ANNUAL REPORT 2005
CIAT Project on Saving Agrobiodiversity
SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses

CIAT
DECEMBER, 2005

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PROJECT SB-1/2: CONSERVATION AND USE OF TROPICAL GENETIC RESOURCES

PROJECT DESCRIPTION

Objective: To conserve the FAO Designated Collections and employ modern biotechnology to identify and use genetic diversity for broadening the genetic base and increasing the productivity of mandate and selected non-mandate 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. Increase efficiency of breeding program using genomics tools
4. Mandate crops conserved, multiplied and distributed as per international standards.
5. Germplasm available, restored, and safely duplicated.
6. Designated Collections made socially relevant.
7. NARS strengthened for conservation and use of Neotropical plant genetic resources.
8. Conservation of Designated Collections linked with on-farm conservation efforts and protected areas.

Milestones:

2005 Efficient transformation system devolved for cassava. Bean with high iron and zinc tested and transferred to CIAT Africa program for bioavailability testing. Survey of cassava germplasm for beta carotene. 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.

2006 Scaling up of marker assisted selection and transformation established for rice bean and cassava. High through put screening for selected tropical fruits initiated. Marker assisted selected for multiple traits implemented in beans, rice and cassava. Target genes for drought identified and tested in beans. High iron and zinc bean lines developed through markers assisted selection released for field testing. Beta carotene cassava tested in Colombia, Brazil and selected countries in Africa.

2007 Data mining (SNIPs) in *ex situ/ in situ* collections of wild relatives of beans, cassava and forages for genes of economic importance (drought, starch). Field testing for transformed cassava. Gene flow studies diffused to NARS. Upgrading Plan completed. Safety duplicates at CIMMYT and CIP. Biofortified bean and cassava varieties in field testing. Methods for rapid multiplication of tropical fruit germplasm diffused to NARS. Genes for drought resistance in beans and cassava compared.

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, INIAA, INIFAP, UCR, INIAs); USDA; AROs (IRD, CIRAD, Danforth Center, CAMBIA, NCGR, and universities—Cornell, Yale, Clemson, Kansas State, Bath, Hannover, Rutgers, Ghent, Gembloux); biodiversity institutions (I. von Humboldt, CONABIO, 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-2:* 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-2:* 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 methods and techniques for gene banks and crop productivity projects. Interspecific hybrids and transgenic stocks for crop productivity and IPM projects.

CIAT: SB-1/2 PROJECT LOG FRAME (2005-2007)

PROJECT: CONSERVATION AND USE OF TROPICAL GENETIC RESOURCES
PROJECT MANAGER: JOE TOHME (BRU)/ D.G. DEBOUCK (GRU)

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute to the sustainable increase of productivity and quality of mandated and other priority crops, and the conservation of agrobiodiversity in tropical countries.	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 mandate and non-mandate 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 for nitrification and Al tolerance in *Brachiaria*.	*Publications, reports, and* project proposals. Germplasm. Availability of a laboratory information management system (LIMS).	Availability of up-to-date genomics equipment, operational funding.
Output 2 Genomes modified: genes and gene combinations used to broaden the genetic base of mandated and nonmandated crops.	Transgenic lines of rice and advances in *cassava, beans, Brachiaria,* and other crops. Cloned genes for iron, zinc and drought traits 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. New partnerships with private sector.	Publications. Training courses and workshops. Project proposals.	Government and industry support national biotech initiatives.
Narrative Summary	**Measurable Indicators**	**Means of Verification**	**Important Assumptions**
Output 4 Mandated crops conserved and multiplied as per international standards.	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 requests 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. CIAT becomes partner to the Treaty.
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 plant 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.	NARS interested in conservation efforts. Farmers interested in conservation efforts.

Annex 2 CG Performance Measurement. Output Template

Project SB1/2 (GRU)	Output	Output target 2005	Category of Output target	Achieved?
1.1.	Backlogs cleared/ introduced	2,000 materials/ year	materials	no (1,636)
1.2.	Materials planted	6,520 materials/ 2005	materials	yes (11,060)
1.3.	Materials regenerated	3,400 materials/ 2005	materials	yes (7,852)
1.4.	Materials processed	2,000 materials/ 2005	materials	yes (8,875)
2.1.	Materials cleaned	4,500 materials/ 2005	materials	yes (4,710)
2.2.	Materials distributed	Unpredictable target	materials	yes (8,480)
2.2.	Data available	New web page	practice (information product)	yes
2.4.	Safety back-ups	2,000 at CIMMYT	materials	yes (3,400)
3.1.	Publications	3 articles in refereed journals	Knowledge	yes
4.1.	Training	Course and NARS trained	Capacity	yes

N.B.: Categories of output targets to be used are materials, policy strategies, practices, capacity, and other kinds of knowledge.

SUMMARY ANNUAL REPORT 2005
Genetic Resources Unit
SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Staff:

Daniel G. Debouck, Head, PhD (80%)
Alba Marina Torres, Biologist, M.Sc. (on study leave in 2005)
Graciela Mafla, Biologist (100%)
Julio C. Roa, Biologist (has retired in 2005)
César Ocampo, Biologist, M.Sc. (100%)
Orlando Toro, Technician (100%)
Arsenio Ciprián, Technician (100%)
Roosevelt Escobar, Biologist, M.Sc. (50%)
Benjamin Pineda, Ing. Agr., M.Sc. (has retired in 2005)
Norma Cristina Flor, Ing. Agr. (has left in 2005)
Ericson Aranzales, Ing. Biotec. (has joined in 2005)
Maria del Socorro Balcazar, Bacteriologist (100%)
Jesús M. Salcedo, Biologist (100%)
Manuel G. Moreno, Ing. Biotec. (has joined in 2005)
Rosa I. González, Bacteriologist, M.Sc. (100%)
Guillermo Enrique Rueda Q., Telematic Engineer (100%)
Carmenza Llano, Administrative Assistant (100%)

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 Jörg 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. Marleni Ramírez, IPGRI – Americas, Colombia
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
Head: Daniel G. Debouck
Sub-Project #1: The International Standards

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To make the FAO Designated Collections complying with international standards	ICER'95 and ICER'97 recommendations met	FAO Commission experts visits	
Purpose Our purpose is to multiply and conserve the Designated 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 Safely Duplicated

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To make the FAO Designated 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 Designated 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 Designated 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 CIMMYT and CIP	Visits to CIMMYT and CIP	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 Designated 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 Designated 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. Designated 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 Designated 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	478,816	52
Carryover from 2004	28,693 (104,000)	3
Sub Total	**507,509**	
Special projects		
Enhancing forages MADR	70,430 (110,000)	7
Gene Flow BMZ	51,280	5
Palms MADR	21,315	2
Upgrading Plan Operations WB	285,242 (350,800)	31
Sub Total	**428,267**	
TOTAL	**935,776**	**100**

3.4. Research Highlights in 2005

Activity area # 1: the International Standards
The Upgrading of the CGIAR genebanks has progressed at full speed during 2005. A total of 10,404 accessions of beans and 5,274 accessions of tropical forages have been planted for seed increase and regeneration because of aging seeds. GRU is now operating four stations: Palmira (9.5 Ha), Quilichao (10.2 Ha), Popayán (1 Ha; 11 mesh-houses) and Tenerife (3,5 Ha), with 75 Staff. A total of 10,229 accessions have been harvested, processed and dried, while 2,444 accessions have been secured in the long-term vault. Viability has been tested for 4,834 seed accessions. To date, 11,695 seed accessions of beans and forages have been shipped to CIMMYT as safety back-up, while 1,184 accessions of cassava have been shipped to CIP (as slow-growth *in vitro*). Almost the entire cassava core collection (630 accessions) is presently conserved in liquid nitrogen, as a test for a security back-up of the entire collection. Bar coding is now being extended to operations in the field (characterization, harvest), and the quality controls labs (viability and germplasm health).

Activity area # 2: the Germplasm and its data available
In 2005, GRU has distributed 8,480 samples of accessions out of the FAO designated collections for the three commodity crops (beans, cassava and tropical forages). This figure is comparable to the one of last year (8,274 samples of accessions), and continues to be on the high side as a clear indication of continuing interest into the FAO designated collections. It would be therefore quite justified to celebrate an agreement with the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture, at the moment of its first meeting (12-16 June 2006, Madrid). A new web site has been designed in order to facilitate consultations of GRU databases by internet users. To date, 21,676 digital images have been taken for seeds, cassava root sections, forage plants in the field, herbarium vouchers, in order to help internet users tailor down their germplasm requests. A total of 83% of the entire cassava collection has been tested and certified against viruses of quarantine importance.

Activity area # 3: the Genetic and Social relevance of the Conservation
After hurricane Katrina devasted several areas of the Caribbean in August 2005, GRU has replied positively to the restoration of cassava clones to Cuba. Contacts have been made with REMERFI to restore farmers' seed stocks in areas of Guatemala, Honduras and Nicaragua affected by hurricane Stann. On the other hand, research has advanced in seed physiology of species of

tropical fruits, namely *Passiflora* and *Carica*, as models of intermediate seed behaviour; this research will help our partners to conserve poorly known species at lower costs and for longer durations. A study has been done in cooperation with Corpoica about extent of diversity and redundancy in the national collection of avocado of Colombia. In view of expanding the cassava collection in the future, while reducing costs of maintenance *in vitro*, a research has been undertaken to track down the duplicated accessions. At the request of MADR of Colombia, a protocol has been developed to successfully conserve seeds of the peach palm in liquid nitrogen, with possibility to apply it also to other palm species.

Activity area # 4: the International cooperation and capacity building
Three courses received input from GRU Staff in 2005. Nine publications were published in 2005. Thirteen lectures and presentations were made by Staff during this year. Two thesis research were supervised by GRU Staff and well concluded. Ten Professionals were given specialized training in GRU facilities. Five posters were presented in national/ international scientific congresses. One workshop was carried out with the network of botanical gardens of Colombia.

Activity area # 5: the Link with in situ Conservation on farm and in the wild
Phase 2 of the Gene Flow project supported by BMZ of Germany has started, along three perspectives. First, the methodology successfully developed to identify cases of gene flow in Costa Rica is now being applied to putative hybrid swarms identified in other countries (e.g. Guatemala, Colombia, Peru, Bolivia) between cultivated common bean and its wild form. Second, although very rare, there seems to be a few cases of introgression with the participation of a sister species belonging to the same evolutionary phylum as *P. vulgaris*. These natural interspecific hybrids were spotted in Colombia and in Costa Rica because of their morphology. Microsatellites screened at 68 loci generated banding patterns shared among all taxa (those of the same evolutive phylum), but also specific to each species and thus found in their putative hybrids. In both localities the natural crossing involves *P. dumosus* and *P. vulgaris*, but might result without effect given the lack of fertility of the natural hybrids. Third, in localities of Costa Rica where hybrid swarms have been identified in the past, and where agriculture has been abandoned, we continued to analyze subsequent generations of weedy forms in order to see whether past gene flow has any lasting effect.

This year we have collated information about the geographic distribution of wild bean populations in the following herbaria: BR, CR, CICY, ENCB, K, INB, M, SI and USJ. These data will help us to build up the pilot for the GEF 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

As indicated last year, for the upgrading of the facilities (e.g. new lighting system in the *in vitro* subculturing room, shelving system, alarms), problems in delivery on time and as per agreed terms have been faced with high frequency. Apart from contracting in the US or Germany (but possibly at higher costs), 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). On the other hand, continuing reductions in the core (US$ 36,414 to operate in 2005!, the Upgrading Plan apart) raise doubts about operating the GRU without external special funding, and make planning difficult.

3.6. Plans for next year

- Continue to clear backlogs, namely that of the bean collection
- continue with regeneration of bean and tropical forage collections
- continue the shipments of the security back-ups to CIMMYT and CIP
- continue with the documentation of the 'institutional memory' by recovering elite germplasm released by CIAT and partners in the past in the countries
- update the web site, namely with evaluation and herbarium data
- expand the cryoconservation to a set of cassava clones beyond the core collection through vitrification technique
- continue Phase 2 of the Gene Flow Project
- publish in full results of Phase 1 of the Gene Flow Project, and of floral biology
- make appropriate follow-up to the pdf-B process for the GEF project
- prepare for the ratification of the International Treaty within SGRP (e.g. automatic documentation of MTAs)
- run international courses as it may be required (e.g. electronic distance education on *ex situ* conservation in Spanish 2nd version, and 1st version in English for Africa and Asia)

3.7. Executive summary

The Upgrading Plan of the CGIAR Genebanks ('Rehabilitation of International Public Goods', phase 1) has progressed at full speed in 2005. A total of 10,404 accessions of beans and 5,274 accessions of tropical forages have been planted for seed increase and regeneration because of aging seeds in four stations in Colombia. A total of 10,229 accessions have been harvested, processed and dried, while 2,333 accessions have been secured in the long-term vault. A 20% of the designate collections has been shipped to CIMMYT for the seed collections of beans and forages, and to CIP for the *in vitro* cassava collection, respectively, as safety back-ups. In 2005, GRU has distributed 8,480 samples of accessions out of the FAO designated collections for the three commodity crops. Research has advanced to get a better understanding of behaviour of seeds of *Passiflora* and *Carica* under different conservation conditions. A protocol has been successfully developed for the conservation of seeds of the peach palm in liquid nitrogen. Research has been carried out to identify the level of redundancy (i.e. genetic duplicate accessions) in collections of avocado and cassava, with help of AFLPs and SSRs markers, respectively. Three courses at national/ international level received input from GRU Staff in 2005. The special project on gene flow supported by BMZ of Germany has started its Phase 2, with emphasis on the participation of related species in the gene flow events, the ocurrence of gene flow over a large geographic range, and the persistence of its effects through time.

4. Project performance indicators

1.FLOWS, TECHNOLOGIES, METHODS & TOOLS

1.1. Backlogs cleared: 1,636 accessions cleared
1.2. Accessions regenerated: 4,550 of beans, 3,302 of tropical forages
1.3. Accessions secured in long-term: 2,333 accessions secured
1.4. Accessions in security back-up: Shipment this year 3,400 seed accessions (CIMMYT) and 1,184 *in vitro* accessions (CIP)
1.5. Accessions characterized 12,854 (field/ lab) + 6,640 (image bank)

1.6. Accessions distributed with passport data: 8,480 accessions distributed
1.7. Support Tools (software in germplasm management; databases available from internet) see www.ciat.cgiar.org
1.8. Data Bases united/ improved, same

2. PUBLICATIONS

2.1. Refereed Journals: published: 3

Chacón M.I., Pickersgill B. & D.G. Debouck. 2005. Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. Theor. Appl. Genet **110** (3): 432-444.

Ocampo, C. H., Martín, J. P., Sánchez-Yélamo, M. D., Ortiz, J. M. & O. Toro. 2005. Tracing the origin of Spanish common bean (*Phaseolus vulgaris* L.) cultivars using biochemical and molecular markers. Genet. Resources & Crop Evol. **52**: 33-40.

Torres González A.M. & C.M. Morton. 2005. Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae). Molec. Phylogenet. Evol. **37**: 36-44.

2.2. Refereed Journals: submitted (accepted indeed): 2 (1 Crop Sci.; 1 NOVON)

Taxonomy of Tepary Bean (*Phaseolus acutifolius*) and Wild Relatives as Determined by Amplified Fragment Length Polymorphism (AFLP) Markers. L.C Muñoz, M.C Duque, D.G. Debouck & M.W. Blair. Crop Science **46**: *in press*.

Phaseolus novoleonensis, a new species (Leguminosae, Phaseolinae) from the Sierra Madre Oriental, Nuevo León, Mexico. J. Salcedo C., J. A. Arroyave, O. Toro Ch. & D. G. Debouck. NOVON **16:** 106-112.

2.3. Published Proceedings: published articles: 3

Balcázar, M.S., Rivera, Á. L. & Pineda L., B. 2005. Actividad antagónica de bacterias aisladas de semillas de *Brachiaria y* asociadas con hongos de *Brachiaria.* In: Memoria XLV Congreso annual de la Sociedad Americana de Fitopatologia Division Caribe, VI Congreso nacional de Fitopatologia y I Congreso Nacional de Fitoprotección. San José, Costa Rica, @7 Junio-1 De Julio de 2005. p. 89.

González-Torres, R.I., Araya-Villalobos R. & D.G. Debouck. 2005. Gene flow and its effect on biodiversity: common bean as model for future considerations of biosafety. Deutscher Tropentag 2005: The Global Food & Product Chain-Dynamics, Innovations, Conflicts, Strategies. October 11 – 13, 2005, University of Hohenheim, Stuttgart, Germany. p. 406.

Salcedo, J.M & D.G. Debouck. 2004. Comparación de areas en la region del estigma entre las formas silvestres y cultivadas (tradicionales y modernas) de *Phaseolus vulgaris* L. III Congreso Colombiano de Botánica. Noviembre 2004, Popayán, Colombia. p. 52.

2.4. Scientific Meeting Presentations: presentations: 13
2.5. Working Papers, Other Presentation or Publications: 3
(see under 6 in full report)

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

3.1.Training Courses : 3

3.2. Individualized Training : 10

3.3. PhD, MSc. and pregraduate thesis students: 2

4.0 RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted

- Sustainable utilization of the weregue palm in the Chocó of Colombia.
- Rehabilitation of International Public Goods: the Upgrading of CGIAR Genebanks, extension 2007-2009.
- Out-scaling of a multi-institutional e-learning venture on ex-situ conservation of plant genetic resources to Eastern African countries, together with E. Hess, for extension of distance education to African countries.

4.2. Ongoing special projects in 2005

Studies of gene flow in the bean model, Phase 2, supported by Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany, US$51,280 (to CIAT) and Euro21,330 (to University of Costa Rica).

Development of cryoconservation protocols for palm species, supported by Ministerio de Agricultura Tropical of Colombia, US$ 21,315.

CGIAR Genebank Upgrading, supported by the World Bank, US$ 285,242.

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, summing to 17,640 images for the bean collection, 2,208 images of seed and plants in the field for the forages, and 1,648 images of cassava for the descriptor "root pulp color", to date (total 21,496), accessed through CIAT web site or ready to be loaded into it.

This year the GRU has added 180 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, namely botanists, will find it as a useful taxonomic tool.

Contributors: O. Toro, G. Mafla, J.C. Roa, J.M. Salcedo, A. Ciprián, G. Rueda.

Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into the genebank processes

A total of 436 accessions of the backlog of bean germplasm was introduced in the multiplication cycles in 2005; 758 additional materials were obtained through internal separations of bean mixtures, and were multiplied. We also introduced 1,200 germplasm accessions of tropical legumes received from CSIRO, Australia. At this time, 1,990 accessions of forages and 9,274 of beans are in the backlog. One should note that many accessions in the bean backlog already exist in the designate collection, and should thus not be introduced.

Contributors: O. Toro, A. Ciprian

Output 1.2. Backlogs of materials pending on multiplication multiplied

Activity 1.2.1. Multiplication of materials cleared by quarantine authorities.

In 2005 a total of 436 bean accessions were cleared by quarantine authorities, and planted either in Palmira or in Popayán. On the other hand, a total of 2,849 bean plants for the Gene Flow Project were handled as pure lines (for phaseolin and DNA analysis) and planted in Popayán. Similarly, 1,200 accessions of forages were cleared by ICA authorities and directed to the fields in Palmira and Quilichao after a hardening period in the glass-houses in Palmira.

Contributors: O. Toro, A. Ciprian, J.M. Salcedo, R. González

Output 1.3 Materials pending on regeneration

Activity 1.3.1. Multiplication of materials with aging seeds.

Table 1 indicates the total numbers of bean accessions that were regenerated because seed viability reached the lower threshold.

Table 1. *Phaseolus* bean germplasm processed for regeneration (and characterized during the process) under greenhouse/mesh-house conditions (number of accessions).

Localities	Greenhouse/meshouse
Palmira	146
Popayán	4,525
Tenerife	5,733
Total	10,404

Out of these materials, 3,824 accessions filled once all requirements for the five conservation purposes. Similarly, 3,841 accessions of forages were planted in 2005 for regeneration purposes (Tables 2, 3). Table 2 includes them, as well as materials planted in previous years.

Table 2. Forage germplasm planted for multiplication and regeneration under greenhouse/mesh-house and field conditions (number of accessions).

Localities	Legumes	Grasses	Total
Greenhouse/ Mesh-house	1,591	83	1,674
Quilichao	2,241	140	2,381
Palmira	1,034	158	1,192
Popayan	105	227	332
Tenerife	26	64	90
Total	4,997	672	5,669

Table 3. Forage germplasm processed during 2005.

	Legumes	Grasses	Total
Backlog	1,200	0	1,050
Regenerated (because of aging seeds)	2,486	5	2,491
Characterized during the process	2,465	65	2,450
Designated to FAO	2,569	46	2,615

Table 4. Forage germplasm installed during 2005.

	Palmira	Quilichao	Popayan	Total
Australia	802	365	9	1,176
Regeneration	1,266	1,387	20	2,673
Total	2,068	1,752	29	3,849

Contributors: O. Toro, A. Ciprian, J.M. Salcedo

Status of designated germplasm at the GRU in 2005.

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 in 2006, the status of designated accessions is currently as follows:

Manihot cassava: 5,941 (out of which 5,535 of *M. esculenta*)
Phaseolus beans: 34,617 (out of which 29,849 of *P. vulgaris*)
Tropical forages: 20,753
Total: 61,311

Ouput 1.4. Materials processed into final packing

Activity 1.4.1. Final drying and temporary storage

Table 5 indicates the amount of accessions for beans (9,261) and forages (3,358), respectively, (total 12,619), which have been harvested, cleaned, dried, and stored at 5°C, awaiting the results from viability and health tests.

Table 5. Seed germplasm processed during 2005.

	Beans	Forages
Seed selection / temporal storage	9,261	3,358
TOTAL	9,261	3,358

Contributors: J.M. Salcedo, A. Ciprian, O. Toro

Activity 1.4.2. Viability testing

Table 6 indicates flows of materials during 2005 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 figures of viability higher than 65% do allow seed distribution and of viability higher than 85% do allow long term seed conservation.

In order to support multiplication activities, for very old seeds, the viability lab pre-germinated 1,950 accessions of the backlog of forages, and 750 accessions of CSIRO, Australia, and 1,062 accessions of bean germplasm. Several techniques of pre-germination have been used for successful results such as sand beds, petri dishes and germination paper.

Literature cited

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

Table 6. Viability testing for *Phaseolus* beans and tropical forages during 2003

Class	BEANS		FORAGES	
	Germination (%)	No. Accesions	Germination (%)	No. Accesions
Already stored materials	1-64 65-84 85-100	120 173 647	1-64 65-84 85-100	51 19 33
Sub-total		**940**		**103**
Recently multiplied materials	1-64 65-84 85-100	28 137 3,184	1-64 65-84 85-100	38 91 1,379
Sub-total		**3,349**		**1,508**
TOTAL		*4,289*		*1,611*

Contributors: J.M. Salcedo, F. Gil, H. García

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

Once cleared by the Viability Lab (for viability above 85%) and by 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 (Tables 7-8).

Table 7. Final storage and packing of *Phaseolus* beans processed during 2005 (number of accessions)

	Beans
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	1,190
SHORT TERM only (Distribution)	4,848
Total	6,977

Table 8. Final storage and packing of tropical forages processed during 2005 (number of accessions)

	Total
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	1,143
SHORT TERM only (Distribution)	755
Total	1,898

Contributor: J.M. Salcedo

Activity 1.4.4. Complement drying process and new packing

During 2005, 3,836 accessions (1,880 of beans and 1,956 of forages) were given a complement drying process (poorly dried in the past) and packed in new plastic aluminum pouches.

Contributor: J.M. Salcedo

Activity 1.4.5. Monitoring viability of conserved seed germplasm of beans and forages

This year we tested as monitoring the viability of seed lots of *Phaseolus vulgaris* L. after 5 years of conservation in long term storage, namely for four groups of seeds: Ten1997A, Ten1997B, Ten1998A and Ten1999A.

The seed lots Ten1997A, B and Ten1998A were tested in 2002 and 2003 with a health problem in the test, and thus repeated for confirmation during this year. A total of 361 accessions were tested and the results are shown in Table 9.

The mean germination decreased 3.8 units for Ten1997A, 1.5 for Ten1997B and 0.6 for Ten1998A after conservation (Table 11). These results contrast with those reported in the years 2002 and 2003, showing a smaller loss in the germination percentages. For the lots Ten1997B and 1998A in this occasion, no significant statistical differences are reported in contrast with the lot Ten1997A (Table 9). However the registered difference for germination loss is acceptable for the period of monitoring.

The above-mentioned results confirm that there were sanitary problems in the first monitoring and that the physiological quality of the seeds is still good.

In the seed lots Ten1999A the mean germination decreased 2.2 units after 5 years of conservation. The difference is statistically significant in this case with confidence interval of 95%. The decrease in germination seems to be the normal reduction through time. Perhaps such a decrease is really a low value.

Similarly, the monitoring test was done for forage germplasm conserved after a 5 years period for a group of 199 forages species stored in 2000. The results are shown in Table 10. The difference in the germination mean was of 1.6 units. This difference is not statistically significant with a confidence interval of 95%.

According to these results, for the monitored seed lots a total of 15 accessions of forages and 24 accessions of beans have to be refreshed by seed multiplication.

Table 9. 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
Ten1997A	Initial	97.33	3.903	0.350	160	2.169	5.428	4.61	0.000
	Monitored	93.53	9.580	0.860					
Ten1997B	Initial	95.66	4.44	0.47	105	-0.290	3.394	1.67	0.098
	Monitored	94.11	8.39	0.90					
Ten1998A	Initial	97.12	4.95	0.56	96				
	Monitored	96.48	6.47	0.73		-1.061	2.343	0.75	0.456
Ten1999A	Initial	96.81	3.912	0.260					
	Monitored	94.58	8.156	0.541	227	1.098	3.360	3.88	0.000

Table 10. 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	95.47	4.400	0.343					
Monitored	93.85	6.234	0.485	199	0.465	2.783	2.77	0.060

Table 11. Comparison of loss of germination for the lots tested in 2002, 2003 and 2005

	Loss of germination		
Seeds lots	2002	2003	2005
TEN1997A	10,3	--	3,8
TEN1997B	--	3.4	1,5
TEN1998A	--	1,6	0,6

In 2005 embryo culture was used to rescue seed of wild *Manihot* species kept in storage since 1992-1994. The results of recovered plants are shown in Table 12.

Table 12. Viability testing for wild *Manihot* germplasm.

Species	Populations (No.)	Seeds (No.)	Plants recovered(No.)/Populations
M. angustiloba	1	4	0/0
M. caerulescens	3	10	2/1
M. carthaginensis	1	4	0/0
M.dichotoma	2	14	0/0
M. epruinosa	2	20	9/2
M.flamingiana	1	10	0/0
M. flabellifolia	60	1,360	38/19
M. janiphoides	2	17	6/1
M. peruviana	16	293	4/4
M. quinquepartita	4	8	0/0
M. tripartita	1	7	0/0
M.violacea	3	34	0/0
Total	96	1,781	59/27

Contributors: J.M. Salcedo, G. Mafla, V. Nuñez.

Output 1.5. Improved conservation techniques

Activity 1.5.1. Protocol for seed conservation of germplasm of tropical fruits

1. Dormancy and germination of seeds of *Passiflora edulis* and *P. maliformis*: effect of constant and alternating temperatures

Introduction

We found in 2004 that scarified seeds of *Passiflora edulis* and *P. maliformis* germinated better at alternating temperatures. However, the maximum promotion increased germination to only 74% in *P. maliformis* suggesting that more investigation is required in this species.

In consequence, this research reports a systematic investigation of the response of seed germination to constant and alternating temperatures for *P. edulis* and *P. maliformis*. This was achieved using a two-dimensional temperature-gradient plate where several characteristics of diurnal temperature alternation could be experimentally and separately tested (Murdoch *et al.*, 1989; Roberts, 1988). This allows a very broad range of different constant and alternating temperature regimes, amplitude (difference between maximum and minimum temperature) and two diurnal cycles in order to assess their effect on germination.

Materials and methods

Experiment 1: *Passiflora edulis*
One seed lot of *P. edulis* forma *flavicarpa* was used in this experiment. Seed size is comparatively large and so there were only 10 seeds per cell. Seeds were pre-treated in groups of 200. Each group was soaked in 60 ml of boiled water (96 °C) for 24 hours (as the water cooled). The temperature on the plate varied among cells from a minimum of 13 to a maximum of 43 °C. The first germination count was made on the 14th day and the total test duration was 35 days. In order to control fungal contamination, from the 18th day infected seeds were removed from the cells, rinsed in 1% sodium hypochlorite solution (Hong and Ellis, 1996), and returned to the same cell. At the end of the test non-germinated seeds were cut with a scalpel to discount empty seeds from the seed sample. Results are presented as the percentage of fully developed seeds.

Experiment 2: *Passiflora edulis*
The same seed lot of *P. edulis* f. *flavicarpa* as in Experiment 1 was used. Ten seeds were placed in each cell of the plate after first soaking in 1% sodium hypochlorite solution for 5 minutes. After drying the seeds with paper towels, the seeds were manually scarified with a scalpel. Consequently any empty seed was discovered and discarded before germination tests began. The temperature on the plate had a variation from a minimum of 13.3 to a maximum of 44.3 °C. The first seed germination count was made on the 7th day and the total duration of the experiment was 28 days. Fungal contamination was controlled, as in the previous experiment, from the 7th day.

Experiment 3: *Passiflora maliformis*
The seed lot of *P. maliformis* also has a comparatively large seed size and so each cell on the plate contained only 10 seeds. Seeds were first soaked in 1% sodium hypochlorite solution for 5 minutes. After that, seeds were dried with paper towels and scarified manually with a scalpel. Germination counts started on the 7th day and the total duration was 28 days. Fungal control began on day 14, as above.

Data analysis

The results for total germination (angular transformed percentage) were mapped as contour plots against the temperature applied in each thermo-period on a plan of the temperature-gradient plate. Thus the germination is identified for the particular constant and/or alternating temperature and amplitude. The data were mapped using SigmaPlot 7.0.

The pattern of results from investigations on the plate could be the result of random error not withstanding the systematic design. Accordingly a simple, arbitrary, multiple regression model was fitted to the results using Genstat (Genstat-5-Committee, 1997). Results were fitted to the equation:

$$G = a + bT + cA + dT^2 + eA^2 + fAT + gA^2T^2$$

Where G is germination (after angular transformation of percentages), A is amplitude (°C), T is mean temperature and a, b, c, d, e, f, g are parameters estimated in the model. The fitted model was drawn out as a germination contour plot on a plan of the plate. This simple model comprises a linear response to temperature (b), a quadratic term to allow deviation from a linear response (d), a linear amplitude term (c), and a similar non-linear quadratic term for amplitude (e), and an interaction between their two linear (f) and quadratic (g) terms.
More complicate models including cubic terms could be justified statistically in several cases, while on some occasions some of the individual terms in the fitted models have comparatively high errors. Nevertheless, the above model explained the majority of variation, and enabled the major responses to be identified and the data smoothed for rapid identification from the outstanding points. Finally, the single model allows comparison of the smoothed responses among the seed lots.

Results

Experiment 1: *Passiflora edulis*
Greater germination occurred at alternating temperatures, although maximum germination was no more than 78%. The most promotory areas in the contour graphs are the same after 28 or 35 days in test and are 33.0-35.5/25.5-28 and 18.0-20.5/28.0-30.0 °C for 16/8 h, which is confirmed by the fitted model (Figure 1 a,b,c,d).

As mentioned above, there was little variation in the results after 28 or 35 days in test. A similar pattern of low promotion of germination was found at both thermo-periods in response to temperature and amplitude at 35 days, albeit with only about half the variation explained (16h: $p<0.05$, $r^2 = 0.431$, 54 d.f.; 8h: $p<0.05$, $r^2 = 0.573$, 66 d.f.). In addition, there was not a big effect of amplitude on germination (Figure 2 a,b). Consequently, the individual terms related to amplitude in the fitted model have comparatively high errors.

Experiment 2: *Passiflora edulis*
The manually scarified seeds provided greater germination for *P. edulis* than in the previous experiment. The area with the greatest germination was found in the range 26.2-34.0/28.0-34.0 °C for 16/8 h after 21 and 28 days in test, which crosses the diagonal line of constant temperature (Figure 3 a,b,c,d). The contour graphs provided by the fitted model show more clearly the absence of a strong response of germination to alternating temperature.

The model describing the response of germination to alternating temperature after 21 days in test was justified for both thermo-periods (Figure 4 a,b; 16h: $p<0.05$, $r^2 = 0.731$, 66 d.f.; 8h: $p<0.05$, $r^2 = 0.656$, 77 d.f.). But, the terms related to amplitude have relatively high error values. In neither the longer nor the shorter thermo-period did amplitude have any benefit.

Experiment 3: *Passiflora maliformis*
The best regime to promote germination of scarified seeds of *P. maliformis* was 24.0-31.7/31.7-34.3 °C for 16/8 h after 14 or 28 days in test (Figure 5 a,b,c,d). This range includes the constant temperature of 31.7 °C where germination was as high as in any alternating temperature regime.

The fitted model after 14 days in test (16h: $p<0.05$, $r^2 = 0.583$, 63 d.f.; 8h: $p<0.05$, $r^2 = 0.564$, 77 d.f.) was better than that at 28 days. As can be seen in Figure 6 a,b for the larger and shorter thermo-period, respectively, a similar pattern of germination response was found where increasing amplitude up to 13 °C increased germination at the same mean temperature.

Discussion

Block to germination in the *Passiflora* seeds studied was related to hard-seedness. Seed treatment affected the response of germination to temperature. The same seed lot of *P. edulis* provided greater germination for scalpel-scarified seeds than boiled water seeds treated in the same temperature regimes. A minority of the population did not germinate in the scalpel scarification treatment in all the regimes tested, while in the boiled water treatment the majority of the population did not germinate whether at alternating or constant temperatures.

Temperature fluctuation can affect hard-seed breakdown in natural conditions, for instance heating from solar radiation and night time cooling (Probert, 2000). It can be seen in slower break-down of hard-seededness of *Trifolium* and *Medicago* species when buried at 5-10 cm than those seed on the soil surface (Russi *et al.*, 1992). In laboratory conditions, this has been tested for *Abutilon theophrasti*, a typical hard-seeded species, in which soaking at 60-90 °C or immersion in hot water reduced the proportion of hard seeds (Horowitz & Taylorson, 1984). In another species, exposure for few minutes at 140-150 °C for seeds of four species of *Stylosanthes* was successful in hard-seed breakdown (Mott, 1979). However, this short exposure was less effective in *P. edulis,* because it is possible that part of the seed population does not germinate due to high temperature exposure reducing viability, or to the contrary, failing to break the hard seed coat.

In addition, the onset of germination was delayed and much slower in seeds treated with boiled water. This suggests that some non-scarified seeds when naturally dispersed germinate only slowly.

Apart from the effect of mean temperature, neither amplitude nor thermo-period in each diurnal cycle affected the germination of *P. edulis*. In *Passiflora maliformis* there was a relatively greater response to alternating temperature than in P. *edulis,* increasing slightly when amplitude increased.

The cumulative germination of *P. edulis* at different constant temperatures shows that boiled water treated seeds did not germinate when temperature was lower than 24 °C, while scalpel scarified seeds showed germination at temperatures as cool as 13 °C. Similar results were obtained by Severin et al. (2003) for *in vitro* germination of *Passiflora caerulea*, obtaining high germination at constant (23 ± 2 °C) as well as at alternating temperature (25/35 °C). Likewise, the optimal temperature for germination of seeds of *Passiflora incarnata* was a constant temperature of 35 °C (Bienvenuti *et al.*, 2001).

The determination of the best temperature conditions to germinate *Passiflora* seeds will allow future research to test tolerance to desiccation and storage at low temperatures. Because the seed storage behaviour of several *Passiflora* species is considered intermediate (Flynn *et al.*, 2004), confusion of seed dormancy (hard-seedness) with loss of viability should be avoided.

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Contributor: A. M. Torres

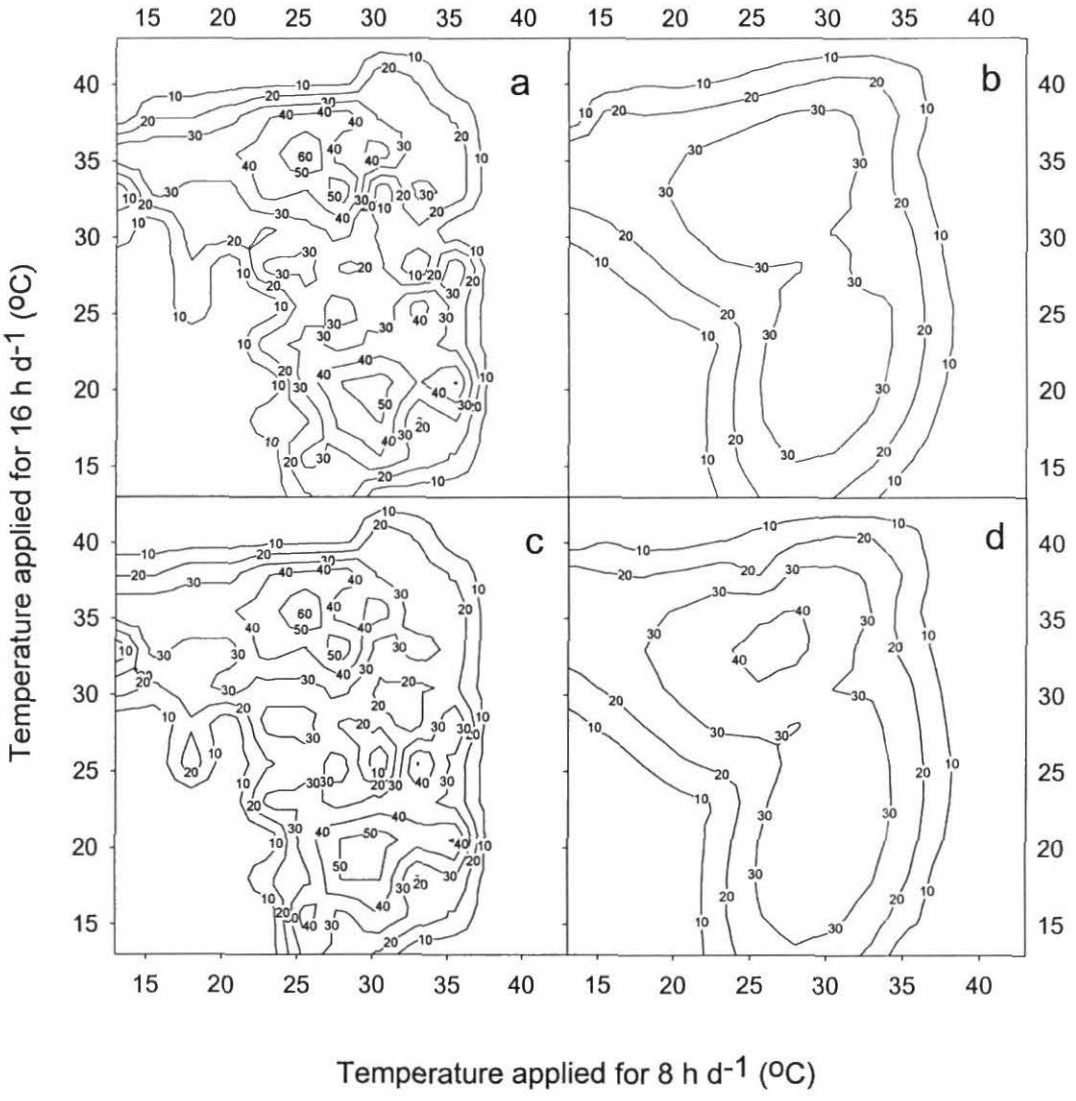

Figure 1. Contour plots of the germination (transformed to angles, °) of boiled water scarified seeds of *Passiflora edulis* at constant and alternating temperatures. a: angular transformed raw data, b: fitted model, both after 28 days in test, c: angular transformed raw data, d: fitted model, both after 35 days in test.

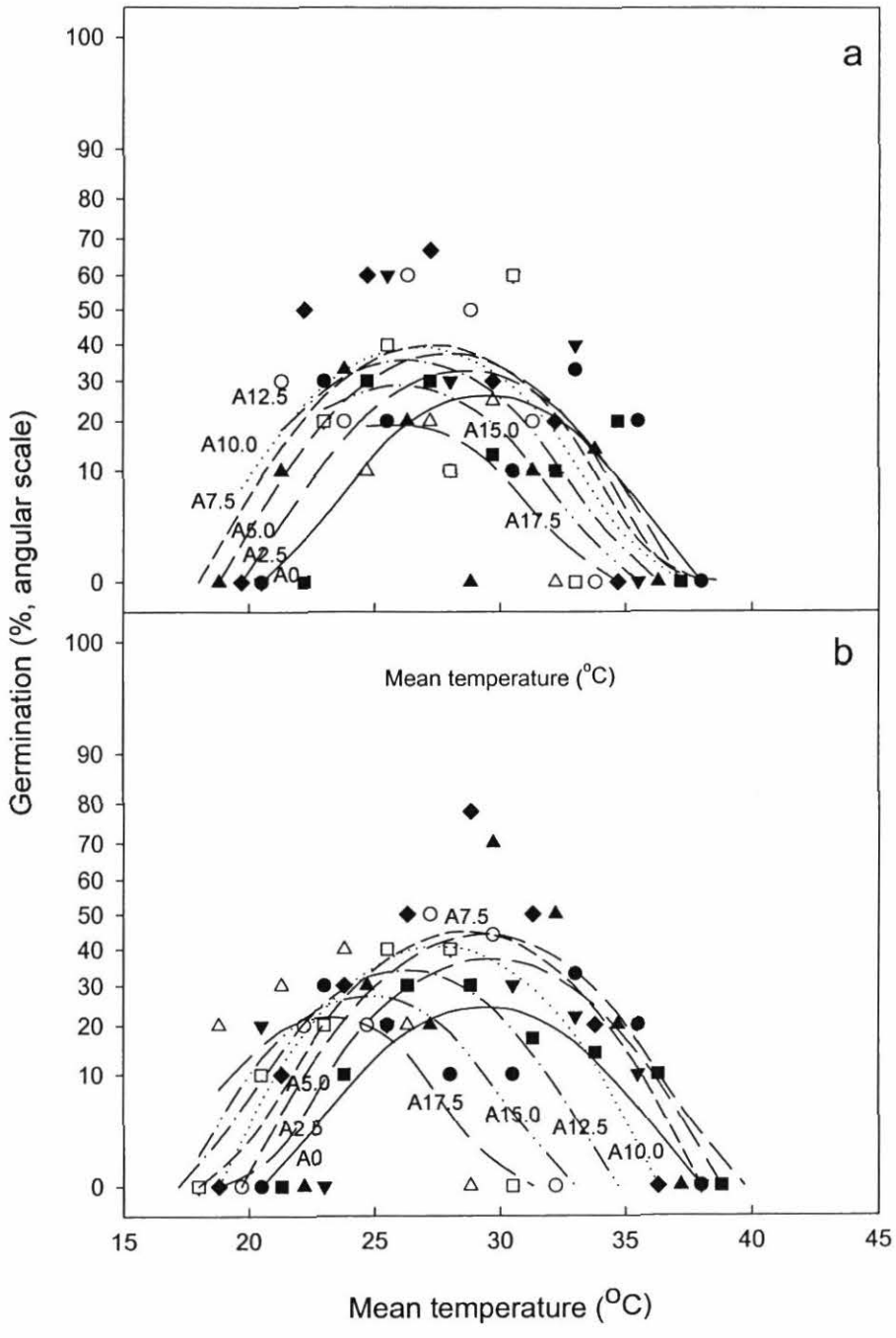

Figure 2. Fitted model of seed germination of *Passiflora edulis* (boiled water scarification) at different temperatures and amplitudes at 35 days. Amplitudes were 0 °C (●), 2.5 °C (■), 5.0 °C (▲), 7.5 °C (▼), 10.0 °C (◆), 12.5 °C (○), 15.0 °C (□), 17.5 °C (△). a: 16 h thermo-period, b: 8 h thermo-period.

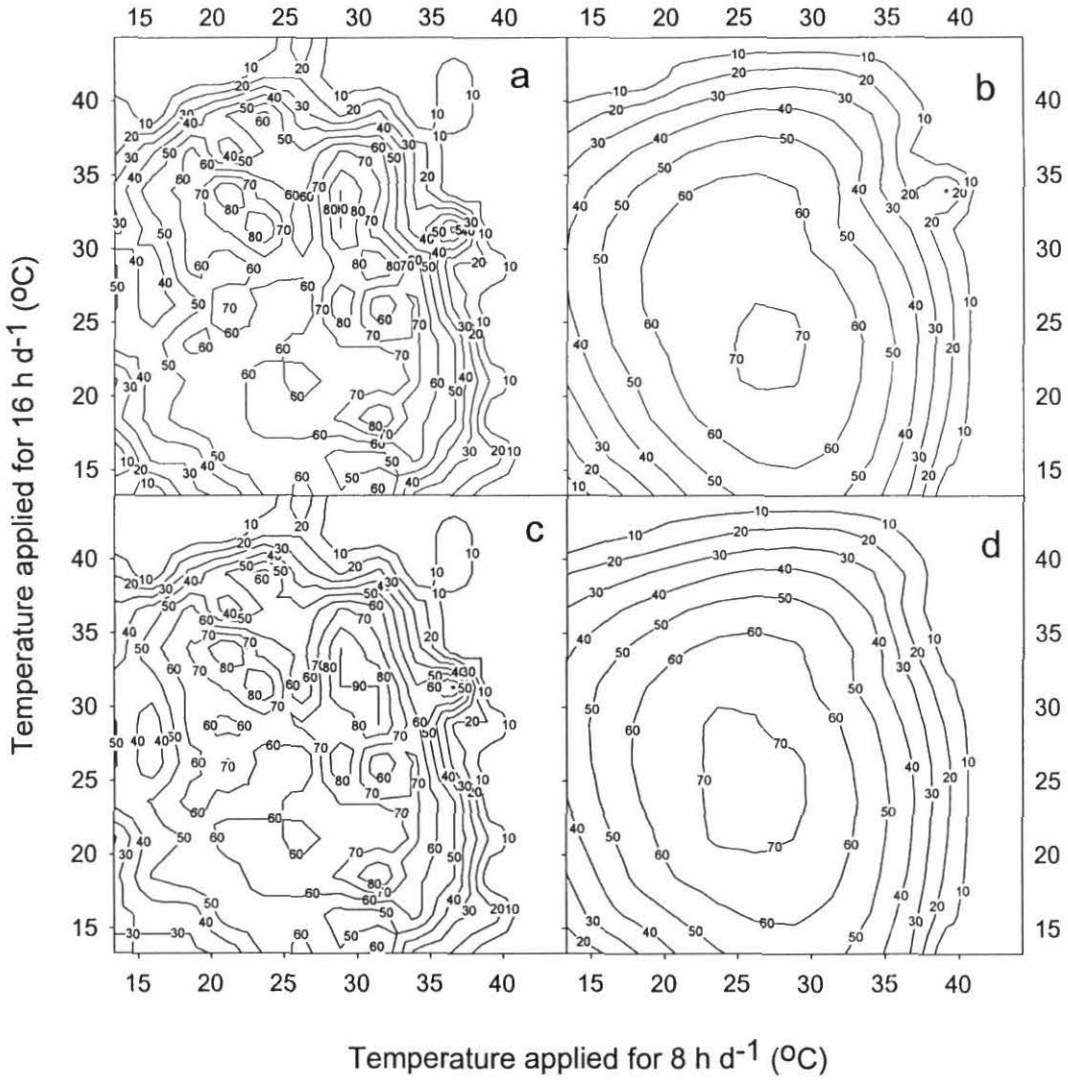

Figure 3. Contour plots of the germination (transformed to angles, °) of scalpel scarified seeds of *Passiflora edulis* at constant and alternating temperatures. a: angular transformed raw data, b: fitted model, both after 21days in test, c: angular transformed raw data, d: fitted model, both after 28 days in test.

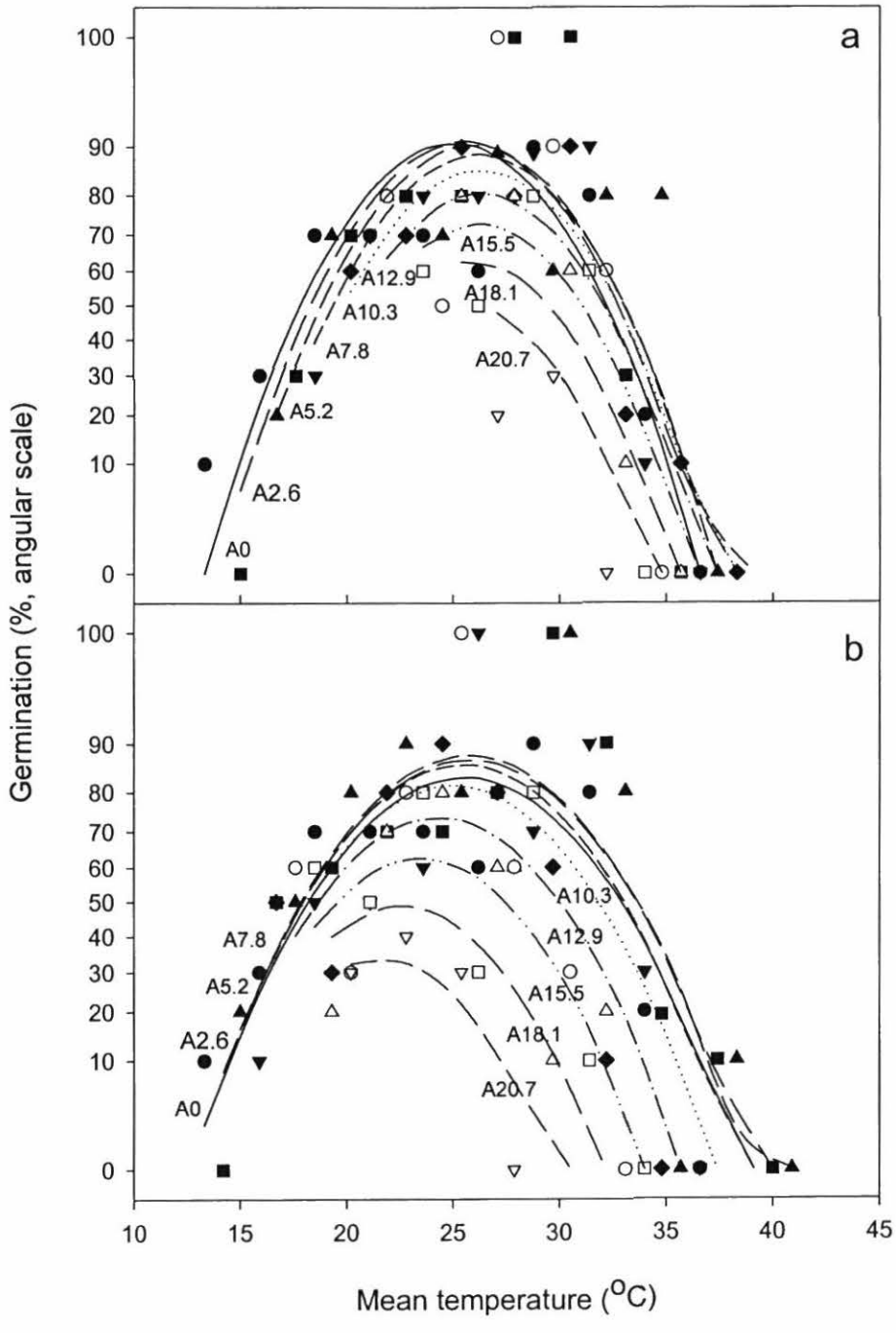

Figure 4. Fitted model of seed germination of *Passiflora edulis* (scalpel scarification) at different temperatures and amplitudes at 21 days. Amplitudes were 0 °C (●), 2.6 °C (■), 5.2 °C (▲), 7.8 °C (▼), 10.3 °C (◆), 12.9 °C (○), 15.5 °C (□), 18.1 °C (△), 20.7 °C (▽). a: 16 h thermo-period, b: 8 h thermo-period.

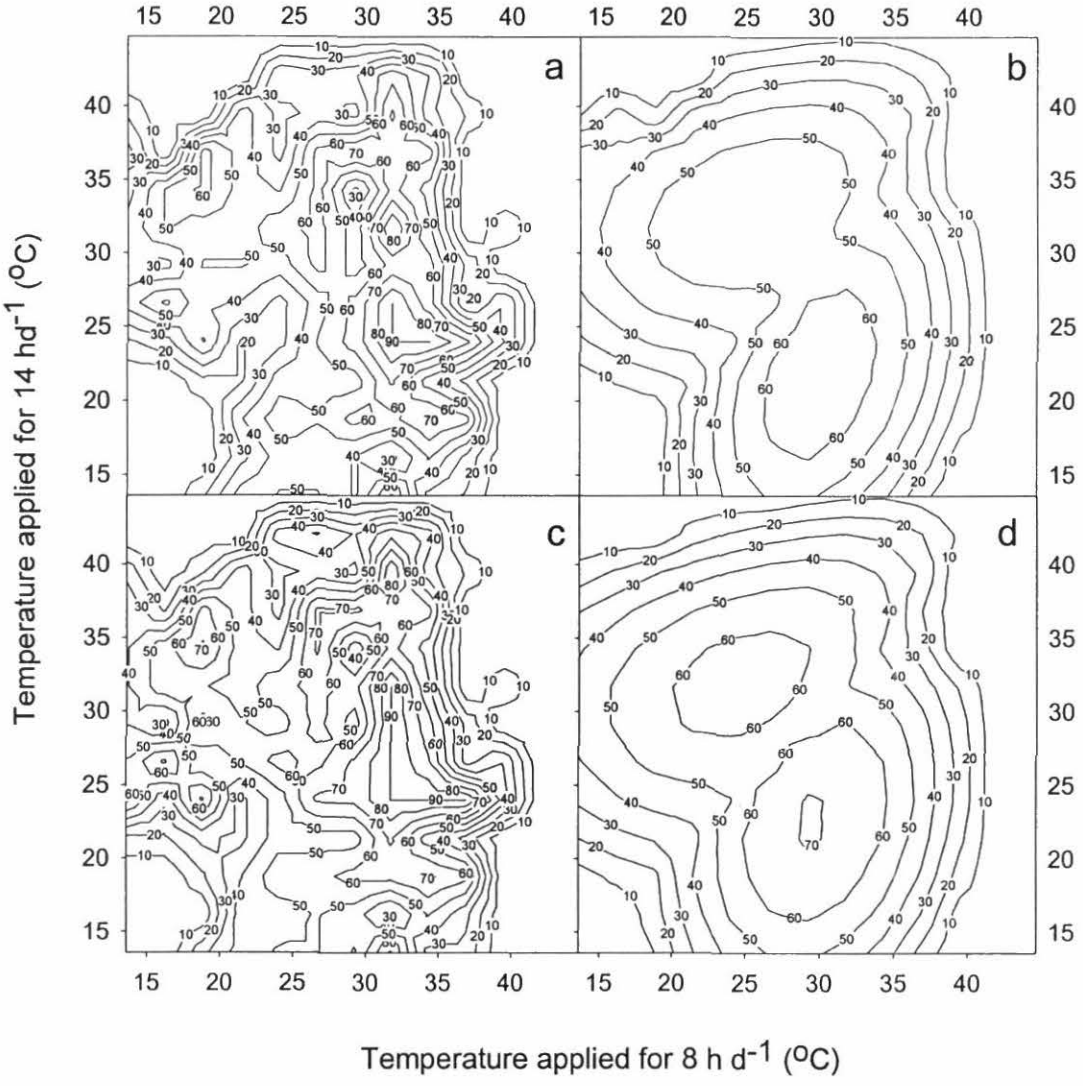

Figure 5. Contour plots of the germination (transformed to angles, °) of *Passiflora maliformis* at alternating and constant temperature. a) angular transformed raw data, b) fitted model, both after 14 days in test, c) angular transformed raw data, d) fitted model, both after 28 days in test.

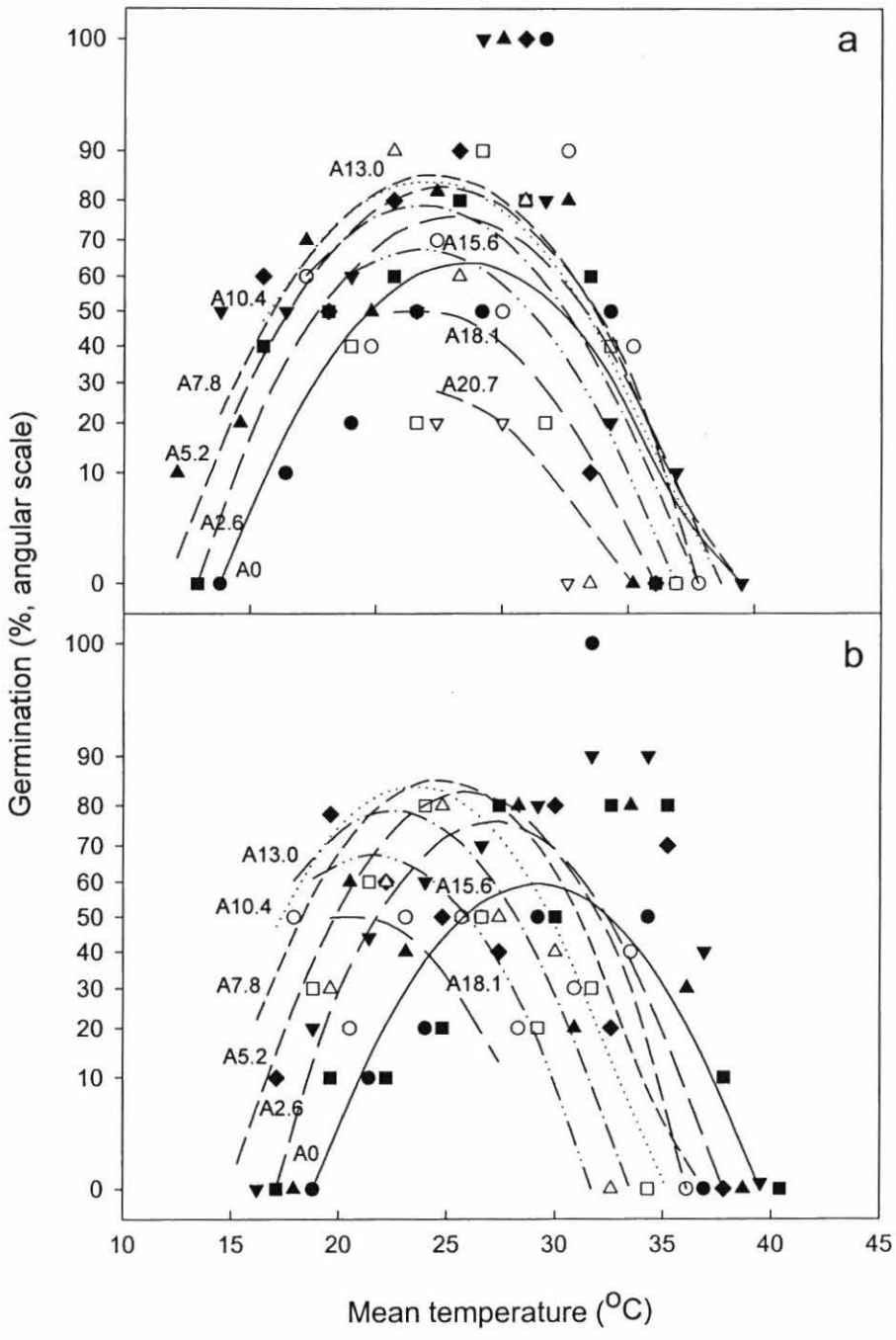

Figure 6. Fitted model of seed germination of *Passiflora maliformis* at different temperatures and amplitudes at 14 days. Amplitudes were 0 °C (●), 2.6 °C (■), 5.2 °C (▲), 7.8 °C (▼), 10.4 °C (◆), 13.0 °C (○), 15.6 °C (□), 18.1 °C (△). a: 16 h thermo-period, b: 8 h thermo-period.

2. Seed survival of *Carica papaya* storage in a wide range of environments from fruit harvested at different times of development

The seed storage behaviour of *Carica papaya* is considered as intermediate due to rapid loss of viability at –20 °C, but seeds can be stored at cooler or warmer temperatures without injury to the seeds (Ellis *et al.*, 1991). Seeds exposed to -196 °C for 24-72 hours showed good survival (Becwar *et al.*, 1983; Salomao & Mundim, 2000).

Wood *et al.* (2000) found that seeds of *C. papaya* were tolerant to desiccation but that dormancy induction occurred during desiccation. In that investigation, dormancy was removed with the application of a heat shock treatment at 36 °C for 4 hours to re-hydrated seeds.

In 2004, good tolerance to desiccation was shown for seeds of three species of Caricaceae: *Carica papaya, Vasconcellea goudotiana* and *V. cauliflora.* However, in all these species loss of viability was detected for seeds stored at –20 °C, while at warmer or cooler temperatures seeds maintained their ability to germinate over three months in hermetic storage.

This research reports a detailed investigation on the effect of fruit development on the subsequent seed survival in a large range of different storage temperatures, seed moisture contents and periods for *C. papaya*.

Materials and methods

Twenty one shrubs in a commercial crop plantation of *C. papaya* cv.Tainung growing in Bolivar, Valle, Colombia (4°20' N, 76°11' W, 1000 masl) were selected for study. The hermaphrodite flowers were labelled at anthesis between 29 of January and 25 of March 2004 and fruits harvested on different dates. Harvest was done on 28 of July and 11 of August of 2004 and we obtained fruits at stages 126, 140, 154, 168, 175 days after anthesis (DAA) from the shrubs, and also seven days extra from the soil (fruits had naturally fallen by then) and recorded as 182 DAA.

Seeds were extracted from the fruits one day after harvesting. Seeds completely exotesta free were dried in silica gel environment to moisture content between 8.2 and 9.0% (w.b.) and stored hermetically at +5 °C on 27 August 2004. Seed moisture content of every development stage was determined on wet basis (ISTA, 2005) and dried in silica gel environment to four moisture contents as shown in Table 13. Subsamples for each moisture content and development stage were hermetically sealed in laminated aluminum foil bags each 12 x 8.5 cm and subsequently stored on 10 of November at five different temperatures: 15, 5, 0, -20 and -86 °C for six months (183 days). Each treatment combination was represented by 100 seeds and stored in a separate laminated-aluminum foil packet.

Table 13. Seed moisture content for each harvest date of *Carica papaya* cv. Tainung

Harvest day (DAA)	Seed moisture content (%, w.b.)			
126	3.5	5.9	7.4	9.1
140	3.0	5.3	7.0	9.3
154	3.7	5.0	6.8	9.6
168	3.3	5.7	7.6	9.1
175	2.6	5.5	7.2	9.8
182	3.5	5.6	7.0	9.1

Before storage, the initial germination was determined for samples of seeds at all combinations of fruit collection date and seed moisture content. After that, seeds were sampled from each storage environment after 1, 3 or 6 months. Samples were left overnight (24 hours) in their sealed packets at +20 °C to adjust temperature.

After storage, each sample of 100 seeds was pre-treated with 40 ml of GA3 at 2,000 ppm for 24 hours and then split up into replicates of 25 seeds to be tested in rolled paper towels within polythene bags. On the 22nd day after the germination test was begun, a heat shock (36 °C for 4 hours) was applied to the seeds in test. This was an additional dormancy-breaking treatment following the protocol of Wood *et al.* (2000). The germination tests lasted a total of 49 days. Normal and abnormal seedlings (ISTA, 2005) were evaluated and counted once a week for all treatments. Firm non-germinated seeds were assessed for viability by a tetrazolium test. The progress of germination (radicle protrusion) was recorded twice a week and rate of germination was calculated.

The results for normal germination (percent) were angular-transformed and then subjected to analysis of variance (ANOVA). The initial germination following desiccation to different moisture contents was analysed as a factorial design (6 harvests x 4 moisture contents). These analyses were repeated for results after heat shock and with viable (firm) seeds included. A similar analysis was carried out for the initial rate of germination.

Further analyses of variance were carried out on the results following storage, a factorial design of 3 periods of storage, 4 moisture contents, 5 temperatures and 6 fruit harvest dates.

Results

The ability to germinate normally was affected slightly by desiccation at lower moisture content in harvests between 126 and 182 DAA before the heat shock (36 °C for 4 h) was applied ($p<0.05$, Figure 7a). After application of heat shock, germination increased in average 7.8% in all harvest dates, and there was no difference in germination among moisture contents ($p>0.05$, Figure 7b), as breaking dormancy occurred in seeds that did not respond fully to the effect of gibberellic acid. Once we added figures of abnormal germination and viable seeds, total viability increased 1.7% more and no difference between seed moisture content was observed ($p>0.05$, Figure 7c). In contrast to what happened for harvest dates between 140 and 182 DAA, the ability to germinate and tolerate desiccation is not fully developed for seeds harvested at 126 DAA ($p<0.05$, Figure 7 a,b,c).

We found that the rate of germination of *C. papaya* was affected by desiccation, collection date, and their interaction being slowest at moisture contents of about 3.0% and for the earliest harvest date ($p<0.05$, Figure 8a). The interaction was largely due to results at 126 DAA. When mean is calculated across all harvest dates, the moisture content about 3.0% provided the slowest germination and this increased progressively when seed moisture content increased to attain the most rapid germination at about 9.0% (Figure 8b).

All main factors and almost all interactions had significant effects on germination of *C. papaya* seeds. The general results (i.e. 1, 3 and 6 months storage) clearly show that the first harvest (126 DAA) provided poorer viability and germination than later harvests (Figure 9a). Late collection dates, from 140 to 182 DAA, exhibited similar viability with lower ability to germinate at 168 and 175 DAA, that the heat shock treatment and longer germination test duration enabled dormancy to be broken. Generally speaking in overall, seed germination was slightly affected at

lower moisture content for all tests during storage (Figure 9b), before and after heat shock was applied.

Storage at -20 °C resulted in rapid loss of viability in all lots at all moisture contents, since the first month in storage, while the colder (-86 °C) and warmer temperatures (0, +5, +15 °C) maintained viability over the 6 months of this investigation. Any of these four temperatures gave evidence to be problematic (Figure 10).

The variation in speed of germination during six months in storage was related to the response to seed dormancy. When overall of tests during the period in storage (e.g. 1, 3 and 6 months) is calculated, the harvest collections before shedding were more dormant than the over-ripped harvest (Figure 11a). In addition, in all treatments germination was affected gradually by desiccation to low moisture content, at a decreasing rate from about 9 to 3% moisture content (Figure 11b).

Discussion

Seeds of *C. papaya* tolerate dehydration to moisture content between 2.6-9.8% without reduction in viability, lower than values determined for *C. papaya* by Ellis et al (1991) of 7.9-9.4% moisture content. The range of moisture content investigated in this study (2.6-9.8%) is equivalent to relative humidity (RH) between 10 and 45%, located in water sorption zones I and II where no free water is found (Priestley, 1986). Thus, no crystallization of free water could occur to cause embryo damage. The seed dormancy acquired probably as response to desiccation was released by the effect of gibberellic acid, alternation of temperature and heat shock (36 °C for 4 h), the latter in agreement with the study of Wood et al. (2000). This desiccation tolerance results in confers the partially orthodox character to the seed storage behavior of *C. papaya*.

However, *C. papaya* seeds were intolerant to –20 °C but tolerant to warmer temperatures of +15, +5, 0 and colder -86 °C. Pritchard (2004) described this behaviour as Type II (or intermediate storage behavior) where seeds tolerate desiccation to about 30% RH and storage at certain sub-zero temperatures but not all, e.g. toleration of orchids seeds at –20, -70 and –196 °C but less at –30 and –50 °C. The response to certain temperatures (e.g. 0 °C) had variation among harvest times as it was described for several seed populations of *Caricaceae* species exposed at sub-zero temperatures in the previous year and for another intermediate species such as *Coffea arabica* (Hong & Ellis, 1992).

The tolerance to sub-zero temperature also varies between species of Type II seeds, but in general dehydration of seeds to a moisture content about 12% (50-60% RH) exhibits glass transition temperature (T_g) of around 5 °C and safe storage at temperature of T_g –70 (Pritchard, 2004). It explains the reason why *C. papaya* seeds studied are not sensitive to –86 °C but are sensitive at warmer temperatures of –20 °C, when probably the glassy state is lost. The stability of the glassy state depends on carbohydrates and proteins which form a non-symmetric network (Hoekstra, 1995), and it has been found in orthodox and recalcitrant seeds (Sun *et al.*, 1994; Sun & Leopold, 1993). However, the real presence of this state has to be investigate in *C. papaya* using the appropriate techniques (Bruni & Leopold, 1992; Sun & Leopold, 1993; Williams, 1994).

In summary, the intermediate seed storage behavior of *C. papaya* seeds is confirmed in a broad range of temperatures and moisture contents. And as it was suggested above, the research must be focused on the glassy state of this species, to understand the main biochemical properties of the seeds with intermediate storage behavior.

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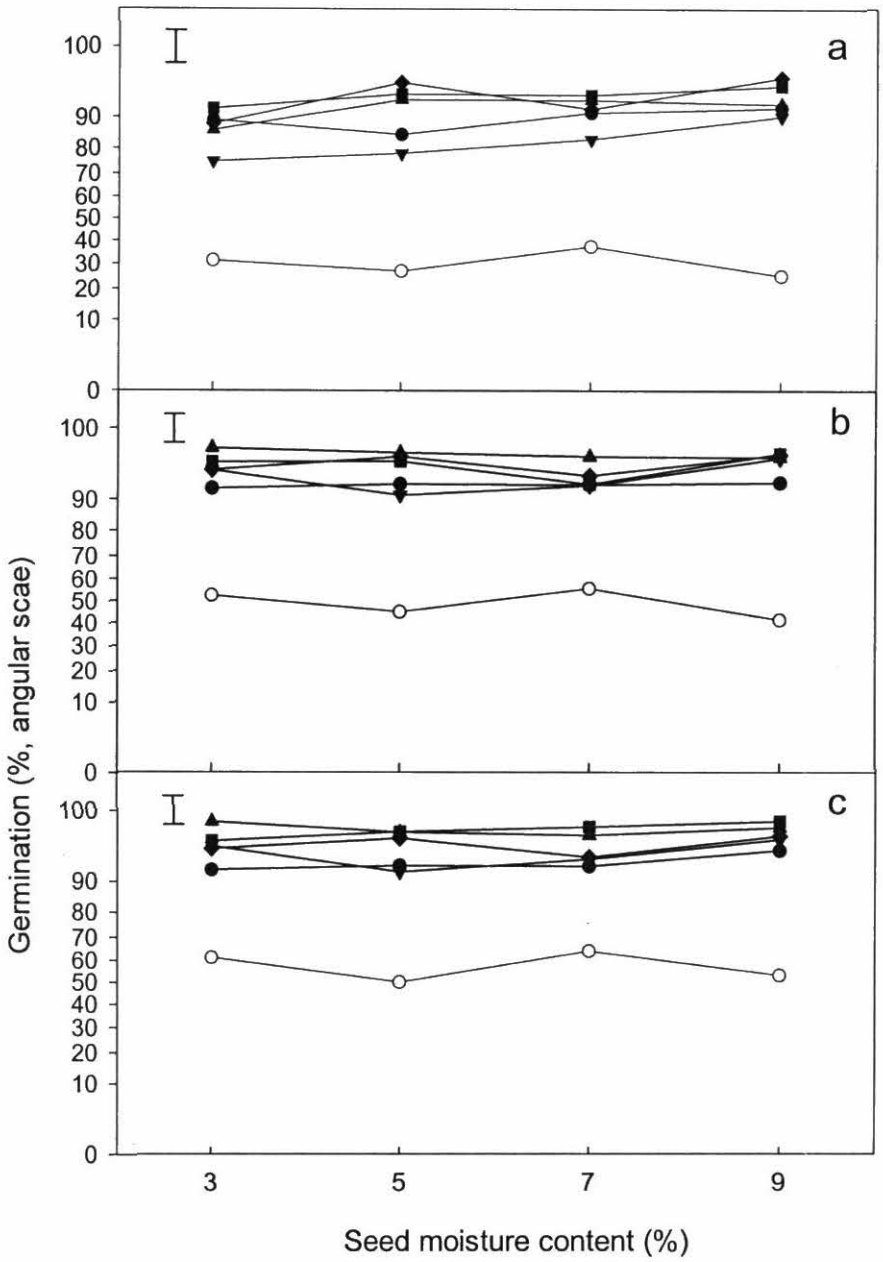


Figure 7. Initial germination (%, angular-transformed scale) of seeds of *Carica papaya* dried to four moisture contents, extracted from fruits harvested at 126 (O), 140 (●), 154 (■),168 (▲), 175 (▼) and 182 (◆) days after anthesis (DAA). a) Normal germination after 21 days without heat shock, b) normal germination after 49 days and application of heat shock, c) total germination and viable seeds, d) mean of moisture contents (□) before heat shock, (△) after heat shock, (▽) total viability. Vertical bar at top left of each box is standard error of the difference.

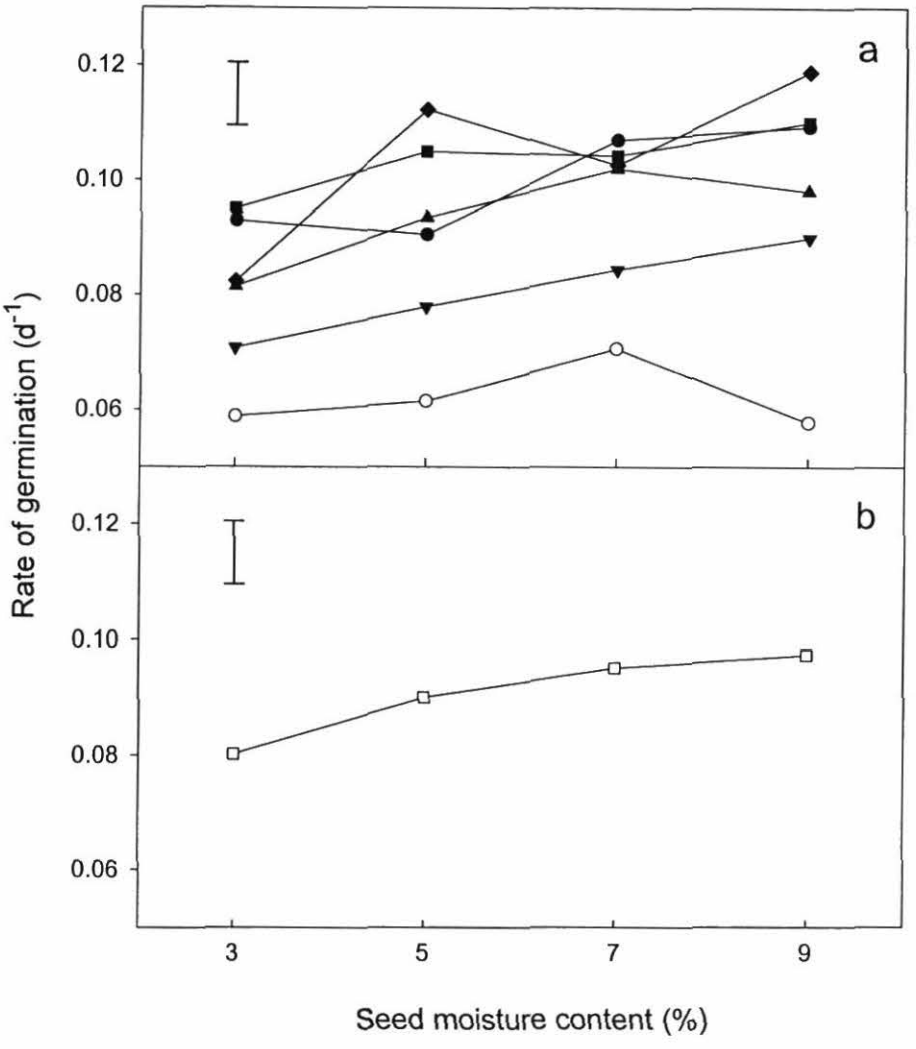


Figure 8. Initial rate of germination of *Carica papaya* dried to four moisture contents, extracted from fruits harvested at a) 126 (○), 140 (●), 154 (■), 168 (▲), 175 (▼) and 182 (◆) days after anthesis (DAA), b) mean of all moisture contents (□). Vertical bar at top left of each box is standard error of the difference.

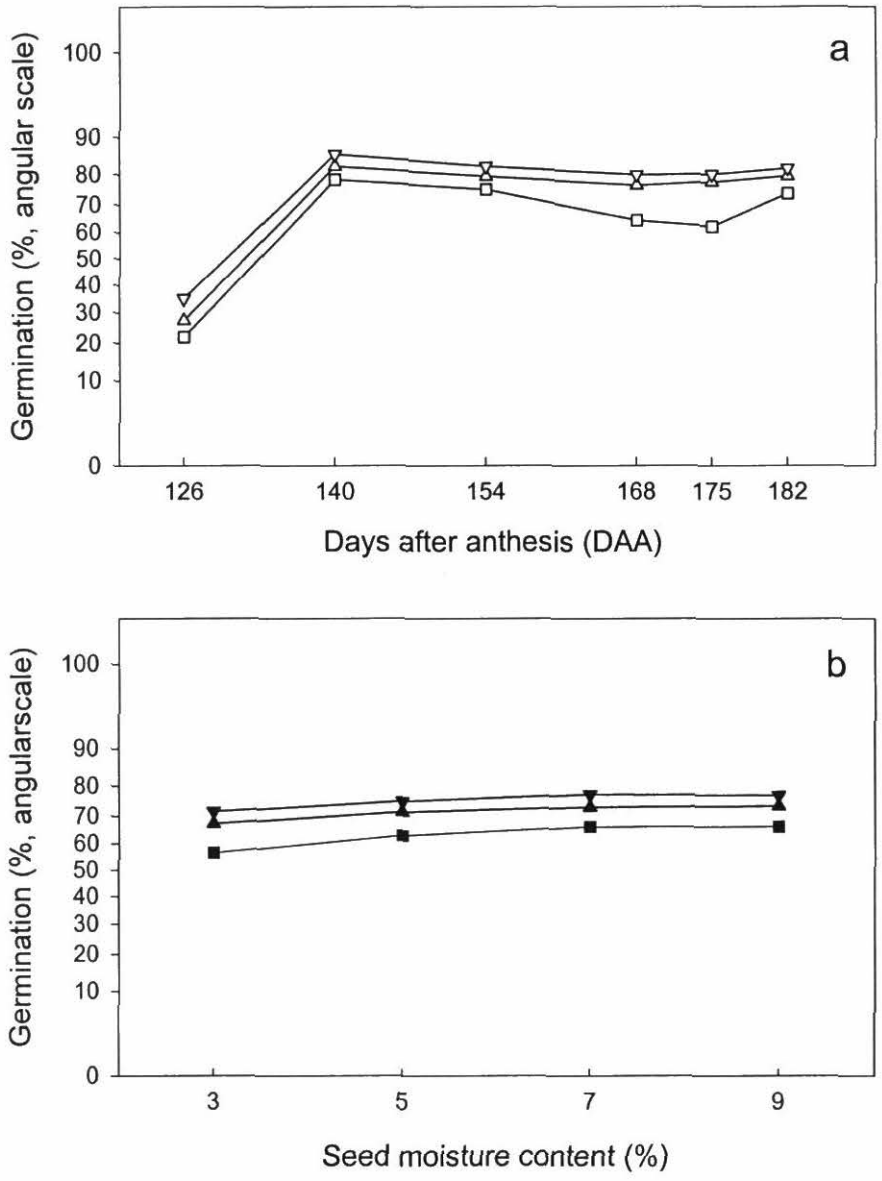


Figure 9. Germination (%, angular-transformed scale) of seeds of *Carica payaya* a) mean of normal germination for collection dates and test after 1, 2 and 3 months in storage, without heat shock and 21 days in test (□), with heat shock and 49 days in test (△), viability with heat shock and 49 days in test (▽); b) mean of normal germination for seed moisture content and test after 1, 2 and 3 months in storage, without heat shock and 21 days in test (■), with heat shock and 49 days in test (▲), viability with heat shock and 49 days in test (▼).

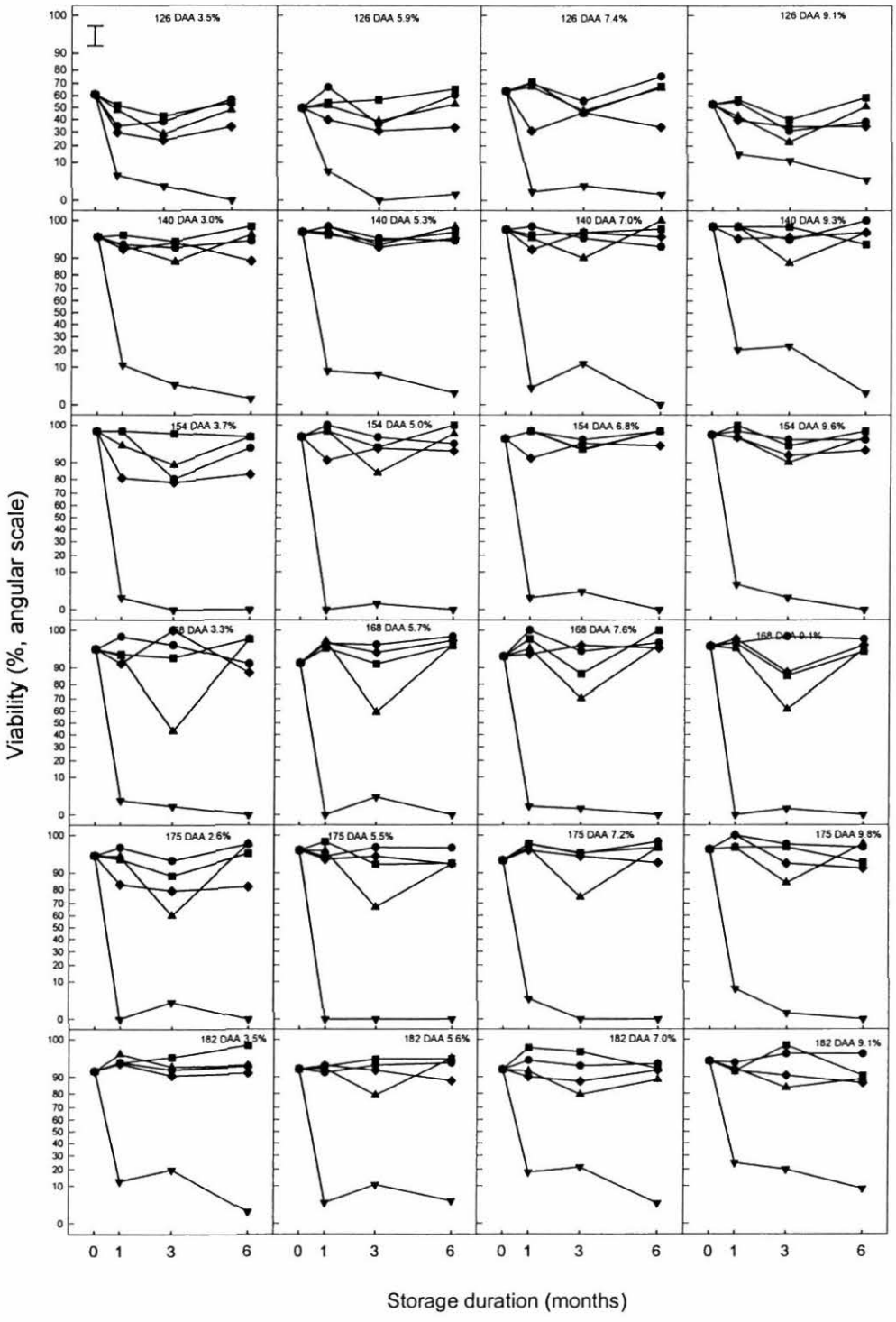


Figure 10. Total seed viability (%, angular scale) of *Carica papaya* after 49 days in test (with heat shock: 36 ºC for 6h), including normal and abnormal seedlings and viable seeds for six fruit harvest dates and stored hermetically at the moisture contents shown with the following storage temperatures: +15 (●), +5 (■), 0 (▲), -20 (▼) and -86 (◆) ºC. Vertical bar at top left is standard error of the difference.

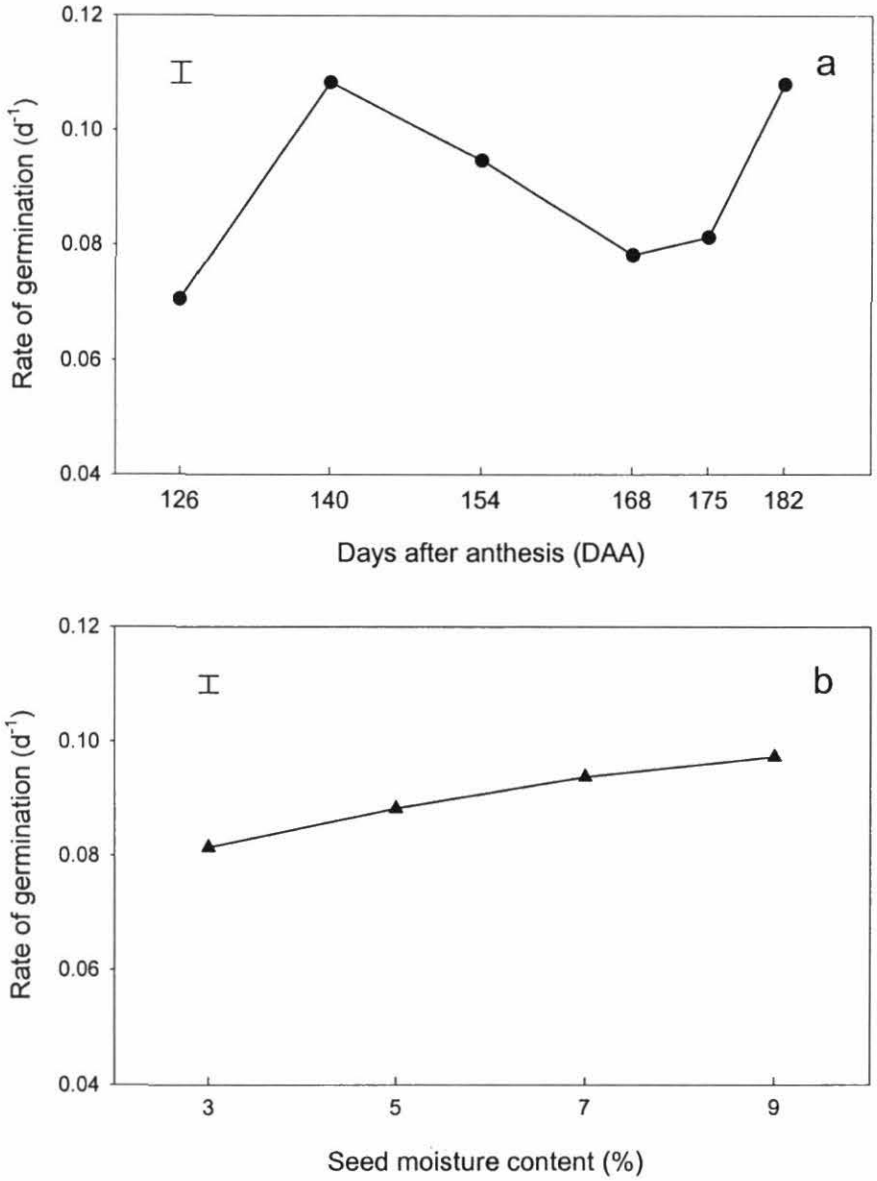


Figure 11. Rate of germination of seeds of *Carica payaya* a) mean of rate of germination for collection dates and test after 1, 2 and 3 months in storage (●); b) mean of rate of germination for seed moisture content and test after 1, 2 and 3 months in storage (▲). Vertical bar at top left of each box is standard error of the difference.

Activity 1.5.2. Development of *in vitro* conservation protocols in palms, using *Bactris gasipaes* Kunth ('chontaduro') as model.

Introduction

Following up on the request by the Ministry of Agriculture and Rural Development of Colombia (agreement 046), we initiated activities of conservation with 'chontaduro' as experimental

material, with prospects to establish methodologies applicable to materials of African palm (*Elaeis guineensis* Jacq.) and other species of Palmae reported in the Red List of Phanerogams of Colombia.

Two objectives were proposed in this study: 1) to establish conditions of management for cryopreservation of chontaduro (*Bactris gasipaes* Kunth), and 2) to establish a method of *in vitro* conservation of *B. gasipaes* under minimal slow growth.

Materials and methods

a) Cryopreservation of chontaduro

The seeds we used came from mature fruits (four months after the pollination), obtained from a farmer in Armenia, Colombia (Figure 12). Six moisture content (MC) levels were tested (4, 8, 10,12, 20 and 40 %); these levels were reached in dry rooms (20 °C and 40% RH) and the drying curve is presented in Figure 13. The seeds were then vacuum packed in aluminum foil bags (10 seeds per package for every MC). The bags were placed in liquid nitrogen (-196 °C) and remained for 7 and 30 days under these conditions. This test was repeated twice. The packages of seed were extracted from the liquid nitrogen, defrosted slowly, and viability and regeneration were evaluated using rescue of embryos.

Figure 12. a) Collection of fruits of *Bactris gasipaes.* b) Mature fruits of approximately 4 months after pollination.

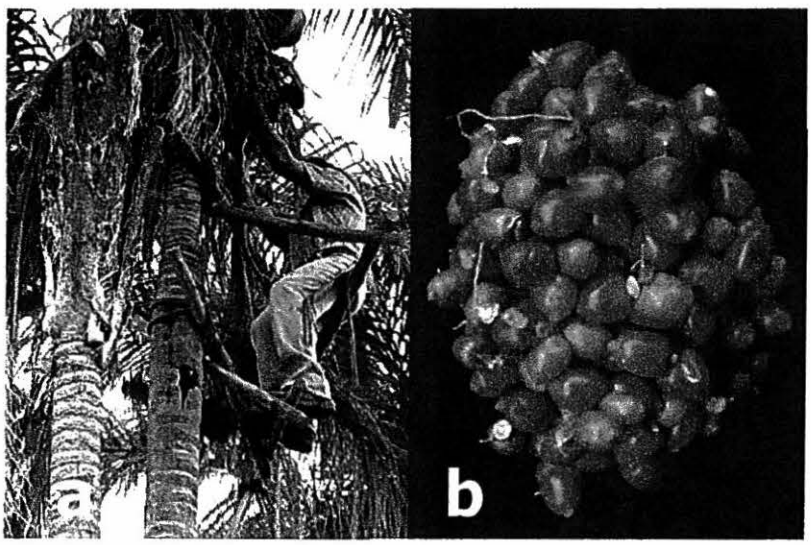
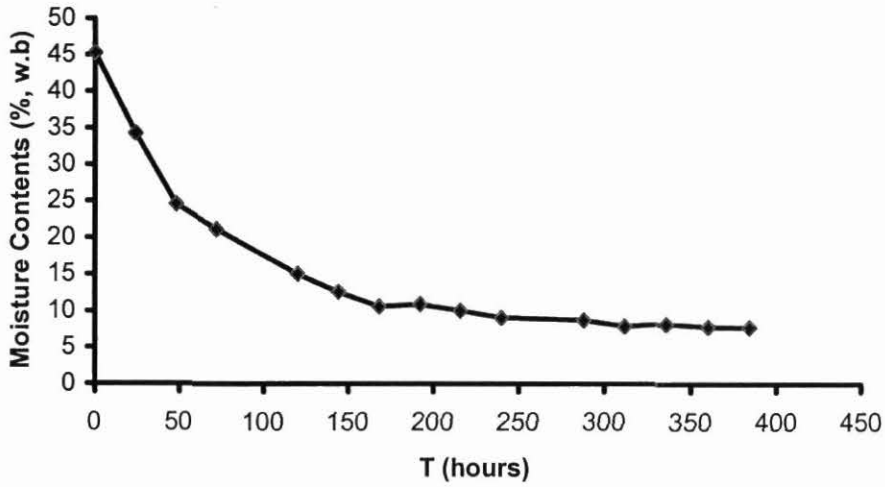


Figure 13. Drying curve for seeds of *B. gasipaes* placed at 20°C and 40% of relative humidity.



b) Methods of *in vitro* conservation of *B.gasipaes* under minimal slow growth.

Two conditions of temperature and two culture media were evaluated (28°C and 24°C/ NP and 8S) for minimal slow growth. The cultures were incubated under a 12-h photoperiod (1,000 lux). Each treatment included 10 replicates. After 8 months of storage, observations were recorded on stem length (mm), number of leaves and roots.

Results and Discussion

a) Cryopreservation of chontaduro

It was possible to observe 100% of plants regenerated from seeds with 40% MC that had not been in liquid nitrogen (N.L.), while for seeds with a 10 and 20% MC the averages were 50 and 48%, respectively. The previous result indicates that after lowering the percentages of MC of the seed, the percentage of regeneration of *in vitro* plants descends due to possible damages caused in the structures of the embryo that do not allow its later germination (Table 14).

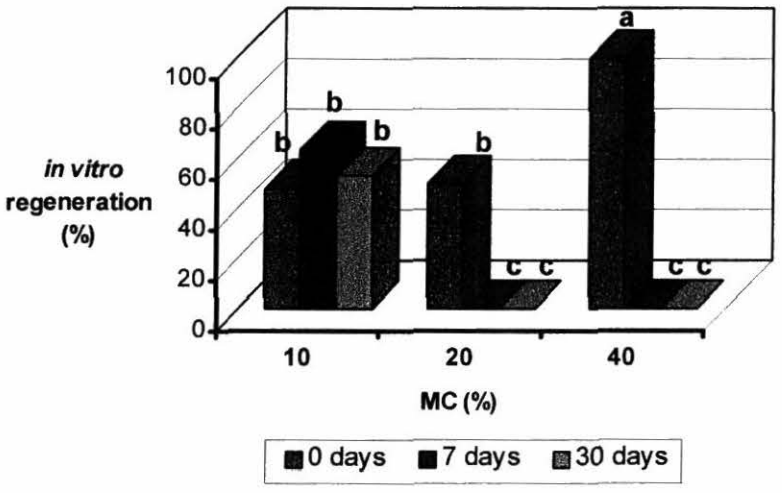
Table 14. Means of viability and regeneration *in vitro* of seeds of chontaduro for three times of conservation in liquid nitrogen (-196 °C).

	Moisture content (%) **					
Time NL (days)	10		20		40	
	%Via.	% Reg.	%Via.	% Reg.	%Via.	% Reg.
0 (control)	64 b*	48 b	70 b	50 b	100 a	100 a
7	80 b	63 b	0 c	0 c	0 c	0 c
30	80 b	53 b	0 c	0 c	0 c	0 c
Viab. CME 0.02 – Regen. CME 0.01 DF 22						

For the embryos extracted from cryopreserved seeds with 20 and 40 % MC, there was no response in viability nor *in vitro* regeneration, whereas at 10 % of MC we registered percentages of viability of 80 % after 7 and 30 days of conservation and percentages of regeneration of 63 and

53 %, respectively. These results suggest that contents of moisture higher than 20 % are not appropiate for the establishment of cryopreservation protocols for seeds of chontaduro (Figure 14).

Figure 14. Effect of the cryopreservation in N.L. on the regeneration of seeds of *B. gasipaes* for three contents of humidity (10, 20 and 40%); the same letter indicates no significant difference at 95% confidence.



The analysis showed statistical differences for the percentage of regeneration and *in vitro* viability in the interaction of the two principal effects: MC (%) and time of storage in N.L. (days). This indicates that MC is a determinant factor, in the response of the seeds to cryo-conservation; at lower MC the probability of intracellular freezing is minor, thus reducing the possible damages in the structure of the embryo.

With the purpose of adjusting the ideal MC for the cryopreservation of chontaduro, new ranges of contents of moisture were evaluated. The MC of seed evaluated in this second phase were 12, 8 and 4 %, for which we determined viability and *in vitro* regeneration. The results show that a reduction in the MC of the seeds down to 4 % turns out to be too drastic. These results indicate that the ideal range is about 8-12 %.

After 7 days of conservation in N.L. we observed a low percentage of viability (17 %) and of regeneration (2 %) for cryopreserved seeds with moisture content of 4 %, values that were not significant in relation to other times of conservation evaluated. This result can be attributed to an effect of the lot of seeds used as control.

For the seeds in liquid nitrogen with 8 % MC we registered high percentages of viability (80 %) and regeneration (60 %) for two times of conservation, unlike the seeds with 12 % MC that had low answers for these variables (9 % viability and 5 % regeneration) (Figures 15 and 16).

Figure 15. Percentages of *in vitro* viability for seeds of *B. gasipaes* with three moisture contents (4, 8 and 12%) and three durations of cryopreservation (0, 7 and 30 days).

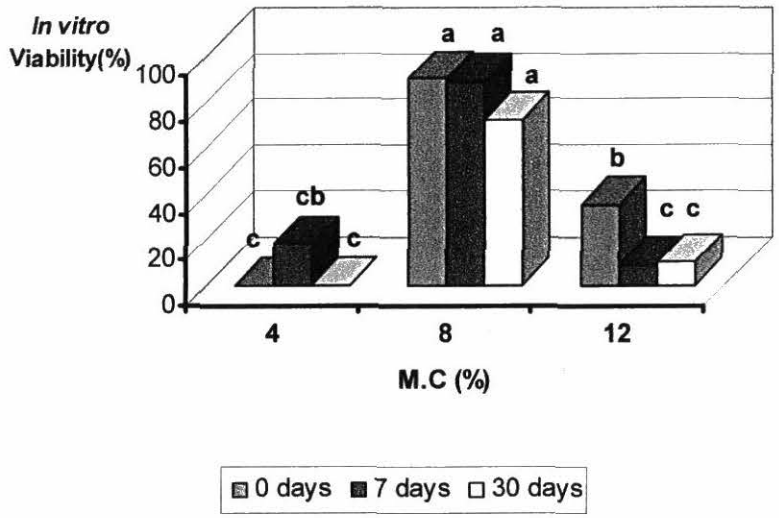
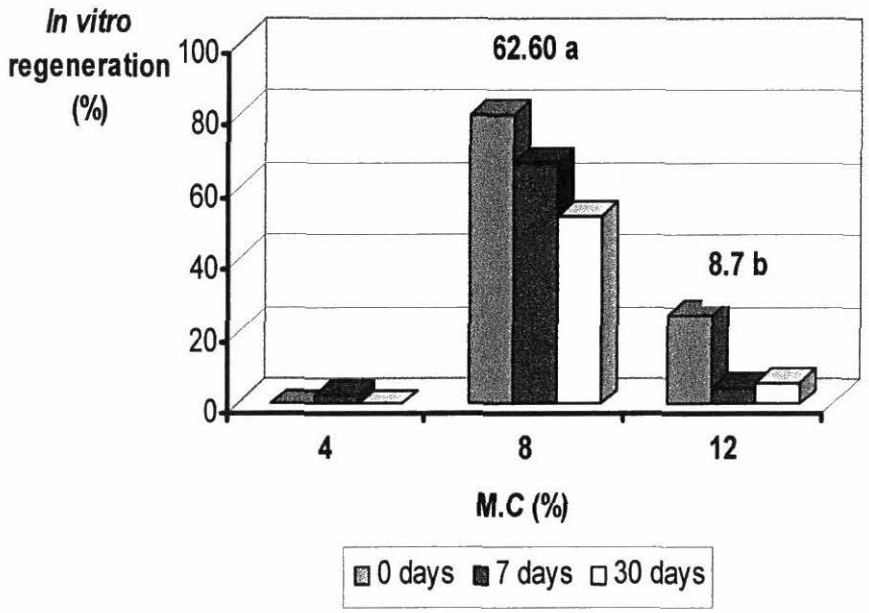


Figure 16. *In vitro* regeneration (%) of seeds of *B. gasipaes* cryopreserved with three MC (4, 8 and 12%) for three durations (0, 7 and 30 days).



The information about the rate of regeneration and viability up to 30 days of conservation in liquid nitrogen suggest that there is no effect of the time of conservation on the parameters of response for the moistures evaluated. This would allow to state that the seeds can remain for longer periods without affecting the regeneration of the same seeds under *in vitro* conditions, because under these conditions all metabolic activity stops (Stanwood, 1985).

The cryo-preservation of seeds could be a good alternative for long term conservation of different species of the family *Palmae* reducing the possibility of genetic changes. For the two times of evaluation we observed that a good tolerance to reduction in the MC for the seeds of chontaduro was about 8-10 %. Under these levels of moisture it was possible to verify *in vitro* regeneration before and after the conservation in liquid nitrogen.

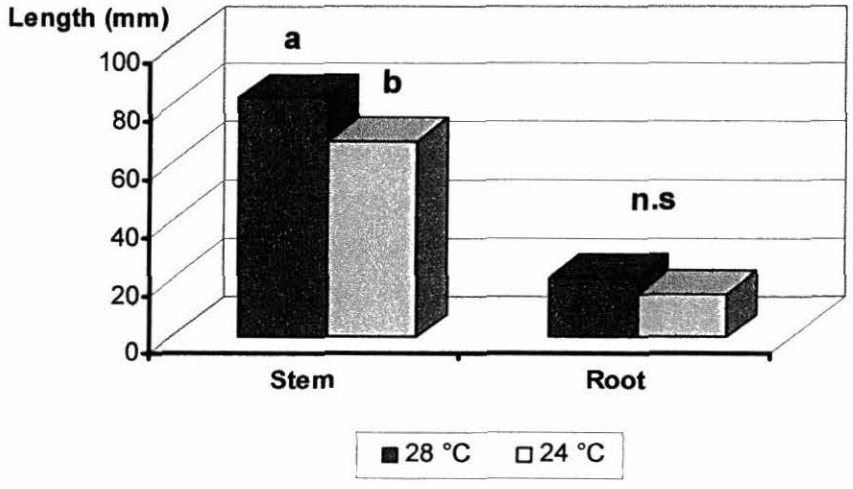
It is expected that these protocols are not genotype dependent so that their application could be possible to a wide range of accessions maintained in the collections and in the field in Colombia.

b) Conservation under minimal slow growth

The analyses of variance showed that our conservation conditions (24°C and 28°C) had a greater effect on stem elongation and number of green leaves, while no significant differences were observed between the different medias.

A reduction of growth was obtained when the material remained at a temperature of 24°C, indicating that the physical factor (temperature) has an effect on the *in vitro* growth of chontaduro. At 24°C the plants presented 82% of the growth achieved by those maintained at 28°C (Figure 17).

Figure 17. Effect of temperature on stem elongation and length of roots (mm) for the *in vitro* conservation of *B. gasipaes* (time of conservation: eight months).



For the material maintained at a temperature of 24 °C in the 8S media, we could observe some yellowing in the leaves, indicating the beginning of a possible process of deterioration; this response was not observed in the *in vitro* plants grown in N.P. (silver nitrate) media.

This reaction observed in the NP could be due to the presence of a major quantity of nitrogen source (NO_3) which favors the increase and proliferation of leaves, or to the effect of inhibition of the ethylene caused by the silver nitrate.

It is important to emphasize that after eight months of evaluation of the media and conditions of conservation we have not seen any senescence. Avoiding or lowering down senescence is an important factor to consider when evaluating conservation under minimal growth (Barceló et al., 1992).

After eight months of conservation under the evaluated conditions one can state that this duration has not been sufficient to determine the total possible time of conservation, but it has been possible to demonstrate the importance of the physical conditions for the *in vitro* conservation of chontaduro, especially the conditions of temperature where there is a marked decrease of the rate of growth at lower temperatures.

The germplasm conservation of palms under minimal growth is highly desirable since these are species with generally woody and perennial stems, also they present periods of little known or poorly described level of latency in the stage of vegetative growth. This latency added to an appropiate management of the sensitiveness to the cold temperatures showed by the tropical plants could be reflected in the obtaining of longer periods of conservation (Engelmann, 1991).

Conclusions

This experiment constitutes the first successful report of cryo-preservation of seeds of a member of the genus *Bactris spp.* The method of cryo-conservation of seeds developed in this research can be used as a strategy for the conservation of species of palms reported in the Red List of Phanerogams of Colombia.

Seeds of *B. gasipaes* seem to tolerate the drying until 8% of MC showing high percentages of viability (80%) and regeneration (62.6%) for the times of cryo-conservation evaluated. Extracted embryos of seeds cryo-conservated with a 4% MC presented low percentages of viability and *in vitro* regeneration.

After eight months of conservation *in vitro* a decrease in the temperature has allowed to reduce the rate of growth of the plants of *B. gasipaes.* Media with silver nitrate have not shown an effect on the decrease in the rate of growth, however we observed that the number of leaves and their color has been lightly higher than those of the media 8S. After eight months of *in vitro* conservation in the medias and under the evaluated conditions of temperature, we have 95% of survival.

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Activity 1.5.3. Cryopreservation of seed of African palm (*Elaeis guineensis* Jacq.)

Once we adjusted the methodology for the cryopreservation of seeds of chontaduro, we applied it to seeds of the African palm coming from mature fruits collected in Norte de Santander, Colombia. Seeds were extracted from fresh fruits with a MC of 27%; they were treated in a dry room (20 °C and 40% RH) to obtain seeds with ranges of MC near those evaluated for chontaduro. Embryos obtained from seeds of African palm were adjusted to three MC (4.9, 7.5 and 13%). Although they were viable, not all embryos however had the capacity to regenerate plants under *in vitro* conditions. Only for seeds with 7.5% of moisture a full 100% regeneration was achieved after two months of establishment (Figures 18 and 19).

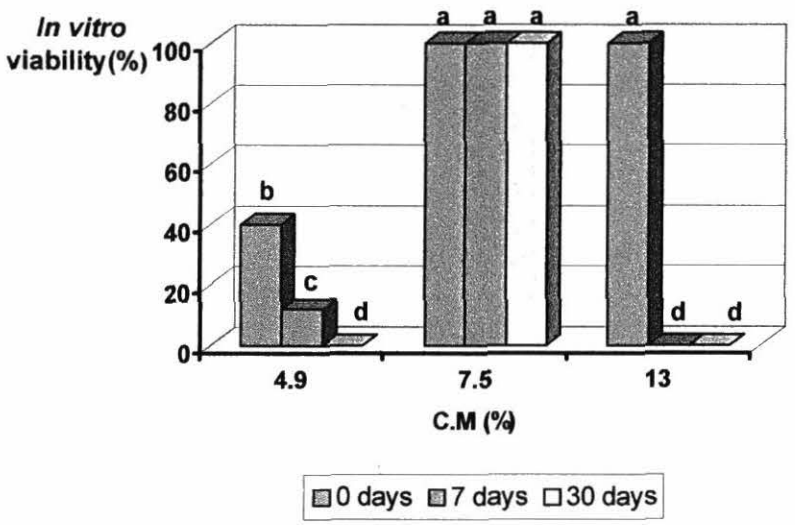

Figure 18. *In vitro* viability for seeds of *E. guineensis* cryopreserved at three MC (4.9, 7.5 and 13%) for three durations (0, 7 and 30 days).

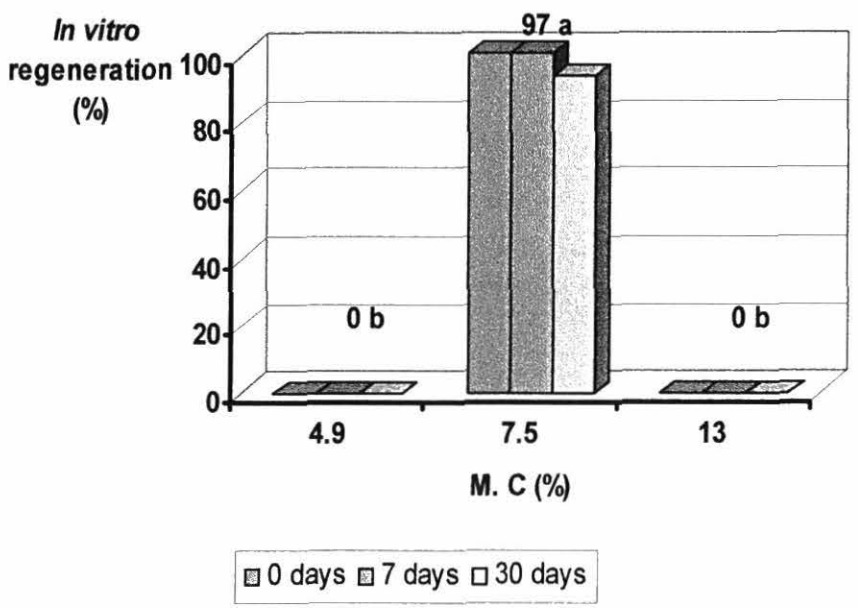

Figure 19. *In vitro* regeneration for seeds of *E. guineensis* cryopreserved at three MC (4.9, 7.5 and 13%) for three durations (0, 7 and 30 days).

The statistical analysis indicated significant differences in terms of viability for the main parameters such as moisture content (%) and time of cryopreservation (days), and for the interaction among them. Means marked with the same letter indicate no significant differences at the confidence level of 95% (variables transformed for the analysis with arcosinus). Differences were significant only for the moisture contents for the variable "percentage of regeneration" and the analysis didn't show differences in the durations of cryopreservation. Embryos coming from seeds with a moisture content of 7.5% and cryopreserved for 30 days, reached percentages of regeneration of 93%-100% (Figure 20).

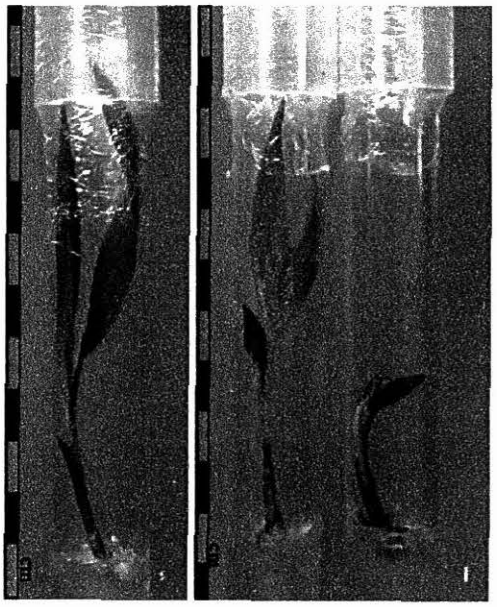

Figure 20. Regenerated *in vitro* plants of African palm starting from zygotic embryos: a) extracted embryos of seeds with 7.5% MC; b) extracted embryos of seeds cryopreserved for 7 and 30 days, respectively.

An intermediate behaviour of stored seeds of this species in relation to dessication had been already reported by some authors who carried out research on cryopreservation of embryos and seeds without endocarp (Hong et al. 1996).

References

Hong, T.D., Linington, S. & Ellis, R.H. 1996. Seed storage behaviour: a compendium. Handbooks for Genebanks: N° 4. International Plant Genetic Resources Institute (IPGRI), Rome, Italy.

Contributors: E. Aranzales, G. Mafla, R. Escobar, D.G. Debouck

Activity 1.5.4. Establishing a protocol for seed conservation of the chontaduro *Bactris gasipaes*

The behaviour of *Bactris gasipaes* seeds has not been established. However, in the classification of the family Palmae carried out by León (1961), its seeds are considered as intermediate with

respect to their behaviour towards dessication (Hong et al., 1996). In this study three moisture contents (40, 20 and 10%) and two temperatures (+5 °C and -20 °C) were evaluated, and the evaluation was realized three months later. The tests for viability and regeneration were done using embryo rescue.

Table 15 shows the effect of moisture content (MC) on the viability and *in vitro* regeneration of chontaduro seeds. The statistical analysis showed significant differences in the three moisture contents, presenting a direct relationship between the decrease in the moisture content and the observed variables, an effect possibly due to the damages in the structure of the embryo caused by the decrease in the MC.

Table 15. Effect of MC on the viability and regeneration *in vitro* in seeds of *B. gasipaes*.

	Moisture content (%)		
	10	20	40
Viability (%)	64 b	70 b	100 a
Regeneration (%)	48 b	50 b	100 a

Means marked with the same letter indicate no significant differences at the confidence level of 95% (variables transformed for the analysis with arcosinus $\sqrt{\%/100}$).

With respect to the conservation temperatures, seeds kept at +5 °C had a regeneration average of 34% and at -20 °C the regeneration average was of 15%, showing an effect of the conservation temperature in the regeneration of extracted embryos for the three MC evaluated. For the moisture contents of seeds conserved at 10% there was no statistical differences after three months of conservation in none of the two evaluated temperatures. In seeds with 20% moisture we registered a total loss of regeneration after three months of conservation under the two temperature conditions. The seeds conserved with moisture of 40% presented significant differences in relation of the two temperatures, obtaining a percentage of regeneration of 67% for +5 °C and absence of regeneration at -20 °C. This suggests an effect of the conservation temperature on the response parameters in seeds at this MC (Figure 21).

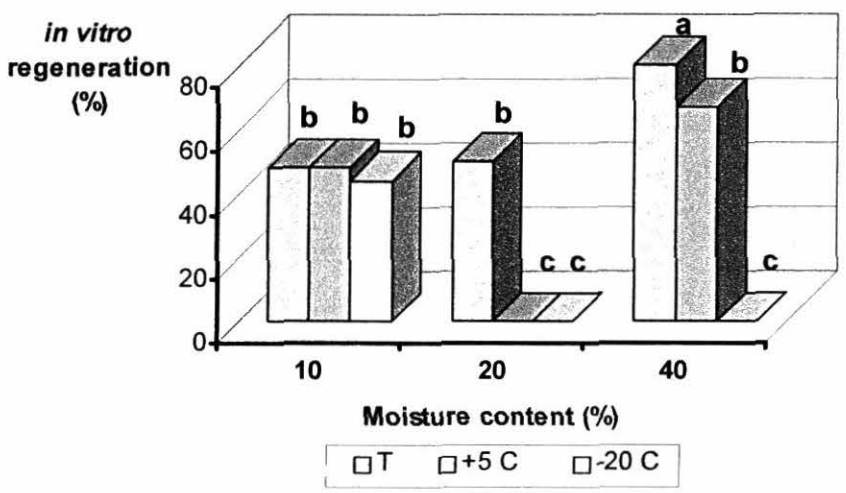

Figure 21. *In vitro* regeneration of seeds of *B. gasipaes* with three MC conserved at +5 °C and -20°C during 3 months.

Seeds at 10% of MC can be conserved at +5 and -20 °C without remarkable effects on the response parameters. However there is a need to analyze longer durations under these conditions.

Our data suggest a possible intermediate behavior, since it was possible to conserve seeds with MC of 8-10% for conservation temperatures of +5 °C and -20°C without a significant loss of the viability which allows the subsequent regeneration of *in vitro* plants.

References

Hong, T.D., S. Linington & R.H. Ellis. 1996. Seed storage behaviour: a compendium. Handbooks for genebanks: No. 4. International Plant Genetic Resources Institute, Rome, Italy.

Activity 1.5.5. Implementation of cassava cryopreservation protocol using the Core Collection as a model.

Background

During the period 2003-2005, different activities were carried out in order to know the logistical aspects involved in the management of an IVBG cassava collection. The following critical points are being studied, namely how much effort and manpower are necessary for the implementation of the cassava cryopreservation protocol on the entire collection.

Materials and Methods

The encapsulation-dehydration methodology was implemented (Annual Report, 2000) using *in-vitro* plants supplied by GRU. It was necessary to recover a copy of core collection coming from clones maintained at BRU to make a Cryo-core II.

Results

We currently maintain under liquid nitrogen (L.N.) conditions 621 clones of the core collection. For each clone we maintain 6 tubes with 10 beads. This year, we initiated with the duplication of the Cryo-core, but high contamination and logistical problems in the lab with agar lots and autoclave did not allow us to go farther with any of the copies (Cryo-core I and II) (Table 16).

Table 16: Groups of response and copies established under L.N. conditions using the cassava core collection as a model.

Collection Type			No Clones	% of response based on
Cryo-Core I		Frozen	621	98.57% of core collection
		Evaluated	599	96.5% of frozen clones
	Group of response*	Lowest	191	Less than 30% plant recovery
		Intermediate	245	Between 30-70% plant recovery
		Highest	163	Up to 70% plant recovery
Cryo-Core II		Frozen	84	

* Clones are distributed into response's groups based on n=599 clones evaluated.

On the basis of the currently frozen clones, 96.5% have been recovered and grouped according to their responses in low, intermediate or highest group. More than 68% (intermediate and highest group) of the clones showed up to 30% recovery considered as the base line for maintaining one clone into the cryo-tank (Figure 22).

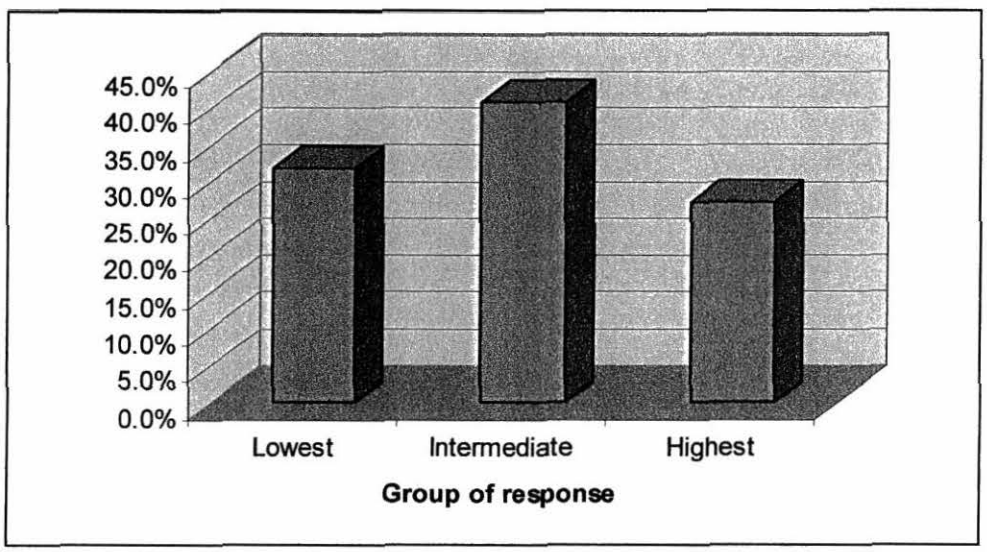

Figure 22: Grouping of clones after freezing procedure (<30%, 30<x>70%, >70% of response as the lowest, intermediate and highest group, respectively). n = 599 clones tested.

We consider that a critical point during the process is the propagation scheme that produces explants for cryo, because it is time consuming and requires a lot of manpower. In the same sense, encapsulation-dehydration has a lot of steps that are not practical for its implementation on a big collection as that of cassava (based on number of clones conserved per month).

For that reason we initiated activities with a vitrification protocol using buds as explants. Probably, these procedures would allow us to make all freezing activities in one day and to put more clones into the tank per month. Panis *et al.* (2005) at INIBAP adjust a droplet-vitrification protocol and tested with the *Musa* and *Ensete* genera, with view of applying it to all banana accessions.

Conclusions

We conserved under L.N. condition 98,6% of core collection (Cryo-core I). As a duplicate copy of Cryo-core II, we included 84 clones during 2005.

On the basis of the core collection, 68% of clones tested showed plant formation up-to 30% (245 and 163 clones as intermediate and highest responding groups, respectively). Propagation activities and contamination aspects occurring during the process could be a critical point for routine of cryopreservation. It could delay the process. It is necessary to freeze 22 more clones to complete the entire core collection under L.N. conditions.

Future activities

- Establish a critical point scheme for monitoring cryopreservation activities. Hazard Analysis and Critical Control Point (HACCP) analysis will be useful to make control activities.
- Adjust procedure to use nodes as explants that allow us to reduce propagation activities.
- Adjust the vitrification procedure for the lowest responding clones.

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.

Panis B., Piette B. and R. Swennen. 2005. Droplet vitrification of apical meristems: a cryopreservation protocol applicable to all Musaceae. Plant Science 46, 168: 45–55.

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. pp. 269-301.

Contributors

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[1] Agrobiodiversity and Biotechnology Project-CIAT; [2] Genetic Resources Unit-CIAT

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 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 clones at national and international levels. We have been working on the indexing for three viruses: CsCMV, CsXV and FSDA. For the indexing three diagnosis techniques are used: ELISA for CsCMV and CsXV, and grafting with a hypersensitive clone for the causal agent of Frog Skin Disease (FSDA).

The total number of accessions available at this moment and tested against viruses CsCMV, CsXV and FSDA, thus ready for distribution, is 5,114 clones (83 % of the entire collection) (Table 20).

The total number of accessions available at this moment and tested against viruses CsCMV, CsXV and FSDA, thus ready for distribution of the FAO collection is 4,830 clones (84,1 %) (Table 4). The referred amount of clones was taken from a total of 5,740 clones (cultivated, hybrids and wild species).

Indexing for CsCMV

The number of clones evaluated against CSCMV until August 2005 is shown in Table 17.

Table 17. Clones of the entire collection indexed against CsCMV.

Source	Indexed clones	Negative clones
Argentina	11	8
Brasil	7	7
Colombia	5	5
Paraguay	2	2
Perú	1	1
VNM	1	1
CM	2	2
SM	1	1
TOTAL CLONES	30	27

These results indicate that to date 90 % of clones presented negative results for this virus and 10 % positive results.

Indexing for CsXV

The number of clones evaluated against CsXV until August 2005 is shown in Table 18.

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

Source	Indexed clones	Negative clones
Argentina	12	8
Brasil	24	23
Colombia	20	18
Costa Rica	3	2
Panamá	1	1
Perú	2	2
Venezuela	3	3
CG	2	2
CM	3	3
SM	1	1
VNM	1	1
TOTAL CLONES	72	64

The results indicate that 88,8 % of the evaluated clones present negative results for this virus and 11,1 % positive results.

Indexing for FSDA

The number of clones evaluated against FSDA until August 2005 is shown in Table 19.

Table 19. Clones of the whole collection indexed against FSDA.

Source	Indexed clones	Negative clones
Argentina	8	4
Brasil	32	33
Colombia	22	18
Guatemala	1	1
Indonesia	1	1
Malasia	1	1
México	2	1
Paraguay	6	5
Perú	1	0
Philipinas	2	1
Venezuela	6	4
Wild species		
BLO	1	1
CHL	1	1
CTH	1	1
FLA	2	0
PER 417-005	1	0
VIO	1	1
Costa Rica	1	1
TOTAL CLONES	90	74

The results indicate that 82,2 % of materials presented negative results in the indexing for this virus and 17,8 % presented positive results.

We still experience some problems with the growth of wild clones (as shown in last year Annual Report) and also with some cultivated ones.

The current status of the entire 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 20.

Table 20. Indexing status of the Cassava Germplasm Collection kept in GRU by December 2005.

Source	In vitro clones	INDEXED CLONES			Index 2005
		CsCMV	CsXV	FSDA	
Argentina	116	91	97	75	70
Bolivia	7	7	7	5	5
Brasil	1,325	1,310	1,306	1,195	1,187
China	2	2	2	2	2
Colombia	2,044	2,001	1,962	1,778	1,757
Costa Rica	148	148	144	140	137
Cuba	77	77	77	77	77
Estados Unidos	9	9	9	8	8
Ecuador	116	114	113	107	106
Fiji	6	5	5	5	5
Guatemala	91	91	88	76	76
Indonesia	51	51	51	43	43
Malasia	67	67	66	57	57
México	102	101	98	88	87
Nigeria	19	19	19	17	17
Panamá	43	39	38	37	36
Paraguay	209	204	205	164	160
Perú	406	405	404	384	379
Philipinas	6	5	5	5	5
Puerto Rico	15	15	15	13	13
Rep. Dominicana	5	5	5	4	4
Salvador	8	7	7	6	6
Tailandia	31	30	30	19	19
Venezuela	244	237	234	217	216
CG	90	81	79	77	73
CM	443	418	419	411	407
SG	46	45	43	41	40
SM	88	84	84	78	77
HMC	4	4	4	4	4
KM	9	7	6	4	4
CT	1	1	1	1	1
SUBTOTAL	5,828	5,680	5,623	5,138	5,078
WILD SPECIES					
30 spp in vitro					
3 Undefined spp					
AES.	4	0	0	0	0
ALT.	8	3	2	1	1
ANM.	3	0	0	0	0
BLO.	1	1	1	1	1
CAE.	24	5	4	0	0
CEC.	6	1	1	0	0
CHL.	6	4	5	5	3
CTH.	94	22	17	12	9
EPR.	1	0	0	0	0
FLA.	32	14	14	13	12
FMT.	5	4	4	4	4
FRU.	1	0	0	0	0
GLA.	5	1	1	0	0
GUT.	36	1	1	0	0

Table 20. Indexing status of the Cassava Germplasm Collection kept in GRU by December 2005.

HAS.	4	0	0	0	0
IRW.	2	0	0	0	0
JAC.	14	2	0	0	0
LON.	6	0	0	0	0
ORB.	10	1	0	0	0
PEL.	1	0	0	0	0
PER 413-003	1	0	0	0	0
PER 417-003	1	0	0	0	0
PER 417-005	1	1	1	0	0
PIL.	2	1	1	0	0
PNT.	1	0	0	0	0
PSE.	1	0	0	1	0
PUR.	1	0	0	0	0
QPT.	1	0	0	0	0
RUB.	15	3	2	0	0
SPR.	2	0	0	0	0
TPH.	15	1	0	0	0
TST.	23	10	7	10	4
VIO.	4	2	2	2	2
WV.	1	0	0	0	0
SUB TOTAL	332	77	63	49	36
TOTAL	6,160	5,757	5,686	5,187	5,114

As one can see in the previous Table, 93,4% of the cassava collection is negative to CsCMV, 92,3% to CsXV and 84,2 % to FSDA.

Table 21 shows progress in the indexing of the cassava collection (materials negative for three viruses) obtained between 1998 and 2005.

Year	Percentage of negative clones for CsCMV	Percentage of negative clones for CsXV	Percentage of negative clones for FSDA	Index %
1998	35,8	34,7	9,77	602 (9,77)
1999	42,6	40,1	17,4	1,073 (17,4)
2000	71,0	63,7	39,3	2,346 (38)
2001	82,9	79,5	58,3	3,452 (56)
2002	91,3	88,9	76,8	4,559 (74)
2003	92,3	90,7	80,6	4,823 (78,2)
2004	93,1	91,7	83,1	5,067 (82,2)
2005	93,4	92,3	84,2	5,114 (83,0)

As one can see, this process is getting slow, as we experience some problems during the establishment of the remaining materials. We looked for passport data using the ORACLE database, 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.

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

Activity 2.1.3. Indexing wild materials of cassava for GRU

We continued with the growing-out activities 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 from the *in vitro* collection where they are currently kept.

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

Activity 2.1.4. Establishment of a "Bonsai" collection as safety back-up of the whole Cassava Collection

Since October 2001 we began to establish one copy of the whole cassava collection under greenhouse conditions, because most of materials delivered to the field genebank became infected with FSDA, and the Cassava Breeding Project no longer provided the service. Along our current agreement with FAO, this back-up is necessary before the entire collection is safely maintained under cryopreservation.

At the beginning of this process, we wanted to maintain the plants under conditions of much reduced growth (like a "bonsai"); several plants however did not thrive at all under those conditions. Currently, plants are allowed to grow normally (as it can see in Figure 23) in order to maintain a reliable back-up of the cassava *in vitro* collection.

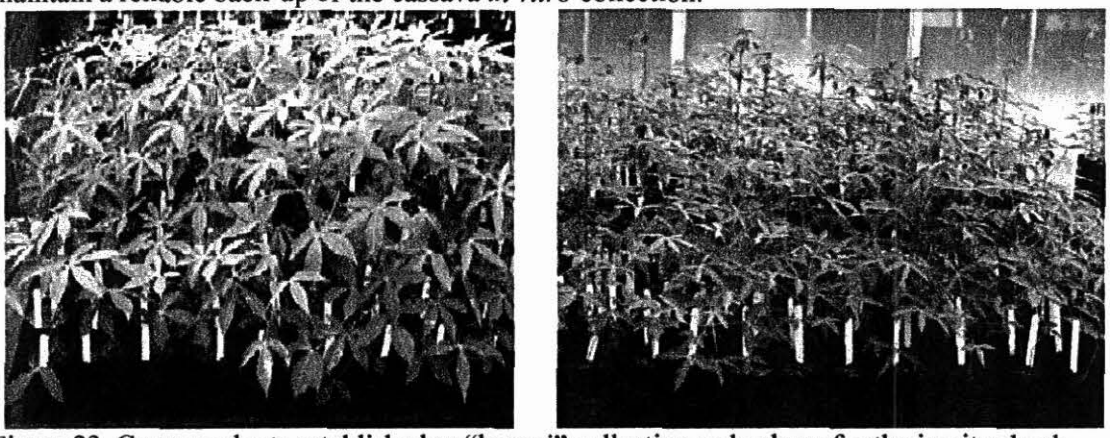


Figure 23. Cassava plants established as "bonsai" collection as back-up for the in vitro bank.

However, most of them have deteriored, after being under the same conditions over time (the same bag, soil, environmental conditions and so forth). In order to preserve those materials, we began a "renovation process", taking new stakes and replanting again in a new substrate, but under the same greenhouse conditions in order to avoid infection by viruses transmitted by white flies.

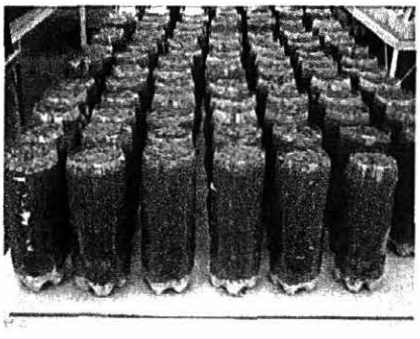
Figure 24. Stake planted during the process of renovation of the "Bonsai" collection.

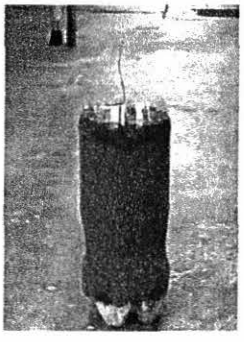
Figure 25. On the rigth a regenerated plant; on the left an ill plant

Contributor: N.C. Flor.

Activity 2.1.5. Utilization of plastic bottles for establishement of *in vitro* plants as back-up to 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 a lot of plants in plastic bottles to observe their growth. So far, the plants still show the same growth characteristics as in the last methods (bag and soil).





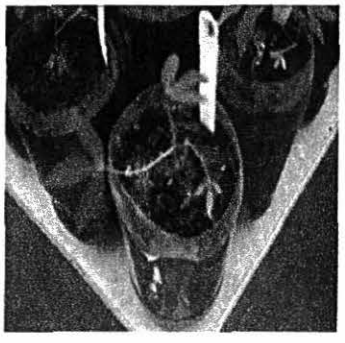


Figure 26. Plastic bottles with soil and granite *In vitro* plants

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

Activity 2.1.6. Updating the Cassava ORACLE database

We continued updating the ORACLE database with the new results of indexing (to CsCMV, CsXV and FSDA) and with the data about the new 'bonsai' clones.

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

Activity 2.1.7. 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) of GRU carried out 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 January 2005- December 2005, the GHL tested a total of 5,412 seed samples, distributed as follows: 3,454 bean seed samples, 1,036 legume forages and tropical grasses seed samples from GRU, and 829 samples of bean seeds and 93 of tropical grasses and legumes from GD-01 (Bean Germplasm Improvement) and IP5 (Tropical grasses and legumes) projects, respectively.

Materials and Methods

Phytosanitary inspections are carried out in multiplication plots (field and glass-houses) of Popayán, Tenerife, Quilichao and CIAT Palmira. Accesions are tested in the GHL using accepted methodologies to identify seed-borne pathogens as fungi, bacteria and viruses according with the pathogens recorded in the seed production areas (Annual Report 1997). The procedures used in GHL have been described in Annual Report of 1999.

Testing for some genera of seed borne 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. Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli,* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp., in tropical pastures) are tested. The GHL 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 the pathogenicity biological test. Testing for seed borne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and or seedling-symptom test.

Results

Beans (*Phaseolus* spp.)

Seed samples of 3,454 accesions of beans (3,400 of *Phaseolus vulgaris* and 54 of *P. lunatus*) produced by GRU were tested, some of them with destination to foreign countries, and all others for conservation in the genebank. Their health status showed 77.6 % samples without pathogens of quarantine importance (Figure 27).

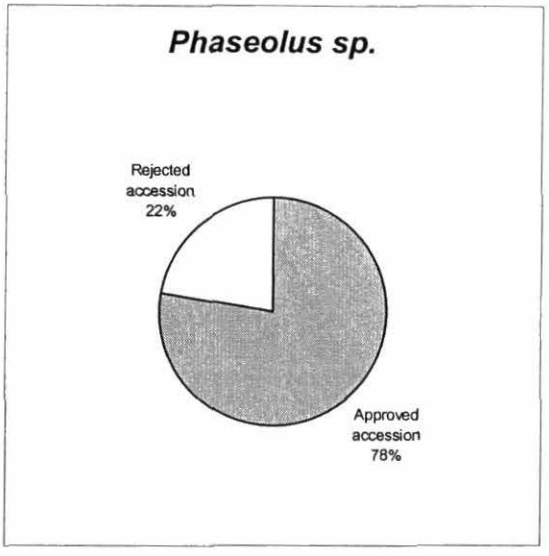


Figure 27. Number of *Phaseolus* sp. seed samples rejected or accepted after seed health analysis.

Samples with pathogens (22.4 %), considered as factors for rejection, showed in general low percentages of the fungi *Macrophomina phaseoli, Colletotrichum lindemuthianum, Phomopsis* sp., *Pestalotiopsis* sp., *Sclerotinia* sp., and *Rhizoctonia* spp. Seedborne viral infections by BCMV (Potyvirus) and Southern mosaic virus (SBMV) were also detected. It is important to note that BCMV (Potyvirus) was the quarantine pathogen detected with higher frequency alone and in mixed infections (Table 22).

Table 22. Factors for rejection of *Phaseolus* seed samples analyzed by GHL.

Factors	*Affected Samples*	
	Number	***Percentage***
Macrophomina	96	2.8
Macrophomina, Colletotrichum	1	0.03
Macrophomina, Rhizoctonia	2	0.06
Pestalotiopsis	3	0.09
Phomopsis	2	0.06
Rhizoctonia	3	0.09
Sclerotinia	3	0.09
Poty	503	14.50
SBMV	51	1.5
$Gram^+$ Bacteria	18	0.52
Pseudomonas	16	0.46
Xanthomonas	1	0.03
Poty, *Colletotrichum*	1	0.03
Poty, *Macrophomina*	21	0.62
Poty, *Phomopsis*	1	0.03
Poty, *Rhizoctonia*	2	0.06
Poty, SBMV	23	0.66
Poty, $Gram^+$ Bacteria	4	0.11
Poty, *Pseudomonas*	5	0.15
Poty, *Xanthomonas*	1	0.03
SBMV, *Macrophomina*	6	0.18
Poty, *Macrophomina,* SBMV	3	0.09
Poty, *Macrophomina, Pseudomonas, SBMV*	1	0.03
Poty, SBMV, Weevils (Bruchides)	1	0.03
Weevils (Bruchidae)	5	0.15
Total	773	22.4

The presence of Gram positive bacteria was checked and only one accession of *P. vulgaris* showed colonies of these kinds of bacteria after subculturing on YDCA, and incubation under high temperature (36-37°C). Their identification with complementary tests using a Gram-Positive ID Kit (Becton Dickinson BBL Crystal™, Nippon Becton Dickinson Company Ltd.) showed Coryneform plant bacteria, but not *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*.

About insects such as weevils (Bruchidae), these were detected in some seed samples at very low percentages (Table 22).

Tropical grasses and legumes

Seed samples of 1,036 of tropical grasses and legumes from GRU production, distributed as 1,014 accessions of 43 genera of tropical legumes pastures, 2 of Bignoniaceae and 20 of Poaceae of 5 genera were tested (Table 23).

Table 23. Number of analyzed seed samples of tropical grasses and legumes by GHL.

FAMILY	GENUS	# Analysed Accesion	Accepted	Rejected
POACEAE	Setaria	4	1	3
	Axonopus	1	0	1
	Paspalum	1	0	1
	Brachiaria	13	1	12
	Eragrostis	1	1	0
FABACEAE	Abrus	2	2	0
	Senna	3	2	1
	Vigna	44	27	17
	Acacia	2	2	0
	Lablab	1	1	0
	Mimosa	1	1	0
	Mucuna	1	0	1
	Uraria	6	4	2
	Zornia	65	49	16
	Cajanus	6	4	2
	Dioclea	18	3	15
	Christia	1	1	0
	Clitoria	8	4	4
	Cratylia	1	1	0
	Dolichos	1	1	0
	Dunbaria	1	1	0
	Galactia	12	10	2
	Leucaena	7	6	1
	Pueraria	2	1	1
	Sesbania	4	4	0
	Tadehagi	4	2	2
	Teramnus	7	6	1
	Canavalia	42	28	14
	Desmodium	153	117	36
	Flemingia	9	8	1
	Tephrosia	11	9	2
	Calliandra	2	2	0
	Centrosema	117	98	19
	Crotalaria	35	25	10
	Desmanthus	8	5	3
	Dysolobium	1	1	0
	Indigofera	1	1	0

FAMILY	GENUS	# Analysed Accesion	Accepted	Rejected
	Neonotonia	1	0	1
	Phyllodium	2	2	0
	Pycnospora	3	3	0
	Rhynchosia	4	3	1
	Alysicarpus	20	18	2
	Aeschynomene	65	52	13
	Calopogonium	19	16	3
	Chamaecrista	5	3	2
	Macroptilium	38	36	2
	Psophocarpus	1	1	0
	Stylosanthes	280	238	42
BIGNONIACEAE	*Crescentia*	1	1	0
	Parmentiera	1	1	0
Total		1036	803	233

The health status showed 77.5 % of seed samples of legume and tropical grasses without pathogens of quarantine importance. In rejected samples we detected some seedborne fungi of quarantine importance (*Colletotrichum* spp., *Drechslera* spp., *Macrophoma* sp., *Macrophomina* sp., *Pestalotia* spp., *Phoma* spp, *Phomopsis* spp., *Rhizoctonia* sp.), and a very low frequency of Poty virus, and SBMV on legumes. Bacteria (*Xanthomonas* spp.) were detected only in legumes at very low frequency, also a Coryneform Gram positive bacteria (Table 24).

Table 24. Factors for rejection of legume and tropical grasses seed samples analysed by GHL.

Rejected Factors	*Affected Samples*	
	Number	***Percentage***
Colletotrichum	26	2.51
Colletotrichum, Rhizoctonia	1	0.10
Macrophomina	1	0.10
Macrophoma	20	1.93
Macrophoma, Phomosis	1	0.10
Macrophoma, Poty	4	0.38
Macrophoma, Phoma	1	0.10
Macrophoma, Phomosis	4	0.38
Drechslera	11	1.06
Drechslera, Phoma	6	0.6
Pestalotia	4	0.38
Pestalotia, Phoma	1	0.10
Pestalotia, Poty	1	0.10
Phoma	13	1.25
Phoma, Phomosis	1	0.10
Poma, Poty	3	0.29
Phomopsis	20	1.93
Phomosis, Macrophoma	1	0.10
Phomosis, Macrophoma, Poty	2	0.19
Phomosis, Phoma	2	0.19
Phomosis, Poty	3	0.29
Phomosis, SBMV	1	0.10
Poty	71	6.85
Gram^{+} Bacteria	3	0.29
Poty, *Colletotrichum*	2	0.19
Poty, SBMV	2	0.19
Rhizoctonia	6	0.6
Rhizoctonia, Phomosis	1	0.1
Rhizoctonia, Poty	1	0.1
SBMV	20	1.90
Total	233	22.5 %

The results of phytosanitary status of seed samples analysed during the period showed that the methods for disease control under field production conditions were effective, especially with tropical pasture legumes. About grasses, namely of *Brachiaria*, it is necessary to refine the procedures of seed production under field conditions, with a research program to ensure that the germplasm is free of seed borne pathogens.

Service of germplasm health certification for other CIAT projects

Seed samples from GD-01 and IP-5 projects were analyzed (Table 25). In *Phaseolus vulgaris* 75 % of samples did not show pathogens of quarantine importance. In the rejected samples *Macrophomina phaseoli* was the fungus detected with higher frequency. We also detected the bacteria *X. campestris* pv *phaseoli* at low frequency, and bean common mosaic virus (BCMV)

and SBMV in intermediate frequency. In tropical pastures germplasm near 80% of seed samples of *Brachiaria* spp. showed the presence of the seed borne fungi *Drechslera* spp. and *Phoma* spp. In legumes such as *Lablab* spp., we detecteted *Macrophomina* spp. in low percentages.

Table 25. Number of analyzed samples of beans, tropical grasses and legumes for germplasm sent abroad, for Projects GD-01 and IP-5.

Specie	Samples number	Project
Phaseolus vulgaris	658	GD-01 SB
Phaseolus vulgaris	171	GD-01 MB
Subtotal	829	
Arachis spp	1	IP-5
Desmodium spp	5	IP-5
Brachiaria spp	21	IP-5
Calliandra spp	5	IP-5
Canavalia spp	6	IP-5
Centrosema spp.	2	IP-5
Cratylia spp.	7	IP-5
Lablab spp.	11	IP-5
Leucaena spp	3	IP-5
Mucuna spp.	7	IP-5
Pueraria spp	1	IP-5
Stylosanthes spp	1	IP-5
Vigna spp	23	IP-5
Subtotal	93	

Contributors: B. Pineda, M.S. Balcazar

Output 2.2. Germplasm, passport and characterization data available to users

Activity 2.2.1. Design of the computerized system of GRU for quality control, flow monitoring and web consultation.

Introduction

The information system implemented at the GRU is in continuing evolution; therefore it presents periodically additions, changes and improvements. The most recent one is the development of an internal web portal. The intention is to bring in the future some extra functionality; for now it offers the ease of search and uploads of all available images for bean, cassava and tropical forages, by only giving the accession number; this work facilitates control of seed flows, which are displayed with passport information.

At the same time, in 2005 we worked towards the development of a completely new web portal available to everyone, inside and outside CIAT. In this portal, we tried to increase easiness for seed search and germplasm request of bean, cassava and tropical forages; we also add some new functionalities such as news and access to GRU publications and internal documents.

Another important improvement achieved in 2005 was the introduction of bar code labels in the field, for characterization/ evaluation purposes. Our next step is to include more areas, like the printing of labels in the field in order to label the bags at harvest.

Materials and Methods

In order to make the changes in the internal information system of GRU we used the tool called Developer 2000 from Oracle. To generate reports and statistics, we used the Oracle Discoverer tool. Java language program was used to build GRU's Web portal, and Tomcat Application Server is used to keep it online.

For bar coding, we are using Zebra printers (S600, TLP 2742 and TLP2742z) and right now we are acquiring a Zebra TLP 2844z. For work in the field, we are using PSION WorkAbout PRO handhelds with a laser barcode reader and Printek MT 300 printers. This equipment was acquired because of its modularity, portability, ease of use, and link with Java capabilities.

Results

The internal information system has been updated with new information and reports, a lot of new images have been added to be used with the new internal Web portal, helping to a fast identification of the material and avoiding confusions during seed multiplication processes.

The new Web portal is almost ready in English and Spanish versions with its new functionalities, as well as news, list of publications, documents and training opportunities. One of the improvements on this new approach is the effectiveness of new search parameters and the visualization and downloading of the search results, also a new fast way to make requests in just a few clicks.

The introduction of bar coding has been done gradually as proofs progress with very good results so far. The acquisition of the new field equipment let us to speed up our development process and also test our improvements in the methods of field evaluation/ characterization. The next step includes the possibility to read and print bar code labels in the field, and thus a considerable amount of errors will be avoided. Once this goal has been reached, we intend to include the bar code technology in the pre-drying, viability testing and health processes.

Contributor: G.E. Rueda

Activity 2.2.2. Distribution of germplasm from designate collections to end-users

Achievement: 8,480 accessions of the three commodity FAO designate collections distributed to germplasm users.

As it can be seen in Tables 26 and 27 and Figures 28 to 33, a total of 8,480 accessions were distributed, through 335 requests honoured in 2005 for cassava, bean and forages. The main recipients were CGIAR Centers with 4,729 accessions (3,483 to CIAT recipients and 1,246 to others CGIAR Centers). NARS and Universities were another important recipients. Pending on recipient type, the main purposes of the requests were agronomy, breeding, applied and basic research.

Table 26. Distribution of germplasm during 2005, by purpose

	Beans		Forages		Cassava	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	24	865	1	2	9	83
Agronomy	10	1,111	141	327	24	435
Applied research	9	849	16	189	10	47
Basic research	42	1,483	7	125	29	2,639
Training	1	30	7	32	--	--
Other	1	231	2	26	2	6
Total	87	4,569	174	701	74	3,210

Table 27. Distribution of germplasm during 2005, by kind of institution

	Beans		Forages		Cassava	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	42	1,818	17	139	50	2,762
Commercial companies	--	--	22	35	3	150
Farmers	--	--	106	119	--	--
Gene banks	1	39	--	--	--	--
NARS	14	1,936	4	63	11	203
NGOs	1	24	5	11	2	5
Regional Organizations	--	--	--	--	4	68
Universities	24	752	20	334	4	22
Total	87	4,569	174	701	74	3,210

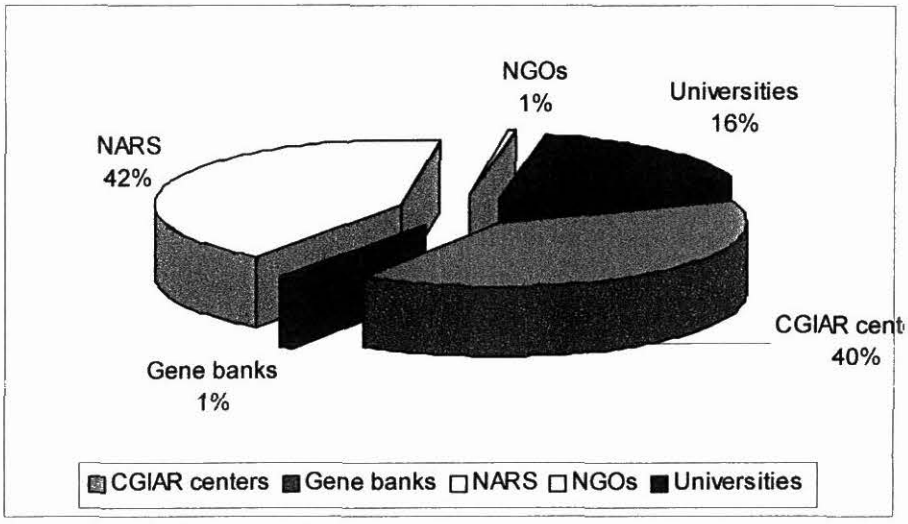


Figure 28. Distribution of bean seed germplasm by users

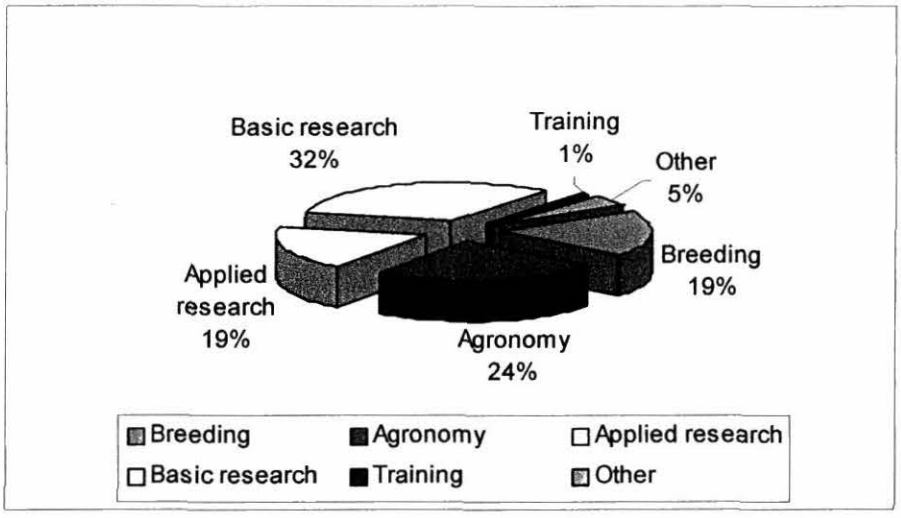


Figure 29. Distribution of bean seed germplasm by purposes

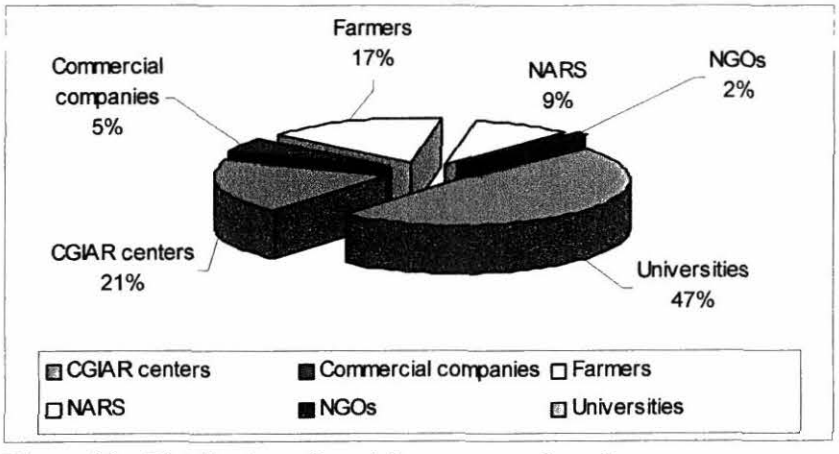


Figure 30. Distribution of seed forage germplasm by users

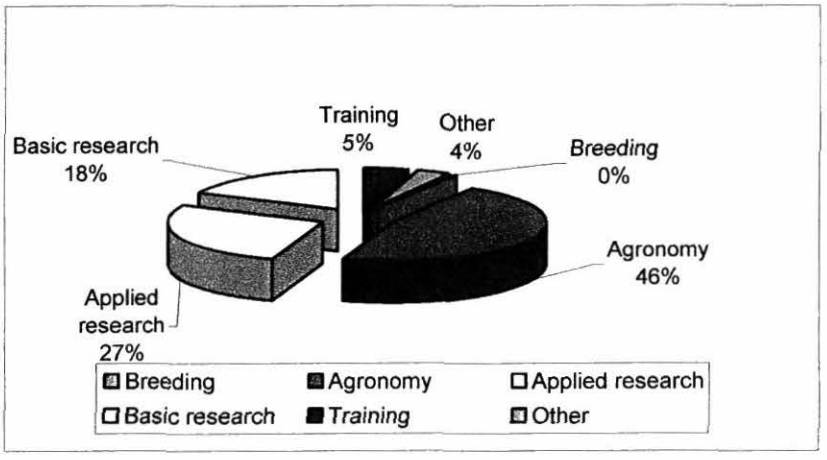


Figure 31. Distribution of seed forage germplasm by purposes

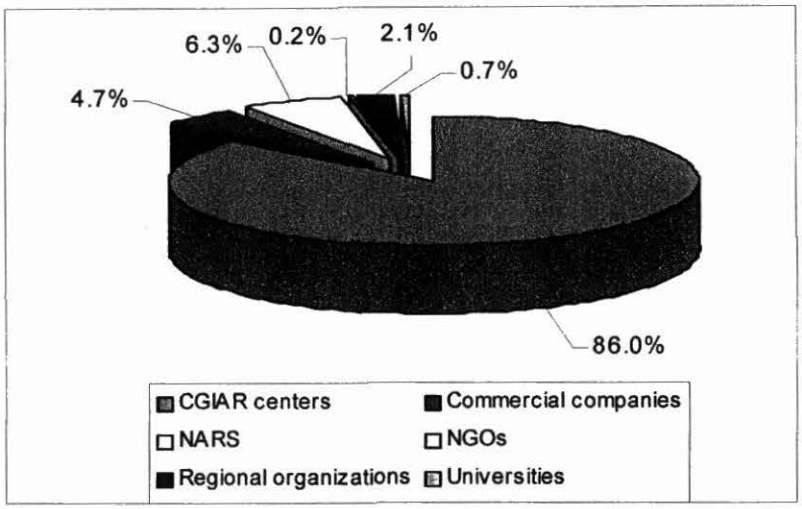


Figure 32. Distribution of *in vitro* cassava germplasm by users

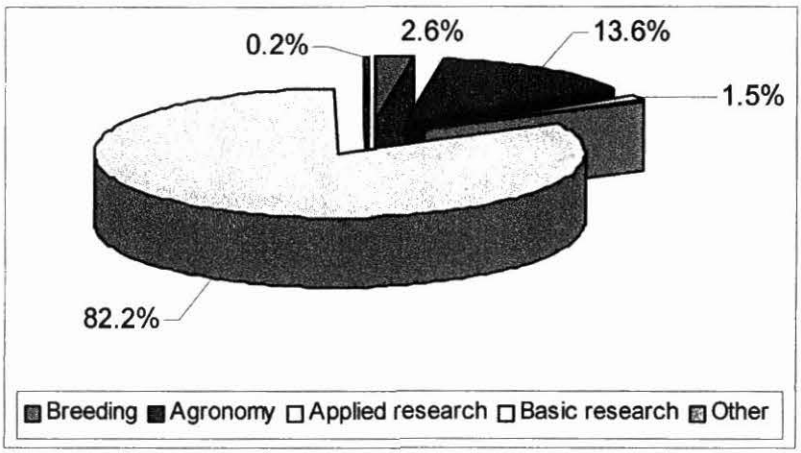


Figure 33. Distribution of *in vitro* cassava germplasm by purposes

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

Activity 2.2.3. Checking the validity of names of forages and other wild species through a reference herbarium

A total of 315 specimens of tropical forages and wild beans were added to CIAT Herbarium in 2005 (Table 28). While accessions are multiplied in the field, voucher specimens were taken for taxonomic research purposes. Donation of duplicated herbarium specimens was done towards several institutions. One holotype and 14 isotypes of the new species *Phaseolus novoleonensis* Debouck were sent to several herbaria (IBUG, ANSM, BR, COL, ENCB, F, G, K, MEXU, MICH, MO, P, SI, UC, US) in order to follow the rules for publication of new species.

Table 28. Specimens of tropical forages and wild beans added to CIAT Herbarium in 2005

	Number of species	Number of accessions
Wild beans	5	5
Legumes	83	280
Grasses	10	30
Total	98	315

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

Output 2.3. National collections restored to NARS

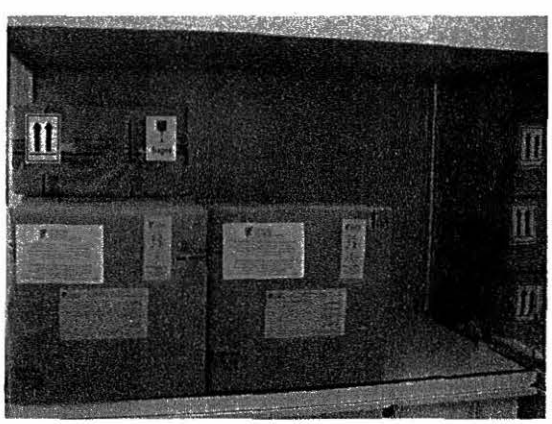
During 2005 GRU did not receive any request for the restoration of germplasm collections.

Output 2.4. FAO designated collections safely duplicated

Activity 2.4.1 Shipment of germplasm collections for security backups

A letter of agreement was signed by CIAT and CIMMYT (Sept 24, 2004) regarding to maintaining a safe duplicate of the FAO designate seed collections of beans and forages. Two shipments were sent to CIMMYT in December 2004. A total of 8,284 accessions (6,025 beans and 2,259 forages) in 57 boxes arrived in excellent conditions. In 2005 3,411 accessions of bean (1,836) and forages (1,575) were sent for continuing the safety duplication at CIMMYT (11,695 accessions to date, or 21% of the designated seed collections).

A letter of agreement was signed by CIAT and CIP (Jan 10, 2005) regarding to maintaining a safe duplicate of the FAO designate collections of cassava and sweet potato. In 2005 we have shipped to CIP 1,184 accessions of the *in vitro* cassava collection (19,9% of the designated *Manihot* collection), and we have received 240 accessions of sweet potato *in vitro* sent by CIP. A total of 3,000 additional accessions of cassava are ready to be sent in 2006 (Figure 34).



In vitro cassava

In vitro sweet potato

Figure 34. Security backups of *in vitro* cassava and sweet potato.

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

Output 2.5. Refined core collections

During 2005 GRU did not carry out special activity for this output, apart from some morphoagronomic and biochemical characterization; such a characterization will be used later on for refining the core collections of beans and selected forages.

Output 2.6. Improved disease indexing techniques

Activity 2.6.1. Preliminary evaluation about antagonistic activities of some bacterial metabolites against seed-associated *Phoma* sp. on *Brachiaria brizantha* (Panicoidea, Poaceae)

Introduction

Phoma species are cosmopolitan in nature and are common plant pathogens. Some species as *Phoma sorghina* (telemorph of *Phaeosphaeria maydis*) are important fungi being reported as affecting the Gramineae. We have reported the presence of *Phoma* sp (probably *P. sorghina*) associated with seeds produced in Popayán, where multiplication and regeneration of *Brachiaria* spp. germplasm are carried out (Garcia & Pineda 2000; Garcia *et al*, 2001; CIAT, 2001; CIAT, 2002). Trials for chemical control of this fungus and other fungi species using fungicides showed low percentages of control disease (CIAT, 2001; CIAT, 2002; CIAT, 2003; CIAT, 2004). Since it is necessary produce *Brachiaria* spp seeds with good phytosanitary quality, GHL has realized some testing of biological control using some bacterial isolates (CIAT, 2004; Balcazar *et al*, 2003; Balcázar *et al*, 2005). The biological control using bacteria could be addressed through growth competence with the target fungi or through antagonistic effects of bacterial metabolites. Recently, GHL conducted some preliminary trials using filtrates of a Gram- negative (G3) bacteria isolated from *Brachiaria* spp. seeds, against *Phoma* sp. Results are as follows.

Materials and Methods

To assess the antagonistic potential of bacterial metabolites obtained from a Gram- negative (G3) bacteria isolated from *Brachiaria* spp. seeds, a preliminary trial was conducted. Isolates of bacteria were plated in Nutrient Agar and PDA culture media. They were streaked on culture media and incubated for 48 h at 28-30°C, then they were increased in Nutrient Broth Culture media under agitation on a Shaker; afterwards, the liquid phase was filtrated through 0.22µ Millipore membranes. The filtrate free of bacterial cells was finally stored in a sterile bottle. An isolate of *Phoma* sp. obtained from *Brachiaria* seed was increased in Petri dishes with PDA culture media. Petri dishes were incubated at 27^0 C for a week. After this period the mycelium was collected to obtain spores for its further use as control.

Seeds of the *B. brizantha* accessions 16290, 2664, and 16288 susceptible to *Phoma* were germinated inside 15 cm Petri dishes using humid paper towels. When the plantlets were ready 15-20 days after germination, they were used in the following treatments (Table 29), using 4 petri dishes each one with 25 plantlets.

Table 29. Treatments used in order to test the antagonistic effect of a bacterial filtrate from a Gram- negative (G3) bacteria isolated from *Brachiaria* spp. seeds.

Accession	Treatment
Brachiaria brizantha 16290	*Phoma* sp + Bacterial filtrate*
Brachiaria brizantha 16290	*Phoma* sp
Brachiaria brizantha 26641	*Phoma* sp + Bacterial filtrate
Brachiaria brizantha 26641	*Phoma* sp
Brachiaria brizantha 16288	*Phoma* sp + Bacterial filtrate
Brachiaria brizantha 16288	*Phoma* sp

*$5x10^6$ spores/mL + 5 mL of bacterial filtrate for each Petri dish (100mL /treatment)

Results

The results showed that there were low effects of the treatments if they were compared with the number of affected plantlets after inoculation with *Phoma* sp., without bacterial filtrates (Table 30). It is necessary to repeat the trials after improving the production of bacterial filtrates because there were evidences in other *in vitro* trials that the filtrates were effective against *Phoma* and other fungi species (Balcázar *et al* 2003; Balcázar *et al*, 2005; CIAT, 2004).

Table 30. Results obtained after some treatments used in order to establish the antagonistic effect of a bacterial filtrate from a Gram- negative (G3) bacteria isolated from *Brachiaria* spp. Seeds

Accession	Treatment	Petri dish number			
		1	2	3	4
Brachiaria brizantha 16290	*Phoma* sp + Bacterial filtrated*	3/25**	3/25	3/25	2/25
	Phoma sp	5/25	6/25	4/25	5/25
Brachiaria brizantha 26641	*Phoma* sp + Bacterial filtrated	4/25	2/25	2/25	0/25
	Phoma sp	7/25	6/25	4/25	4/25
Brachiaria brizantha 16288	*Phoma* sp + Bacterial filtrated	5/25	6/25	4/25	5/25
	Phoma sp	5/25	6/25	4/25	5/25

*25 mL for each Petri dish (100mL /treatment) ** Number of affected plantlets/ number of inoculated plantlets

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

Output 3.1. Designate Collections better characterized

Activity 3.1.1. Single sequence repeat (SSR) markers to confirm possible genetic duplicates in the cassava germplasm collection of Colombia.

Introduction

Ex situ conservation plays a major role in the preservation of genetic diversity of cassava. The collection held at CIAT *in vitro* is by far the largest in the world. A large percentage of duplicates was identified using passport, morphological and isozyme characterization (Ocampo *et al.* 1993; Jiménez, 1994; Sumarani *et al.* 2004). Such a redundancy makes the maintenance and management of the existing collection expensive, and slows down the introduction of new germplasm. CIAT has implemented a model to detect possible groups of genetic duplicates using morphological descriptors and aß-esterase isozyme patterns (Hershey *et al.* 1991). The combination of molecular markers with morphology/isozymes can give a high degree of confidence to identify duplicates (Ocampo *et al.* 1995). A research project is under way using DNA fingerprinting to confirm 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. In this study we have 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 we know the level of redundancy, to study the distribution of the resulting genetic diversity in the different agroecological zones of Colombia.

Materials and Methods

Plant material. This work has been initiated on the cassava germplasm collection of Colombia maintained at CIAT as FAO Designate Collection, consisting of 1,986 accessions (the largest collection by country). The *in vitro* Cassava Laboratory (GRU) provided the accessions for characterization according to morphological and isozymatic similarities. Priority has been given to groups including three and more accessions, resulting in more efficient procedure to identify duplicates.

Molecular Markers. One type of molecular markers that may be suitable for cassava germplasm characterization are the microsatellites (SSR). Microsatellites are considered more sensitive in detecting genotypic differences as compared to morphological and isozymatic descriptors. Microsatellites, like RFLPs, are considered codominant markers. Their high polymorphism makes microsatellites suitable markers in order to identify redundancies in the cassava world collection (Chavarriaga *et al.* 1998).

Results and Discussion

Four SSR markers [SSR59 (249), SSR105 (506), SSR100 (498), SSR109 (521)] showed identical patterns in 31 evaluated groups (94 accessions) (Table 31). Polymorphisms were displayed among different groups, but there are no differences among accessions within these groups. The mean number of alleles in the identical groups was 7. This level of polymorphism is low due to similarity in morpholgy among accessions within the same group. In addition, they have identical isozyme patterns.

In conclusion, the fact that these groups of possible genetic duplicates were confirmed by four SSR markers suggests that the model developed at CIAT to detect these duplicates is reliable. However, more SSR markers are necessary to confirm with high level of confidence these possible genetic duplicates.

Table 31. Possible genetic duplicates in the Colombian cassava collection showing identical fingerprints with a set of four SSR markers [SSR59 (249), SSR105 (506), SSR100 (498), SSR109 (521)].

Group No.	Accessions
1	COL 25 and 896
2	COL 45, COL 948C, COL 1008, COL 1431
3	COL 61, COL 978
4	COL 70, COL 78B
5	COL 76B, COL 912A, COL 927, COL 1962
6	COL 81, COL 647, COL 1067, COL 106, COL 1538
7	COL 93, COL 1044
8	COL 134, COL 138
9	COL 137, COL 140, COL 145
10	COL 207, COL 1485
11	COL 240, COL 281
12	COL 261, COL 547
13	COL 376, COL 380
14	COL 436, COL 2617
15	COL 437[a], COL 1934
16	COL 467, COL 1720
17	COL 942, COL 958, COL 1955
18	COL 1043, COL 1057, COL 1065
19	COL 1092, COL 1602, COL 1616, COL 1821
20	COL 2239, COL 1830, COL 1828A, COL 1518, COL 1516, COL 151
21	COL 1601, COL 1990,
22	COL 2282, COL 2297, COL 2375, COL 2390
23	COL 2286, COL 2292, COL 2298, COL 2300, COL 2313
24	COL 1672, COL 1673, COL 1678
25	COL 1711, COL 1764A, COL 1764B
26	COL 1772, COL 1777, COL 1781, COL 1895
27	COL 1786, COL 1879, COL 2023
28	COL 1889, COL 1893, COL 1894
29	COL1896, COL 1900, COL 2062
30	COL 1901, COL 1902, COL 1903
31	COL 2358, COL 2362, COL 2407

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Activity 3.1.2. Genetic diversity and redundancy level in the Colombian collection of avocado (*Persea americana* Mill.) (collaborative project with CORPOICA, funded by the Ministerio de Agricultura y Desarrollo Rural, Colombia).

Introduction

The cultivated avocado (*Persea americana* Mill.) belongs to the genus *Persea* (subgenus *Persea*) of the laurel family (Lauraceae). It is a sub-tropical fruit tree, diploid with 2n = 24 chromosomes and a moderate genome size of 883 Mb (8.83 x 10^8 bp). Genome analysis and breeding of avocado are 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).

This fruit was called by the Aztecs ahuacatl from the nahuatl language ahuacatl ("testicle"), referring to its shape. The avocado probably originated in a broad geographical area, stretching from the eastern and central highlands of Mexico through Guatemala to the Pacific coast of Central America (Costa Rica). However, it was cultivated from the Rio Grande to central Peru before the arrival of the Europeans (Bergh 1992). Based on their presumed center of origin, the species *P. americana* has been commonly divided into three distinguishable ecotypes or horticultural races, known as the Mexican, Guatemalan and West-Indian (Antillean) races and designated as *P. americana var. drymifolia, P. americana var. guatemalensis and P. americana var. americana,* respectively (Bergh & Ellstrand, 1986). Recently, a new race (*P. americana var. costaricensis*) native from the mountain region of Costa Rica, has been described (Ben-Ya'acov1 *et al.*, 2003). *P.americana* presents a wide variety of genetic diversity, probably because avocados evolved in a part of North America, Central America, Colombia and the Caribbean region itself characterized by considerable diversity in climates, in relation to the varied topography of these regions. The diversity of avocado germplasm has tremendous impact on the development of the avocado industry worldwide (Bufler & Ben-Ya'acov, 1992). The molecular markers promise to help overcome some 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 the isozymes, RFLPs (chloroplast DNA and ribosomal DNA) DNA fingerprints (DFP), RAPDs, microsatellites and AFLPs (Clegg *et al.* 1999).

In this study we have proposed the following objectives: (1) to know the extendt of genetic diversity present in an avocado collection maintained ex-situ, using molecular markers (AFLPs), and (2) to know the level of redundancy (possible genetic duplicates) present in the characterized collection.

Materials and Methods

Plant material consisted of 60 accessions of *Persea americana* Mill. maintained by CORPOICA in Palmira (Colombia). These accessions represent a wide geographical and botanical diversity from Colombia, Mexico, Guatemala and Trinidad and Tobago. In addition, two wild species of *Persea* were included as outgroup: *Persea caerulea* (young leaves collected on native trees from Valle del Cauca, Colombia) and *Persea rigens* (young leaves collected on native trees from Quindio, Colombia) (Table 32).

Table 32. Identification of avocado varieties and wild species of *Persea* used in AFLP analysis with indication of their horticultural races.

Accession	Horticultural races/wild species	Accession	Horticultural races/wild species
Persea caerulea	Wild species	Nativo 2011	Antillean
Persea rigens	Wild species	Booth 5	Hybrid GuatemalanxAntillean
Trappica (C)	Antillean	Hulumana	Antillean
Lorena (C)	Antillean	1607	Mexican
Trappica (PF)	Antillean	Bacon	Mexican
Lorena (PF)	Antillean	135 27	Hybrid GuatemalanxMexican
Oriente	Antillean	Collin Red	Hybrid GuatemalanxAntillean
Hass	Hybrid GuatemalanxMexican	Booth 7	Hybrid GuatemalanxAntillean
Jim	Hybrid GuatemalanxMexican	Waldin	Antillean
HX 48	Hybrid GuatemalanxMexican	Semil 44	Hybrid GuatemalanxAntillean
135 – 15	Hybrid GuatemalanxMexican	Monroe	Hybrid GuatemalanxAntillean
Papelillo	Antillean	135-21	Hybrid GuatemalanxMexican
Simonds	Antillean	Trapp	Antillean
Peterson	Antillean	Pollock	Antillean
Itzamna	Guatemalan	Booth 1	Hybrid GuatemalanxAntillean
Nabilico	Guatemalan	Lula	Guatemalan
Kanola	Guatemalan	G 755	Guatemalan
Tumaco	Antillean	Puebla	Mexican
Winslowson	Hybrid GuatemalanxAntillean	Gottfried	Mexican
Hayes	Hybrid GuatemalanxMexican	La selva	Hybrid GuatemalanxMexican
Fuerte	Mexican	Booth 8	Hybrid GuatemalanxAntillean
135 –20	Hybrid GuatemalanxMexican	Dr. Sardi	Antillean
Accession	Horticultural races/wild species	Accession	Horticultural races/wild species
Persea caerulea	Wild species	Nativo 2011	Antillean
Costa Rica	Hybrid GuatemalanxMexican	Zutano	Mexican
143 - 77	Hybrid GuatemalanxMexican	Ibague	Antillean
Trinidad	Hybrid GuatemalanxAntillean	Los Silos	Antillean
Choquette	Hybrid GuatemalanxAntillean	Gwent	Hybrid GuatemalanxMexican
Gripiña	Hybrid GuatemalanxAntillean	Marcus	Antillean
Mayapan	Guatemalan	Ruelhe	Antillean
Duke 7	Hybrid GuatemalanxMexican	Ecuatoriano	Mexican
Marzala	Antillean	Fairchild	Hybrid GuatemalanxAntillean
Oculta 1	Antillean		

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. The AFLP fingerprinting was performed basically as described in the protocol provided by Vos et al. 1995, with some additions and changes made in our Laboratory.

For the amplification a total of sixteen primer combinations EcoR1/Mse1 were selectively tested to identify at least four that can show polymorphism among accessions: E-AG/M-CAC, E-AG/M-CAA, E-AG/M-CAG, E-AG/M-CAT, E-AG/M-CTA, E-AG/M-CTC, E-AG/M-CTG, E-AG/M-CTT, E-AC/M-CAA, E-AC/M-CAC, E-AC/M-CAG, E-AC/M-CAT, E-AC/M-CTA, E-AC/M-CTC, E-AC/M-CTG, E-AC/M-CTT. As a result, the four most polymorphic primer combinations (E-AC/M-CAC, E-AC/M-CTC, E-AG/M-CTA, E-AG/M-CAT) produced clearly readable fragments, and overall reproducibility of the AFLP amplification patterns was good. These four primer combinations were selected for the subsequent analysis of the complete avocado collection. AFLP markers were manually scored as binary data with presence as "1" and absence as "0". Cluster analysis was performed on the similarity matrix employing the "unweighted pair group method using arithmetic means" (UPGMA) algorithm provided in the computer program NTSYSpc, version 2.11S (Exeter Software Co., New York).

Results and Discussion

Genetic diversity analysis. The four primer pairs used in this study (E-AC/M-CAC, E-AC/M-CTC, E-AG/M-CTA, E-AG/M-CAT) resulted polymorphic. These combinations have on average 43 polymorphic and 62 monomorphic fragments across the germplasm of avocado (Fig. 35).

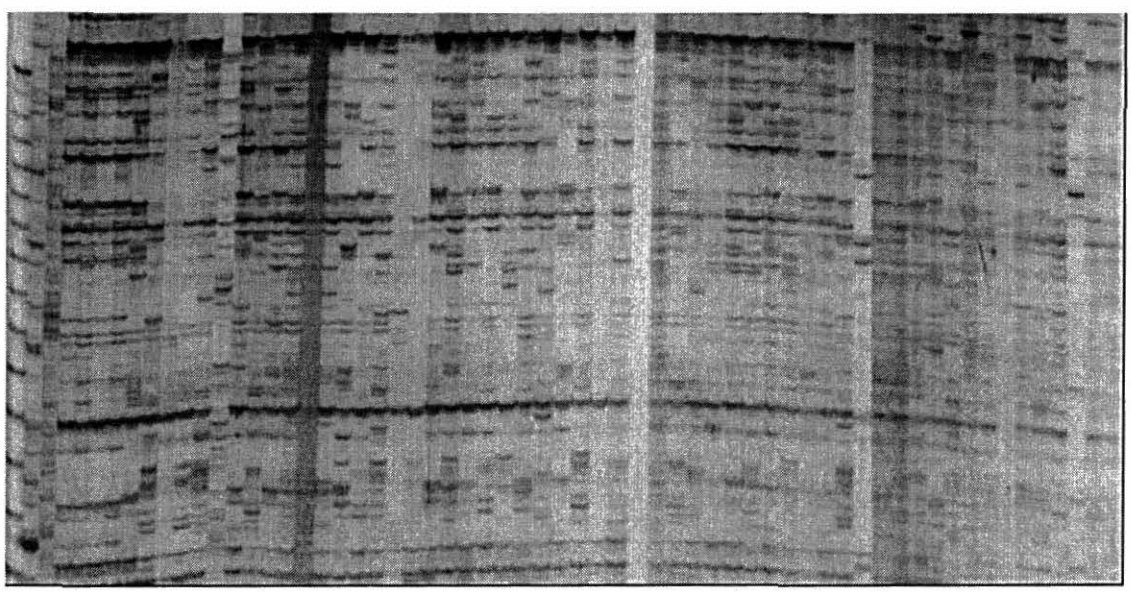

Figure. 35 Polymorphisms detected by AFLP with primer combination E-AC/M-CAC between avocado varieties and two wild *Persea* species (*P. caerulea* and *P. rigens*).

The UPGMA dendrogram obtained using the primer combination E-AC/M-CAC scored in all genotypes, fails only to separate two accessions, identifying 97 % of studied genotypes. This confirms the usefulness of this type of marker for genotype identification (Fig. 36). Though no AFLP primer pair was capable to identify all studied genotypes, by combining data from at least two or more AFLP primer pairs, each genotype could be characterized by specific band patterns and is therefore certainly identified. These results agree with those obtained by Fiedler *et al.*

(1998) using RAPD markers. In order to investigate the relationships among the different avocado accessions, a dendrogram based on AFLP data was built and showed that the accessions could be grouped according to the three known horticultural races of avocado. This way, possible ecological groups were adequately represented in the dendrogram according to their putative origin, since Antillean plus hybrid GuatemalanxAntillean, Guatemalan plus hybrid GuatemalanxMexican and Mexican accessions were positioned in the different groups. The dendrogram based on AFLP data however failed in grouping two putative Guatemalan (PT37 and Linda) and one Antillean accession (Oculta), which grouped independently. This dendrogram based on AFLP data supported the idea that Guatemalan and Antillean races are more closely related one another than either of them is to the Mexican race, although a larger sample of Mexican ecotypes should be studied to confirm this hypothesis. These results are concordant with those obtained by Fiedler *et al.* (1998) and Ramirez *et al.* (2004).

Possible genetic duplicates. Among the 60 characterized accessions, two genotypes (Lorena and Trappica) were grouped together: the banding patterns were identical both in terms of number of bands and position. This was also confirmed by analyzing the Similarity Index and plotting a dendrogram showing the genetic distance (Fig. 36). This identical grouping was present in the four primers combinations used in this study. They represent a possible genetic duplicate. The data from at least the AFLP primer pair (E-AC/M-CAC) show that each genotype could be characterized by specific banding patterns and therefore is certainly identified, with the exception of the previous duplicated genotypes (Lorena and Trappica).

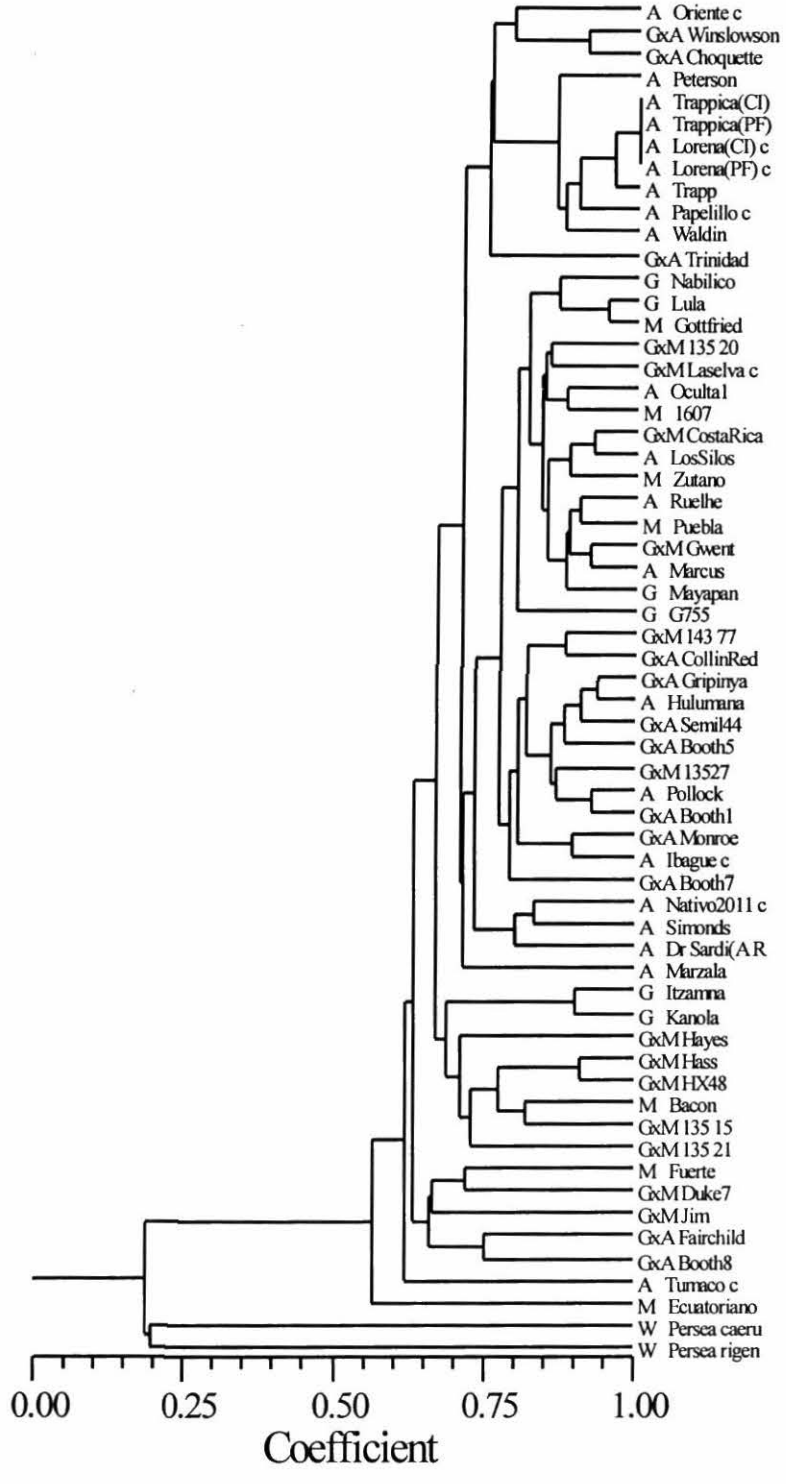

Figure 36. Dendrogram of 57 accessions of *Persea americana* Mill, and one *P. caerulea* and *P. rigens* generated by UPGMA clustering analysis based on AFLP data (primer combination E-AC/M-CAC). The race or the species to which belongs each of the analyzed materials is indicated to the left w: wild species, A: Antillean race, G: Guatemalan race, M: Mexican, GxM: Hybrid GuatemalanxMexican, and GxA: Hybrid GuatemalanxAntillean.

Several conclusions derived from this study: (1) a high degree of variability can be detected in the Colombian avocado collection maintained at CORPOICA using AFLP markers, confirming the usefulness of this type of marker for genotype identification, (2) 97 % of the studied accessions are genotypically unique, (3) there is a possible redundancy level of only 3 % in the characterized collection, and (4) the cultivars could cluster inside racial groups in such a way that the genetic data confirm the ecological and/ or botanical designation.

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Activity 3.1.3. Three new phaseolin types found in populations of *Phaseolus vulgaris* L. collected in its primary center of diversity.

Introduction

Phaseolin (Phs), the major seed stotage protein of common bean, has proved to be an excellent, cheap and polymorphic marker in evolutionary studies (Gepts, 1988). Although this globulin has a narrow range of molecular weights (45-52 kD) and isoelectric points (5.6-5.8) (Brown *et al.* 1981), up to now several types and subvariants of phaseolin have been found in wild, landraces and improved bean genotypes (Gepts & Bliss, 1986; Gepts *et al.*, 1986; Koenig *et al.*, 1990; Debouck *et al.*, 1993; Tohme *et al.*, 1995; Beebe *et al.*, 1997). The present report indicates several Phs types not reported previously, and establishes standards for Phs morphotypes which are available internationally as genetic stocks (Ocampo & Toro, 2005).

Materials and Methods

The accessions reported here were obtained from the worldwide collection held in CIAT (Table 33). The samples were analyzed in 1D-SDS-PAGE (Brown *et al.* 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975).

Results and Discussion

New phaseolin diversity found in beans from Mesoamerica. Using 1D/2D-IEF-SDS-PAGE, two new phaseolins were identified in wild and cultivated accessions from Mesoamerica (Figure 38). The first phaseolin was found only in G11027, a wild population from Mexico, in the state of Durango. We suggest that this pattern be designated as "Durango" pattern (abbreviated "Dur") making reference to the name of the Mexican state where it was found. The second new phaseolin type was first observed among wild accessions from Honduras (G50722B) and Costa Rica (G51062A). However, it was later found in several cultivated accessions (for example G18970) from the Costa Rican locality of Telire (Table 33). This is the first time that the Mesoamerican cultivars are reported to have a phaseolin type other than the "S", "CH" and "B" types (Gepts and Bliss, 1986; Koenig et al., 1990). Accordingly we suggest that this phaseolin be designated as "Telire" ("Tel").

A novel simple phaseolin found in beans from Colombia. Using 1D/2D-IEF-SDS-PAGE, a simple phaseolin type was observed for the first time and exclusively in a Colombian cultivar (G24674) (Figure 38). Along with the previous result, we suggest that this phaseolin be designated as "Quincho" ("Qui") making reference to the name of the cultivar where it was found. This study confirms again that germplasm from Middle America and Colombia has the greatest amount of phaseolin types (Gepts & Bliss, 1986; Gepts *et al.*, 1986; Koenig *et al.*, 1990; Beebe *et al.*, 1997).

Table 33. The molecular weight (MW) of bands in 1D-SDS-PAGE and isoelectric focusing (IEF) of peptides within each band are described for the new phaseolin types.

Phaseolin Types	Biological population			1D-SDS-PAGE (bands)		2D-IEF-SDS-PAGE (peptides)	
	Identification	Status	Gen. St[A]	Number	MW (kD)	Number	Total
Durango (Dur)	G11027	Wild	Mexdu-01	2	58.94	5	10
					56.00	5	
Quincho (Qui)	G24674	Cult.	Fi-4421	2	56.00	6	11
					53.20	5	
Telire (Tel)	G18970	Cult.	Fi-5791	2	54.12	5	9
	G51062A	Wild	Fi-5765		50.55	4	
	G50722B	Wild	Fi-3925				

A: The accessions which are reported here as the source of each Phs morphotype will be maintained in CIAT as genetic stocks.

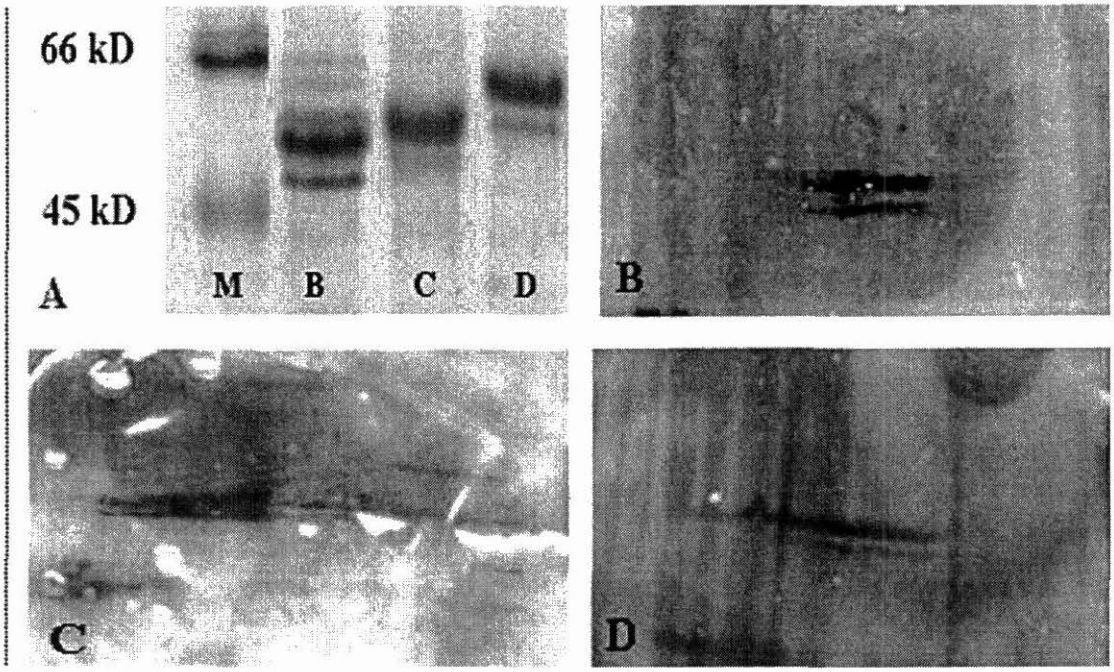

Figure 37. 1D-SDS-PAGE (A) and 2D-IEF-SDS-PAGE (B-D) electrophoresis of newly found phaseolins. A: molecular weight marker (M) with its value of kilo-Daltons (kD), B: "Tel" phaseolin type, C: "Qui" phaseolin type, D: "Dur" phaseolin type.

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Contributors: C. H. Ocampo and O. Toro

Activity 3.1.4. Two novel globulin variants found in the wild teparies beans (*Phaseolus acutifolius* A. Gray)

Introduction

In teparies, the major seed storage protein is called globulin and has similar chemical characteristics (molecular weight, isoelectric points, serological cross-reactivity, etc.) as the phaseolin of common bean (*Phaseolus vulgaris* L.) (Sathe *et al.*, 1994). However the sequence homology between its coding genes has not yet been verified. Schinkel and Gepts (1988) report fourteen different patterns among wild forms, whereas only one pattern was identified in the cultivars. Another study contributed by Toro and Debouck (1989) increases the number of reported patterns to 25; 23 patterns were identified among wild teparies and two patterns in the domesticated form. Later Florez (1996) confirms these results and increases the number of patterns found to 27 (25 patterns among wild teparies and two patterns among the domesticated form). We are here reporting two novel globulin types found in wild populations of the tepary bean (Ocampo & Toro, 2005).

Materials and Methods

We made a further screening of the seed storage protein (globulin) variability, focusing on the material that was not worked out by the previous investigators: we analyzed 68 accessions (50 wild and 18 cultivated) of *P. acutifolius* from the worldwide collection held in CIAT. This variation was first analyzed using one-dimensional SDS/PAGE electrophoresis (Brown *et al.* 1981) and later confirmed in two-dimensional IEF-SDS-PAGE electrophoresis (O'Farrel, 1975).

Results and Discussion

Finding new globulin variants. Two new patterns were observed among wild populations of *var. acutifolius* collected in Mexico. The first pattern (XXVIII) was found in G40103, a population collected in Sinaloa by D. G. Debouck, which showed previously the pattern XVII (Schinkel and Gepts, 1988), and the second pattern (XXIX) was found in the population G40298 (collected in Guerrero by Robert Reid). These patterns can be identified by one-dimensional electrophoresis parameters (Table 34): the pattern XXVIII by the presence of a pair of equally strained bands and with an average molecular weight (42.52 and 46.22 kD). The second pattern (XXIX) reveals a stronger band with a molecular of 44.70 kD. These patterns also can be observed in two-dimensional electrophoresis (Figure 38), using the isoelectric focus parameter. A single band in 1D-SDS-PAGE is often composed of peptides with slight differences in molecular weight but with some overlaping, creating a smear of peptides, which can be observed in 2D-IEF-SDS/PAGE. The IEF peptides within each band are presented for both patterns in Table 34. These results confirm the presence of two new globulin types for teparies beans. In addition the fact that these new globulin patterns have been found only in wild material confirms the previously described trend of a strong reduction in genetic diversity in the cultivated form of *P. acutifolius* Gray.

Table 34. Description of new patterns: the molecular weight (MW) of bands in 1D-SDS-PAGE and isoelectric focusing (IEF) of peptides within each band are presented for the globulins XXVIII and XXIX.

Pattern	Populations	1D-SDS-PAGE (bands)		2D-IEF-SDS-PAGE (peptides)	
		Number	MW (kD)	Number	Total
XXVIII	G40103	2	42.52	4	8
			46.22	4	
XXIX	G40298	1	44.70	4	4

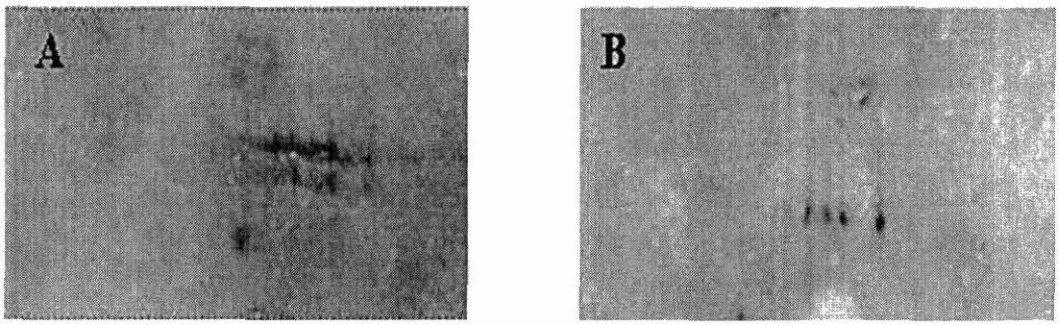

Figure 38. Two-dimensional IEF-SDS-PAGE electrophoresis of newly found globulins. A, pattern XXVIII (G40103) and B, pattern XXIX (G40298).

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Contributors: C. H. Ocampo and O. Toro

Activity 3.1.5. Biochemical characterization of the *Phaseolus vulgaris* L. germplasm collection held at CIAT were analyzed for seed storage proteins (phaseolin) using ID-SDS-PAGE electrophoresis. This step together with morphoagronomic characterization is a requisite for improving the representativeness of the designate collection.

Output 3.4. Unique genes better sampled and characterized

Activity 3.4.1. Use of the different phaseolin types for broadening the genetic base of crops: the case of the interspecific hybridization between common and tepary beans (service operation for the Biotechnology Unit).

Through this collaborative activity with the Biotechnology Unit we are trying to transfer multiple desirable traits from selected genotypes of tepary bean into lines elite of common bean. To assess introgression of tepary alleles in the common bean, the parents and its offspring were analyzed for seed storage protein of common bean (phaseolin), using the 1D-SDS-PAGE technique. This year, 130 individuals of offsprings have been analyzed. Additional studies on introgression of tepary DNA fragments were carried out using molecular markers.

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Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

The staff of the Genetic Resources Unit joined with Universidad Nacional de Colombia, Palmira headquarters, to support the Master of Science in Genetic Resources Conservation, giving theoretical and practical instruction on 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: J. Salcedo, G. Mafla, R. Escobar, B. Pineda, D.G. Debouck

Output 4.2. Conferences in national/ international for a

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 a calendar in collaboration with the Red Nacional de Jardines Botánicos de Colombia and Cali's Zoo. The rationale was to show the importance of *ex situ* conservation efforts to better know the characteristics of individual species and genotypes, in view of their survival and utilization. This perspective is shared among the three *ex situ* conservationists. In order to improve legibility, the calendar was split in two for each semester, stressing the message of connectivity and interdependence.

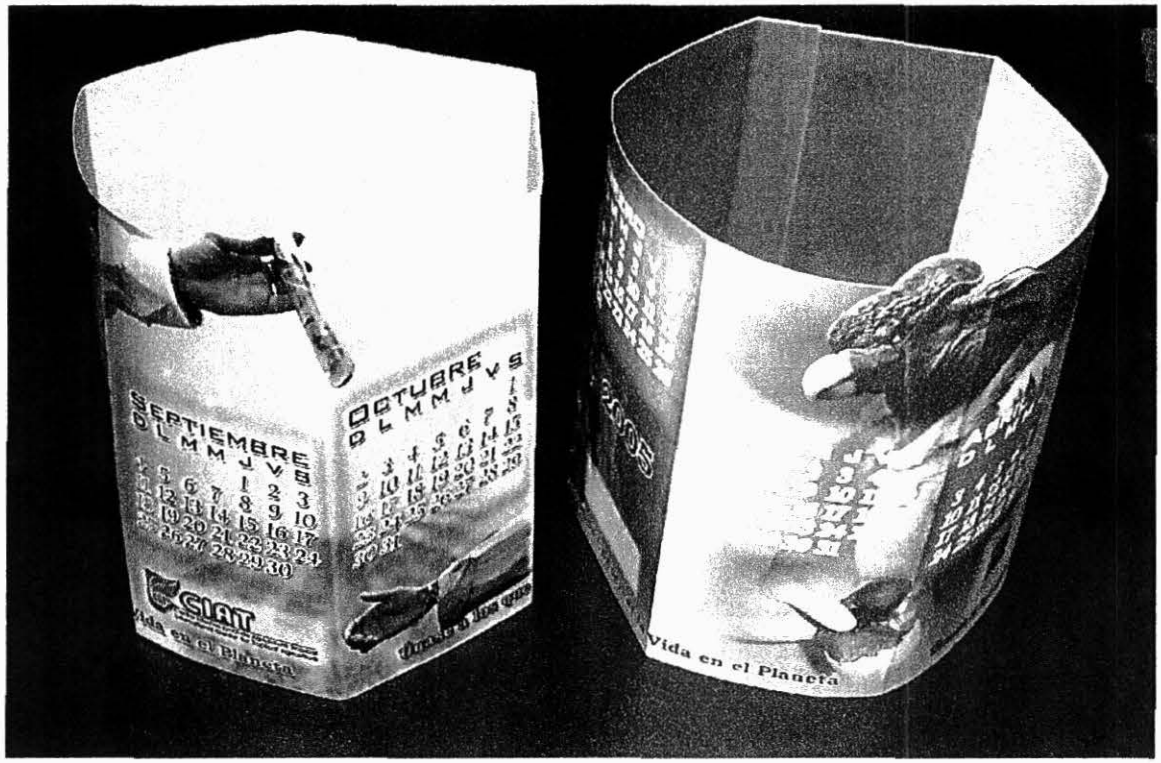

Figure 39. Calendar 2005

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

Output 5.1. 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). An output of this work is the taking of digital images of vouchers and to make them available 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: BR, CR, CICY, ENCB, K, INB, M and USJ. 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, 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

Output 5.2. Studies of gene flow with help of biochemical and molecular markers (special project supported by BMZ, Germany)

Activity 5.2.1. Determination of gene flow among bean species (*Phaseolus* ssp.) from Colombia and Costa Rica using microsatellites markers.

After using microsatellite markers to successfully establish gene flow events in weedy forms of common bean, we were interested in testing the hypothesis of participation of alien species in such a gene flow. We tested six rare forms possibly resulting from interspecific hybridizations in natural conditions of Colombia (with *P. dumosus*) and Costa Rica (with *P. costaricensis* and *P. dumosus*) and the species controls (*P. costaricensis, P. coccineus, P. dumosus, P. albescens, P. vulgaris*) (Table 35). We tested microsatellites screened at 68 loci to evaluate the level of participation of nuclear genes in this natural event (Gaitán Solís et al. 2001). The atypical materials were initially selected as potential interspecific hybrids due to their phenotypic characteristics like shrivelled seed, infertility and other genetic abnormalities associated to the plant growing and common in artificial man-made interspecific hybrids (Figure 40).

Table 35. Identification of *Phaseolus* ssp. materials used in the evaluation with microsatellites.

CIAT Identification	Species	Country	Department	Biological status
G24765 (Pop9072)	*P.* x *vulgaris*	Colombia	Boyacá	Weedy
G24666A (Pop9077)	*P.* x *vulgaris*	Colombia	Cundinamarca	Weedy
FI7031 (S34124)	*P.* x *vulgaris*	Costa Rica	Cartago	Weedy
FI7033 (S34124)	*P.* x *vulgaris*	Costa Rica	Cartago	Weedy
FI7034 (S34124)	*P.* x *vulgaris*	Costa Rica	Cartago	Weedy
FI7035 (S34124)	*P.* x *vulgaris*	Costa Rica	Cartago	Weedy
S29699	*P. costaricensis*	Costa Rica	San José	Wild
G36285 (Coc-1718)	*P. coccineus*	Guatemala	Quezaltenango	Wild
G36290 (Coc-1440)	*P. dumosus*	Colombia	Caldas	Cultivated
PL3592	*P. albescens*	Mexico	Jalisco	Wild
FI6846 (G23418)	*P. vulgaris*	Costa Rica	Cartago	Wild

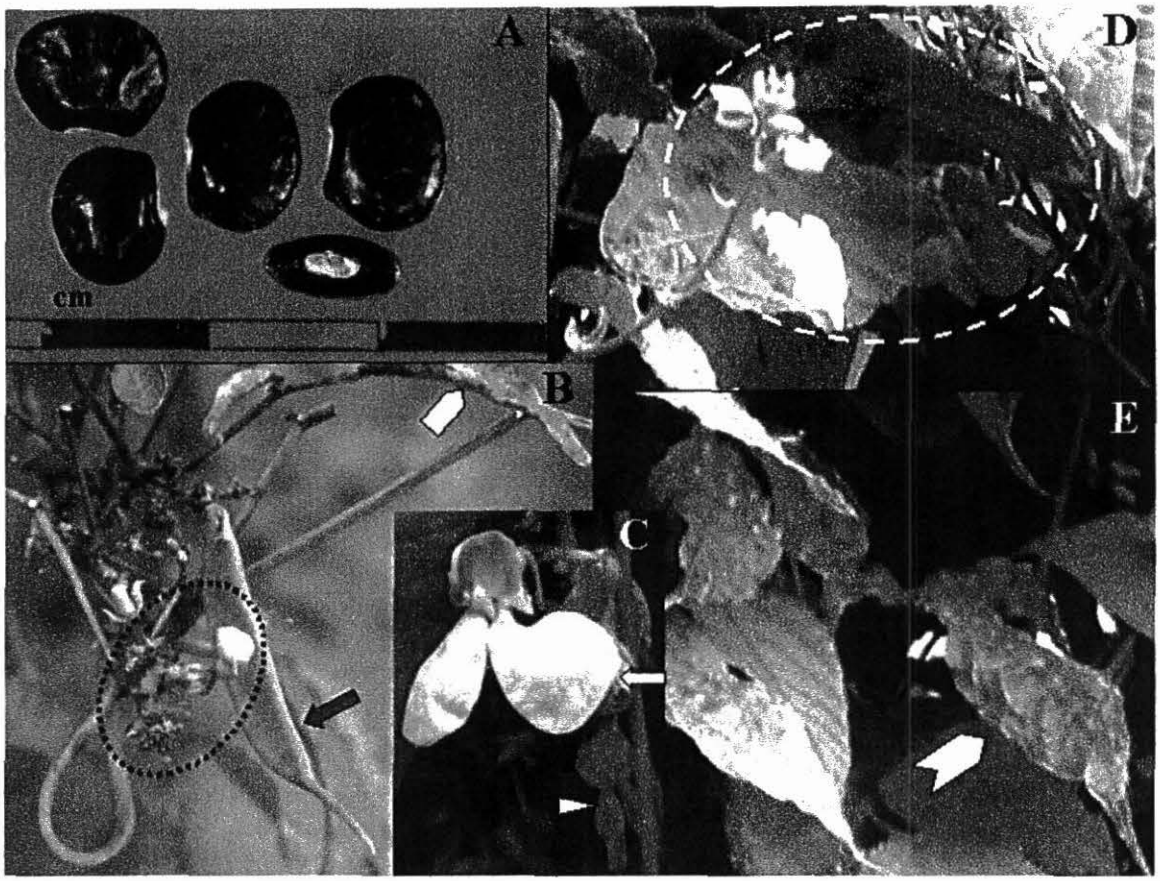

Figure 40. Phenotypic markers for natural interspecific hybrids.

A. Original seeds of individual FI7031
B. Individual FI7031 presents the *cripple* phenomenon and atypical floral buds.
C. Individual G24765 shows bigger size of bracteoles, flowers and the position of wings similar to those of *P. dumosus*.
D. Two different flower colors were expressed in G24666A material.
E. *Cripple* phenomenon in population G24666A.

The data analysis was carried out considering each band as an allele, using multiple correspondence analysis (MCA), also in order to understand the population structure and individual dispersion. The resulting graphic representation permitted to locate the materials according to their genetic similarity in a multidimensional plane using CORRESP module of NTSYS v. 2.10Y.

Results

The analysis of results was realized putting together specific descriptions using molecular markers and morphoagronomic characters of each species involved in possible gene flow events. These characteristics were displayed by hybrid individuals (Figure 40).

The evaluated microsatellites were of high discrimination power, so that each genotype was singled out in this evaluation, and this condition allows a detailed characterization of each species (Figure 41).

The control material of *P. costaricensis* was linked (using DICE coefficient) to the rest of individuals with a similarity coefficient of 16%; *P. coccineus* and *P. albescens* had 34%, *P. vulgaris* and *P. dumosus* grouped together with similarity coefficients of 55% and 60%, respectively (Figure 42). The molecular characterization allowed to establish that some alleles belong to the ancestral evolutive *phylum*, while others are characteristic of one species. The high differentiation obtained for the individuals studied with help of SSR permitted to attribute a gene flow event in the putative hybrids, and the identity of the species involved. The interspecific gene flow event among bean species has a low frequency and finding possible hybrids in the field is rare. The changes in the frequency of the alleles suggest generation differences; in other words, the studied individual hybrids might not belong to the same generation (Figure 43).

The results confirm that the *loci* evaluated using microsatellites could be employed for the differentiation and characterization of the different bean species according to Gaitán-Solís *et al.* (2002), including *P. costaricensis* and *P. albescens* evaluated here for the first time (Figure 41).

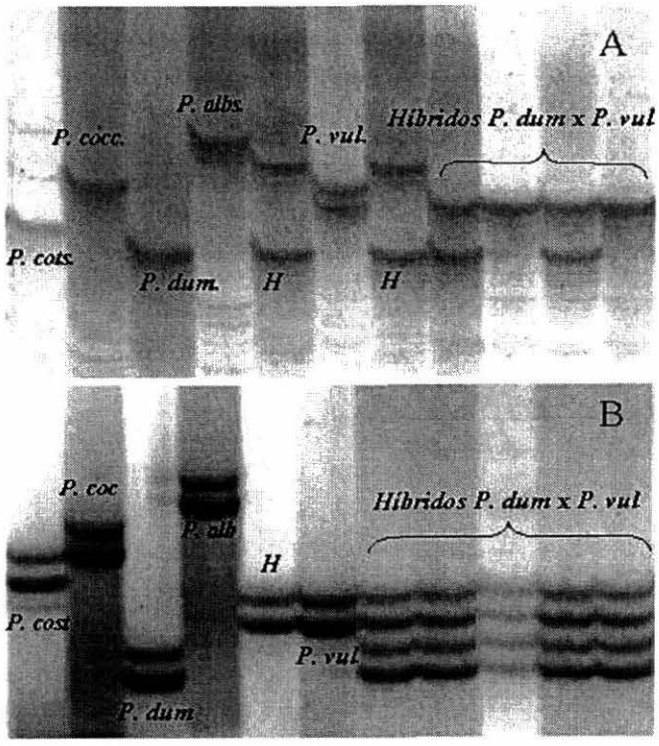

Figure 41. Specific microsatellites obtained to characterize each species and observed in the evaluated interspecific hybrids.

A. *Loci* of microsatellites GATS11 show different allelic forms in all species and the shared alleles in hybrids individuals between *P. vulgaris* and *P. dumosus*.
B. *Loci* of microsatellites BM181.

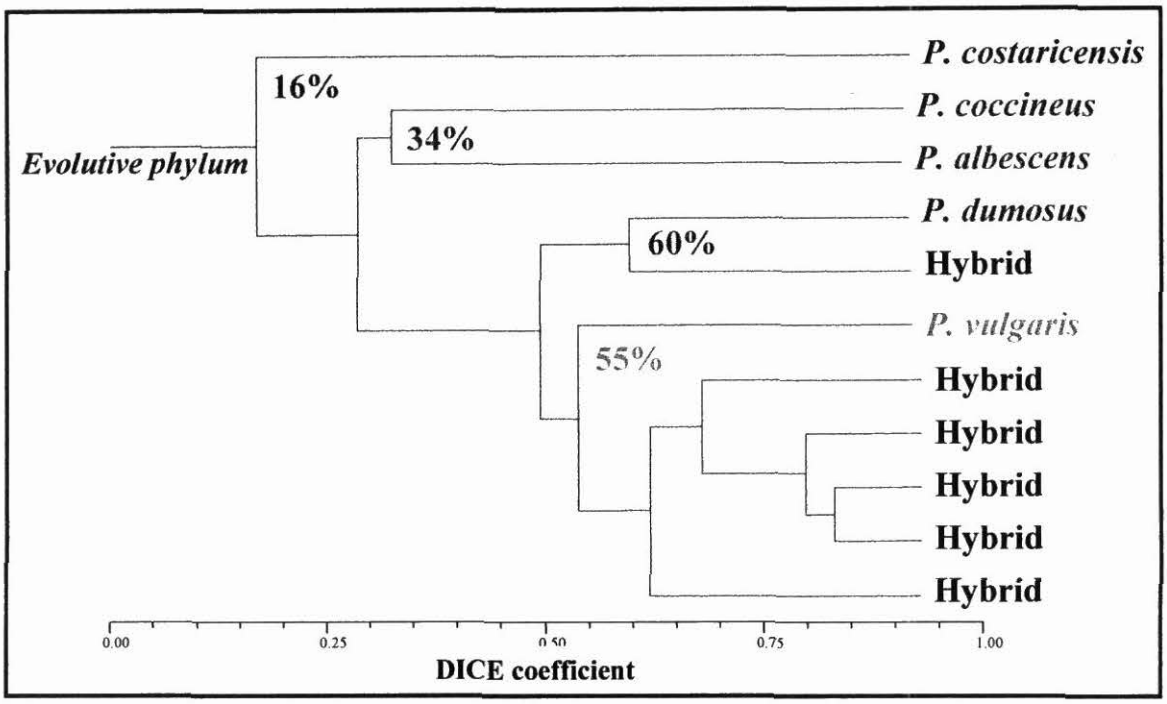

Figure 42. Cluster of evaluated individuals of their genetic similarity using coefficient of DICE.

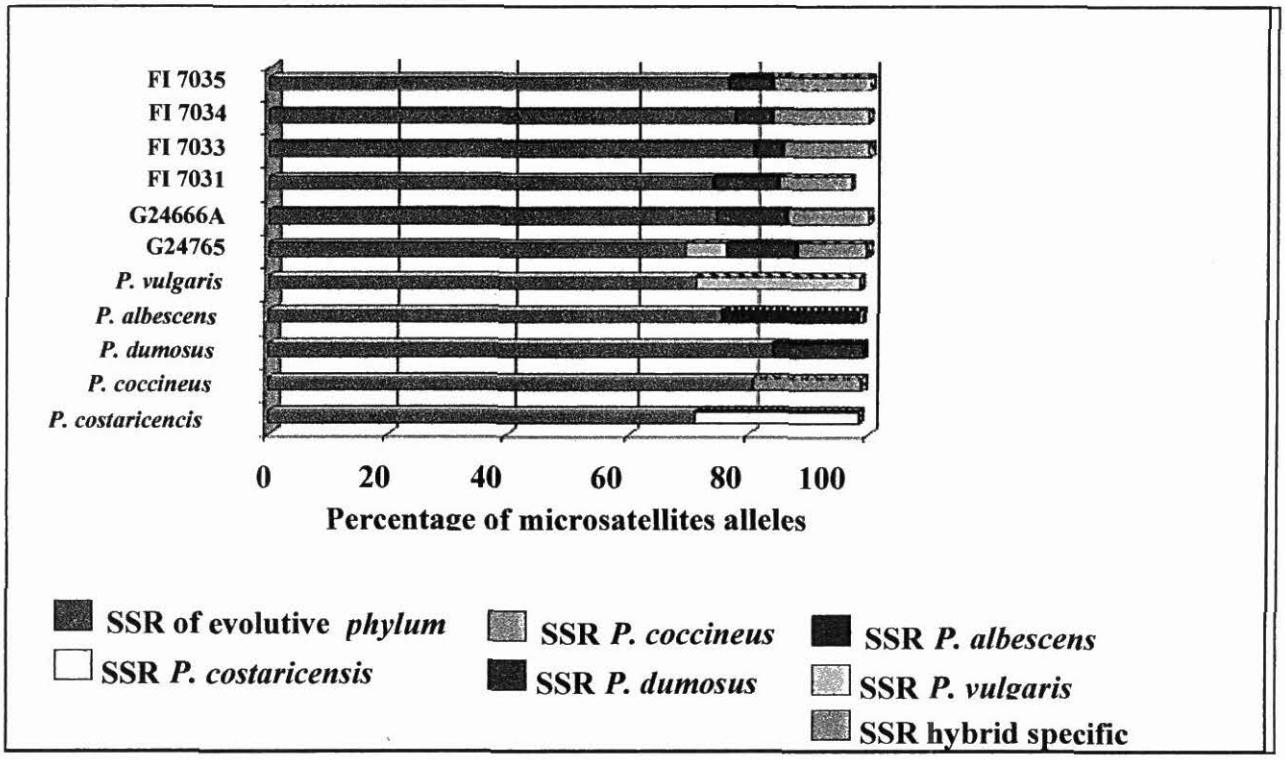

Figure 43. Graphic representation of microsatellites alleles found in the evaluated individuals.

The cluster realized with multiple correspondence analysis shows the structure of the hybrids (Figure 44). This group is spatially closed to *P. vulgaris* and *P. dumosus* suggesting that the SSR *loci* had been object of recombination by gene flow among these species. In that way, the nuclear genome of hybrids is sharing specific alleles of the evaluated species. Figure 44 displays *P. costaricensis* presenting a remote position in relation to all individuals. In an opposite position is *P. coccineus*, which has near values to *P. albescens* in the dim1 and dim2; however the last is completely contrastable in its dim3 value. Dim3 magnifies differences of *P. albescens* with regard to other individuals, as well as the dim1 increases the dissimilarity between *P. costaricensis* and the others. Dim2 separates *P. coccineus* and facilitates the discrimination of *P. albescens*. *P. vulgaris* and *P. dumosus* are closer in terms of dimensions 1 and 3 with a little divergence in dim2 (Figure 44).

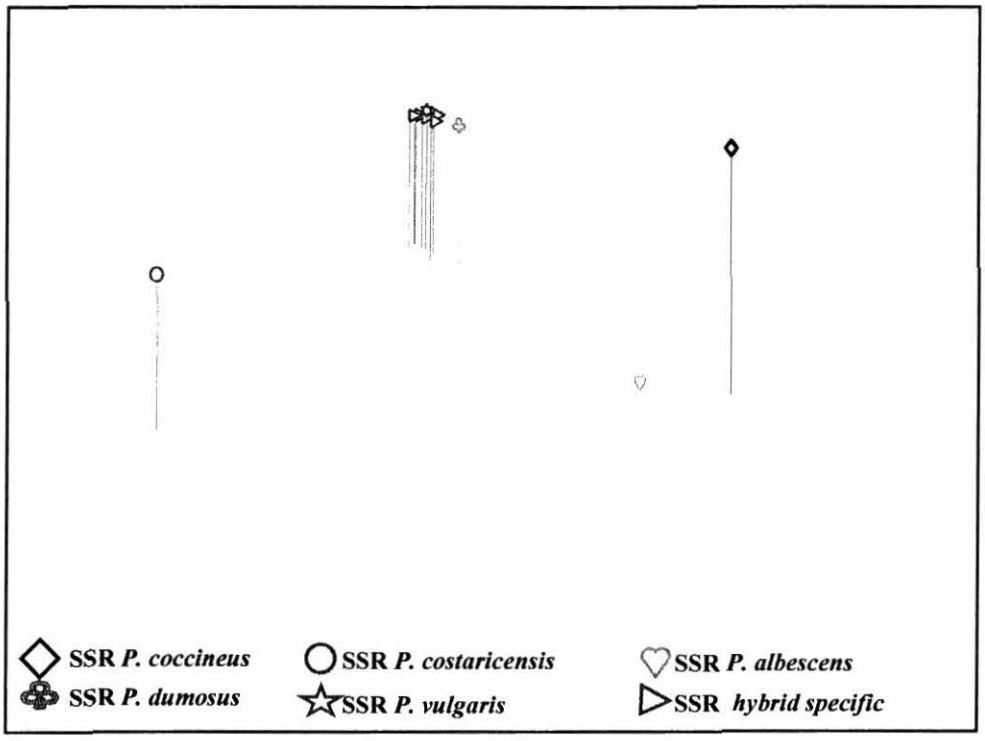

Figure 44. Spatial distribution generated by MCA analyses of hybrid individuals with respect to control species.

The hybrid group has been sharing spatial positions nearer to *P. vulgaris*, mainly, and with *P. dumosus*; the hybrid identified as G24666A is among them indicating a recent gene flow (Figure 45). The results indicate that the evaluated hybrids are indeed the result of gene flow events between common bean and its near species *P. dumosus* in the Central Valley of Costa Rica as well as in Boyacá, Colombia. In addition, this information implies that these species could be affected by outcrossing pollination in sympatric conditions. Besides, the direction of gene flow was mainly realized by pollen of *P. dumosus* as donor towards forms of *P. vulgaris* since the majority of specific alleles found in the hybrids were those of *P. vulgaris* as compared to the ones of *P. dumosus*.

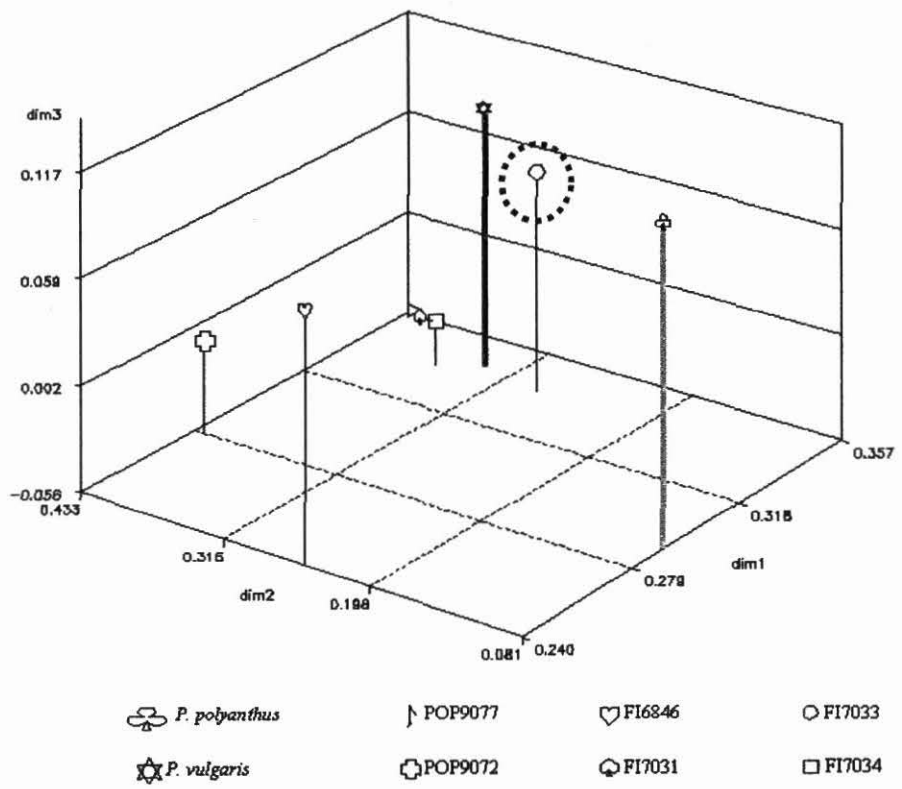

Figure 45. Spatial distribution generated by MCA analyses of hybrid identified as G24666A. This hybrid has been sharing spatial positions nearer to *P. vulgaris*, mainly, and with *P. dumosus*, indicating a recent gene flow.

References

Gaitán-Solís E, Duque MC, Edwards KJ & Tohme J. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris)*: isolation, characterization, and cross-species amplification in *Phaseolus ssp.* Crop Sci. 42: 2128-2136.

Contributors: R.I. González-Torres, D.G. Debouck, O. Toro, M.C. Duque.

Activity 5.2.2. Estimation of gene flow on common bean: the case of the Central Valley of Costa Rica

The genetic compatibility between crops and their wild relatives has important implications for the conservation of genetic diversity and for the introduction and management of transgenic crops. The gene flow event, distribution of receptor populations, gene transfer effectiveness, pollination activity and long-term effects (namely in fitness) are being studied in natural populations of common bean in a center of origin and genetic diversity.

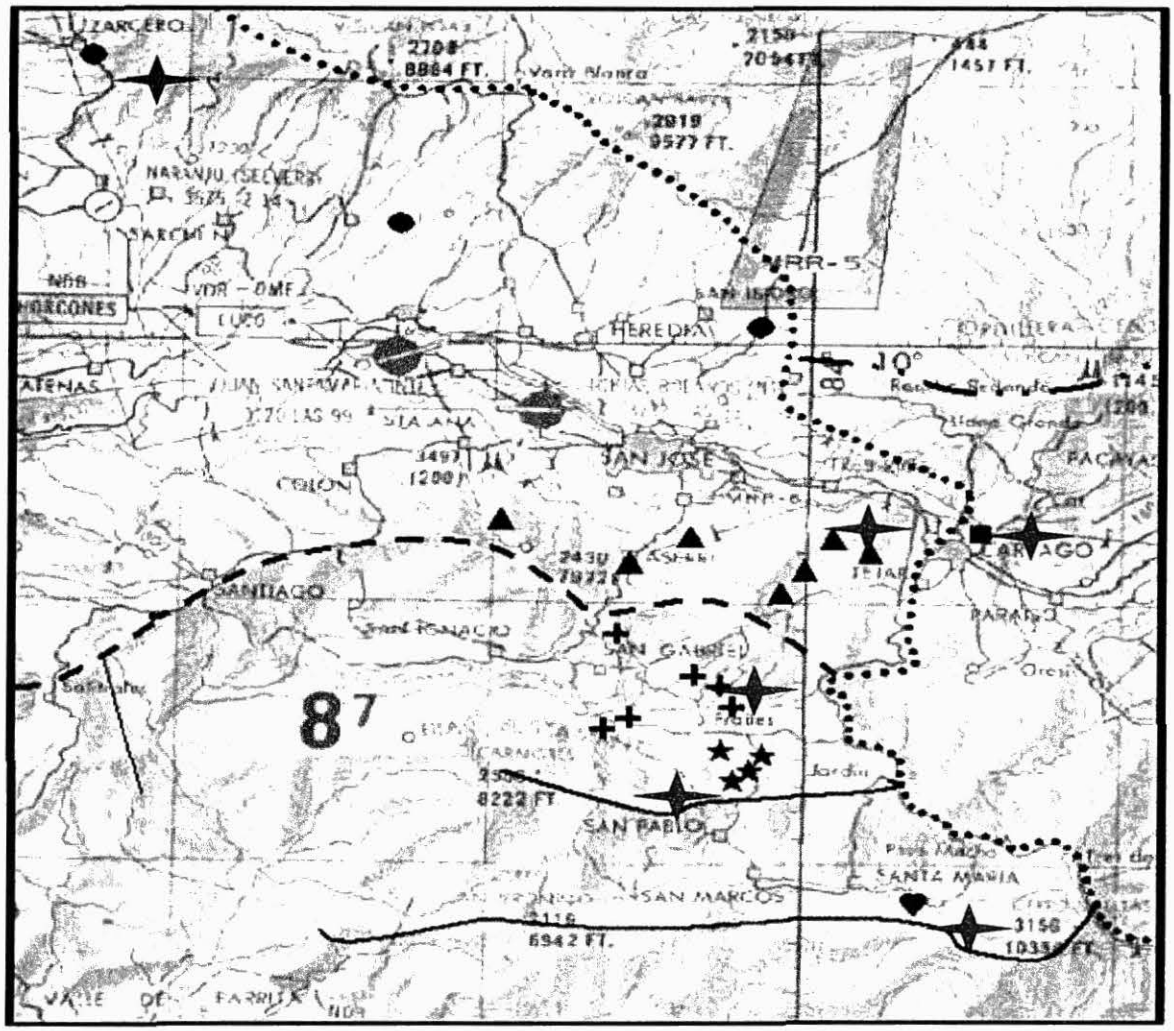

Figure 46. Distribution of wild common bean in the Central Valley of Costa Rica (base map: IGN-DGAC, 1991). Solid square: Reventazón, one population; solid heart: Pirrís, one population; closed circles: Virilla north, 3 populations; solid triangles: Virilla south, 7 populations; crosses: Candelaria north, 6 populations; and closed stars: Candelaria south, 4 populations. Dotted line represents the continental divide, while the other lines limit the different watersheds. Red closed stars: distribution of complexes of wild-weedy-cultivated.

We documented the geographical distribution of each wild population as well as the biological complexes of "wild-weedy-cultivated" materials (Figure 46). Twenty-two populations of wild common bean are known for Costa Rica, and distributed in four watersheds in the central part of that country representing at least 90% of the found populations (González Torres *et al.* 2004).

For the disclosure of wild populations, we applied a technique of ecogeographic surveying. For the molecular characterization, 1,232 individuals were selected based on a morphoagronomic evaluation, and 417 of them were weedy types possibly resulting from gene flow event. We used phaseolin, isozymes, and microsatellites as markers of nuclear genes. The gene flow direction was detected by RFLPs-PCR, sequencing and SNPs on chloroplast-DNA (Table 36).

Table 36. Morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter

Biological form	Seed average weight (g)	Phaseolin type	Isozymes		Microsatellites		Chloroplast haplotype
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 (2.5-7) N=1399	"S-4" "S" "M1" "S-3" N=907	DIA -1 N=229	PRX 100 N=197	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=316	160 80 164 110 165 147 138 122 224	H N=540
Weedy	13 (8-21.3) N=794	"C" *"CH"* "H" "S" *"X-7"* "S-4" N=548	DIA-1 *DIA-2* *DIA-4* N=157	PRX 100 *PRX 98* N=182	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=408	160, *177* 80 164, *185* 110 165, *189* 147, *150* 138, *148* 122, *136* 224, 243	*G*, H J, K, L, F N=669
Cultivated	23 (22-46) N=225	"S" "T" *"X-7"* "CH" N=205	*DIA -2* *DIA -4* N=64	*PRX 98* N=29	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=67	*180* 80 *183* 110 *189* *150* *148* *136* 243	*J, K, L* N=53

The results obtained in the characterization of the populations are summarized in Table 36. The red underline fonts refer to 'wild' characteristics and the green fonts are 'cultivated' characteristics. The blue fonts are specific characteristics found only in a biological status. The data analysis showed that 98% of the putative hybrids were indeed hybrid. The direction of gene flow was mainly wild pollen towards the cultivated materials (82%). However, the other direction was observed at lower frequency but as significant percentage. The gene flow was mostly in materials belonging to the Mesoamerican gene pool (the one prevailing in the area). However, outcrossing between Mesoamerican and Andean gene pools were evidenced in 8% of the weedy individuals (Figure 47).

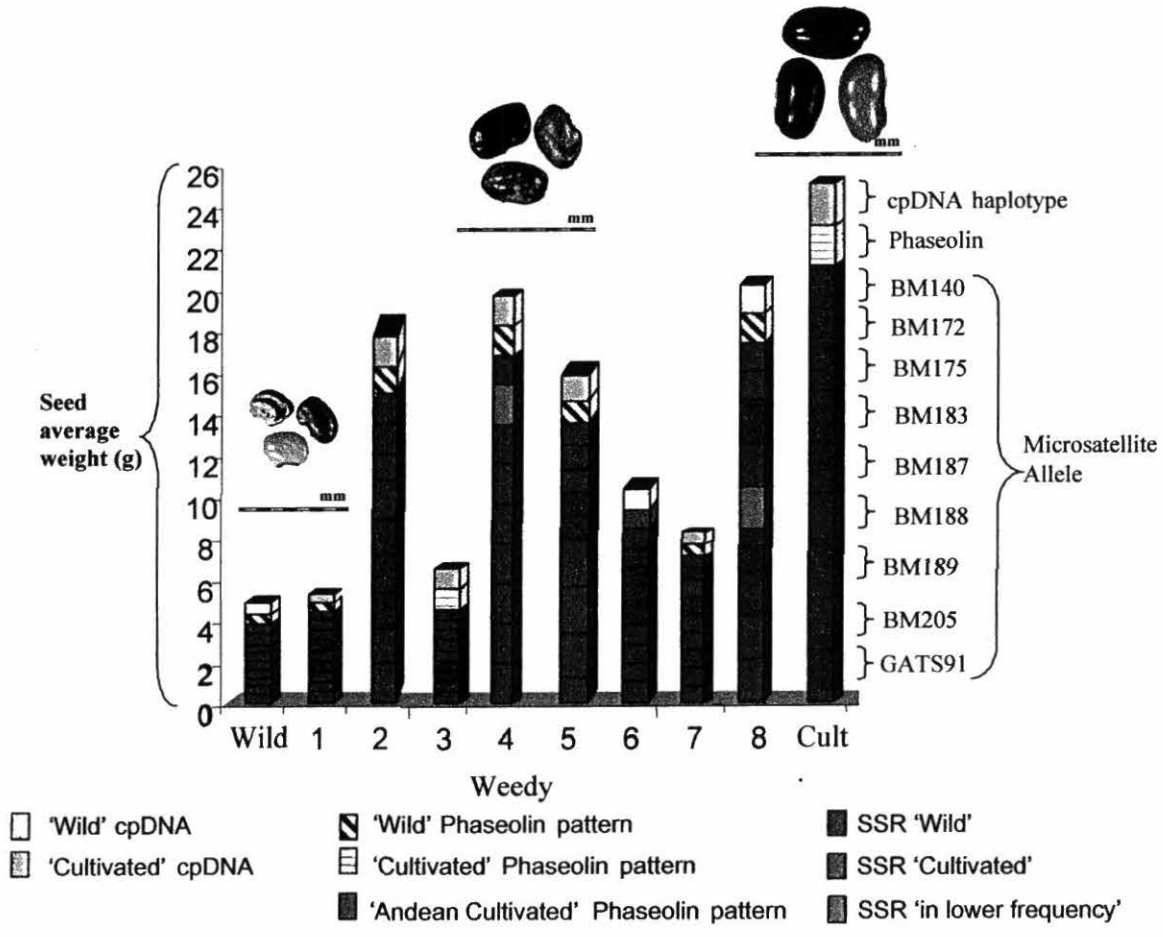

Figure 47. Graphic representation of markers used on a selection of individuals.

Figure 47 shows repeated events of gene flow of wild pollen towards cultivated forms (individuals 1 to 3). Individual 4 indicates events of repeated outcrossings resulting into a weedy form with a nuclear genome of a wild form with cytoplasmic genome of the cultivated (chloroplast capture); however, it keeps a high seed weight as in a cultivated type. Individual 6 illustrates a case of outcrossing between Andean and Mesoamerican genepools. It has a chloroplast haplotype as in the Mesoamerican genepool and an Andean phaseolin type. In contrast, its SSR alleles are 'wild' and 'cultivated'.

Our results provide an update about the distribution of wild common bean in Costa Rica, its ecology and conservation status. In addition, they allowed us to reliably establish the existence of simple or complex events of gene flow among different biological forms. Obviously, domestication has not yet altered the reproductive system of common bean up to prevent gene exchange between such forms. This in turn is also important for the production of certified seeds, or the management of genetic resources on-farm.

Contributors: R.I. González-Torres , D.G. Debouck, O. Toro, M.C. Duque.

Activity 5.2.3. Estimation of gene flow of complex "wild-weedy-cultivated" of common bean in its geographical range.

The complexes of forms "wild-weedy-cultivated" of common bean have been observed phenotypically in Oaxaca (México), El Progreso (Guatemala), San José (Costa Rica), Boyacá (Colombia), Azuay (Ecuador), Apurimac (Perú) and Tarija (Bolivia). The natural populations chosen from different countries and quantity of selected individuals are described in Table 37. The same methodology reported by González-Torres *et al.* (2003, 2004) was used to evaluate the participation of nuclear and chloroplast genomes in gene flow events on common bean in the range of geographical distribution.

Table 37. Identification of selected materials involved in the complex "wild-weedy-cultivated" from different countries.

Country	Province	**Biological Status**	**Gnumber**	**No. of selected individuals**
Bolivia	Tarija	Wild	G23445	71
		Weedy	G23445 G23446 G23447	408
		Cultivated	G23446 G23447 G23609 G24014 G24532	72
Argentina	Salta	Wild	S-512 S-513 S-514 S-515 S-515A S-516 S-517	7
		Weedy	G50015B G50015C	56
		Cultivated	G50015 G50015A G50015B G13995	105
Ecuador	*Azuay*	Wild	G23579 G23580	47
		Weedy	G23580	85
		Cultivated	G23580	50
Guatemala	El Progreso	Wild	G23434	26
		Weedy	G23434	77
		Cultivated	G23434 G3109	36
Perú	Apurimac	Wild	G21245 G23425	89
		Weedy	G23425	320
		Cultivated	G23425	90
Colombia	Choachi Salamina	Wild	G50879 G50983	90
		Weedy	G50879 G50983	148
		Cultivated	G50879 G50983 G51126	90

During 2005 2,849 seeds (334 seeds of 'wild' type; 1,377 seeds of 'landrace' type and 1,138 seeds of 'weedy' type) have been selected for planting in greenhouses, to obtain the phaseolin pattern and to realize the DNA extraction. In addition to this planting, aprox. 150 seeds of possible interspecific hybrids were germinated. To date half of all materials have been processed for phaseolin and molecular markers.

Contributors R.I. González-Torres, D.G. Debouck , O. Toro, A. Hernández, C. Córdoba.

Activity 5.2.4. Estimation of gene flow of complex "wild-weedy-cultivated" of common bean in Bolivia.

The evaluation of the complex of common bean from Bolivia using RFLP-PCR of cpDNA resulted in determining three different haplotypes as A, J, C for 233 individuals. The haplotypes A, J and C have been reported in wild and cultivated materials, respectively, from South America according to a methodology developed by Chacón (2001). This evaluation permitted a specific characterization of the each population depending on their biological status. The wild population shows only haplotype "A"; the cultivated materials exhibit hapotypes: "A", "J" and "C", and the weedy population displays shared haplotypes between wild and cultivated individuals (Table 38).

Table 38. Haplotypes of cpDNA identified in the complex "wild-weedy-cultivated" from Bolivia.

Biological Status	Haplotype	Haplotype frequency
Wild	A	15/15
Weedy	J	165/191
	C	14/191
	A	7/191
Cultivated	C	9/16
	J	5/16
	A	2/16

Contributors R.I. González-Torres, D.G. Debouck, M. Carvajal, O. Toro, M. C.Duque.

References

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.Bean Improvement Cooperative (USA) 46: 1-2.

6. Annexes

6.1. List of publications by Project Staff in 2005

A. In refereed journals:

Chacón M.I., Pickersgill B. & D.G. Debouck. 2005. Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. Theor. Appl. Genet 110 (3): 432-444.

Ocampo, C. H., Martín, J. P., Sánchez-Yélamo, M. D., Ortiz, J. M. & O. Toro. 2005. Tracing the origin of Spanish common bean (*Phaseolus vulgaris* L.) cultivars using biochemical and molecular markers. Genet. Resources & Crop Evol. 52: 33-40.

Segura, S. D., Coppens, G., Ocampo, C. H. & P. Ollitrault. 2005. Isozyme variation in Passiflora subgenus Tacsonia: geographic and interspecific differentiation among the three most common species. Genet. Resources & Crop Evol. 52: 455-463.

Torres González A.M. & C.M. Morton. 2005. Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae). Molec. Phylogenet. Evol. 37: 36-44.

B. In non-refereed Journals:

Ocampo, C.H. & O. Toro. 2005. New sources of phaseolin variation found in populations of *Phaseolus Vulgaris* L. collected in its primary center of diversity. Annu. Rept. Bean Improvement Coop. (USA) 48: 22-23.

Ocampo, C.H. & O. Toro. 2005. Two novel globulin variants found in the wild teparies beans (*Phaseolus acutifolius* A. Gray). Annu. Rept. Bean Improvement Coop. (USA) 48: 30-31.

Salcedo, J.M & D.G. Debouck. 2005 Comparison of stigma areas among wild and cultivated forms (landrace and modern cultivars) of *Phaseolus vulgaris* L. Annu. Rept. Bean Improvement Coop. (USA) 48: 34-35.

C. As Conference Proceedings:

Balcázar, M.S., Rivera, Á. L. & Pineda L., B. 2005. Actividad antagónica de bacterias aisladas de semillas de *Brachiaria y* asociadas con hongos de *Brachiaria.* In: Memoria XLV Congreso annual de la Sociedad Americana de Fitopatologia Division Caribe, VI Congreso nacional de Fitopatolgia y I Congreso Nacional de Fitoprotección. San José, Costa Rica, @7 Junio-1 De Julio de 2005. p. 89.

González-Torres, R.I., Araya-Villalobos R. & D.G. Debouck. 2005. Gene flow and its effect on biodiversity: common bean as model for future considerations of biosafety. Deutscher Tropentag 2005: The Global Food & Product Chain-Dynamics, Innovations, Conflicts, Strategies. October 11 – 13, 2005, University of Hohenheim, Stuttgart, Germany. p. 406.

Salcedo, J.M & D.G. Debouck. 2004. Comparación de areas en la region del estigma entre las formas silvestres y cultivadas (tradicionales y modernas) de *Phaseolus vulgaris* L. III Congreso Colombiano de Botánica. Noviembre 2004, Popayán, Colombia. p. 52.

6.2. List of thesis research supervised by Project Staff in 2005

Aranzales, E. 2005. Desarrollo de protocolos de conservación *in vitro* para especies de palma, usando la palma de chontaduro (*Bactris gasipaes* Kunth) como modelo. Engineer of Biotechnological Production. Universidad Francisco de Paula Santander, Cucuta, Colombia.

Martínez Castillo, J. 2005. Diversidad intraespecífica de *Phaseolus lunatus* L. e intensificación de la agricultura tradicional en la Península de Yucatán, México. Doctorado en Ciencias y Biotecnología de Plantas. Centro de Investigaciones Científicas de Yucatán, Mérida, Yuc., México. Final exam, April 2005.

6.3. List of conferences and scientific communications presented by Project Staff in 2005

Aranzales, E. San José de Cúcuta, Colombia, 10 Noviembre 2005, presentation during the VI Simposio de Biotecnología y el sector productivo y V Jornada biotecnológica: "Desarrollo de protocolos de conservación *in vitro* para especies de palma usando como modelo la palma de chontaduro (Bactris gasipaes Kunth)".

Debouck, D.G. Montevideo, Uruguay, 24 November 2005, invited conference in the V Simposio de Recursos Genéticos de América Latina y Caribe: "Cuantas veces vino el poroto a nuestra Terra Australis? Reflexiones sobre la formación de los Recursos Fitogenéticos".

Debouck, D.G. Chapingo, Mexico, 17 November 2005, invited conference at the Universidad Autónoma de Chapingo in an international workshop organized by SOMEFI: "Las plantas viajeras: sus sueños, sus oportunidades, y sus limites".

Debouck, D.G. Bogotá, Colombia, 2 September 2005, invited conference in the 1st Regional Workshop about Biopiracy in Latin America: "Observaciones sobre un frijol amarillo, o cuando un sistema de DPI extraviado llega a ser contraproduciente".

Debouck, D.G. San José, Costa Rica, 10 August 2005, invited conference: "Gallo pinto en horas de la mondialización: cómo añadir valor a la agrobiodiversidad?".

Debouck, D.G. Mérida, Mexico, 12 April 2005, invited seminar at the Centro de Investigaciones Científicas de Yucatán: "El fríjol peregrino: sus viajes desde el Pliocénico tardío".

Debouck, D.G. Lima, Peru, 29 March 2005, invited conference in the symposium to honour Dr Miguel Holle: "La importancia de lo no-planificado en la planificación de trabajos en recursos fitogenéticos: layos del zorro y pichupa numías para los tiempos de filogeografía y genómica".

Debouck, D.G. Popayán, Colombia, 8 November 2004, invited opening conference at the 3rd National Botanical Congress of Colombia: "Trenta mil años de tolerancia hacia ciertas plantas malezas en el Neotrópico".

González, R.I., Cali, Colombia, 13 October 2005. Presentation during the XL National Congress of Biological Sciences. "Determinación de flujo de genes entre especies de frijol (*Phaseolus ssp.*) en Colombia y Costa Rica mediante marcadores microsatélites".

González, R.I., Palmira, Colombia, 21 May 2005. Presentation during Workshop on Genebanks operations for Botanical Gardens. "Estudio y caracterización de la diversidad genética". CIAT.

González, R.I.,Ciudad de Panama, Panama, 5 May 2005. Presentation realized by Rodolfo Araya during Workshop LI of PCCMCA. "Flujo genético en frijol común: consideraciones para el manejo de cultivos genéticamente modificados".

Mafla Bohórquez, G. 18 April 2005. Invited presentation at 'Curso Internacional sobre sistemas modernos de producción, procesamiento y utilización de yuca': Conservación del germoplasma *in vitro* de la yuca. Palmira, Colombia.

Mafla, G. 10 Noviembre 2005, presentation "El valor de los recursos genéticos, conservación *in vitro*" during the VI Simposio de Biotecnología y el sector productivo, and V Jornada biotecnológica, San José de Cúcuta, Colombia,:.

Ocampo, C.H. May 21, 2005. Presentation during the meeting of the Botanic Gardens Network in Colombia: Marcadores bioquímicos y moleculares para la caracterización genética del germoplasma vegetal. CIAT.

Salcedo, J.M. 21 April 2005. Invited presentation at 'Curso: Biodiversidad: conocer, conservar y utilizar'. "Bancos de semillas como una alternativa de conservación *ex situ*". Universidad Nacional, sede Palmira, Colombia.

6.4. List of international and national courses with input from Project Staff in 2005

Debouck, D.G. Master's Degree programme in Plant Genetic Resources. Use of molecular markers for germplasm characterization. Universidad Nacional Agraria La Molina. Lima, Peru. March 2005.

Mafla G., Curso Internacional sobre sistemas modernos de producción, procesamiento y utilización de yuca': Conservación del germoplasma *in vitro* de la yuca. CIAT, Cali, Colombia. 18 – 27 April, 2005.

Mafla G, Master's Degree programme in Plant Genetic Resources, Universidad Nacional de Colombia. October 2005.

6.5. List of trainees trained by Project Staff in 2005

In Seed Conservation

The staff of MAGFOR/Nicaragua, José Mauricio Jean, José Luis Rodriguez and Reynaldo Cajina, were trained in seed conservation at the Genetic Resources Unit, 22 –25 February, 2005.

Biol. Rosa Melania Espinoza from INIA, Venezuela was trained in seed and *in vitro* conservation at the Genetic Resources Unit, 5-14 September 2005.

In vitro Lab

Rodríguez, Fátima. Training in conservation and management of *in vitro* cassava germplasm INTA, Nicaragua, 17 November 2005.

Mc Donald, Rohan. Conditioning and establishment of micropropagated plants Ministry of Agriculture St. Vincent and The Granadines, 28-30 September 2005.

Electrophoresis Lab

Sandra Miranda. Training in SDS-PAGE technique for phaseolin. Instituto Nacional de Ciencias Agrícolas de Cuba. 19-30 September 2005.

Danilo Moreta. Training in 2D-IEF-SDS-PAGE technique for phaseolin. Centro de Investigaciones Biotecnológicas del Ecuador (CIBE-ESPOL), 15-30 November 2005.

In Gene Flow

B.Sc. Mónica Carvajal. Training in molecular markers of *Phaseolus vulgaris* L. 21 July-21 Sept-2005.

St.B.Sc. Carlos Andrés Martinez. Universidad del Tolima. Training in biochemical and molecular markers of *Phaseolus vulgaris*. Sept 2005-January 2006.

St.B. Sc. Marcela Gómez. Universidad del Tolima. Training in biochemical and molecular markers of *Phaseolus ssp.* Sept 2005-January 2006.

6.6. Posters

Aranzales, E., Mafla, G., Escobar, R. & D.G. Debouck. 2005. Establecimiento de protocolos para la conservación in vitro de especies de la familia Palmae: modelo de la palma de chontaduro (*Bactris gasipaes* Kunth.). V SIRGEALC –Simposio de Recursos Genéticos para América Latina y el Caribe. November 23-25, Montevideo, Uruguay.

Balcázar, M.S., Rivera, Á. L. & Pineda L., B. 2005. Actividad antagónica de bacterias aisladas de semillas de *Brachiaria y* asociadas con hongos de *Brachiaria.* XLV Congreso annual de la Sociedad Americana de Fitopatologia Division Caribe, VI Congreso nacional de Fitopatolgia y I Congreso Nacional de Fitoprotección.7 June-1 July 2005. San José, Costa Rica.

González-Torres, R.I., O. Toro, M. Carvajal & D.G. Debouck. 2005. Presencia de complejos "Silvestre –Malez-Cultivo" de *Phaseolus vulgaris* L. en Costa Rica y Bolivia utilizando marcadores de AND nuclear y cloroplástico. V SIRGEALC –Simposio de Recursos Genéticos para América Latina y el Caribe. November 23-25, Montevideo, Uruguay.

González-Torres, R.I., Araya-Villalobos R. & D.G. Debouck. 2005. Gene flow and its effect on biodiversity: common bean as model for future considerations of biosafety. Deutscher Tropentag 2005: The Global Food & Product Chain-Dynamics, Innovations, Conflicts, Strategies. October 11 – 13, 2005, University of Hohenheim, Stuttgart, Germany.

Salcedo, J.M & D.G. Debouck. 2004. Comparación de areas en la region del estigma entre las formas silvestres y cultivadas (tradicionales y modernas) de *Phaseolus vulgaris* L. III Congreso Colombiano de Botánica. November 2004, Popayán, Colombia.

6.7. Awards

Aranzalez, E. Thesis awarded as meritorious by the Universidad Francisco de Paula Santander, Cucutá-Colombia, for the degree of Engineer of Biotechnological Production.

Tofiño, A. P. & C. Ocampo. Award at the Professional level for the research work "Introgresión del acervo mesoamericano en el germoplasma de la habichuela *Phaseolus vulgaris* L. cultivada en los centros secundarios de domesticación". IX Congreso de la Asociación Colombiana de Fitomejoramiento y Producción de Cultivos. Palmira, Colombia. 11 May, 2005.

R.I. González and the SB-01/02 Project: Gene flow for assessing the safety of bioengineered crops. Outstanding Team Year Award for 2004 at CIAT.

González-Torres, R.I., O. Toro, M. Carvajal & D.G. Debouck. 2005. Presencia de complejos "Silvestre -Malez-Cultivo" de *Phaseolus vulgaris* L. en Costa Rica y Bolivia utilizando marcadores de ADN nuclear y cloroplástico. V SIRGEALC -Simposio de Recursos Genéticos para América Latina y el Caribe. November 23-25, Montevideo, Uruguay. Best Poster Award.

6.8. Visitors

The Professional Staff of the Genetic Resource Unit attended the visit of 223 people from 60 different government bodies, institutions, companies, etc. A total of 240 students from 12 different universities of Colombia visited the Genetic Resource Unit, on May and October 2005 through the Open House day coordinated by CIAT's Training Office.

6.9. Donors

CIAT Core Budget.

Ministerio de Agricultura y Desarrollo Rural of Colombia.

Ministerio de Agricultura y Desarrollo Rural of Colombia (special project: Development of cryopreservation techniques for palm species in view of developing cost effective genetic banks).

World Bank (special project: Rehabilitation of International Public Goods; CGIAR Genebanks Upgrading Project, Global Public Goods, phase 1).

Bundesministerium fuer Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany (special project: studies of gene flow in the bean model).