the minimum time.

Materials and Methods
Thermotherapy applied to shoot tips has been the main method to produce plants free of virus. Shoot tips (0.5 cm) were cultured for 12 days in the propagation medium 4E (Roca et al. 1984) at a temperature of 35°C, 1,000 lux and 12-h photoperiod. After this time, they were transferred under the same conditions after completing 3 cycles of thermoterapy. At the end of the treatment, the materials were subcultured, and a sample of each variety was taken to the greenhouse for the pertinent indexing tests (ELISA and minigrafting). The Germplasm Health Laboratory (GHL) carries these out.

Results
A total of 4,054 varieties of cassava has been processed until now through the in vitro thermoterapy technique, which corresponds to 70% of the collection designated to FAO. Out of these, 1,308 varieties have presented negative results for the tests of CCMV, CsXV, and FSD, making them therefore available for exchange. They can also replace clones in the field genebank. In addition, 221 varieties are free of FSD, but still pending for ELISA testing (Table 8).

Future goal
To conclude the process of in vitro thermoterapy for the all in vitro collection, and continue to deliver plants to GHL for their indexing

References
Frison, E.A. and Feliu, E. (eds.). 1991. FAO/IPGRI Tecnichal guidelines for the safe movement of Cassava Germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board for Plant Genetica Resources, Rome

Roca, W.M., Rodriguez, J.A., Mafla, G., Roa, J.C. 1984. Procedures for recovering cassava clones distributed in vitro. CIAT, Cali, Colombia. 8p.

Activity 1.5.5. Evaluation of the regeneration of in vitro cassava plants after long storage in presence of silver nitrate

G.Mafla, J.C.Roa and C.Ocampo

Introduction
This work whose principal objetive was to create an alternative medium supplemented with silver nitrate for slow-growth in vitro for cassava was started in 1997. We wanted to extend the intervals between each subculturing to lower our costs, make possible a safety backup of the in vitro collection, and also have possibility to expand reasonably the cassava clonal collection (CIAT, 1997).

Six varieties were evaluated in different mediums containing several concentrations of silver nitrate, an inhibitor of ethylene. A decrease in stem length and an increment in the period of conservation up to 20 months in all the evaluated varieties were observed.

We needed to confirm the viability and the genetic stability by means of morphological and isoenzymatic descriptors. In the case of conservation of vegetatively propagated germplasm a detailed analysis of genetic stability after in vitro culture is vital. Clonal germplasm storage involves the maintenance of specific gene combinations. If a plantlet should come out of storage with a different gene combination to that wich it entered with, then the validity of the storage method must be questioned.

Results
The medium 4E (Roca et al. 1984) developed in order to stimulate the growth of cassava was utilized for the recuperation of the plants that had been conserved for 20 months. After 60 days of growing explants in this medium, one could observe that all explants survived. They were placed subsequently in the rooting medium, and transferred to the greenhouse. The plants recovered from the different mediums were similar in height after 90 days, and checked through for the morphological descriptors they were evaluated in the past.

The genetic stability is being analyzed by means of biochemical markers such as isozymes. We are using 15 systems that present a good activity in cassava, and also show high polymorphism between varieties. Twenty genes are involved in these systems, which represent a tiny coverage of the genome, but an easy and cheap comparison to start with.

References
CIAT. 1997. Annual Report. Project on saving Agrobiodiversity SB-01, Genetic Resources Unit. Pp. 18-19.

Activity 1.5.6. Cryopreservation of cassava shoot tips using the encapsulation-dehydration technique.

R. H. Escobar, N. C. Manrique and W. M. Roca

Background.
We have developed the encapsulation-dehydration technique as a way to cryopreserve the cassava germplasm collection. We are exploring this conservation method for cassava in order to 1) have security backups of the

cassava world collection, and 2) reduce because of cost implications the field gene bank to clones under actual evaluation. This methodology was established by Palacio (1997) and adjusted by Rangel (1998). However, some clones showed a low response to the technique. In 1999, through the adjustment of the bead quality and dehydration time, it was possible to test a sub-core collection of cassava 45 clones.

Methodology

Adjustments were made in the quality of beads (3% alginate with 75mM CaCl2). In spite of the fact that the concentration of 2-3% alginate with 50mM CaCl2 gives us good response after freezing, the beads could not be established; they become deformed and shoots were lost under sucrose treatment. Dehydration time was increase to 24 hours. We tested 45 clones with this protocol.

Results and discussion

Adjustment of beads, associated with dehydration time (24 hours) improved the response after freezing. Only 4.5% of the 45 clones tested showed low response (MNga 1 and MPar 110). Some tissues have potential for improved shoot recovery after freezing (up to 5-10%). Some shoots were small and grew slowly. Adjusting GA3 and light treatments increased total shoots per freezing experiment. We observed that clones from Brazil and Colombia (the most representative countries on the cassava collection) have a consistent response after freezing (46% and 49.9% on average respectively). This is the first time that a larger group of clones were cryopreserved (Table 14).

Conclusions and future activities

- 45 cassava clones were cryopreserved and shoots were recovered after freezing in liquid nitrogen
- 42 cassava clones showed recovery rates up to 20%
- It is possible to increase shoot recovery rate up to 5-10% by modification of growing conditions and recovery media
- The number of cassava clones for cryopreservation will be increased to 105.

References

Palacio J.D. 1997. Crioconservación de ápices de yuca (*Manihot esculenta* Crantz) utilizando la técnica de encapsulación deshidratación. Tesis CIAT.

Rangel M.P. 1998. Ajuste y aplicación de la metodología de encapsulación-deshidratación para crioconservar un amplio rango de variedades de yuca (*Manihot esculenta* Crantz). Tesis CIAT.

Table 14. Response of 45 sub-core cassava clones cryopreserved in liquid nitrogen using encapsulation-dehydration technique

Varieties	% response	
	Viability	Shoot formation
CG 1141-1	83.8	83.8
CM 4063-6	74	29.6
CM 523-7	72.1	53.8
CM 6082-1	90	50
M Bra 12	100	57.1
M Bra 69	86.2	23.7
M Bra 191	76.6	53.3
M Bra 325	90	60
M Bra 328	83.3	50
M Bra 337	56	50
M Bra 383	49.1	28.3
M Bra 474	-	-
M Bra 507	85.9	71
M Bra 514	53.6	26.6
M Bra 590	87.5	62.5
M Bra 632	80.5	64
M Bra 691	67.6	43.3
M Bra 698	63.3	20
M Bra 759	80	30
M Bra 769	83.3	43.3
M Bra 839	85.3	58.8
M Bra 881	92.8	32.14
M Bra 894	96.4	50
M Col 1468	35.7	28.5
M Col 1505	79.4	76.4
M Col 2016	70	20
M Col 22	95	95
M Col 32	33.3	23.3
M Col 40	-	-
M Col 511	93.7	56.2
M CR 113	60.7	42.8
M Cub 29	72.7	54.5
M Cub 39	-	-
M Cub 55	80	66.6
M Ecu 117	81.5	48.1
M Mal 2	71.1	55
M Mex 71	75	40.62
M Nga 1	45	15
M Nga 5	72.7	54.5
M Pan 51	100	84.8
M Par 110	46	0
M Par 71	96	96
M PTR 19	77.5	21.8
M Ven 232	94.4	50

Activity 1.5.7. Establishment of protocols for the in vitro conservation of tropical fruits

G. Mafla

We are interested to evaluate the methodology of slow-growth established for *Manihot esculenta* for some selected tropical fruits, such as lulo (*Solanum quitoense*), sapote costeño (*Pouteria sapota*), avocado (*Persea americana*) and guayava (*Psidium guajava*). In vitro culture could be a choice method of conservation of many them, as they are multiplied vegetatively, are sterile, or have seed unresponsive to standard seed storage techniques (i.e. recalcitrant seed). The idea is to develop protocols for a safe and cheap conservation in vitro, and thus to reduce costs of maintenance of field gene banks and arboreta. We have started with preliminary trials to have germplasm of these species established and growing under in vitro conditions (kind of plant material for micropropagation, sterilization techniques, growing media).

5.2 Sub-Project 2: Objective: to make the FAO Designate Collections and their pertinent information fully available

Output 2.1. Designate collections cleaned against seed borne diseases

Activity 2.1.1.Germplasm health control

To make the FAO designate collections fully available to users it is necesary to establish the seed health status of each material. The seed health assessment activity is conducted with the purpose of reducing the accidental introduction of plant pathogens along with seeds or vegetative plant parts. In order to manage this risk the Germplasm Health Laboratory (GHL) applies indexing procedures to ensure that distributed materials are free of pathogens of quarantine importance.

Materials and Methods

GHL uses accepted methodologies to intercept seed-borne pathogens as fungi, bacteria and viruses according with those pathogens recorded in seed production areas (Annual Report 1997). In seed health testing, sample reception and registration, preparation of working samples, preparatory work to put the working samples into final format for

testing, and analysis are the activities routinely carried out. Testing for fungi includes blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar.

Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp., in tropical pastures) are tested using dilution and plating on semiselective culture media such as MXP or King B, in addition to immunoprecipitation test with specific antisera or pathogenicity tests. Testing *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* in tropical pastures (*Zornia* spp.) is achieved by subculturing on YDCA, by Gram staining and incubation under high temperature (36-37° C). Testing for seed borne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and/ or seedling-symptom test.

Results

Seed samples produced during the 95b, 96a, 96b, 97a and 97b semesters, once approved for optimum seed viability were obtained from Tenerife multiplication plots for analysis. Their health status, analyzing each period, showed 58.1% (95b), 77.8% (96a) and 64.8% (96b), 84.8% (97a), and 47.9% (97b) samples without pathogens of quarantine importance (Figure 4).

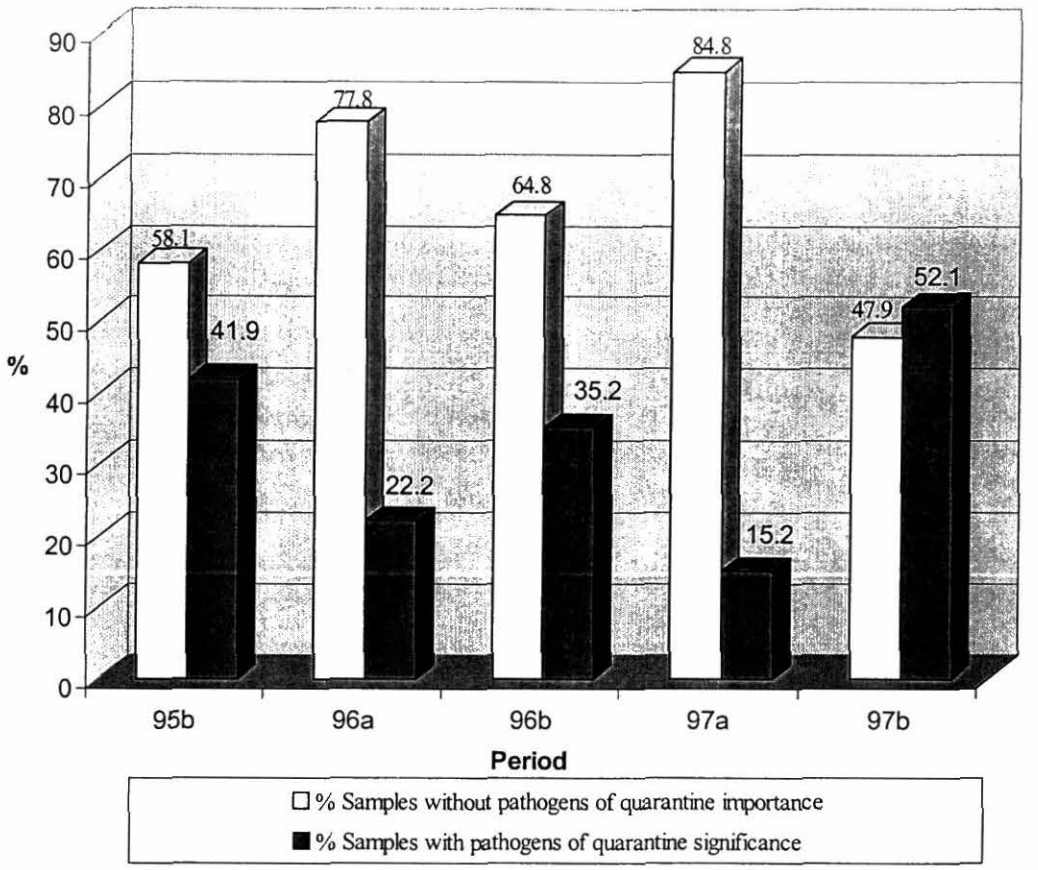

Figure 4. Seed health status of *Phaseolus vulgaris* germplasm from Tenerife during five production periods.

In infected materials fungal and bacterial infections were low in all periods while viral infections were relatively high, particularly BCMV reaching percentages as52.1% during semester 97B. Looking at the general trend of health status, it is possible conclude that seeds produced during 95B, 96A, 96B, and 97A have better status than those produced during semester 97B (Figure 5).

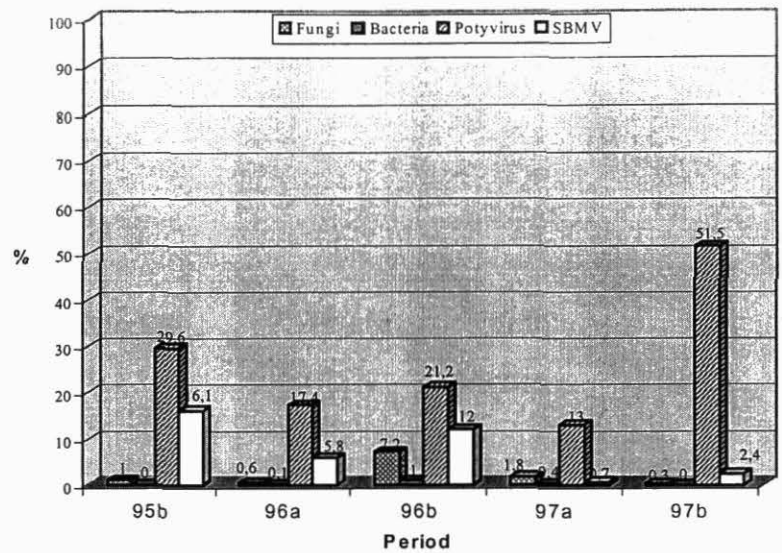

Figure 5. Percentage of *Phaseolus vulgaris* seed samples from Tenerife infected with pathogens in five production periods.

In general the fungal infections were very low (0.6 – 7.2 %), although it was possible detect in some samples the presence of *Colletotrichum lindemuthianum, Rhizoctonia solani, Phomopsis* spp., *Macrophomina phaseoli* and *Phoma exigua.* Seed borne infection by *Xanthomonas campestris* pv *phaseoli* was detected at low percentage (0.8%) in samples from 97A period, *Pseudomonas syringae* pv *phaseolicola* was detected in 1% of samples from 96b production period. Viral infections by Southern mosaic virus (SBMV) and Poty virus (BCMV) were the most conspicuous infections detected in seeds (Figure 5).

Seed health testing carried out on 1,059 seed samples of *Phaseolus vulgaris* from GRU stocks prepared for international exchange showed 515 accesions (4 8.6%) without pathogens of quarantine importance, and 544 infected materials (51.4%) showed the following pathogens: *Macrophomina phaseoli* (1.2%), *Xantthomonas campestris* pv. *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* (0.8%), BCMV (42.3%), and SBMV (10.4%).

In 103 samples of *Phaseolus* spp (*P. lunatus* 93 samples, *P. coccineus* 10), 67 % of them did not have pathogens of quarantine significance, meanwhile 33 % of those samples showed BCMV (25.2%), SBMV (5.8%) and fungi (0.8%) as *Colletotrichum* spp and *Macrophomina phaseoli.*

A total of 216 of bean seed samples from the Germplasm Characterization Project were tested during 1999. A total of 86.6% of those samples did not have any pathogen of quarantine significance. In infected materials *Macrophomina phaseoli* (7.9%) was the most frequent fungus. Virus such as BCMV (4.6 %) and SBMV (1.8%) were detected. Samples from the tropical forage Projects such as *Arachis pintoi (1), Desmodium* spp. (3) and *Centrosema* spp. (3) samples were tested and they showed potyvirus. Seed samples of tropical pastures (324) of 25 genera for which seed health status was checked in the GHL showed 230 samples without pathogens of quarantine significance, meanwhile 94 of them showed accessions with some pathogens (Table 16).

Table 16. Phytosanitary status of Tropical pastures seed samples analyzed during 1999 in the GHL.

Genera	Samples number	Admitted	Non admitted	Virus	Bacteria	Fungi	Family plant
Andropogon spp	11	5	6			4 (Helm), 2 (Phoma)	Graminae
Brachiaria spp	15	13	2			1 (Helmn), 1 (Dresch)	Graminae
Echinocloa sp	1	1					Graminae
Panicum spp	7	6	1			1 (Dresch)	Graminae
Paspalum spp	2		2			2 (Phoma)	Graminae
Pennisetum spp	4	4					Graminae
Arachis spp	2		2			2 (Rizoc)	Leguminosae
Cajanus spp	5	4	1			1 (Phomop)	Leguminosae
Calopogonium spp	14	12	2	1 (SBMV)		1 (Pythom)	Leguminosae
Centrosema spp	46	38	8	5 (Poty), 3 (SBMV)		1 (Phomop)	Leguminosae
Clitoria spp	5	4	1	1 (SBMV)			Leguminosae
Dendrolobium spp	5	3	2	2 (SBMV)			Leguminosae
Desmanthus s pp	5	4	1	1 (Poty)			Leguminosae
Desmodium spp	9	5	4	2 (Poty), 2 (SBMV)			Leguminosae
Flemingia spp	5	2	3	3 (SBMV)		1 (Phomop)	Leguminosae
Galactia spp.	36	22	14	9 (poty), 6(SBMV)			Leguminosae
Lablab spp	5	5					Leguminosae
Leucaena spp	2	2					Leguminosae
Macroptilium spp	15	9	6	5 (Poty), 1 (SBMV)	1 (Ps)		Leguminosae
Prosopis spp	3	3					Leguminosae
Pueraria ssp	18	13	5	3 (Poty), 2 (SBMV)			Leguminosae
Rhynchosia spp	1	1					Leguminosae
Sesbania spp	5	5					Leguminosae
Stylosanthes spp	91	64	27	24 (Poty), 2 (SBMV)		1 (Collec)	Leguminosae
Vigna spp	12	5	7	5 (poty), 1 (SBMV)		1 (Phomop)	Leguminosae
	324	230	94				

Activity 2.1.2. Disease indexing of Cassava (*Manihot esculenta* Crantz) Germplasm Collection

The objective of this part is to continue the evaluation of the whole cassava collection maintained in vitro against four viruses of quarantine importance: CCMV (Cassava Common Mosaic Virus), CsXV (Cassava X virus), CVMV (Cassava Vein Mosaic Virus) y FSDA (Frog Skin Disease Agent). The number of clones available for national and international distribution will thus continue to increase, thus fulfilling the FAO/IPGRI criteria set for international genebanks.

To date there is a total of 1,073 (17 %) cassava clones available for distribution both at national and international levels. However, there is a total of 41, 6 % of the collection already evaluated for CCMV, 39, 2 % of the collection evaluated for virus X (CsXV), and 17 % evaluated for FSDA. As it can be seen in (Table17), a high number of clones has been already evaluated for viruses CCMV and CsXV, since the indexing technique is fast (ELISA). As presented in another part of this report, a new technique has been proposed to double the number of clones evaluated for FSDA.

Table 17. Indexing status of the Cassava Germplasm Collection in Genetic Resources Unit by September 1999.

Source	In vitro clones	INDEXED CLONES			Available for distribution to 1999
		CCMV (-) / C. I *	CSXV (-) / C. I *	FSDA (-) / C. I *	
Argentina	101	15 / 10	23 / 25	8 / 8	8
Bolivia	7	6 / 7	7 / 7	2 / 3	2
Brasil	1341	616 / 632	616 / 631	252 / 260	252
China	2	2 / 2	2 / 2	2 / 2	2
Colombia	2009	1163 / 1207	1051 / 1142	329 / 346	329
Costa Rica	148	34 / 34	30 / 34	17 / 22	17
Cuba	77	21 / 21	21 / 21	20 / 20	20
Estados Unidos	9	5 / 5	5 / 5	4 / 4	4
Ecuador	116	90 / 96	83 / 96	42 / 44	42
Fiji	6	2 / 2	2 / 2	2 / 2	2
Guatemala	91	16 / 17	15 / 17	12 / 13	12
India	51	11 / 11	11 / 11	9 / 9	9
Malasia	67	18 / 18	17 / 18	15 / 15	15
México	102	48 / 50	47 / 50	26 / 28	26
Nigeria	19	4 / 4	4 / 4	3 / 3	3
Panamá	43	19 / 19	18 / 19	11 / 11	11
Paraguay	209	56 / 57	56 / 57	38 / 38	38
Perú	406	171 / 175	164 / 175	89 / 98	89
Philipinas	6	3 / 3	3 / 3	3 / 3	3
Puerto Rico	15	12 / 12	12 / 12	5 / 5	5
Rep. Dominicana	5	4 / 5	5 / 5	3/ 5	3
Salvador	8	2 / 2	1 / 1		
Tailandia	31	10 / 10	10 / 10	4 / 4	4
Venezuela	244	131 / 135	119 / 135	38 / 43	38
Hibridos					
CG	116	24 / 24	22 / 24	19 / 22	19
CM	430	84 / 87	81 / 86	76 / 80	76
HMC	4	1 / 1	1 / 1	1 / 1	1
SG	46	12 / 12	9 / 12	8 / 11	8
SM	49	44 / 45	38 / 45	35 / 38	35
SUBTOTAL	**5839**	**2627**	**2475**	**1073**	**1073**
Crossing for G. Mapping	147	6 / 6	6 / 6		
WILD SPECIES					
30 spp in vitro	330				
3 Undefined spp	3				
TOTAL	**6319**	**2633**	**2481**	**1073**	**1073**

- *clones with negative reaction/ number of indexed clones.*

Indexing for FSDA

We use a grafting technique with a hypersensitive clone COL 2063 (also called Secundina), being grafted on the rootstock of the clone to be tested. All materials are raised in glass-house till they get the appropriate age and diameter for grafting. Once grafted, the materials are transferred to a growth chamber 25°C, 12 hour light) to obtain the symptoms of the virus, in case it is present in the rootstock. Evaluation is performed 45 days after the grafting. (Table 18) shows results of the indexing against FSDA. Currently there are 141 clones pending for evaluation.

Table 18. Results of indexing against FSDA

Source	Indexed clones	Positive clones
Brasil	87	7
Colombia	111	2
Cuba	1	
Ecuador	22	1
Guatemala	1	
México	9	1
Panamá	2	
Paraguay	3	
Perú	29	1
Rep. Dominicana	3	
Venezuela	12	
CG	2	
CM	11	1
SG	2	
SM	4	4
TOTAL	**299**	**17**

Indexing for CCMV

We use the ELISA (Sandwich) technique for its sensibility and swiftness. Results are shown in (Table 19)

Table 19. Results of indexing for CCMV

Source	Indexed clones	Positive clones
Brasil	58	10
Colombia	28	9
Costa Rica	1	
Ecuador	15	6
México	1	1
Perú	5	
Puerto Rico	1	
Rep. Dominicana	1	1
Venezuela	6	1
CG	1	
CM	5	3
KM	3	
TOTAL	**125**	**31**

Indexing for CsXV

Indexing for this virus is done by the same method as for CCMV. Results are presented in (Table 20).

Table 20. Results of indexing for CsXV

Source	Indexed clones	Positive clones
Brasil	59	3
Colombia	36	21
Costa Rica	1	1
Ecuador	25	7
México	1	
Panamá	1	
Perú	6	2
Venezuela	7	2
CG	2	
CM	3	
KM	3	1
SM	1	1
TOTAL	**145**	**38**

Updating the cassava ORACLE data base

To date, for activivites realized in 1998-1999, 2,200 data related to indexing for viruses CCMV and CsXV, and 1,073 data about FSDA have been introduced into the cassava ORACLE data base.

Indexing materials of the K family for the Cassava Genetics Project

For materials of the K family for the Cassava Genetics Project (Dr. Martin Fregene), approximately 100 clones have been indexed for FSDA, and 117 clones for CCMV and CsXV.

Output 2.2: *Germplasm*, Passport and Characterization data available to users

Activity 2.2.1. Upgrading the GRU databases

The GRU has continued the upgrading of its databases with view of fusing all old databases into a single ORACLE format in graphic mode. This fusion will allow: i) a much better monitoring of flows along all operations and across commodities, ii) consequently to i) a much better tracking of problems and monitoring of expenses along the process, and iii) the preparation of standard reports to FAO, countries, etc. Given the unresolved staff imbalance mentioned by the ICERs, this activity was started with a student (with specialty in computer systems) under the joint supervision of the CIAT Information Unit and GRU. GRU Staff has started the full revision of all current descriptors about germplasm and about flows in view of uniformity across the 3 groups of commodities. The novelty is that the reform of the GRU system as it will appear on the computer screens fully matches the flow of operations. Status: the modules "Introduction", "First Multiplication", and "Distribution" have been fully developed. The modules "Quarantine", "Viability", and "Seed Health" are currently under development. This activity has suffered some delays from a health accident of the student and limited time from Information Unit Staff.

Activity 2.2.2: Distribution of germplasm from the FAO Designate Collections.

Table 21. Distribution of germplasm during 1999

	PHASEOLUS	FORAGES	MANIHOT (in vitro)
	Number of accessions (No. of requests)	Number of accessions (No. of requests)	Number of accessions (No. of requests)
CIAT Projects	8,327 (48)	235 (15)	422 (10)
NARS, Universities and other insitutions worldwide	1,273 (17)	290 (18)	38 (3)
TOTAL	9,600 (65)	525 (33)	460 (13)

A couple of requests of cassava germplasm have been temporarily retained, pending on the performing of all indexing. This year, we have put in place the shipment of germplasm with the new CGIAR Material Transfer Agreement, using specially printed plastic bags.

Activity 2.2.3. Establishment of a reference Herbarium for forage germplasm identification

The herbarium has a total of 13,086 accessions, namely legumes (11,924) and grasses (1,162). A total of 663 accessions were included in the herbarium in 1999. The main porpose of the herbarium is to obtain the correct identification of the forage germplasm which belong mainly to wild species. It is also used by Colombian institutions in their taxonomical and floristic investigations.

Table 22. Specimens of forage accessions added to the CIAT Herbarium in 1999

	# Species	# Accessions
Legumes	111	654
Grasses	7	9
Total	118	663

Activity 2.2.4: Characterization of Germplasm Accessions

Using the IBPGR standard descriptor lists and the revised ones, the characterization went on for 3,995 bean accessions and for 867 forage accessions in 1999.

The following table indicates numbers for which characterization data have been recorded. Growth habit, plant height at flowering, time to flowering and maturity, pests and diseases were particularly recorded.

Table 23. Characterization data

Materials	Palmira	Quilichao	Tenerife	Popayan
Field				
Forages				
Gramineae	0	0	0	15
Leguminoseae	231	636	0	0
Beans	1,084		2,559	352

Output 2.4. FAO Designate Collections safe duplicated

Activity 2.4: Safe duplication of FAO Designate Collections in different genebanks

The GRU has two on-going agreements about safe-duplicating its entire seed collections, with CATIE (Costa Rica) and CENARGEN (Brazil). During 1999 we have prepared 3,000 samples of bean germplasm to be shipped to CENARGEN. Contacts have been made with this institution for the shipment to Brasilia under black-box. As detailed in another part of this report, research is going on to improve cryoconservation protocols that would allow the safe duplication in liquid nitrogen in at least two different places (earlier contacts with CIP indicated the interest of CIP in this scheme under terms of reciprocity).

Output 2.6. Improved disease indexing techniques

Activity 2.6.1. Detection of *Xanthomonas campestris* pv *phaseoli* in bean seeds using immunofluorescent staining

María S. Balcazar, Benjamín Pineda L.

Introduction

Common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli* ***(Xanthomonas axonopodis* pv. *Phaseoli)*** is a major seed borne disease of beans, *Phaseolus vulgaris* L., and its causal agent is considered as a pathogen of quarantine significance. The detection of the bacteria in seeds is done by using semiselective culture media (Claflin et al., 1987), and other procedures which have a weakish sensitivity. Indirect immunofluorescent staining is a high sensivity test and also used to detect *X. canpestris* pv *phaseoli* with successful results (Malin et al., 1983).

We present here some advances in the adjustment of this test in order to use it as a GHL routine test to identify *X. capestris* pv *phaseoli* in bean germplasm.

Materials and methods

Bacterial isolate (Xcp 123 lyophilized) used to produce antisera was obtained from CIAT Bean Pathology Laboratory; adjusting bacterial concentration at $5x10^8$. c. f. u. /ml. Cells (0.2 ml of bacterial suspension) were injected into marginal ear veins of two New Zealand rabbits. Repeated injections using antigen (0.2ml) as prepared along above indications were made at weekly intervals. One week after 5^{th} injection blood was extracted and the antisera agglutination titer determined. Fluorescent antibody staining procedure was crried out using the technique described by Malin and co-workers (1983).

Results

At least 30 ml of specific antiserum was collected with a good titer (1:2560). Observations through the epifluorescence microscope showed that it was possible to detect the target bacteria in high dilutions. Comparing this detection results with tests using YDC orl MXP culture media it was no possible detect the bacteria in high

References

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Activity 2.6.2. Survey of seed fungal diseases in regeneration of *Brachiaria* germplasm under CIAT Santa Rosa station (Popayan) conditions.

Sandra X. Garcia (Pontificia Universidad Javeriana), Benjamín Pineda L.

Introduction

The productivity of tropical pastures can be substantially increased by using seeds of high quality, but one of the current bottlenecks to increase such productivity is the lack of knowledge about seed-born diseases that reduce yields. Particularly in *Brachiaria* spp., one of the most important tropical pastures, the available information about diseases is very restricted under Colombian conditions. We want to study seed fungal diseases affecting the *Brachiaria* spp. germplasm under regeneration in CIAT Santa Rosa station (Popayan).

Materials and Methods

Thirty accessions of three *Brachiaria* species (*B. brizantha*, *B .decumbens* and *B. jubata)* were selected from GRU germplasm collections with enough seeds and produced during the period 98-99 under Santa Rosa station conditions. To detect seedborne fungal diseases, seed samples were plating on Petri dishes using blotter test and PDA incubation methods. After plating the dishes were placed in a growth chamber under 20-27 ^{0}C, 12 hours darkness and 12 hours cool light and near UV light illumination. After 5-6 days the samples were examined through stereomicroscope and light microscope to identify seedborne pathogens. Under field conditions, plots of the same 30 accessions were selected to examine growth and progress of diseases on panicles. We also wanted to recheck the results obtained in the GHL with seed samples harvested during 98-99 period.

Results

To date 12,000 seeds of the *Brachiaria* species have been evaluated. We found the following fungi:
Drechslera spp, *Phoma* spp, *Fusarium* spp, *Epicoccum* spp, *Curvularia* spp, *Alternaria* spp, *Cladosporium* spp, *Nigrospora* and 10 unidentified species. Pathogenicity tests are in progress.

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Activity 2.6.3. Standardization of the PCR technique to identify Cassava Vein Mosaic Virus CVMV) in cassava germplasm.

Maritza Cuervo Ibañez, Norma Cristina Flor Payán, Lee Calvert.

The objective of this research is to evaluate and standardize a PCR based test for the detection of the cassava vein mosaic in the FAO cassava designate collection, and thus to guarantee its safety for distribution at both national and international levels. Four sets of primers designed after the sequencing of the viral genome (Calvert L., Ospina M. & Shepherd R., 1995) were evaluated in order to define which one would allow to establish certainly the presence of the virus.

Results

Four sets of primers (SST, RBD, RT and HS) were evaluated using cassava material coming from glasshouse material as well as from the field.

After amplification with the RBD primer on glasshouse plants, bands were observed in a few clones. Bands however were not of the same molecular weight as our plasmid check (CVMV 141). With the SST primer, bands were noted with size as expected, for clones for which there was amplification (Figure 6). With the HS primer, only clones PTR 102, TAI 3, BRA 137 and BRA 31 presented bands, although of a different molecular weight as compared to the

plasmid check. With RT primer, only clones PTR 102, C.RICA 149 and BRA 137 presented bands, though of different molecular weight as compared to the plasmid check. Some consistency has been observed throughout all tests realized with SST and RBD primers in a few clones, and amplification was observed consistently with such primers. We noted however inconsistency with another set of clones, with irregular amplification (Tables 24 and 25).

Table 24. Results of amplication with SST primer for glasshouse and in vitro grown cassava materials.

SST	First DNA extraction						Second DNA extraction	IN VITRO
	1	2	3	4	5	6	7	8
Puerto Rico 102	X	X			X	X	X	X
Tailandia 3	X	X						X
Venezuela 284 B								X
Perú 385							X	X
Costa Rica 149	X	X	X		X	X	X	X
Secundina								X
Brasil 29								X
Brasil 246	X							X
Brasil 274								X
Brasil 31			X					
Brasil 88							X	X
Brasil 181	X	X	X	X	X	X		X
Brasil 86								X
Brasil 81		X				X		
Brasil 76								X
Brasil 156				X				
Brasil 137	X		X	X	X	X		X

X = bands present in the clone.

Table 25. Results of amplication with RBD primer for glasshouse and in vitro grown cassava materials.

RBD	First DNA extraction									Second DNA extraction	IN VITRO
	1	2	3	4	5	6	7	8	9	10	11
Puerto Rico 102	X	X	X	X	X	X	X	X	X	X	X
Tailandia 3	X	X	X							X	X
Venezuela 284 B										X	X
Perú 385										X	X
Costa Rica 149	X	X	X	X	X	X	X	X	X	X	X
Secundina											X
Brasil 29	X										X
Brasil 246		X									X
Brasil 274			N	N	X						X
Brasil 31	X		X				X				X
Brasil 88			N	N						X	X
Brasil 181	X	X	X	X	X		X	X	X		X
Brasil 86											X
Brasil 81		X	X		X			X	X		
Brasil 76			N	N							X
Brasil 156			N	N							X
Brasil 137	X	X	N	N	X	X	X	X	X		X

X = Bands present in the clone. N = Clone not evaluated in this test.

Activity 2.6.4. Standardization of the DNA amplification programme (concentrations of reagents, buffers, primers, nucleotides and enzymes for the amplification profile)

Results
Through different tests we standardized the amplification profile and appropriate concentrations to run the PCR reaction. A temperature of 42°C was preferred for annealling. DNA concentrations of 1ul to 3 ul were used indiscriminantly without marked differences.

Activity 2.6.5. Testing liability of PCR based diagnostic test for CVMV for cassava clones grown in vitro and raised in glasshouse

We wanted to test our current PCR based protocol for the identification of CVMV on identical materials maintained in vitro and raised in glasshouse conditions, with the view of testing materials straight from the in vitro collection.

Results
The majority of materials evaluated with SST and RBD primers presented bands. Cassava materials either coming from glasshouse conditions or from the in vitro collection presented with SST primer bands of similar molecular weight as the control plasmid CVMV 141 (Figure 7). The same group of cassava materials was next evaluated with RT and HS primers. All cassava materials from both source conditions displayed noteworty amplification, with bands of different molecular weights, specially with HS primer. All cassava clones with the exception of BRA 29 displayed bands with molecular weight identical to the control. With RT primer only the cassava clone C. Rica 149 showed band of molecular weight identical to the check CVMV 141 (Figure 8).

We also evaluated a group of cassava materials from Ecuador as well as Elite germplasm from the in vitro collection with primers SST, RBD, HS and RT. Amplification was obtained for all cassava materials in all our trials. With SST and HS primers, bands observed have molecular weight similar to the check CVMV 141.

Activity 2.6.6. Confirmation of results obtained in gels through hybridization of samples obtained with the four primers with plasmid check CVMV 141 as probe

Results
Under high astringency (65 °C and high salt concentration), we tried to hybridize PCR products of glass-house raised cassava clones evaluated with the four primers with plasmid check CVMV 141 as probe. None of the materials did hybridize. DNA filters from gels of in vitro grown cassava materials were hybridized with plasmid CVMV 141 as probe under low astrigency (55 °C and low salt concentration). Bands were observed for all materials tested.

Activity 2.6.7. Cloning, transformation and sequencing

Results
As we obtained high consistency of bands present in cassava clones C. Rica 149 and BRA 137, we carried out a ligation test using PCR-Script using a commercial vector with products obtained with primer SST, and we obtained seven fragments of different molecular weight. These fragments were automatically sequenced by Luisa Fernanda Fory and were compared with the virus genome. None of these fragments presented sequences similar to the sequences of the virus nor to the cassava genome. One could tentatively conclude that this primer and amplification conditions under low astringency for hybridization would not allow us to safely identify the presence of CVMV in cassava clones.

Activity 2.6.8. Comparison of DNA extraction from fresh and dried cassava material for glasshouse and in vitro raised plants

Results

We extracted DNA from fresh tissue and from vacuum dried tissue of a new group of cassava clones from Colombia, Costa Rica, Venezuela, Brazil and Peru raised in vitro and in glasshouse, in order to see whether this processing would affect the amount of extracted DNA. A higher amplification as measured by the number of bands was observed for in vitro raised materials as compared to glasshouse materials. For glasshouse raised materials amplification was observed only in one material (MEXICO 70). We concluded that drying process has no influence on DNA amplification. There was a high correspondence between results obtained with amplifications of fragments from fresh tissue as well as from dried tissue, from plants raised in glasshouse or raised in vitro.

PRIMER SST

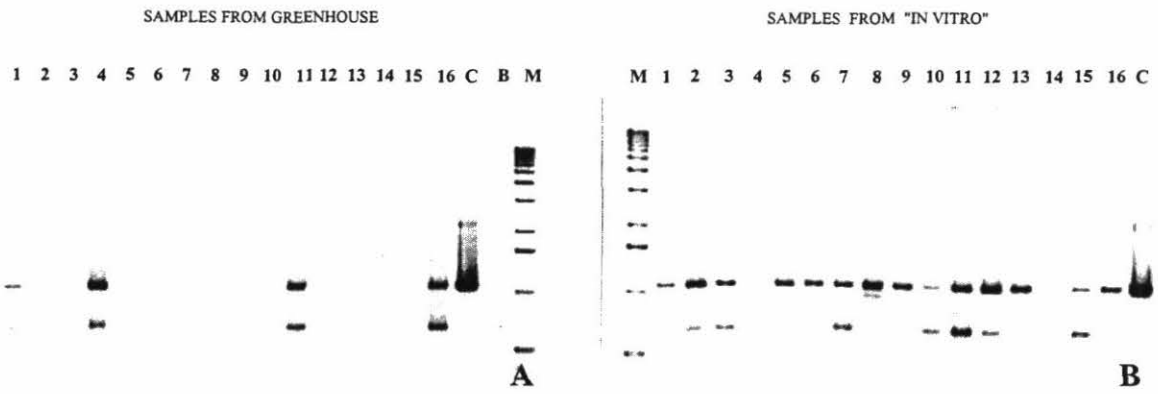

Figure 6.

Figure 7.

PRIMER RT

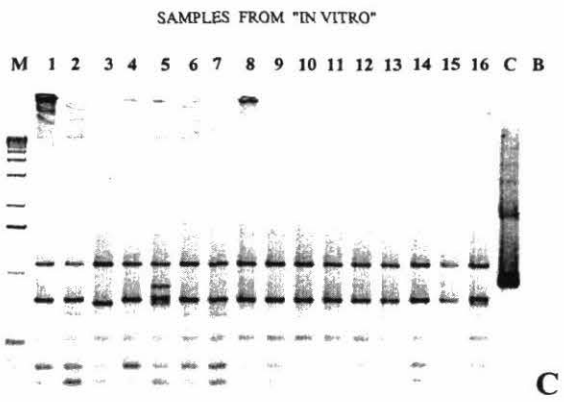

Figure 8.

A	B - C
1. PUERTO RICO 102	M. MARKERS
2. TAILANDIA 3	1. PUERTO RICO 102
3. PERU 385	2. TAILANDIA 3
4. COSTA RICA 149	3. VENEZUELA 284 B
5. SECUNDINA	4. PERU 385
6. BRASIL 29	5. COSTA RICA 149
7. BRASIL 246	6. SECUNDINA # 24
8. BRASIL 274	7. BRASIL 29
9. BRASIL 31	8. BRASIL 246
10. BRASIL 88	9. BRASIL 31
11. BRASIL 181	10. BRASIL 88
12. BRASIL 86	11. BRASIL 181
13. BRASIL 81	12. BRASIL 86
14. BRASIL 76	13. BRASIL 81
15. BRASIL 156	14. BRASIL 76
16. BRASIL 137	15. BRASIL 156
C. POSITIVE CONTROL (CVMV 141)	16. BRASIL 137
B . BLANKING	C. POSITIVE CONTROL (CVMV 141)
M. MARKERS	B. BLANKING

5.3. Sub-Project 3: Objective: to make the FAO Designate collections genetically and socially Relevant

Output 3.1. Designate Collections better characterized

Activity 3.1.1. Measure of infraespecific genetic variability and phylogenetic relationships of key species of *Capsicum* in Colombia

Mario A. Garcia (Universidad Nacional de Colombia, Palmira), César H. Ocampo and D. G. Debouck

Introduction

The genus *Capsicum* contains five domesticated taxa and over 20 wild species. These species are native to the warmer regions of the Americas, extending from the southern United States to northern Argentina. Peppers are good sources of vitamins, particularly vitamins A and C, have uses in medicine, and as ornamentals (Heiser, 1976). The current understanding of intraspecific genetic diversity and evolutionary relationships of the species of *Capsicum* is unsatisfactory. Its domestication patterns is also poorly understood (Eshbaugh et al., 1983). We plan to use different kinds of biochemical markers (seed proteins and metabolism enzymes) to gain additional evidence about patterns of diversity and of domestication within *Capsicum* in Colombia. We choose the germplasm of Colombia given the geographic location of the *annuum- frutescens- chinense* complex.

Results

Protocols for 15 enzyme systems have been developed in *Capsicum*. These systems reveal good activity, repeatibility and polymorphism. There are present 24 polymorphic loci with 69 alleles. In addition, we developped an electrophoresis in 1D-SDS-PAGE for seed proteins. We observed at least four different banding patterns between species. Preliminary results show genetic variation at inter- and intraspecific levels within accessions of *Capsicum* in Colombia. Specific alleles (metabolism enzymes and seed proteins) separate species within this genus as well as natural populations within wild species of *Capsicum*.

References

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Activity 3.1.2. Reproductive mode in *Brachiaria* spp.

A.M. Torres, C.H. Ocampo

Once the morphological characterization was completed, we focused on the reproductive mode of the field collection of *Brachiaria* germplasm.

The purpose of knowing the floral biology of the speciesof *Brachiaria* is to conserve the genetic integrity of each accession. Thus, the apomictic accessions produce their own seeds asexually without pollination. On the other hand, accessions with sexual reproduction can be autogamous or allogamous in their pollination. The apomictic facultative accessions can exhibit both breeding systems, apomixis and sexual reproduction.

The reproductive mode of 17 accessions were determined by microscopy analysis of the embryonary sac. Thus, the entire field collection of *Brachiaria* was assessed for reproductive mode. As final result, only 15% of the collection has sexual reproduction, the remained has fully or facultative apomixis.

In order to determine the allogamy in sexual and facultative apomictic accessions, a preliminary study of the progeny of 6 lines of *Brachiaria* was carried out. The maternal material (6 individuals) and offsprings (106 individuals) were planted. For all individuals, morphological traits were noted and alfa and beta esterase isoenzymes were essayed in PAGE-electrophoresis.

The results of morphological analysis and isoenzyme essays show a high level of allogamy for sexual accessions and a very low level of allogamy in facultative apomictic accessions. On the base of these results, the sexual accessions of *Brachiaria* in the field collection are multiplied in mesh-cages, while apomictic materials are multiplied in open field without any protection against wind pollination.

Activity 3.1.3 Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae)

A.M. Torres, C. Morton (Department of Botany, University of Reading, Reading, UK), S. Renvoize (Head of Grass Section, Herbarium of the Royal Botanic Gardens, Kew, UK)

Research objective

The main goal of this research was to clarify the species relationships within the grass genera *Brachiaria* and *Urochloa*. These genera are intensively used as forage grasses. However, there is a taxonomic problem of unclear delimitation between them.

Research outputs

The results show a continuous gradation between the two genera previously found in several morphological studies. The lack of clear-cut separation between the two genera rejects the current nomenclature distinction.

Research Progress

Several authors have discussed the weakness of morphological characters currently used to separate the genera *Brachiaria* and *Urochloa* (Webster, 1987, 1988; Morrone & Zuloaga, 1992, Veldkamp, 1996). More than 40 species of *Brachiaria* have been transferred to *Urochloa* following the priority principle of the Code of Botanical Nomenclature (Greuter, 1996). Our main inquiries were: What do happen with the species still named as *Brachiaria*? How close are the species once transferred to *Urochloa* and the species originally described as within *Urochloa*?

A phylogenetic analysis was carried out using twenty-one species of *Brachiaria* and *Urochloa* (*Poaceae*). These species were included in the study as ingroup and four species of Paniceae were chosen as outgroups. The molecular data were generated by sequencing the ITS (Internal Transcribed Spacer) region of nuclear ribosomal DNA. This region has proven to be useful for evaluation of relationships at generic and species level in other grasses, such as *Sorghum* (Sun et al., 1994) and *Zea* (Buckler & Holtsford, 1996). The characterization of all morphological traits of of the spikelet for each taxon produced the coded morphological data. Eight of the 17 characters used by Renvoize et al. (1996) for grouping the morphological variations were chosen.

The aligned sequences of the ingroup and outgroup species had 601 characters including gaps due to insertion-deletion events. The cladistic analysis was carried out using the software package PAUP for Macintosh, version 4.0.0d64. The molecular and morphological data were analized separately and in a combined way. Heuristic search for maximum parsimony of the combined analysis yielded three maximally parsimonious trees. The combined phylogenetic analyses had higher statistical supported values as compared to the molecular and morphological analysis considered separately. Furthermore, the combined analyses had more stabilized topology producing a lower number of trees (3), while the morphological analysis had 126 and the molecular analysis had five trees.

None of the morphological groups of *Brachiaria* (*sensu lato*) (Renvoize et al, 1996) were found to be monophyletic in the cladistic analysis. Molecular data and morphological traits did not show high levels of congruency. This result together with the difficulty of coding morphological traits into discrete characters for each species show the need of a revision of the morphology of these taxa.

The analyses support the fact that neither *Brachiaria* nor *Urochloa* are monophyletic. There is a continuous gradation in the species of both genera. The clades or groups in all phylogenetic trees contain a miscellaneous representation of current species of *Brachiaria* and *Urochloa*. Thus, the separation of both taxa is not justified in the botanic nomenclature.

Prospects

The results obtained in this study strongly support that a systematic treatment using a larger number of taxa and more morphological and molecular characters is needed for *Brachiaria* and *Urochloa*. There is a need to avoid confusions with the naming of the species of these genera. Thus, using the results of this study, we are claiming that the name *Brachiaria* should be conserved against the name*Urochloa*. This claim has been addressed directly to the Commitee of Flowering Plants at the Royal Botanic Gardens, Kew.

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Activity 3.1.4. Variability of phaseolin types found in populations of *Phaseolus vulgaris* L. collected in its primary centers of diversity

C. H. Ocampo, O. Toro, D. G. Debouck

Introduction

Phaseolus vulgaris L., the common bean, contains a large amount of native genetic variability in its populations. Identification of the kinds and amounts of genetic variability is necessary to insure that all variability is conserved and available to users. Phaseolin, the major seed storage protein of common bean (Osborn, 1988), has proved to be an excellent - cheap and polymorphic - marker in evolutionary studies (Gepts, 1988). The description of phaseolin types is also becoming a routine descriptor and powerful for the bean germplasm characterization. Given the usefulness and practicability of such marker, the characterization of genetic variation at the population level for accessions of both wild and cultivated *of Phaseolus vulgaris* L. collected in its primary centers of diversity and maintained in CIAT gene bank was felt necessary.

Results

Several original seeds were sampled from each wild population and landraces of *Phaseolus vulgaris* L. representing its range geographic (from Mexico to Argentina). These samples were analyzed in one di-SDS-PAGE electrophoresis for seed protein Phaseolin (Table 24). The variation obtained shows several phaseolin types within these populations and landraces. Mesoamerican populations show 'S', 'CH' and several 'M' types. One Mexican population (DGD-274) displays a novel and apparently unique phaseolin. In contrast, the Andean populations show

the T, C, H, I, Ko, Pa, and J_4 types. The Colombian populations display two interesting features. First, they display phaseolin types that are present in both Mesoamerican and Andean centers. Second, the phaseolin types such as Mu (found in a wild form), several 'X' types and the 'L' (found in cultivated materials) are novel and apparently unique to Colombia.

Prospects

These results show the presence of intrapopulational genetic variability at least in these characterized populations. The above aproach could be extended to the whole bean germplasm as routine characterization, also in order to better define existing core collections.

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Table 24. Phaseolin types found in populations of *Phaseolus vulgaris* L. colected in its primary center of diversity.

Country of origin	Identification	Biological Status	No. of sampled seeds	Phaseolin Types
Mexico	DGD-274	Wild	36	M9, M15, M1, M10, S Nueva
Guatemala	DGD-2423	Wild	173	M16, CH, S, Sd, M13
	CAP-EM 1265	Wild/Weedy	15	S, M13, M26
	CAP-EM 1266	Weedy/Wild	10	S, M13,
	Norvell 3356	Wild	51	S, M5, M13, M25
Honduras	SB-6	Wild	13	S, CH, Nueva
	SB-7	Wild	29	S, CH
	SB-10	Wild	12	S, M16
Colombia	OT-491	Weedy/Weedy	87	CH
	OT-174	Weedy (Segreg.)	61	CH, L, M6
	OT-122	Weedy	85	CH, B, Mu
	Cargamanto (several popul.)	Cultivated	135	S, T, Ca, Ca_1, H_1, H_2, LI, CAR, HE
Perú	DGD-1956	Wild	138	I
	DGD-2635	Cultivated	10	Pa, T
	DGD-2235	Weedy	17	T, H_1, H_2
	DGD-2239	Cultivated	8	Ko, B
Argentina	DGD-621	Wild	138	C, H_1, J_4, T

Activity-3.1.5. Phylogenetic studies of some *Phaseolus* wild species on the basis of biochemical polymorphisms and palynological analysis

I. P. Solarte (Universidad del Valle, Colombia), O. Toro, C. Ocampo, D. G. Debouck

Introduction

The genus *Phaseolus* L. in its contemporary circumscription comprises about 50 species of American distribution (Maréchal et al., 1978; Delgado Salinas, 1985). The genus includes five economically important cultivated species and with their respective wild ancestral form. The current understanding of evolutionary relationships within *Phaseolus* is unsatisfactory. We have useful tools (biochemical markers: polymorphic and cheap) to help us define natural groups within *Phaseolus*, and so to better reflect natural affinities. This will also contribute to determine practical possibilities of widecrossing, instead of making crosses at random as it has been done majorily so far. Delgado Salinas (1985) establish four sections: *Chiapasana, Phaseolus, Minkelersia* and *Xanthotricha*.

Results

The following techniques were particularly useful in revealing good level of polymorphisms and repeatability between all 53 Mesoamerican wild species (including *P. lunatus, P. vulgaris,* and a *Macroptilium* as a test)

characterized. The results of one di-SDS-PAGE electrophoresis on seed storage proteins show that most Mesoamerican wild species display seed protein patterns close to *Phaseolus vulgaris* L. These proteins have a narrow range of molecular weights (46-59 KD). Furthermore, *P. glabellus* and *P.grayanus* display a narrow range between 37 and 43 KD. In contrast, *P. lunatus* shows a wide range between 23 and 43 KD. These data are confirmed by Sullivan and Freytag (1986), and Baudoin et al. (1991). The two accessions of *P. acutifolius* (*tenuifolius and lattifolius*) display a similar pattern. The same similarity is present between *P. altimontanus* and *P. neglectus*. The Mesoamerican wild species were evaluated by 10 enzyme systems (Table 25).

The enzyme data (16 loci) display five groups: *P. leptostachyus* is a taxon lying outside a Mesoamerican pool of species. The dendogram obtain with enzyme and protein data yielded several groups. The most important of these groups is formed by *P. vulgaris, P. coccineus, P. polyanthus, P. costaricensis and P. purpurascens*. Another consistent group is formed by *xanthotrichus* related species. This figure also shows a *P. leptostachyus* (n=20) as a taxon outside the evaluated species by these markers. The most undetermined species are close relative of *P. marechalii and P. polystachyus* (tertiary gene pool of *P. lunatus*).

Prospects

These results are useful to help define natural groups within *Phaseolus* and so to better reflect natural affinities, and practical possibilities of widecrossing. These data are complementary to the ones generated by palynological analysis.

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Outputs

Solarte, I. P. 1999. Relaciones Filogenéticas de algunas especies silvestres de *Phaseolus* sobre la base del polimorfismo bioquímico y análisis palinológico. Tesis Biólogo. Universidad del Valle, Cali, Colombia.

Table 25. Quaternary structure, loci, tissue source, gel and buffer of isoenzymes revealed in Centroamerican wild species of *Phaseolus*.

Enzyme	Tissue Source	Gel	Buffer	Quaternary Structure	No. Loci/Enzyme
Esterases	Root	PAA[1]	TB[2]	Monomer/dimer	3
Diaphorase	Root	STARCH	LB[3]	Tetramer	2
Peroxidase	Root	PAA	TB	Monomer	2
GOT (AAT)	leaf	PAA	TB	Dimer	1
Isocitric dehydorgenase	Leaf	STARCH	HC[4]	Dimer	1
Malate dehydrogenase	Leaf	STARCH	HC	Dimer	2
Malic enzyme	Root	STARCH	LB	Tetramer	1
Shikimate dehydrogenase	Leaf	STARCH	HC	Monomer	1
6-phosphogluconate dehydrogenase	Leaf	STARCH	HC	Dimer	1
Phosphoglucomutase	Leaf	STARCH	HC	Monomer	2

1 Polyacrylamide gel
2 Tris-borate buffer system
3 Lithium-borate buffer system
4 Histidine-citrate buffer system

Output 3.3. Genetic erosion monitored and documented

No particular activity was carried out on this aspect in 1999.However we reported on specific cases of genetic erosion in different species of Phaseolus beans for Costa Rica in a paper submitted to the journal *Genetic Resources and Crop Evolution*.

Output 3.4. Unique genes better sampled and characterized

No activity has been planned in 1999 for this output as the progress on backlogs (i.e. novel accessions and characterization of recently multiplied materials) is still beyond schedule to identify with BRU and IP projects specific genes in the entire collections, and ensure their representativity in different genetical backgrounds.

5.4. Sub-Project 4: Objective: to contribute to the formation of human resources in conservation sciences and techniques in the region

Annexe 6 shows results of training activities, and outputs as thesis, courses, etc.

5.5. Sub-Project5: Objective: to provide scientific input in *in situ* conservation of farmers' landraces and wild relatives

In situ work by Project Staff was reflected by one training course in Bolivia together with the IPGRI RegOff (see annex), one expert consultation meeting for the Plan Verde of the Ministry of Environment of Colombia in cooperation with the von Humboldt Institute. One seminar reflecting on issues was presented at CIAT (see annex).

5.6. Regional developments and systemwide activities

The GRU participated into several information exchange sessions with the Contact Group of the government of Colombia in view of the re-negotiation of the International Undertaking of Plant Genetic resources.

The GRU is hosting the national programme of plant genetic resources of CORPOICA, and has initiated cross presentations of respective activities in order to develop future collaborations.

The GRU participated into the following systemwide initiatives (not logframed and not planned!):
+ costing of the Upgrading Plan for FAO Designate Collections (presented to TAC in March 1999)
+ revision of SGRP objectives, strategy and logframe of activities
+ attendance to the 8th Session of the FAO Commission on GRFA
+ attendance to the CGIAR International Workshop on Genebanks and Genomics
+ participation into the IPGRI/CGN Workshop on Genebank Management
+ participation into the Investment Plan consultation meeting organized by SGRP
+ input into the SGRP Public Awareness initiative

6. Annexes

6.1. SB-01 Project and Genetic Resources Unit Staff

1. Conservation Group:

C. L. Guevara, Ph.D. *	Specialist, Germplasm Conservation
R. Escobar, Biologist	Research Assistant (Cryobiology)
G. Mafla, Biologist	Research Assistant (In vitro Cassava)
H. Velasquez, Biologist	Technician (In vitro Cassava)
J. C. Roa, Biologist	Expert (In vitro Cassava)

2. Production Group:

O. Toro, Tech.	Expert (Bean Germplasm)
A. M. Torres, Biologist	Research Assistant (Tropical Forages)
A. Ciprián, Tech.	Technician (Tropical Forages)

3. Service:

D. G. Debouck, Ph.D.	Head, Genetic Resources Unit
B. Pineda, M.Sc.	Research Associate (Seed Health Testing)
S. Balcázar, Bacteriologist	Lab. Technician (Seed Health Testing)
N. C. Flor, Ing. Agr.	Research Assistant (Seed Health Testing)
A. Valderrama, Biologist**	Research Assistant (Seed Health Testing)
C. Ocampo, Biologist	Research Assistant (Electrophoresis Lab.)
S. Albarracín	Bilingual Secretary

4. Pathology

E. Alvarez, Ph.D.	Pathologist

* Left June, 1999
** Leth December, 1998

6.2. List of publications by Project Staff in 1999

A. In refereed journals:

Fofana, B., Baudoin, J. P., Vekemans, X., **Debouck, D. G.** & du Jardin, P. 1999. Molecular evidence for an Andean origin and a secondary gene pool for the Lima bean (*Phaseolus lunatus* L.) using chloroplast DNA variation. Theor. Appl. Genet. 98: 202-212.

Guevara, C., J.A. Ospina, G.Mafla & V. Verdier. 1998. Zygotic embryo culture of Manihot esculenta Crantz: A practical approach for the safe international movement of cassava seed stocks. Revista Brasileira de mandioca. 17:52.

Mafla, G., J.C. Roa & C. L. Guevara. 1998.Advances on the in vitro growth control of cassava, using silver nitrate. Revista Brasileira de Mandioca. 17: 53.

Maass, B.L. & **Torres, A.M.** 1998. Off-types indicate natural outcrossing in five tropical forage legumes in Colombia. Tropical Grasslands 32:124-130.

B. In non-refereed journals:

Chacón Sánchez M.I., Pickersgill B. and **Debouck D.G.** 1999. Intraspecific chloroplast DNA diversity in common bean (*Phaseolus vulgaris*) for domestication studies. Annu. Rept. Bean Improvement Coop. (USA) 42: 79-80.

C. In books:

Debouck D.G. 1999. Diversity in *Phaseolus* species in relation to the common bean. *In:* S.P. Singh (ed.), Common bean improvement in the twenty-first century. Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp. 25-52.

Pineda-L, B., Huertas C. A., Iwanaga, M. and Morales F. 1999. International Centre for Tropical Agriculture (CIAT). *In*: Kahn, Robert P. and Mathur, S.B. (eds). Containment Facilities and Safeguards, for Exotic Plant Pathogens and Pests, American Phytopathological Society, St. Paul, Minnesota, USA. Pp 23-32.

D. In proceedings:

Balcázar, M. S., Pineda, B., Guzmán, M. L. 1999. Detección de *Xanthomonas axonopodis* pv. *Phaseoli* en Semilla de Fríjol mediante inmunofluorescencia. In: Memorias XX Congreso Nacional de Fitopatología, ASCOLFI, Manizales, 30 Junio, 1 y 2 de Julio de 1999. p. 1.

Debouck, D.G. 1999. Cuando la lingüística anticipa la genética molecular: importancia del conocimiento tradicional para orientar los estudios de biosistemática. *In*: Memorias del Primer Congreso Colombiano de Botánica, Universidad Nacional, Santafé de Bogotá D. C., Abril 26 al 30 de 1999. Pp. 97-98.

Ocampo, C. H., Torres, A. M. & Maass, B. L. 1999. Evidencia bioquímica de patrones geográficos y taxonómicos de variación en *Stylosanthes guianensis* (Aublet) Swartz. *In*: Memorias del Primer Congreso Colombiano de Botánica, Universidad nacional, Santafé de Bogotá D. C., Abril 26 al 30 de 1999. Pp 114.

Ospina, J. A., Guevara, C. L., Caicedo, L. E., Cardozo, C.I. 1999. Rompimiento de latencia determinación de viabilidad con Tetrazolio en semillas de Passifloras. *In:* Memorias VI Congreso Sociedad Colombiana de Fitomejoramiento y Producción de Cultivos. Villavicencio, Meta, 14-16 Julio, 1999. Pp. 54.

Ospina, J. A., Guevara, C. L., Caicedo, L. E., Barney V. 1998. Effects of moisture content on Passiflora seed viability after immersion in liquid nitrogen. *In*: Abstracts of Cryopreservation of Tropical Plant Germplasm. Joint International Workshop. JIRCAS/IPGRI. Tsukuba, Japan. Pp15.

Velásquez E., Mafla, G. 1999. Mejoramiento de la técnica de micropropagación in vitro para algunas especies silvestres de *Manihot* spp. (Euphorbiaceae). *In:* Memorias VI Congreso Sociedad Colombiana de Fitomejoramiento y Producción de Cultivos. Villavicencio, Meta, 14-16 Julio, 1999. p 1.

6.3. List of thesis research supervised by Project Staff in 1999

1. Guzman, F. A. 1999. Desarrollo de estrategias de manejo óptimo para reducir la deriva y la erosión genética en accesiones silvestres de frijol común (*Phaseolus vulgaris* L.) conservadas en el banco de germoplasma del CIAT. Tesis Biólogo. Universidad del Valle, Cali, Colombia. 85p.

2. Marquez, M. M. 1998. Variabilidad morfológica, patogénica y bioquímica de *Alternaria tenuissima* (Ness & Ness: Fries) Wiltshire en Tangelo Minneola. Tesis Magister en Fitopatologia. Universidad de Caldas, Manizales, Colombia. 128 p.

3. Segura, S. D. 1999. Diversidad genética de passifloras andinas (sugénero *Tacsonia* and *Passiflora manicata*). Tesis Doctorado. Ecole Nationale Supérieure Agronomique, Montpellier, France. 120p.

4. Solarte, I. P. 1999. Relaciones Filogenéticas de algunas especies silvestres de *Phaseolus* sobre la base del polimorfismo bioquímico y análisis palinológico. Tesis Biólogo. Universidad del Valle, Cali, Colombia. 80p.

5. Torres, A.M. 1998. Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae. Thesis for the degree M. Sc. in applied and pure taxonomy of plants, University of Reading, United Kingdom.51p.

6.4. List of conferences and scientific communications presented by Project Staff in 1999

Debouck, D. G.

1. Leticia, Colombia, 21 May 1999, invited address at the information network meeting of the Tratado de Cooperación Amazónico, at Instituto SINCHI: "Agrosilvi-culturas amazónicas: experiencias humanas para conservación y uso".

2. Reading, United Kingdom, 12 April 1999, invited seminar at the School of Plant Sciences: "Domestication of neotropical crops: selection for food versus aesthetics".

3. Palmira, Colombia, 7 April 1999, invited seminar at the Centro Internacional de Agricultura Tropical: "*In situ* conservation of plant genetic resources for food and agriculture: *ex situ* conservation was easier!".

4. State College PA, USA, 2 February 1999, invited seminar at PennState University: "Between hickory and goosefoot: views about origin of an old South Appalachian crop".

5. Bogotá, Colombia, 24 November 1998, invited opening lecture for the XXXVth anniversary of the Faculty of Agricultural Sciences of Universidad Nacional de Colombia: "Los Recursos Genéticos de las Américas: Contribución a la Seguridad Alimentaria Mundial".

6. Chinchiná, CENICAFÉ, Colombia, 16 October 1998, invited conference: "Patrones de Diversidad Genética en Cultivos Neotropicales: Síntesis de Factores Físicos, Biológicos y Antrópicos".

Guevara, C. L.

1. Ospina, J. A., Guevara, C. L., Caicedo, L. E., Cardozo, C.I. 1999. Rompimiento de latencia determinación de viabilidad con Tetrazolio en semillas de Passifloras. *In:* Memorias VI Congreso Sociedad Colombiana de Fitomejoramiento y Producción de Cultivos. Villavicencio, Meta, 14-16 Julio, 1999. p. 54.

Flor, N. C.

1. Flor P. N. C., Correa V, F , Escobar, F., Duque, M.C., Flor, C.A. 1999. Dinámica de la virulencia y estructura genética de *Pyricularia grisea* Sacc. Del Arroz *Oryza sativa* L. en el Tiempo. Trabajo presentado en el XX Congreso de Fitopatología y Ciencias Afines – ASCOLFI – Manizales, 30 Junio y 1-2 de Julio de 1999. Trabajo seleccionado para el Premio "GONZALO OCHOA", Categoria Estudiantes, previa selección por la presentación oral.

6.5. List of international and national courses with input from Project Staff in 1999

Debouck D.G. International course on *in situ* conservation, design and management. International Plant Genetic Resources Institute, and Agencia de Cooperación Internacional de España, Centro de Capacitación Regional, Santa Cruz de la Sierra, Bolivia, 15-19 de Marzo de 1999.

Mafla, G. Fourth International Scientific Meeting Cassava Biotechnology Network, Salvador, Bahía, Brazil. 03-07 November 1998.

Mafla, G. National Course of Tropical Fruits, Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias- Sede Palmira. September 27- October 2 1999.

Ocampo, C. H. International course on molecular markers for assessing agrobiodiversity. Institute Alexander Von Humboldt of Colombia and Smithsonian Institute of USA, CIAT, 31 May-12 June 1999, Palmira (CIAT), Colombia.

Torres, A.M. Análisis filogenético molecular y morfológico de *Brachiaria* y *Urochloa* (Poaceae). I Congreso Colombiano de Botánica, Bogotá, 26-30 April 1999.

Torres, A.M. Relaciones filogenéticas en Poaceae. XX Reunión de la Asociación Colombiana de Herbarios, Pasto, 29 August-5 September, 1999.

6.6. List of trainees trained by Project Staff in 1999

Electrophoresis Lab

1. Cadona, Gladys. Development of protocols for isoenzymes in Capsicum. Microbiologist. Instituto amazónico de investigaciones científicas (Sinchi), Projecto de Recursos Genéticos, Santafé of Bogota D. C., 18-28 May, 1999.

2. Ligarreto, Gustavo. Biochemical Caracterizatión of a common bean collection of Colombia. Ing. Agr. CORPOICA, Tibaitata Seat (Programa Nacional de Recursos Fitogenéticos), Student of Doctorate of the National University, Host Santafé of Bogota, 11-30 October, 1998.

3. Matheus, Juan. Introduction to techniques of Biochemical Characterization. IDEA, Venezuela, March 03-15/99.

4. Medina, Clear Ines. Development of a model of genetic diversity in Phaseolus lunatus L. Biologist. CORPOICA (Programa Nacional de Recursos Genéticos), CI La selva, Rionegro Seat, 25 Octubre-05 November, 1999.

5. Puche, Francisco. Techniques of electroforesis 1D-SDS-PAGE/2D-SDS-IEF-PAGE for of Lima bean seed proteins. Universidad de Córdoba, Monteria, 10- Mayo, 1999.

6. Solorzano, Jose R. Biochemical characterization (isoenzymes) of cassava germplasm. Technical university of Manabí. Portoviejo, Ecuador. 17-28 Mayo, 1999.

In vitro Lab

1. Calderín, Marcos. Training in conservation **in vitro** techniques of Cassava germplasm. ALMAGRO Ranching & Farming. Bogotá, Colombia.

2. Matheus, Juan. Training in techniques of Conservation and Management of in vitro Cassava germplasm. IDEA, Venezuela, June 1999.

3. Méndoza, Alma... Training in conservation **in vitro** techniques of Cassava germplasma Instituto Nacional de Investigaciones Agropecuarias – INIAP -, Portoviejo, Ecuador.

4. Kai Mian, Li.. Training in conservation **in vitro** techniques of Cassava germplasm Tropical Field Crops and Animal Husbandry Research Institute CATAS. October, 1999.

5. Solorzano, José R.. Training in conservation **in vitro** techniques of Cassava germplasm Universidad Técnica de Manabí. Portoviejo, Ecuador.

Viability Lab

1. Matheus, Juan. Training in techniques of viability assessment of seeds of Tropical Forages. IDEA, Venezuela, June 1999.

2. Cano, Magnolia. Agrosemillas, Medellin. Colombia, June 1999

Seed Health Lab

1. Kai Mian, Li. Training in Cassava virus indexing techniques. Tropical Field Crops and Animal Husbandry Research Institute CATAS. October, 1999.

2. Méndoza, Alma. Training in Cassava virus indexing techniques. Instituto Nacional de Investigaciones Agropecuarias – INIAP -, Portoviejo, Ecuador.

3. Solorzano, José R. Training in Cassava virus indexing techniques. Universidad Técnica de Manabí. Portoviejo, Ecuador.

6.7. Posters

1. Ocampo, C. H., Torres, A. M. & Maass, B. L. 1999. Evidencia bioquímica de patrones geográficos y taxonómicos de variación en *Stylosanthes guianensis* (Aublet) Swartz. Primer Congreso Colombiano de Botánica, Universidad Nacional, Centro de Convenciones Alfonso López Pumarejo, Abril 26 al 30 1999, Santafé de Bogotá D. C., Colombia.

2. Velásquez E, Mafla, G. 1999. Conservación in vitro: Una alternativa segura para preservar especies silvestres de *Manihot* spp. (Euphorbiaceae). *In:* Memorias VI Congreso Sociedad Colombiana de Fitomejoramiento y Producción de Cultivos. Villavicencio, Meta, 14-16 Julio, 1999. p 1.

6.8. Visitors

The professional staff of the Genetic Resources Unit attended the visit of 63 institutions of different countries, for a total of 450 people during the period from October 1998 to September 1999.

6.9. Donors

CIAT Core budget, CIAT Capital Fund, CIAT Fondo para el Desarrollo del Recurso Humano

Ministerio de Agricultura y Desarrollo Rural, Colombia

Systemwide Programme on Information for Plant Genetic Resources (SINGER), CGIAR

6.10 Awards

Ing. Agr. Juan Manuel Osorno G.. Winner of the National Competition "Otto de Greiff" for the Best Thesis at graduate level in Colombia. Thesis research "Estudio de la diversidad genética y distribución natural de una colección de *Centrosema macrocarpum* Bentham (Fabaceae: Papilionoideae), mediante técnicas de electroforesis", carried out in GRU in 1997.

Annex 1. SB-01 Project Log-Frame

Project: Saving Biodiversity, Genetic Resources Conservation and Characterization
Manager: Daniel G. Debouck

Sub-Project # 1: the International Standards

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To make the FAO Designate Collections complying with the International Standards	ICER'95 and ICER'97 recommendations met	FAO Commission experts visits	
Purpose Our purpose is to multiply and conserve the Designate Collections under the highest standards of quality and cost-effectiveness	Germination rates for long stored materials Costs per accession, per year as compared to other genebanks	Visits to GRU multiplication substations and conservation facilities	Sustained and appropriate funding Staff security guaranteed Services delivered on time Support in documentation delivered
Output 1.1 Backlogs of introduced materials processed	Backlog materials presented to ICA and multiplied in quarantine glass-houses	Visits to quarantine glass-houses On-line consultations of GRU system	Agreement ICA-CIAT renewed and funded Quarantine glass-house space available in different altitudes
Output 1.2 Backlogs of materials pending on multiplication multiplied	Multiplication glass-houses/ plots with backlog materials	Visits to multiplication plots in different substations	Availability of manpower and field equipment
Output 1.3 Materials pending on regeneration regenerated (incl. In vitro)	Regenerated accessions/ year	Visits to regeneration plots in different substations/ in vitro Lab	Availability of manpower and field equipment
Output 1.4 Materials processed into final packing	Processed accessions/ year	Visits to cold store facilities On-line consultations of GRU System	Availability of manpower and lab equipment
Output 1.5 Improved conservation techniques	Savings in maintenance costs Longer periods between regenerations	Publications in refereed journals	Availability of students and Staff time

Sub-Project # 2: the Germplasm Available, Restored and Safe Duplicated

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To make the FAO Designate Collections available to users, inside and outside CIAT	ICER'95 and ICER'97 recommendations met Distribution records	FAO experts visits Consultations of users	
Purpose Our purpose is to distribute the Designate Collections to any *bona fide* user through MTAs	Number of germplasm requests received and satisfied annually	Checks of correspondence about MTAs	Sustained and appropriate funding Agreement with FAO goes on Services delivered on time Support in documentation delivered
Output 2.1 FAO Designate Collections cleaned against seed borne diseases (incl. In vitro)	Accessions tested in *SHL and cleaned* in special multiplication plots/ glasshouses	Visits to SHL/ multiplication plots Reports of external experts	Participation of CIAT virologists and pathologists
Output 2.2 Germplasm, passport and characterization data available to users	Users receive germplasm and data Users ask for novel germplasm and data	On-line consultations on the InterNet	CIAT Information Unit contributes to the re-engineering of databases Budget for recovering databases
Output 2.3 National collections restored to NARS	Accessions of national collections dispatched	Checks in genebank(s) of original country	Agreements with quarantine authorities allow effective shipments GRU enabled to multiply all collections
Output 2.4 FAO Designate Collections safe duplicated (incl. In vitro)	Accessions sent annually to CATIE and CENARGEN	Visits to CATIE and CENARGEN	Agreements with quarantine authorities allow effective shipments GRU enabled to multiply all collections
Output 2.5 Refined core collections	Breeders and agronomists use wider germplasm through core collections	Requests for core collections Core collections multiplied and shipped	GRU enabled to multiply all collections Cooperation with BRU for molecular assessment
Output 2.6 Improved disease indexing techniques	Savings in SHL costs Higher numbers of accessions processed by SHL	Publications in refereed journals	Availability of students Participation of CIAT virologists and pathologists

Sub-Project # 3: the Genetic and Social Relevance of the Conservation

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To make the FAO Designate Collections genetically and socially relevant	Farmers recover landraces from GRU Breeders find novel genes in collections	Surveys of landrace diversity	
Purpose Our purpose is to conserve Designate Collections that meet users' needs today and tomorrow	Landrace diversity restored back to farmers (e.g. Seeds of Hope project)	Comparisons of landrace diversity over time Genes included in novel varieties	Sustained and appropriate funding Staff security guaranteed International collecting possible Support in documentation delivered
Ouput 3.1. Designate Collections better characterized	Genepools and species relationships further defined	Germplasm catalogs On-line consultations on the InterNet Publications	Collaborations with AROs, CIAT BRU and IP projects Support in documentation
Output 3.2 Novel materials acquired or collected	Recently acquired/ collected materials in quarantine glass-houses	Visits to quarantine glass-houses On-line consultations of GRU system Publications	Agreement between country of origin and CIAT Quarantine matters cleared
Output 3.3 Genetic erosion monitored and documented	Endangered populations/ varieties identified/ mapped	Comparative mapping Publications	Collaboration with CIAT GIS laboratory and regional projects
Output 3.4 Unique genes better sampled and characterized	Farmers use new varieties Breeders use novel genes	Plant Variety registration acts and national catalogs	Collaboration with CIAT BRU, IP projects and GIS

Sub-Project # 4: the International Cooperation and Capacity Building

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute through training to capacity building in conservation sciences and techniques in the region	National capacities for conservation and utilization established and improved	FAO State of the World report FAO Commission and CBD COP reports	
Purpose Our purpose is to strengthen the NARS for conservation and utilization of Neotropical plant genetic resources	NARS germplasm collections conserved NARS scientists trained Networks strengthened	Visits to national GRUs Country questionnaires FAO/ IPGRI surveys	Sustained and appropriate funding NARS and networks willing and enabled to cooperate
Output 4.1 NARS human resources trained	Trainees trained in CIAT Courses at CIAT and in the region	Visits to training sites Research Theses	Cooperation of Regional Cooperation Office Participation of IPGRI
Output 4.2 Conferences in national/ international for a	Conferences held	Publication of proceedings	Interest of NARS
Output 4.3 Public awareness products	Public supportive to CIAT role in conservation	Press releases, TV emissions, press articles	Cooperation with CIAT Public Information Office
Output 4.4 Education and training materials	Universities, academia using training materials	Distribution/ sales of training materials	Cooperation of Regional Cooperation Office Participation of IPGRI

Sub-Project # 5: the Link with In situ Conservation on Farm and in the Wild

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To develop in situ methodologies for farmer landraces and wild relatives	Wider gene pools conserved *in situ*	List of taxa in protected areas	
Purpose Our purpose is to link the conservation of Designate Collections with on-farm conservation efforts and protected areas	Case studies and pilot *in situ* conservation projects	Contacts with Farmers' associations and Ministries of Environment	Sustained and appropriate funding International surveying possible Support in documentation delivered
Ouput 5.1. Project proposals prepared	Concept Notes distributed to potential donors	Concept Notes in Project/ Business Offices	Collaboration with CIAT Project Office
Output 5.2 Contribution made towards protected areas in Latin America	Wild relatives of CIAT crops included in protected areas	Publications	Interest by NARS and Conservation Agencies
Output 5.3 Practices on on-farm conservation documented	Participation of Farmers, NGOs and NARS in documentation of conservation practices	Publications Catalogs of landraces	Collaboration with CIAT GIS laboratory and regional projects