ANNUAL REPORT 2000

CIAT Project on Saving Biodiversity SB-01

Genetic Resources Unit
Report on Achievements and Progresses

CIAT
OCTOBER, 2000

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1. Project Overview

Project SB-1: Integrated Conservation of Neotropical Plant Genetic Resources

Objectives: FAO Designated Collections complying with the International Standards and Available to users.

Outputs:

1. Mandate corps conserved and multiplied as per international standards.
2. Germplasm available, restored and safely duplicated.
3. Designate Collections made socially relevant.
4. Strengthen NARS for conservation and utilization of Neotropical plant genetic resources.
5. Conservation of Designate Collections linked with on-farm conservation efforts and protected areas.

Gains: Small farmers of Latin America, sub-Saharan Africa, and Southeast Asia will use dozens of germplasm accessions conserved by the gene bank, as such or after improvement. Sources of disease and pest resistance will be identified for current and future efforts in germplasm enhancement and plant breeding.

Milestones:

2000 Procedures developed for conservation of wild species and landraces, based on studies of seed biology and physiology. Safe-duplication and restoration continued.

2001 Protocols for cryoconservation of seeds and tissue germplasm established. Germplasm collections regenerated. Safe-duplication and restoration continued.

2002 Links with consservation efforts in protected areas and on farm established. Germplasm collections regenerated. Safe-duplication and restoration continued

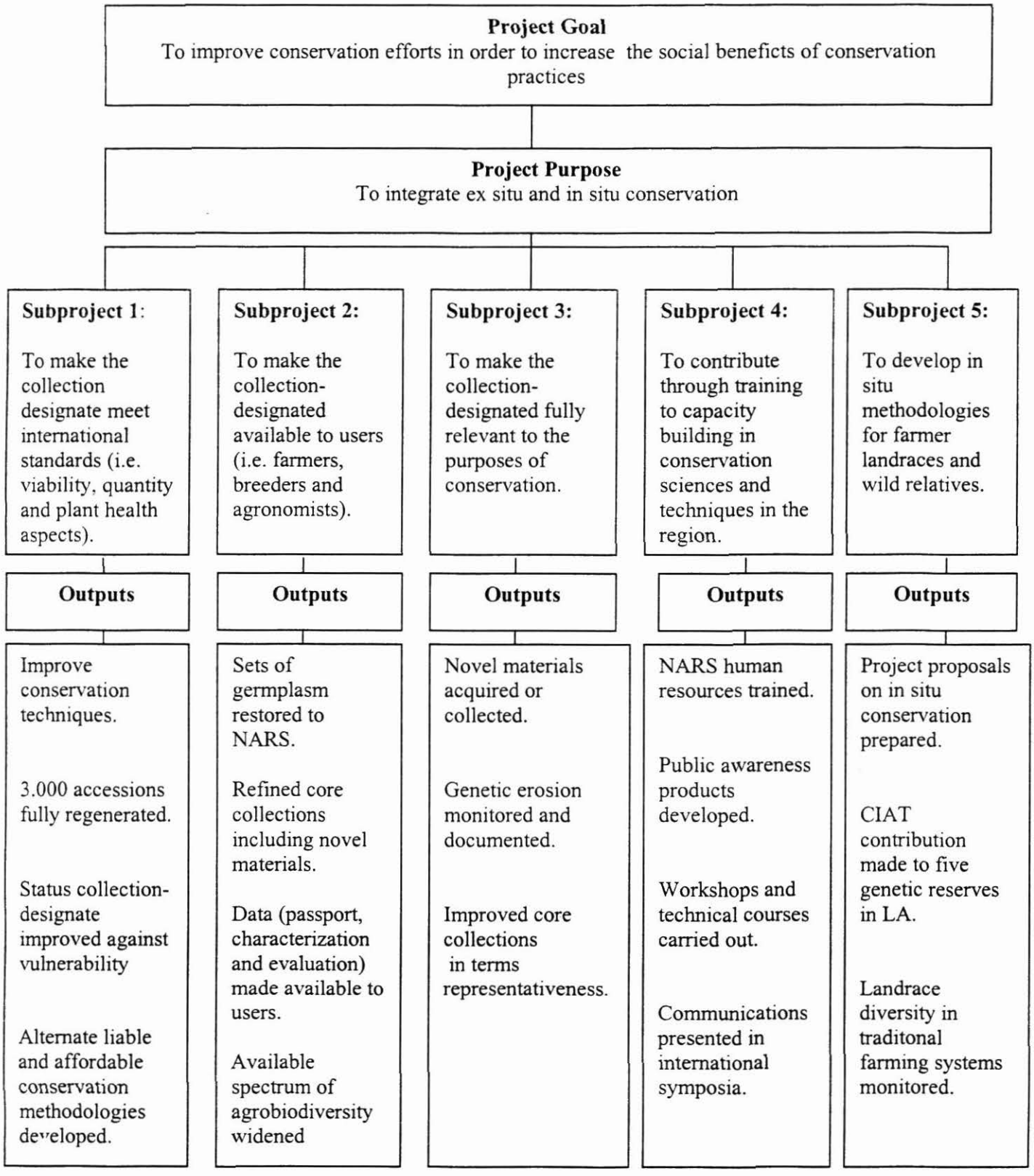
Users: Plant breeding and agronomy programs throughout the tropics and subtropics. Extension services. Farmer associations. Universities and biodiversity institutes in research and training.

Collaborators: *Research:* CATIE, CIMMYT, CIP, CORPOICA, EMBRAPA, INIAA, INIFAP, IPGRI, USDA, and Colombian NGOs, universities, and institutes. *Distribution, safe-duplication, and restoration:* CORPOICA, EMBRAPA, INIAA, INIAP, and INIFAP.

CGIAR system linkages: Saving Biodiversity (80%); Enhancement and Breeding (15%); Training (5%). Participates in Systemwide Genetic Resources Program and SINGER.

CIAT project linkages: Works in methods with SB-2 and PE-4. Provides conserved germplasm and data to breeding in IP-1, IP-2, IP-3, IP-4, and IP-5.

2. Project work breakdown structure



3. Project Logframe

SB-01 Project Logical Framework

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

4. 1. Highlights

Activity area # 1: the International Standards

- A new informatics system in ORACLE Graphics helps to the monitoring of flows and operations in GRU
- Regeneration of 8,381 accessions of beans and forages with ageing seeds
- Seeds of *Carica papaya* can be successfully stored in liquid nitrogen
- Progress made on minimal growth *in vitro* and cryopreservation of cassava germplasm opens ways to reduce conservation costs

Activity area # 2: the Germplasm and its data available

- 40% of the cassava designate collection indexed and cleaned against diseases of quarantine importance
- 6,949 accessions out of the designate collections of three commodity germplasms distributed to end users
- Data compiled about distribution of germplasm (384,204 accessions) by CIAT GRU over the period 1973-2000 for SINGER 2
- Monitoring of fungi of possible quarantine importance initiated on *Brachiaria* forage germplasm

Activity area # 3: the Genetic and Social relevance of the Conservation

- New light on the evolution of Lima bean in Colombia and its affinities with wild populations from the Americas
- New sources of phaseolin variation found in populations of common bean in its primary centers of diversity.
- Molecular taxonomy defines of the major evolutionary groups within the genus *Phaseolus* by ITS sequencing
- A model of genetic simulation helps to control drift and genetic erosion in genebank management practices

Activity area # 4: International Cooperation and Capacity building

- 3 international courses for 65 professionals of Latin America run by Project Staff
- 7 technical conferences given for the Colombian national plan to control FrogSkin disease in cassava
- Cost studies together with IFPRI and SGRP for all conservation methods used in cassava germplasm
- 16 invited conferences presented by Project Staff, namely for ASCOLFI, and 602 visitors attended

4. 2. Executive Summary

Following up on the recommendations of the ICER of 1995, 1997, and the EPMR of 2000, the GRU has continued its upgrading operations for the designate collections, in spite of financial limitations and security problems. Important refreshment of aging seed germplasm of beans and forages has been done. A proper multiplication facility has been settled in Quilichao for forages, but some field machinery is still needed in order to process more materials, particularly in view of the multiplication of forages received from Australia. Security has been a serious constraint to several basic activities of the Project. The site of Tenerife, where about 4,000 accessions of beans were regenerated yearly under good plant health conditions, has to be abandoned. Activities were re-oriented to Popayán, where multiplication is done under plastic covers. Security and access are not guaranteed for the Popayan site either. One way out would be to operate a second multiplication site in Mosquera close to Bogota, but the Project does not have the operational money to properly operate such a multiplication facility. An important reform of the informatics system of GRU has been put in operation (in collaboration with ISU), and the establishment of management of several operations through bar coding has started. An accurate inventory of all seed stocks has been performed, also reflected into the new database system. Important progress has been made to clean the cassava *in vitro* collection during this year; it should be completed in early 2001. From it, the field genebank will be completely re-established. Different grafting techniques have been tested in order to speed up the indexing against FrogSkin disease of cassava. A new development was on disease indexing techniques for forage grasses, with the identification of bacteria and fungi of possible quarantine importance. First results show that minimal growth *in vitro* would not induce somaclonal variation; this should be tested further with the help of molecular markers. Minimal growth *in vitro* is also being tested on several Neotropical fruit species. Another set of cassava clones has been successfully cryoconserved in 2000. Capitalizing on early results with passion fruits, cryoconservation protocols have been successfully developed for *Carica papaya* seed (in collaboration with IPGRI). A total of 6,949 accessions from the three commodity germplasm FAO designate collections were distributed to users. Germplasm characterization went on as regeneration cycles took place for 5,848 accessions; descriptors are under development for *Brachiaria*. Significant progress has been made to understand better the structure of genetic diversity in the genus *Phaseolus* through ITS sequencing (in collaboration with GRU), and in Lima bean. Several new phaseolin types have been disclosed in common bean. Service in electrophoresis techniques has been provided to CIAT Bean and Forage Projects. A study was developed to monitor effects of drift in genebank practices. A costing study of all conservation methods using cassava as model has been developed with Finances and IFPRI. The input in SINGER2 concentrated mainly on distribution data for the period 1973-2000. Three international courses involving collaborations with IPGRI, the Project Office, and CLAYUCA, were run in 2000. Technical assistance has been provided to ICA for the national plan of FrogSkin disease control. Technical assistance has been provided on the Enola case.

Plans for next year include:

- Expand the bar coding to the management of seed germplasm
- Revise the modules of the GRU computed system and the reporting tables and automatic feedback schemes
- Allow internet users to make their germplasm requests through internet after consulting GRU databases
- Continue to clear backlogs, especially in bean germplasm
- Continue to regenerate accessions of forage and beans
- Introduce the forage germplasm received from Australia
- Complete the cleaning of the cassava in vitro collection and install it as field genebank in CIAT
- Complete and publish the protocols about minimal growth *in vitro* for cassava and for fruit germplasm
- Expand the cryoconservation of cassava germplasm to more accessions of the core collection
- Establish the cassava core collection in Thailand and its evaluation scheme
- Complete and publish the protocols for cryoconservation of seed of *Carica* fruit germplasm
- Complete and publish the protocols for the safe multiplication of *Brachiaria* germplasm
- Complete and publish the molecular taxonomy of *Phaseolus*
- Complete and publish the cost analysis of cassava germplasm conservation with IFPRI
- Run international courses as it may be required
- Continue to train the Staff for the use of the new computerized system and the bar coding
- Implement the experimental part of the BMZ Gene Flow Project in Peru and Costa Rica

5. Progress Report

Sub-project # 1. The International Standards

Output 1.0. A computerized management system

Activity 1.0.1. Migration of data from previous databases into a single new one

A new database system ORACLE Graphics has been implemented for all the processes of the Genetic Resources Unit. It has been designed over the last two years and most of the tables have been migrated from the previous ORACLE system and from old d-Base databases. Thus, the information of passport, multiplication, cleaning, temporary and final conservation, viability and distribution is now being updated in the new database. We have also initiated to link certain modules with a bar coding system to register all movements of germplasm along the GRU flow of operations; in particular we have initiated the bar coding for the cassava *in vitro* collection. Training has also been provided to Staff in order to use it.

The implementation of a new informatics system for GRU gave us the opportunity to update (create in some cases) the basic inventory of all accessions. In particular we wanted to record the precise number of seeds already conserved in the different cold rooms; such an inventory process has started at the end of 1999, and shall be completed in 2000 for the bean and forage seed collections. The inventory has focused on seed harvested before year 1995 because these materials were reported with unexact quantities of stored materials. Materials harvested after 1995 year are well documented and no further inventory was required. In the bean seed collections, 9,112 accessions of 14,501 (63 %) were recorded. Similarly, in the forage seed collections, 8,578 (100%) accesions were recorded in the new ORACLE system. Coded variables were: source of seeds, weight of seeds per source, weight of 100 seeds, and total number of seeds.

Contributors: all GRU Professional Staff, C. Meneses, F. Rojas, S. Ramírez, J.C. Orozco (bar coding)

Output 1.1. Backlogs of introduced materials processed

Activity # 1.1. Introduction of germplasm into GRU process

This activity refers to the processing of germplasm obtained in the past either through germplasm explorations or donations by NARS. In beans, a large survey of the 14,542 accessions pending in 1999 has been undertaken, and from a first analysis a total of 492 accessions were estimated duplicates, and thus not introduced into the GRU process. In forages, from a total of 2,227 accessions pending in 1999, 560 were introduced in post-quarantine glasshouses in 2000. In cassava, a collection of 179 accessions of wild species is still pending on introduction.

Table 1. Status of materials introduced into process (number of accessions)

	Beans	Forages	Cassava	Total
Germplasm pending in 1999	14,542	2,227	179	16,769
Processed in 2000	644	560	---	1,204
Germplasm pending	13,898	1,667	179	15,744

Contributors: O. Toro, A. Ciprian, A.M. Torres

Output 1.2. Backlogs of materials pending on multiplication multiplied

Activity # 1.2.1. Multiplication of materials cleared by quarantine authorities.

In 2000, multiplication of seed has been done for 560 accessions of forages. 123 accessions of common bean. In addition, 195 accessions of 34 wild species of *Phaseolus* that were multiplied over the past years were included in the germplasm bank with CIAT number assignated.

Contributors: O. Toro, A. Ciprian, A.M. Torres

Output 1.3. Materials pending on regeneration regenerated

Activity # 1.3.1. Multiplication of materials with ageing seeds

Achievement: 8,381 accessions of beans and forages of designate collections with ageing seeds being multiplied

Table 2 shows a total of 5,223 accessions of beans regenerated in 2000 in three localities. The main purpose of regeneration was refreshment due to commitments towards the FAO designate collection. However, for a large number of accessions we could achieve full multiplication, because the quantities of seeds required for final packing for long-term conservation were already obtained. In 1999, 11,768 bean materials were pending on regeneration; 6,545 are pending now. Due to security problems, the Tenerife site has been discontinued at the beginning of the year, and altitude operations have been concentrated in Popayán with the building of three additional mesh-houses. For the forage collection (Table 3), 3,158 accessions have been/ are being (several woody species will produce enough seed only by 2001-2002!) multiplied; 8,403 accessions are pending now.

Table 2. *Phaseolus* beans germplasm processed for regeneration under greenhouse/meshouse and field conditions (number of accessions).

Localities	Greenhouse/meshouse	Field	Total
Palmira	1,200	---	1,200
Popayán	750	---	750
Tenerife	155	3,118	3,273
Total	2,105	3,118	5,223

The data include material designated to FAO in 1999 and material to be designated in a near future.

Table 3. Forage germplasm processed for multiplication and regeneration under greenhouse/meshouse and field conditions (number of accessions)

	Greenhouse / Meshouse		Field / Conditions		Total
Localities	Legumes	Grasses	Legumes	Grasses	
Quilichao	N.A.	N.A.	1,257	218	1,475
Palmira	434	405	211	21	1,071
Popayán	N.A.	N.A.	57	555	612
Total	434	405	1,525	794	3,158

Contributors: O. Toro, A. Ciprian, A.M. Torres

Activity # 1.3.2. Periodical subculturing of the FAO designate cassava collection

This year, 4,290 clones of *Manihot* were subcultured by the nodal cutting technique. Subculturing clones constitutes the most time and labour demanding activity in the In Vitro Lab. The accessions multiplied represents 75% of the collection, and 1,848 Secundina plants (cloneCOL 2063) were propagated for indexing work by GRU Staff and other CIAT Cassava projects.

Contributors: G. Mafla, J.C. Roa

Ouput 1.4. Materials processed into final packing

Activity # 1.4.1. Final drying and temporary storage

Tables 4 and 5 show the amount of accessions for beans (5,669 accessions) and forages (2,314 accessions) respectively, which have been cleaned, dried to 7% moisture, and stored at 5°C awaiting results from viability and health tests.

Table 4. Bean germplasm in seed processing

	TEN1999A	TEN1999B
Seed selection / temporal storage	---	3,111
Final Drying / awaiting for viability and health tests results	2,558	---
Total	2,558	3,111

Table 5. Tropical Forages in seed processing

	Legumes	Grasses
Seed selection / temporal storage	1,520	729
Final Drying / awaiting for viability and health tests results	47	18
Total	1,567	747

Contributors: A. Ciprian, O. Toro, A.M. Torres

Activity # 1.4.2. Viability testing

The following table (Table 6) indicates flows of materials during 2000. It shows the importance of good drying and other improved procedures established since 1996.

Table 6. Viability testing for *Phaseolus* beans and tropical forages during 2000

	PHASEOLUS				**FORAGES**		
	Germination %	*P.vulg.* # acces	*P. lun.* # acces	*P.Acut.* # acces.	Germination %	LEGUMINOSAE # acces.	POACEAE # acces.
Already stored materials	1-50	725	2	38	1-50	27	28
	51-84	289	5	50	51-84	31	9
	85-100	183	5	44	85-100	37	5
TOTAL		1,197	12	132		95	42
Recently multiplied materials	1-50	69	-	-	1-50	18	4
	51-84	162	-	-	51-84	23	13
	85-100	1,067	-	-	85-100	171	14
TOTAL		1,298				212	31

Contributors: H. Velásquez, A.M. Torres

Activity # 1.4.3. Final packing and germplasm orientation into the five targets of conservation

Once cleared by the Viability Lab (for viability above 85%) and the Germplasm Health Lab (for absence of diseases of quarantine importance), the materials are processed into the five conservation purposes: long-term, safe duplicates, restoration, periodical monitoring, and distribution (Table 7).

Table 7. Final storage and packing of *Phaseolus* beans processed during 2000 (number of accessions)

	TEN1998A	PAL1998B
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	2,069	891
SHORT TERM only (Distribution)	44	193
Total	2,113	1,084

The germplasm processed from PAL1998B, besides *Phaseolus vulgaris* accessions, included 28 accessions of the complex of perennial species *Phaseolus coccineus/ Phaseolus polyanthus*.

Table 8. Final storage and packing of tropical forages processed during 2000 (number of accessions)

	Legumes	Grasses
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	143	4
SHORT TERM only (Distribution)	137	966
Total	280	970

Contributor: A.M. Torres

Activity # 1.4.4. Revision of seed size samples for forage conservation purposes

Sample size of seed for conservation of forage germplasm has been revised. For several years the number of accessions conserved into the five purposes has been very low, because of the large quantity of seeds required, with a range from 5,000 to 30,000.

The revision includes several considerations for each of the 720 species. The biological considerations were the reproductive biology system (namely outcrossing rate) and the amount of seeds introduced as original population. The technical considerations are time required for regeneration, life cycle and type plant, seed production and number of seeds required for testing seed moisture, and later on viability. All these traits were cross-referred for each species in order to have an average amount of seeds to be conserved by species category

Contributors: A.M. Torres, H. Velásquez, A. Ciprian

Output 1.5. Improved conservation techniques

Achievement: morphological stability of cassava genotypes under minimal growth *in vitro*.

Activity 1.5.1. Morphological study of cassava maintained under minimal growth conservation in silver nitrate media.

Introduction

Maintenance of genotypic stability is of great importance in any *in vitro* conservation approach, no *in vitro* culture system will be acceptable if it induces a high risk of genetic instability and/ or selection among genotypes (Withers 1980). Isozyme and DNA analysis as well as examination of the morphology of cassava plants regrown in the field did not reveal any modification after 10 years of storage under slow growth (Angel et al. 1996). Since 1997 we have been trying to reduce growth *in vitro* in order to open ways to safe-duplicate the collection and increase time span between regenerations. In order to determine if the plants of cassava regenerated from silver nitrate medium

(minimal growth) show variation as compared to plants conventionally maintained *in vitro*, a screen using morphological descriptors has been performed.

Materials and methods

Six cassava varieties were evaluated: ARG 2, BRA 337, COL 2056, NGA 16, VEN 329A and CM 2177-2. They were maintained as *in vitro* plants for 20 months on medium containing 58.8 $_{u}$M (T1) and 70.6 $_{u}$M (T2) of silver nitrate and *in vitro* storage medium 8S(C) (Roca et al. 1984). The materials were regenerated and propagated *in vitro*, greenhouse acclimatized and eventually replanted in the field. Four plants from each treatment for each of the six varieties were studied. Ten morphological descriptors were evaluated, which are used for the characterization of cassava accessions from the active germplasm bank (CIAT-CNPMF, 1995); the evaluations were done six months after transplanting in the field.

Results

The results showed that seven of the qualitative descriptors: colour of apical leaves, colour of expanded leaf, pubescence of young leaves, leaf vein colour, number of leaf lobes, shape of central lobe and petiole color remained constant in the different treatments. We observed significant variation only in the length of the central lobe and petiole length for the variety BRA 337, which presented an average significantly higher in the treatment 2 (Table 9). The environment (light, soil, and plant vigor) has considerable effect on the expression of these morphological characters (CIAT-CNPMF, 1995).

In general, using morphological descriptors we have shown that cassava plants remain true to type after *in vitro* conservation under minimal growth conditions during 20 months with silver nitrate. It is necessary to analyze the other descriptors that will be noted up to harvest. We will also perform an analysis of esterase isoenzyme activity, the polymorphism of which has proven very useful in cassava, before concluding to the genetic stability of the materials.

Table 9. Evaluation of traits of cassava *in vitro* plants regenerated after culturing in contact with silver nitrate.

	Width of lobe* (Cm)			Length of lobe* (Cm)			Length of petioles* (Cm)		
Varieties	8S	T1	T2	8S	T1	T2	8S	T1	T2
ARG 2	31.2^{A**}	35.0^{A}	28.7^{A}	16^{A}	17.5^{A}	16.5^{A}	28.5^{A}	32.5^{A}	23.5^{A}
BRA 337	25.2^{A}	24.2^{A}	31.0^{A}	17.5^{B}	18.2^{B}	22.0^{A}	24.2^{B}	26.0^{AB}	30.0^{A}
COL 2056	45.7^{A}	46.5^{A}	48.2^{A}	19^{A}	18.7^{A}	18.7^{A}	31.5^{A}	29.5^{A}	32.7^{A}
NGA 16	58.7^{A}	55.7^{A}	51.5^{A}	20.5^{A}	18.7^{A}	17.2^{A}	30.5^{A}	28.7^{A}	27.5^{A}
VEN 329A	41.0^{A}	31.0^{A}	45.7^{A}	18.2^{A}	13.2^{A}	19.5^{A}	32.2^{A}	22.0^{A}	34.2^{A}
CM 2177-2	27.2^{A}	24.2^{A}	24.7^{A}	23.7^{A}	22.2^{A}	23.0^{A}	35.7^{A}	34.2^{A}	33.5^{A}

* Average of 4 replications/treatment

** Averages with the same letter do not show significant differences at $P< 0.05$ level According to Duncan test.

Contributors: G. Mafla, J.C. Roa, and G. Jaramillo

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

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Activity # 1.5.2. Cryopreservation of cassava shoot tips using the encapsulation-dehydration technique.

Achievement: 66 cassava clones were cryopreserved and recovered after being frozen in liquid nitrogen

Introduction

There are at least seven different procedures for vitrification: encapsulation-dehydration, vitrification *per se*, encapsulation-vitrification, desiccation, pre-growth, pre-growth-desiccation and droplet freezing (Engelmann 2000). In vitrification-based procedures, cell dehydration is performed prior to freezing by exposure of samples to concentrated cryoprotection media and/or air desiccation. The encapsulation-dehydration technique (Palacio 1998) was established as an alternative to slow (Escobar *et al* 1997) and rapid freezing (Escobar *et al* 1997) for cassava. We adjusted the encapsulation-dehydration methodology, and used a wider range of genotypes to test these modifications.

Methodology

Adjustments were made on the growth conditions of plants that served as source of shoots. The reproducibility of the response after freezing across time (different freezing experiments) was tested. We tested 66 clones with the adjusted protocol.

Results and Conclusions.

It was determined that the conditions of *in vitro* plants that served as source of tissue could affect the response after freezing. Source material with the following conditions responded better:

- Plants with no previous cuttings
- Three to 3,5 months old plants
- Plants grown on 4E medium
- Fifteen to 20 plants per magenta

From 66 clones tested, ten (10/66 or 15%) showed shoot recovery at less than 30% after freezing (Table 10). Some cassava clones, e.g. MPer205 and MPar110, were recalcitrant to cryopreservation. Supplementing the recovering medium with other growth regulators (Kinetin + IAA or Kinetin + IBA) improved the response after freezing.

The reproducibility of the methodology is summarized in Table 11.

Table 10 Response of 55 cassava clones from the core collection, cryopreserved in liquid nitrogen using encapsulation dehydration technique.

Cassava clone	Survival (%)	Shoot Recovery (%)	Cassava clone	Survival (%)	Shoot recovery (%)
M Arg12	84.4	42.2	M Dom 2	68.8	68.8
M Arg 13	61.9	48.2	M Dom 3	92.3	76.9
M Arg2	100	88.8	M Dom 4	100	90
M Bra 124	69.6	42.2	M Dom 5	93.3	93.3
M Bra 125	77.4	71.5	M Ecu 165	46.9	9.5
M Bra 130	69.8	43.3	M Ecu 171	93.6	56
M Bra 132	88.8	60	M Ecu 31	35	15
M Bra 18	90	62.3	M Ecu 41	60.9	45
M Bra 258	73.8	73.8	M Ecu 82	53.3	30
M Bra 311	13.3	3.3	M Gua 15	92.3	83
M Bra 329	57.5	33.8	M Ind 8	65	50
M Bra 404	100	46.7	M Mal 1	75	35
M Bra 542	91.9	56.7	M Mal 13	96.7	53.3

M Bra 691	82.9	37.7	M Mal 48	65	35
M Bra 698	95.8	90.1	M Mal 63	73.3	50
CM 4733-4	35.8	19.2	M Mex 8	81.7	54.2
M Col 185	81.8	58.1	M Mex 92	68.1	20.8
M Col 1438	93.3	28.1	M Nga 5	69.7	49.5
M Col 1522	40	20	M Pan 7	96.7	73.3
M Col 1752	90	75.5	M Pan 97	97	84.6
M Col 1805	100	71.2	M Par 100	95	90
M Col 2173	91.2	34.2	M Par 105	100	91.1
M Col 2215	50	22.7	M Par 41	90	85
M Col 2361	60.9	56.4	M Par 68	87.9	87.9
M Col 40	66.7	44.4	M Per 205	43.8	6.3
M Cr 35	100	63.12	M Per 255	97	41.5
M CR 59	100	73.3	M Per 295	93	20.7
M Cr 63	82.5	65.7	M Per 436	94.1	79.7
M Cub 16	89.6	89.6	M Per 597	90	73.3
M Cub 23	97.4	97.4	M Tai 1	75.6	62.2
M Cub 29	97.5	97.5	M Ven 117-b	73.3	26.2
M Cub 39	80.9	39.4	M Ven 90	76.7	50
M Cub 55	85	80			

Table 11. Reproducibility of the response of six cassava clones cryopreserved in liquid nitrogen across time.

Cassava clones	Survival (%) Plot 1	Plot 2	Plot 3	Control	Shoot recovery (%) Plot 1	Plot 2	Plot 3	Control
CM 4063-6	100	90.9	72.5	73.4	33	19.5	5	31.5
M Bra 69	100	96.6	91.6	86.3	50	60	73.3	33.7
M Bra 881	100	100	100	91.4	75	85	60	28.2
M Ecu 117	66.5	80	91.5	81.9	36.5	37.2	34	44.8
M Mal 2	86	94.4	80	71.1	67.5	78.3	60	61.7
M Par 71	100	100	100	96.7	85	100	95	96.7

Conclusions

- 66 cassava clones were cryopreserved and were recovered after being frozen in liquid nitrogen.
- The adjusted protocol (described above) showed reproducible results over time.
- Using recovery media with IBA or IAA one can reduce callus induction.
- Standardizing the growth conditions of plants that served as source of shoots in material such as MNga 1, previously reported as recalcitrant (Annual Report 1999), improved their response after freezing. Other accessions such as MPar 101 and MPer 205 were still recalcitrant.

Future activities

- GRU provided us with another 100 cassava clones from the core collection to test them for cryopreservation with the adjusted protocol.
- We will work on recalcitrant materials with different desiccation procedures.
- We will test different cryopreservation times (2, 4, and 6 months) with selected materials.

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

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Activity # 1.5.3. Establishing cryoconservation methods for seed of highland papayas.

Achievement: seed of *Carica papaya* can be successfully conserved in liquid nitrogen

Introduction

The family Caricaceae comprises four genera (Brummitt 1992) and 31 species (Mabberley 1987) of small trees or shrubs. It is native to the warm tropical regions of America and Africa. Many species such as *Carica papaya* L. and *C. candamarcencis* Hook have edible fruits (Hong et al. 1996). *C. papaya* seeds show an intermediate behavior and can be stored at temperatures between 5°C and 10°C. This suggests that other species of *Carica* can also be conserved at temperatures between 5°C and 10°C, with a moisture content between 8% and 10%, or at a relative humidity of 40% to 50%.

Hong et al. (1996) reported that *C. papaya* seeds tolerate desiccation when their moisture content is as low as 9.8%. Seed longevity is reduced under storage at 0°C (Teng & Hor 1976, cited in Hong et al. 1996). With moisture content of 10%, they can also survive 24 h in liquid nitrogen (Becwar et al. 1983).

Caricaceae species, particularly wild species, have high value as a plant genetic resource for improving papaya, especially for virus resistance. *Carica pubescens, C. cauliflora*, and *C. horovitziana* are all resistant to viruses that attack *C. papaya.* All these cultivars are of great economic importance to many countries. However, their production and conservation have several limiting factors. The fruits are short lived under tropical conditions. They are highly susceptible to mechanical damage and to diseases, particularly anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Sankat and Maharaj 1997). For all these reasons, this valuable germplasm must be conserved under optimal storage conditions, in accordance with its biological characteristics, to guarantee a high level of viability over time.

One objective of the present work is to understand the dynamics behind the viability of *C. papaya* seeds under different conservation conditions over time. Such understanding will help to establish protocols for evaluating the seed physiological quality, their tolerance to desiccation, and the best conditions for long-term conservation, with a view to later conserving wild species of high importance to the genetic improvement of this crop.

Objectives

- To evaluate tolerance to desiccation when seed moisture content is at 9%, 5%, or 2%.
- To evaluate tolerance to four contrasting methods of conserving *C. papaya* seeds.
- To compare the effect of exposure time when seeds are exposed to liquid nitrogen through cryoconservation for 1 h, 4 months, and 1 year.

Methodology

Healthy and mature fruits of *C. papaya* were obtained from the local market, then conditioned to one of three moisture contents: 9%, 5%, and 2%. Once the level of moisture content was reached, the seed was packed in tri-laminar aluminum bags sealed under vacuum. The temperatures and relative humidities used are presented in Table 12.

Table 12. Environmental conditions of the four methods of conservation.

Place	Environmental conditions	
	Temperature (°C)	RH (%)
Laboratory (Lab)	22	50-60
Short-term cold room (STCR)	7	50
Long-term cold room (LTCR)	-20	-
Liquid nitrogen (LN)	-196	-

The slow method was used to thaw seeds immersed in liquid nitrogen, by which the seeds were removed from the liquid nitrogen and left at room temperature for 24 h. The seed physiological quality was assessed at 0, 4, and 12 months, testing for germination on a substrate of paper rolls. Those seeds that did not germinate were tested for viability, using chromatography with tetrazolium triphenyl salt. The seed moisture content was also evaluated according to the method described by international norms for seed testing (ISTA 1999).

Statistical Analysis

Testing was carried out according to a random complete bloek design, in a factorial arrangement with four replicates (replicates of the germination test). An analysis of variance (ANOVA) was carried out, where the sources of variation were individual factors and the respective interactions. The sources that yielded significant differences at the 5% level of probability according to the F test, were further tested by a separation of means, according to Duncan's test.

Results

Overall, total viability of seeds declined significantly over time, with an important reduction (25.3%) in the first 4 months, and 19.8% in the following 8 months. For every conservation method, the moisture content affected total viability of seeds over time. In the short-term cold-room method (7°C), viability loss was 14.5% for seeds with a moisture content of 2%, while viability loss was about 7.0% for seeds with a moisture content of either 5% or 9% (Figure 1a).

Figure 1b shows that, under laboratory conditions (T = 22°C), a moisture content of 2% drastically affected the seed total viability, with a loss of 21%. The highest viability losses were seen in the long-term cold-room conservation method, where temperatures were as low as –20°C. Losses were as high as 58.5% when the seed moisture content was either 2% or 5% (Figure 1c). Ellis et al. (1991) reported a similarly reduced seed longevity for this genus when conserved at this temperature. For the liquid nitrogen conservation method (T = -196°C), the seed viability losses were smaller, regardless of initial moisture content, showing that a moisture content of 9% did not present viability loss (Figure 1D).

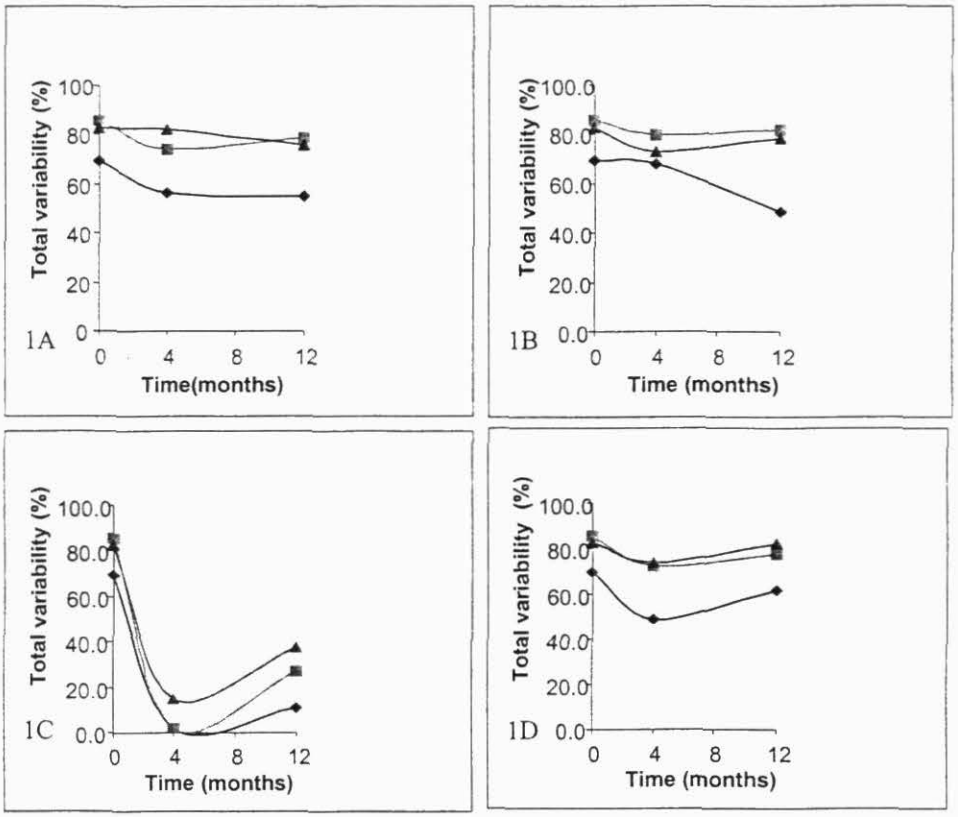

Figure 1. Effect of moisture content over time for each of four conservation methods: (a) short-term cold room, (b) laboratory conditions, (c) long-term cold room, and (d) immersion in liquid nitrogen. (–◆–2%; –■– 5%; –▲– 9% moisture content.)

The way by which seed moisture content affects its viability therefore depends on the conservation method used, although the results also indicate that a moisture content of 2% drastically affects seed viability in all methods. This is most critical in the long-term cold-room method (-20°C), where viability was reduced by as much as 58.5%. The best method for conserving *Carica papaya* seeds is immersion in liquid nitrogen, with the seeds having a moisture content of 9%. Overall, this species does not tolerate low levels of moisture content. Future research should therefore test at moisture contents higher than 5%, obtaining them by using moisture curves on silica gel.

Recommendations

The findings of this research led to the development of a second phase with some modifications to the initial project. The idea was to improve aspects that would create optimal conditions of long-term storage.

Methodology

For the project second phase, homogeneous fruits of two varieties of *Carica papaya* were selected from the local market to evaluate the effect of variety. Some preliminary tests carried out with the two varieties, showed differences in the percentage of full seeds, their weight, and total viability. This finding is borne out by Hartman and Kester (1968) and Pérez et al. (1980), who reported that the variety of the fruit influenced germination. Our preliminary tests also showed that small fruits have seeds of smaller weight and less viability than do large fruits. This confirms Nagao and Furutani's report (1986) that seed weight determines germination in that most seeds weighing less than 12 mg each float in density tests and also show an absence of embryos. To achieve the desired levels of moisture content (11%, 9%, and 5%), the moisture curve presented in Figure 2 was used.

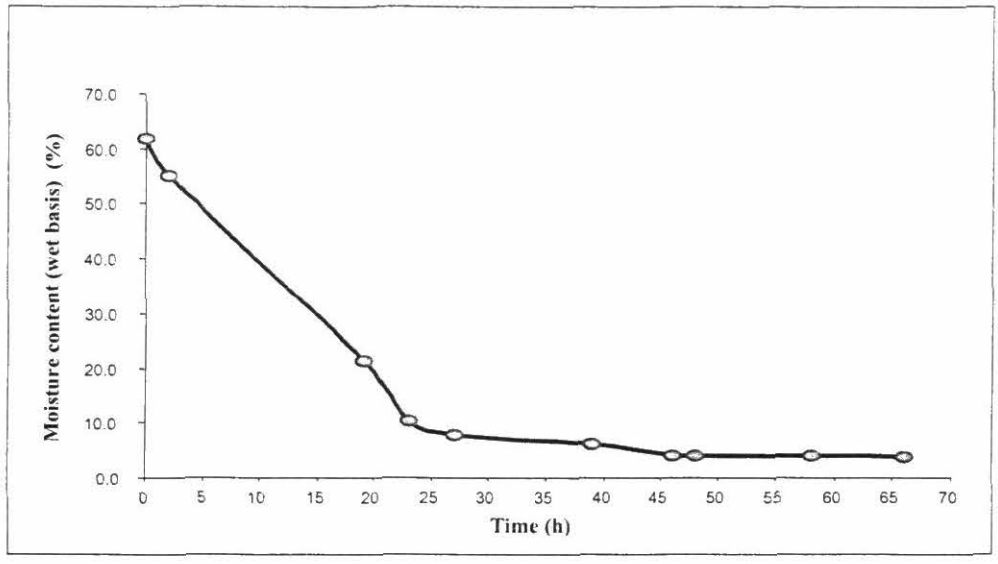

Figure 2. Drying curve for *Carica papaya* seeds placed on silica gel.

Breaking dormancy

To decide on a specific treatment to break the dormancy of a seed, the class of dormancy must first be determined, because on this method depends the rate of germination. *Carica papaya* seeds present a certain degree of dormancy, requiring some treatment to break it.

For the experiment first phase, seed dormancy was broken by immersion in hot water (45°C) for 10 min, then in a solution of GA_3 (500 ppm) for 24 h, with alternate temperatures (35/25°C), a thermoperiod of 8/16 h, and a photoperiod of 12 h light. Because this treatment did not increase the speed of germination, other methods, reported in the literature by Riley (1980) and Pérez et al. (1980), were tried (Table 13).

Table 13. Methods for breaking dormancy of *Carica papaya* seeds.

Treatment	Germination (%)	
	8 days	17 days
Imbibition in water at room temperature for 24 h	13.4	69.0
H_2SO_4 concentrate for 5 min	60.0	64.0
Imbibition in water + agitation for 24 h	58.5	100.0
Imbibition in 1.5% solution of KNO_3 + KH_2PO_4 + agitation for 24 h	100.0	
Imbibition in 1.5% solution of KNO_3 + KH_2PO_4	100.0	
Imbibition in water at 70°C for 10 min + imbibition in water at room temperature for 24 h	35.1	100.0
Imbibition in water at 40°C for 10 min + imbibition in GA_3 (500 ppm) solution for 24 h	70.2	100.0

Similarly, in order to understand the dynamics of imbibition in seeds and, after confirming that dormancy of *C. papaya* seeds is not associated with the seed's external coverings, a curve of imbibition was carried out over time (Figure 3).

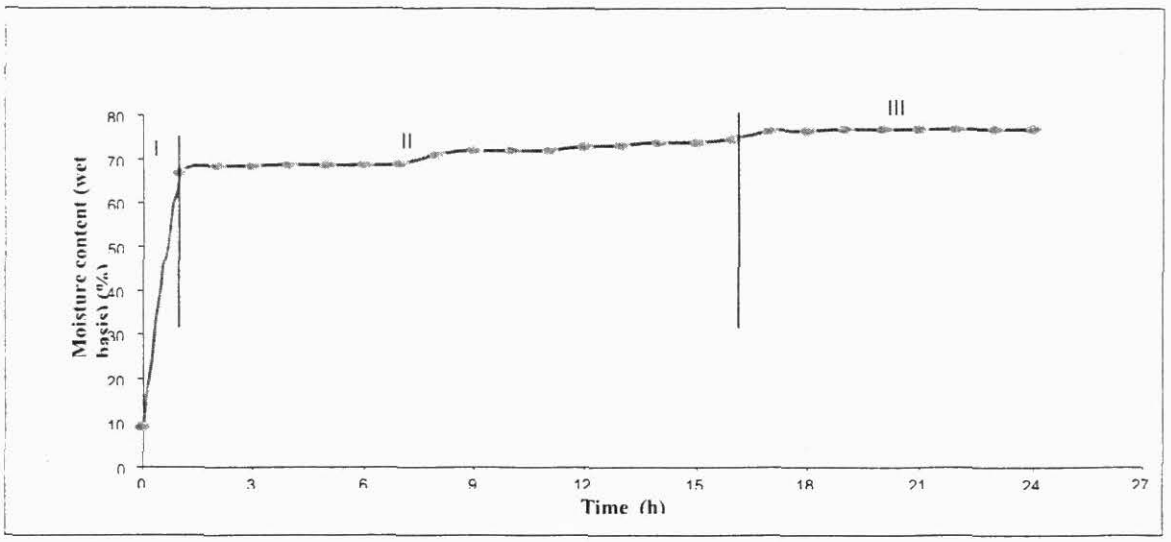

Figure 3. Imbibition curve for *Carica papaya* seeds over time. Three phases, imbibition (I), activation (II), and growth (III) are indicated for the curve imbibed.

Bradford (1995) reported that seeds with permeable external coverings usually show three phases of imbibition: (1) imbibition, (2) activation of germination, and (3) growth. Figure 3 shows that seeds absorb water very quickly during the first 24 h, reaching a moisture content of 77%, a level at which any seed without dormancy problems would germinate. This suggests that seed dormancy in *C. papaya* is associated with endogenous factors and not to external factors such as external coverings.

Contributors: H. Velásquez, J.A. Ospina (with a grant from FUNTAGRO – IPGRI Regional Project on Caricaceae)

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Activity # 1.5.4. Use of locus of the PGM isozyme to estimate the rate of allogamy in CIAT *Arachis pintoi* Krap.& Greg. *nom. nud.* germplasm collection.

Arachis pintoi includes 69 registered accessions at the moment, proceeding from Brazil and with a great potential as tropical forage species (Krapovickas & Gregory, 1994). Very little is known about its floral biology and allogamy (Myles, personal communication). Therefore we are carrying out a study, using a polymorphic genetic marker in this species, such as the phosphoglucomutase isozyme (PGM),in order to determine the rate of allogamy of *A. pintoi* in Palmira. We have determined for the phosphoglucomutase isozyme (PGM) the genetic determinism by inheritance (parentals and first descendants or hybrids) and we found a locus with 2 simple and codominant alleles and a monomeric quaternary structure. This locus is called locus PGM. With this simple and codominant genetic marker, we will be measure the rate of allogamy of *A. pintoi* in Palmira, a key trait to determine appropriate size of populations in different germplasm operations.

Contributors: C.H. Ocampo, J. Myles (Forage Breeding Program)

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Sub-Project # 2: the FAO Designate Collections and their pertinent information fully available, and safe duplicated

Output 2.1. FAO designate collections cleaned against seed borne diseases

Activity # 2.1.1. Indexing and cleaning the cassava collection

Achievement: 40% of cassava designate collection indexed and cleaned against viruses of quarantine importance

Indexing activities have been continued on the clones of the world collection of cassava maintained under *in vitro* conditions. The final objective is to cleaning the whole collection for the four viruses of quarantine importance, following the FAO/IPGRI recommendations for the safe movement of clones at national and international level. The total of accessions available at this moment (tested against viruses CCMV, CsXV, and FSD) and ready for distribution are 2,285 clones (40% of FAO Designate Collection). According to the plans, the cleaning and indexing of the entire cassava *in vitro* collection should be completed by May 2001. The indexing is carried out once thermoterapy has been completed. Two diagnosis techniques are used: ELISA for CCMV and CsXV, and grafting with a hypersensitive clone for the causal agent of the Frog Skin Disease (FSDA).

Indexing for CCMV

The number of clones evaluated during this period is shown in the Table 14.

Source	Indexed clones	Negative clones
Argentina	31	18
Brasil	100	85
Colombia	130	90
Costa Rica	1	1
Cuba	1	1
Ecuador	1	1
Fiji	1	1
Guatemala	1	1
México	11	10

Panamá	1	1
Paraguay	6	3
Perú	18	14
Puerto Rico	1	0
Salvador	1	1
Venezuela	18	17
CG	1	1
CM	5	4
SG	1	1
SM	1	1
KM	1	1
TOTAL CLONES	**331**	**252**

The above mentioned indicates that 76,1 % of clones presented negative results for this virus and 23,86 % positive results.

Indexing for CsXV

The number of clones evaluated during this period is shown in the Table 15.

Source	Indexed clones	Negative clones
Argentina	11	10
Brasil	69	68
Colombia	104	96
Costa Rica	16	13
Ecuador	1	1
Guatemala	9	9
México	7	7
Panamá	5	5
Paraguay	2	2
Perú	20	20
Puerto Rico	2	2
Salvador	1	1
Venezuela	16	16
CG	2	0
CM	7	5
SG	1	1
SM	2	1
TOTAL CLONES	**275**	**257**

The results indicate that 93,4 % of the evaluated clones presented negative results for this virus and 6,54 % presented positive results. According to the previous data we see that the presence of CCMV has stronger incidence in the collection as compared to CsXV.

Indexing for FSDA

The number of clones evaluated during this period is shown in the Table 16.

Source	Indexed clones	Negative clones
Argentina	13	11
Bolivia	1	1
Brasil	339	323
Colombia	492	467
Costa Rica	6	6
Cuba	2	1
Ecuador	29	27
Guatemala	11	11
India	1	1

Malasia	8	8
México	28	26
Nigeria	2	2
Panamá	8	7
Paraguay	17	17
Perú	81	79
Puerto Rico	4	4
Salvador	3	3
Tailandia	4	3
Venezuela	63	62
CM	5	5
SG	1	1
SM	4	4
TOTAL CLONES	**1,122**	**1,069**

As it is observed in Table 16, 95,2 % of materials presented negative results in the indexing for this virus, being presented in a very low percentage of positive clones.

In summary, the current state of the cassava collection according to the number of negative clones for each virus and to the number of clones available at the moment (negatives for the three virus) for distribution is presented in the following table.

Table 17. Indexing status of the cassava germplasm collection in GRU by September 2000.

Source	**In vitro clones**	**INDEXED CLONES**			**Available for distribution to 2000**
		CCMV	**CSXV**	**FSDA**	
Argentina	101	39	36	19	17
Bolivia	7	6	7	3	3
Brasil	1341	1095	1031	576	559
China	2	2	2	2	2
Colombia	2011	1591	1412	866	778
Costa Rica	148	138	51	111	39
Cuba	77	77	76	73	74
Estados Unidos	9	9	9	4	4
Ecuador	116	97	91	77	71
Fiji	6	3	2	2	2
Guatemala	91	84	55	27	27
India	51	50	50	12	13
Malasia	67	67	64	24	27
México	102	88	85	56	54
Nigeria	19	18	18	5	5
Panamá	43	35	28	18	19
Paraguay	209	192	188	69	69
Perú	406	358	320	179	168
Philipinas	6	4	4	3	3
Puerto Rico	15	12	13	9	7
R.Dominicana	5	4	5	3	4
Salvador	8	3	2	3	3
Tailandia	31	27	27	7	8
Venezuela	244	203	191	126	127
Híbridos					
CG	116	25	22	19	32
CM	433	89	86	81	174
HMC	4	1	1	1	1
SG	46	13	10	9	20

SM	51	45	39	39	35
KM	81	1	1	0	1
SUBTOTAL	**5.846**				
Crossing for G.Mapping	144	142	140	132	130
WILD SPECIES					
30 spp in vitro	330				
3 Undefined spp	3				
TOTAL	**6.323**	**4.518**	**4.066**	**2.555**	**2.476**

Contributors: N.C. Flor, G. Mafla, J.C. Roa, J.L. Ramírez.

Activity 2.1.2. Cleaning the cassava field genebank

The elimination of FSD on CIAT campus was another objective in 2000. The field gene bank is being replaced with indexed plants from the *in vitro* collection. To date, a total of 5,227 accessions have been duplicated in the field and/or greenhouse (91%). Each cassava clone with negative results for the three virus of importance quarantine was given to the field genebank established in the experimental center of the Universidad Nacional – headquarters Palmira (CEUNP), to maintain a copy of the *in vitro* collection, and for using them in different assays and experimental evaluations.

Table 18 shows the accessions established the period 1999-2000.

Source	**Accessions installed into the field genebank**
Argentina	9
Bolivia	1
Brasil	446
China	0
Colombia	505
Costa Rica	9
Cuba	8
Estados Unidos	1
Ecuador	47
Fiji	1
Guatemala	15
India	3
Malasia	11
México	35
Nigeria	1
Panamá	9
Paraguay	19
Perú	111
Philipinas	0
Puerto Rico	1
Rep.Dominicana	4
Salvador	2
Tailandia	5
Venezuela	88
Híbridos	
CG	6
CM	27

HMC	0
SG	4
SM	9
KM	1
TOTAL	**1,378**

Contributors: N.C. Flor, G. Mafla, J.C. Roa, J.L. Ramírez.

Activity 2.1.3. Updating the Cassava ORACLE database

During the period 1999-2000, 7,232 records have been introduced into the Cassava ORACLE database, upgrading it into its new format. These data correspond to the results of indexing of cassava clones for the three virus of quarantine importance.

Table 19 indicates the data introduced according to the period in wich the results were obtained.

VIRUS	PERIOD 1999-2000	PERIOD 1996-1998	TOTAL
CCMV – CSXV	703	4,645	5,348
FSDA	1,397	487	1,884
TOTAL	2,100	5,132	**7,232**

Contributor: N.C. Flor

Activity # 2.1.4. Indexing materials of the K family for the Cassava Genetics Project

We continue with the indexing of materials belonging to the family K of the Genetic Cassava Project. Table 20 shows the number of material indexed for the virus CCMV, CsXV and FSDA during the period 1999-2000.

Source	CCMV	CSXV	FSDA	Available for Distribution in 2000
147 K	142	140	132	117

Contributor: N.C. Flor

Activity 2.1.5. Indexing cassava clones for the Breeding Cassava Project

The indexing of eight cassava clones coming from field plants for a later quick multiplication was carried out for the Cassava Improvement Project. The evaluated clones were: BRA 383, PER 183, SM 1460-1, SM 1219-9, SM 909-25, SM 1741-1, CM 7514-7, and CM 7951-5. For each clone approximately ten plants were indexed, and for each plant two or three cuttings, resulting in a total of 175 graftings. The cuttings of the indicative clone for this evaluation were obtained from an experimental plot of Cenicaña.

Contributor: N.C. Flor

Activity # 2.1.6. Germplasm health control in seed germplasm

The safe movement of germplasm requires checking health status of propagules since they are usually pathogen carriers. With the purpose of reducing the risk of accidental introduction of plant pathogens along with seeds or vegetative plant parts, the seed health assessment conducted its activities. In order to manage these risks 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). The procedures utilized in the Germplasm Health Laboratory have been described in Annual Report 1999.

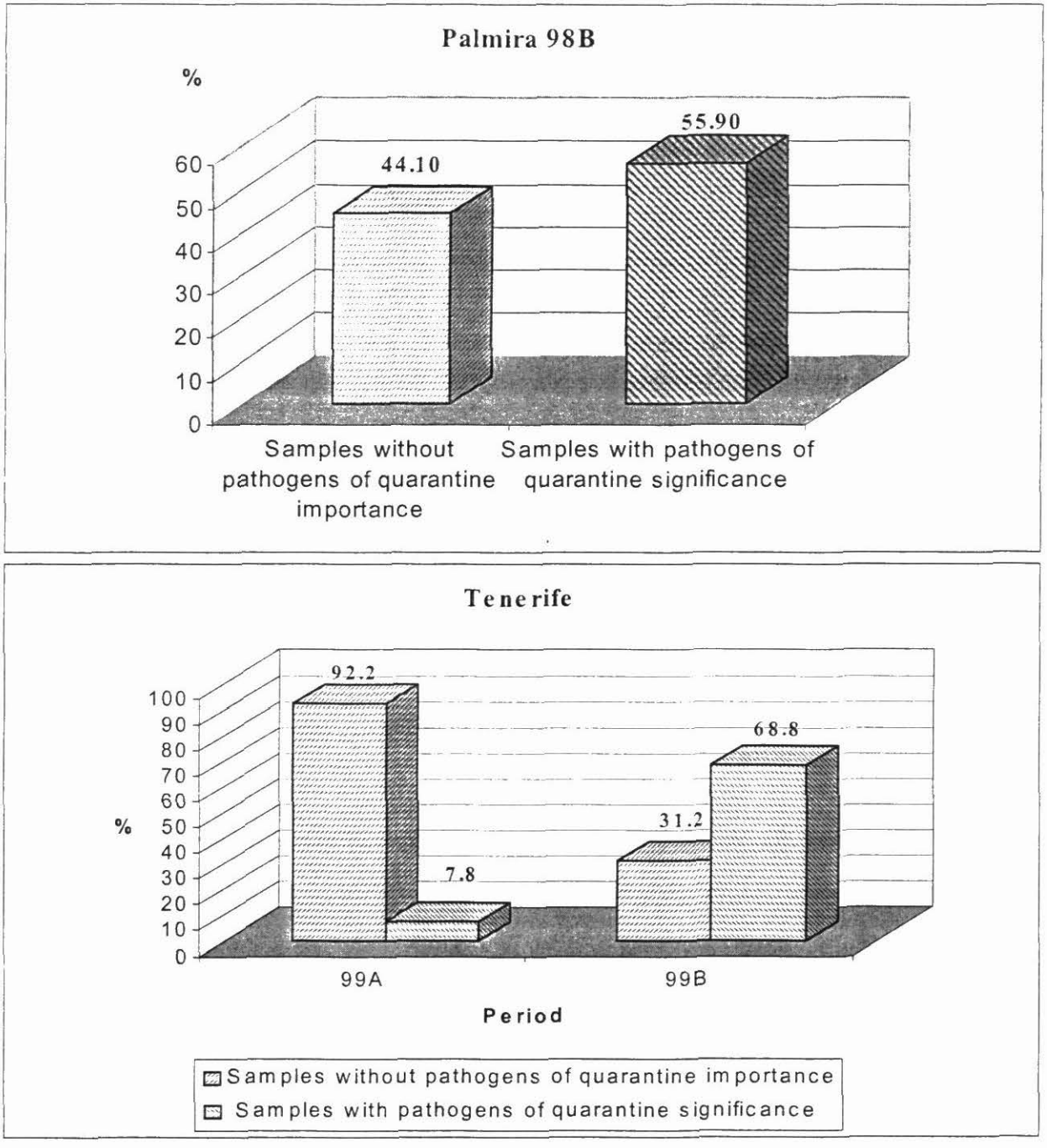
Testing for fungi includes blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar. Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp., in tropical pastures) are tested. The SHL uses dilution and plating on semiselective culture media such as MXP or King B, in addition to immunoprecipitine test with specific antisera or pathogenicity tests. The lab also uses immunofluorescent techniques. Testing *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* 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 98 B, 99A and 99B semesters, once approved for optimum seed viability, were obtained from Palmira and Tenerife multiplication plots for analysis. Their health status, analyzing each place and period, showed 44.1% (Pamira 98B), 92.2% (Tenerife 99A) and 31.2% (Tenerife 99B) samples without pathogens of quarantine importance, respectively (Figure 4).

In general the fungal infections were low although the samples from Palmira showed the presence of *Macrophomina phaseoli* and *Rhizoctonia solani* in percentages of 21.2 and 16.9, respectively. It was possible detect in some samples the presence of *Colletotrichum lindemuthianum, C. truncatum*, and *Phoma sp.* Seed borne infection by *Xanthomonas campestris* pv *phaseoli* was detected in 5.6 % of the samples, *Pseudomonas spp* was detected in 1.5% of samples. Additional tests are in progress to confirm if that pathogen is *Pseudomonas syringae* pv *phaseolicola*. Viral infections by Southern mosaic virus (SBMV) and Poty virus (BCMV) were present in middle percentages (Figure 5).

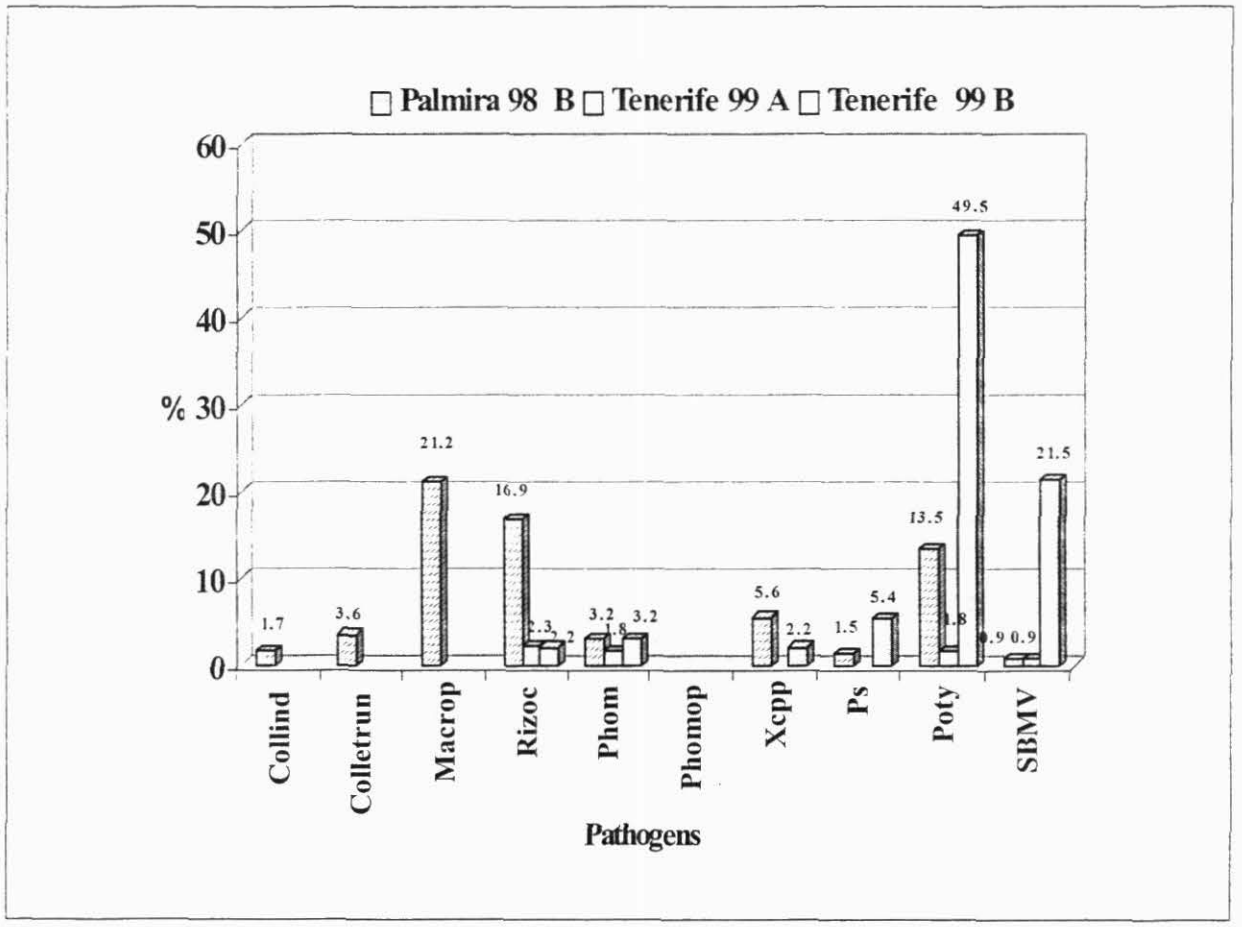
Seed samples of *Phaseolus vulgaris* from Tenerife 99A showed low percentages of diseases (Figure 4), but samples



from 99B showed high percentages of virus; presumably the seed source contains originally high percentages of these pathogens. It is known that BCMV, a potyvirus, is a virus transmitted by seed in percentages above 30 %. To obtain seeds without virus it is necessary to establish a special program under greenhouse conditions.

Other analysis carried out on 138 samples of *Phaseolus acutifolius* and 16 of *P. lunatus* from stocks prepared for international exchange, showed that 39.1 % of *P. acutifolius* and 93.8% of *P. lunatus* samples did not have pathogens of quarantine importance. Pathogens as Potyvirus, SBMV, *X. campestris* pv *phaseoli, Rhizoctonia* spp. *Macrophomina phaseoli* and *Phoma* spp were the pathogens registered at low percentages in non admitted samples.

A total of 172 bean seed samples from the Germplasm Characterization Project were tested during 1999. A total of



83.7% of those samples did not have any pathogen of quarantine significance. In infected materials *Macrophomina phaseoli* was the most frequent fungus. Virus such as BCMV and SBMV were detected.

The phytosanitary status of 143 samples of 18 genera of tropical legumes pastures, analyzed in the GHL, are shown in Table 21 and Table 22. One can see that virus and fungi are the most frequent pathogens present in legumes, while the fungi was the only group of pathogens detected on tropical grasses. Bacteria were detected only in legumes with very low frequency. In general 52.4 % of the legumes and 40.2% samples of grasses did not have pathogens of quarantine importance (Figure 6).

Table 21. Phytosanitary status of tropical legumes seed samples analyzed during 2000 in the GHL

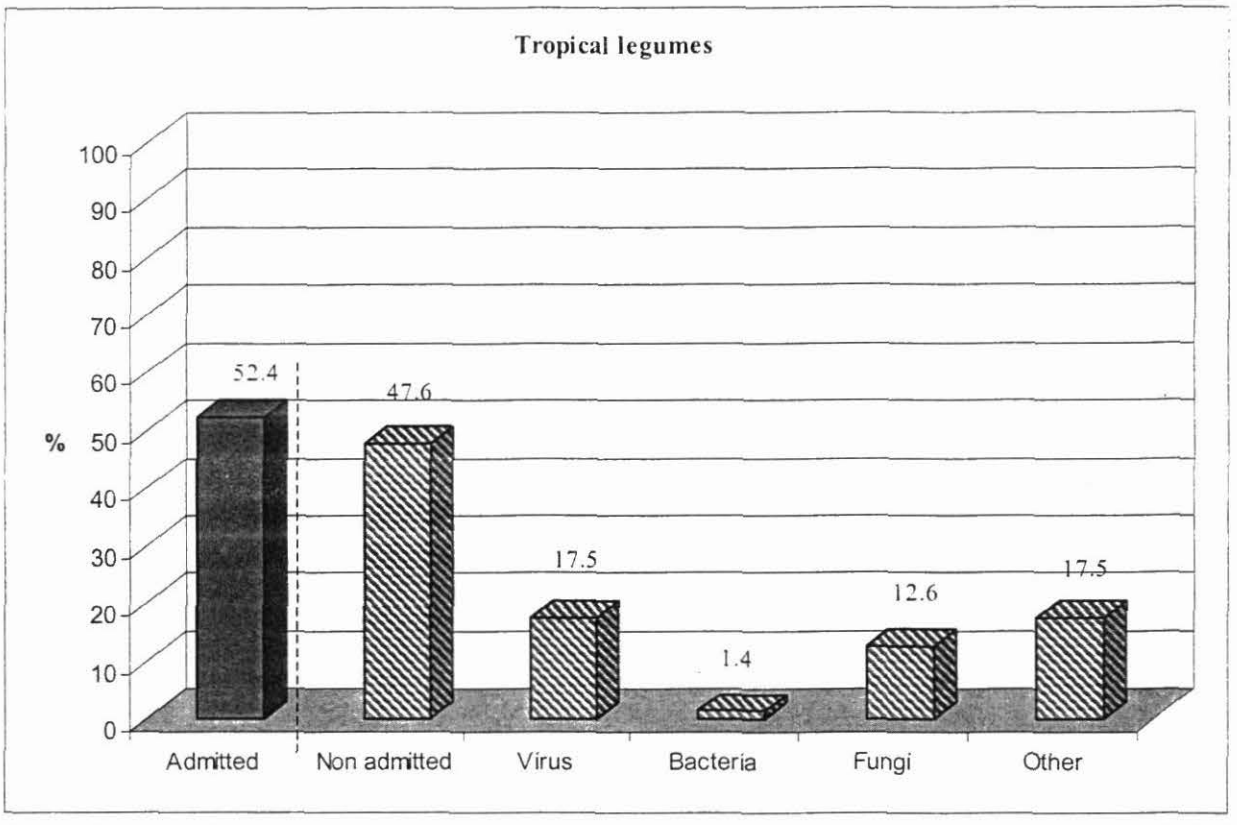
Genera	Samples number	Admitted	Non admitted	Virus	Bacteria	Fungi	Other
Aeschinoneme spp	6	5	1	0	0		1
Cajanus spp	1	0	1	1 (SBMV)			
Calopogonium spp	7	5	2	1 (Poty)	1(Xanth)		
Centrosema spp	25	17	8	3(Poty), 2(SBMV)		2(Phoma), (Phomopsis, 1(Rhizoc),	
Clitoria spp	5	3	2	2(SBMV)			
Cratilia spp	8	1	7	1(SBMV)		3(Collect), 4(Phomop), 1(Macroph)	
Chamaecrista spp	4	0	4			1(Macroph)	3
Desmodium spp	1	1					

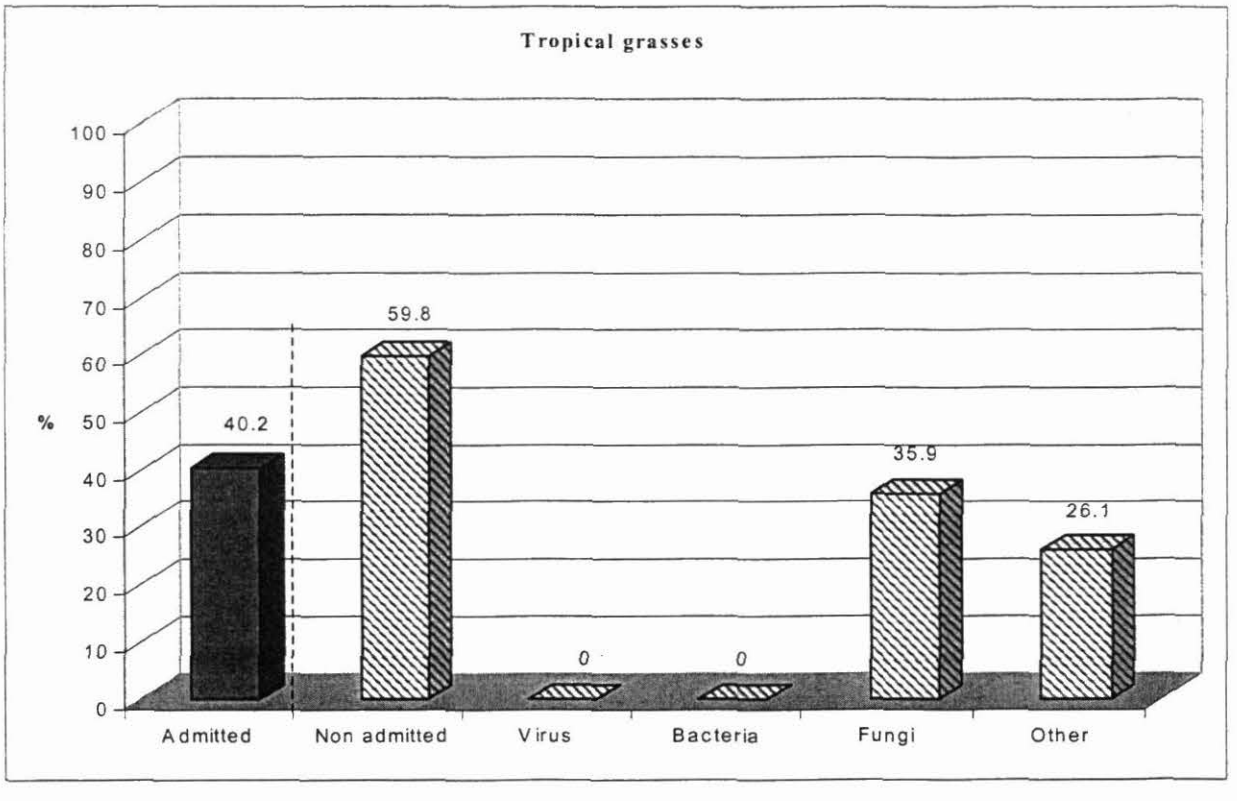
Flemingia spp	1		1	1(Poty, SBMV)		1 (Phomop)	
Galactia spp.	8	7	1	1 (Poty),			
Gliricidia spp	1	1					
Lablab spp	9	8	1	1 BSMV			
Leucaena spp	2	2					
Macroptilium spp	16	6	10	3 (Poty), 4(SBMV)			
Neonotonia spp	8	3	5	2(SBMV, Poty), 1 (PsfII) 1(SBMV)		1 (Phoma)	
Pueraria ssp	6	5	1			1 (Collet)	
Stylosanthes spp	34	10	24	1(Poty) 1(SBMV)		2(Phoma),	21
Tephrosia spp	1	1					
Total	**143**	**75**	**68**	**25**	**2**	**18**	**25**

Table 22. Phytosanitary status of tropical pastures (Poaceae) seed samples analyzed during 2000 in the GHL

Genera	Samples number	Admitted	Non admitted	Virus	Bacteria	Fungi	Other
Andropogon spp	11	2	9	0	0	5(Dresch), 3 (Phoma)	
Brachiaria spp	53	33	20	0	0	4 (Phoma), 18 (Dresch)	
Chloris spp	3	1	2	0	0	1(Drech)	1
Panicum spp	20	1	19	0	0	1(Dresch), 1(Phoma	18
Paspalum spp	5	0	5	0	0		5
Total	**92**	**37**	**55**	**0**	**0**	**33**	**24**

Contributors: B. Pineda, M.S. Balcazar, J.L. Ramírez





Activity # 2.1.7. Survey of seed fungal diseases in refreshing *Brachiaria* germplasm under CIAT Santa Rosa station (Popayan) conditions.

Achievement: *Drechslera* and *Phoma* detected as important fungi on covered seeds of *Brachiaria*

Introduction

The *Brachiaria* germplasm collection maintained in CIAT contains more than 700 accessions from 27 identified species. The process of maintaining, cultivating and rejuvenating the germplasm is carried out under CIAT Santa Rosa Station located at 2° 25'N and 76° 40'W. Under field conditions seed-borne fungi could affect production of seed. These pathogens affect yield and quality of seeds, could produce deterioration during storage, reduce germination, and prevent safe movement of germplasm. The available information about *Brachiaria* seed borne diseases is very limited for Colombian conditions, and in order to increase seed productivity of the germplasm of *Brachiaria* spp. it is necessary to go ahead with pathological research work. The objective of this study was to determine the seed-borne fungi that were affecting the seed production of the species *B. brizantha,* (18 accessions), *B. decumbens* (7 acc.) y *B. jubata* (5 acc.).

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. The laboratory studies were done in the Germplasm Health Laboratory (GHL). To detect seed-borne fungal diseases, seed samples were planted in Petri dishes using blotter test and PDA methods. After planting the dishes were placed in a growth chamber with 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 under 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. The results obtained were rechecked in the GHL with seed samples harvested during 98-99 period.

Results

To date 12,000 seeds of the *Brachiaria* species have been evaluated. The fungi were identified on the basis of conidial characteristics through microscope observation. The results show that the seedborne fungi with highest incidence were *Drechslera* spp. and *Phoma* spp. (Table 23 and Table 24). Pathogenicity test indicates that *Drechslera* spp. and *Phoma* sp. cause severe symptoms in seedlings. Additionally, *Fusarium* spp., *Epicoccum* spp., *Curvularia* spp., *Alternaria* spp., *Cladosporium* spp., *Nigrospora* spp. and 10 unidentified species were found. The results of this study should be important for determining the risk these fungi pose to the safe movement of *Brachiaria* germplasm.

Table 23. Incidence of fungi affecting seeds of 30 B*rachiaria* spp. accessions determined using the Blotter Test

Especie	No CIAT	*Alternaria* sp.	*Aspergillus* sp.	*Cladosporium* sp.	*Curvularia* sp.	*Chaetomium* sp.	***Drechslera* sp.**	*Epicoccum* sp.	*Fusarium* sp.	*Penicillium* sp.	***Phoma* sp.**	*Nigrospora* sp.
B. brizantha	16154	0*	0	1.5	1.5	0	8	1	0.5	14	7.5	0
B. brizantha	16290	0.5	0	0	0.5	0.5	14	1.5	0.5	5.5	22	0
B. brizantha	16348	0.5	0	1	2.5	0	18	1	0.5	1.5	5	0.5
B. brizantha	6682	0	0	0	0.5	0	11	1	1	0	12	2
B. brizantha	16125	0	0	0	2	0	16	0	4	22	0	0
B. brizantha	16470	0	0	0	1	0	8	0	1	1.5	10	0.5
B. brizantha	16288	0.5	0	0	1	0	5	1	1	0.5	12	0.5
B. brizantha	16828	1.5	0.5	0	1.5	0	4.5	0	6.5	0.5	8	0
B. brizantha	16335	0.5	0	0	0.5	0	0.5	0.5	2	0.5	2	0
B. brizantha	16331	1	0	0.5	2.5	0	24	0	7	0	3	0
B. brizantha	16156	1	0	1.5	1	0	14	0	4	0	8.5	0.5

B. brizantha	26131	0.5	0	12	1.5	0	31	3	4	2	1	0.5
B. brizantha	26124	2	0	18	1.5	0	9	3	6.5	2	3.5	1.5
B. brizantha	16830	1.5	0.5	5	5	0.5	5.5	2	2	2	14	2
B. brizantha	26991	0	0	0.5	0.5	0	4	3.5	0	0.5	2.5	0
B. brizantha	26745	0	0	0	0.5	0	6.5	0	0	0	7.5	0
B. brizantha	26641	0	0	0	1	0	20	0.5	2	0	16.5	0
B. brizantha	16462	1	0	3.5	9	0	15	0	1	0	3	0.5
B. decumbens	16500	0	0	3	2.5	0	11	0.5	1	0	6	0
B. decumbens	16502	2.5	0	7	3	0	9	0	0	0	12	0
B. decumbens	16504	1.5	0	9.5	0	0	7.5	5	2	1.5	2	2.5
B. decumbens	664	0.5	0	3	0.5	0	3.5	1.5	1	0	6.5	0
B. decumbens	16133	0	0	0.5	0	0	11	0.5	0	0	3	0
B. decumbens	16177	3.5	0	1	0.5	0	2.5	1.5	0	0	2	0
B. decumbens	26187	0	0	1	0	0	3.5	2	0	3	4.5	0
B. jubata	16468	0	0	1.5	0	0	1	0	0	0.5	4.5	0
B. jubata	16176	0	0	2	3.5	0	7	0	0	0	6.5	0
B. jubata	16532	0	0	0.5	0	0	0	0	0.5	0.5	6	0
B. jubata	26891	1	0	4	0	1	3	0	0	0	24	0.5
B. jubata	16459	0	0	0	0	0	0	0	0	0	4	0

* % of affecting seeds using samples of 200seeds

Table 24. Incidence of fungi affecting seeds of 30 B*rachiaria* spp. accesions using PDA-LA incubation test.

Specie	No CIAT	*Alternaria* sp.	*Aspergillus* sp.	*Chaetomium* sp.	*Cladosporium* sp.	*Curvularia* sp.	*Drechslera* sp.	*Epicoccum* sp.	*Fusarium* sp.	*Nigrospora* sp.	*Phoma* sp.	*Penicillium* sp.
B. brizantha	16154	0*	0	0	1	0	4	0.5	0.5	0	7.5	0
B. brizantha	16290	0	0	0	1	0.5	6.5	0.5	3	0	25	0
B. brizantha	16348	1	0	0	0	0	5	1	7.5	0	9	0
B. brizantha	6682	0.5	0	0	0	0	9.5	0	3.5	0	6	0
B. brizantha	16125	0.5	0	0	0.5	0	9	0.5	6.5	0	8	0
B. brizantha	16470	0	0	0	0.5	0.5	4	0	0	0	7	0
B. brizantha	16288	0	0	0	0	0.5	2.5	0.5	2.5	0	22	0
B. brizantha	16828	0	0	0	0	0	1.5	1	5	0	15	0
B. brizantha	16335	0	0	0	0	0.5	0.5	0	0	0	6.5	0
B. brizantha	16331	1.5	0	0	1.5	0.5	8.5	0	7.5	0	6	0
B. brizantha	16156	0	0	0	1	0	15	1	1	0	11.5	0
B. brizantha	26131	0	0	0	0.5	0	12.5	0	2.5	0	3	0
B. brizantha	26124	0	0	0	2	0.5	5	3	6	0.5	10	0
B. brizantha	16830	0	0	0	0	0.5	2.5	5.5	2.5	0.5	22	0
B. brizantha	26991	0	0	0	0	0	3	1.5	1	0	7	0
B. brizantha	26745	0	0	0	0.5	0	3.5	0.5	2.5	0	11.5	0.5
B. brizantha	26641	0.5	0	0	0	0	11	1	2.5	0	41.5	0
B. brizantha	16462	0	0	0	0	0	2.5	4.5	1	0	3.5	1.5
B. decumbens	16500	0	0	0	0	0	9.5	2	0.5	0	10	0
B. decumbens	16502	0	0.5	0.5	0.5	0	9	3	2	0	12	0
B. decumbens	16504	1	0	0	0	0.5	7.5	1	3	0	16.5	0
B. decumbens	664	0.5	0	0	0	0	6.5	1.5	1	0	5.5	0
B. decumbens	16133	0	0	0	0	1	5	0	10	0	12.5	0.5
B. decumbens	16177	0	0	0	0	0	6	1	0.5	0	3.5	0.5
B. decumbens	26187	1	0	0	0	0.5	4.5	2	0.5	0	3.5	2
B. jubata	16468	1.5	0	0	0	0	0.5	0	0	0	4	0
B. jubata	16176	0.5	0	0	0	0	0.5	1.5	1	0	5.5	0
B. jubata	16532	0	0	0	0	0	0.5	0	0	0	11.5	0.5

B. jubata	26891	0	0	0	0	0	0	0.5	0	0	3	0
B. jubata	16459	0	0	0	0.5	0	0	0	0	0	4	0

* % of affecting seeds using samples of 200 seeds

Contributors: Sandra Ximena Garcia (Pontificia Universidad Javeriana), Benjamín Pineda L

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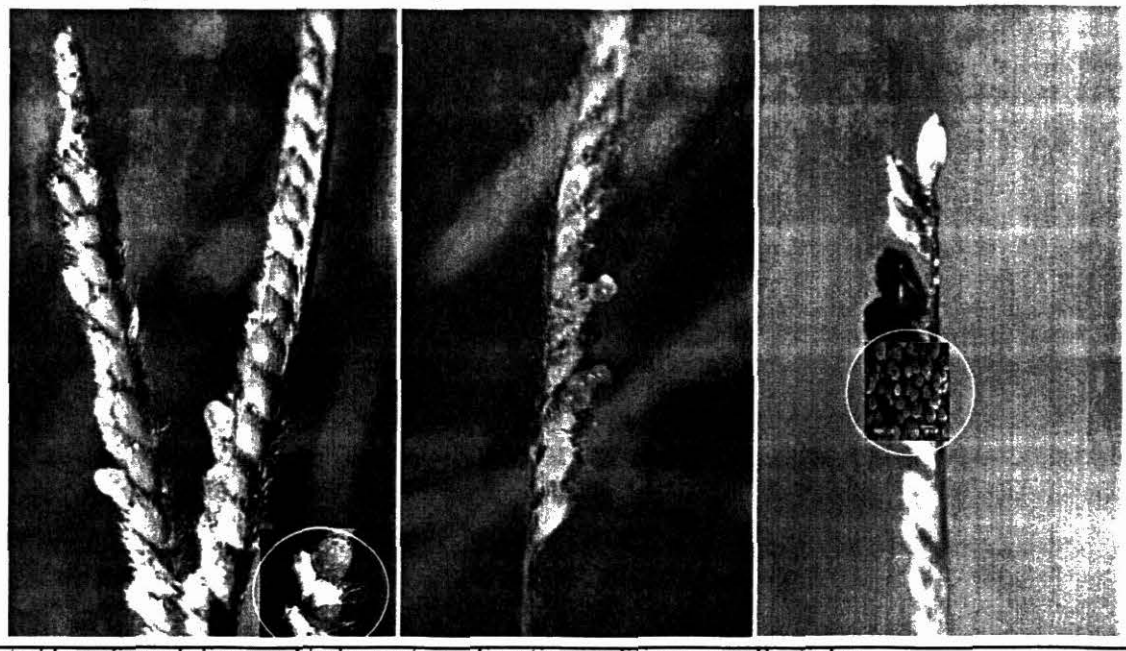
Activity # 2.1.8. Etiology studies about the honey dew disease affecting germplasm of *Brachiaria* spp.

Introduction

The increasing and refreshing of the germplasm of *Brachiaria* is carried out, under conditions of field, in Popayán (2o 25'N; 76o 40'W) where the production of seed for 700 accesions of 27 species is relatively good. Nevertheless under the conditions of precipitation (more than 2,000mm) and temperature (16-24^0 C) of this location, the production of seeds seems to be affected by a fungal disease. Under certain climatic conditions, small, sticky,

Figure 7. Exudation of sticky, colorless honey dew droplets , white mycelium and the black fungi *Epicoccum* sp. (*Cerebella* sp) on infected *Brachiaria* spikelets.



Materials and Methods

During the second semester of 1999 and the first of the 2000 a disease survey was conducted. Observations in the field, on the affected *Brachiaria* accessions, when the flowering state was started, allow to see an exudation of sticky, colorless honey dew droplets sweat of viscous consistency and sweet flavor on infected inflorescences. Small drops of clear color, almost transparent, appeared in early hours of the morning, and became progressively opaque during the day. Subsequently, on the surface of the spikelets a white mycelium was observed. When the infection was severe, the affected inflorescences could be recognized at naked eye particularly on the plants of the plot borders. Once the white growth invaded the inflorescence, the apparition of fungal growths of black color, green or pink in individual or combined form was observed (Figure 7).

Seed samples of 17 accesions of *B. brizantha*, 5 of *B. decumbens* and 3 of *B. jubata* were gathered in order to verify the association of seed and fungi. The seeds were conditioned according to the procedures established in the URG for the gramineae germplasm. The analysis of seed health was carried out in the laboratory utilizing microscopic analysis of the ooze, isolation starting from ovary of affected flowers and incubation methods of seeds on PDA and Blotter test or analysis using "washing test" and microscopic observations after incubation.

Results

The studies carried out in the field and in the Germplasm Health Laboratory (GHL), allowed to determine that the diseases was the "Honey dew" caused by a species of *Sphacelia*. This fungus produces several types of hyaline spores: oblong to oval macroconidia (13.8x5.2 microns), spherical microconidia (4.6 microns), and pear- shape secondary conidia (14x5.5microns). The analysis yields positive outputs for all studied accesions (Table 25). Studies are carried out in order to determine if *Sphacelia* attacks the covers of the seed (lemma and palea), the cariopside or the embryo.

The field observations of the plots of multiplication of the *Brachiaria brizantha*, *Brachiaria decumbens* and *Brachiaria jubata* allowed to determine that the disease affected the inflorescence with several grades of severity. The color fungal growths, according to the analysis of laboratory, were fructifications of *Cerebella* sp (*Epicoccum* sp), *Cladosporium* sp, and *Fusarium* spp.

Contributors: Sandra Ximena Garcia (Pontificia Universidad Javeriana), Benjamin Pineda L.

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Table 25. Number of spores of *Sphacelia* sp. Observed in spore suspensions after observations using seed-washing test

Species	CIAT Number	Spores per microscopic field*
B. brizantha	16154	87
B. brizantha	16290	22
B. brizantha	16348	27

B. brizantha	6682	7
B. brizantha	16125	37
B. brizantha	16470	389
B. brizantha	16288	4
B. brizantha	16828	55
B. brizantha	16331	93
B. brizantha	16156	52
B. brizantha	26131	368
B. brizantha	26124	120
B. brizantha	16830	206
B. brizantha	26991	80
B. brizantha	26745	44
B. brizantha	26641	598
B. decumbens	16500	23
B. decumbens	16504	0
B. decumbens	664	78
B. decumbens	16133	22
B. decumbens	16177	20
B. brizantha	16462	236
B. jubata	16176	96
B.jubata	16468	91
B.jubata	26891	122

* average per 10 microscopic fields

Output 2.2 Germplasm, passport and characterization data available to users

Activity # 2.2.1. Distribution of germplasm from designate collections to end users

Achievement: 6,949 accessions of the three commodity FAO designate collections distributed to end users

As it can be seen in the Table 26 and Table 27, distribution of germplasm out of the designate collections continues at a relatively high rate as compared to the multiplication rates of GRU (namely for bean germplasm). One should mention that distribution is quite unpredictable in relation to project planning. It is also unpredictable in terms of kinds of end users (Figures 8-10); although many breeding activities have been reduced over the past few years, CGIAR and CIAT projects continue to be very important end users of germplasm from CIAT GRU.

Table 26. Distribution of germplasm during 2000 by purpose

	Beans		Forage		Cassava	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	6	307	---	---	13	1,581
Agronomy	6	181	14	257	14	109
Applied research	16	2,549	1	5	14	182
Basic research	23	1,219	8	255	13	304
Training	---	---	---	---	---	---
Total	51	4,256	23	517	54	2,176

A total of 2,176 cassava accessions was distributed, through 54 requests (summing to 4,531 samples). The main recipient were CGIAR Centres (mainly CIAT Projects), who received over 89% of total of samples. Special requests were sent to CORPOICA (Tibaitatá-Cundinamarca, Turipaná-Costa Norte, and Tulenapa-Antioquia) for multiplication and subsequent distribution to farmers.

Table 27. Distribution of germplasm during 2000 by kind of institution

	Beans		Forages		Cassava	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	26	1,468	6	136	34	1,936
Commercial companies	2	11	---	---	3	10
Farmers	---	---	---	---	---	---
Gene banks	---	---	---	---	---	---
NARS	8	2,275	6	73	10	201
NGOs	---	---	---	---	2	3
Regional organizations	2	15	1	3	1	2
Universities	13	487	10	305	4	24
Germplasm networks	---	---	---	---	---	---
Others	---	---	---	---	---	---
Total	51	4,256	23	517	54	2,176

The main purposes of distribution in cassava were breeding with 73% of the total, while the basic research (cryopreservation, embryogenesis and classic biochemistry) with a 14%, apply research (pathology and entomology) with 8% and finally agronomy with 5% (Figure 8).

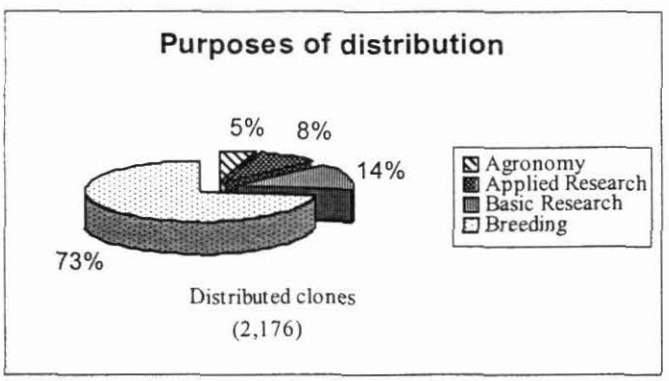

Figure 8. Purposes of distribution of in vitro cassava germplasm.

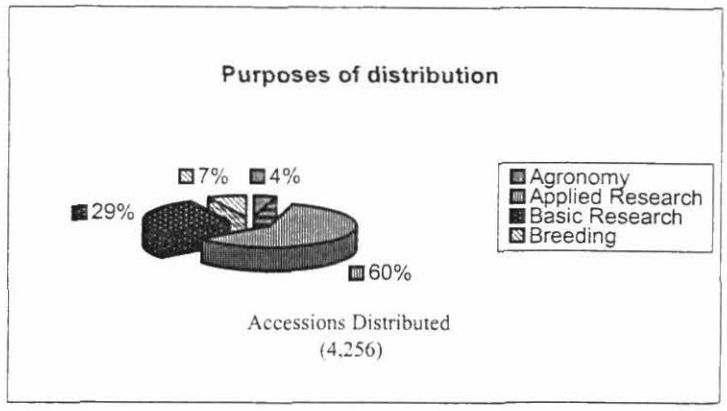

Figure 9. Purposes of distribution of seed of bean germplasm

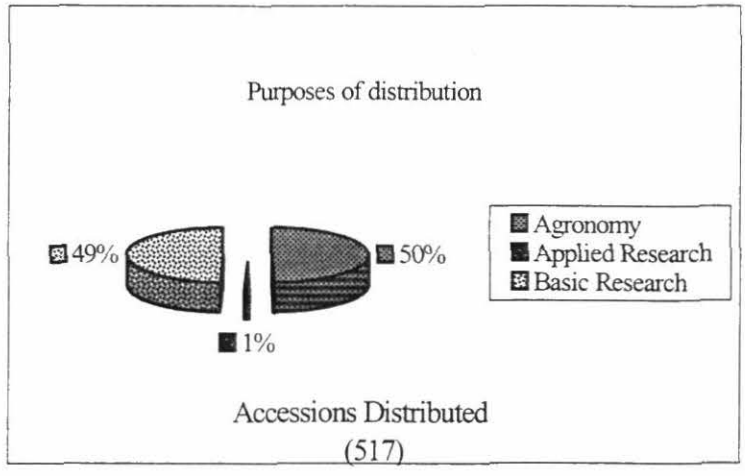

Figure 10. Purposes of distribution of seed of forages germplasm

Contributors: A.M. Torres, G. Mafla, O. Toro, A. Ciprian, D.G. Debouck

Activity # 2.2.2. Making distribution data available on the Internet (SINGER Project, phase 2)

Achievement: data about distribution by GRU computed over 1973-2000 for CGIAR SINGER 2 Project

As part of a systemwide initiative (the SINGER Project, phase 2), GRU with the participation of the Information Support Unit of CIAT has revised all distribution data since the beginning of its activities. The shipment of bean, cassava, and forage germplasm covers the period 2000 backwards to 1973 (Table 28), where germplasm distribution activities started to be formally recorded. We are still computing forage data for distribution of designate accessions from CIAT programme/ projects different from GRU. The least one can say is that these collections have been used, perhaps too much as compared to GRU capacity to multiply and conserve the designate

collections (its prime responsibilities towards FAO and the countries). For each shipment, the following seven data were recorded: names of recipient and recipient institution, type of recipient, date of shipment, accessions and numbers of propagulas shipped, and request purpose.

Table 28. Numbers of accessions (number of requests) distributed by GRU in the period 1973-2000.

End users	Beans	Cassava	Forages	Total
CIAT projects	219,812 (1,994)	6,110 (141)	25,296 (798)	251,218 (2,933)
External institutions	106,527 (1,300)	5,394 (389)	21,065 (876)	132,986 (2,565)
Total	326,339 (3,294)	11,504 (530)	46,361 (1,674)	384,204 (5,498)

Contributors: A.M. Torres, O. Toro, G. Mafla, A. Ciprian, A. Franco, F. Rojas, S. Ramirez, and D.G. Debouck

Activity # 2.2.3. Checking validity of forage and other wild species names through a reference herbarium

In line with having the germplasm well characterized for users, it is an important requisite to have the germplasm botanically well identified. The forage collections and other wild species collections are supported by a reference working herbarium containing 13,861 specimens. The herbarium follows international standars for collection and organisation. This year, 419 herbarium specimens of 96 species belonging to the legume family were added to the herbarium (Table 29).

Table 29. Specimens of tropical forages and other wild species added to CIAT Herbarium in 2000

	Number of species	Number of accessions
Legumes	96	419
Grasses	0	0
Total	96	419

Contributors: A.M. Torres, A. Ciprian, O. Toro

Output 2.3 National collections restored to NARS

Activity # 2.3.1. Conditioning and shipment of particular collections to NARS as part of restoration

One restoration activity took place this year: a shipment of *Desmodium* germplasm and other forage legumes towards Venezuela.

Contributors: A.M. Torres, D.G. Debouck

Output 2.4 FAO designated collections safe duplicated

Activity # 2.4.1. Preparation of germplasm collections for security backups

Achievement: 2,181 bean accessions prepared for safety duplication

The Genetic Resources Unit has two on-going agreements about safe-duplicating its entire seed collections, namely with CATIE (Costa Rica) and CENARGEN (Brazil). During 2000 we have prepared 1,163 samples of beans to be shipped to Brazil and 1,018 samples of beans to be shipped to Costa Rica. During 2000, contacts have been made with the Ministry of Agriculture of Thailand to keep under *in vitro* conditions a duplicate set of the cassava core collection (630 clones).

Contributors: A.M. Torres, D.G. Debouck

Output 2.5 Refined core collections

See under activity # 3.1.5. part of the work done in order to improve representativity of the bean core collection.

Output 2.6 Improved disease indexing techniques

Activity # 2.6.1. Production of antiserum to detect the bacterium *Pseudomonas syringae* pv *phaseolicola* in seeds of *Phaseolus vulgaris* for broader applicability

Introduction

Halo blight of beans is caused by the bacterium *Pseudomonas syringae* pv *phaseolicola.* The bacterium is found in regions with moderate temperatures. The halo blight causes yield losses of 23%-43 % and seeds transmit its causal agent (Schwartz, 1989). *P. syringae* pv *phaseolicola* is considered as a pathogen of quarantine significance. In GHL the detection of the bacteria in seeds is done by using semi-selective KB culture media (Hildebrand et al, 1988), and serology. But there are other techniques (Saettler et al, 1989), as indirect immunofluorescent staining, and ELISA which, will be possible to adapt in our lab if obtain an available quantity and quality antiserum. We present here some advances in the production of antiserum to use it as a GHL reactive in routine test to identify *Pseudomonas syringae* pv *phaseolicola* in bean germplasm.

Materials and methods

Lyoplilized bacterial isolate PsP286COL to produce the antiserum was obtained from CIAT Bean Pathology Laboratory. The procedure used was the one recommended by plant pathologists of CIAT Bean Pathology project. The content of a vial was sowed in nutrient agar media, upon the previous dilution of the its content in a solution of peptone (10%) and sucrose (20%). After48 hours of incubation to 28°C the colonies were pealed to King B media Culture. Petri dishes were incubated for 24 hours to 26°C. With the bacteria obtained from the Petri dish a bacterial suspension was prepared for centrifugation at 2500 rpm during 10 minutes. After centrifugation the pellet was washed with 3 to 4ml of saline solution (NaCl 0.85%); it was resuspended and finally the bacterial suspension adjusted to a concentration of $5x10^8$. c. f. u. /ml. Cells (0.2 ml of bacterial suspension) were injected into marginal ear veins of two New Zealand rabbits 4 months old. 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 antiserum agglutination titer determined. The analyzed titles were: 1: 10, 1: 20, 1: 40, 1: 80, 1: 160, 1: 320, 1: 640, 1: 1280, and 1: 2560.

Results

At least 30 ml of specific antiserum was collected with a good titer (1:1280). The titles were positives until the dilution 1: 1280, getting the better title in 1: 640. Adaptation of the immunofluorescent staining, and ELISA test to detect *Pseudomonas syringae* pv *phaseolicola* in bean germplasm are in progress.

Contributors: M. S. Balcazar, J. L. Ramirez, B. Pineda L.

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Activity 2.6.2. Evaluation of the distribution of frog skin viral complex in cassava plants using grafting techniques.

Introduction

Fifteen clones with symptoms of frog skin disease and with three different degrees of severity (light, moderate and severe) were used in this work. Two different techniques of grafting were evaluated: bud grafting and English grafting (Roa, J.C. et al., 2000). Stakes of approximately 15 cm of length of three different parts of the mature plant: apical, middle and basal were tested for each clone and for each technique; two stakes were prepared for each section as repetitions, evaluating a total of 6 stakes for each clone.

The Secundina clone (COL 2063), highly susceptible to viruses and properly certified, was used as indicator plant. Grafts of Secundina on Secundina were used as controls. The evaluation was carried out to 8 days after grafting, and the information was registered every 8 days. The presence or absence of symptoms and deformations in the leaves of the indicator clone were analyzed.

Results and Discussion

The results showed that both techniques detected the viral complex in the three types of stakes used. They indicated that differences in the distribution of the virus in the affected plants didn't exist, and either one should be used for indexing frog skin disease. Also all the grafted parts expressed symptoms in the three levels of severity of the illness of the mother plant (Table 30). These procedures are so sensitive that can be applied with low virus concentration. About incubation of the virus the presence of symptoms in all the grafted parts usually was noted 8 to 15 days after grafting.

The results showed that viral complex can be found distributed along in the entire plant, although information exists that most viruses invade all cells of their hosts. However, at a given time of the infection cycle, their concentration is not equal in all host organs, as for most viruses higher concentration is usually found in leaves in the middle part of the plants (Salazar, 1994).

Table 30. Effect of the type the stake in the evaluation from frog skin disease.

Clone	Symptoms of frogskin in the roots	Position of the stakes in cassava plants					
		Apical		Middle		Basal	
		Buds graft	English graft	Buds graft	English graft	Buds Graft	English graft
Col 175	Light	+	N.E	N.E	+	+	+
Col 184	Light	+	N.E	+	+	+	+
Col 534 B	Ligth	+	+	+	+	+	+
Col 1177	Ligth	+	+	+	+	+	+
Per 481	Ligth	N.E	+	+	N.E	+	+
Bra 359	Moderate	+	+	N.E	+	+	+
Col 246	Moderate	+	N.E	N.E	+	+	+
Col 543 B	Moderate	+	+	+	+	+	N.E
Col 1173	Moderate	+	+	+	+	+	+
Mex 66	Moderate	+	+	+	+	+	+
CM 4777-2	Severe	+	+	+	+	+	N.E
Col 498	Severe	+	+	+	+	+	+
Col 560	Severe	+	+	+	N.E	+	+
Col 561	Severe	+	+	+	+	+	+
Col 562	Severe	N.E	+	+	+	+	+

N.E: Material not evaluated
+ : presence of symptoms

Contributors: Flor, N.C., Roa, J.C. and Mafla, G.

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

Introduction

We have continued with the tests to conclude the process of standardization of the PCR technique for the identification of the Cassava Vein Mosaic Virus (CVMV) in cassava germplasm in order to begin the evaluation of the entire collection in a routine and reliable way. Problems showed up with reproducibility of data with some of the evaluated clones; since in some tests these clones amplify and in others no. In order to discard the presence of possible contaminants in the stocks of the primer, positive samples of CVMV coming from three states of Brazil were requested (Sao Vicente, Lagoa and Sao Joao Do Piaot) from the Empresa Brasileira de Pesquisa de Mandioca y Froticultura Tropical (EMBRAPA). The three positive samples (BGM 1577, BGM 1410 and BGM 1387) were evaluated with the group of four available primers (SST, RBD, RT, and HS). For the extraction of DNA the same protocol of Gilbertson et. al (1991) was used in all previous tests. For the amplification of the materials the same conditions standardized previously were used (concentration of reagents, buffers, primers, nucleotides, enzyme and amplification profile).

Results

The sample BGM 1577 didn't amplify with none of the 4 low primers with the suitable conditions. The samples BGM 1410 and BGM 1387 amplified with all the primers presenting bands of similar molecular weight to the positive control (Plasmid CVMV 141). The clone M Col 2063 (Secundina) used as negative witness didn't amplify in none of the four tests. The clones Costa Rica 149 and Brazil 137 belonging to the collection of URG used in previous tests and known for their constant amplification presented bands of the expected size with the SST primer. When carrying out the repetition of this test, a great uniformity was observed in the obtained results. However, with the primer RBD two samples Bra 137 and C.Rica 149 amplified with bands of similar size to the plasmid CVMV 141.

From the above mentioned results one can conclude that the stocks of the currently available primers detect in a reliable way the presence of CVMV, being SST and RBD the most appropriate primers for the evaluation of the samples. With these two primers a lower number of unspecific bands was obtained.

To test further the reproducibility of results a new group of materials were evaluated including one of the positive samples of Brazil (BGM 1387) besides the plasmid CVMV 141. The samples were evaluated with the group of four primers, and so far two tests have been carried out; for each an a new extraction of DNA of the plant material has been obtained using the same protocol mentioned previously.

Table 31 shows the obtained results.

	PRIMER SST		PRIMER RBD		PRIMER HS		PRIMER RT	
CLONES	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
C.rica 149	+	+	-	+	-	-	-	-
BGM 1387	+	+	-	+	+	+	+	+
Bra 137	+	+	-	+	+	-	-	-
Bra 53	+	+	-	-	+	+	-	-
P.Rico 102	+	-	+	-	-	-	-	-
Bra 14	-	+	-	-	-	-	-	-
Bra 1270	-	+	-	+	+	+	-	-
Sec.No.1	-	+	-	+	-	-	-	-

Bra 15	+	+	-	+	-	-	-	+
Bra 24	-	+	-	-	+	-	-	-
Bra 278	-	-	-	-	+	-	-	-
Bra 395	+	+	+	-	+	-	-	-
Bra 198	-	+	+	-	-	-	-	+
Sec.No.2	-	-	+	-	-	-	-	+
Bra 66	-	-	+	-	-	-	-	-
CVMV 141	+	+	+	+	-	+	+	+

A high inconsistency in the results can be seen in Table 31: when the same group of samples is evaluated with the four different primers, different reactions are noted, even in repetitions with the same primer. We don't know the reasons for such variations, because we have checked the cleaning and the effectiveness of each one of the stocks of primers and the blind control (that contains only reagents) that doesn't amplify in none of the tests.

Thus, for routine evaluations, two of the available primers (i.e. SST and RBD) can be used, as they give more specific amplifications as compared to the others. However, we recommend to repeat the test again in those samples that don't amplify with none of the primers in order to obtain reliable results and so to avoid pointing to false negatives.

Contributors: M. Cuervo I. (Virology Unit), N. C. Flor (GRU), and L. Calvert (Virology Unit)

Activity # 2.6.4. Evaluation of three types of grafting to improve cassava virus indexing

Introduction

Obtaining plants free of virus is of great importance in the establishment of the cassava crop (Lozano et al, 1982), for the conservation of germplasm, and its distribution and exchange (Frison & Feliu 1991). Systems of rapid and accurate indexing that guarantee their health are thus important requisites (Roca & Mroginski, 1991).

In the certification of health of the propagules, obtained from *in vitro* plantlets by means of thermotherapy and meristem tip culture or from field plants, it is possible to utilize some special techniques. Usually serological, biochemical or molecular diagnosis techniques are used, provided they are available. For Frogskin disease (FSD) these techniques are not available: only the biological grafting technique is at hand, using MCol 2063 as a hypersensitive grafted indicator.

In CIAT, the grafting of stakes has been used as routine procedure for indexing against FSD with 100% efficiency in grafting establishment (Pineda, B, Bedoya, A. and Rodríguez, C. T, data not published). However it resulted not very practical for its utilization in massive form. One constraint is the time to produce in the field the stakes of the indicator guaranteed clone (8-12 months). Other constraints include the level of risk of re-infection by the vector *Bemisia tuberculata*, the space and the quantity of soil required for the execution of the technique.

In order to find out more efficient and rapid grafting techniques, we planned to evaluate three variations of the technique. These techniques were: *in vitro* micrografting, grafting of "vitro plants" on sprouts of stakes, and the "English graft" on young thin cuttings rooted in water.

Material and Methods

In the three techniques we evaluated the incubation period (minimum and maximum), the percentage of establishment of grafting, the mean age of the materials for the grafting (rootstock, graft), and the sensibility of detection of the technique. In addition, we considered the easiness of execution, the demand of special skills and preparation of the workers, the requirements of space and installations, and other tricks related with the practicability and dependability of the systems of indexing.

Cuttings of 25 clones of cassava that showed symptoms of FSD on the roots with several grades of severity were used as rootstocks. As grafted parts we used cuttings from young plants of MCol 2063 (highly susceptible to viral

diseases), properly certificated free of any virus. The grafted materials and their respective controls were maintained under adequate conditions for expression of symptoms (20-27 °C, low illumination). During the study relating scores were taken as to period of incubation (minimum and maximum), expression of symptoms, and percentage of establishment of grafting. The methodologies of the three techniques of grafting used in this research are illustrated in the Figures 11, 12, 13 and are described hereafter:

In vitro Micrografting Technique

The mother stakes planted in "icopor" pots with sterilized soil were placed in a growth chamber with a temperature of 30 °C until the appearance of sprouts (at approximately at 20 days). Under conditions of laboratory, and within a laminar flow cabinet the sprouts were sectioned to obtain buds carrying out the following steps: the buds contained in a beaker were disinfected with 70 % ethanol and then with 0,5% sodium hypochlorite, washing them with distilled sterile water between each step. From these buds tips of eight primordias were extracted. These were planted in tubes that contained half of Murashige-Skoog medium (Roca et al., 1984). They were then raised in a growth chamber with 28 °C, photoperiod of 12 hours, and light intensity of 2,000 lux. After 90 days plantlets with vigorous and lignified stems were gotten; we then micro-grafted the Secundina clone with an incision in the apex of the rootstock at 0.5 cm of depth. The micro-grafts were raised in a growth chamber with 24° C progressively raised to 28 °C, humidity of the 60%, photoperiod of 12 hours, and light intensity of 2,000 lux.

Technique of grafting of "vitroplants" on sprouts

The mother stakes planted in "icopor" pots with sterilized soil were grown at 25 °C until of the axillary buds grew out forming sprouts. Subsequently a section was done in the terminal part of the young stems to a height of 5-7 cm with a disinfested knife. Then, a cut an incision of 1 cm of depth was made and all axillary buds were eliminated. Then a portion of the stem of *in vitro* Secundina plantlets was cut off to implant it like a graft on the rootstock sprout. The graft was inserted in the fissure of the rootstock by subjecting it with ribbons of "parafilm". Finally the inplants were covered with a transparent plastic bag in order to limit dehydration, and were raised in a growth chamber at 25 °C, photoperiod of 12 hours, and intensity of 2,000 lux.

Technique of the "English Graft"

The technique of "English graft", after some modifications, was carried out according the description by Hartmann and Kester (1971). The "mother" stakes planted in "icopor" pots with sterilized soil were placed under conditions of growth room until lignified sprouts were obtained (about 25 days later). Then the sprouts were cut in order to utilize them as rootstocks. A transversal cut in the upper part was done in each of them. A cut of the same longitude was done in stakes of similar diameter of the indicator clone; on these the cut was carried out in the lower part. All buds present in the rootstocks were eliminated in order to avoid their development. Once the cuts were done, both sectioned extremes were jointed strongly with ribbons of "parafilm" in order to secure the establishment of the implant.

The grafted clones were placed in flasks of sterile glass containing distilled water and located in a growth chamber at 26°C and a photoperiod of 12 hours. Two repetitions for each clone were carried out in each technique.

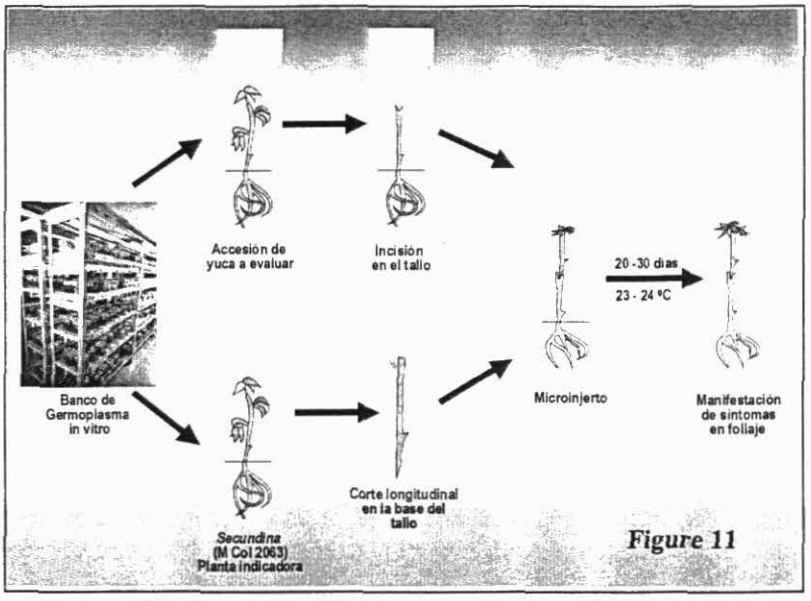

Figure 11

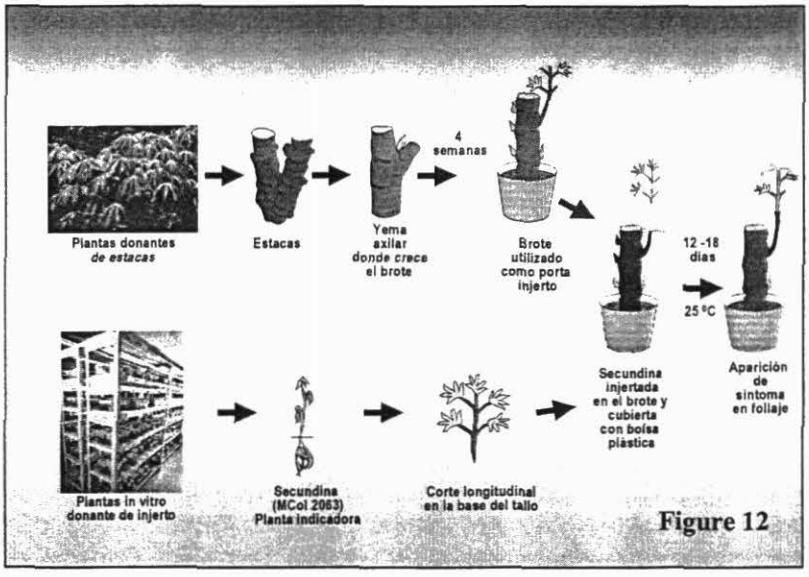

Figure 12

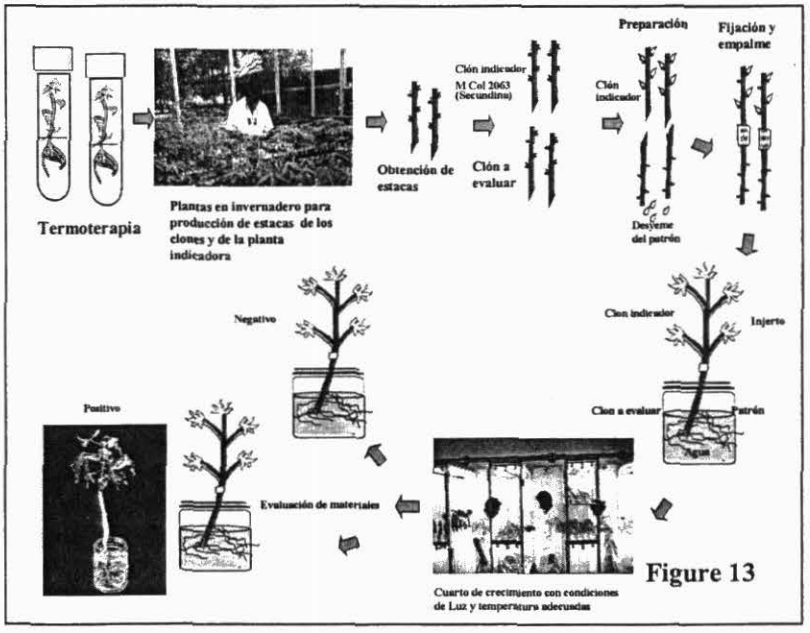

Figure 13

Results and discussion

Our results show that the three techniques of grafting detect the materials infected with FSD, at the three levels of disease severity, with some differences. The evaluation of the percentage of establishment of the three techniques showed that, in spite of the mortality of some implants, the percentage of establishment were: 100% for micrografting, a 92% for the grafting on sprouts, and 93,6% for the English grafting. Symptom expression was obtained at 48% with the *in vitro* micrografting, at 76% with the grafting of "vitroplants" on sprouts, and at 100% with the "English graft" technique on sprouts rooted in water.

If the three techniques of implant were evaluated with the traditional grafting (English grafting on stakes), the results show an increased efficiency as per the total time required for the diagnosis (Table32). There are also fewer risks of re-infection with the FSD by presence of B. *tuberculata*. So far the English grafting on sprouts rooted in water is the most efficient method. However it is worth waiting for the verifications and fittings of the other two techniques, as they might be more efficient and reliable than traditional grafting.

Table 32. Comparison among different grafting techniques evaluated in this research

Method	Utilization age in weeks		Establishment %	Symptoms expression %	Incubation period	Total Diagnostic Time (weeks)	Accurate level	Risk level[2]
	Rootstock	Graft						
In vitro micrografting	17	3	100	48	3-4	24	A.V.P[1]	Very low or negligible
Grafting of "vitroplants" on sprouts	3-5	4	92	76	2-3	12	A.V.E.	Low to middle
English grafting on sprouts rooted in water	3-4	10	93,6	100	0,3-1,3	15,3	High	Low to middle
English grafting (Traditional grafting)	34	34	100	...	3-4	72	High	Middle to high

* Reference: Pineda, B; Bedoya, A y Rodríguez, C.T, unpublished data [1], Additional Verification Progressing [2] Risk of grafting reinfection by FSD vector *B. turberculata*

Conclusions

1. All the techniques utilized in this experiment corroborated the presence of viral disease observed in the materials harvested in field, possibly associated to the complex of virus of FSD.

2. The techniques generate good outputs when the environmental conditions for the expression of symptoms are adequate; as much material of the indicator clone and that of the rootstock are required.

3. The three types of grafting are functional and have applicability according to the requirements of indexing for type and class of materials and they rely on the skills and availability of resources.

4. The percentage of establishment with the three evaluated techniques was high, however a directed training may be necessary.

5. The technique of grafting of "vitroplants" on sprouts could be the most adequate alternative in order to evaluate material from field, in less time as compared to the traditional grafting.

6. The technique of the English implant on buds taken root in water was the most efficient, keeping in mind that it detected the entirety of the clones affected with the FSD at one time relatively acceptable.

Contributors: J. C. Roa, N. C. Flor P., J. L. Ramírez, G. Mafla , B. Pineda L, and D.G. Debouck

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Subproject # 3. The genetic and social relevance of the conservation

Output 3.1. Designate Collection Better Characterized

Activity # 3.1.1. Characterization of Designate collections

Increasing knowledge about agro-morphological characters of the designated collections is done during the multiplication steps in greenhouses, and in the field at each locality. The main traits recorded are: growth habit, plant height at flowering time, flowering time, beginning and finishing time of harvest, flower colour and presence of pests and/ or diseases (Table 33).

Table 33. Characterization of beans and tropical forages germplasm

Materials	Palmira	Quilichao	Popayán	Tenerife	Total
Beans	1,200	---	750	3,118	5,068
Forages	224	546	10		780

Contributors: O. Toro, A. Ciprian

Activity #3.1.2. Morphological characterization of *Brachiaria* spp.

The list of 57 morphological descriptors have been revised and used in nine accessions of *Brachiaria* spp. planted in field plots in Palmira. Each accession had 5 plants. Two measures of each descriptor were taken per plant. The descriptors included vegetative and reproductive traits, as well as qualitative and quantitative characters. Some descriptors were measured in the field and others were measured at the Herbarium Lab with stereo microscope. Seven of nine accessions have been fully characterised.

The main goal of testing these descriptors was to have them available to test germplasm and breeding lines. There are no published descriptors for this important genus. After finishing the nine accessions, descriptive statistics will be analysed to test the most useful descriptors. In the mean time we are able to publish the *Brachiaria* descriptors.

Contributors: A.M.Torres, J.W. Miles, P.A. Sotelo

Activity # 3.1.3. Characterization of gene pools in Lima bean

Introduction

Two gene pools with different wild ancestral forms have been demonstrated for *Phaseolus lunatus* L. on the basis of biochemical (Gutiérrez Salgado et al., 1995) and molecular (Nienhuis et al., 1995) markers. The large-seeded cultivars had been domesticated from the large-seeded wild lima beans in western South America. However, for the small-seeded lima bean cultivars, a precise domestication center in the Americas is not known (Debouck et al., 1987). Colombia appears to be more than a place of contact between gene pools of cultivated materials. Using novel materials (121 accessions) and biochemical markers, we tried to gain additional evidence for answering key questions on the evolution of Lima bean in Colombia: from wich material did this crop arise?, where ?, how ?, when ?, by whom ?, all of them of critical importance for the shaping of genetic diversity, and thus in the decision making process for the conservation of such a genetic diversity (Debouck, 1996).

Results

In the wild progenitor proceeding from the Americas, eight Mesoamerican and five Andean known lectin patterns were found. In Colombia, the wild forms from Northern Coast and Caldas were all M1 (the most abundant protein pattern in the small-seeded germplasm of Lima bean), while wild forms from Boyaca and Cundinamarca were M1 and M12, the latter being exclusive of this country (Table 36). In cultivated Colombian forms, two Mesoamerican patterns (M1 and M7) and three Andean patterns (A3, A4, and A5) were found (Table 37). In Colombia, a weedy population presented both Mesoamerican and Andean patterns, as well as a hybrid pattern (Table 35).

From the 33 enzyme systems assayed by electrophoresis and obtained from cotyledonary tissues, 15 showed suitable and reproducible resolution (Zoro Bi et al, 1999). Twenty-five polymorphic and 7 monomorphic loci with 75 alleles were revealed in the 15 enzymes systems (Table 34). The allele 94 of Mdh-3 and allele 90 of Skdh were found novel and single in the Colombian wild progenitor. However, as it has been shown elsewhere on Costa Rican wild forms (Maquet et al., 1996), several loci (Mdh-2, Idh-2, Me-1, Pgi-3, Pgm-2) with alleles common among Andean materials were also found in Colombian populations with Mesoamerican protein patterns. This would indicate that as it is the case for some Costa Rican materials Colombia too would be a contact zone between the two major gene pools. A slightly higher diversity has been observed on the basis of allozyme polymorphism in the Colombian small-seeded wild progenitor in comparison to the large-seeded wild progenitor from the northwestern Andes (Table 38).

These results reinforce the hypothesis presented elsewhere about the situation of Lima bean in Colombia (Debouck, 1996; Tohme et al., 1996) as a place of contact, and introgression, but maybe also early origin before the formation of gene pools. However, on the basis of the allozyme and seed protein polymorphism, evidence of domesticacion in Colombia does not exist. These results should be expanded with other molecular markers; and collecting of additional wild forms particularly in southwestern Colombia is badly needed.

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Table 34. Locus and allele designations, and factor combinations producing a reliable resolution of enzymatic systems resolved from the cotyledonary tissues of the Lima bean (Zoro Bi et al, 1999) populations selected for this study.

Enzyme	System	Buffer	Locus Designation	Migration Orientation (6)	Alleles
Esterases	PAGE (1)	TB (3)	Est-1 Est-2	A A	88, 94, 100, 105 100
Diaphorase	SGE (2)	LB (4)	Dia-1 Dia-2	A A	88, 91, 100, 112 100, 120, 173
Peroxidase	PAGE	LB	Prx-1 Prx-2	A C	100 100
Aspartate aminotransferase	PAGE	TB	Aat-1 Aat-2	A A	Null, 94, 100, 95, 100
Acid phosphatase	PAGE	TB	Acp-1 Acp-2 Acp-3	A A A	87, 90, 96, 100 94, 100, 105 100
Malate dehydrogenase	SGE	HC	Mdh-2 Mdh-3	A A	100, 140 94, 100
Malic enzyme	SGE	HC (5)	Me-1 Me-2	A A	100, 130 100
Shikimate dehydrogenase	SGE	HC	Skdh	A	90, 100, 110, 118
6-phosphogluconate dehydrogenase	SGE	HC	6-Pgdh-1 6-Pgdh-2	A A	100, 110 100, 109
Phosphoglucomutase	SGE	HC	Pgm-1 Pgm-2	A A	95, 100, 110 85, 100, 110
Glucose-6-phosphate isomerase	SGE	HC	Gpi-1 Gpi-2 Gpi-3	A A A	90, 96, 100, 130 80, 100, 112, 124 Null, 100
Isocitric dehydrogenase	SGE	HC	Idh-1 Idh-2 Idh-3	A A A	100, 120 75, 100 100
Fumarate hydratase	SGE	HC	Fum-1 Fum-2	A A	100, 110 68, 100
Alcohol dehydrogenase			Adh-1 Adh-2	A A	100, 116 61, 100
Formate dehydrogenase	SGE	HC	Fdh-1 Fdh-2	A A	100 88, 96, 100
N (7) = 15	N = 2	N = 3	N = 32	N = 2	N = 75

(1) Polyacrylamide gel electrophoresis
(2) Starch gel electrophoresis
(3) Tris-borate buffer system
(4) Lithium-borate buffer system
(5) Histidine-citrate buffer systm
(6) Isozyme migrating to the anodal side A or to the cathodal side C relative to the origin
(7) Different elements found in the sample size

Table 35. Geographic distribution of seed protein patterns in wild, weedy, and cultivated *P. lunatus* L. proceeding from Colombia.

Origin	Middle American patterns			Hybrid Pattern	Andean patterns			Total
	M1	M7	M12	H	A3	A4	A5	

Wild	10		21					31
Weedy			1	1	1			3
Cultivated	27	1			20	2	1	51
Total	37	1	22	1	21	2	1	85

Table 36. Geographic distribution of seed protein patterns of wild *Phaseolus lunatus* L. proceeding from the Americas.

Origin	Middle American patterns								H ★	Andean patterns								Total
	M1	M 2	M 3	M 6	M 7	M 9	M 10	M 12		A 1	A 2	A 3	A 6	A 8	A *	A **	A ***	
México	2	1	1	1		2	1											8
Guatemala	1			3														4
Belize	1																	1
Honduras	2			1														3
Salvador	1																	1
Costa Rica	5																	5
Panama				1														1
Cuba	1																	1
Colombia:	**10**							**21**										**31**
Magdalena	3																	
Atlántico	2																	
Córdoba	1																	
Boyacá	3							19										
Cundinamarca								2										
Caldas	1																	
Peru					1				1	1		1	1				1	6
Ecuador										2	1		1	1	2	1		8
Argentina	1																	1
Total	24	1	1	6	1	2	1	21	1	3	1	1	2	1	2	1	1	70

★ Hybrid pattern (Middle American + Andean)
* Novel Andean pattern (so far designated like A? 1)
** Novel Andean pattern (so far designated like A? 2)
*** Novel Andean pattern (so far designated like A? 4)

Table 37. Geographic distribution of seed protein patterns of cultivated *Phaseolus lunatus* L. proceeding from Colombia.

Origin	Middle American patterns		H ★	Andean Patterns			Total
	M1	M7		A3	A4	A5	
Guajira	1						1
Magdalena	1						1
Atlántico	1						1
Bolivar	1						1
Sucre	2						2

Córdoba	1						1
Antioquia	5			5	1		11
Boyacá				2			2
Cundinamarca				2			2
Tolima	3			1			4
Huila	2			1			3
Caldas	2			1			3
Risaralda	1			1			2
Valle del Cauca	1			1			2
Cauca	3			1			4
Nariño	3	1		4	1	1	10
Putumayo				1			1
Total	27	1		20	2	1	51

★ Hybrid pattern (Middle American + Andean)

Table 38. Allozyme distribution (polymorphic loci) among the small-seeded wild progenitor proceeding from Colombia and the Americas, the domesticated form cultivated in Colombia and the large-seeded wild progenitor collected on the western slope of the Andes.

Locus	Alleles				
	Wild progenitor from Colombia (small seed) (N = 31)	**Wild progenitor from the Americas (small seed) (N = 26)**	**Domesticated form proceeding from Colombia**		**Wild progenitor From the Americas (large seed) (N = 13)**
			Small Seed (N = 28)	**Large Seed (N = 23)**	
Got-1	94, 100. Null	94, 100, Null	100, 94	100	100
Got-2	100	100	100	100	95, 100
Acp-1	90, 100.	87, 90, 100	90, 96, 100	96	87, 96
Acp-2	100, 105	100, 105	100, 105	94, 105	105
Dia-1	88, 91, 100, 112	88, 91, 100	88, 91, 100, 112	91, 112	91, 100, 112
Dia-2	100, 173	100, 120	100, 173	173	173, 100
Est-1	88, 94, 100	88, 94, 100, 105	88, 94, 100	88	88
Mdh-2	100, 140	100, 140	100, 140	140	140
Mdh-3	94, 100	100	100	100	100
Me-1	100, 130	100, 130	100, 130	130	130
Pgi-1	90, 100	90, 96, 100	90, 100	96	96
Pgi-2	80, 100, 112	80, 100, 112, 124	100, 112, 124	100, Null	100
Pgi-3	100, Null	100	100	100	100, Null
Skdh	90, 100. 110, 118	100	100, 118	100, 118	100, 110, 118
Pgm-1	95, 100	95, 100	95, 100	95, 100	100
Pgm-2	85, 100	85, 100	100, 110	85	85, 100
Fum-1	100	100	100	100, 110	100, 110
Fum-2	68, 100	68, 100	100	100	100
Idh-1	100	100	100	100	100
Idh-2	75, 100	75, 100	100, 75	75	75

Fdh-2	88, 96, 100	88, 96, 100	88, 100	88, 96	88, 96
Adh-1	100, 116	100, 116	100	100	100
Adh-2	61, 100	61, 100	100	100	100
Pgdh-1	100	100	100	110	100
Pgdh-2	100	100	100	109	100, 109

N = Amount of sampled populations

Activity # 3.1.4. SDS-PAGE analysis of seed storage proteins for assessment of evolutionary affinities between *Phaseolus lunatus* L., and related South American wild species.

Introduction

A group of wild species with morphological similarities with the wild Andean Lima bean (*Phaseolus lunatus* L.) exists in the Andes south of Colombia. Three species, *P. augusti* growing from Ecuador to Argentina, *P. pachyrrhizoides* found in Peru only, although sometimes difficult to separate from the former, and *P. bolivianus* growing only in Bolivia, seem to be valid species (Brako and Zarucchi, 1993). The three Andean species have been proposed by Fofana et al, 1999, as the secondary gene pool of *P. lunatus*, while its companion allies of Mesoamerican distribution would be members of its tertiary gene pool. It is therefore useful to clarify the phyletic position of the Andean wild species between themselves and in relation to Lima bean.

Results

Using one-dimensional SDS-PAGE technique for seed storage proteins, we have analysed all the populations available for wild allies of Lima bean of Andean origin and maintained in CIAT genebank. (Table 39). According to results by Maquet et al, 1999, *P. augusti*, *P. pachyrrhizoides* and *P. bolivianus* posses similar seed protein patterns to the ones of Lima bean and more precisely to its Mesoamerican gene pool. Nevertheless, our results show that in addition to the Mesoamerican protein pattern (with new and well-known patterns), an Andean pattern exists in the *P. augusti* species proceeding from Ecuador (Figure 14). The Mesoamerican protein pattern is revealed by bands of molecular weights ranging from 24.5 kDa to 26.5 kDa, while the Andean protein pattern shows a first band around 23 kDa and a second one around 25.7 kDa. For the Mesoamerican protein, we found known patterns (M1 and M7) in the Lima bean. We also found new patterns exclusive in two Andean species, *P. augusti* and *P. pachyrrhizoides*, which seem to be exclusive, since they do not exist in the Lima bean.

Based on these observations and other dates (molecular) is suggested for the genus *Phaseolus*, an second centre of diversification in the Andes, which includes *P. lunatus*, *P. augusti*, *P. bolivianus* and *P. pachyrrhizoides*. The last three species could constitute the secondary gene pool of Lima bean. A palynological analysis including these species under development could help to support this hypothesis. The results of this study, along with others (molecular and palynological), should result in a better selection of parental materials in breeding programs and stress the importance of collecting and conserving Andean *Phaseolus* germplasm.

Contributors: O. Toro, C. Ocampo, D. G. Debouck

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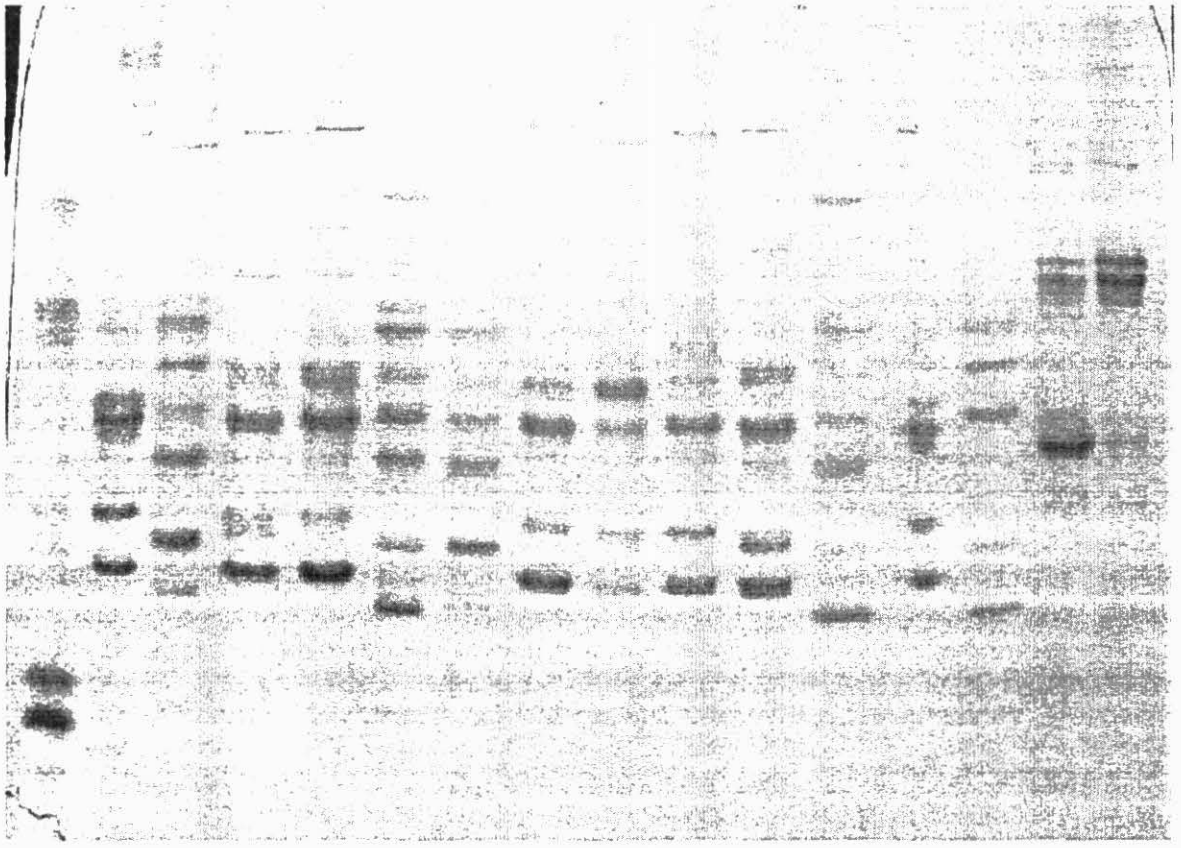

Figure 14. One-dimensional SDS-PAGE of seed storage proteins of the South American wild related species of *Phaseolus lunatus* L. (lanes 2 to 16). MM protein standard with molecular weights indicated at the left side (lane 1). Material identifications are as follows:

Lane 2: M1, P. lunatus, DGD-453, Mexico Jalisco (G25704), wild Mexican form as check.
Lane 3: A1, P. lunatus, DGD-1945, Peru, Cajamarca (G25914), wild Peruvian form as check.
Lane 4: M7, P. pachyrrizoides, DGD 2149 (S30323), Peru, Junin.
Lane 5: M7, P. augusti, DGD 2146 (S30320), Peru, Junin.
Lane 6: A2, P.augusti, DGD 2766 (S31773), Ecuador, Azuay.
Lane 7: A1, P.lunatus, DGD 2863 (G26606), Ecuador, Chimborazo.
Lane 8: M7, P.augusti, DGD 638 (S29354), Argentina, Tucuman.
Lane 9: M7, P. bolivianus, DGD 3001 (S33681), Bolivia, Cochabamba.
Lane 10: M12, P. lunatus, OT-435 (G26618B), Boyaca, Colombia. Wild. Colombian form as check.
Lane 11: M7, P. bolivianus, PI 260 412 (S5257), Peru, Cuzco.
Lane 12: A2, P. augusti, DGD 2764 (S31771), Ecuador, Azuay.
Lane 13: M1, P. lunatus, Koelz 8867 (G25237A), India, Maharashtra, cultivated form as check.
Lane 14: A3, P. lunatus,NI 18 (G26193),Zaire, Ituri, cultivated form as check.
Lane 15: M4, P. vulgaris characterised by its phaseolin (arrow-head), DGD 451 (G11051), Mexico, Jalisco, wild Mexican form as check.
Lane 16: J3, P. vulgaris characterised by its Phaseolin (arrow-head), DGD1711 (G21197), Argentina, Jujuy, wild Argentinan form as check.

Table 39. The seed storage protein patterns of the South American wild related species of *Phaseolus lunatus* L.

SPECIES	Sampled Populations	Sampled Seed by population	Country of origin	Departament	Seed Protein Patterns
P. augusti	15	5	Peru	Cuzco	M1, M2, M7
P.augusti	1	5	Peru	Piura	M7, Novel 5
P.augusti	2	7, 5	Ecuador	Azuay	A2
P.augusti	1	5	Ecuador	Loja	M7
P.augusti	3	5	Bolivia	Cochabamba	M7, Novel 2
P.augusti	3	5	Bolivia	Chuquisaca	M7
P.augusti	1	5	Bolivia	Norcinti	M7
	3	5	Argentina	Salta	M1, M7
	1	5	Argentina	Tucuman	M7
P. bolivianus	2	2	Bolivia	Cochabamba	M7
P. pachyrrizoides	15	5	Peru	Apurimac	M7, Novel 3, Novel 6
	5	5	Peru	Junin	M7
	1	4	Peru	Amazonas	M7, Novel 1
	2	5	Peru	Cajamarca	M7, Novel 2
	1	5	Peru	Cuzco	M7

Activity # 3.1.5. New sources of phaseolin variation found in populations of *Phaseolus vulgaris* L. collected in its primary center of diversity

Achievement: 61 phaseolin patterns have been found so far in the American gene pools of common bean

Introduction

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 type is also becoming a routine powerful descriptor for bean germplasm characterization, namely to define relationships to gene pools and races. Given the usefulness and practicability of such marker, its characterization at the population level, where great amounts of native genetic variability exist, is needed, so that this variability is conserved and available to users (Gepts et al, 1986). We have initiated a systematic search for new phaseolin types in the accessions of both wild and cultivated *Phaseolus vulgaris* L. collected in its primary centers of diversity and maintained in CIAT gene bank.

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 of phaseolin (Table 40). Although this globulin has a narrow range of molecular weights (45-52 kD) and isoelectric points, a total of 61 banding patterns has been found so far, 29 being present in Mesoamerican materials and 32 in the Andean region, be wild or cultivated. In Mesoamerican materials all 29 patterns are present in wild forms, while only four exist in cultivated forms so far. A contrasting situation prevails in the Andes where 15 patterns have been found in cultivated forms (11 with no counterpart in the wild forms so far), and 17 types exclusive of wild forms.

The observed variation shows several phaseolin types within these populations and landraces. In the Mesoamerican populations show 'S', 'CH' and several 'M' types. A Mexican population (DGD-274) displays a novel and apparently unique phaseolin, and three Costa Rican populations also display a novel and apparently unique type. In contrast, the Andean populations show the types T, C, H, I, Ko, Pa, and J_4. The Colombian populations display two interesting features. First, they display phaseolin types (novel and known) that are present in both Mesoamerican and Andean centers. Second, the phaseolin types such as novel Phaseolin No. 1, several 'X' types and the 'L' (in cultivated) that are novel and apparently unique to Colombia. The variation within wild populations and landraces would call for a cautionary approach in handling the germplasm; simultaneously it could help to monitor changes over time.

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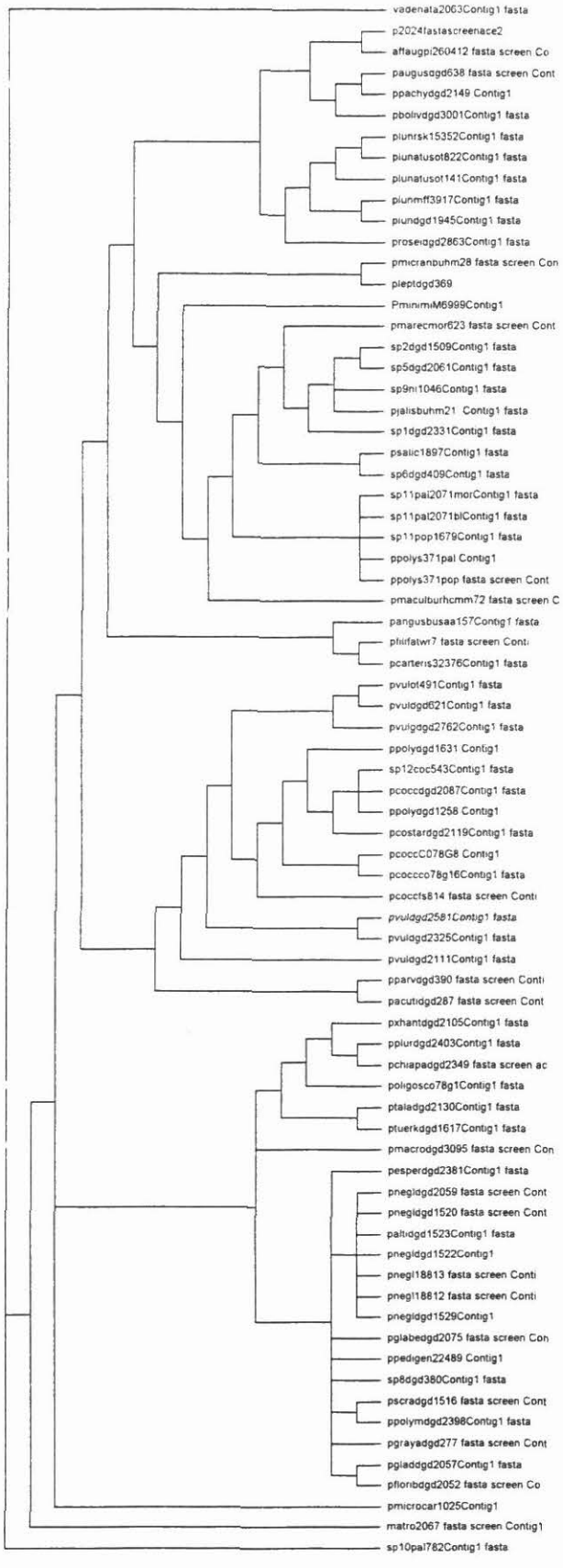
Table 40. New and known types of phaseolin found in populations of *Phaseolus vulgaris* L. collected in its primary centers of diversity.

Country of origin	Identification	Biological Status	No. of sampled seeds	Phaseolin Types
Mexico	DGD-274	Wild	36	S, M1, M9, M10, M15,
	DGD-274	Wild	30	Novel and simple phaseolin No.
Guatemala	DGD-2423	Wild	173	S, Sd, M13, M16, CH
	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,
	SB-6	Wild	13	Novel phaseolin No. 2
	SB-7	Wild	29	S, CH
	SB-10	Wild	12	S, M16
Costa Rica	OT-3106	Wild	5	Novel and simple phaseolin No.
	OT-3126	Weedy	15	S, Novel phaseolin No. 2, Novel phaseolin No. 4
	OT-3131	Wild	15	S, Novel and simple phaseolin N
Colombia	OT-491	Wild/Weedy	87	CH
	OT-174	Weedy (Segreg.)	61	CH, L, M6
	OT-122	Weedy	85	CH, B, Novel phaseolin No. 2
	Cargamanto (several popul)	Cultivated	135	B, T, Ca, Ca_1, H_1, H_2, LI, CAR, HE
	OT-519	Cultivated/Weedy	10	B, CH
	OT-252	Cultivated	2	Novel and simple phaseolin No.
	OT-712	Cultivated	75	B, C, H1, T
	OT-819	Cultivated	40	B, C, H1
	OT-70	Cultivated	6	C, T
		Weedy	7	B, C, T
	OT-83	Wild	5	B, T
		Weedy	3	B, C
		Cultivated	8	B, C, T
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
	Ayacucho-90	Cultivado	5	A1
Argentina	DGD-621	Wild	138	C, H_1, J_4, T

Activity 3.1. 6. Molecular taxonomy of the genus *Phaseolus* through ITS sequencing.

Main achievements

- 74 OTUs representing 60 taxa (species, subspecies and botanical varieties) included into a ITS sequencing
- at least six natural groups defined within the genus
- composition of secondary gene pools of the two major bean cultigens
- species relationships in view of future interspecific hybridization



Results and Discussion

ITS sequencing shows us the organization of the genus *Phaseolus* into several phyla (Figure 15). As expected, the American Phaseolinae *Vigna adenantha* and *Macroptilium atropurpureum* fall outside the genus, and serve as

outgroups. Check is needed about sp10, a Mexican Phaseolinae with hooked hairs that has not bloom so far. The phylum that includes the Lima bean and its Andean and Mesoamerican gene pool also comprises the secondary gene pool of Andean species, confirming early results (Caicedo et al. 1999). The tertiary gene pool of Lima bean with taxa such as *maculatus, jaliscanus, polystachyus*, indeed links with the group of *P. lunatus* (Fofana et al. 1999; Maquet et al. 1999). If the link is through *P. leptostachyus* and *P. micranthus* remains however a point to be checked. Because they share the unique trait of rugose testa (Debouck 2000), it was expected that *P. filiformis, P. angustissimus* and *P. carteri* would be grouped together at some distance between the *P. lunatus* phylum and that including the common bean, and this is confirmed by ITS sequencing. The phylum of P. vulgaris includes *P. vulgaris, P. polyanthus, P. costaricensis,* and *P. coccineus*. The parsimony analysis thus confirms many former molecular studies (Schmit et al. 1993; Llaca et al. 1994; Hervieu et al. 1994; Jacob et al. 1995; Delgado et al. 1999). We need however to check further through bootstrap analysis the robustness of the present analysis, because the differences between cpDNA (e.g. Schmit et al. 1993) and nuclear DNA (Llaca et al. 1994; Jacob et al. 1995; Delgado et al. 1999) analyses are not fully revealed as such. Interspecific hybridization has long shown the remote but existing linkage between the common bean phylum and the tepary; this is also confirmed by ITS sequencing. Interestingly, *P. parvifolius* that was thought to belong to the secondary gene pool of *P. acutifolius* is indeed shown as such. The last group would need additional bootstrap analysis: it includes species as morphologically diverse as *P. xanthotrichus, P. chiapasanus*, or *P. neglectus*. Certain affinities revealed in previous studies (Delgado et al. 1993, 1999) appeared once again. For instance, *P. glabellus* appears close to the *P. pedicellatus* group; we know it to be quite separate from the *P. coccineus* group (Schmit et al. 1996). The differences between the *P. pedicellatus* group and the *P. neglectus* group although morphologically important would not be very strong on the basis of ITS sequencing data; both are however confirmed as groups including a couple of taxa (Debouck 2000). Some synonymy that was inferred from morphological studies would be validated by ITS sequencing.

As compared to a similar recent study (Delgado et al. 1999), our work 1) confirms the different phyla existing in the genus such as the *P. lunatus* phylum, the *P. vulgaris* phylum, etc, 2) validates the identity and position of several new species discovered by one of us mainly in Mexico, and 3) indicates better species relationships as a preamble to successful future breeding.

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References

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8. Llaca V, Delgado A, Gepts P. 1994. Chloroplast DNA as an evolutionary marker in the *Phaseolus vulgaris* complex. Theor. Appl. Genet. **88:** 646-652.
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10. Schmit V, du Jardin P, Baudoin JP, Debouck DG. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. **87:** 506-516.
11. Schmit V, Debouck DG, Baudoin JP. 1996. Biogeographical and molecular observations on *Phaseolus glabellus* (Fabaceae, Phaseolinae) and its taxonomic status. Taxon **45:** 493-501.

Output 3.2 Novel materials acquired or collected

Activity # 3.2.1. Multiplication of recently acquired germplasm collection

The agreement suscribed in 1999 with CSIRO, Australia, has come into effect with the receipt at CIAT of two shipments of 920 and 358 accessions, summing 1,278 accessions. After comparisons with materials shared in the past with CSIRO, estimates of seed viability and amounts received, a first group of 50 accessions of *Stylosanthes* have been planted in Quilichao.

Contributors: A. Ciprian, A.M. Torres, D.G. Debouck

Output 3.3. Genetic erosion monitored

Research Activity # 3.3.1. A model of genetic simulation helps to control drift and erosion in management practices of genebanks

Introduction

The main function of genebanks is to maintain the genetic variability of the species against losses due to genetic erosion, destruction of wild habitats and uniformization of agriculture, and to make it available as such to end users (Roos, 1988). Once a new accession arrives at CIAT genebank, its original seed is multiplied in a quarantine glass-house, and the next generations in a field. During this process, a serious genetic drift (i.e., change of gene frequency) could occur. The main objective of this project is to establish a model of genetic simulation to help to control genetic drift in the conserved accessions. In order to develop this strategy it is necessary to understand the genetic structure of the original accession and the effects of different methods of initial seed increase on the genetic contents of that accession. Using wild common bean (*Phaseolus vulgaris* L.) as a model this work aims at suggesting methods that allow to control the genetic erosion during the typical activities of a genebank in order to avoid the loss of genes. In order to promote the action of genetic drift, wild populations were evaluated in three environments that displayed conditions different from those of their original environment. The environments were: –A (1,000 msnm, 24 °C), –B (1,750 msnm, 17 °C); in this site the rainfall was eliminated with a plastic ceiling to simulate another environment called C. Original seed was cultivated and from each plant four cuttings were obtained. In each environment, a cutting was cultivated, and its production expressed as number of seeds registered. The fourth cutting was used to establish, by means of the isozyme systems electrophoresis, the frequencies of alleles in the Diaforase (DIA), Isocitrato deshidrogenase (IDH) and Peroxidase (PRX) systems (Figure 16). Previous work (Koenig & Gepts 1989) did show activity and polymorphism in wild common bean with these enzyme systems. The calculated frequencies were obtained on the basis of the parental genotype, taking into account the amount of seeds that were produced in each environment.

Results

The populations show allozyme variation in the three evaluated environments (Table 41). The comparison between the initial frequency and the frequencies calculated in each environment emphasizes the need to control the loss of genes in genebanks, because the changes in allele frequencies eventually breaks balance between allelic forms and promotes the genetic uniformity of the multiplied material. The number of multiplied individuals is an important aspect to control the genetic erosion because it determines the probability of maintaining allelic variants in a population. In addition one must not harvest at random because such practice would favour those characters present in individuals with high production. Therefore is recommendable to establish a fixed amount of seed that must be

harvested in each producing individual in order to reduce the effect of the differences in productivity, and so to control the genetic erosion.

The variance analysis indicated significant differences by effect of the environment on the seed production of the populations. ANOVA averages (Duncan) were compared and it appears that the mean production in environment C was significantly (α=0.05) different from the average in the other two environments. In the histograms (Figure 17) ione can see that in the environment C, the number of individuals that did not produce seed diminished in relation to the other two environments. This indicates that this environment was ecologically close to the original environment of the evaluated population; therefore more individuals have contributed reproductively to the filial generation. Then in this environment some characteristics at low frequency in the parental population had more opportunity to happen to the filial generation as compared to the other two environments. The multiplication site must offer an environment ecologically close to the original one to reduce the loss of individuals because of poor adaptation. The number of cultivated individuals and the individual production of seed are equaaly important if it is to control effectively the genetic erosion in activities of a genebank. In a germplasm collection one must ensure that all the factors contributing to the loss of genetic variability are under control in order to meet its conservation objectives.

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

References

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2. 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.

3. Roos, E. E. 1988. Phaseolus seed storage. *In* Genetic Resources of *Phaseolus* beans. P. Gepts (Ed.). Kluwer Academic Publishers. Dordrecht, Holland. Pp. 31-49.

Table 41. Frequencies initial and calculated of the alleles found in the three evaluated systems.

Enzymes	Allele	Frecuency Initial	Frecuency Calculated in A	Frecuency Calculated in B	Frecuency Calculated in C
Diaphorase	95	0.405	0.730	0.401	0.282
	100	0.595	0.270	0.599	0.718
Isocitric dehydrogenase	98	0.013	0	0.021	0.019
	100	0.987	1	0.979	0.981
Peroxidase	98	0.937	1	0.986	0.957
	100	0.063	0	0.014	0.043

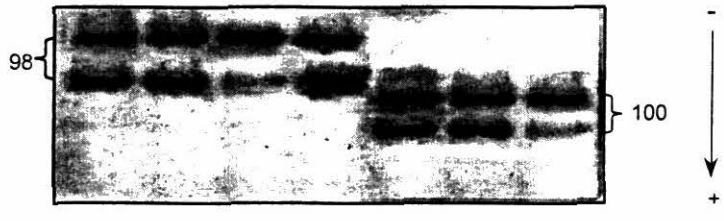

Figure 16. Alleles shown in the peroxidase enzyme system.

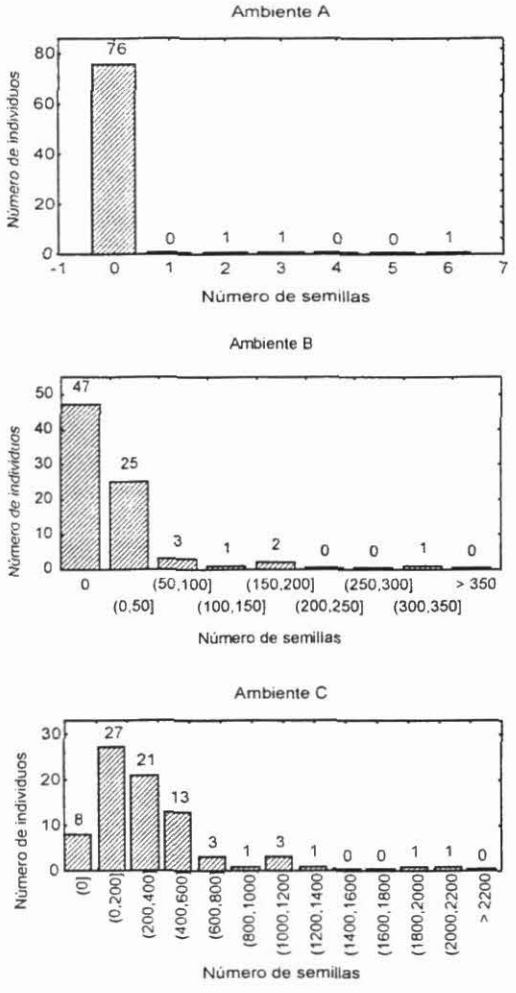

Figure 17. Individual production in the evaluation environments.

Output 3.4 Unique genes better sampled and characterized

Activity # 3.4.1. Selection of backcross in common bean using phaseolin I as genetic marker

With the purpose to improve common bean improvement through backcrossing with genetic marker, a cross between a Peruvian wild common bean (G23585) with 'I' phaseolin and the recurrent parental line with faseolina S (DOR 390) was done. Lines of backcross with the phenotype of the recurrent parental and with the 'I' phaseolin were developed. The evaluation was done using 1D-SDS- PAGE seed protein electrophoresis for about 200 lines of these backcrosses.

Contributors: C.H. Ocampo, St. Beebe (Bean Breeding Program)

Subproject # 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

Three international courses were held with the participation of GRU Staff.

1. Course on "Conservación *ex situ* de Recursos Fitogenéticos IPGRI-CIAT-ICA", CIAT, 13-17 December, 1999.

This course was organized together with Staff of the Regional Office of IPGRI Americas for 20 participants from Colombia, Paraguay, Peru, and Venezuela; it also served to test audiotutorial materials prepared by IPGRI.

2. Course on "Gerencia de Proyectos y Herramientas para la Conservación y Valorización *ex situ* de la Agrobiodiversidad", CIAT, 6-23 June, 2000.

This course was organized together with Staff of CIAT Project Office and Universidad Nacional de Colombia for 18 participants from Colombia, Mexico, and Venezuela.

3. "Curso Internacional sobre Sistemas Modernos de Producción y Procesamiento de Yuca", CIAT, 23 Octubre - 10 November 2.000.

This course sponsored by CLAYUCA included 27 professionals from Bolivia, Colombia, Cuba, Ecuador, Haiti, Nicaragua, Paraguay, Peru, and Venezuela.

On the other hand, on 7th October 2000, the Universidad Nacional de Colombia approved the Magister Scientiae in Neotropical Plant Genetic Resources, an initiative started in 1993 with Staff of the Regional Office of IPGRI Americas.

Output 4.2. Conferences in national/ international fora

A total of sixteen conferences/ lectures has been presented by Project Staff in 1999-2000. These were:

4.2.1. Debouck, D.G. "Genes en tiempo oportuno ... la carrera a las resistencias", invited opening lecture, XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, Colombia, 30 August 2000.

4.2.2. Debouck, D.G. "Recursos genéticos de *Phaseolus*: patrones en el tiempo, el espacio, y la gente", invited opening lecture, 2do Seminario de Judía de la Península Ibérica, Villaviciosa, Asturias, Spain, 5-7 September 2000.

4.2.3. Debouck, D.G. "Plantas mochicas, sus origenes, viajes e impactos", Seminarios Internos, Centro Internacional de Agricultura Tropical, Palmira, Colombia, 20 September 2000.

4.2.4. Debouck, D.G. "El pallar peruano, de la apaitalla moche a una contribución a la alimentación del mundo", invited opening lecture, Día Mundial de la Alimentación, Universidad Privada Antenor Orrego, Trujillo, Perú, 16 October 2000.

4.2.5. Escobar, R. "Preservando y conservando la diversidad del germoplasma", invited communication at IV Congreso Colombiano de Genética, Popayán, Colombia, 21-25 February 2000.

4.2.6. Escobar, R. "El uso de la biotecnología en la conservación y multiplicación de la yuca", invited communication at Seminario comemorativo de 15 años de trabajos del GETEC: Tópicos actuales de investigación en genética, Universidad del Valle, Cali, Colombia, 6-7 June, 2000.

4.2.7. Escobar, R. (with P. Chavarriaga, J. Tohme and W.M. Roca) "El potencial de la biotecnología para contribuir al manejo de las plagas en yuca", XXVII Congreso de la Sociedad Colombiana de Entomología, Medellín, Colombia, 26-28 July 2000.

4.2.8. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Sincelejo (Sucre), 29-30 March 2000.

4.2.9. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Villavicencio (Meta), 28 April 2000.

4.2.10. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Quilichao (Cauca), 2 June 2000.

4.2.11. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Barranquilla (Atlántico), 18-19 July 2000.

4.2.12. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Espinal and Neiva (Tolima), 25-27 July 2000.

4.2.13. Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Saravena (Arauca), 10-11 October 2000.

4 2.14. Torres, A.M. "Evidencia bioquímica de patrones fitogeográficos y taxonómicos en *Stylosanthes guianensis*", Reunión de la Asociación Colombiana de Herbarios (ACH). Manizales, Colombia, 14 September 2000.

4.2.15. Roa, J.C. "Estudios preliminares sobre el uso de la técnica de microinjertación para indizar germoplasma de yuca (*Manihot esculenta* Crantz)." , XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, 1 September 2000.

4.2.16. Velásquez, E. "Conservación *in vitro*: una alternativa segura para preservar especies silvestres de *Manihot"*, II Congreso Nacional de Conservación de la Biodiversidad. Pontificia Universidad Javeriana, Bogotá, 19-22 October 2000.

Output 4.3 Public awareness products

Two newspaper articles in Spain and in Peru have been published in relation to work of GRU Staff. While visiting the central genebank of INIA, Spain, one member of the Staff has presented the outline of a video about the work of genebanks of Iberoamerica. This shall be pursued further with Television Española during 2001.

Output. Costing studies

Activity: costing all expenses about the different methods to conserve cassava germplasm

As part of a systemwide intiative, the GRU together with IFPRI has initiated an analysis of all costs (i.e. capital, personnel, services, supplies, etc) for the conservation of cassava genetic resources, using the different methods: seed, field genebank, *in vitro*, and cryopreservation. The advantages of the cassava model are in the including of all conservation methods. The purposes are: 1) inform countries about conservation and distribution costs, 2) fix the amount of money required for an endowment, and 3) determine the most cost effective methods in view of their respective spin-offs and drawbacks.

Contributors: M.C. Bedoya, G. Mafla, P. Pardey (IFPRI), B. Koo (IFPRI), and D.G. Debouck

Subproject # 5. The link with in situ conservation on farm and in the wild

Output 5.3 Practices on on-farm conservation documented

Activity 5.3.1. Monitoring gene flow between wild relatives and landraces in common bean

This activity, planned by one of us (DGD) since germplasm explorations carried out in the Mayan area of Yucatan Peninsula in January-February 1979, has been started very recently thanks to a grant from BMZ of Germany. The field work for the part to be developed in Apurimac and Cuzco in Peru has been discussed with the Peruvian counterpart.

6. Annexes

6.1. SB-01 Project and Genetic Resources Unit Staff

1. Conservation Group:

R. Escobar, Biologist	Research Assistant (Cryo-conservation)
G. Mafla, Biologist	Research Specialist (In vitro Conservation)
J. C. Roa, Biologist	Expert (In vitro Conservation)
A.M. Torres, M. Sc.	Research Associate (Seed Conservation)
H. Velásquez, Biologist	Research Assistant (Viability Testing)

2. Production Group:

O. Toro, Tech.	Expert (Bean Germplasm)
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	Reseach Assistant (Seed Health Testing)
N. C. Flor, Ing. Agr.	Research Assistant (Seed Health Testing)
C. Ocampo, Biologist	Research Assistant (Electrophoresis Lab.)
C. Llano	Bilingual Secretary
S. Ramirez	Student, Informatics Systems
J.C. Orozco	Student, Informatics Systems

6.2. List of publications by Project Staff in 2000

A. In refereed journals:

Caicedo A.L., Gaitán E., Duque M.C., Toro Ch. O., Debouck D.G. & J. Tohme. 1999. AFLP fingerprinting of *Phaseolus lunatus* L. and related wild species from South America. Crop Sci. **39** (5): 1497-1507.

Thro, A.M., Roca, W.M., Restrepo, J., Caballero, H., Poats, R., Escobar, R., Mafla, G. and Hernandez, C. 1999. Can in vitro biology have farm-level impact for small-scale cassava farmers in Latin America? In Vitro Cell. Dev. Biol.-Plant. **35**: 382-387

B. In books:

Debouck, D. G. 2000. Biodiversity, ecology and genetic resources of *Phaseolus* beans – Seven answered and unanswered questions. *In*: "Wild legumes", Oono, K. (ed.), National Institute of Biological Resources, Tsukuba, Japan, pp. 95-123.

Debouck, D.G. 2000. Genetic resources of *Phaseolus* beans: patterns in time, space, and people. *In*: "La judía en un nuevo marco de calidad", 2nd Seminario de Judía de la Península Ibérica, M.A. Fueyo Olmo, A..J. González Fernández, J.J. Ferreira Fernández, R. Giraldez Ceballos-Escalera (eds.), Asturgraf, Villaviciosa, Asturias, Spain, pp. 17-39.

C. In proceedings:

Escobar, R.H., Debouck D.G. & Roca W.M. 2000. Development of cassava cryopreservation –I. *In:* Cryopreservation of tropical plant germplasm – Current research progress and application, F. Engelmann and H. Takagi (eds.), JIRCAS, Tsukuba, Japan, Pp. 222-226.

Escobar, R.H., Mafla G. & Roca W.M. 2000. Cassava cryopreservation –I. *In:* Cryopreservation of tropical plant germplasm – Current research progress and application, F. Engelmann and H. Takagi (eds.), JIRCAS, Tsukuba, Japan, Pp. 404-407.

Escobar, R.H., Palacio J.D., Rangel M.P.. & Roca W.M. 2000. Cassava cryopreservation –II. *In:* Cryopreservation of tropical plant germplasm – Current research progress and application, F. Engelmann and H. Takagi (eds.), JIRCAS, Tsukuba, Japan, Pp. 408-410.

García D, S. X., Pineda L. B ; Debouck, D & Varela, A. 2000. Reconocimiento de enfermedades fungosas transmitidas por semilla asociadas al incremento de germoplasma de *Brachiaria* spp.). *In:* Memorias XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, August 30 - September 1, 2000. Pp. 29.

García D, S. X., Pineda L. B ; Debouck, D & Varela, A. 2000. Presencia del mal de azúcar (*Sphacelia* spp) afectando germoplasma de *Brachiaria* spp. *In:* Memorias XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, August 30 - September 1, 2000, Pp. 28.

Roa, J.C., Mafla, G., Debouck, D. 2000. Estudios preliminares sobre el uso de la técnica de microinjertación para indizar germoplasma de yuca *(Manihot esculenta* Crantz). *In:* Memorias XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, August 30 - September 1, 2000. Pp. 47.

Roa, J.C., Flor, N.C., Ramirez, J.L., Mafla, G., Pineda, B., Debouck, D. 2000. Evaluación de tres tipos de injertos utilizados en la indización de yuca. *In:* Memorias XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira August 30 - September 1, 2000. Pp.48.

Roca W.M., Debouck D.G., Escobar R.H., Mafla G. & Fregene M. 2000. Cryopreservation and cassava germplasm conservation at CIAT. *In:* Cryopreservation of tropical plant germplasm – Current research progress and application, F. Engelmann and H. Takagi (eds.), JIRCAS, Tsukuba, Japan, Pp. 273-279.

6.3. List of thesis research supervised by Project Staff in 2000

García Diaz, S.X. 2000. Reconocimiento de enfermedades fungosas transmitidas por semilla asociadas al incremento de germoplasma de *Brachiaria* spp (Poaceae, Paniceae). Pontificia Universidad Javeriana, Bogotá, 178 p.

Guzmán Díaz, F.A. 2000. Estudio de la erosión genética durante las actividades del banco de germoplasma del CIAT utilizando como modelo el fríjol común silvestre. Universidad del Valle, Cali, Colombia, 105p.

Manrique Carpintero, N.C. 2000. Respuesta varietal de 95 genotipos de la colección núcleo de yuca (*Manihot esculenta* Crantz) a la crioconservación usando la técnica de encapsulación-deshidratación, Universidad Nacional, Palmira, Colombia. 88p.

Solarte Pérez, I.P. 2000. Estudio de las relaciones interespecíficas de un grupo de especies silvestres mesoamericanas pertenecientes al género *Phaseolus*. Universidad del Valle, Cali, Colombia, 115p.

6.4. List of conferences and scientific communications presented by Project Staff in 2000

Debouck, D.G. "Genes en tiempo oportuno ... la carrera a las resistencias", invited opening lecture, XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, 30 August 2000.

Debouck, D.G. "Recursos genéticos de *Phaseolus*: patrones en el tiempo, el espacio, y la gente", invited opening lecture, 2do Seminario de Judía de la Península Ibérica, Villaviciosa, Asturias, Spain, 5-7 September 2000.

Debouck, D.G. "Plantas mochicas, sus origenes, viajes e impactos", Seminarios Internos, Centro Internacional de Agricultura Tropical, Palmira, Colombia, 20 September 2000.

Debouck, D.G. "El pallar peruano, de la apaitalla moche a una contribución a la alimentación del mundo", invited opening lecture, Día Mundial de la Alimentación, Universidad Privada Antenor Orrego, Trujillo, Perú, 16 October 2000.

Escobar, R. "Preservando y conservando la diversidad del germoplasma", invited communication at IV Congreso Colombiano de Genética, Popayán, Colombia, 21-25 February 2000.

Escobar, R. "El uso de la biotecnología en la conservación y multiplicación de la yuca", invited communication at Seminario comemorativo de 15 años de trabajos del GETEC: Tópicos actuales de investigación en genética, Universidad del Valle, Cali, Colombia, 6-7 June, 2000.

Escobar, R. (with P. Chavarriaga, J. Tohme and W.M. Roca) "El potencial de la biotecnología para contribuir al manejo de las plagas en yuca", invited communication at XXVII Congreso de la Sociedad Colombiana de Entomología, Medellín, Colombia, 26-28 July 2000.

Pineda, B. "Enfermedades virales de la yuca con énfasis en Cuero de Sapo", invited conference presented in the joint activity ICA-CIAT to alert about FrogSkin disease in Colombia in Sincelejo (Sucre), 29-30 March 2000, Villavicencio (Meta), 28 April 2000, Quilichao (Cauca), 2 June 2000, Barranquilla (Atlántico), 18-19 July 2000, Espinal and Neiva (Tolima), 25-27 July 2000, and Saravena (Arauca), 10-11 October 2000.

Torres, A.M. "Evidencia bioquímica de patrones fitogeográficos y taxonómicos en *Stylosanthes guianensis*", Reunión de la Asociación Colombiana de Herbarios (ACH), Manizales, 14 September 2000.

Roa, J.C. "Estudios preliminares sobre el uso de la técnica de microinjertación para indizar germoplasma de yuca *(Manihot esculenta* Crantz)." XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, 1 September 2000.

Velásquez, E. "Conservación *in vitro*: una alternativa segura para preservar especies silvestres de *Manihot"*, II Congreso Nacional de Conservación de la Biodiversidad. Pontificia Universidad Javeriana, Bogotá, 19 – 22 October 2000.

6.5. List of international and national courses with input from Project Staff in 2000

- "Conservación *ex situ* de Recursos Fitogenéticos IPGRI-CIAT-ICA", CIAT, 13-17 December, 1999.
- "Gerencia de Proyectos y Herramientas para la Conservación y Valorización *ex situ* de la Agrobiodiversidad", CIAT, 6-23 June, 2000.
- "Curso Internacional sobre Sistemas Modernos de Producción y Procesamiento de Yuca", CIAT, 23 Octubre - 10 November 2.000.

6.6. List of trainees trained by Project Staff in 2000

Cryoconservation Lab (with Biotechnology Research Unit)

Arzuza, Maria Cristina, Training in cassava tissue culturing, Corn Product International, Barranquilla, Colombia, 9-20 October 2000.

Gámez, Rocio Margarita, Training in conservation and management of *in vitro* cassava germplasm. CORPOICA-Tibaitatá, Colombia. 26-28 July, 2000.

Hernández, Carlos, Low cost multiplication systems, FIDAR, Cauca, Colombia, 16 May-31 December 2000.

Salgado, Catalina, Training in tissue culturing, Universidad del Cauca, Popayán, Colombia, 14-19 February, 2000.

Villada, Daniel, Low cost multiplication systems, FIDAR, Cauca, Colombia, 16 May-31 December 2000.

In vitro Lab

Fuenmayor, Francia. Training in conservation and management of *in vitro* cassava germplasm. FONAIAP, Venezuela. 19-26 October 1999.

Gámez, Rocio Margarita, Training in conservation and management of *in vitro* cassava germplasm. CORPOICA-Tibaitatá, Colombia. 26-28 July, 2000.

Pricien, Joseph. In vitro cassava germplasm. ORE. Haití. Agosto 1, 2000|

Electrophoresis Lab

Medina, Clara Inés, CORPOICA, Programa Nacional de Recursos Fitogenéticos), CI La Selva, Rionegro, 25 Octubre-05 Diciembre, 1999.

Fuenmayor, Francia. Training in Conservation and Management of In Vitro Cassava Germplasm. FONAIAP, Venezuela. 19-26 October 1999.

Seed Conservation and Viability Labs

- Technical staff of the conservation and viability group. "Producción y conservación de semillas", CIAT, 14-19 May, 2000.

- Sánchez, Rodolfo and Quiroz, Luís, CATIE. Conservation and viability techniques of ortodox seeds. CIAT, 8-18 July 2000.

Seed Health Lab

Fuenmayor, Francia. Training in conservation and management of in vitro cassava germplasm. FONAIAP, Venezuela. 19-26 October 1999.

Gámez, Rocio Margarita, Training in conservation and management of in vitro cassava germplasm. CORPOICA-Tibaitatá, Colombia. 26-28 July, 2000.

6.7. Posters

Roa, J.C., Flor, N.C., Ramirez, J.L., Mafla, G., Pineda, B. & Debouck, D.G. 2000. Evaluación de tres tipos de injertos utilizados en la indización de yuca. Memorias XXI Congreso Nacional de Fitopatología, ASCOLFI, Palmira, 31 August 2000.

Guzmán, F.A., Sánchez I., Cárdenas H. & Debouck, D.G. 2000. Control de la erosión genética en un banco de germoplasma. XVIII Congress of SOMEFI, Irapuato, Guanajuato, México, 15-20 October 2000.

6.8. Visitors

The Professional Staff of the Genetic Resources Unit attended the visit of 73 institutions of different countries, for a total of 604 people during the period from October 1999 to September 2000.

A total of 326 people from 16 institutions visited the tissue culture laboratory.

6.9. Donors

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

Ministerio de Agricultura y Desarrollo Rural, República de Colombia

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

Directorate of International Cooperation for Development DGIS, Belgium

6.10. Awards

Ing. Agr. Norma Cristina Flor, 3rd rank in the Otto de Greiff award for her thesis research "Dinámica de la virulencia y estructura genética de *Pyricularia* grisea sacc. del arroz *Oriza sativa* L. en el tiempo".