ANNUAL REPORT 2004
CIAT Project on Saving Agrobiodiversity
SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses

CIAT
OCTOBER, 2004

Table of Contents Page

Project SB-01/02: Conservation and Use of Neotropical Genetic Resources

1. Project Description

Objective: To preserve the Designated Collections and employ modern biotechnology to identify and use genetic diversity for broadening the genetic base and increasing the productivity of mandated and selected non-mandated crops.

Outputs:
1. Improved characterization of the genetic diversity of wild and cultivated species and associated organisms.
2. Genes and gene combinations used to broaden the genetic base.
3. Mandated crops conserved and multiplied as per international standards.
4. Germplasm available, restored, and safely duplicated.
5. Designated collections made socially relevant.
6. Strengthen NARS for conservation and use of Neotropical plant genetic resources.
7. Conservation of Designated 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 through biotechnology tools. Sources of disease and pest resistance will be identified for current and future efforts in germplasm enhancement and plant breeding.

Milestones:

2003 Efficient transformation system devolved for beans. Transgenic cassava tested for resistance to stemborer. Bioreactor technology implemented for cassava and rice. Markers developed for iron and zinc in beans. Collaboration with public and private partners strengthened. Advanced backcross populations of rice characterized. Protocols for cryoconservation of seeds and tissue germplasm established. Germplasm collections regenerated. Safe-duplication and restoration continued.

2004 High throughput screening of germplasm bank and breeding materials implemented, using microarray technology. Aluminum tolerance in Brachiaria characterized. Marker-assisted selection for ACMV and whitefly resistance initiated. Transgenic rice resistant to a spectrum of fungal diseases. Development of insertion mutagenesis population in rice, using Ac/Ds. Gene flow studies for bean and rice completed. Links with conservation efforts in protected areas and on farms established. Germplasm collections regenerated. Initiation of DNA banks for core collections. Safe-duplication and restoration continued.

2005 Efficient transformation system devolved for cassava. Bean with high iron and zinc tested and transferred to CIAT Africa program. SNP markers developed for bean and implemented for MAS. Targeted sequencing of cassava genome. Isogenic of QTL in rice developed and tested. Gene expression studies. Technology transfer for rapid propagation system to NARS. Testing of Ac/DS population for gene identification

Users: CIAT and NARS partners (public and private) involved in germplasm conservation and crop genetic improvement and agrobiodiversity conservation; AROs from DCs and LDCs, using CIAT technologies.

Collaborators: IARCs (IPGRI through the Systemwide Genetic Resources Program, CIP, and IITA through root and tuber crop research, IFPRI through biofortification proposal and CATIE); NARS (CORPOICA, ICA, EMBRAPA, IDEA, INIA, INIFAP, UCR, INIAs); AROs (USDA-ARS, IRD, CIRAD, Danforth Center, CAMBIA, NCGR, and universities—Cornell, Yale, Clemson, Kansas State, Bath, Hannover, Rutgers, Ghent, Gembloux; biodiversity institutions (A von Humboldt, INBIO, SINCHI, Smithsonian); corporations and private organizations.

CGIAR system linkages: Saving Biodiversity (40%); Enhancement & Breeding (55%); Training (4%); Information (1%).

CIAT project linkages: Inputs to SB-01/02: Germplasm accessions from the gene bank project. Segregating populations from crop productivity projects. Characterized insect and pathogen strains and populations from crop protection projects. GIS services from the Land Use Project. Outputs from SB-01/02: Management of Designated Collections (gene banks); genetic and molecular techniques for the gene bank, crop productivity, and soils (microbial) projects. Identified genes and gene combinations for crop productivity and protection projects. Propagation and conservation method and techniques for gene banks and crop productivity projects. Interspecific hybrids and transgenic stocks for crop productivity and IPM projects.

CIAT:SB-01/02 Project Log Frame (2003-2006)
Project: Conservation and Use of Neotropical Genetic Resources
Project Manager: Joe Tohme (BRU:J.Tohme; GRU: D.G. Debouck)

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumption
Goal: To contribute to the sustainable increase of productivity and quality of mandated and other priority crops, and the conservation of agrobiodiversity	CIAT scientists and partners using biotechnology information and tools in crop research. Genetic stocks available to key CIAT partners.	CIAT and NARS publications. Statistics on agriculture and biodiversity.	
Purpose: To conserve the genetic diversity and ensure that characterized agrobiodiversity, improved crop genetic stocks, and modern molecular and cellular methods and tools are used by CIAT and NARS scientists for improving, using, and conserving crop genetic resources	Information on diversity of wild and cultivated species. Mapped economic genes and gene complexes. Improved genetic stocks, lines and populations.	Publications, reports, and project proposals.	Pro-active participation of CIAT and NARS agricultural scientists and biologists.
Output 1: Genomes characterized of wild and cultivated species of mandated and non-mandated crops and of associated organisms.	Molecular information on diversity of mandated and nonmandated crops species, and related organisms. Bioinformatic techniques implemented. QTLs for yield component in rice, for nutrition traits in beans and cassava and fo Al tolerace in *Brachiaria*.	Publications, reports, and project proposals. Germplasm Availabilty of laboratory information management system (LIMS)	Availibilty of up-to-date genomics equipment and operational funding.
Ouput 2: Genomes modified: genes and gene combination used to broaden the genetic base of mandated and non mandated crops.	Transgenic lines of rice and advances in cassava, beans, *Brachiaria*, and other crops. Cloned genes and preparation of gene constructs. Information on new transformation and tissue culture techniques.	Publications, reports and project proposals. Germplasm.	IPR management to access genes and gene promoters. Biosafety regulations in place.
Output 3: Collaboration with public-and private-sector partners enhanced.	CIAT partners in LDCs using information and genetic stocks. News partnerships with private sector.	Publications. Training courses and workshops. Project proposals.	Government and industry support national biotech initiatives.
Output 4: Mandated crops conserved and multiplied as per international standars.	Germination rates for long-stored materials. Cost per accession/year, compared with other gene banks.	Visits to GRU substations and conservation facilities.	Absence of uncontrolled diseases. Quarantine greenhouse space available at different altitudes.
Output 5: Germplasm available,restored and safely duplicated.	Number of germplasm request received and satisfied annually. Users received germplasm and data. Users asked for novel germplasm and data.	Visits to multiplication plots. Reports on requests and delivery. Number of core collections multiplied and shipped.	Agreement with CIAT holds.
Output 6: Designated collections made socially relevant.	Landrace diversity restored to farmers. Farmers use new varieties. Breeders use novel genes.	Germplasm catalogs. Plant variety registration logs. National catalogs	International collecting possible. Quarantine matters cleared.
Output 7: Strengthen NARS for conservation and use of neotropical plants genetic resources.	NARS germplasm collections conserved. Number of trainees trained at CIAT. Number of universities and NARS using training materials.	Country questionnaires. Courses registered. Distribution and sales of training materials.	NARS and networks willing to cooperate.
Output 8: Conservation of Designated Collections linked with on-farm conservation efforts and protected areas.	Number of case studies and pilot *in situ* conservation projects.	Project documentation. Publications	NARS interested in conservation efforts. Farmers interested in conservation efforts.

SUMMARY ANNUAL REPORT 2004
Genetic Resources Unit
SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Researchers: Daniel G. Debouck, Head, PhD
Alba Marina Torres, Biologist, M.Sc.
Graciela Mafla, Biologist
Julio C. Roa, Biologist
César Ocampo, Biologist, M.Sc.
Orlando Toro, Technician
Arsenio Ciprián, Technician
Roosevelt Escobar, Biologist
Benjamin Pineda, Ing. Agr., M.Sc
Norma Cristina Flor, Ing. Agr.
Maria del Socorro Balcazar, Bacteriologist
Jesús M. Salcedo, Biologist
Danny Mauricio Montero, System Engineer
Rosa I. González, Bacteriologist

3.2. Partners/ Cooperators:

Within CIAT:

Steve Beebe (IP-1), Matthew Blair (IP-1), Lee Calvert (IP-2), Hernán Ceballos (IP-3), Elizabeth Alvárez (IP3-IP1), Andrew Jarvis (PE-4), Carlos Lascano (IP-4), Zaida Lentini (SB-02), John Miles (IP-4), Michael Peeters (IP-4), Joe Tohme (SB-02).

Outside CIAT:

MSc. Rodolfo Araya, University of Costa Rica, Costa Rica
Dr. Hans Jorg Jacobsen, University of Hannover , Germany
Dra. Inés Sánchez, CORPOICA, Colombia
Dr. Mario Lobo, CORPOICA, Colombia
Dr. Samy Gaiji, SINGER, IPGRI, Italy
Dr. Jane Toll, SGRP, IPGRI, Italy
Dr. Jean Henson, ILCA, Ethiopia
Dr. Bonwoo Koo, IFPRI, USA
Dr. Ramón Lastra, IPGRI – Americas, Colombia
Dr. M. Hermann, IPGRI – Americas, Colombia
Dr. Patricia Kolef Osorio, CONABIO, Mexico
Dr. Ana Lorena Guevara, INBio, Costa Rica
Dr. Katy Williams, USDA, USA
Dr. Molly Welsh, USDA, USA

Genetic Resources Unit work breakdown structure

Project Goal
To improve conservation efforts in order to increase the social benefits of conservation practices

Project Purpose
To integrate ex situ and in situ conservation

Subproject 1:	**Subproject 2:**	**Subproject 3:**	**Subproject 4:**	**Subproject 5:**
To make the collection designate meet international standards (i.e. viability, quantity and plant health aspects).	To make the collection-designated available to users (i.e. farmers, breeders and agronomists).	To make the collection-designated fully relevant to the purposes of conservation.	To contribute through training to capacity building in conservation sciences and techniques in the region.	To develop in situ methodologies for farmer landraces and wild relatives.
Outputs	**Outputs**	**Outputs**	**Outputs**	**Outputs**
Improve conservation techniques. 3.000 accessions fully regenerated. Status collection-designate improved against vulnerability Alternate liable and affordable conservation methodologies developed.	Sets of germplasm restored to NARS. Refined core collections including novel materials. Data (passport, characterization and evaluation) made available to users. Available spectrum of agrobiodiversity widened	Novel materials acquired or collected. Genetic erosion monitored and documented. Improved core collections in terms representativeness.	NARS human resources trained. Public awareness products developed. Workshops and technical courses carried out. Communications presented in international symposia.	Project proposals on in situ conservation prepared. CIAT contribution made to five genetic reserves in LA. Landrace diversity in traditonal farming systems monitored.

Genetic Resources Unit Logical Framework
DebouckSub-Project #1: the International Standards
Head: Daniel G. Debouck

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

3.3. Financial Resources

Source	Amount (US$)	Proportion (%)
Unrestricted core	318,346	39
Carryover from 2003	115,021	14
Sub Total	**433,367**	
Special projects		
Gene Flow BMZ	25,199	3
Upgrading Plan Operations	343,800	42
Sub Total	**368,999**	
TOTAL	**802,366**	**100**

3.4. Highlights in 2004

Activity area # 1: the International Standards

In 2004 GRU has dubbled its investment in digital images to be made available through CIAT web site. The association of botanists of Colombia has welcomed the idea of including images of voucher specimens of species previously identified by specialists. The entire collection of cassava designated germplasm (5,759 clones) is now being tested in presence of a growth retardant in view of sending a back-up at CIP and reducing operation costs at HQ in the future. 88% of the cassava core collection is now being kept in liquid nitrogen, as as step towards the full collection maintained in cryoconservation. In line with the Upgrading Plan supported by the World Bank (and coordinated by SGRP of the CGIAR), GRU has increased substantially its operations in Quilichao, Popayán and Tenerife. An upgrading of the facilities (moving shelving system in cold store, improved drying room, growing room with controlled conditions for *in vitro*) also took place.

Activity area # 2: the Germplasm and its data available

A total of 8,274 accessions were distributed during 2004. This figure is slightly higher as compared to last year, because of important initiatives by CIAT commodity projects (e.g. the Biofortification Project, the Generation Project) and partners. It shows that interest into the Designate Collections maintained by GRU continues on the high side, and that it will be highly justified to sign an agreement with the Governing Body of the International Treaty (international law since June 29, 2004). Improvements made to CIAT web site (namely the increase of the descriptors and data fields for consultation) to speed up consultations by internet users will facilitate access to the information on germplasm and from there access to the collections themselves. 83% of the entire cassava collection is now certified against viruses of quarantine importance (and thus fully available), while research to bio-control seed-borne fungi of quarantine importance in forage grasses has progressed.

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

An important research effort has been launched to trace duplicated accessions in the entire cassava collection, starting with the collection from Colombia (1,986 clones). This is for money

saving reasons, considering maintenance costs in the mid-/ long-term, but also in view of future acquisitions of cassava germplasm in line with the International Treaty. Once the methodology has been tested successfully on the cassava collection, it will then be applied to the common bean collection.

Activity area # 4: the International cooperation and capacity building

Through a partnership involving GRU, IPGRI Americas, Universidad Nacional of Colombia and RedCapa of Brazil, CIAT has launched a new way of training: distance education through internet, about *ex situ* conservation (over 100 applicants, in Spanish). Conferences and scientific communications were presented in 17 fora, namely the 20th anniversary of SOMEFI, Mexico and the 25th anniversary of ASCOLFI, Colombia. Four courses with input of GRU Staff were run in 2004. Ten publications by Staff were published during the year. Input has been provided to the design of the new system of gene banks of Mexico.

Activity area # 5: the Link with *in situ* Conservation on farm and in the wild

Phase 1 of the Gene Flow Project supported by BMZ of Germany has concluded. Phase 1 has shown that in the bean model in Costa Rica gene flow between the crop and its wild relative occurs, though preferentially from the wild form into the cultivated. The results were obtained through molecular markers based on both nuclear and cpDNA genes. Gene flow on station in Costa Rica using commercial lines was demonstrated to be low, usually below 1%; ways of computing the data seem significant with higher percentages when one computes the number of hybrid plants instead of hybrid seeds. In farmers'plots a higher outcrossing was found when using landraces as compared to commercial lines. A morphological study of stigma areas between wild forms, traditional landraces and modern cultivars has shown a larger terminal area in the wild while cultivated forms have a bigger internal area.

We have continued to invest in the collation of data about geographic distribution of populations of wild relatives of bean, cassava and rice in the New World tropics. During this year, we visited CHAPA, CR, ENCB, F, INB, and P. These data will be used during the pdf-B preparation of the pilot for the GEF starting project "Conservation and sustainable use of Neotropical wild relatives of crops through an integrated understanding of functional diversity".

3.5. Problems encountered and their solution

Limiting factors (personnel, finances) mentioned in last year Annual Report persist to a large extent. For the upgrading of the facilities (e.g. new *in vitro* subculturing room, shelving system, alarms), problems in delivery on time and as per agreed terms have been faced with high frequence. Apart from contracting in the US or Germany, it is not clear what GRU can do, as these services are not often required on the Andean market. Imports (reagents for the lab, equipment for the Upgrading, official mail) from Miami have been noted with delays (frequency of delays, duration of delays).

3.6. Plans for next year

- Complete the database consultation process from the field stations (Quilichao, Popayán)
- continue to clear backlogs, namely that of the bean collection
- continue with regeneration of bean and tropical forage collections
- send security back-ups to CIMMYT and CIP
- update the web site, namely with evaluation and herbarium data
- expand the cryoconservation to a set of cassava clones beyond the core collection
- initiate Phase 2 of the Gene Flow Project
- publish in full results of Phase 1 of the Gene Flow Project
- make appropriate follow-up to the pdf-B process for the GEF project
- anticipate consequences of the ratification of the International Treaty (e.g. actions towards Peru)
- run international courses as it may be required (e.g. electronic distance education on *ex situ* conservation in English)

3.7. Executive summary

The Upgrading Plan of the CGIAR gene banks is on at CIAT, with 6,777 accessions with aging seeds regenerated. Backlogs have diminished substantially with 2,974 accessions cleared, a priority in view of the entry into force of the agreement with the International Treaty. Special attention has been put on the safety back-ups with agreements with CIMMYT and CIP, also in the research efforts with the entire cassava collection now under slow-growth, 59% of the entire collection kept as 'bonsai', and 88% of the core in cryoconservation. Protocols developed in the past for the conservation *in vitro* or of seed germplasm of wild species of CIAT mandate crops have been successfully applied to native fruit species of Colombia (lulo, papaya, tree tomato), and recently at the request of MADR of Colombia to palm species. Four international/ national courses benefited from inputs by Staff, while a new way of distance education in *ex situ* conservation was launched in the CGIAR, thanks to a novel partnership CIAT- IPGRI Americas- Universidad Nacional of Colombia- RedCapa of Brazil. A special project supported by BMZ of Germany has provided results about gene flow between a crop and its immediate wild relative, with important consequences for the collection and mangement of genetic resources *in situ*, and the handling of transgenical crops.

4. Project performance indicators

1.FLOWS, TECHNOLOGIES, METHODS & TOOLS

1.1. Backlogs cleared
2,974 accessions cleared
1.2. Accessions regenerated
4,083 of beans, 2,694 of tropical forages = 6,777
1.3. Accessions secured in long-term
947 accessions secured
1.4. Accessions in security back-up
No shipment this year (but agreement signed with CIMMYT), 947 accessions added

1.5. Accessions characterized
6,644 (field/ lab) + 6,663 (image bank) = 13,307
1.6. Accessions distributed with passport data
8,274 accessions distributed
1.7. Novel germplasm acquired
139 accessions acquired
1.8. Novel genes identified
One novel phaseolin type discovered
1.9. Support Tools (software in germplasm management; databases available from internet)
see www.ciat.cgiar.org
1.10. Data Bases united/ improved
same

2. PUBLICATIONS

2.1. Refereed Journals: published: 2
2.2. Refereed Journals: submitted: 5 (1 TAG; 2 GRACE; 1 NOVON; 1 PlSystEvol)
2.3. Book Chapters: published: 1
2.4. Published Proceedings: published articles: 7
2.5. Scientific Meeting Presentations: presentations: 17
2.6. Working Papers, Other Presentation or Publications: 6
(see under 6 in full report)

3. STRENGTHENING NARS
(see also under 6 in full report)

3.1. Training Courses: 4
3.2. Individualized Training: 10
3.3. PhD, MSc. and pregraduate thesis students: 5

4.0 RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted

"Gene flow analysis for environmental safety in the tropics", submitted to the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany on May 31, 2004.

"Conservation and sustainable use of Neotropical wild relatives of crops through an integrated understanding of functional diversity", presented to the World Bank for the GEF on August 18, 2004.

5. Progress Report

Sub-project 1. The International Standards

Output 1.0. A computerized management system

Activity 1.0.1. Development of an image bank as support for CIAT website

We have continued with the gathering of digital images of seed types (Figure 1), summing to 11,000 images for the bean collection, accessed through CIAT web site or through the CIAT network (Fig. 2, and Table 1). In 250 cases the variation within accessions has been illustrated too.

During this year 200 images of cassava have been captured with the descriptor "root pulp color"; these are being added to the database for access by internet users. Table 1 shows the distribution of materials represented in the image bank.

Figure 1. Diversity in seed types of *Phaseolus* L. spp.

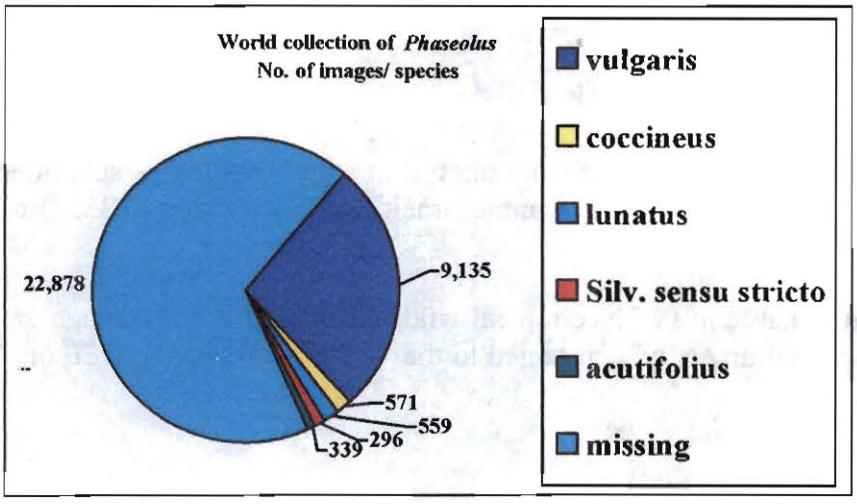


Figure 2. Distribution of materials represented in the image bank.

This year the GRU has added 357 digital images of herbarium voucher specimens to the web site. During the coming months we will continue with this task in order to have the 800 different species available from our collection represented in the web site. Internet users will find a useful taxonomic tool that will help them with the correct identification of the requested material.

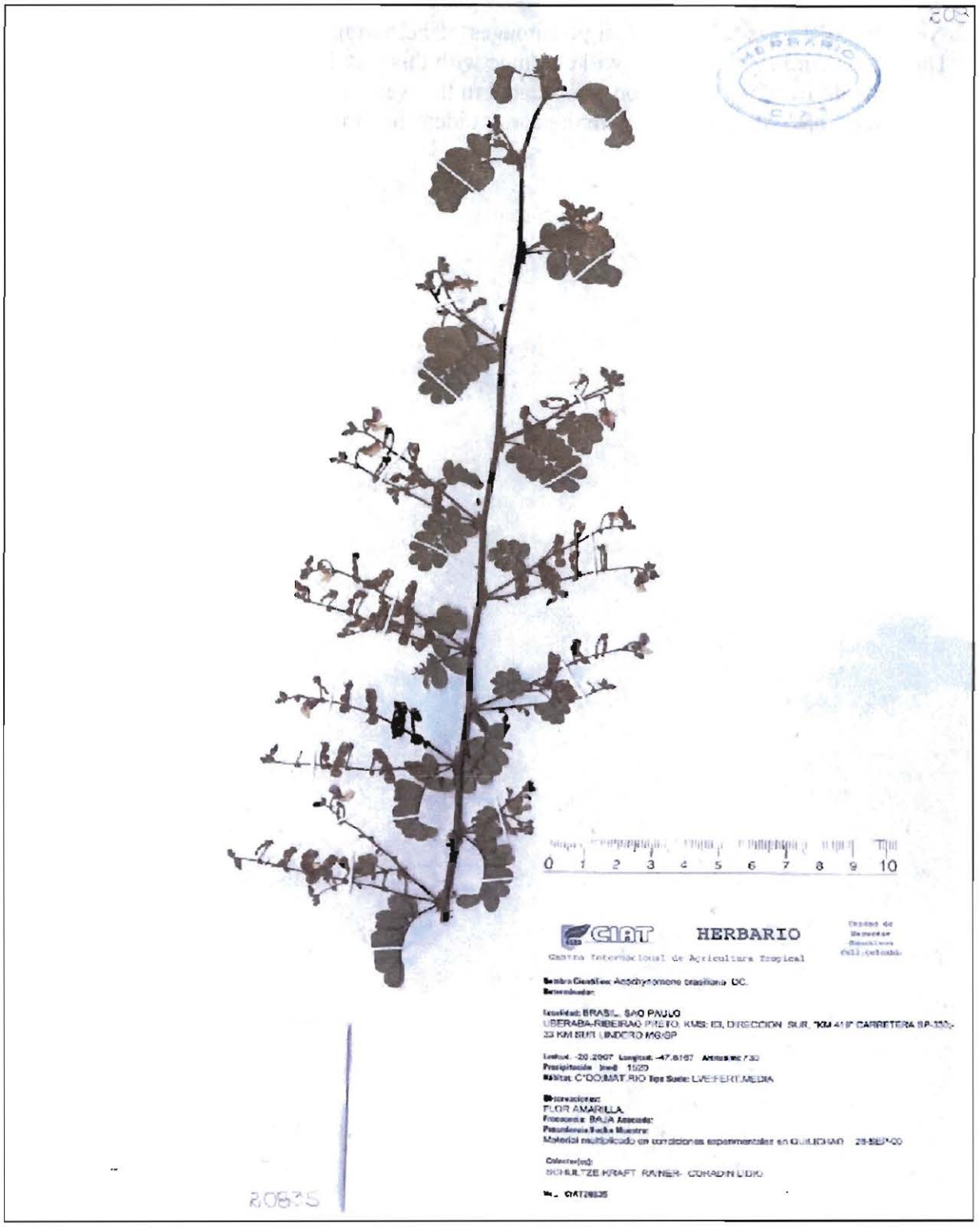


Figure 3. Digital image of the legume forage herbarium material (*Aeschynomene brasiliana*).

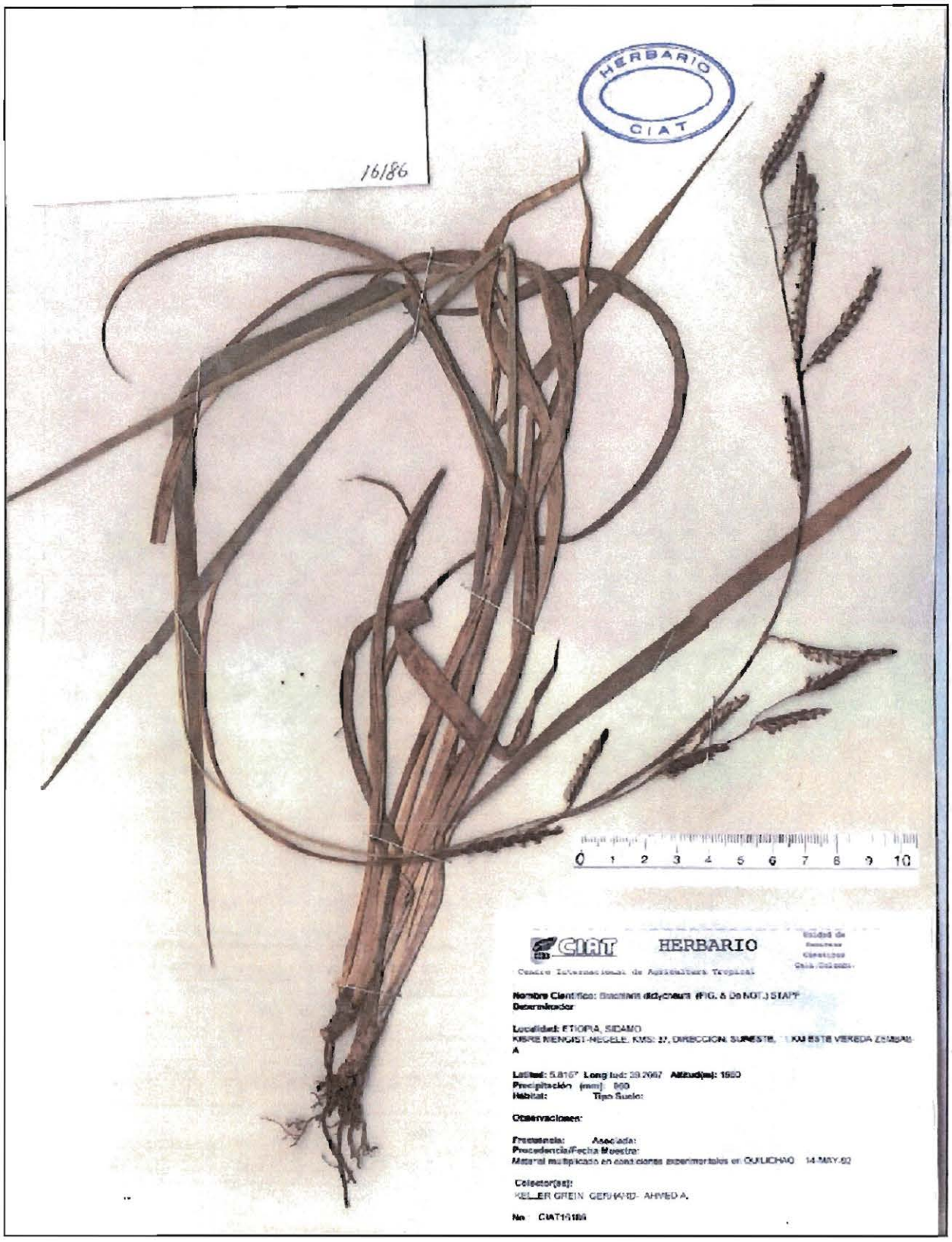


Figure 4. Digital image of the grass forage material herbarium (*Brachiaria dictyoneura*).

Figure 5. Digital image of an herbarium voucher of *Phaseolus lunatus*.

Table 3. Bean backlog processed in 2004.

Description	Bean
Germplasm pending in 2003	12,283
Germplasm processed in 2004	2,573
Pending germplasm	9,710

Contributors: O. Toro, A. Ciprian

Activity 1.1.2. In vitro culturing for rare *Phaseolus* L. spp.

For rare and difficult materials, we use an *in vitro* subculturing technique (done for 22 accessions so far) in order to obtain quickly a higher number of rooted cuttings ready for multiplication in glass-houses (158 plantlets obtained so far) (Fig. 6).

Figure 6. In vitro plants of *Phaseolus* spp.

Contributors : J. C. Roa, O. Toro, G. Mafla.

Output 1.2 Backlogs of materials pending on multiplication multiplied

Activity 1.2.1. Multiplication of materials cleared by quarantine authorities.

In 2004, a total of 2,092 bean accessions were cleared by quarantine authorities, and planted either in Palmira or in Popayán. On the other hand, a total of 1,550 bean plants for the Gene Flow Project were handled as pure lines (for phaseolin and DNA analysis) planted in Popayán. Similarly, 401 accessions of forages were cleared by ICA authorities and directed to the fields in Palmira and Quilichao after a hardening period in glass-houses in Palmira.

Table 1. Number of images available in CIAT web page.

Species	No. of images
P. vulgaris	9,135
P. coccineus	571
P. lunatus	559
P. acutifolius	339
silv. sensu stricto	296
Additional vulgaris (Nuñas & others)	161
Other lunatus	2
Other coccineus	26
Other wild forms	9
Other stocks	14
Tropical forages (plants, seeds)	1,271
Cassava	200
Herbarium material	357
TOTAL	12,940

Contributors: O. Toro, G.Mafla, J.C. Roa, Jesús M. Salcedo, A. Ciprián, D.M. Montero.

Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into the genebank processes

Nine accessions corresponding to elite lines from CIAT Bean Program were registered in the genebank during 2004 (Table2). These materials correspond to differential materials for rust, anthracnose and *Ascochyta* blight that the pathologists use in their research. A total of 130 bean materials coming from separation of segregants were also registered. The backlog was substantially reduced (Table 3) - by 2,573 materials – through multiple checks of passport data and comparisons of morphoagronomic data, and those found of interest (viz. not already existing in the gene bank) were planted. On the other hand, during 2004 we introduced 3,995 germplasm accessions received from CSIRO, Australia.

Table 2. New accessions introduced in the genebank in 2004.

ENT.	GNUMBER	IDENTIFICATION
1	G51308	MEX 222
2	G51309	PAN 72
3	G51310	AND 1005
4	G51311	BAT 1373
5	G51312	CAL 149
6	G51313	DOR 482
7	G51314	DRK 47
8	G51315	FT 83-120
9	G51316	ABA 2

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

Output 1.3 Materials pending on regeneration

Activity 1.3.1. Multiplication of materials with ageing seeds.

Table 4 indicates the total numbers of bean accessions that were regenerated because seed viability reached the threshold.

Table 4. *Phaseolus* bean germplasm processed for regeneration under greenhouse/meshouse (number of accessions).

Localities	Greenhouse/meshouse
Palmira	565
Popayán	1,527
Palmira/Popayán	1,991 (Isotypes-FI)
Total	4,083

Similarly, 2,694 accessions of forages were planted for regeneration purposes (Tables 5, 6).

Table 5. Forage germplasm planted for multiplication and regeneration under greenhouse/meshouse and field conditions (number of accessions).

Localities	Legumes	Grasses	Total
Greenhouse/Meshouse	921	140	1,061
Quilichao	1,619	148	1,767
Palmira	943	207	1,150
Popayan	64	222	286
Total	3,547	717	4,264

Table 6. Forage germplasm processed during 2004.

	Legumes	Grasses	Total
Backlog	2,879	11	2,890
Multiplied from CSIRO, Australia	401	0	401
Regenerated (because of aging seeds)	2,582	112	2,694
Characterized during the process	2,128	41	2,169

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

Activity 1.3.2. Multiplication and Characterization in the field (Tenerife)

A total of 2,248 accessions of bean with growth habit I and II were planted in September 2004 at Tenerife station (2,100 masl) (Fig. 7), corresponding to seed regeneration processes for FAO designated materials.

Figure 7. Planting in Tenerife (Sept. 29, 2004).

Contributors: O. Toro, A.M. Torres

Activity 1.3.3. Periodical subculturing of the FAO designate cassava collection.

This year, 6,543 materials (5,345 accessions) of *Manihot* were subcultured by the nodal cutting technique; the multiplied accessions represent 93.1% of the collection. A total of 9,825 plants (3,094 accessions) were propagated for distribution to users.

Contributors: G. Mafla, J.C. Roa

Status of designated germplasm at the GRU in 2004

In view of progress of ratification of the International Treaty on Plant Genetic Resources for Food and Agriculture, and of a possible agreement with its Governing Body, the status of designated accessions is currently as follows:

Manihot cassava: 5,759
Phaseolus beans: 33,778
Tropical forages: 18,138
Total: 57,705

Ouput 1.4. Materials processed into final packing

Activity 1.4.1. Final drying and temporary storage

Table 7. shows the amount of accessions for beans (4,883) and forages (2,176) respectively, which have been produced, cleaned, dried, and stored at 5°C awaiting results from viability and health tests.

Table 7 . Germplasm in seed processing during 2004.

	Beans	Forages
Seed selection / drying/ temporal storage	4,883	2,176
Total	4,883	2,176

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

Activity 1.4.2. Viability testing.

Table 8 indicates flows of materials during 2004 for viability testing. It shows the importance of good drying and other procedures following the genebank standards (FAO/IPGRI, 1994). Ranges of germination were chosen because viability higher than 65 do allow seed distribution and viability higher than 85 not allow long term seed conservation.

In order to support multiplication activities for very old seeds the viability lab pre-germinated 368 accessions of the backlog of forage, and, 2,164 and 179 accessions of the already seed storaged of forages and beans, respectively, to transplant to the multiplication field lots. Several techniques of pre-germination has been used for success results such us sand beds, petri dishes and germination paper.

Table 8. Viability testing for *Phaseolus* beans, tropical forages and cassava during 2004.

Class	BEANS		FORAGES		CASSAVA	
	Germination (%)	No. Accesions	Germination (%)	No. Accesions	Germination (%)	No. Accesions
Already stored materials	1-64	144	1-64	28	1-64	41
	65-84	152	65-84	17	65-84	0
	85-100	798	85-100	45	85-100	1
Sub-total		1,094		90		42
Recently multiplied materials	1-64	4	1-64	46	1-64	---
	65-84	20	65-84	108	65-84	---
	85-100	203	85-100	651	85-100	---
Sub-total		227		805		
TOTAL		1,321		895		

References:

FAO/IPGRI 1994. Genebank standards. Rome, Food and Agriculture Organization of the United Nations and International Plant Genetic Resources Institute, Rome, Italy: 17 p.

Contributor: A.M. Torres

Activity 1.4.3. Final packing and seed 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 9, 10).

Table 9. Final storage and packing of *Phaseolus* beans processed during 2004 (number of accessions).

	Beans
LONG TERM (Base, duplicates, repatriation, monitoring) and SHORT TERM (Distribution)	274
SHORT TERM only (Distribution)	3,175
Total	3,449

Table 10 . Final storage and packing of tropical forages processed during 2004 (number of accessions).

	Total
LONG TERM (Base, duplicates, repatriation, monitoring) and SHORT TERM (Distribution)	673
SHORT TERM only (Distribution)	969
Total	1,642

Activity 1.4.4. Final packing of seeds of rice materials

A group of 946 rice breeding lines was received from the CIAT Rice Program, dried and conserved in temporary storage. A viability test was done in order conserve this material in long term storage. A total of 539 accessions reached germination between 85 and 100%. The total 946 accessions of *Oryza sativa* were vacuum packed and sealed in aluminium foil bags and conserved in long term (-20 °C).

Contributor: A.M. Torres

Activity 1.4.5. Monitoring viability for wild *Manihot* germplasm stored in the past

This year embryo culture and pre-germination in sand was used to rescue seed of wild *Manihot* species maintained in storage since 1992-1994. Several techniques for seed hydration, desinfection and different culture medium were tested, and results are shown in Table 11.

Table 11. Viability testing for wild *Manihot* germplasm.

Species	Populations (No.)	Plants recovered(No.)/Populations
M. flabellifolia	82	28/21
M. peruviana	13	2/2
M. janiphoides	1	1/1
M. violacea	1	0/1
Total	97	31/25

Contributors: A.M. Torres, G. Mafla

Activity 1.4.6 Monitoring viability of conserved germplasm of seeds of beans and forages

This year we performed the monitoring of seed viability for two groups of seeds of *Phaseolus vulgaris* after 5 years of conservation in long term storage: Ten1996A and Ten1996B. These seed lots were tested in 2002 showing a germplasm health problem in the test and thus repeteated for confirmation. A total of 1,666 accessions were tested and the results are shown in Table 14.

The mean of germination decreased 2.6 units for Ten1996A and 2.4 for Ten1996B after conservation. The difference is statistically significant in all cases with confidence intervals of 95%. The decrease in germination is reducing through the source time. However, the reduction in germination was minor as compared with the test applied for the two seed lots in 2002, with 12.3 and 17.2, for Ten1996A and Ten1996B, respectively. Similarly, the monitoring was done for forage germplasm conserved after 5 years period for a group of 209 forages species conserved during 1999. The results are shown in Table 15. The difference of the mean germination was of 9.3 units. This difference is statistically significant with confidence intervals of 95%. The seed quality of forage had more reduction as compared with bean. The conservation conditions are the same for both seed crops, probably indicating that the source of this variation is to be looked for in some factor of the harvest process.

According to these results, for the seed lots monitored a total of 41 (19%) accessions of forages and 109 (6.5%) accessions of beans have to be refreshed by seed multiplication following the protocols for seed conservation with a germination rate above 85%.

Table 12. Paired T-Test for monitoring bean seeds after 5 years of long-term conservation.

Source	% Germ	Mean	StDev	SE Mean	N	Difference	Std.Dv.Diff	T-value	P-Value
Ten1996A	Initial	97.301	3.523	0.117					
	Monitored	94.743	10.400	0.346	904	2.558	10.750	7.15	0.000
Ten1996B	Initial	97.004	3.752	0.136					
	Monitored	94.556	7.576	0.274	762	2.448	7.741	8.73	0.000

Table 13. Paired T-Test for monitoring forage seeds after 5 years of long-term conservation.

% Germ	Mean	StDev	SE Mean	N	Difference	Std.Dv.Diff	T-value	P-Value
Initial	98.18	5.07	0.35					
Monitored	88.89	14.78	1.02	209	9.29	14.86	9.04	0.000

Contributor: A. M. Torres

Output 1.5. Improved conservation techniques

Activity 1.5.1. Protocol for seed conservation of tropical fruits.

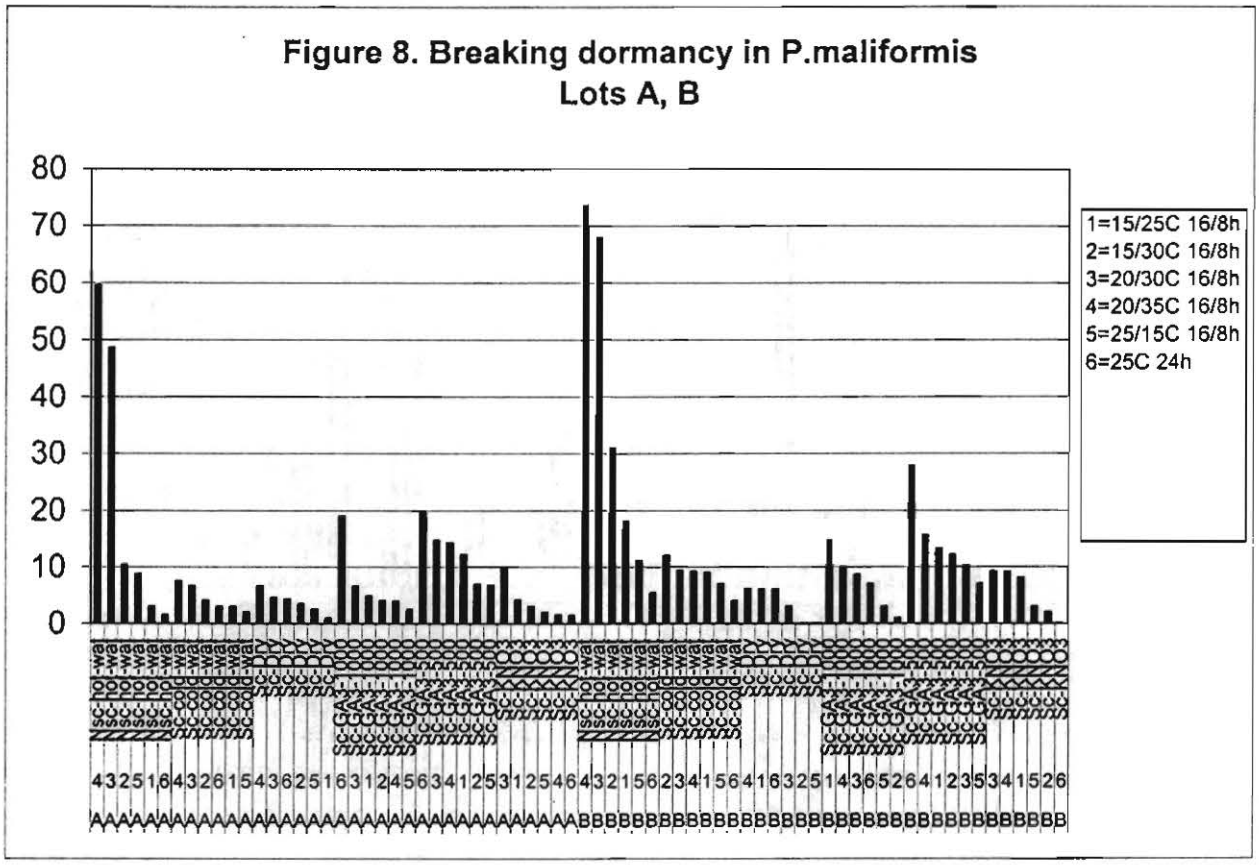
1. Breaking dormancy of *Passiflora maliformis*

The figures for the germination of *P. maliformis* in last year experiments were very low and the hypothesis that germination of this species can be obtained only by chemical treatments and alternating temperatures cannot be accepted. Thus, a new experiment was carried out with two seed lots, following the same procedure for extraction and drying of seeds and the same treatments. One temperature was added (25/15 °C for 16/8 h) to widen the range of temperature regimes up to a total of six. Each non-germinated seed was dissected to discount empty seeds from the total seed sample.

The analysis of variance of the germination shows statistical differences in lot, temperature, treatment and interaction between all factors (Table 14). The highest figure for germination was obtained for the treatment hot water at 20/35 (59.5 and 73.6%) followed by 20/30 °C for 16/8 h (48.6 and 68.0%) in seed lots A and B, respectively (Figure 8). None of these results coincides with the results previously reported for this species.

Table 14. ANOVA procedure for germination (angles) of *P. maliformis* Lots A, B

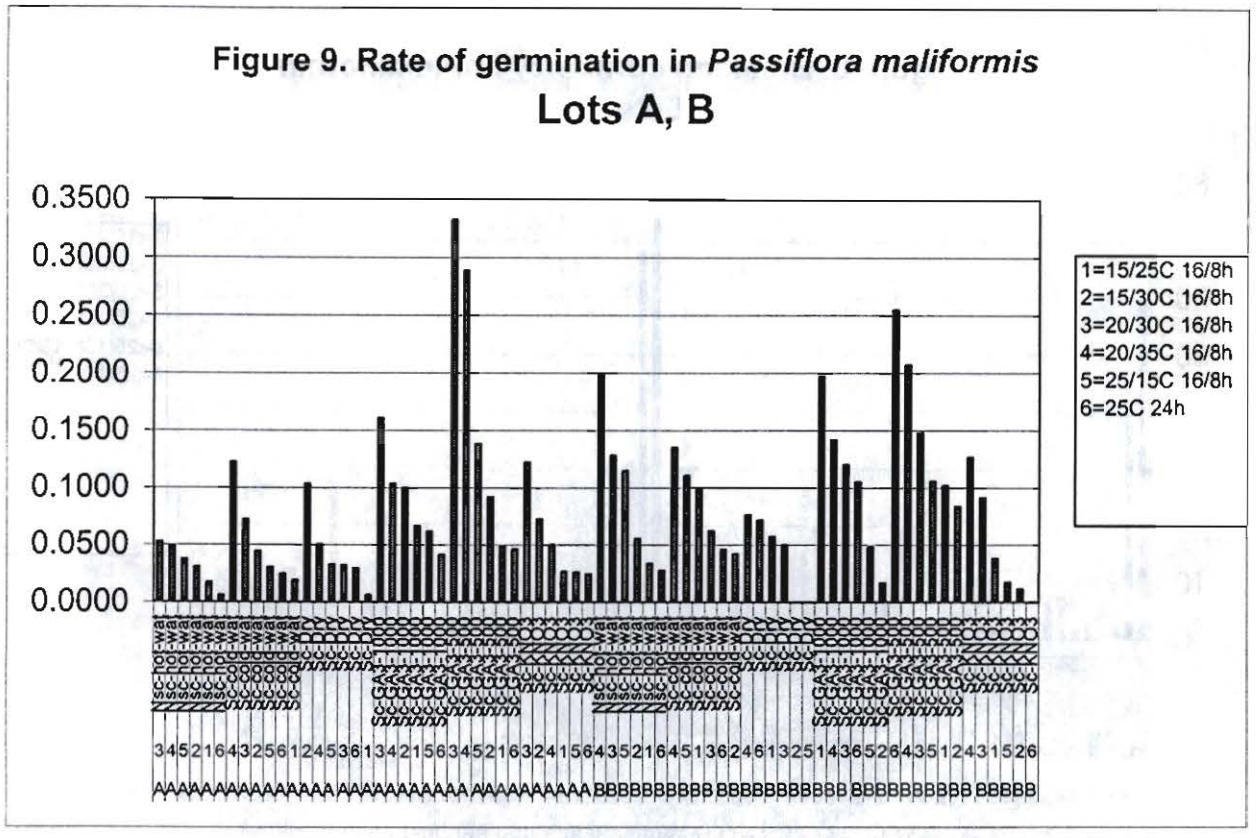
Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	0.13531978	0.13531978	12.05	0.0006
temp	5	1.73873629	0.34774726	30.96	<.0001
treat	5	4.18809044	0.83761809	74.58	<.0001
lot*temp	5	0.13971807	0.02794361	2.49	0.0325
lot*treat	5	0.37059913	0.07411983	6.60	<.0001
temp*treat	25	3.86635965	0.15465439	13.77	<.0001
lot*temp*treat	25	0.46429128	0.01857165	1.65	0.0308



Statistical differences were found in the rate of germination for each seed lot, temperature, treatment and interaction between all factors (Table 15). The highest rate of germination was found for the treatment manually scarified seeds with GA3 500 p.p.m. at 20/30 °C for 16/8 h for seed lot A, and, GA3 500 p.p.m. at 25 °C for 24 h for seed lot B (Figure 9).

Table 15. ANOVA procedure for rate of germination of *P. maliformis* Lots A, B

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	0.01820950	0.01820950	5.94	0.0156
temp	5	0.26289366	0.05257873	17.16	<.0001
treat	5	0.39641296	0.07928259	25.87	<.0001
lot*temp	5	0.10546995	0.02109399	6.88	<.0001
lot*treat	5	0.04218853	0.00843771	2.75	0.0196
temp*treat	25	0.19073714	0.00762949	2.49	0.0002
lot*temp*treat	25	0.22085332	0.00883413	2.88	<.0001

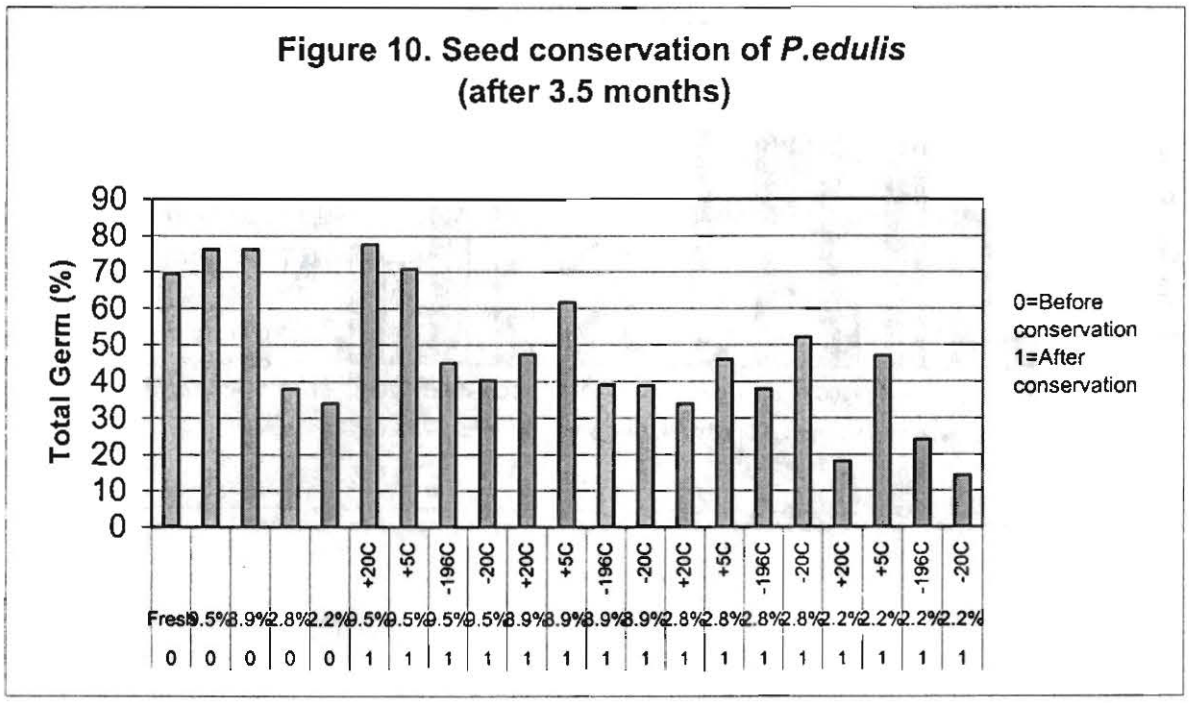


2. Seed conservation of *Passiflora edulis and Caricaceae*

The hypothesis to test was related to the storage behaviour of *Passiflora edulis* and three species of Caricaceae. The seeds of these fleshy fruits can be dried to moisture contents lower than 8% without loosing viability, and can be conserved at low temperatures (+20, +5, -20 and –196 °C) without loosing viability.

Several groups of seeds of these species were extracted and dried at different moisture content to be conserved at four temperature conditions. Germination test was done between roll paper, using 4 replicates of 50 seeds each. Normal and total germination was recorded, every seven days, during 35 days. The figures 3 to 8 show initial germination before conservation and the final germination after 3.5 months of conservation at each temperature.

The results of conservation of *P. edulis* suggest no tolerance to dehydration with low percent of germination for seeds between 2.2 and 2.8% moisture content, before and after conservation (Figure 10). However, the reduction in germination of seeds conserved at 8.9% moisture content in all temperature conditions could indicate a not fully resolved problem, perhaps due to the physical barrier of the hard testa during the germination process. This result coincides with the intermedium storage behaviour suggested with uncertainty for this category (Hong, Linington et al. 1996).



The behaviour of three seed lots of *Carica papaya* (Figures 11, 12 and 13) shows an homogeneous physiological reaction for the seeds at +5, +20 and –196 °C, maintaining the viability reasonably good. However, seeds with low and high moisture content died at –20 °C. Some seeds of each sample stained with tetrazolium salt suggesting a degree of dormancy induced by freezing effect or showing vital but not vigorous seeds.

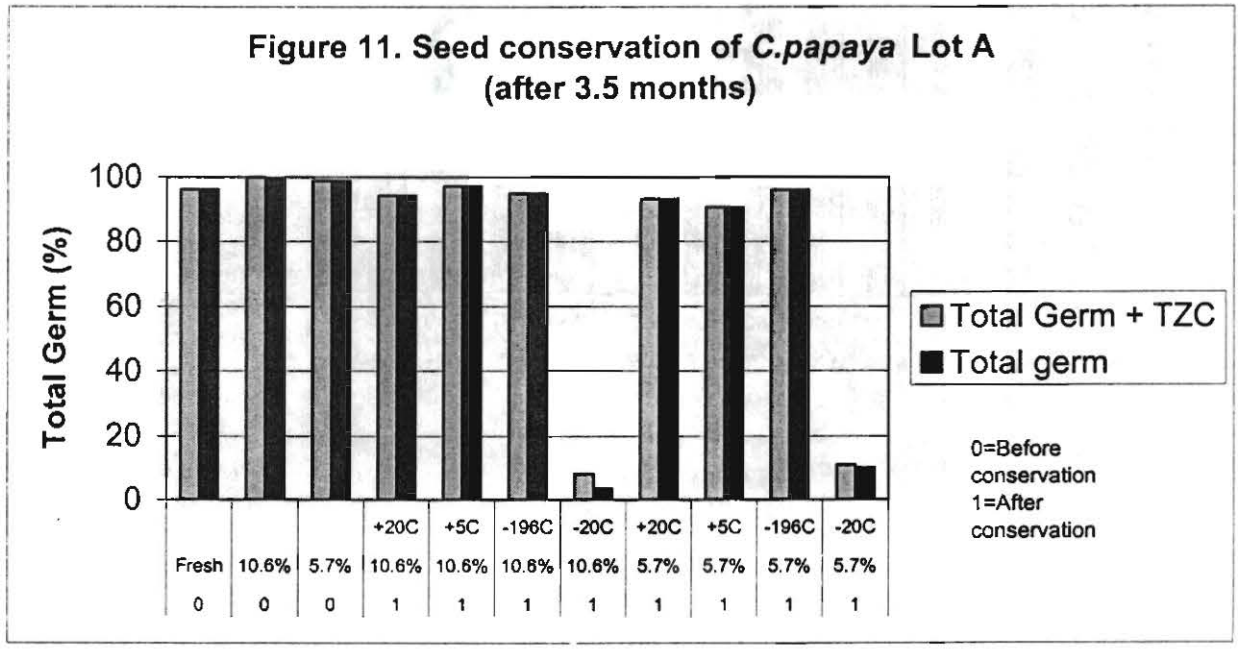


Figure 12. Seed Conservation of *C.papaya* Lot B (after 3.5 months)

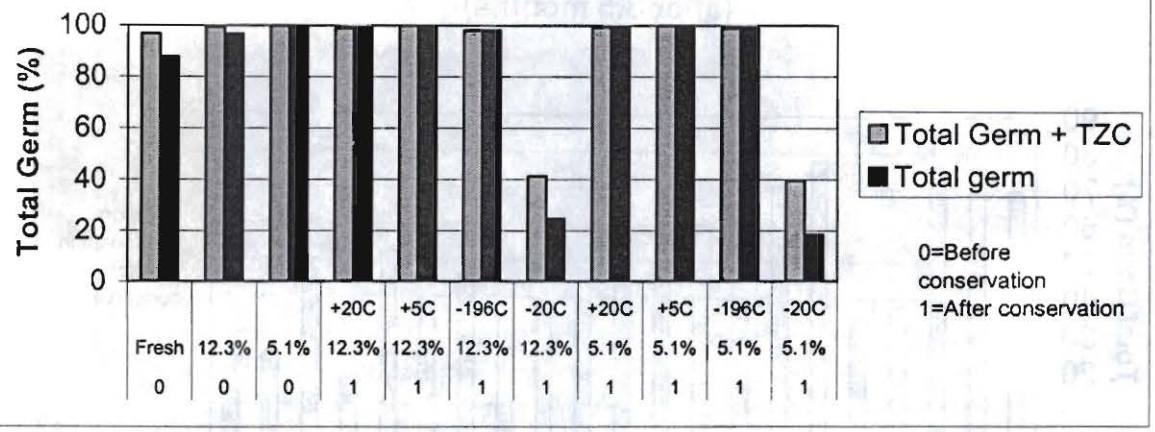
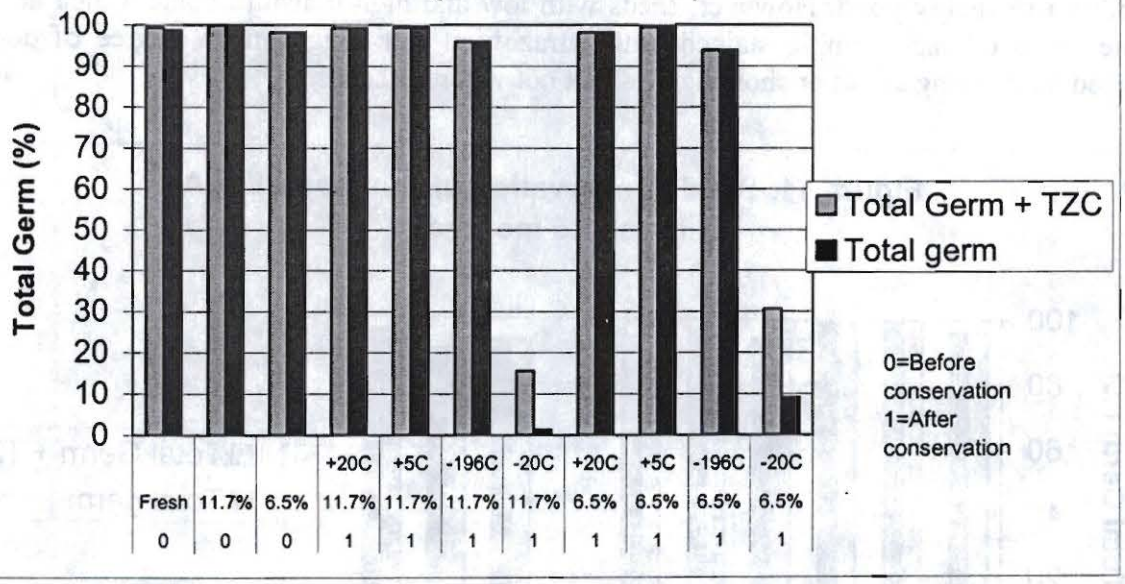
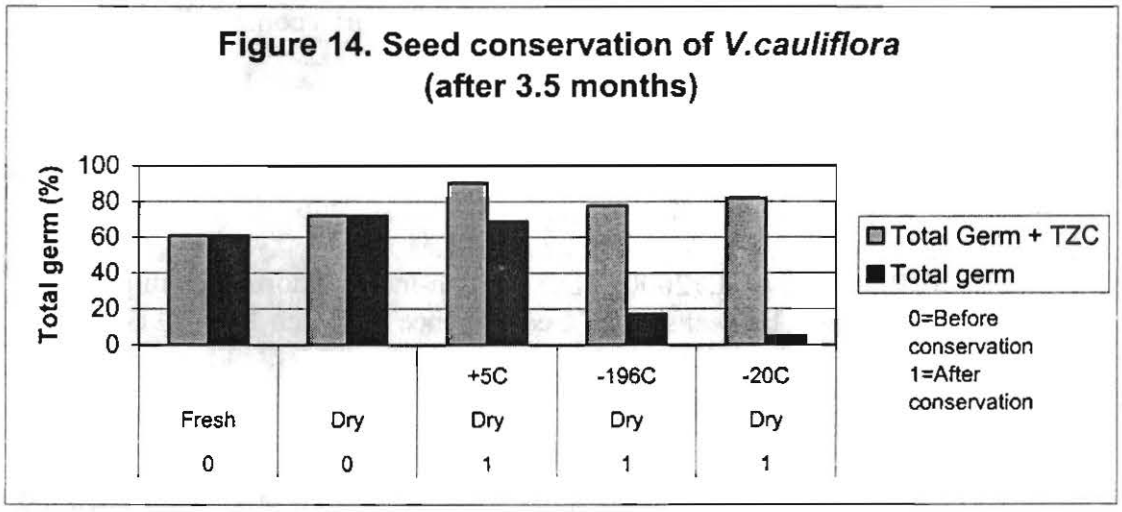
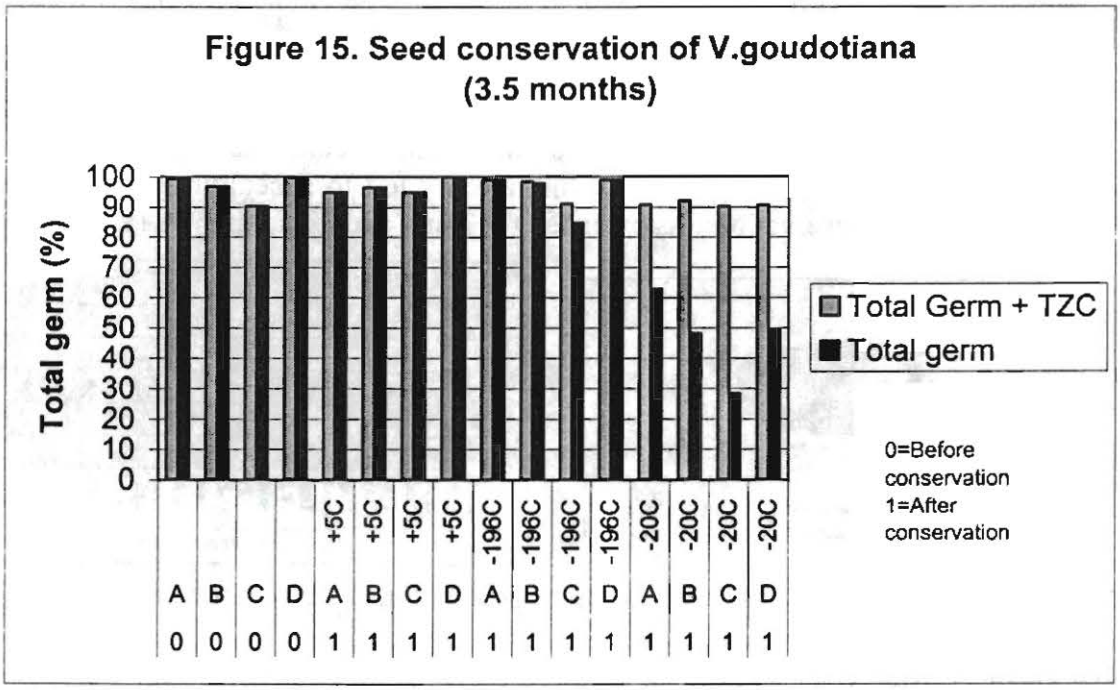


Figure 13. Seed conservation of *C.papaya* Lot C (after 3.5 months)



The same pattern of seed storage behaviour was found for the wild Caricaceae species, *Vasconcellea cauliflora* and *V. goudotiana*, Figures 14 and 15, respectively. For those wild species the conservation at –20 °C shows a decrease in the viability of the seeds. Going along with the cultivated species *C. papaya*, part of the population seems to survive the cooling but does not respond to germination stimuli, while staining positively with tetrazolium salt.





The results of conservation of *C. papaya* confirm the intermediate storage behaviour of seeds of this species found by some authors (Ellis, Hong et al. 1991; Sangakkara 1995; Wood, Pritchard

et al. 2000). The reaction of seeds of *Vasconcellea* species is a new record of intermediate storage behaviour of the wild relatives of the paw paw fruit in the plant family Caricaceae.

References

Ellis, R. H., T. D. Hong, et al. (1991). "Effect of storage temperature and moisture on the germination of papaya seeds." Seed Science Research **1**: 69-72.

Hong, T. D., S. Linington, et al. (1996). Seed storage behaviour: a compendium. Rome, International Plant Genetic Resources Institute.

Sangakkara, U. R. (1995). "Influence of seed ripeness, sarcotesta, drying and storage on germinability of papaya (*Carica papaya* L.) seed." Pertanika Journal Tropical Agriculture Science **18**(3): 193-199.

Wood, C. B., H. W. Pritchard, et al. (2000). "Desiccation-induced dormancy in papaya (*Carica papaya* L.) seeds is alleviated by heat shock." Seed Science Research **10**: 135-145.

Contributor: A.M. Torres

Activity 1.5.2. Determining the effectiveness of silver nitrate on slow growth of the cassava collection.

A total of 2,879 cassava varieties from 26 countries were planted in 2003 in AgNO3 medium in order to see whether silver nitrate was able to extend the time of conservation between subculturings, or not (Mafla et al, 2003).

We have found that silver nitrate allowed to extend the time of conservation in 36% of the selected varieties. Until now these clones are still in this medium and have not yet gone to subculturing. The other 64%, in spite of having already left to subculturing, always showed a better appearance and conservation was extended for 3 or 4 additional months (Figure 16).

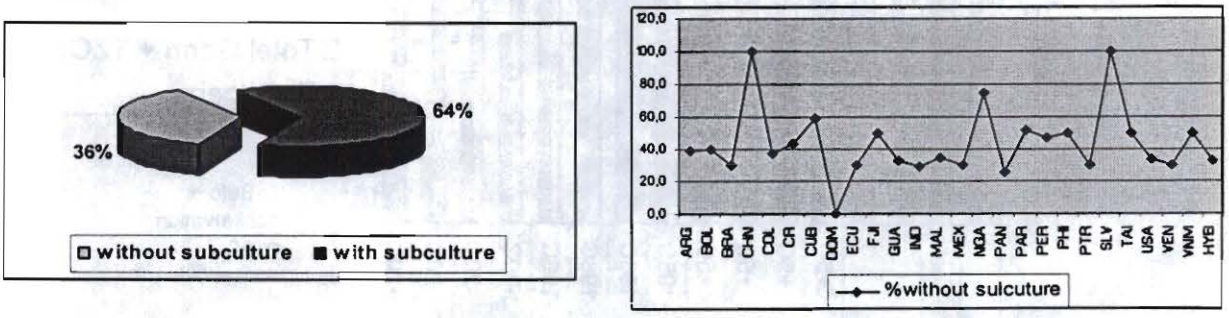


Figure 16. Response of part of the in vitro cassava collection to silver nitrate.

Contributors: G, Mafla and J.C. Roa

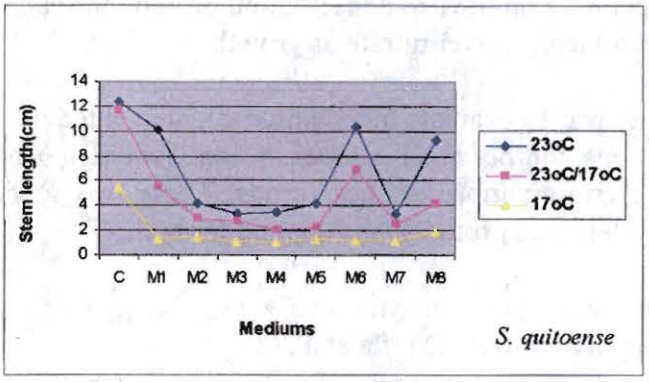
Activity 1.5.3. Effect of temperature on *in vitro* conservation of lulo (*Solanum quitoense*) and tree tomato (*Solanum betaceum*) using silver nitrate as growth retardant.

The main purpose of this study was to evaluate the medium supplemented with silver nitrate with different temperature conditions for *Solanum quitoense* and *Solanum betaceum*, in order to extend the period of *in vitro* conservation for these crops. These two species are reported as moderately cold tolerant, and the same protocol was applied for both.

- Minimal growth medium was based on Murashige and Skoog (MS) supplemented with different concentrations of silver nitrate (Mafla et al, 2002).
- The temperature regimes were: a) 23 °C (continuing), b) 23°C (for 4 weeks after planting) and then 17°C (continuing), and c) 17°C (continuing).

Results

As expected, the growth *in vitro* of the plantlets was higher with the increase of temperature during conservation. The plantlets maintained at 23°C showed growth rate higher than rate for plantlets kept under 17°C. Figure 10 shows the growth retardant effects of silver nitrate and temperature on Lulo (*Solanum quitoense*) and tree tomato (*Solanum betaceum*) microplants cultured for 9 months at 17°C. The silver nitrate in combination with temperature was effective in gradually decreasing stem length in relation to the control (23°C). Storage temperature at 17°C inhibited more the growth of *Solanum quitoense* as compared to *Solanum betaceum*.
After 9 months of conservation, the plantlets of *Solanum betaceum* showed strong green color, low level of browning, normal root development when grown at 17°C and in presence of silver nitrate (M6) and the plants were perfectly healthy to be kept under the same conditions without subculturing.
The evaluations continue in order to see when subculturing will be required.



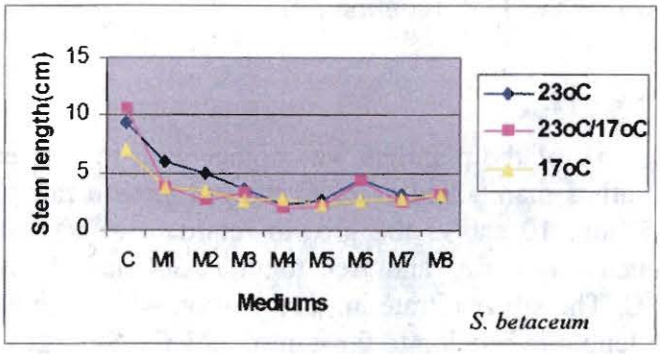


Figure 17. Effect of temperature and silver nitrate on stem elongation (cm) in *S. quitoense and S. betaceum*.

References

Mafla, G., Roa, J.C. and Debouck D.G. 2002. In vitro conservation of tree tomate (*Solanum betaceum*) and lulo (*Solanum quitoense*) using ancymidol and silver nitrate as growth retardants, Annual Report 2002, CIAT Project SB-1, pp 39-41.

Contributors: G. Mafla, J.C. Roa

Activity 1.5.4. A comparison of different culture vessels for shipment of *Manihot* security backups.

We started this study last year in order to set the most effective system for shipping germplasm abroad. Five systems of storage were tested with two different mediums (8S, NP). After twelve months we observed that the medium with addition of silver nitrate (NP) and any vessel described in this study resulted in longer conservation periods.

The storage system using polypropilene tubes (30 x 115 mm) and silver nitrate medium (NP) was evaluated with another 68 varieties and after 12 months we observed that 97% of the materials were in good condition.

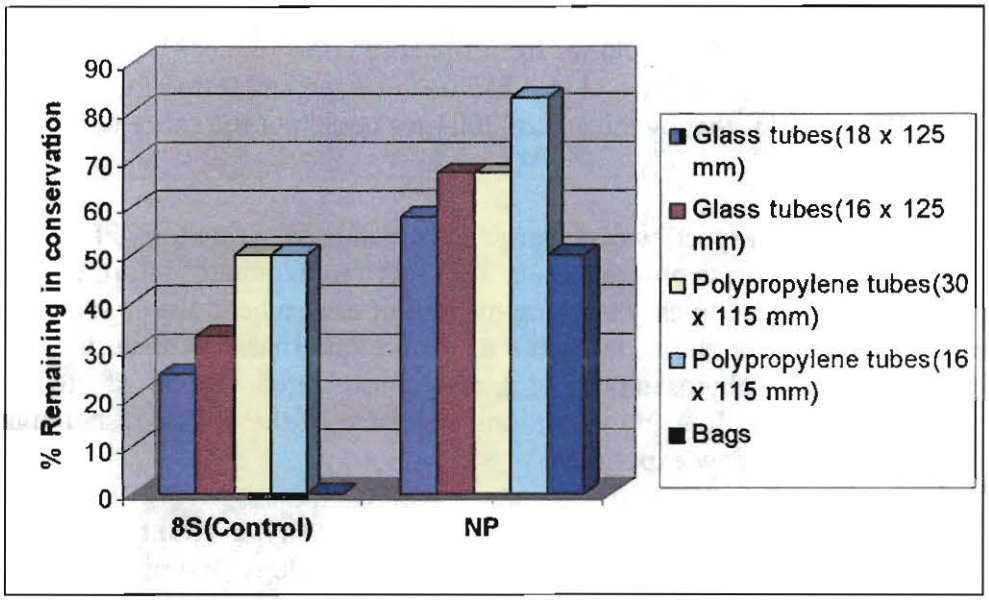


Figure 18. In vitro cassava stored for 12 months in different culture vessels.

Contributors: G. Mafla, J. Roa

Activity 1.5.5. Logistic aspects involved in the management of an *in vitro* cassava collection in liquid nitrogen.

Introduction:

Before putting the cryopreservation protocol into practice on the entire collection we carried out a pilot project on the core collection. This allowed us to adjust the logistical aspects involved in the management of a complex collection and to give a general idea about how much effort, input, resources and manpower will be necessary for its full implementation.

Material and Methods

The encapsulation-dehydration methodology was implemented (Annual Report, 2000) using *in vitro* plants supplied by GRU; and previously intensively micropropagated on 4E medium (Roca, 1984). This process must be done until enough shoots/clone, 80-100 explants, are obtained.

Results

This year we received 153 clones from GRU. Starting with this material and after 2-3 subcultures we obtained 15,120 new explants to be incorporated in the freezing scheme. We maintain a continuous process that incorporates 8-12 new clones into the process per week. Currently we normally include 6 tubes per clone with 10 shoots per tube. Two of these tubes are used as controls (to be taken out after 1 and 3 months of conservation in liquid nitrogen) and 4 tubes for long-term conservation.

Five hundred and fifty-seven (557) clones from the core collection (88%) were maintained in a cryo-tank. Four hundred and fifty-two clones had been frozen and tested for conservation during different times. We consider that by the end of 2004 we could put the entire core collection under L.N. conditions.

As part of the logistical aspect, we constructed a database written in PHP and JavaScript languages with Mysql data base programming. This software facilitates information management across experiments and gives an easy tracking method of each tube in the cryotank. Additionally, it gives specific information about the status of each clone (passport data, morphological and isozyme characterization, phytosanitary, etc) and consolidated reports of stock per freezer, behavior of each clone after freezing conditions and a list of the lowest-responding clones that must be repeated again in a new experiment.

Nine CEF clusters from African clones supplied by Nigel Taylor (Danford Center. Mo-USA) were put under liquid nitrogen conditions following methodology described by Santos in 2002. Currently those clusters are growing on recovery media to test the recovery rate after 1 month on L.N. Controls are growing on GD250Pi.

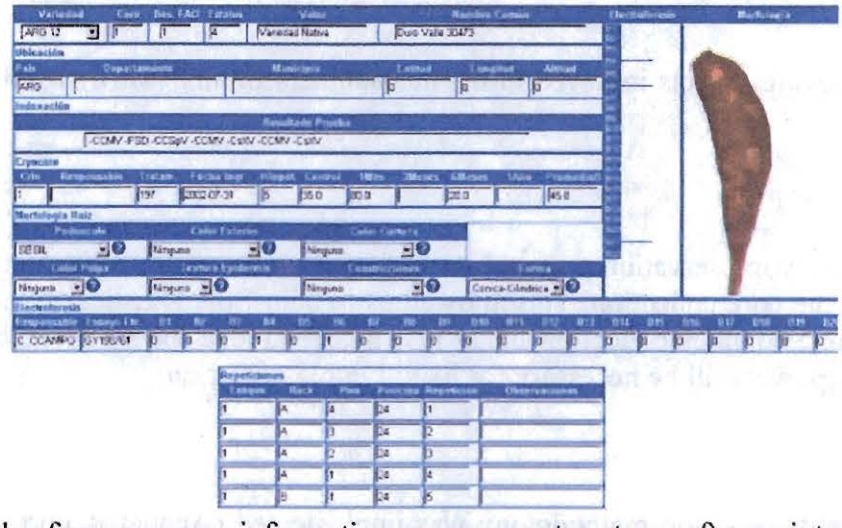


Figure 19: Details of cassava cryo-information management system software interface.

DNA analysis by AFLP was done using frozen tissues [they were maintained under L.N for different periods (from 1hour to 12 months) of conservation] and their control without freezing. Non-significant differences were observed with 6 polymorphic combinations (Figure 13). Clones have more than 96% similarity between non- and frozen tissues.

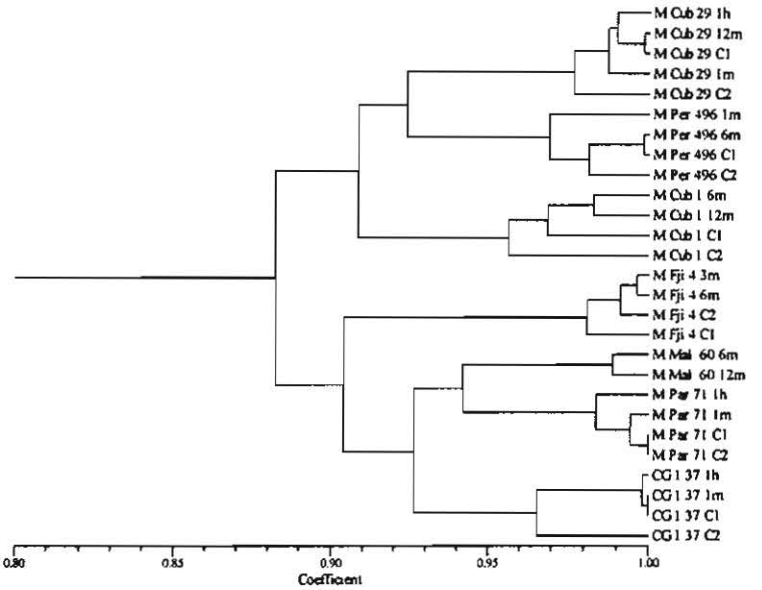


Figure 20: Dendrogram of similarity among frozen clones recovered after different times and its control without freezing.

Conclusions:

- If putting the entire cassava collection under L.N. conditions is under consideration, how to adjust the propagation facilities must be given through.
- We maintain more than 91.6% of the core collection in a state of cryopreservation.
- It was possible to establish a basic protocol for the management of a cryobank using the core collection as a model.
- Different markers were analyzed (morphology, isozyme and DNA based markers) and did not show changes associated with frozen steps per se.

Future activities:

- Discuss the implementation of cryopreservation on the entire cassava collection.
- Adjust the protocol to the lowest responding clones.
- Recover CEF after freezing.

References:

Escobar, R.H.; Manrique, N.C.; Debouck, D.G.; Tohme, J.; Roca, W.M..2000. Cryopreservation of cassava shoot tips using encapsulation-dehydration technique. In: Annual Report, Project SB-01: Assesing and utilizing agrobiodiversity through biotechnology. CIAT (Centro Internacional de Agricultura Tropical), Cali, CO. p. 178-181.

Roca W.M.1984. Cassava: In: Sharp, W.R.; Evans, D.A.; Ammirato, P.; Yamada, Y. (eds). Handbook of plant cell culture. V.2. Crops species. MacMillan Publishing Co. New York. P. 269-301

Santos L.G. Estudio exploratorio para desarrollar una metodologia de crioconservacion de callo embriogenico friable (CEF) de yucca (Manihot esculenta Crant) variedades Mcol 2215 y Mnig 11.2002, Tesis Universidad Nacional, Sede Palmira.

Contributors: R. Escobar, N. Manrique, A. Rios, A. Velasco, G. Mafla, M. Duque & J. Tohme

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

We continued with the indexing activities of clones of the Cassava World Collection maintained under *in vitro* conditions at CIAT. The final objective of this activity is to clean and certifiy the whole collection for the three viruses currently known of quarantine importance, following the FAO/IPGRI recommendations for the safe movement of cassava germplasm at national and international levels. We have been working on the indexing for three viruses: CCMV, CsXV and FSDA. For the indexing three 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).

The total of accsesions available at this moment and tested against viruses CCMV, CsXV and FSDA, thus ready for distribution are 5,067 clones (82,8 % of the entire collection).

Indexing for CCMV

The number of clones evaluated against CCMV in 2004 is shown in Table 16.

Table 16. Clones of the whole collection indexed against CCMV.

Source	Indexed clones	Negative clones
Argentina	16	12
Brazil	7	6
Colombia	18	17
Costa Rica	2	2
Paraguay	2	1
Peru	2	2
Venezuela	1	1
CG	1	1
CM	2	2
SM	3	3
Wild species		
BLO.	1	1
CHL.	1	1
CTH.	2	2
FLA.	2	2
PER 417-005	1	1
TST.	1	1
VIO.	1	1
TOTAL CLONES	63	56

These results indicate that to date 88,8 % of clones presented negative results for this virus and 11,1 % positive results. The country with high number of positive clones was Argentina with four positive clones; however this number is low. We have seen over the past few years that this country frequently shows the highest number of positive clones for CCMV and also that is still possible to find positive clones after the second or third thermoterapy treatment.

Indexing for CsXV

The number of clones evaluated against CsXV in 2004 is shown in Table 17.

Table 17.. Clones of the whole collection indexed against CsXV.

Source	Indexed clones	Negative clones
Argentina	7	6
Brazil	15	12
Colombia	39	28
Costa Rica	4	4
Ecuador	2	2
Mexico	3	1
Paraguay	1	1
Peru	4	4
CG	3	3
CM	5	5
SG	1	1
SM	9	8
Wild species		
BLO.	1	1
CHL.	2	2
CTH.	5	3
FLA.	2	1
TST.	1	1
VIO.	1	-
TOTAL CLONES	105	83

The results indicate that 79 % of the evaluated clones present negative results for this virus and 20,9 % positive results.

The countries where this virus was present were Colombia (28,2%) and Brazil with 20 % of incidence. Again, the presence of CsXV in the Collection is stronger than the presence of CCMV and FSDA, according with the previous data.

Indexing for FSDA

The number of clones evaluated against FSDA in 2004 is shown in Table 18.

Table 18. Clones of the whole collection indexed against FSDA.

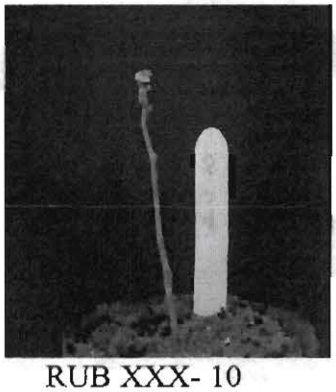
Source	Indexed clones	Negative clones
Argentina	5	3
Brazil	61	55
Colombia	76	67
Costa Rica	3	3
Cuba	2	2
Indonesia	3	3
Malasia	4	4
Mexico	5	5
Nigeria	1	1
Paraguay	15	13
Peru	11	11
Puerto Rico	1	1
Venezuela	9	9
CG	6	6
CM	15	15
SG	2	2
SM	8	7
Wild species	-	-
TOTAL CLONES	227	207

The results indicate that 91,1 % of materials present negative results in the indexing for this virus and 8,8% present positive results. The countries where this virus was present were Colombia (11,6%) and Brazil with 9,83 % of incidence.

We have had some problems with the growth of wild clones, because as one can see in the next pictures, most of them don't grow under our greenhouse conditions. So, for this coming year, we will have to evaluate them under diiferent environmental conditions (Figure 21).

CAE XXX- 29
(2 years after planting)

CAE XXX- 49
(2 years after planting)



RUB XXX- 10
(2 years after planting)

CTH XXX- 195
(2 years after planting)

The current status of the whole Cassava Collection (number of negative clones for each virus and number of clones currently available for distribution, negative for the three viruses) is presented in Table 19 .

Table 19. Indexing status of the Cassava Germplasm Collection in GRU by October 2004.

Source	In vitro clones	INDEXED CLONES			Available for distribution in 2004
		CCMV	CsXV	FSDA	
Argentina	114	84	92	70	66
Bolivia	7	7	7	5	5
Brazil	1324	1341	1329	1183	1165
China	2	2	2	2	2
Colombia	2016	1987	1947	1790	1759
Costa Rica	148	148	144	142	138
Cuba	77	77	77	77	77
United States	9	9	9	8	8
Ecuador	116	114	113	108	105
Fiji	6	5	5	5	5
Guatemala	91	91	88	75	75
Indonesia	51	51	51	42	42
Malasia	67	67	67	56	56
Mexico	102	102	100	87	86
Nigeria	19	19	19	17	17
Panama	43	39	37	37	35
Paraguay	210	204	207	160	154
Peru	406	404	402	385	382
Philippines	6	5	5	4	4
Puerto Rico	15	15	15	13	13
Dominican Rep.	5	5	5	4	4
Salvador	8	7	7	6	6
Thailand	31	30	30	19	19
Venezuela	244	237	234	213	210
CG	89	86	82	78	72
CM	442	427	426	414	407
SG	46	46	44	42	40
SM	87	84	84	79	75
HMC	4	4	3	4	3
KM	7	1	1	1	1
CT	1	1	1	1	1
SUBTOTAL	5793	5699	5633	5127	5032
WILD SPECIES					
30 spp in vitro	321				
3 Undefined spp					
ALT.		3	2	1	1
CAE.		5	4	-	
CEC.		1	1	-	
CHL.		4	5	4	3
CTH.		21	17	11	9
BLO.		1	1	-	
FLA.		14	14	13	12
FMT.		4	4	4	4
GLA.		1	1	-	
GUT.		1	1	-	
JAC.		2	-	-	
ORB.		1	-	-	
PSE.		-	-	1	
PIL.		1	1	1	1
PER 417-005		1	1	-	
RUB.		3	2	-	
TPH.		1	-	-	
TST.		10	7	11	4
VIO.		2	1	1	1
SUB TOTAL		76	62	47	35
TOTAL	6,114	5,775	5,695	5,174	5,067

As one can see in the previous table, 94,4 % of the Cassava Collection are negative to CCMV, 93,1 % to CsXV and 84,6 % to FSDA.

The next Table 20 shows progress in the indexing of the Cassava Collection (materials negative for three viruses) obtained between 1998 and 2004.

Total de clones	Year	Percentage of negative clones for CCMV	Percentage of negative clones for CsXV	Percentage of negative clones for FSDA	Available for distribution
6114	1998	36,7	35,5	10	602 (9,87%)
6114	1999	42,6	40,1	17,4	1,073 (17,6%)
6114	2000	70,8	63,5	39,2	2,346 (38,4%)
6114	2001	84,2	80,8	60	3,453 (56,6%)
6114	2002	90	87,6	75,6	4,559 (74,7%)
6114	2003	93	91,1	81	4,823 (79,1%)
6114	2004	94,4	93,1	84,6	5,067 (82,8%)

As one can see, we are reporting progress in the indexing process during the years indicated, although this process is slow, as we experiment some problems during the establishement of the last materials. We looked for passport data using the ORACLE date base, because we thought that some materials needed different growing conditions; in some cases we found those data, in other cases, the data do not exist. As trial and error, places with higher temperatures have been tested, but with limited success.

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

In the next tables (21, 22) one can see the number of materials that still need to be evaluated in order to finish with the indexing process. We make the comparison between the materials designate to FAO and not designate.

Table 21. Whole Collection: number of total clones: 6,114 (cultivated, hybrids and wilds).

Cultivated clones	Without CCMV	Without CsXV	Without FSDA	Not available
5,793	94	160	666	761
Wild clones				
321	245	259	274	286
6,114	339	419	940	1,047

As one can see in the previous Table, 17 % (1,047) clones of the whole Cassava colecction are not yet available. But 86,8% (5,032) clones cultivated and hybrids and 11 % (35) of the wild species are available.

Table 22. FAO Designate Collection: Number of total clones: 5,740 (cultivated, hybrids and wild species).

Cultivated clones	Without CCMV	Without CsXV	Without FSDA	Not available
5,419	117	176	677	720
Wild clones				
321	249	251	276	285
5,740	366	427	953	1,005

As one can see in the previous Table, 17,5 % (1,005) clones of the FAO Cassava colecction are not yet available. But 86,7% (4,699) clones cultivated and hybrids and 11,2 % (36) of the wild species are available.

Activity 2.1.2. Indexing wild materials of Cassava for GRU.

We continued with the growing-out of the wild materials of Cassava in order to advance the indexing activities. The growth of these plants is very slow, erratic and difficult, and a lot of materials has been lost during the growing-out phase in the greenhouse. We are testing different environmental conditions in order to overcome these shortfalls.

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

Activity 2.1.3. Establishement of a "Bonsai" Collection as safe back-up of whole Cassava Collection

Since October 2001 we have established a copy of the whole Cassava collection under greenhouse conditions, because most of materials maintained in the field as field genebank became infected with FSDA, and the Cassava Breeding Project no longer provided the back-up service to the in vitro collection. Along our current agreement with FAO, this back-up is necessary before the entire collection is safely maintained under cryopreservation. We currently have 4,094 plants in four different greenhouses. Out of this number (4,094), 3,411 are clones from different countries as it can be seen in Table 23, and the other ones (683) are duplicates (two plants of the same clone). These materials are by-products of the certification process against FSDA and thus certified against viruses of quarantine importance.

Table 23. Composition of the 'Bonsai' collection.

Source	Accesions installed under greenhouse conditions
Argentina	68
Bolivia	2
Brazil	857
Colombia	1,137
Costa Rica	93
Cuba	53
Ecuador	72
United States	7
Fiji	3
Guatemala	55
Indonesia	37
Nigeria	11
Malasia	36
Mexico	73
Panamá	27
Paraguay	130
Peru	260
Philippines	3
Puerto Rico	6
Dominican Rep.	3
Salvador	4
Thailand	18
Venezuela	118
CG	38
CM	146
SG	18
SM	56
CT	1
Wild species	79
TOTAL	3,411

At the begin of this process, we wanted to maintain the plants under conditions of much reduced growth (like a "bonsai"); several plants however do not thrive at all under those conditions. Currently, plants are allowed to grow normally (as it can see in Figure 22) in order to maintain a reliable back-up of the Cassava *in vitro* collection.

Figure 22. Cassava plants established as 'bonsai' collection as back-up for the in vitro bank.

The 'bonsai' collection is regenerated through cuttings of the same materials (Fig. 23).

Figure 23. Stake during the process of renovation of the 'bonsai' collection.

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

Activity 2.1.4. Utilization of recycled plastic bottles for the establishement of in vitro plants as back-up of the in vitro bank.

In order to be able to run the safety back-up at low cost, we need to extend the period between each subculturing, and thus to test the best conditions to that end. So, we planted 25 in vitro plants in plastic bottles to observe their growth. So far, the plants have showed the same growth characteristics (see figure 24).

Figure 24. Plastic bottles with soil and granite In vitro plants.

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

Activity 2.1.5. Updating the Cassava ORACLE database.

During 2003-2004 we continued with the activities of implementing the database (ORACLE) for the cassava 'Bonsai' collection; all data about the materials currently kept under greenhouses conditions have been compiled (Table 24).

Table 24. Example of presentation of data in the data base.

Identification	Entry date	Exit date	Reason for exiting	Greenhouse	Table	Location
COL2340						
	28 Jan 2003	04-MAR-2003	death	3	3	57
	31 Dec 1998	02-MAY-1999	to the field	3	3	57

Contributors: N.C.Flor, G. Mafla, D.M. Montero

Activity 2.1.8. Germplasm health control in seed germplasm

Introduction

In agreement with FAO–IPGRI genebanks standards, seeds for storage in germplasm collections should be as clean and free from weed seeds, pests, and diseases as possible. To that purpose the Germplasm Health Laboratory (GHL) practiced phytosanitary inspections on multiplication plots (fields and glass-houses), and applied indexing procedures in the laboratory to ensure that the germplasm was free of seed borne diseases that could affect its longevity during the storage and prohibit its distribution to users.

During the period september 2003- September 2004, the GHL tested 3,592 seed samples from projects SB-1 (Integrated Conservation of Neotropical Plant Genetic Resources), 1,938 *Phaseolus* seed samples from GD-01 (Bean Germplasm Improvement) and 97 tropical forages seed from IP5 (Tropical grasses and legumes) project.

Materials and Methods

Phytosanitary inspections are carried out in multiplication plots (field and glass-houses) of Popayán, Santander de Quilichao and CIAT Palmira. Accessions are tested in the GHL using accepted methodologies to identify seed-borne pathogens such as fungi, bacteria and viruses, also taking into account those pathogens recorded in seed production areas (Annual Report 1997). The procedures utilized in the Germplasm Health Laboratory have been described in Annual Report of 1999.

Testing for some genera of seedborne 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 culture media. Seedborne 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, incubation under high temperature (36-37° C). Also complementary tests using a Gram-Positive ID Kit and Gram- negative (Becton Dickinson BBL Crystal™, Nippon Becton

Dickinson Company Ltd.) containing different enzymatic and biochemical substrates are carried out. In addition, we used biological tests using hypersensibility reactions on *Mirabilis jalapa* after bacterial infiltration and pathogenicity. Testing for seedborne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and/or seedling-symptom test.

Results

Beans (*Phaseolus* spp.)
Seed samples of 839 accesions of beans project SB-1 were tested, some of them with destination to international germplasm exchange, and the other one to conservation in the Bean Germplasm Bank. Their health status showed 88.9% samples without pathogens of quarantine importance (Figure 18). Samples with pathogens (11.1 %) showed in general very low percentages of fungi and bacteria. Fungi as *Macrophomina phaseoli,* and *Colletotrichum lindemuthianum* were detected. Seedborne viral infections by bean common mosaic virus (member of viruses family potyviridae), and Southern bean mosaic virus (SBMV) were detected in 9.8% of analyzed samples (Figure 25).

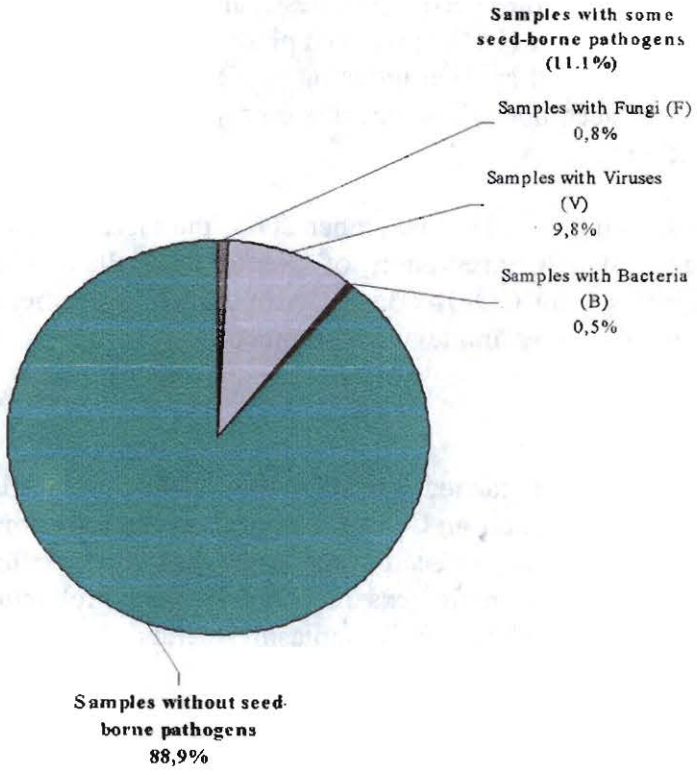


Figure 25. Phytosanitary status of Bean germplasm determined at SHL from 839 seed samples during September 2003- 2004 period..

The presence of Gram positive bacteria was checked and only one accession of *Phaseolus vulgaris* showed colonies of these kinds of bacteria after subculturing on YDCA, and incubation

under high temperature (36-37°C). However, their identification with complementary tests using a Gram-Positive ID Kit (Becton Dickinson BBL Crystal™, Nippon Becton Dickinson Company Ltd.) showed a Coryneform plant bacteria, but not *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*.

Tropical grasses and legumes

Seed samples of 2,753 of tropical grasses and legumes from SB-1 project, distributed in 2,440 accesions of 35 genera of tropical legumes pastures, and 313 of Poaceae were analyzed in the Germplasm Health Laboratory (GHL), during the period September 2003- September 2004 (Table 25, 26). Their health status showed 72.6 % of samples without pathogens of quarantine importance (Figure 26).

Health status of tropical legumes pastures showed 72.2 % of samples without pathogens of quarantine importance (Table 25). In the rejected samples of forage legumes, we detected the following seedborne fungi of quarantine importance: *Ascochyta* spp., *Colletotrichum* spp., *Drechslera* spp., *Phoma* spp., *Phomopsis* spp., *Macrophoma* sp., *Macrophomina* sp., *Pestalotia* spp., and *Rhizoctonia* (Table 25, 27). Seedborne viral infections by Potyviruses were detected in 65 samples (Table 27) and Southern bean mosaic virus (SBMV) were detected in 29 samples (Table 27). Bacteria (*Xanthomonas* spp.) were detected at very low frequency; a Coryneform Gram-positive bacteria was detected too (Table 27).

Health status of tropical grass pastures showed 12.1 % of samples without pathogens of quarantine importance (Table 27). In the rejected samples of forage grasses seedborne fungi of *Drechslera* spp. and *Phoma* spp. were detected (Table 26, 27). *Brachiaria* spp was the member of Poaceae most affected by *Drechslera* spp. (Table 26). Bacterial and viral seed infections were detected at very low frequency (Table 26, 27).

Table 25. Phytosanitary status of seed samples from forage legumes (Fam: Fabaceae) analyzed in the GHL during September 2003- 2004 period.

Genera	Number of Tested Samples	Percentage of Samples Without PwoQI*	Number of Affected Samples	Samples with Fungi (F)	Fungi Frequency ( )	Samples with Viruses (V)	Virus Frequency ( )	Samples with Bacteria	Bacteria Frequency ( )	Samples with F+B	Samples F+V
Abrus	5	80.0	1	1	*Macrophoma* (1)						
Acacia	6	100.0	0								
Aeschynomene	139	75.5	34	19	*Macrophoma*, (10) *Phomopsis* (7) *Phoma*, (4) *Colletotrichum*,(2) *Pestalotia* (1)	9	SBMV (5), Poty (3)			5	6
Alysicarpus	93	82.8	16	12	*Rhizoctonia* (2), *Macrophoma* (1), *Phoma* (7), *Colletotrichum* (2), *Phomopsis* (1)	3	SBMV (1), Poty (2)		Corynef Gram + (1)	1	
Arachis	6	50.0	3	2	*Macrophomina* (10), *Rhizoctonia* (1), *Phoma* (1)			1	Corynef Gram + (1)		
Bauhinia	1	100.0	0								
Cajanus	35	88.6	4	4	*Macrophomina*(2), *Rhizoctonia* (1), *Phomopsis* (1)						
Calopogonium	104	67.3	34	27	*Macrophoma* (6), *Phoma* (4), *Phomopsis* (25), *Colletotrichum* (1),		Poty (4)		Corynef Gram + (2), *Xanthomonas*	3	4

Table 25. Phytosanitary status of seed samples from forage legumes (Fam: Fabaceae) analyzed in the GHL during September 2003- 2004 period.

Genera	Number of Tested Samples	Percentage of Samples Without PwoQI*	Number of Affected Samples	Samples with Fungi (F)	Fungi Frequency ( )	Samples with Viruses (V)	Virus Frequency ( )	Samples with Bacteria	Bacteria Frequency ( )	Samples with F+B	Samples F+V
					Ascochyta (1)				(1)		
Canavalia	45	82.2	8	3	*Colletotrichum* (3), *Macrophoma* (1)	4	Poty (2), SBMV (5)				1
Centrosema	399	80.4	78	60	*Rhizoctonia* (3), *Macrophoma* (2), *Phoma* (11), *Colletotrichum* (8), *Phomopsis* (36), *Macrophomina*(2)	11	Poty (11), SBMV (3)	2	Corynef Gram + (4)	2	3
Chamaecrista.	14	78.6	5	5	*Macrophoma* (3), *Phomopsis* (3)						
Clitoria	6	50.0	3	3	*Phoma* (2), *Colletotrichum* (2)						
Codariocalyx	24	75.0	6	6	*Macrophoma* (4), *Phoma* (1), *Phomopsis* (1), *Macrophomina*(1)						
Coursetia	1	100.0	0								
Cratylia	21	28.6	15	12	*Colletotrichum* (1), *Macrophoma* (10), *Phomopsis* (2)	3	Poty (3)				
Crotalaria	53	77.3	12	7	*Macrophoma* (3), *Phoma* (1), *Colletotrichum* (3), *Phomopsis* (1)	3	Poty (3)	1	*Xanthomonas* (1)		
Dendrolobium	2	100.0	0								
Desmanthus	31	80.6	6	2	*Phoma* (1), *Colletotrichum* (1)	2	SBMV (2)				
Desmodium	451	89.3	48	36	*Macrophoma* (5), *Phoma* (20), *Colletotrichum* (9), *Ascochyta* (2), *Pestalotia*(3)	9	Poty (6), SBMV (3)	3	Corynef Gram+(1)		
Dioclea	68	55.9	30	21	*Macrophoma* (9), *Phoma* (4), *Phomopsis* (8), *Pestalotia*(2), *Drechslera* (1)	6	Poty (7)				1
Dolichos	1	100.0	0								
Dumbaria	3	66.7	1	1	*Macrophoma*(1)						
Eriosema	6	66.7	2	1	*Phoma* (1), *Rhizoctonia*(1), *Phomopsis*(1)		Poty (1)				1
Erytrina	1	100.0	0								
Flemingia	37	78.4	8	8	*Macrophoma* (4), *Phomopsis* (2), *Macrophomina*(2), *Pestalotia* (1)						
Galactia	24	95.8	1	1	*Phomopsis* (1)						
Glycine	1	100.0	0								
Indigofera	90	86.7	12	10	*Macrophoma*, (3) *Phomopsis* (2) *Phoma*, (2) *Colletotrichum*,(2) *Pestalotia*(1), *Drechslera*(1)	1	Poty (1)				
Lablab	15	86.6	2	2	*Macrophoma* (1), *Phomopsis* (1)						
Leucaena	14	78.5	3	1	*Phomopsis* (1)	2	Poty (2)				
Lotonis	1	100.0	0								
Macroptilium	47	87.2	6	6	*Macrophoma* (1), *Phoma* (3), *Pestalotia* (1), *Colletotrichum* (1)						
Macrotyloma	1	100	0								
Medicago	1	0	1	1	*Drechslera* (1)						
Mimosa	9	100.0	0								

Table 25. Phytosanitary status of seed samples from forage legumes (Fam: Fabaceae) analyzed in the GHL during September 2003- 2004 period.

Genera	Number of Tested Samples	Percentage of Samples Without PwoQI*	Number of Affected Samples	Samples with Fungi (F)	Fungi Frequency ( )	Samples with Viruses (V)	Virus Frequency ( )	Samples with Bacteria	Bacteria Frequency ( )	Samples with F+B	Samples F+V
Mucuna	5	80.0	1	1	*Macrophoma* (1)						
Neonotonia	15	86.7	2	2	*Colletotrichum* (2)						
Neptunia	1	100.0	0								
Parosela	1	0.0	1			1	SBMV (1)				
Phyllodium	5	100.0	0								
Prosopis	1	0.0	1			1					
Pseudarthria	1	100.0	0								
Psophocarpus	2	100 0	0								
Pueraria	11	81.8	2			1	SBMV (1)	1	Corynef Gram+ (1)		
Pycnospora	10	80.0	2	2	*Phoma* (2)						
Rhynchosia	89	83.1	17	12	*Macrophoma* (2), *Phoma* (7), *Colletotrichum* (1), *Macrophomina* (1), *Phomopsis*(1)	4	Poty (3), SBMV (3)	1	Corynef Gram+(1)		1
Senna	2	100.0	0								
Sesbania	9	88.9	1		*Phoma* (1)				*Xanthomonas* (1)	1	
Soemmeringia	1	0 0	1			1	Poty (1)				
Stylosanthes	259	85.7	37	26	*Phoma* (18), *Colletotrichum* (5), *Phomopsis*(2), *Pestalotia*(3)	6	Poty (5), SBMV (4)	2	*Xanthomonas* (1), Corynef Gram+ (1)		1
Tadehagi	1	100	0								
Tephrosia	21	61.9	8	5	*Macrophoma* (2), *Phoma* (1), *Phomopsis* (4), *Rhizoctonia* (1),		Poty (2)		Corynef Gram+ (1)	1	2
Teramnus	96	68.7	30	22	*Macrophoma* (17), *Phoma* (3), *Colletotrichum* (2), *Phomopsis*(7)	3	Poty (5), SBMV (1)				3
Uraria	10	70.0	3	3	*Phoma* (1)						
Vigna	63	71.4	18	12	*Macrophoma* (6), *Phomopsis* (5), *Colletotrichum*(1)	3	Poty (3)	3	Corynef Gram+ (3)		
Zornia	82	81.7	15	12	*Phoma* (10), *Phomopsis* (2), *Pestalotia* (2)	1	Poty (1)	2	*Xanthomonas* (1), Corynef Gram+ (1)		
Total	2440	72.2	478	346		74		16		13	23

* PwoQI = Pathogens without quarantine importance

Table 26. Phytosanitary status of seed samples from forage grasses (Fam: Poaceae) analyzed in the GHL during September 2003- 2004 period

Genera	Number of Tested Samples	Percentage of Samples Without PwoQI*	Number of Affected Samples	Samples with Fungi (F)	Fungi Frequency ( )	Samples with Viruses (V)	Virus Frequency ( )	Samples with Bacteria	Bacteria Frequency ( )	Samples with F+B	Samples F+V
Andropogon	7	0.0	7	7	*Drechslera* (5), *Phoma* (7)						
Brachiaria	275	7.3	255	251	*Drechslera* (244), *Phoma* (112)				Corynef Gram + (4)	4	
Chloris	1	0.0	1	1	*Drechslera* (1), *Phoma* (1)						
Echinochloa	2	0.0	2	2	*Phoma*(1), *Drechslera* (1)						
Enteropogon	1	0.0	1	1	*Phoma* (1)						
Eragrostis	7	100.0	0								
Hyparrhenia	1	100.0	0								
Leptochloa	1	100.0	0	1	*Drechslera*(1)						
Melinis	2	0.0	2	2	*Drechslera*(2)						
Panicum	12	75.0	3	3	*Phoma*(1), *Drechslera*(2)						
Paspalum	2	50.0	1		*Phoma* (1)		Poty (1)		Corynef Gram+ (1)	1	1
Setaria	1	0.0	1	1	*Phoma*(1), *Drechslera*(2)						
Sorghum	1	0.0	1	1	*Drechslera*(1)						
Total	**313**	**12.14**	275	268						5	1

* PwoQI = Pathogens without quarantine importance

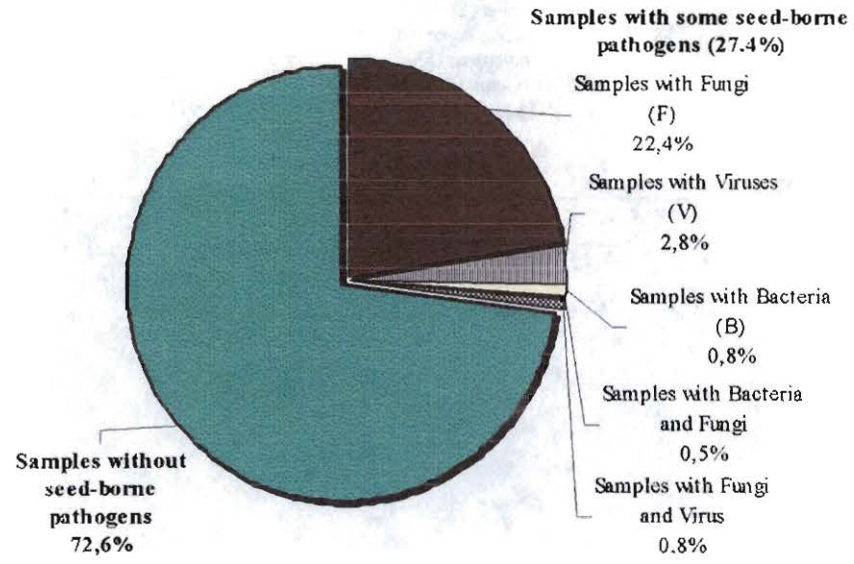


Figure 26. Phytosanitary status of Tropical Pastures germplasm determined at SHL for 2,753 seed samples (2,440 legumes, 313 grasses) during September 2003- 2004 period.

Table 27. Seedborne pathogens detected in tropical forages seed samples analyzed in the GHL during September 2003- 2004 period.

Tropical Forages Family	Pathogen	Number of Tropical Forages Genera affected	Number of seed affected samples
Fabaceae	Ascochyta	2	3
	Colletotrichum	17	45
	Drechslera (Helminthosporium)	3	3
	Phoma	22	67
	Phomopsis	22	122
	Macrophoma	21	83
	Macrophomina	6	16
	Pestalotia	8	14
	Rhizoctonia	5	7
	Potyvirus	19	65
	SBMV (Southern Bean Mosaic Virus)	11	29
	Bacteria Coryneforme Gram+	11	17
	Xanthomonas	5	5
Poaceae	Drechslera	9	259
	Phoma	7	125
	Potyvirus	1	1
	Bacteria Coryneforme Gram+	2	5

Service of germplasm health certification for other projects

Seed samples from GD-01 (Bean's Germplasm development) and IP5 (Tropical grasses and legumes) projects were analyzed (Table 28). In *Phaseolus vulgaris* 60 % of samples did not show pathogens of quarantine importance. In the rejected samples *Macrophomina phaseoli* was the fungus detected with highest frequency. We also detected the bacteria *X. campestris* pv *phaseoli* in low percentage and bean common mosaic virus (BCMV) and SBMV in intermediate percentages. In tropical pastures germplasm 60% of seed samples of *Brachiaria* spp. showed the presence of the seed borne fungi *Drechslera* spp. and *Phoma* spp.. In legumes such as *Vigna* spp., we detecteted *Macrophomina* spp. in low percentages.

Table 28. Number of analyzed samples of beans, tropical grasses and legumes for germplasm sent abroad, projects GD-01 and IP5

Specie	Samples number	Project
Phaseolus vulgaris	867	GD-01 (S. Beebe)
Phaseolus vulgaris	1,071	GD-01 (M.Blair)
Total	1,938	
Legumes	74	IP5 (J. Myles)
Grasses	23	IP5 (J. Myles)
Total	97	

Contributors: B. Pineda, M.S. Balcazar, N.C. Flor

Activity 2.1.9. Design of the computerized system of GRU for quality control, flow monitoring and web consultations.

Introduction

The GRU information system keeps valuable information of the germplasm bank; this information is required by GRU Staff and is consulted by external people through CIAT web page. The web page offers the chance to consult a portion of the information, and external users can also make a germplasm request of the different materials (bean, cassava, tropical forages).

During this year we improved the web site and introduced images of high quality of herbarium voucher specimens. We continued with the introduction of bean images, while we finished with the introduction of images of tropical forages (field and seed). The possibility of accessing the web site in Spanish and English has been habilitated, allowing a faster request, introducing new information, publishing news and documents regarding the GRU.

Another important improvement made during the present year is the introduction of field bar coding. Very soon the GRU will introduce the use of portable printers and data collectors in the field.

Materials and Methods

In order to make the changes in the internal information system we used the tool called Developer 2000 from Oracle. The program language 'Java' is the tool used to make germplasm requests via the web site. With regard to the bar coding we are using printer ZEBRA S600 but due to high cost and for its easy operation right now we use printer ZEBRA TLP 2844Z.

To print bar codes in the field we are using Zebra PT400 printers and a data collector Symbol SPT 1800 which is programmed in NS-Basic language. This equipment will be replaced because its poor compatibility and instead we will use Symbol PPT 8800 which allows to run 'Java' language that is the most common language currently in use at CIAT.

Results

The internal information system has been updated with new information, and new pictures have been included. We have put in service procedures for massive data loading through Excel files, which allow us to speed up the actualization of data. Also, a new section for the managing of the cassava bonsai collection has been implemented in the GRU data system.

The introduction of bar coding has been done gradually as proofs progress with very good results so far. The acquisition of new bar coding equipment will allow us to have more printing points and to adopt bar coding in every step of the work flow.

The web application put in English and Spanish will be ready by the end of the year with new documents, news such list of publications, training opportunities. On the other hand it is possible to make a detail zoom over the herbarium images.

Contributor: D.M. Montero V.

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: 8,274 accessions of the three commodity FAO designate collections distributed to germplasm users.

As it can be seen in Tables 29, 30 and Figures 20,21, 22, 23 , a total of 8,274 accessions were distributed, through 181 requests for bean, forage and cassava. The main recipient was CGIAR Centres (mainly CIAT Projects). NARS and Universities were another important recipients. As consequence of the type institutions requesting the germplasm the main purposes of the requests were agronomy, applied and basic research.

This year clones of the in vitro cassava core collection were distributed for further evaluations in the field and to support the activities under Sub Program 1 Cluster 2 of the 'Generation' Challenge Program.

Table 29. Distribution of germplasm during 2004 by purpose.

	Beans		Forages		Cassava	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	12	536	1	1	3	49
Agronomy	6	453	34	767	32	1,020
Applied research	16	1,463	2	20	5	20
Basic research	30	795	6	63	23	2,005
Training	2	41	8	1,037		
Other	---	---	1	4		
Total	66	3,288	52	1,892	63	3,094

Table 30. Distribution of germplasm during 2004 by kind of institution

	Beans		Forages		Cassava	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	37	2,249	13	1,182	43	2,460
Commercial companies	---	---	3	19	2	13
Farmers	1	1	14	44	1	5
Gene banks	---	---	---	---		
NARS	9	542	9	240	10	320
NGOs	---	---	3	273	2	241
Regional organizations	---	---	---	---	4	37
Universities	19	496	10	134	1	18
Germplasm networks	---	---	---	---		
Others	---	---	---	---		
Total	66	3,288	52	1,892	63	3,094

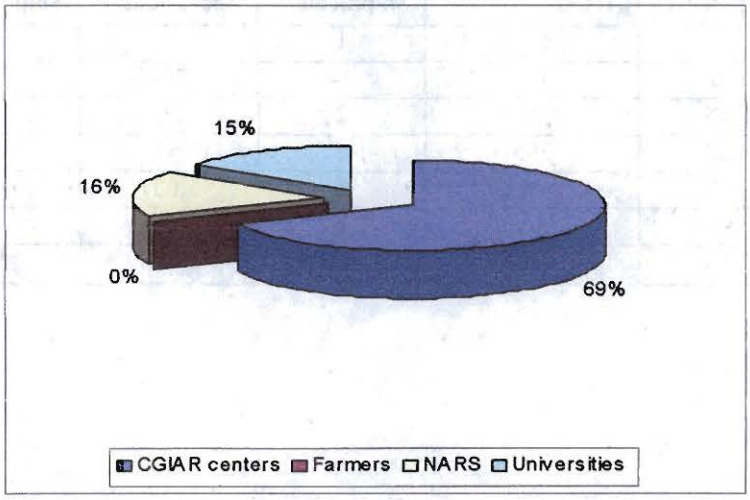


Figure 27. Distribution of bean seed germplasm sorted out by user type

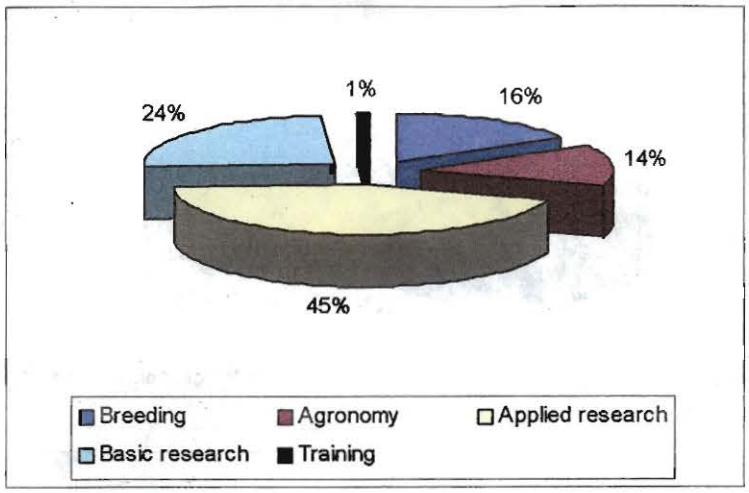


Figure 28. Distribution of bean seed germplasm sorted out by kind of purposes.

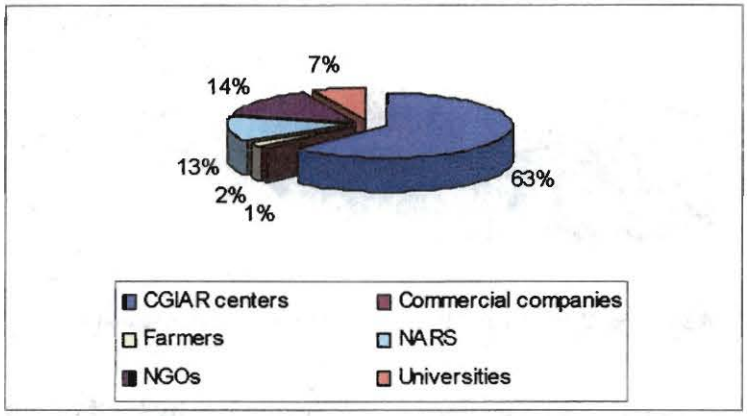


Figure 29. Distribution of forage seed germplasm sorted out by user type.

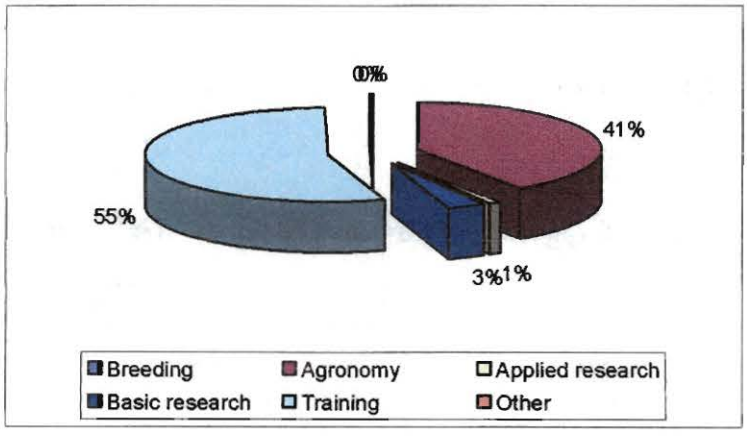


Figure 30. Distribution of forage seed germplasm sorted out by kind of purposes.

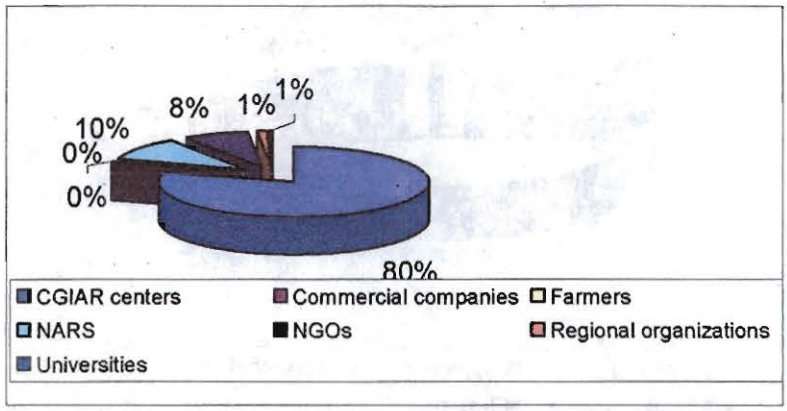


Figure 31. Distribution of *in vitro* cassava germplasm sorted out by users.

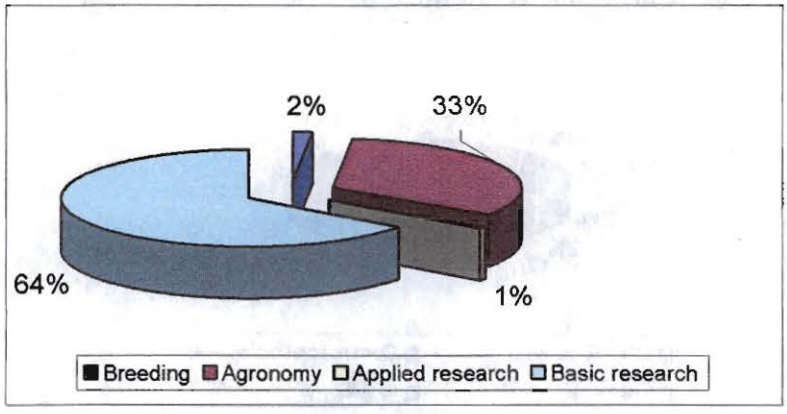


Figure 32. Distribution of *in vitro* cassava germplasm sorted out by kind of purposes.

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

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

Table 31. Specimens of tropical forages and wild beans added to CIAT Herbarium in 2004.

	Number of species	Number of accessions
Wild beans	10	48
Legumes	117	418
Grasses	12	19
Total	139	485

While accessions are multiplied in field, voucher specimens were taken for taxonomic research purposes. Exchange of herbarium specimens was done with other national and international herbaria. Herbarium specimens were sent as donation to: the National Herbarium of Colombia

COL (13 of *Phaseolus* spp), the Herbarium of Universidad del Valle CUVC (21 of *Galactia* spp.), the Herbarium of Universidad del Quindio HUQ (52 of legumes forages), the Herbarium of Conservatoire Botanique de la Ville de Genève G (13 of *Phaseolus* spp.), the Royal Botanical Gardens at Kew K (13 of *Phaseolus* spp.), and the Herbarium of the Field Museum of Natural History F (9 of *Phaseolus* spp.).

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

Output 2.3. National collections restored to NARS

During 2004, a shipment of 257 accessions has been done towards NPGR, India, as per the request of that country. This restoration work includes 231 accessions of beans, 24 of tropical forages, and 2 accessions of cassava.

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

Output 2.4. FAO designated collections safe duplicated

Activity 2.4.1. Preparation of germplasm collections for security backups

Achievement: 8,284 accessions of bean and forages were prepared for safety duplication at CIMMYT. A special box has been designed according to specifications given by CIMMYT Staff. A Letter of Agreement has been prepared during the summer of 2004, and signed between both institutions to that end.

This year we have shipped to Thailand 257 clones of the *in vitro* cassava core collection, in order to keep a duplicate in that country, and for future evaluation of agronomic performance at the Rayong Field Crop Research Center.

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

Output 2.5. Improved disease indexing techniques

Activity 2.5.1. Preliminary evaluation about antagonistic activities of a bacterial isolate against seed-associated fungi affecting *Brachiaria brizantha* (Panicoideae, Poaceae)

Introduction

The increase and regeneration of *Brachiaria* spp. germplasm are carried out, under field conditions at "Santa Rosa" experimental station near Popayan where the conditions for seed production are relatively favorable. Nevertheless under these conditions (more than 2,000 mm rainfall, temperature 16-24 ^{0}C) the production of seeds can be affected by fungal diseases.
Recent GHL studies (Garcia & Pineda 2000; Garcia et al, 2001; CIAT, 2001; CIAT, 2002) showed that *Drechslera* spp., *Phoma* sp., *Curvularia* spp. *Sphacelia.* sp., *Cerebella* sp., were the

most frequent fungi affecting *Brachiaria* spp. seed production. In field evaluations carried out with fungicides we found that the tested products had low control against the above mentioned fungi (CIAT, 2001; CIAT, 2002). Preliminary studies in the GHL using antagonistic bacteria isolated from *Brachiaria* seeds tested *in vitro* showed antagonistic potential against the fungi *Drechslera* sp., *Phoma* sp., *Curvularia* sp., and *Fusarium* sp., isolated from *Brachiaria* spp. (CIAT 2003).

Nowadays as an environmentally benign alternative it is necessary to find out forms of biological control, for example the use of antagonistic bacteria, frequently present as epiphytic flora on plants or on plant organs. Some preliminary studies with the G3 bacterial isolate from *Brachiaria* spp. seeds were initiated under field conditions. Here are the results.

Materials and Methods

To assess the antagonistic potential of G3 bacterial isolate obtained from *Brachiaria* spp seeds, previously tested for fungi growth inhibition under *in vitro* conditions, a field trial was carried out in CIAT Santa Rosa Research Station during November 2003-February 2004. A group of 21 accesions of *Brachiaria brizantha* with plants in flowering state were selected. The trial included three treatments (G3 Bacterial suspension, Propiconazole (Tilt 250EC) 2.5 mL/L and Serike distilled water), each one with seven plots. In the GHL bacterial isolate G3 was plated in Nutrient Agar culture media. It was streaked on culture media and incubated for 48 h at 28-30°C. Bacterial suspensions were prepared by removing the colonies from the media using a sterile solution of 0.85% sodium chloride. Bacterial suspension was standardized to approximately 10^8 colony forming units (cfu)/mL using a Spectronic 20 at an absorbance value of 0.1 at 660 nm. In the field trial we used 800mL of bacterial suspension.

The field trial was initiated in November 2003. G3 bacterial inoculum was prepared diluting 800mL of concentrated bacterial suspension in 5L of sterile distilled water and then sprayed on plots, using a garden Matabi sprayer. Propiconazole 2.5 mL/L, and sterile distilled were also sprayed.

The experiment was harvested in February 2004, following GRU standard procedures. After harvesting seed samples were conditioned to establish their health status in the GHL. Of each accession samples of 200 seeds were analyzed using incubation in blotter test (Neergard, 1977; Agarwal and Sinclair et al, 1987). Presence of fungi was observed through the stereoscope and light microscope, and their identification to genera was made by comparing descriptions and pictures in specialized references (Barnnet and Hunter, 1998; Zillinsky, 1983; Ahmed and Ravinder Reddy, 1993)

Results

The evaluation of the treatments showed some interesting results (Table 32). Comparing the observed percentages of *Drechslera* spp., and *Phoma* sp., after G3 bacteria and propiconazole treatments , against the control with distilled water, differences could be noted. Observations with *Curvularia* sp., *Epicoccum* sp. and *Fusarium* sp. did not show differences (percentages are very similar). These results may be promising, nevertheless we need to do more research under field conditions. One of the major problems associated with biological control is variable efficacy in the field that limits agronomic application. Variability is due to any factor interfering

with either the population size of a biocontrol agent or its expression of activity to compare the inhibitory capacity of each bacterial isolate against selected fungal pathogens.

Table 32. Percentage of fungi observed on seeds after field treatments with G3 bacteria isolate and then fungicide Propiconazole (Tilt 250 EC)

Accesion	Treatment	*Drechslera* spp.	*Phoma* sp.	*Curvularia* sp.	*Epicoccum* sp.	*Fusarium* sp.
16113		34.0	29.0	8.5	22.0	9.0
16470	160mL/L	46.0	35.5	6.0	18.5	8.0
16449		17.0	41.5	2.0	22.0	6.0
16478	G3 Bacterial suspension	50.2	37.5	12.0	23.5	9.0
16460		36.5	42.5	6.0	14.5	5.0
16327		35.0	42.0	7.5	24.0	20.5
16149		45.5	36.5	5.5	33.5	8.0
Average		37.7	37.8	6.8	22.5	9.3
16467		33.0	39.0	5.0	44.0	9.5
26562	2.5 mL/L	43.0	32.5	13.5	17.0	7.5
26641		19.5	26.5	5.5	22.5	4.5
16471	Propiconazole (Tilt 250EC)	21.0	31.0	4.5	19.0	3.5
16458		49.5	40.0	10.5	30.5	13.5
16809		24.0	34.5	4.5	50.0	9.0
16160		57.5	43.0	9.0	22.5	13.0
Average		35.3	35.2	7.5	29.3	9.9
16549	Sterile distilled	45.5	44.0	9.5	20.5	10.5
16457	Water	75.0	31.0	6.0	20.0	7.5
16329	0.7mL/Plot	71.5	64.5	10.5	24.0	12.5
16300		54.5	26.0	2.0	28.0	4.5
16163		60.0	35.0	2.0	12.5	8.5
16461		57.5	51.5	12.0	21.0	14.5
17170		46.0	39.0	8.0	27.0	9.0
Average		58.6	41.5	7.1	21.8	9.6

References

Agarwal , K, V and Sinclair, B. 1987. Principles of seed pathology (Vol II). CRC,Press. Boca Raton, Florida. p 34-37

Amhed, K. M. and Ravinder Reddy, CH. 1993. A Pictorial Guide to the Identification of Seedborne fungi of Sorghum, Pearl Millet, Finger Millet, Chckpea, Pigeonpea and Groundnut.
ICRISAT Information Bulletin No 34. 192 pp

Barnett, H.L; Hunter, B.B. 1998. Illustrated genera of imperfect fungi. Fourth edition. The American Phytopathological Society. APS Press.. St. Paul Minnesota. USA. 218pp

CIAT. 2003. Genetic Resources Unit. Annual Report 2003. CIAT Project on Saving Biodiversity SB-01. Genetic Resources Unit. Report on Achievements and Progresses.

Dunn, R. T; Lewis, S. A and Papavizas, G.. 1983. Production and fermentation of two biological control agents from liquid fermentation. Phytopathology 73:165.

García, S. X., Pineda, B. 2000. Reconocimiento de enfermedades fungosas transmitidas por semillas en germoplasma de Brachiaria spp. Fitopatología Colombiana. 24(2): 39-46.

García, S. X., Pineda, B., Salazar, S. M. 2001. Presencia de la enfermedad del mal de azúcar (*Sphacelia* spp) en tres especies del pasto Brachiaria (Panicoidea, Poaceae). Fitopatología Colombiana. 25 (2). pág 1 – 8.

Kim, Ds; Cook, R. J; and Wellr,D M.1997. *Bacillus* sp. L 324-92 for biological control of three root disease of wheat grown with redeced tillage, Phytopathology 87: 551-558

Neergard, P. 1977. Seed Pathology. Halted Press, a division of John Wiley and sons, Inc, New York .p 738-754

Zillinksky, F.J. 1983. Common diseases of small grain cereals. A guide to identification. Centro Internacional de Mejoramiento de Maiz y Sorgo. CIMMYT. 141 pp.

Contributors : M.S. Balcazar, A.L. Rivera, B. Pineda

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

Output 3.1: Designate Collections better characterized.

Activity 3.1.1. Molecular fingerprinting of the cassava germplasm Colombian collection held at CIAT as a FAO Designate Collection. (project funded by Ministerio de Agricultura y Desarrollo Rural, Colombia)

In an asexually maintained germplasm collection, duplicate accessions may be common. In the case of the cassava collection at CIAT, 20 % to 25 % is estimated as duplicated. The presence of genetic duplicates in a germplasm collection has serious implication for germplasm conservation, as well as for a breeding program. Such redundancy makes the collection expensive to maintain and manage, and slows down the introduction of new germplasm (Hershey et al. 1991). For cassava, a large percentage of these duplicates were identified using passport, morphological and isozyme characterization (Ocampo et al. 1993; Jiménez, 1994; Sumarani et al. 2004). A research project is under way to study the use of DNA fingerprinting to confirm suspected groups of possible genetic duplicates; that is, to detect genotypic differences among these groups that otherwise appear identical in their morphology and isozyme-banding patterns. The combination of molecular markers with morphology/isozymes can give a high degree of confidence to identifying duplicates (Ocampo et al. 1995). These data on the molecular fingerprinting also can be used to reach the following objectives: (1) to develop a description of

each accession based on its molecular pattern (fingerprinting) as a criterion to avoid genetic duplicates when new germplasm is introduced in the cassava world collection held at CIAT. (2) Once known the level redundancy, to study the distribution of the resulting genetic diversity in the different agroecological zones of Colombia.

Materials and Methods

Plant material and DNA extraction. This work has been initiated with the cassava germplasm Colombian collection maintained at CIAT, consisting of 1986 accessions (the largest collection by country). The *in vitro* Cassava Laboratory (GRU) provided the accessions to characterize according to morphological and isozymatic similarities. Priority will be given to groups conformed by more than three accessions, which will improve the procedure to identifying duplicates. The first group is conformed by 94 accessions grouped in 31 groups of possible genetic duplicates, the number of accessions per group varied between 2, 3, 4, 5 and 6. The genomic DNA extractions for *in vitro* material (using young leaves) were carried out out according to Dellaporta et al. (1983), with the modifications reported by González et al. (1995) for cassava.

Molecular Markers. One type of molecular markers that may be suitable for cassava germplasm characterization is the microsatellite (SSR). Microsatellites are considered more sensitive in detecting genotypic differences as compared with morphological and isozymatic descriptors, because of higher polymorphism (Chavarriaga et al. 1998). Microsatellites, like RFLPs, are considered codominant markers.

Results and Discussión

A proposed methodology to confirm possible genetic duplicates in cassava. The following procedure represents an effective methodology for genetic duplicates identification, wich could be applied in other asexually maintained germplasm collections. It is based a three-step procedure:

(1) The first stage involved grouping the accessions according to biochemicals descriptors showing the highest levels of confidence (αß-esterase isozyme), (Table 33).

(2) The second stage was to apply cluster análisis with the morphological descriptors, wich had a lower degree of confidence, but which helped separate different accessions formed by the first level of grouping.

(3) The third stage involves the use of DNA fingerprinting (SSR markers) as an additional confirmation tool for duplicate identification. These markers helped to separate or to confirm the groups formed in the two first stages.

These grouping consider only the materials that have complete passport data. Priority will be given to groups conformed by materials with the same geographic origin. The combined analysis of all this information increases the confidence level.

Microsatellite Markers. Microsatellites are more expensive and more complex than morphological and isozyme characterization. Therefore, this project also aims to (a) search means of reducing operational costs, perhaps by selecting an adequate SSR primers, and (b) overcome the technical difficulties of producing enough high quality DNA on the one hand, and enough good quality gels, with high banding definition, on the other. For the microsatellite marker, a set of fifteen SSR markers, carefully chosen to represent a broad coverage of the cassava genome with moderate to high polymorphism information content (PIC) and robust amplification, were used in this study (Table 34). The methodogy to test its variation, PCR amplification, polyacrylamide gel electrophoresis, and silver staining stablished by Fregene et al. (2002) was used in this study. Sufficient quantities of high quality DNA (4-7 µg/ per sample) have been obtained from *in vitro* material. It was also possible to select the most adequate SSR loci that can be useful for discriminating between closely related individuals. In addition these different SSR loci are being selectively tested to identify at least ten wich can show polymorphism among accessions that are similar morphologically and isozymatically (Table 34).

Table 33. Description of groups with similar isozyme patterns (possible genetic duplicates) observed in the cassava Colombian collection maintained at CIAT.

No. of access. For each distinct group	Total No. of each distinct group	Total No. of acces. of each distinct group	Percent (%) of each distinct group	Percent (%) of access. of each distinct group
1	872	872	71	44
2	196	392	16	20
3	77	231	6	12
4	30	120	2.4	6
5	26	130	2.1	6
6	11	66	0.89	3.3
7	7	49	0.57	2.5
8	3	24	0.24	1.2
9	5	45	0.40	2.2
10	2	20	0.16	1.0
11	1	11	0.08	0.5
12	1	12	0.08	0.6
14	1	14	0.08	0.7
TOTAL	1232	1986	100	100

Table 34. Different primer combinations evaluated in this study. Number of alleles per locus and polymorphism information content (PIC) are included (Marin et al 2003).

Primer (*)	Alleles per locus	Polymorphism information content (PIC)	Primer (*)	Alleles per locus	polymorphism information content (PIC)
SSR2	14	0.765	SSR506	13	0.538
SSR198	17	0.672	SSR507	14	0.784
SSR249	17	0.672	SSR521	---- * ----	---- * ----
SSR279	10	0.677	SSRGA-16	6	0.208
SSR313	16	0.729	SSR283	13	0.46
SSR381	11	0.813	SSR188	10	0.539
SSR498	17	0.828	SSR542	---- * ----	---- * ----
SSR503	11	0.725			

References

Chavarriaga A., P.; Maya, M.M.; Bonierbale, M.W; Kresovich, S.; Fregene, M.A.; Tohme M., J.; Kochert, G. 1998. Microsatellites in cassava (*Manihot esculenta Crantz*): Discovery, inheritance and variability. Theoretical and Applied Genetics 97(3):493-501.

Dellaporta, S. L., Wood, J. and Hicks, J. B. 1983. A plant DNA minipreparation: version II. Plant Mol Bio Rep. 1:19-21.

Fregene, M.A.; Gutiérrez A., J.P.; Buitrago R., Ch. (eds.). [2002]. Protocolos genética molecular de yuca. Centro Internacional de Agricultura Tropical (CIAT), Cali,Colombia. 55 p.

González DO, Palacios N, Tohme J (eds) (1995) Protocolos para Marcadores Moleculares. Unidad de Investigación en Biotecnologia, Centro Internacional de Agricultura Tropical (CIAT), Cali-Colombia, Publicación CIAT No. 258.

Hershey, C., Ocampo, C., Jaramillo, G., Ramírez, O. E.,Iglesias, C. and Iwanaga, M. 1991. A proposed procedure for duplicate identification in cassava. Working Document for Internal CIAT discussion.

Jimenez, A. 1994. Identificación de duplicados del banco de germoplasma de yuca (*Manihot esculenta* Crantz) del CIAT. Universidad Nacional de Colombia, Sede Palmira. 115p.

Marin, J., Ospina, C., Barrera, E., Santos, L., Moretta, D., Moreno, Y., and M. Fregene. 2003. Development and use of biotechnology tools for cassava improvement. Annual Report CIAT (Project IP3).

Ocampo, C., Hershey, C., Iglesias, C. and Iwanaga, M. 1993. Esterase isozyme fingerprinting of the cassava germplasm collection held at CIAT. In: Roca, W. and Thro, A.M. (eds.) 1993. Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network

(CBN), Cartagena, Colombia, 25-28 August 1992. Cali, Colombia: Centro Internacional de Agricultura Tropical, 1993. Working Document 123.

Ocampo, C. H., Angel, F., Jímenez, A., Jaramillo, G., Hershey, C., Granados, E. & C. Iglesias. 1995. DNA fingerprinting to confirm possible genetic duplicates in cassava germplasm. *In*: Roca, W. and Thro, A. M. (eds.). Proceedings of the Second International Scientific Meeting of the Cassava Biotechnology network. Bogor, Indonesia, 22-26 August 1994. Working Document No. 150. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia, pp. 145-151.

Sumarani, G.O., Pillai, S.V., Harisankar, P. and S. Sundaresan. 2004. Isozyme analysis of indigenous cassava germplasm for identification of Duplicates. *Genetic Resources and Crop Evolution* 51: 205–209, 2004.

Contributors: C.H. Ocampo (CIAT, GRU), G. Mafla (CIAT, GRU), A. Bohórquez (CIAT, BRU).

Activity 3.1.2. Genetic diversity analysis in an avocado (*Persea americana* Mill.) collection using molecular markers (collaborative work with CORPOICA, funded by Ministerio de Agricultura y Desarrollo Rural, Colombia).

Introduction

Persea americana Mill. presents a wide variety of genetic diversity, probably because avocados evolved in a part of North America, Central America, Colombia and the Caribbean region characterized itself by considerable diversity in climates, related to the varied topography of these regions (Bufler and Ya' acov, 1992; Ramirez et al. 2004). Genome analysis and breeding of avocado is quite difficult mainly because of the size of the trees, a long juvenile phase, and a lack of satisfactory genetic knowledge (Fiedler et al., 1998). The molecular markers promise to help overcome of the obstacles of conventional breeding and genome analysis. In the last few years, several types of molecular markers have been used for the genome analysis of avocado, such as isozymes, RFLPs (chloroplast and ribosomal DNA), DNA fingerprints (DFP), RAPDs, microsatellites and AFLPs (Clegg et al. 1999). In this study we have the following objectives: (1) Standardization of a molecular fingerprinting method to know the genetic diversity in avocado (2) to know the genetic diversity present in an avocado collection conserved ex--situ, using for it molecular markers (AFLPs). (3) to know the redundancy level (possible genetic duplicates) present in the characterized collection. (4) to produce data that can be stored and readily used again for future analysis.

Materials and Methods

The collection of 62 avocado accessions maintained by CORPOICA in Palmira (Colombia) were studied. These accessions represent many landraces from Colombia, Mexico, Guatemala and Trinidad y Tobago. The molecular marker selected was the AFLP technique because of the magnitude of genome coverage. A typical AFLP fingerprint contains between 50 and 100 amplified and analyzable fragments (Vos et al. 1995). The total genomic DNA was extracted from young leaves collected of plants coming from field. In a later stage we will follow the method of AFLP Analysis System I with modifications for avocado.

Results and Discussion

Standardized procedure for the DNA extraction. With the present study, it has been standardized the procedure for the DNA extraction of avocado, so that for this species its extraction is enough difficult, by the high content and activity of polyphenoloxidases (that causes that the extract acquires a gray or brown coloration) that interferes with the DNA extraction (Ramirez et al. 2004). This standardization has been based on the protocol of Dellaporta modified for extraction of DNA of rice for microsatellites (Dellaporta (1983). The modifications made for avocado consist basically of the use of polyvinilpirrolidone (Pvp-40) to eliminate polyphenoloxidases and to avoid the DNA degradation (Figure 1). In addition the application to a washing with phenol: chloroform: octanol (25:24:1) and two washings with chloroform: octanol (24:1), consecutively. Once the DNA has been washed, becomes to precipitate with isopropanol, pelleted and to wash with ethanol, finally to dissolve with TE (10 mM Tris-HCl /1mM EDTA pH 8.0). These washings are essential to be able to make the digestion, and later the DNA amplification.

Standardization of AFLP assays. We selected DNA from six different accessions to test the reliability of AFLP fingerprints. The extracted DNA was digested at an enzyme-to-DNA ratio of 1.5 unit/μg (EcoR1), the Fig. 2 show an completely digested DNA. Six hundred nanograms of DNA each avocado accession were double digested (enzymes EcoR1/Mse1) and and later amplified. For the amplification a total of ten primer combinations EcoR1/Mse1 were selectively tested to identify at least three-four that can shows polymorphism among accessions: E-AT/M-CAC, E-AT/M-CAA, E-AC/M-CTA, E-AC/M-CAG, E-AAC/M-CTG, E-AAC/M-CTC, E-AGG/M-CAT, E-AGG/M-CTT, E-AGC/M-CTT and E-AGC/M-CAT. The amplified fragments were electrophoresed in 6% denaturin polyacrylamide sequencing gels on Sequi-Gen (BioRad) sequencingapparatus.The gels were dyed with silver nitrate.The most-useful primer combinations were those having the highest polymorphism, reproducibility and scorability of AFLP patterns, and that also generate a reasonable number of clearly detectable total fragments. As a result, the four most-polymorphic primer combinations (E-AT/M-CAC, E-AGG/M-CAT, E-AGG/M-CTT, E-AGC/M-CTT), producing clearly readable fragments and overall reproducibility of the AFLP amplification patterns was good. These four primer combinations were selected for the subsequent analysis with the avocado complete collection.

Figure 33. Genomic DNA extracted from young leaves. To the left (first four tracks): a high quality DNA (extracted with the modified procedure described in this study). To the right (tracks 5-8): a degraded DNA (extracted without the modified procedure).

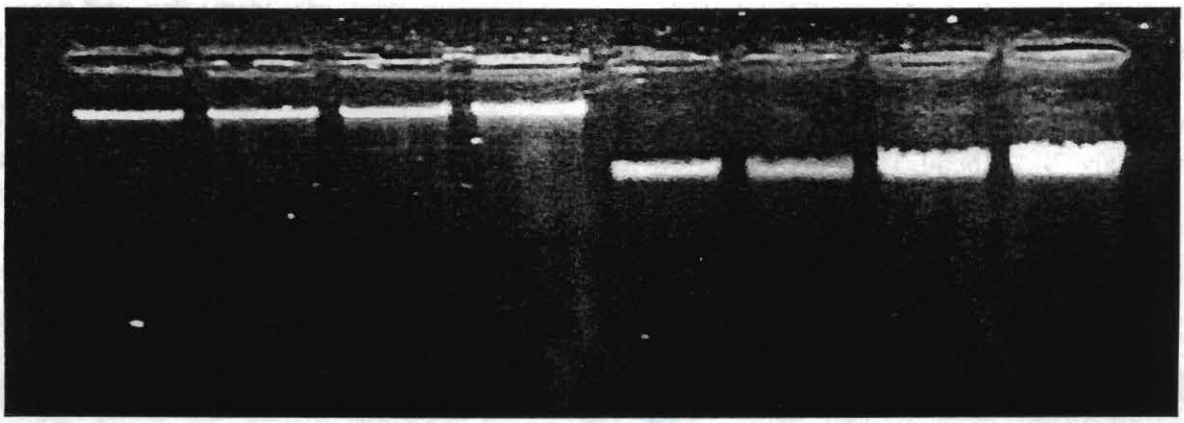
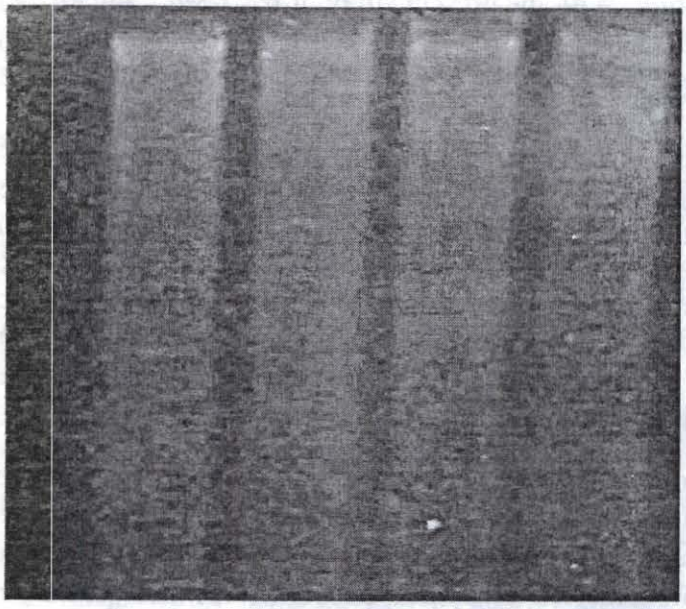


Figure 34. Genomic DNA (extracted with the modified procedure in this study) completely digested with EcoR1.



References

Bufler G. and Ben-Ya'acov A. 1992. A study of the avocado germplasm resources 1988-1990. Proceeding of 2 World Avocado Congress, Orange, California.

Clegg M. T., Kobayashi M., Zhong-Lin J. 1999. The use of molecular markers in the management and improvement of avocado (*Persea americana* Mill.). Revista Chapingo Serie Horticultura 5, 227-231.

Dellaporta, S. L., Wood, J. and Hicks, J. B. 1983. A plant DNA minipreparation: version II. Plant Mol Bio Rep. 1:19-21.

Fiedler, J., Bufler, G. and F. Bangerth. 1998. Genetic relationships of avocado (*Persea americana* Mill.) using RAPD markers. Euphytica 101: 249-255.

Ramírez, I.M., Fuentes J. L., Rodríguez, N.N., Coto, O., Cueto, J., Becker, D. and W. Rohde. 2004. Genetic diversity análisis of cultivated avocado (*Persea americana* Mill.) in Cuba based on agromorphological and molecular markers data. Submitted to plant Breeding.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, A., Pot, J., Peleman, J., Kuiper, M. and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407-4414.

Contributors: César H. Ocampo (CIAT, GRU), Inés Sánchez (CORPOICA), Gerardo Gallego (CIAT, BRU).

Activity 3.1.3 Two-dimensional electrophoresis (2D-IEF-SDS-PAGE) of seed storage proteins of common bean (*Phaseolus vulgaris* L.): I. *Optimization of the technique for the obtaining of improved 2D-Gel proteins and confirmation of the new phaseolin types.*

Introduction

Two-dimensional electrophoresis (2-D electrophoresis) is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. This technique sorts proteins according to two independent properties in two discrete steps: the first-dimension step, isoelectric focusing (IEF), separates proteins according to their isoelectric points (pI); the second-dimension step, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), separates proteins according to their molecular weights (Mr, relative molecular weight) (Dunn & Corbett, 1996).

Materials and Methods

For the 2D methodoly, the protocol has not been changed essentially, compared to classical 2-D electrophoresis with carrier ampholytes (O'Farrell 1975). However, with the use of IPG-Dalt, significant improvements in 2-D electrophoretic separation have come up, permitting higher resolution, especially with narrow-range IPGs and reproducibility of 2-D patterns both within a laboratory and, more importantly, between laboratories (Corbett et al. 1994; Blomberg et al. 1995). This 2D modern technology (IPG-Dalt) is not available at the Electrophoresis Laboratory of GRU, we only have the classical 2D electrophoresis. Therefore the purpose of this work is to present modifications of sample preparation and electrophoretic parameters of the classical 2-D electrophoresis. This analysis was applied for the seed storage protein of common bean (*Phaseolus vulgaris* L.) to confirm the new phaseolin types observed in its primary center of diversity.

Results

Modified sample preparation. Non-protein impurities in the sample can interfere with separation and subsequent visualization of the 2-D result, so sample preparation can include steps to get rid of these substances. The extraction was made on seeds common bean (*Phaseolus vulgaris* L.). These seeds have a high content of polysaccharides. Samples should be supplied free of salts, nucleic acids, polysaccharides and lipids, which interfere with the first dimension isoelectric focusing step. Salt is a particular problem that leads to burning of IPG strips, we used the TCA/acetone precipitation as desalting method. TCA (trichloroacetic acid) is a very effective protein precipitant. The TCA precipitation helps to eliminate the polysaccharides and other interfering substances.

Modified isoelectric focusing (IEF) parameters. Careful casting of the tube gels is extremely important for gel to gel reproducibility. Casting and running of three gels per sample were made, to avoid that some gels are lost during the procedure. The tube gels were run initiating with a pre-electrophoresis: 200 V during 15 minutes, followed with 300 V during others 15 minutes and finishing with 400 V during 30 minutes. After pre-electrophoresis the samples were loaded, applying 1.5 µL by tube. The run was done at 500 V during 10 minutes, then increase the voltaje

to 750 V for 3.5 hours. High voltajes are not recommended because the unit may overheat. After the electrophoresis the gel was stained directly without equilibration; the staining and destaining were similar for 1D-SDS-PAGE.

Modified second-dimension (SDS-PAGE) parameters. Modifications were made in the following steps: Preparation of the stacking and separation gels, removing gel from the tube and loading onto the slab gel, and run conditions for the slab gel. These new 2D electrophoretic conditions allow to obtain improved *2D-Gel* (Figure 3).

2D Characterization of the new phaseolin types. We began applying the modified classical 2-D electrophoresis with the five novel phaseolin types previously reported by Ocampo and Toro (2003). The results show that the novel phaseolin types are different among them. However, its confirmation applying the 2D modern technology (IPG-Dalt) would be recommended.

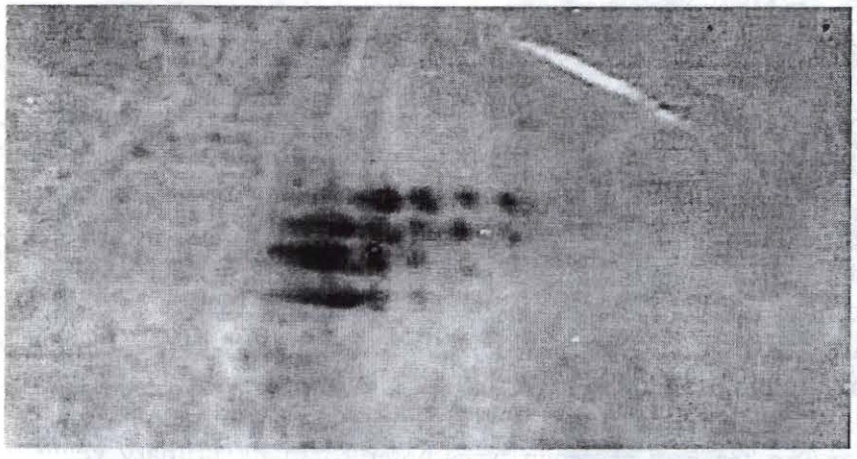


Figure 35. Two-dimensional IEF-SDS-PAGE of the new LI phaseolin from Liborina (Colombia).

References

Blomberg A, Blomberg L, Fey SJ, Larsen PM, Roepstorff P, Degand P, Boutry M, Posch A, Görg A 1995. Interlaboratory reproducibility of yeast protein patterns analyzed byimmobilized pH gradient two-dimensional gel electrophoresis. *Electrophoresis* 16: 1935-1945.

Corbett J, Dunn MJ, Posch A, Görg A (1994) Positional reproducibility of protein spots in two-dimensional polyacrylamide gel electrophoresis using immobilized pH gradient isoelectric focusing in the first dimension: An interlaboratory comparison. *Electrophoresis* 15: 1205-1211.

Dunn, M.J., and J. M. Corbett. 1996. 2-dimensional polyacrylamide gel electrophoresis. *Methods Enzymol.* **271**, 177–203.

O'Farrel, P. H. 1975. The Journal of Biological Chemistry. 250 (10): 4007-4021.

Ocampo, C. H. and O. Toro. Two-dimensional electrophoresis for the seed proteins identification in common bean (*Phaseolus vulgaris* L.): the case of new phaseolin types. Genetic Resources Unit (CIAT). Annual Report 2003.

Contributors: C. H. Ocampo, O. Toro.

Activity 3.1.4. Biochemical characterization of *Phaseolus* germplasm bank for improved and refined collections.

In 2004, 372 genotypes of tepary bean from the *Phaseolus* germplasm bank held in CIAT were analyzed for seed storage proteins (globulins) using ID-SDS-PAGE electrophoresis. This step with morphoagronomic characterization is a requisite for improving the representativeness of the collection.

Contributors: C H. Ocampo and O. Toro.

Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

The staff of the Genetic Resources Unit joined to Universidad Nacional de Colombia, Palmira headquarters, to support the Master of Science in Genetic Resources Conservation, giving theoretical and practical instruction in the Subject of "Management and Conservation of Plant Genetic Resources". The presentations included Conservation of seeds, in vitro conservation and cryoconservation.

A detailed list of courses, other training events and individual trainees can be found in Annex 6.

Contributors: A.M. Torres, G. Mafla, R. Escobar, B. Pineda, D.G. Debouck

Output 4.2. Conferences in national/ international fora

Please see list in Annex 6.

Output 4.3 Public awareness products

Given the high number of visitors and cooperators, the GRU has started the preparation of public awareness materials. This year we have prepared book marks in both Spanish and English (Fig. 36).

Figure 36. Book marks about GRU work in Spanish and English.

Contributors: C. Llano, J.C. Martínez (CIAT Graphic Arts Unit), D.G. Debouck.

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

Output 5.1. Practices on on-farm conservation documented; gene flow studies in the bean plant model (special project supported by BMZ, Germany).

Part 1: field work: Ecogeographic survey of the target species

We present here evidence about the distribution and ecology of the target species in Costa Rica. A risk – real or potential – with transgenical crops is the movement of artificial transgenes to the natural flora through pollen, namely to genetically compatible species (Rissler & Mellon 1996). These are species belonging to the same biological entity or gene pool, or related species usually belonging to the same evolutionary phylum. An important step related to the introduction and management of transgenical crops is thus in the precise mapping of all populations of such species through ecogeographic surveys; the field work will also bring evidence about synchrony of flowering and vectors of pollen.

The genetics of the common bean is such that in case of cross between cultivated and wild forms traits of wild forms will be dominant in the early generations (Bassett 1996; Leakey 1988). In order to establish the direction of the cross (important in the case of transgene from transgenical crops to wild relatives), we will thus need to study traits controlled by nuclear genes and polymorphisms of cpDNA. The hybrid swarms – sometimes called wild-weed-crop complexes (Beebe et al. 1997) - are also worth studying as they could behave as temporary reservoirs of transgenes or as bridges towards non transgenical landraces. Part 2 examines with help of molecular markers materials collected in the field and thought to result from past hybridizations.

It aims at certifying cases of gene flow, its directions, and at providing some insight about the persistence through time.

Given our model crop, under our present knowledge (Freytag & Debouck 2002), we have to consider only the Phaseoli section of the genus. The four related species are thus: *P. albescens, P. costaricensis, P. dumosus*, and *P. vulgaris* (itself, as wild form and as landraces). In the context of Costa Rica, *P. albescens* is not to be considered, since this species is restricted to western Mexico (Ramírez Delgadillo & Delgado Salinas 1999). *P. dumosus* as cultivated species is grown as 'cuba' apparently in several places in Costa Rica, although poorly reported by agronomic authorities and agricultural statistics. As feral it is widely present in many mountainous ranges of the country. It has been shown to belong to the *P. vulgaris* phylum through cpDNA analysis (Schmit et al. 1993). However, it would with very great difficulties be crossed with the common bean as pollen receiver (Camarena & Baudoin 1987), since the hybrid embryos would survive and result into mature plants only through embryo rescue. *P. dumosus* would thus not be a species to consider in this part of the ecogeographic survey.

Although not much wide crossing has been done so far, *P. costaricensis* – shown to belong to the phylum of the common bean (Delgado Salinas et al. 1999; Schmit et al. 1993) – seems genetically compatible with the common bean (Singh et al. 1997). In contrast with *P. dumosus*, hybrids between *P. vulgaris* and *P. costaricensis* (as donor of pollen) have been easily obtained; to our knowledge the key cross with *P. costaricensis* as cytoplasm (pollen receiver) parent has not been attempted. It is thus good cautionary practice to include *P. costaricensis* in our ecogeographic survey; progress in the mapping of all populations has been significant (Araya Villalobos et al. 2001; Debouck et al. 1989) since the discovery of the species (Freytag & Debouck 1996). Similarly, for the wild common bean, the mapping of all populations has made progress (Araya Villalobos et al. 2001) since the first report of presence for this country (Debouck et al. 1989).

Results and Discusion

Phaseolus costaricensis Freytag & Debouck

With the collections done this year (Table 35, 36), we know about 24 populations of this species, mainly distributed on the southern slope of the Volcanic Cordillera in the Central Valley, from N Zarcero (the Tapezco population # 3120) to Río Birrís (the easternmost population # 3128). It is also present on the northern slope of Cerros de Escazú (south of the Central Valley), from Bebedero (# 3138) to Hda. Tres Ríos (# 3144). This range includes three watersheds: Río Virilla, Río Reventazón and Río San Carlos. *P. costaricensis* is also present in the extreme W of Cordillera de Talamanca, in the watersheds of Río Pirrís (# 2122, 2135), Río Naranjo-Savegre (# 2132), and Río Chirripó Pacífico (# 2126, 2128, 3111, 3112, 3113). Four populations have been added to the total reported in 2001 (Araya Villalobos et al. 2001). Our searches for it to the northwest of Costa Rica (e.g. Cordillera de Tilarán, Monteverde), and to the southeast (e.g. Fila Cotón, Sierra de Coto Brus) have failed; one should note that the vegetation type bmh-MB where it thrives is absent from these regions (Araya Villalobos et al. 2001).

This species is usually found in humid montane forests bmh-MB (Araya Villalobos et al. 2001); it is the vicariant species replacing wild *P. vulgaris* in such vegetations at a slightly higher altitude and rainfall, with a dry season of three months. We have found two places where the local populations of *P. costaricensis* would have hybridized, and tentatively with *P. dumosus*: one in Aserrí (# 2114) and three in Quircot (# 3149, 3157, 3158). Interestingly, these two places were the ones where *P. costaricensis* and wild *P. vulgaris* were found together (also in Bebedero and Zarcero). Pollination is by humming-birds (# 3120, 3127, 3144) and carpenter bees (# 2116, 2132, 3127), as indicated by the field notes.

Phaseolus vulgaris L.

With the collections done this year (Table 35, 37), we know of 22 populations of wild common bean distributed in four watersheds at intermediate altitude in Costa Rica. Ten populations have been found in the Central Valley or watershed of Río Vírilla (ending in Río Grande de Tarcoles), seven on the southern slope (#2097 Tarbaca, 2111 Aserrí, 3136 San Miguel Desamparados, 3137 Bebedero, 3140 Parque Iztarú, 3143 Hda. Tres Ríos, 3178 Guatuso), and three on the northern slope (#3106 Chagüite, 3132 Zarcero, 3133 Sabana Redonda). Ten populations have been found in the upper valley of Río Grande de Candelaria: six on the northern slope (# 3131 Jérico, 3134 Tranquerillas, 3135 Chirogres, 3147 El Tigre, 3148 Manzano, 3190 Vuelta de Jorco), and four on the southern slope (# 3184 Río Tarrazú, 3186 in Bajo Los Angeles, and 3188 and 3189 in the surroundings of San Andrés). The mountainous range that separates these two watersheds – Cerros de Cedral o de Escazú – has thus the largest number of populations: thirteen (7+6). One population has been found in the upper valley of Río Reventazón (# 3126 Quircot), which is the only one so far on the Atlantic slope of the continental divide. One population has been found in the upper valley of Río Pirrís (# 3168 Copey), the southernmost population to the southeast of the country. Our attempts to find wild common bean in other parts of Costa Rica, namely the upper Río Savegre, Río División and Río Chirripó Pacífico have failed so far. There might still be one population in the watershed of Río Pirrís (the slope north of San Marcos de Tarrazú, but heavily cleared for coffee plantations), and one on the slope of Fila Bustamante. All vegetations where it thrives reported on maps of life zones (Bolaños M. & Watson C. 1993; Gómez Pignataro 1986; Tosi, 1969) have been visited.

Wild common bean is usually found in subhumid montane forests (bmh-P, bh-MB and bmh-MB: (Araya Villalobos et al. 2001)) at intermediate altitude (Table 36), now largely cleared for coffee plantations and urban areas. In this habitat, the end of the rainy season coincides with the flowering period, and mists are not frequent; bean plants thus escape pressures from diseases such as anthracnose and root rots, as well as drought stresses, and seed dispersal will occur during the dry season (3-4 months) (Araya Villalobos et al. 2001). Germination of wild bean will occur from July onwards, with flowering in September-December anticipating thus synchronization of flowering with landraces during that period. Synchronization in the case of bush varieties, usually planted in October-November, will take place only during a short period (late November- December). Carpenter bees, bumble bees and honey bees have been seen as active in the pollination at Quircot, Jérico, and Sabana Redonda, as indicated by the field notes.

On the other hand, the continuing contact between wild forms and cultivars of common bean is going down, because of the use of herbicides or the practice of weeding. In Quircot the use of

atrazin in maize has virtually eliminated the wild bean from certain plots. In El Manzano and in Chirogres, weeding has seriously reduced the wild bean to just a very few plants. Coffee plantations and use of herbicides therein have eliminated the population # 3131.

For economic reasons (competitive prices of grain commodities), the traditional association of maize and beans ('fríjol tapado') is much lesser planted in the Central Valley of Costa Rica as compared to decades ago. As a matter of fact, the association has almost disappeared from the valley; the contact between the wild relative and the bean crop is nowadays scarce. During the course of the project and during previous years to it, we were able to identify in the field five places (Table 38) where hybrid swarms or wild-weed-crop complexes (Beebe et al. 1997) have existed. These were: Tarbaca (alt. 1750 masl), Aserrí (1560 masl), Quircot (1540 masl), Zarcero (1610 masl), and possibly Manzano (1370 masl). Out of these sites in 1998, the only one with sufficient planting of common bean and acreage was Quircot, and thus chosen for additional studies. In the period 1987-1998, the site of Tarbaca disappeared as original because of housing and road expansion, and that of Zarcero was converted into a quarry in 1998-2000. In Aserrí the planting of common bean has been greatly reduced, and so were the weedy forms. At Quircot and Manzano, planting of common bean has been switched to that of *P. dumosus* 'cuba'; some traditional landraces have been present at Quircot since 1998 (e.g. 'Higuerilla' # 3152, 'Vainica amarilla' # 3153), sometimes as escape from cultivation.

The fact that large planting of common bean has disappeared at the five sites is a problem inasmuch we cannot infer about the importance of variants and weedy forms that can be generated by recent/ continuing gene flow as seen elsewhere (Beebe et al. 1997). It is however useful as it would allow to see the permanence over time of effects of gene flow once demonstrated. In Table 38 about the others reported as "none", a survey is however being done every time intermediate types possibly resulting from gene flow have been observed. One can imagine that it is very difficult to certify that any of the wild common bean population has not been in contact with landraces in the past. Indeed before 1850 and the increase of coffee plantations in Central Costa Rica it is likely that the acreage of common bean landraces was much higher than at present. For instance, Puriscal is today well-known for its coffee, while 'purisco' means 'bean blossom' in rural language in that part of Costa Rica.

Table 35 – List of materials found in 2003-2004, sites and coordinates.

Collectors' Number	Species	Province, district, closest site	Longitude	Latitude	Altitude (masl)
3165	*Leptostachyus*	Cartago, San Nicolás, Quircot	83°56'W	9°54'N	1540
3166	*Oligospermus*	Cartago, Tobosí, Tablón	84°02'W	9°49'N	1470
3167	*Leptostachyus*	Cartago, Tobosí, Tablón	84°02'W	9°49'N	1470
3168	*vulgaris* silv.	San José, Sta. María de Dota, Copey	83°57'W	9°39'N	1600
3169	*lunatus* silv.	San José, Sn Marcos Tarrazú, Sn Lorenzo	84°03'W	9°38'N	1420
3170	*Tuerckheimii*	San José, Sta. María de Dota, Los Angeles	83°58'W	9°38'N	1860
3171	*Tuerckheimii*	San José, Sta. María de Dota, Cima de Dota	83°55'W	9°40'N	1980
3172	sp.	San José, Sn Isidro, Sta. Eduviges	83°45'W	9°30'N	1520
3173	sp.	San José, Sn Isidro, Las Nubes	83°46'W	9°28'N	1570
3174	*Manihot brachyloba*	San José, San Pedro, Sta Teresa	83°34'W	9°21'N	1110
3175	*lunatus* silv.	San José, San Pedro, Sn Jerónimo	83°30'W	9°21'N	1190
3176	*Xanthotrichus*	San José, Alajuelita, Llano de Alajuelita	84°07'W	9°52'N	1400
3177	*lunatus* silv.	San José, Alajuelita, Llano de Alajuelita	84°07'W	9°52'N	1470
3178	*vulgaris* silv.	San José, Desamparados, Guatuso	84°02'W	9°51'N	1380
3179	*Oligospermus*	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3180	*Xanthotrichus*	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3181	*lunatus* silv.	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3182	*Talamancensis*	San José, Sta María de Dota, *do.*	83°59'W	9°39'N	1490
3183	*Tuerckheimii*	San José, San Pablo, *do.*	84°02'W	9°43'N	1720
3184	*vulgaris* silv.	San José, San Pablo, Sn Cristobal Sur	84°01'W	9°44'N	1450
3185	*lunatus* silv.	Puntarenas, Buenos Aires, Dúrika	83°16'W	9°15'N	1080
3186	*vulgaris* silv.	San José, San Gabriel, Bajo Los Angeles	84°05'W	9°44'N	1200
3187	*Leptostachyus*	San José, San Gabriel, Bajo Los Angeles	84°05'W	9°44'N	1200
3188	*vulgaris* silv.	San José, San Gabriel, Sn Andrés León Cor.	84°05'W	9°44'N	1250
3189	*vulgaris* silv.	San José, San Gabriel, Sn Andrés León Cor.	84°06'W	9°44'N	1300
3190	*vulgaris* silv.	San José, San Ignacio, Vuelta de Jorco	84°07'W	9°49'N	1480

Table 36 – List of populations of *P. costaricensis* found, sites, watershed, coordinates and year.

Collectors' Number	Province, district, closest site	Watershed	Longitude	Latitude	Altitude (masl)	Year found
1. 2093	Cartago, San Nicolás, Cta Chinchilla	Reventazón	83°53'W	9°54'N	1650	1987
2. 2095	San José, Aserrí	Virilla sur	84°06'W	9°51'N	1470	1987
3. 2102	San José, Alajuelita, San Miguel	Virilla sur	84°07'W	9°52'N	1620	1987
4. 2116	San José, Aserrí, Piedra	Virilla sur	84°07'W	9°52'N	1590	1987
5. 2118	Cartago, La Unión, La Carpintera	Virilla sur	83°58'W	9°53'N	1600	1987
6. 2119	Cartago, Tres Ríos, Dulce Nombre	Virilla sur	83°57'W	9°57'N	1750	1987
7. 2122	San José, Sta. María de Dota, Copey	Pirrís	83°57'W	9°40'N	1660	1987
8. 2126	San José, Sn Isidro, Pueblo Nuevo	Chirripó P.	83°40'W	9°26'N	1550	1987
9. 2128	San José, Sn Isidro, Herradura	Chirripó P.	83°37'W	9°30'N	1690	1987
10. 2132	San José, Sn Isidro, Providencia	Naranjo	83°51'W	9°34'N	1990	1987
11. 2135	San José, Sn Isidro, Copey	Pirrís	83°55'W	9°37'N	2080	1987
12. 3111	San José, Herradura, Herradura	Chirripó P.	83°37'W	9°29'N	1550	1998
13. 3112	San José, Buena Vista, La Piedra	Chirripó P.	83°40'W	9°31'N	1500	1998
14. 3113	San José, Buena Vista, N La Piedra	Chirripó P.	83°41'W	9°31'N	1880	1998
15. 3115	Cartago, Sn Rafael, hacia Cot	Reventazón	83°54'W	9°53'N	1560	1998
16. 3118	Cartago, Pacayas, Cervantes	Reventazón	83°47'W	9°54'N	1570	1998
17. 3120	Alajuela, Zarcero, Tapezco	San Carlos	84°24'W	10°13'N	1710	1998
18. 3122	Alajuela, Zarcero, Río Seco	Virilla norte	84°23'W	10°10'N	1610	1998
19. 3127	Cartago, Cartago, Quircot	Reventazón	83°56'W	9°54'N	1510	1998
20. 3128	Cartago, Pacayas, Río Birrís	Reventazón	83°47'W	9°55'N	1520	1998
21. 3138	San José, Escazú, Bebedero	Virilla sur	84°10'W	9°54'N	1700	2002
22. 3139	San José, Sn Rafael, Vista de Mar	Virilla norte	83°58'W	9°58'N	1790	2002
23. 3142	Cartago, San Nicolás, Río Tarras	Reventazón	83°55'W	9°55'N	2000	2002
24. 3144	Cartago, La Unión, Hda Tres Ríos	Reventazón	83°59'W	9°54'N	1630	2002

Table 37 – List of populations of wild common bean found, sites, watershed, coordinates and year.

Collector s' Number	Province, district, closest site	Watershed	Longitude	Latitude	Altitude (masl)	Year found
1. 2097	San José, Tarbaca	Virilla sur	84°07'W	9°49'N	1750	1987
2. 2111	San José, Aserrí	Virilla sur	84°07'W	9°52'N	1560	1987
3. 3106	Alajuela, Carrizal, Chagüite	Virilla norte	84°10'W	10°06'N	1510	1998
4. 3126	Cartago, San Nicolás, Quircot	Reventazón	83°56'W	9°54'N	1540	1998
5. 3131	San José, Desamparados, Jérico	Candelaria n	84°03'W	9°49'N	1540	1998
6. 3132	Alajuela, Alfaro Ruíz, Zarcero	Virilla norte	84°23'W	10°10'N	1610	2002
7. 3133	Alajuela, Poas, Sabana Redonda	Virilla norte	84°14'W	10°07'N	1380	2002
8. 3134	San José, Aserrí, Tranquerillas	Candelaria n	84°07'W	9°48'N	1500	2002
9. 3135	San José, Aserrí, Chirogres	Candelaria n	84°06'W	9°48'N	1480	2002
10. 3136	San José, Desamparados, Sn Miguel	Virilla sur	84°04'W	9°51'N	1370	2002
11. 3137	San José, Escazú, Bebedero	Virilla sur	84°10'W	9°54'N	1600	2002
12. 3140	Cartago, La Unión, Pque Iztarú	Virilla sur	83°58'W	9°54'N	1750	2002
13. 3143	Cartago, La Unión, Hda Tres Ríos	Virilla sur	83°59'W	9°54'N	1500	2002
14. 3147	San José, Aserrí, El Tigre	Candelaria n	84°06'W	9°49'N	1450	2003
15. 3148	San José, Desamparados, Manzano	Candelaria n	84°05'W	9°49'N	1370	2003
16. 3168	San José, Sta. María de Dota, Copey	Pirrís	83°57'W	9°39'N	1600	2003
17. 3178	San José, Desamparados, Guatuso	Virilla sur	84°02'W	9°51'N	1380	2003
18. 3184	San José, San Pablo, Sn Cristobal Sur	Candelaria s	84°01'W	9°44'N	1450	2003
19. 3186	San José, San Gabriel, Bajo Los Angeles	Candelaria s	84°05'W	9°44'N	1200	2004
20. 3188	San José, San Gabriel, Sn Andrés León Cor	Candelaria s	84°05'W	9°44'N	1250	2004
21. 3189	San José, San Gabriel, Sn Andrés	Candelaria s	84°06'W	9°44'N	1300	2004
22. 3190	San José, San Gabriel, Vuelta de Jorco	Candelaria n	84°07'W	9°49'N	1480	2004

Table 38 – List of populations of wild common bean found, possible weedy hybrid forms, sites, and year.

Collectors' Number for Wild forms	Collectors' Number for Weedy forms	Province, district, closest site	Year found
2097	2098	San José, Tarbaca	1987
2111	2115	San José, Aserrí	1987
3106	none	Alajuela, Carrizal, Chagüite	1998
3126	3151, 3155, 3158	Cartago, San Nicolás, Quircot	1998
3131	none	San José, Desamparados, Jérico	1998
3132	3121	Alajuela, Alfaro Ruíz, Zarcero	2002
3133	none	Alajuela, Poas, Sabana Redonda	2002
3134	none	San José, Aserrí, Tranquerillas	2002
3135	none	San José, Aserrí, Chirogres	2002
3136	none	San José, Desamparados, Sn Miguel	2002
3137	none	San José, Escazú, Bebedero	2002
3140	none	Cartago, La Unión, Pque Iztarú	2002
3143	none	Cartago, La Unión, Hda Tres Ríos	2002
3147	none	San José, Aserrí, El Tigre	2003
3148	possibly one	San José, Desamparados, Manzano	2003
3168	none	San José, Sta. María de Dota, Copey	2003
3178	none	San José, Desamparados, Guatuso	2003
3184	none	San José, San Pablo, Sn Cristobal Sur	2003
3186	none	San José, San Gabriel, Bajo Los Angeles	2004
3188	possibly one	San José, San Gabriel, Sn Andrés León Cor	2004
3189	none	San José, San Gabriel, Sn Andrés	2004
3190	none	San José, San Gabriel, Vuelta de Jorco	2004

References

Araya Villalobos, R., W.G. González Ugalde, F. Camacho Chacón, P. Sánchez Trejos & D.G. Debouck. 2001. Observations on the geographic distribution, ecology and conservation status of several *Phaseolus* bean species in Costa Rica. Genet. Resources & Crop Evol. **48:** 221-232.

Bassett, M.J. 1996. List of genes - *Phaseolus vulgaris* L. Annu. Rept. Bean Improvement Coop.(USA) **39:** 1-19.

Beebe, S., O. Toro Ch., A.V. González, M.I. Chácon & D.G. Debouck. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Resources & Crop Evol. **44:** 73-91.

Bliss, F.A. 1980. Common bean. *In:* W. R. Fehr and H. H. Hadley (eds.) Hybridization of crop plants. American Society of Agronomy, Crop Science Society of America, Madison, Wisconsin, USA. pp. 273-284.

Bolaños M., R.A., V. Watson C. 1993. Mapa ecológico de Costa Rica. Centro Científico Tropical. Scale 1:200.000. 9 sheets, San José, Costa Rica.

Camarena, F. & J.P. Baudoin. 1987. Obtention des premiers hybrides interspécifiques entre *Phaseolus vulgaris* et *Phaseolus polyanthus* avec le cytoplasme de cette dernière forme. Bull. Rech. Agron. Gembloux **22:** 43-55.

Debouck, D.G., R. Araya Villalobos, R.A. Ocampo Sánchez & W.G. Gonzalez Ugalde. 1989. Collecting *Phaseolus* in Costa Rica. FAO/IBPGR Plant Genet. Resources Newsl. **78/79:** 44-46.

Delgado Salinas, A., T. Turley, A. Richman & M. Lavin. 1999. Phylogenetic analysis of the cultivated and wild species of *Phaseolus* (Fabaceae). Syst. Bot. **24:** 438-460.

Freytag, G.F. & D.G. Debouck. 1996. *Phaseolus costaricensis*, a new wild bean species (Phaseolinae, Leguminosae) from Costa Rica and Panama, Central America. NOVON **6:** 157-163.

Freytag, G.F. & D.G. Debouck. 2002. Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Botanical Research Institute of Texas. SIDA Botanical Miscellany 23, Fort Worth, Texas, USA, 300p.

Gómez Pignataro, L.D. 1986. Vegetación de Costa Rica - Apuntes para una biogeografía costarricense. Vol. 1. Editorial Universidad Estatal a Distancia, San José, Costa Rica, 327p.

Ramírez Delgadillo, R. & A. Delgado Salinas. 1999. A new species of Phaseolus (Fabaceae) from west-central Mexico. SIDA **18:** 637-646.

Rissler, J. & M. Mellon. 1996. The ecological risks of engineered crops. Massachusetts Institute of Technology Press, Cambridge, Massachusetts, USA, 168p.

Schmit, V., P. du Jardin, J.P. Baudoin & D.G. Debouck. 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.

Singh, S.P., D.G. Debouck & W.M. Roca. 1997. Successful interspecific hybridization between *Phaseolus vulgaris* L. and *P. costaricensis* Freytag & Debouck. Annu. Rept. Bean Improvement Coop. (USA) **40:** 40-41.

Tosi, J.A. 1969. Mapa ecológico, República de Costa Rica, según la clasificación de zonas de vida del mundo de L.R. Holdridge. Centro Científico Tropical, San José, Costa Rica, 1 hoja.

Contributors: D.G. Debouck (CIAT Genetic Resources Unit), and R. Araya Villalobos (Estación Experimental FBM, Universidad de Costa Rica).

Part 2: work in the lab

We present here evidence on gene flow events among biological forms of common bean in Costa Rica, in addition to our previous work (González-Torres et al. 2003; González-Torres et al. 2004). The analysis was carried out on six natural populations of common bean, using two seed sets collected in the Central Valley over different years: a) 1987, 1998, 2003, and b) 2004. After an in-depth characterization of wild forms and a couple of landraces still found in the Central Valley, we focus on weedy or intermediate forms, the characteristics, namely 100-seed weight, of which are intermediate between the two biological forms.

Figure 37 graphically shows three results found in the first seed set: a) **Repeated events of gene flow of wild pollen towards the cultivated forms**. This is so far the dominant direction evidenced in our work. Individual 2 there has small seeds as does the wild form, and two micro sats alleles obtained from the cultivated form through gene flow. Individual 10 shows a 'wild' cp DNA haplotype in contrast with some 'cultivated' characteristics (a bigger seed for instance, as compared to the wild forms) and one infrequent SSR allele (gray wave). b) **Events of Chloroplast capture** (obtained through repeated outcrossing, resulting in individuals with the nuclear genome of a particular biological form with cytoplasmic genome of the other). Individual 1 displays mainly 'wild' characteristics; however it has its chloroplast haplotype and two SSR alleles typical of 'cultivated' forms. Individual 4 has hybrid isozyme patterns and one hybrid SSR locus. Individuals 5 and 6 have all characteristics as found in wild forms, but their chloroplast haplotype is that of 'cultivated' form.

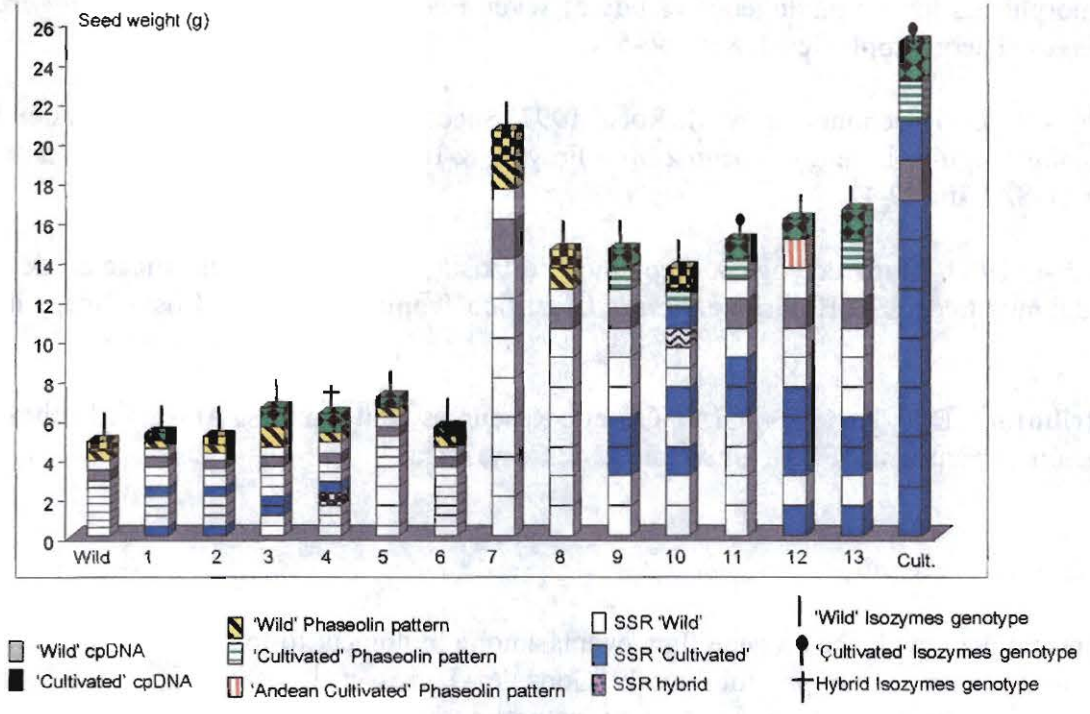


Figure 37. Graphical representation of weedy materials with the different markers used to indicate evidence of gene flow. The gray boxes are common SSR loci among biological forms.

c) Outcrossing between Andean and Mesoamerican genepools: in Figure 38, individual 12 displays chloroplast haplotype typical of 'Mesoamerican cultivated form', and a phaseolin type as found in Andean materials. In contrast, its SSR alleles are of mixed nature, 'wild' and 'cultivated'. This situation could be understood along the introduction of Andean materials into Costa Rica, as to avoid pathogen damage and to have a more attractive seed type (color and size) for sale on market. The results obtained in the characterization of the populations are summarized in Table 39.

Table 39. Morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter. The underlined fonts refer to 'wild' characteristics, while the italic fonts refer to 'cultivated' characteristics.

Biological form	Seed average weight (g)	Phaseolin type	Isozymes		Microsatellites		Chloroplast haplotype
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 (2.5-7) N=443	"S-4" "S" "M1" "S-3" N=392	DIA -1 N=229	PRX 100 N=197	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=134	160 80 164 110 165 147 138 122 224	H N=210
Weedy	13 (8-21.3) N=226	"C" "CH" "H" "S" "X-7" "S-4" N=196	DIA-1 DIA-2 DIA-4 N=157	PRX 100 PRX 98 N=182	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=226	160, 177 80 164, 185 110 165, 189 147, 150 138, 148 122, 136 224, 243	G, H J, K, L N=170
Cultivated	23 (22-46) N=198	"S" "T" "X-7" "CH" N=198	DIA -2 DIA -4 N=64	PRX 98 N=29	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=58	180 80 183 110 189 150 148 136 243	J, K, L N=53

The discrimination among some chloroplast haplotypes (namely, 'H' or 'G'), key as to determine the direction of gene flow in the individuals of the first set, requires the sequencing of rps14-psaB gene fragment, and then a SNPs analysis using a fluorescent method.

The figure 30 shows the presence of Adenine nucleotide (highest columns) or Thiamine (shortest columns) in the gene fragment NdhA intron of chloroplast indicating the haplotype identification of wild type or cultivated type, respectively. The identification of haplotype 'G' in some evaluated individuals suggests the introduction of cultivated materials from México and Guatemala to Costa Rica and crossing of these materials with local wild materials, since this haplotype seems not original in Costa Rica (Chacón 2001).

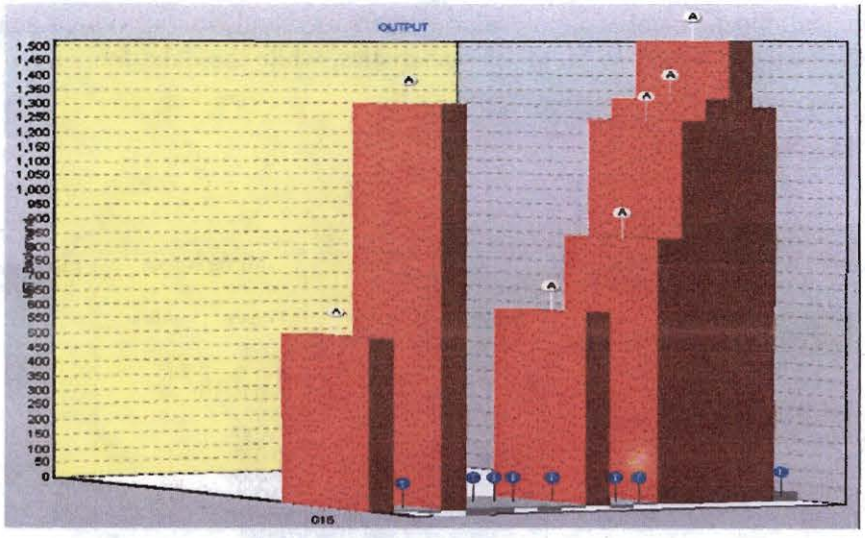


Figure 38. Result of the SNPs analysis used to determine chloroplast haplotypes in a selection of individuals.

The SNPs analysis indicates evidence of chloroplast capture of haplotype 'G' into wild materials. This result suggests a complex and different model of outcrossing in natural populations of local materials of Costa Rica with cultivated materials from Guatemala. In addition, we have seen many individuals with haplotypes 'J' and 'L', usually found in landraces of countries near Costa Rica, that are used by Costa Rican farmers.

On the other hand, out of the collection realized during 2004, we selected 657 seeds (600 seeds of 'wild' type; 27 seeds of 'landraces', and 30 seeds of 'weedy' types; see Fig. 39 for our selection criteria, based on 100-seed weight). In addition, we germinated 894 seeds from previous years for further analysis.

Table 40. Individual analyzed collection realized during 2004.

Number of seeds	Number of plants collected	Extraction of DNA	Determination of Chloroplast haplotype
1551	1176	1176	345 individuals have chloroplast haplotype 'J' or 'L' 644 individuals show chloroplast haplotype 'F', 'G', or 'H' 38 individuals have a possible chloroplast haplotype 'A', 'B', 'C' or 'D' (to verify) 149 individuals have still an indeterminate chloroplast haplotypes

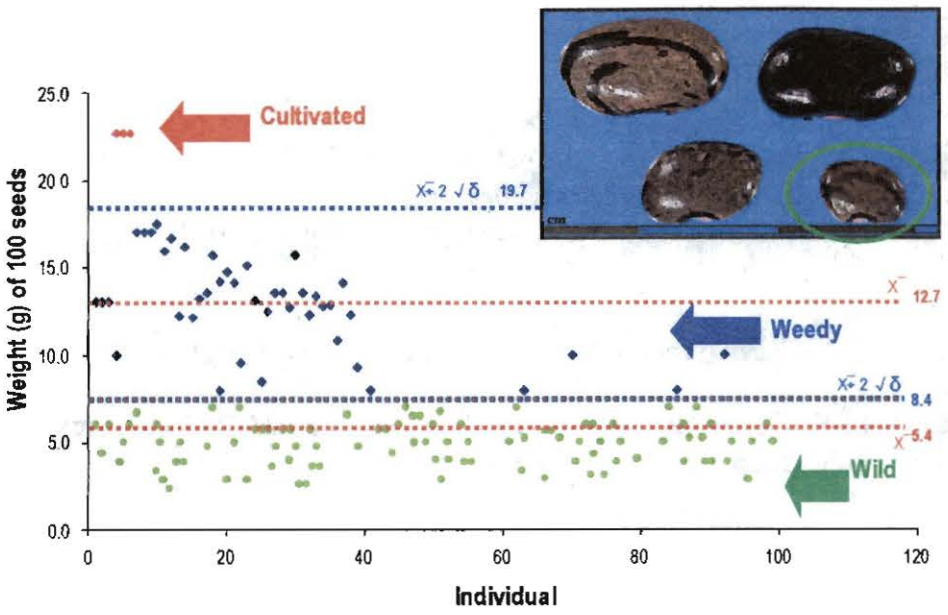


Figure 39.

The methodology used for the characterization of the wild and cultivated populations (biochemical and molecular tools) presented an ample discrimination range among individuals. Microsatellites proved to be the most useful tool for this study, since some microsatellites differentiate up to 83%, cpDNA showed a 69% differentiation, and the isozymes were the markers showing the lowest differentiation potential. Though the phaseolin marker exhibited an average differentiation power (54%), it is an easy-to-implement marker that fortifies the analysis in combination with the rest of the markers exhibiting higher differentiation.

These previous results allowed us to reliably establish the existence of gene flow in common bean in the Central Valley of Costa Rica. This affirmation is endorsed with 214 cases of gene flow from a total of 217 selected individuals, after a careful study of the diversity present in the different wild bean populations. The direction of gene flow in the evaluated individuals (184, with complete data, for Quircot) was mainly directed from wild material to the cultivated type (82.1%). However, 34 individuals (17.9%) represent cases of gene flow from cultivated materials to the wild form. Given the long extent of the flowering period in the wild form as compared to the cultivated form, namely the modern bush cultivars, the former result was expected. Although the flow of 'cultivated' pollen to the wild form is not as high as the one reported by Papa & Gepts (2003) for complexes studied in Mexico, the phenomenon in the Costa Rican conditions is not negligible. As observed in other conditions in Latin America (Beebe et al. 1997), this flow could explain the remanent genetic diversity often present in landraces.

The gene flow events were performed mainly between common bean belonging to the Mesoamerican gene pool; nevertheless 8% of the cases were between different gene pools (Mesoamerican vs Andean). The direction of pollen for this 8% was in all cases from wild forms towards cultivated forms. This crossing between gene pools can be related to the disclosure of the 'cripple' phenomenon in the plant material grown in greenhouses (confirming early

observations: Shii et al. 1980; Singh & Molina 1996). For these we observed a 30% mortality of the plants and better adaptation of the plants to temperature conditions between 4-16 °C.

References

Beebe, S., O. Toro Ch., A.V. González, M.I. Chácon & D.G. Debouck. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Resources & Crop Evol. **44:** 73-91.

Chacón-Sánchez, M.I. 2001.PhD Thesis,Univ. of Reading, United Kingdom.

González-Torres, R.I., E. Gaitán, R. Araya, O. Toro, J. Tohme & D.G Debouck. 2004. Annu. Rept. Bean Improvement Coop. (USA) 47:167-168.

González-Torres, R.I., E. Gaitán, M.C. Duque, O. Toro, J. Tohme & D. Debouck. 2003. Annu.Rept.BIC. (USA) 46:1-2

Papa, R. & P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theor. Appl. Genet. **106:** 239-250.

Shii, C.T., M.C. Mok, S.R. Temple & D.W.S. Mok. 1980. Expression of developmental abnormalities in hybrids of *Phaseolus vulgaris* L.. Interaction between temperature and allelic dosage. J. Hered. **71:** 218-222.

Singh, S.P. & A. Molina. 1996. Inheritance of crippled trifoliate leaves occurring in interracial crosses of common bean and its relationship with hybrid dwarfism. J. Hered. **87:** 464-469.

Contributors: R.I. González-Torres, E. Gaitán Solís, O. Toro, J. Tohme & D.G. Debouck.

Output 5.2. Contribution made towards protected areas in Latin America: databases about distribution of wild relatives of crops

We have continued with the establishment of databases about the geographic distribution of wild relatives for the so-called CIAT mandate crops. The objectives of that work are:

i. correct identification of materials collected and kept in *ex situ* conservation facilities (namely CIAT genebank, and other collaborating institutions). Another output of this work is the taking of digital images of vouchers and their access through our web site (a service acclaimed by the Botanical Society of Colombia).
ii. geographic distribution of wild relatives of direct interest in breeding activities (namely acquisition of germplasm useful to the breeders).

iii. distribution of wild relatives genetically compatible with the crop, in view of introduction and management of transgenical crops.
iv. monitoring of modification/ destruction of natural habitats and disappearance of populations.

This year we have collated information in the following herbaria: CHAPA, CR, ENCB, F, INB, and P. These data will help us to build up the pilot for the component of threat analysis for a regional project in preparation with the Natural Resources Division of the World Bank. This pilot was agreed upon with the six partners of the project (CONABIO of Mexico, I. von Humboldt of Colombia, INBio of Costa Rica, CIAT, Cornell University and Smithsonian Institute) at first meeting in February 2004. Agreements to 'repatriate' that information to CONABIO of Mexico and INBio of Costa Rica were also made.

Contributor: D.G. Debouck

Activity 5.2.1.:Comparison of areas in the region of the stigma among wild and cultivated forms (landrace and modern) of *Phaseolus vulgaris* L.

Introduction

In this study we analyzed the differences in the area of the stigma among cultivated (landrace and modern) and wild forms (which corresponds to three biological forms) of *Phaseolus vulgaris* L. of the Meso-American and Andean gene pools. The objective was to compare the proportions corresponding to the total, terminal and internal areas of the stigma (Figure 40). A preliminary analysis of 30 flowers for one introduction of cultivated and wild showed that it is not necessary to analyze a high number of stigmas to get additional accuracy in the statistical treatment.

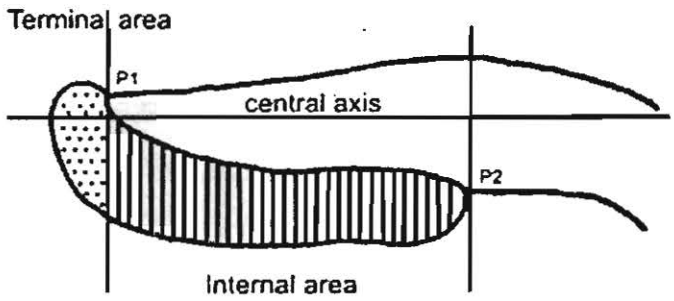


Figure 40. Division of areas in the stigma region.

Materials and methods

Two completely colored flower buds of 30 introductions were collected for each biological status in the two gene pools. These flower buds were immersed in an AFA solution (Bridson & Forman 1992). The dissection consisted of a cut of the style in the part proximal to the stigma. Diagrams of the stigma zone were made with a camera lucida adapted to a stereomicroscope, using a 4.0X increase. The diagrams were inked with black color, in order to make a scan of the images and to use the program WinRhizo.Pro.V2002c for obtaining the exact data of areas (the data were analyzed with a transformation of 1mm =3 cm).

Results and Discussion

A variance analysis was done where significant statistical differences were observed with a probability of 95% in three major differences: 1. Total area among gene pools, 2. Total Area between biological forms, and 3.Differences among forms in the proportions of terminal and internal areas (Figure 32).

1. Comparing the total areas there was a difference among the size of stigmas for the two gene pools showing a bigger size for the material belonging to the Andean pool irrespective of the biological state (3.13 cm2 vs. 2.66 cm2).
2. Comparing each biological form, a bigger size of stigmatic area has been found in the cultivated materials as compared to the wild. For the Andean gene pool the average values of total area in modern and traditional were of 3.15 cm2 and 3.42 cm2, respectively, in contrast with 2.88 cm2 in the wild materials. For the Meso-American gene pool the mean values were of 2.79 cm2 and 2.70 cm2 in comparison with 2.43 cm2; the same pattern has been seen in the Andean gene pool.
3. Very significant statistical differences were observed about the proportions between the terminal and internal areas among the biological forms. This might be due to a bigger proportion of stigmatic area towards the terminal region in the case of the wild material for both gene pools. In the Andean gene pool the proportion of the terminal area of the stigma is 46.03% and in the Meso-American is 49.76%, which contrasts with an average proportion of 22.23% for the cultivated materials in both gene pools. These differences in the proportions of the stigma showed preliminarily a bigger area of the cultivated material toward the internal part of the flower. This spatial balance of stigmatic areas promotes self-pollination, and by this way wanted characteristics in the cultivated materials can be conserved. The terminal area of the stigma is probably associated to processes of crossed pollination, itself associated with increased genetic diversity, as shown by studies conducted on wild materials (e.g. Koenig & Gepts 1989). The morphological variations in style and stigma of *Phaseolus vulgaris* can be associated with gigantism resulting from the domestication process (Smartt 1988).

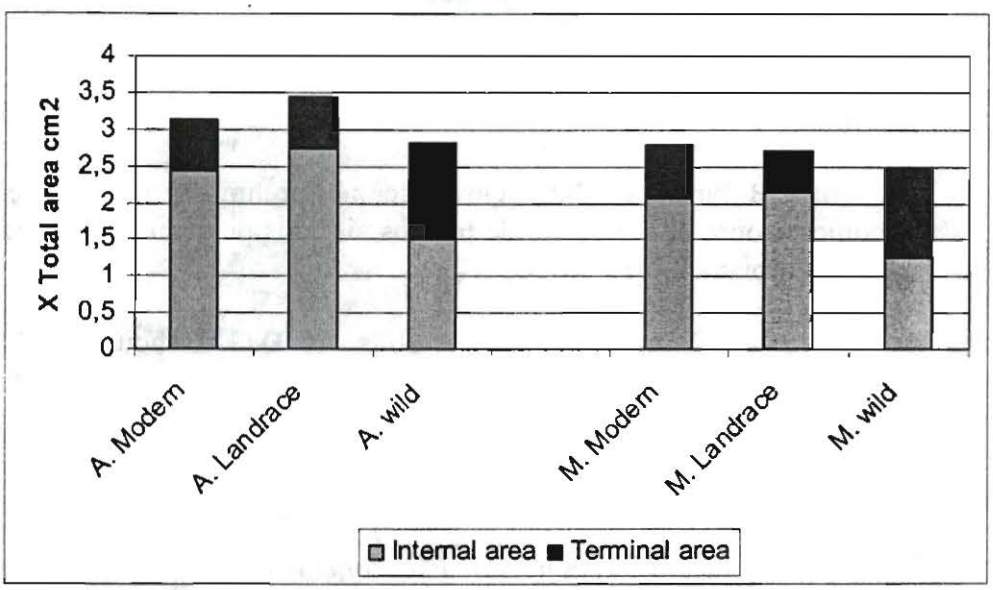


Figure 41. Comparison of stigma areas in Andean (left sided bars) and Mesoamerican (right sided bars) gene pools.

Contributors: J. M. Salcedo, D.G. Debouck

References

Bridson, D & L. Forman (eds.). 1992. The herbarium handbook, pp. 228-229. Royal Botanical Garden Kew

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

Smartt, J. 1996. morphological, physiological and biochemical changes in phaseolus beans under domestication. complex. in: p. gepts (ed.). genetic resources of phaseolus beans, pp. 143-161. kluwer academia publishers.

6. Annexes

6.1. List of publications by Project Staff in 2004

A. In refereed journals:

Bálcazar, M.S., A.L. Rivera & B. Pineda L.. 2003. Observaciones preliminares sobre el efecto *in vitro* de bacterias antagónicas sobre el desarrollo de hongos aislados de semillas de *Brachiaria brizantha*. Fitopatología Colombiana 27 (1): 33-36.

González-Torres R.I., R. Araya Villalobos, E. Gaitán Solís & D.G. Debouck. 2004. Wild common bean in the Central Valley of Costa Rica: ecological distribution and molecular characterization. Agron. Mesoam. 15(2): 145-153.

B. In non-refereed journals:

Debouck D.G. . 2004. Phylogeographic migrations of *Phaseolus* beans in the New World, and consequences for taxonomy, conservation and breeding. Annu. Rept. Bean Improvement Coop. (USA) 47: 29-30.

González-Torres, R.I., E. Gaitán, R. Araya, O. Toro, J. Tohme & DG Debouck. 2004. Additional evidence on gene flow events in Phaseolus vulgaris in Costa Rica. Annu. Rept. Bean Improvement Coop. (USA) 47:167-168.

Ocampo, C. H. & O. Toro. 2004. Biochemical evidence supporting the existence of a weedy form in tepary bean (*Phaseolus acutifolius* A. Gray). Annu. Rept. Bean Improvement Coop. (USA) 47: 165-166.

Tofiño, A., Ocampo, C. H. & O. Toro. 2004. Determination of genetic diversity of snap beans *Phaseolus vulgaris* L. cultivated at secondary centers of domestication, using morphological and biochemical descriptors. Annu. Rept. Bean Improvement Coop. (USA) 47: 169-170.

Tofiño A., Ocampo, C. H., Toro, O. & V. H. Garcia. 2004. Introgresión Mesoamericana en el germoplasma de habichuela (*Phaseolus vulgaris* L.) de centros secundarios de domesticación. Fitotecnia Colombiana 4(1): 66-79.

Toro, O. & C.H. Ocampo. 2004. Additional evidence about wild-weed-crop complexes of common bean in different parts of Colombia. Annu. Rept. Bean Improvement Coop. (USA) 47: 171-172.

C. as Book Chapters

D.G. Debouck, J. Engels & L. Guarino. 2004. Domestication and development of plant cultivars. Encyclopedia of Life Support Systems developed under the auspices of the UNESCO. V. Squires (ed.). EOLSS Publishers, http://www.eolss.net., Oxford United Kingdom. Pp. 1-18.

D. as Conference Proceedings

Araya Villalobos R. & D.G. Debouck. 2003. Observaciones sobre poblaciones de frijol silvestre (*Phaseolus vulgaris* L.) en Costa Rica. Programa de Investigación y Transferencia en Tecnología Agropecuaria. VII Reunión Annual, Santo Domingo, Heredia, Costa Rica. Ministerio de Agricultura y Ganaderia de Costa Rica. San José, Costa Rica. pp. 29-34.

Chaves N., R. Araya Villalobos & D.G. Debouck. 2003. Polinización natural del frijol común en Costa Rica. Programa de Investigación y Transferencia en Tecnología Agropecuaria. VII Reunión Annual, Santo Domingo, Heredia, Costa Rica. Ministerio de Agricultura y Ganaderia de Costa Rica. San José, Costa Rica. pp. 35-40.

Escobar, R.H., Manrique, N.C., Rios, A., Mafla, G., Debouck, D., Tohme, J. 2004. Implementation of the encapsulation-dehydratation cryopreservation method for the cassava core collection. In: Abstracts Sixth International Scientific Meeting of the Cassava Biotechnology Network. March 8-14 CIAT, Cali, Colombia. p. 129.

González-Torres, R.I., E. Gaitán, M.C. Duque, O. Toro, J. Tohme & D.G. Debouck. 2004. Analyzing gene flow on Common bean using molecular markers. Memorias II Congreso Colombiano de Biotecnología. Bogotá, Colombia. pp. 210-211.

Mafla G., Roa, J.C., Ocampo, C., Gallego, G., Jaramillo, G. & D.G. Debouck. 2004. Efficacy of silver nitrate for slow growth conservation of cassava (Manihot esculenta Crantz). Determination of viability and genetic stability. In: Abstracts of the Sixth International Scientific Meeting of the Cassava Biotechnology Network. March 8-14 CIAT, Cali, Colombia. p. 134.

Pickersgill B., M.I. Chacón Sánchez & D.G. Debouck. 2003. Multiple domestications and their taxonomic consequences: the example of Phaseolus vulgaris. In: Knüpffer H. and J Ochsmann (eds.), "Rudolf Mansfeld and Plant Genetic Resources". Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld, Gatersleben, Germany, 8-9 October 2001. Schriften Genet. Ressourcen 22: 71-83.

Pineda-L., B. 2004. Características generales de los virus de plantas. In :ASCOLFI Memorias del seminario Taller Diagnóstico de enfermedades causadas por virus y Fitoplasmas.CIAT, Palmira Agosto 9-10 de 2004. pp. 9-17.

6.2. List of thesis research supervised by Project Staff in 2004

A.M. Ayala. 2004. Endurecimiento y caracterización fenotípica de la colección Core del CIAT. Universidad del Valle, Cali, Colombia.

R.I. González. Universidad Nacional de Colombia, Bogotá, Colombia. MSc. thesis. October 2003-April 2004.

J. Martínez Castillo. Centro de Investigaciones Científicas de Yucatán, Mérida, México. PhD thesis. October 2003-September 2004.

M.C. Vélez Escobar, Aspectos morfológicos y anatómicos de 5 especies del género Canavalia (Fabaceae, Faboideae) de Colombia y sus relaciones filogenéticas. Thesis to obtain the M.Sc. degree, Universidad del Valle, Cali, Colombia.

M.P. Sepúlveda. 2004. Endurecimiento y caracterización fenotípica de la colección Core del CIAT. Universidad Nacional Sede Palmira, Colombia.

6.3. List of conferences and scientific communications presented by Project Staff in 2004

M.S. Bálcazar. Palmira, Colombia, 13 August 2004, communication at the 25th anniversary of ASCOLFI: "Observaciones sobre el efecto *in vitro* de bacterias antagónicas sobre el desarrollo de hongos aislados de semillas de *Brachiaria brizantha*".

D.G.Debouck, Palmira, Colombia, 4 October 2004, invited seminar for the Biosafety GEF Colombia project: "Cultivos transgénicos y no transgénicos, biodiversidad y conservación de recursos genéticos: pueden co-existir?".

D.G. Debouck, Toluca, México, 21 September 2004, invited presentation at the 20th national congress of phytogenetics: "Reflexiones y opciones para un sistema nacional de recursos genéticos vegetales".

D.G. Debouck, Palmira, Colombia, 11 August 2004, invited conference for the 25th anniversary of ASCOLFI: "De las curiosidades encontradas en el mercado de Tlatelolco con relación a una agenda de investigación en agricultura tropical".

D.G. Debouck, Montecillos, México, México, 7 July 2004, invited conference at the Colegio de Postgraduados: "Es *Phaseolus* parte de la flora andina? – Nuevos aportes a un viejo debate".

D.G. Debouck, Santo Domingo, Costa Rica, 30 June 2004, invited conference for the 15th anniversary of INBio: "Parientes silvestres de plantas cultivadas del Neotrópico, oportunidades para agregar valor a la biodiversidad y multiplicar el esfuerzo de conservación".

D.G. Debouck, Palmira, Colombia, 12 March 2004, invited presentation at the 6th congress of the Cassava Biotechnology Network: "Biodiversity of cassava: challenges and innovations for its conservation".

D.G. Debouck, Paris, France, 13 February 2004, invited seminar at the Muséum National d'Histoire Naturelle: "*Phaseolus*: un faux retour à la case départ . . . vers un grand genre?".

D.G. Debouck, Santo Domingo, Costa Rica, 13 January 2004, invited presentation in the IPGRI-INBio Regional Workshop on access and benefit-sharing in PGRFA: "A viewpoint from CIAT".

D.G. Debouck, Sacramento, California, USA, 27 October 2003, invited conference to the 17th biennial meeting of the Bean Improvement Cooperative (USA): "Phylogeographic migrations of *Phaseolus* beans in the New World and consequences for taxonomy, conservation and breeding".

D.G. Debouck, Berkeley, California, USA, 24 October 2003, invited seminar: "Observations about *Phaseolus carteri*: endemisms, phylogeography, and breeding" (in honour to the late Dr Annetta Carter).

D.G. Debouck, Pasto, Colombia, 16 October 2003, invited conference on the World Food Day: "Recursos fitogenéticos andinos: construcción, unicidad, y desafíos".

R.I. González-Torres. Bogotá, Colombia, presentation during the precongress of Second Congress of the Colombian Biotechnology, 31 August 2004: "SNP, Polimorfismo de un nucléotido". Universidad Nacional de Colombia.

R.I. González-Torres. Palmira, Colombia, 5 October 2004. Invited seminar at the Regional Workshop of Biosafety: "Flujo de genes en frijol común". CIAT.

R.I. González-Torres. Palmira, Colombia, 7 October 2004.Final results presentation at the Workshop of assesing the safety of bioengineered crops."The bean/wild/weedy/land race complex. Gene flow analysis and biodiversity implications".CIAT

G. Mafla Bohorquez. Palmira, Colombia, 31 May 2004, invited presentation at 'Curso internacional sobre sistemas modernos de producción, procesamiento y utilización de yuca': 'Conservación in vitro del germoplasma de *Manihot'*.

A.M. Torres. Workshop in Systematic of Legumes. Course for the M.Sc. in Biology. Universidad del Quindío, Armenia, Colombia, May 22, 2004.

6.4 List of international and national courses with input from Project Staff 2004

Curso internacional sobre sistemas modernos de producción, procesamiento y utilización de yuca. 31 May- 12 June, 2004. CIAT, Cali, Colombia.

Distance e- course on *ex situ* conservation with RedCapa, IPGRI-Americas, and Universidad Nacional de Colombia. August – November 2004.

Master's Degree programme in Plant Genetic Resources, Universidad Nacional de Colombia. September-November 2003.

Master's Degree programme in Plant Genetic Resources, Universidad Nacional de Colombia. September-November 2004.

6.5. List of trainees trained by Project Staff in 2004

In vitro Lab

Prapit Wongtiem. Training in conservation and management of in vitro cassava germplasm. Thailand, 15-19 March, 2004.

Juan Francisco Casas. Training in conservation and management of in vitro cassava germplasm. México. 3- 4 March, 2004.

Fanny Cruz. Training in conservation and management of in vitro cassava germplasm. México. 12 May, 2004

Christina Holmes. Training in conservation and management of in vitro cassava germplasm. Canada. 13-15 September, 2004.

In Germplasm Health Lab

Prapit Wongtiem. Training in Cassava virus indexing techniques (Elisa Test and grafting). March, 2004.

Christina Holmes. Training in Cassava virus indexing techniques (Elisa Test and grafting). September, 2004.

Electrophoresis Lab

Dr. Juan Francisco Casas Salas. Universidad de Guadalajara, México. 1-5 March 2004.

In Seed Conservation

B.Sc. Paola Andrea Acero, 1-2 July, 2004.

The staff of CORPOICA, Marina Cardona and Iván Valbuena, 1-2 September, 2004.

6.6 Posters

González-Torres, R.I., E. Gaitán, M.C. Duque, O. Toro, J. Tohme & D.G. Debouck. 2004. Estimation of gene flow on Phaseolus vulgaris L. using molecular markers: microsatellites and polymorphisms of chloroplast DNA. V Latin American and Caribbean Meeting on Agricultural Biotechnology. REDBIO. Bocachica, Dominican Republic.

Mafla G., Roa, J.C., Ocampo, C., Gallego, G., Jaramillo, G. & D.G. Debouck. 2004. Efficacy of silver nitrate for slow growth conservation of cassava (*Manihot esculenta* Crantz). Determination of viability and genetic stability. In: Abstracts Sixth International Scientific Meeting of the Cassava Biotechnology Network. March 8-14 CIAT, Cali, Colombia.

Escobar, R.H., Manrique, N.C., Rios, A., Mafla, G., Debouck, D.G. & J. Tohme. 2004. Implementation of the encapsulation-dehydratation cryopreservation method for the cassava core collection. In: Abstracts Sixth International Scientific Meeting of the Cassava Biotechnology Network. March 8-14 CIAT, Cali, Colombia.

6.7. Awards

González-Torres, R.I. Master of Science Thesis awarded with Merit recognition by Universidad Nacional de Colombia.

González-Torres, R.I., E. Gaitán, M.C. Duque, O. Toro, J. Tohme & D.G. Debouck. 2004. First Award for best poster, Measure of gene flow on Common bean using molecular markers. Memorias II Congreso Colombiano de Biotecnología. Bogotá, Colombia. 210-211.

6.8 Visitors

The Professional Staff of the Genetic Resource Unit attended the visit of 489 people from 60 different government bodies, institutions, companies, etc. A total of 120 people from 6 universities visited the in Vitro Lab.

6.9. Donors

CIAT Core Budget.

Ministerio de Agricultura y Desarrollo Rural of Colombia.

World Bank (specialproject: Rehabilitation of International Public Goods; CGIAR Genebank Upgrading Project).

Bundesministerium fuer Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany.