ANNUAL REPORT 2006
CIAT Project on Saving Agrobiodiversity
SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses

CIAT
DECEMBER, 2006

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

PROJECT DESCRIPTION

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

Outputs:

1. Improved characterization of the genetic diversity of wild and cultivated species and associated organisms.
2. Genes and gene combinations used to broaden the genetic base.
3. Increase efficiency of breeding program using genomics tools
4. Mandate crops conserved, multiplied and distributed as per international standards.
5. Germplasm available, restored, and safely duplicated.
6. Designated Collections made socially relevant.
7. NARS strengthened for conservation and use of Neotropical plant genetic resources.
8. Conservation of Designated Collections linked with on-farm conservation efforts and protected areas.

Milestones:

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

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

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

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

Collaborators: IARCs (IPGRI through the Systemwide Genetic Resources Program, CIP, 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 gene bank project. Segregating populations from crop productivity projects. Characterized insect and pathogen strains and populations from crop protection projects. GIS services from the Land Use Project. *Outputs from SB-2:* Management of Designated Collections (gene banks); genetic and molecular techniques for the gene bank, crop productivity, and soils (microbial) projects. Identified genes and gene combinations for crop productivity and protection projects. Propagation and conservation methods and techniques for gene banks and crop productivity projects. Interspecific hybrids and transgenic stocks for crop productivity and IPM projects.

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

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

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

Annex: 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	yes (2,808)
1.2.	Materials planted	6,520 materials	materials	yes (11,752)
1.3.	Materials regenerated	3,400 materials	materials	yes (5,346)
1.4.1.	Materials processed	2,000 materials	materials	yes (8,656)
1.4.3.	Materials secured	4,000 materials	materials	yes (5,016)
2.1.	Materials cleaned	4,500 materials	materials	yes (7,448)
2.2.	Materials distributed	Unpredictable target	materials	yes (5,046)
2.2.	Data available	New web page	practice (information product)	yes (June 2006)
2.4.	Safety back-ups	2,000 at CIMMYT	materials	yes (5,917)
3.1.	Publications	3 articles in refereed journals	Knowledge	Yes
4.1.	Training	Course and NARS trained	Capacity	Yes

Proof. of achievement: Final Report CGIAR Genebank Upgrading (SGRP, 2007); Report of the External Review of GPG1 at CIAT GRU of October 2006; 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 2006
Genetic Resources Unit
SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Staff:

Daniel G. Debouck, Head, PhD (80%)
Alba Marina Torres, Biologist, M.Sc., PhD (has left in 2006) (100%)
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 Balcazar, Bacteriologist (100%)
Manuel G. Moreno, Ing. Biotec. (has left in 2006) (100%)
Rosa I. González, Bacteriologist, M.Sc. (100%)
Guillermo Enrique Rueda Q., Telematic Engineer (100%)
Carmenza Llano, Administrative Assistant (100%)
Eliana Urquijo, Secretary (has joined in 2006) (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 Alvárez (IP3-IP1), Andrew Jarvis (PE-4), Carlos Lascano (IP-4), Zaida Lentini (SB-02), John Miles (IP-4), Michael Peeters (IP-4), Joe Tohme (SB-02).

Outside CIAT:

MSc. Rodolfo Araya, University of Costa Rica, Costa Rica
Dr. Hans Jörg Jacobsen, University of Hannover , Germany
Dra. Inés Sánchez, WARDA, Africa
Dr. Mario Lobo, CORPOICA, Colombia
Dr. Samy Gaiji, SINGER, IPGRI, Italy
Dr. Jane Toll, SGRP, IPGRI, Italy
Dr. Jean Henson, ILCA, Ethiopia
Dr. Bonwoo Koo, IFPRI, USA
Dr. Marleni Ramírez, IPGRI – Americas, Colombia
Dr. Katy Williams, USDA, USA
Dr. Molly Welsh, USDA, USA

Genetic Resources Unit work breakdown structure

Project Goal

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

Project Purpose

To integrate ex situ and in situ conservation

Subproject 1:	**Subproject 2:**	**Subproject 3:**	**Subproject 4:**	**Subproject 5:**
To make the collection designate meet international standards (i.e. viability, quantity and plant health aspects).	To make the collection-designated available to users (i.e. farmers, breeders and agronomists).	To make the collection-designated fully relevant to the purposes of conservation.	To contribute through training to capacity building in conservation sciences and techniques in the region.	To develop *in situ* methodologies for farmer landraces and wild relatives.
Outputs	**Outputs**	**Outputs**	**Outputs**	**Outputs**
Improve conservation techniques. 3,000 accessions fully regenerated. Status collection-designate improved against vulnerability Alternate liable and affordable conservation methodologies developed.	Sets of germplasm restored to NARS. Refined core collections including novel materials. Data (passport, characterization and evaluation) made available to users. Available spectrum of agrobiodiversity widened	Novel materials acquired or collected. Genetic erosion monitored and documented. Improved core collections in terms representativeness.	NARS human resources trained. Public awareness products developed. Workshops and technical courses carried out. Communications presented in international symposia.	Project proposals on *in situ* conservation prepared. CIAT contribution made to five genetic reserves in LA. Landrace diversity in traditonal farming systems monitored.

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

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

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

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

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

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

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

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

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

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

3.3. Financial Resources

Source	Amount (US$)	Proportion (%)
Unrestricted core	478,816	55.8
Sub Total	**478,816**	
Special projects		
Gene Flow BMZ	21,706	2.5
Upgrading Plan Operations WB	358,186	41.7
Carry over from 2005	-	-
Sub Total	**379,892**	
TOTAL	**858,708**	**100.0**

3.4. Research Highlights in 2006

Activity area # 1: the International Standards
The Upgrading of CGIAR genebanks ('Reabilitating International Public Goods' Phase 1) has progressed according to prefixed milestones (most of them exceeded), and has come to completion in 2006. A total of 11,752 materials were planted in the stations, and 5,346 materials have been regenerated in 2006. A total of another 5,016 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 7,448 seed accessions (4,937 of beans and 2,511 of forages) have been tested for absence of diseases of quarantine importance, while another 159 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 (30% of total collections), while 3,544 accessions of cassava *in vitro* has been shipped to CIP as security backup (85%). Bar coding has been successfully installed in the Viability Lab.

Activity area # 2: the Germplasm and its data available
In 2006, GRU has distributed 5,046 samples of accessions registered into the Multilateral System of the International Treaty. On October 16, 2006, CIAT has signed an agreement of cooperation with the Governing Body of the International Treaty, and has registered 64,870 accessions into the Multilateral System (35,231 of *Phaseolus* beans, 23,140 of tropical forages, and 6,499 of *Manihot* cassava). This registering in May and again in October 2006 was part of a collective action of the System-wide Program of Genetic Resources of the CGIAR (SGRP) together with the pther nine genebanks and SINGER. 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 has been implemented in 2006 in order to facilitate access to information and in-trust germplasm collections. Steps have been taken towards the documentation of SMTAs agreed through the 'click-wrap' consultation (i.e. germplasm requests done through CIAT GRU web site). The amount of digital images of seeds, roots, plant habits, herbarium specimens available to users and for internal checks now sums up to 25,363. A total of 88% of the entire collection of cassava has been tested against diseases of quarantine importance and is thus available for international distribution.

Activity area # 3: the Genetic and Social relevance of the Conservation
Research has advanced in the definition of seed conservation protocols for *Carica papaya*, the tree tomato and some of their wild relatives. Seed physiology studies have defined the best time

for harvesting seed in view of long-term conservation, in relation fruit development on the mother plant. Research done on the duplicates existing in the Colombian collection of cassava with help of seven unlinked SSR markers has identified a set of 90 redundant accessions. The research done in cooperation with Corpoica on the Colombian collection of avocado has shown a low level of redundancy (only two duplicates). A synthesis research has been done on phaseolin with 62 unique banding patterns revealed so far by SDS-PAGE electrophoresis, and the reference materials are maintained and distributed as genetic stocks by GRU.

Activity area # 4: the International cooperation and capacity building
One international course received input from GRU Staff in 2006. Two thesis research were supervised by GRU Staff in 2006. Three articles were published in international refereed journals, one in a non-refereed journal, and two conference proceedings have also been published. Seven presentations in conferences were made by GRU Staff, while four posters were presented in scientific congresses and fora. Twelve Professionals/ students were trained in different disciplines in GRU facilities.

*Activity area # 5: the Link with in situ Conservation on farm and in the wi*ld
Phase 2 of the Gene Flow project supported by BMZ of Germany is coming to completion with results in population genetics, reproductive biology, assessment of gene flow between species, between the crop and its wild relative in space and over time. Limited introgression has been found between *Phaseolus vulgaris, P. costaricensis* and *P. dumosus* in some places of the range. All evidence accumulated so far has shown that the gene flow occurs from the wild relative to the cultivated forms, though the reverse direction might be significant in some places. Gene flow between the wild relative and landraces is not a rare event but occurs in several countries along the range of distribution of the wild relative when the two forms come into contact. The methology developed to identify hybrid swarms in common bean has been successfully applied to the Lima bean.

Information has been gathered about the taxonomy and geographic distribution of wild relatives of crops in the following herbaria: A, BAA, BM, ECON, FHO, GH, HNMN, K, MA, NA, NEBC, OXF, and SI, as background information for the GEF project in preparation "Conservation and sustainable use of wild relatives of Neotropical crops through an integrated understanding of functional diversity".

3.5. Problems encountered and their solution

CIAT has experienced severe reductions in its core income in 2006 and this has affected GRU and the normal delivery of outputs of the last year of Phase 1 of the Genebank Upgrading. Since August 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, and plantings in Popayan have been re-oriented towards regeneration of common bean only.

The Upgrading of CGIAR genebanks (Phase 1) has been the subject of two external reviews in 2nd semester of 2006: a financial audit (September 2006) and a technical review (October 2006). While the reviews mentioned the progresses achieved against all output targets of Phase 1, issues of fragility (one Staff phenomenon) and sustainability (during and after Phase 2) were raised.

Clearly, there is a mismatch between an expectation to have international public goods as germplasm and its related information immediately available and the capacity to fulfill it.

3.6. Plans for next year

- Continue to clear backlogs, namely that of the bean collection
- continue with regeneration of bean and tropical forage collections
- continue the shipments of the security back-ups to CIMMYT and CIP (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
- make appropriate follow-up to the pdf-B process for the GEF project
- run international courses as it may be required

3.7. Executive summary

The Upgrading of CGIAR genebanks ('Reabilitating International Public Goods' Phase 1) has progressed according to prefixed milestones, and has come to completion in 2006; the positive results achieved and tasks still pending have provided justification for an approved Phase 2. A total of 11,752 materials were planted in the stations, and 5,346 materials have been regenerated in 2006. A total of 5,016 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 7,448 seed accessions (4,937 of beans and 2,511 of forages) have been tested for absence of diseases of quarantine importance, while another 159 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 3,544 accessions of cassava *in vitro* has been shipped to CIP as security backup (now at 85%). In 2006, GRU has distributed 5,046 samples of accessions registered into the Multilateral System of the International Treaty. On October 16, 2006, CIAT has signed an agreement of cooperation with the Governing Body of the International Treaty, and has registered 64,870 accessions into the Multilateral System (35,231 of *Phaseolus* beans, 23,140 of tropical forages, and 6,499 of *Manihot* cassava). By January 3, 2007, GRU has started the distribution of in-trust material under the new SMTA.

Research has advanced in the definition of seed conservation protocols for *Carica papaya*, the tree tomato and some of their wild relatives. Seed physiology studies have defined the best time for harvesting seed in view of long-term conservation, in relation fruit development on the mother plant. Research done on the duplicates existing in the Colombian collection of cassava with help of seven unlinked SSR markers has identified a set of 90 redundant accessions. The research done

in cooperation with Corpoica on the Colombian collection of avocado has shown a low level of redundancy (only two duplicates). A synthesis research has been done on phaseolin with 62 unique banding patterns revealed so far by SDS-PAGE electrophoresis, and the reference materials are maintained and distributed as genetic stocks by GRU.

Phase 2 of the Gene Flow project supported by BMZ of Germany is coming to completion with results in population genetics, reproductive biology, assessment of gene flow between species, between the crop and its wild relative in space and over time. Limited introgression has been found between *Phaseolus vulgaris, P. costaricensis* and *P. dumosus* in some places of the range. All evidence accumulated so far has shown that the gene flow occurs from the wild relative to the cultivated forms, though the reverse direction might be significant in some places. Gene flow between the wild relative and landraces is not a rare event but occurs in several countries along the range of distribution of the wild relative when the two forms come into contact. The methology developed to identify hybrid swarms in common bean has been successfully applied to the Lima bean.

4. Project performance indicators

1.FLOWS, TECHNOLOGIES, METHODS & TOOLS

1.1. Backlogs cleared: 2,808 accessions cleared
1.2. Accessions regenerated: a total of 5,346, as 4,059 of beans, and 1,287 of tropical forages
1.3. Accessions secured in long-term: 5,016 accessions secured
1.4. Accessions in security back-up: Shipment this year of 5,917 seed accessions (CIMMYT) and 3,544 *in vitro* accessions (CIP)
1.5. Accessions characterized: 8,353 (field/ lab) + 3,867 (image bank)
1.6. Accessions distributed with passport data: 5,046 accessions distributed
1.7. Support Tools (software in germplasm management; databases available from internet) see www.ciat.cgiar.org
1.8. Data Bases united/ improved, same

2. PUBLICATIONS

2.1. Refereed Journals: published: 3

Ceballos, H., T. Sánchez, A.L. Chávez, C. Iglesias, D.G. Debouck, G. Mafla & J. Tohme. 2006. Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots. Journal of Food Composition and Analysis 19: 589-593.

Muñoz, L.C., M.C. Duque, D.G. Debouck & M.W. Blair. 2006. Taxonomy of tepary bean and wild relatives as determined by amplified fragment length polymorphism (AFLP) markers. Crop Science 46 (4): 1744-1754.

Salcedo C., J., Arroyave J.A., O. Toro Chica & D.G. Debouck. 2006. *Phaseolus novoleonensis*, a new species (Leguminosae, Phaseolinae) from the Sierra Madre Oriental, Nuevo León, Mexico. Novon 16 (1): 105-111.

2.2. Refereed Journals: submitted (accepted indeed): 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.: *in press*.

2.3. Published Proceedings: published articles: 2

González-Torres, R.I., O. Toro, M. C. Duque, R. Araya & D. G. Debouck. 2006. Gene flow events among bean species of section *Phaseoli* in Colombia and Costa Rica using microsatellites markers. LII *PCCMCA* scientific committee 2006 (Programa Cooperativo Centroamericano de Mejoramiento de Cultivos y Animales). April 24-28. Managua, Nicaragua. p. 221.

Ocampo, C.H., Gallego G., Duque M.C., Sánchez I., Rios-Castaño D. & D.G. Debouck. 2006. Diversidad Genética de la Colección Colombiana de Aguacate (*Persea americana* Mill.). *In*: Memorias del Primer Congreso Colombiano de Horticultura, Universidad Jorge Tadeo Lozano. Bogotá, D.C., Colombia. 17-22 October 2006. p. 78.

2.4. Scientific Meeting Presentations: presentations: 7
(see under 6 in full report)

2.5. Working Papers, Other Presentation or Publications: 7
(see under 6 in full report)

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

3.1.Training Courses: 1

3.2. Individualized Training : 13

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

4. RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted

- Rehabilitation of International Public Goods: the Upgrading of CGIAR Genebanks, extension 2007-2009 approved (US$ 740, 540, not summing incomes from collective activities).

- Roadsides as *in situ* conservation areas for forages in Central and South America (with Drs M. Peters and P. Jones).

4.2. Ongoing special projects in 2006

Studies of gene flow in the bean model, Phase 2, supported by Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) of Germany, US$ 21,706 (to CIAT) and US$ 9,278 (to University of Costa Rica).

CGIAR Genebank Upgrading, Phase 1, supported by the World Bank, US$ 358,186.

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 3,867 digital images, summing to 22,853 images for the bean collection, 2,510 images of seed and plants in the field for the forages to date (total 25,363), accessed through CIAT web site or ready to be loaded into it.

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

Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into the genebank processes

A total of 823 accessions of the backlog of bean germplasm was introduced in the multiplication cycles in 2006; 743 additional materials were obtained through internal separations of bean mixtures, and were multiplied. At this time, 8,451 accessions of beans are still in the backlog. One should note however that many accessions in the bean backlog already exist in the designate collection, and should thus not be introduced. A total of 1,985 materials from Australia were introduced into multiplication in 2006.

Tabla 1. New elite lines from CIAT Bean Program introduced in the genebank in 2006.

GNUMBER	NAME	CODE
G51404	EMCAPA 404-SERRANO (BRA)	A 230
G51405	RIO DOCE (BRA)	A 247
G51406	A 262 (ETH)	A 262
G51407	BR-IPA 11 BRIGIDA (BRA)	A 281
G51408	MANTEQUILLA MAIRANA (BOL)	A 295
G51409	NORTHIMBO (BDI), A 321 (COG)	A 321
G51410	VULINDLELA (ZAF), A 344 (MWI)	A 344
G51411	INIAP 419-CHAUPEÑO (ECU)	AFR 585
G51412	ROJO CASARABE (BOL)	CAL 144
G51413	DARK 54 (PER)	DRK 54
G51414	DRK 57 (UGA & MWI)	DRK 57
G51415	DRK 70 (UGA)	DRK 70
G51416	ROJO LLANERO (BOL)	DRK 112
G51417	CARIOCA ANTOFAGASTA (BOL)	FEB 188
G51418	INIAP 421-BOLIVAR (ECU)	LAS 298
G51419	AZUFRADO TAPATIO (MEX)	MAM 13
G51420	RAA 15 (PER)	RAA 15
G51421	NABE 5 (UGA), SUG 73 (UGA)	SUG 73
G51422	OURO BLANCO (BRA), SEHIRALI 90 (TUR)	WAF 16
G51423	VICTORIA (VEN)	WAF 18
G51424	LALE (ESP)	WAF 30
G51425	TATA (ESP)	WAF 137
G51426	ZAA 2 (ARG)	ZAA 2

Contributors: O. Toro, A. Ciprian

Output 1.2. Backlogs of materials pending on multiplication multiplied

Activity 1.2.1. Multiplication of materials cleared by quarantine authorities.

The quarantine authorities were inspectioning in three times about 10,000 bean materials planted either in Tenerife or in Popayán. On the other hand, a total of 1,735 bean plants for the Gene Flow Project were handled as pure lines (for phaseolin and DNA analysis) and planted in Popayán. Similarly, 1,985 accessions of forages were cleared by ICA authorities and directed to the fields in Palmira and Quilichao after a hardening period in the glass-houses in Palmira.

Table 2. Bean backlog pending for processing and procesed in 2006.

Description	Frijol
Germplasm pending for processing in 2005	9,274
Germplasm processed in 2006	823
Pending germplasm for processing	8,451

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

Output 1.3. Materials pending on regeneration

Activity 1.3.1. Multiplication of materials with aging seeds.

Table 3 indicates by locations and species the total numbers of bean accessions that were regenerated because seed viability reached the lower threshold.

Species	Palmira		Popayán		Tenerife		Total	
	Accessions received	Accesions regenerated	Accessions received	Accesions regenerated	Accessions received	Accesions regenerated	Accessions received	Accesions regenerated
P.vulgaris	312	20	1,515	380	5,923	3,984	7,750	4,384
Complex coccineus			13	12	158	158	171	170
P.lunatus	721	366	108	48			829	414
Other spp.	255	38			3	2	258	40
Total	1,288	424	1,636	440	6,084	4,144	9,008	5,008

Some of the accessions planted have not finished with full regeneration. Out of these materials, 5,008 accessions filled once for all requirements for the five conservation purposes. Similarly, over 4,000 accessions of forages were planted in 2006 for regeneration purposes (Tables 4,5). Table 6 includes them, as well as materials planted in previous years.

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	975	30	1,005
Quilichao	3,001	128	3,129
Palmira	872	176	1,048
Popayan	132	185	317
Tenerife	-----	-----	----
Total	4,005	489	4,494

Table 5. Forage germplasm processed during 2006.

	Legumes	Grasses	Total
Backlog	-----	-----	------
Regenerated (because of aging seeds)	6,897	103	7,000
Characterized during the process	4,249	29	4,278
Designated to FAO	2,662	17	2,679

Table 6. Forage germplasm installed during 2006.

	Palmira	Quilichao	Popayan	Total
Australia	481	1,480	40	2,001
Regeneration	422	1,343	8	1,773
Total	903	2,823	48	3,774

Contributors: O. Toro, A. Ciprian

Status of designated germplasm at the GRU in 2006.

In view of entry into force of the International Treaty on Plant Genetic Resources for Food and Agriculture, and the agreement with its Governing Body on October 16, 2006, GRU updated the figures of in-trust materials, and the status of accessions currently registered in the Multilateral System of Access and Benefit Sharing is as follows:

Manihot cassava: 6,499 (for 33 taxa, out of which 5,568 of *M. esculenta*)
Phaseolus beans: 35,231 (for 44 taxa)
Tropical forages: 23,140 (for 668 taxa)
Total: 64,870 accessions in-trust

Activity 1.3.2. Periodical subculturing of the FAO designate cassava collection

This year, 6,268 accessions of *Manihot* were subcultured by the nodal cutting technique; the accessions multiplied represents 96.4% of the collection. A total of 3,476 accessions (5,134 *in vitro* plants) were propagated for the distribution to users and 1,532 accessions (3,368 *in vitro* plants) were propagated for indexing test.

Contributors: G. Mafla, E. Aranzales

Activity 1.3.3. Embryo culture as a means to rescue *Phaseolus* spp. seeds.

Introduction

A total of 8,417 accessions of bean backlog (264 are *sensu stricto*) have not been included in the active collection because of low viability, phytosanitary problems or low seed numbers. The *in vitro* culture can be considered as an alternative to recover and increase some of these accessions, and this would allow their introduction in the multiplication cycles afterwards. Embryo rescue has been developed to recover embryos of interspecific hybrids, although these hybrids have low fertility and most are sterile (Santalla et al., 1998).

Materials and methods

Seed samples of 92 populations (20 species) that had been conserved, in average, for 27 years were obtained from the germplasm bank. Whole seeds were superficially sterilized by inmersion in alcohol to 70% for 10 seconds, then for 10 minutes in 0.5% of benlate, 5 minutes in 2.5% sodium hypochlorite, and finally washed three times with sterile distilled water. The seeds were after scarified and pre-treated with GA3 at 200 ppm for 8 hours. Zygotic embryos were excised under aseptic conditions and placed on 4E media (Roca, 1984). Cultures were maintained at 27 ° C in the dark for 7 days and then transferred to light conditions (1,000 lux) for 12 h per day.

Results

A total of 61 populations (20 species) of *Phaseolus* germplasm backlog were established by embryo rescue (Table 1). The protocol of desinfection has been useful in the elimination of the superficial contaminants and the step of pre-treatment in GA3 has facilitated the extraction of embryonic axis, avoiding possible mechanical damages. The materials are being multiplied in order to increase the number of plantlets. Once increased the materials will be temporarily conserved *in vitro* and other parts of them planted for multiplication and regeneration under greenhouse and field condition (Figure 1). A total of 140 *P. hygrophilus* plants have been obtained by micropropagation, and 24 plants have been transplanted in the greenhouses in Palmira, Popayán and Tenerife.

Table of Contents Page

Table 7. Populations of *Phaseolus* spp. recovered by embryo rescue.

Species	No.Populations	Populations recovered(No.)
P. ritensis	14	12
P. maculates	20	10
P. reticulates	1	1
P. pauciflorus	2	1
P. polystachyus	2	1
P. grayanus	3	2
P. pedicellatus	5	3
P. pluriflorus	2	2
P. metcalfei	8	5
P. vulgaris	2	1
P. leptostachyus	18	13
P. xanthotrichus	4	1
P. ovatifolius	2	2
P. macrolepis	1	1
P. perplexus	1	1
P. neglectus	1	1
P . talamancensis	1	1
P . hygrophilus	1	1
P. scabrellus	1	1
P. hintonii	3	1
TOTAL	92	61

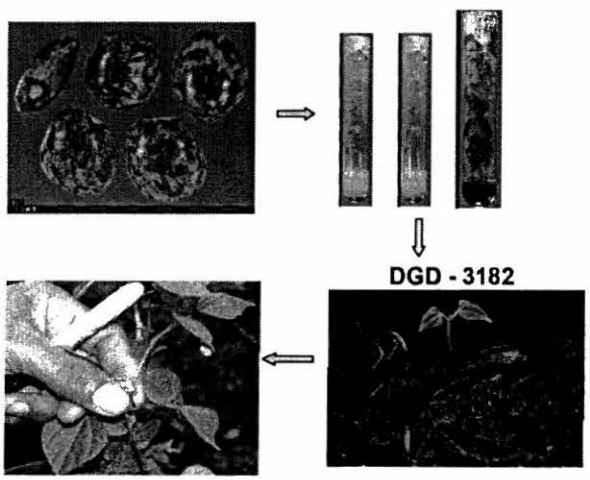

Figure 1. *In vitro* plants of *Phaseolus* spp regenerated by embryo rescue.

References

Santalla, M., Brian Power, J. & Davey, M.R. 1998. Efficient in vitro shoot regeneration responses of *Phaseolus vulgaris* and *P. coccineus*. Euphytica 102(2): 195-202.

Contributors: E. Aranzales, G. Mafla, O. Toro

Activity 1.3.4. Monitoring viability of conserved seed germplasm of beans, forages and cassava.

In 2006 embryo culture was used to rescue seed of wild *Manihot* species kept in storage since 1992-1994. The results of recovered plants are shown in Table 8.

Table 8. Viability testing for wild *Manihot* germplasm.

Populations (No.)	Populations	Seeds (No.)	Plants recovered(No.) /Populations
M. alutacea	1	1	0/0
M. angustiloba	1	17	0/0
M. anomala	4	6	0/0
M. caerulescens	10	85	28/5
M. chorosticta	4	18	0/0
M. crassisepala	2	10	0/0
M. carthaginensis	1	21	56/6
M. davisiae	1	1	0/0
M. dichotoma	3	59	8/2
M. epruinosa	6	76	20/4
M. flamingiana	3	35	0/0
M. flabellifolia	90	5576	238/43
M. grahami	4	19	0/0
M. janiphoides	2	106	24/2
M. maracasensis	1	30	0/0
M. oaxacana	2	8	0/0
M. peruviana	18	2089	133/21
M. purpureo-costata	2	4	0/0
M. quinquepartita	14	205	5/2
M. salicifolia	1	5	0/0
M. tripartita	3	70	0/0
M. triphylla	1	1	0/0
M .violacea	5	301	7/1
M. websterae	4	8	0/0
TOTAL	183	8751	519/86

Ouput 1.4. Materials processed into final packing

Activity 1.4.1. Final drying and temporary storage

Table 9 indicates the amount of accessions for beans (6,092) and forages (2,919), respectively, (total 9,011), which have been harvested, cleaned, dried, and stored at 5°C, awaiting the results from viability and health tests.

Table 9. Germplasm in seed processing during 2006.

	Beans	**Forages**
Seed selection / temporal storage	6,092	2,919
Total	6,092	2,919

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

Activity 1.4.2. Viability testing

Table 10 indicates flows of materials during 2006 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 CIRO, Australia. Several techniques of pre-germination have been used for successful results such as sand beds, petri dishes and germination paper.

Table 10. Viability testing for *Phaseolus* beans and tropical forages during 2006.

Class	**BEANS**		**FORAGES**	
	Germination (%)	No. Accesions	Germination (%)	No. Accesions
Already stored materials	1-64	33	1-64	30
	65-84	34	65-84	12
	85-100	215	85-100	71
Sub-total		**282**		**113**
Recently multiplied materials	1-64	19	1-64	21
	65-84	86	65-84	145
	85-100	3,403	85-100	1,630
Sub-total		**3,508**		**1,796**
TOTAL		*3,790*		*1,909*

Literature cited

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

Contributors: A.M. Torres, C. Lima, F. Gil, H. García

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

Once cleared by the Viability Lab (for viability above 85%) and by the Germplasm Health Lab (for absence of diseases of quarantine importance), the materials are processed into the five conservation purposes: long-term, safe duplicates, restoration, periodical monitoring, and distribution (Tables 11-12).

Table 11. Final storage and packing of *Phaseolus* beans processed during 2006 (number of accessions)

	Beans
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	3,402
SHORT TERM only (Distribution)	5,229
Total	***8,631***

Table 12. Final storage and packing of tropical forages processed during 2006 (number of accessions)

	Total
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	2,140
SHORT TERM only (Distribution)	2,929
Total	***5,069***

Contributors: A.M. Torres, C. Lima

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

This year we tested as regular monitoring the viability of 725 accessions of *Phaseolus vulgaris* after 5 years of conservation in long-term storage, namely Ten 1999B and POP2001B. The seed lot PAL1996B was tested previously but with a health problem in the germination test. Thus, the test was repeated for confirmation for 130 accessions of this seed lot.

The mean germination of beans was stable after conservation during five years (Table 13, $p=0.567$). It means that there is no statistical difference between the means before and after conservation. Thus, seeds of these seed lots of beans can be stored for another five years to be monitored after 10 years of conservation.

For the forages, we tested 321 accessions of legumes and grasses stored during five years. The seed lots are named PAL1998A, PAL1999A, PAL2000A, PAL2001A, POP1998A, POP199A, POP2000A, QUI1998A, QUI1999A, QUI2000A and QUI2001A.

The mean germination of forages increased in 0.5 units indicating that some accessions have reduction in seed dormancy, and no decrease in viability is registered. There is no statistical difference between the means before and after conservation (Table 13, p=0.335). Consequently, seeds can be kept in cold rooms for conservation and be monitored after 10 years of conservation.

As a general conclusion the viability of seeds of both crops has been maintained in conservation following the FAO standards (FAO/IPGRI 1994) between year 2001 and year 2006. This result shows that improving drying facilities and packing in vacuum in the Genetic Resources Unit has ensured the stability of the seed viability for long-term conservation.

Table 13. Paired t-test for monitoring viability of bean and forage seeds after 5 years of long term conservation

Crop	Mean of Initial germination	Mean of Monitoring germination	N	Standard deviation of difference	Standard Error of Mean	P value
Bean	97.7	97.7	725	4.340	0.1612	0.567
Forage	93.0	93.6	321	9.650	0.5386	0.335

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.

Contributors: A.M. Torres, G. Mafla, V. Nuñez.

Output 1.5. Improved conservation techniques

Activity 1.5.1. Development and maturation of fruits and seeds, and changes in seed quality in *Solanum betaceum, Carica papaya* and *Vasconcellea cauliflora*

Introduction

The highest seed quality is the principal objective when determining the appropriate time to harvest seed crops or to collect wild species. This objective will also ensure high seed viability during subsequent storage. According to Harrington (1972), physiological maturity -when seeds reach their maximum dry weight- is when seeds achieve maximum quality and thereafter seed ageing begins and deterioration increases.

However, and in contrast to the above hypothesis, several studies have demonstrated that seed quality continues to increase after maximum seed dry weight is reached (Pieta Filho & Ellis 1991; Demir & Ellis 1992a; Demir & Ellis 1992b; Demir & Ellis 1993). In consequence, the term mass maturity is now preferred to that of physiological maturity to describe the end of the seed-filling

period of seed development, since the term physiological maturity is misleading with regard to seed physiology (Demir & Ellis 1992a; Ellis & Pieta Filho 1992).

Seed quality development has been studied in several fleshy fruits. For instance, in tomato *(Solanum lycopersicon =Lycopersicon esculentum)* maximum seed quality was attained about 23-40 days after mass maturity (Demir & Ellis 1992a). The potential longevity of seeds is quantified by the value of the seed lot constant K_i of the seed viability equation (Ellis & Roberts 1980). This value has been used to determine seed quality in several seed development studies. Thus, the maximum potential longevity in pepper (*Capsicum annuum*) was found at 10-12 days after mass maturity (Demir & Ellis 1992b), and in marrow (*Cucurbita pepo*) 24-31 days after mass maturity (Demir & Ellis 1993). Demir and Samit (2001) suggest that the attainment of maximum seed quality in tomato is related to the changes in fruit colour that occur much later than mass maturity, when fruit colour is red, more than 20-30 days after mass maturity. This duration is very similar to that of 23-40 days reported by Demir and Ellis (1992a).

The objective of this chapter was to determine when maximum seed quality was attained during the development of seeds of *Solanum betaceum, Carica papaya* and *Vasconcellea cauliflora* by testing the hypothesis Ho: Maximum seed quality in these fleshy fruits is attained coincident with the end of the seed-filling phase (i.e. at mass maturity). In the event of this hypothesis being disproved, my objective was to determine if other aspects of fruit and seed development are associated with the attainment of maximum seed quality.

Materials and methods

One plantation of each of *Solanum betaceum, Vasconcellea cauliflora* and *Carica papaya* was used in three different localities in Colombia during 2003 and 2004 in order to produce fruits and study seed development.

Solanum betaceum (common variety)

Twenty-five shrubs from a commercial plantation placed in Popayán, Cauca, Colombia (2°25' N, 76°29' W, 2000 masl) were used to produce the fruits for this study. Shrubs were chosen for flower tagging uniformly across the whole plot to accommodate the variation in the shrubs within the plot. The crop was planted on 13 April 2002 with seedlings 20 cm in height. Flowers at anthesis began to be labelled on 6 February 2003. This was continued every 7 days; flowers at opening were identified and labelled until 22 May 2003. Labels were placed in the pedicel of the flower between 9:00 and 11:00 a.m. The total number of flowers labelled each day varied between 100 and 300, but very few developed into fruits.

During the period of flower labelling, fertilizers were applied once a month to the soil (350 g of 17-6-18-2 [N-P-K-Mg] and 50 g of Agrimins for each plant) and to the leaves (mixture spray: 10 cc of elosal, 80 g of vitrifoliar, 30 g of boro and 30 g of zinc, 300 cc of molasses in 20 L of water/0.1 Ha). Application of insecticides was avoided to allow pollination by insects. As a result, the larvae of the moth *Neoleucinodes elegantalis* attacked many fruits resulting in early fruit drop. Thus biological control was introduced during February and March, e.g. traps of plastic bottles containing molasses hanging from the branches. Due to the continued and persistent loss of fruits, insecticides (mixture spray: 40 cc of karate, 40 cc of Inex A in 20 L of water) were applied on 21 April and 5 May 2003. All developed, tagged fruits were harvested on two dates: 26 June and 25 July 2003. This provided developmental durations of 105 to 154 days after anthesis (DAA). Due to low quantity of seeds for each development stage in the first harvest date and the similarity in seed weight and germination percentage between harvests, seeds

of the same developmental duration from each tagging date were combined in a proportion of 1:3 from first and second harvests, respectively.
Seeds from fruits collected between 105 and 154 DAA were extracted the day after harvesting. Seed moisture content of fresh seeds was measured immediately after extraction, using the two-stage procedure, i.e. with pre-drying (ISTA 2005). After that, seeds were dried over silica gel at 20 °C for 48 hours and hermetically stored overnight at -20 °C before estimating moisture content (ISTA 2005).

After 154 DAA the fruits began to drop naturally. These fallen fruits were collected every day from the soil between 11 September and 2 October 2003 and corresponded to durations between 154 and 161 DAA. Fruits were kept in cotton bags to enable further maturation, until just before the fleshy part of the fruits started to spoil, at 23.1-27.1 °C and 43-63% RH, minimum and maximum, respectively. Four durations of this *ex planta* maturation were provided: i.e. 154-161 DAA and after that 7, 14 and 21 days more. Hence these treatments are designated: (154-161 DAA), (161 DAA +7), (161 DAA +14 d) and (161 DAA +21 d). Seeds were extracted and treated in the same way as for those collected from the crop between 105 and 154 DAA.

The total fresh weight, length, diameter and colour, using the colour chart of the Royal Horticultural Society (RHS 1966), of all fruits were recorded.

To determine ability to germinate, seeds were pre-treated in GA_3 2,000 ppm at 20 °C for 24 hours, divided into four replicates of 25 seeds each and tested between moist rolled paper towels, at 20/30 °C (16/8 h) for 35 days. Normal and abnormal seedlings were counted every 7 days. Finally, firm seeds at the end of the tests were checked for viability according to the ISTA rules (ISTA 2005). These tests were carried out in seeds freshly extracted from fruits and after subsequent drying.

Dried seeds from fruits collected between 112 and 154 DAA and from ex-planta additional maturation between 154-161 DAA and to 161 DAA +21d were hermetically stored at -20 °C with 3.2-4.2 % of moisture content (wet basis) before starting the experimental storage to determine longevity. On 9 December 2003, sub-samples of 100 seeds each of all these collection treatments were hermetically stored in laminated aluminium foil bags at 40 °C at the moisture contents indicated above. Germination was assessed before this storage treatment and then tested at regular intervals during 190 days of storage. The germination tests were carried out as described above.

Carica papaya var. *Tainung*

A total of 21 shrubs were used from a commercial crop of *C. papaya* var. Tainung to obtain the fruits for this study. With the exception of the border shrubs (not sampled), shrubs were chosen for flower tagging uniformly across the whole plot to accommodate the variation in the shrubs within the plot. The plantation was located in Bolivar, Valle del Cauca, Colombia (4°20' N, 76°11' W, 1000 masl). The crop was transplanted on 15 November 2003 from seedlings 45 days old. After that, fertilisers, fungicides and water were applied weekly in accordance with normal local practices. The variety Tainung is high yielding with hermaphrodite and female fruits with long and rounded shapes, respectively (Anonymous 2005).

Hermaphrodite fruits produce more seeds than female fruits, thus hermaphrodite flowers were chosen for labelling. Between 2 and 17 flowers on each shrub were labelled every 14 days between 29 January and 25 March 2004. Labels were placed in the pedicel of the flower, between

10:00 and 13:00. No artificial pollination was required. The flowers were either self pollinated or cross-pollinated by bees.

The harvest of the marked fruits was done on two dates: 28 July and 11 August 2004 to provide the following durations of fruit and seed development: 126, 140, 154, 168, 175 DAA. Seeds of both harvest dates and belonging to the same development duration (DAA) were combined. In addition, fallen fruits were collected from the ground on 11 August 2004, and correspond to developmental durations between 175 and 182 DAA. Fruit colour was recorded for all harvest dates using the colour chart of the Royal Horticultural Society (RHS 1966).

Seeds were extracted the day after harvesting following the same procedure. Seed moisture content of fresh seeds was recorded using the two-stage procedure, i.e. with pre-drying (ISTA 2005). Subsequently, seeds were dried to 8.2 to 9.0% in laboratory conditions at 22 °C and 50% RH over 72 hours.

Seeds were pre-treated with gibberellic acid 2,000 ppm to avoid confounding viability with dormancy in germination tests. These were done using four replicates of 50 seeds, in moist rolled paper towels in an alternating temperature regime of 20/30 °C for 16/8 h, for fresh and dried seeds during 57 and 35 days, respectively. Normal and abnormal seedlings were counted every seven days and finally tetrazolium test was made on firm ungerminated seeds (ISTA 2005).

Electrical conductivity tests were carried out to assess seed vigour (ISTA 1981). Each replicate of 70 seeds was weighed and placed in glass beakers with 250 ml deionised water at 20 °C and soaked for 24 hours at this temperature. Beakers were covered with cling film to avoid contamination with dust particles or evaporation. One beaker with deionised water (only) was set up as a control. Finally, the seeds were separated with a sieve and the water transferred to a clean beaker. The electrical conductivity of this water was measured using the dip cell. The electrolytes leached into water were measured with an electrical conductivity meter CMD 750 (WPA, Linton, Cambridge, U.K.).

Dry seeds at 8.2 to 9.0% moisture content (wet basis) were hermetically stored at +5 °C from 27 August 2004, until experimental storage. Hermetic packages containing the seeds were removed from storage on 25 September 2004 and sent to Reading by courier, where they arrived on 1 October 2004 and were returned to storage at +5 °C. In order to estimate potential longevity, on 6 October 2004, sub-samples of 200 seeds of each treatment were sealed in laminated aluminium foil bags and stored hermetically at 40 °C at moisture contents between 9.1 and 9.8%. Germination was tested on samples before storage and regularly during storage until 169 days. The same pre-treatment and germination procedures as explained above were used.

Vasconcellea cauliflora

Twenty shrubs of this species were planted in Palmira, Valle del Cauca, Colombia (3°30' N, 76° 19' W, 1000 masl). Seeds of the accession ILS-1780 (CORPOICA number) were planted on 7 April 2004 and seedlings were maintained in greenhouses for two months and then transplanted to the field in early June 2004. Each plant was placed on a grid 30 x 40 cm and fertilisers (100 g of 17-6-18-2 [N-P-K-Mg] and 30 g of Agrimins for each plant) were added to the soil. Plants were watered frequently until the seedlings had established and the same fertilisers were applied monthly.

This species is dioecious. Hence, ten male shrubs were maintained in the plantation to provide pollen and ten female shrubs were maintained to provide the fruits. Between 1 and 12 flowers per

shrub at anthesis were marked every seven days from 11 September to 13 November 2004 between 9:00 and 11:00 a.m. Tags were placed in the pedicel of each flower. Fruits were harvested on 1 April 2004, giving the following durations: 161, 168, 175, 182, 189 and 196 DAA. In addition, fruits that fell after 196 DAA were collected from the ground at 203 DAA, thus representing durations on the plants between 196 and 203 DAA.

Fruit colour was recorded for each harvest date using the colour chart of the Royal Horticultural Society (RHS 1966). The procedure for seed extraction was similar to that developed for *C. papaya* seeds. Moisture content of fresh seeds was determined immediately after extraction, using the two-stage procedure (ISTA 2005). After that seeds were dried over silica gel at 20 °C during 24 hours to moisture contents between 7.4 and 10.8% (w.b.).
To break seed dormancy seeds were pre-treated with GA_3 2,000 ppm at 20 °C and the subsequent germination tests made on four replicates of 25 seeds each in the alternating temperature regime of 20/30 °C, 16/8 h. Germination tests lasted 77 and 35 days for fresh and dry seeds, respectively. Due to the limited quantity of seed for this species it was not possible to determine potential longevity.

Calculation and analysis

Germination was angular transformed to enable t-test comparisons between the germination of fresh and dry seeds (Genstat-5-Committee 1997). Non-transformed germination percentage was used to produce all graphs with Sigma Plot 7.0.
Seed survival curves with normal germination as the criterion of survival were fitted for each seed development treatment in *S. betaceum* and *C. papaya*, by probit analysis (Genstat-5-Committee 1997), in accordance with the viability equation:

$$v = K_i - p/\sigma$$

where, v is probit percentage viability after p days in storage, σ is the standard deviation of the frequency distribution of seed deaths in time (days), and K_i is the intercept of the seed survival curve (Ellis & Roberts 1980).
The electrical conductivity of seed steep water was calculated according to the following formula (ISTA 1981).

Results

Solanum betaceum

The fruits maintained similar size (length and diameter) between 105 and 154 DAA, while the fresh weight of fruits increased slightly and progressively during this period ($p<0.05$, $r^2 = 0.051$) (Figure 2 a,b). The fruits collected from the ground between 154 and 161 DAA, and allowed to mature further tended to be smaller (than those collected at 154 DAA) but increased in size and weight thereafter.

The colour of the fruits changed during development and maturation (Figure 3, Table 14). Fruits were classified in the purple and green groups of the RHS between 105 and 119 DAA and mainly in the red group between 126 and 154 DAA, and all longer durations. Vertical green lines were found in all fruits between 105 and 140 DAA.

Table 14. Colour description of fruits of *Solanum betaceum* during maturation according to The Royal Horticultural Society Colour Chart (RHS 1966)

Duration (DAA)	Fruit colour from the base to middle	Fruit colour of the vertical lines	Fruit colour from apex to the middle
105	Purple group 79A, Green group 137B	Green group 136A	Purple group 79A, Green group 137B
112	Purple group 79A	Green group 136A	Red group 53A
119	Purple group 79A	Green group 133A	Red group 53A
126	Red group 42 A-B (95%), Red group 53 A-B (5%)	Green group 137A	Red group 42 A-B
133	Red group 42 A	Green group 137A	Red group 34 A-B
140	Red group 42 A	Green group 137A-B	Red group 34 A-B
147	Red group 42 A-B	Red group 42A	Red group 34 A-B
154	Red group 42 A		Red group 34A
154-161	Red group 34A, 33A, 31A		Orange group 26A
154-161 (+7d)	Red group 34A, 33A, 32A		Red group 34A
154-161 (+14d)	Red group 34A		Red group 34A
154-161 (+21d)	Red group 34A-B		Red group 34B

Seed moisture content decreased rapidly from 76.5 to 64.3% between 112 and 126 DAA and stabilised at about 60% from 147 DAA onwards (Figure 4a). Seed dry weight increased to a maximum value of 530 mg at 126 DAA where mass maturity was reached. Seed dry weight was more or less maintained constant thereafter until shedding of the fruits.

The normal germination of fresh and dry seeds (Figure 4b) did not differ within a single duration of development ($p>0.05$). Maximum normal germination was attained at 126 DAA, i.e. at the same time as when the heaviest seeds were collected, and was maintained thereafter. The germination of seeds from fruits collected from the ground was slightly reduced compared to those at 154 DAA harvested from the plants. Similarly, mean times to germinate were greater, having been very similar from 126 to 154 DAA, but these were progressively reduced with *ex planta* maturation: after 21 days *ex planta,* mean germination time was similar to that for fruits harvested from the plants (Figure 4b).

Seed survival curves were constructed for seven seed lots from *in planta* maturation and four seed lots from additional *ex planta* maturation (Figure 4). The results could be described well by negative cumulative normal distributions (e.g. 119, 147, 154 DAA), although some patterns were quite variable (e.g. 133 DAA, 154-161 +14d). The results of the early harvests between 112 and 126 DAA for no experimental storage (i.e. zero duration in Figure 5) were similar to the germination test results shown in Figure 4, where ability to germinate developed over the period to 126 DAA.

The standard deviation (σ) of the 11 survival curves did vary significantly ($F_{10, 74} = 3.19$, $p<0.005$) with duration of development (Table 15). The highest K_i value was attained at 140 DAA (Figure 6): from 112 to 140 DAA potential longevity increased substantially and consistently, and then stop improvement to decrease gradually after 140 DAA. The seeds from fruits that had

undergone *ex planta* maturation continued this general pattern of decreasing potential longevity among later harvests.

Table 15. Estimates of K_i, $1/\sigma$ and σ for seeds of *Solanum betaceum* stored at 40 °C with 3.2-4.4% moisture content

Duration after anthesis (DAA)	K_i (s.e.)	$1/\sigma$ (s.e.)	σ
112	-1.044 (0.150)	0.0239 (0.0070)	41.79
119	0.116 (0.187)	0.0292 (0.0077)	34.27
126	1.565 (0.184)	0.0223 (0.0072)	44.94
133	1.617 (0.180)	0.0171 (0.0071)	58.58
140	2.777 (0.236)	0.0460 (0.0076)	21.73
147	2.492 (0.227)	0.0461 (0.0076)	21.71
154	2.095 (0.214)	0.0425 (0.0075)	23.51
154-161	1.900 (0.196)	0.0324 (0.0073)	30.91
154-161 +7d	1.451 (0.181)	0.0208 (0.0072)	48.10
154-161 +14d	1.093 (0.172)	0.0124 (0.0071)	80.78
154-161 +21 d	1.394 (0.192)	0.0170 (0.0072)	58.86

Carica papaya

Fruit colour changed during development, from completely green at 126 DAA, to green and yellow at 140 DAA, and to yellow and orange from 154 DAA onwards (Table 16, Figure 7). Seeds harvested at 126 DAA were either cream or brown in colour and some lacked ribs, while seeds from harvests from 140 DAA onwards were all brown and their *testae* all exhibited small ribs (Figure 7 and 8).

Table 16. Colour description of fruits of *Carica papaya* during maturation according to The Royal Horticultural Society Colour Chart (RHS 1966)

Days after anthesis (DAA)	Colour
126	Green group 140A,B
140	Green group 140A, Yellow green group 154B, Yellow group 7A,B
154	Yellow group 14A, Yellow orange group 21B, and dots Green group 141C
168	Yellow orange group 21A,B, Orange group 26A, and dots Green group 141C
175	Yellow orange 23A and Orange red group 30C
175-182	Orange group 26A, Yellow orange 21B and Orange red group 30C

Seed moisture content decreased from 126 to 140 DAA and after that was stable at about 66% (Figure 8a). Seed dry weight increased between 126 and 140 DAA, and attained the maximum value at this time (1.55 g), remaining stable thereafter. Hence, mass maturity was close to 140 DAA.

Germination of fresh seeds was lower than dry seeds in the majority of harvest dates ($p<0.05$) (Figure 8b). This was also the case for viability, i.e. when abnormal and viable seeds were added. Hence, I assume that most fresh seeds died in the germination test. Thus, the germination of dried seeds was the more useful measure of seed quality. Maximum normal germination was obtained at 140 DAA and this value was maintained until 175 DAA. Seeds extracted from fallen fruits (175-182 DAA) showed a slight reduction in germination. Mean germination time decreased substantially between 126 and 140 DAA and was constant thereafter (Figure 8c).

The survival curves were plotted for seed germination from five harvest dates and fruits shed from the mother plant (Figure 9). There was limited but significant variation ($F_{5, 56} = 3.28$, $p<0.05$) among the estimates of σ (Table 17). Potential longevity (K_i) increased substantially between 126 and 140 DAA and continued to increase slightly until the greatest value was recorded at 175 DAA and decreased after that for seeds from shed fruits (Figure 9a).

Table 17. Estimates of K_i, $1/\sigma$ and σ for seeds of *Carica papaya* stored at 40 °C with 3.2-4.4% moisture content

Duration after anthesis (DAA)	K_i (s.e.)	$1/\sigma$ (s.e.)	σ
126	0.288 (0.145)	0.0560 (0.0072)	17.86

140	2.161 (0.173)	0.0256 (0.0073)	39.08
154	2.549 (0.184)	0.0298 (0.0073)	33.56
168	2.541 (0.184)	0.0328 (0.0073)	30.47
175	2.724 (0.196)	0.0431 (0.0076)	23.21
175-182	1.815 (0.166)	0.0229 (0.0072)	43.67

The electrical conductivity of seed-steep water decreased substantially between 126 and 140 DAA (Figure 9b). There was little change thereafter, but the lowest value was obtained at 175 DAA which coincided with the highest value of K_i. Fruits shed from the mother plant provided seeds with greater conductivity than seeds from the last harvests.

Vasconcellea cauliflora

Fruit colour was green and yellow between 161 and 175 DAA and completely orange from 182 DAA onwards (Table 18, Figure 10). Seeds harvested at 161 DAA were either cream or brown and *testa* ribs were sometimes not pronounced. Thereafter, all seeds were brown and their *testae* exhibited ribs.

Table 18. Colour description of fruits of *Vasconcellea cauliflora* during maturation according to The Royal Horticultural Society Colour Chart (RHS 1966)

Days after anthesis (DAA)	Colour
161	Green group 141B (95%) and Yellow green 151C (5%)
168	Yellow green group 144B and Yellow green 151C
175	Yellow orange group 14A (95%), 15A and Green group 140A (5%)
182	Orange group 26A
189	Orange group26A
196	Orange red group 31A
196 (+7d)	Orange red group 32A

Seed moisture content started to decrease progressively from 78.2% at 168 DAA to a minimum value of 57.7% at 196 DAA (Figure 13a). Seed dry weight increased throughout the period of study to a maximum value of 25.9 mg at 196 DAA (Figure 13a).

The normal germination of fresh and dry seeds did not differ within each fruit collection date ($p>0.05$). Ability to germinate increased between 161 and 168 DAA and then stabilised at about 90% (Figure 13b). Mean germination time (Figure 13c) was greatest at 168 DAA and declined thereafter, although differences between 175 and 196 DAA were slight.

Discussion

Solanum betaceum

The results showed that once mass maturity was attained (e.g. maximum seed dry weight) at 126 DAA, normal germination reached maximum values and mean germination time minimum values. These findings coincide with the study of Demir and Ellis (1992b) in pepper where mass maturity coincided with the onset of germinability and desiccation tolerance. After mass maturity was reached seed dry weight and normal germination were stabilised in maximum and mean germination time in minimum for 28 days more, just until before shedding. As in other fleshy fruits e.g. pepper, tomato and marrow, there was no decline in viability and vigour immediately after attaining mass maturity (Demir & Ellis 1992a; Demir & Ellis 1992b; Demir & Ellis 1993). The limited change in moisture content after mass maturity was also similar to the observations in other fruits with moist environments such as pepper, tomato and marrow. Little change in moisture content immediately after mass maturity was also found in maize seeds and attributed to the isolation provided by the husk (Egli & Tekrony 1997).

The seed survival curves showed an unexpected variation among the slopes ($p<0.05$). This may have been due to the heterogeneity of fruits at different positions in the shrubs receiving different sun light, temperature and wind. For example, variation in seed development was shown by Demir and Ellis (1992a) in tomato among the 1st, 2nd and 3rd trusses. Moreover, the combination of different harvests corresponding to same duration of development (different tagging dates) could cause variation because there was some difference in environmental conditions between the two harvest dates. Finally, seed moisture content varied by over 1% and this alone could have accounted for the variation recorded.

The maximum potential longevity (K_i) was found 14 days after mass maturity (140 DAA). This finding also coincides with those for other fleshy fruits, such as pepper where maximum potential longevity was attained 10-12 days after mass maturity (Demir & Ellis 1992b) and in marrow at 24-31 days after mass maturity (Demir & Ellis 1993). Potential longevity declined considerably after 140 DAA. Clearly that duration of development and maturation was therefore optimal for seed quality in *Solanum betaceum*.

Collections from ground and extra maturation *ex planta* gave greater heterogeneity in the survival curves (i.e. σ bigger and curves shallower). This *ex planta* (in fruit) maturation gave some improvement for part of the population to make the extreme with good individuals of much longer lived. Seeds from fruits over all 154-161 and *ex planta* maturation were poorer quality (e.g. initial viability) but because the longest-lived seeds were improved by the period in fruit *ex planta*, survival curves are shallower (σ bigger). For instance, the slope of the survival curve of the period 154-161 DAA +21 d was almost the same as the period 133 DAA where maximum longevity was predicted.

Greater germination for some seeds from *ex planta* maturation which were maintained imbibed in the fruit at 60% of moisture content is explained by the hypothesis of Villiers (1973; 1975) of continuing repair and maintenance to remove the damage in fully hydrated tissues. It has been found that repairing occurs in lettuce seeds fully hydrated, in the presence of oxygen and at high moisture content (between 15 and 44%), (Ibrahim & Roberts 1983; Ibrahim *et al.* 1983; Roberts & Ellis 1989).

Seed physiological changes were not clearly related with fruit dimensions, which had similar size during the study period *in planta*, while fruit weight increased slightly and progressively until

shedding. After shedding, moisture content condensed on fruits overnight so increasing in fruit weight and bringing more moisture to the repair mechanism. In contrast, the acquisition of red colour by fruits at 126 DAA coincided with mass maturity, achievement of maximum normal germination and mean germination time. Demir and Samit (2001) found that maximum tomato seed quality was related to the change from a pink fruit colour in to red and firm.

Hence, the change in fruit colour from purple to red is a morphological marker associated with the achievement of maximum seed weight and germinability in *Solanum betaceum*, but for maximum quality a further 14 days *in planta* is required.

Carica papaya

Mass maturity was attained 140 DAA and coincided with the achievement of maximum ability to germinate for the dry seeds and the minimum mean germination time. High germination and minimum germination time were both maintained for a further 35 days after mass maturity while the fruits remained *in planta*. This conclusion coincides with other investigations of fleshy fruits where maximum seed quality was maintained for a long time after mass maturity e.g. pepper, tomato and marrow (Demir & Ellis 1992a; Demir & Ellis 1992b; Demir & Ellis 1993) and tree tomato in this chapter.

Maximum potential longevity as estimated by K_i was found at 35 days after mass maturity (175 DAA) and also coincided with the lowest electrical conductivity of seed-steep water. It is therefore confirmed that maximum potential longevity is attained in this fleshy fruit some time after mass maturity, as it has been shown in others e.g. 14 days after mass maturity in *Solanum betaceum* in this chapter, 10-12 days after in *Capsicum annum* (Demir & Ellis 1992b) and 24-31 after days in *Cucurbita pepo* (Demir & Ellis 1993).

The fact that half the seeds from completely green fruits at 126 DAA were able to germinate is somewhat surprising. However, the presence of some yellow colour in the fruit and all seeds completely brown and with ribs already developed, probably coincided with mass maturity. For maximum seed quality, however, the achievement of the orange colour in fruits is required.

The relationship of onset of germinability and development of maximum seed quality in *C. papaya* with decrease in moisture content and increase in seed dry weight and further development after mass maturity shows that the earlier findings in orthodox seeds apply to intermediate seeds too (Ellis *et al.* 1987; Pieta Filho & Ellis 1991; Demir & Ellis 1992a; Demir & Ellis 1992b; Ellis & Pieta Filho 1992; Demir & Ellis 1993; Egli & Tekrony 1997). In fact, similar patterns were also shown in recalcitrant species such as *Acer pseudoplatanus* (Hong & Ellis 1990).

Vasconcellea cauliflora

It was not possible to determine precisely when mass maturity occurred, but it probably occurred shortly before 161 DAA. Maximum ability to germinate normally was attained at 168 DAA and then maintained for 28 days longer *in planta*. This is in agreement with results for *C. papaya*. Given the fact that there was no evidence of damage over this 28 days period, and the previous results, it is suggested that fruits be collected when their skin colour is orange.

Conclusions

In all three species studied, seed quality did not decline immediately after mass maturity. The Harrington (1972) hypothesis is therefore rejected. Seed quality was greatest some 14 to 35 days after mass maturity.

Repairing and turnover mechanism occurred in *S. betaceum* and *C. papaya* seeds from fruits shed or maintained in fruit for *ex planta* maturation, due to seeds at about 60% and 70%, respectively, were exposed some time at hydrated environment inside the fruit. This finding is in agreement with Villiers (1973) hypothesis for seeds from shedding or extra ripen fruits. It is recommended that germplasm collectors collect fruits and extract seeds of *Solanum betaceum* 7 days after the fruit colour has changed from purple to red, *Carica papaya* when fruit colour changes from yellow and yellow green to yellow orange, and in *Vasconcellea cauliflora* when fruits are just changing from orange to orange red.

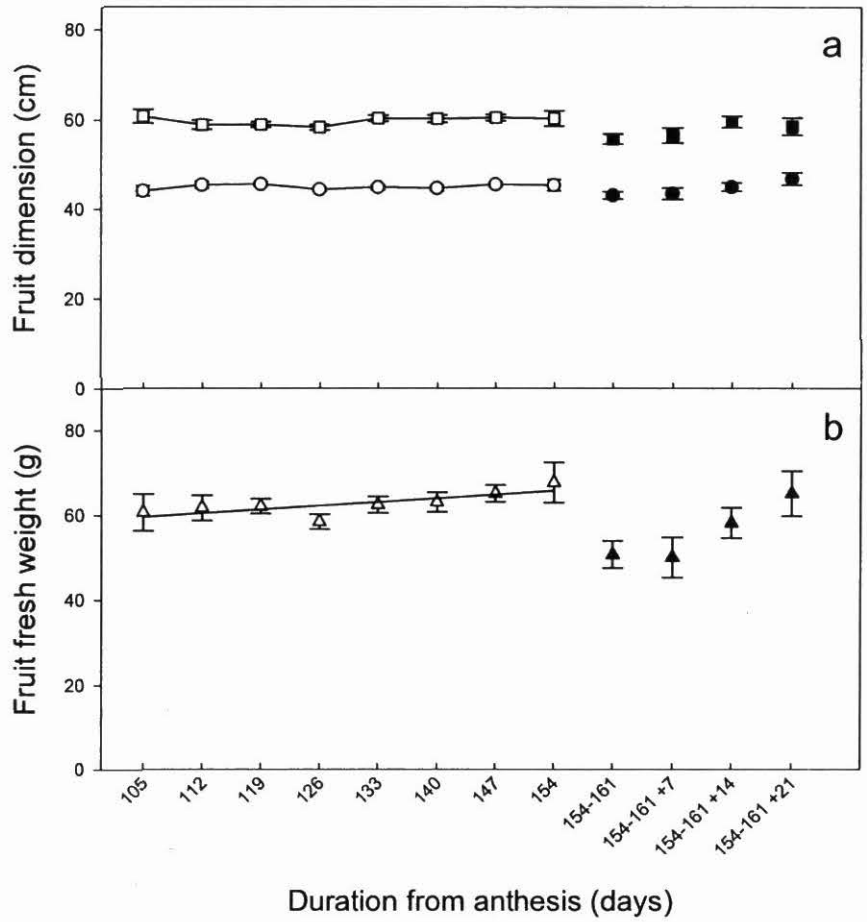

Figure 2. Mean fruit length (□, ■, a), mean fruit diameter (○,●, a) and mean fruit fresh weight (△,▲, b; fitted line $p<0.05$, $r^2=0.512$) of lots of *Solanum betaceum* common cultivar harvested at different durations of development and maturation (open symbols) or fruits collected from the ground at 154-161 DAA and allowed to mature *ex planta* for the periods shown (+7, +14, +21 days) (closed symbols). Bars indicate means ± standard error.

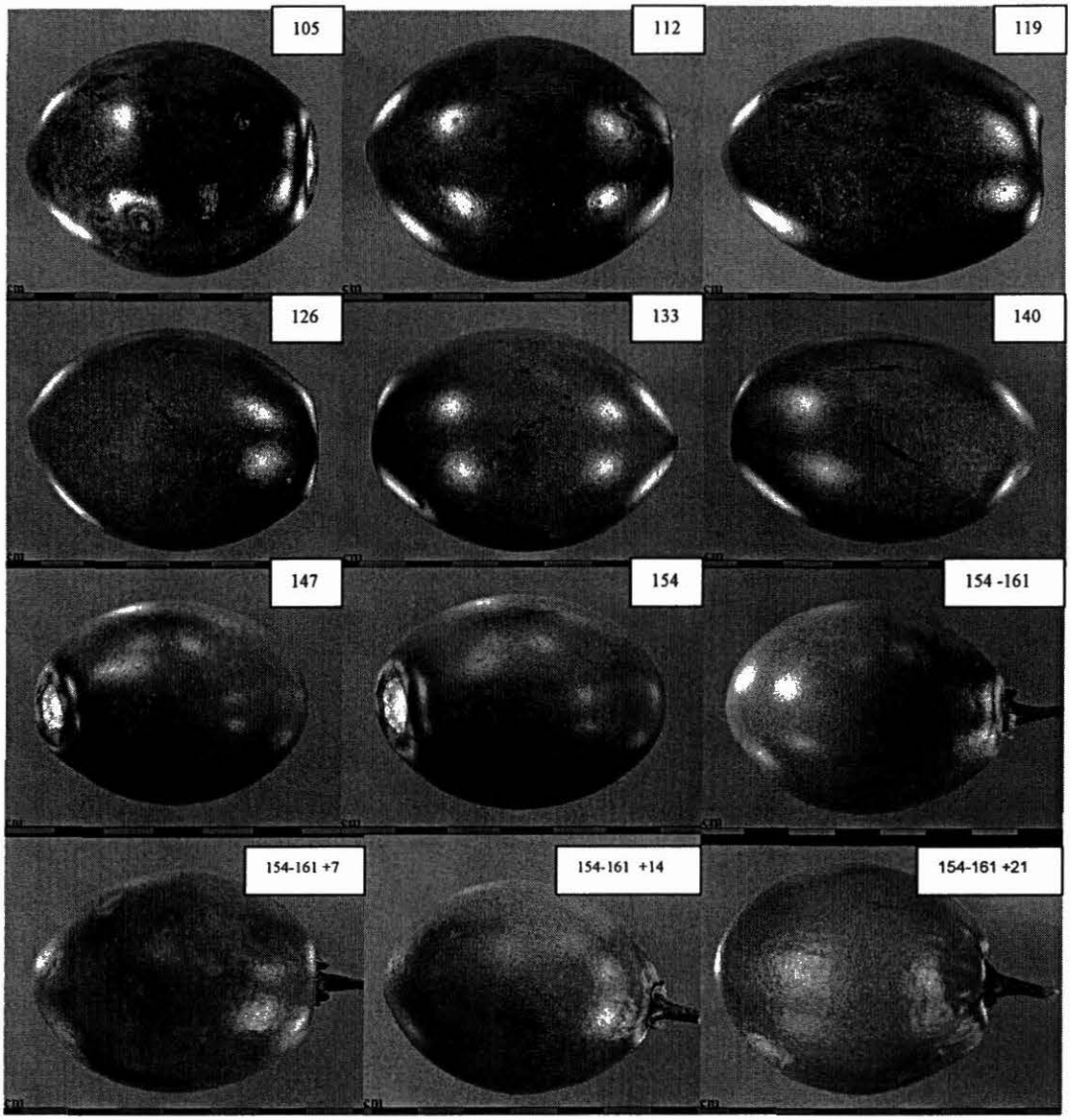

Figure 3. Fruits of *Solanum betaceum* harvested at different durations of development and maturation (durations in days after anthesis shown) or collected from the ground and allowed to mature *ex planta* (+7, +14, +21 days).

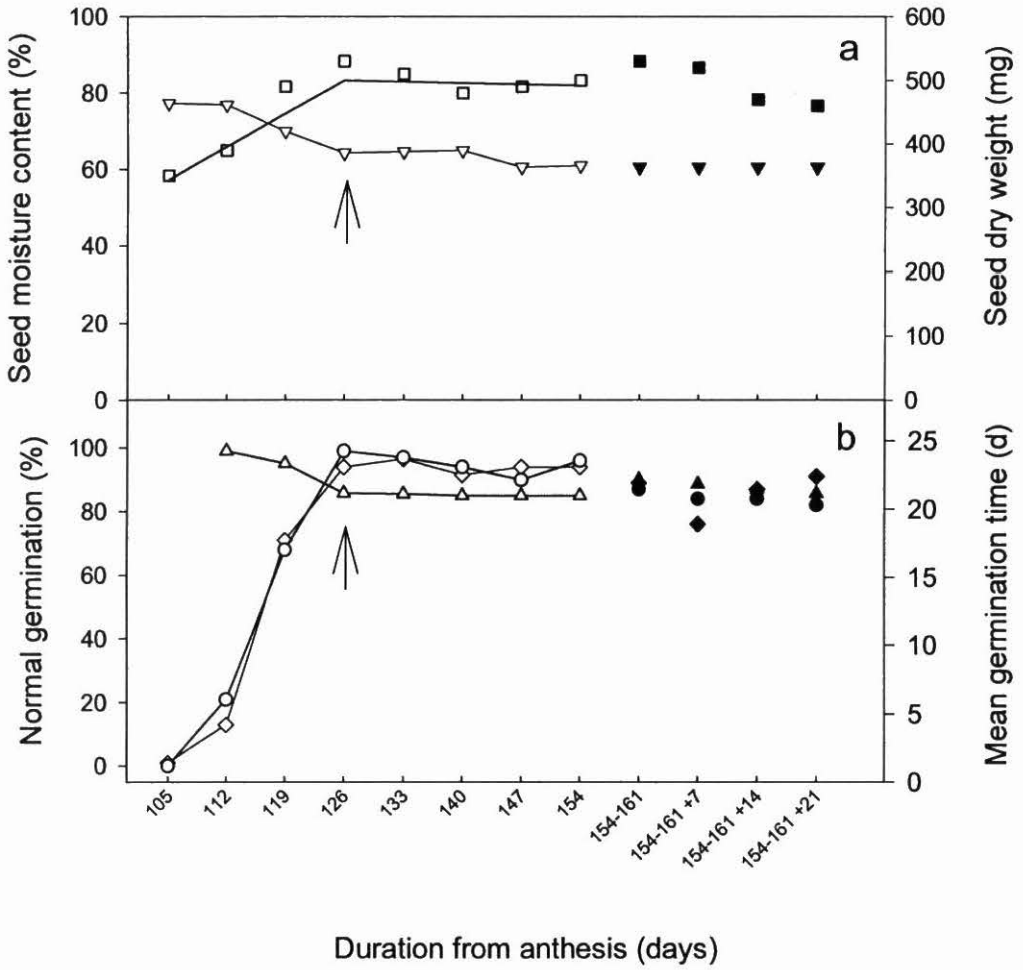

Figure 4. Mean seed dry weight (□, ■, a), seed moisture content (▽,▼, a), normal germination of fresh (◇,◆, b) and dry (○,●, b) seeds, and mean germination time of dry seeds (△,▲, b) of seed lots of *Solanum betaceum* extracted from fruits harvested at different durations of development and maturation (open symbols) or collected from the ground at 154-161 DAA and allowed to mature *ex planta* for the periods shown (+7, +14, +21 days) (closed symbols). The arrow indicates mass maturity and is the point of intersection between the line of positive slope (105-126 DAA) and the subsequent horizontal line (to 154 DAA). The positive slope was significant ($p<0.05$) with intercept –616 (± 140) mg and slope 9.14 (± 1.21) mg d^{-1} ($r^2=0.949$). Between 133 and 154 DAA there was no significant trend ($p>0.25$) with mean value 495 (± 6.5) mg.

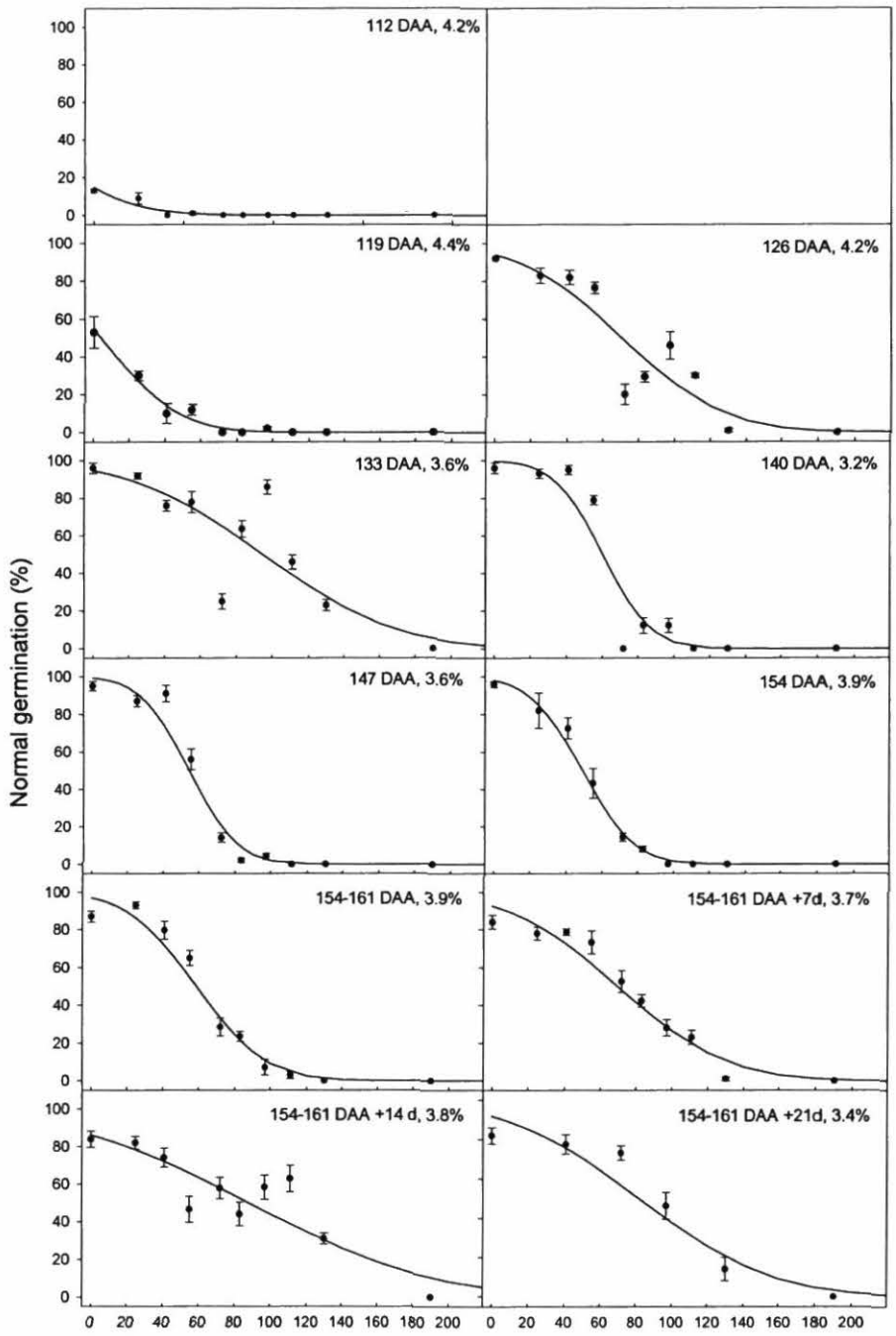

Figure 5. Seed survival curves for seed lots of *Solanum betaceum* stored hermetically at 40 °C with 3.2-4.4% m.c. (w.b.) collected after different durations of development and maturation as indicated in days after anthesis (DAA), or collected from the ground at 154-161 DAA and allowed to mature *ex planta* for the periods shown (+7, +14, +21 days). Bars indicate means ± standard error. The curves were fitted by probit analysis: estimates of Ki and $1/\sigma$ are provided in Table 16.

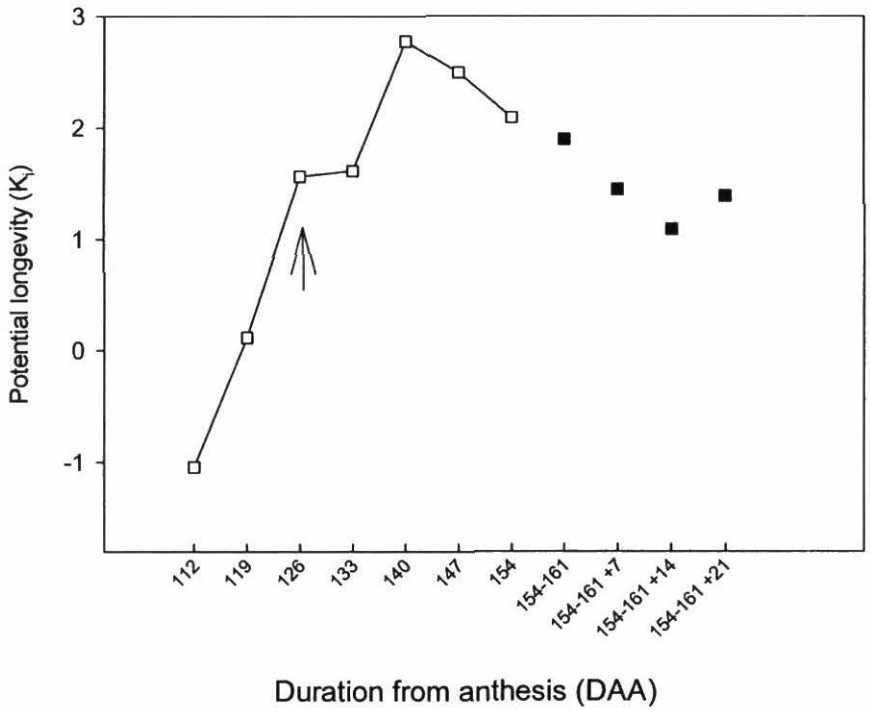

Figure 6. Potential longevity (K_i) for seeds of *Solanum betaceum* extracted from fruits harvested at different durations of development and maturation (open symbols) or collected at 154-161 DAA from the ground and allowed to mature *ex planta* for the periods shown (+7, +14, +21 days) (closed symbols), stored at 40 °C with 3.2-4.4% moisture content. The arrow indicates mass maturity.

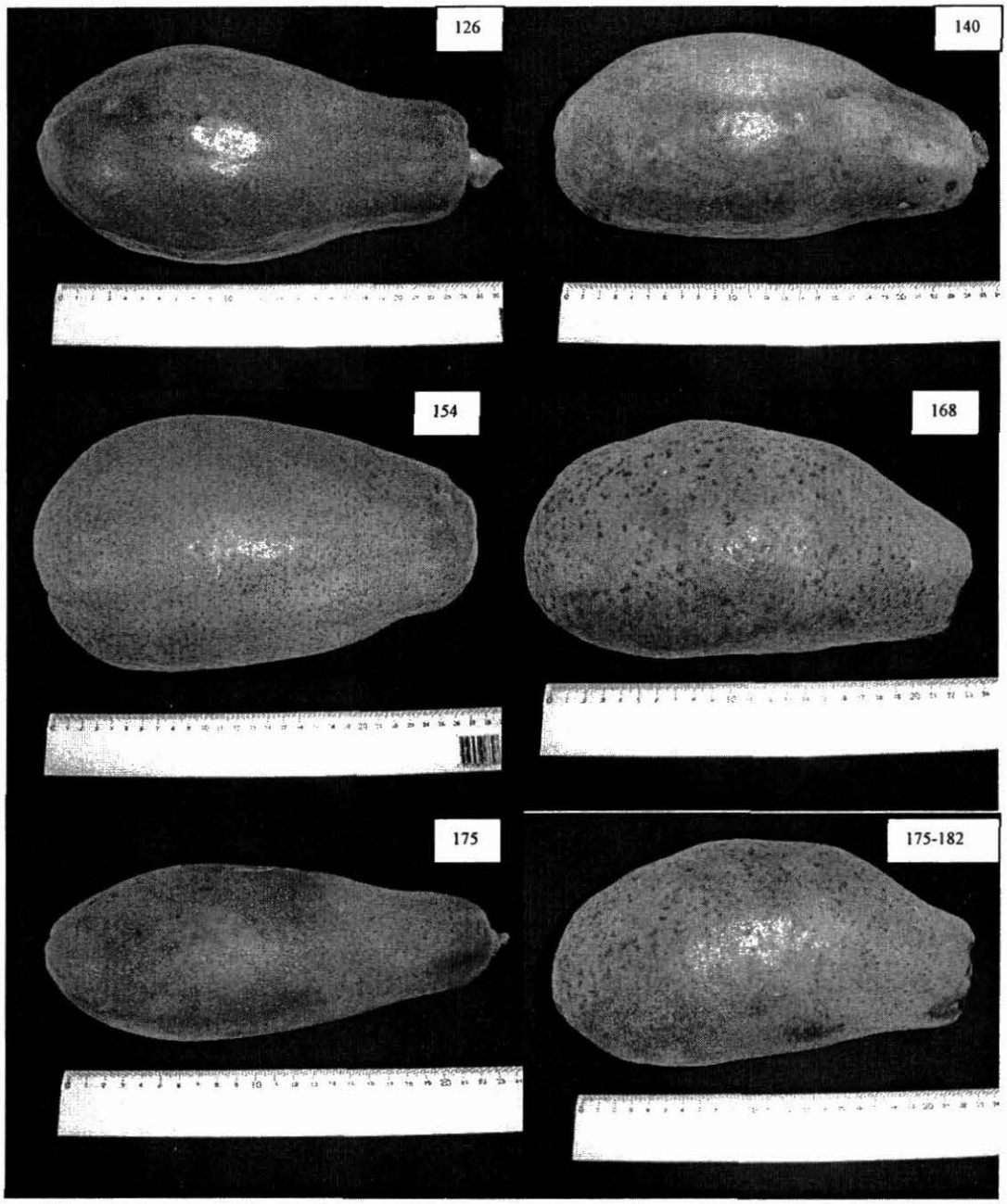

Figure 7. Fruits of *Carica papaya* cv. Tainung harvested at different durations of development and maturation, as indicated by days after anthesis (DAA), or collected from the ground at 175-182 DAA.

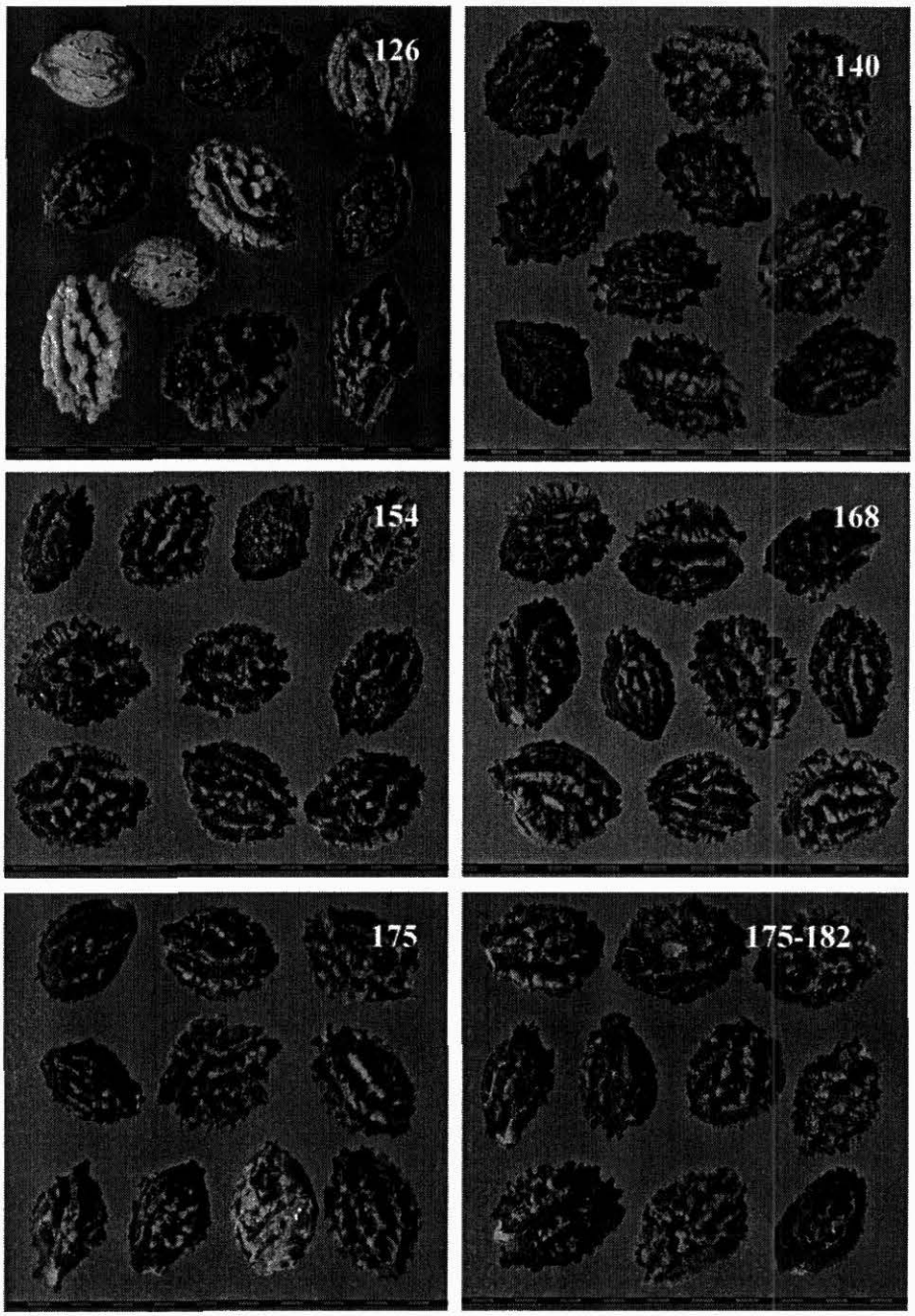

Figure 8. Seeds of *Carica papaya* cv.Tainung harvested at different durations of development and maturation, as indicated by days after anthesis (DAA), or collected from the ground at 175-182 DAA.

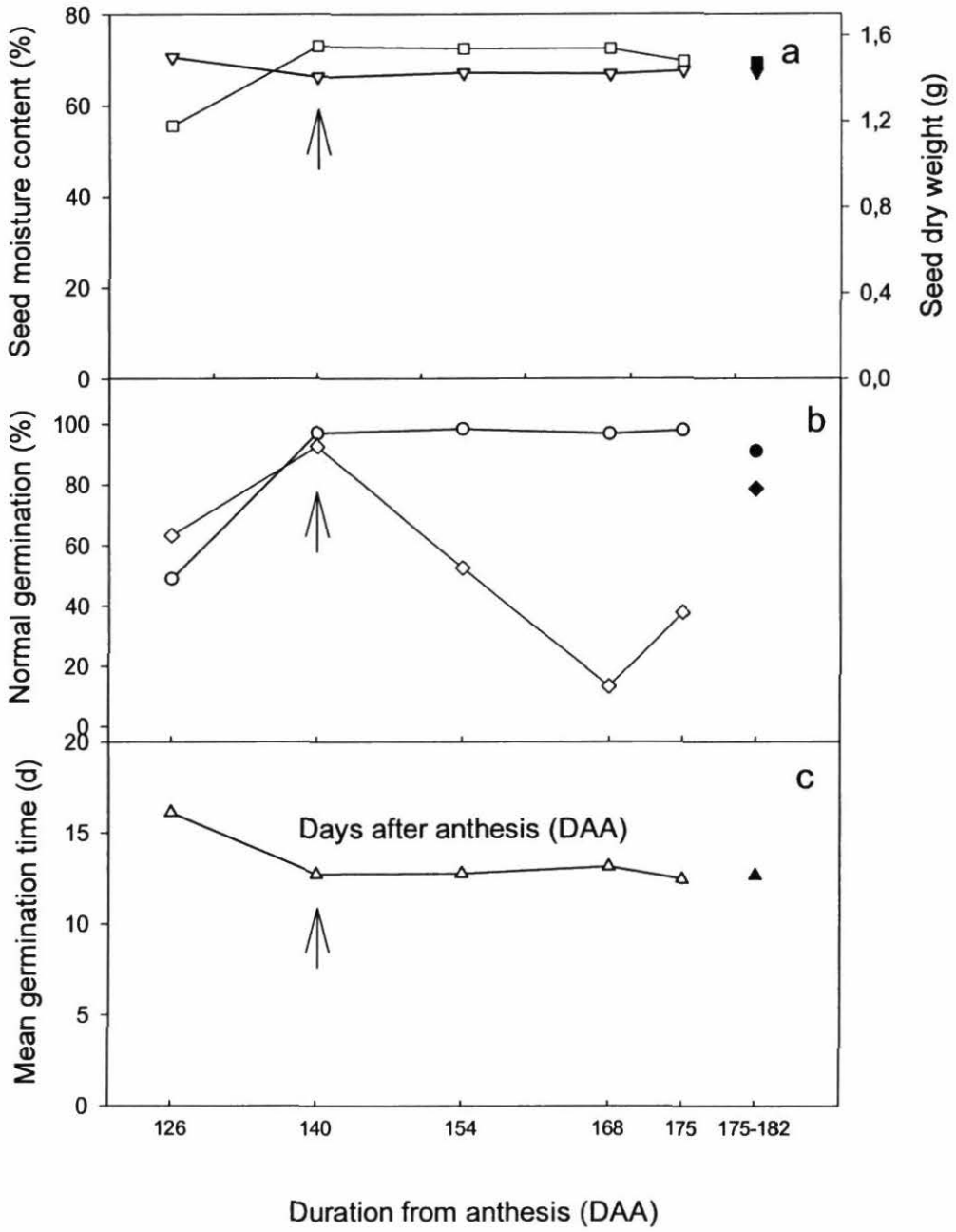

Figure 9. Mean seed dry weight (□, ■, a), seed moisture content (▽,▼, a), normal germination of fresh (◇,◆, b) and dry (O,●, b) seeds, and mean germination time of dry seeds (△,▲, b) of seed lots of *Carica papaya* cv. Tainung extracted from fruits harvested at different durations of development and maturation (*open* symbols) or collected from the ground (closed symbols). The arrow indicates mass maturity.

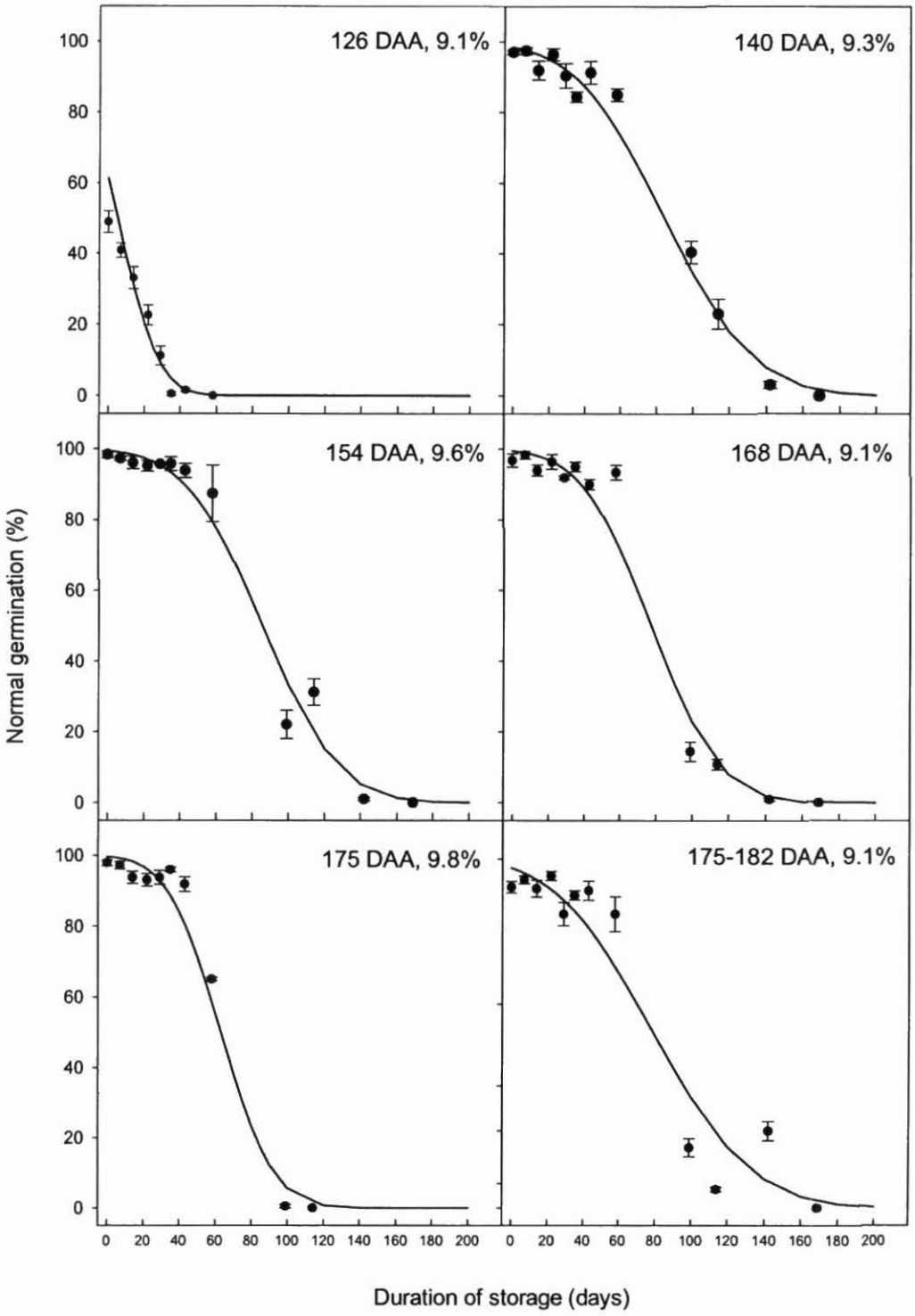

Figure 10. Seed survival curves for seed lots of *Carica papaya* cv. Tainung stored hermetically at 40 °C with 8.2-9.0% m.c. (w.b.) collected after different durations of development and maturation as indicated in days after anthesis (DAA), or collected from the ground at 175-182 DAA. Bars indicate means ± standard error. The curves were fitted by probit analysis: estimates of K_i and $1/\sigma$ are provided in Table 15.

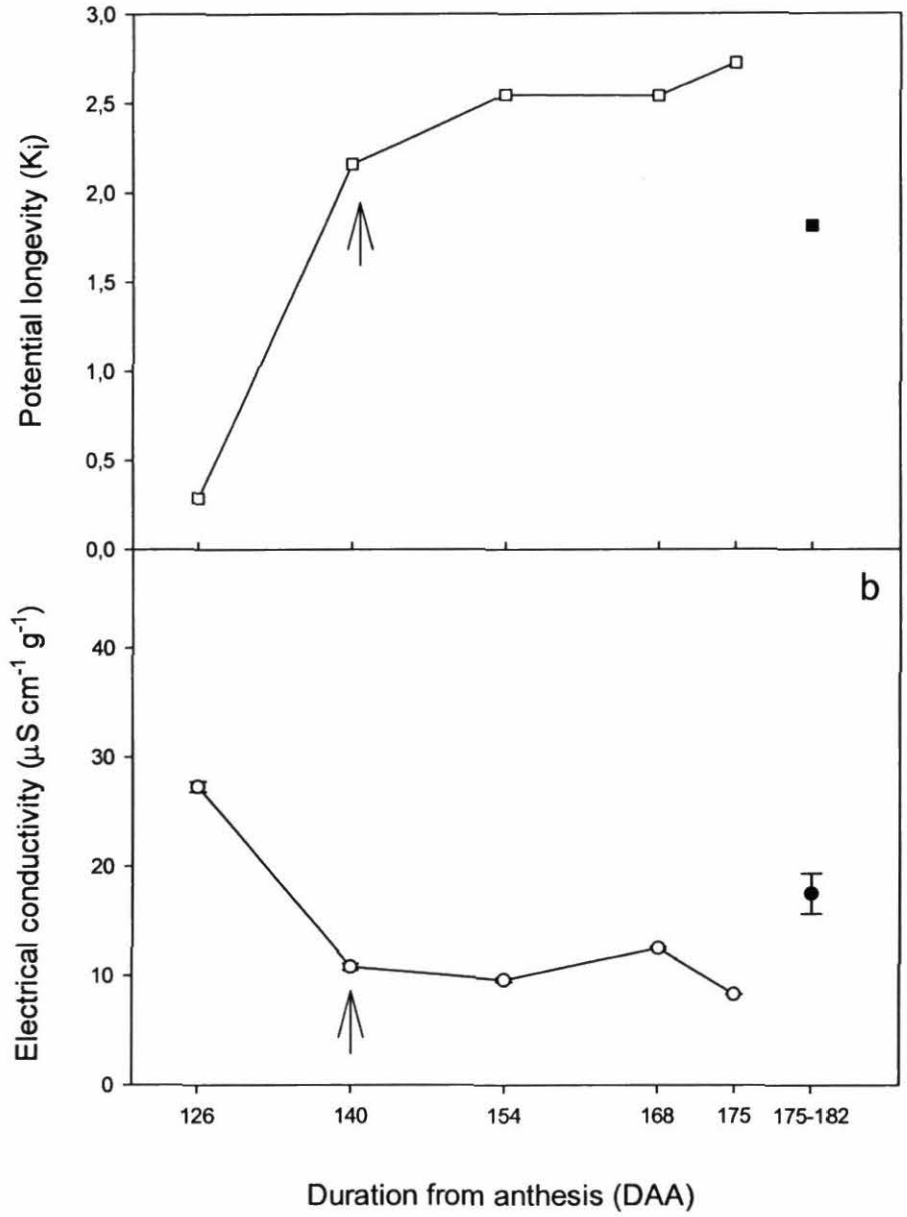

Figure 11. Mean seed dry weight (□, ■, a), seed moisture content (▽,▼, a), normal germination of fresh (◇,◆, b) and dry (O,●, b) seeds, and mean germination time of dry seeds (△,▲, b) of seed lots of *Carica papaya* cv. Tainung extracted from fruits harvested at different durations of development and maturation (open symbols) or collected from the ground (closed symbols). The arrow indicates mass maturity.

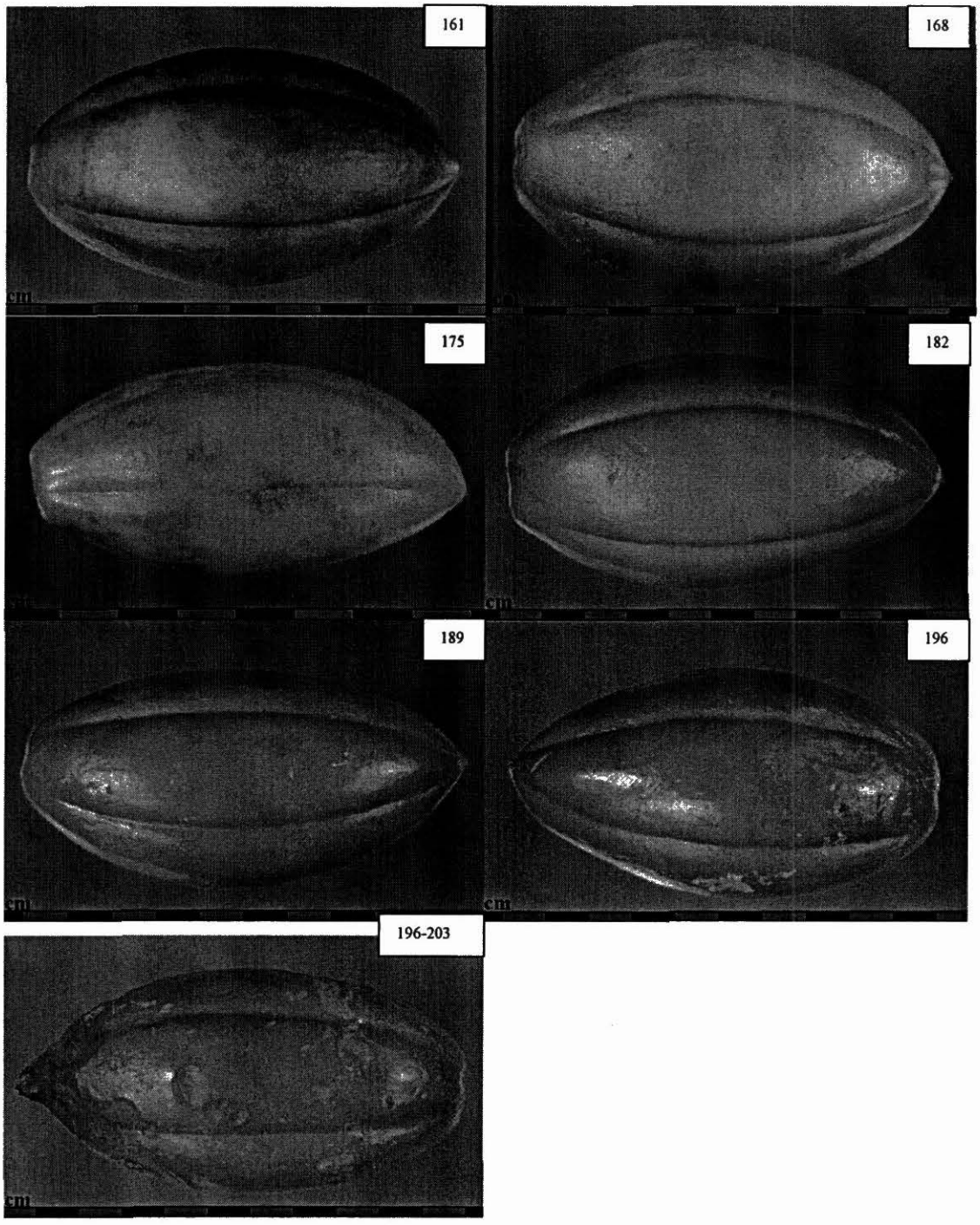

Figure 12. Fruits of *Vasconcellea cauliflora* harvested at different durations of development and maturation, as indicated by days after anthesis (DAA), or collected from the ground at 196-203 DAA.

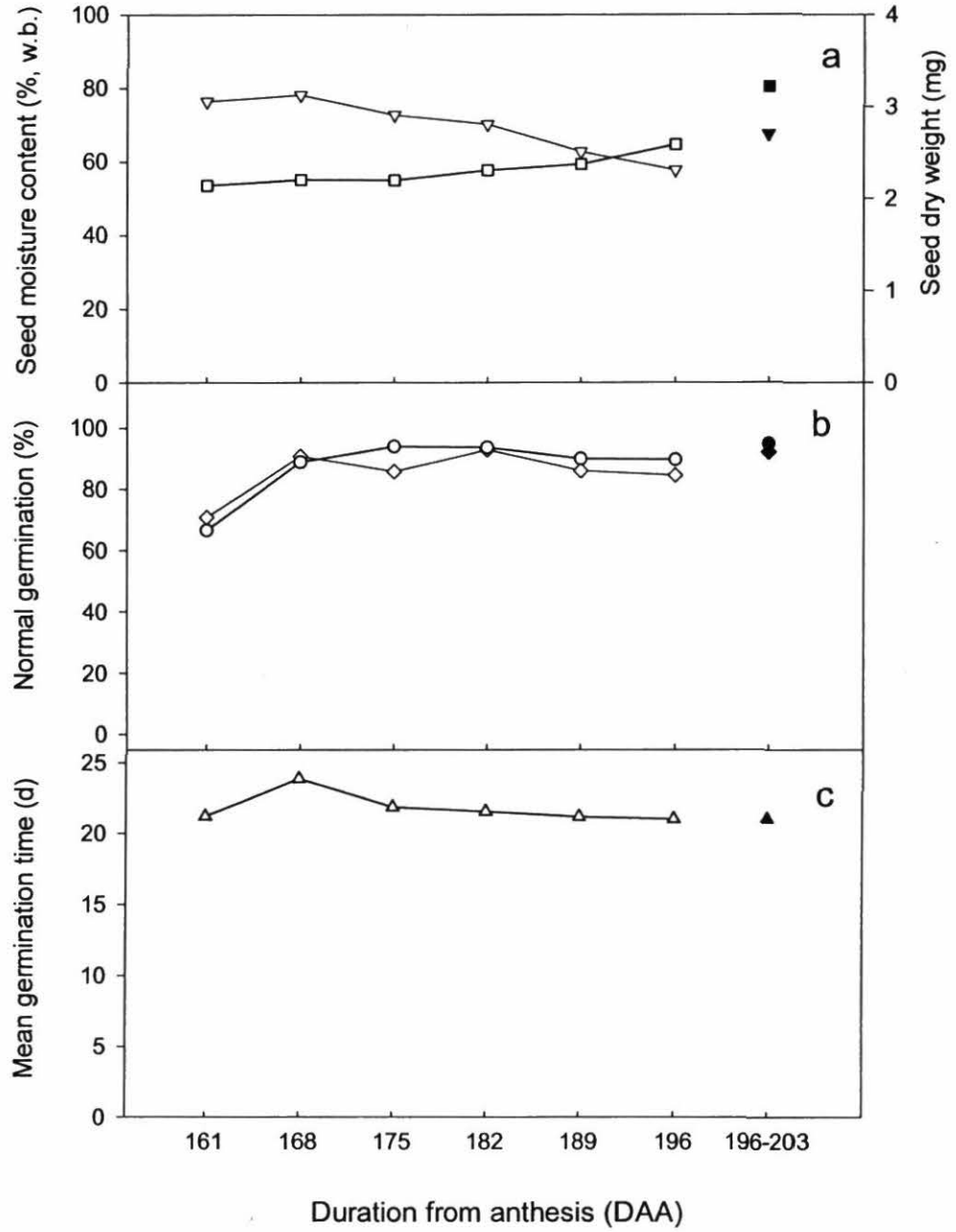

Figure 13. Seed moisture content (▽, a), mean seed dry weight (□, b), mean germination time of dry seeds (△, b), normal germination of fresh (◇,◆, c) and dry (○,●, c) seeds for *Vasconcellea cauliflora* extracted from fruits harvested at different durations of development and maturation (open symbols) or collected from the ground (closed symbols).

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Contributor: A.M. Torres

Activity 1.5.2. Implementation of the cryopreservation technique's using a cassava core collection as a model.

Introduction

After adjusting a basic cryopreservation encapsulation-dehydration technique with a few cassava clones, a core collection (640 clones) has been included in the experiments to test genotypic effects. Partial data have shown three groups of response after freezing phase: Lowest response (less than 30% plant recovery), Intermediate (more than 30 and less than 70 % plant recovery) and Highest responding group (more than 70% plant recovery). Logistical considerations have been considered to make a duplicate of Core collection under frozen conditions.

Material and methods

The encapsulation-dehydration technique has been implemented (Annual Report 2000) using *in-vitro* cassava plants supplied by GRU. Last year we start to make a duplicate of the core under liquid nitrogen conditions (called Cryo-Core II). *In-vitro* cassava material used for this second copy comes from clones maintained under BRU lab conditions.

Results

During the 2005-2006 period we started a Cryo-Copy II (Annual Report 2005). To develop these activities it was necessary to make a subculture of plants coming from clones maintained at BRU on 4E medium (Roca 1984). Due to growing conditions (temperature of the growth-room and culture media), those materials need a subculturing every 4-8 months. Basically those materials suffered a rapid deterioration at BRU laboratory (fast growth rate and quit aging of the material).

In 2006 we included only 5 new clones to the Cryo-Core I. Nowadays its collection just waits for 4 clones to be completed. More than 99% of the core clones are maintained in liquid nitrogen conditions. This collection could be considered as unique in the world. Our results show that more than 60% of the clones tested respond up-to 30% as plant recovery (intermediate and highest group) (Table 19).

For the Cryo-Core II we included 182 new clones in the 2005-2006 period (Table 19). It represents twice the number of clones included during the last year (Annual Report 2005). Nevertheless, data obtained by group responses by clones are not consistent with the first copy (Table 20). Based on our experiments the management of aged tissue has a strong influence on the response after freezing. Additionally, in some cases when a new subculture is initiated, some bacterial contaminations appear, making difficult the including of tissue in the liquid nitrogen tank.

Table 19. Establishment of Cassava core collection under liquid nitrogen conditions.

	Cryo-Core I		Cryo-Core II	
	No. Clones	% of the core	No. Clones	% of the core
Frozen	626	99.4	266	41.5
Evaluated	623	98.8	242*	38.4

* Clones without evaluation: 18. It's on running experiments

Some clones from Intermediate group change their behavior to the Lowest group. The Highest group is more consistent in its response.

Table 20. Groups of response of copies of the Core collection maintained under liquid nitrogen conditions.

		% Clones based on grouping responses	
		Cryo-Core I	Cryo-Core II
Group of response*	Lowest	33.6%	59.6
	Intermediate	40.5	17
	Highest	25.8	23.4

CIAT has been using Humboldt's Cryopreservation-tanks to maintain its copies in L.N. Inputs to the collections, from both institutions, are very active. For space reason, we may have soon to stop our including into their tanks and consider other options for the Cryo-Core II.

CIAT made a safety duplication of its in vitro collection to CIP, Peru. In the same way, CIAT will consider that, when cryopreservation technique is fine-tuned, CIP (at Peru) or INIBAP (at Belgium) could act as depositary of the copy of the Cryo collection under a black-box option.

Conclusions

CIAT has an entire copy of the Cassava Core-collection under liquid nitrogen. More than 98% of the clones have been tested after freezing step. Actually a duplicate of this collection has been built (41.5% of the core). Tissue aging affects the response after freezing. It is necessary maintain younger tissue to ensure the best grouping responses after freezing.

Future activities

Determine a procedure to make a duplicated collection under L.N. Recover plants from different periods of conservation and transfer to the field conditions to observe its behavior.

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[1]Agro-biodiversity and Biotechnology Project-CIAT; [2] Genetic Resources Unit-CIAT.

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

Output 2.1 FAO designate collections cleaned against seed borne diseases

Activity 2.1.1 Indexing and cleaning the cassava collection

We continued with indexing activities of clones of the Cassava World Collection maintained under *in vitro* conditions at CIAT. The final objective of this activity is to make all accessions available for distribution. This is obtained through cleaning and certifying the whole collection for the three viruses currently known of quarantine importance, following the FAO/IPGRI recommendations for the safe movement of cassava clones at national and international levels. We have been working on the indexing for three viruses: Cassava Common Mosaic Virus (CsCMV), Cassava X Virus (CsXV) and Cassava Frog Skin Virus (CsFSV). For the indexing, three diagnosis techniques are used: ELISA for CsCMV and CsXV, and grafting with a hypersensitive clone (MCOL 2063) for the CsFSV.

The total Cassava World Collection kept in GRU designated by the FAO is of 6,499 accessions, of which 5,184 (77%) correspond to the cultivated species, 384 (5,9%) hybrids and 931 (14,3) wild species. Of these, 4,995 clonal materials are available for distribution corresponding to 77% (Table 21 and Figure 14).

The number of clones evaluated against CsCMV (325), CsXV (366) and CsFSV (130) from November 2005 until October 2006 and the comparison of progress with previous years, is shown in Figure 14.

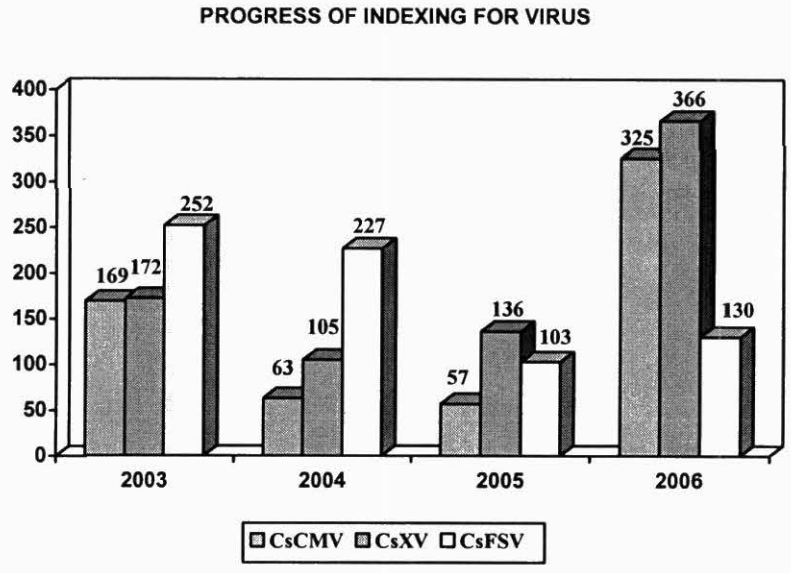

Figure 14. Number of Negative Clones evaluated for each virus 2003 – 2006.

The total of negative clones evaluated for the three viruses is of 862 from November 2005 until October 2006, this result is equivalent to 201,4% of average of the three previous years, product of the continuous work of indexing for three viruses, this result and the comparison of progress with before years is shown in Figure 15.

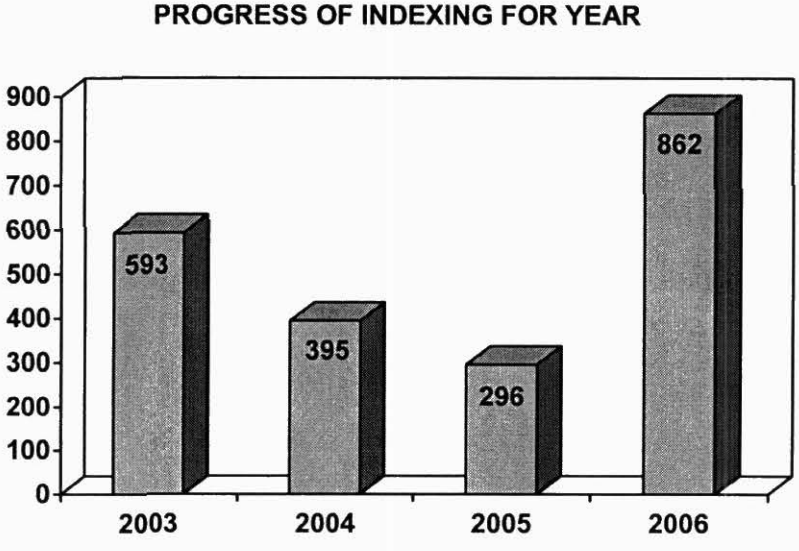

Figure 15. Negative clones evaluated in the period 2003 - 2006

The current status of the hybrid cassava collection designated to the Multilateral System of the Treaty (number of negative clones for each virus and number of clones currently available for distribution, negative for the three viruses) is presented in the following Table.

Table 21. Indexing status of the Hybrid Cassava Germplasm Collection.

HYBRIDS					
Source	FAO	INDEXATION OF NEGATIVES CLONES			Available
		CsCMV	CsXV	CsFSV	
CG	78	77	75	71	70
CM	235	234	225	220	220
CT	1	1	1	1	1
HMC	4	4	3	4	3
SG	43	43	42	40	40
SM	23	23	23	21	21
Sub. Total	**384**	**382**	**369**	**357**	**355**
Sub. %	**100%**	**99%**	**96%**	**93%**	**92%**

CULTIVATED SPECIES					
Source	FAO	INDEXATION OF NEGATIVES CLONES			Available
		CsCMV	CsXV	CsFSV	
ARG	122	108	104	86	82
BOL	7	7	7	5	5
BRA	1317	1312	1308	1209	1207
CHN	2	2	2	2	2
COL	1995	1988	1979	1816	1814
CR	148	148	145	144	141
CUB	77	77	77	77	77
DOM	5	5	5	5	5
ECU	116	116	114	108	106
FJI	6	6	5	5	5
GUA	91	91	90	77	77
IND	135	121	89	43	43
MAL	67	67	67	57	57
MEX	102	102	100	89	89
NGA	19	19	19	17	17
PAN	43	43	39	37	37
PAR	208	207	208	167	167
PER	405	405	405	386	385
PHI	6	6	5	5	5
PTR	15	15	15	13	13
SLV	8	7	7	6	6
TAI	31	30	30	19	19
USA	9	9	9	8	8
VNM	9	6	5	4	3
VEN	241	241	237	218	217

Sub. Total	5,184	5,138	5,071	4,603	4,587
Sub. %	100%	99,1%	98,0%	89,0%	88,5%

Total	6,499	5,838	5,740	5,016	4,995
Total %	100%	90,0%	88,3%	77,2%	77,0%

Progress on indexing is also shown in Figures 16 and 17

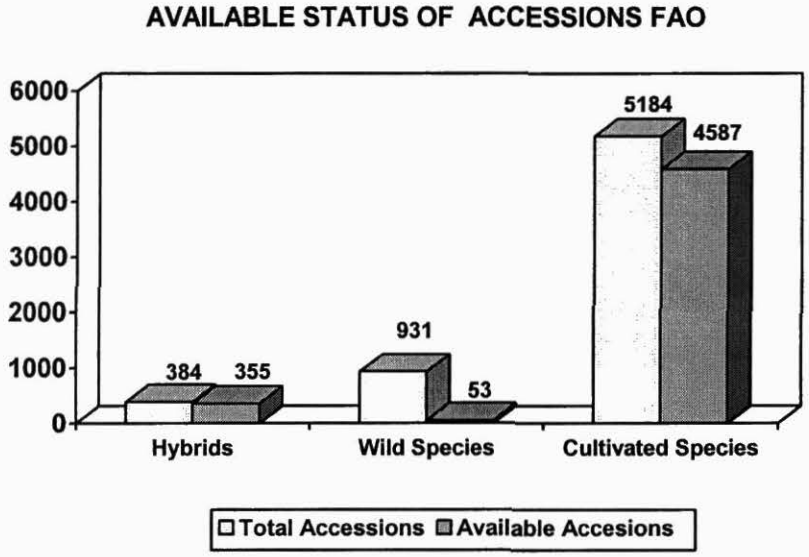

Figure 16. Status of availability of the Cassava Germplasm Collection.

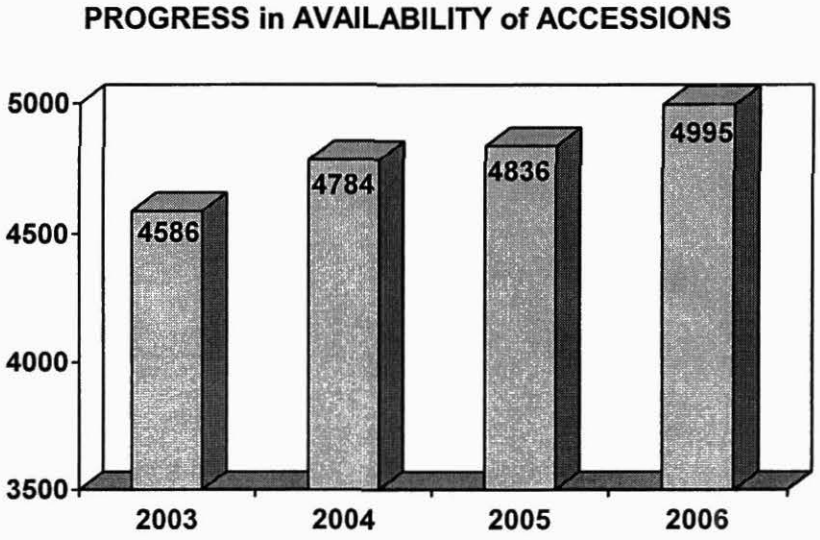

Figure 17. Available Accessions of the Cassava Germplasm Collection kept in CIAT GRU.

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

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

At the moment, the detection of Cassava Frog Skin Virus (CsFSV), has been made possible by grafting. This process causes a delay of three months on an average as the plants must have the size for grafting, and tree weeks are necessary to observe the characteristic symptoms in the secundina clones (MCOL 2063 highly sensitive to the virus). This duration can be longer if problems persist on the establishment, as reported previously (Flor et al. 2003). In order to decrease this duration, we implemented a molecular methodology (RT-PCR) for the detection of CsFSV developed in the Virology Lab of CIAT, that requires tree days and seems more sensible and specific (Cuervo 2006).

Out of 180 samples, from the Cassava Collection the CsFSV was detected in four In Vitro plants (Table 22), and the correlation between the positive and negative controls is of 100% (Figure 18).

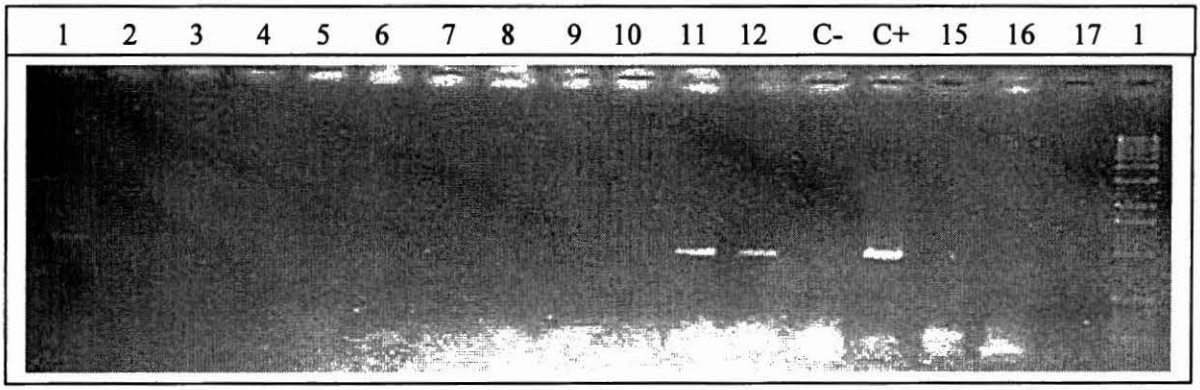

Figura 18. Electrophoresis RT-PCR CsFSV in Agarose Gel 1%. *In Vitro* Plants positives for CsFSV (1, 11 y 12), In Vitro plants negatives for CsFSV (2, 3 y 4), Bonsai collection (5-10), Negative control (Virology and GRU) (C-, 13), Positive control (Virology and GRU) (C+,14), White RT (15), White PCR (16), Weight marker (1Kb, 18)

Table 22. Comparison of indexing methods: grafting *versus* molecular testing by RT-PCR.

No. Samples	Source	RT-PCR
10 Negative by Grafting	In Vitro	10 Negative
5 Positive by Grafting	In Vitro	2 Positive
40 Negative by Grafting	Bonsai	40 Negative
9 Negative by Grafting	Secundina	9 Negative
10 Positive by Grafting	Secundina	10 Positive
106 without Grafting	In Vitro	2 Positive

Contributors: M. Cuervo., M. G. Moreno, G. Mafla.

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

In October 2001 we started establishing one copy of the whole cassava collection under greenhouse conditions. The material is coming from the indexing for FSDV, and sticks are planted in recycled bottles instead of being thrown away, and is thus free of viruses if kept in insect proof glass-house. Along our current agreement with FAO, this back-up is necessary and complements that kept at CIP. At the moment, in the Bonsai Greenhouse 631 clones are maintained (Figure 19).

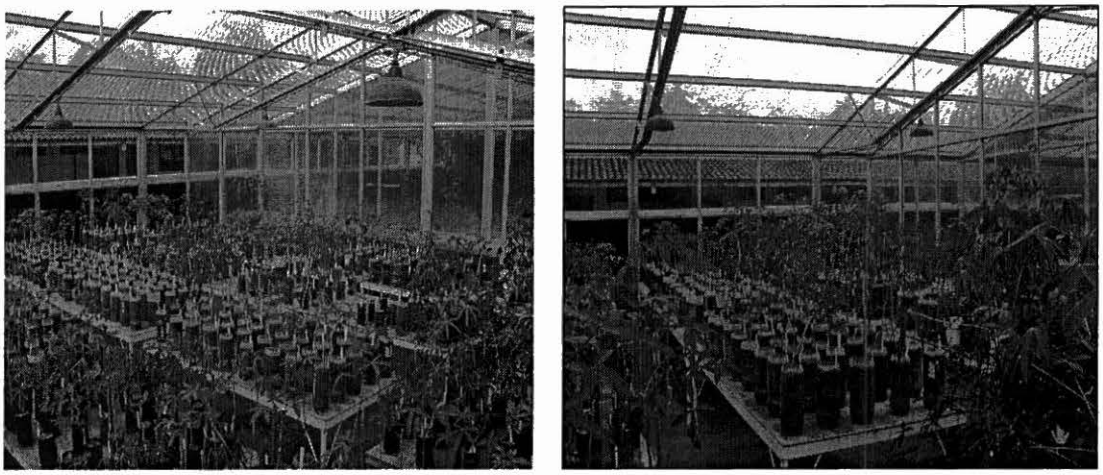

Figure 19. Cassava plants established as "Bonsai" collection as back up for the In Vitro Bank.

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

Activity 2.1.5 Updating the Cassava ORACLE database

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

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

Activity 2.1.7. Germplasm health control in seed germplasm

Introduction

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

In 2006, the GHL tested 7,448 seed samples (4,937 bean seed samples, 2,511 seed samples of legume forages and tropical grasses from the project Integrated Conservation of Neotropical Plant Genetic Resources, including 541 samples of bean seeds and 91 of tropical grasses and legumes from CIAT projects Mesoamerican Bean Genetics, Andean Bean Genetics and Tropical grasses and legumes, respectively.

Materials and Methods

Phytosanitary inspections are carried out in multiplication plots. Accessions are tested in the GHL using standard methodologies to identify seed-borne pathogens such as fungi, bacteria and viruses according to the pathogens recorded in seed production areas. To detect pathogens of quarantine significance in the commodity crops, the GHL uses the methodologies recommended by CIAT pathologists and virologists. When a recipient country requests additional controls, the GHL carries out additional tests whenever possible to comply with the specific quarantine regulations of the recipient country.

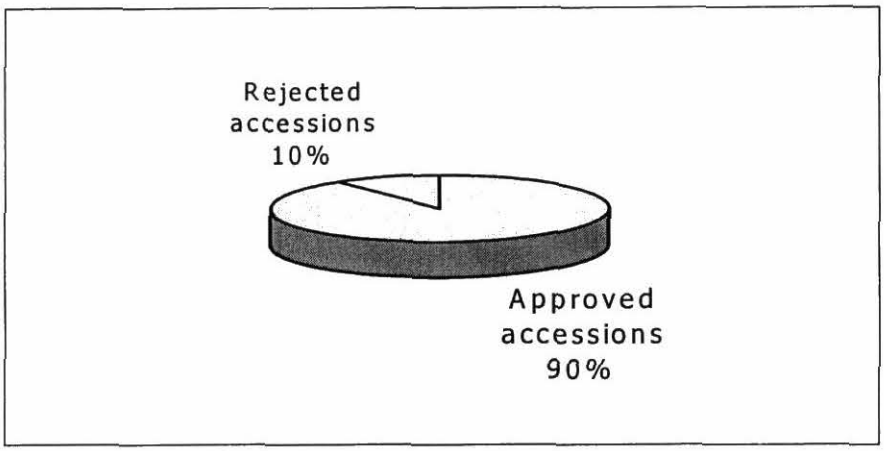
Testing for some genera of seed borne fungi includes blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar culture media. Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli,* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp., in tropical pastures) are also tested. The SHL uses dilution and plating on semiselective culture media such as MXP or King B, in addition to immunoprecipitine test with specific antisera or pathogenicity tests. The lab also uses immunofluorescent techniques. Testing *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* is achieved by subculturing on YDCA, by Gram staining, incubation under high temperature (36-37° C). Also complementary tests using a Gram-Positive ID Kit and Gram- negative (Becton Dickinson BBL Crystal™, Nippon Becton Dickinson Company Ltd.) containing different enzymatic and biochemical substrates are carried out. In addition, we use the pathogenicity biological test. Testing for seed borne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and or seedling-symptom test.

Results

Beans (*Phaseolus* spp.)

Seed samples of 4,937 accessions of beans were tested, some of them for shipment to foreign countries, and the other ones for conservation in the Bean Germplasm Bank, distributed among 23 species (Table 19). Their health status showed 90.0 % samples without pathogens of quarantine importance (Figure 20).

Figure 20. Number of *Phaseolus* sp. seed samples rejected or accepted after seed health laboratory analysis.



Ten percent of samples of *Phaseolus* sp. showed pathogens of quarantine importance (Table 20, Figure 21). The seedborne fungi such as *Macrophomina* sp, *Botrytis sp., Ascochyta* spp., *Macrophoma* sp., *Colletotrichum* spp., *Rhizoctonia*, *Phomopsis* spp., *Curvularia sp., Phytomyces sp., Sclerotina y Verticillium sp.* were detected.

Seedborne viral infections by potyviruses were detected in 71 samples and Southern bean mosaic virus (SBMV) was detected in 43 samples. This year *Pseudomonas syringae* pv *phaseolicola* were detected at high frequency (109), as compared to previous years and *Xanthomonas* spp. were detected in only one accession. The *Corynobacterium* Gram-positive bacteria were not detected.

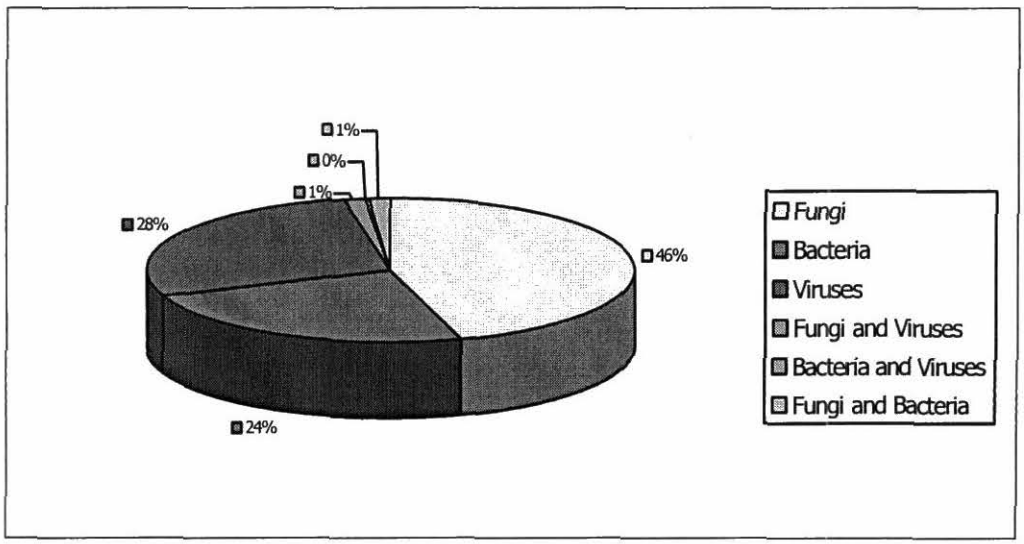


Figure 21. Factors for rejection of *Phaseolus sp.* seed samples analyzed at GHL.

Table 23. Factors for rejection of *Phaseolus* seed samples analyzed by GHL.

Factors for rejection	Number of affected accessions	Percentage
Macrophomina	107	23,8
Pseudomonas	106	23,6
Potyvirus	71	15,8
SBMV	43	9,6
Botrytis	24	5,3
Macrophomina, Colletotrichum	13	2,9
Ascochyta spp.	12	2,7
Macrophoma	11	2,4
Potyvirus, SBMV	11	2,4
Macrophomina, Rhizoctonia	10	2,2
Colletotrichum	9	2,0
Rhizoctonia	8	1,8
Macrophomina, Poty	3	0,7
Macrophomina, Xanthomonas	3	0,7
Potyvirus, Pseudomonas	3	0,7
Macrophomina, Rhizoctonia	2	0,4
Phomosis	2	0,4
Botrytis, Rhizoctonia	1	0,2
Curvularia	1	0,2
Sclerotina	1	0,2
Macrophomina,Curvularia, Phytomyces	1	0,2
Macrophomina,Rhizoctonia, Pseudomonas	1	0,2
Macrophomina, Sclerotina	1	0,2
Verticillium	1	0,2
Pseudomonas, Macrophoma	1	0,2
Pseudomonas, Macrophomina	1	0,2
Pseudomonas, SBMV	1	0,2
Xanthomonas	1	0,2

Tropical grasses and legumes

Seed samples of 2,511 accessions of tropical grasses and legumes were tested, distributed among 62 genera. Their health status showed 80.0% samples without pathogens of quarantine importance (Figure 22).

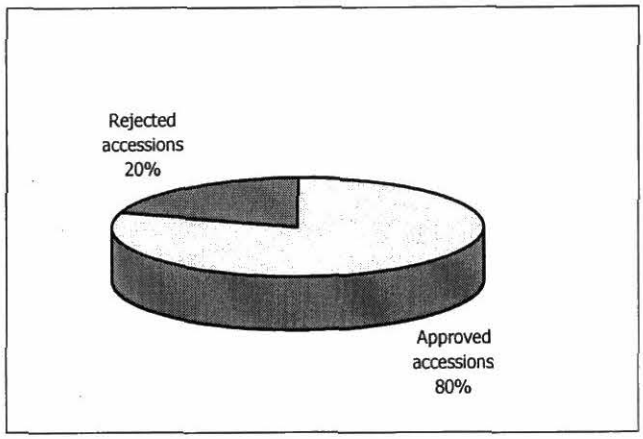


Figure 22. Number of seed samples of rejected Tropical grasses and legumes or accepted after seed health analysis.

In the rejected samples we detected some seedborne fungi of quarantine importance (*Curvularia sp.*, *Colletotrichum* spp., *Drechslera* spp., *Phomosis spp.*, *Macrophoma* sp., *Macrophomina* sp., *Pestalotia* spp., *Phoma* spp, *Rhizoctonia* sp.) (Table 21, Figure 25). Seedborne viral infections by Potyviruses and Southern bean mosaic virus (SBMV) were detected in 18 samples (Table 22). *Pseudomonas fluorescens* was detected in 48 accessions and *Xanthomonas* spp. in only one accession. The *Corynobacterium* Gram-positive bacteria were detected in 4 samples.

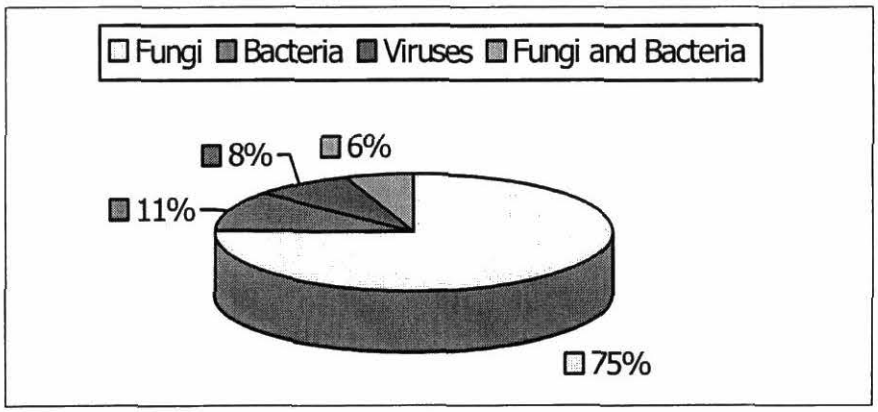


Figure 23. Rejection factors of *Phaseolus sp.* seed samples analyzed by GHL

Table 24. Disapproval factors of legume and tropical grasses seed samples analyzed by GHL.

Rejection Factors	Affected accesion	%
Curvularia	52	11,61
Colletrotrichum	45	10,04
Pseudomonas	45	10,04
Drechslera	40	8,93
Phomosis	33	7,37
Macrophoma	31	6,92
Rhizoctonia	25	5,58
SBMV	18	4,02
Potyvirus	18	4,02
Phoma sp.	17	3,79
Drechslera, Pseudomonas	12	2,68
Drechslera, Curvularia	11	2,46
Pestalotia	11	2,46
Drechslera, Phoma	9	2,01
Macrophomina	8	1,79
Colletotrichum, Pseudomonas	5	1,12
Curvularia, Phoma	5	1,12
Macrophoma, Phomosis	5	1,12
Colletotrichum, Phoma	4	0,89
Corynebact Gram+	4	0,89
Colletotrichum, Phomosis	3	0,67
Curvularia, Rhizoctonia	3	0,67
Pestalotia, Phomosis	3	0,67
Ascochyta	2	0,45
Colletotrichum, Curvularia	2	0,45
Colletotrichum, Curvularia, Pestalotia	2	0,45

Rejection Factors	Affected accesion	%
Curvularia, Phomosis	2	0,45
Drechslera, Curvularia, Phoma	2	0,45
Drechslera, Rhizoctonia	2	0,45
Macrophomina, Curvularia	2	0,45
Macrophomina, Pestalotia	2	0,45
Pestalotia, Curvularia	2	0,45
Pestalotia, Macrophoma	2	0,45
Phomosis, Pseudomonas	2	0,45
Rhizoctonia, Phomosis	2	0,45
Colletotrichum, Curvularia, Phomosis	1	0,22
Colletotrichum, Rhizoctonia	1	0,22
Drechslera, Curvularia, Pseudomonas	1	0,22
Dreschlera, Curvularia	1	0,22
Dreschlera, Pseudomonas	1	0,22
Macrophoma, Macrophomina, Phomosis	1	0,22
Macrophoma, Pseudomonas	1	0,22
Macrophoma, Rhizoctonia	1	0,22
Macrophomina, Pseudomonas	1	0,22
Phomosis, Curtobacterium	1	0,22
Phomosis, Macrophomina	1	0,22
Pseudomonas, Ascochyta	1	0,22
Rhizoctonia, Curvularia, Phomosis	1	0,22
Rhizoctonia, Pestalotia, Curvularia	1	0,22
Phytomyces	1	0,22
Rhizoctonia, Phomosis, Macrophoma	1	0,22
Xanthomonas spp.	1	0,22

Service of germplasm health certification for other projects

Seed samples of 564 accessions from CIAT Projects Andean Bean Genetics, Mesoamerican Bean Genetics and IP5 (Tropical grasses and legumes) were analyzed (Figure 24).

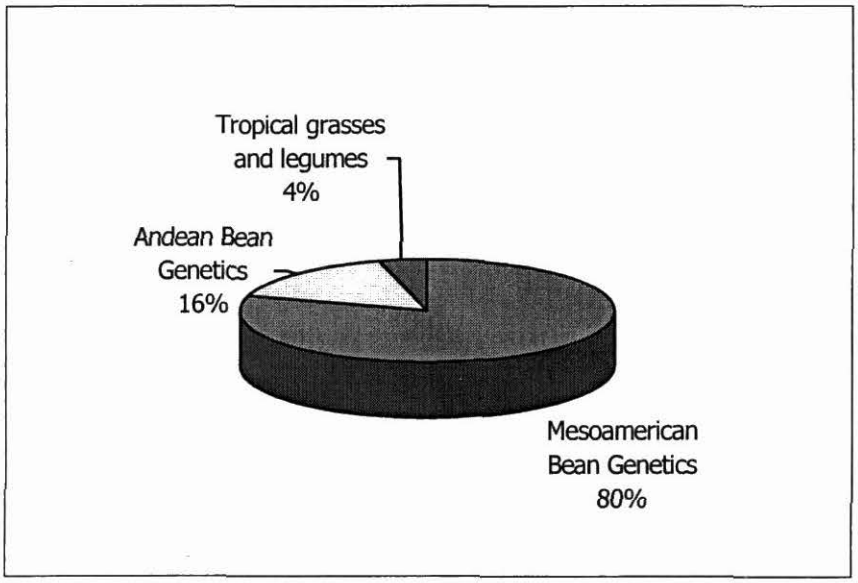


Figure 24. Number of accessions tested for other CIAT projects in 2006.

Their health status showed 75.9 % samples without pathogens of quarantine importance (Figure 25). In the rejected samples (24.2 %) we detected some seedborne fungi of quarantine importance (in 118 accesssions *Macrophomina* sp., *Colletotrichum* spp., *Rhizoctonia* sp.); *Pseudomonas fluorescens* were detected in 13 accessions and *Xanthomonas* spp. in only one accession (Figure 25).

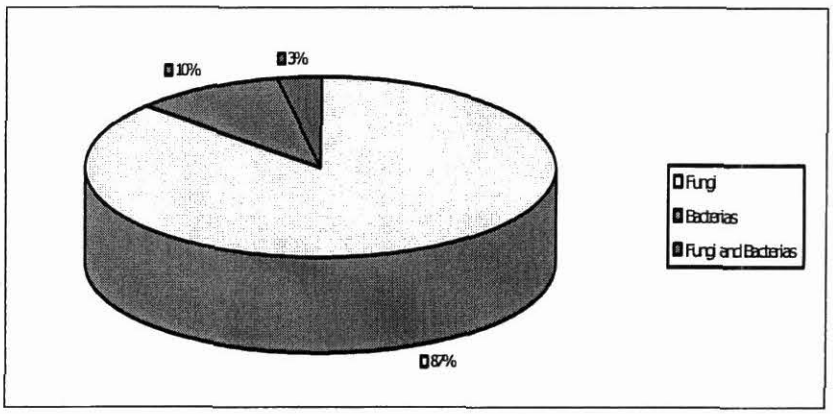


Figure 25. Factors for rejection of samples from other projects analyzed by GHL

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

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.

Introduction

The information system implemented at the GRU is periodically updated with additions, changes and improvements.

At the same time, in 2006, we worked hard in implementing a totally new GRU Web portal, completely available for everyone, inside and outside CIAT. In this portal, we give to the user a lot of new functionalities, such as news, list of publications, documents and training opportunities. One of the improvements of this new approach is the effectiveness of new search parameters and the visualization and downloading of the search results, also a new fast way to make requests in just a few clicks. In this portal, we tried to improve a user friendly search for seed germplasm and the ordering of samples of bean, cassava and tropical forages.

Another important improvement we achieved in 2006 was the introduction of barcode labels and mobile computing in the field, for characterization/evaluation purposes, and the introduction in the Viability Laboratory. Our next step is to cover some extra areas, like printing labels on fields to label the bags in harvesting and implementing in health laboratory a similar system as the one in the Viability Lab.

Materials and Methods

Our database is entirely built and maintained in Oracle.

In order to make the changes in the internal information system we use the tool called Developer 2000 from Oracle. To generate reports and statistics, we use Oracle Discoverer tool and to access the database and make advanced queries we use Oracle SQL Developer.

To build the GRU Web portal, the Java language (object oriented) program was used, also the J2EE architecture, the Struts Framework, the Hibernate object/relational persistence and query service and AJAX development technique. The Tomcat Application Server was used to test it and is currently used to keep it online. This portal is worldwide accessible, because it is published in internet, thus available for everyone and everywhere.

For bar coding, we are using Zebra printers (S600, TLP 2742, TLP2742Z and TLP2844Z). For the work in the field, we are using PSION WorkAbout PRO handhelds with a laser barcode reader and Printek MT 300 printers. This equipment was acquired due to its modularity, portability, ease of use and Java capabilities. For the work in the laboratories, we are using Symbol SPT1800 handheld and PSION WorkAbout MX with barcode scanner. We already bought the PSION WorkAbout PRO handhelds that we are planning to use in the seed reception from field process and we are forecasting to buy some HP iPAQ PDAs to implement mobile computing and barcode in seed conservation process.

Results

The internal information system has been updated with new information and reports, and lots of new images has been added to be used with the new internal Web portal, helping to a fast identification of the material and avoiding confusions during seed multiplication processes. There is one computer in each step of the germplasm flow chart and several parts of the building, where the user can insert or update the information.

The new Web portal has been installed, in English and Spanish versions, with its new functionalities:

- Keep public and up to date our Information Access Agreement (IAA) and Material Transfer Agreement (MTA)
- User registration. To keep records and statistics of the users and to make it simple for them when they search for information or request for materials in our portal.
- Bean, cassava or forages information search by accession or by descriptors (after the acceptance of the IIA, and where the user can choose which descriptors he/she wants to use as a filter). After the search has been done, the user can improve it or perform a new one and even make a material request (after the acceptance of the MTA). In the results page, not only the information is displayed, also the seed, plant and herbarium photo (when they are available). The user can download the search results into Excel, so he/she can make a deeper evaluation of the results later and offline the internet.
- Bean, cassava or forages direct material request by accession (after the acceptance of the MTA and without having to accept the IIA) with just a few clicks
- View the up to date list of publications of the GRU, since 1986
- List and download the GRU's unit files, which are a set of not formal publications that don't lose importance with time
- Read the latest news of the GRU and see some photos
- Meet the GRU staff, with our key information, like studies, jobs and interests
- Write feedbacks, comments and suggestions

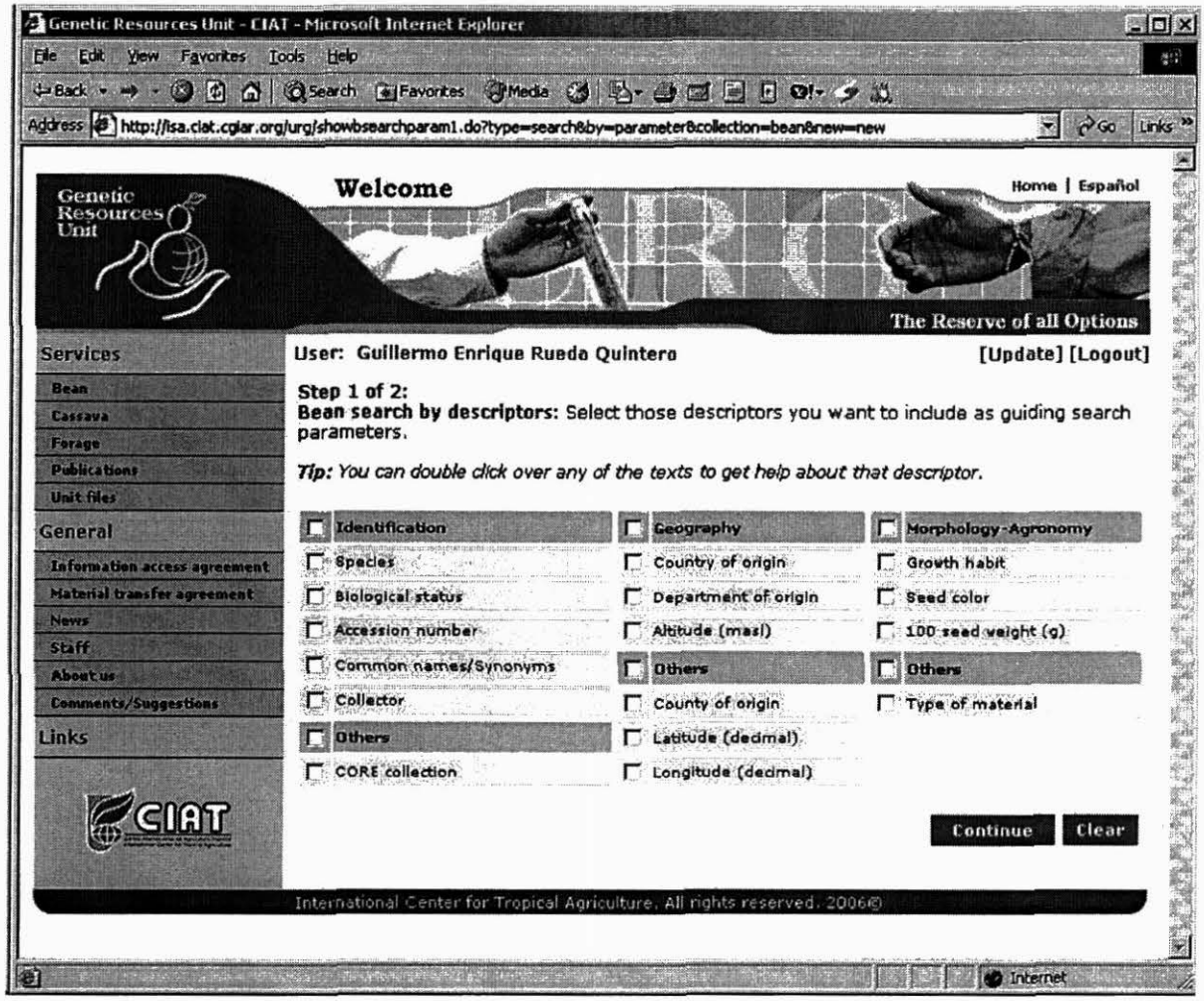


Figure 26. New web portal as it appears at www.ciat.cgiar.org

The introduction of bar coding has been done gradually as proofs progress with very good results so far. During 2006, several tests where made on field evaluation/characterization, and right now we are working hard to replace the beta version of the developed software with the version we hope is going to be the final release. Next step is to develop a software to read and print barcode labels in the field, therefore a significant amount of errors could be avoided (Figure 27).

In the Viability Laboratory, the implementation of the barcode system and mobile computing has proven to be an excellent combination in order to be more efficient, the bottleneck has disappeared and the speed and quality of the data gathering have been improved (Figure 28). We already analyzed seed conservation and health laboratory processes, and one of the next-year plans is to bring processing by barcode there. After this goal has been reached, we intend to include barcode technology in the seed reception from field processes, particularly multiple harvests.

Figure 27. Field evaluation/characterization bar coding system

Figure 28. Viability barcode system

Contributor: G.E.Rueda

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

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

Achievement: 5,046 accessions of the three commodity FAO designate collections distributed to germplasm users.

As it can be seen in Tables 25 and 26 and Figures 1 to 6, a total of 5,046 accessions were distributed, through 223 requests attended during 2006 for beans, forages and cassava. The main recipients were CGIAR Centers with 3,372 accessions and 1,674 to others institutions. NARS and universities were another important recipients. Pending on recipient type, the main purposes of the requests were: breeding, basic research and agronomy.

Table 25. Distribution of germplasm during 2006 by kind of institution.

	Beans		Forages		Cassava	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	27	396	10	142	32	2,834
Commercial companies	3	5	10	36	1	13
Farmers	1	1	74	82		
Gene banks						
NARS	4	324	7	69	46	354
NGOs			7	20		
Regional organizations			1	1	4	31
Universities	12	427	15	67	9	244
Germplasm networks						
Others						
Total	47	1,153	124	417	52	3,476

Table 26. Distribution of germplasm during 2006 by purpose

	Beans		Forages		Cassava	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	7	70	1	20	8	2,018
Agronomy	8	97	105	304	10	199
Applied research	6	76	4	15	5	12
Basic research	23	886	8	41	27	1,225
Training	3	24	4	32	2	22
Other			2	5		
Total	47	1,153	124	417	52	3,476

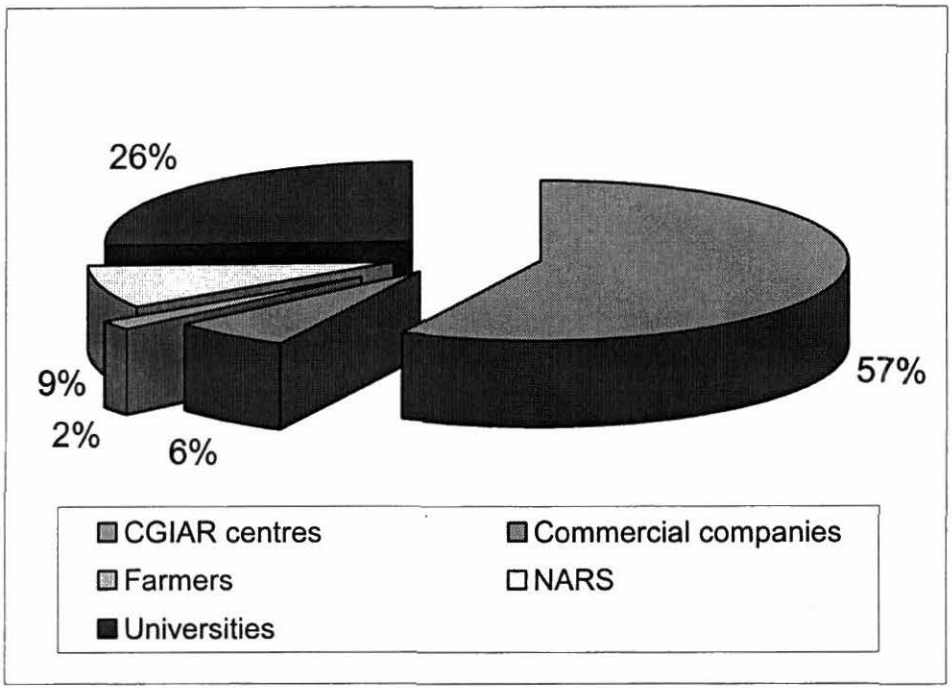


Figure 29. Distribution of bean seed germplasm by kind of users

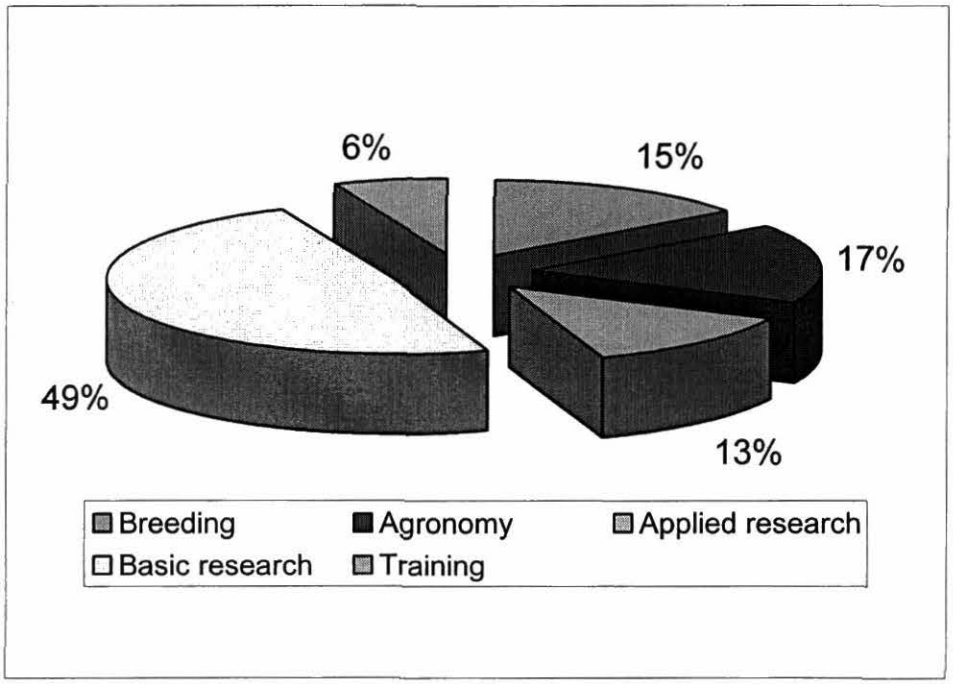


Figure 30. Distribution of bean seed germplasm by purposes

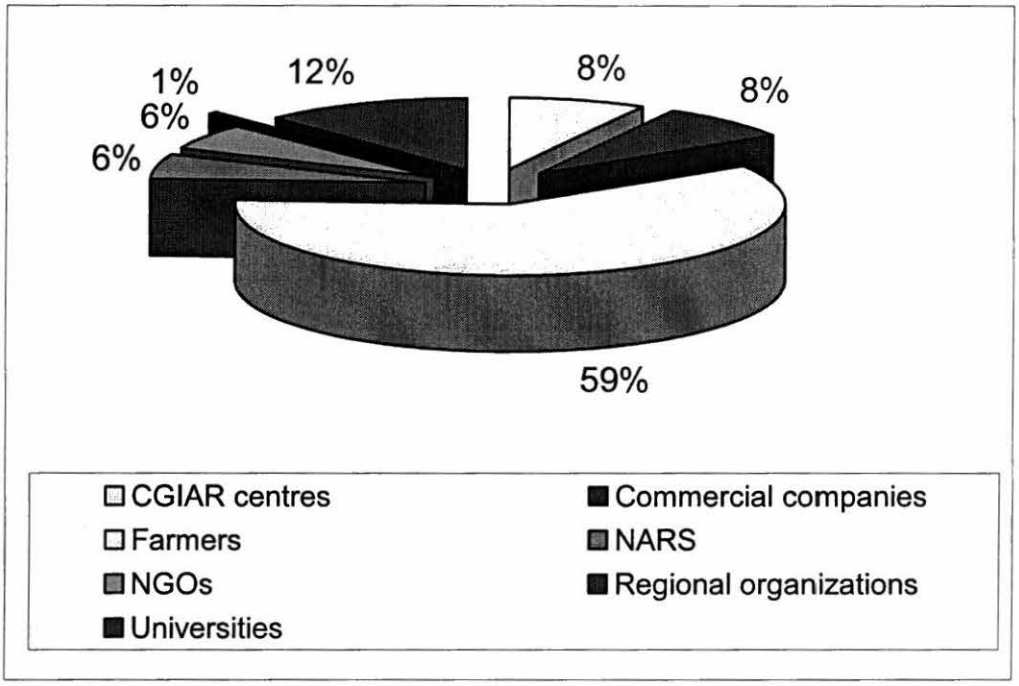


Figure 31. Distribution of forage seed germplasm by kind of users

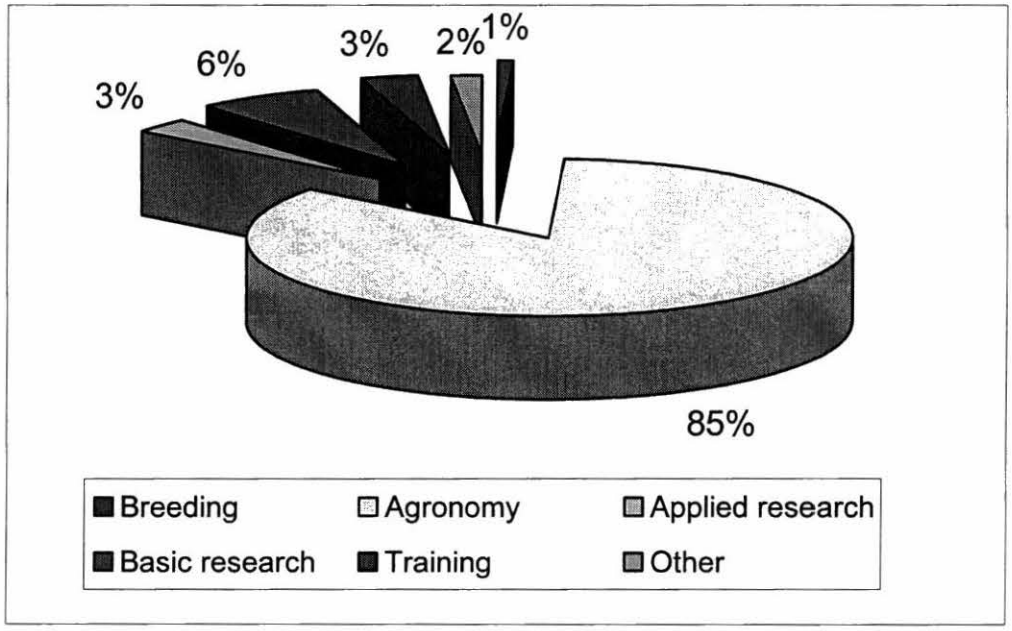


Figure 32. Distribution of forage seed germplasm by purposes.

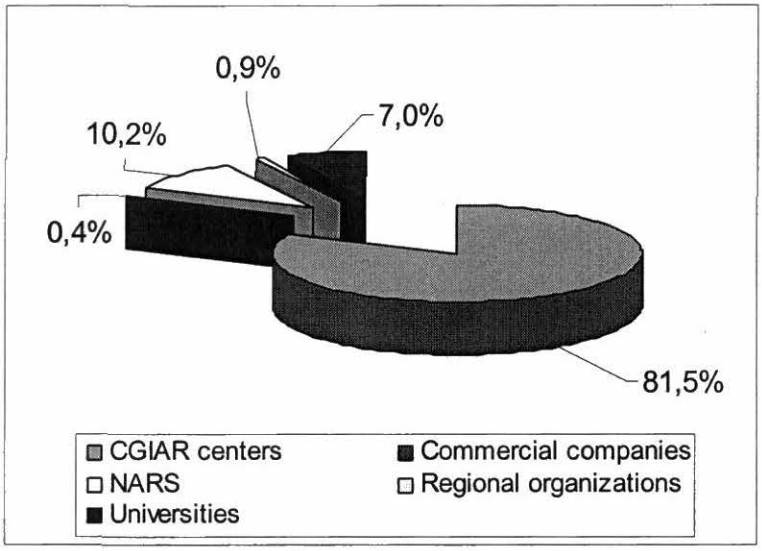


Figure 33. Distribution of *in vitro* cassava germplasm by kind of users

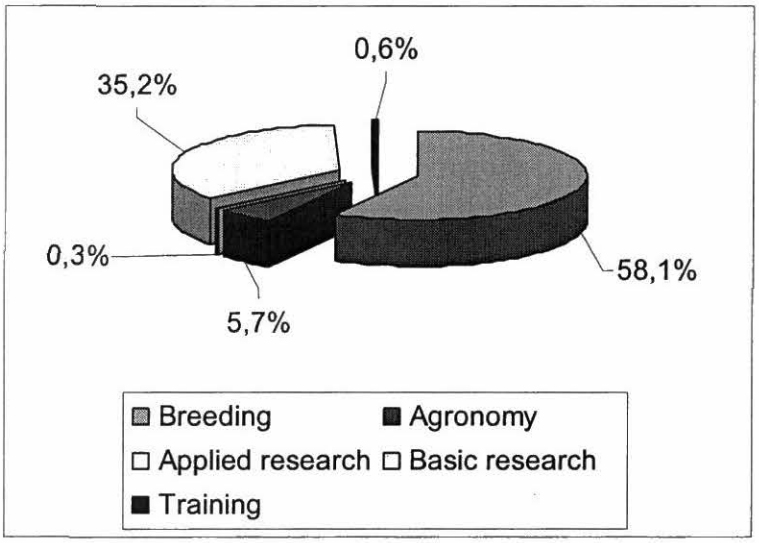


Figure 34. Distribution of *in vitro* cassava germplasm by purposes

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

Output 2.3. National collections restored to NARS

During 2006, 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 security backups

In 2006 we have shipped to CIP 3,544 accessions (7,088 tubes) of the *in vitro* cassava collection (4,728 accessions to date, or 84,9% of the designated *Manihot esculenta* collection), and we have received 4,183 accessions of *in vitro* sweet potato sent by CIP. On the other hand, we shipped 5,917 seed accessions of beans and forages as security backup to CIMMYT. Finally, we celebrated a cooperation agreement with ILRI to host a security backup of the forage collection in the long-term cold store (-20°C) of CIAT GRU.

Contributors: G. Mafla, E. Aranzales, C. Llano & 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 2006, 1,606 genotypes of *Phaseolus vulgaris* L. and others *Phaseolus* species ("Project gene flow in *Phaseolus*", *Phaseolus* germplasm characterization and phaseolin variability) were analyzed for seed storage proteins using ID-SDS-PAGE electrophoresis. The analyzed accessions belong to the *Phaseolus* germplasm collection held at CIAT. This step with morphoagronomic characterization is a requisite for improving the representativeness of the designate collection.

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

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

Output 3.1. Designate Collections better characterized

Activity 3.1.1. Single sequence repeat marker diversity in cassava: study of the level of genetic redundancy and identification of genotypes.

Introduction

In an asexually propagated germplasm collection, duplicate accessions may be common. In the case of the cassava collection at CIAT, 20 % to 25 % is estimated to be as internal duplicated. The presence of genetic duplicates in a germplasm collection has serious implication for germplasm conservation, as well as for a breeding program. Such redundancy makes the existing collection more expensive to maintain and manage, and slows down the introduction of new germplasm (Hershey et al. 1991). For cassava, a large number of these 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 into identifying duplicates (Ocampo et al. 1995). Now we propose to confirm these groups of possible genetic duplicates, using the technology of DNA fingerprinting (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). The additional objectives are: (1) to develop a description of each accession based on its molecular pattern (fingerprinting) as a criterion to avoid genetic duplicates when new germplasm is introduced in the cassava

collection; (2) once known the level of redundancy, to study the distribution of the resulting genetic diversity in the different agroecological zones of Colombia.

Materials and Methods

Plant material. This work has been initiated on the 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. One type of molecular markers that may be suitable for cassava germplasm characterization is the microsatellite (SSR). Microsatellites are considered more sensitive in detecting genotypic differences as compared to morphological and isoenzyme descriptors. Microsatellites, like RFLPs, are considered codominant markers. Their high polymorphism makes microsatellites suitable markers in order to identify redundancies in cassava (Chavarriaga et al. 1998). 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 (Table 27).

Table 27. Different primer combinations evaluated in this study. Parameters of number of alleles per locus and polymorphism information content (PIC) are included (Marin et al. 2003).

Primer		Alleles per locus	Polymorphism information content (PIC)
SSRY100	498	17	0.828
SSRY82	381	11	0.813
SSRY106	507	14	0.784
SSRY69	313	16	0.729
SSRY59	249	17	0.672
SSRY105	506	13	0.538
SSRY109	521	----------	----------

Results and Discussion

The molecular variation was assessed at seven unlinked SSR markers (Fregene et al. 2003) in 188 accessions of cassava landraces grouped into 78 groups of possible genetic duplicates according to their biochemical and morphological similarities. The number per group varied between 2, 3, 4, 5 and 6 accessions, showing 110 redundant accessions among the 188 duplicated accessions (Tables 27 and 28). The molecular grouping obtained on these morphobiochemical groups shows that the SSR polymorphisms obtained were displayed principally among different groups and in a moderate proportion within these groups. This level of polymorphism is moderate due to high similarity among accessions, which are closely related as previously determined by morphological and biochemical markers (Ocampo et al. 1993; Jimenez 1994). The molecular fingerprinting analysis confirms 91 % of the morphobiochemical groups, showing 71 different molecular groups (between 2 and 5 accessions within of each group). Additionally 27 accessions that are uniquely separated from other accessions, represent unique genotypes. Therefore the molecular grouping forms 98 groups, including 27 unique genotypes (Table 27). There is an important reduction of 18 % in the level of genetic redundancy: 90 redundant accessions versus the 110 original redundant accessions, which imply changing 188 duplicated accessions to 161

accessions with the molecular grouping (Table 28). 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 28. Accessions involved as morphobiochemical duplicates versus molecular duplicates of the Colombian cassava collection held at CIAT as a FAO Designate Collection.

Group No.	Morphobiochemical duplicates (Accessions)	Group No.	Molecular duplicates (Accessions)
1	COL 25, 896	1	COL 25, 896
2	COL 45, COL 948C, COL 1008, COL 1431	2	COL 45, COL 948C,
		3	COL 1008, COL 1431
3	COL 61, COL 1978	4	COL 61, COL 978
4	COL 70, COL 78B	5	COL 70, COL 78B
5	COL 76B, COL 912A, COL 927, COL 1962	6	COL 76B, COL 912A, COL 927, COL 1962
6	COL 81, COL 647, COL 1067, COL 106, COL 1538	7	COL 81, COL 647, COL 1067, COL 106, COL 1538
7	COL 93, COL 1044	8	COL 93, COL 1044
8	COL 134, COL 138	9	COL 134, COL 138
9	COL 137, COL 140, COL 145	10	COL 137, COL 140, COL 145
10	COL 207, COL 1485	11	COL 207, COL 1485
11	COL 240, COL 281	12	COL 240,
		13	COL 281
12	COL 261, COL 547	14	COL 261,
		15	COL 547
13	COL 376, COL 380, COL 588A, COL 727	16	COL 376, COL 380
14	COL 436, COL 2617	17	COL 436, COL 2617
15	COL 437A, COL 1934	18	COL 437[a], COL 1934
16	COL 467, COL 1720	19	COL 467, COL 1720
17	COL 942, COL 958, COL 1955	20	COL 942,
		21	COL 958, COL 1955
18	COL 1043, COL 1057, COL 1065	22	COL 1043, COL 1057, COL 1065
19	COL 1092, COL 1602, COL 1616, COL 1821	23	COL 1092, COL 1602,
		24	COL 1616, COL 1821
20	COL 2239, COL 1830, COL 1828A, COL 1518, COL 1516, COL 151	25	COL 2239, COL 1830, COL 1828A, COL 1518,
		26	COL 1516, COL 151
21	COL 1601, COL 1990,	27	COL 1601, COL 1990,
22	COL 2282, COL 2297, COL 2375, COL 2390	28	COL 2282,
		29	COL 2297, COL 2375, COL 2390
23	COL 2286, COL 2292, COL 2298, COL 2300, COL 2313	30	COL 2286, COL 2292, COL 2298, COL 2300, COL 2313
24	COL 1672, COL 1673, COL 1678	31	COL 1672, COL 1673, COL 1678
25	COL 1711, COL 1764A,	32	COL 1711

	COL 1764B	33	COL 1764A, COL 1764B
26	COL 1772, COL 1777, COL 1781, COL 1895	34	COL 1777,
		35	COL 1781, COL 1895, COL 1772,
27	COL 1786, COL 1879, COL 2023	36	COL 1879,
		37	COL 1786, COL 2023
28	COL 1889, COL 1893, COL 1894	38	COL 1889,
		39	COL 1893, COL 1894
29	COL1896, COL 1900, COL 2062	40	COL1896,
		41	COL 1900, COL 2062
30	COL 1901, COL 1902, COL 1903	42	COL 1901, COL 1902, COL 1903
31	COL 2358, COL 2362, COL 2407	43	COL 2358, COL 2362, COL 2407
32	COL 275, COL 290	44	COL 275, COL 290
33	COL 280, COL 2542	45	COL 280,
		46	COL 2542
34	COL 286, COL 328	47	COL 286, COL 328
35	COL 303, COL 306	48	COL 303, COL 306
36	COL 344, COL 386	49	COL 344, COL 386
37	COL 475, COL 1452	50	COL 475, COL 1452
38	COL 476, COL 494	51	COL 476, COL 494
39	COL 487, COL 509	52	COL 487, COL 509
40	COL 488, COL 490	53	COL 488, COL 490
41	COL 654, COL 667A	54	COL 654,
		55	COL 667A
42	COL 661, COL 663	56	COL 661, COL 663
43	COL 671, COL 673A	57	COL 671, COL 673A
44	COL 683, COL 1442	58	COL 683,
		59	COL 1442
45	COL 777, COL 778	60	COL 777, COL 778
46	COL 796, COL 1486	61	COL 796, COL 1486
47	COL 800, COL 803	62	COL 800, COL 803
48	COL 902A, COL 902B	63	COL 902A, COL 902B
49	COL 844, COL 845A	64	COL 844, COL 845A
50	COL 948A, COL 1967	65	COL 948A, COL 1967
51	COL 957B, COL 957C	66	COL 957B,
		67	COL 957C
52	COL 978, COL 974A	68	COL 978,
		69	COL 974A
53	COL 1019, COL 1023	70	COL 1019, COL 1023
54	COL 1231, COL 1347	71	COL 1231, COL 1347
55	COL 1409, COL 1413	72	COL 1409, COL 1413
56	COL 1440, COL 1917	73	COL 1440, COL 1917
57	COL 1463, COL 2189	74	COL 1463, COL 2189
58	COL 1471, COL 1472	75	COL 1471, COL 1472
59	COL 1478, COL 2305	76	COL 1478, COL 2305
60	COL 1504, COL 1632	77	COL 1504, COL 1632
61	COL 1505, COL 2054	78	COL 1505, COL 2054
62	COL 1513, COL 1514	79	COL 1513, COL 1514
63	COL 1552, COL 1553	80	COL 1552, COL 1553
64	COL 1563, COL 1564	81	COL 1563, COL 1564

65	COL 1566, COL 1717	82	COL 1566, COL 1717
66	COL 1607, COL 10	83	COL 1607,
		84	COL 10
67	COL 1613, COL 1614	85	COL 1613, COL 1614
68	COL 1630, COL 1745	86	COL 1630,
		87	COL 1745
69	COL 1667, COL 1671	88	COL 1667, COL 1671
70	COL 1823, COL 2229	89	COL 1823, COL 2229
71	COL 1868A, COL 1868B	90	COL 1868A, COL 1868B
72	COL 1884, COL 1898	91	COL 1884, COL 1898
73	COL 1912, COL 1915	92	COL 1912, COL 1915
74	COL 2004, COL 2007	93	COL 2004, COL 2007
75	COL 2025, COL 2033	94	COL 2025,
		95	COL 2033
76	COL 2082, COL 2203	96	COL 2082, COL 2203
77	COL 2107, COL 2114	97	COL 2107, COL 2114
78	COL 2143, COL 2147	98	COL 2143, COL 2147

Table 29. Description of the morphobiochemical duplicates versus molecular duplicates of the colombian cassava germplasm collection held at CIAT as a FAO Designate Collection.

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	59	118	59	59	118	59	27
Groups containing 3 similar accessions	10	30	20	7	21	14	
Groups containing 4 similar accessions	6	24	18	3	12	9	
Groups containing 5 similar accessions	2	10	8	2	10	8	
Groups containing 6 similar accessions	1	6	5	0	0	0	
Total	78	188	110	71	161	90	27

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

Activity 3.1.2. Patterns of genetic diversity in the Colombian collection of avocado (*Persea americana* Mill.) using molecular markers (collaborative project with CORPOICA, initially funded by Ministerio de Agricultura y Desarrollo Rural, Colombia).

Introduction

The Colombian collection of avocado (*Persea americana* Mill.) with 60 accessions, maintained *ex situ* by CORPOICA in Palmira, is the major collection of this fruit in Colombia. *P. americana* is a subtropical diploid (2n=24) tree and has been commonly divided into three distinguishable ecotypes or horticultural races, known as Mexican, Guatemalan and West-Indian (Bergh & Ellstrand 1986; Storey et al. 1986). Being the avocado with open pollination, it contains a large genetic variability in its germplasm. This diversity has had a great impact on the development of the crop and the avocado industry worldwide (Bergh 1992). To make an efficient use and a suitable management of the Colombian collection of avocado we proposed to know its patterns of diversity and the levels of genetic redundancy present in this collection, using DNA molecular PCR based markers.

Materials and Methods

Plant material. 60 accessions of *Persea americana* Mill. maintained by CORPOICA in Palmira, of which we report here the analysis for 56 accessions. In addition, two wild species: *Persea*

caerulea and *P. rigens* sampled in Colombia were included as outgroup. In Table 30 these accessions are reported with their geographical origin and horticultural race.

Methodology. The DNA molecular markers used were AFLPs. This technology was selected because of the magnitude of genome coverage and high reproducibility. The AFLP fingerprinting was performed basically as described in the manual protocol provided by Vos et al. 1995, as well as additions and changes made in our lab for avocado. Due to the moderate size of the avocado genome (8,83 x 10^8 bp) (Bergh 1992), primer combinations of type 2/3 (EcoR1/Mse1) were used for the AFLPs. For the amplification a total of sixteen primer combinations were selectively tested to identify at least four that can show polymorphism and quality of the amplified fragments, which are in descending order: E-AC/M-CAC, E-AC/M-CTC, E-AG/M-CTA, E-AG/M-CAT.

Table 30. Avocado accessions and wild species of *Persea* from Colombia included in this study.

Accession	Race[1]	Origin[2]	Accession	Race[1]	Origin[2]
P. caerulea	W	Valle, COL	Nativo 2011	WI	Valle, COL
P. rigens	W	Quindio, COL	Booth 5	GxWI	Florida, USA
Trapica	WI	Valle, COL	Hulumana	WI	Canal Zone, PAN
Lorena	WI	Valle, COL	1607	M	California, USA
Oriente 1	WI	Valle, COL	Bacon	GxM	California, USA
Hass	G	California, USA	135–27	GxM	California, USA
Jim	GxM	California, USA	Collinred	GxWI	Florida, USA
HX 48	GxM	California, USA	Booth 7	GxWI	Florida, USA
135–15	GxM	California, USA	Waldin	WI	Florida, USA
Papelillo	WI	Valle, COL	Semil 44	GxWI	Rio Piedras, PRI
Simmonds	WI	Florida, USA	Monroe	GxWI	Florida, USA
Peterson	WI	Florida, USA	Trapp	WI	Florida, USA
Itzamna	G	Santa MJ, GTM	135-21	GxM	California, USA
Nabilico	G	***Desconocida***	Pollock	WI	Florida, USA
Kanola	G	Antigua, GTM	Booth 1	GxWI	Florida, USA
Tumaco	WI	Nariño, COL	Lula	GxWI	Florida, USA
Winslowson	GxWI	Florida, USA	G755	G	Coban, GTM
Hayes	GxM	California, USA	Puebla	M	Puebla, MEX
Fuerte	GxM	Atlixco, MEX	Gottfried	M	Florida, USA
135–20	GxM	California, USA	La Selva	GxM	Antioquia, COL
Costa Rica	GxM	Antioquia, COL	Booth 8	GxWI	Florida, USA
143–77	GxM	California, USA	Dr. Sardi	WI	Valle, COL
Trinidad	GxWI	Canal Zone,PAN	Zutano	GxM	California, USA
Choquette	GxWI	Florida, USA	Ibague	WI	Tolima, COL
Gripiña	GxWI	Rio Piedras, PRI	Los Silos	WI	Antioquia, COL
Mayapan	G	Purula, GTM	Gwen	GxM	California, USA
Duke 7	M	California, USA	Marcus	WI	Florida, USA
Marzala	WI	Canal Zone,PAN	Ruehle	WI	Florida, USA
Oculta 1	WI	Antioquia, COL	Fairchild	GxWI	Florida, USA

[1] Designation of the avocado botanical races: W (wild species); WI (West Indian); G (Guatemalan); M (Mexican); GxA (Hybrid between the Guatemalan and West Indian races) y GxM (Hybrid between the Guatemalan and Mexican races).

[2] The origin corresponds first to the province and second to the country (according to FAO).

To report the botanical race and the origin, the following sources were consulted:

CORPOICA, Colombia (Bernal y Diaz, 2005; CORPOICA, 2004); PROFRUTALES LTDA, Colombia (Rios-Cataño y col, 2005); "Instituto de Investigación de Recursos Biológicos Alexander von Humboldt", Colombia (Vargas and Palacios, Pers. Com.).
Also the following WEB pages were consulted:
Virtual library on avocado: http://avocadosource.com
Florida avocados (USA): http://edis.ifas.ufl.edu/HS284
California avocados (USA): http://www.avocado.org/about/varieties.php

Results and Discussion

Assessment of the genetic diversity. The dendrogram derived from a UPGMA cluster analysis (using the four selected primers combinations), shows only a 1.8 % of genetic redundancy (two accessions as a possible genetic duplicate) in 56 analyzed accessions (Fig. 35). The possible genetic duplicate reported here corresponds to the accessions "Lorena" and "Trapica", which are similar in fruit, and plant habit, are of West Indian race and were originated by mass selection in the Valle. However, there are some morphoagronomic differences among them (Rios-Castaño, pers. com.; Rios-Castaño et al. 2005). The level of genetic redundancy in the collection is minimal, which facilitates the management and utilization. This analysis also shows that all the accessions are different with a similarity level of 95 % with exception of Lorena and Trapica. Therefore most of the accessions (98.2 %) could be characterized by specific molecular prints and then certainly identified (Fig. 35). Consequently, these results indicate the presence of genetic variability with a high similarity among the 56 analyzed accessions, suggesting that the genetic diversity should be increased, especially, with alleles of interest for avocado improvement.

Genetic diversity patterns. The pattern of genetic variability shows clearly the separation, without any relation, of the two *Persea* wild species sampled in Colombia from the rest of analyzed germplasm (Figure 36). The West Indian race is most adapted to the climatic conditions of Colombia, to the extent that some authors suggest that the West Indian race originated in South America, with the North coast of Colombia, as the most probable place (Morton 1987; Patiño 2002). The UPGMA cluster analysis (Fig. 35) as well as the Analysis of Multiple Correspondence (Fig. 37) show the compact grouping of most of the West Indian avocados. From 12 original accessions of Colombia, 10 are West Indian and of these, 8 form the compact West Indian group. In order to investigate the relationship among the different avocado accessions, the racial distribution of the analyzed material shows the domain of the interracial hybrids with a 48 % (GxM = 25 % and GxWI = 23 %) and of the West Indian race with a 34 %. On the other hand, the Guatemalan and Mexican have respectively 11 % and 7 % of the analyzed accessions (Table 28). Relating these racial/ ecological designations with the genetic variability of the collection, we find a pattern of constant distribution without a clear differentiation among the races, with the presence of the interracial hybrids in between (Figure 35). These patterns possibly result from gene flow caused as much by the management of the accessions as by their improvement. These results question the racial/ ecological designation for most of the analyzed accessions; nevertheless it was not possible to have this designation confirmed *a priori*. On the base of these results, it is advisable to preserve most of the accessions of the Colombian avocado collection as a new gene pool of *Persea americana* Mill germplasm.

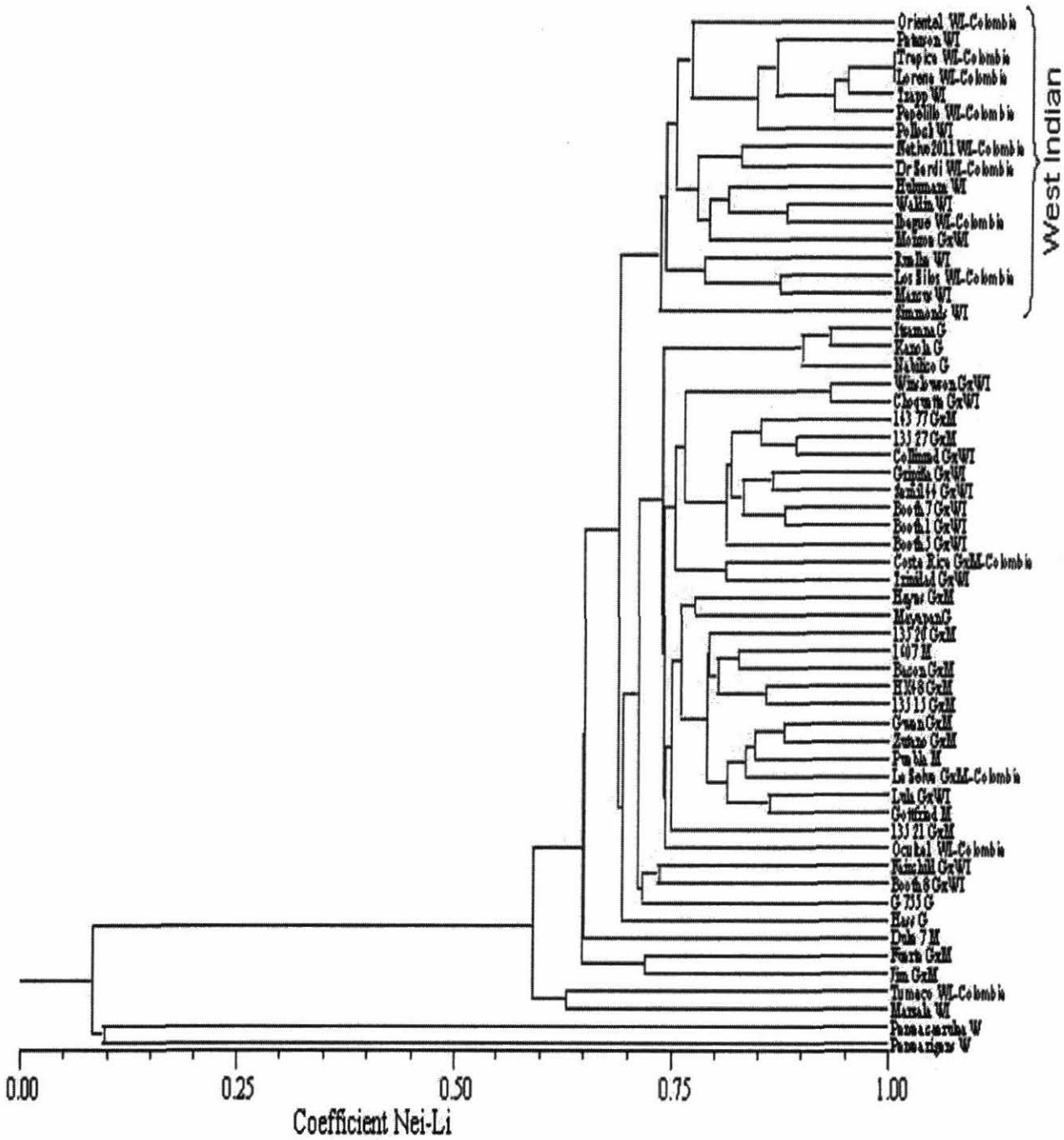
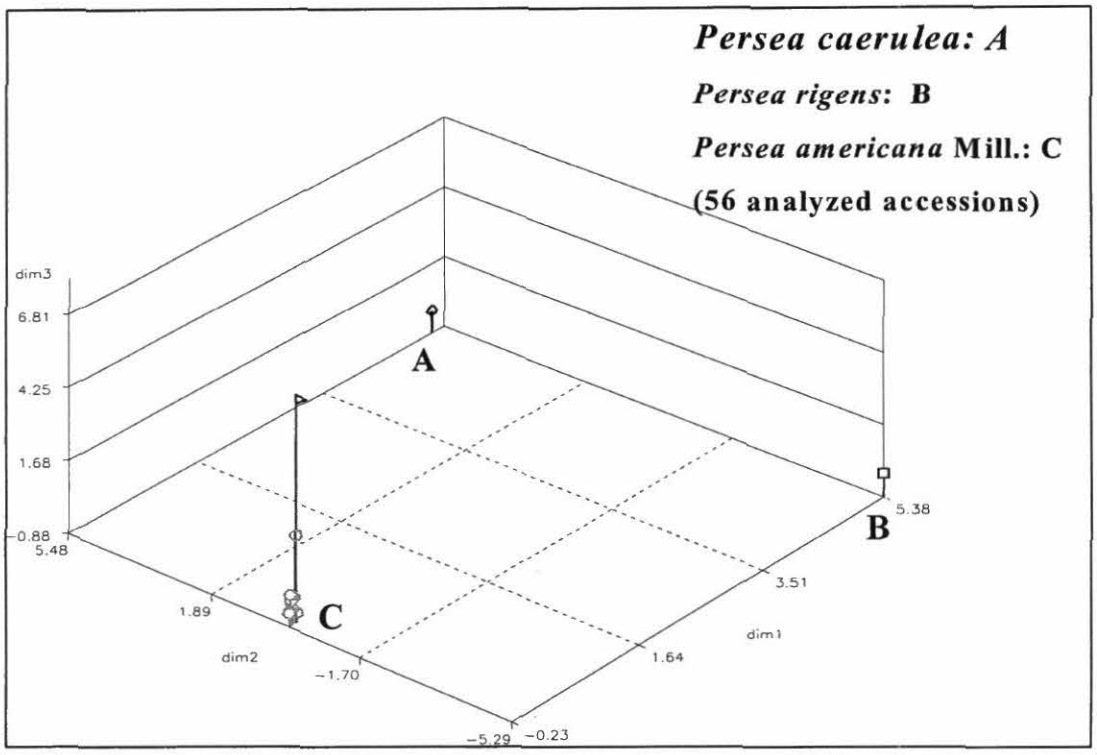


Figure 35. Dendrogram (derived from a UPGMA cluster analysis) showing the 56 analyzed accessions of *P. American*, one of *P. caerulea* and other of *P. rigens*.

Figure 36. Three-dimensional graph based on multiple correspondence analysis (MCA).



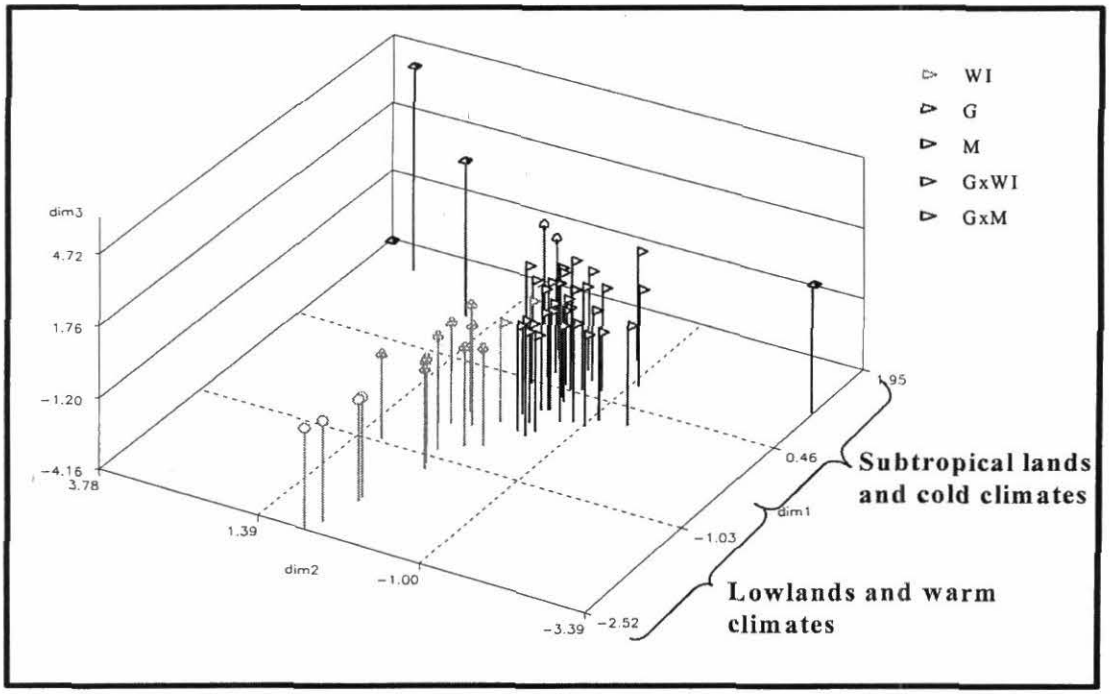


Figure 37. Three-dimensional graph based on MCA. Grouping done according to the horticultural race. The Tumaco and Marzala accessions were not included (see conventions in Table 27).

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Activity 3.1.3. Phaseolin: variability and standards in wild and cultivated common beans.

Introduction

Phaseolin – the most important seed storage protein in common bean – has been used as an easy-to-detect marker in evolutionary studies and assessment of genetic diversity. We report on phaseolin types not published previously and we establish standards for phaseolin morphotypes which are available internationally as genetic stocks.

Materials and Methods

The accessions which are reported here were obtained from the world collection held in CIAT (Table 30). The samples were analyzed in one dimension-SDS-PAGE (Brown et al. 1981) and confirmed later in two dimension-IEF-SDS-PAGE (O'Farrel 1975).

Results and Discussion

Variability in banding patterns of phaseolin has been found in the Mesoamerican and the Andean centers of diversity, among wild, weedy and cultivated accessions. Even though this globulin has a narrow range of molecular weights (45-52 kD) and isoelectric points, a total of 63 banding patterns has been found so far, 31 being present in Mesoamerican materials, 21 in the Andean region, 8 in Colombia, 2 in Mesoamerica and Colombia, and 1 in the Mesoamerican and Andean regions (Table 31). More phaseolin types have been found in wild accessions as compared to the cultivated bean, suggesting a founder effect upon domestication. However, the founder effect may not be as severe as previously thought. The accessions reported here as the source of each phaseolin morphotype is maintained at CIAT, and small quantities of seed are available for research.

Table 31. *Diversity of phaseolins and reference materials in wild, weedy and cultivated common bean.*

No.	Phaseolin Types	Number *G*	Standards (*Phs morphotypes*)	Biological Status[1]	Genetic Pool[2]	Country of *origin*[3]
1	S	G12853	FI-2380	WILD	M	GTM
2	Sb	G12952	FI-5416	WILD	M	MEX
3	Sd	S33761	FI-2881	CULT	M	COL
4	M1	G23418	FI-5824	WILD	M	CRI
5	M2	G23652	FI-1930	WILD	M	MEX
6	M3	G12865	FI-1389	WILD	M	MEX
7	M4	G23678	FI-1697	WILD	M	MEX
8	M5	G12851	FI-4068	WILD	M	GTM
9	M6	G24365	FI-1712	WILD	M	MEX
10	M7	G12869	FI-1415	WILD	M	MEX
11	M8	G12879	FI-4414	WILD	M	MEX
12	M9	G12878	FI-1457	WILD	M	MEX
13	M10	G11034	FI-1363	WILD	M	MEX
14	M11	G50869	FI-3657	WDY	M	COL
15	M12	G10002	FI-1304	WILD	M	MEX
16	M13	G23439	FI-3144	WILD	M	GTM
17	M14	G12853		WILD	M	GTM
18A	M15	G24365	FI-1714	WILD	M	MEX
18B	M15	G11027A	XX-61	WEEDY	M	MEX
19	M16	G50726	FI-3976	WILD	M	HND
20	M17	G12882A	FI-1504	WILD	M	MEX
21	M18	G12855A	FI-2390	WILD	M	GTM
22A	M19	G12854	FI-3349	WILD	M	GTM
22B	M19	G19907	FI-1273	WILD	M	GTM
23	M20	G24584	FI-5419	WILD	M	MEX
24	M21	G2721	FI-4045	CULT	M	PER
25	M22	G23511A	FI-1629	WILD	M	MEX
26	M23	G12890	FI-4089	WILD	M	MEX
27	M24	G12949	FI-1923	WILD	M	MEX
28	M25	G12851	FI-4070	WILD	M	GTM
29	M26	G23434A	FI-28	WDY	M	GTM
30	T	G50015B	FI-2838	WDY	A	ARG
31	To1	G24776	FI-4454	CULT	A	COL
32	To2	G23786B	FI-4456	CULT	A	PER

33	Ta	G23445	FI-1029	WILD	A	BOL
34	Tcaj	G23600	XX-72	CULT	A	PER
35	K	G23422	FI-4105	CULT	A	PER
36	Ko	G23814	FI-4655	CULT	A	PER
37	H1	G51049	FI-2753	CULT	A	COL
38	H2	G50401	FI-2514	CULT	A	COL
39	C	G21194	FI-4188	WILD	A	ARG
40	Ca	G12857	FI-1747	WILD	A	PER
41	Ca1	G50850	FI-3847	CULT	A	COL
42	B	G24717	FI-2038 F2	CULT	M/COL	COL
43	J1	G19895	FI-998	WILD	A	ARG
44	J2	G23592	FI-934	WILD	A	ARG
45	J3	G19902	FI-976	WILD	A	ARG
46	J4	G21194	FI-4190	WILD	A	ARG
47	CH	G50886	FI-3716 F_2	WDY	A/M	COL
48	P1	G23423	FI-1805	WILD	A	PER
49	Pa	G23455	FI-1831	WILD	A	PER
50	L	G24408	FI-2121	WILD	COL	COL
51	LI	G51019	FI-2493	CULT	COL	COL
52	CAR	G50843	FI-2432	CULT	COL	COL
53	HE	G51006	FI-2490	CULT	COL	COL
54	TI1	G51048	FI-2849	CULT	COL	COL
55	TI2	G51036	FI-2896	CULT	COL	COL
56	I	G21244	FI-1	WILD	A	PER
57	A	G12857	FI-1748	WILD	A	PER
58	A1	G12078	FI-4842	CULT	A	PER
59	Qui	G24674	FI-4421	CULT	COL	COL
60	Mu	Ent 12, retrocruza	FI-7118	CULT	COL	COL
61	Dur	G11027	MEXDU-01	WILD	M	MEX
62	Tel	G18970	FI-5791	CULT	M/COL	CRI
63	Nvo1?	DGD3195	FI-12661	HYBRID	M	CRI[4]

[1]Biological Status: WILD (Wild), WDY (Weedy), CULT (Cultivated)
[2]The common bean genetic pools: M (Mesoamerican); A (Andean); COL (Colombia).
[3]Country of origin: MEX (Mexico), GTM (Guatemala), HND (Honduras), CRI (Costa Rica), COL (Colombia), PER (Peru), BOL (Bolivia), ARG (Argentina).
[4]The germplasm analyzed to find this new phaseolin is part of the project "Gene flow in *Phaseolus*".

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

Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

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

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

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

Output 4.2. Conferences in national/ international for a

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

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: A, BAA, BM, ECON, FHO, GH, HNMN, K, MA, NA, NEBC, OXF, and SI. These data will help us to build up the pilot for the component of threat analysis for a regional project in preparation with the Natural Resources Division of the World Bank. This pilot was agreed upon with the six partners of the project (CONABIO of Mexico, I. von Humboldt of Colombia, INBio of Costa Rica, CIAT, and Smithsonian Institute) at first meeting in February 2004. Agreements to 'repatriate' that information to CONABIO of Mexico and INBio of Costa Rica were also made. The information

'Cahiers de Phaséologie' has been put on CIAT web site for the sections: *Chiapasana, glabellus, microcarpus*, and *Revoluti*.

Contributor: D.G. Debouck

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

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

In the course of our project on gene flow on the bean model, because of past field work, we were wondering whether any other closely related bean species would take part in the natural introgression. We present hereafter evidence on interspecific gene flow events among bean species (*Phaseolus* ssp.) in natural conditions of Colombia and Costa Rica using molecular markers along our previous work (González-Torres et al. 2006). We selected a total of 150 individuals possibly resulting from interspecific hybridizations and 21 individuals as controls of the possible species involved (Table 32 and 33, respectively). The individuals were analyzed as reported by González-Torres et al. (2006) including for the first time the analysis of chloroplast DNA.

Table 32. Identification of *Phaseolus* ssp. materials and number of individuals analyzed in the interspecific gene flow evaluation.

Country	Department	Accesion	Species	
COL	Nariño	G 12709	*P.* x *vulgaris*	N= 1
COL	Cundinamarca	G24628	*P.* x *vulgaris*	N= 3
COL	Cundinamarca	G24628B	*P.* x *vulgaris*	N= 1
COL	Cundinamarca	G24628E	*P.* x *vulgaris*	N= 3
COL	Cundinamarca	G24661	*P.* x *vulgaris*	N= 3
COL	Cundinamarca	G24661A	*P.* x *vulgaris*	N= 1
COL	Cundinamarca	G24666	*P.* x *vulgaris*	N= 1
COL	Cundinamarca	G24666A	*P.* x *vulgaris*	N= 5
COL	Cundinamarca	G24666D	*P.* x *vulgaris*	N= 2
COL	Boyacá	G24764	*P.* x *vulgaris*	N= 7
COL	Boyacá	G24765	*P.* x *vulgaris*	N=15
COL	Boyacá	G24765A	*P.* x *vulgaris*	N= 8
COL	Boyacá	G24765B	*P.* x *vulgaris*	N=11
COL	Boyacá	G24765C	*P.* x *vulgaris*	N=12
COL	Boyacá	G24765D	*P.* x *vulgaris*	N= 9
COL	Boyacá	G24765E	*P.* x *vulgaris*	N=14
COL	Boyacá	G24765F	*P.* x *vulgaris*	N=10
COL	Boyacá	G24765G	*P.* x *vulgaris*	N= 9
COL	Boyacá	G24765H	*P.* x *vulgaris*	N= 2
COL	Boyacá	G24765I	*P.* x *vulgaris*	N= 3
COL	Boyacá	G24765J	*P.* x *vulgaris*	N= 5
COL	Boyacá	G24765K	*P.* x *vulgaris*	N= 4

COL	Boyacá	G24766	*P.* x *vulgaris*	N= 2
COL	Boyacá	G24767	*P.* x *vulgaris*	N=14
COL	Boyacá	G24767A	*P.* x *vulgaris*	N= 1
COL	Boyacá	G24767B	*P.* x *vulgaris*	N= 1
COL	Boyacá	G24767C	*P.* x *vulgaris*	N= 1
COL	Boyacá	G24767D	*P.* x *vulgaris*	N= 2

Table 33. Identification of *Phaseolus* species used as control for comparisons with the possible hybrids.

Identification	Specie
COC1634.	*P. dumosus*
COC 1440	*P. dumosus*
G35758	*P. dumosus*
G35877	*P. dumosus*
COC 1396	*P. dumosus*
11280	*P. vulgaris*
11429	*P. vulgaris*
9590	*P. vulgaris*
11390	*P. vulgaris*
6744	*P. vulgaris*
COC 1653	*P. coccineus*
COC 1531	*P. coccineus*
COC 1533	*P. coccineus*
COC 1280	*P. coccineus*
COC 1718	*P. coccineus*
DGD 2095	*P. costarricensis*
DGD 2102	*P. costarricensis*
DGD 2116	*P. costarricensis*
DGD 3120	*P. costarricensis*
S 29699	*P. costarricensis*
PL 3592	*P. albescens*

Results

In our previous work (González Torres et al. 2006) we found that the evaluated microsatellites were of high discriminatory power (PIC), in spite of the fact that many *loci* belong to the ancestral evolutive *phylum*, and are thus shared. For that reason, we proposed to evaluate another 39 SSR *loci* (with high PIC) using fluorescent techniques that increase the resolution of alleles. Some technical aspects of PCR conditions were improved as shown in Figure 38.

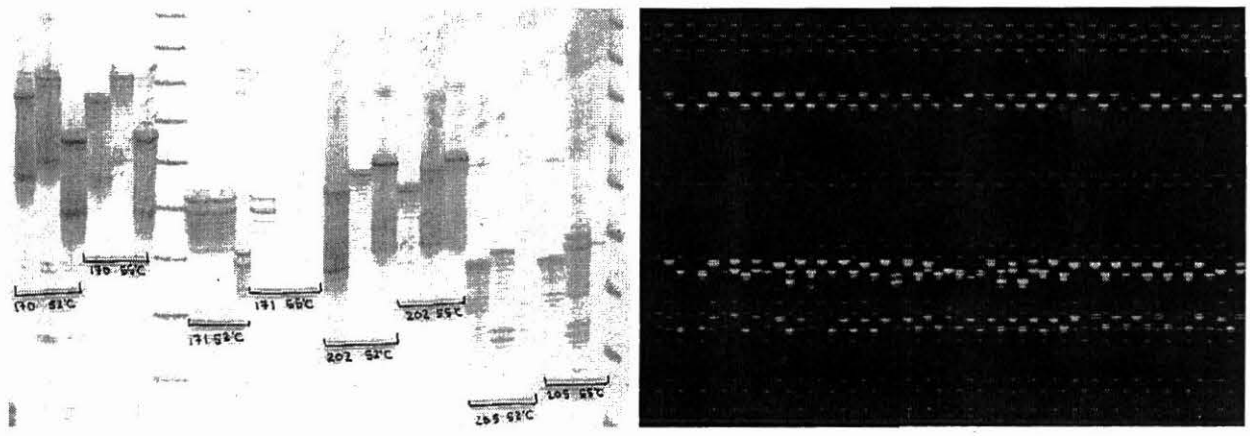

Figure 38. Left: polymorphisms of microsatellite using silver staining; Right: polymorphisms of microsatellite using primer fluorescents.

At the moment, we have run the electrophoresis of all SSR *loci* of the selected set of individuals and the analyses of the gels using GeneScan and Genotyper softwares have been realized. The objective is to analyze parameters of genetic diversity using POPGENE and TFPGA softwares and the contribution of each species to the hybrid population using ADMIX software and MCA with SAS.

The determination of chloroplast haplotypes has been conducted using polymorphism of ten non-coding regions of chloroplast DNA. The PCR restriction fragments of length polymorphism (RFLP) were analyzed using the chloroplast regions proposed by Chacón (2001). The restriction site patterns found in the interspecific hybrids and alien species were significantly different from the ones disclosed in *P. vulgaris* materials (Table 34) by Chacón et al. (2005).

Table 34. Description of ten chloroplast haplotypes found into *Phaseolus* species and interspecific hybrids.

		AccD-psaI	***NdhA intron***	***Rps14-psaB***		***TrnL intron***		***TrnL-trnF***			***TrnT-trnL***	***RpL16 intron***	
Haplotype	***Species and frequency***	***DdeI***	***DraI***	***Tsp509I***	***AluI***	***HphI***	***MseI***	***Tsp509I***	***VspI***	***SspI***	***RsaI***	***DraI***	***PacI***
H1	*Weedy (1/148)*	1	1	1	1	0	1	1	1	0	1	1	0
H2	*Weedy (1/148)*	0	1	1	0	0	0	1	1	0	1	0	0
H3	*P.vulgaris (1/5) P.dumo (5/5) P.costar. (4/5) P.coccin. (5/5) P.albesc (1/1)*	0	1	1	0	0	1	1	1	0	1	0	0
H4	*Weedy (8/148)*	1	1	1	0	0	0	1	1	0	1	0	0
H5	*P.vulgaris (1/5) Weedy (100/148)*	1	1	1	0	0	0	1	1	0	1	0	1
H6	*P.costar.*	1	1	1	0	0	0	1	1	1	1	0	0

	(1/5)												
H7	*Weedy (5/148)*	1	1	1	0	0	1	1	1	0	1	0	0
H8	*P.vulgaris (3/5)*	1	1	1	0	0	1	1	1	0	1	0	1
H9	*Weedy (1/148)*	1	1	1	0	0	1	1	1	0	1	1	0
H10	*Weedy (1/148)*	1	1	1	0	0	1	1	1	0	1	1	1

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Contributors: Rosa González, Carlos Martínez, Harold Suárez, D.G. Debouck

Activity 5.2.2. Determination of gene flow in common bean in the Central Valley of Costa Rica

The gene flow event was evidenced in materials of a newly collected year (2006) in Quircot (Central Valley- Costa Rica) using morphological, biochemical and molecular markers as mentioned in our previous works (González –Torres et al. 2003, 2004). In this case, a total of 153 individuals of common bean were evaluated belonging to the Costa Rican complex (43 weedy, 47 cultivated and 63 wild materials) from different locations of Quircot as shown in Table 35.

Table 35. Identification of evaluated populations.

No. Population	Biological status	Geographical localization
1	Weedy	Sector 2
2	Weedy	Sector 3
3	Weedy	Sector 4
4	Weedy	Sur quebrada
5	Weedy	*P.costaricensis*
6	Cultivated	Parcela Tali
7	Cultivated	Parcela Tali
8	Wild	Sector 2
9	Wild	Sector 3
10	Wild	Sur quebrada
11	Wild	Sector 5
12	Wild	Sector 4
13	Wild	Sector 2
14	Wild	Sector 1

The tendency of the population structure and the dispersion of the individuals, were made by an analysis of multiple correspondence (ACM), by the CORRESP procedure of SAS (SAS Institute, 1989), from a data matrix with different combinations of all the used markers: morpho-agronomic evaluation, phaseolin, nine microsatellite *loci*, and cpDNA polymorphisms by PCR- RFLPs. The contribution of each one of the variables (markers) to the main tendency of the wild-weedy-cultivated complex was considered. The graphical representation allowed to observe spatial localization in a multidimensional plane, of the different biological forms evaluated by their genetic similarity. In the context of the analysis of data for genetic interpretation the MCA is quite sensitive, this fact permits to detect subtle patterns of similarity based on rare alleles that held in common among genotypes.

Results

The clusters obtained from the multiple correspondence analyses, using all the markers, allowed to establish five groups that explain 91.5% of the total variation of the evaluated individuals (Figure 39). Figure 39 shows two defined groups of cultivated materials (1 and 5); cluster 5 involves landraces collected from a farm. The majority of wild forms were grouped into two clusters from three sectors. Cluster 3 is conformed by wild and weedy types; these materials were collected in different sectors, with sector 5 being occupied only by weedy types. The resulted descriptors for each cluster are shown in Table 35. All *P. costaricensis* materials, possibly weedy forms, exhibited haplotype of chloroplast "A", as well as 14 weedy forms of *P. vulgaris* from Quircot sector 2 and sur de la quebrada (Table 35).

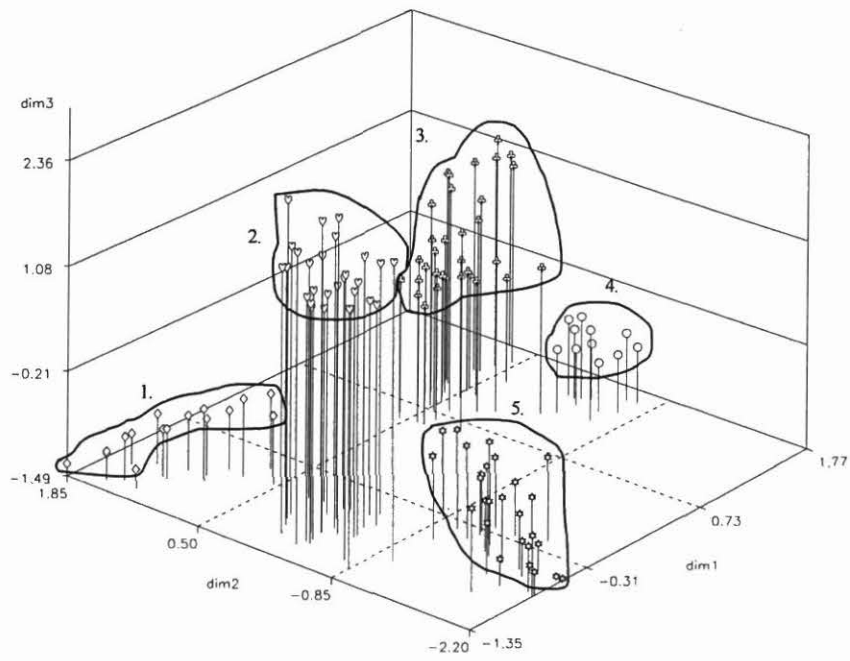

Figure 39 Spatial distribution of evaluated materials from Costa Rica using MCA.

Table 36. *Description of cluster* obtained by MCA analysis of SSR

Cluster	Biological status	Localization or material type	Cp haplotype	Seed size
1	Cultivated	Higuerilla	"J"	
		Vainica de palo		Large
		Frijol Amarillo		Medium
		Higuerilla sistema tapado		
2	Weedy	Quircot sector 2	"L"	
		Quircot sector 3	"A"	Very Large
		Quircot sector 4		Medium
		Quircot sector sur quebrada		Small
		Quircot sector- *P. costaricensis* weedy		
3	Wild Weedy	Quircot weedy	"J"	Small
		Quircot sector sur quebrada		Medium
		Quircot sector 5		Large
		Quircot sector 4		
		Quircot sector 2		
		Quircot sector 1		
4	Wild	Quircot sector 2	"L"	Small
		Quircot sector 3	"H"	Medium
		Quircot sector sur de la quebrada		
5	Cultivated	Quircot Farm of Mr. Tali	"A", "L", "J"	Medium

Figure 40 shows the same 153 individuals according to their biological status in red, blue, and green as cultivated, weedy, and wild, respectively. It exhibits the differentiation of two complexes, in which weedy forms are closer to cultivated forms, possibly because of the higher weight given by the MCA to descriptors such as cpDNA (none of the weedy was of 'H' haplotype) and 100-seed weight. The haplotypes of chloroplast DNA in the weedy populations indicated the following gene flow direction: pollen of wild materials towards cultivated forms.

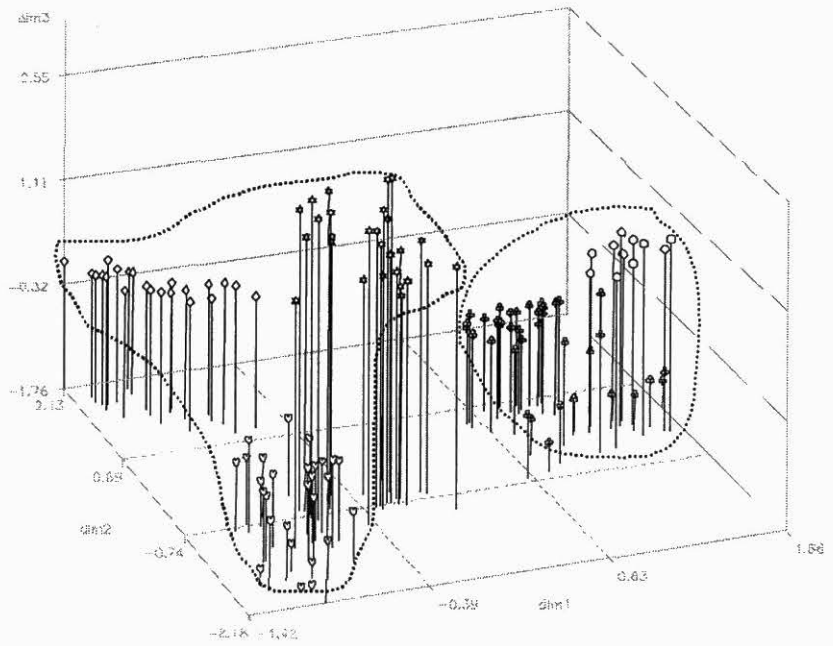

Figure 40. Spatial distribution of evaluated materials from Costa Rica using MCA by biological status. In red: cultivated types, blue: wild forms, and green for weedy population.

Table 37 summarized results for each evaluated population. Wild population shows an average seed weight of 6.85 g and displays mainly haplotypes "L" and "J"; however the haplotype "H" was found at lower frequency. The cultivated population presents an average of 22.1 g, and we principally find haplotype "J". In addition, weedy forms exhibit 21.73 g as mean weight, and shared haplotypes as "L" and "J". However, they also show haplotype "A" in four hybrids, this haplotype was found in 5/5 individuals selected out of *P. costaricensis*. This data suggests a gene flow event between *P. vulgaris* and *P. costaricensis*, where common bean was the pollen receptor.

Table 37. Description of evaluated markers on wild, cultivated and weedy individuals.

Population N=136	Biological Status	Mean weight (g/100 seeds)	Phaseolin patterns					Haplotype of chloroplast			
			S	TEL	CH	M1	Novel Pattern	H	L	J	A
Sector 1 N=2	Wild N=45 X=6.85g	5.8								2/2	
Sector 2 N=25		7.5						7/25	8/25	10/25	
Sector 3 N=13		6.2						3/13	10/13		
Sector 5 N=5		7.9								5/5	
Sector 2 N=9	Weedy N=31 X=21.73g	17.9					9/9				9/9
Sector 3 N=5		19.2				5/5			5/5		
Sector 4 N=17		21.6			1/2				2/2	15/15	
Sur quebrada N=13		28.25				4/10	4/10		3/3	4/10	6/10
Sector 1 N=5	Cultivated N=47 X=22.1g	21.2	5/5							5/5	
Parcela Tali N=42		23	27/42	14/42					8/42	32/42	

According to proposed model by Bertorelle & Excoffier (1998), and used by Papa and Gepts (2003), the relative contribution to the weedy population of the wild and cultivated populations were estimated using ADMIX 1_0 software (California University, Berkeley, URL: http://web.unife.it/progetti/genetica/Giorgio/giorgio_soft.html). The contribution is expressed as coefficient of mixing mY (Table 36). We found the SSR alleles frequency for each population and locus, using POWERMAKER software. These results provide data to build a molecular distance matrix to analyze the coefficient of mixing mY (Tables 36, 37, 38). The main difference between mY and the other two estimators is that mY also takes into account the molecular distance between the different alleles. The analysis of contribution was conducted with 1,000 randomly resampling.

The admixture coefficient M_Y was calculated, based on SSR alleles frequencies and the molecular distances between them. The term *Mw* is the relative contribution of wild forms and *Mc* that of cultivated forms. The admixture coefficient for wild forms was slightly higher than that cultivated which means *a priori* that the contribution of wild forms to the weedy types is 8.107 times greater (Table 38). These results suggest that the direction of the gene flow was pollen of wild populations towards cultivated types inferred in weedy forms.

Table 38. Estimation of admix distribution among wild and cultivated forms using SSR alleles.

Weedy				
Mwild		Mcultivated		Mc/Mw
Estimated	SD	Estimated	SD	
0,8902	0,0595	0,1098	0,0593	0.123

On the other hand, the selection of individuals for evaluation involve some individuals with interspecific characteristics such as in crosses with *P. costaricensis*, therefore an analysis of admix was realized using these individuals (Tables 38, 39). The estimate of the contribution of the wild population to the cultivated population was significantly higher than the estimate of the contribution of cultivated to the wild in the interspecific individuals.

Table 39. Estimation of admix distribution among wild and cultivated forms with possible interspecific hybrids using SSR alleles.

P X vulgaris				
Mwild		Mcultivated		Mc/Mw
Estimated	SD	Estimated	SD	
0,9504	0,0473	0,0496	0,0525	0.052

Table 40. Estimation of admix distribution among wild and cultivated forms with possible *P. costaricensis* individuals using SSR alleles.

P. costaricensis				
Mwild		Mcultivated		Mc/Mw
Estimated	SD	Estimated	SD	
0,7966	0,0876	0,2034	0,0502	0.255

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Activity 5.2.3. Estimation of gene flow of "wild-weedy-cultivated" complexes of common bean along its range of distribution

Although common bean has long been considered as an autogamous plant, it can outcross naturally with its wild relative or even with sister species leading to the formation of complexes "wild-weedy-cultivated" (Gonzalez-Torres et al. 2006). These complexes have been phenotypically observed in the states/ departments of Oaxaca (México), El Progreso (Guatemala), San José (Costa Rica), Boyacá (Colombia), Azuay (Ecuador), Apurimac (Perú) and Tarija (Bolivia). We applied molecular markers using cpDNA (SNPs) and nuclear DNA (SSRs) to individuals putatively resulting from gene flow events under natural conditions (González-Torres et al. 2003, 2004) from this geographic range.

Results

The results shown in Table 10 display the diversity of chloroplast haplotypes in elements of the complexes and the direction of the flow. The wild and cultivated populations were characterized with such markers first allowing then to infer about the weedy individuals. The main direction

was that of wild pollen towards cultivated materials, although the other direction was also evidenced at significant frequency in many places.

Table 41. Chloroplast haplotypes found in the complex "wild-weedy-cultivated" and their frequencies.

	Chloroplast haplotypes found			
Country	***Wild***	***Weedy***	***Cultivated***	***Pollen flow direction and frequency***
Costa Rica	H (540/540)	G (3/481) H (179/481) J (98/481) K (1/481) L (199/481) F (1/481)	J (18/56) K (23/56) L (15/56)	Wild to C ultivated (98+1+199 /481)
Guatemala	I (6/16) J (10/16)	C (1/32) J (31/32)	K (16/16)	Cultivated to W ild (31/32)
Colombia	J (50/68) L (18 /68)	L (64/96) J (28/96) A (2/96) C (2/96)	L (14/158) C (3/158) J (140/158)	Wild to C ultivated Or Cultivated to W ild
Ecuador	F (28/28)	F (47/51) A (3/51) J (1/51)	C (15/15)	Cultivated to W ild (47/49)
Perú	C (51/51)	C (126/181) I (1/181) J (38/181) L (11/181) F (4/181) A (1/181)	C (70/105) J (18/105) L (17/105)	Wild to C ultivated Or Cultivated to W ild
Bolivia	A (6/6)	A (1/20) C (2/20) I (1/20) J (16/20)	C (6/12) J (6/12)	Wild to Cultivated (16 +2 /20)
Argentina	D (6/6)	C (40/40)	C (51/51)	Wild to Cultivated (40/40)

The data for Colombia and Peru seem to indicate cpDNA haplotypes shared between wild, weedy and cultivated forms, in the same places of domestication (Chacón 2001), preventing thus to infer about direction of flow. SSR analysis with admixture estimation will be used to solve out this limitation.

The results obtained for the evaluated populations from Ecuador (Azuay) and Guatemala (El Progreso) in Table 41 suggest that the direction of gene flow was from pollen of cultivated materials towards wild forms, since according to Chacón (2001) the haplotypes dominant in the wild forms are 'F' and 'J', respectively.

The Colombian population involves a set of 26 individuals selected as "escape", therefore an analysis of genetic similarity of Nei 1978 of escape population was required. This study was using POPGEN software, to group this set with their putative genetic counterparts (wild, cultivated or weedy individuals) (Figure 41). The tree displays a genetic similarity of 80.6% between cultivated forms and escaped individuals. For that reason, in the following analysis of genetic contribution (ADMIX) these populations were joined.

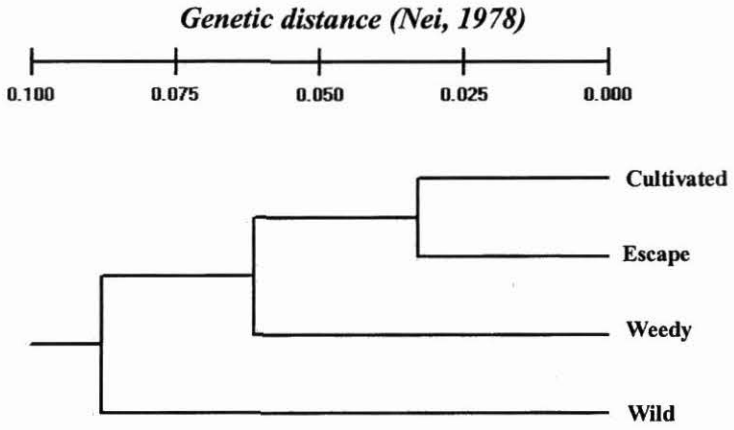

Figure 41. Cluster of genetic distance of evaluated biological forms, according to Nei (1978).

The admixture coefficient for wild forms was similar than cultivated population, indicating that the gene flow has occurred in both directions (wild pollen towards cultivated types and vice versa) almost symmetrically in Colombian populations (Table 42).

Table 42. Estimation of admix distribution among wild and cultivated forms on weedy population using SSR alleles.

Weedy				
Mwild		Mcultivated		Mc/Mw
Estimated	SD	Estimated	SD	
0,4206	0,0551	0,5794	0,0549	0.143

We were also interested in examining gene flow events over time by sampling materials at the same localities in different years (1987, 1998, 2003, 2004, and 2006), in San José and Cartago, Costa Rica. We evaluated 9 nuclear SSR *loci* in a total of 520 weedy individuals to evidence the gene transfer and its quantification. We determined characteristic alleles for each population belonging to the complex and these were detected in the weedy populations suggesting that the evaluated hybrids are real cases of gene flow. The weedy individuals were confirmed by morphoagronomic and biochemical markers found in wild or cultivated populations (Table 43).

Table 43. Number of real hybrids revealed for each year screened in Quircot and Jerico populations in Costa Rica.

Year / Population	1987	1998	2003	2004
Cartago (Quircot)	29	40	123	50
San José (Jerico)	--	9	50	40

The chloroplast analysis provided evidence on the effects of the evolutionary forces of domestication and gene flow on the levels of genetic diversity in this crop. On the one side, the few domestication events induced a strong founder effect, thus reducing crop genetic diversity. On the other side, the gene flow events have worked against this consequence, over millennia and across the range of the wild relative, contributing to the richness of the common bean gene pools (nuclear and chloroplast genomes). These two forces, among others, have taken part in the

formation of domesticated races (Singh et al. 1991; Beebe et al. 2000), an unexpected result in a reported autogamous (!) crop.

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Contributors: R. I. González, C.A. Martínez, H. Suárez, O. Toro, D.G. Debouck

Activity 5.2.4. Using SNP haplotypes to study gene flow in a *P. vulgaris* wild-weedy-crop complex from Colombia.

Chloroplast haplotypes have been successfully used to determine gene flow direction in *P. vulgaris* populations from Costa Rica (González-Torres et al 2003, 2004). The study of some "wild-weedy-cultivated" complexes along the range of common bean distribution in the Americas seems to show no preferential gene flow direction in Colombia, Ecuador and Peru (Chacón et al. 2006). We conducted a pilot study in the wild-weedy-crop complex G50879 from Colombia (19 wild, 32 weedy, and 34 cultivated individuals), previously evaluated with biochemical markers and SSRs, to test the potential of nuclear SNP markers as an additional tool for the identification of gene flow events and inference about their direction.

A total of 85 individuals from the G50879 wild-weedy-crop complex were genotyped with 20 SNPs located in linkage groups B01 and B07 (where most genes and markers related to the domestication syndrome are located: Koinange et al. 1996). The single base extension method on a Luminex-100 platform (Quintero et al 2004) was used for SNP allele scoring. As codominant markers, SNPs were useful in identifying heterozygotes. SNPs blocks and haplotypes were inferred using HAP software (Halperin & Eskin 2004; URL: http://research.calit2.net/hap/WebServer.htm).

First, we addressed some characteristics of population structure and we found that the percentage of polymorphic *loci*, heterozygosity and diversity were considerably higher in the biological forms of the complex than in wild populations used as checks (Table 44), the weedy forms having the highest values (except for diversity index).

Table 44. Polymorphic SNPs, heterozygosity and diversity of biological forms in the wild-weedy-cultivated complex G50879.

Gnumber	Status	Polymorphic Loci (%)	h^1	I^2
G21117	Wild check	10	0.010	0.041
G23996	Wild check	20	0.045	0.096
G50879	Wild	80	0.129	0.413
	Weedy	95	0.182	0.445
	Domesticated	80	0.148	0.455

[1]Observed heterozygosity.
[2]Shannon (1949) diversity index.

Block partition and haplotype prediction within each block was done using HAP software. Both linkage groups (B01 and B07), each having 10 SNPs, were divided in three blocks of limited diversity. Total number of inferred haplotypes was 43, block 3 (B07) having the least number of haplotype variants and block 2 (B01) having the highest diversity of haplotypes (Table 45).

Polymorphism information content for each block was calculated and three of them were found to have values close to 0.7, similar to the SSRs used by González (2004), studying gene flow in Costa Rica.

Table 45. Haplotype blocks inferred in linkage groups B01 and B07.

LG	Block	SNPs (#)	Haplotypes (#)	PIC
B01	1	3	8	0,702
B01	2	5	12	0,756
B01	3	2	4	0,310
B07	1	6	11	0,731
B07	2	3	6	0,498
B07	3	1	2	0,288

Common and rare variants of haplotypes were found. Wild checks G21117 and G23996 were again very uniform: no more than two haplotypes per block were observed, a result that agrees with their uniformity in seed size and color. In contrast, all 43 SNP haplotypes were observed in the wild-weedy-crop complex: 84% in weedy forms, 77% in cultivated forms and 70% in the wild forms.

With the admixture model proposed by Bertorelle & Excoffier (1998), and used by Papa & Gepts (2003), we estimated the relative contribution of both wild and cultivated forms, to the weedy types, with ADMIX1_0 software developed by G. Bertorelle (California University, Berkeley, URL: http://web.unife.it/ progetti/ genetica/ Giorgio/ giorgio_soft.html).

Then the admixture coefficient M_Y was calculated, based on haplotype frequencies and the molecular distances between them. We named *Mw*, the relative contribution of wild forms and *Mc* that of cultivated forms. The admixture coefficient for wild forms was slightly higher than that for cultivated forms which meant *a priori* that the contribution of wild forms to the weedy types was 1.34 times greater (Table 46).

Table 46. Admixture analysis of SNP haplotypes and SSRs in the wild-weedy-crop complex G50879.

M_W		M_C		M_W / M_C
Estimated[1]	SD[2]	Estimated	SD	
0.58	0.12	0.42	0.16	1.34

[1]1000 resampling events
[2]Standard deviation

According to Hurles et al. (2003), the confidence of the estimated coefficient relies on our ability to clearly identify distinct parental populations that have been involved in the admixture process. Since we observed that the standard deviation of calculated coefficients was considerably higher than that reported by Papa & Gepts (2003), we decided to look at the distribution of SNP haplotypes within and between biological forms, to refine parental populations. We selected the three most informative (PIC values around 0.7) haplotype blocks: blocks 1 and 2 in B01, and block 1 in B07 (Table 46).

Differences between haplotype frequencies allow us to identify those that describe better each of the parental biological forms. In most cases, haplotypes were classified as wild types when their frequency was at least twice the one observed in cultivated forms, and vice versa (Figure 42).

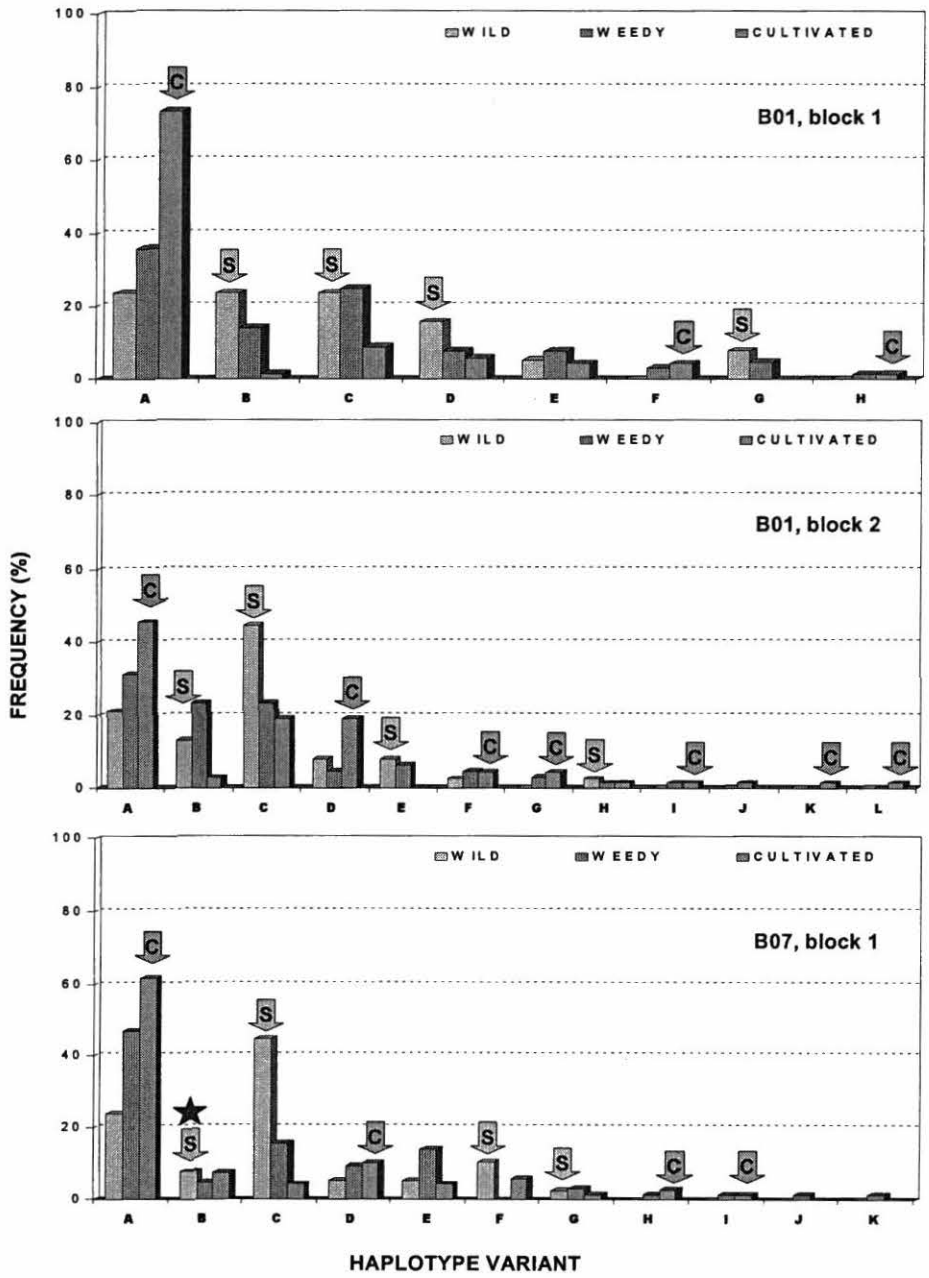

Figure 42. Haplotype frequency in the wild-weedy-crop complex G50879.

Inside the wild and cultivated forms, not all the individuals coincide in phenotype (seed weight) and genotype (SNP haplotypes). We define as truly wild those individuals that had wild type haplotypes in the three blocks, together with low seed weight (less than 14g/100seeds). Similar criteria were applied to the cultivated materials, and then a truly cultivated parental population was defined.

When we looked at weedy forms, some materials were added to the corresponding parental population if they were close in phenotype and coincided in genotype.

We propose that parental truly-wild and truly-cultivated selected as above, could give rise to three hybrid subpopulations in this wild-weedy-crop complex (Table 47).

Table 47. Assumed hybrid subpopulations in the wild-weedy-crop complex G50879.

Name	Phenotype (P100s)	Genotype (SNP haplotype)
Wild-hybrid	Wild	Wild-Cultivated
Cultivated-hybrid	Cultivated	Wild-Cultivated
Weedy	Weedy	Wild-Cultivated

Admixture analysis was then performed considering them as three separate cases, and the relative contributions of truly wild and cultivated were estimated (Table 48).

Table 48. Admixture analysis of the wild-weedy-crop complex G50879 considering three possible hybrid subpopulations.

1. Weedy				
Ms		*Mc*		*Mc/Ms*
Estimated[1]	DS[2]	Estimated	DS	
0,4458	0,0436	0,5518	0,043	1,2
2. Wild-hybrid				
Ms		*Mc*		*Mc/Ms*
Estimated	DS	Estimated	DS	
0,4669	0,055	0,5281	0,0588	1,1
3. Cultivated-hybrid				
Ms		*Mc*		*Mc/Ms*
Estimated	DS	Estimated	DS	
0,3237	0,0501	0,6766	0,0495	2,1

[1]1000 resampling events
[2]Standard deviation

In the weedy and wild-hybrid subpopulations, the contribution of truly-cultivated was slightly higher than that of truly-wild meaning that both gene flow directions are possible and almost symmetric. For the cultivated-hybrid, the contribution of truly-cultivated was twice that of the

truly-wild, suggesting that more pollen flows from the cultivated types. Since the sample size of this pilot study is quite low we took a cautious opinion and stay with the evidence that suggests that both gene flow directions are possible. Thus the study of more Colombian wild-weedy-crop complexes though SNP haplotypes is required.

The use of biallelic SNPs for genetic diversity studies has been controversial since they are less informative than multiallelic SSRs (Brumfield et al. 2003; Morin et al. 2004). Although we have looked only at one wild-weedy-crop complex from Colombia our results show that SNP haplotypes are informative enough to provide evidence about gene flow dynamics.

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Activity 5.2.5. Determination of gene flow events in the Andean and Mesoamerican genepools of *Phaseolus lunatus*

After using molecular and biochemical markers to successfully establish gene flow events in weedy forms of *Phaseolus vulgaris* and to indicate pollen direction from the wild into the cultivated and vice versa (González-Torres et al. 2004), we were interested in testing the hypothesis of gene flow in populations of *Phaseolus lunatus*. Lima bean has an interesting reproductive system with a facultative allogamy up to 48% (Baudoin et al. 1998). Wild Lima beans have a high level of HCN glucoside, that can be easily evaluated, allowing thus to assess consequences of gene flow in a food and crop domestication perspective. In this study we propose to evaluate nuclear genome with lectin data, microsatellites, and the analysis of seed cyanide acid. The contribution of the chloroplast genome is evaluated using RFLPs of non-coding regions in order to set the direction of gene flow.

Initial evaluations

The preliminary evaluations of a set of individuals belonging to both gene pools (Andean and Mesoamerican) with all markers were conducted to determine the analysis conditions (Table 49).

Table 49. Populations of *Phaseolus lunatus* evaluated in the preliminary studies.

Accession Biological status		Average seed weight (g) (n=5)	Color testa of seed	Country
WILD	G25704	11,5	Wild type	Mexico
	G25737	8,3	Wild type	
	G25759	11,5	Wild type	
	G25789	10,3	Wild type	
	G25913	11,7	Wild type	Perú
	G25914	11	Wild type	
	G25916	18	Wild type	
CULTIVATED	G25705	47,1	Colored	Mexico
	G25733	31,1	Colored	
	G25760	39,7	Colored	
	G25778	22,2	Colored	
	G25786	25	Colored	
	G25787	37,8	Colored	
	G25826	47,5	Colored	Perú
	G25831	175,3	Colored	
	G25919	99,7	Colored	
	G25930	50	Colored	
	G25933	83,4	Colored	
	G25943	34,8	Colored	
	G25955	106,4	Colored	
WEEDY	G25706	18,6	Wild type	Mexico
	G25736	22	Colored	
	G25778	22,2	Colored	
	G25915	28	Wild type	Perú
	G25944	44,5	Colored	
	G25947	53,1	Wild type	
	G25948	55,3	Colored	
	G25952	36,3	Colored	
	G25952A	31,4	Colored	

Biochemical markers

Quantification of cyanide acid

The amount of linamarin compound (as the anti-nutritional marker) was determined using the colorimetric method reported by Essers et al (1993), using a lower amount of seed tissue than previously reported. For that reason, one and two grams were evaluated with this method to compare results.

Results

Table 50 shows cyanide concentration (ppm) obtained for each biological status and gene pool. These results fall in line with levels reported for *P. lunatus* by Baudoin et al. (1997) who found very high HCN contents in the wild populations, markedly greater than those found in the cultivated types, as a possible result of domestication. In addition, we found no correlation between seed coat color and cyanide content. Weedy forms exhibited a content of the cyanogenic glycoside intermediate between wild and cultivated types, as a possible result of gene flow events.

Table 50. Cyanide concentration (ppm) and its average for each biological status of *P. lunatus*.

	Cyanide concentration (ppm)		
	Wild	***Cultivated***	***Weedy***
Mesoamerican	2345 2296 1547 1421 **X=1902,3**	69 72 38 107 **X=71,5**	 1881 356 **X=1118,5**
Andean	 2747 3210 3684 **X=3213,7**	232 94 485 335 114 34 379 **X=239**	 1625 643 1386 582 2077 2451 **X=1460,7**

Lectins or reserve proteins

Seed storage proteins or lectins are localized between 31 y 45 kDa, and were analyzed for individuals listed previously using the electrophoretic method one di-SDS-PAGE described by Gutiérrez-Salgado et al. (1995). We used accessions G25916 (Andean genepool) and G25704 (Mesoamerican genepool) as electrophoretic controls of protein patterns reported by Gutiérrez-Salgado et al. (1995).

Results

The obtained patterns of lectins for each gene pool were similar to those of used controls. The Mesoamerican population shows two different patterns in the cultivated form, while the wild and weedy individuals have the same M1 lectin like the control (Figure 42 white arrows).

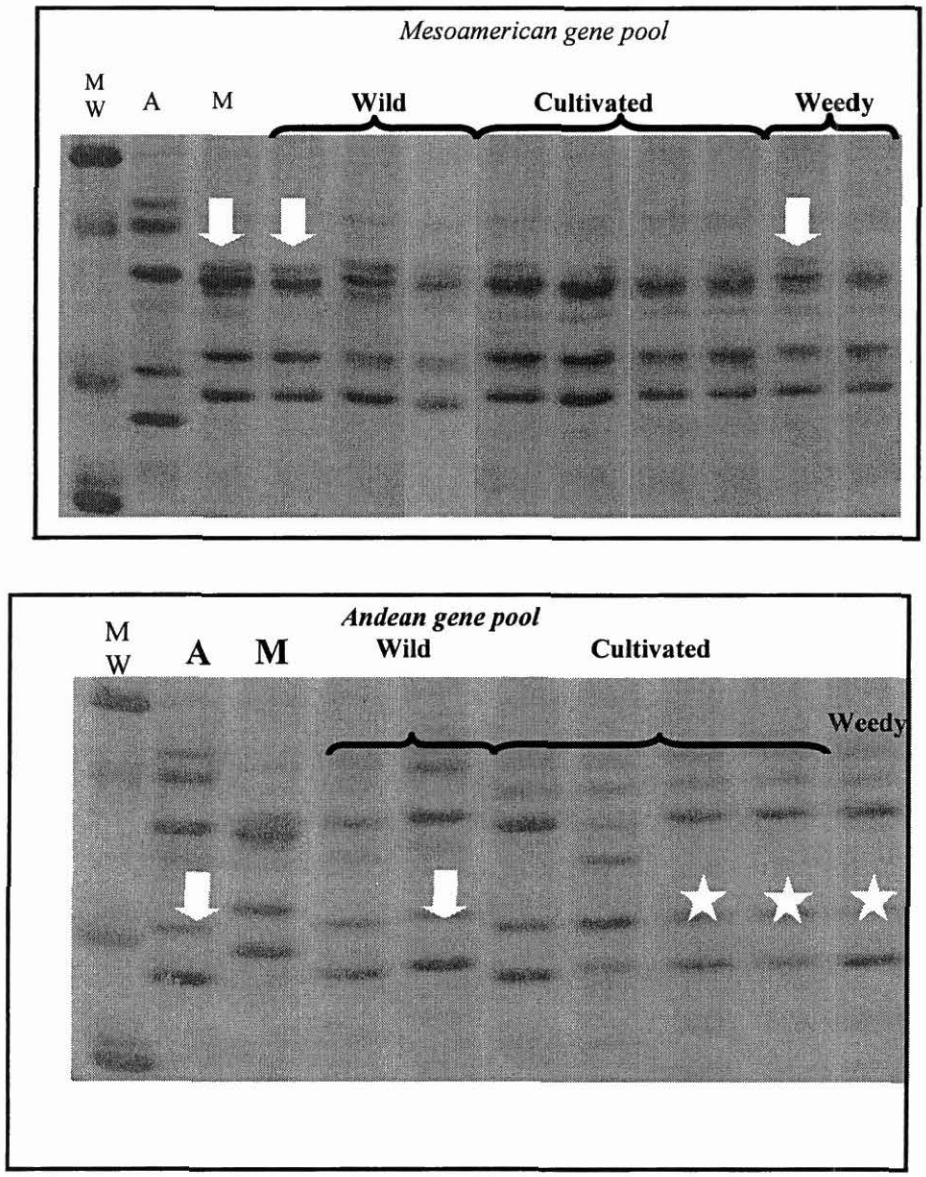

Figure 43. Lectins patterns obtained using SDS-PAGE. A: Andean control, M: Mesoamerican control, MW: molecular weight marker (Kda).

Andean individuals displayed four different patterns of lectins, two of them are shown in Figure 43 as white arrows and stars. One wild individual has the same lectin as the control.

Molecular markers

Microsatellites

A total of 68 SSR loci reported by Gaitán et al (2002) for *P. vulgaris* were evaluated on the selected population of *P. lunatus* to evidence polymorphism and improve amplification conditions. The microsatellites were evaluated with silver staining (Figure 44).

Results

Polymorphism information content for microsatellite was calculated using POWERMARKER software V3.0, and 20 of the microsatellites were found to have values higher than 0.374 (Table 51), similar to the SSRs used by González (2004), studying gene flow in common bean of Costa Rica. These *loci* will be evaluated to evidence gene flow events in the complex "wild-weedy-cultivated" of *P. lunatus*.

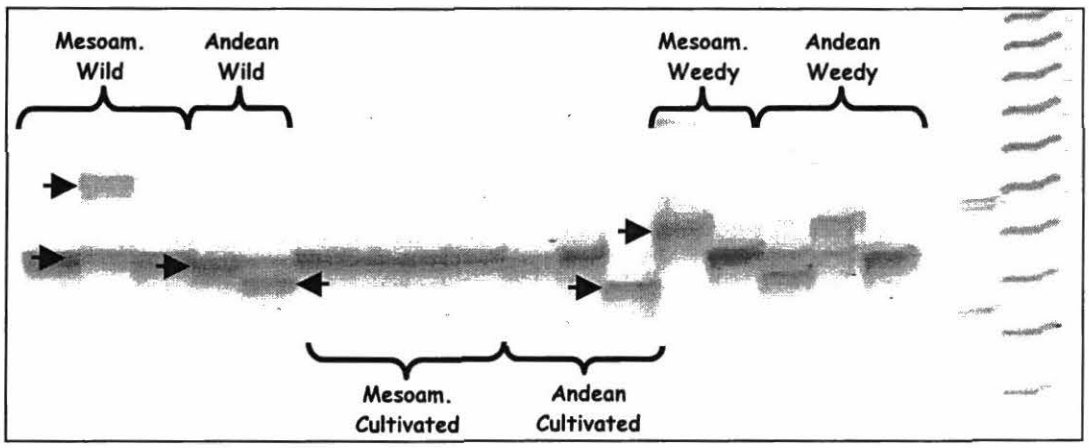

Figure 44. Microsatellies alleles obtained for locus BM 187 in the evaluated population

Table 51. Description of polymorphic SSR with a high PIC.

SSR	*Polymorphic alleles*	*PIC*
BM209	9	0,776
GATS91	7	0,768
BM211	6	0,746
BM143	6	0,669
BM140	5	0,641
BM170	6	0,611
BM181	5	0,564
BM154	5	0,563
BM156	3	0,551
BM202	3	0,546
BM183	6	0,526
BM148	5	0,515
BM171	4	0,508
BM212	4	0,508
BM201	4	0,481
BM141	3	0,397
BM155	3	0,383
BM153	2	0,374
AG1	2	0,374
BM146	3	0,374

RFLPs of chloroplast DNA

We are interested in finding specific haplotypes of chloroplast DNA in the *P. lunatus* populations with the objective of determining gene flow direction, as it is maternally inherited. Hence, twelve intergenic regions of cpDNA reported by Fofana et al. (1999) and Demesure et al. (1995) were evaluated to determine the amplification conditions (Table 52).

Table 52. Regions of cpDNA evaluated in this study.

CpDNA Region	PRIMER SEQUENCE (5`-3)	SIZE (pb)	REFERENCE
atpB-rbcL spacer	GTGTCAATCACTTCCATTCC GTAAAATCAAGTCCACCGCG	1700	1
rps14-psaB spacer	CATTTCACGAAGTATGTGTCCG TGGCGTGGATATTGGCAGGA	700	1
petA-psbE region	GCATCTGTTATTTTGGCACA TACCTTCCCTATTCATTGCG	1200	1
psbC-tRNAser spacer	GGTCGTGACCAAGAAACCAC GGTTCGAATCCCTCTCTCTC	1700	1
tRNAser-tRNAfmet spacer	GAGAGAGAGGGATTCGAACC CATAACCTTGAGGTCACGGG	1600	2
trnT-trnL spacer	CATTACAAATGCGATGCTCT TCTACCGATTTCGCCATATC	800	2
trnL Intron	CGAAATCGGTAGACGCTACG GGGGATAGAGGGACTTGAAC	630	2
trnL-trnF spacer	GGTTCAAGTCCCTCTATCCC ATTTGAACTGGTGACACGAG	530	2
rpl16 intron	GCTATGCTTAGTGTGTGACTCCGTT CGTACCCATATTTTTCCACCACGAC	1210	2
ndhA intron	GGWCTTCTYATGKCRGGTATRGMTC CTGYGCTTCMACTATATCAACTGTAC	1500	2
accD-psaI spacer	GGAAGTTTGAGCTTTATGCAAATGG AGAAGCCATTGCAATTGCCGGAAA	700	2
rpoC1-rpoC2 spacer	GAAGTTCACTATGAATCTTTNGGTACC TAGACATCGGTACTCCAGTGC	1700	2

1. Fofana et al. 1997
2. Demesure et al. 1995

References

Baudoin, J. P., Degreef, O. Hardy, F. Janart, & I. Zorro Bi. 1998. Development of an *in situ* conservation strategy for Lima bean (*Phaseolus lunatus* L.) populations in the Central Valley of Costa Rica, p. 417-426. In S.J. Owens and P.J. Rudall (eds.) Reproductive biology, Royal Botanic Garden Press, Kew, England.

Essers, S.A.J.A., Bosveld, M., Grift, van der R.M. & Voragen, A.G.J. 1993. Studies on the cuantification of specific cyanogens in cassava products and introduction of a new chromogen. J. Sci. Food Agric. 63 287-296.

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

Gaitán-Solís. E., Duque. M. C., Edwards. K. J. & Tohme. J. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterizacion, and cross-species amplification in *Phaseolus* ssp. Crop Science. 42: 2128-2136.

Gutierrez-Salgado, A., Gepts, P, & D.G. Debouck. 1995. Evidence for two gene pools of Lima bean, *Phaseolus lunatus* L. in the Americas. Genetic Resources and Crop Evolution 42: 15-28.

Lioi, C. Lotti. & I. Galasso. 1998. Isozyme diversity, RFLP of the rDNA and phylognetic affinities among cultivated lima beans, *Phaseolus lunatus* (Fabaceae). Plant Systematic and Evolution 213: 153-164.

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6. Annexes

6.1. List of publications by Project Staff in 2006

A. In refereed journals:

Ceballos, H., T. Sánchez, A.L. Chávez, C. Iglesias, D.G. Debouck, G. Mafla & J. Tohme. 2006. Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots. Journal of Food Composition and Analysis 19: 589-593

Muñoz, L.C., M.C. Duque, D.G. Debouck & M.W. Blair. 2006. Taxonomy of tepary bean and wild relatives as determined by amplified fragment length polymorphism (AFLP) markers. Crop Science 46 (4): 1744-1754.

Salcedo C., J., Arroyave J.A., O. Toro Chica & D.G. Debouck. 2006. *Phaseolus novoleonensis*, a new species (Leguminosae, Phaseolinae) from the Sierra Madre Oriental, Nuevo León, Mexico. Novon 16 (1): 105-111.

B. In non-refereed Journals:

González Torres, R.I., Carvajal M., Toro O., Duque M.C., Araya R. & D.G. Debouck. 2006. Evidence of gene flow among bean species of section *Phaseoli* in Colombia and Costa Rica using microsatellite markers. Annu. Rept. Bean Improvement Coop. (USA) 49: 135-136.

C. As Conference Proceedings:

González-Torres, R.I., O. Toro, M. C. Duque, R. Araya & D. G. Debouck. 2006. Gene flow events among bean species of section Phaseoli in Colombia and Costa Rica using microsatellites markers. LII PCCMCA scientific committee 2006 (Programa Cooperativo Centroamericano de Mejoramiento de Cultivos y Animales). April 24-28. Managua, Nicaragua. p. 221.

Ocampo, C.H., Gallego G., Duque M.C., Sánchez I., Rios-Castaño D. & D.G. Debouck. 2006. Diversidad Genética de la Colección Colombiana de Aguacate (*Persea americana* Mill.). In: Memorias del Primer Congreso Colombiano de Horticultura, Universidad Jorge Tadeo Lozano. Bogotá, D.C., Colombia. 17-22 octubre 2006. p. 78.

6.2. List of thesis research supervised by Project Staff in 2006

Martínez, C.A. 2006. Evaluation of gene flow among populations of cultivated and wild Lima beans (*Phaseolus lunatus*) belonging to the Andean genepool. Biologist degree. Universidad del Tolima, Ibague, Colombia.

Suárez Barón, H.G. 2006. Evaluation of gene flow in wild –weedy- crop complexes in *Phaseolus lunatus* L., of the Mesoamerican gene pool, with help of morpho agronomic, biochemical and molecular markers. Biologist degree. Universidad del Quindio, Armenia, Colombia.

6.3. List of conferences and scientific communications presented by Project Staff in 2006

Debouck D.G. 2006. "Germplasm banks and the phytosanitary risks of introducing pests", invited conference in the 2nd International Course on Phytosanitary Risks for the Colombian Agriculture MADR-ICA-CIAT, Palmira, Colombia, 15 November 2006.

Debouck D.G. 2006. "Comentarios sobre Mecanismos de Protección de Propiedad Intelectual en Plantas", Regional Seminar "Derechos de Propiedad Intelectual en el ámbito de los Recursos Filogenéticos" organizad by FAO-RedBio-FOFEPAL-Universidad de Buenos Aires, Buenos Aires, República Argentina, 18-20 October 2006.

Debouck D.G. 2006. "Genetics of plant domestication: the basket and the clay pot challenging the PCR", invited conference at the international MolConnect workshop, Bogotá, Colombia, 5 September 2006.

Debouck D.G. 2006. "Information build trust: how existing information systems can support the Multilateral System of the Treaty", presentation at the 1st meeting of the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture, Madrid, Spain, 13 June 2006.

Debouck, D.G. 2006. Invited conference in the 52th annual congress of the Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales: "Los recursos genéticos en construcción: flujo de genes y selecciones de campesinos", Montelimar, Nicaragua, 24 April 2006 (awarded as the best conference of the congress).

Ocampo, C. H. 2006. Presentation during the CIAT internal seminars: Molecular markers and genetic diversity in *Persea americana* Mills: the case of the Colombian collection of avocado. CIAT, Palmira, Colombia. November 29 2006.

Rueda, G. E. 2006. Invited at III national meeting of Investigators in Informatics and Computation ParqueSoft - Universidad del Cauca: "Dispositivos Móviles en la Investigación y la Industria Agrícola", *Cali Octubre de 2006.*

6.4. List of international and national courses with input from Project Staff in 2006

2nd International Course on Phytosanitary Risks for the Colombian Agriculture MADR- ICA-CIAT, 14-16 November 2006.

6.5. List of trainees trained by Project Staff in 2006

In Seed Conservation

Trujillo, Iselen. Training in seed conservation and management. Universidad Nacional Experimental Simón Rodríguez, Venezuela. 11-15 September 2006.

In vitro Lab

Jiménez, Pablo Edgar . Training in conservation and management of *in vitro* cassava germplasm. CORPOICA, Tibaitatá, Colombia. 27-31 March 2006.

Triana, Alba Lucía. Training in conservation and management of *in vitro* cassava germplasm. CORPOICA, Tibaitatá, Colombia. 27-31 March 2006.

Gordillo, Erika Lucia. Training in conservation and management of *in vitro* cassava germplasm. Universidad de la Amazonía, Colombia. 24-28 July 2006.

Marchant, Alejandro. Training in conservation and management of *in vitro* cassava germplasm.Universidad Adventista de Chile. 24-28 July 2006.

Trujillo, Iselen. Training in conservation and management of *in vitro* cassava germplasm. Universidad Nacional Experimental Simón Rodríguez, Venezuela. 11-15 September 2006.

Zhu, Wenli. Training in conservation and management of *in vitro* cassava germplasm Chinese Academy of Tropical Agricultural Sciences, China. 23-27 October 2006.

Electrophoresis Lab

St. M.Sc. Wilfredo Pantoja. Training in the SDS-PAGE and 2D-IEF-SDS-PAGE techniques for phaseolin. CIAT, Bean Project. Novembre 2005-January 2006.

Profesora Erika Lucia Gordillo (Universidad de la Amazonia, Colombia) and Profesor Alejandro Marchant (Universidad Adventista de Chile). Training in biochemical and molecular markers. 27-28 July 2006.

Profesora Iselen Trujillo. Universidad Nacional Experimental Simón Rodríguez, Venezuela. Training in biochemical and molecular markers. 27-28 July 2006. 15 September 2006.

In Gene Flow

Martínez, C.A. Evaluation of gene flow among populations of cultivated and wild Lima beans (*Phaseolus lunatus*) belonging to the Andean genepool. Thesis for biologist degree. Universidad del Tolima, Ibague, Colombia, January-December 2006.

Suárez Barón, H.G. 2006. Evaluation of gene flow in wild –weedy- crop complexes in *Phaseolus lunatus* L., of the Mesoamerican gene pool, with help of morpho agronomic, biochemical and molecular markers. Thesis for biologist degree. Universidad del Quindio, Armenia, Colombia, January-December 2006.

Gómez, Marcela. Universidad del Tolima. Training in biochemical and molecular markers of *Phaseolus* ssp. for gene flow assessment. Sept 2005-March 2006.

Ing. Walter Barrantes. University of Costa Rica, San José, Costa Rica. Training in molecular markers of *Phaseolus vulgaris* L. for the assessment of genetic diversity, gene flow events, and in seed conservation. 17 April- 16 June, 2006.

6.6. Posters

1. Chacón M.I., R.I. González & D.G. Debouck. 2006. When gene flow counteracts domestication: the case of common bean (Phaseolus vulgaris L.). Visit of Science Council, CIAT-HQ, Palmira, Colombia, 11-13 September 2006.

2. Mafla G., J.C. Roa, N.C. Flor, E. Aranzales & D.G. Debouck. 2006. Distribution of cassava germplasm from an international genebank: a service to the global agriculture. First meeting of the Governing Body, ITPGRFA, Madrid, Spain, 12-16 June 2006.

3. Ocampo C.H., G. Gallego, M.C. Duque, I. Sánchez, D. Rios-Castaño & D.G. Debouck. 2006. Diversidad genética de la colección colombiana de aguacate (*Persea americana* Mill.). Primer Congreso Colombiano de Horticultura, Bogotá, D.C., Colombia, 17-22 octubre 2006.

4. Torres A.M., A. Ciprian, O. Toro & D.G. Debouck. 2006. Distribution of bean and tropical forage germplasm from an international genebank: a service to the global agriculture. First meeting of the Governing Body, ITPGRFA, Madrid, Spain, 12-16 June 2006.

6.7. Awards

Debouck D.G. Programa Centroamericano Cooperativo para el Mejoramiento de Cultivos y Animales 52, Mesa de Biotecnología y Recursos Naturales, Nicaragua, 2006, award for the best conference.

Debouck D.G. 2006 CGIAR Science Award for Outstanding Partnership, Washington DC, USA.

6.8. Visitors

The Professional Staff of the Genetic Resource Unit attended the visit of 583 people from different government bodies, institutions, companies, etc. A total of 250 students from 12 different universities of Colombia visited the Genetic Resources Unit, on May and October 2006 through the 'Open House' day coordinated by CIAT's 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; US$ 358,186).

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; US$ 21,706).