ANNUAL REPORT 2003

CIAT Project on Saving Agrobiodiversity SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses

CIAT
OCTOBER, 2003

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

1. Project Description

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

Outputs:

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

Gains:

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

Milestones:

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

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

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

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

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

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

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

CIAT:SB-01/02 Project Log Frame (2003-2006) Project: Conservation and Use of Neotropical Genetic Resources Project Manager: Joe Tohme (BRU:J.Tohme; GRU: D.G. Debouck)			
Narrative Summary	Measurable Indicators	Means of Verification	Important Assumption
Goal: To contribute to the sustainable increase of productivity and quality of mandated and other priority crops, and the conservation of agrobiodiversity	CIAT scientists and partners using biotechnology information and tools in crop research. Genetic stocks available to key CIAT partners.	CIAT and NARS publications. Statistics on agriculture and biodiversity.	
Purpose: To conserve the genetic diversity and ensure that characterized agrobiodiversity, improved crop genetic stocks, and modern molecular and cellular methods and tools are used by CIAT and NARS scientists for improving, using, and conserving crop genetic resources	Information on diversity of wild and cultivated species. Mapped economic genes and gene complexes. Improved genetic stocks, lines and populations.	Publications, reports, and project proposals.	Pro-active participation of CIAT and NARS agricultural scientists and biologists.
Output 1: Genomes characterized of wild and cultivated species of mandated and non-mandated crops and of associated organisms.	Molecular information on diversity of mandated and nonmandated crops species, and related organisms. Bioinformatic techniques implemented. QTLs for yield component in rice, for nutrition traits in beans and cassava and fo Al tolerace in *Brachiaria*.	Publications, reports, and project proposals. Germplasm Availabilty of laboratory information management system (LIMS)	Availibilty of up-to-date genomics equipment and operational funding.
Ouput 2: Genomes modified: genes and gene combination used to broaden the genetic base of mandated and non mandated crops.	Transgenic lines of rice and advances in cassava, beans, *Brachiaria,* and other crops. Cloned genes and preparation of gene constructs. Information on new transformation and tissue culture techniques.	Publications, reports and project proposals. Germplasm.	IPR management to access genes and gene promoters. Biosafety regulations in place.
Output 3: Collaboration with public-and private-sector partners enhanced.	*CIAT partners in LDCs* using information and genetic stocks. News partnerships with private sector.	Publications. Training courses and workshops. Project proposals.	Government and industry support national biotech initiatives.
Output 4: Mandated crops conserved and multiplied as per international standars.	Germination rates for long-stored materials. Cost per accession/year, compared with other gene banks.	Visits to GRU substations and conservation facilities.	Absence of uncontrolled diseases. Quarantine greenhouse space available at different altitudes.
Output 5: Germplasm available,restored and safely duplicated.	Number of germplasm request received and satisfied annually. Users received germplasm and data. Users asked for novel germplasm and data.	Visits to multiplication plots. Reports on requests and delivery. Number of core collections multiplied and shipped.	Agreement with CIAT holds.
Output 6: Designated collections made socially relevant.	Landrace diversity restored to farmers. Farmers use new varieties. Breeders use novel genes.	Germplasm catalogs. Plant variety registration logs. National catalogs	International collecting possible. Quarantine matters cleared.
Output 7: Strengthen NARS for conservation and use of neotropical plants genetic resources.	NARS germplasm collections conserved. Number of trainees trained at CIAT. Number of universities and NARS using training materials.	Country questionnaires. Courses registered. Distribution and sales of training materials.	NARS and networks willing to cooperate.
Output 8: Conservation of Designated Collections linked with on-farm conservation efforts and protected areas.	Number of case studies and *pilot in situ conservation* projects.	Project documentation. Publications	NARS interested in conservation efforts. Farmers interested in conservation efforts.

SUMMARY ANNUAL REPORT 2003
Genetic Resources Unit
SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Researchers:

Daniel G. Debouck, Head, PhD
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3.2. Partners/ Cooperators:

Within CIAT:

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

Outside CIAT:

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

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

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

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

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

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

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

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

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

3.3. Financial Resources

Source	Amount (US$)	Proportion (%)
Unrestricted core	481,046	59,73
Carryover from 2002	147,244	18,28
Sub Total	**628,290**	----
Special projects		
Gene Flow BMZ	16,989	2,10
Upgrading Plan Operations	160,000 (260,500)	19,86
Upgrading Plan Capital	190,000 (217,000)	----
Sub Total	**176,989**	----
TOTAL	**805,279**	100

3.4. Highlights in 2003

Activity area # 1: the International Standards

In 2003 the GRU has widened the consultation possibilities of its databases by internet users, and has continued to invest in digital images. Significant progress (4,827 clones or 84% of the designated collection) has been made in testing the slow-growth method on the entire cassava collection with view of replicating it at CIP as security back-up. This is in line with the current effort at the level of the System-wide Genetic Resources Programme, to use existing facilities of IARCs to provide each other with security back-up services. The GRU thanks to a grant from the World Bank for the Upgrading of International Germplasm Collections in 2003-2006 has increased its regeneration facilities in Quilichao and Popayan. Progress has been made as to improve protocols for the long-term conservation of grass germplasm, and from there applications for the conservation of seed germplasm of some tropical fruit species (e.g. tree tomato, mountain papaya) have been developed.

Activity area # 2: the Germplasm and its data available

The distribution of germplasm out of the FAO designate collections continues on the high side (8,179 accessions in total). This is a demonstration of the strong interest by CIAT projects, partners and other institutions world wide to these collections maintained in the public domain, and a solid justification to enlist them in an agreement with the International Treaty on Plant Genetic Resources for Food and Agriculture (now in the final step towards its ratification). As a result of the cleaning and indexing effort launched in 1996 81% of the cassava collection is now indexed against viruses of quarantine importance and available for distribution. As a by-product of this work 2,595 clones have been established as a 'bonsai' collection that may result in a low-cost alternative to the field genebank before the work on the cryocollection is completed. Progress has been made in methods to control fungi of quarantine importance in *Brachiaria* germplasm with the use of antagonistic epiphytic bacteria

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

A 14 years effort to better understand the taxonomy of *Phaseolus* has been completed, while wild weed crop complexes in common bean have been studied from different parts of Colombia.

Activity area # 4: the International cooperation and capacity building

The diffusion of research results continues to be a priority for GRU Staff. The participation of the GRU in the The Epcot International Flower & Garden Festival, Orlando, Florida, has broken records of visitors 196,760. Scientific presentations were made in nine conferences, workshops and other fora. GRU Staff provided input in five international/national courses. Nine papers were published during 2003.

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

The results of the BMZ (Germany) supported project on Gene Flow in the bean model in Costa Rica indicate that gene flow through pollen movements can be substantial, although quite variable according to locations and possibly seasons. While gene flow measured in commercial lines on experimental station in Costa Rica is low, it seems to be important in the wild and in rural habitats where weedy races and landraces still coexist. So far, our results indicate that gene flow occurs principally from the wild relatives of common bean into the landraces; we have evidence however of a few cases of gene flow from the cultivated varieties into the wild materials.

We have continued to invest into the databases about distribution of wild relatives of *Manihot, Oryza* and *Phaseolus* in the New World with visits to the herbaria COL, EBUM, INB, NY, QCA and US. These data will serve as background information to projects of introduction and management of transgenical crops in the Neotropics, as well as to a regional initiative in functional genomics in a few crop genera of economic importance.

3.5. Problems encountered and their solution

After many years waiting, the GRU has obtained a grant from the World Bank to participate into an Upgrading Plan together with the other ten genebanks of the CGIAR under the auspices of the System-wide Genetic Resources Programme. After three months waiting, CIAT Finances has authorized the disbursement, and most of the Capital Equipment approved by the Plan has been purchased or ordered. GRU has started many activities and will continue to carry them out as approved by the donor and as per the work plan of the Upgrading.

CIAT Finances has without consultation with the Project Manager quitted the budget allocated to the Cassava Field genebank (an obligation under the FAO-CIAT agreement for the designated collection). Although the Field genebank has been discontinued by the Cassava Breeding project because of the high incidence of FrogSkin disease, the GRU has however established a 'bonsai' collection, free of disease of quarantine importance. As compared to cassava germplasm obtained from *in vitro*, the 'bonsai' collection can provide germplasm to CIAT research projects with a sure net gain of 2-3 months; this service has a cost that can be met if that budget line is restored to the GRU.

As mentioned previously, a limiting factor is the lack of flexibility on the personnel side, as continuing contracts seem to be against work productivity. Moving Staff from Finances to help in the daily management of budgets would help to alleviate the burden of work for Project Staff, while diminishing duplicate tasks at Center level.

3.6. Plans for next year

- Finish with the implementation of the bar coding in Quilichao and Popayan
- Refine the *management* modules of the GRU computer system (namely control of flows)
- Continue to clear backlogs, especially in bean germplasm
- Continue to regenerate accessions of forage and beans
- Continue with the introduction of the forage germplasm received from Australia
- Finish with the installation of the 'bonsai' cassava collection in glass-house at CIAT
- Complete and publish the protocols about minimal growth *in vitro* for cassava and for fruit germplasm
- Expand the cryoconservation of cassava germplasm to the entire core collection
- Start with the evaluation of the cassava core collection established in Thailand
- Complete and publish the protocols for cryoconservation of seed of *Carica* fruit germplasm
- Complete and publish the protocols for the safe multiplication of *Brachiaria* germplasm
- Run international courses as it may be required
- Continue to train the Staff for the use of the new computerized system and the bar coding
- Conclude the experimental part of the BMZ Gene Flow Project in Peru and Costa Rica
- Anticipate consequences of the ratification of the International Treaty in the region (training)

3.7. Executive summary

Thanks to a grant from the World Bank for the 'Rehabilitation of International Public Goods', the GRU has initiated the Upgrading Plan, an ambitious work plan to clear all backlogs and shortfalls, namely in the seed collections by 2006. Seed rejuvenation has been carried out for aging seed stocks (3,566 and 3,377 accessions of beans and forages, respectively). The cleaning and certification of the cassava collection against viruses of quarantine importance has made significant progress, as 81% of the collection is now ready for safe distribution. An indirect result of that work is the availability of 2,595 certified clones that are kept as a 'bonsai' collection, as a low-cost alternative to the field genebank. In line with the modernization of its computer system, the GRU has added a substantial number (6,277) of images to the databases available from CIAT website in order to enable users to make more precise requests of information and germplasm. A total of 8,179 accessions were distributed this year to CIAT projects and outside institutions, as a clear indication of the importance of that service to agricultural development worldwide. Protocols developed for seed conservation of tropical forages and other wild species were successfully applied with minor modifications to fruit germplasm, namely papaya and tree tomato. Five international/ national courses benefited from GRU Staff input, and several trainees were personally attended. Input was provided at different times to a Working Group coordinated by the Von Humboldt Institute of Colombia for the establisment of a policy on access and benefit sharing of genetic resources for the Andean Region. In relation to this a consultancy on mechanisms
of follow-up and control has been carried out.

4. Project performance indicators

1.FLOWS, TECHNOLOGIES, METHODS & TOOLS

1.1. Backlogs cleared
1,208 accessions cleared
1.2. Accessions regenerated
3,566 of beans, 3,377 of tropical forages
1.3. Accessions secured in long-term
953 accessions secured

1.4. Accessions in security back-up
No shipment this year, 953 accessions added
1.5. Accessions characterized
3,266 (field/ lab) + 6,277 (image bank)
1.6. Accessions distributed with passport data
6,943 accessions distributed
1.7. Novel germplasm acquired
56 accessions acquired
1.8. Novel genes identified
2 novel phaseolin types discovered
1.9. Support Tools (software in germplasm management; databases available from internet)
see www.ciat.cgiar.org
1.10. Data Bases united/ improved
same

2. PUBLICATIONS

2.1. **Refereed Journals:** published: 3
2.2. **Refereed Journals:** submitted: 2
2.3. **Book Chapters:** published: 0
2.4. **Published Proceedings:** published articles: 2
2.5. **Scientific Meeting Presentations:** presentations: 9
2.6. **Working Papers, Other Presentation or Publications:** 1
(see under 6 in full report)

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

3.1.**Training Courses :** 3

3.2. **Individualized Training :** 10

3.3. **PhD, MSc. and pregraduate thesis students:**
M.Sc. : 1
B. Sc. : 1

4.0 RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted
- Rehabilitating International Public Goods (2nd round, with SGRP)
- Investigating functional diversity for investments in agrobiodiversity
- Changing wasted - and expensive to maintain - roadsides into conservation areas for wild relatives of crops and forage species in Latin America.

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

The image bank has been increased with near 5,000 images, which allows website users a better utilization of the data base. The GRU has added an image for some accessions with particular purposes e.g. popping beans, showing how they crack and the volume that can reach after the cooking process (Figure 1). Table 1 shows progress achieved during this year for the image bank. We also initiated the including of images of biochemical characterization, e.g. globulins in tepary bean (Figure 2); this will allow an evaluation of the extent of genetic diversity of the accessions. Also during this year 526 images of cassava have been captured with the descriptor "root pulp color"; these are being added to the database for access to internet users. Table 3 shows the distribution of materials represented in the image bank.

Figure 1. Popping beans

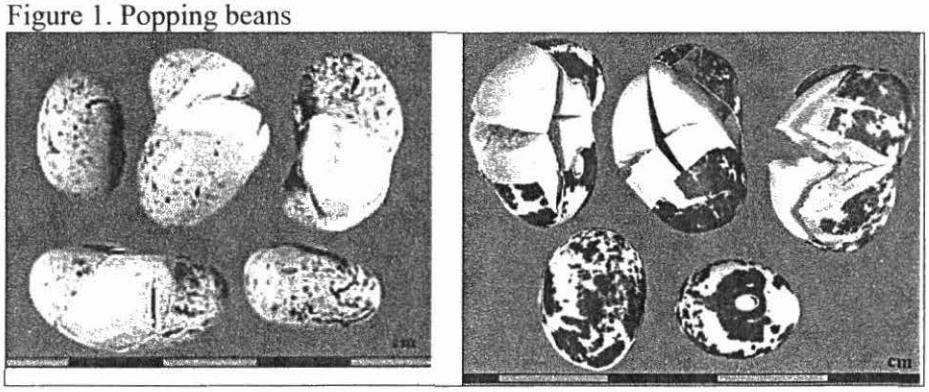


Figura 2. Types of globulins in *Phaseolus acutifolius*

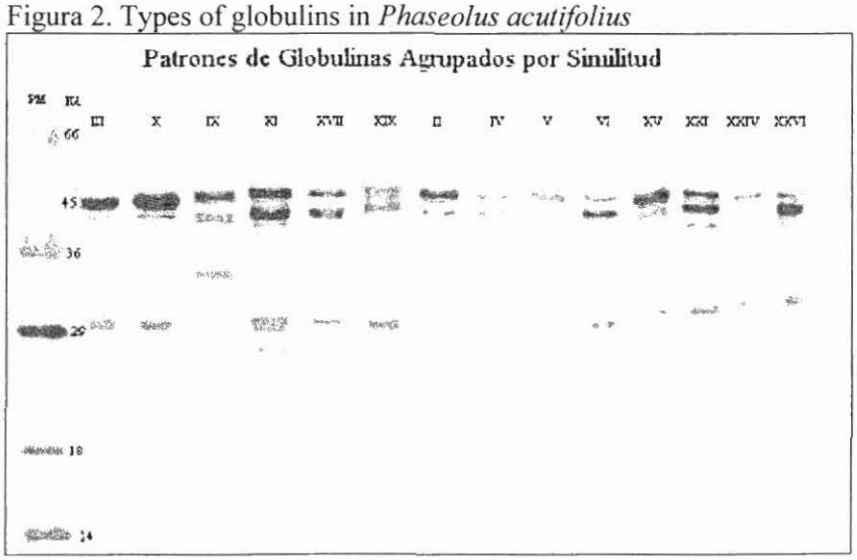
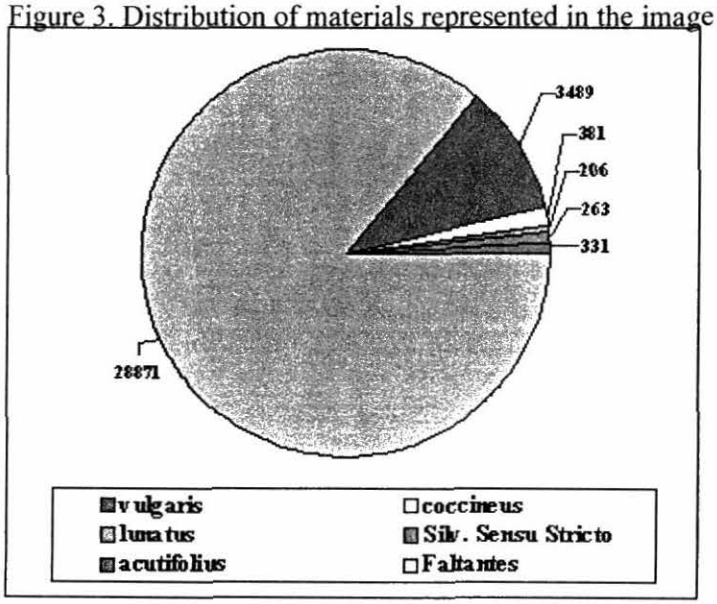


Table 1. Number of images available in CIAT's Web page.

Species	Number of images	Percentage
P.vulgaris	3,489	12.1%
P. coccineus and related	381	30.8%
P.lunatus	206	7.7%
P.acutifolius	331	100.0%
Other wild species	263	100.0%
Adittional (Popping beans)	128	
Tropical forrajes	1,479	
Total	6,277	

Figure 3. Distribution of materials represented in the image bank



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Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into genebank processes

The GRU continued in 2003 with the introduction of new materials into the germplasm bank. A total of 62 bean and 1,146 forage (1,021 legumes and 125 grasses) accessions were included during this process (Table 2). The materials of Costa Rica belongs to an exploration trip made in December 2002 and January 2003 in the Central Valley of Costa Rica, within the frame of a project on bean gene flow. We have introduced lines from CIAT breeding activities, namely lines form interspecific crosses with resistance to bacterial blight and ascochyta bligth.

Tabla 2. New accessions introduced to the germplasm bank in 2003

ORIGIN	SPECIES	STATUT	TOTAL
various	Forage legumes	WILD	1,021
various	Forage grasses	WILD	125
CRI	P.vulgaris	CULTIVATED	7
CRI	P.vulgaris	WEEDY	5
CRI	P.vulgaris	WILD	23
CRI	P.costaricensis	WILD	1
CRI	P.leptostachyus	WILD	1
CRI	P.hybrid	WEEDY	3
CRI	P.xanthotrichus	WILD	2
COL	P.vulgaris	CULTIVATED	20
TOTAL			1,208

Contributors: O. Toro, A. Ospina, A. Ciprian

Output 1.2 Backlogs of materials pending on multiplication multiplied

Activity 1.2.1. Multiplication of materials cleared by quarantine authorities

Once cleared by ICA plant quarantine authorities, germplasm is introduced into the genebank processes. Usually, the germplasm is increased in glass/ mesh-houses facilities in Palmira. In 2003, 1,150 bean materials and 1,146 forage materials were multiplied. We have also initiated the multiplication of 318 materials obtained from CSIRO, Australia (310 accessions of legumes and 8 accessions of grasses).

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

Output 1.3 Materials pending on regeneration regenerated

Activity 1.3.1. Multiplication of materials with ageing seeds

Table 3 shows a total of 3,566 accessions of beans regenerated in 2003 in two localities. The regeneration of beans was done exclusively in greenhouses and meshouses due to the impossibility of multiplying seeds in the field for security reasons. The main purpose of regeneration was seed stock refreshment given CIAT's commitments to ensure the availability of FAO designate collections in the future. For the forage collection (Table 4), 3,377 accessions have been multiplied at three localities in 2003. The major collection of legumes is multiplied in Quilichao, while the major collection of grasses is multiplied in Popayán.

Table 3. *Phaseolus* beans germplasm processed for regeneration under greenhouse/meshouse (number of accessions)

Localities	Greenhouse/meshouse
Palmira	1,420
Popayán	2,146
Total	3,566

Table 4. Forage germplasm processed for multiplication and regeneration under greenhouse/meshouse and field conditions (number of accessions)

	Greenhouse /Meshouse		Field		
Localities	Legumes	Grasses	Legumes	Grasses	Total
Quilichao	N.A.	N.A.	965	190	1,155
Palmira	603	250	814	248	1,915
Popayán	N.A.	N.A.	61	230	291
Total	603	250	1,840	668	3,361

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

Status of designated germplasm at the GRU in 2003
In view of progress of ratification of the International Treaty on Plant Genetic Resources for Food and Agriculture, and of a possible agreement with its Governing Body, the status of designated accessions is currently as follows:

Manihot cassava: 5,742
Phaseolus beans: 33,450
Tropical forages: 18,138

Activity 1.3.2. Periodical subculturing of the FAO designate cassava collection

This year, 7,847 materials (5,706 accessions) of *Manihot* were subcultured by the nodal cutting technique; the accessions multiplied in 2003 represents 99.6% of the collection. A total of 2,640 accessions were propagated for the establishment of a 'Bonsai' collection as safety back up of the entire Cassava Collection, since due to the Frog Skin Disease the collection cannot any longer be maintained in the field.

Contributors: G. Mafla, J.C. Roa

Ouput 1.4 Materials processed into final packing

Activity 1.4.1. Final drying and temporary storage

Table 5 shows the numbers of accessions for beans (3,112) and forages (1,864), respectively, that have been produced, cleaned, dried, and stored at 5°C awaiting results from viability and health tests.

Table 5 Germplasm in seed processing during 2003

	Beans	Forages
Seed selection / temporal storage	3,112	1,864
Total	3,112	1,864

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

Activity 1.4.2. Viability testing for recently multiplied materials

Table 6 indicates flows of materials for viability testing during 2003. It shows the importance of good drying and other improved procedures established since 1996. Ranges of germination were chosen

because viability lower than 65% does not allow seed distribution and viability lower than 85% does not allow long term seed conservation.

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

Class	BEANS		FORAGES	
	Germination (%)	No. Accessions	Germination (%)	No. Accessions
Already stored materials	1-64	5	1-64	12
	65-84	4	65-84	19
	85-100	40	85-100	37
Sub-total		49		68
Recently multiplied materials	1-64	19	1-64	54
	65-84	63	65-84	170
	85-100	388	85-100	479
Sub-total		470		703
TOTAL		519		771

As a support to multiplication activities for very old seeds the viability lab pre-germinated seed of 2,123 accessions of forages and 95 accessions of beans for direct transplant in the field multiplication plots. Several techniques of pre-germination such as sand beds, petri dishes and germination paper have been used successfully.

Contributor: A.M. Torres

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

Once cleared by the Viability Lab (for viability above 85%) and the Germplasm Health Lab (for absence of diseases of quarantine importance), the materials are processed into the five conservation purposes: long-term, safe duplicates, restoration, periodical monitoring, and distribution (Tables 7-8).

Table 7. Final storage and packing of *Phaseolus* beans processed during 2003 (number of accessions).

	Beans
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	360
SHORT TERM only (Distribution)	1,991
Total	2,351

Table 8. Final storage and packing of tropical forages processed during 2003 (number of accessions).

	Legumes	Grasses	Total
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	446	147	593
SHORT TERM only (Distribution)	1,323	203	1,526
Total	1,769	350	2,119

Contributor: A.M. Torres

Activity 1.4.4 Monitoring viability of conserved germplasm of seeds of beans and forages

In this year we performed the monitoring of viability after 5 years of conservation of *Phaseolus vulgaris* seed kept in long term storage, for three groups of seeds: Ten1997B, Ten1998A and Pal1998B that were conserved during 1998. A total of 2,252 accessions were tested and the results are shown in Table 9.

The mean germination decreased 3.4 units for Ten1997B, 2.6 for Ten1998A and in 1.6 for Pal1998B, after 5 years of conservation. The difference is statistically significant in all cases with confidence interval of 95%. The decrease in germination slows as the time of harvests, perhaps reflecting improvements in pre-conservation seed treatments such as drying.

Similarly, the monitoring was done for forage germplasm after a period of 5 years for a group of 581 forages species packed during 1998. The results are shown in Table 10 where the difference of the mean germination was of 9.2 units. This difference is statistically significant with confidence interval of 95%. The decrease is significant for conservation purposes; an explanation might be in the packing of seeds with some viability deterioration (lots up to 18 years old when packed).

Accordingly, given our standard of germonation above 85%, for the seed lots monitored, a total of 223 (38%) accessions of forages and 241 (10%) accessions of beans have to be refreshed by seed multiplication.

Table 9. Paired T-Test for monitoring bean seeds after 5 years of long-term conservation

Source	% Germ	Mean	StDev	SE Mean	N	Difference	Std.Dv.Diff	T-value	P-Value
Ten1997B	Initial	97.025	3.559	0.128					
	Monitored	93.623	10.317	0.371	775	3.401	10.294	9.2	0.000
Ten1998A	Initial	97.864	4.074	0.142					
	Monitored	95.217	9.416	0.328	825	2.647	9.910	7.67	0.000
Pal1998B	Initial	96.181	3.895	0.153					
	Monitored	94.534	6.907	0.270	652	1.647	7.351	5.72	0.000

Table 10. Paired T-Test for monitoring forage seeds after 5 years of long-term conservation

% Germ	Mean	StDev	SE Mean	N	Difference	Std.Dv.Diff	T-value	P-Value
Initial	93.976	6.418	0.266					
Monitored	84.733	13.589	0.564	581	9.243	13.397	16.38	0.000

Activity 1.4.5 Monitoring viability of germplasm of seeds of sorghum and rice

A group of *Sorghum* accessions has been multiplied by ICRISAT in a regional program based at CIAT headquarters. An agreement was made with ICRISAT to conserve a duplicate of these seeds at the Genetic Resources Unit of CIAT. Before long-term conservation we proposed a viability test. Only accessions with germination values higher than 85% would be conserved under long term (-20 °C). We tested germination of 475 accessions of *Sorghum* spp. in paper rolls at 20/30 °C during 14 days. As shown in Table 11 none of the accessions could be conserved under long term due to poor quality of seeds.

Table 11

Group of Sorghum	Accession numbers	Germination range (%)
Hybrids	66	0-36
Original seed -1995	311	0-71
Millets -1999	98	0-16

Over the past two years, the CIAT Rice program approached the GRU for long-term conservation of their breeding materials and stocks. A total of 946 rice accessions were received, dried and conserved under temporal storage. A viability test was done in order conserve this material under long term storage (-20 °C). The germination of 946 accessions of *Oryza sativa* was tested in paper rolls at 20/30 °C during 14 days. Before the germination test the seeds were heat-treated (65 °C, for 15 minutes) for control of seed nematods, and to break dormancy. A total of 539 accessions reached germination levels between 85 and 100%. These accessions are ready to be packed and conserved under long term. A total of 385 accessions had poor quality seeds with germination lower than or equal to 84% and could not be conserved under long term storage.

Contributor: Alba M. Torres

Output 1.5. Improved conservation techniques

Activity 1.5.1. Establishing a protocol for seed conservation of tree tomato (Solanum *betaceum*) *and Brachiaria humidicola*

The purpose of this study was to establish a protocol to conserve seeds of *Solanum betaceum* (tree tomato) and *Brachiaria humidicola*. These two species are reported as of ortodox behaviour (Hong et al. 1996), thus the same protocol was applied for both: three moisture contents (4, 8, 12%) and three temperatures: (5, -18 and –196 °C). The conservation time was six months with three monitorings (at 0, 3 and 6 months).

Results and discussion

A total of 54 treatments were evaluated (factorial 2x3x3x3) in a ramdomized model of a factorial arrangement of four replicates. An analysis of variance (ANOVA; Table 12, 13) was done for the germination registered at 0, 3 and 6 months (Figures 4, 5 and 6), where the variation source was the individual factors (time, temperature, moisture content, and kinds of material for *B. humidicola* and cultivar for *S. betaceum*) and the interactions.

In the case of significant differences at probability of 5% of F test, a DUNCAN test for mean comparison was done by using the package STATISTICA 6.0.

Conservation of *Solanum betaceum*

Table 12. ANOVA for S. *betaceum*

Effect	D.F Effect	M.S Effect	D.F Error	M.S Error	F	p-level
1 var. cultivate	1	0.00485507	162	0.00313	1.551216	0.214754477
*2 time	2	0.686763406	162	0.00313	219.4238	0
*3 temperature	2	0.062723525	162	0.00313	20.04043	1.67176E-08
*4 M. content	2	0.044507198	162	0.00313	14.22024	2.04275E-06
12	2	0.003388025	162	0.00313	1.082489	0.341188848
13	2	0.009533316	162	0.00313	3.045935	0.050284348
*23	4	0.017181551	162	0.00313	5.489579	0.000360206
*14	2	0.014275813	162	0.00313	4.561183	0.011826801

*24	4	0.057672948	162	0.00313	18.42675	1.69427E-12
34	4	0.004307901	162	0.00313	1.376393	0.244400039
123	4	0.004638538	162	0.00313	1.482033	0.210009813
124	4	0.006514454	162	0.00313	2.081396	0.085598692
134	4	0.003620876	162	0.00313	1.156885	0.33192879
234	8	0.002610182	162	0.00313	0.833964	0.57393533
1234	8	0.002202242	162	0.00313	0.703626	0.688032746

The analysis does not show differences between cultivar (F=1.551, p=0.214), thus the behaviour of both cultivars in relation to the conservation factors is similar (Figure 4). However, in the interactions there were statistical differences between cultivar and moisture content (F=4.561, p=0.011). The cultivar 'Tamarillo' had better germination values over the cultivar 'Common' in all moisture contents of the seeds. Total germination during the time of conservation had a significant decrease of 4.9% showing the major loss in the first three months (3.8%). During the following three months the decrease was only 1.1%.

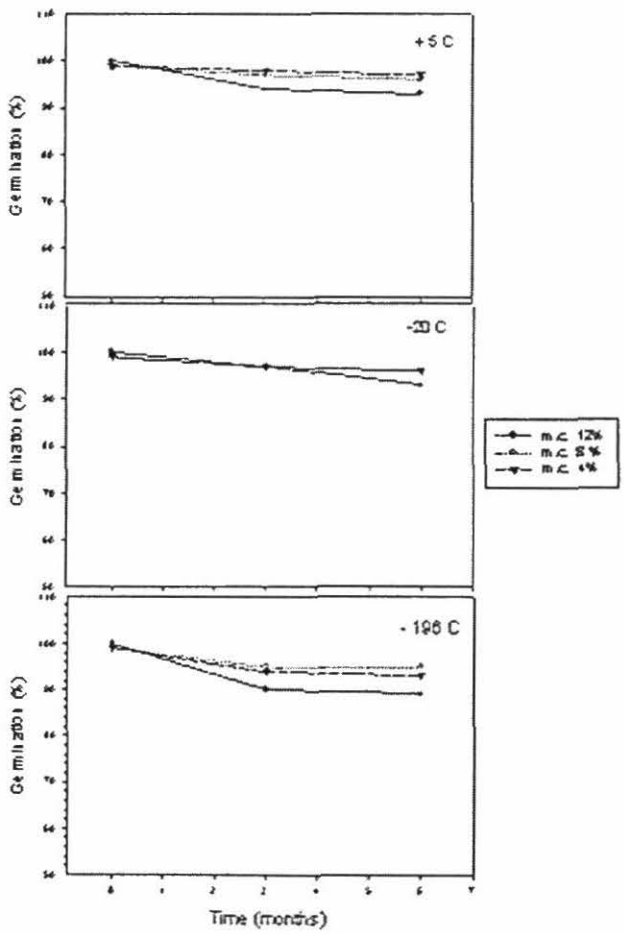


Figure 4. Behaviour of *S. betaceum* seeds during 6 months of conservation for three factors (moisture content, time and temperature).

There was no significant difference between +5 and -20 °C (p=0.932), while at -196 there was a drastic reduction of 6.7% in germination. Thus the temperature of liquid nitrogen was the one causing a lower germination during the conservation period evaluated for this species.

There was no statistical difference between 4 and 8% moisture content (p=0.112). In contrast, there was difference for 12% moisture content affecting the total germination of seeds. A reduction of 8% in germination during the six months of conservation for a moisture content of 12%, versus 3.1 and 3.4% for 4 and 8% of moisture contents, respectively.

If we check the behaviour through time exclusively, we would see in the last three months of conservation for all temperatures a tendency of establishment, specially at –196°C. In the last period at this temperature there was no significative decrease in the germination for all moisture content levels, even at 12%. Between the second and third monitoring, at 12% moisture content, there was a decrease in germination of 0.5% at –196°C, of 1.6% at +5, and of 0.8% at –20°C. This stability of seed viability in liquid nitrogen is an indication of a decrease in the metabolic functions of the seeds preventing the natural deterioration caused by fisiological activities of the seeds through the time.

Vigor test for *S. betaceum*
The vigor test was evaluated by using a chamber for accelerated aging after two periods of time conservation (3 and 6 months).

Both cultivars show statistical differences in the germination loss (p<0.001). This loss was higher in the cultivar 'Common', 62.7% against 62% for cultivar 'Tamarillo'. This is an indication that cultivar 'Tamarillo' has higher vigor a compared to cultivar common.

In addition, at +5 °C temperature we obtained the lowest vigor in both cultivars (61.4% for 'Common' and 69.5% for 'Tamarillo'). At lower temperatures (-20 and –196°C) we obtained higher vigour values of 78.2% for cultivar 'Tamarillo' and 61.1% for cultivar 'Common' at –20 °C (Figure 3).

The moisture content of 12% showed the lower values in the vigor test of 60.5 and 48.4% for cultivar 'Tamarillo' and 'Common', respectively, as compared to higher values of 70% for the moisture contents of 8 and 4% (Figure 2).

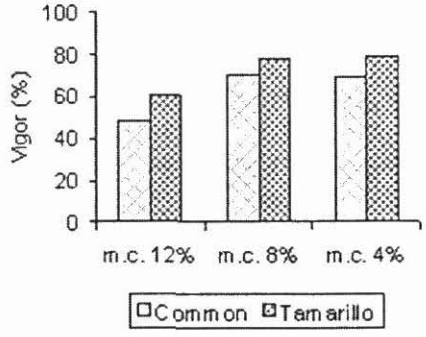


Figure 5. Total vigour through time at three moisture contents of *S. betaceum* seeds.

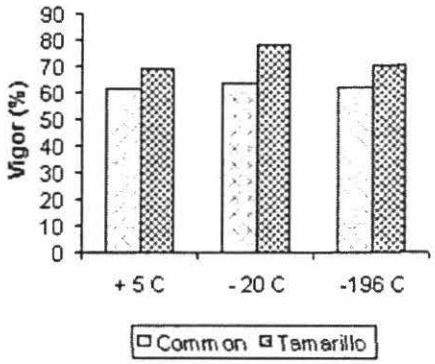


Figure 6. Total vigour through time at thre temperatures of conservation of *S. betaceum* seeds

Conservation of *Brachiaria humidicola*

The analysis of figures 7, 8 and Table 12 does not reveal significative differences between the factors time, condition and moisture content. There was no difference between temperatures, as an indication of seed tolerances to low and extreme temperatures (liquid nitrogen).

Table 13.ANOVA for *B. humidicola*

Effect	F.D Effect	M.S Effect	F.D Error	M.S Error	F	p-level
*1 Condition	1	0,082793	162	0,001647	50,25972	3,94E-11
*2 Time	2	0,083357	162	0,001647	50,60189	8,45E-18
3	2	0,004905	162	0,001647	2,977776	0,0537
Temperature						
*4 M. content	2	0,071417	162	0,001647	43,35397	8,31E-16
12	2	0,003701	162	0,001647	2,246882	0,109013
13	2	0,000923	162	0,001647	0,560196	0,572199
23	4	0,003186	162	0,001647	1,934162	0,107213
14	2	0,001963	162	0,001647	1,191407	0,306441
24	4	0,003862	162	0,001647	2,344251	0,056953
34	4	0,003024	162	0,001647	1,83571	0,124449
123	4	0,002113	162	0,001647	1,282834	0,278911
124	4	0,001933	162	0,001647	1,173629	0,324427
134	4	0,000247	162	0,001647	0,149925	0,962811
234	8	0,00112	162	0,001647	0,679918	0,708758
1234	8	0,000433	162	0,001647	0,263056	0,976786

The total germination during the first three months of conservation had a decrease of 4.3%. However, during the last three months, the germination had higher values, resulting in a decrease in germination of only 0.7% at six months.

During the six months of conservation, the seeds without glumes had an advantage of 2.5% in the germination over the seeds conserved without glumes. However, the behaviour of the germination during the time of conservation in all factors was the same for both sorts of materials (with and without glumes) (Figures 7, 8).

For both seed conditions at the temperature of +5°C, the moisture content of 8 and 12% had similar germination between each other and lower as compared to the moisture content of 4%. At lower temperatures (-10 and –196°C) the moisture content of 8% gave a germination rate similar to the moisture content of 4%, and both were higher than the germination for a moisture content of 12%.

These results indicate that moisture content and temperature are independent factors. Thus, the optimal moisture content has a particular behaviour at any temperature.

A reduction in germination occurred in the first three months followed by an increase as shown by the final monitoring (Figures 7, 8); that variation could be generated by many traits such as genetic or physiological variations among the seed lots.

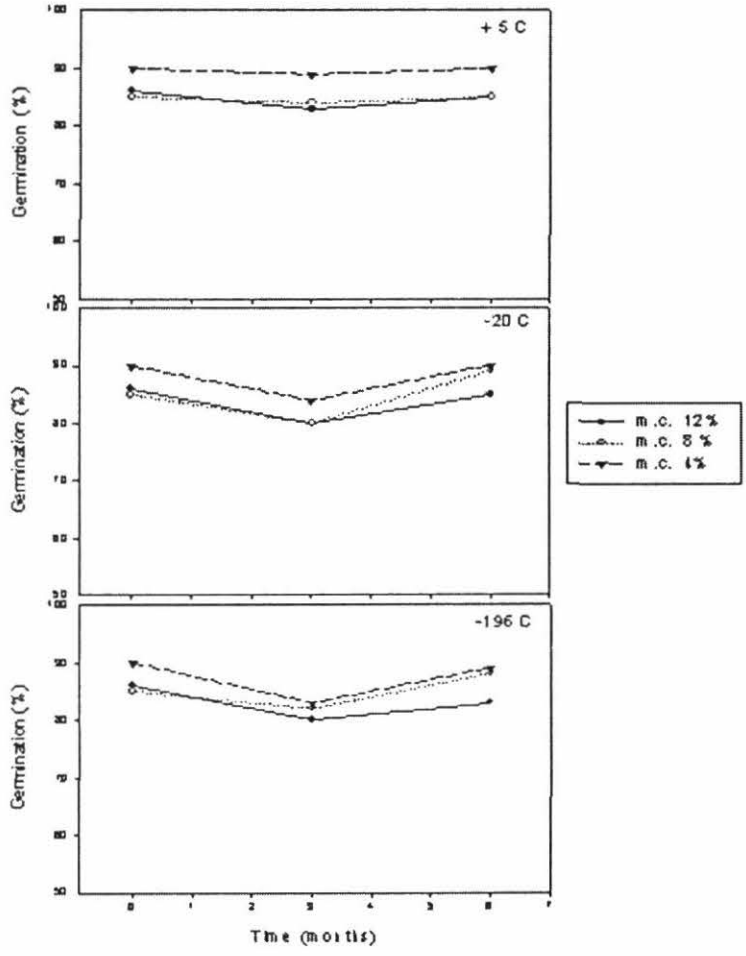


Figure 7. Behaviour of *B. humidicola* seeds with glums in six months of conservation

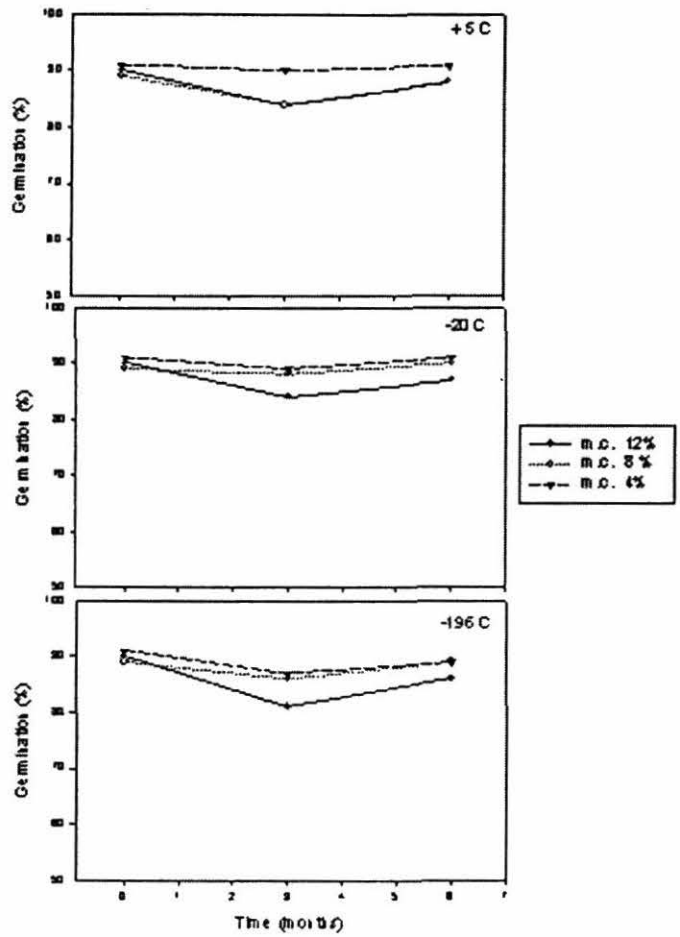


Figure 8. Behaviour of *B. humidicola* seeds without glumes in six months of conservation.

Vigor test for *U. humidicola*

The vigor test for *B. humidicola* seeds showed an increase of 3.6% in the germination during the evaluation periods (3 and 6 months) in both kinds of seeds. However, there were differences between both staes (F-19.313, $p<0.001$). In the state without glumes the vigor was 88.% and with glumes the vigor was 87.7. Once again there is an advantage of 1.2% in the seeds withouth glumes. For the seeds conserved at the temperature –196°C we observed the lowest vigour in both states of seeds with and without glumes, 86.8 and 88% , respectively. In the other temperatures the vigour was higher,specially at +5°C in both seed states (Figure 9).

As found in *S. betaceum*, the vigour was lower at the moisture content of 12% with 85.7% (media) for both states, against 87.8% and 98.8% obtained for the moisture content of 8 and 4, respectively (Figure 10).

The higher value of germination in this vigour test as compared to the germination of the monitoring shows the value of this test as an alternative to promote germination in *Brachiaria humidicola*. The high temperature of this test (40°C) and the high relative humidity (100%) help to soften the physic barriers of the seed cover, allowing the air interchange and water imbibition at the germination time.

The heat treatment could work as a sort of stratification, condition very similar to the geographic origin of this species, which come from tropical savannahs, humid and hot areas, climatic conditions very similar to the seed- aging test.

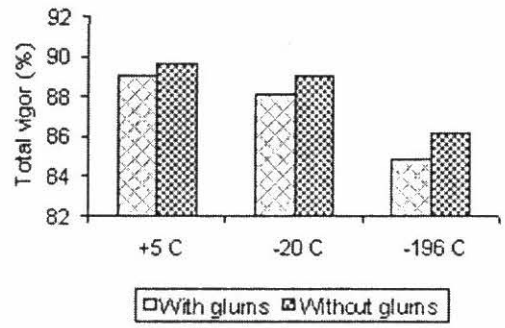


Figure 9. Total vigour through the time at three moisture contents of *B. humidicola* seeds.

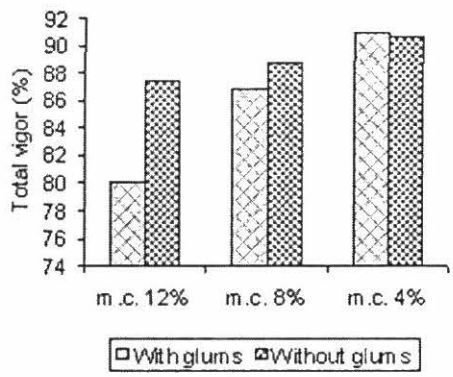


Figure 10. Total vigour through the time at thre temperature of conservation of *B. humidicola* seeds.

References:

Hong, T.D., Linington, S. & Ellis, R.H. 1996. Seed storage behaviour: a compendium.

Handbooks for genebanks: No. 4. International Plant Genetic Resources Institute, Rome.

Contributors: Jesús Salcedo, Alba M. Torres

Activity 1.5.2. Protocol for seed conservation of Tropical fruits

The conservation of the genetic diversity of fruits in tropics is very important because many natural habitats have been reduced and many natural populations are in risk of disappearance.

However, the knowledge of the physiology and behaviour of their seeds in conservation is not well known for a large diversity of tropical species. This project is focused on three groups of fruits, all are fleshy fruits and commonly used in tropical America, where they are coming from.

1. Solanaceae: Tree tomato (*Solanum betaceum*), lulo (*Solanum quitoense*) and a wild relative (*Solanum hartwegii*).
2. Passifloraceae: Passion fruit (*Passiflora edulis*) and a wild relative (*Passiflora maliformis*)

3. Caricaceae: Paw-paw fruit (*Carica papaya*) and a wild relative (*Vasconcellea cauliflora and V. goudotiana*)

The hypotheses to test for these fruits during 2003 were:
Ho: The seeds of these fleshy fruits have a positive response in germination when we apply methods such as physical conditioning, alternate temperatures, shocks of temperatures and chemical treatments (nitrates and gibberellins)

Ho: The seeds of the cultivated species of these fleshy fruits are more suitable for germination and conservation as compared to the wild species.

1. Thermogradient plate
A way to test the response by germination of seeds to different temperatures was done by using the thermogradient plate developed by the University of Reading (Murdoch, Roberts et al. 1989). The thermogradient contains 64 cells of different temperatures in a range of 12 to 40 °C in a combination of different amplitudes. Fifty seeds were placed in each cell without any additional treatment. Germination was check every two days for 21 days and recorded by protrusion of the radicle (2 mm long).

A fresh seed lot of *S. betaceum* cultivar 'Tamarillo' (Figure 11) and a seed lot of *S. quitoense* common fruit (Figure 12) were tested. *S. betaceum* had positive response to constant temperatures between 25 and 30 °C, and to alternate temperatures between 20 and 30 °C at 16 hours of thermo period and between 12 and 30 °C at 8 hours of thermo period. It is an indication of the low dormancy. In contrast, *S. quitoense* does not have a positive response to constant temperatures. In alternate temperatures the germination was relatively low (up to 80%) as compared to *S. betaceum* (up to 100%) with good response between 22 and 28 °C at 16 and 8 hours of thermo period in the major amplitude (difference between lowest and highest temperature).

Figure 11.

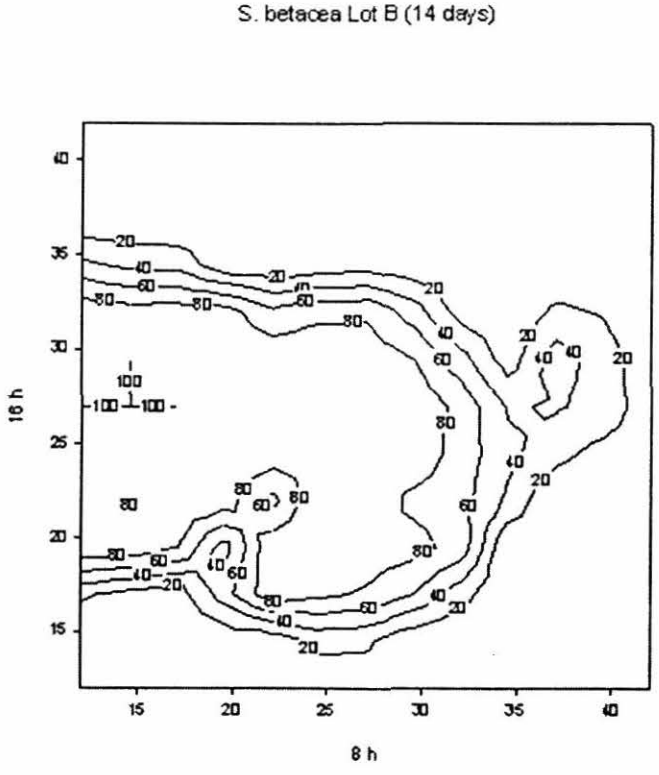
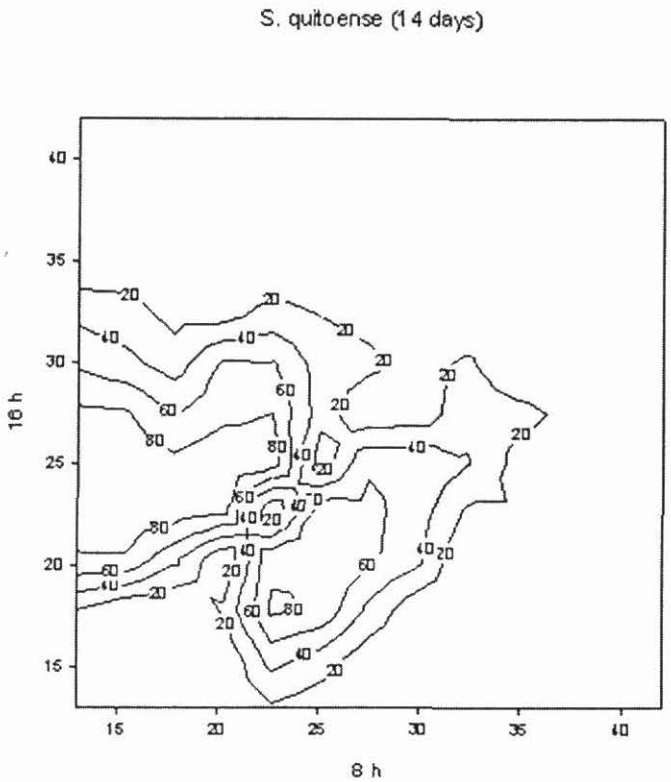


Figure 12.



2. Breaking dormancy treatments

The extraction of the seeds for all fruits was done by soaking the fruits in water without fermentation, and by cleaning all fleshy cover adhering to the seed. The seed immersion in water and hormones was for 24 hours according to ISTA (ISTA 1999). Dry seeds were used as control and immersion in water was used as another control to discard the response of increasing seed water potential in the hormones treatments. Alternate and constant temperatures were tested in different ranges for each species. Radicle protrusion (2 mm long) was recorded as germination. For all test four replicates of 50 seeds each were used. In order to have more precise results in the analysis of variance the variable germination (percent) was transformed by the arcsine function of the square root of germination/100.

2.1 Solanaceae

2.1.1 *Solanum betaceum* Cav.

The germination test in Petri dishes was applied to four seed lots of *S. betaceum* in five conditions of germination and four treatments of breaking dormancy (Annex 1, Figures 13, 14). The analysis of variance of the germination transformed data shows statistical differences between lots, conditions, treatments and interactions between all of them. The Tukey test for mean comparison was done giving the higher results for germination to the seed lot A ("Tamarillo" fresh seeds, over "Tamarillo 6 months old", "Common 6 months old" and "Common fresh"), to the germination condition of 25/15 °C for 16/8 hours of thermo period and to the treatments GA_3 2,000 p.p.m. and KNO_3 0.01 M (without differences between these two). The results show an endogenous dormancy in both cultivars of this species, better removed by gibberellins due to the natural deficit of this hormone in the seeds.

Table 14. ANOVA procedure for germination transformed to Angles of *S. betaceum*

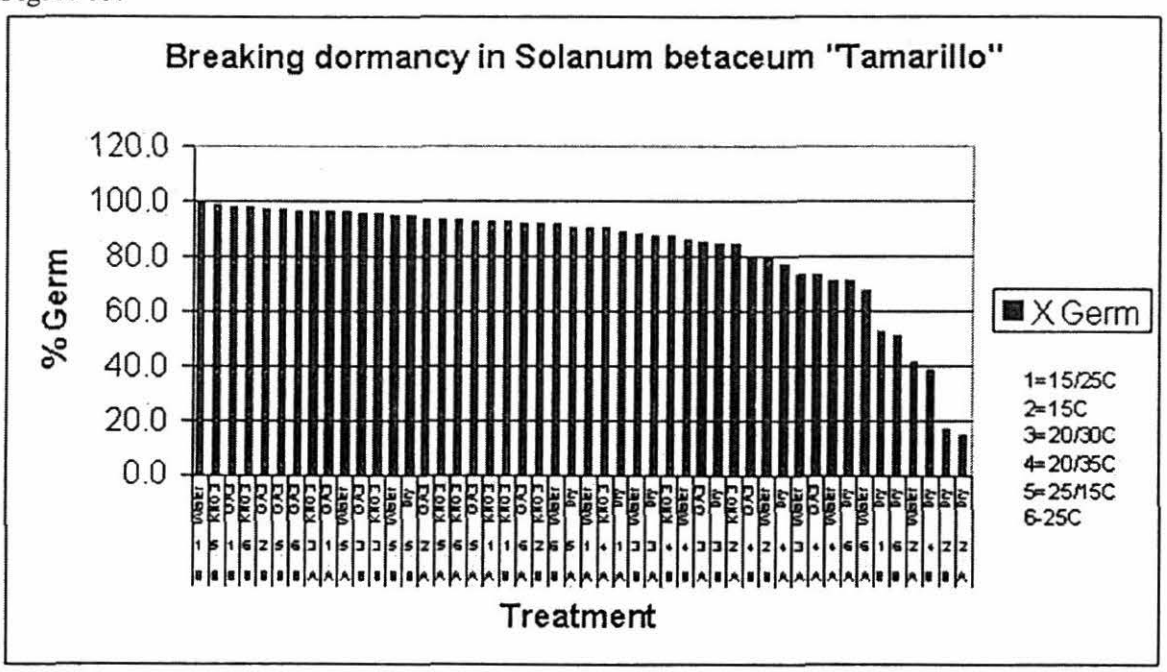
Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	3	0.04850943	0.01616981	63.14	<.0001
cond	5	0.71012101	0.14202420	554.59	<.0001
lot*cond	15	0.08594641	0.00572976	22.37	<.0001
treat	3	0.34670411	0.11556804	451.28	<.0001
lot*treat	9	0.02171441	0.00241271	9.42	<.0001
cond*treat	15	0.21151469	0.01410098	55.06	<.0001
lot*cond*treat	45	0.07257506	0.00161278	6.30	<.0001

The rate of germination was recorded by checking the germination test every 2 days during 21 days. There were statistical differences between seed lots, condition, treatment and interaction between all factors. Tukey test for mean comparisons shows the highest germination rate for lot A ("Tamarillo" 6 months old), at the condition of 25/15 °C for 16/8 hours of thermo period and in the treatment GA_3 2,000 p.p.m.

Table 15. ANOVA procedure for rate of germination of *S. betaceum*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	3	0.04850943	0.01616981	63.14	<.0001
cond	5	0.71012101	0.14202420	554.59	<.0001
lot*cond	15	0.08594641	0.00572976	22.37	<.0001
treat	3	0.34670411	0.11556804	451.28	<.0001
lot*treat	9	0.02171441	0.00241271	9.42	<.0001
cond*treat	15	0.21151469	0.01410098	55.06	<.0001
lot*cond*treat	45	0.07257506	0.00161278	6.30	<.0001

Figure 13.



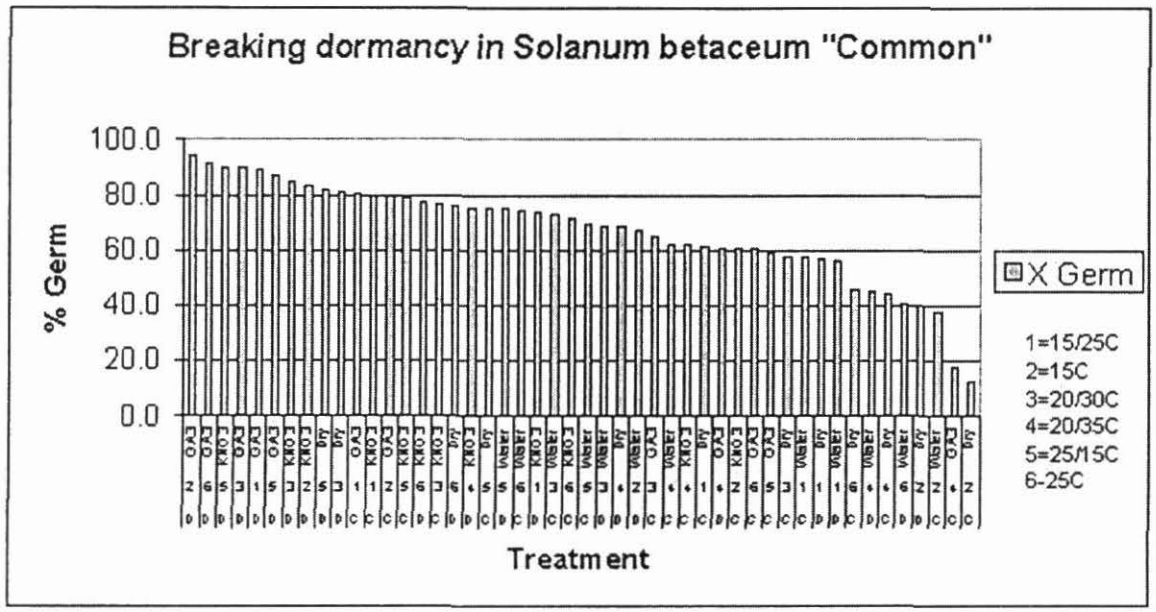


Figure 14.

2.1.2 Solanum quitoense Lam.

The germination test in Petri dishes was applied for two seed lots of *S. quitoense*, six combinations of temperatures and four treatments to break seed dormancy (Annex 2, Figure 15). The analysis of variance of the factorial using the germination data transformed to angles shows statistical differences between lots, condition and treatment. There was interaction between lot, condition and treatment only. The Tukey test for mean comparisons show the highest germination for lot E ("Common lulo" over "Lulo La Selva"), the temperature condition 25/15 °C for 16/8 hours of thermo period and the treatment GA_3 2,000 p.p.m. The results confirm the deep dormancy in both cultivar of this species.

Table 16. ANOVA procedure for germination transformed to Angles of *S. quitoense*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	2.99681631	2.99681631	129.52	<.0001
cond	5	10.06709133	2.01341827	87.02	<.0001
lot*cond	5	0.17391752	0.03478350	1.50	0.1924
treat	3	13.31685147	4.43895049	191.85	<.0001
lot*treat	3	0.13299313	0.04433104	1.92	0.1296
cond*treat	15	4.16106359	0.27740424	11.99	<.0001
lot*cond*treat	15	0.97748754	0.06516584	2.82	0.0007

The rate of germination was measured by applying a germination test every two days for 21 days. The analysis of variance shows the highest rate of germination for lot E ("Common lulo"), temperature of germination 25/15 °C for 16/8 hours of thermo period and for the treatment GA_3 2,000 p.p.m.

Table 17. ANOVA procedure for rate of germination of *S. quitoense*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	0.06285769	0.06285769	98.90	<.0001
cond	5	0.26646979	0.05329396	83.85	<.0001
lot*cond	5	0.03564400	0.00712880	11.22	<.0001
treat	3	0.21328102	0.07109367	111.86	<.0001
lo					
t*treat	3	0.01080860	0.00360287	5.67	0.0011
cond*treat	5	0.16489717	0.01099314	17.30	<.0001
lot*cond*treat	15	0.04870971	0.00324731	5.11	<.0001

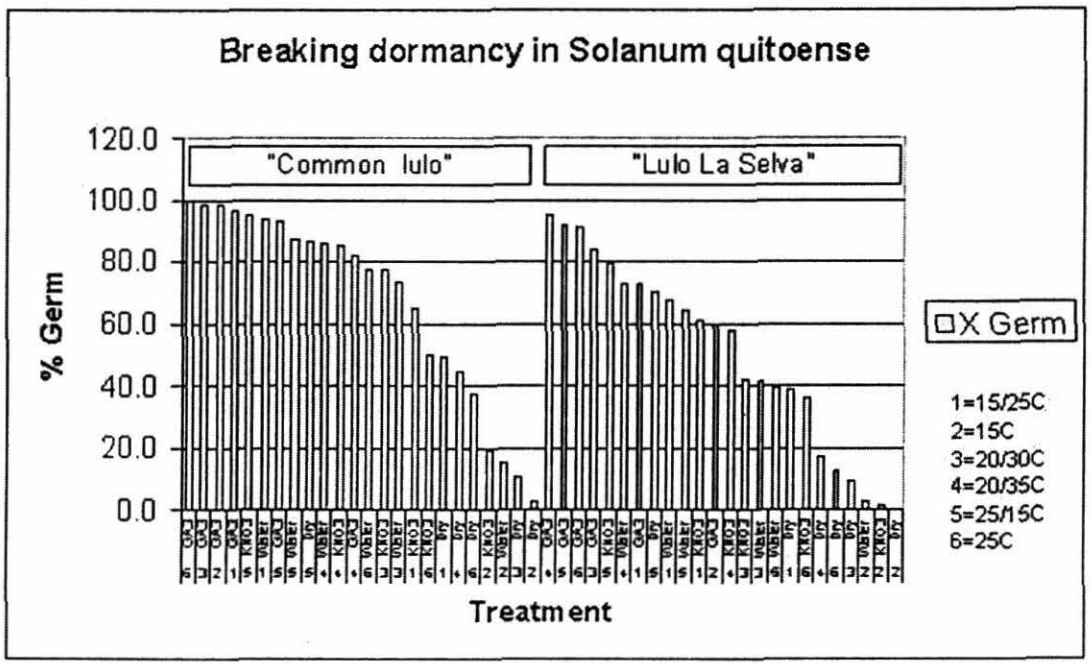


Figure 15.

2.1.3 Solanum hartwegii

One seed lot of *S. hartwegii* was tested in six temperature conditions and four treatments to break dormancy treatments were applied (Annex 3, Figure 16). The analysis of variance of the germination transformed to angles show statistical differences between condition, treatment and interaction between both factors. Tukey test for mean comparisons yields the best germination for three conditions in the same grouping: 20/30, 15/30 and 15/25 °C for 16/8 hours of thermo period, and, to the chemical treatment GA_3 2,000 p.p.m.

Table 18. ANOVA procedure for germination transformed to Angles of *S. hartwegii*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	5	3.90445604	0.78089121	56.79	<.0001
treat	3	6.82966331	2.27655444	165.56	<.0001
cond*treat	15	0.55358481	0.03690565	2.68	0.0027

The germination rate was recorded by checking germination every two days for 21 days. There were statistical differences in the rate of germination between condition and treatment but no interaction

between both factors. The highest rate of germination was found in the constant temperature (25 °C for 24 hours) and in the treatment of GA_3 2,000 p.p.m. This species shows the same trend in dormancy as the cultivated species of *Solanum* (*S. betacea* and *S. quitoense*).

Table 19. ANOVA procedure for rate of germination of *S. hartwegii*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	5	0.00267988	0.00053598	10.60	<.0001
treat	3	0.00419238	0.00139746	27.63	<.0001
cond*treat	15	0.00113337	0.00007556	1.49	0.1306

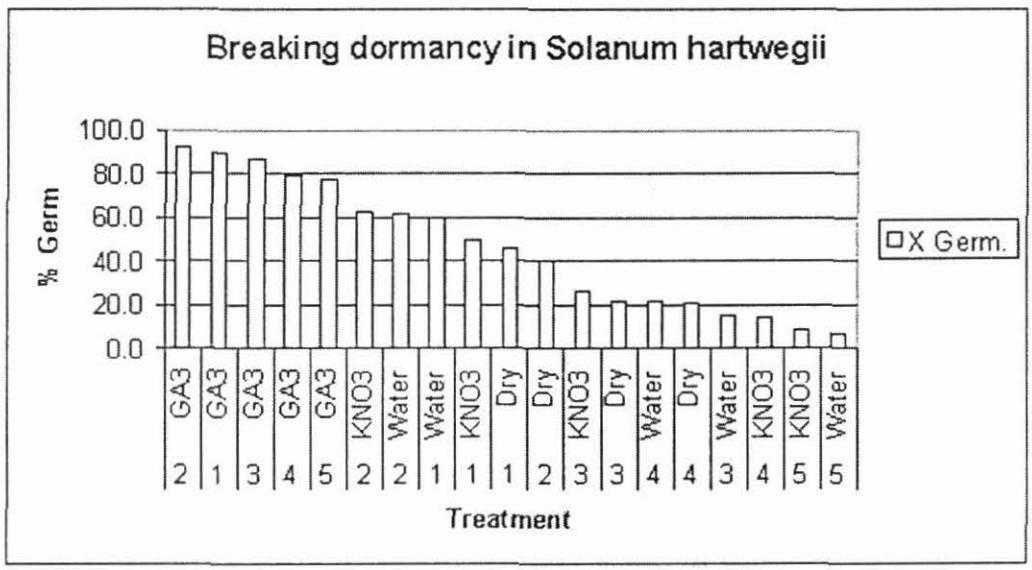


Figure 16.

2.2 Passifloraceae

2.2.1 *Passiflora edulis* Sims

Germination test of scarified seeds of *P. edulis* was done in paper rolls for five temperature different conditions and six treatments (Annex 4, Figure 17). All seeds were scarified manually and one set of seeds was scarified by a boiling water treatment (96 °C for 15 minutes). The analysis of variance of the germination data transformed to angles shows statistical differences in condition, treatment and interaction between both factors. The Tukey test for mean comparisons indicates that the best condition for germination is the highest temperature (20/35 °C for 16/8 hours of thermo period) and the treatment of manual scarification using dry seeds. This result confirms the physic barrier of testa as the main trait preventing germination of these seeds. The use of hormones for this species is unnecessary due to the absence of endogenous dormancy.

Table 20. ANOVA procedure for germination transformed to Angles of *P. edulis*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	3.68687723	0.92171931	114.72	<.0001
treat	5	0.96387718	0.19277544	23.99	<.0001
cond*treat	20	3.25922950	0.16296147	20.28	<.0001

The rate of germination was measured by applying a germination test every seven days during 30 days. No statistical differences were found in the rate of germination for any condition or treatment.

Table 21. ANOVA procedure for rate of germination of *P. edulis*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	0.00000122	0.00000030	0.03	0.9978
treat	5	0.00005734	0.00001147	1.27	0.2818
cond*treat	20	0.00002928	0.00000146	0.16	1.0000

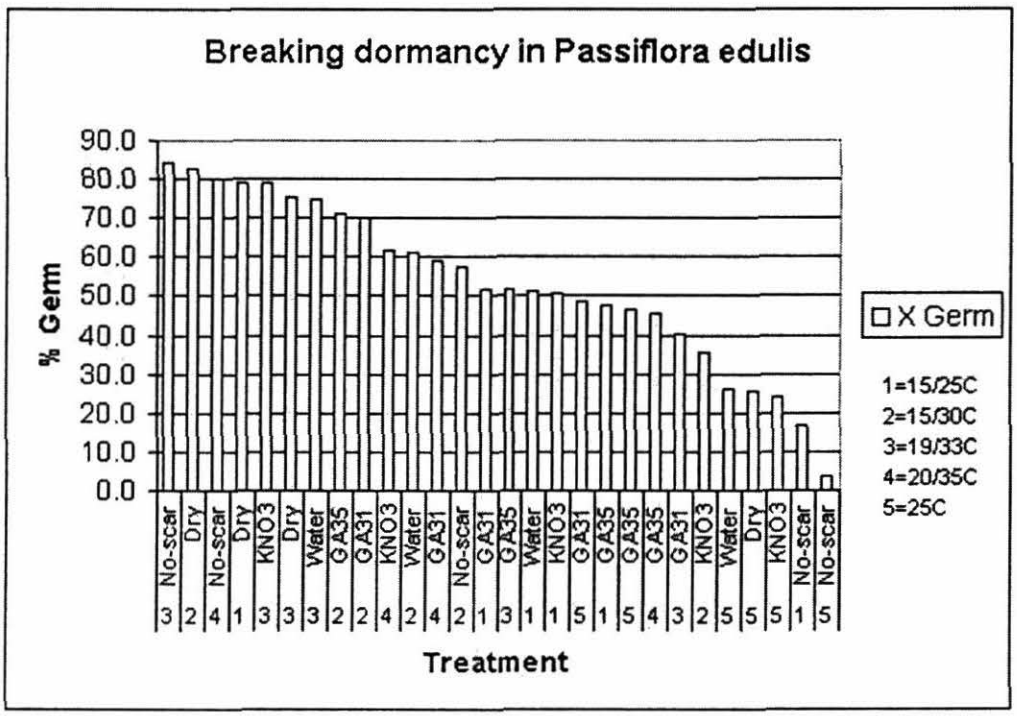


Figure 17.

2.3 Caricaceae

2.3.1 *Carica papaya* L.

Germination test of two lots of seeds of *C. papaya* was done in paper rolls for five different temperature conditions and four treatments (Annex 5, Figure 18). One seed lot consisted of fresh seed without sclerotesta and another seed lot consisted of one-year seed with some pieces of sclerotesta. The analysis of variance of the germination data transformed to angles shows statistical differences in lot, condition, treatment and interaction between all factors. The Tukey test for mean comparisons indicates that the lot without sclerotesta has better germination. The best temperature for germination was 20/30 and 20/35 °C for 16/8 hours of thermo period, and the best treatment was GA_3 2,000 p.p.m. The results confirm that the main factor blocking germination in *C. papaya* is the sclerotesta followed by endogenous factors.

Table 22. ANOVA procedure for germination transformed to Angles of *C. papaya*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	1.71649395	1.71649395	178.90	<.0001
cond	4	5.30083472	1.32520868	138.12	<.0001
lot*cond	4	1.36057674	0.34014419	35.45	<.0001
treat	3	4.01240976	1.33746992	139.39	<.0001
lot*treat	3	0.10935854	0.03645285	3.80	0.0121
cond*treat	12	1.59423432	0.13285286	13.85	<.0001
lot*cond*treat	12	1.41122047	0.11760171	12.26	<.0001

The rate of germination was recorded by applying a germination test every seven days during 35 days. The analysis of variance shows statistical differences between lot, condition and treatment. Interaction was found between lot and treatment, and condition and treatment. The Tukey test yielded the best rate in lot A (without sclerotesta), the constant temperature (30 °C for 24 hours) and the treatment GA_3 2,000 p.p.m. + KNO_3 + KH_2PO_4 (1.5%).

Table 23. ANOVA procedure for rate of germination of *C. papaya*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lot	1	0.05616378	0.05616378	21.30	<.0001
cond	4	0.04234573	0.01058643	4.02	0.0043
lot*cond	4	0.01534086	0.00383522	1.45	0.2203
treat	3	0.19373919	0.06457973	24.50	<.0001
lot*treat	3	0.03821705	0.01273902	4.83	0.0033
cond*treat	12	0.13750373	0.01145864	4.35	<.0001
lot*cond*treat	12	0.02034000	0.00169500	0.64	0.8017

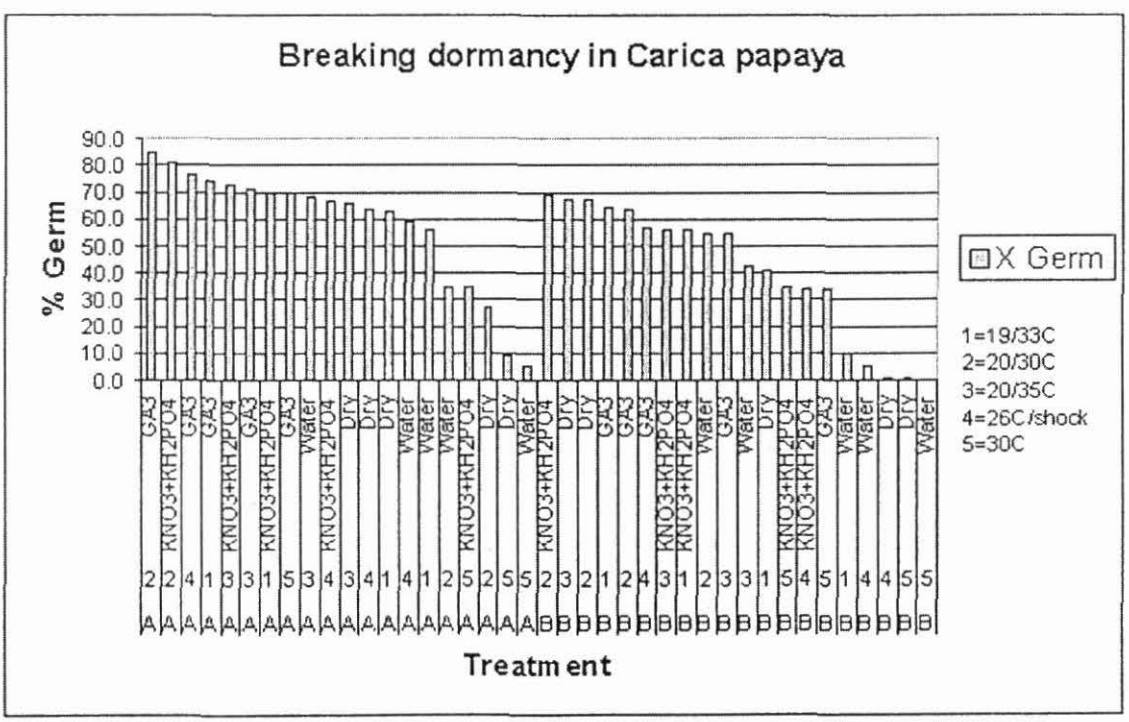


Figure 18.

2.3.2. *Vasconcellea cauliflora (Jacq.) A. DC.*

Germination test of one lot of *V. cauliflora* in paper rolls was done for five different temperature conditions and four treatments (Annex 6, Figure 19). The sclerotesta was fully removed manually by soaking in water. The analysis of variance of the germination data transformed to angles shows statistical differences in condition. No statistical differences were found between treatments. These results indicate no dormancy in seeds of this species. The Tukey test for mean comparisons indicates that the worst temperature was constant temperature (25 °C) and heat shock for 24 hours (35 °C); however all other temperatures gave good germination rates. The heat shock is recommended for *Carica papaya* as the best treatment (Wood, Pritchard et al. 2000); however for this wild close relative it is not working.

This result rejects the hypothesis of easier germination in cultivated species as compared to wild species, because no dormancy has been found in this wild species of Caricaceae, while it is found in *Carica papaya*.

Table 24. ANOVA procedure for germination transformed to Angles of *V. cauliflora*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	0.29223831	0.07305958	11.61	<.0001
treat	3	0.04599586	0.01533195	2.44	0.0734
cond*treat	12	0.08766894	0.00730575	1.16	0.3314

The rate of germination was recorded by applying a germination test every seven days during 35 days. The analysis of variance shows statistical differences between condition and treatment and interaction between both factors. The Tukey test yielded the best rate of germination for the temperature 19/33 °C for 16/8 hours of thermo period and for the treatment GA_3 2,000 p.p.m.

Table 25. ANOVA procedure for rate of germination of *V.cauliflora*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	0.00049994	0.00012498	14.86	<.0001
treat	3	0.00369707	0.00123236	146.52	<.0001
cond*treat	12	0.00255715	0.00021310	25.34	<.0001

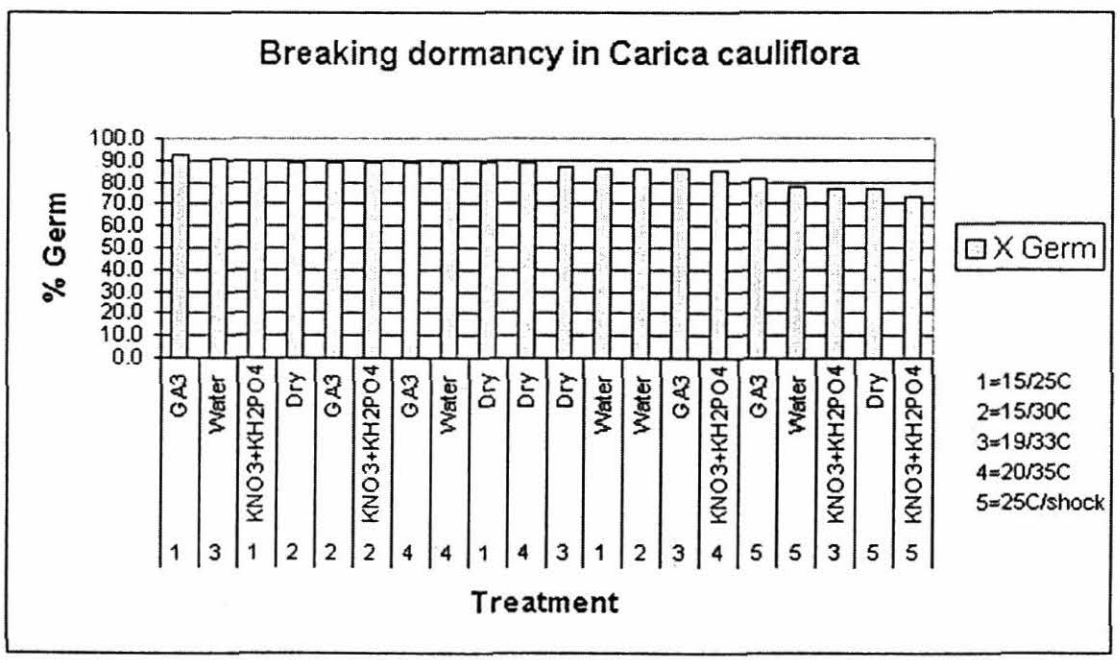


Figure 19.

2.3.3. *Vasconcellea goudotiana* Triana & Planch.

Germination test of one lot of *V goudotiana* in paper rolls was done for five different temperature regimes and four treatments (Annex 7, Figure 20). The sclerotesta was fully removed manually by soaking in water. The analysis of variance of the germination data transformed to angles shows statistical differences in condition, treatment and interaction between both factors. The Tukey test for mean comparisons indicates that the best temperature regimes were 19/33, 20/35 and 15/25 °C for 16/8 hours of thermo period and the best treatment was GA_3 2,000 p.p.m.

Table 26. ANOVA procedure for germination transformed to Angles of *V.goudotiana*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	0.40764471	0.10191118	15.13	<.0001
treat	3	1.74447599	0.58149200	86.33	<.0001
cond*treat	12	.50393054	0.04199421	6.23	<.0001

The rate of germination was recorded by checking the germination every seven days during 35 days. The analysis of variance shows statistical differences between condition, treatment and interaction between both factors. The Tukey test yielded the best rate of germination for the constant temperature (25 °C) and heat shock (35 °C for 24 hours) and for the treatment GA_3 2,000 p.p.m. This species of Caricaceae shows dormancy in contrast to the close wild relative *V. cauliflora*. This is an indication of the independent evolution of dormancy in different species of the same genus or family.

Table 27. ANOVA procedure for rate of germination of *V. goudotiana*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
cond	4	0.00126162	0.00031541	39.29	<.0001
treat	3	0.00215822	0.00071941	89.62	<.0001
cond*treat	12	0.00068395	0.00005700	7.10	<.0001

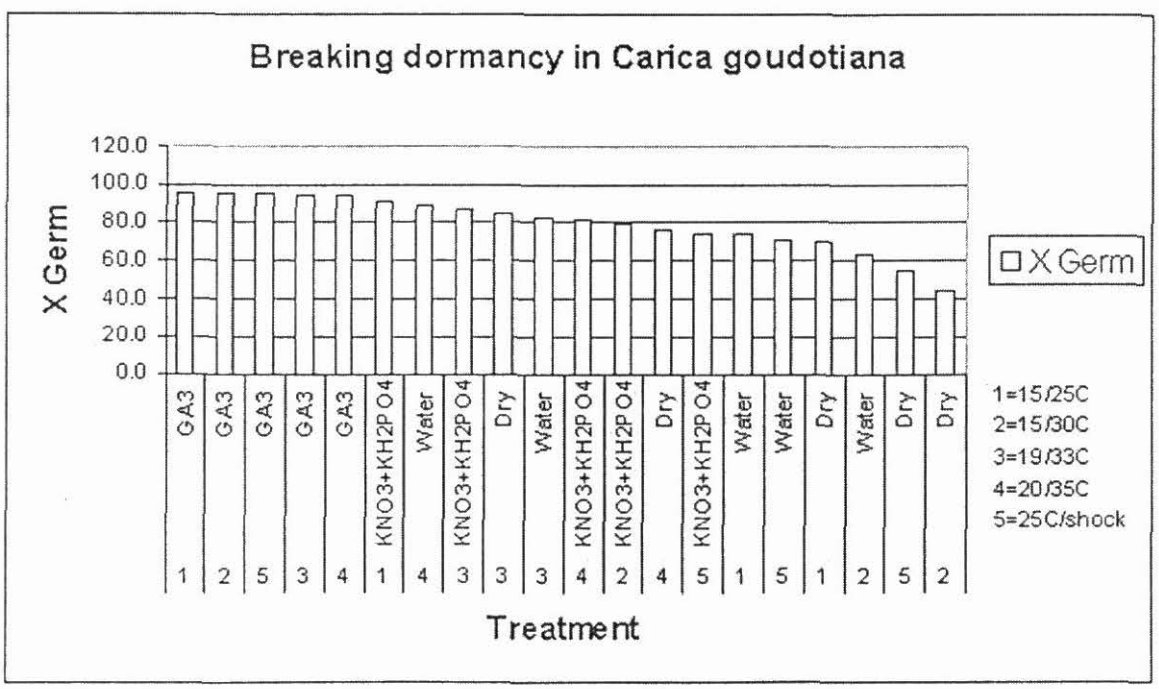


Figure 20.

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Contributor: A.M. Torres

Output 1.5. Improved conservation techniques

Activity 1.5.1 Implementation of the encapsulation-dehydration cryopreservation method for the cassava core collection

Introduction

Cryopreservation techniques can be divided into classical and new. CIAT has developed alternatives for both kinds of techniques (Escobar et al., 1997, Escobar et al., 2000). New techniques based on encapsulation-dehydration, which are simple and rapid, would be useful for preserving large germplasm collections such as that of cassava. Not too many steps are involved as in the classic methods. Using these techniques with a wider number of clones will give a general idea about how safe this conservation method is in the long term with respect to repeatability and consistency after freezing.

Materials and methods

The encapsulation-dehydration method was implemented using materials from the in vitro bank. These materials were propagated using 4E medium (Roca, 1984) for the bud/node explants. When we tried scaling up, we considered increasing the number of beads per container (recycled baby food jars). Normally each jar with 50 g of silica gel holds 20 beads; we tested 40 beads per jar.

To determine if a clone is amenable to being cryopreserved, it has to form shoots from at least 30% of the beads after freezing. This is considered the threshold (Escobar et al., 2001).

Results and discussion

To date we have received 447 cassava clones, which are being propagated to increase them up to 100-150 plants/clone to produce enough new shoots (2-3 months old and without cuttings) to use in the cryopreservation process.

During the last 6 months we had a problem with dust mites infecting the production and lost 187 clones (42% of the material received). This attack made it necessary to increase propagation activities, reducing the number of clones put under cryopreservation. From the material received, we cryopreserved 348 clones (78% of material received, which corresponds to 55.2% of the core collection).

Of the total clones cryopreserved so far, 68% have surpassed the threshold value (Table 28). Of the 145 clones tested in 2002-2003, 43% reached the threshold (Table 29).

Table 28. Summary of response of 348 cassava clones cryopreserved from the core collection.

Group response*	No. of Clones	% Response
Highest (>70%)	91	26.15
Intermediate (30-70%)	146	41.95
Lowest (<30%)	111	31.90
No. of clones tested	348	

*Represented as shoot formation after freezing in liquid nitrogen

Table 29: Response of cassava clones cryopreserved during 2002-2003.

Group response*	No. of Clones	% Response
Highest (>70%)	16	11.03
Intermediate (30-70%)	46	31.72
Lowest (<30%)	83	57.25
No. of clones tested	145	

*Represented as shoot formation after freezing in liquid nitrogen

The 40 beads in the baby food jar did not dehydrate sufficiently or homogeneously; thus encapsulated shoots did not survive freezing. In contrast, when the 40 beads were placed in a petri dish, they reached similar humidity levels to the 20 beads in a baby food jar (Fig. 1). These results indicate that dehydration, as expected, depends on the number of beads, the type of container and the sucrose pretreatment. Ideally, larger containers should be used to handle more beads; i.e., glass petri dishes with a larger numbers of beads. However, the cost of a glass petri dish is 250 times higher than a baby food jar.

A routine was established to clean the growth room. It permits mite control by combining chemical treatments specific for adults and eggs. Every 15 days we also apply mint or eucalyptus oil scents on the floor as a preventive measure to keep mites away.

Future activities

- Recover clones lost by mite attack
- Continue freeze-testing using core collection
- Analysis of clone behavior and group definitions after freezing

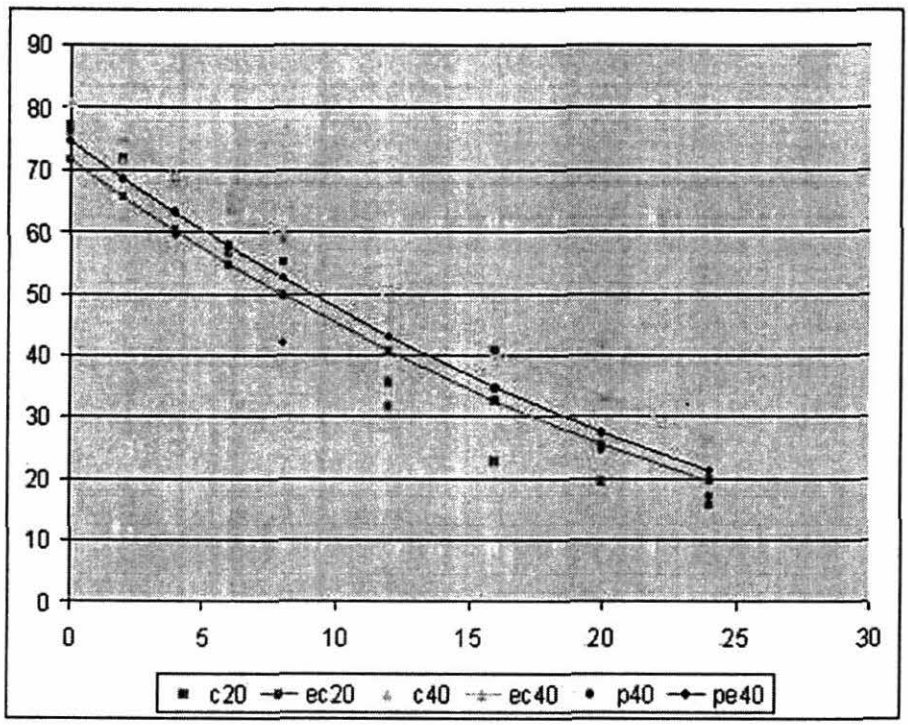

Figure 21. Behavior of beads treated in 0.75 M silica gel for 3 days across different dehydration times. Two types of container were tested: Baby food jars (c) and petri dishes (p)

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

Introduction

Over the past few years, efforts have been made to develop more efficient methods for *in vitro* conservation of cassava. Research has been focussed to develop a conservation system that permits to reduce growth using an ethylene inhibitor.

The use of silver nitrate has so far extended the subculturing time in six varieties (Mafla et al, 2000). These varieties corresponded to a small sample of the collection but are representative of behavior in conservation, as they grouped according to the subculturing lag. The study presented here was, therefore, undertaken to know whether or not silver nitrate is effective to reduce growth and increase the conservation lag on the rest of the collection.

Materials and methods

A total of 4,827 varieties have been subcultured in presence of silver nitrate (2 tubes for variety). A sample of 2,870 cassava varieties of 24 regions has been included in this group for further evaluation; this sample represents the 50% of the total collection.

These clones were maintained on medium consisting of MS, 0.02 mg/l BAP, 0.1 mg/lt GA, 0.01 mg/l ANA, 2% sucrose, 0.7% agar (8S). We compared this medium with a silver nitrate-supplemented medium at 10 mg/lt (NP). Two explants for tube (25 x 150 mm size, covered with aluminum foil) were cultured at 23-24 ^{0}C under a 12-h- photoperiod and lighting of 1,000 lux.

After 2 months of conservation, observations were recorded on stem length; each treatment included two replicates and data of stem length were averaged. Obviously we need to continue the evaluation of these varieties in order to determine for how long silver nitrate is able to extend the time of conservation for this wide range of varieties.

Results

We have found that silver nitrate with concentration of 10 mg/lt reduced the growth three times less as compared to the standard medium. The general average for the 2,870 cassava varieties in silver nitrate medium was 2.7 cm and 8.7 cm in the standard medium (8S).

Figure 22 show the principal effect of silver nitrate on slow growth in 2,870 cassava varieties, which represent germplasm coming from 23 different countries and a group of breeding lines.

The average stem length varied from 6.0 cm to 11.7 cm when the microplants were conserved in media not supplemented with silver nitrate. Addition of silver nitrate to standard medium reduced stem length in all varieties, the stem length was between 1.2 – 4.0 cm. The beneficial effect of silver nitrate on microplants growth was evident in the wide range of varieties.

After 7 months of conservation a total of 167 varieties that were cultivated without silver nitrate began to deteriorate showed defoliation, thus requiring carry out the subculture. It is very important to mention that these varieties have presented a behavior of quick deterioration with an average of 9.9 months to subculture. The plants that were in silver nitrate media even continued in conservation under good conditions and had a higher number of green leaves and better aspect than those growing in standard medium.

The present study has shown that the effect of silver nitrate was uniform over all varieties evaluated. In general, slow growth was observed in the plants conserved in silver nitrate medium. The evaluation is still in progress with the purpose of determining the extent of increase in the conservation time in wide range of varities tested.

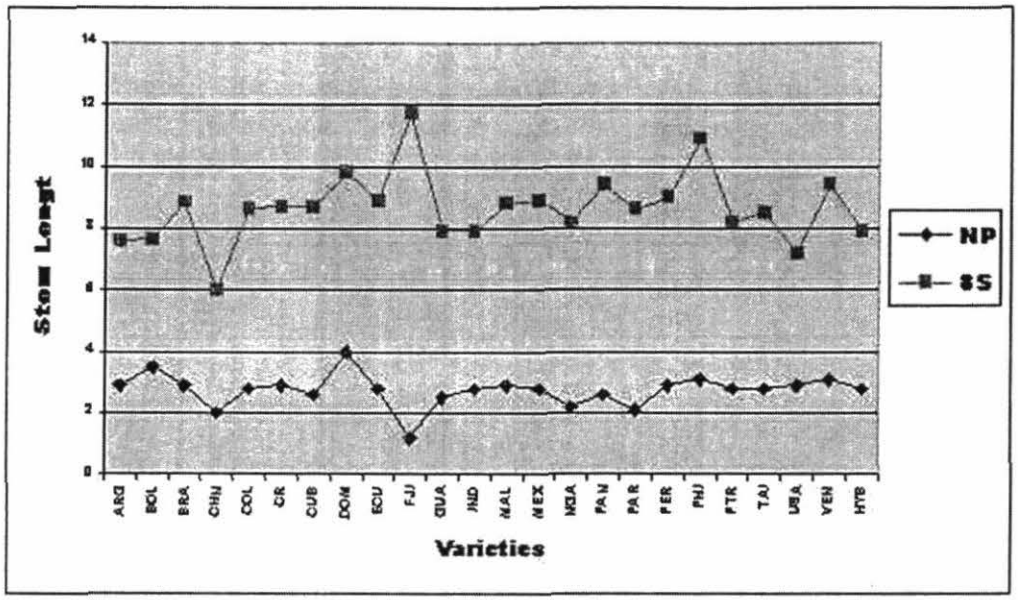

Figure 22. Effect of silver nitrate on *in vitro* growth of cassava varieties.

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Contributors: G. Mafla, J.C. Roa, D.G. Debouck

Activity 1.5.3. Simple strategy for the *in vitro* conservation of palms

Introduction

Palms (Arecaceae) are one of the most important multi-purpose plant families, with around 2,600 species; the multiple useful products obtained from them make palms an important resource. Many of the species are threatened with extinction making them obvious candidates for *ex situ* conservation. The primary method by which all palms are propagated is by seed. Germination of seeds for many species is slow and erratic, and germination percentage can be very low. Production of somatic embryos in callus tissue derived from shoot tips or in vitro culture of sexual embryos have been used to propagate a range of economically important species of palms (Reynolds, 1985).

To the best of our knowledge, protocols for *in vitro* conservation under slow growth of palms have not been reported, and we though of them as strategies for the conservation of this group of plants. With the purpose of beginning the research on slow growth this first step was to establish a stock of *in vitro* plants obtained from zygotic embryos of *Bactris gasipaes* ('chontaduro'), selected as a representative of the palms group.

Materials and methods

A total of eighty fruits from *Bactris gasipaes* were used. The fruits were washed in running water and immersed in soap for five minutes. After removing the pulp, the entire seeds were washed again with soap by 5 minutes, later were submitted to the desinfection procedure in laminar air- flow transfer hood. The seeds were immersed in ethanol at 70% for ten seconds followed by inmersion in benlate at 0.5 gr/lt for 15 minutes and then sodium hypochloride at 2.5% for 15 minutes and finally three times in sterile distilled water. *Bactris gasipaes* embryo is a small conical body (about 3mm long), embedded in the endosperm, near the base of the seed (Figure 1). Forty embryos were cultured in the proliferation medium (4E) and forty embryos in rooting medium (17N) (Roca, 1984). The embryos were cultured for five days in darkness and then were transferred to 1000 lux of light intensity, 27^0 C and 12-h-photoperiod.

Results and discussion

Table 1 summarizes the processes from the moment of the disinfection to the obtaining of viable embryos. The protocol used for the disinfection was effective since no contamination appears. It is probable that those seeds that did not germinate were affected by mechanic damage to the embryo. The used mediums had a very similar response in the obtaining of viable embryos, although it is important to mention that in 4E medium the development of the plants was much higher as compared to the other growth media.

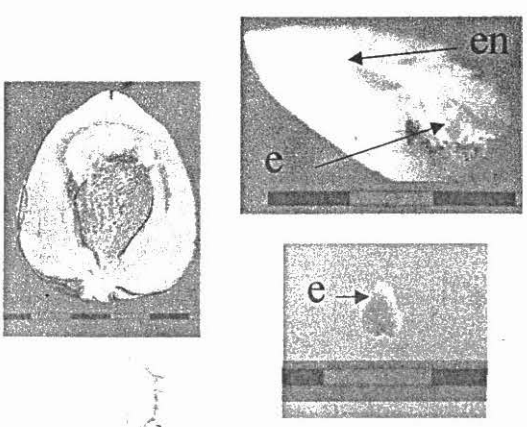
In culture, viable embryos (Figure 23) swelled and elongated, between 3-4 weeks coleoptile elongation and protrusion of the first root occurred. After 6 weeks in culture completely developed plants were obtained. These plants are now to be used in the research on slow growth.

The aim of this study, besides to obtain plants for initiate slow-growth, was to develop a protocol which would give consistently high germination rates for 'chontaduro' embryos. In vitro germination can used to improve the germination rates of palms. Zygotic embryos were chosen as explants because of the ease with which they can be transported from their place of origin and because embryos are the best source of somatic embryos in many species.

Table 30. *In vitro* culture of zygotic embryos of *Bactris gasipaes.*

Medium	Seeds available (No.)	Number of embryos		Viable embryos (No.)
		Contaminated	Not germinated	
4E	40	0	8	32
17N	40	0	10	30
Total	80	0	18	62

Figure 23. Section of the fruit and seed of *Bactris gasipaes.*



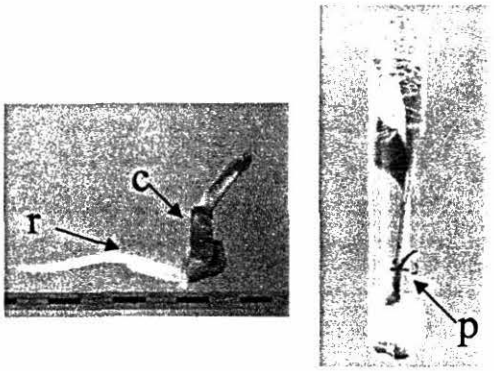


Figure 24. Embryo germination of *Bactrisgasipaes*.
(e-embryo, en-endosperm) (r-root, c-coleoptile, p-plant after 6 weeks).

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Roca, W.M. 1984. Cassava . In : Sharp W.R., Evans D.A., Ammirato P.V. and Yamada Y. (eds) Handbook of Plant Cell Culture; 2: MacMillan Publishing, New York. Pp 269-301.

Contributors: G. Mafla, J.C. Roa, D.G. Debouck

Activity 1.5.4. *In vitro* conservation of tree tomato (*Solanum betaceum*) and lulo (*Solanum quitoense*) using ancymidol and silver nitrate as growth retardants.

Introduction

The purpose of this study is to establish a protocol to conserve under slow growth tree tomato (*Solanum betaceum*) and lulo (*Solanum quitoense*) elite germplasms. Different culture mediums were used (Mafla et al, 2002). After 10 months of storage, observations were recorded on microshoot stem length (cm), number of retained leaves and dead leaves. Other evaluation was made to confirm the viability of the cultures when they exit the treatments (micropropagation capacity).

Materials and Methods

Materials and methods have been described in Annual Report (SB-01) 2002, and modifications have not been made since then.

Results

The effect of ancymidol and silver nitrate on the stem length, number of retained leaves, dead leaves and recovery explants in *Solanum quitoense,* 'Ginebra' clon and 'La Selva'clon, after 10 months are shown in Figure 25a-b. Significant differences were observed between the treatments in all evaluated variables.
A better quality of the material was observed when different concentrations of silver nitrate were used for both clones. The average stem length varied from 11.6 cm to 15.1 cm with ancymidol whereas with silver nitrate varied from 4.0 cm to 15.1 cm in the 'Ginebra' clon.

The similar response was observed in 'La Selva' clon; the rate of growth measured in terms of stem length presented significant differences and varied between 11.5 cm to 12.3 cm with ancymidol whereas with silver nitrate was from 4.3 cm to 12.3 cm. This result shows that silver nitrate has an effect on the rate of growth.

A significant increase in the number of retained leaves was observed when the microplants were conserved in media containing silver nitrate in the different concentrations. In relation to recovery rates explants were different for both clones. Silver nitrate was more effective in improving the recovery of explants in the 'La selva'clon.

Although it was not possible to obtain a significant increase in conservation time is important to consider that silver nitrate could favorably be used for *in vitro* conservation of *Solanum quitoense* microplant. The strategy to follow is evaluate the silver nitrate with other temperature conditions because *Solanum quitoense* is a moderately cold climate crop and in this way we can determine if it is possible to increase the time of conservation.

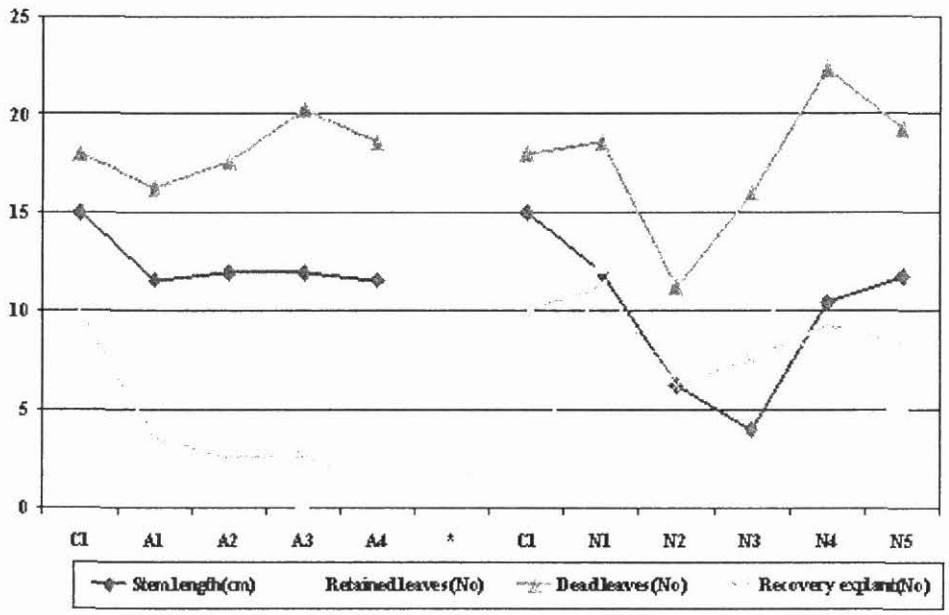


Figure 25a. Effect of ancymidol (A) and silver nitrate (N) on stem length (cm), number of retained leaves(No.), dead leaves(No.) and recovery explant(No.) during the *in vitro* culture of *Solanun quitoense* ('Ginebra'clon).

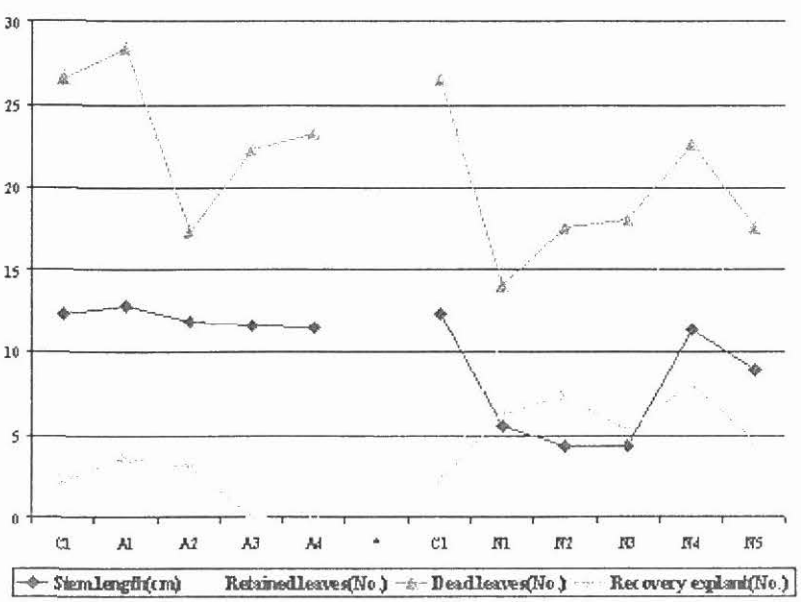
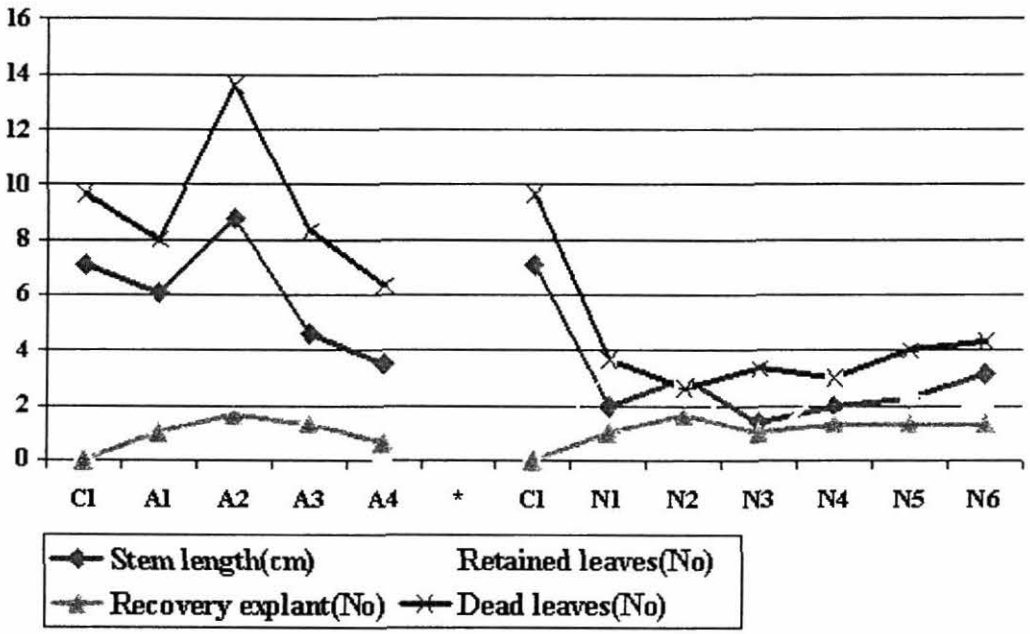


Figure 25b. Effect of ancymidol (A) and silver nitrate (N) on stem length (cm), number of retained leaves (No.), dead leaves (No.) and recovery explant (No.) during the *in vitro* culture of *Solanun quitoense* ('La Selva'clon).



The analysis of variance showed that ancymidol and silver nitrate in tree tomato had effect on all the characters studied with relation to control (C1). In tree tomato differences in the growth of microplants were found; a decrease in the stem length was observed in mediums containing different levels of ancymidol and silver nitrate. After 10 months we observed a beneficial effect on recovery of explants in mediums with ancymidol and silver nitrate. As for *Solanum quitoense* it is necessary to evaluate other mediums and different temperature conditions with the purpose of extending the conservation time.
Figure 26. Effect of ancymidol and silver nitrate on the stem length (cm), retained leaves (No.), dead leaves (No.) and recovery explants (No.) during the *in vitro* culture of tree tomato.

References

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

Contributors: G. Mafla, J.C. Roa, D.G. Debouck

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

Introduction

One of the priorities for next year is to have a security backup of *Manihot in vitro* collection under slow growth in another place. We must find a system that provides even or additional security for *Manihot* germplams as compared to the tubes normally used. Other advantages such as easy handling, reduced storage space, resistance to breakage, easiness of inventory and a safer method for shipping germplasm must be looked at.

Materials and methods.

Eighty varieties of *Manihot esculenta* were multiplied on medium with silver nitrate. Two tubes of each variety were placed in each one of different types of culture vessels. The same conservation room conditions were used for each one.

Looking for a good storage system, the follow culture vessels were used:
Control : Glass test tubes (25 x 150 mm), without silver nitrate (- SN), with silver nitrate (+ SN)

a. Glass test tubes (16 x 125 mm)
b. Polypropylene culture tube (16 x 115 mm)
c. Polypropylene culture tube (16 x 9 mm)
d. Polypropylene culture tube (30 x 115 mm)
e. Bags (CultuSak, Becton Dickinson, Lincoln Park, NJ)

Results

This study examines the longevity using different storage systems. The systems vary in several parameters such as composition of container and size. Figure 27 shows the growth retardant effects of silver nitrate in all the containers. After of three months the polypropylene tubes (b, c) produce a desiccation of the medium that in the long term can be considered as undesirable. The shoots stored in glass test tubes (a), polypropylene culture tube (d) and bags (e) are better because desiccation is lower. Other 250 varieties were cultured in polypropylene culture tube (d) with the purpose to evaluate them under a longer period and to be able to make a decision on the matter.

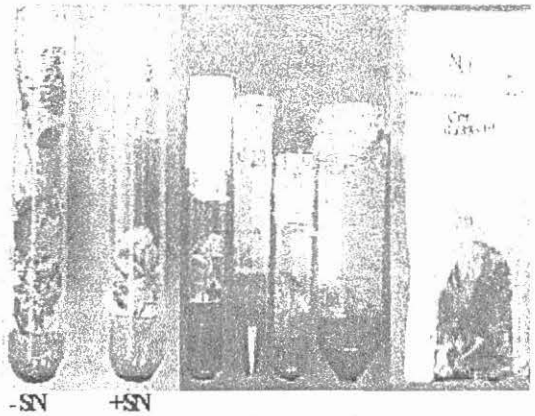


Figure 27. Culture vessels with different sizes

Contributors: G. Mafla, J.C. Roa

Output 1.6 Germplasm, passport and characterization data available to users

Activity 1.6.1..Distribution of germplasm from designate collections to end users

A total of 1,820 *Manihot* accessions were distributed through 56 requests (summing to 10,259 samples of *in vitro* plants). The main recipients were CGIAR Centers (mainly CIAT Projects), who received over 79.1% of total of samples, and then the NARS with 13.8% (Table 31, Figure 28). The main purposes of distribution in cassava were basic research (cryopreservation and classic biochemistry) with 71.6% of the total, while agronomy was of 18.7% (Table 32, Figure 29).

Table 31. Distribution of germplasm during 2002 by kind of institution.

	Cassava	
Institution type	Shipments	Accessions
CGIAR centers	36	1,440
Commercial companies	2	16
Farmers	-	-
Gene banks	1	29
NARS	9	251
NGOs	1	5
Regional organizations	1	10
Universities	6	69
Germplasm networks		
Others		
Total	56	1,820

Table 32. Distribution of germplasm during 2002 by purpose.

	Cassava	
Purpose	Shipments	Accessions
Breeding	13	151
Agronomy	18	340
Applied research	6	26
Basic research	19	1,303
Training		
Other		
Total	56	1,820

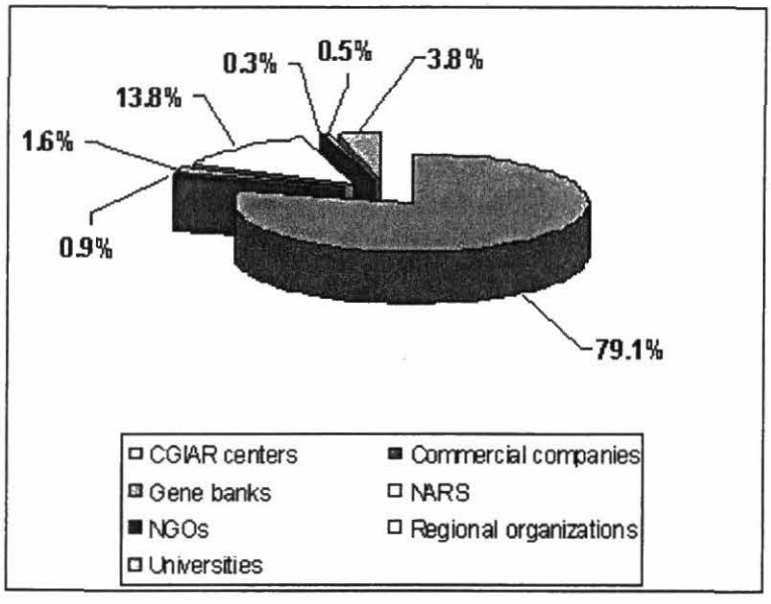


Figure 28. Distribution of *in vitro* cassava germplasm by users

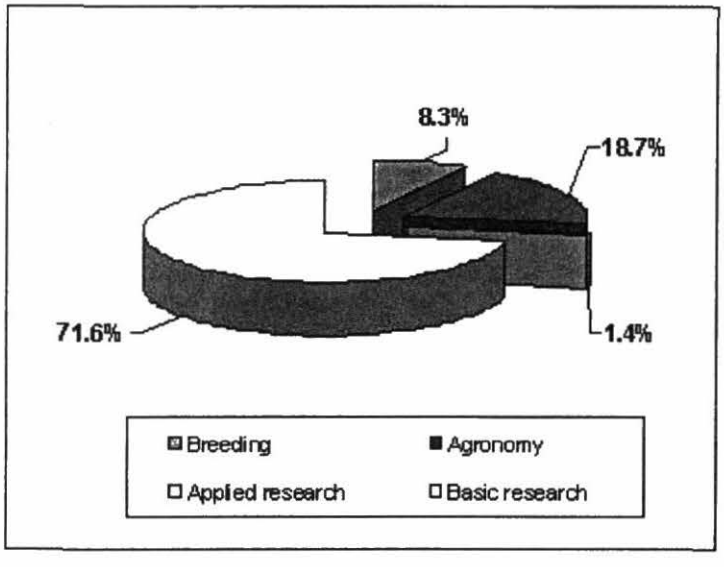


Figure 29. *Distribution* of *in vitro* cassava germplasm by purposes

Contributors: G. Mafla, D.G. Debouck

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

Output 2.1. FAO designate collections cleaned against seed borne diseases

Activity 2.1.1. Indexing and cleaning the Cassava Collection

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

For the indexing three diagnosis techniques are used: ELISA for CCMV and CsXV and grafting with a hypersensitive clone for the causal agent of the Frog Skin Disease (FSDA).

The total of accesions available at this moment and tested against viruses CCMV, CsXV and FSDA, thus ready for distribution are 4,823 clones (84,2 % of FAO Designate Collection).

Indexing for CCMV
The number of clones evaluated against CCMV in 2003 is shown in Table 33.

Table 33. Clones of the FAO Designate collection indexed against CCMV in 2003.

Source	Indexed clones	Negative clones
Argentina	2	1
Brasil	30	24
Colombia	48	48
Costa Rica	1	1
Ecuador	1	1
Paraguay	6	4
Perú	8	8
Nigeria	1	1
Salvador	1	1
Venezuela	2	2
CG	6	6
CM	13	13
SG	1	1
SM	6	6
Wild species		
ALT.	2	2
CAE.	5	5
CEC.	1	1
CTH.	16	16
FMT.	2	2
GLA.	1	1
GUT.	1	1
JAC.	2	2
ORB.	1	1
PIL.	1	1
RUB.	3	3
TPH.	1	1
TST.	7	7
TOTAL CLONES	169	160

These results indicate that to date 94,7 % of clones presented negative results for this virus and 5,3 % positive results.

The countries with high numbers of positive clones were Brazil (3,52 %), Paraguay (1,17 %) and Argentina (0,58 %); however these numbers are low.

Indexing for CsXV

The number of clones evaluated against CsXV during this year is shown in Table 34.

Table 34. Clones of the FAO Designate collection indexed against CsXV in 2003.

Source	Indexed clones	Negative clones
Brasil	29	29
Colombia	59	56
Costa Rica	7	7
Malasia	1	-
México	1	-
Nigeria	1	1
Panamá	1	1
Paraguay	5	5
Perú	8	8
Salvador	1	1
Tailandia	1	1
Venezuela	1	1
CG	7	4
CM	11	10
SG	2	1
SM	5	5
Wild species		
ALT.	1	1
CAE.	4	4
CEC.	1	1
CHL.	1	1
CTH.	12	11
FMT.	2	2
GLA.	1	1
GUT.	1	1
PIL.	1	1
RUB.	2	2
TST.	6	6
TOTAL CLONES	172	161

The results indicate that 93,6 % of the evaluated clones present negative results for this virus and 6,4 % present positive results.

The countries where this virus was present were Colombia (1,74 %), a couple of hybrid clones CG (1,74 %), and Malasia, México, CM and SG (hybrids) with 0,58 % of incidence.

Again, the presence of CsXV in the collection is stronger than the presence of CCMV, also taking into account last year data.

Indexing for FSDA

The number of clones evaluated against FSDA during this year is shown in Table 35.

Table 35. Clones of the FAO Designate collection indexed against FSDA in 2003.

Source	Indexed clones	Negative clones
Argentina	5	4
Brasil	54	53
Colombia	117	113
Costa Rica	1	1
Ecuador	5	5
Estados Unidos	1	1
Guatemala	2	2
Indonesia	1	1
Malaysia	2	1
México	4	4
Paraguay	12	12
Perú	28	28
Venezuela	5	5
HMC	1	1
CG	1	1
CM	4	4
SG	1	-
SM	1	1
Wild species		
CTH.	5	5
GLA.	1	1
PIL.	1	1
TOTAL CLONES	252	244

The results indicate that 96,8 % of materials presented negative results in the indexing for this virus and 3,17% presented positive results.

A low incidence of FSDA was observed in the Collection; only in clones of Colombia and Argentina (1,58 %), Brasil, Malaysia and SG hybrids (0,39 %) we observed the presence of this virus.

The previous data indicate that the thermoterapy applied to erradicate the viruses mentioned in the clones has been effective.

The current status of the Cassava Collection (number of negative clones for each virus and number of clones currently available for distribution, negative for the three viruses) is presented in Table 36.

Table 36. Indexing status of the Cassava Germplasm Collection held in GRU (by October 2003).

Source	In vitro clones	INDEXED CLONES			Available for distribution in 2003
		CCMV	CsXV	FSDA	
Argentina	125	72	86	67	59
Bolivia	7	7	7	5	5
Brasil	1,324	1,308	1,291	1,122	1,098
China	2	2	2	2	2
Colombia	2,016	1,964	1,920	1,714	1,668
Costa Rica	148	147	141	140	133
Cuba	77	77	77	75	75
United States	9	9	9	8	8
Ecuador	116	114	112	109	105
Fiji	6	5	5	5	5
Guatemala	91	91	88	75	75
Indonesia	51	51	51	40	40
Malaysia	67	67	67	53	53
México	102	101	98	85	82
Nigeria	19	19	19	16	16
Panamá	43	39	37	37	35
Paraguay	229	223	206	153	146
Perú	405	402	399	378	371
Philippines	6	5	5	4	4
Puerto Rico	15	15	15	12	12
Dominican Rep.	5	5	5	4	4
Salvador	8	7	7	6	6
Thailand	31	30	30	19	19
Venezuela	250	236	234	205	201
CG	89	86	78	73	67
CM	442	418	415	396	389
SG	47	47	44	39	38
SM	86	81	75	70	63
HMC	4	4	4	4	4
KM	9	7	7	4	4
CT	1	1	1	1	1
SUBTOTAL	5,830	5,640	5,535	4,921	4,787
WILD SPECIES					
30 spp in vitro	300				
3 Undefined spp	3				
ALT.		3	2	1	1
CAE.		5	4	-	-
CEC.		1	1	-	-
CHL.		3	3	4	3
CTH.		19	14	13	9
FLA.		12	13	13	12
FMT.		4	4	4	4
GLA.		1	1	1	1
GUT.		1	1		-
JAC.		2	-	-	-
ORB.		1	-	-	-
PIL.		1	1	1	1
RUB.		3	2	-	-
TPH.		1	-	-	-
TST.		9	6	11	4
VIO.		1	1	1	1
PSE.		-	-	1	-
TOTAL	6,133	5,707	5,588	4,971	4,823

As one can see in the previous Table, 93 % of the Cassava Collection are negative to CCMV, 91,1 % to CsXV and 81 % to FSDA.

The next Table 37 shows progress in the indexing of the Cassava Collection (materials negative for three viruses) obtained between 1998 and 2003.

Total of clones	Year	Percentage of negative clones for CCMV	Percentage of negative clones for CsXV	Percentage of negative clones for FSDA	Available for distribution
6,133	1998	36,7	35,5	10	602
6,133	1999	42,6	40,1	17,4	1,073
6,133	2000	70,8	63,5	39,2	2,346
6,133	2001	84,2	80,8	60	3,453
6,133	2002	90	87,6	75,6	4,559
6,133	2003	93	91,1	81	4,823

As one can see, we are reporting progress in the indexing process during the years indicated, although this process is slow, as we experiment some problems during the establishement of the last materials. Maybe, some materials need different conditions for growth as compared to our environmental conditions at the CIAT's greenhouses.

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

Activity 2.1.2. Indexing wild materials of Cassava for the GRU

We continued with the growing activities of the wild materials of Cassava in order to finish with the indexing activities. The growth of these plants is very slow, atypic and difficult, and a lot of materials has been lost during the growing out phase in the greenhouses. However, we obtained the first results for CCMV, CsXV and FSDA in materials from the past year.

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

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

Since October 2001, we begin to establish one copy of the whole Cassava Collection under greenhouse conditions, because most of materials delivered to the field genebank became infected with FSDA, and the Cassava Breeding Project no longer provided the service. Along our current agreement with FAO, this back-up is necessary before the entire collection is safely maintained under cryopreservation. We currently have 2,595 clones from different countries as it can be seen in Table 38. These materials are by-products of the certification process against FSDA, and thus certified against viruses of quarantine importance.

Table 38.

Source	Accesions installed under greenhouse conditions
Argentina	34
Bolivia	5
Brasil	619
Colombia	921
Costa Rica	65
Cuba	40
Ecuador	56
Estados Unidos	5
Fij	2
Guatemala	49
Indonesia	26
Nigeria	10
Malasia	30
México	45
Panamá	18
Paraguay	73
Perú	199
Philipinas	1
Por encima	1
Puerto Rico	8
Rep. Dominicana.	3
Salvador	4
Tailandia	11
Venezuela	116
CG	36
CM	130
SG	20
SM	36
HMC	1
CT	1
KM	1
Wild species	
ALT.	1
CHL.	3
CTH.	9
FLA.	8
FMT.	4
TST.	4
TOTAL	2,595

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

Activity 2.1.4. Updating the Cassava ORACLE database

During the period 2002-2003 we continued to introduce new data about the testing for CCMV, CsXV and FSDA into the new system ORACLE, and to check previous data for mistakes.

During 2003 we have been working on the new "Bonsai" database (ORACLE), where we have introduced all datas about the materials that we have under greenhouses conditions under reduced growth (the so-called Cassava 'bonzai' collection). The data include: accession number, number of table in the greenhouse, location on the table, planting time.

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

Activity 2.1.5. Germplasm health control in seed germplasm

Introduction

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

During the period October 2002- September 2003, the GHL tested 827 seed samples from projects SB-1 (Integrated Conservation of Neotropical Plant Genetic Resources), GD-01 (Bean Germplasm Improvement) and IP5 (Tropical grasses and legumes).

Materials and Methods

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

Testing for some genera of seed borne fungi includes blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar culture media. Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp., in tropical pastures) are tested. The SHL uses dilution and plating on semiselective culture media such as MXP or King B, in addition to immunoprecipitine test with specific antisera or pathogenicity tests. The lab also uses immunofluorescent techniques. Testing *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* is achieved by subculturing on YDCA, by Gram staining, incubation under high temperature (36-37° C). Also complementary tests using a Gram-Positive ID Kit and Gram- negative (Becton Dickinson BBL Crystal™, Nippon Becton Dickinson Company Ltd.) containing different enzymatic and biochemical substrates are carried out. In addition, we used biological tests using hypersensibility reactions on *Mirabilis jalapa* after bacterial infiltration and pathogenicity. Testing for 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 386 accesions of beans project SB-1 were tested, some of them with destination to Bulgaria, China, India, and Peru, and the other one to conservation in the Bean Germplasm Bank (Table 39). Their health status showed 90% samples without pathogens of quarantine importance. Samples with pathogens (10 %) showed in general very low percentages of the fungi *Macrophomina phaseoli,* and *Rhizoctonia* spp. Seedborne viral infections by Southern mosaic virus (SBMV) were also detected. During the seed health analysis it was very evident that *Phaseolus vulgaris* seed samples from Popayan showed high seed quality as a result of the very careful seed production process and handling improved over the past few years.

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

Table 39 . Number of analysed samples of *Phaseolus* spp. for germplasm sent abroad or Germplasm Conservation, project SB-1

Species	Samples number	Destination
Phaseolus vulgaris	145	Peru, Bulgaria, India, China
Phaseolus vulgaris	84	Germplasm bank (conservation)
Phaseolus spp.	86	USA, China
Phaseolus spp.	71	Germplasm bank (conservation)
Total	386	

Tropical grasses and legumes

Seed samples of 441 of tropical grasses and legumes from SB-1 project, distributed in 342 accesions of 35 genera of tropical legumes pastures, and 99 of Poaceae (Figure 30; Table 40). Their health status showed 53,4 % of samples without pathogens of quarantine importance. In rejected samples we detected some seedborne fungi of quarantine importance (*Ascochyta* spp., *Colletotrichum* spp., *Drechslera* spp., *Macrophomina* sp., *Pestalotia* spp., *Phoma* spp, *Phomopsis* spp.), and a very low frequency of Poty virus, but a higher percentage of SBMV on legumes. Bacteria (*Xanthomonas* spp.) were detected only in legumes at very low frequency, also a Coryneform Gram positive bacteria.

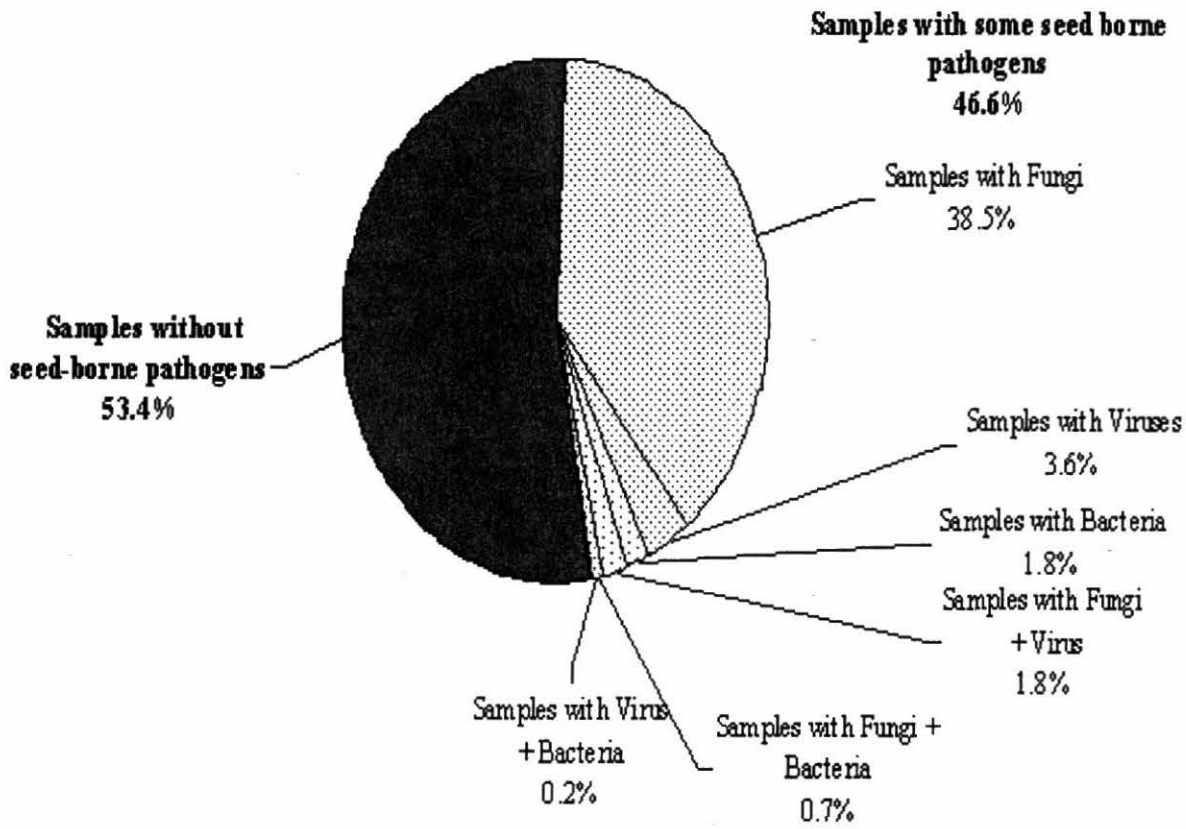


Figure 30. Phytosanitary status of Tropical Pastures germplasm determined at SHL from 441 seed samples (342 legumes, 99 grasses)

Table.40 Number of analyzed samples of tropical grasses and legumes for germplasm sent abroad and for germplasm conservation.

Family	Species	Samples number	Destination
Poaceae	*Andropogon* spp.	5	Germplasm bank (conservation)
	Axonopus sp.	1	Germplasm bank (conservation)
	Eragrostis sp.	89	Germplasm bank (conservation)
	Brachiaria spp	2	Germplasm bank (conservation)
	Panicum spp.	1	Germplasm bank (conservation)
	Paspalum sp	1	Germplasm bank (conservation)
Fabaceae	*Abrus* sp.	1	Germplasm bank (conservation)
	Acacia sp.	1	Germplasm bank (conservation)
	Aeschinomene spp.	35	Germplasm bank (conservation)
	Alysicarpus spp.	12	Germplasm bank (conservation)
	Arachis sp.	1	Germplasm bank (conservation)
	Calopogonium spp.	3	Germplasm bank (conservation)
	Canavalia spp.	31	Paraguay (21)
	Centrosema spp.	71	Thailanadia (23)
	Chamaecrista spp.	2	Germplasm bank (conservation)
	Clitoria sp.	1	Germplasm bank (conservation)
	Crotalaria spp.	18	India (11)
	Desmodium spp.	42	Paraguay (14)
	Dioclea spp.	2	Germplasm bank (conservation)
	Dolichus sp.	1	Germplasm bank (conservation)
	Galactia spp.	2	Germplasm bank (conservation)
	Gliricidia sp.	1	Brasil (1)
	Indigofera spp.	7	Germplasm bank (conservation)
	Leucaena spp.	12	Brasil (12)
	Macroptilium sp.	1	Germplasm bank (conservation)
	Medicago sp.	1	Germplasm bank (conservation)
	Mimosa sp.	1	Germplasm bank (conservation)
	Neonotonia sp.	1	Germplasm bank (conservation)
	Periandra sp.	1	Germplasm bank (conservation)
	Phyllodium sp.	1	Germplasm bank (conservation)
	Prosopis sp.	1	Germplasm bank (conservation)
	Pseudarthelia sp.	1	Germplasm bank (conservation)
	Pueraria spp.	3	Germplasm bank (conservation)
	Rhynchosia spp.	13	Germplasm bank (conservation)
	Stylosanthes spp.	41	Germplasm bank (conservation)
	Tephrosia spp.	6	Germplasm bank (conservation)
	Teramnus spp.	3	Germplasm bank (conservation)
	Uraria sp.	1	Germplasm bank (conservation)
	Vigna spp.	11	Germplasm bank (conservation)
	Zornia spp.	13	Germplasm bank (conservation)
Total		441	

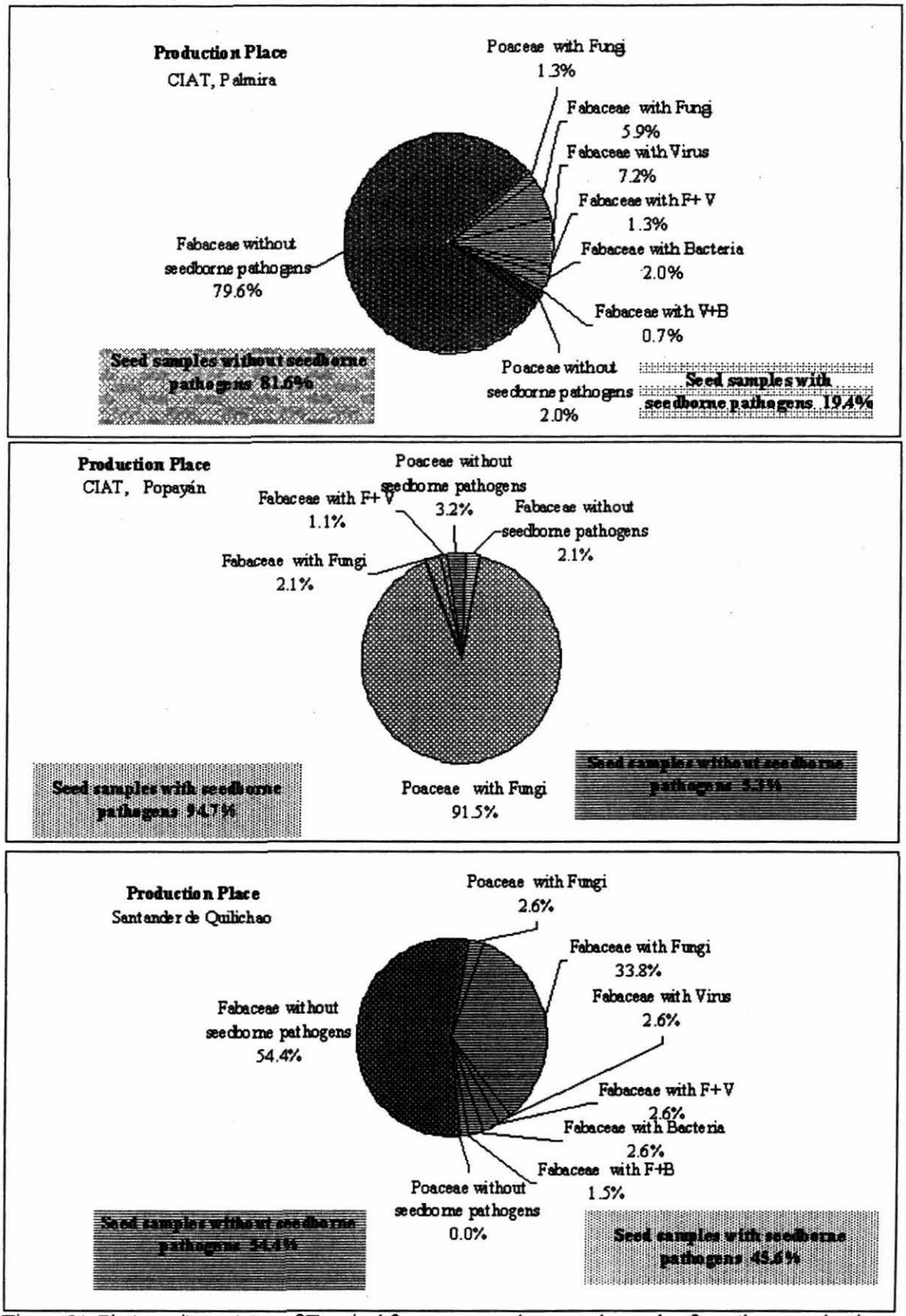


Figure 31. Phytosanitary status of Tropical forages germplasm seed samples from three production places.

According to our results and keeping in mind the source of seed samples, which are produced under field conditions (CIAT Palmira, Popayán, Santander de Quilichao), it was possible to establish that each place had been under different disease pressure at germplasm increasing plots. The phytosanitary status of seeds produced at CIAT Palmira station showed that 81.6% of them were not affected by seedborne pathogens; while seeds of Poaceae germplasm from Popayan showed high percentage of infection (94.7 %). The seeds produced at Santander de Quilichao Sation showed 54.4 % of samples with seedborne pathogens (Fig 31). Our results showed that fungi were of high incidence, while percentages of viruses and bacteria were low (Fig 30).

We see these phytosanitary status results as very important information to make decisions. Specially to refine the procedures of seed production under field conditions, also as a research tool in the development and application of control disease methods to ensure that the germplasm is free of seed borne diseases that could affect its longevity during the storage and prohibit its distribution to users.

Service of germplasm health certification for other projects

Seed samples from GD-01 (Bean's Germplasm development) and IP5 (Tropical grasses and legumes) were analyzed (Table 41). In *Phaseolus vulgaris* 58.0 % of samples did not show pathogens of quarantine importance. In rejected samples *Macrophomina phaseoli* was the fungus detected with higher frequency. We also detected the bacteria *X. campestris* pv *phaseoli* in low percentage and bean common mosaic virus (BCMV) and SBMV in intermediate percentages. In Tropical pastures germplasm 50% of seed samples of *Brachiaria* spp. showed the presence of the seed borne fungi *Drechslera* spp. and *Phoma* spp. In legumes such as *Lablab* spp., we detecteted *Macrophomina* spp. in low percentages.

Table. 41 Number of analyzed samples of beans, tropical grasses and legumes for germplasm sent abroad, projects GD-01 and IP5

Specie	Samples number	Project
Phaseolus vulgaris	344	GD-01 SB
Phaseolus vulgaris	100	GD-01 MB
Total	444	
Arachis spp	1	IP5
Desmodium spp	1	IP5
Brachiaria spp	8	IP5
Calliandra spp	2	IP5
Canavalia spp	1	IP5
Cratylia spp	1	IP5
Lablab spp	15	IP5
Leucaena spp	1	IP5
Mucuna spp	1	IP5
Pueraria spp	1	IP5
Total	32	

Contributors: B. Pineda, M.S. Balcazar

Output 2.2 Germplasm, passport and characterization data available to users

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

A total of 1,820 *Manihot* accessions were distributed through 56 requests (summing to 10,259 samples of *in vitro* plants). The main recipients were CGIAR Centers (mainly CIAT Projects), who received over 79.1% of total of samples, and then the NARS with 13.8% (Table 42, Figure 32).

The main purposes of distribution in cassava were basic research (cryopreservation and classic biochemistry) with 71.6% of the total, while agronomy was of 18.7% (Table 43, Figure 33).

Table 42. Distribution of germplasm during 2003 by kind of institution

	Beans		**Forages**		**Cassava**	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	33	2,655	15	56	36	1,440
Commercial companies	3	118	5	63	2	16
Farmers	---	---	33	107	-	-
Gene banks	1	6	---	---	1	29
NARS	11	1,507	13	77	9	251
NGOs			3	29	1	5
Regional organizations	1	1	2	5	1	10
Universities	23	1,625	10	93	6	69
Germplasm networks	---	---	---	---		
Others	---	---	1	17		
Total	72	5,912	82	447	56	1,820

Table 43 Distribution of germplasm during 2003 by purpose

	Beans		**Forages**		**Cassava**	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	3	353	---	---	13	151
Agronomy	12	1,419	66	302	18	340
Applied research	24	2,441	5	17	6	26
Basic research	28	644	8	108	19	1,303
Training	2	243	3	20		
Other	3	812	---	---		
Total	72	5,912	82	447	56	1,820

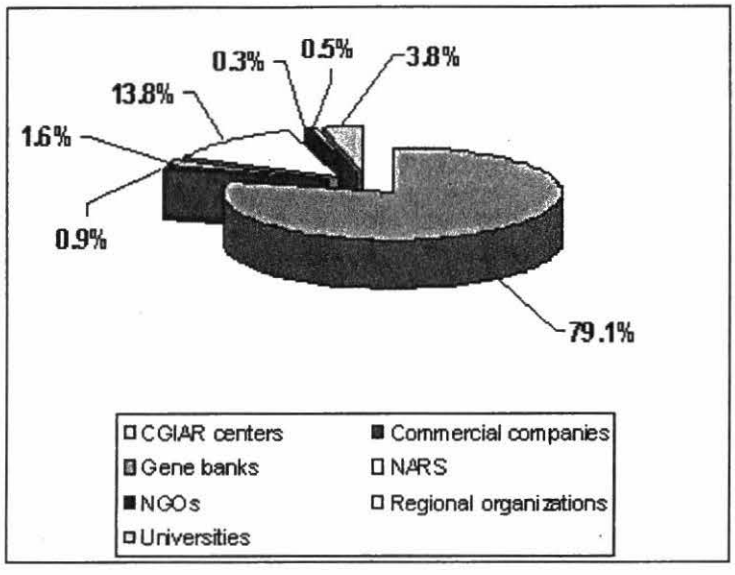


Figure 32. Distribution of *in vitro* cassava germplasm by users

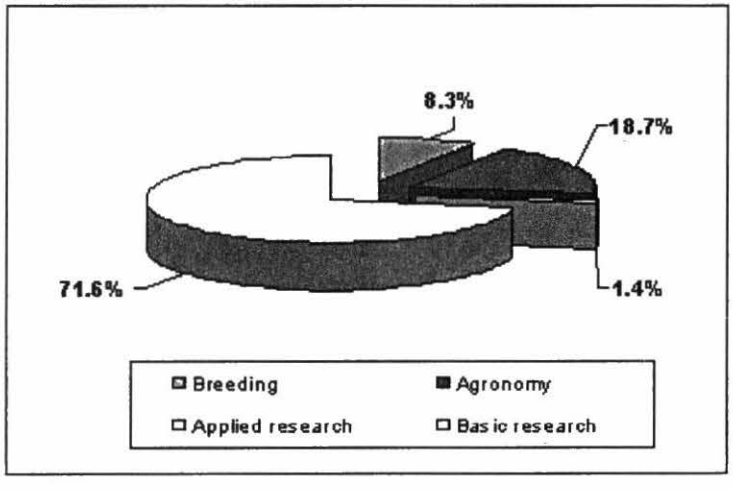


Figure 33. Distribution of *in vitro* cassava germplasm by purposes

Figure 34 . Distribution by users of seed bean germplasm

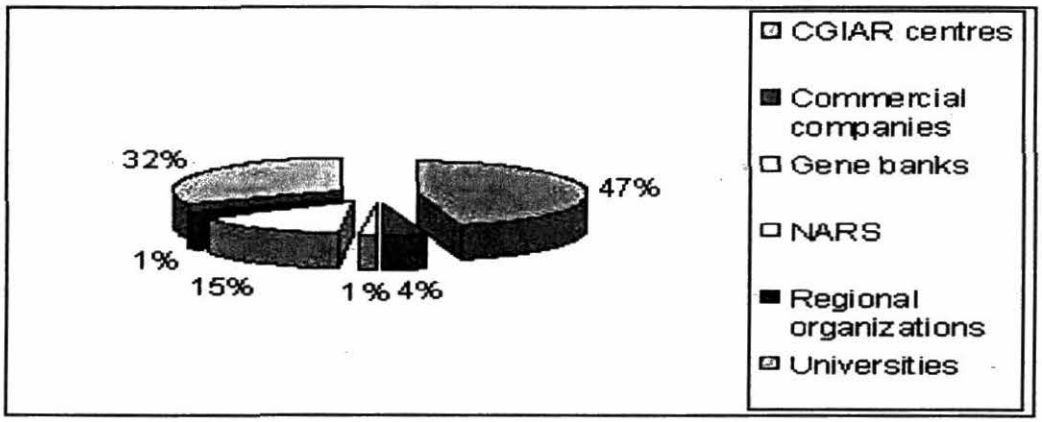


Figure 35. Distribution by purposes seed of bean germplasm

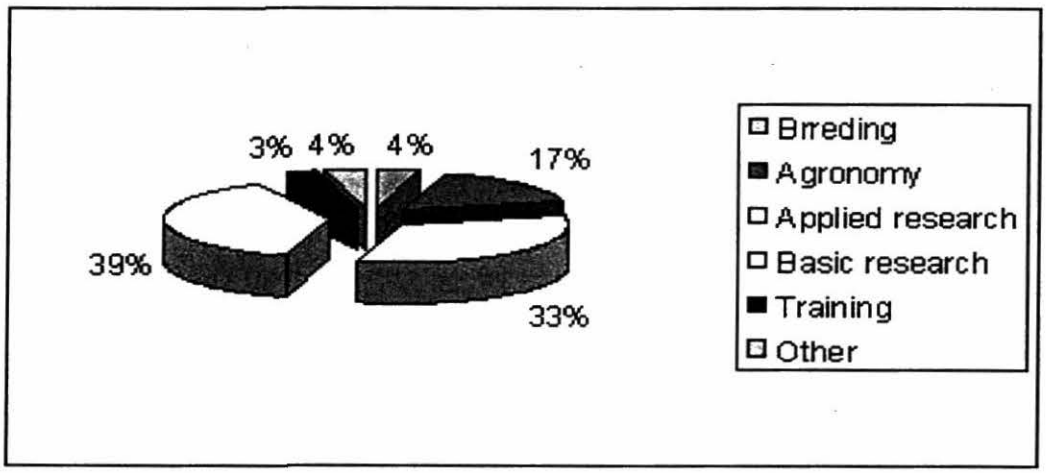


Figure 36 . Distribution by users of seed forage germplasm

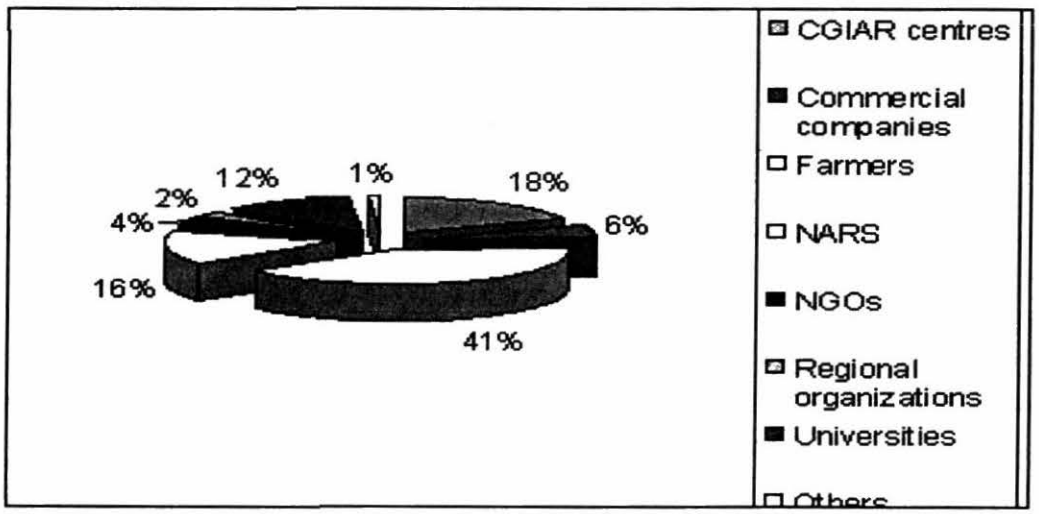
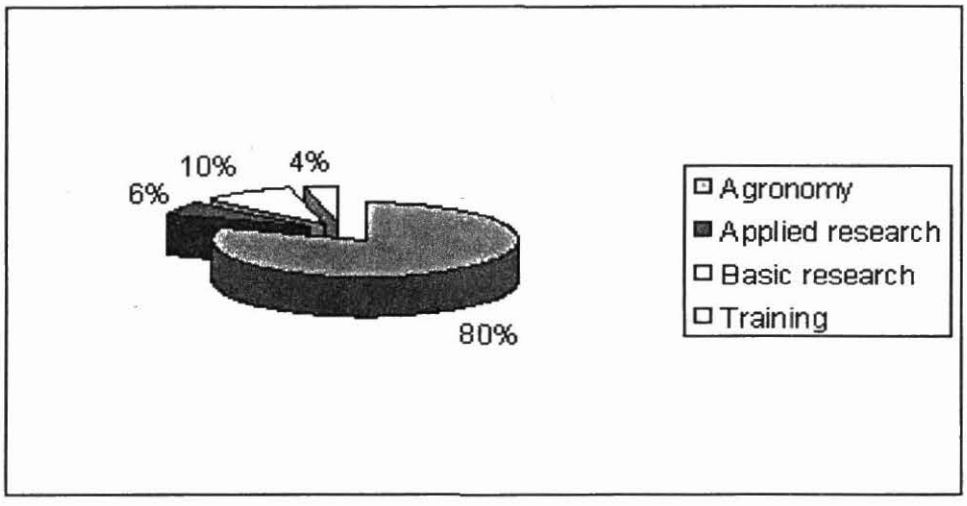


Figure 37. Distribution by purposes of seed forage germplasm



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

Activity 2.2.2. Design of the computerized system of GRU for quality control, flow monitoring and web consultations

Introduction

The information system implemented at the GRU is in constant evolution, therefore presents changes and improvements periodically. An example of this is the introduction of new reports, and also the introduction process of passport data and glass-house information for bean crop. This information had not been migrated in its totality. In addition to this, we improved the procedure of germplasm requests by users of CIAT web site by adding new search parameters and a considerable amount of digital pictures.

Another important activity to report is the introduction of the bar code in the information flow from field to conservation. The introduction of bar coding in the field is still in process, given the need of special printers and labels. The labels should be resistant to different climatic conditions; in fact the GRU has been testing different types of labels for almost two years with good results.

Materials and Methods

In order to carry out the changes in the internal information system of GRU we used a tool called Developer 2000 from Oracle. To make germplasm requests from CIAT Web site we used the programming language called Java. For the bar coding we used Zebra S600 printers, moving to Eltron printers due to the low cost and the easy way to operate them. For bar code impression in the field we are currently testing two printing options: Zebra or Paxar.

Results

The internal information system shows updated information and lets visualize seed images, helping to the identification of the material, and avoids confusions during seed multiplication

processes. The introduction of a bar code on the plastic aluminum bags that contain the seeds allows an easy and fast identification of its content, which avoids to reopen and to repack the seed material (with risk of increased seed moisture content).

The consultation of CIAT germplasm databases and the request procedure have been made ligther. Our web site has more new search parameters which permit to do refined searches. Around 4,000 additional images of bean and tropical forages accessions have been incorporated. The introduction of bar codes in the field and glass-house operations is in process; if appropriate portable printers can be purchased soon it can be operational at the end of year 2003.

Contributor: D. M. Montero

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

Table 44. Specimens of tropical forages and wild beans added to CIAT Herbarium in 2003.

	Number of species	Number of accessions
Wild beans	3	9
Legumes	117	815
Grasses	22	144
Total	142	968

While accessions are multiplied in the field, voucher specimens were taken for taxonomic research purposes. Exchange of herbarium specimens was done with other national herbaria of Colombia. Fifteen specimens of native grasses were received as donation from Herbario MEDEL, from Universidad Nacional de Colombia, Medellín. And 38 herbarium specimens of *Phaseolus* spp. were sent as donation to the National Herbarium, COL, at Universidad Nacional de Colombia, Bogotá.

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

Output 2.4. FAO designated collections safe duplicated

Activity 2.4.1. Preparation of germplasm collections for security backups

Achievement: 360 accessions of bean and 593 accesions of forages were prepared for safety duplication at CIMMYT. A special box has been designed according to specifications given by CIMMYT Staff.

This year we have shipped to Thailand 87 clones of the *in vitro* cassava core collection (630 clones), in order to keep a duplicate and for future evaluation of agronomic performance at the Rayong Field Crop Research Center.

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

Output 2.5. Improved disease indexing techniques

Activity 2.5.1. Evaluation of fungicides to control the fungous complex of *Drechslera* spp., and other genera of fungi affecting *Brachiaria brizantha* inflorescences.

Introduction

Regeneration of germplasm is one of the most important activities in genebanks operation, but it must be conducted under conditions with a minimum phytosanitary risk in order to obtain good quality seeds. At regeneration fields of *Brachiaria* ssp. in Santa Rosa Station in Popayan some seed borne fungi are of common occurrence and they affect yield and health quality of the germplasm (Garcia y Pineda 2000; Garcia et al, 2001). One of the control methods of those fungi is the use of fungicides. Evaluations carried out at Germplasm Health Laboratory (GHL) and in Santa Rosa Station showed that the fungicides tested had low effectivity against *Drechslera* spp, one of the most important quarantine fungi (CIAT, 2001; CIAT, 2002). Then it was necessary to test some other fungicide products in a trial in Santa Rosa Station, of which results are presented in this report.

Materials and Methods

A study was conducted in CIAT Santa Rosa station in 2002-2003 to test the efficacy of five fungicides for the control of a fungous complex affecting *Brachiaria* spp. inflorescences, resulting in seed yields of low phytosanitary quality. The fungicides are listed in Table 4. Untreated plots were included as a check in the test.

The trial was initiated in 29 November 2002 using plots with *Brachiaria brizantha* accession 6387, and plants were in booting state (beginning with inflorescence initiation). Experimental design was a randomized complete block with 6 treatments and 4 replications. Fungicide applications were made every two weeks from November 2002 to January 2003, using a back Agrolaura sprayer. All treatments were applied in 400 L/ha.

Disease ratings were taken by plot before each fungicide application, and a final rating was done two weeks after the last fungicide spraying. Evaluation of the fungi infection progress after fungicide sprays was scored using a scale with six severity grades of symptoms (CIAT, 2002).

The experiment was harvested in February 2003, following GRU standard procedures. After harvesting seed samples were conditioned to establish their health status in the GHL. Of each accession samples of 100 seeds were analyzed using two methods: seed-washing test and incubation in blotter (Neergard, 1977; Agarwal and Sinclair et al, 1987). Presence of fungi was observed through the stereoscope and light microscope, and their identification to genera was made by comparing descriptions and pictures in specialized references (Barnnet and Hunter, 1998; Zillinsky, 1983; Ahmed and Ravinder Reddy, 1993)

Results

The results of evaluations under field conditions about the infection progress of the fungous complex associated with *Brachiaria brizantha* inflorescences, after fungicide spraying on plots showed variability. The treatment efficacy considering the percentage of inflorescences affected

and the affection grade are given in Table 45. Disease infection grade was more or less high, and there were no significant differences in disease among treatment plots. However, the fungicides Trifloxystrobin + Propiconazol, and Iprodione + Bromuconazol were the products with some treatment efficiency (Table 46).

Table 45. Selected fungicides to evaluate their effect on fungi affecting seed quality production of *Brachiaria brizantha,* accession 6387

Fungicide	Commercial name	Formulation	Concentration (i. a %)	Dose - /Ha
Mancozeb	Manzate 200	Wet Powder	80%	2.5 kg
Trifloxystrobin + Propiconazole	Stratego DC 250	Dispersion Concentrated	12.5 + 12.5	1L
Iprodione + Bromuconazole	Brodione SC	Suspension Concentrated	26.6 + 13.3	1L
Prochloraz	Octave 50 WP	Wet Powder	50	0.3 Kg
Propiconazole	Tilt 250 EC	Emulsion Concentrated	25	0.6L

Table 46 Treatment Efficacy in the control of a fungal disease complex affecting inflorescences of *B. brizantha,* accesion 6387.

Treatment	Maximun affection grade	Percentage of inflorescences affected	Percentage of treatment Efficacy
Mancozeb (Manzate 200)	4a*	75,25 a	7.35 d
Trifloxystrobin + propiconazole (Stratego DC 250)	3a	50.00 a	41.18 a
Iprodione + Bromuconazole (Brodione SC)	3a	53.25 a	37.35 b
Prochloraz (Octave 50 WP)	4a	78.75 a	7.35 d
Propiconazole (Tilt 250 EC)	4a	65.00 a	23.53 c
Untreated check	4a	85.00 a	0.00 e

* Average values with the same font do not have significant differences at 0.05 probability, according with Tukey's test

Seed health analysis using samples of seed harvested from inflorescences after treatments under field conditions showed that Iprodione + Bromuconazole (Brodione SC) and Prochloraz (Octave 50 WP) were the fungicides somewhat effective against *Drechslera* spp. but not against other genera of fungi (Table 47).

Now we need to conduct more research about control measures against *Phoma* spp., another important fungus affecting *Brachiaria* spp. seed production.

Table 47. Percentage of *Brachiaria brizantha* seeds infected by fungi, after fungicide sprayed under field conditions .

Treatments	Percentage of seeds with Fungi					
	Drechslera	*Phoma*	*Curvularia*	*Cerebella*	*Cladosporium*	*Fusarium*
Mancozeb (Manzate 200)	9.0 ab*	32.2 a	5.8 a	85.2 a	28.2 a	23.5 a
Trifloxystrobin + Propiconazole (Stratego DC 250)	17.5 a	48.8 a	10.0 a	92.2 a	29.5 a	19.8 a
Iprodione + Bromuconazole (Brodione SC)	3.5 b	37.0 a	0.0 b	82.5 a	34.5 a	39.5 a
Prochloraz (Octave 50 WP)	7.5 ab	19.3 a	2.5 ab	89.8 a	30.3 a	29.5 a
Propiconazole (Tilt 250 EC)	10.5 ab	27.8 a	13.8 ab	88.3 a	41.0 a	40.0 a
Untreated check	13.8 a	34.0 a	2.8 ab	75.0 a	36.5 a	40.0 a

* Average values with the same font do not have significant differences at 0.05 probability, according with multiple range Ryan-Einot–Gabriel-Welsch test

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

Activity 2.5.2. Preliminary evaluation about antagonistic activities of some bacterial isolates against seed-associated fungi on *Brachiaria brizantha* (Panicoidea, Poaceae)

Introduction

The increase and regeneration of *Brachiaria* spp. germplasm are carried out, under field conditions at "Santa Rosa" experimental station near Popayan where the seed production conditions are relatively favorable. Nevertheless under its environmental conditions (precipitation more than 2000mm, temperature 16-24 ^{0}C) the production of seeds can be affected by fungal diseases.

Recent GHL studies (Garcia y Pineda 2000; Garcia et al, 2001; CIAT, 2001); CIAT, 2002) showed that *Drechslera* spp., *Phoma* sp., *Curvularia* spp. *Sphacelia.* sp., *Cerebella* sp., were the most frequent fungi affecting the *Brachiaria* spp. seed production. In other field evaluations carried out with fungicides it was found that those tested products had low control against above mentioned fungi (CIAT, 2001; CIAT, 2002).

Actually as an environmentally benign alternative is necessary to find out forms of biological control; in example using antagonist bacteria, frequently presents as epiphytic flora on plants or on its organs. On this way some preliminary studies with bacterial isolates from *Brachiaria* spp. seeds in the GHL were initiated. The obtained results are including in this report.

Materials and Methods

To assess the antagonistic potential of some bacterial isolates obtained from *Brachiaria* spp seeds, a screening with three groups of isolates (G1and G2 , Gram- positives; G3, Gram-negative) *in vitro* trials was used. Isolates of bacteria were plating in Nutrient Agar and PDA culture media. They were streaked on culture media and incubated for 48 h at 28-30°C. Bacterial suspensions were prepared by removing the colonies from the media using a sterile solution of 0.85% sodium chloride. Bacterial suspensions were standardized to approximately 10^8 colony forming units(cfu)/mL using a Spectronic 20 at an absorbance value of 0.1 at 660 nm.

For inhibition of growth trial Potato Dextrose Agar blocks (PDA) (4 mm diameter) from fresh cultures of *Drechslera* sp., Phoma sp., *Curvularia* sp., *Fusarium* sp., and *Epiccocum* sp (*Cerebella* sp) were plated in one border of Petri dishes of (84mm diamater) with PDA ; on the other border exactly placed in front of the PDA block bacteria tested were streaked onto PDA media. All cultures were randomly placed and incubated at 27°C during one week. The zone of inhibition between the bacteria and the leading edge of the fungal colony was measured with a metric ruler after 3, 5 and 8 days . The experiment was performed with three replications. Inhibition growth values were calculated using the following mathematical ecuation:

$$\text{Growth inhibition } \% = \frac{\text{MGP - GAT}}{\text{MGP}} \text{x100}$$

MGP = Maximum Growth Possible (Check) GAT= Growth in Antagonistic Test

After first screening, above described, other experiments were established using all bacterial groups. Also a trial to measure the inhibition activity of culture bacteria extracts was carried out.

Results

There were interactions between bacteria - fungi growth with notable differences among the three groups of bacteria used in the first screening trial (Table 48). Inhibition zones were observed against selected fungi on PDA. Bacterial isolates group G3 (Gram-negative Bacillus) were the most effectives against all genera of fungi used in this experiment (Table 48). Nevertheless the most significant interactions were when culture bacteria extracts were used (Table 49, Figure 38).

On the other hand, *Drechslera* sp., Phoma sp., *Curvularia* sp., and *Fusarium* sp., were the most inhibited fungi whereas *Epicoccum* sp.was the least inhibited (Table 49).

Inhibition growth results applying the calculated values showed that all genera of fungi had high levels of inhibition when were plating on PDA with bacterial filtrated extracts (Table 50). Inhibition growth percentages were ranged between 88.7 – 98.9, values considered as very important because they are indicating that the antagonistic acivities of the GHL bacterial isolates can be use in biologivcal control of fungi affecting Brachiaria seed production. Nevertheless we need to do more research activities under field conditions. One of the major problems associated with biological control agents is variable efficacy in the field that limits agronomic application. Variability is due to any factor interfering with either the population size of a biocontrol agent or its expression of activity. to compare the inhibitory capacity of each bacterial isolate against selected fungal pathogens.

Table 48. Average Inhibition Distance (cm) of fungi growth in PDA plating with Bacterial isolates.

Bacterial Isolates	*Drechslera* sp.	*Phoma* sp.	*Curvularia* sp.	*Fusarium* sp.	*Epicoccum* sp.
G1.1	1.2[1]	0.9[2]	1.2	1.2	0.8[3]
G1.2	1.4	0.7	1.0	1.3	0.4
G1.3	1.1	0.8	1.1	1.1	0.2
G1.4	1.2	0.5	0.6	1.2	0.4
G2.1	1.3	0.9	0.7	1.4	0.4
G2.2	0.8	0.5	0.5	1.3	0.4
G2.3	0.7	0.8	0.6	1.3	0.5
G2.4	0.4	0.7	0.4	1.0	0.8
G2.5	0.2	0.5	0.1	0.9	0.7
G2.6	0.3	0.5	0.3	0.9	0.8
G3.1	3.2	3.2	3.2	3.2	3.1
G3.2	3.4	3.5	3.0	3.1	3.4
G3.3	3.1	3.6	3.1	3.3	3.2
G3.4	3.2	3.1	3.2	3.3	3.2
G3.5	3.2	3.2	3.0	3.1	3.1
Control[1]	7.0	6.3	7.5	6.5	5.2 c

[1] Fungi plating on PDA without bacterial colonies.

Table 49. Maximum average fungi growth (cms) in PDA with filtrated bacterial extracts

Bacterial Isolates	*Drechslera* sp.	*Curvularia* sp.	*Phoma* sp.	*Fusarium* sp.	*Epicoccum* sp.
G1.1	3.1	3.2	4.4	4.7	2.4
G1.2	3.8	4.8	4.6	4.8	2.5
G1.3	4.0	4.5	4.8	4.7	2.4
G1.4	5.3	5.0	4.9	4.7	2.5
G2.1	6	5.6	5.1	4.6	2.3
G2.2	5.8	5.9	5.5	4.7	2.5
G2.3	6	6.0	5.4	4.7	2.4
G2.4	6.2	5.8	6.3	4.6	2.3
G2.5	6.2	6.0	5.4	4.8	2.5
G2.6	6.4	5.8	5.1	4.7	2.5
G3.1	1.3	1.8	1.3	1.5	1.6
G3.2	1.2	1.7	1.3	1.6	1.6
G3.3	1.3	1.9	1.2	1.5	1.6
G3.4	1.2	1.7	1.3	1.5	1.5
G3.5	1.3	1.8	1.2	1.6	1.5
Control[1]	7.0	8.2	5.3	6.5	2.5

[1] Fungi plating on PDA without with filtrated bacterial extracts.

Table 50. Growth inhibition percentage of five genera of fungi grew on PDA with bacterial filtrate extracts.

Bacterial Isolate	Fungi Genera				
	Drechslera sp.	*Phoma* sp.	*Curvularia* sp.	*Fusarium* sp.	*Cerebella* sp.
G3.1	98.5	90.0	96.5	98.9	97.4
G3.2	98.5	88.7	96.4	96.9	97.4
G3.3	97.1	90.0	96.6	98.4	98.6
G3.4	98.5	88.7	96.5	98.4	96.1
G3.5	97.1	90.0	96.5	96.9	97.4

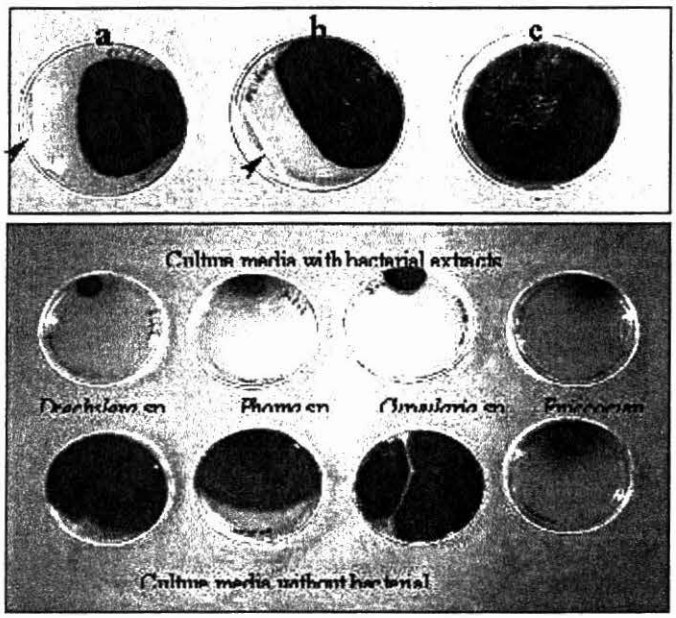

Figure 38. Effect of antagonistic bacterial isolates against some Brachiaria seed associated fungi. Upper picture **a, b** inhibitory growth effect of bacterial colonies on *Drechslera* sp., **c**. *Drechslera* sp. control colony. Lower picture: Inhibitory effect of a bacterial culture filtrated extract on fungi growth, compared with fungi grew in PDA culture media without bacterial extracts.

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

Output 3.1: Designate Collections better characterized

Activity 3.1.1 Two-dimensional electrophoresis of seed proteins of common bean (*Phaseolus vulgaris* L.): the case of new phaseolin types.

Two dimension electrophoresis (2D-IEF-SDS-PAGE) is one of the best experimental tools for the reliable separation of thousands of proteins in a single gel. 2D consists of a tandem pair of electrophoretic separations (Anderson, 1988; Blum et al. 1987):

- In the first dimension, proteins are resolved in according to their isoelectric points (pIs) using immobilized pH gradient electrophoresis (IPGE), isoelectric focusing (IEF), or non-equilibrium pH gradient electrophoresis (NEPHGE). Under standard conditions of temperature and urea concentration, the observed focusing points of the great majority of proteins using IPGE (and to a lesser extent IEF) closely approximate the predicted isoelectric points calculated from the proteins' amino acid compositions.
- In the second dimension, proteins are separated according to their approximate molecular weight using sodium dodecyl sulfate poly-acrylamide-electrophoresis (SDS-PAGE). This technique can provide molecular weight approximations (+/- 10%) for most proteins, with some dramatic exceptions.

Phaseolin, the major seed stotage protein of common bean, has proved to be an excellent –cheap and polymorphic – marker in evolutionary studies. Analysis of phaseolin by 1D-SDS-PAGE and 2D-IEF-SDS-PAGE, reveals characteristic profiles of polypeptide subunits due to their microheterogenety in molecular weight (MW) and isoelectric point (pI) (Anderson, 1988). Although this globulin has narrow range of molecular weights (45-52 kD) and isoelectric points, so far at least 61 types and sub variants of phaseolin have been found in wild, landraces and improved bean genotypes: 29 being present in Mesoamerican materials and 32 in the Andean region. In Mesoamerican materials all 29 patterns are present in wild forms, while only four exist in cultivated forms so far. A contrasting situation prevails in the Andes where 15 patterns have been found in cultivated forms (11 with no counterpart in the wild forms so far), and 17 types exclusive of wild forms. The new phaseolin types were found in the primary center of diversity of *Phaseolus vulgaris* L., using only the 1D-SDS-PAGE technique (Ocampo et al. 2000). With this study we have initiated a systematic confirmation for the new phaseolin types, using two-dimensional isoelectric focusing SDS-PAGE (2D-IEF-SDS-PAGE). In addition reported modifications of electrophoretic parameters for Two-dimensional electrophoresis.

Materials and Methods

Plant material. The germplasm analyzed in this study consisted of wild and cultivated genotypes of *Phaseolus vulgaris* L. collected in its primary center of diversity and maintained in CIAT ' s gene bank (Table 51). These genotypes (seeds) were analyzed as isotypes of phaseolin type found for each analyzed seed (nondestructive test of seed for proteins extraction). Later these seeds were planted in greenhouse, which made possible to conserve its genotype and to guarantee repeatability of the results obtained with this study . The work beginning with the new and simple phaseolin types reported by Ocampo et al (2000).

Table 51. New types of phaseolin that we found in populations of *Phaseolus vulgaris* L. collected in its primary center of diversity.

Country of origin	Identification	Biological Status	No. of sampled seeds	Phaseolin Types
Mexico	DGD-274	Wild	30	Novel and simple phaseolin No. 3
Honduras	SB-6	Wild	13	Novel phaseolin No. 2 (nonsimple type)
Costa Rica	DGD-3106	Wild	5	Novel and simple phaseolin No. 4
	DGD-3126	Weedy	15	Novel phaseolin No. 2 (nonsimple type) Novel and simple phaseolin No. 4
	DGD-3131	Wild	15	Novel and simple phaseolin No. 4
Colombia	OT-122	Weedy	85	Novel phaseolin No. 2 (simple type)
	OT-252	Cultivated	2	Novel and simple phaseolin No. 1

Initially the proteins new were separated according to their molecular weight using one-dimensional sodium dodecyl sulfate polyacrilamide gel electrophoresis. The slab gels were 1.5 mm thick with 15.0 % acrylamide in the running gel and 6.38 % acrylamide in the stacking gel (Brown et al. 1981). Two-dimensional isoelectric focusing SDS-PAGE (2D-IEF-SDS-PAGE) was done according to the method described by O'Farrel (1975), using 15 % acrylamide slab gel for SDS-PAGE, except that no stacking gel was included. For first-dimension separation (isoelectric focusing), the proteins are resolved in according to their isoelectric points (pIs) using 5.0 % acrylamide in the tube gels (6,5 cm in length and 0,75 mm in thickness) and 2.0 % carrier ampholytes (pH 5-7).

Results and Discussion

These new phaseolin types were first observed in 1D-SDS-PAGE and confirmed later in 2D-IEF-SDS-PAGE (Table 51). For the two-dimensional electrophoresis, we began with the simple and non simple novel phaseolin No.2, wich have been in the weedy population OT-122. In this population also reported the "Muisca" phaseolin by Chacón (1996). This approach allows comparisons between these three phaseolin types from the same population. In addition the novel phaseolin No. 2 (non simple type), previously has been reported with the name of the heterozygote phaseolin (S + I) in the SB-6 (Honduras), DGD-3126 (Costa Rica) and OT-122 (Colombia) populations (data non published). The results show that the novel simple and non simple phaseolin types No. 2 are different by 1D-SDS-PAGE (Figure 39) and also by electrophoresis 2D. The "Muisca" phaseolin shows a 2D fingerprint very different from novel phaseolin No. 2 (Figure 40). This evidence defines that the non simple type of the novel phaseolin No. 2 (previous heterozygote phaseolin) is a novel phaseolin different from the known "Muisca" phaseolin.

Figure 39. One dimension SDS-PAGE gel of the novel phaseolin types (simples and non simples) found in common bean.

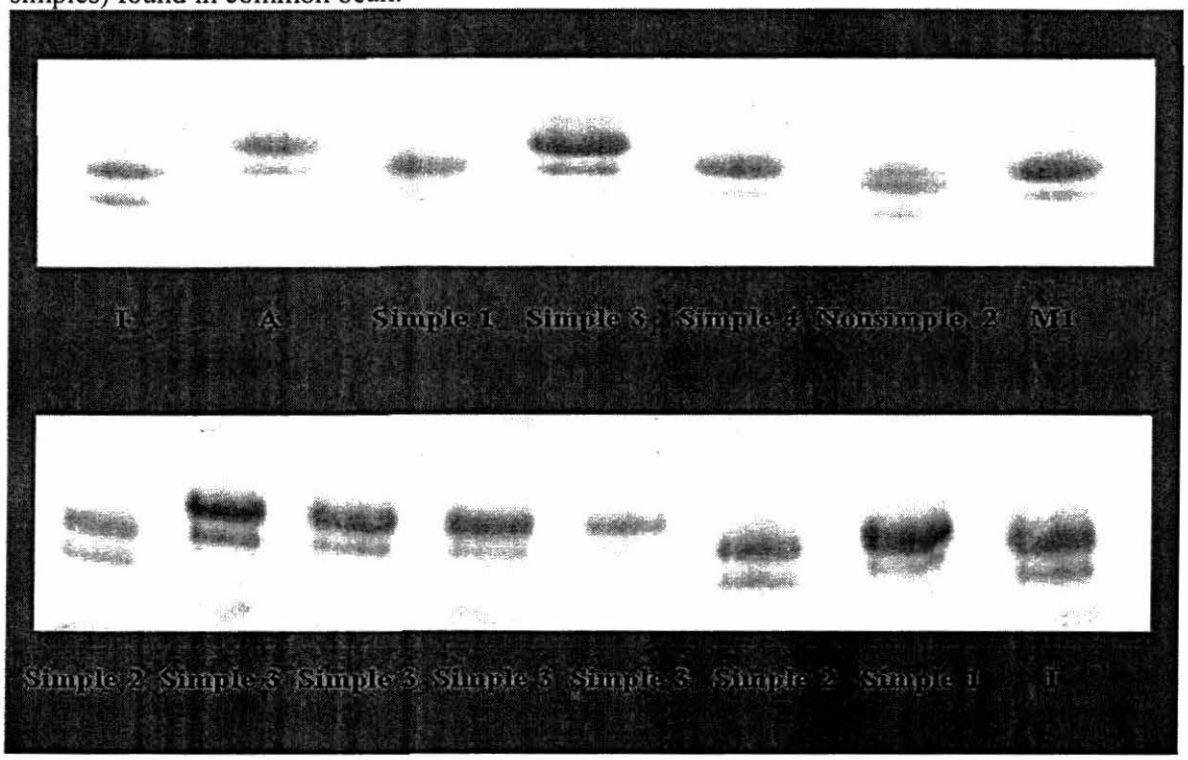
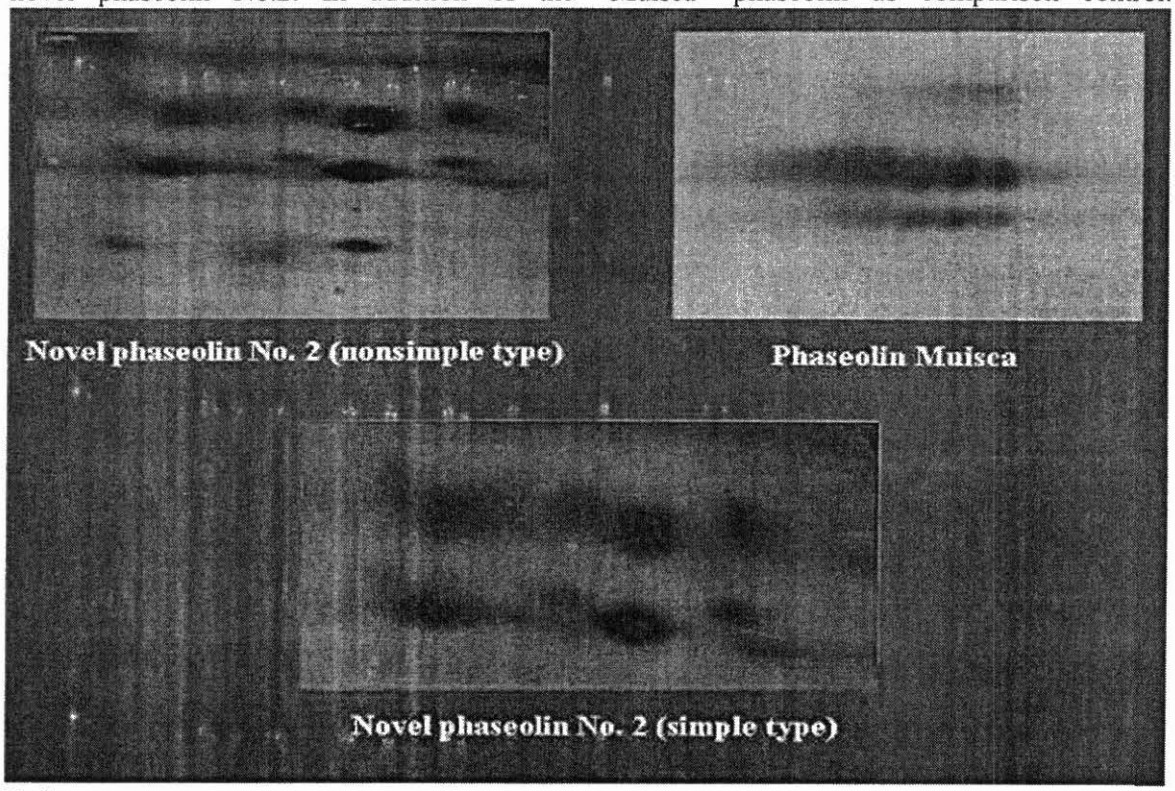


Figure 40. Two dimensional IEF-SDS-PAGE electrophoresis of the the simple and nonsimple novel phaseolin No.2. In addition of the "Muisca" phaseolin as comparison control.



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Activity 3.1.2 Biochemical evidence supporting the existence of a weedy form in the tepary bean (*Phaseolus acutifolius* A. Gray).

Commonly known as tepary beans by the native peoples of the southwestern United States and has long been known as a domesticated bean species with particular adaptation to dry areas. As indicated by Freytag and Debouck (2002), four forms of *P. acutifolius* are known: domesticated variety *latifolius*, wild variety *latifolius*, wild variety *acutifolius*, and wild variety *tenuifolius*.

However, until now an intermediate form between the wild and cultivated form has not been identified. These forms are found growing wild in dry gullies, stream beds and the foothills in and around the deserts or drier areas of the southwestern United States and northern Mexico and sparsely scattered south through the dry central highland areas to central Chiapas and Guatemala (Freytag and Debouck, 2002). The cultivated teparies are distributed in arid Mesoamerica. From results of phaseolin (Schinkel & Gepts, 1988) and isozyme (Garvin & Weeden, 1994; Manshardt & Waines, 1983; Schinkel & Gepts, 1989) variability, it seems that the tepary might have been domesticated in only a very few places of arid Mesoamerica, and thus has limited probabilities of further genetic improvement. The potential for future tepary breeding might thus rely mostly on wild forms (Debouck, 1992; Garvin & Weeden, 1994). The aconitase isozyme pattern (ACO-2 allozyme) found in tepary bean suggests that this species was domesticated in a single geographic region. Two ACO isozymes were detected . Whereas ACO-1 was monomorphic, two allozymes were detected for ACO-2. The allele encoding the slower migrating ACO-2 allozyme was present in domesticated tepary bean at a frecuency of 0.997, whereas the frecuency of the fast allele in wild tepary bean was found to be 0.96 (Garvin and Weeden, 1994). This allozyme may therefore serve as an evolutionary marker for help identifying wild and domesticated teparies, and by extension, in order to help to identify the intermediate form. In addition the weedy has not been reported for the teparies. As the interbreeding complexes (wild-weedy-cultivated) may be important mechanisms for the generation of genetic variability in landraces, we analyzed a complex (wild-weedy-crop) from a wild population of teparies beans from a biochemical (seed proteins-globulins and aconitase isozyme) and morphological viewpoint in order to find evidence that suggests the existence of a true intermediate form in the bean tepary.

Materials and Methods

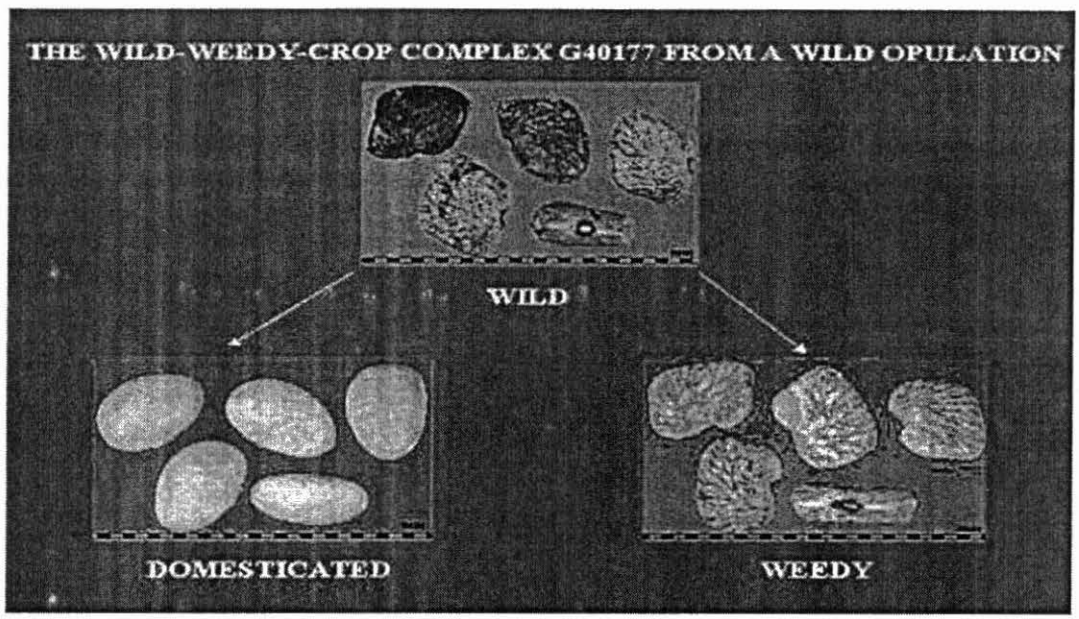
We studied a population (G40177) whose original seed was collected and classified as wild in the North American state of Arizona. However, during the seed multiplication in the *Phaseolus* germplasm bank greenhouses maintained in the CIAT (In this place was introduced as increased seed), we observed segregation in seed size and colors indicating a possible wild-weedy-crop complex. For this study, we only took multiplied seed in greenhouse, being in an advanced stage of increase, which once stabilized the biological status of the seed, the materials were classified as cultivated, intermediate and wild. In addition, two accessions were chosen as comparison controls for the aconitase isozyme analysis: a cultivated (G40064) from the Arizona (USA), which has the slow allele of ACO-2, and a wild (G40090) from the Mexican state of Durango with the fast allele of the ACO-2 gene. For the seed morphological traits analysis, we study the nature of the analyzed material from a morphological viewpoint is provided, according to the evaluation of the size seed, its color and pattern. Using starch gel electrophoresis (Garvin and Weeden, 1994), we examined aconitase variation in 98 seeds of the population G40177, which 87 were analyzed as isotypes of aconitase isozyme type (non destructive test of seed for isozyme extraction) and 11 were analyzed with destructive test of seed. In addition we included a seed proteins analysis (globulins) by ID-SDS-PAGE (Brown et al. 1981), using a destructive test of seed.

Results and Discussion

Stabilized the biological status of the seeds in an advanced stage of increase, the isotypes (87 in total) were classified as cultivated [48 (55 %)], intermediate [29 (33 %)] and wild [10 (12 %)]

(Table 52). This segregating population was considered to be a complex, since they involve wild, domesticated and weedy forms (Figure 41).

Figure 41.Morphological variation in the wild-weedy-crop complex G40177 from a wild population.



This confirm that a considerable amount of natural hybridization occurs in the zone (Pima County in the state of Arizona, USA) where this wild population was collected, where both wild types and landraces are locally distributed. In addition this complex has shown a great diversity in seed size (from small to large) and color. The aconitase isozyme analysis shows two alleles of ACO-2 in all phases of the complex, from typical wild seeds to fully domesticated forms. In addition three analyzed seeds by destructive test, display a heterozygote allozyme (Fast/Slow). Only two patterns (IX and IV) of globulins were found also in all phases of the complex (Table 52).

Table 52. Globulin types, ACO-2 allozyme constitution and seed size of the wild-weedy-crop complex G40177 from a wild population of teparies beans. The aconitase alleles and globulin types frequencies is in parenthesis.

Biological status	Analyzed Seeds		100 Seed weight (g)	Globulin type	ACO-2 Allozyme		
	isotypes	Destructive test			Fast	Slow	Fast/Sslow
Cultivated	48		< 6 g	XI (32) IV (16)	28	20	
Weedy	29		6-9 g	XI (20) IV (9)	19	10	
Wild	10		>10 g	XI (4) IV (6)	8	2	
Weedy ?		11	6-9 g	IV (11)	5	3	3
Total	87	11	---- * ----	XI or XVII (56) IV (42)	60	35	3

The "XI" type is present only in domesticated accessions, whereas that the other globulin type (IV) is present exclusively in the wild teparies. However, for this study these patterns were observed only with the 1D-SDS-PAGE technique. In addition, there is a much larger variability in globulin patterns in wild tepary populations, while only two patterns are found in landraces (Schinkel & Gepts 1988). The higher frequency of introgression between the wild and domesticated forms suggested by isozyme data correlates well with the higher frequency contributed by globulin analysis. This process of introgression has happened by seed size, seed color and pattern, globulin type and aconitase isozyme constitution of the 87 analyzed isotypes. In conclusion, these evidences suggest the existence of a true intermediate form in the bean tepary.

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Activity 3.1.3 A biochemical trait helps to recognize *Phaseolus parvifolius* Freytag in the genepool of tepary bean.

Introduction

The section of *Phaseolus* currently including the tepary bean, i.e. the *Acutifolii*, consists of two species: *Phaseolus acutifolius* A.Gray (with three varieties: var. *acutifolius*, var. *latifolius* and var. *tenuifolius*) and *P. parvifolius* Freytag (Freytag & Debouck 2002). Schinkel & Gepts (1989) could not separate these variants by nine allozyme assays. Garvin & Weeden (1994) reported

limited polymorphism for aconitase, apparently with no relationship with foliar attributes. Jaaska (1996) found a unique electromorph for three out of six accessions of var. *tenuifolius*, now classified as '*parvifolius*'. In a study of 91 accessions with ten enzyme systems, Florez (1996) found that the allele *Aat-2*^{95} uniquely separates the '*parvifolius*' materials (12) from the rest of wild teparies. Zink & Nagl (1998) reported a minor difference in banding pattern of microsatellites between *P. parvifolius* and accessions of *P. acutifolius*. Muñoz et al. (2002) found in a diversity study with help of AFLPs that *P. parvifolius* forms a group separating from other wild teparies at the level of separation of common bean genepools. The purpose of this study was to find a biochemical marker ("diagnostic isoenzyme") for the recognition of either one of the varieties of tepary bean.

Materials and Methods

A total of 100 accessions (26 cultivated, 72 wild and 2 "escaped") of *P. acutifolius* from the world collection held at CIAT were analyzed. These accessions represent the geographic, ecological, and morphoagronomic variability, as well as the variation of seed proteins found in tepary bean. Ten enzyme systems assayed by means of polyacrylamide and starch gel electrophoresis from different tissues were evaluated. The methodology for isozyme extraction, running and staining was the one reported by Ramirez et al. (1987). Globulins patterns (seed proteins) were analyzed by SDS-PAGE as in Gepts et al. (1986). For each allozyme, loci and alleles were designated as described by Koenig & Gepts (1989).

Results and Discussion

Out of all enzymatic complexes analyzed, the aspartate aminotransferase (AAT; E. C. 2.6.1.1) system obtained from root tips and polyacrylamide gel electrophoresis displayed alleles in *P. parvifolius* that were absent in the other varieties (Figure 42).

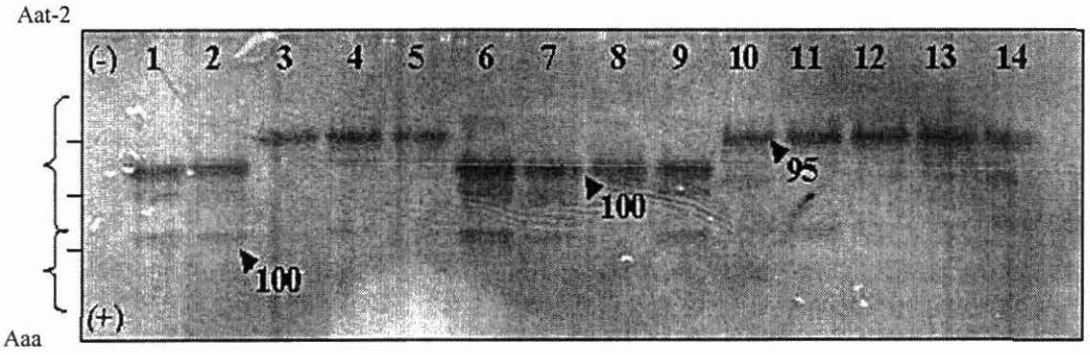

Fig. 42. Polyacrylamide gel phenotypes observed for aspartate aminotransferase (AAT). Individuals in lanes 1 and 2 are cultivated (var. *acutifolius*), individuals 6 and 7 are wild var. *acutifolius*, and individuals 8 and 9 are wild var. *tenuifolius*. The rest are classified as *P. parvifolius* (lane 3, 4, 5, 10, 11,12, 13, and 14).

In agreement with genetics of Aat isozyme (Garvin & Weeden 1994; Garvin et al. 1989), the Aat-2 locus has three alleles (93, 95 and 100), all of them homozygous in the accessions evaluated. The allele *Aat-2*^{95} is present exclusively in *P. parvifolius* (Table 53).

Table 53. Distribution of the allozymes found for AAT isozyme[1] in the three varieties of *Phaseolus acutifolius* A. Gray

Botanical variety	**Biological Status**	**Allozymes/individuals**				
		Aat-1		**Aat-2**		
		100/	**n/n**[2]	**93/100**	**95/95**	**100/100**
Var. *Acutifolius*	Cultivated	12	14	1	-	25
Var. *acutifolius*	Wild	23	5	-	-	28
Var. *tenuifolius*	Wild	21	3	1	-	23
Var. *parvifolius*	Wild	20	-	-	20	-
Weedy (wild/cultivated)	Intermediate	2	-	-	-	2

[1] The genetics of AAT isozyme variants has been reported by Garvin and Weeden (1994), with three zones of migration observed. Nevertheless, for the present study, only two zones of migration were observed (Florez, 1996).

[2] A null allele has been reported in tepary bean.

Only three patterns (IX, X and XII) of globulins were found in *P. parvifolius* (Table 54). The "XII" type is dominant (present in all accessions), whereas in the other botanical varieties it appears with low frequency (4,1 % in wild var. *acutifolius* and 9,3 % in wild var. *tenuifolius*) (Florez, 1996). In addition, there is a much larger variability in globulin patterns in wild tepary populations, while only two patterns are found in landraces (Schinkel & Gepts 1988).

Table 54. Descriptors of the var. *parvifolius* of tepary bean, distribution in its primary center of domestication in the Americas and alleles found for AAT isozyme.

Gnumber	**Biological status**	**Botanical variety**	**Country**	**Province**	**Seed Size**	**Globulins (patterns)**	**Loci/Alleles**	
							Aat-1[a]	**Aat-2**
G40090	Wild	Parvifolius	MEX	DURANGO	1.2	XII	100/	95
G40102	Wild	Parvifolius	MEX	DURANGO	1.8	XII	100/	95
G40109	Wild	Parvifolius	MEX	NAYARIT	1.3	XII, IX	100/	95
G40167	Wild	Parvifolius	MEX	JALISCO	1.6	XII	100/	95
G40170	Wild	Parvifolius	MEX	JALISCO	1.4	XII	100/	95
G40181A	Wild	Parvifolius	USA	ARIZONA	1.2	XII	100/	95
G40182	Wild	Parvifolius	MEX	OAXACA	1.3	XII	100/	95
G40183	Wild	Parvifolius	MEX	GUERRERO	1.3	XII	100/	95
G40184	Wild	Parvifolius	MEX	GUERRERO	1.2	XII	100/	95
G40185	Wild	Parvifolius	GTM	JALAPA	1.5	XII	100/	95
G40186	Wild	Parvifolius	GTM	JALAPA	1.2	XII	100/	95
G40195	Wild	Parvifolius	USA	TEXAS	2.4	XII	100/	95
G40240	Wild	Parvifolius	MEX	DURANGO	1.1	XII	100/	95
G40241	Wild	Parvifolius	MEX	DURANGO	1.4	XII, X	100/	95
G40264	Wild	Parvifolius	MEX		1.2	XII, X	100/	95
G40266	Wild	Parvifolius	MEX	DURANGO	1.7	XII, X	100/	95
G40267	Wild	Parvifolius	MEX	DURANGO	1.7	XII, X	100/	95
G40268	Wild	Parvifolius	MEX	JALISCO	2.7	XII, X	100/	95
G40292	Wild	Parvifolius	MEX	SINALOA	1.2	XII, X	100/	95
G40293	Wild	Parvifolius	MEX	SINALOA	1.8	XII, X	100/	95

[a] For this polymorphic locus it has been reported null allele in tepary bean, with which they are showed like heterozygote (Florez, 1996).

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Activity 3.1.4 Introgression in the wild-weed-crop complexes from domesticated populations of common bean (*Phaseolus vulgaris* L.) in Colombia

Introduction

The ocurrence of introgression between the domesticated form and their wild ancestor or between the Middle American and Andean gene pools seem very probable and has been propose for the common bean (*Phaseolus vulgaris* L.), as a consequence of intraspecific hybridisation (Paredes and Gepts, 1995; and Chacón et al. 2002). The variability in these two gene pools overlap by between-gene pool introgressive hybridization, scene that probably has happened in Colombia, because is a place of particular relevance by its intermediate geografical posicición between these two major gene pools (Debouck, 1996). Extensive wild-weedy-cultivated complexes of common bean were observed during collection expeditions in regions of Colombia where wild and cultivated beans are sympatric (Beebe et al. 1997). The single gene markers (seed proteins-phaseolins, isozymes or microsatellite) can provide valuable information to better understand gene flow. Debouck et al. 1993; Freyre et al. 1996; Beebe et al. 1997; Papa and Gepts. 2003 and González et al. 2003., documented several examples of hybridization between wild and domesticated bean plants. Hybridization has been found between the Middle American and Andean gene pools in cultivated common bean from Chile (Paredes and Gepts, 1995) and Spain (Ocampo et al. 2002). In the present study, we analyzed the complexes (wild-weedy-cultivated) from domesticated Colombian populations of common bean, from a biochemical (phaseolin and isozyme markers) and morphological viewpoint to test the hypothesis of introgression between the Middle American and Andean gene pools in Colombia.

Materials and Methods

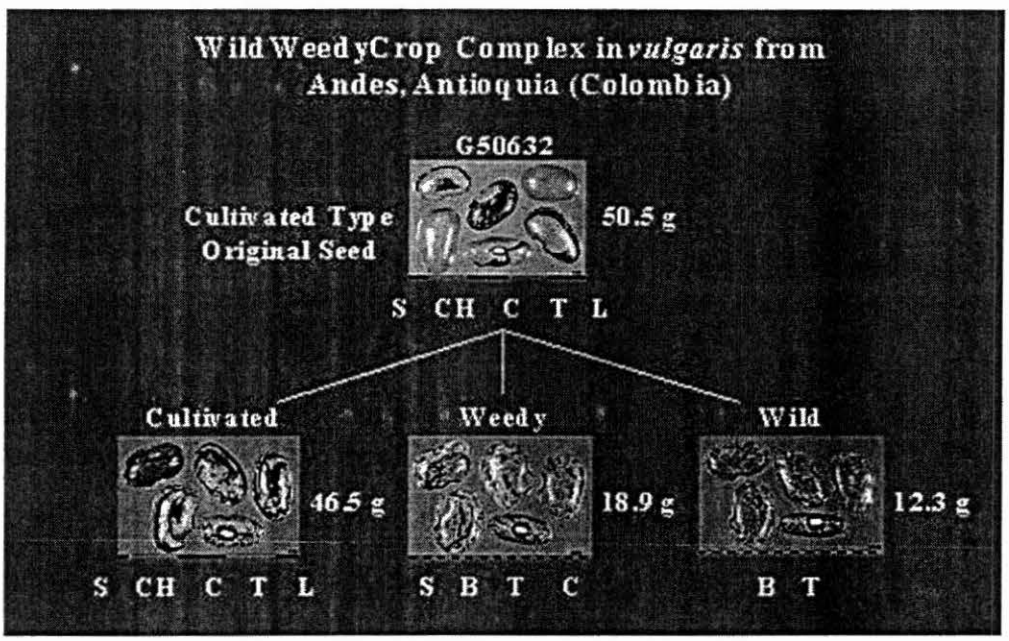
Plant material. For this study ten wild-weedy-crop complex were selected with base in a geographic sampling in Colombia. These populations were collected in some Colombian departments where wild and cultivated beans are sympatric (Cundinamarca and Boyaca) or in departments that are highly producing for common bean (Antioquia, Caldas, Tolima and Cauca) (Table 55). For this study, we only took multiplied seed in greenhouse, being in an advanced stage of increase (conserved in in the *Phaseolus* germplasm bank held in CIAT). In addition three accessions were chosen as comparison controls for the phaseolin and isozyme analysis: two cultivated (improved) *P. vulgaris* from the Andes and Mesoamerica (G4494 and G5773, respectively) and a Colombian wild (G24408) (Table 56).

Seed morphological traits analysis. For the seed morphological traits analysis, we study the nature of the analyzed material from a botanical and evolutionary viewpoint is provided, according to the evaluation of the size seed, its color and pattern. *Phaseolin analysis.* Using one-dimensional SDS-PAGE (Brown et al. 1981) and 2D-IEF-SDS-PAGE (O'Farrel, 1975) techniques for seed storage proteins, the seeds were analyzed as isotypes of phaseolin type found for each analyzed seed (nondestructive test of seed for proteins extraction). Later these seeds were planted in greenhouse, which made possible to conserve its genotype and to guarantee repeatability of the results obtained with this study. *Isozyme analysis.* The same seeds or isotypes utilized for the phaseolin determination were used for isozyme analysis, which allows its comparison with the phaseolin analysis. Were used in this study, only two enzymatic complexes: peroxidase (PRX; 1.11.1.7) and diaphorase (DIA; 1.6.4.1) obtained from root tips and polyacrylamide gel electrophoresis, which displayed alleles that belong to both Middle American and Andean gene pools (Koenig and Gepts, 1989). The methodology for isozyme extraction, running and staining was the one reported by Ramirez et al. (1987).

Results and discussion

Seed morphological variation of the complexes. The original seed of these populations was collected and classified as cultivated materials (Table 55). However, using the original seed and observations in greenhouse during the first generation of seed multiplication we observed segregation seed size and colors indicating possible wild-weedy-crop complexes. This allowed comparisons with the following seed increases, which once stabilized the biological status of the seed, the materials (1182 in total) were classified as cultivated [642 (54%)], intermediate [432 (37 %)] and wild [108 (9 %)] (Table 55). These segregating populations were considered to be complexes, since they involve wild and weedy forms. This confirm that a considerable amount of natural hybridization occurs in the areas where were collected these populations. These complexes showed a great diversity in seed size (from small to large) and color (Figure 43).

Figure 43. Example of seed morphological variation and biochemical for wild-weedy-crop complex G50632. In the right side of image shows 100 seed weight and down side is the phaseolin types.



Biochemical analysis. A great diversity within these complexes for phaseolin types was found (Table 55). This variation was first observed in 1D-SDS-PAGE and confirmed later in 2D-IEF-SDS-PAGE (Figure 44). The found patterns were, five Andean (C, T, H1, H2 and Ca), two Mesoamerican-Colombian (B, CH), a exclusive Mesoamerican (S) and three exclusively Colombian (L, Car and Mμ), with a frequency of 55%, 20%, 21% and 4% respectively.
The "C" phaseolin was present at the highest frequency (30%), followed by the "S" type with 21%, "T" type with 20% and "B" type with 17%. Colombia also display phaseolin types such as "L" (Beebe et al. 1997), "Mμ" and "Car" (data non published), which are apparently unique to Colombian materials (Table 55).

Table 55. Phaseolin types variation in the wild-weedy—crop complexes from domesticated Colombian populations of common bean.

CIAT No.	Seed original descriptors		Phaseolin Variation in the wild-weed-crop complexes (increased seed)		
	Department	Gene pool	Biological Status	Isotypes	Phaseolin types (frecuency in parenthesis)
G50711	Antioquia	Andean cultivated	Cultivated Weedy Wild	9 14 8	S (1), B (2), C (4), CAR (2) S (6), B (2), C (5), H_1 (1) S (5), C (3)
G50849	Antioquia	Andean cultivated	Cultivated Weedy Wild	91 8 6	S (37), C (41), H_1 (6), H_2 (3), T (4) S (15), C (6), H_1 (2), H_2 (1) S (6), C (3)
G50632	Antioquia	Andean cultivated	Cultivated Weedy Wild	138 24 7	S (36) , CH (5), C (41), T (55), L (1) S (3), B (17), C (3), T (1) B (6), T (1)
G50646	Antioquia	Andean cultivated	Cultivated Weedy Wild	80 27 7	S (14), B (2), CH (1), T (37), C (24), H_1 (1), H_2 (1) S (13), T (9), C (5) T (1), C (6)
G50785	Antioquia	Andean cultivated	Cultivated Weedy Wild	136 118 29	S (16), B (3), CH (1), C (41), T (67), H_1 (8) S (19), B (12), CH (10), T (30), C (45), H_1 (2) S (4), B (4), CH (3), T (5), C (13)
G50879	Caldas	Andean cultivated	Cultivated Weedy Wild	87 24 4	B (13), C (49), T (2), H_1 (22), H_2 (1) B (16), C (4), H_1 (4) B (2), C (1), H_1 (1)
G50983	Cundinamarca	Andean weedy	Cultivated Weedy Wild	9 129 6	S (6), C (2), Mu (1) S (24), B (48), CH (13), C (9), H_2 (1), Mu (34) S (3), B (2), Mu (1)
G50988	Boyaca	Andean cultivated	Cultivated Weedy Wild	20 16 12	S (3), T (2), C (10), H_1 (5) S (10), C (4), H_1 (2) S (5), C (6), H_1 (1)
G50797	Tolima	Andean cultivated	Cultivated Weedy Wild	1 13 15	S (1) S (6), C (3), H_1 (4) S (9), C (2), H_1 (4)
G50859	Cauca	Andean cultivated	Cultivated Weedy Wild	71 43 11	S(5),B (24),T(10),C(18),Ca_1(4),H_1(2),H_2(1),Car (7) B (36), C (6), H_1 (1) B (11)

For the isozyme analysis only a complex was selected, being used for it thirty seeds (as phaseolin isotypes). Most of these seeds (77%) were generally characterized by a great and medium seed size and an Andean cultivated phenotype introgressed with the Middle American gene pool (Table 56). The isotypes of this complex had different phaseolin patterns, but a high percentage of them had Andean phaseolin types (67%). The rest had a phaseolin constitution representative of the Mesoamerican gene pool (including the "B" phaseolin and "CH"). The selected isozyme loci Carry alleles from both Middle American and Andean gene pools : The Dia-1^{95}, PRX^{98} alleles are considered to be Mesoamerican and the Dia-1^{100}, PRX^{100} alleles are of Andean origin (Koenig and Gepts, 1989) (Figure 45). The PRX locus shows a higher frequency of the Mesoamerican allele (68%) and an intermediate frequency for the Andean allele (32%). However, the Dia-1 locus shows a higher frequency of the Andean allele (80%) and a lower frequency of the Mesoamerican allele (20%). In addition seven genotypes (23%) display a heterozygote allozyme (PRX^{100}/ PRX^{98}).

Figure 44. One-dimensional SDS-PAGE (upper line) and 2D-IEF-SDS-PAGE (lines second, third and fourth) gels of wild-weed-crop complexes showing phaseolin types found in them . For the 2D gels, arrows point to key peptides (Figure 2).

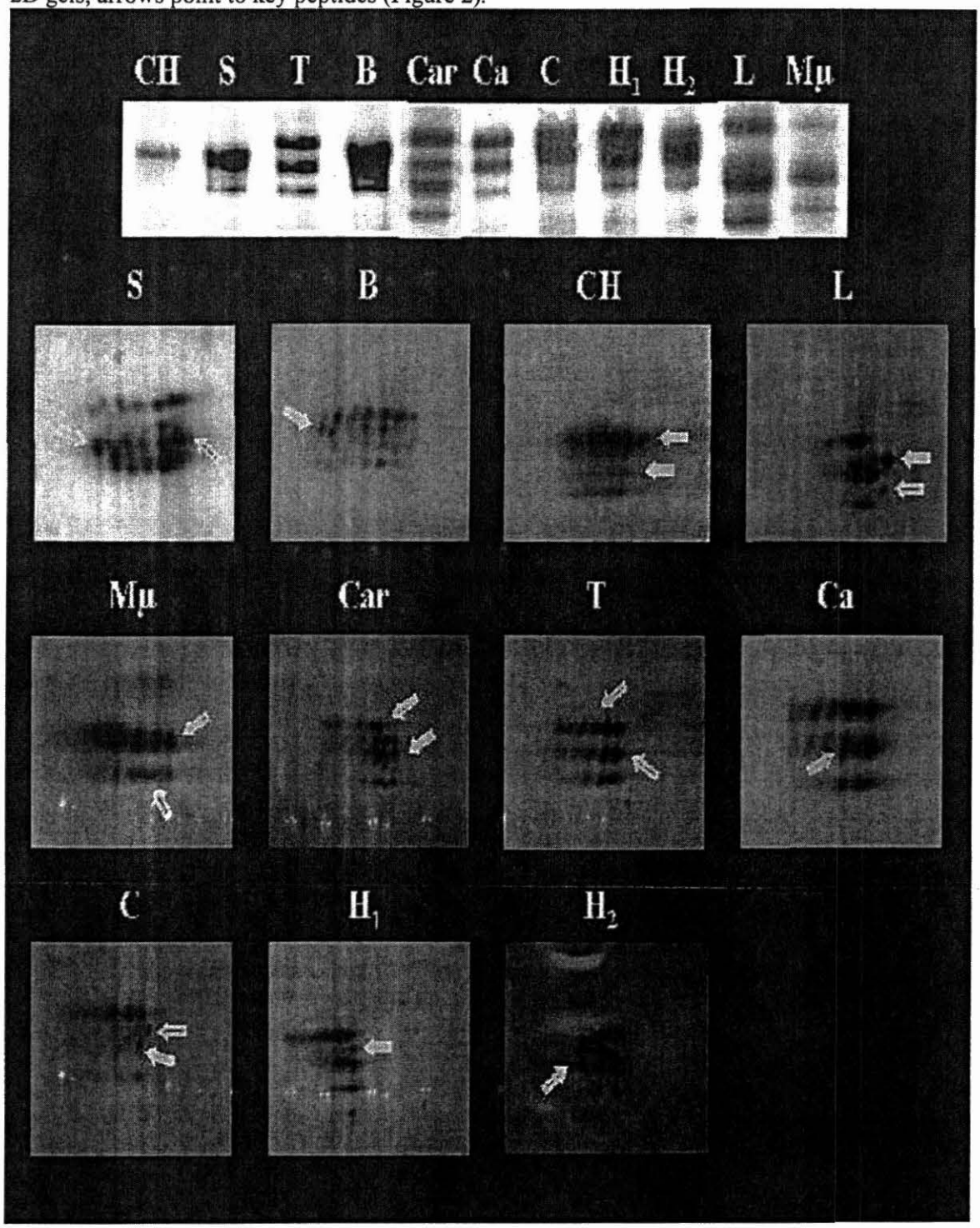


Table 56. Phaseolin, allozyme constitution and seed size of the wild-weedy-crop complex G50849. In addition three accessions were chosen as controls.

Biological Material	Analyzed isotypes	100 seed weight (g)	Phaseolin	Isozyme loci	
				Prx	Dia-1
G4494 (Control)	----- * -----	54.8	T	--- o ---	100
G5773 (Control)	----- * -----	24.0	S	98	95
G24408 (Control)	----- * -----	10.6	L	100	95
G50849 Cultivated	23	23.4-47.8	S (3), B (3), T (2), C (7), H1 (4), H2 (4),	100 (5) 98 (14) 100/98 (4)	100 (17) 95 (6)
G50849 Weedy	4	10.0-24.0	C (2), B (2)	100 (0) 98 (3) 100/98 (1)	100 (4) 95 (0)
G50849 Wild	3	5.3-7.2	C (1), S (1), B (1)	100 (1) 98 (0) 100/98 (2)	100 (3) 95 (0)

Phenotypical and biochemical evidence suggest a substantial genetic interchange between Middle American and Andean gene pools in these wild-weedy-crop complexes. These evidences are:
(1) Original seed in the complexes with Andean cultivated phenotype that originated genotypes with "S" phaseolin type and isozyme alleles that are typical of the Middle American gene pool.
(2) A high proportion of complexes with an biochemical pattern characteristic (including the "B" phaseolin and "CH") of the Mesoamerican gene pool.
(3) Presence of Andean phenotypes with Andean phaseolin pattern and Mesoamerican isozyme constitution.
(4) Genotypes with "S" phaseolin and "B" are found in all phases of the complex, from typical wild seeds to fully domesticated forms.

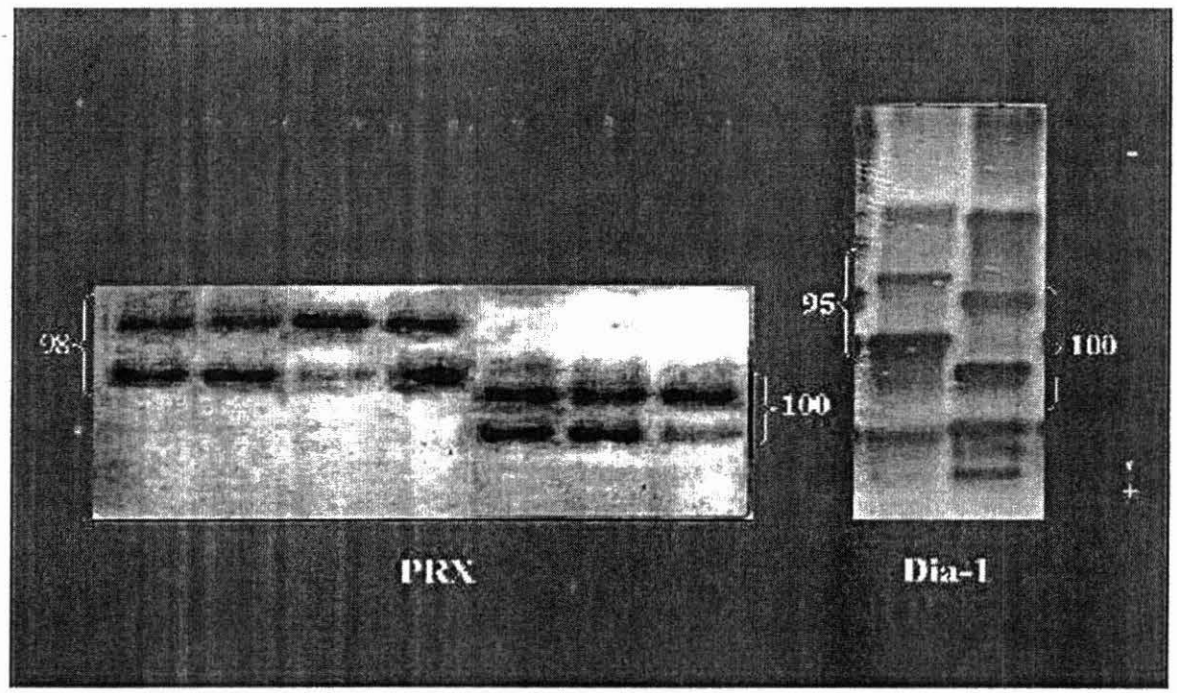


Figure 45. Loci and alleles of the peroxydase (left image) and diaphorase isozymes (right image).

This process of introgression has happened in all biological states of the complex as revealed by seed size, seed color and pattern, phaseolin type and isozyme constitution of these wild-weedy-crop complexes from Colombia. In addition the existence of these complexes that are apparently hybrids between the two gene pools is explained by the existence of true wild forms and landraces in Colombia with "S" phaseolin and "CH" (present elsewhere in Mesoamerica) and "T" (Andean). Assuming that both wild types and landraces are local in origin, these probably have been naturally hybridized to result in the introgressed populations (Beebe et al. 1997).

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Contributors: O. Toro, C. H. Ocampo

Activity 3.1.5 Possible contribution of Mesoamerican phenotype in snap beans cultivated in secondary centres.

Introduction

Snap bean genotypes grown in Colombia are often affected by several biotic stresses. In order to carry out a pre-breeding process, we wanted to know the extent of genetic variability of the parental materials to be used (Kami and Gepts, 1991; Myers and Baggett, 1999). Phaseolin type is an important evolutionary genetic marker. In this paper, we wanted to compare, using morphological descriptors and biochemical markers (phaseolin), the snap bean varieties cultivated in secondary centers with the genetic pools distributed in the Americas, i.e. the Mesoamerican, Colombian or Andean domestication centers.

Materials and methods

One hundred and sixteen snap beans genotypes from URG-CIAT bean germplasm bank coming from Europe, Asia, Africa and America, two commercial genotypes of snap beans [Blue lake Ferry and Milenio (G51158)]. As controls four common bean accessions, G4494 (cultivated material proceeding from the Andes), G23725 (Ecuadorian wild type), G21117 (Colombian wild type) and G5733 (cultivated genotype coming from Mesoamerica) were used, in order to make a comparison to the American genetic pools. Pod characteristics (fiber content 15% humidity, length, shape, seed number) and seed characteristics (color and weight) were used as morphological descriptors (Muñoz et al, 1993). Phaseolin patterns were analyzed by SDS-PAGE as in Gepts et al. (1986).

Results and discussion

In previous reports (Gepts 1988; Ocampo et al. (2002) most of the European materials studied by them, are Andean in origin. However, data obtained in this study are not in agreement with those results, namely with respect to data from Europe, Africa and USA (Table 57). Seven phaseolin types were found. The "S" type was present at the highest frequency (53% of the genotypes), followed by "T" type (17%), "C" type (19%), "CH" type (6.9 %), "Sb" type (2%), "H_1" type (0.86%) and heterozygotic "H(S+I)" type (0.86%). We found that twenty-two accessions (28%) were atypical, showing phenotypical characteristics of Andean materials (seed type) and

Mesoamerican "S" phaseolin type. These genotypes with contrasting morphological and biochemical characteristics were found only in traditional varieties (n=78). These genotypes have creamy and spotted seeds, medium size, and rounded or kidney shape seeds (Table 58).
Fourty six genotypes out of sixty-two phaseolin S materials are traditional varieties. Nevertheless, in spite that the sample from USA is the most numerous and contains more S phaseolin genotypes, none of the accessions coming from USA showed morphotypes originated by hybridization between the American origin centers excepted the material with heterozygote phaseolin type [H(S+1)]. This type has been reported in common bean by Ocampo et al. (2000). These results suggest that the contribution of Mesoamerican types to the secondary centers of domestication and diversification of snap bean, is higher than the value accepted currently.

Table 57. Geographical distribution of phaseolin types found in snap bean.

Continental region	Number of accessions	S	T	C	CH	H_1	Sb	H(S+1)
Mediterranean Europe	18	2	1	11	2	1	1	
Central-Eastern Europe	16	10	1	2	3			
Western Europe	6	5		1				
Asia	6	3	3					
China*	17	12	2		2		1	
Turkey*	16	5	6	4	1			
USA*	27	18	6	2				1
Rest of America[1]	8	5	1	3				
África	3	3						
Total accessions	118	63	20	23	8	1	2	1

*Countries with highest contributions to the sample. [1]South America contribution was only two samples.

Table 58. Description of atypical "S" phaseolin type snap bean landraces found in this study.

Accession	Country	Seed weight	Seed color	%Fiber	Seeds per pod	Pod length	Pod diameter ratio	Phaseolin type
G621	TUR	23.0	Cream	21.54	4,1	7,5	0,48	S
G10134	NLD	42	Color mix*	13.59	5,5	12,63	0,48	S
G10214	PRT	45	Color mix*	13.12	5,5	11.8	0,74	S
G10220	PRT	48	Cream	17.94	6,1	14,64	0,62	S
G10222	PRT	38.7	Cream	12.35	7,6	15,5	0,7	S
G10233	PRT	38	Cream	8.04	7,9	11,71	0,52	S
G13431	CHN	40	Brown	11.63	5,8	11,56	0,57	Sb
G14722	ITA	23	Cream	18.36	6,3	11,15	0,94	S
G15300	ZMB	26	Color mix*	6.98	7,5	11,49	0,76	S
G15660	MEX	30	Spotted	22.77	4,3	10,33	0,53	S
G15913	NLD	39.1	White	16.69	5,4	13,55	0,68	S
G17861	HUN	32.5	Cream	17.54	6,5	12,5	0,97	S
G18212	ESP	29	Cream	5.14	6,2	13,12	0,68	Sb
G19268	SUN	41	Spotted*	13.25	4,9	11,4	0,74	S
G19279	CHN	44	Color mix*	7.75	4,7	9,4	0,77	S

G20401	CHN	29	Cream	15.54	5,8	11,03	0,54	S
G20624	IND	31	Cream	14.87	7,6	15,4	0,87	S
G23952	CHN	39	Cream	10.24	6,9	14,5	1,12	S
G24544	CHN	41.4	Spotted*	8.29	6,4	14,66	0,69	S
G50638	CHN	35.9	Spotted*	5.5	5,2	10,35	0,71	S
G50639	CHN	30.5	Spotted*	20.14	6,1	12	0,64	S
G50640	CHN	30	Spotted*	10.84	6,7	14,75	0,6	S

*Includes creamy seeds

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Contributors: A. Tofiño (Universidad Nacional de Colombia, Palmira, Colombia), C.H. Ocampo and O. Toro (Genetic Resources Unit, CIAT).

3.1.6 Biochemical caracterization of *Phaseolus* germplasm bank for improved and refined collections.

In 2003, 959 genotypes of the *Phaseolus* germplasm bank held in CIAT (768 genotypes of common bean, 5 of Lima Bean and 186 of tepary bean) were analyzed for seed storage proteins (phaseolins, globulins and total proteins) using ID-SDS-PAGE electrophoresis. This step with morphoagronomic characterization is a requisite for improving the representativeness of the bean core collection.

Contributors: C H. Ocampo and O. Toro

Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

Activity 4.1.1. Lecturing in specialized courses.

The list of lectures, namely in the MSc degree course in plant genetic resources provided by Universidad Nacional de Colombia can be found in the Annex 6.

Output 4.2. Conferences in national/ international fora

Please see list in the Annex 6.

Output 4.3 Public awareness products

As part of a system-wide initiative on public awareness at Epcot Center, Orlando, Florida, CIAT nominated one professional of GRU to be CIAT Ambassador in 2003.

The Future Harvest Centers represented this year included CIAT, CIP, ICARDA, ILRI, the World Agroforestry Center, and the World Fish Center. CIP sent two ambassadors, one who was sponsored by the World Bank Institute (WBI). Other WBI-sponsored Ambassadors include a scientist from the Department of Agriculture in the Philippines and an independent animal health scientist from Malawi

The Epcot International Flower & Garden Festival held in Orlando, Florida began on April 25th and ended on June 8th. The total number of guests that went through the three exhibits, Gardening for Food in Africa, Gardening for Food in Asia, and Gardening for Food in Latin America was 196,760 during the 2003 festival. This sets a new record for GFFAW, breaking last year's record guest attendance of 178,291 for the three exhibits (a 9.4% increase in guest attendance over last year.) Some of the stories/messages shared with Epcot guests by the Latin America's Ambassadors were: Nuna popping beans as a high-protein food; research to adapt it to lowlands, effects of 'Mulato' grass on increased milk production, cassava production & processing; importance of a new white fly-resistant variety, quality protein maize: impact on nutrition around the developing world, biodiversity, seed banks, development of sweet potatoes

processing; importance of a new white fly-resistant variety, quality protein maize: impact on nutrition around the developing world, biodiversity, seed banks, development of sweet potatoes high in vitamin A for Africa research to reduce late blight in potatoes value and uses of yacon and arracacha, the "lost crops of the Incas", new products from potatoes and tubers: exotic potato chips, syrups, desserts.

Contributors: C. Llano, with help of D.G. Debouck; Professional Staff (CIAT GRU Unit) N.Russell (CIAT Communications Unit)

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

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

1. Field work

The field work is part of the monitoring of wild forms of common bean, genetically compatible with the cultivated form (landraces, commercial cultivars). It includes: the mapping of all populations in one geographic area (either through collection consultations or direct visits), the spotting of observed cases of gene flow, and the study of the conditions by which gene flow is created and maintained.

During this field work (December 2002- January 2003), we were interested in 1) searching more populations of wild common bean in the Valle Central of Costa Rica, 2) verifying the stability of wild-weed-crop complexes spotted in previous field works (1987, 1998), 3) finding more 'intermediate' materials that would deserve study by molecular markers (see section 3).

The methodology followed was the one defined elsewhere to look for wild *Phaseolus* species (Debouck 1988). The results of the field work can be seen in table 59 and figure 46.

Table 59.

Número	Especie	Fecha d/m/a	Provincia, Distrito	Sitio cercano	Coordenadas
2111	vulg s	15/12/2002	San José, Aserrí	Aserrí	84°07'W 9°52'N 1550 m
3106	vulg s	13/12/2002	Alajuela, Carrizal	Chagüite	84°10'W 10°06'N 1510 m
3132	vulg s	14/12/2002	Alajuela, Zarcero	Zarcero	84°23'W 10°10'N 1610 m
3133	vulg s	14/12/2002	Alajuela, Sabana Red.	Sabana Redonda	84°14'W 10°07'N 1380 m
3134	vulg s	15/12/2002	San José, San Gabriel	Tranquerillas	84°07'W 9°48'N 1500 m
3135	vulg s	15/12/2002	San José, Tarbaca	Chirogres	84°06'W 9°48'N 1480 m
3136	vulg s	15/12/2002	San José, San Miguel	Sn Miguel Desamp.	84°04'W 9°51'N 1370 m
3137	vulg s	16/12/2002	San José, San Antonio	Bebedero	84°10'W 9°54'N 1600 m
3138	costar	16/12/2002	San José, San Antonio	Bebedero	84°10'W 9°54'N 1700 m
3139	costar	16/12/2002	San José, Vista de Mar	Vista de Mar	83°58'W 9°58'N 1790 m
3140	vulg s	17/12/2002	Cartago, San	Parque Iztarú	83°58'W 9°54'N 1750 m

3141	leptos	17/12/2002	Cartago, San Rafael	Parque Iztarú	83°58'W 9°54'N 1640 m
3142	costar	18/12/2002	Cartago, San Nicolás	Río Taras	83°55'W 9°55'N 2000 m
3143	vulg s	18/12/2002	Cartago, San Rafael	Hda. Tres Ríos	83°59'W 9°54'N 1500 m
3144	costar	16/01/2003	Cartago, San Rafael	Cerros Carpintera	83°59'W 9°54'N 1630 m
3145	xantho	15/01/2003	San José, San Antonio	Bebedero	84°10'W 9°54'N 1650 m
3146	leptos	15/01/2003	San José, Aserrí	Piedra de Aserrí	84°07'W 9°52'N 1550 m
3147	vulg s	15/01/2003	San José, Tarbaca	El Tigre	84°06'W 9°49'N 1450 m
3148	vulg s,w	15/01/2003	San José, San Miguel	El Manzano	84°05'W 9°49'N 1370 m
3149	sp. (X)	16/01/2003	Cartago, San Nicolás	Quircot, sitio 31	83°56'W 9°54'N 1520 m
3150	vulg c	16/01/2003	Cartago, San Nicolás	Quircot, sitio 26	83°56'W 9°54'N 1560 m
3151	vulg w	16/01/2003	Cartago, San Nicolás	Quircot, sitio 29	83°56'W 9°54'N 1510 m
3152	vulg c	16/01/2003	Cartago, San Nicolás	Quircot, sitio 29	83°56'W 9°54'N 1510 m
3153	vulg c	16/01/2003	Cartago, San Nicolás	Quircot, sitio 29	83°56'W 9°54'N 1510 m
3154	vulg w	17/01/2003	Cartago, San Nicolás	Quircot, sitio 26	83°56'W 9°54'N 1560 m
3155	vulg s,w	17/01/2003	Cartago, San Nicolás	Quircot, sitio 26	83°56'W 9°54'N 1560 m
3156	vulg s,w	17/01/2003	Cartago, San Nicolás	Quircot, sitio 30	83°56'W 9°54'N 1530 m
3157	sp. (X)	17/01/2003	Cartago, San Nicolás	Quircot, sitio 30	83°56'W 9°54'N 1530 m
3158	sp. (X)	17/01/2003	Cartago, San Nicolás	Quircot, sitio 30	83°56'W 9°54'N 1530 m
3159	xantho	13/01/2003	Alajuela, Carrizal	Chagüite	84°10'W 10°06'N 1510 m

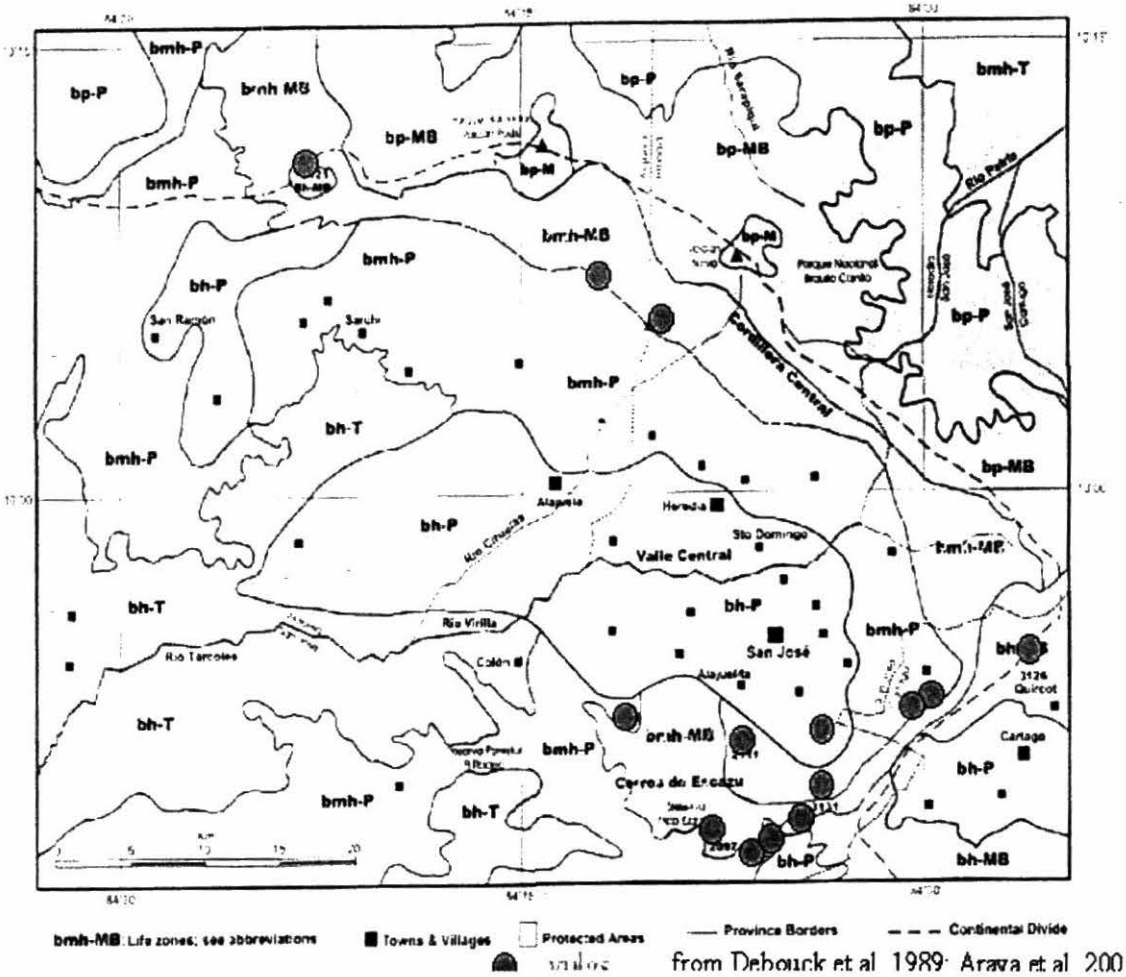

Figure 46 – Map of Valle Central with all populations known for wild *P. vulgaris*.

The number of known populations for wild *Phaseolus vulgaris* L. has been doubled as compared to previous works (Debouck et al. 1989; Araya et al. 2001), and currently covers most of the Central Valley.

The field work has revealed the low permanency of wild-weed-crop complexes once the planting of bean crop has been discontinued, as in Aserrí and Tarbaca, and to a lower extent Quircot. Of equal importance for the permanence of a complex is the maintenance of a 'weedy' environment: if farmers are using herbicides as in some plots in Quircot, wild and weedy forms disappear. If the original humid forest vegetation is allowed to come back as in Tres Ríos, complexes and populations of wild forms tend to regress as well. If farmers are switching to cash crops (such as the year-bean, *P. dumosus*) instead of maintaining traditional landraces, then the complexes regress. Interestingly, the field work has revealed natural hybrids with *P. costaricensis*, perhaps of value in future breeding. Finally, it seems important to report that while farmers know about the presence of the wild and intermediate forms, in our case study site (= Quircot) they do not longer make selections in the segregating populations resulting from natural hybridizations. This situation is in contrast with that observed in certain parts of the Colombian and Peruvian Andes (Beebe et al. 1997). There, in the late 1980s and 1990s, farmers were still

actively selecting forms of interest to them in the hybrid swarms resulting from natural hybridizations between wild and cultivated forms and among weedy and cultivated forms. Our interviews with farmers at Quircot would indicate that the proximity of markets and access to new sources of variability would not incite to make use of the variants generated through the w-w-c complexes.

We plan to extend the field observations in December 2003-January 2004, in order to validate the above described conditions allowing the permanency of the complexes.

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Contributors : D.G. Debouck (CIAT-GRU), R. Araya (University of Costa Rica)

2. Studies of gene flow under field station conditions.

In this part, gene flow is studied in the field in Costa Rica under conditions at the experimental stations of Alajuela (840 m) and Fraijanes (1850 m). The design (plot planted with beans, of 15 x 15 m) has been standardized and is used planting after planting at these stations. Two subplots of white-flowered and white-seeded bean variety are planted on both sides of a plot with lilac-flowered and black-seeded bean variety. The white-seeded variety cumulates recessive characters such as green hypocotyl and green stems, while the black-seeded variety has dominant characters such as purple hypocotyl and colored internodes. Intensity of gene flow is measured by countings of purple hypocotyls on seedlings from seed harvested in the plots with recessive lines. If gene flow through pollen carried by bees or other insects has occurred, the seed harvested on the white-flowered lines is hybrid, and with purple hypocotyl being dominant over the green recessive hypocotyl, these hybrids can be easily picked up quickly after germination.

The % of outcrossing has been of 0.06 and 0.03% at Alajuela for two trials, while it has been of 0.03% at Fraijanes for one trial. There is a marked effect of the dominant wind at Alajuela, while it is less marked at Fraijanes. Main pollinators observed were bees (*Apis*) and carpenter bees (*Xylocopa*). There is also a marked distance effect, since hybrid plants are observed in rows 1-10, and mainly in rows 1-5 (row 1 is the closest to the pollen donor line, while row 10 is at the extreme, or 6 m from the border of the pollen donor line).

Contributor: R Araya (University of Costa Rica)

3. Studies of gene flow with help of biochemical and molecular markers

We present here evidence on gene flow between wild and cultivated forms of common bean in Costa Rica in addition to our previous work (González-Torres et al. 2003).

Seeds were collected from natural populations in the Central Valley of Costa Rica as previously reported (González-Torres et al. 2003). We focus on 226 'weedy' or 'intermediate' materials initially selected on morpho-agronomic characteristics, which phenotype is inherited from possible hybridization between wild and cultivated materials. A similar procedure has been used by Papa & Gepts (2003). The analyses were conducted on: 1) morpho-agronomic evaluation; 2) biochemical analysis of phaseolin by SDS-PAGE (Gepts et al. 1986), and isozymes: diaphorase (DIA) and peroxidase (PRX) according to Ramírez et al. (1987), and 3) molecular marker analysis: eight microsatellite primers reported by Gaitán-Solis et al. (2002), and cpDNA polymorphisms by PCR- RFLPs following the protocol of Chacón-Sánchez (2001).

The wild populations showed mainly two phaseolin patterns, S-4 and S (Table 60: morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter). In cultivated materials, the phaseolins T, Sb and S-4 were also observed although in low frequency.

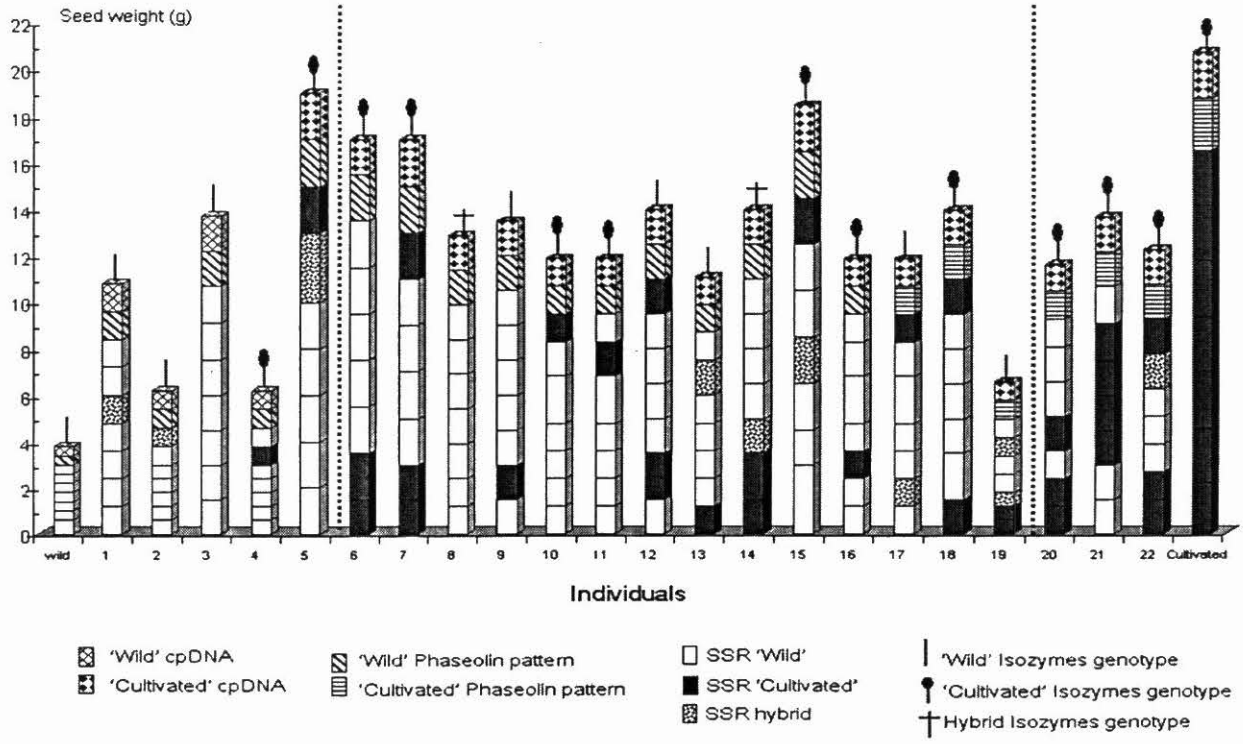
Table 60.

Biological status	Seed average weight (g)	Phaseolin Type	Isozymes		Microsatellites		cpDNA haplotypes
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 **N=443**	"S-4" "S" **N=402**	DIA –1 N=227	*PRX 100* N=204	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=134	160 ***80*** 162 110 163 146 137 122	*G, H* **N=97**
Weedy	13 **N=226**	"C" "CH" "H" ***"S"*** "X-7"[3] "S-4" **N=191**	*DIA-1* DIA-2 DIA-4 **N=170**	*PRX 100* PRX 98 N=170	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=142	160, 177 ***80*** 162, 183 110, 106 163, 189 146, 150 137, 174 122, 135	*G, H* J, K, L **N=100**
Cultivated	23 **N=188**	**"S"** "X-7" "CH" **N=186**	DIA –2 DIA –4 **N=150**	PRX 98 **N=150**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=35	177 ***80*** 183 106 189 150 174 135	J, K, L **N=33**

[1] According to Sprecher (1988); [2]according to Koenig & Gepts (1989); [3] Phaseolin pattern to be checked.

The figure 47 is a representation of markers used on a selection of individuals; bar height shows the weight (g) of 100 seeds. The shortest bar represents mainly wild characteristics and the longest bar is a description of cultivated materials. The other bars show exchange among individuals of the following markers: shared SSR alleles, change in cpDNA haplotypes, seed weight, isozymes and phaseolin patterns. In individuals 1 and 2, all the evaluated parameters are "wild" and they have a hybrid SSR locus, which suggests a recent crossing of wild material with pollen of cultivated material. Seed size of individual 3 could be a phenotypic consequence of more than one past event of gene flow from cultivated material into the wild form, because all evaluated parameters are "wild" including hypocotyl color (purple), purple flower, 85 days to flowering and growth habit IV. Besides, its F2 displays a weight of 10.3 g, which suggests that it has kept "wild" characteristics and acquired a "cultivated" seed size. Individual 8 has hybrid isozymes, "wild" microsatellites and phaseolin, but it has a "cultivated" chloroplast haplotype. Individual 9 has the same characteristics as individual 8 but it has "wild" isozymes. These materials may represent cases of repeated gene flow of cultivated materials crossed with wild forms. Individual 14 is hybrid (PRX enzyme and one SSR locus), meaning that it comes from recent flow of "wild" pollen into a cultivated form. The evaluation of 22 cases from Costa Rica indicates that all materials are indeed product of a hybridization showing that the methodology implemented in the selection of the intermediate materials was the appropriate one. Papa et al. (2003) found in intermediate materials of Mexico that the contribution of cultivated parental population was significantly higher than the wild parental one. So far, for these materials of Costa Rica, we have found a more important gene flow from wild material into the cultivated type.

Figure 47



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Contributors: R.I. González-Torres (U. Nal.), E. Gaitán (CIAT, BRU), J. Tohme (CIAT, BRU) & D.G. Debouck (CIAT, GRU)

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

We have continued with the establishment of databases about the distribution of wild relatives of beans (*Phaseolus*), cassava (*Manihot*) and rice (*Oryza*) in the Neotropics. This year we have done an inventory at the following herbaria: COL, EBUM, INB, NY, QCA, and US. The data basically include: taxonomic identification, location, date, phenology and notes. For easy consultation, types are in red, collectors in blue, and state/ province in green (examples below).

To date, the following herbaria have been visited: ARIZ, BAA, BM, BR (part), BRIT, COL, CR, CUZ, DES, ENCB, F (part), G, HAO, HUT, K, LIL, LPB, MA, MEXU, MICH, MO, MSC, NY, QCA, SGO, SI, US, USCG, USJ, and USM. This work has been developed bearing in mind the following perspectives:

i. correct identification of materials collected and kept in *ex situ* conservation facilities (namely CIAT genebank, and from there collaborating institutions).
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.

Along perspective # 1, a major outcome of that work has been a revision of *Phaseolus* (Freytag & Debouck 2002).

Example 1
Flora de Costa Rica, Fabaceae/ Pap. *Vigna umbellata* (Thumb.) Ohwi & Ohashi, det. N Zamora, enero 1996. San José, cantón de Aserrí. Z.P. Cerros de Escasú. Cerros de Escasú – La Carpintera. Cedral. Bosque primario y secundario en la falda norte del Cerro Pico Alto. Cuenca del Río Poás. 09°50'57"N 84°08'25"W. 1600-2300 m. Planta rastrera, flores fucsia. JF Morales 194. 13 December 1991. Instituto Nacional de Biodiversidad, en colaboración con el Missouri Botanical Garden (MO). /// Instituto Nacional de Biodiversidad. Fabaceae/ Pap. JF Morales 194. *Phaseolus coccineus* L. ssp. *darwinianus* Hern-Xol & Miranda, identifica N Zamora, octubre 1996. /// DGD: *costaricensis*, 1 racème, début floraison, exemplaire pauvre non typique, peu vigoureux. [INB; 21-VIII-2003].

This example shows the correct identification for a population of a new species described for Costa Rica (Freytag & Debouck 1996), that has been shown to belong to the phylum of the common bean (Schmit et al. 1993), thus widening possibilities for wide crossing.

Example 2
Trabajo de recolección de germoplasma de Phaseolus. Misión colaborativa entre el Centro Internacional de Agricultura Tropical (Cali, Colombia) y la Estación Experimental Fabio Baudrit de la Universidad de Costa Rica, con el apoyo de Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung de Alemania. *Phaseolus costaricensis* Freytag & Debouck, det. DG Debouck 18 Diciembre 2002. Costa Rica, Cartago, cantón La Unión, distrito San Rafael, Hacienda Tres Ríos en Cerros de La Carpintera. 83°59'W 9°54'N. 1630 m. Fecha 16 Enero 2003.

En abras del bosque montano húmedo con *Dahlia imperialis, Heliconia*, varias plantas trepadoras (Cucurbitaceae, *Ipomoea*), Araceae, *Ficus, Begonia*, helechos. Semi soleado. Grupo aislado, sólo en abras. En floración y vainas verdes; flor rosado fuschia oscuro. Plantas volubles 3-4 m alto. Col. DG Debouck & R Araya Villalobos no. 3144. [CR].

This example shows how the survey of populations can increase value of efforts of *in situ* conservation. The landlord of Hacienda Tres Ríos is converting an old coffee plantation into a natural reserve protecting water sources for the cities of Cartago and San José east; he is now aware of the presence of wild relatives of bean.

Example 3
EBUM7394. Fam, Euphorbiaceae. *Manihot intermedia* Weatherby. n.v. "teyapu". Loc. Rancho Galeana. Edo. Michoacán. Mpio. Apatzingán. Hab. Terreno plano, rocoso basáltico, suelo arcilloso. 310 msnm. Selva baja caducifolia. Col. X Madrigal Sánchez no. 3167. Obs. Latex muy irritante. Det. X Madrigal Sánchez. Fecha Dic 6/ 1978. /// DGD: *tomatophylla*, végétatif, fe palmatilobées, à 5 lobes arrondis, pétioles 12-16 cm long. [EBUM; 11-II-2003].

This example shows an additional record to the few known for this wild species of cassava, the distribution of which seems restricted to Michoacán, Mexico. It does not exist in genebanks.

Example 4
Plants of Louisiana. Herbarium of Southern Methodist University. *Oryza sativa* L.. Allen Parish, 1.7 miles northeast of Oberlin. Shallow water, roadside ditch, silty clay; bordering rice field. Several plants. Lloyd H Shinners 22092. 8 October 1955. /// Herbarium of Northern Kentucky University (KNK), det. John W Thieret 1984. /// DGD: *sativa*, la panicule a perdu pratiquement tous ses épillets, certains épillets ont les glumes brunâtres, type 'red rice' ! [BRIT; 10-VI-2002].

This example opens the possibility of the presence of 'red rice' on borders of rice fields in the southern USA, as early as 1955.

Example 5
Herb. Le Jolis [s.n.], *Phaseolus*, Mexique Occidental, Acapulco, [Guerrero], presqu'île Griffon, Legumin. Oct. 1866. /// Herbier Barbey-Boissier. /// Durand 1913 (acquisition ou cession?!). /// DGD: *mcvaughii*, go vertes, 3 racèmes, dimorphisme dans les gousses! [G; 15-II-2002].

This case shows how modification of habitats affects wild populations, because this site has now been converted into a tourist resort. It has been shown elsewhere (Bayuelo et al. 2002) that this species is promising for salinity tolerance. Collecting for conservation in genebanks might perhaps be the best conservation strategy in this case.

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Schmit, V, P du Jardin, JP Baudoin & DG Debouck. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. 87: 506-516.

Contributor: D.G. Debouck

6. Annexes

6.1. List of publications by Project Staff in 2003

A. In refereed journals:

Bayuelo, JS, DG Debouck & JP Lynch. 2002. Growth, gas exchange, water relations, and ion composition of *Phaseolus* species grown under saline conditions. Field Crops Research 80: 207-222.

Freytag GF & DG Debouck. 2002. Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. SIDA Bot. Misc. 23: 1-300.

Segura, S. D., Coppens, G. d'E., Ocampo, C. H. & P. Ollitrault. 2002. Isozyme variation in *Passiflora* subgenera *Tacsonia* and *Manicata*. Relationships between cultivated and wild species. Genet. Resources & Crop Evol. 50: 417-427.

B. In non-refereed journals:

Balcazar, M del S. & Pineda-L, B. 2002.Diagnóstico preliminar de bacterias coryneformes asociadas a Germoplasma de *Zornia* spp. Fitopatología Colombiana 26(1): 27-31

Flores, C. P., Ocampo, C.H. & O. Toro. 2003. A biochemical trait helps to recognize *Phaseolus parvifolius* Freytag in the genepool of tepary bean. Annu. Rept. Bean Improvement Coop. (USA) 46: 23-24.

González, R.I., Gaitán, E., Duque, M. C., Toro, O., Ocampo, C., Tohme, J. & D.G. Debouck. 2003. Monitoring gene flow between wild relatives and landraces of common bean in Costa Rica. Annu. Rept. Bean Improvement Coop. (USA) 46: 1-2.

Huertas D., Carlos A., Sarria V., Graicy & Pineda-L. 2002. Evidencias del mildeo velloso *Peronosclerospora sorghi* (W. Weston & Uppal) c. G. Shaw en cultivos de maíz y sorgo en Colombia. Fitopatología Colombiana 26(2): 55-60.

Pineda-L, B, Rivera, A. L. & Balcazar, M del S. 2002. Evaluación de fungicidas para el control de hongos en inflorescencias de Brachiaria brizantha (Panicoidea, Poaceae). Fitopatología Colombiana 26(1): 13-19.

Tofiño, A. and C. H. Ocampo. 2003. Possible contribution of Mesoamerican phenotype in snap beans cultivated in secondary centres. Annu. Rept. Bean Improvement Coop. (USA) 46: 127-128.

D. In proceedings:

Balcazar, M del S. & Pineda-L, B. 2003. Diagnóstico preliminar de bacterias coryneformes asociadas a germoplasma de *Zornia* spp. In: Memorias XXIV Congreso Ascolfi. Junio 25-27 de 2003. Armenia. p 21. (Abstract)

Rivera. A..L., Pineda, B., Balcazar, Maria del S. & Ramírez J. L. 2003. Evaluación de Funguicidas para el control del complejo fungoso *Drechslera* spp. y otros géneros de hongos en inflorescencias de *Brachiaria brizantha.* In: Memorias XXIV Congreso Ascolfi. Junio 25-27 de 2003. Armenia. p 1.
(Abstract)

E. As working paper

Torres., E & Debouck.,D. G., 2003. Sistemas de Seguimiento y Control de los Recursos Genéticos. Report of the consultancy carried out for the Instituto Alexander von Humboldt, Bogota. Colombia.

6.2. List of thesis research supervised by Project Staff in 2003

J. Salcedo. Universidad del Valle, Colombia. BSc thesis. October 2002-September 2003.

R. González. Universidad Nacional de Colombia. MSc. thesis. October 2002-September 2003.

6.3. List of conferences and scientific communications presented by Project Staff in 2003

Palmira, Colombia , 7 December, 2002, Beneficios de la investigación y conservación *in vitro*. Día de Puertas Abiertas. Centro Internacional de Agricultura Tropical.

Palmira, Colombia, presentation during the Open House at the Genetic Resources Unit, 7 December 2002: "A race, a revolution, a treaty".

Fort Collins, 31 January 2003, invited seminar at the Genetic Resources Conservation Center of USDA: "Searching for new bean germplasm in the Americas".

Mexico, 10 February 2003, invited conference at the University of Morelia: "Evolución temprana del frijol en el Occidente de México".

Mexico, 13 February 2003, invited seminar at the University of Guadalajara: "Entre Maguey y Cempoalxochitl. Observaciones sobre los recursos fitogenéticos del Occidente de México".

New York, USA, 9 June 2003, invited seminar at the New York Botanical Garden: "Bees and beans: diversity of sweets, tongues, mutualisms, and consequences".

Lima Peru, 17 July 2003, invited presentation (with Dr W Roca of CIP) at the Regional Workshop on Access to Genetic resources in the Andean Region: "Mecanismos de intercambio de germoplasma de los Centros del CGIAR".

Washington, USA, 26 August 2003, invited seminar at the Smithsonian Institution, National Museum of Natural History: "*Phaseolus* beans in the Pliocenic transit lounge of Panamá: last findings of markers and cpDNA analyses".

Bogotá, Colombia, 26 September 2003, invited presentation (with Dr E Torres of IvH) at the Regional Workshop on Access to Genetic resources and protection of indigenous knowledge: "Propuesta de mecanismo de seguimiento y control, luego de un contrato de acceso a recursos genéticos".

6.4 List of international and national courses with input from Project Staff 2003

International Course on Bean Production and Breeding, CIAT, October 2002.

Master's Degree Programme in Plant Genetic Resources, Universidad Nacional de Colombia, October 2002.

International course on genebanks management (with IPGRI RefOff Americas), Universidad Austral de Chile, Valdivia, January 2003.

Curso manejo integrado de la enfermedad del Moko en platano. CIAT-Ministerio de Agricultura de Colombia. CIAT, Palmira, June 09-13, 2003

Course for the Colombian Association of Herbaria (ACH), annual meeting, Ibagué, Colombia, June, 2003.

6.5. List of trainees trained by Project Staff in 2003

In vitro Lab

Pereira da Paz, Oswaldo. Training in conservation and management of *in vitro* cassava germplasm. Brasil, 26 Octubre 2002.

Aguilar, Lesbia. Training in conservation and management of *in vitro* cassava germplasm. República Dominicana, 4 March 2003.

Mejia, Julio. Training in conservation and management of *in vitro* cassava germplasm. República Dominicana, 4 March 2003.

In Germplasm Health Lab

Osvaldo Pereira da Paz. EMBRAPA- Cruz das Almas, Bahía. Brasil.Training in Cassava virus indexing techniques (Elisa Test and grafting). December 2002.

María Margarita Hernández. INCA.. La Habana - Cuba. Training in Cassava virus indexing techniques (Elisa Test and grafting).December 2002.

Lesbia Aguilar. INTA. Nicaragua. Training in Cassava virus indexing techniques (Elisa Test and grafting).March 2003.

Julio Mejía. IDIAF. República Dominicana. Training in Cassava virus indexing techniques (Elisa Test and grafting).March 2003.

Carlos Andrés Hidalgo. Escuela Politécnica del Ejército del Ecuador. Training in Cassava virus indexing techniques (Elisa Test and grafting). September 2003.

Adrián A. Herrera. Escuela Politécnica del Ejército del Ecuador. Training in Cassava virus indexing techniques (Elisa Test and grafting). September 2003.

César Augusto Vera.. Escuela Politécnica del Ejército del Ecuador. Escuela Politécnica del Ejército del Ecuador. Training in Cassava virus indexing techniques (Elisa Test and grafting). September 2003.

Students of Universidad del Amazonas, Magister of Universidad Nacional de Colombia- Palmira, October 2002.

Students of Universidad Nacional de Colombia- Palmira, Novembe 2002, Universidad de Caldas, May 2003, Universidad Nacional de Colombia- Palmira, June 2003.

6.6 Visitors

The Professional Staff of the Genetic Resource Unit attended the visit of 558 people from 54 different government bodies, institutions, companies, etc. A total of 214 people from 12 institutions visited the in Vitro Lab (universities, schools, and farmers).

6.7. Donors

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

Ministerio de Agricultura y Desarrollo Rural, República de Colombia