

ANNUAL REPORT 2007
CIAT Project on Saving Agrobiodiversity
SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses



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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:

- 2006 Scaling up of marker assisted selection and transformation established for rice bean and cassava. High throughput screening for selected tropical fruits initiated. Marker assisted selection 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 marker 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.
- 2008 Upgrading Plan advanced. Safety duplicates at CIMMYT, Svalbard and CIP. DNA bank established for beans and cassava. Data from previous agronomic evaluations (pests, diseases, abiotic stresses) made available on CIAT web site to request germplasm electronically. Common registries established for beans, cassava and tropical forages. Bean and cassava strategies advanced for the Global Crop Diversity Trust. Automatic reporting of all SMTAs handled by CIAT Staff at HQ and in the regions. 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, WARDA 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 genebank 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 (genebanks); genetic and molecular techniques for the genebank, crop productivity, and soils (microbial) projects. Identified genes and gene combinations for crop productivity and protection projects. Propagation and conservation methods and techniques for genebanks 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 <i>Brachiaria</i> .	Publications, reports, and project proposals. Germplasm. Availability of a laboratory information management system (LIMS).	Availability of up-to-date genomics equipment, and 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, <i>Brachiaria</i> , 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 genebanks.	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 <i>in situ</i> conservation projects.	Project documentation.	NARS interested in conservation efforts. Farmers interested in conservation efforts.

Annex: CG Performance Measurement of GRU. Output Template: achievement of output targets.

Activity Area (number in Annual Report)	Output	Output target 2006	Category of Output target	Achieved ?
1.1.	Backlogs cleared/ introduced	2,000 materials/ year	materials	no (1,663)
1.2.	Materials planted	6,520 materials	materials	yes (7,660)
1.3.	Materials regenerated	3,400 materials	materials	yes (5,255)
1.4.1.	Materials processed	2,000 materials	materials	yes (3,773)
1.4.3.	Materials secured	4,000 materials	materials	yes (5,917) (extra 30,911 Svalbard)
2.1.	Materials cleaned	4,500 materials	materials	no (4,076 seed; 596 in vitro)
2.2.	Materials distributed	Unpredictable target	materials	yes (4,882)
2.2.	Data available	New web page	practice (information product)	Yes
2.4.	Safety back-ups	2,000 at CIMMYT	materials	yes (5,917, and 30,911 into the Svalbard Global Seed Vault)
3.1.	Publications	2 articles in refereed journals	Knowledge	Yes
4.1.	Training	MSc thesis and NARS trained	Capacity	Yes (2 and 30, respectively)

Proof of achievement: Final Report CGIAR Genebank Upgrading (SGRP, 2007); Report of the CGIAR Audit Committee February 2007; this report.

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

SUMMARY ANNUAL REPORT 2007

Genetic Resources Unit

SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Staff:

- Daniel G. Debouck, Head, PhD (80%)
- Celia Lima, Agr. Engineer, M.Sc. (has joined in 2007) (100%)
- Graciela Mafla, Biologist (100%)
- Maritza Cuervo, Agr. Engineer, M.Sc. (100%)
- César Ocampo, Biologist, M.Sc. (100%)
- Orlando Toro, Technician (100%)
- Arsenio Ciprián, Technician (100%)
- Roosevelt Escobar, Biologist, M.Sc. (50%)
- Ericson Aranzales, Ing. Biotec. (100%)
- Maria del Socorro Balcázar, Bacteriologist (100%)
- Rosa I. González, Bacteriologist, M.Sc. (has left in 2007) (10%)
- Guillermo Enrique Rueda Q., Telematic Engineer (has left in 2007) (100%)
- Carmenza Llano, Administrative Assistant (has left in 2007) (100%)
- Josefina Martínez, Secretary (has joined in 2007) (100%)
- Eliana Urquijo, Secretary (100%)

3.2. Partners/Cooperators:

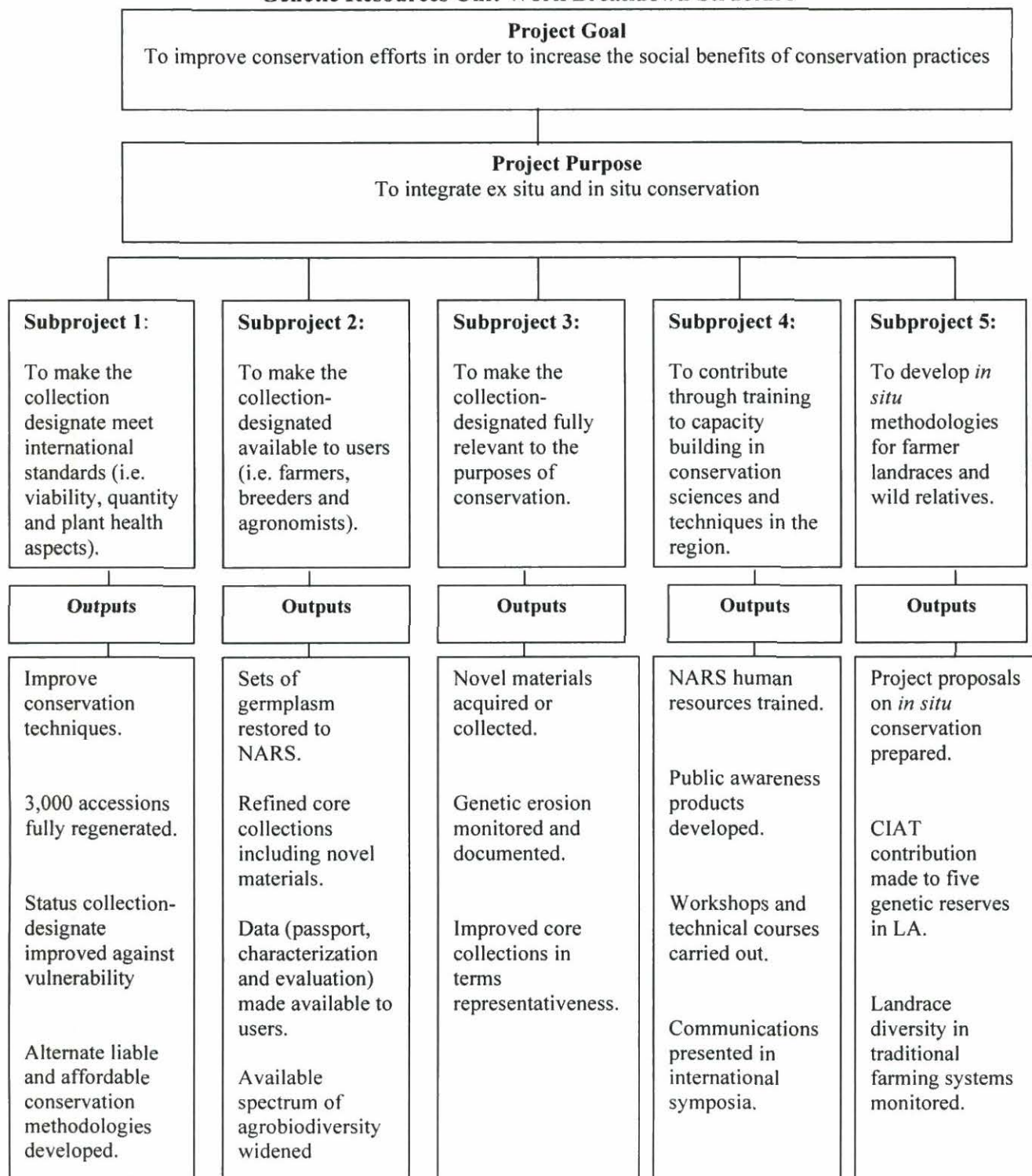
Within CIAT:

Steve Beebe (IP-1), Matthew Blair (IP-1), Lee Calvert (IP-2), Hernán Ceballos (IP-3), Martin Fregene (IP-3), Elizabeth Álvarez (IP3-IP1), Andrew Jarvis (PE-4), Carlos Lascano (IP-4), Zaida Lentini (SB-02), John Miles (IP-4), Michael Peters (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, WARDA, Africa
Dr. Mario Lobo, CORPOICA, Colombia
Dr. Samy Gaiji, SINGER, IPGRI, Italy
Dr. Jane Toll, SGRP, IPGRI, Italy
Dr. Jean Hanson, 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



Genetic Resources Unit Logical Framework

Head: Daniel G. Debouck

Sub-Project #1: The International Standards

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
<i>Goal</i> To make the FAO Designated Collections complying with international standards	ICER'95 and ICER'97 recommendations met	FAO Commission experts visits	
<i>Purpose</i> 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
<i>Output 1.1</i> 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
<i>Output 1.2</i> 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
<i>Output 1.3</i> 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
<i>Output 1.4</i> 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
<i>Output 1.5</i> 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
<i>Goal</i> To make the FAO Designated Collections available to users, inside and outside CIAT	ICER'95, ICER'97, EPMR'07 recommendations met Distribution records	FAO experts visits Consultations of users	
<i>Purpose</i> 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 seedborne 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, Svalbard, and CIP	Visits to CIMMYT, Svalbard, 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
<i>Goal</i> 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	
<i>Purpose</i> 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
Output 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 Bioversity
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 Bioversity

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

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
<i>Goal</i> To develop in situ methodologies for farmer landraces and wild relatives	Wider gene pools conserved in situ	List of taxa in protected areas	
<i>Purpose</i> 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
<i>Output 5.1</i> Project proposals prepared	Concept Notes distributed to potential donors	Concept Notes in Project/ Business Offices	Collaboration with CIAT Project Office
<i>Output 5.2</i> Contribution made towards protected areas in Latin America	Wild relatives of CIAT crops included in protected areas	Publications	Interest by NARS and Conservation Agencies
<i>Output 5.3</i> 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	514,522	60.1
Sub Total	514,522	
<i>Special projects</i>		
Gene Flow BMZ	2,459	0.3
Upgrading Plan Operations 1 WB	130,579	15.3
Upgrading Plan Operations 2 WB	208,010	24.3
Sub Total	341,048	
TOTAL	855,570	100.0

3.4. Research Highlights in 2007

Activity area # 1: the International Standards

The Upgrading of CGIAR genebanks ('Rehabilitating International Public Goods' Phase 2) has started in 2007. A total of 5,255 materials were planted in the stations, and have been regenerated in 2007. A total of another 2,913 seed materials were secured in the long-term storage (-20°C), while the entire cassava core collection of 630 clones is maintained in liquid nitrogen. A total of 4,076 seed accessions (2,992 of beans and 1,084 of forages) have been tested for absence of diseases of quarantine importance, while another 596 clones were added to the certified cassava collection available for distribution (77% of total collection). A total of 30,911 accessions of beans and tropical forages has been sent to the Svalbard Global Seed Vault (52% of total collections), and a total of 5,917 seed accessions of beans and forages have been shipped to CIMMYT as security backup (30% of total collections), while 3,330 accessions of cassava *in vitro* has been shipped to CIP as security backup (85%).

Activity area # 2: the Germplasm and its data available

In 2006, GRU has distributed 4,882 samples of accessions registered into the Multilateral System of the International Treaty. CIAT has registered 65,290 accessions into the Multilateral System (35,683 of *Phaseolus* beans, 23,140 of tropical forages, and 6,467 of *Manihot* cassava). By January 3, 2007, GRU has started the distribution of in-trust material under the new Standard Material Transfer Agreement approved by the Governing Body in Madrid in June 2006. A new web portal implemented in 2006 has been upgraded further in 2007 in order to facilitate access to information and to germplasm of the in-trust collections. The documentation of SMTAs (i.e. germplasm requests done through CIAT GRU web site) has been the starting point for the handling of all SMTAs by CIAT Staff. The amount of digital images of seeds, roots, plant habits, herbarium specimens available to users and for internal checks now sums up to 28,025. A total of 87% of the entire collection of cassava has been tested against diseases of quarantine importance and is thus available for international distribution. The RT-PCR diagnostic method developed for indexing Frog Skin Disease in cassava seems promising because of the matching between this method and the 'classic' grafting with a hypersensitive clone.

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

Research done on the duplicates existing in the Colombian collection of cassava (1,986 accessions) with help of seven unlinked SSR markers has identified a set of 202 redundant accessions. The diversity of phaseolin types was assessed in a collection of 148 landraces of Nicaragua. A new map of wild-weed-crop complexes of common bean with additional cases of gene flow between wild forms and traditional landraces was produced for Colombia. A germplasm exploration carried out in five departments of

northwestern Nicaragua brought 24 populations of *Phaseolus* beans new to Science, including four populations of wild common bean. Another highlight has been the understanding of patterns of genetic diversity in wild common bean, where for the first time geographic patterns are explained in terms of past migrations between Mesoamerica and the Andean region during the late Tertiary - early Quaternary. If one can hypothesize the origin of the common in southern Central America before an early migration into the Andean region, another migration took place later from NW Peru towards Colombian and again into Central America.

Activity area # 4: the International cooperation and capacity building

Two MSc thesis research were supervised by GRU Staff in 2007. One article was published in an international refereed journal; two articles in a non-refereed journal, and one conference proceeding have also been published. GRU Staff made fifteen presentations in conferences, while ten posters were presented in scientific congresses and fora. Twenty-three Professionals were trained in different disciplines in GRU facilities.

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 come to conclusion with the preparation of several articles in population genetics, reproductive biology, assessment of gene flow between species, between the crop and its wild relative in space and over time. While the model has been common bean, a limited but informative study has been carried out on the Lima bean model.

Information has been gathered about the taxonomy and geographic distribution of wild relatives of crops in the following herbaria: AGUAT, BR, COL, L, MEXU, MICH, O, PH, and WIS, as background information for several future developments (such as germplasm explorations – an example being the exploration carried out in December 2007 in Nicaragua, or GIS analysis such as the work developed with the University of Birmingham). In this last work, one of the questions addressed was to see how far populations known to exist (either because of a germplasm collection, or because of a voucher herbarium specimen) are indeed conserved, either *ex situ* in a genebank or in a protected area. In order to have contrasting situations, the species selected were those of the sections *Acutifolii*, *Coriacei*, *Minkelersia* and *Rugosi*, in Aridoamerica of northwestern Mexico and southwestern United States.

3.5. Problems encountered and their solution

CIAT has experienced severe reductions in its core income in 2007 and this has affected GRU and the normal delivery of outputs of the last year of Phase 1 and the first year of Phase 2 of the Genebank Upgrading (disbursements were authorized in May 2007 while Phase 2 was supposed to start on January 3, 2007!). Given financial uncertainties for 2008, since August 2007 some contingency plans were developed, concentrating on harvesting seed accessions already installed in the field in Palmira and Quilichao. The Tenerife station has been closed for security reasons, and plantings in Popayán have been re-oriented towards the regeneration of common bean only. For materials requiring cool temperatures and thus high altitude, contacts have been made with two institutions in the Bogotá area. A smooth execution of the different budget lines would be implemented if the project manager would know from day 1 which resources are actually available, if expenses can be reflected immediately in the budget accounts, and if cost centers can be charged by approved expenses only from the project.

The uncertainties affecting CIAT core budget have had some weight in the decisions by some members of GRU Staff to leave the Center. It would be timely to find a satisfactory solution between contracts

continuing up to retirement and contracts limited to one year. Apart from contractual aspects, it is also timely to plan for a training of the Staff in meeting both personal and institutional interests.

3.6. Plans for next year

(under the assumption of availability of funds as stated in GPG2 Plan)

- 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, Svalbard and CIP (for the latter after a review a slight modification in number of explants will increase efficiency and security of the backup)
- continue with the documentation of the 'institutional memory' by recovering elite germplasm released by CIAT and partners in the past in the countries, and evaluation data
- continue to update the web site, namely with evaluation, herbarium data and digital images
- expand the cryoconservation to a set of cassava clones beyond the core collection through vitrification technique
- assess the feasibility to have three cryoconserved collections of cassava, and if appropriate make the pertinent institutional agreements
- launch the DNA bank initiative if resources are available
- close Phase 2 of the Gene Flow Project with full publication of project results
- participate in the different collective inter-Centre activities of Global Public Goods Phase 2
- run international courses as it may be required

3.7. Executive summary

The Upgrading of CGIAR genebanks ('Rehabilitating International Public Goods' Phase 2) has started with delay and under financial uncertainties. A total of 6,520 materials were planted in the stations, and 5,255 materials have been regenerated in 2007. A total of 2,913 seed materials were secured in the long-term storage (-20°C), while the entire cassava core collection of 630 clones is maintained in liquid nitrogen. A total of 4,076 seed accessions (2,992 of beans and 1,084 of forages) have been tested for absence of diseases of quarantine importance, while another 596 clones were added to the certified cassava collection available for distribution (77% of total collection). A total of 5,917 seed accessions of beans and forages have been shipped to CIMMYT as security backup (now at 30%), while 3,330 accessions of cassava *in vitro* has been shipped to CIP as security backup (now at 85%). In 2006, GRU has distributed 4,882 samples of accessions registered into the Multilateral System of the International Treaty. CIAT has registered 65,290 accessions into the Multilateral System (35,683 of *Phaseolus* beans, 23,140 of tropical forages, and 6,467 of *Manihot* cassava). By January 3, 2007, GRU has started the distribution of in-trust material under the new SMTA; no cases of non-acceptance have been registered.

Research has advanced in the detection of Frog Skin Disease, where a RT-PCR diagnostic method has been tested against the 'classic' grafting technique. Detection of genetic copies within the cassava *in vitro* collection of Colombia has resulted in the detection of 202 redundant accessions. The compilation of world herbaria for wild *Phaseolus* species has had two outcomes in 2007: a germplasm exploration carried out in December 2007 in Nicaragua, and a GIS analysis for a work on *in situ* conservation developed with the University of Birmingham. Phase 2 of the Gene Flow project supported by BMZ of Germany has come to conclusion with the preparation of several articles in population genetics, reproductive biology, assessment of gene flow between species, between the crop and its wild relative in

space and over time. While the model has been common bean, a limited but informative study has been carried out on the Lima bean model.

4. Project performance indicators

1. FLOWS, TECHNOLOGIES, METHODS & TOOLS

- 1.1. Backlogs cleared: 463 accessions cleared
- 1.2. Accessions regenerated: a total of 5,255, as 4,055 of beans, and 1,200 of tropical forages
- 1.3. Accessions secured in long-term: 2,913 accessions secured
- 1.4. Accessions in security back-up: Shipment this year of 30,911 seed accessions (Svalbard), 5,917 seed accessions (CIMMYT) and 3,330 *in vitro* accessions (CIP)
- 1.5. Accessions characterized: 4,976 (field/ lab) + 1,852 (image bank)
- 1.6. Accessions distributed with passport data: 4,882 accessions distributed
- 1.7. Support tools (software in germplasm management; databases available from internet): see www.ciat.cgiar.org/urg
- 1.8. Data Bases united/improved, same

2. PUBLICATIONS

2.1. Refereed Journals: published: 1

Chacón M.I., Pickersgill B., D.G. Debouck & J.S. Arias. 2007. Phylogeographic analysis of the chloroplast DNA variation in wild common bean (*Phaseolus vulgaris* L.) in the Americas. *Plant Syst. Evol.* **266** (3-4): 175-195.

2.2. Refereed Journals: submitted (accepted indeed): 1

Motta-Aldana J.R., M.L. Serrano-Serrano, J. Hernández Torres, G. Castillo-Villamizar, D.G. Debouck & M.I. Chacón S. Identification of chloroplast and nuclear DNA markers useful for inter and intraspecific studies in wild Lima beans (*Phaseolus lunatus* L.) and related species. *Molecular Ecology Notes*.

2.3. Published Proceedings: published articles: 1

Toro, O., C.H. Ocampo & D.G. Debouck. 2007. Additional evidence suggests a new map for the distribution of wild-weed-crop complexes of common bean in Colombia. VI Simposio Internacional sobre los Recursos Genéticos de América Latina y el Caribe (SIRGEALC). 13-16 November 2007, Mexico City, D.F., México. p. 28.

2.4. Scientific Meeting Presentations: presentations: 15

(see under 6 in full report)

2.5. Working Papers, Other Presentation or Posters: 10

(see under 6 in full report)

3. STRENGTHENING NARS

(see also under 6 in full report)

3.1. Training Courses: none in 2007.

3.2. Individualized Training: 30 training events for Professionals as detailed in Annex 6.

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

4. RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted

- Rehabilitation of International Public Goods: the Upgrading of CGIAR Genebanks, Phase 2, extension 2007-2009 approved (US\$ 740,540, not summing incomes from collective activities).
- Conservation and availability of genetic resources of cassava and common bean, with the Global Crop Diversity Trust (US\$ 270,000, "in perpetuity").

4.2. Ongoing special projects in 2007

Studies of gene flow in the bean model, Phase 2, supported by Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany, US\$ 2,459.

CGIAR Genebank Upgrading, Phase 1, supported by the World Bank, US\$ 130,579.

CGIAR Genebank Upgrading, Phase 2, supported by the World Bank, US\$ 208,010.

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 1,852 digital images, summing to 24,705 images for the bean collection, 3,320 images of seed and plants in the field for the forages to date (total 28,025), accessed through CIAT web site or ready to be loaded into it.

Contributors: O. Toro, A. Ciprián, G. Rueda.

Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into the genebank processes.

Within the process of documenting the 'Institutional Memory', twenty-five new accessions were added to the bank from the elite lines of the Bean Improvement project of CIAT. Of these, ten correspond to non-nodulating mutants, which along with nodulating ones allow to quantify the response to nitrogen fixation. Six are drought-tolerant lines, other six are materials that have been released as commercial varieties in different countries of the world, and the remaining lines are very promising for resistance to BCMV, rust, anthracnose, angular leaf spot, in addition to good adaptation and performance. Table 1 reports the new introductions.

Table 1. New elite lines from CIAT Bean Improvement Program introduced in the genebank in 2007.

GNumber	Code	Release name or other identification	Observation
G 4445A	EX RICO 23 NN		Non nodulation mutant
G19696A	DGD-1049 NN	GLORIABAMBA NN	Non nodulation mutant
G51105A	DOR 364 NN		Non nodulation mutant
G51137A	PVA 773 NN		Non nodulation mutant
G51295A	BAT 477 NN		Non nodulation mutant
G51396A	A 285 NN	A 285 NN	Non nodulation mutant
G51491	PVA 1111	PVA 1111 (ARG)	Good adaptation and performance
G51492	WAF 132	MYRTOU (CYP)	Good adaptation and performance
G51493	AFR 361		Res. rust, als and bcmv
G51493A	AFR 361 NN		Non nodulation mutant
G51494	DOR 60	NEGRO HUASTEKO 81 (MEX) HUASTEKO (CUB) NEGRO HUASTEKO (CRI) HUASTEKO (BOL)	Good adaptation and performance
G51494A	DOR 60 NN		Non nodulation mutant
G51495	DRK 24		Res. anthracnose and ALS

GNumber	Code	Release name or other identification	Observation
G51495A	DRK 24 NN		Non nodulation mutant
G51496	INIAP 404		Good adaptation and performance
G51496A	INIAP 404 NN		Non nodulation mutant
G51497	AND 620		Res. rust, anthracnose, ALS and BCMV
G51498	AFR 180	TUC 180 (ARG)	Good adaptation and performance
G51499	BAT 1514	REVOLUCION 84 (NIC)	Good adaptation and performance
G51500	LSA 54	INIAP 418-JE.MA (ECU)	Good adaptation and performance
G51501	SEA 4		Drought tolerant
G51502	SEA 5	SEA 5 Reg. No. GP-206 PI 613166	Drought tolerant
G51503	SEA 9		Drought tolerant
G51504	SEA 13	SEA 13 Reg. No. GP-207 PI 613167	Drought tolerant
G51505	SEA 15		Drought tolerant

Contributors: O. Toro, A. Ciprián.

Output 1.2. Backlogs of materials pending on multiplication multiplied

Activity 1.2.1. Multiplication of materials cleared by quarantine authorities.

In 2007, a total of 463 bean accessions of backlog were handled following scarification methods - disinfection - pregermination, combined with substrates such as paper germination - soil - sand - petri-dishes and others. Similarly, in vitro cultivation methods were applied, with embryo rescue for wild and cultivated accessions with germination problems. The management of the remaining Backlog will continue to be a high priority for the coming years. Table 2 shows the progress in the 2007 period.

Table 2. Backlog pending and processed in 2007.

<i>Description</i>	<i>Bean</i>
Germplasm pending for processing in 2006	8,451
Germplasm processed in 2007	463
Pending germplasm for processing	7,988

Contributors: O. Toro, E. Aranzales.

Output 1.3. Materials pending on regeneration regenerated

Activity 1.3.1. Multiplication of materials with aging seeds.

Table 3 shows the number of bean accessions managed in the different locations. Activities in Tenerife were discontinued for security reasons. Figure 1 shows part of the processes of regeneration and multiplication in the field and in greenhouses in Popayán and in Palmira, respectively. Tables 4 and 5 indicate the movements for tropical forage germplasm.



Figure 1. Multiplication and regeneration of germplasm in Palmira and Popayán.

Table 3. Number of bean accessions by species and locality in 2007.

Species	Palmira		Popayán		Tenerife		Total	
	Planted	Goal Fulfilled	Planted	Goal Fulfilled	Planted	Goal Fulfilled	Planted	Goal Fulfilled
<i>P. vulgaris</i>	46	8	2,807	1,538	394	258	3,247	1,804
Complex <i>coccineus</i>			226	83	124	94	350	177
<i>P. lunatus</i>	25	23	419	132	4	1	447	156
Other spp.	5	3	5	1	---	---	11	4
Total	76	34	3,457	1,754	522	353	4,055	2,141

Table 4. Forage germplasm planted for multiplication and regeneration under greenhouse/mesh-house and field conditions (number of accessions).

Localities	Legumes	Grasses	Total
Greenhouse/ Mesh-house	1,067	39	1,106
Quilichao	1,416	87	1,503
Palmira	453	237	690
Popayán	129	177	306
Total	3,065	540	3,605

Table 5. New forage germplasm installed during 2007.

	Legumes	Grasses	Total
Sown during 2007	1,198	2	1,200
Characterized during the process	858	63	921

Contributors: O. Toro, A. Ciprián.

Status of designated germplasm at the GRU in 2007.

The status of accessions currently registered in the Multilateral System of Access and Benefit Sharing at CIAT is as follows:

Manihot cassava: 6,467 (out of which 5,584 of *M. esculenta*)

Phaseolus beans: 35,683 (for 44 taxa)

Tropical forages: 23,140 (for 668 taxa)

Total: 65,290

Activity 1.3.2. Periodical subculturing of the FAO designated cassava collection.

This year, 7,997 accessions of *Manihot* were subcultured by the nodal cutting technique. A total of 2,039 accessions (8,055 *in vitro* plants) were propagated for the distribution to users, 400 accessions (536 *in vitro* plants) were propagated for disease indexing tests, and 3,330 accessions (8,730 *in vitro* plants) were propagated for security backups.

Contributors: G. Mafla, E. Aranzales

Output 1.4. Materials processed into final seed packing

Activity 1.4.1. Final seed drying and temporary storage.

Table 6 indicates the amount of accessions for beans (2,393) and forages (1,380), respectively, (total 3,773), which have been harvested, cleaned, dried, and stored at 5°C, awaiting the results from viability and health tests.

Table 6. Germplasm in seed processing during 2007.

	Beans	Forages
Seed selection/temporal storage	2,393	1,380
Total	2,393	1,380

Contributors: C. Lima, A. Ciprián, O. Toro.

Activity 1.4.2. Viability testing.

Table 7 indicates flows of materials during 2007 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 632 accessions of the backlog forages from CSIRO, Australia. Several techniques of pre-germination have been used for successful results such as sand beds, petri dishes and germination paper.

Table 7. Viability testing for *Phaseolus* beans and tropical forages during 2007.

Class	BEANS		FORAGES	
	Germination (%)	No. Accessions	Germination (%)	No. Accessions
Already stored materials	1-64	27	1-64	27
	65-84	68	65-84	23
	85-100	314	85-100	108
Sub-total		409		158
Recently multiplied materials	1-64	25	1-64	30
	65-84	92	65-84	168
	85-100	2,480	85-100	952
Sub-total		2,597		1,150
TOTAL		3,006		1,308

Literature cited

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

Contributor: C. Lima.

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, repatriation, periodical monitoring, and distribution (Tables 8-9).

Table 8. Final storage and packing of *Phaseolus* beans processed during 2007 (number of accessions).

	Beans
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	1,998
SHORT TERM only (Distribution)	1,188
Total	3,186

Table 9. Final storage and packing of tropical forages processed during 2007 (number of accessions).

	Total
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	915
SHORT TERM only (Distribution)	464
Total	1,379

Contributor: C. Lima.

Activity 1.4.4. Monitoring the viability of conserved seed germplasm of beans and forages.

This year we have done the monitoring test for the germplasm conserved after 10 and 5 years. A group of 331 species of forage legumes were packed during 1997, and 330 were packed during 2002. The results of the first group (conserved since 1997) are shown in Table 10, and the difference of the mean germination was of 6.483. This difference is statistically significant with a confidence interval of 95%.

The results of the second group (conserved since 2002) are shown in the Table 11, and the difference of the mean germination was of 6.623. This difference is statistically significant with the confidence interval of 95%. This decrease over a period of 5 years can be seen as high for conservation purposes, and is being investigated along some possibilities such as delayed harvest, disease late infection, or poor drying.

According to these results, from the seed lot put into conservation during 1997 a total of 64 (19%) accessions have to be refreshed by seed multiplication following the protocols for seed conservation with germination rate above 85% (FAO/IPGRI 1994). From the seed lot put into conservation during 2002, a group of 89 accessions (23 %) need to be refreshed following the same protocols.

Table 10. Paired T-Test for monitoring forage seeds after 10 years of long-term conservation

% Germ	Mean	StDev	SE Mean	N	Difference	Std.Dv.Diff	T-value	P-Value
Initial	95.568	4.670	0.257					
Monitored	89.085	9.148	0.503	331	6.483	9.039	13.05	0.000

Table 11. 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	91.633	4.037	0.204					
Monitored	85.010	18.464	0.935	390	6.623	17.973	7.28	0.000

Literature cited

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

Contributor: M.C. Lima.

Output 1.5. Improved conservation techniques

Activity 1.5.1. Fine-tuning of the encapsulation-dehydration technique for cassava cryopreservation

Introduction

Many authors agree that although many protocols are available in the literature, not all researchers could follow them because they are often difficult to interpret for everyday use (Towill, 2002). Most of the protocols contain components, which are usually developed empirically using plant-specific strategies for survival enhancement (Benson 2008). For that reason although the procedures themselves are not difficult, the initial implementation of cryopreservation procedures can be daunting where financial and human resources (number and skills) are limited (Reed, 2008).

Materials and methods

- 1) Request GRU for cassava germplasm (core collection)
 - a To obtain a new clones to include in the process
 - b To replace lost clones
- 2) Plants propagated on 4E (Roca, 1984) conventional solid medium.
 - a Scaling-up of the shoots-tips number with continuous propagation cycles
 - i) At least 4-5 cycles to produces 100 units
 - (1) New clone sent by GRU
 - (2) Other clones that BRU maintains from last shipments
- 3) Encapsulation-dehydration methodology established at BRU (Manrique, 2000; Escobar 2005)
- 4) Frozen and recovery steps after different duration times on L.N.
- 5) Propagation and evaluation of responses (viability and shoot recovery)
 - a Evaluation of the response (based on 10 beads/1 tube) after freezing
 - b To decide if the clone/ tubes could be maintained on L.N.
 - c For its group allocation (lowest-intermediate or highest)
 - d Establishment of Cryo-Core I and Cryo-Core II (duplicate)

Results

1. How to make a duplicate of the collection: If we consider which institute, in the region or outside, could serve as a depository of a cryopreserved cassava collection, not many options exist. CIP (Peru) or INIBAP (Belgium) have the expertise and equipment to allow transfer of the copy when it has been established. For that reason, we made a preliminary test, and based on that a certified courier to Peru or Belgium could spent 3-5 days, we initiated to test if the procedure should stop on dehydration and make the freezing step later (when collection arrives to the final place). For good responding clones such as MBra 856, it could maintain its response just for 3 at room conditions and on refrigerator (4°C), responses could maintain until 7 days, allowing movement of tubes. However, these conditions it was not able to obtain with the lowest responding-materials. That kind of clones shows more sensibility to this management and probably this could be involved on factors such as explants (buds), sugars different to sucrose (type and contents) and dehydration pathway. Until now PVS solutions could be consider as an option but it is necessary to adjust it to try to reduce tissue toxicity. Preliminary data shown that this option could be possible.

Table 12. Conditions for maintenance of the beads/ tissue, before to freeze phase to simulate a shipment until final destination (1-7 days). Clone MBra 856.

	At growth room (days)				At 4°C/cooler (days)			
	1	3	5	7	1	3	5	7
- Frozen	96.6	66.66	0	0	100	100	0	93.33
+ Frozen	100	82.5	0	0	100	100	100	92.6

2. Responses after freezing of the recalcitrant clones to the modifications of growth media: The nitrogen content and its relationships ($\text{NO}_3^-/\text{NH}_4^+$) on the growth medium affect response on tissue culture and on its treatments. However, for recalcitrant clones this conditions it did not work after freezing procedure. For most of the clones tested those conditions it not allow improvement of the responses.

Table 13. Response after freezing of the recalcitrant materials grown on different $\text{NO}_3^-/\text{NH}_4^+$ relationship.

Genotype	Growth media	Propagation time before use (week)			
		2	3	4	5
M Col 1939	Control	17.8%	16.7%	0.0%	21.9%
	Modified	30.3%	16.7%	0.0%	43.8%
M Bol 3	Control	38.1%	73.9%	0.0%	31.1%
	Control	ND	65.0%	20.8%	0.0%
	Modified	16.7%	20.0%	0.0%	
	Modified	ND	77.8%	4.6%	28.2%
M Col 1438	Control	0.0%	0.0%	0.0%	0.0%
	Modified	0.0%	-	0.0%	0.0%
M Col 1468	Control	37.5%	0.0%		0.0%
	Control	ND	50.0%	13.7%	0.0%
	Modified	ND	0.0%	0.0%	0.0%
	Modified	ND	35.0%	16.7%	0.0%
M Ecu 165	Control	30.0%	0.0%	0.0%	3.3%
	Modified	6.7%	0.0%	0.0%	0.0%
M Ecu 31	Control	ND	69.5%	82.2%	83.3%
	Modified	ND	33.2%	10.0%	8.4%

Determination of the critical points of cryopreservation procedure: Most of the critical points have been defined and include: how old/ young the tissue is, health status, precedence's or origin of the clone/tissue/explants, pregrowth conditions (medias and time duration), accumulative stress during cryopreservation procedure (sensibility to media sugar content and duration, dehydration stress), regrowth conditions (growth regulator type and doses, temperature and darkness/light relationship). For most of the clones, the dehydration stresses (directly -by osmotic agents- and accumulative -on silica gel) are the most affecting factor on the response before and after freezing.

Conclusions

- 1) It is necessary to make a balance among research and implementation protocols.
- 2) The dynamic to establish the duplicate methodology it not easy, such as it were considered at the beginning-many factors needs to be include. The beads, containing cassava shoots, not allows extra-time on tubes without freezing. A consensus to design al logic scheme to make a duplicate of the collection it need s to be consider, including manpower, equipment, spaces and goals (clones to be include in a pilot test).
- 3) Based on the non-tolerance of the tissue to continue process without it freezing, it needs to consider that probably the entire collection must be frozen in one plot and then will be transferred to the final site. Cost and logistic need to be considered before start to implement any alternative.
- 4) Tissues grown on modified medium not gave us alternatives to improve shoots responses of the material with lowest response. It should consider that the responses its more influence by other factors
- 5) The application of cryopreservation protocols can be consider as a good alternative for gene-bank management when its average response after freezing have been included the responses as much

plants, clones, varieties or accessions of the same species of the same genus. Comparison made among different crops, that use encapsulation-dehydration on cryopreservation research, concluded that the most advanced development and application is with cassava (Engelmann et al 2008). However if CIAT considers the implementation of a black-box with the entire collection using this technique it needs to consider that when a protocol it have been developing and implementing at the same time some of the research could be relegate. For that reason it could be more reasonable that we focus on fine-tuning the actual protocol on recalcitrant materials until its modeling, and then move to the implementation.

Future activities

- a. Concentrate on recalcitrant and lowest responding material
- b. Establish a group of no more than 10-15 clones including 2 of the best responses (MCol 22 and MBra 856).
- c. Establishment of a test with these clones to make comparison among:
 - i) Buds vs. Shoots as explants
 - ii) Vitrification-dehydration technique Vs Encapsulation-dehydration
 - iii) Use of other sugars in the process (namely Trihalose)
 - iv) Adjust the dehydration step (duration and method)
 - v) Implement the best conditions found on a wider recalcitrant group's of clones (approximately 95/640 of the core collection).

References

1. Benson E. (2008). Cryopreservation Theory. In: Reed B (ed) Plant Cryopreservation-A practical guide. Springer-Verlag, USA. pp 15-32
2. Engelmann F., Arnao MT, Wu Y. and Escobar R. (2008) The development of encapsulation dehydration. In: Reed B (ed) Plant Cryopreservation-A practical guide. Springer-Verlag, USA. pp 59-76
3. Escobar-Perez R.H. (2005) Aspectos logísticos de manejo y determinación de la estabilidad genética de materiales crioconservados de yuca (*Manihot esculenta* Crantz) MSc. Thesis. Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Sede Palmira. Colombia
4. Manrique N. (2000) Respuesta varietal de 95 genotipos de la colección núcleo de yuca (*Manihot esculenta* Crantz) a la crioconservación usando la técnica de Encapsulacion-deshidratacion. BSc. Thesis Universidad Nacional de Colombia, Sede Palmira. Colombia
5. Reed B. (2008) Cryopreservation-Practical considerations In: Reed B (ed) Plant Cryopreservation-A practical guide. Springer-Verlag, USA. pp 3-11
6. Roca W. (1984) Cassava In: Sharp WR, Evans DA, Ammirato P, Yamada Y (eds). Handbook of plant cell culture V2. Crops species. MacMillan Publishing Co. New York pp 269-301
7. Towill LE. (2002) Cryopreservation of plant germplasm. In: Towill LE, Bajaj YPS (eds). Biotechnology in Agriculture and Forestry: Cryopreservation of plant germplasm II. Springer-Verlag, Berlin, pp 3-21.

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Sub-Project 2. The in-trust Collections and their pertinent information fully available and safely duplicated

Output 2.1. In-trust collections cleaned against seedborne 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 make available the accessions for distribution, following the FAO/IPGRI recommendations for the safe movement of cassava -clones at national and international levels. The plants developed *in vitro* are tested for cassava virus diseases: Cassava Common Mosaic Virus (CsCMV), Cassava X Virus (CsXV), and Cassava Frog Skin Disease (CFSD). For the indexing, the diagnosis techniques were: ELISA for CsCMV and CsXV, and grafting with a hypersensitive clone (MCOL 2063) for the CFSD. We also tested a molecular method for indexing CFSD with the analysis by RT-PCR.

The total Cassava World Collection kept in GRU and registered into the Multilateral System of the Treaty is of 6,467 accessions, of which 5,184 (80.2%) corresponds to landraces species, 400 (6.2%) breeding material, and 883 (13.6%) wild species. Of these, 5,613 are available for distribution corresponding to 86.8 % (Table 14 and Figure 4).

The total of negative clones evaluated for the three viruses in 2007 is of 596.

The number of clones evaluated against CsCMV (78), CsXV (138) and CsFSV (380) from January 2007 until December 2007 and the comparison of progress with before years, is shown in Figure 2.

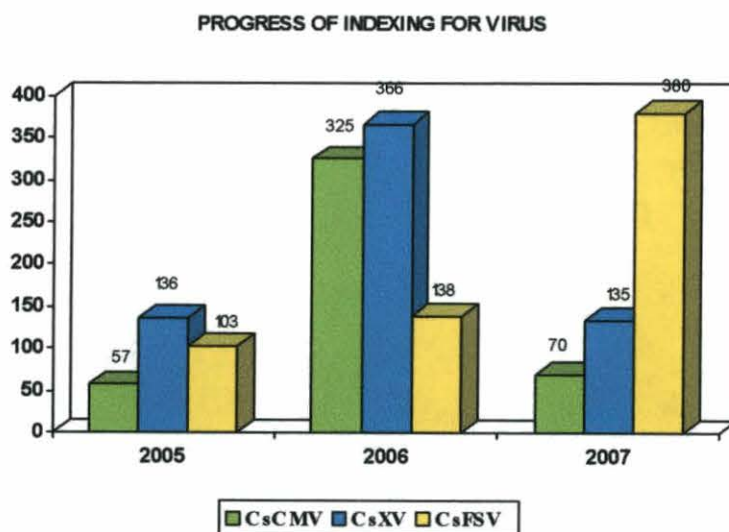


Figure 2. Number of Negative Clones evaluated for each virus 2005 – 2007.

The total progress in the indexing of the cassava collection (negatives clones for three viruses) over the last three years is shown in the Figure 3.

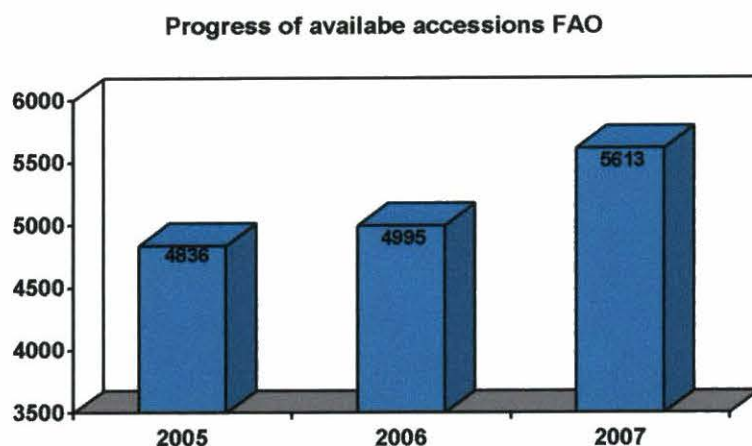


Figure 3. Available accessions of the cassava germplasm collection kept in GRU.

Activity 2.1.2. Application of a molecular technique for the detection of Cassava Frog Skin Virus (CsFSV).

Up to 2006, the detection of Cassava Frog Skin Virus (CsFSV) has been done by grafting. This process takes on average three months so the plants have the appropriate size for grafting, and tree weeks to observe the characteristic symptoms in the Secundina clones (MCOL 2063 is highly susceptible to the virus). In some cases the process takes more time as some varieties recover slowly after the *in vitro* subculturing (Flor et al. 2003). With the purpose of decreasing this time, we have implemented a molecular methodology (RT-PCR) for the detection of CsFSV developed in cooperation with the Virology Laboratory of CIAT, that takes three days and seems to be more sensible and specific (Cuervo 2006).

Of 285 samples evaluated from the Cassava Collection we found 281 samples negative by grafting test and RT-PCR, 2 samples positive by grafting test and RT-PCR, and 2 samples negative by grafting test but positive by RT-PCR (Table 14).

Table 14. Comparison of results between 'classic' grafting and indexing with RT-PCR.

Landraces/ materials		Grafting result	RT-PCR result
Argentina	22	22 Negative	22 Negative
Brazil	59	56 Negative	56 Negative
		2 Positive	2 Positive
		1 Negative	1 Positive
Colombia	50	50 Negative	50 Negative
		1 Negative	1 Positive
Costa Rica	2	2 Negative	2 Negative
Ecuador	6	6 Negative	6 Negative

Landraces/ materials		Grafting result	RT-PCR result
FJI	3	3 Negative	3 Negative
Guatemala	5	5 Negative	5 Negative
Indonesia	69	69 Negative	69 Negative
Malaysia	4	4 Negative	4 Negative
Mexico	8	8 Negative	8 Negative
Panama	4	4 Negative	4 Negative
Paraguay	2	2 Negative	2 Negative
Peru	1	1 Negative	1 Negative
Venezuela	1	1 Negative	1 Negative
Wild species	24	24 Negative	24 Negative
Breeding material	25	25 Negative	25 Negative
Negative by Grafting test and RT-PCR	281		
Positive by Grafting test and RT-PCR	2		
Negative by Grafting test and Positive by RT-PCR	2		
Total evaluated	285		

Activity 2.1.3. Establishment of a "Bonsai" collection as safety backup for the whole Cassava Collection.

Since October 2001 we began to establish one copy of the whole cassava collection under greenhouse conditions; the planting material is the one tested negative after the indexing for Frog Skin Disease. Along our current agreement with the Treaty, this backup is useful until a complete backup is established in liquid nitrogen; it also serves as original material for the cassava DNA bank. At the moment, in the Bonsai Greenhouses 798 clones of different origins are kept (Figure 4).



Figure 4. Cassava plants established as "Bonsai" collection as back up for the In Vitro Bank.

Activity 2.1.4. Updating the Cassava ORACLE database.

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

References

Flor. N.C., Mafla G., Danny M. Montero. Annual Report 2003 CIAT Project on Saving Agrobiodiversity SB 01/02. 2003.

Cuervo, M. Masters Thesis: Caracterizacion Molecular de algunos aislamientos del virus del cuero de sapo de la yuca recolectados en diferentes zonas de Colombia. Universidad Nacional de Colombia, sede Palmira. 2006.

Contributors: M. Cuervo, G. Mafla, E. Aranzales.

Activity 2.1.5. Germplasm health control in seed germplasm.

Introduction

To minimize the phytosanitary risks associated with the movement of germplasm, especially concerning the inadvertent transport of pathogens and pests of significance quarantine, CIAT is following a regulatory and quarantine program, in close cooperation with the plant quarantine authority of its host country Instituto Colombiano Agropecuario (ICA).

To that purpose the Germplasm Health Laboratory (GHL) practiced phytosanitary inspections on multiplication plots (fields and glass-houses), and applied indexing procedures in the laboratory to ensure that the germplasm was free of seedborne diseases that could affect its longevity during the storage and prohibit its distribution to users.

During the period January 2007-December 2007, the GHL tested 4,767 seed samples (2,992 bean seed samples, and 1,084 seed samples of tropical forage legumes and grasses) produced by GRU. It also tested 622 samples of bean seeds, and 69 of tropical grasses and legumes from CIAT projects 'Mesoamerican Bean Genetics', 'Andean Bean Genetics', and 'Tropical grasses and legumes'.

Materials and Methods

Accessions are tested in the GHL using accepted methodologies to identify seed-borne pathogens as fungi, bacteria and viruses according with those pathogens recorded in seed production areas. To detect pathogens of quarantine significance, the GHL uses the methodologies recommended by CIAT pathologists and virologists. When a recipient country requests additional statements, the GHL carries out additional tests whenever possible to comply with the specific quarantine regulations of the recipient country.

Testing for some genera of seedborne fungi includes blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar culture media.

Seedborne bacteria (*Xanthomonas axonopodis* pv. *Phaseoli*, and *Pseudomonas syringae* pv. *Phaseolicola* in beans, and *Xanthomonas campestris* pv. *Graminis* and *Pseudomonas fluorescens*, in tropical pastures) are tested.

For the detection of seedborne bacteria the GHL uses methods such as isolation in culture media and serology. The method used in the detection of *Xanthomonas axonopodis* pv. *phaseoli* is the agar plate dilution technique. It also uses the MXP semiselective culture medium, the bacteria hydrolyzes starch around the colonies. A first identification focuses on color and morphology of the colonies, and the confirmation is done with serological reactions with a specific antiserum.

The detection of *P. syringae* pv. *phaseolicola* has an extraction phase like *X. axonopodis* but the spreading is onto the surface of King B medium. The *P. syringae* pv. *phaseolicola* is identified by presence of the fluorescent pigment and with the serologic agglutination test.

The detection of *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* (*Corynebacterium flaccumfasciens* fsp. *flaccumfasciens*, has the same extraction phase. Then the bacterias are identified using color and morphology of the colonies and the gram coloration. There are only gram-positive bacteria of quarantine importance. The GHL considers information in relation to the presence of this kind of pathogens around the production areas and makes decisions as to use specific methods of detection.

The ELISA test is used for the examination of bean and tropical legumes seeds in order to check absence of the seedborne viruses: bean common mosaic virus (BCMV) and bean southern mosaic virus (BSMV) (Clark et al. 1977). For the detection of common mosaic virus we use the protocol with a monoclonal antiserum against the potyvirus group. In order to check the samples against BSMV we use the DAS Elisa Reagent set.

Results

Beans (*Phaseolus* spp.)

Seed samples of 2,992 accessions of beans were tested, some of them for export to some countries, and all the rest for conservation in the genebank. Their health status showed 70% samples without pathogens of quarantine importance (Figure 5).

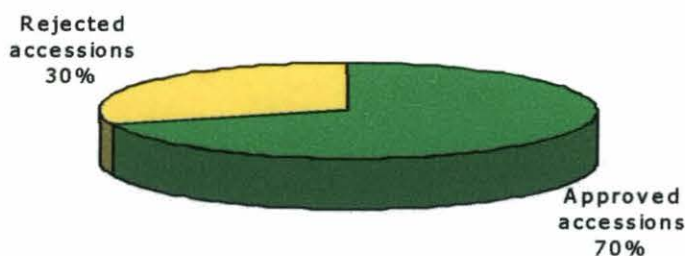


Figure 5. Number of *Phaseolus* sp. seed samples rejected or accepted after seed health laboratory analysis.

The 30% of samples of *Phaseolus* sp. showed pathogens of quarantine importance (Table 15, Figure 5 and 6).

Seedborne fungi such as *Macrophoma* sp., *Ascochyta* spp., *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phomopsis* sp., *Botrytis* sp., *Sclerotium rolfsii*, *Colletotrichum truncatum*, *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum* were detected.

Seedborne viral infections were detected at high frequency in this year. The Potyviruses were detected in 580 samples, and Southern bean mosaic virus (SBMV) was detected in 424 samples (Table 15).

This year *Pseudomonas syringae* pv. *phaseolicola* were detected in 67 samples and *Xanthomonas axonopodis* pv. *phaseoli* were detected in one accession only. The *Corynebacterium* Gram-positive (*Curtobacterium flaccumfasciens* pv. *flaccumfasciens*) were detected in two samples.

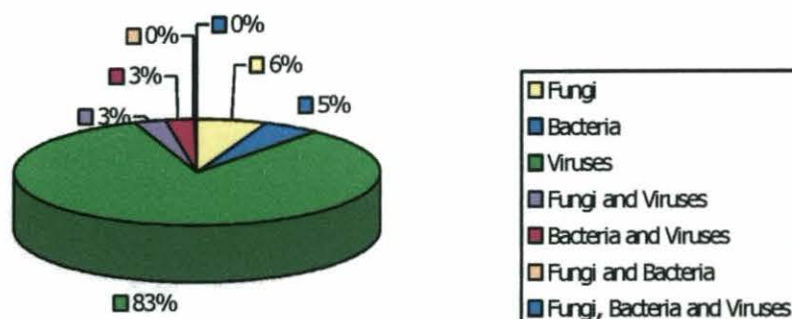


Figure 6. Reject factors of *Phaseolus* sp. seed samples analyzed by GHL.

Table 15. Reject factors of *Phaseolus* seed samples analyzed by GHL.

Factors	Affected accessions	%
Potyvirus	368	40,9
SBMV	203	22,6
Potyvirus, SBMV	179	19,9
<i>Pseudomonas syringae</i> pv. <i>Phaseolicola</i>	43	4,8
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> , Potyvirus, SBMV	22	2,4
<i>Macrophoma</i> sp.	13	1,5
<i>Ascochyta</i> spp.	10	1,1
<i>Macrophomina phaseolina</i>	10	1,1
<i>Rhizoctonia solani</i>	5	0,56
<i>Phomopsis</i> sp.	5	0,56
<i>Botrytis cinerea</i>	4	0,44
<i>Botrytis cinerea</i> , SBMV	3	0,33
<i>Ascochyta</i> spp., SBMV	2	0,23
<i>Curtobacterium flaccumfasciens</i> pv. <i>flaccumfasciens</i>	2	0,23
<i>Macrophoma</i> sp., Potyvirus	2	0,23
<i>Macrophoma</i> sp., SBMV	2	0,23
<i>Macrophomina phaseolina</i> , Potyvirus, SBMV	2	0,23

Factors	Affected accessions	%
<i>Rhizoctonia solani</i> , SBMV	2	0,23
<i>Sclerotium rolfsii</i>	2	0,23
<i>Ascochyta</i> spp., <i>Colletotrichum</i>	1	0,11
<i>Ascochyta</i> spp., <i>Colletotrichum</i> sp., Potyvirus	1	0,11
<i>Ascochyta</i> spp., <i>Macrophomina phaseolina</i>	1	0,11
<i>Botrytis cinerea</i> , <i>Ascochyta</i> spp., SBMV	1	0,11
<i>Botrytis cinerea</i> , Potyvirus, SBMV	1	0,11
<i>Botrytis cinerea</i> , <i>Sclerotium rolfsii</i> , Potyvirus	1	0,11
<i>Colletotrichum</i> sp.	1	0,11
<i>Colletotrichum</i> sp., <i>Botrytis cinerea</i> , Potyvirus	1	0,11
<i>Colletotrichum</i> sp., <i>Phomosis</i> sp., SBMV	1	0,11
<i>Colletotrichum</i> sp., Potyvirus	1	0,11
<i>Colletotrichum</i> sp., <i>Pseudomonas</i>	1	0,11
<i>Macrophoma</i> sp., <i>Phomosis</i> sp., SBMV	1	0,11
<i>Macrophoma</i> sp., Potyvirus, SBMV	1	0,11
<i>Phomosis</i> sp., <i>Colletotrichum</i> sp.	1	0,11
<i>Phomosis</i> sp., SBMV, Potyvirus	1	0,11
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> , Potyvirus	1	0,11
<i>Rhizoctonia solani</i> , SBMV, Potyvirus	1	0,11
<i>Sclerotinia sclerotiorum</i>	1	0,11
<i>Sclerotium rolfsii</i> , <i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> , SBMV	1	0,11
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> , SBMV, Potyvirus	1	0,11

Tropical grasses and legumes

Seed samples of 1,084 accessions of tropical grasses and legumes were tested. Their health status showed 77.0% samples without pathogens of quarantine importance (Figure 7).

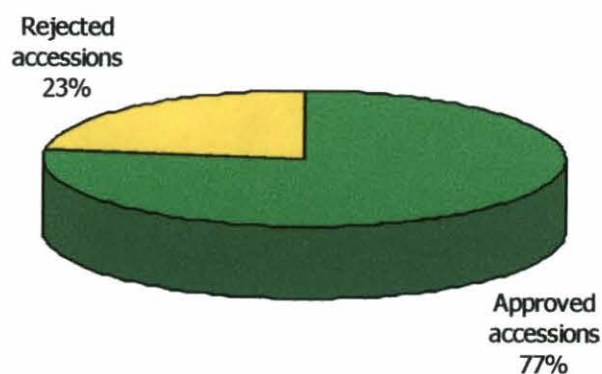


Figure 7. Number of Tropical grasses and legumes seed samples rejected or accepted after seed health laboratory analysis.

In the rejected samples we detected some seedborne fungi of quarantine importance such as *Colletotrichum* sp., *Curvularia* sp., *Macrophoma* sp., *Phomosis* sp., *Rhizoctonia solani*, *Drechslera* spp., *Phoma* sp., *Ascochyta* spp., *Colletotrichum* sp., *Pestalotia* sp. (Table 16, Figure 8).

The Potyviruses were detected in 42 samples, and Southern bean mosaic virus (SBMV) was detected in 41 samples (Table 16).

Pseudomonas fluorescens was detected in 16 accessions, and the *Corynebacterium* Gram-positive (*Curtobacterium flaccumfasciens* pv. *flaccumfasciens*) was detected in one accession only. The *Xanthomonas campestris* pv. *graminis* were not detected.

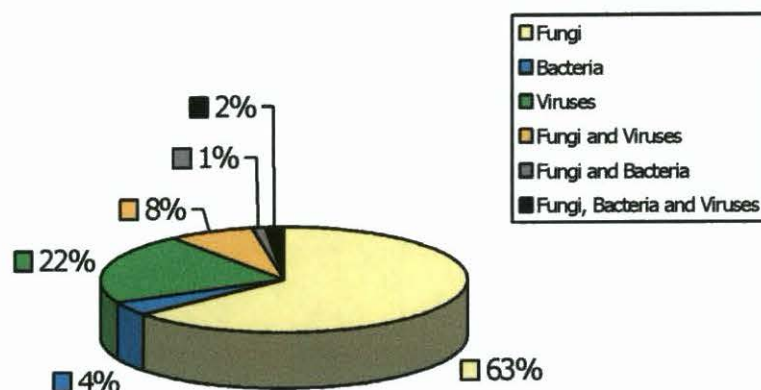


Figure 8. Reject factors of Tropical grasses and legumes seed samples analyzed by GHL.

Table 16. Reject factors of legume and tropical grasses seed samples analyzed by GHL.

Factors	Affected accessions	%
<i>Colletotrichum</i> sp.	33	13,2
<i>Curvularia</i> sp.	27	10,8
Potyvirus	25	10
SBMV	24	9,6
<i>Phomosis</i> sp.	20	8
<i>Macrophoma</i> sp.	12	4,8
<i>Macrophoma</i> sp., <i>Phomosis</i> sp.	10	4
<i>Rhizoctonia solani</i>	10	4
<i>Pseudomonas fluorescens</i>	9	3,6
<i>Drechslera</i> spp.	7	2,8
<i>Phoma</i> sp.	7	2,8
Potyvirus, SBMV	5	2
<i>Ascochyta</i> spp.	3	1,2
<i>Curvularia</i> sp., <i>Phoma</i> sp.	3	1,2
<i>Curvularia</i> sp., Potyvirus	3	1,2
<i>Macrophoma</i> sp., Potyvirus	3	1,2
<i>Macrophoma</i> sp., SBMV	3	1,2
<i>Colletotrichum</i> sp., <i>Curvularia</i> sp.	2	0,8
<i>Colletotrichum</i> sp., <i>Phomosis</i> sp.	2	0,8

Factors	Affected accessions	%
<i>Curvularia</i> sp., <i>Phomosis</i> sp.	2	0,8
<i>Drechslera</i> spp., <i>Phoma</i> sp.	2	0,8
<i>Drechslera</i> spp., <i>Phoma</i> sp., <i>Colletotrichum</i> sp.	2	0,8
<i>Phomosis</i> sp., SBMV	2	0,8
<i>Rhizoctonia solani</i> , <i>Phomosis</i> sp.	2	0,8
<i>Ascochyta</i> spp., <i>Phoma</i> sp., Potyvirus	1	0,4
<i>Colletotrichum</i> sp., <i>Macrophoma</i> sp.	1	0,4
<i>Colletotrichum</i> sp., Potyvirus	1	0,4
<i>Colletotrichum</i> sp., SBMV	1	0,4
<i>Curtobacterium flaccumfasciens</i>	1	0,4
<i>Curvularia</i> sp., <i>Ascochyta</i> spp.	1	0,4
<i>Curvularia</i> sp., <i>Colletotrichum</i> sp., Potyvirus	1	0,4
<i>Curvularia</i> sp., <i>Drechslera</i> sp.	1	0,4
<i>Curvularia</i> sp., <i>Macrophoma</i> sp., <i>Pseudomonas fluorescens</i> , SBMV	1	0,4
<i>Curvularia</i> sp., <i>Macrophoma</i> sp., <i>Pseudomonas fluorescens</i> , SBMV, Potyvirus	1	0,4
<i>Curvularia</i> sp., <i>Phomosis</i> sp., <i>Phoma</i> sp.	1	0,4
<i>Curvularia</i> sp., <i>Rhizoctonia solani</i>	1	0,4
<i>Curvularia</i> sp., SBMV	1	0,4
<i>Macrophoma</i> sp., <i>Phomosis</i> sp., <i>Curvularia</i> sp.	1	0,4
<i>Macrophoma</i> sp., <i>Phomosis</i> sp., Potyvirus	1	0,4
<i>Macrophoma</i> sp., <i>Phomosis</i> sp., SBMV	1	0,4
<i>Macrophoma</i> sp., Potyvirus, SBMV	1	0,4
<i>Macrophoma</i> sp., <i>Pseudomonas fluorescens</i>	1	0,4
<i>Macrophomina</i> sp., <i>Phomosis</i> sp.	1	0,4
<i>Pestalotia</i> sp.	1	0,4
<i>Pestalotia</i> sp., <i>Curvularia</i> sp.	1	0,4
<i>Phoma</i> sp., <i>Ascochyta</i> spp.	1	0,4
<i>Phoma</i> sp., <i>Curvularia</i> sp., <i>Drechslera</i> sp.	1	0,4
<i>Phomosis</i> sp., <i>Curtobacterium flaccumfasciens</i>	1	0,4
<i>Phomosis</i> sp., <i>Macrophoma</i> sp., <i>Rhizoctonia solani</i>	1	0,4
<i>Phomosis</i> sp., <i>Pseudomonas fluorescens</i> , SBMV	1	0,4
<i>Pseudomonas fluorescens</i> , <i>Colletotrichum</i> sp.	1	0,4
<i>Rhizoctonia solani</i> , <i>Colletotrichum</i> sp.	1	0,4
<i>Rhizoctonia solani</i> , <i>Curvularia</i> sp., <i>Phomosis</i> sp., Potyvirus	1	0,4
<i>Rhizoctonia solani</i> , <i>Phoma</i> sp.	1	0,4
<i>Rhizoctonia solani</i> , <i>Pseudomonas fluorescens</i>	1	0,4
<i>Rhizoctonia solani</i> , <i>Pseudomonas fluorescens</i> , SBMV	1	0,4

Service of germplasm health certification for other projects

Seed samples of 691 accessions from CIAT projects 'Andean Bean Genetics', 'Mesoamerican Bean Genetics', and IP5 (Tropical grasses and legumes) were analyzed (Figure 9).

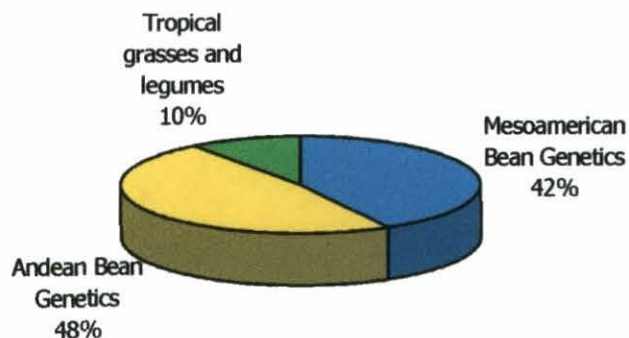


Figure 9. Number of accessions tested for other projects in 2007.

Their health status showed 75.0% samples without pathogens of quarantine importance (Figure 10). In rejected samples (25.0%) we detected some seedborne fungi of quarantine importance in 59 accessions (*Ascochyta* spp., *Colletotrichum* sp., *Macrophomina phaseolina*).

The Potyviruses were detected in 83 samples, and Southern bean mosaic virus (SBMV) was detected in 47 samples (Table 16).

Pseudomonas fluorescens was detected in one accession and the *Xanthomonas campestris* pv. *graminis* was detected in one accession only. The *Corynebacterium* Gram-positive (*Curtobacterium flaccumfasciens* pv. *flaccumfasciens*) were not detected.

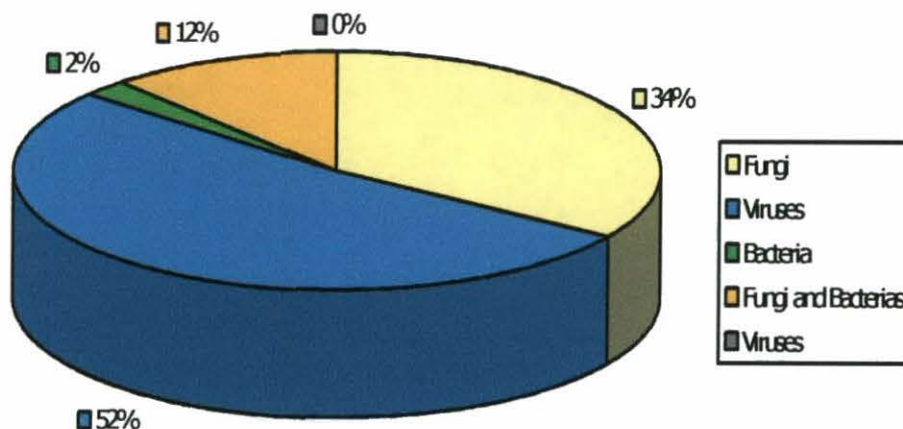


Figure 10. Reject factors of other projects seed samples analyzed by GHL.

Table 17. Reject factors of other projects seed samples analyzed by GHL.

Factors	Affected accessions	%
SBMV	19	11
Potyvirus	52	30,3
SBMV, Potyvirus	19	11
<i>Ascochyta</i> sp., <i>Colletotrichum</i> sp.	2	1,16
<i>Colletotrichum</i> sp.	2	1,16
<i>Macrophomina phaseolina</i>	52	30,2
<i>Macrophomina phaseolina</i> , Potyvirus	8	4,65
<i>Macrophomina phaseolina</i> , Potyvirus, SBMV	4	2,32
<i>Macrophomina phaseolina</i> , SBMV	6	3,5
<i>Ascochyta</i> spp., Potyvirus	1	0,59
<i>Ascochyta</i> spp., SBMV	1	0,59
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i>	2	1,16
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	1	0,58
<i>Ascochyta</i> spp.	3	1,74

Contributors: M. Cuervo I., M.S. Balcazar.

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

Activity 2.2.1. Development and implementation of the computerized system of GRU for quality control, flow monitoring and Web consultation.

A system for data capturing in the field station of Popayán (Figure 11) has been implemented in 2007, that allows to take quickly data of characterization (growth habit, flower color), and harvest, and to check their validity. With this system the risks of error in data capturing by the Staff in charge of field operations is much reduced. The transfer of data to the central database is also made easy, and immediately updated.

Technical input:

Hardware:

- Handheld PSION WorkAbout PRO
- Printer Zebra S600
- Printer Printek MtP300

Software

- Windows CE Operation System
- Java and SWT custom developing
- HSQLDB database
- Synchronization through cradle

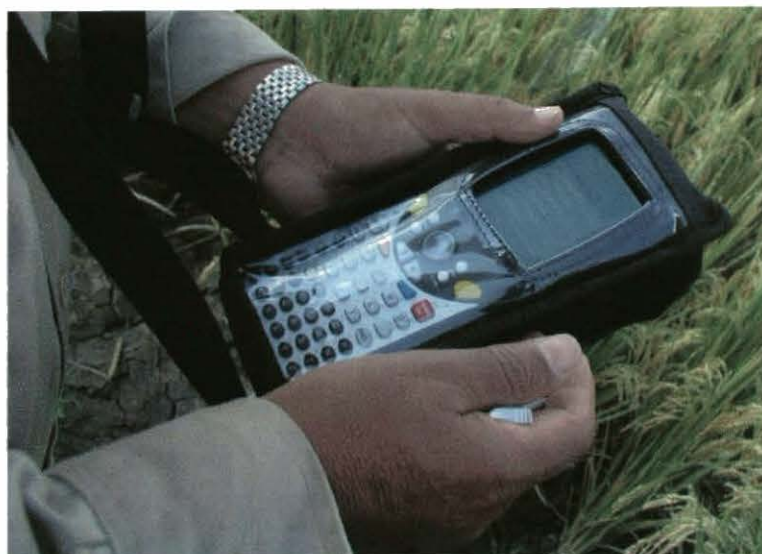


Figure 11. Data collecting in the field.

The web site of GRU with respect to germplasm distribution has been updated in line with the coming requirements of the Governing Body of the International Treaty and standards set by other CGIAR Centers such as IRRI. This was necessary in view of the coming automatic reporting.

A migration of Oracle Discoverer to its web version was done. This migration will allow the preparation and updating of reports about flows of germplasm throughout all processes, and the computing of statistics (e.g. number of consultation of GRU web site, and for which products). These reports and statistics were defined according to users' needs.

Technical input:

- Oracle database
- Oracle Discoverer 4i

The system developed for the management of the Cassava in-trust Collection kept at CIAT was presented in the Workshop on Best Practices for the management of clonal crops, CIP, Lima, 12-16 November 2007, (inter-centre collaborative activity in the project 'Rehabilitation of International Public Goods' Phase 2).

The purchase of additional equipment was done in order to complete the process of data capturing in the field and in the Germplasm Health Lab, and to facilitate the harvest in the field stations (Figure 12).



Figure 12. Mobile hardware for printing bar codes.

Contributor: G.E.Rueda

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

Achievement: 4,882 samples of accessions of the three commodity FAO designate collections distributed to germplasm users.

As it can be seen in Tables 18 and 19 and Figures 13 to 18, a total of 4,882 samples of accessions were distributed, through 197 requests attended during 2007 for beans, forages and cassava. The main recipients were CGIAR Centers with 2,688 samples of accessions and 2,194 to others institutions. NARS and universities were another important recipients. Pending on recipient type, the main purposes of the requests were: basic research, breeding, and applied research.

Table 18. Distribution of germplasm during 2007 by kind of institution (01/01/2007-31/12/2007).

Institution type	Beans		Forages		Cassava	
	Shipments	Samples	Shipments	Samples	Shipments	Samples
CGIAR centers	23	1,106	10	52	23	1,530
Commercial companies	3	23	12	13	3	250
Farmers	1	1	71	220		
Genebanks						
NARS	7	274	12	72	2	23
NGOs			2	4	2	5
Regional organizations					2	12
Universities	8	925	8	153	8	219
Germplasm networks	---	---	---	---	---	---
Others	---	---	---	---	---	---
Total	42	2,329	115	514	40	2,039

Table 19. Distribution of germplasm during 2006 by purpose (01/01/2007–31/12/2007).

Purpose	Beans		Forages		Cassava	
	Shipments	Samples	Shipments	Samples	Shipments	Samples
Breeding	7	165	1	3	10	934
Agronomy	1	1	96	279	6	45
Applied research	7	925	7	30		
Basic research	27	1,238	8	158	22	1,055
Training	---	---	3	44	2	5
Others	---	---	---	---	---	---
Total	42	2,329	115	514	40	2,039

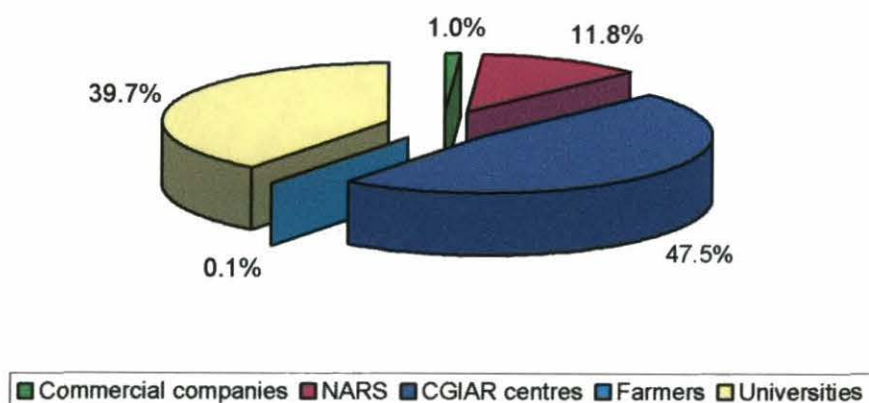


Figure 13. Distribution of bean seed germplasm by kind of users.

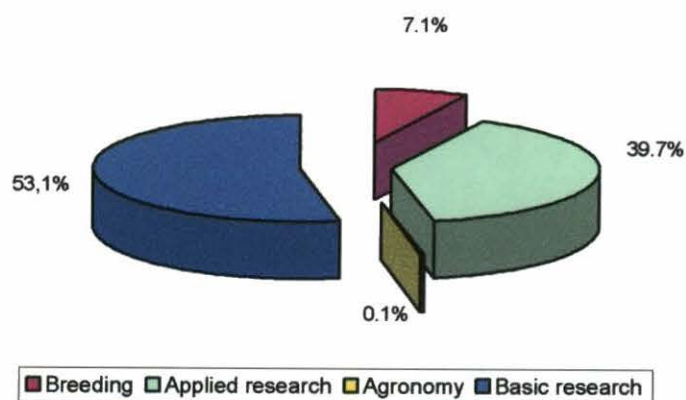


Figure 14. Distribution of bean seed germplasm by purposes.

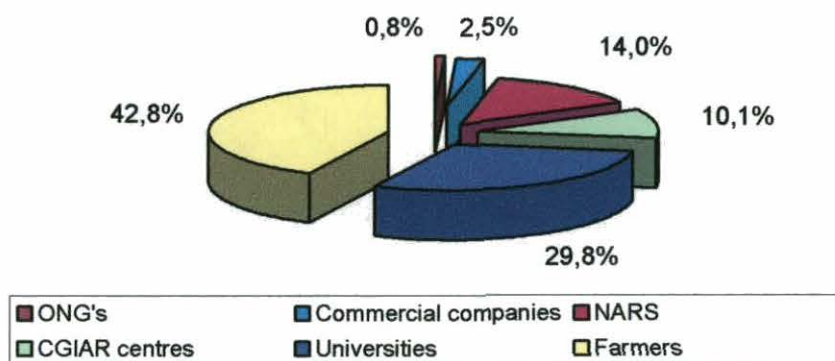


Figure 15. Distribution of forage seed germplasm by kind of users.

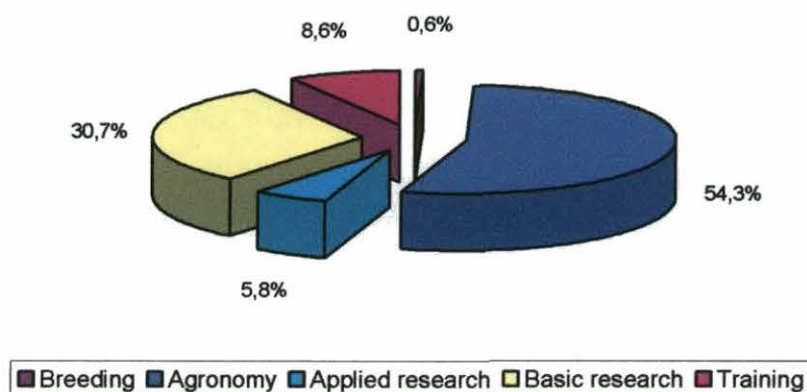


Figure 16. Distribution of forage seed germplasm by purposes.

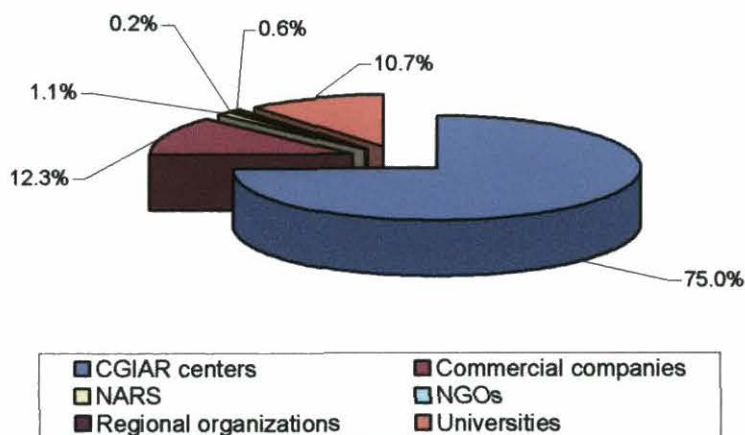


Figure 17. Distribution of *in vitro* cassava germplasm by kind of users.

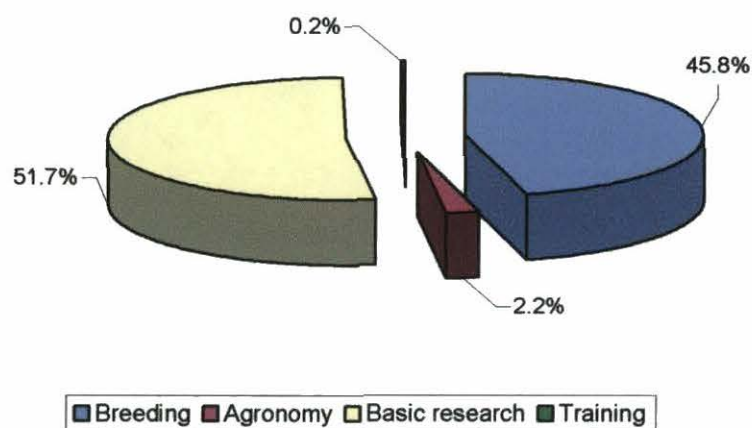


Figure 18. Distribution of *in vitro* cassava germplasm by purposes.

Contributors: C. Lima, G. Mafla, E. Aranzales, D.G. Debouck

Output 2.3. National collections restored to NARS

During 2007, the 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 safety backups

In 2007 we have shipped to CIP a total of 3,330 accessions of the *in vitro* cassava collection (3,044 accessions were re-shipped) (8,730 tubes), and we have received 1,620 accessions (3,240 tubes) of *in vitro* sweet potato sent by CIP for its safe keeping in Palmira. During the fall of 2007 a large shipment of seed collections (21,698 accessions of *Phaseolus* beans and 9,213 accessions of tropical forages, for a total of 30,911 accessions, or 52% of the seed accessions registered into the Multilateral System of the International Treaty) was prepared for its long-term conservation at the Svalbard Global Seed Vault (please see specific report); the shipment arrived in Svalbard on time for the opening of the Vault on February 26, 2008. An agreement between the Ministry of Agriculture and Food of the Kingdom of Norway and CIAT was signed on December 19, 2007. GRU has continued with its shipments of seed collections to CIMMYT, with 5,917 accessions sent in 2007 (3,725 accessions of beans and 2,192 accessions of tropical forages), summing up 17,601 accessions to date kept at El Batán.

Contributors: C. Lima, M. Cuervo, G. Mafla, M.S. Balcázar, E. Aranzales, D.G. Debouck

Output 2.5. Refined core collections

Activity 2.5.1. Biochemical characterization of *Phaseolus* germplasm bank for improved and refined collections.

In 2007, 2,056 genotypes of *Phaseolus vulgaris* L. and others *Phaseolus* species were analyzed for diversity of seed storage proteins using ID-SDS-PAGE electrophoresis. The analyzed accessions belong to the *Phaseolus* germplasm collection held at CIAT. This step together with morphoagronomic characterization is a requisite for improving the representativeness of the designated collection.

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

Subproject 3. The genetic and social relevance of the conservation

Output 3.1. Designated Collections better characterized

Activity 3.1.1. Marker-assisted reduction of genetic redundancy in germplasm collections: the case of the cassava world-wide collection held at CIAT.

Introduction

For cassava, a large number of possible genetic duplicates were identified using passport, morphological, and isozyme characterization (Ocampo et al. 1993; Jiménez 1994; Sumarani et al. 2004). The combination of molecular markers with morphology/ passport/ isozymes can give a high degree of confidence in identifying duplicates (Ocampo et al. 1995). We proposed to confirm these groups of possible genetic duplicates, using the technology of the single sequence repeat markers or SSR; that is, to detect genotypic differences among these groups that otherwise appear identical in their morphology and isozyme-banding patterns (Chavarriaga et al. 1999).

Materials and Methods

Plant material. This work has been initiated on the designate cassava germplasm collection of Colombia, consisting of 1,986 accessions (the largest collection by country). The *in vitro* Cassava Laboratory (GRU) provided the accessions to characterize according to their morphological and isozymatic similarities.

Molecular Markers. A set of seven SSR markers, carefully chosen to represent coverage of the cassava genome with moderate to high polymorphism information content (PIC) and robust amplification, were used in this study (Chavarriaga et al. 1998; Marin et al. 2003).

Results and Discussion

The molecular variation was assessed at seven unlinked SSR markers (Fregene et al. 2003) in 365 accessions of cassava landraces grouped into 133 groups of possible genetic duplicates according to their biochemical and morphological similarities. The number per group varied between 2, 3, 4, 5, 6, 8, 9 and 10 accessions, showing 232 redundant accessions among the 365 duplicated accessions (Tables 20 and 21). The molecular fingerprinting analysis confirms 93% of the morphobiochemical groups, showing 124 different molecular groups (between 2 and 9 accessions within of each group). There is an important reduction of 23% in the level of genetic redundancy: 202 redundant accessions versus the 232 original redundant accessions, which imply changing 365 duplicated accessions to 326 accessions with the molecular grouping (Table 21). Additionally, 39 accessions that are uniquely separated from other accessions represent unique genotypes. In conclusion, if these accessions are indeed genetically identical, they could be pooled together with no loss in the overall amount of genetic variation. Furthermore, the fact that most of these groups of possible genetic duplicates were confirmed by seven SSR markers, suggests that the model developed at CIAT to detect these duplicates is reliable. However it might be desirable to test more SSR markers to confirm with high reliability these possible genetic duplicates.

Table 20. Accessions involved as morphobiochemical duplicates versus molecular duplicates of the Colombian cassava collection held at CIAT.

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
1	COL 2150, COL 2191	1	COL 2150, COL 2191
2	COL 2241, COL 2245	2	COL 2241, COL 2245
3	COL 2249, COL 2545	3	COL 2249, COL 2545
4	COL 2283, COL 2295, COL 2348	4	COL 2283, COL 2295, COL 2348
5	COL 2303, COL 2503	5	COL 2303, COL 2503
6	COL 2308, COL 2356	6	COL 2308, COL 2356
7	COL 2498, COL 2499	7	COL 2498, COL 2499
8	COL 2512, COL 2514	8	COL 2512, COL 2514
9	COL 2523, COL 2533	9	COL 2523, COL 2533
10	COL 1647, COL 2120	10	COL 1647, COL 2120
11	COL 2341, BRA 730	11	COL 2341, BRA 730
12	COL 583, COL 646, COL 670 COL 70A, VEN 149	12	COL 583, COL 646,
		13	COL 670, COL 70A, VEN 149
13	COL 685, VEN 65	14	COL 685, VEN 65
14	COL 979, IND 11	15	COL 979
		16	IND 11
15	COL 1624, VEN 72, VEN 73, VEN 184	17	COL 1624, VEN 72, VEN 73, VEN 184
16	COL 1681, VEN 319	18	COL 1681, VEN 319
17	COL 1819, CUB 49	19	COL 1819, CUB 49
18	COL 2112, PER 327	20	COL 2112, PER 327
19	COL 2148, PER 198	21	COL 2148, PER 198
20	COL 159, BRA 495	22	COL 159, BRA 495
21	COL 25, COL 896	23	COL 25, COL 896
22	COL 45, COL 948C, COL 1008, COL 1431	24	COL 45, COL 948C
		25	COL 1008, COL 1431
23	COL 61, COL 1978	26	COL 61, COL 978
24	COL 70, COL 78B	27	COL 70, COL 78B
25	COL 76B, COL 912A, COL 927, COL 1962	28	COL 76B, COL 912A, COL 927, COL 1962
26	COL 81, COL 647, COL 1067, COL 106, COL 1538	29	COL 81, COL 647, COL 1067, COL 106, COL 1538
27	COL 93, COL 1044	30	COL 93, COL 1044
28	COL 134, COL 138	31	COL 134, COL 138
29	COL 2212, MAL 49	32	COL 2212
		33	MAL 49
30	COL 2609, CR 53	34	COL 2609, CR 53
31	COL 137, COL 140, COL 145	35	COL 137, COL 140, COL 145
32	COL 207, COL 1485	36	COL 207, COL 1485
33	COL 240, COL 281	37	COL 240
		38	COL 281

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
34	COL 261, COL 547	39	COL 261
		40	COL 547
35	COL 376, COL 380, COL 588A, COL 727	41	COL 376, COL 380, COL 588A, COL 727
36	COL 436, COL 2617	42	COL 436, COL 2617
37	COL 437A, COL 1934	43	COL 437 ^a , COL 1934
38	COL 467, COL 1720	44	COL 467, COL 1720
39	COL 942, COL 958, COL 1955	45	COL 942,
		46	COL 958, COL 1955
40	COL 1043, COL 1057, COL 1065	47	COL 1043, COL 1057, COL 1065
41	COL 1092, COL 1602, COL 1616, COL 1821	48	COL 1092, COL 1602,
		49	COL 1616, COL 1821
42	COL 2239, COL 1830, COL 1828A, COL 1518, COL 1516, COL 151		COL 2239, COL 1830, COL 1828A, COL 1518,
		50	COL 1516, COL 151
43	COL 684, COL 1515, COL 1831, COL 2256, COL 2288, COL 2644, COL 2645, COL 2656		COL 684, COL 1831, COL 2645, COL 2256, COL 2644,
		52	COL 2656, COL 1515, COL 2288
44	COL 724, COL 978, COL 265, COL 418A, COL 623, COL 588, COL 398, COL 278, COL 1791, COL 1807		COL 724, COL 265, COL 623, COL 588
		54	COL 398, COL 278
		55	COL 418A, COL 1807, COL 978, COL 1791
45	COL 971, COL 2241, COL 2245, COL 2547, COL 2627, COL 534B, COL 483, COL 485, COL 1793		COL 971, COL 2241, COL 2245, COL 2547, COL 2627, COL 534B, COL 483, COL 485, COL 1793
		56	
46	COL 87, COL 154, COL 155, COL 986, COL 1055, COL 1636	57	COL 87, COL 154, COL 155, COL 986, COL 1055, COL 1636
47	COL 2454, COL 2584, COL 2587	58	COL 2454, COL 2584, COL 2587
48	COL 466, COL 532, COL 1190	59	COL 466, COL 532, COL 1190
49	COL 2508, COL 2474, COL 2480	60	COL 2508, COL 2474, COL 2480
50	COL 225, COL 655, COL 656, COL 718, COL 445B	61	COL 225, COL 655, COL 656, COL 718, COL 445B
51	COL 2434, COL 2446, COL 2459, COL 689B	62	COL 2434, COL 2446, COL 2459, COL 689B
	COL 1691, COL 2457, COL 2468, COL 1356B	63	COL 1691, COL 2457, COL 2468, COL 1356B
52		64	COL 2438, COL 2506
53	COL 2438, COL 2506		COL 1140, COL 1241, COL 2020, COL 2439
54	COL 1140, COL 1241, COL 2020, COL 2439	65	
55	COL 638, COL 1148	66	COL 638, COL 1148
56	COL 2057, COL 1040	67	COL 2057, COL 1040
57	COL 211, COL 222A	68	COL 211, COL 222A
58	COL 2588, COL 2069	69	COL 2588, COL 2069
59	COL 1690, COL 2600	70	COL 1690, COL 2600

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
60	COL 1695, COL 2081	71	COL 1695
61	COL 1688, COL 1696, COL 2568	72	COL 2081
62	COL 1601, COL 1990	73	COL 1688, COL 1696, COL 2568
63	COL 2282, COL 2297, COL 2375, COL 2390	74	COL 1601, COL 1990
64	COL 2286, COL 2292, COL 2298, COL 2300, COL 2313	75	COL 2282,
65	COL 1672, COL 1673, COL 1678	76	COL 2297, COL 2375, COL 2390
66	COL 1711, COL 1764A, COL 1764B	77	COL 2286, COL 2292, COL 2298, COL 2300, COL 2313
67	COL 1772, COL 1777, COL 1781, COL 1895	78	COL 1672, COL 1673, COL 1678
68	COL 1786, COL 1879, COL 2023	79	COL 1711
69	COL 1889, COL 1893, COL 1894	80	COL 1764A, COL 1764B
70	COL 1896, COL 1900, COL 2062	81	COL 1777,
71	COL 1901, COL 1902, COL 1903	82	COL 1781, COL 1895, COL 1772,
72	COL 2358, COL 2362, COL 2407	83	COL 1879,
73	COL 275, COL 290	84	COL 1786, COL 2023
74	COL 280, COL 2542	85	COL 1889,
75	COL 286, COL 328	86	COL 1893, COL 1894
76	COL 303, COL 306	87	COL 1896,
77	COL 344, COL 386	88	COL 1900, COL 2062
78	COL 475, COL 1452	89	COL 1901, COL 1902, COL 1903
79	COL 476, COL 494	90	COL 2358, COL 2362, COL 2407
80	COL 487, COL 509	91	COL 275, COL 290
81	COL 488, COL 490	92	COL 280,
82	COL 654, COL 667A	93	COL 2542
83	COL 661, COL 663	94	COL 286, COL 328
84	COL 671, COL 673A	95	COL 303, COL 306
85	COL 683, COL 1442	96	COL 344, COL 386
86	COL 777, COL 778	97	COL 475, COL 1452
87	COL 796, COL 1486	98	COL 476, COL 494
88	COL 800, COL 803	99	COL 487, COL 509
89	COL 902A, COL 902B	100	COL 488, COL 490
90	COL 844, COL 845A	101	COL 654,
91	COL 948A, COL 1967	102	COL 667A
92	COL 957B, COL 957C	103	COL 661, COL 663
93	COL 978, COL 974A	104	COL 671, COL 673A
		105	COL 683,
		106	COL 1442
		107	COL 777, COL 778
		108	COL 796, COL 1486
		109	COL 800, COL 803
		110	COL 902A, COL 902B
		111	COL 844, COL 845A
		112	COL 948A, COL 1967
		113	COL 957B,
		114	COL 957C
		115	COL 978,
		116	COL 974A

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
94	COL 1019, COL 1023	117	COL 1019, COL 1023
95	COL 1231, COL 1347	118	COL 1231, COL 1347
96	COL 1409, COL 1413	119	COL 1409, COL 1413
97	COL 1440, COL 1917	120	COL 1440, COL 1917
98	COL 1463, COL 2189	121	COL 1463, COL 2189
99	COL 1471, COL 1472	122	COL 1471, COL 1472
100	COL 1478, COL 2305	123	COL 1478, COL 2305
101	COL 1504, COL 1632	124	COL 1504, COL 1632
102	COL 1505, COL 2054	125	COL 1505, COL 2054
103	COL 1513, COL 1514	126	COL 1513, COL 1514
104	COL 1552, COL 1553	127	COL 1552, COL 1553
105	COL 1563, COL 1564	128	COL 1563, COL 1564
106	COL 1566, COL 1717	129	COL 1566, COL 1717
107	COL 1607, COL 10	130	COL 1607,
		131	COL 10
108	COL 1613, COL 1614	132	COL 1613, COL 1614
109	COL 1630, COL 1745	133	COL 1630,
		134	COL 1745
110	COL 1667, COL 1671	135	COL 1667, COL 1671
111	COL 1823, COL 2229	136	COL 1823, COL 2229
112	COL 1868A, COL 1868B	137	COL 1868A, COL 1868B
113	COL 1884, COL 1898	138	COL 1884, COL 1898
114	COL 1912, COL 1915	139	COL 1912, COL 1915
115	COL 2004, COL 2007	140	COL 2004, COL 2007
116	COL 2025, COL 2033	141	COL 2025,
		142	COL 2033
117	COL 2082, COL 2203	143	COL 2082, COL 2203
118	COL 2107, COL 2114	144	COL 2107, COL 2114
119	COL 2143, COL 2147	145	COL 2143, COL 2147
120	COL 1667, COL 1671, COL 1672, COL 1678, COL 1675, COL 1703, COL 1775, COL 2448,	146	COL 1667, COL 1671, COL 1672, COL 1678, COL 1675, COL 1703, COL 1775, COL 2448,
		147	COL 870
121	COL 870, COL 1046	148	COL 1046
		149	COL 2312, COL 2313
122	COL 2312, COL 2313	150	COL 214, COL 964, COL 1098, COL 1429, COL 543A
123	COL 214, COL 964, COL 1098, COL 1429, COL 543A		COL 115, COL 440, COL 1890,
			COL 2414, COL 511,
124	COL 115, COL 511, COL 440, COL 1890, COL 2414	151	COL 115, COL 440, COL 1890,
125	COL 1478, COL 1479	152	COL 2414, COL 511,
126	COL 213, COL 217, COL 1061, COL 2609, COL 894A, COL 1822B	153	COL 1478, COL 1479
		154	COL 213, COL 217, COL 1061, COL 2609, COL 894A, COL 1822B
127	COL 960, COL 962, COL 963		155

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
128	COL 1424, COL 2034, COL 2055, COL 1828B	156	COL 1424, COL 2034, COL 2055, COL 1828B
129	COL 2585, COL 1698	157	COL 2585
130	COL 1138, COL 548B	158	COL 1698
131	COL 436, COL 2061, COL 1787B	159	COL 1138, COL 548B
132	COL 763, COL 1112A	160	COL 436, COL 2061, COL 1787B
133	COL 1692, COL 1676	161	COL 763
		162	COL 1112A
		163	COL 1692, COL 1676

Table 21. Description of the morphobiochemical duplicates versus molecular duplicates of the Colombian cassava germplasm collection held at CIAT.

Type of Group	Morphobiochemical duplicates			Molecular duplicates			
	No. of each group	Duplicated accessions	Redundant accessions	No. of each group	Duplicated accessions	Redundant accessions	Single accessions
Groups containing 2 similar accessions	92	184	92	88	176	88	39
Groups containing 3 similar accessions	16	48	32	16	48	32	
Groups containing 4 similar accessions	11	44	33	9	36	27	
Groups containing 5 similar accessions	6	30	24	5	25	20	
Groups containing 6 similar accessions	4	24	20	4	24	20	
Groups containing 8 similar accessions	2	16	14	1	8	7	
Groups containing 9 similar accessions	1	9	8	1	9	8	
Groups containing 10 similar accessions	1	10	9	0	0	0	
Total	133	365	232	124	326	202	39

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Activity 3.1.2. Phaseolin variability of the Nicaraguan bean collection held at CIAT, as a contribution to its conservation and use.

Introduction

Tapia (1987) reported the presence of three species of *Phaseolus* for Nicaragua, namely *P. vulgaris*, *P. acutifolius* and *P. lunatus*. Out of these three species, *P. vulgaris* is the most important and is distributed in all agroecological zones of the country. There is a large number of common bean landraces in Nicaragua. Numerous collection missions have been carried at different points in time (1952, 1960, 1980s, 1990s) and most of the landraces collected are stored in genebanks of different international and national institutions. Common bean landraces are an important component of the cropping systems of the Nicaraguan small-scale farmers. They have also been used in breeding programs for the development of improved cultivars. The general purpose of this work was to complement the knowledge already available

about the Nicaraguan bean (Gómez et al. 2004). A specific goal was to know the phaseolin variability of the Nicaraguan collection held at CIAT as a contribution to their conservation and use. Additionally, this information can help to plan new collections of Nicaraguan germplasm.

Materials and Methods

Three hundred thirty five accessions of common bean (*Phaseolus vulgaris* L.) are available from the Nicaraguan collection held in CIAT, which include 329 landraces, 5 commercial varieties and one bred line. To facilitate the characterization and later use of Nicaraguan germplasm, a “tentative core collection” was formed. In total, 148 accessions of common bean were selected using three criteria to represent the total diversity of the species in this country. These criteria are: (1) All the cultivars selected are originally from Nicaragua and are representative of the geographic distribution of the species in this country, mainly in the most important regions of bean production. (2) The most common land races. (3) The agromorphological and agroecological data were utilized as indicators of genetic diversity. Finally, the passport data were compiled where it was possible in order to select these 148 accessions. The seed storage proteins were analyzed using the 1D-SDS-PAGE technique (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O’Farrel, 1975).

Results and Discussion

Four electrophoretic types of phaseolin were found among the 148 Nicaraguan common bean accessions analyzed. The ‘CH’ phaseolin was present at the highest frequency (64%), followed by the ‘S’ type with 35%, and the ‘T’ and ‘C’ types with 1% (Table 22). This diversity in the phaseolins possibly indicates that the bean cultivated in Nicaragua has not experienced an important genetic interchange between the Mesoamerican and Andean gene pools, as it has happened in other American regions (Paredes and Gepts, 1995; González et al. 2003; and Toro and Ocampo, 2004). In addition, a founder effect is suggested by the fact that greater variability (99%) in the phaseolins, only happens with the ‘CH’ and ‘S’ patterns. The ‘CH’ phaseolin was present in 94 accessions, reporting an extensive distribution in the most important regions for Nicaraguan bean production, especially the zones that surround the Nicaraguan lakes (Managua and Nicaragua) and the North region that borders with Honduras (Figure 19). The ‘S’ and ‘CH’ types are typical of the small and medium seeded Mesoamerican beans. These are of particular interest to breeders and agronomists since they may represent more than 65% of world bean production (CIAT data). Therefore, understanding genetic diversity within the Nicaraguan germplasm collection has important implications for genetic improvement of the common bean in the Mesoamerican lowlands.

Table 22. Geographic distribution of the types of phaseolin found in 148 accessions of *Phaseolus vulgaris* L. collected in Nicaragua.

Department/Types of phaseolin	CH	S	T	C	Total
Boaco	4	6			10
Carazo	6	8			14
Chinandega	12	3			15
Chontales	8	2			10
Esteli	8	2			10
Granada	16	6			22
Jinotega	18	2		1	21
Madriz	8				8
Masaya	2	2			4
Matagalpa	6	4			10
Leon		2			2

Department/Types of phaseolin	CH	S	T	C	Total
Managua		6	2		8
Nueva Segobia		4			4
Rivas	6	4			10
Total	94	51	2	1	148



Figure 19. Geographic distribution of the phaseolins present in 148 accessions of the Nicaraguan common bean collection held at CIAT.

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Activity 3.1.3. Introducing new germplasm in the collection held in CIAT: the case of the Chilean common bean collection.

Introduction

It is desirable for a genebank to introduce new germplasm accessions so it keeps up to date. Before sensible conservation decisions can be made (as the introduction of new germplasm in the collection), we need a basic understanding of the genetic diversity of the plant group awaiting introduction. In the present study, we analyzed new Chilean common bean germplasm acquisitions, from a biochemical (phaseolin marker) and morphological viewpoint to estimate their variability and compare with the phaseolin variability reported for the Chilean common bean germplasm (Paredes and Gepts, 1995).

Materials and Methods

The common bean materials analyzed here were obtained from the collected populations throughout all Chile by O. Voysest, S. Singh and F. Morales. The samples were analyzed in 1D-SDS-PAGE (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975). For the analysis of morphological traits of seed phenotypes, we focused on seed size, seed shape, 100-seed weight, color and color pattern.

Results and Discussion

The genetic diversity of 1,144 representative Chilean common bean seeds was analyzed using phaseolin marker. Six types of phaseolin were found, 'H1', 'S', 'T', 'C', 'Sb' and 'Sd', in decreasing order of frequency. The 'H1' phaseolin was present at the highest frequency (33.6 %—383 seeds), followed by the 'S' type with 32% (363 seeds), 'T' type with 23% (269 seeds), 'C' type with 11% (125), 'Sb' type with 0.3% (3 seeds) and 'Sd' type with 0.1% (1 seed). Comparing these results with those obtained by Paredes and Gepts (1995), one sees that phaseolin variability is different between these two research works. Paredes and Gepts found the following phaseolin frequencies in 95 analyzed Chilean common bean landraces: The 'C' phaseolin was present at the highest frequency (59.9%), followed by the 'T' type with 11.4%, 'S' type with 9.2% and 'H1' type with 3.9%. The rest of the percentage corresponds to accessions that were heterogeneous for the phaseolin type. The most outstanding fact is that the 'H1' phaseolin for us has the greater frequency, however for Paredes and Gepts (1995), had the smaller frequency. Also for our results, the phaseolin introgression of Middle American germplasm into Chilean common bean was greater than the obtained by Paredes and Gepts (1995). However, the phaseolin analysis of these 1,144 Chilean common bean seeds confirmed a previous report (Gepts et al. 1986) that there are four basic types of phaseolin among Chilean landraces: 'C', 'T', 'S' and 'H'. Finally, the new phaseolin variants for the Chilean beans ('Sb' and 'Sd') can be introduced in the Chilean collection. Therefore, our results confirm the Andean origin of the collected populations throughout all Chile by O. Voysest, S. Singh and F. Morales, with an extensive phaseolin introgression of Middle American germplasm into Chilean common bean.

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

Activity 3.1.4. A new map for the distribution of wild-weed-crop complexes of common bean in Colombia.

Introduction

The wild-weedy-cultivated complexes may be important mechanisms for the generation of genetic variability in landraces. Such complexes have also been observed in *Phaseolus vulgaris* L, in both Middle American and Andean gene pools (Beebe et al. 1997; González et al. 2003; and Toro and Ocampo, 2004; Toro et al. 2007a). In the present study, we report the finding of new complexes (wild-weedy-cultivated) of common bean in Colombian regions where these have not been reported previously. We analyzed these complexes from a biochemical (phaseolin and isozyme markers) and morphological viewpoint to estimate the variability as a contribution to their conservation and use.

Materials and Methods

Plant material. Ten wild-weedy-crop complexes were selected after a geographic sampling in Colombia. In addition, three accessions were chosen as controls: two cultivated *P. vulgaris* from the Andes and Mesoamerica (G4494 and G5773, respectively) and a Colombian wild (G24408). For the morphological and biochemical analysis, we only took the multiplied and conserved seed in the *Phaseolus* germplasm bank held in CIAT (Table 23).

Morphological analysis. For the analysis of morphological traits of seed phenotypes, we focused on seed size, seed shape, 100-seed weight, color and color pattern.

Biochemical analysis. The seed storage proteins were analyzed as selfed materials of phaseolin type found for each analyzed seed. This variation was first analyzed in 1Di-SDS-PAGE (Brown et al. 1981) and confirmed later in 2Di-IEF-SDS-PAGE (O'Farrel, 1975). For the isozyme analysis only a complex was selected (G50849), being used for it thirty selfed materials of phaseolin type. We used only two polymorphic enzymatic complexes: peroxidase (PRX; 1.11.1.7) and diaphorase (DIA; 1.6.4.1). The methodology for isozyme analysis was the one reported by Ramirez et al. (1987).

Results and Discussion

Seed morphological variation of the complexes. The original seed of these populations was collected and classified as cultivated materials. However, during the initial seed increase, we observed segregation for seed size and colors indicating possible wild-weedy-crop complexes. The materials (1,182 in total) were classified as cultivated [642 (54%)], intermediate [432 (37%)] and wild [108 (9%)] (Table 23). These

segregating populations were considered to be complexes, since they involve wild and weedy stabilized forms. These complexes showed a great diversity in seed size (from small to large) and color.

Seed protein variation of the complexes. A great diversity for phaseolin types was found within these complexes. The patterns found so far were: five Andean (C, T, H1, H2 and Ca), two Mesoamerican-Colombian (B, CH), a Mesoamerican (S) and three Colombian (L, Car and Mu), with a frequency of 55%, 20%, 21% and 4% respectively. In these complexes, the 'S', 'B', 'C', 'T', and 'Mu' phaseolins form a continuum across the full range of biological status. The 'C' phaseolin was present at the highest frequency (30%). Followed by the 'S' type with 21%, 'T' type with 20% and 'B' type with 17%. The higher occurrence of Andean phaseolin types (55%) compared to that of Middle American 'S' phaseolin type (21%) might have resulted from a selection for larger seed size in the sampled Colombian regions for this study (Table 23). The phaseolin types such as 'L' (Beebe et al. 1997), 'Mu' and 'Car' (Toro et al. 2007b) have been found so far only in Colombian materials.

Table 23. Description of the wild-weed-crop complexes from domesticated Colombian populations of common bean.

CIAT No.	Department	Generation Go (seed original)		Generation advanced (increased seed)	
		S. W. ¹	Gene pool	B. S. ²	Phaseolin types (frequency in parenthesis)
G50711	Antioquia	64.2 g.	Andean	Cultivated Weedy Wild	S (1), B (2), C (4), CAR (2) S (6), B (2), C (5), H ₁ (1) S (5), C (3)
G50849	Antioquia	31.0 g.	Mesoamerican	Cultivated Weedy Wild	S (37), C (41), H ₁ (6), H ₂ (3), T (4) S (15), C (6), H ₁ (2), H ₂ (1) S (6), C (3)
G50632	Antioquia	50.5 g.	Andean	Cultivated Weedy Wild	S (36), CH (5), C (41), T (55), L (1) S (3), B (17), C (3), T (1) B (6), T (1)
G50646	Antioquia	64.8 g.	Andean	Cultivated Weedy Wild	S (14), B (2), CH (1), T (37), C (24), H ₁ (1), H ₂ (1) S (13), T (9), C (5) T (1), C (6)
G50785	Antioquia	60.6 g.	Andean	Cultivated Weedy Wild	S (16), B (3), CH (1), C (41), T (67), H ₁ (8) S (19), B (12), CH (10), T (30), C (45), H ₁ (2) S (4), B (4), CH (3), T (5), C (13)
G50879	Caldas	62.5 g.	Andean	Cultivated Weedy Wild	B (13), C (49), T (2), H ₁ (22), H ₂ (1) B (16), C (4), H ₁ (4) B (2), C (1), H ₁ (1)
G50983	Cundinamarca	21.0 g.	Mesoamerican	Cultivated Weedy Wild	S (6), C (2), Mu (1) S (24), B (48), CH (13), C (9), H ₂ (1), Mu (34) S (3), B (2), Mu (1)
G50988	Boyacá	35.4 g.	Andean	Cultivated Weedy Wild	S (3), T (2), C (10), H ₁ (5) S (10), C (4), H ₁ (2) S (5), C (6), H ₁ (1)
G50797	Tolima	61.0 g.	Andean	Cultivated Weedy Wild	S (1) S (6), C (3), H ₁ (4) S (9), C (2), H ₁ (4)
G50859	Cauca	33.0 g.	Andean	Cultivated Weedy Wild	S(5), B(24),T(10),C(18),Ca ₁ (4),H ₁ (2),H ₂ (1),Car (7) B (36), C (6), H ₁ (1) B (11)

¹S. W.: Is the seed weight derived from 100 seeds.

²B. S.: Biological Status.

Isozyme variation of the complexes. For the isozyme analysis, both allozymes (Mesoamerican and Andean) are found in the analyzed complex (G50849). The selected isozyme loci carry alleles from both Mesoamerican and Andean gene pools: The Dia-1 (95), PRX98 alleles are considered to be Mesoamerican and the Dia-1 (100), PRX100 alleles are of Andean origin (Koenig and Gepts, 1989;

Debouck et al. 1993). Nevertheless, only two allozymes were found in all phases of the complex: a heterozygote allozyme (PRX 100/98) and an Andean allozyme (Dia-1 [100/100]) (Table 24).

Table 24. Allozyme constitution and seed size of the wild-weedy-crop complex G50849.

Biological material	Analyzed "selfed materials"	100 seed weight (g)	Isozyme loci	
			Prx	Dia-1
G50849 Cultivated	23	23.4-47.8	100 (5) 98 (14) 100/98 (4)	100 (17) 95 (6)
G50849 Weedy	4	10.0-24.0	100 (0) 98 (3) 100/98 (1)	100 (4) 95 (0)
G50849 Wild	3	5.3-7.2	100 (1) 98 (0) 100/98 (2)	100 (3) 95 (0)

Conclusions

The variability at the phaseolin and isozyme levels suggests an important genetic interchange in the study area in Colombia between Mesoamerican and Andean materials. The presence of these complexes that are apparently hybrids between the two gene pools, can be explained by the existence of true wild forms and landraces in Colombia with "S" phaseolin and "CH" (Mesoamerican) and "T" (Andean). These results are concordant with those obtained by Debouck et al. (1993); Paredes and Gepts (1995) and Beebe et al. (1997), using morphological and biochemical markers, and those obtained by Tohme et al. (1996), Chacón et al. (2002), and Ocampo et al. (2005), using molecular markers. However, we are reporting an extensive distribution of these introgressed complexes in Colombia, much more than those reported by Beebe et al. (1997). This distribution includes some departments where wild and cultivated beans are sympatric (Cundinamarca and Boyacá), or in departments where the common bean is an important crop (Antioquia, Caldas, Tolima and Cauca). These results suggest a new map in Colombia for the distribution of these biological complexes of common bean and confirm that a considerable amount of natural hybridization occurs in the areas where these populations were collected (Figure 20).

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Toro, O., Ocampo, C.H., D.G. Debouck. 2007b. Annu. Rept. Bean Improvement Coop. (USA) 50: 69-70.

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

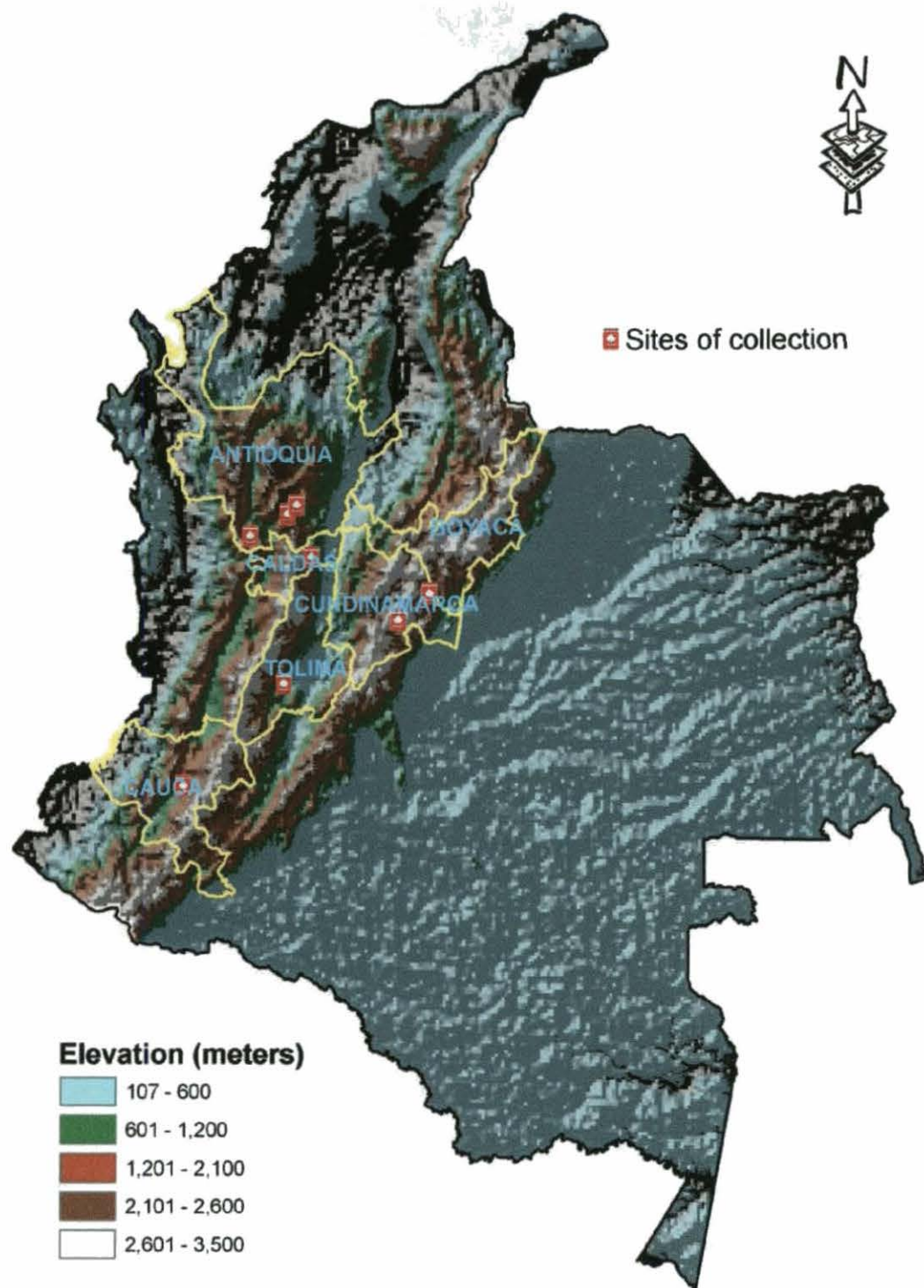


Figure 20. New map for the distribution of the wild-weedy-crop biological complexes of common bean in Colombia.

Activity 3.1.5. Use of a “diagnostic isoenzyme” (aspartate aminotransferase) for broadening the genetic base of crops: the case of the interspecific hybridization between *Phaseolus acutifolius* A. Gray and *P. parvifolius* Freytag.

Introduction

The section of *Phaseolus* currently including the tepary bean, i.e. the *Acutifolii*, consists of two species: *Phaseolus acutifolius* A. Gray (with three varieties: var. *acutifolius*, var. *latifolius* and var. *tenuifolius*) and *P. parvifolius* Freytag (Freytag & Debouck 2002). In a study of 100 accessions with ten enzyme systems, Flores et al. 2003 found that the allele *Aat-2*⁹⁵ uniquely separates the twenty ‘*parvifolius*’ materials from the rest of wild teparies. The tepary bean (*P. acutifolius*) is known to possess high levels of resistance to several abiotic and biotic stresses. To assess introgression of *P. parvifolius* alleles in the tepary bean, the parents (*P. parvifolius* and *P. acutifolius* var. *tenuifolius*) and its offspring were analyzed for the aspartate aminotransferase isoenzyme as a “diagnostic marker” to verify this introgression.

Materials and Methods

The parents (*P. parvifolius* and *P. acutifolius* var. *tenuifolius*) and its offspring (F1 and F2) were analyzed for the aspartate aminotransferase isoenzyme (AAT; E. C. 2.6.1.1). The methodology for isozyme extraction, running and staining was the one reported by Ramirez et al. (1987). For each allozyme, loci and alleles were designated as described by Koenig & Gepts (1989). Additionally, an agromorphological analysis was made on the plant architecture to compare them with the isozyme data.

Results and Discussion

In agreement with genetics of Aat isozyme (Garvin & Weeden 1994; Garvin et al. 1989), the Aat-2 locus has three alleles (93, 95 and 100) with diploid inheritance. However, in the present study we only found the alleles 95 (*P. parvifolius*) and 100 (*P. tenuifolius*) (Figure 21). The allele *Aat-2*⁹⁵ is present exclusively in *P. parvifolius* (Flores et al. 2003).

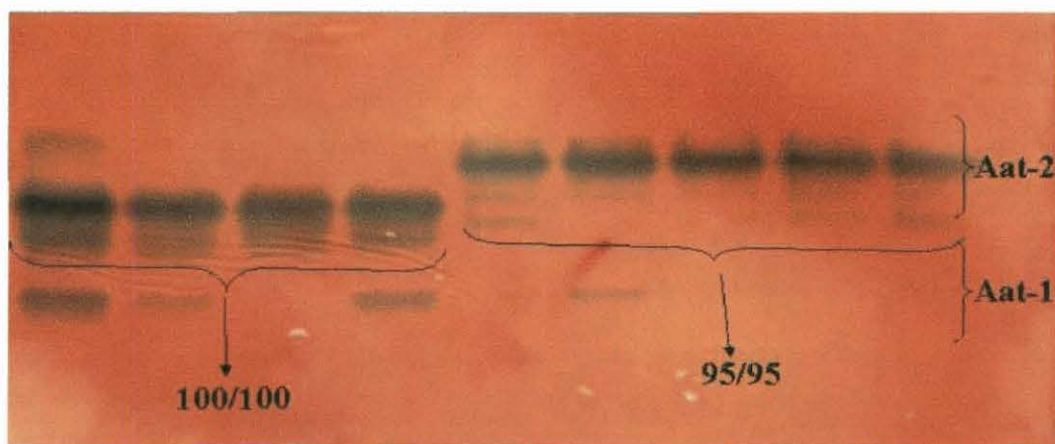


Figure 21. Polyacrylamide gel phenotypes observed for aspartate aminotransferase (AAT). Individuals in the first four lanes are *P. acutifolius* var. *tenuifolius* (genotype Aat-2: 100/100). The rest are classified as *P. parvifolius* (genotype Aat-2: 95/95).

Verification of initial hybrids and evidence of gene introgression. Monitoring of introgression of genes into the progenies coming from the crossing between *P. acutifolius* var. *tenuifolius* and *P. parvifolius*, was carried out by analyzing the distribution of alleles found for the analyzed AAT isozyme loci (Table 25). This isozyme was used as a “diagnostic marker” to verify this introgression. The Aat-2 locus is homozygous in both parental (Flores et al. 2003), which guarantees the verification of hybrids.

Introgression into the hybrid progenies or F1 was ascertained in all individuals, except one (Table 25), (Figure 22). The presence of both parental alleles (Aat-2⁹⁵ and Aat-2¹⁰⁰) in the progenies F1 [Aat-2 (95/100)], confirms their hybrid origin. To assess the stability of introgression, lineages F2 also were analyzed (locus Aat-2). Assuming a free combination of alleles for the expected frequencies, in the progeny F1 there is a high-observed frequency of the alleles in heterozygotes, but very low in homozygotes. In contrast, in the F2 progenies, the observed allelic frequency in heterozygotes decreases, but there is an increase in the homozygotes (Table 25). Therefore, the observed values are close to the ideal values to be expected if the alleles had combined freely after a cross. These genetic results suggest a successful introgression between *P. acutifolius* var. *tenuifolius* and *P. parvifolius*. In this work we have generated the largest number of *P. acutifolius* var. *tenuifolius* and *P. parvifolius* fertile hybrids to date genetically. We have also demonstrated high frequencies of *P. parvifolius* alleles introgression. Finally, these genetic data are due to compare with the agromorphological information to confirm this successful genetic introgression

Table 25. Distribution of electromorphs found for AAT isozyme¹ in the produced lineages of the crossing between *P. acutifolius*, var. *tenuifolius* and *P. parvifolius*.

Biological material (Parents and progenies)	Biological Status	¹ Loci/alleles/individuals				
		Aat-1		Aat-2		
		100/ n/n ²		95/95	100/95	100/100
<i>P. parvifolius</i> (Parent) ³	Wild	X	---	X	---	---
<i>P. acutifolius</i> var. <i>tenuifolius</i> (Parent) ³	Wild	X	---	---	---	X
Progeny F1-1 (cross <i>P. acutifolius</i> var. <i>tenuifolius</i> X <i>P. parvifolius</i>)	Soon would be determined	11	1	0	11	1
Progeny F1-2 (cross <i>P. parvifolius</i> X <i>P. acutifolius</i> var. <i>tenuifolius</i>)	Soon would be determined	2	0	0	2	0
Progeny F2 (cross F1-1 X F1-1)	Soon would be determined	12	1	4	6	3
Progeny F2 (cross F1-2 X F1-2)	Soon would be determined	13	0	3	8	2

¹ The genetics of AAT isozyme has been reported by Garvin and Weeden (1994), with three zones of migration observed. Nevertheless, we observed only two zones of migration (Flores et al. 2003).

² A null allele has been reported in tepary bean.

³ These are female parents as much male.

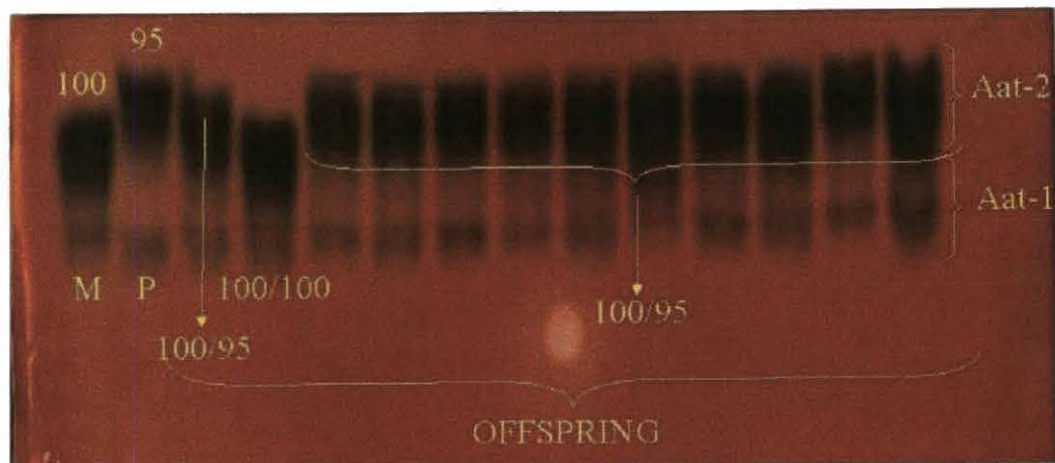


Figure 22. Aat-2 analysis in the cross *P. acutifolius*, var. *tenuifolius* X *P. parvifolius* and its progeny. Alleles involved are Aat-2⁹⁵ and Aat-2¹⁰⁰. Inheritance data are shown in Table 25.

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

Output 3.2. Novel materials acquired or collected

Activity 3.2.1. Germplasm exploration carried out in Nicaragua in December 2007.

In the framework of a cooperation on in situ conservation between a NGO (CIPRES, Nicaragua), the University of Costa Rica, and CIAT, and thanks to the support of Utviklingsfondet of Norway, a germplasm exploration was carried out in five departments of northwestern Nicaragua in December 2007. In one week of fieldwork, the team found 24 populations for four wild species of *Phaseolus* (*P. leptostachyus*, *P. lunatus*, *P. oligospermus*, and *P. vulgaris*), all new floristic records for Nicaragua. In addition, 116 herbarium specimens were collected for their distribution to HNMN, O, BM, EAP, F and MEXU. Each population was evaluated for its vulnerability and the practical possibilities of *in situ* conservation versus the possibilities of conservation in a genebank (please see specific report).

Contributors: D.G. Debouck (CIAT), R. Araya (UCR), R. Herrera (CIPRES).

Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

A detailed list of individual trainees who received specialized training can be found in Annex 6.

Contributors: C. Lima, G. Mafla, C. Ocampo, D.G. Debouck

Output 4.2. Conferences in national/international fora

A total of fifteen conferences were presented in national/international fora. Please see full list in Annex 6.

Output 4.3. Public awareness products

With the shipment of a safety backup of 30,911 accessions to the Svalbard Global Seed Vault, technical elements were communicated to the written media (11), radios (5), videos (4), and television (3).

Contributor: D.G. Debouck (together with E. Hess and E. Figueroa of CIAT Communications Unit)

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: AGUAT, BR, COL, L, MEXU, MICH, O, PH, and WIS. That information has been 'repatriated' to CONABIO of Mexico and INBio of Costa Rica. The information 'Cahiers de Phaséologie' has been put on CIAT web site for the sections: *Acutifolii*, *Chiapasana*, *Coriacei*, *glabellus*, *microcarpus*, *Minkelersia*, *Revoluti*, and *Rugosi*.

Contributor: D.G. Debouck

6. Annexes

6.1. List of publications by Project Staff in 2007

A. In refereed journals:

Chacón S. M. I., Pickersgill B., Debouck D.G. & Salvador Arias J. 2007. Phylogeographic analysis of the chloroplast DNA variation in wild common bean (*Phaseolus vulgaris* L.) in the Americas. *Plant Systematics and Evolution* 266 (3-4): 175-195.

B. In non-refereed journals:

Tofiño, A., C.H. Ocampo & M. Blair. 2007. Association between biochemical descriptors and the pod fibrousness in the characterization of snap bean germplasm for Latin American fresh consumption. *Annu. Rept. Bean Improvement Coop. (USA)* 50: 63-64.

Toro, O., C.H. Ocampo & D.G. Debouck. 2007. Phaseolin: variability and reference materials in wild and cultivated common bean. *Annu. Rept. Bean Improvement Coop. (USA)* 50: 69-70.

C. As conference proceedings:

Toro, O., C.H. Ocampo & D.G. Debouck. 2007. Additional evidence suggests a new map for the distribution of wild-weed-crop complexes of common bean in Colombia. VI Simposio Internacional sobre los Recursos Genéticos de América Latina y el Caribe (SIRGEALC). 13-16 November 2007, Mexico City, D.F., México. p. 28.

6.2. List of thesis research supervised by Project Staff in 2007

Khoury, Colin Kahlil. 2007. An ecogeographic survey and conservation analysis for arid North American *Phaseolus* L. (Fabaceae), sections *Acutifolii*, *Coriacei*, *Minkelersia* and *Rugosi* species. Master of Science degree. University of Birmingham, School of Biological Sciences, Birmingham, United Kingdom.

Mina Vargas, Angela. 2007. Phylogeny of the section *Phaseoli* of the genus *Phaseolus* with help of microsatellite markers. University College, Cork, Cork, Ireland.

6.3. List of conferences and scientific communications presented by Project Staff in 2007

1. Cuervo M., N. Villareal, I. Lozano, L.A. Calvert. 2007. Caracterización molecular de algunos aislamientos del virus de cuero de sapo de la yuca recolectados en diferentes zonas de Colombia. Congreso ASCOLFI, CIAT, Palmira, Colombia, October 4 2007.

2. Debouck, D.G. Managua, Nicaragua, 10 December 2007, invited seminar: "Perspectivas abiertas por el Tratado Internacional de Recursos Fitogenéticos para Alimentación y Agricultura y reflexiones".

3. Debouck, D.G. Managua, Nicaragua, 10 December 2007, invited seminar: "Colecta de frijoles cultivados como fuente de opciones".

4. Debouck, D.G. Managua, Nicaragua, 10 December 2007, invited seminar: "Exploración de germoplasma silvestre para aumentar la variabilidad genética".
5. Debouck, D.G. Mexico, D.F., Mexico, 15 November 2007, invited lecture in the 6th SIRGEALC: "Los caminos de los Mazatl, Ayotochtli, Ahuatl, Yetl y Etl: flujos genéticos de largo alcance y sus consecuencias".
6. Debouck, D.G. Madison, Wisconsin, USA, 2 November 2007, invited seminar at the Botany Department: "Travels of beans since the late Tertiary: why it matters".
7. Debouck, D.G. Philadelphia, Pennsylvania, USA, 14 September 2007, invited seminar at the Academy of Natural Sciences: "Migrations of *Phaseolus* beans, to the delight of plant breeders and crop evolutionists".
8. Debouck, D.G. Palmira, Colombia, 15 June 2007, presentation in the final workshop of BMZ project on gene flow analysis for environmental safety in the tropics: "Gene flow analysis and biodiversity implications in the wild-weed-crop complex of common bean".
9. Debouck, D.G. Palmira, Colombia, 7 March 2007, invited internal seminar: "Implications of the International Treaty on Plant Genetic Resources for Food and Agriculture for the work of the Center".
10. Mafla, G. 2007. "Alternativas para la conservación de Recursos Genéticos en bancos de germoplasma", invited conference in the XVII Congreso Venezolano de Botánica, Maracaibo, Venezuela, 21-26 May 2007.
11. Mafla, G. 2007. In vitro conservation of *Manihot* in CIAT-GRU. Presentation during the "Workshop on clonal crops germplasm management", held at the International Potato Center (CIP) in Lima, Peru, 12-16 November 2007.
12. Cuervo, M. 2007. Safe Movement of Germplasm (Seed Propagated Crops). Presentation during the "Workshop on seed crops germplasm management", held at the International Maize and Wheat Improvement Center, CIMMYT, Mexico, 20-23 August 2007.
13. Cuervo, M. 2007. Safe Movement of Germplasm (Clonal propagated crops). Presentation during the "Workshop on clonal crops germplasm management", held at the International Potato Center (CIP) in Lima, Peru, 12-16 November 2007.
14. Rueda, G. 2007. Development and Implementation of an Inventory Management Systems for the in-trust Cassava Collection at CIAT. Presentation during the "Workshop on clonal crops germplasm management", held at the International Potato Center (CIP) in Lima, Peru, 12-16 November 2007.
15. Rueda, G. 2007. Foros Tecnocimiento – CINTEL y Tigo (Colombia Móvil S.A.). Universidad EAFIT, in Medellín-Colombia, 28 September 2007.

6.4. List of trainees trained by Project Staff in 2007

In Seed Conservation

1. Nayari Camacaro. Training in seed drying, handling, conservation and viability testing. INIA-CENIAP (Venezuela). 3-18 May 2007.

2. Adriana Tofiño. CORPOICA, Colombia, Training in seed drying, handling, conservation and viability testing. 15- 22 August 2007.
3. Ing. Oscar Antonio Terenti. E.E.A. San Luis, INTA-Argentina. Training in seed drying, handling, conservation and viability testing. 22-31 May 2007.
4. Ing. Alexander Benavidez R. INTA, Nicaragua. Training in seed drying, handling, conservation and viability testing. 12-16 November 2007.
5. Ing. Liliana Alexandra Pila. Training in seed drying, handling, conservation and viability testing. La Frabril, Ecuador, 28 August 2007.
6. Prof. Invest. Rogelio Lépiz. Centro Universitario de Ciencias Biológicas y Ambientales de la Universidad de Guadalajara, CUCBA/UDG, Mexico. Training in seed drying, handling, conservation and viability testing. 20-30 November 2007.

In Health Testing

1. Thompson, Ruth. CSIR, Ghana. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 29 January 2007.
2. Ogwok, Enmanuel. NRCRI, Nigeria. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 29 January 2007.
3. Nayari Camacaro. INIA-CENIAP (Venezuela). Training in indexing of seedborne diseases for the safe movement of crop germplasm. 3-18 May 2007.
4. Adriana Tofiño. CORPOICA, Colombia. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 15- 22 August 2007.
5. Ing. Liliana Alexandra Pila. La Frabril, Ecuador. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 28 August 2007.
6. Prof. Invest. Rogelio Lépiz. Centro Universitario de Ciencias Biológicas y Ambientales de la Universidad de Guadalajara, CUCBA/UDG, Mexico. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 20-30 November 2007.
7. Angulo, Catalina. Universidad del Valle, Colombia. Training in indexing of seedborne diseases for the safe movement of crop germplasm. 26-30 November 2007.

In vitro Lab

1. Thompson, Ruth. CSIR, Ghana. Training in conservation and management of *in vitro* cassava germplasm. 29 January 2007.
2. Ogwok, Enmanuel. NRCRI, Nigeria. Training in conservation and management of *in vitro* cassava germplasm. 29 January 2007.

3. Chiedozi, Egesi. NRCRI, Nigeria. Training in conservation and management of *in vitro* cassava germplasm. 29 January 2007.
4. Deusdedit, Peter. LZARDI, Tanzania. Training in conservation and management of *in vitro* cassava germplasm. 29 January 2007.
5. Camacaro, Nayiri. INIA-CENIAP, Venezuela. Training in conservation and management of *in vitro* cassava germplasm. 4 May 2007.
6. Tofiño, Adriana. CORPOICA, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 17 August 2007.
7. Londoño, Claudia Marcela. CASD, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 15 August 2007.
8. Romero, Johana. CASD, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 15 August 2007.
9. Ortiz, Alejandro. CASD, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 22 August 2007.
10. Arias, David Enrique. CASD, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 22 August 2007.
11. Asagi, Nahomi. Ehime University Japan. Training in conservation and management of *in vitro* cassava germplasm. 1 November 2007.
12. Jiang, Shengjun. CATAS, China. Training in conservation and management of *in vitro* cassava germplasm. 15 November 2007.
13. Zhenwen, Zhang. CATAS, China. Training in conservation and management of *in vitro* cassava germplasm. 15 November 2007.
14. Angulo, Catalina. Universidad del Valle, Colombia. Training in conservation and management of *in vitro* cassava germplasm. 26-30 November 2007.

Genetic Quality Lab

1. Marta Isabel Moreno. CIAT, Cassava Project. Training in starch gel electrophoresis techniques for cassava isozymes. 16-27 April 2007.
2. Nayari Camacaro. INIA-CENIAP (Venezuela). Training in Biochemical and Molecular Markers. 3-18 May 2007.
3. Natalia Moreno. CIAT, Bean Project. Training in 1D-SDS-PAGE technique for phaseolin. 7-25 May 2007.

6.5. Posters

1. Toro, O., C.H. Ocampo & D.G. Debouck. 2007. Additional evidence suggests a new map for the distribution of wild-weed-crop complexes of common bean in Colombia. VI Simposio Internacional sobre los Recursos Genéticos de América Latina y el Caribe (SIRGEALC). 13-16 November 2007, Mexico City, D.F., México.
2. Mafla, G., J.C. Roa, N.C. Flor, E. Aranzales & Debouck, D.G. 2007. Distribution of cassava germplasm from an international genebank: a service to the global agriculture. 40th Anniversary of CIAT, Palmira, Colombia, 6-9 November 2007.
3. Mafla, G., J.C. Roa, N.C. Flor, E. Aranzales & D.G. Debouck. 2007. Distribución de germoplasma de yuca desde un banco de genes internacional: un servicio a la agricultura mundial. Aniversario 40 del CIAT, Palmira, Colombia, 6-9 November 2007.
4. Lima, M.C., A. Ciprián, O. Toro & D.G. Debouck. 2007. Distribution of bean and tropical forage germplasm from an international genebank: a service to the global agriculture. 40th Anniversary of CIAT, Palmira, Colombia, 6-9 November 2007.
5. Lima, M.C., A. Ciprián, O. Toro & D.G. Debouck. 2007. Distribución de germoplasma de frijoles y forrajes tropicales desde un banco de germoplasma internacional como un servicio a la agricultura global. 40th Anniversary of CIAT, Palmira, Colombia, 6-9 November 2007.
6. Hanson, J., C. Lima, M. Peters & D.G. Debouck. 2007. A key resource for the improvement of animal productions worldwide: forty thousand options from the in-trust forage collections of CIAT and ILRI. 11th FAO Commission GRFA, Rome, Italy, 11-15 June 2007.
7. Suárez-Barón, H., C. Martínez-Garay, R.I. González-Torres, M.C. Duque, D.G. Debouck & J. Tohme. 2007. Determination of gene flow events in natural "*wild-weedy-cultivated*" complexes in gene pools of *Phaseolus lunatus* L. Knowledge Fair, CIAT, Palmira, Colombia, 14-22 May 2007.
8. Chacón, M.I., B. Pickersgill, D.G. Debouck & J.S. Arias. 2007. The common bean has been part of the *Great American Biotic Interchange*: evidence from the study of cpDNA and implications for conservation and breeding. External Programme and Management Review, CIAT, Palmira, Colombia, 19-25 May 2007.
9. Torres, A.M. & R. Ellis. 2007. Conservación *ex situ* de semillas de frutales jugosos. IV Congreso Colombiano de Botánica, Medellín, Colombia, 22-27 April 2007.
10. Torres, A.M.; Ellis, R. 2007. Latencia de semillas de frutales tropicales. IV Congreso Colombiano de Botánica, Medellín, Colombia, 22-27 April 2007.

6.7. Awards

Best Scientific Poster in Knowledge Sharing Week, International Center for Tropical Agriculture, Colombia, May 2007.

Recognition by the American Journal Experts for article in NOVON 16: 105-111 of 2006, July 2007.

6.8. Visitors

The Professional Staff of the Genetic Resource Unit attended the visit of 331 people from different government bodies, institutions, companies, etc. A total of 114 students from six different universities of Colombia visited the Genetic Resource Unit, on October 5 2007 through the “Open House” day coordinated by CIAT Training Office.

6.9. Donors

CIAT Core Budget.

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

World Bank (special project: Rehabilitation of International Public Goods; CGIAR Genebanks Upgrading Project, Global Public Goods, Phase 2).

Bundesministerium fuer Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany (special project: Gene Flow Analysis for Environmental Safety in the Neotropics (phase 2) studies of gene flow in the bean model).

Utviklingsfondet of Norway, for the germplasm exploration carried out in Nicaragua in December 2007, with the Centro para la Promoción, la Investigación y el Desarrollo Rural y Social de Nicaragua (CIPRES) and the Universidad de Costa Rica (UCR).