ANNUAL REPORT 2002

CIAT Project on Saving Agrobiodiversity SB-01/02

Genetic Resources Unit

Report on Achievements and Progresses

CIAT
OCTOBER, 2002

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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:

2002 Cassava cryopreservation implemented. Gene transfer used to broaden the genetic base and enhance germplasm of rice, cassava, and the forage grass *Brachiaria*. Screening with microarray technology initiated. Marker-assisted selection implemented for rice, beans, and *Brachiaria*. ESTs generated for cassava starch and CBB. A LIMS developed. Procedures developed for conservation of wild species and landraces, based on studies of seed biology and physiology. Safe-duplication and restoration continued.

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 (2003-2005)

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 Assumptions
Goal To contribute to the sustainable increase of productivity and quality of mandated and other priority crops, and the conservation of agrobiodiversity in tropical countries.	CIAT scientists and partners using biotechnology information and tools in crop research. Genetic stocks available to key CIAT partners.	**CIAT and NARS publications.** Statistics on agriculture and biodiversity.	
Purpose To conserve the genetic diversity and ensure that characterized agrobiodiversity, improved crop genetic stocks, and modern molecular and cellular methods and tools are used by CIAT and NARS scientists for improving, using, and conserving crop genetic resources.	Information on diversity of wild and cultivated species. Mapped economic genes and gene complexes. Improved genetic stocks, lines, and populations.	Publications, reports, and project proposals.	Pro-active participation of CIAT and NARS agricultural scientists and biologists.
Output 1 Genomes characterized of wild and cultivated species of 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 for Al tolerance in *Brachiaria*.	Publications, reports, and project proposals. Germplasm. Availability of a laboratory information management system (LIMS).	Availability of up-to-date genomics equipment, and operational funding.
Output 2 Genomes modified: genes and gene combinations 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. New 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 standards.	Germination rates for long-stored materials. Cost per accession/year, compared with other gene banks.	Visits to GRU substations and conservation facilities.	Absence of uncontrolled diseases. Quarantine greenhouse space available at different altitudes.
Output 5 Germplasm available, restored, and safely duplicated.	Number of germplasm requests received and satisfied annually. Users received germplasm and data. Users asked for novel germplasm and data.	Visits to multiplication plots. Reports on requests and delivery. *Number of core collections multiplied and shipped.*	Agreement with CIAT holds.
Output 6 Designated Collections made socially relevant.	Landrace diversity restored to farmers. Farmers use new varieties. Breeders use novel genes.	Germplasm catalogs. Plant variety registration logs. National catalogs.	International collecting possible. Quarantine matters cleared.
Output 7 Strengthen NARS for conservation and use of Neotropical plant genetic resources.	NARS germplasm collections conserved. Number of trainees trained at CIAT. Number of universities and NARS using training materials.	Country questionnaires. Courses registered. Distribution and sales of training materials.	NARS and networks willing to cooperate.
Output 8 Conservation of Designated Collections linked with on-farm conservation efforts and protected areas.	Number of case studies and pilot *in situ* conservation projects.	Project documentation. Publications	NARS interested in conservation efforts. Farmers interested in conservation efforts.

SUMMARY ANNUAL REPORT 2002

Genetic Resources Unit

SB-01/02 PROJECT

Title: Integrated Conservation of Neotropical Plant Genetic Resources

3.1. Researchers:

Daniel G. Debouck, Head, PhD
Alba Marina Torres, Biologist, M.Sc.
Graciela Mafla, Biologist
Julio C. Roa, Biologist
César Ocampo, Biologist, M.Sc.
Orlando Toro, Technician
Arsenio Ciprián, Technician
Roosevelt Escobar, Biologist
Benjamin Pineda, Ing. Agr., M.Sc
Norma Cristina Flor, Ing. Agr.
Maria del Socorro Balcazar, Bacteriologist

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:

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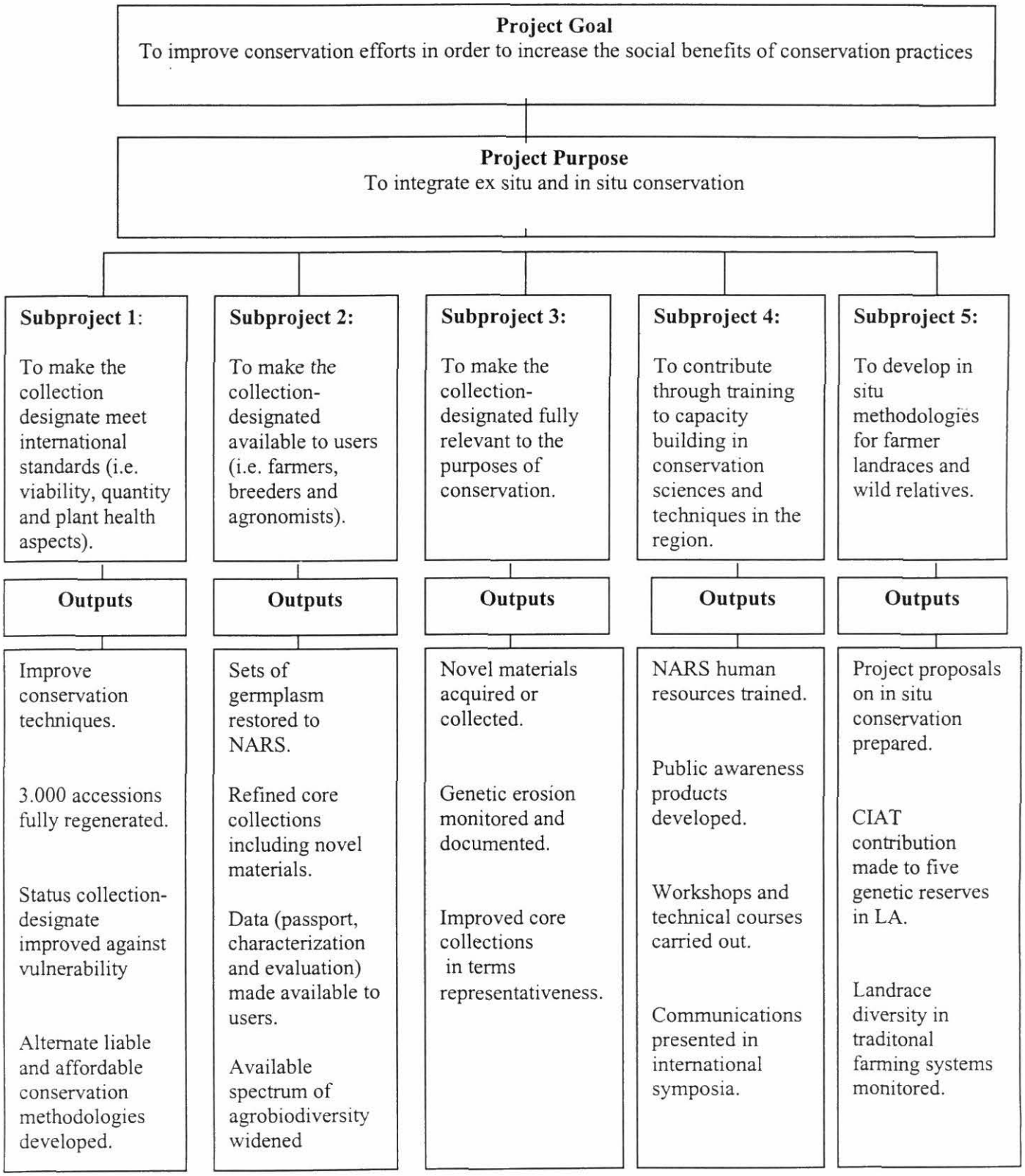
Dr. Hans Jorg Jacobsen, University of Hannover , Germany
Dra. Inés Sánchez, CORPOICA, Colombia
Dr. Mario Lobo, CORPOICA, Colombia
Dr. Samy Gaiji, SINGER, IPGRI, Italy
Dr. Jane Toll, SGRP, IPGRI, Italy
Dr. Jean Henson, ILCA, Ethiopia
Dr. Bonwoo Koo, IFPRI, USA
Dr. Jil Lenné, ICRISAT, India
Dr. Phil Pardey, IFPRI, USA
Dr. Ramón Lastra, IPGRI – Americas, Colombia
Dr. David Williams, IPGRI – Americas, Colombia
Dr. Geo Coppens, CIRAD-FLOHR, Colombia
Dr. Katy Williams, USDA, USA
Dr. George Freytag, USDA, USA

Genetic Resources Unit work breakdown structure



Genetic Resources Unit Logical Framework

Head: Daniel G. Debouck

Sub-Project #1: the International Standards

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
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	506,778	86,5
Carryover from 2001	14,355	2,4
Sub Total	**521,133**	----
Special projects		
SINGER	11,693	2,0
Gene Flow BMZ	51,622 (- 15,000)	8,8
Carica IICA-IPGRI	1,477	0,25
Sub Total	**64,792**	----
TOTAL	**585,925**	100

3.4. Highlights in 2002

Activity area # 1: the International Standards

The GRU continued with the implementation of a digital image bank in order to facilitate access by internet users. On the other hand, the effort to test the cryoconservation of the cassava core collection continued steadily, with 1/3 of the core being maintained in liquid nitrogen and periodically tested. Research on genetic stability of cassava after conservation either in liquid nitrogen or in vitro with silver nitrate yield very positive indications, opening ways to use these technologies to conserve collections at low cost in the future. Preliminary results indicated that the slow growth method could be applied to other vegetatively propagated crops as well (e.g. lulo, tree tomato).

Activity area # 2: the Germplasm and its data available

The distribution of germplasm out of the FAO designate collections continues on the high side (7,557 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. Worth noting in comparison to previous years is the distribution of cassava germplasm (over 2,400 accessions distributed to CIAT projects) and forage germplasm (outside recipient requests are higher than CIAT projects). As a result of the cleaning and indexing effort launched in 1996 73% of the cassava collection is now indexed against viruses of quarantine importance and available for distribution. As a by-product of this work 2,169 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. On the other hand, advances were obtained on the control of fungi diseases of quarantine importance in *Brachiaria* germplasm with systemic fungicides, opening ways to safely deliver that germplasm in the future.

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

Through collaborations, additional information has been obtained on the structure of genetic diversity on highland papayas, avocado and beans from Colombia. Novel germplasm of forages and cassava was obtained from Australia and Argentina, respectively, and introduced in the FAO designate collections. A 14-years effort to better understand the taxonomy of *Phaseolus* has been completed.

Activity area # 4: the International cooperation and capacity building

The diffusion of research results continued to be a priority area for the GRU in 2002. GRU Staff provided input into 5 international/ national courses. Eleven professionals and four groups were given individualized training. Scientific presentations were made in 11 symposiums, congresses, workshops and fora. Fourteen papers were published during 2002.

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

Preliminary results from the BMZ supported project on gene flow and carried out with the University of Costa Rica indicate that gene flow in the bean model can be substantial although with high level of variation between sites and seasons. Such information shall be key for the development of management packages that shall be put in place in future production systems with transgenical crops.

3.5. Problems encountered and their solution

As mentioned in previous reviews ICER'95, '97, and '00, operational funding is a continuing constraint to fully operate the GRU for the FAO in-trust collections (the balance 60% in personnel and 40% in operations is far from being reached). Another limiting factor is the lack of flexibility of the personnel side, as continuing contracts seem to be against work productivity. The Agreement with MADR Colombia is bringing most of the operational money of the Project, but is targeted mostly to cassava and fruits, while most of the operations of the GRU, especially during the Upgrading effort, are in the conservation of beans and tropical forages. The Agreement with MADR has been very positive so far towards this situation, but the operations and commitments towards the FAO in-trust collections cannot be "substituted". Since 1996, the GRU has been seeking additional money from the SGRP for the Upgrading Plan, and recently from the Global Conservation Trust (= endowment fund initiative).

Although formally announced to the BOT as early as 1998, the GRU has not received the full support of the Information Unit. However, this Unit has provided supervision and guidance to students paid by the GRU for the modernization of the computer system. Although slow, noticeable progress has been achieved (ORACLE system, bar coding, link to CIAT website).

3.6. Plans for next year

- Finish with the implementation of the bar coding in the field area and glass-houses
- Refine the management modules of the GRU computer system as required
- 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
- Implement fully the 'bonsai' approach in glass-house at CIAT for a duplicate of the cassava collection
- Complete and publish the protocols about minimal growth *in vitro* for cassava and for fruit germplasm
- Expand the cryoconservation of cassava germplasm to 2/3 of the 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
- Publish the cost analysis of cassava germplasm conservation with IFPRI
- 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

- Continue with 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

Following up on the recommendations of the external reviews of 1995, 1997, 2000, and in order to have the FAO designate collections in line with international standards, the GRU has continued the upgrading effort, in spite of financial constraints and security problems. Seed rejuvenation has been carried out for aging seed stocks (3,935 and 2,753 accessions of beans and forages, respectively). The cleaning and certification of the cassava collection against viruses of quarantine importance has made significant progress, as 73% of the collection is now ready for safe distribution. An indirect result of that work is the availability of 2,169 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 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 7,557 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. Advances were also obtained in genetic diversity studies of highland papayas, mycorrhiza, and avocado. Five international/ national courses benefited from GRU Staff input, and several trainees were personally attended. Presentations about the International Treaty on Plant Genetic Resources for Food and Agriculture were made in several fora.

4. Project performance indicators

1.FLOWS, TECHNOLOGIES, METHODS & TOOLS

1.1. Backlogs cleared
864 accessions cleared
1.2. Accessions regenerated
3,935 of beans, 2,753 of tropical forages
1.3. Accessions secured in long-term
1,087 accessions secured
1.4. Accessions in security back-up
No shipment this year; 1,087 accessions added
1.5. Accessions characterized
6,688 (field) + 2,065 (image bank)
1.6. Accessions distributed with passport data
7,557 accessions distributed
1.7. Novel germplasm acquired
146 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: 1

2.2. **Refereed Journals:** submitted: 2
2.3. **Book Chapters:** published: 1
2.4. **Published Proceedings:** published articles: 10
2.5. **Scientific Meeting Presentations:** presentations: 11
2.6. **Working Papers, Other Presentation or Publications:** 3
(see under 6 in full report)

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

3.1.**Training Courses :** 5

3.2. **Individualized Training :** 16

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

4.0 RESOURCE MOBILIZATION

4.1 Proposals and concept notes submitted
- Global Conservation Trust with SGRP
- Popping beans: an option to save montane forests, and to develop rural enterprises in Colombia

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

Significant advances have been registered in the development of an image bank in the GRU. Table 1 indicates the number of images that have been captured and that are accessible by internet users. Images have been taken for five cultivated species of bean (*P. vulgaris, P. coccineus, P. polyanthus, P. acutifolius and P. lunatus*) and all wild species. The 209 images of common bean represent a very small proportion of the total collection for this species, but rather are a sample of the phenotypic variability that exists in the gene bank. Sample images appear in Figures 1 and 2. Within a 6-8 year time frame, we hope to complete the bank of images for the entire collection. In the future these images will be complemented by other data such as patterns of seed proteins (already done for tepary bean). Apart from making easy many internal operations of GRU (e.g. control of seed purity), these images may also contribute to prevent cases of biopiracy (e.g. 'enola' case). In the case of tropical forages, where very little phenotypical variation is expressed in the seed testa, there is no need to take a picture of all accessions as seed; 174 pictures have been taken basically for internal management purposes. However, images of fully developed plants in the field are useful to users; 331 pictures of such plants have been taken.

Table 1. Numbers of images of gene bank accessions accessible by internet.

Species	Number images	Per cent of accessions
Common bean	209	1%
Coccineus complex	305	25%
Tepary bean	330	100%
Lima bean	33	1,5%
Wild Phaseolus species	261	100%
Tropical forages	505	N.A.
TOTAL	1,643	

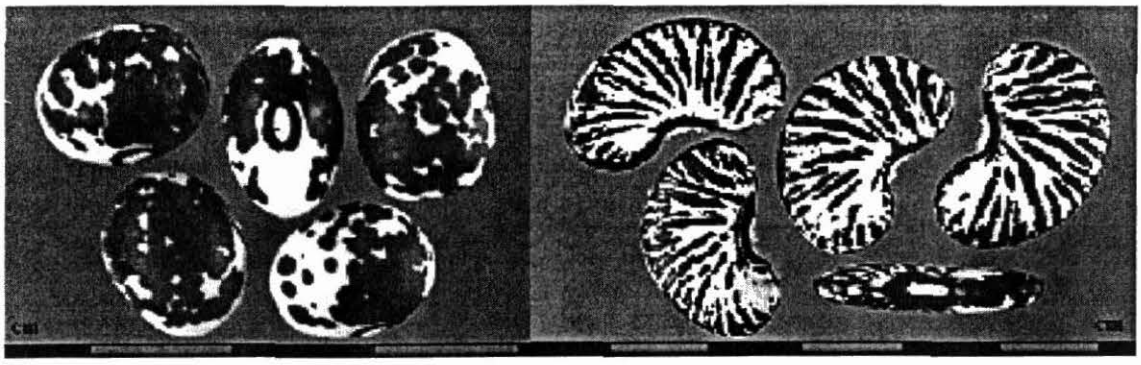


Figure 1.

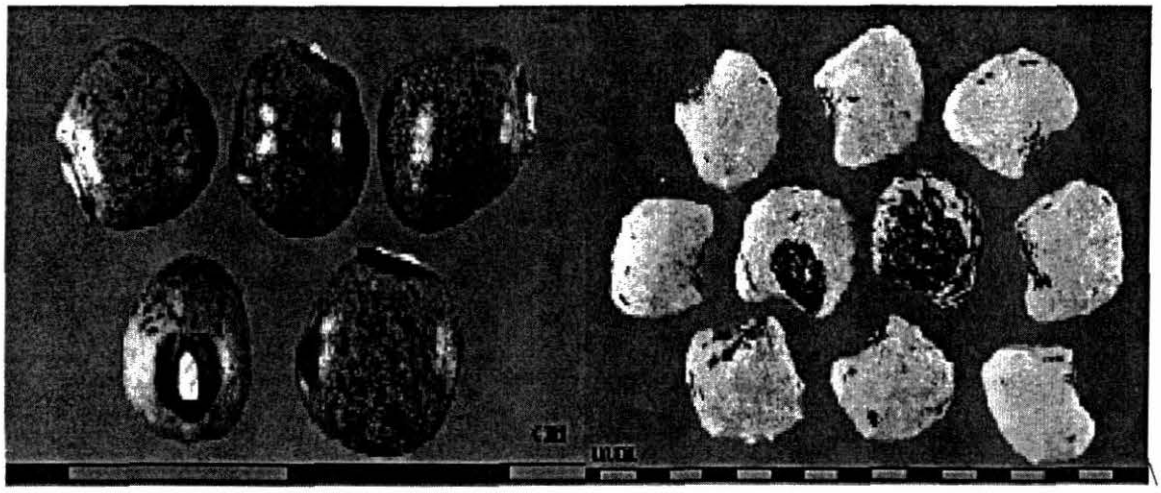


Figure 2.

Similarly, an image bank for cassava (422 images) with the descriptor "root pulp colour" has been started in order to facilitate both internal operations and selection of germplasm by internet users (Figure 3). On the other hand, the bar coding has been implemented for the total collection of cassava (5,728 clones).

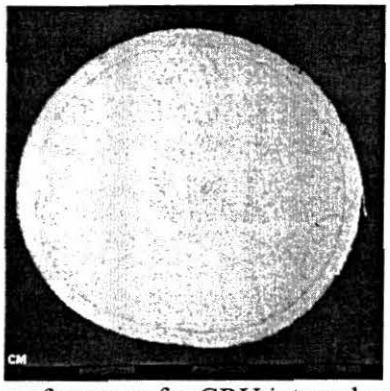


Figure 3. Image of root pulp color of cassava for GRU internal use and internet users.

Contributors: A. Ciprian, G.Mafla, J.C. Roa , D.M. Montero, S. Ramírez, O. Toro.

Output 1.1. Backlogs of received materials processed

Activity 1.1.1. Introduction of germplasm into genebank processes

This activity refers to the processing of germplasm obtained in the past either through germplasm explorations or donations by NARS. In beans, a large survey of the 13,708 pending accessions has been undertaken, and from a first analysis a total of 511 accessions were estimated to be duplicates of accessions already existing in the genebank, and thus not introduced into the GRU processes. In forages, from a total of 1,360 accessions pending on introduction in 2001, 214 were processed.

Out of the collection donated by CSIRO, Australia, 248 accessions were introduced and assigned with G number and planted this year. In cassava, a collection of 179 accessions of wild species is still pending on introduction. See Table 2.

Table 2. *Status of materials introduced into process* (number of accessions)

	Beans	Forages	Cassava	Total
Germplasm pending in 2001	13,708	1,360	179	15,247
Processed in 2002	650	214 (+248)	--	864
Germplasm pending	13,058	1,146	179	14,383

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

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 2002, 507 bean materials and 462 forage materials were multiplied.

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

Output 1.3 Materials pending on regeneration

Activity 1.3.1. Multiplication of materials with ageing seeds

Table 3 shows a total of 3,935 accessions of beans regenerated in 2002 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), 2,753 accessions have been multiplied at three localities in 2002. 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	1,633
Popayán	2,302
Total	3,935

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.	751	235	986
Palmira	395	133	599	288	1,415
Popayán	N.A.	N.A.	46	306	352
Total	395	133	1,396	829	2,753

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

Activity 1.3.2. Periodical subculturing of the FAO designate cassava collection

This year, 6,162 materials (5,016 accessions) of *Manihot* were subcultured by the nodal cutting technique; the accessions multiplied represents 87.5% of the collection. A total of 7,278 plants (30 accessions) were propagated for the IP-03 Project for distribution to users.

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 amount of accessions for beans (1,301) and forages (860) respectively, which have been cleaned, dried, and stored at 5°C awaiting results from viability and health tests.

Table 5. Germplasm in seed processing

	Beans	**Forages**
Seed selection / temporal storage	1,301	860
Total	1,301	860

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

Activity 1.4.2. Viability testing for recently produced germplams and germplasm stored in the past

Table 6 indicates flows of materials during 2002. It shows the importance of good drying and other improved procedures established since 1996. Ranges of germination were chosen because viability lower than 65 do not allow seed distribution and viability lower than 85 do not allow long term seed conservation (FAO standards, 1994).

In order to support multiplication activities for very old seeds the viability lab pre-germinated the accessions of forages using several techniques such as sand beds, petri dishes and germination paper.

Table 6. Viability testing for *Phaseolus* beans and tropical forages during 2002

	PHASEOLUS		FORAGES	
Class	Germination (%)	No. Accesions	Germination (%)	No. Accesions
Already stored materials	1-64	170	1-64	30
	65-84	162	65-84	106
	85-100	210	85-100	71
Sub-total		542		207
Recently multiplied materials	1-64	12	1-64	38
	65-84	8	65-84	130
	85-100	54	85-100	393
Sub-total		74		561
TOTAL		616		768

Contributor: A.M. Torres

Activity # 1.4.3 Monitoring viability of conserved germplasm of seeds of beans and forages

This year we did the monitoring after 5 years of long term conservation of *Phaseolus vulgaris* for 3 groups of seeds: Ten1996A, Ten1996B and Ten1997A. A total of 2,162 accessions were tested and the results are shown in Table 7.

The mean of germination of the three groups changed 10.31 units for Ten1997A, 12.30 for Ten1996A and in 17.18 for Ten1996B, after 5 years of conservation. The difference is statistically significant in all cases, with confidence of 95%; the decrease in germination is on the high side. It is necessary to revise each accession in detail, because the most dramatic decrease is due to unhealthy seedlings.

Table 7. Paired T-Test for monitoring bean seeds after 5 years of long-term conservation

Source	% Germ	Mean	Std.Dv.	N	Diff.	Std. Dv. Diff.	t	Df	p
Ten1996A	1	97.31299	3.498847						
Ten1996A	2	85.00999	22.87446	901	12.303	23.08362291	15.99813	900	0.00
Ten1996B	1	97.01181	3.752335						
Ten1996B	2	79.82415	22.02341	762	17.18766	22.0082461	21.55802	761	0.00
Ten1997A	1	98.20441	2.946358						
Ten1997A	2	87.88778	18.17506	499	10.31663	17.94290207	12.84386	498	0.00

T-test for Dependent Samples (monitoreo1996a.sta)
Marked differences are significant at $p < .05000$

Contributor: A.M. Torres

Activity 1.4.4. Final packing and orientation of germplasm into five conservation purposes

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 (= base), safe duplicates, restoration, periodical monitoring, and distribution (Tables 8-9).

Table 8. Final storage and packing of *Phaseolus* beans processed during 2002 (number of accessions)

	TEN1999B	POP2000-2001
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	546	49
SHORT TERM only (Distribution)	2,610	1,030
Total	3,156	1,079

Table 9. Final storage and packing of tropical forages processed during 2002 (number of accessions)

	Legumes	Grasses
LONG TERM (Base, duplicates, repatriation, monitoring) + SHORT TERM (Distribution)	412	80
SHORT TERM only (Distribution)	1,426	354
Total	1,838	434

Contributor: A.M. Torres

Output 1.5. Improved conservation techniques

Activity 1.5.1 Total amount of seeds to conserve wild bean germplasm

We currently conserve more than 30 species of wild and cultivated beans. The seed production largely varies between species. We have determined the seed quantities along the five conservation purposes (base, viability monitoring, distribution, restoration and safe duplication). The total amount has a range from 400 seeds for species such as *P. grayanus*, *P. macrolepis* and *P. polystachyus*, to 4,000 seeds for species *P. micranthus*, *microcarpus* and *macvaughii*. Fixing these quantities was important in order to process more accessions per year.

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

Activity 1.5.2. Protocol for seed conservation of tree tomato (*Cyphomandra betacea*) and *Brachiaria humidicola*

The purpose of this study is to establish a protocol to conserve seeds of *Cyphomandra betacea* (tree tomato) and *Brachiaria humidicola*. These two species are reported as ortodox (Hong et al. 1996), thus the same protocol was applied to both: three moisture contents 4, 8, 12% and three temperatures: +5, -18 and –196 °C. The conservation started on 8th of April with two monitoring times (3 and 6 months).

Methods

Seeds were obtained by soaping the pulp of mature fruits of two varieties of tree tomato (red and yellow fruit) (Cárdenas, 1998). Floating seeds possibly with ill-formed embryos were discarded. Dry cariopses of *Brachiaria humidicola* were harvested in Popayan CIAT's station between January and March, 2002. A seed blower (Seedburo MO-SB/C) was used to discard empty seeds.

Germination and viability testing

In order to obtain the best substrate to do the germination test for *C. betacea*, three substrates were tested (Table 10):

- Filter paper in petri dishes, using a germinator Hoffman SG-3055 with 35 °C for 8 lighted hours and 20 °C for 16 dark hours and 45% of humidity.
- Germination roll paper and a germinator with the above environment.
- Sand beds at environmental conditions.

Ten treatments were evaluation to determine the best way to break dormancy in tree tomato seeds (Table 11). For Brachiaria humidicola, break dormancy was done according to ISTA (1999) with chemical scarification using H2SO4 96% during 20 minuts and KNO3 0.2% in the first water to the seedlings.

Table 10. Comparison of germination sustrates for *C. betacea* seeds

SUSTRATE	% GERMINATION		ADVANTAGES	SHORTCOMINGS
	Red fruit	Yello fruit	Visual check of number of seeds.	Susceptibility to fungus contamination
Filter paper in petri dishes		25% n = 400	Control of humidity, temperature and light factor in the germinator	High costs of filter paper Large space needed in germinator.
Paper rolls	71% n =400	64% n =400	Visual check of number of seeds Control of humidity, temperature and light factor in the germinator Optimization of space in the germinator	High costs of germination paper
Sand beds	68% n =400	62% n =400	Low cost of sand No germinator is needed Reducing risk of fungus contamination	It is difficult to control the number of seeds It is not posible to control humidity, temperature, light and low repeatibility

Looking for a good color pattern for the viability test with tetrazolium salt, the follow methods were used:

- Seed cut at the oposite side of hilum
- A stitch at the center of the seed
- Seed cut at the same side of hilum
- Seed cut close to the cotyledons

For *Brachiaria humidicola* we follow the germination and viability test protocol of ISTA (1999). Filter paper is recommended for germination and the follow steps for viability: water imbibition during 18 hours, longitudinal cut and imbibition in tetrazolium salt 5% during 4-5 hours at 40 °C.

Table 11. Treatments used to break dormancy

TREATMENT	% Germination 30 days Red fruit n = 200	% Germination 30 days Yellow fruit n = 200
Control	63	59
Mecanic scarification (stitch)	45	48
Mecanif scarification (cut)	--	17
Imbibition in KNO_3 1% 24h.	79	75
Imbibition in GA_3 500ppm 24h	85	82
Imbibition in H_2SO_4 1% 5min.	79	79
Imbibition in KNO_3 + KH_2PO_4 1.5% 24h.	80	73
H_2SO_4 1% 5min + Imbibition in GA_3 500ppm 24h	**100**	**100**
H_2SO_4 1% 5min + Imbibition in KNO_3 1% 24h.	88	84
H_2SO_4 1% 5min + Imbibition in KNO_3 + KH_2PO_4 1.5% 24h	80	79

Desiccation tolerance and conservation

Three moisture contents were tested: 12, 8 and 4%. These levels were reached in dry cabinets in presence of silica gel (23 °C and 21% humidity). The total amount of seeds was then subdivided and vacuum packed in aluminum foil bags. After that, the bags were placed at each temperature for conservation. An initial monitoring of viability was done, and two monitoring tests were performed after 3 and 6 months.

Results

In Table 9 are shown all the sustrates used for the germination of tree tomato seeds. Using DMS test there was no significative differences between the germination rates in paper rolls and sand bed.

For viability testing with tetrazolium salt the best method was the following:

- Imbibition in water at environment temperature for 18 hours
- A stitch at the center of the seed
- Imbibition in tetrazolium salt at 0.5% during 24 hours at 40 °C
- Seed cut longitudinally

Breaking dormancy for seeds of *Cyphomandra betacea*

Seeds of *C. betacea* have dormancy and this was confirmed with 60% germination of seeds five days after extraction without any treatment. It is important to determine the kind of dormancy to do the best treatment to break it down.

Two types of dormancy are found in *C. betacea*: physical dormancy or exogenous linked to the seed coat, which is not fully water resistant. It allows the seed to have moisture content of 50% maximum when it is in water imbibition (Figure 4). Endogenous dormancy or physiological intermediate dormancy associated to inhibitors in

the embryo and other seed structures (Baskin & Baskin, 1998). Table 11 shows the treatments used to break dormancy.

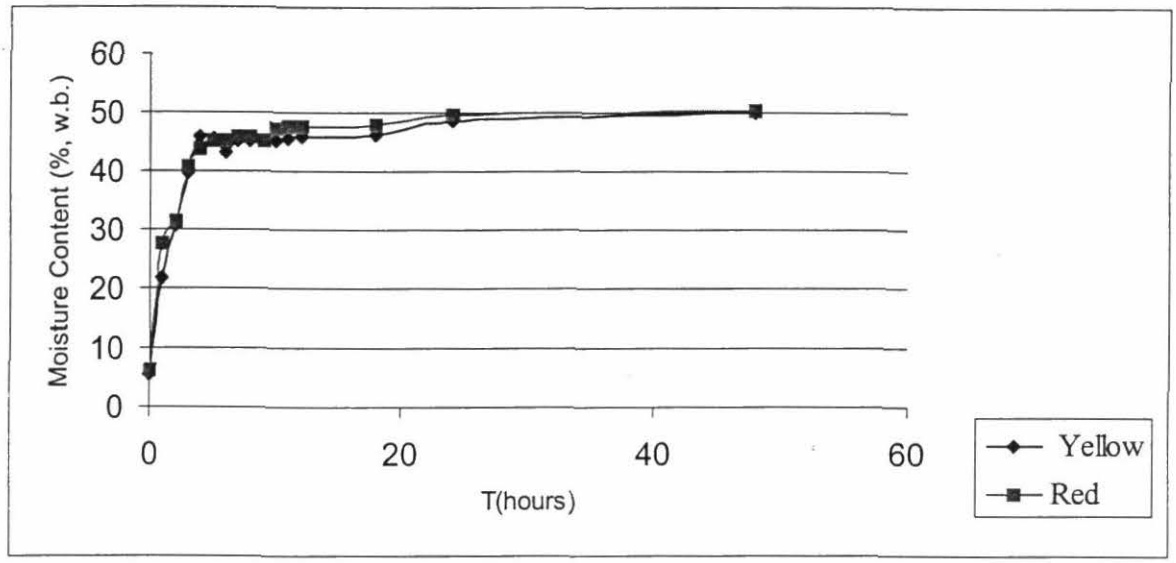


Figure 4 - Seed imbibition dynamics for *Cyphomandra betacea*

Desiccation tolerance

As a final process in the conditioning of seeds were ploted the decreasing of moisture content under dry rooms reaching the moisture content for seed conservation (12, 8 and 4%) (Figures 5 and 6).

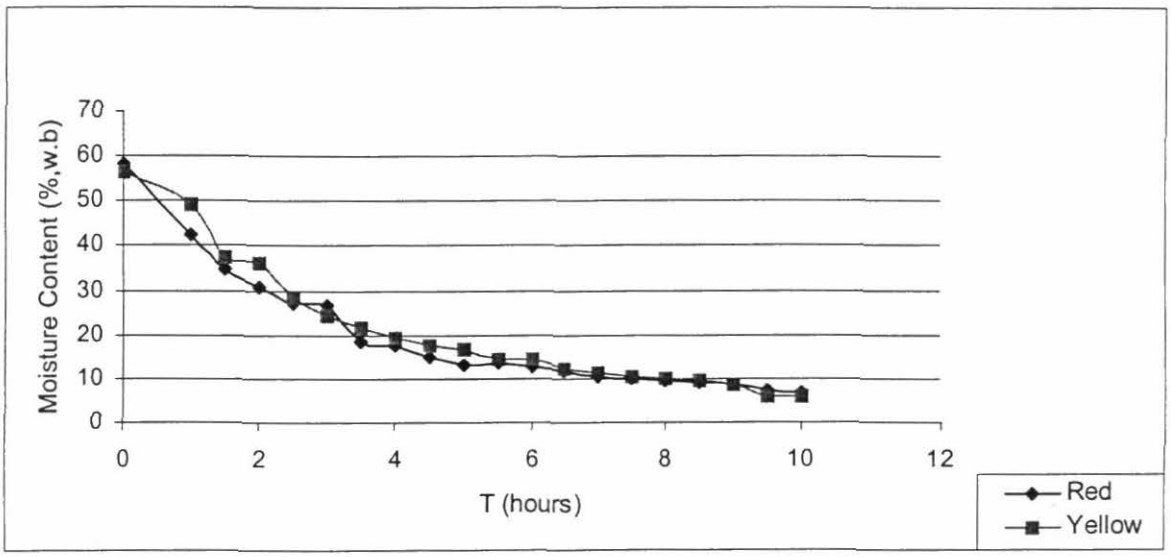


Figure 5. Seed desiccation for *Cyphomandra betacea* (red and yellow fruit)

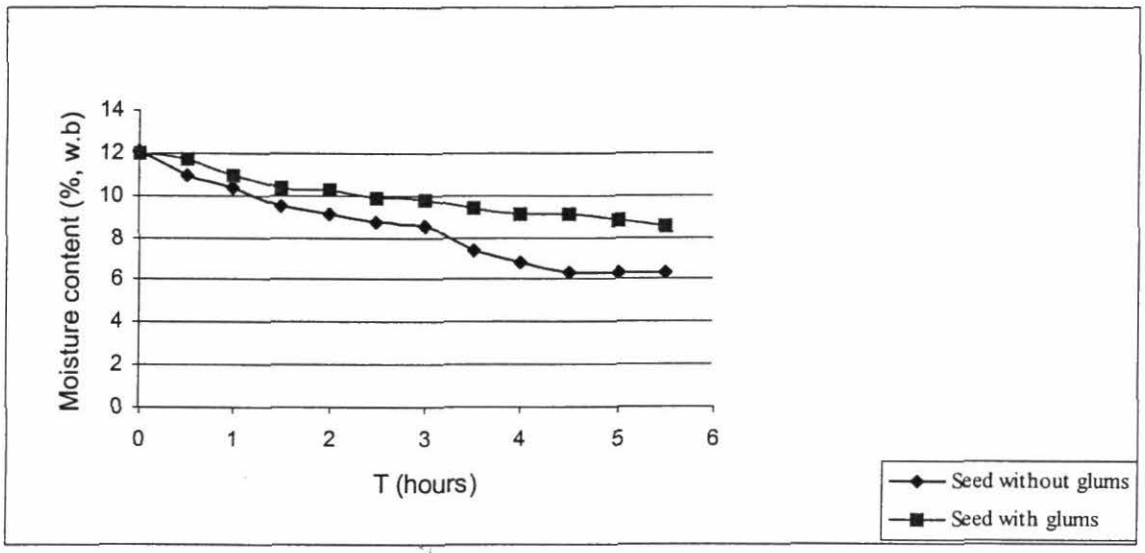


Figure 6. Seed desiccation for *Brachiaria humidicola* (without and with glumes)

Germination test through the time and interaction between conservation factors

Twenty-four treatments were evaluated (2x2x2x3 factorial), in a fully ramdom factorial model with four reptitions. Finally, with the initial germination data, 0 months, (Table 12 and 14) and germination at three months (Tables 13 and 15) a MANOVA was performed where the variation sources were the individual factors (time, temperature, moisture content and conditions for *Brachiaria humidicola* and variedy for *C. betacea*) and the interactions.

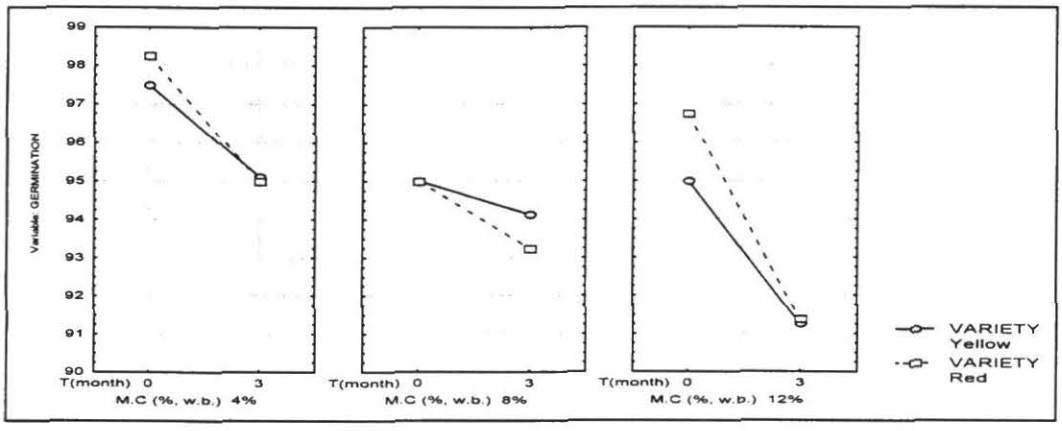


Figure 7. Comparison of factors in seeds of *Cyphomandra betacea*

When significative differences were found at 5% probability with a F test, a comparison of averages was done with Duncan test.

The factor analysis shows that time and moisture content are the factors with significative differences between treatments. Variety and temperature did not show significative differences. The behavior of the two varieties at

different conservation conditions was similar (Figure 7). However, total germination decreased through time and the reduction was 2.9% during the first three months of conservation.

Analysing the second factor with significative differences (moisture content), we see that there is no difference between levels 4 and 8%, but there is a strong difference at level 12%. This indicates that reducing moisture content in seeds of tree tomato decreases the loss of viability.

These results indicate a clear tendency of ortodox behaviour of the seeds of this species as previously observed by Hong & Ellis (1996).

In *Brachiaria humidicola* a decrease of 4.7% in germination through time was observed. Analyzing the interaction of factors it was found that there are significative differences between conditions (whith glumes and whitout glumes), time and moisture content (Figure 8). Our results show that the best conservation condition was without glumes, independently from the temperatures evaluated. This occurs perhaps because low temperatures act as stratification and help seed to react to the chemical treatment with KNO3, as part of the protocol for seed germination.

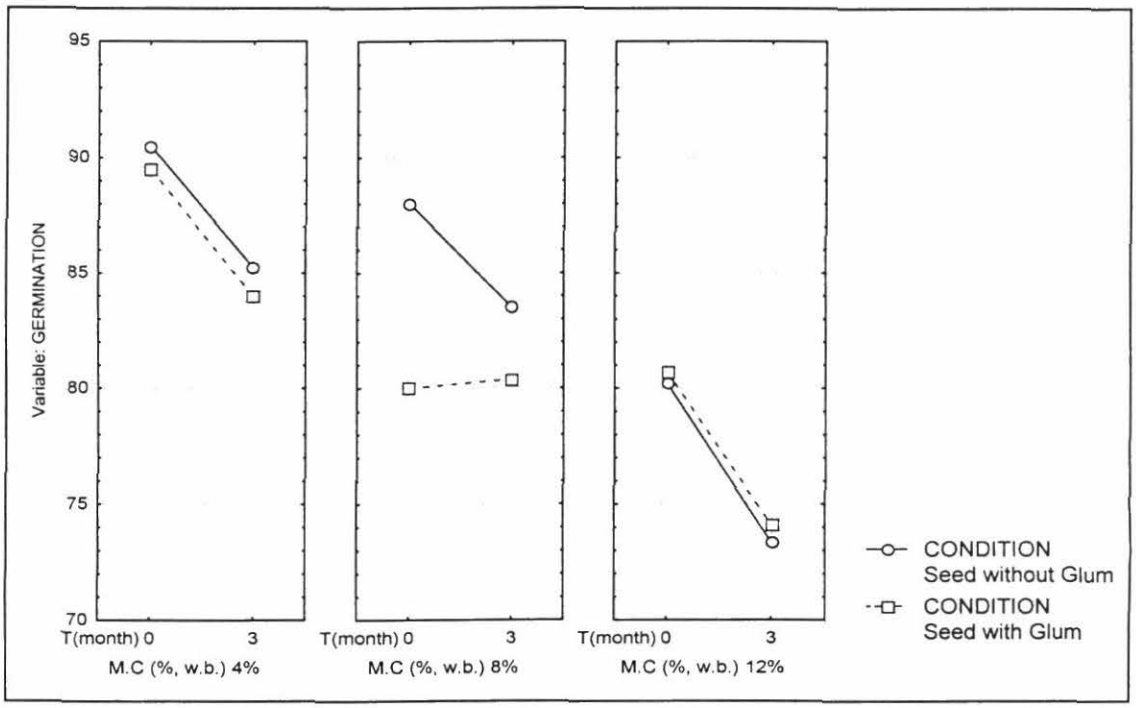


Figure 8. Comparison of factors in seeds of *Brachiaria humidicola*

Table 12. Initial % germination at 0 months for *S. betaceum*

Seed moisture content M.C. (h.b.)	% Germination - Red fruit (15 days after extraction)	% Germination - Yellow fruit (15 days after extraction)
12%	97	95
8%	95	95
4%	97	98

Table 13. Percentage of germination at 3 months for *S. betaceum* varieties with red and yellow fruits

Red fruit			Yellow fruit		
M.C. (h.b.)	Temperature ° C	X %germination	M.C. (h.b.)	Temperature ° C	X %germination
12%	5	91	12%	5	92
	-20	92		-20	90
8%	5	93	8%	5	93
	-20	93		-20	95
4%	5	96	4%	5	94
	-20	94		-20	95

Table 14. Initial % germination at 0 months for *B. humidicola*

Seed moisture content M.C.(h.b.)	% Germination - Seed without glumes (60 days after harvest)	% Germination - Seed with glumes (60 days after harvest)
12%	80	80
8%	88	80
4%	90	89

Table 15. Percentage of germination - 3 months for *B. humidicola* seed without and with glumes

Seed without glums			Seed with glums		
M.C.(h.b.)	Temperature ° C	X %germination	M.C. (h.b.)	Temperature ° C	X %germination
12%	5	73	12%	5	76
	-20	73		-20	72
8%	5	84	8%	5	80
	-20	83		-20	81
4%	5	85	4%	5	85
	-20	85		-20	82

Literature cited

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Contributors: Jesús Salcedo (Universidad del Valle), A. M. Torres Gonzalez

Activity 1.5.3 Protocol for seed conservation of *Carica papaya*

The second phase of this study was focused on testing the seed conservation protocols for seeds of *C. papaya* over one year, testing two varieties, three moisture contents, four temperatures for conservation, and three

evaluation periods. The main purpose was to study the tolerance of *Carica papaya* seeds to desiccation and the conservation through time.

- Evaluate the reaction to desiccation with moisture contents of 11, 9 and 5%.
- The temperatures evaluated were +22, +7, -20 and –196 °C.

Table 16 shows the factors and germination (averages of 4 samples)

FACTORS			(%) GERMINATION	
Moisture content	Time	Temperature	Variety 1	Variety 2
5%	0	+22C	79	100
5%	1	+22C	95	100
5%	2	+22C	99	100
5%	0	+7C	79	100
5%	1	+7C	91	96
5%	2	+7C	98	100
5%	0	-196C	79	100
5%	1	-196C	93	93
5%	2	-196C	91	95
5%	0	-20C	79	100
5%	1	-20C	88	91
5%	2	-20C	82	95
9%	0	+22C	81	100
9%	1	+22C	96	100
9%	2	+22C	98	100
9%	0	+7C	81	100
9%	1	+7C	95	96
9%	2	+7C	93	100
9%	0	-196C	81	100
9%	1	-196C	93	94
9%	2	-196C	94	84
9%	0	-20C	81	100
9%	1	-20C	93	93
9%	2	-20C	93	96
11%	0	+22C	91	98
11%	1	+22C	97	89
11%	2	+22C	96	80
11%	0	+7C	91	98
11%	1	+7C	86	88
11%	2	+7C	94	69
11%	0	-196C	91	98
11%	1	-196C	92	88
11%	2	-196C	97	87
11%	0	-20C	91	98
11%	1	-20C	85	89
11%	2	-20C	88	87

Carica papaya variety 1

Analysing the interaction between factors, statistical differences were found in moisture content, time and temperature. And an interaction was found between the factors moisture content vs. time, and temperature vs. time.

Table 17. Effects of treatments in *Carica papaya* var. 1

1-MOISTURE CONTENT, 2-TIME, 3-TEMPERATURE						
	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	175,900	108	27,60623	6,37175	,002422*
2	2	1384,328	108	27,60623	50,14548	,000000*
3	3	215,086	108	27,60623	7,79120	,000093*
12	4	310,720	108	27,60623	11,25544	,000000*
13	6	32,094	108	27,60623	1,16255	,331798
23	6	67,082	108	27,60623	2,42996	,030483*
123	12	27,091	108	27,60623	,98134	,471307

In figures 9 and 10 we present the interaction of moisture content vs. time, with a large increase of germination for the lowest moisture content. After one year of conservation, the germination increased in all temperatures significatively. It means that low temperatures act as a stratification agent. However, at –20 °C the increase was lower than that at other temperatures, probably due to the intermediate behaviour of *C. papaya* seeds.

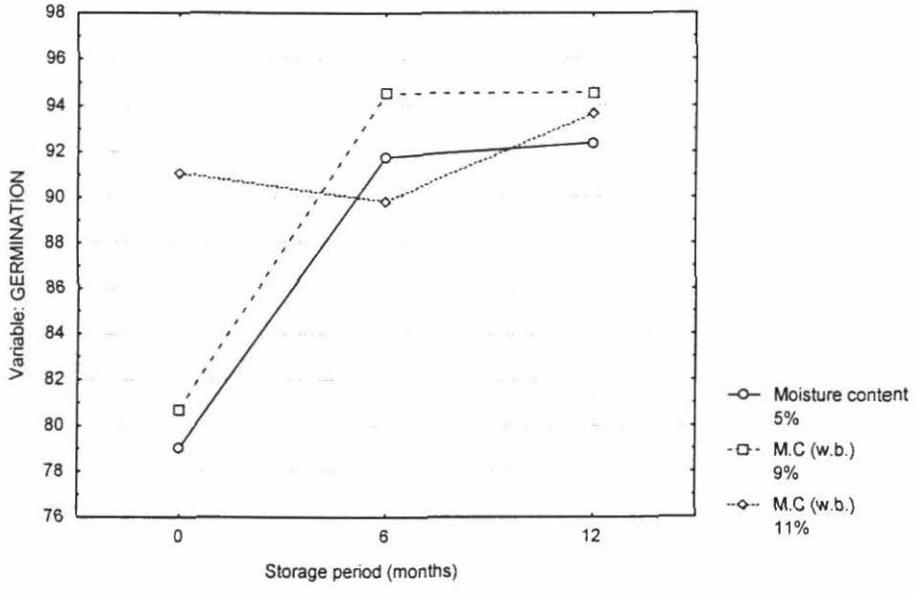

Figure 9. Interaction between moisture content vs. time for *C. papaya* var. 1

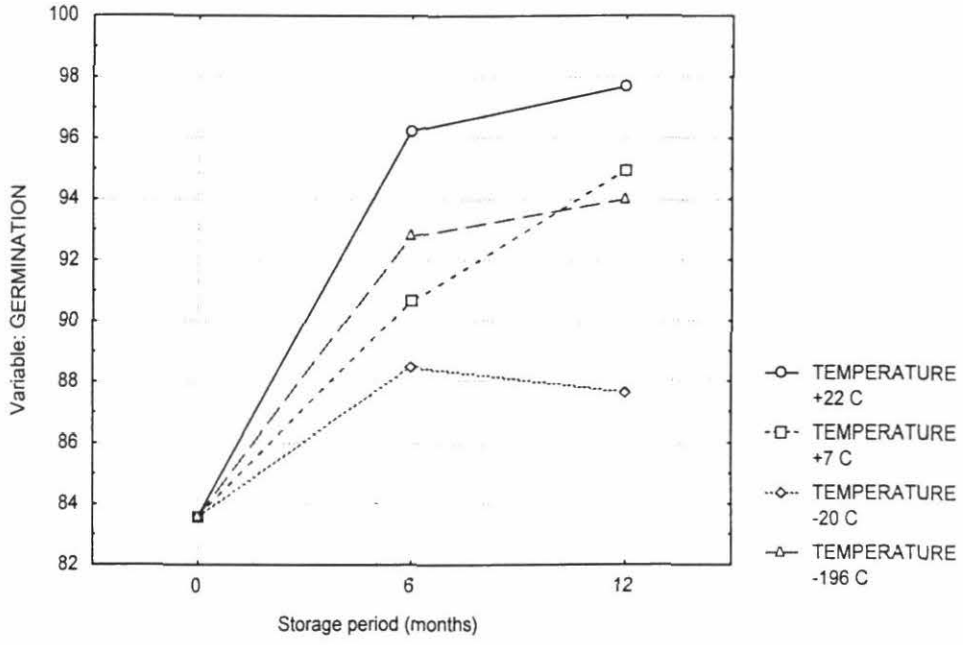

Figure 10. Interaction between temperature vs. time for *C. papaya* var. 1

Carica papaya variety 2

Table 18. Effects of treatments in *Carica papaya* var. 2

1-MOISTURE CONTENT, 2-TIME, 3-TEMPERATURE						
	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	1012,685	108	12,07652	83,85571	,000000*
2	2	898,452	108	12,07652	74,39659	,000000*
3	3	61,245	108	12,07652	5,07145	,002526*
12	4	272,705	108	12,07652	22,58141	,000000*
13	6	112,450	108	12,07652	9,31145	,000000*
23	6	35,063	108	12,07652	2,90343 ,	011559*
123	12	76,555	108	12,07652	6,33920 ,	000000*

Analysing the interaction between factors, significative differences were found between all individual factors and the interactions between them. In Figure 11, we see that variety 2 has lost a higher part of its viability as compared to variety 1. The moisture content of 11% caused a dramatical decrease in viability.

In conclusion, moisture content between 5 and 9% is the best to conserve seeds of *C. papaya* at short and medium term. Besides, there is a genetic variation in the response to several seed conservation conditions.

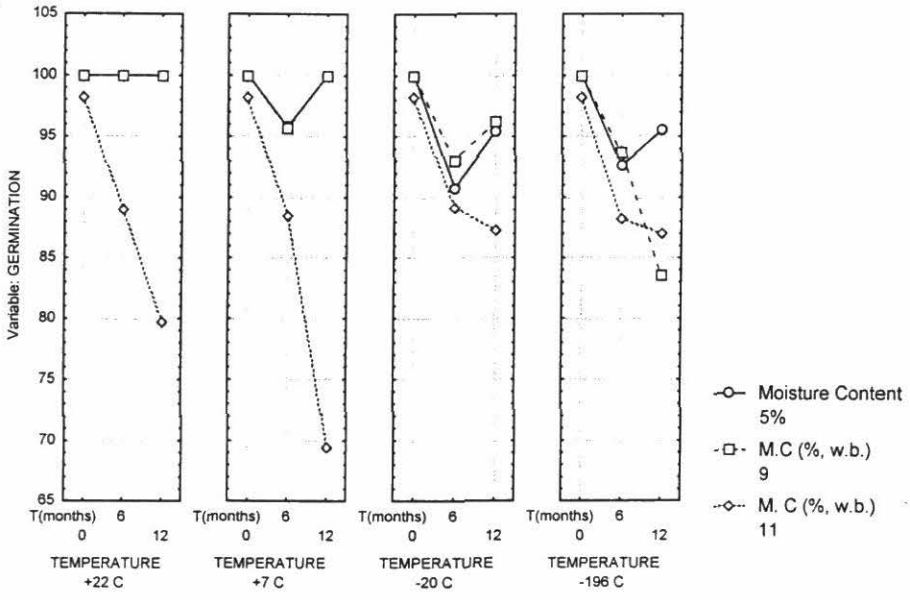

Figure 11

Contributor: A. M. Torres

Activity 1.5.4. Cryopreservation of cassava shoot tips using the encapsulation-dehydration technique.

Achievement: Cryopreservation of cassava shoot tips using the encapsulation- dehydration technique.

Introduction

Cryopreservation could be the safest way to maintain cassava germplasm in the long term. In recent years CIAT developed the encapsulation-dehydration technique (Escobar et al., 2000) that made it possible to increase the percentage of recovered frozen material as compared with classical methods (Escobar et al., 1997). This new technique is not only less costly than the classical methods but also facilitates the handling of beads and drying steps. To test the consistency of the methodology, we initiated activities to cryoconserve the entire core collection. We will thus know how many cryopreservation response groups we have, and what kind of adjustments needs to be implemented.

Materials and Methods

General methods have been described elsewhere (Escobar et al. 2000; Manrique 2000), and modifications have been implemented since then. We have handled 245 clones from the core collection during the last 3 years. At present, we continue to receive clones from the in vitro lab of GRU to complete the collection. We initiated propagation schemes (3-4 cycles/clone) to complete at least 100-150 plants/clone. Frozen and non-frozen plants were planted in CIAT fields and harvested. Yield and root morphology (skin and pulp color) were compared. Leaves were also collected for isozyme and DNA analysis.

Results and Discussion

In 2002 we received 95 clones from the core collection of the GRU. We are maintaining 53% of this collection. Activities this year included a strong propagation scheme and cryopreservation methods (pre-and postfreezing management of tissues).

Preliminary data showed good shoot recovery across time, up to 9 mo, for clones M Cub 16, M Dom 4 and M Pan7. The maximum response observed on M Ven 90 was 10% after one month (Table 19). The initial response with control frozen tissues should be monitored in order to decide whether it is feasible keeping the tissues in liquid nitrogen for longer periods. We estimated that 30% should be the Minimum Shoot Recovery Percentage (MSRP) (Escobar et al., 2001). The lower initial MSRP observed in all clones was usually associated with the use of suboptimal tissues (Escobar et al., 2000). Only M Pan 7 showed an acceptable initial MSRP response after 1 h in liquid nitrogen.

Table 19. Preliminary observation of the response of 4 cassava clones under different conservation periods and liquid nitrogen conditions.

Cassava Clones	M Cub 16		M Ven 90		M Dom 4		M Pan 7	
ConservationTime	% Viability	% Shoots	% Viability	% Shoots	% Viability	% Shoots	% Viability	% Shoots
CONTROL	0	0	0	0	23	0	89.26	30.3
1 month	83.8	32.8	60	10	92.9	82.9	90	60
3 months	70	56.6	16.6	6.6	80	65	93.3	37.7
6 months	92.6	41.1	32.5	6.25	93.9	39.09	93.6	61.1
9 months	87.5	75	16.65	0	92.5	63.3	93	40
First report	89.6	89.6	76.7	50	100	90	96.7	73.3

The procedure allowed us to standardize logistical aspects previous to the freezing step. Good-quality materials (proper age, appearance and shoot size) were used to initiate new experiments. That was why we used M Col 22 and M Per 436, which were in the high-response group as compared to M Ven 90 (the lowest response observed).

In all cases the materials frozen up to 12 months showed up to 80% recovery. The control was consistent in its behavior (Table 20). Differences observed between both experiments confirmed the importance of initial measurement and explant quality for continuing with freezing experiments. Despite the huge amount of labor involved in each experiment, it is better to discard those clones that do not reach at least 30% MSPR during the initial measurements. In Table 20 it can be observed that only 13.8% of the clones (9/65) did not reach 30% MSRP.

Table 20. Response of three cassava clones after four different conservation periods.

Cassava Clone	M Col 22		M Per 436		M Ven 90	
Conservation Time	%Viability	% Shoots	% Viability	% Shoots	% Viability	% Shoots
CONTROL	88.1	88.1	100	87.5	88.85	88.85
1 month	100	100	91.65	78.75	95	95
6 months	96	96	100	83	95	95
12 months	100	100	96	92	92	80
First report	95	85.45	94.1	79.7	76.7	50

Table 21. Response of 65 cassava clones after being frozen in liquid nitrogen.

Cassava Clone	% Viability	% Shoot	Cassava Clones	% Viability	% Shoot
M Bra 356	96.3	92.6	M Col 474	100	56.25
CM 3306-9	55.5	18.15	M Col 590	100	57.7
M Arg 7	100	43.3	M Col 601	50	5
M Arg 9	100	85	M Col 912-B	100	70
M Arg 9	100	94.44	M Col 979	100	67.2
M Bol 3	100	25	M CR 1	81.25	62.5
M Bra 190	90.9	90.9	M CR 100	100	28
M Bra 315	100	88.8	M CR 18	92.9	64
M Bra 584	100	20.3	M CR 25	100	100
M Bra 674	100	58.56	M CR 65	100	90
M Bra 697	100	37.02	M CR 84	100	48.6
M Bra 730	94	46.1	M Cub 29	100	88.8
M Bra 781	100	80.6	M Cub 1	100	70
M Bra 435	93.3	45.2	M Cub 46	82.5	47.4
M Bra 534	100	20.8	M Cub 74	96.3	63.4
M Bra 73	93.3	49.1	M Cub 8	100	90
M Bra 77	100	100	M Ecu 144	100	43
M Bra 897	100	65.1	M Fji 6	93.9	81.9
M Col 1780	100	100	M Gua 44	91.9	55.2
M Col 1795	80.9	26.6	M Mex 49	100	100
M Col 638	93.3	33.3	M Mex 54	95.8	42.5
M Col 1055	67.7	36.6	M Pan 127	100	52.7
M Col 112	88.8	41.6	M Par 7	82.5	31.6
M Col 1186-A	96.6	96.6	M Per 184	100	58.1
M Col 1535	93.93	84.17	M Per 243	84.3	55.6
M Col 1736	76.38	47.2	M Per 333	100	60
M Col 198	100	15	M Ven 173	86.3	42
M Col 2212	96	7.8	M Ven 174	97.2	55
M Col 2409	100	44.24	M Ven 309	88.3	70.5
M Col 2493	37.2	86.9	M Ven 322	100	43.3
M Col 262	100	87.5	M Ven 61	100	42.7
M Col 317	95	65	SG 455-1	87.5	54.2
M Col 32	65.27	38.4			

On the basis of skills and workforce capacity of our group, we did 8 repetitions per clone, with 10 shoots each. Treatments included one control (1 h under liquid nitrogen conditions), 3 different conservation periods (1, 6 and 12 mo), and 4 extra cryo-tubes per clone for medium-term observations (24-36 mo). At present we are maintaining 200 clones under these conditions.

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Contributors: R.H. Escobar, N.C. Manrique, A. Rios, G. Gallego, C. Ocampo, D.G. Debouck, J. Tohme (SB 1/2 project, CIAT); and W.M. Roca (CIP-Peru)

Activity 1.5.5. Implementation of the encapsulation-dehydration method on the cassava core collection: biochemical and morphological analysis of genetic stability.

Introduction

As progress is being made on the cryopreservation of the core collection, we were interested in testing the genetic stability of cassava clones after being maintained in liquid nitrogen. The warranty of genetic stability is obviously a requisite for the successful application of the cryotechniques to the FAO designate collections of *Manihot esculenta*.

Materials and Methods

General methods have been described elsewhere (Escobar et al. 2000; Manrique 2000), and above under Activity 1.5.4. Frozen and non-frozen plants were planted in CIAT fields and harvested. Yield and root morphology (skin and pulp color) were compared. Leaves were also collected for isozyme and DNA analysis.

Results

Eight isozyme systems (DIA, ACP, G6-PDH, GOT, IDH, MDH, SKDH and ß-EST) were used to compare cryopreserved and non-cryopreserved clones (each clone had two lines in the gels, the first corresponding to *in vitro*, non-cryopreserved plants and the second to cryopreserved clones) (Figure 12). For M Bra 691 (first pair, line 1-2) and M Bra 542 (4th pair, line 7-8) the SKDH system showed differences in enzyme patterns, which were later accredited to human error (sample mislabeling or misplacing). The changes observed in both clones most probably did not correspond to the cryopreservation treatment. Ocampo and Hershey (1989) observed similar behavior due to mixing materials in the field bank, using ß-EST system. We are now obtaining DNA for AFLP-fingerprint analyses.

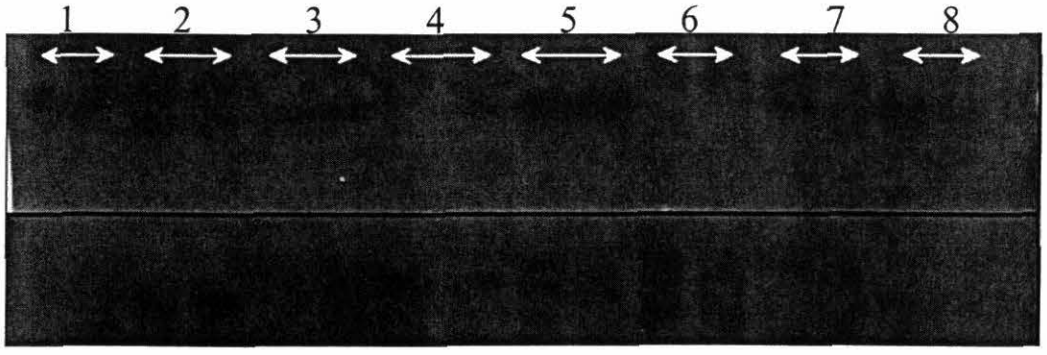

Figure 12. Comparison among eight cassava clones (*in vitro* and frozen) using SKDH. Sample: Young leaves of 6-month-old plants (Pair 1=Bra 691, 2=Bra 698, 3= Bra 759, 4= Bra 542, 5=Bra 769, 6= Bra 830, 7= Bra 881, 8= Bra 894).

The materials after harvest did not show yield differences between the *in vitro* and cryopreserved treatments. Morphological descriptors of the roots were consistent between treatments. As expected, mislabeled materials showed different color patterns (i.e., M Bra 542 and M Bra 691) (Figure 13).

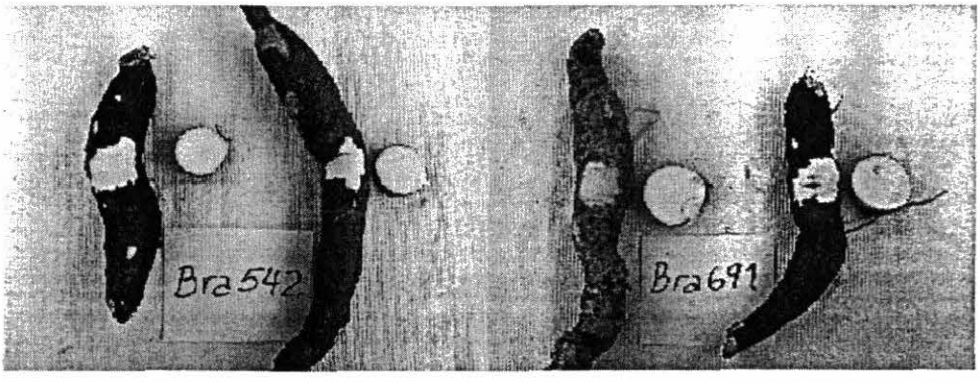

Figure 13. Morphological aspects of roots harvested from cryopreserved and non-cryopreserved cassava plants. The differences observed in the picture for instance, skin color and root shape for clone M Bra 691 are most probably due to mislabeling of clones.

Contributors: R.H. Escobar, N.C. Manrique, A. Rios, G. Gallego, C. Ocampo (SB 1-2 project, CIAT)

Activity 1.5.6. Molecular analysis of genetic stability of cassava (*Manihot esculenta* Crantz) stored *in vitro* after the application of silver nitrate as a growth retardant.

Introduction

The traditional method of *in vitro* tissue culture of cassava (*Manihot esculenta* Crantz) allows the management of 6,000 varieties, with an average conservation period of 12 to 14 months between each subculturing. In order to reduce the laborious task of subculturing and to minimize the possible risks for genetic stability, we need to lower down the growth rate and to increase the conservation lag. In order to achieve this objetive, the first task involves the modification of the culture medium currently used and tests its potential for expanding conservation lag at least to 24 months (Mafla et al., 2000). After applying silver nitrate (an inhibitor of ethylene) as a growth

retardant under two concentrations on six varieties of cassava grown *in vitro*, a decrease in stem length and an increment in the period of conservation up to 20 months in all the evaluated varieties were observed. In the case of conservation of vegetatively propagated germplasm a detailed analysis of genetic stability after *in vitro* culture is crucial. Genetic stability in cultures has long been a matter of concern in the potential application of *in vitro* techniques for germplasm conservation. We needed to confirm the genetic stability using molecular markers deemed suitable tools to that end.

Materials and Methods

The DNA fingerprinting technique selected was the AFLP technique because of the magnitude of genome coverage. This methodology has beeen used to assess the genetic stability of the six cassava varieties (two different treatments and a control for each variety) treated with silver nitrate. In order to guarantee reproducibility of results (as AFLPs generate a great amount of analyzable fragments), two DNA extractions were done on different plants for the same material (Table 22). The total genomic DNA was extracted from young leaves collected of plants coming from field (the same six varieties treated with silver nitrate, processed by meristem micropropagation and moved to the field). This same procedure also was applied for the controls. In a later stage we will follow the method of AFLP Analysis System I with minor modifications.

Table 22. Description of the cultivated cassava accessions and treatments.

IDENTIFICATION FOR THE SAMPLES (*)	Accession	TREATMENTS
1-1 1-2	MARG 2	8S[1]
2-1 2-2	MARG 2	AG3[2]
3-1 3-2	MARG 2	AG4[3]
4-1 4-2	MBRA 337	8S
5-1 5-2	MBRA 337	AG3
6-1 6-2	MBRA 337	AG4
7-1 7-2	MCOL 2056	8S
8-1 8-2	MCOL 2056	AG3
9-1 9-2	MCOL 2056	AG4
10-1 10-2	MNGA 16	8S
11-1 11-2	MNGA 16	AG3
12-1 12-2	MNGA 16	AG4
13-1 13-2	MVEN 329A	8S
14-1 14-2	MVEN 329A	AG3
15-1 15-2	MVEN 329A	AG4
16-1 16-2	CM 2177- 2	8S
17-1	CM 2177- 2	AG3

17-2		
18-1 18-2	CM 2177- 2	AG4

* Two harvests of young leaves for DNA extractions were done on different plants for the same material

[1] 8S: Controls (without treatments)

[2] AG3: Treated plants with silver nitrate (Treatment I: 10 parts by millon)

[3] AG4: Treated plants with silver nitrate (Treatment II: 12 parts by millon)

Results

Initially, three accessions (MArg 2, MCol 2056 and CM 2177-2; only controls) were chosen to test the variation and amplification of the primer combinations (AFLP Starter Primer Kit; Analysis System I). The Applied Biosystems AFLP kit contains eight EcoRI and eight MseI primers for a total of 64 combinations available for amplification reactions. With these accessions the polymorphism and the total number of fragments with 32 primer combinations were evaluated, the primers EcoR1 (E-AAC, E-AAG, E-ACA and E-ACT) with all primers Mse I of this kit (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG and M-CTT). The most useful primer combinations were considered those having the highest polymorphism, reproducibility and scorability of AFLP patterns, also generating a reasonable number of clearly detectable total fragments. The three most polymorphic primer combinations (E-AAC/M-CTA, E-AAG/M-CTG and E-ACA/M-CTG) produced clearly readable fragments and overall reproducibility of the AFLP amplification patterns was good. These three last primer combinations were selected for the subsequent analysis and the fragments produced for each one of them were:

(1) E-AAC/M-CTA: has generated 68 defined, monomorphic and polymorphic bands between varieties (Figure 14).
(2) E-AAG/M-CTG: has generated 61 defined, monomórficas and polymorphic bands between varieties (Figure 15).
(3) E-ACA/M-CTG: has generated 61 defined, monomórficas and polymorphic bands between varieties.

Our results indicate no variation for the evaluated regions of the genome (Figures 14 and 15) for the six cultivated cassava accessions. However, some accessions have displayed differences in few bands (of very tenuous staining) for only one primer combination. This is the case of samples 4-1, 5-1 and 6-1 (MBra 337) for the primer combination E-AAC/M-CTA (Figure 14) and also for the samples of the accessions Mnga 16, Mven 329A and CM-2177-2 (Table 15) for the primer combination E-AAG/M-CTG. These differences are not typical of the samples where they appear, since they are not repeated between the first and second DNA extraction. These differences may be explained by the reproducibility error (RE) of AFLP patterns which has been already reported for cassava by Chavarriaga and co-workers (1999). The error considered in that study was of 2.75 % (average of four determinations), which was estimated as a percentage: [number of non reproducible bands between pairs of duplicated DNA samples]/[number of total bands scored] x 100. An alternative explanation to the small number of bands that might differentiate these accessions (between treatments, controls and/or different extractions of DNA) is the generation of *de novo* genetic variation in plants (genome plasticity), due to, for example, somatic recombination, methylation, transposition, etc (Rasmusson and Phillips, 1997).

1-1,1-2,2-1,2-2, 3-1,3-2 4-1,4-2,5-1,5-2,6-1,6-2 7-1,7-2,8-1,8-2,9-1,9-2 10-1,10-2,11-1,11-

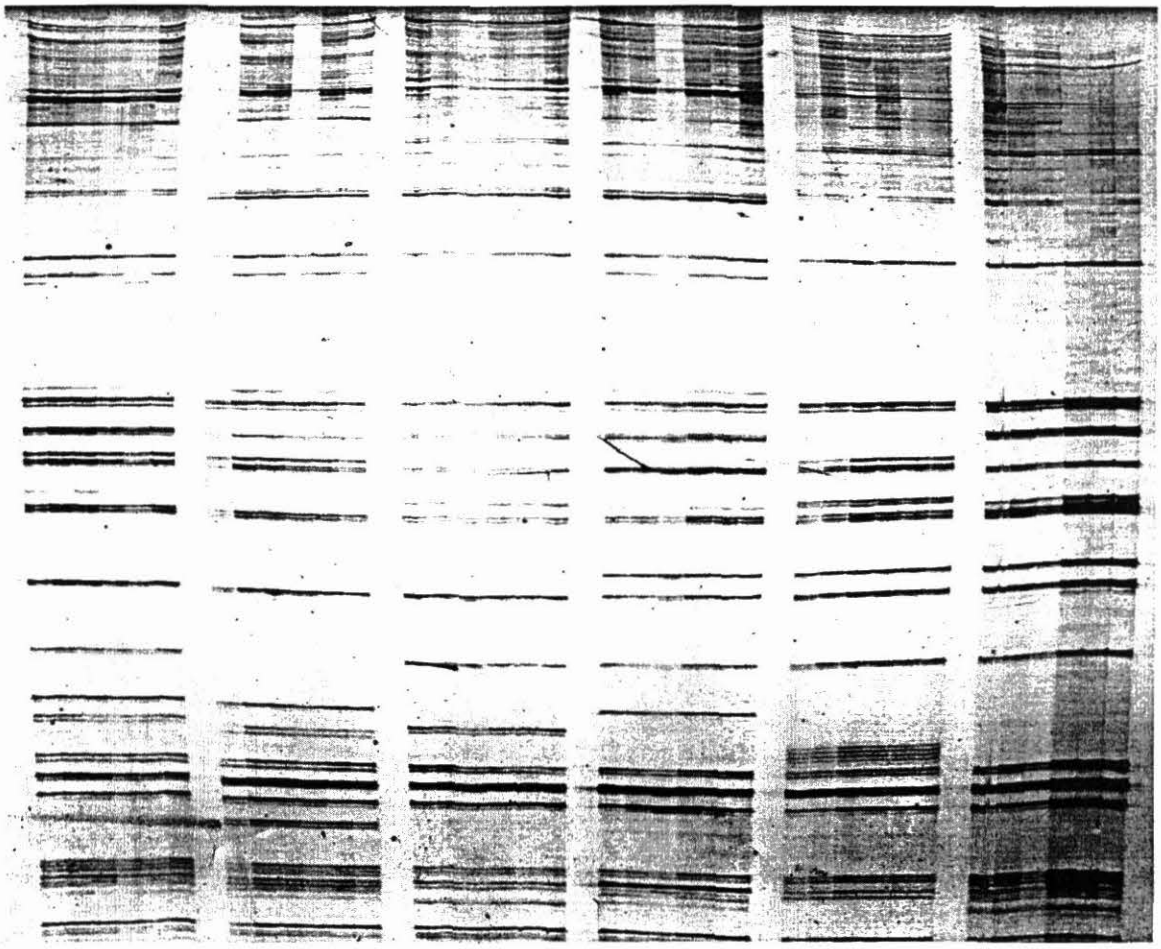

Figure 14. DNA stability monitored with AFLPs (E-AAC/M-CTA, the primer combination used) in six different cassava varieties after its treatment with nitrate silver (the order and identification of the samples according to Table 22).

1-1,1-2,2-1,2-2, 3-1,3-2	4-1,4-2,5-1,5-2,6-1,6-2	7-1,7-2,8-1,8-2,9-1,9-2	10-1,10-2,11-1,11-

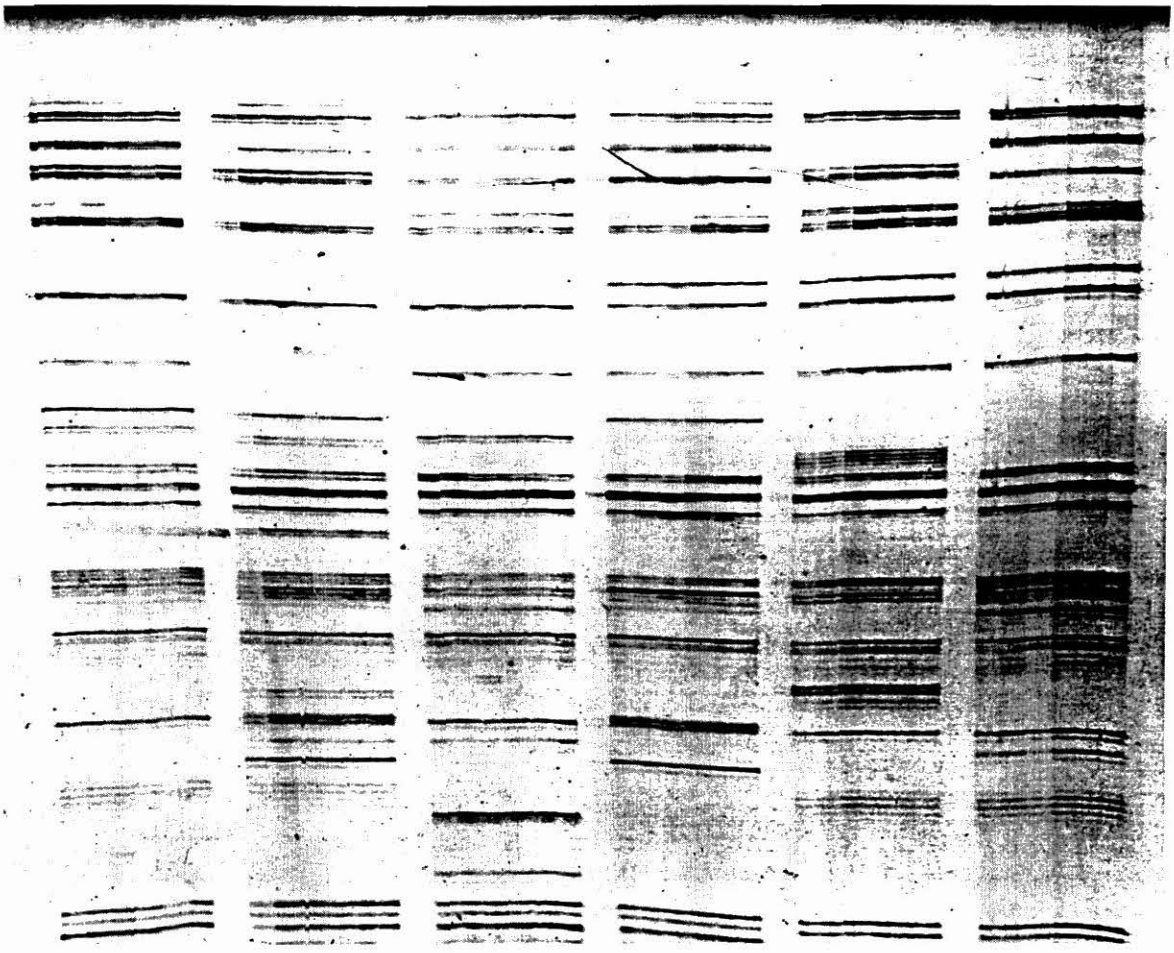

Figure 15. DNA stability monitored with AFLPs (E-AAG/M-CTG, the primer combination used) in six different cassava varieties after its treatment with nitrate silver (the order and identification of the samples according to Table 22).

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Contributors: C. H. Ocampo, G. Mafla, D.G. Debouck (Genetic Resources Unit, CIAT); G. Gallego, J. Tohme (Biotechnology Research Unit, CIAT).

Activity 1.5.7. *In vitro* conservation of tree tomato (*Solanum betaceum*) and lulo (*Solanum quitoense*) using ancymidol and silver nitrate as growth retardants.

Achievement: advances in minimal slow growth in two Soanaeae fruit species lulo and tree tomato.

Introduction

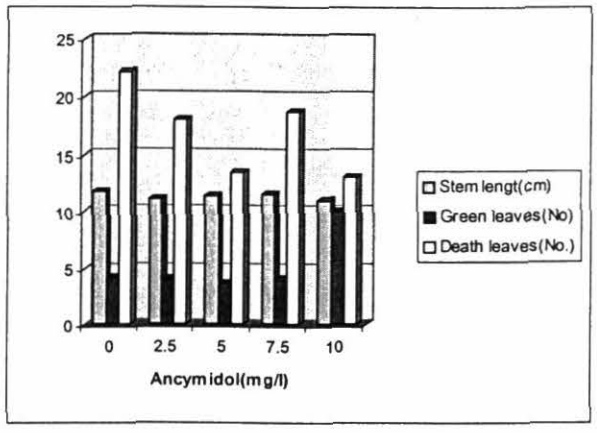
The tree tomato (*Solanum betaceum*) and lulo (*Solanum quitoense*) are important crops for small and medium scale farmers in temperate zones of Colombia. Phytosanitary problems have prevented that planting materials of good quality can be obtained, and stimulated the search of elite materials. The existence of genetic variability in the area makes necessary to apply conservation strategies (Lobo, 2000).
Techniques of *in vitro* culture would allow the reduction of germplasm conservation costs while maintaining materials in good conditions. Ancymidol has been repoorted as a growth regulator inhibiting a series of oxidations in plant tissue. It has been used for the *in vitro* conservation of potato (*Solanum tuberosum*) allowing optimum survival after prolonged periods of storage (16 months), also fostering favorable microplant growth (Sarkar *et al*, 2001). Silver nitrate has been identified as an inhibitor of ethylene and has extended *in vitro* conservation of cassava (*Manihot esculenta*) by 18 months (Mafla *et al*, 2000). We wanted to verify whether these chemicals can extend the *in vitro* maintenance of these fruit trees, since no reports exist.

Materials and Methods

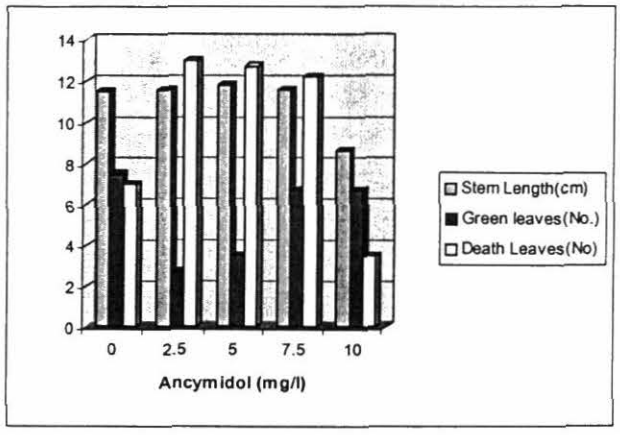
Two genotypes of lulo were used: one material of *Solanum quitoense* from Ginebra (Valle del Cauca, Colombia) and Lulo 'La Selva' (from CORPOICA, Colombia), and one genotype of tree tomato (*Solanum betaceum*)
The experiment was conducted with single node cutting dissected from aseptically grown *in vitro* plantlets. Two node cutting were cultured per tube. For lulo four concentrations of Ancymidol (0, 2,5, 5,0, 7,5 and 10,0 mg/l) were tested. The medium was supplemented with MS (Murashige and Skoog), vitamins and 30 g l-1 of sucrose, and for the silver nitrate treatment 0, 2, 4, 8, 10, 10 * (with regulators of growth) mg/l with 30 g l-1 of sucrose and 20 gr/l manitol were used. The cultures were incubated to 23°C under a 12-h photoperiod (1,000 lux). Each treatment included five replicates. For tree tomato the treatments were the control (0), four concentrations of Ancymidol: 2.5 (1), 5,0 (2), 7.5(3), 10.0(4) mg/l and Nitrate of silver (10mgl, treatment 5). After 5 months of storage, observations were recorded on microshoot heigth (cm), number of green leaves and leaf senescence. Other evaluations will be made to confirm the viability of the cultures when they leave the treatments (micropropagation capacity).

Results

The effect of ancymidol on the stem elongation and number of green and dead leaves in *Solanum quitoense* is shown in Figure 16a. No significant differences were observed between the treatments in relation to the stem length but positively in numbers of green and dead leaves. A better quality of the material was observed when high concentrations of ancymidol were used (10,0 mg/l). The average stem length varied from 8.6 cm to 11.8 cm. The same response was observed in lulo 'La Selva'; the rate of growth measured in terms of stem length did not present significant differences (Figure 16b) but there were changes in the number of green and dead leaves. A significant increase in the number of green leaves was observed only when the microplants were conserved in media containing 10.0 mg/l.



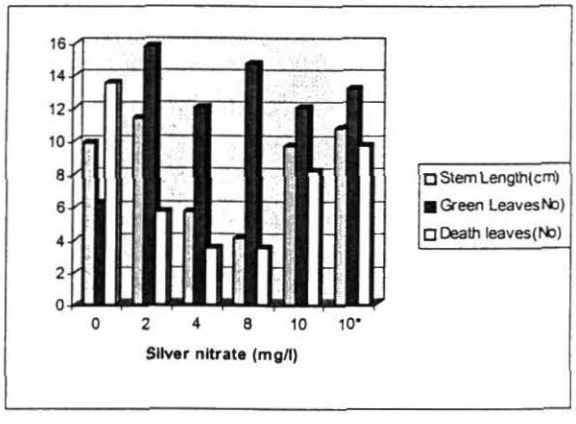
a



b

Figure 16a-b. Effect of ancymidol on stem length (cm), and number of green and dead leaves during the *in vitro* culture in lulo (*Solanun quitoense*) and lulo 'La Selva'.

The analyses of variance showed that silver nitrate had a greater effect in each one of the evaluated characters. The average stem length varied between 4.1 and 11.4 cm in *Solanum quitoense* and 3.6 and 8.7 cm in lulo 'La Selva'. A smaller growth was observed when the medium contained 4,0-8,0mg/l of silver nitrate; the same reduction occurred in dead leaves and the number of green leaves increased (Figura 17a-b). The results presented here show that the materials remain alive up to five months under culture conditions.



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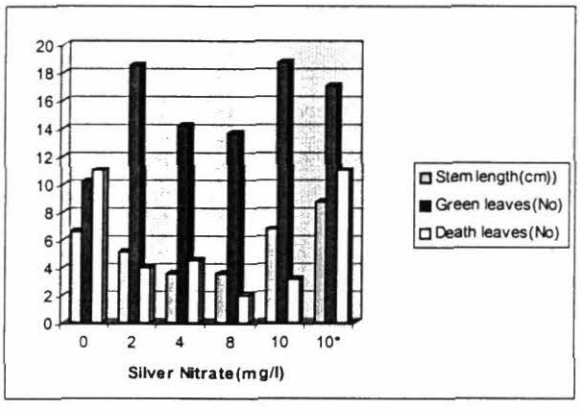

b

Figure 17a-b. Effect of silver nitrate on the stem length (cm) and number of green and dead leaves during the *in vitro* culture of lulo (*Solanun quitoense*) and lulo 'La Selva'.

In tree tomato no differences were found in the growth of microplants in media containing different levels of ancymidol and silver nitrate. We only observed a smaller defoliation when ancymidol to 10,0 mg/l (4) was used (Figure 18).

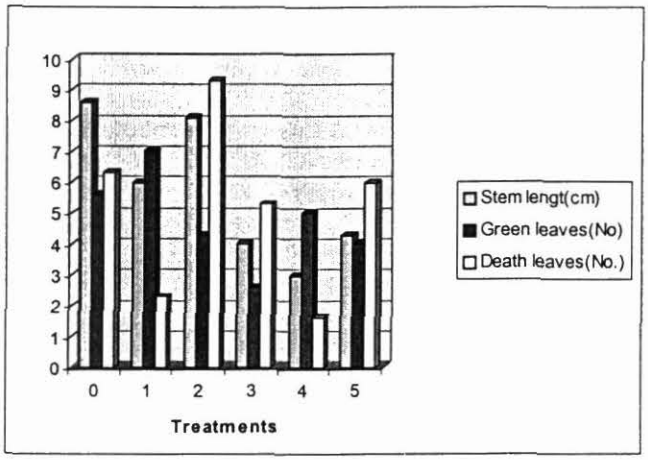

Figure 18. Effect of ancymidol and silver nitrate on the stem length (cm), number of green and dead leaves during the *in vitro* culture of tree tomato.

Conclusions

Silver nitrate in lulo has shown until now a beneficial effect with a reduction of growth; it is necessary to continue with the evaluations and measure the survival rates.

In tree tomato no advantages have been observed so far with the evaluated medium; adjustments in the used concentrations will be done next.

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Contributors: G. Mafla, J.C. Roa, 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 the 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 known of quarantine importance, following the FAO/IPGRI recommendations for the safe movement of cassava clones at national and international levels. We have been working on the indexing for three viruses: CCMV, CsXV and FSDA. As soon as we finish these

activities, we will continue with the indexing of the whole collection for the CVMV virus (done with a PCR technique). For the indexing two 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 accessions available at this moment and tested against viruses CCMV, CsXV and FSDA, thus ready for distribution are 4,559 clones (74,02 % of FAO Designate Collection).

Indexing for CCMV

The number of clones evaluated against CCMV in 2002 is shown in Table 23.

Table 23. Clones of the FAO Designate collection indexed against CCMV.

Source	Indexed clones	Negative clones
Argentina	10	5
Brasil	55	51
Colombia	91	89
Costa Rica	1	1
Ecuador	9	9
Guatemala	2	2
México	3	3
Panamá	1	1
Paraguay	6	5
Perú	10	10
Tailandia	2	2
Venezuela	8	8
CG	4	3
CM	14	14
SG	3	3
SM	11	11
CT	1	1
WILD SPECIES		
ALT	1	1
CHL	4	3
CTH	3	3
FLA	13	12
FMT	2	2
TST	2	2
VIO	1	1
TOTAL CLONES	257	242

The above mentioned indicates that 94,1 % of clones presented negative results for this virus and 5,8 % positive results, a percentage lower than that of last year report. This indicates that the thermoterapy applied to the clones previously reported as positive and to the other clones has been effective.

The countries with high numbers of positive clones were Argentina (1,94 %), Brasil (1,55 %), and Colombia (0,78 %); however these numbers are low. By the time that we have evaluated the entire collection, Argentina could be the country with the highest number of positive clones.

Indexing for CsXV

The number of clones evaluated against CsXV in 2002 is shown in Table 24.

Table 24. Clones of the FAO Designate collection indexed against CsXV.

Source	Indexed clones	Negative clones
Argentina	10	10
Brasil	56	52
Colombia	124	118
Costa Rica	13	13
Ecuador	11	10
Guatemala	5	5
México	3	3
Panamá	1	1
Paraguay	5	4
Perú	23	23
Tailandia	2	1
Venezuela	5	5
CG	7	5
CM	18	17
SG	4	4
SM	13	12
CT	1	1
WILD SPECIES		
ALT	1	1
CHL	2	2
CTH	3	3
FLA	13	13
FMT	2	2
VIO	1	1
TOTAL CLONES	323	306

The results indicate that 94,7 % of the evaluated clones present negative results for this virus and 5,26 % present positive results. The countries where this virus is noticeably present are Brasil (1,23 %) and Colombia (1,85 %).

According to the previous data, we see that the presence of CsXV has stronger incidence in the Collection as compared to CCMV.

Indexing for FSDA

The number of clones evaluated against FSDA in 2002 is shown in Table 25.

Table 25. Clones of the FAO Designate collection indexed against FSDA.

Source	Indexed clones	Negative clones
Argentina	22	13
Bolivia	1	1
Brasil	149	142
Colombia	318	304
Costa Rica	14	13
Cuba	2	1
Ecuador	12	12
Fiji	1	1
Guatemala	28	28
India	18	18

Malasia	9	9
México	6	6
Nigeria	4	4
Panamá	4	4
Paraguay	37	34
Perú	60	57
Puerto Rico	1	1
Philipinas	1	1
Salvador	2	2
Tailandia	5	5
Venezuela	29	29
CG	20	20
CM	39	39
SG	6	4
SM	24	23
CT	1	1
WILD SPECIES		
ALT	1	1
CHL	4	4
CTH	10	9
FLA	13	13
FMT	4	4
PSE	1	1
TST	12	12
VIO	1	0
TOTAL CLONES	859	816

As one can see in the previous table, 95 % of materials presented negative results in the indexing for this virus and 5 % presented positive results. A high incidence of FSDA was observed in clones of Colombia (1,62 %), Argentina (1,04 %) and Brasil (0,81 %) as compared to the whole collection.

The current state 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 26.

Table 26. Indexing status of the Cassava Germplasm Collection in GRU by October 2002.

Source	In vitro clones	INDEXED CLONES			Available for distribution in 2002
		CCMV	CSXV	FSDA	
Argentina	101	72	86	63	56
Bolivia	7	7	7	5	5
Brasil	1342	1314	1290	1061	1039
China	2	2	2	2	2
Colombia	2021	1928	1874	1604	1556
Costa Rica	148	146	135	138	126
Cuba	77	77	77	75	75
Estados Unidos	9	9	9	7	7
Ecuador	116	113	111	106	100
Fiji	6	5	5	5	5
Guatemala	91	91	88	74	71
Indonesia	51	51	51	39	39
Malasia	67	67	67	52	52
México	102	101	99	81	78

Nigeria	19	19	19	16	16
Panamá	43	39	36	37	33
Paraguay	210	201	201	141	129
Perú	406	396	392	350	338
Philipinas	6	5	5	4	4
Puerto Rico	15	15	15	12	12
Rep.Dominicana	5	5	5	4	4
Salvador	8	6	6	6	5
Tailandia	31	29	28	19	19
Venezuela	244	232	234	199	191
Híbridos					
CG	122	82	77	76	73
CM	450	413	408	393	388
HMC	4	4	4	3	3
SG	47	46	43	40	39
SM	86	122	74	71	67
KM	81	7	7	4	4
CT	1	1	1	1	1
SUBTOTAL	5,918		5,456	4,688	4,537
WILD SPECIES					
30 spp in vitro	330	24	22	44	22
3 Undefined spp	3				
TOTAL	6,251	5,629	5,478	4,732	4,559

The next table 27 shows progress in availability for distribution (materials negative for the three viruses) obtained between 2001 and 2002.

Source	In vitro clones	Available for distribution to 2001	Available for distribution to 2002
Argentina	101	42	56
Bolivia	7	4	5
Brasil	1342	841	1039
China	2	2	2
Colombia	2021	1202	1556
Costa Rica	148	106	126
Cuba	77	74	75
Estados Unidos	9	7	7
Ecuador	116	82	100
Fiji	6	5	5
Guatemala	91	43	71
India	51	21	39
Malasia	67	40	52
México	102	67	78
Nigeria	19	12	16
Panamá	43	29	33
Paraguay	210	100	129
Perú	406	282	338
Philipinas	6	3	4
Puerto Rico	15	11	12
Rep.Dominicana	5	4	4
Salvador	8	3	5
Tailandia	31	12	19
Venezuela	244	157	191
Híbridos			

CG	122	40	73
CM	450	180	388
HMC	4	1	3
SG	47	32	39
SM	86	47	67
KM	81	3	4
CT	1	1	1
SUBTOTAL	5,918	3,453	4,537
WILD SPECIES			
30 spp in vitro	330	0	22
3 Undefined spp	3		
TOTAL	6,251	3,453	4,559

As it can be seen in Table 27, there is an increase in the numbers of cassava clones available for distribution in 2002, mainly of clones from Colombia, Brasil, Perú and Venezuela; also of clones from some hybrids (CM). In 2002 we obtained the first results for the wild species. So, this year a total of another 1,106 clones were added 'ready for distribution'.

In Table 28, we report progress obtained in 1998 – 2002 in the indexing of the Cassava Collection.

Total de clones	Year	Percentage of negative clones for CCMV	Percentage of negative clones for CSXV	Percentage of negative clones for FSDA	Available for distribution
6,017	1998	36,7	35,5	10	602
6,172	1999	42,6	40,1	17,4	1,073
6,179	2000	70,8	63,5	39,2	2,346
6,235	2001	84,2	80,8	60	3,453
6,251	2002	90	87,6	75,6	4,559

As one can see, a high number of negative clones have obtained over these years, although the process is slow and some problems have been encountered during the establishement of the materials.

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

Activity 2.1.4. Indexing wild materials of cassava for the GRU

At the moment, we have 246 different wild materials of cassava under greenhouse conditions for indexing (ELISA and grafting) obtained from *in vitro* plants. The materials that we have are: AES, BLO, Esc. Fla, FMT, GLA, HAS, ORB, PIL, PNT, WV JAC, CTH, RUB, LON, GUT, TST, ALT, FLA, EPR, CAE, ANM, CHL, CEC, IRW, TPH, PUR, PSE, VIO, PER 417-003, PER 417-005 and PER 413-003.

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

Activity 2.1.5. 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. There are indications that we could keep the entire FAO Designate collection as "bonsai" in the insect proof glass-house for three years, as a low cost alternative to the field genebank. This back-up is needed before the entire collection is safely maintained under cryopreservation. In addition, GRU has been delivered in

2002 year more than 1,000 stakes from this copy to Cassava Breeding projects for experimental evaluations. We have 2,169 clones from different countries as it can be seen in Table 29; these are by-products of the certification process against FSDA and thus certified against viruses of quarantine importance.

Table 29. Total number of clonal material under glass-house conditions as 'Bonsai'.

Source	Accesions installed under greenhouse conditions
Argentina	26
Bolivia	2
Brasil	420
Colombia	800
Costa Rica	56
Cuba	25
Ecuador	46
Estados Unidos	6
Fiji	2
Guatemala	42
India	23
Malasia	17
México	28
Nigeria	6
Panamá	12
Paraguay	217
Perú	148
Philipinas	1
Puerto Rico	8
Salvador	3
Tailandia	9
Venezuela	76
ALT	1
CG	28
CHL	2
CT	1
CTH	3
FLA	11
FMT	2
CM	91
HMC	1
SG	22
SM	31
KM	1
TST	1
VIO	1
TOTAL	2169

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

Activity 2.1.6 Checking cassava materials for ICA Plant Quarantine Office

The ICA Office asked us for the evaluation of 25 plants obtained from the variety CMC-40 of the Project-Haití-CIAT-HGRP for the CCMV, CsXV and FSDA. During 2002 we evaluated those plants using the test ELISA for CCMV and CsXV and grafting for FSDA. All the materials were negative.

Contributor: N.C.Flor

Activity 2.1.7. Germplasm health control in seed germplasm

Introduction

In agreement with FAO–IPGRI Genebanks Standards, seeds for storage in germplasm collections should be as clean and free from weed seeds, pests, and diseases as possible. To that purpose the Germplasm Health Laboratory (GHL) 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 2002 the GHL tested 1,528 seed samples from projects SB-1 (Integrated Conservation of Neotropical Plant Genetic Resources), GD-01 (Bean Germplasm Improvement), and IP-5 (Tropical grasses and legumes).

Materials and Methods

Phytosanitary inspections on multiplication plots (field and glass-houses) of Popayán, Santander de Quilichao and CIAT Palmira Station are realized. 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 (CIAT Annual Report 1997). The procedures utilized in the Germplasm Health Laboratory have been described in the 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 (Becton Dickinson BBL Crystal™, Nippon Becton Dickinson Company Ltd.) containing 29 enzymatic and biochemical substrates are carried out. In addition a biological test using hypersensibility reactions on *Mirabilis jalapa* after bacterial infiltration is used. . Testing for seed borne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and or seedling-symptom test.

Results

Seed samples of 426 accesions of *Phaseolus* spp. project SB-1 were tested, some of them with destination to Australia, Belgium, Italy, Puerto Rico, and Peru, and the other ones for conservation in the Bean Germplasm Bank (Table 30). Their health status showed 67.1% samples without pathogens of quarantine importance. Samples with pathogens showed in general very low percentages of the fungi *Macrophomina phaseoli.* Seed borne infections by *Xanthomonas campestris* pv *phaseoli* were also detected. Viral infections by Southern mosaic virus (SBMV) and Bean common mosaic virus (BCMV) were present in middle percentages specially on *Phaseolus coccineus* and *P. polyanthus.* 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.

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

Table 30. Number of analysed samples of *Phaseolus* spp. for germplasm sent abroad or to Germplasm conservation, project SB-1

Species	Number of samples	Destination
Phaseolus coccineus	5	Peru
Phaseolus coccineus	224	Puerto Rico
Phaseolus polyanthus	124	Puerto Rico
Phaseolus spp (wild forms)	9	Belgium
Phaseolus vulgaris	2	Australia
Phaseolus vulgaris	18	Italia
Phaseolus vulgaris	44	Germplasm bank (conservation)
Total	426	

Seed samples of 331 of tropical grasses and legumes from SB-1 project, distributed in 227 accesions of 12 genera of tropical legumes pastures, and 104 of *Brachiaria* spp with destination India, Mexico, Ecuador, Venezuela, and Belgium were analysed at SHL (Table 31). Their health status showed 57.7 % of samples without pathogens of quarantine importance. In rejected samples were detected some fungi of quarantine importance (*Colletotrichum* spp., *Drechslera* spp., *Macrophomina* sp., *Pestalotia* spp., *Phoma* spp, *Phomopsis* spp.) and very low frequency of Poty virus, but a higher percentage of SBMV on legumes. Bacteria (*Pseudomonas* spp.) were detected only in legumes with very low frequency, also a Coryneform Gram positive bacteria.

Table 31. Number of analyzed samples of tropical grasses and legumes for germplasm sent abroad (requests to GRU)

Species	Number of samples	Destination Country
Brachiaria spp.	83	India
Brachiaria spp.	21	México
Cajanus spp.	3	Ecuador
Calopogonium spp.	8	Ecuador
Canavalia spp.	11	Ecuador
Centrosema spp.	8	Ecuador
Centrosema spp	58	Venezuela
Crotalaria spp	15	Ecuador
Desmodium spp.	42	Ecuador
Lablab spp.	3	Ecuador
Mucuna spp.	3	Ecuador
Sesbania spp	3	Ecuador
Stylosanthes spp	25	Ecuador
.Tephrosia spp.	11	Ecuador
Vigna spp	27	Ecuador
Vigna spp	10	Belgium
Total	331	

Seed samples from GD-01 (Bean's Germplasm development) and IP-5 (Tropical grasses and legumes) were analyzed (Table 32). In *Phaseolus vulgaris* 61.6 % of samples did not show pathogens of quarantine importance. In rejected samples *Macrophomina phaseoli* was the fungus detected with more frequency. Also was detected the bacteria *X. campestris* pv *phaseoli* in low percentage and bean common mosaic virus (BCMV) and SBMV in intermediate percentage. All seed samples of *Brachiaria* spp. showed the presence of the seed borne fungi *Drechslera* spp. and *Phoma* spp. On legumes as *Cratylia* sp. *Phoma* spp was detected.

Table 32. Number of analyzed samples of beans, tropical grasses and legumes for germplasm sent abroad (projects GD-01 and IP5)

Species	Number of samples	Project
Phaseolus vulgaris	371	GD-01 SB
Phaseolus vulgaris	390	GD-01 MB
Andropogon	1	IP-5
Brachiaria spp	4	IP-5
Arachis spp	1	IP-5
Centrosema sp.	2	IP-5
Cratylia spp	1	IP-5
Vigna spp	1	IP-5
Total	771	

Contributors: B.L. 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

Achievement: 7,557 accessions of germplasm of the FAO designate collections distributed to users

A total of 7,557 accessions (3,077, 2,933 and 1,547 accessions of germplasm of cassava, beans and tropical forages, respectively) were distributed in 2002. A total of 3,077 *Manihot* accessions were distributed through 52 requests (summing to 5,632 samples of *in vitro* plants and 4,290 wild species seeds). The main recipients were CGIAR Centers (mainly CIAT Projects), who received over 79.6% of total of samples, and then the NARS with 16.7% (Table 33, Figure 19). The main purposes of distribution in cassava were basic research (cryopreservation, embryogenesis and classic biochemistry) with 74.7% of the total, while agronomy with 20.1%, applied research (pathology and entomology) with 3.4%, breeding with 1.4% and training with 0.1% (Table 34, Figure 20).

Table 33. Distribution of germplasm during 2002 by kind of institution.

	Beans		Forages		Cassava	
Institution type	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
CGIAR centers	26	1,234	14	394	33	2,450
Commercial companies	--	--	3	21	1	1
Farmers	1	42	51	126		
Gene banks	--	--	1	9		

NARS	11	1,003	11	202	9	516
NGOs	1	54	5	560	1	23
Regional organizations	1	8	3	39	1	5
Universities	21	592	11	196	7	82
Germplasm networks	--	--	--	--		
Others	--	--	--	--		
Total	61	2,933	99	1,547	52	3,077

Table 34. Distribution of germplasm during 2002 by purpose.

	Beans		Forages		Cassava	
Purpose	Shipments	Accessions	Shipments	Accessions	Shipments	Accessions
Breeding	12	229	1	33	1	44
Agronomy	6	949	72	1,115	17	620
Applied research	10	809	7	67	15	107
Basic research	29	843	17	295	18	2301
Training	4	103	2	37	1	5
Other	--	--	--	--		
Total	61	2,933	99	1,547	52	3,077

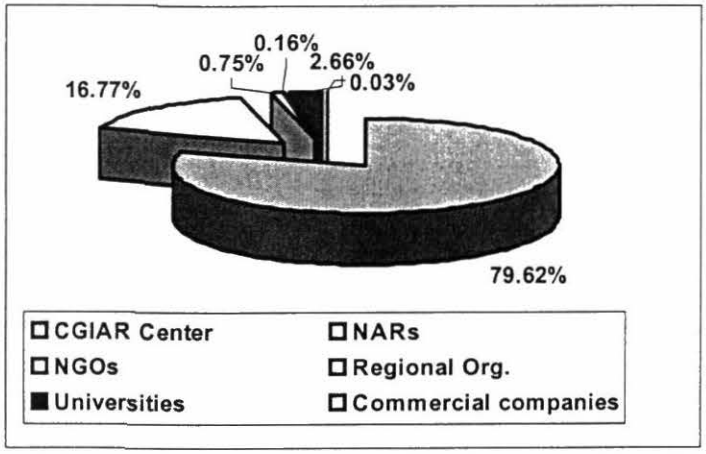

Figure 19. Distribution of *in vitro* cassava germplasm by users

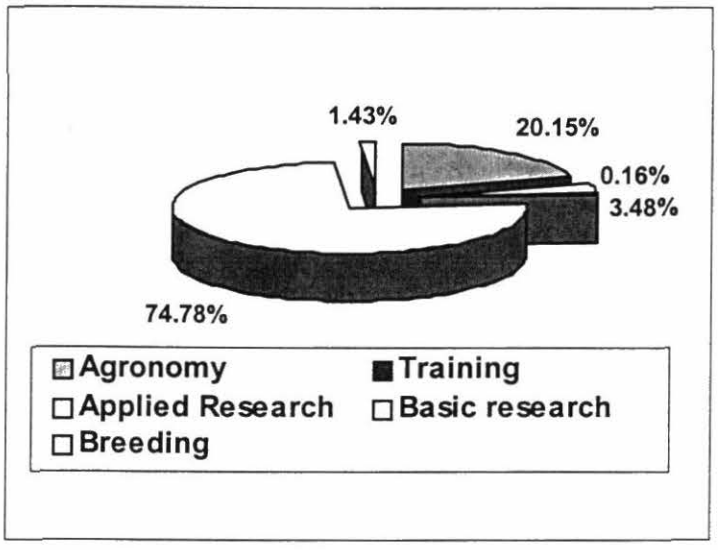

Figure 20. Distribution of *in vitro* cassava germplasm by purposes

As it can be seen in Tables 33-34 and Figures 19-24, distribution of germplasm out of the designate collections continues at a relatively high rate as compared to the multiplication rates of GRU (namely for bean germplasm). While CIAT Projects continue to be the major recipients of germplasm, national programmes are asking for germplasm of cassava and beans, but there are new types of requests coming in for forage germplasm. The numerous requests for *Cratylia argentea* cv. *Veranera* by individual farmers are worth noting.

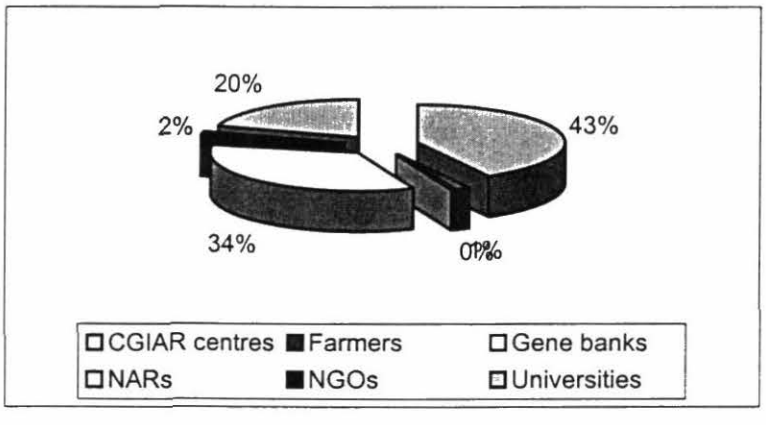

Figure 21. Distribution of bean germplasm by users

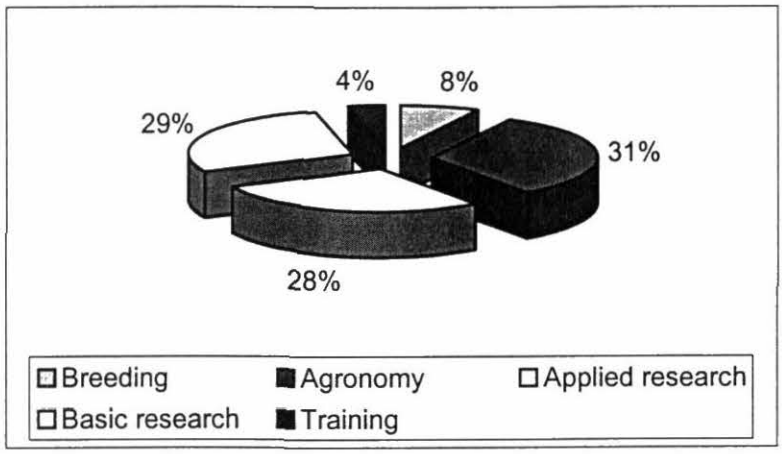

Figure 22. Distribution of bean germplasm by purposes

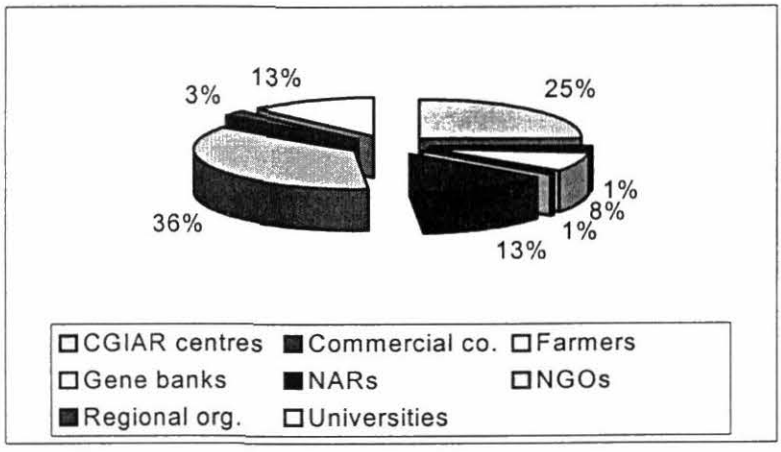

Figure 23. Distribution of forage germplasm by users

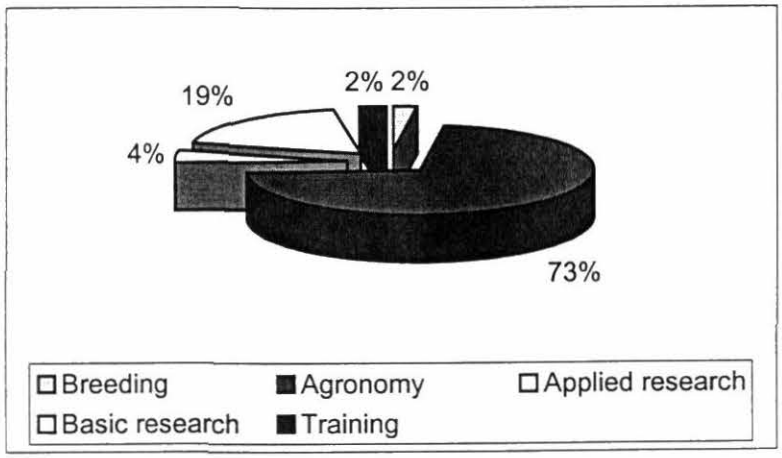

Figure 24. Distribution of forage germplasm by purposes

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

Activity 2.2.2. Checking the quality of passport data

In the framework of an ending phase of the SINGER project, time has been invested to improve the quality of the passport data at the same time that these data fields are made available at CIAT website for consultation by internet users. In the Bean Collection with currently 31,718 accessions in-trust, 253 accessions were spotted with wrong geographic coordinates, and these were corrected. A search is being made over the 19,782 accessions of tropical forages with passport data in order to spot location problems; out of 826 accessions reported for the Meta department of Colombia, 32 were with wrong coordinates, and these have been corrected.

Contributors: A. Ciprian, O. Toro, GIS Project Staff

Activity 2.2.3. Design of the computerized system of GRU for germplasm health data

In 2002 there was additional refinement to the computerized data system of GRU, namely to handle the data collected on aspects of germplasm health during the introduction and production processes. The development of the quarantine module was done with the ICA Plant Quarantine Office in order to speed up the introduction of germplasm in the future. The new module is currently shared between GRU and ICA Staff.

Contributors: S. Ramírez, D.M. Montero (CIAT Informatics Unit); C. Huertas (ICA); A.M. Torres, G. Mafla, B. Pineda, A. Ciprian, O. Toro, D.G. Debouck (CIAT GRU)

Activity 2.2.4. Checking validity of forage and other wild species names through a reference herbarium

Since 1978, a reference herbarium has been established to certify the correct identification of the 861 botanical species kept in the Designate collections. Table 35 sums up additions made in 2002.

Table 35. Specimens of tropical forages and wild beans added to CIAT Herbarium in 2002

	Number of species	Number of accessions
Wild beans	10	93
Legumes	116	587
Grasses	11	13
Total	137	693

Besides, donation of duplicated herbarium specimens was done to several institutions. With the agreement of countries of origin, a total of 254 herbarium were donated to four institutions: 1) 65 vouchers of *Phaseolus* spp. were donated to the Herbarium G, Geneva, Switzerland. 2) 26 vouchers of *Phaseolus* spp. and *Stylosanthes guianensis* were donated to the Herbarium COL, Bogota, Colombia. 3) 94 samples of *Centrosema* spp. were donated to the Herbarium IRBR, Venezuela. 4) 69 samples of *Phaseolus* spp. were donated to the Herbarium M, Munich, Germany.

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

Output 2.3. National collections restored to NARS

Activity 2.3.1. Conditionning and shipment of particular collections to NARS as restoration

In 2002,Venezuela has asked for the restoration of its forage collection, and actions have been undertaken to that end.

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

Output 2.4. FAO designated collections safe duplicated

Activity 2.4.1. Preparation of germplasm collections for security backups

Achievement: Another 595 beans and 492 accesions of forage were prepared; 427 cassava clones already in Thailand

This year have shipped to Thailand 427 clones of the *in vitro* cassava core collection (630 clones) were shipped to Thailand in order to keep a duplicate and for future evaluation at the Rayong Field Crop Research Center. Another 595 beans and 492 accesions of forage were packed for safety backups.

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

Output 2.5. Refined core collections

Activity 2.5.1. Characterization of the reserve collection for refining the core collection

In 2002, 579 genotypes of the Bean Collection were characterized for basic morpho-agronomic descriptors and phaseolin types (using 1diSDS-PAGE electrophoresis) in view of improving the current core collections.

Contributors: C. Ocampo, O. Toro

Output 2.6. Improved disease indexing techniques

Activity 2.6.1. Evaluation of the fungicide Propiconazole to control the fungi complex (*Sphacelia* spp., *Drechslera* spp.,*Phoma* spp., *Cerebella* spp.) on *Brachiaria brizantha* inflorescences.

Introduction

Regeneration of germplasm is one of the most important activities in genebank operations, but it must be conducted under conditions with a minimum phytosanitary risk to obtain good quality seeds. At regeneration fields of *Brachiaria* ssp. in Santa Rosa station, Popayán, Colombia, some seed borne fungi are of common occurrence and they affect yield and health quality of the germplasm (Garcia & Pineda 2000; Garcia et al, 2001). One way to control these fungi is in using fungicides. Evaluations carried out at the Germplasm Health Lab (GHL) under *in vitro* conditions against *Sphacelia* spp., *Drechslera* spp., *Phoma* spp., *Cerebella* isolated from seeds of *Brachiaria brizantha* seeds produced at Santa Rosa showed that the fungicide Propiconazole (Tilt) was one of the most effective product against these fungi (CIAT, 2001). It was necessary to test its efficacy under field conditions. To that purpose a preliminary trial in Santa Rosa Station was made, which results are presented here.

Materials and Methods

Seventy-two *Brachiaria brizantha* accessions were selected at Santa Rosa station. The selection was made completely at random in the regeneration plots using only booting (beginning of inflorescence initiation) or flowering accessions, also some accessions with milky or mature grains (Table 36).

Table 36. Accesions of *Brachiaria brizantha* and its development stages selected beginning to test the efficacy of propiconazole spraying

CIAT Number	Growth stage on Nov 15-01	CIAT number	Growth stage on Nov 15-01	CIAT number	Growth stage on Nov 15-01	CIAT number	Growth stage on Nov15-01
16160	Booting	16151	Full flowering	26314	Full flowering	16479	Full flowering
16303	Booting	16154	Full flowering	26102	Full flowering	26559	Full flowering
6675	Booting	16342	Full flowering	16290	Full flowering	16297*	Full flowering
26321	Booting	16121	Full flowering	16144	Full flowering	16451*	Full flowering
26568	Booting	16296	Full flowering	16153	Full flowering	16442*	Full flowering
16299	Booting	26990	Full flowering	16444	Full flowering	16152*	Full flowering
16315	Early flowering	16811	Full flowering	16809	Full flowering	16461*	Full flowering
26117	Early flowering	16833	Full flowering	16828	Full flowering	16476*	Full flowering
16156	Early flowering	16173	Full flowering	16149	Full flowering	16440*	Full flowering
16120	Early flowering	16457	Full flowering	16163	Full flowering	16447*	Full flowering
26991	Early flowering	16339	Full flowering	16431	Full flowering	26562*	Full flowering
16170	Early flowering	6387	Full flowering	16452	Full flowering	16470*	Full flowering
16327	Early flowering	16289	Full flowering	16328	Full flowering	16096*	Full flowering
16166	Early flowering	16097	Full flowering	26114	Full flowering	16456*	Full flowering
6131	Early flowering	16104	Full flowering	16147	Full flowering	16453	Maturity
16331	Early flowering	26393	Full flowering	16295	Full flowering	16320	Maturity
16164	Full flowering	26565	Full flowering	16492	Full flowering	16829	Maturity
26745	Full flowering	16839	Full flowering	26111	Full flowering	26150	Maturity

* Selected accessions used as control without fungicide spraying

Every two weeks, starting on November 15, 2001, sprays of Propiconazole employing 0.6 Lt/ha, were made using an Agro Laura Sprayer. Evaluation of the fungi infection progress after fungicide sprays was scored using a scale with seven severity grades of symptoms (Table 37). Some full flowering control available plots without fungicide spraying were included in this trial.

Table 37. Evaluation scale in order to establish infection progress of the fungous complex associated with *Brachiaria brizantha* inflorescences after propiconazole spraying.

Infection grades	Symptoms description
0	Inflorescences without visible symptoms
1	Inflorescences with colorless or yellow brown honeydew exudates
2	Inflorescences with honeydew exudates and few white fungi powdery growth
3	Inflorescences only with white fungi powdered growth
4	Inflorescences partially recovered with greenish and/or black growth fungi (*Cladosporium* spp., *Cerebella* spp)
5	Inflorescences completely recovered with greenish and/or black growth fungi (*Cladosporium* spp.,*Cerebella* spp)

Harvest of seeds was made periodically following GRU standard procedures. After harvest seed samples

were conditioned and used to establish their health status in the GHL. Samples of 100 seeds of each accession were analyzed using two methods: seed-washing test and incubation in blotter (Neergard, 1977; Agarwal & Sinclair, 1987). Presence of fungi was observed through the stereoscope and light microscope and the identification of genera was made comparing descriptions and pictures found in specialized literature (Barnnet & Hunter, 1998; Zillinsky, 1983; Ahmed & Ravinder Reddy, 1993)

Results

The evaluations under field conditions about the infection progress of the fungi complex associated with *Brachiaria brizantha* inflorescences, after Propiconazole spraying at different plant growth stages, showed variability (Figure 25). When the fungicide was sprayed at booting state, the infection did not show any progress; all sprayed accessions did not show symptoms of fungi infection at naked eye. Sprays on early flowering accessions, which in the initial evaluation had some infection grades, showed variable infection progress: the number of accessions without symptoms (G0) was increased at the end of the evaluation (28 days after fungicide spraying). Accessions with one (G1), two (G2) or three (G3) infection grades, at the beginning, showed increments after 14 days of fungicide spraying while after 28 days the number was decreased. Sprays on full flowering accessions showed a similar trend as the early flowering ones. No major infection changes were observed when sprays were made on accessions with milky or mature grains (Figure 25). Accessions plots without Propiconazole spraying used as a control showed a light infection increase.

Seed health analysis using samples of seed harvested from inflorescences after treatments under field conditions showed that the Propiconazole sprays at booting stage avoid the incidence of *Sphacelia* spp., while in early flowering and full flowering stages its efficacy was minor. The control effect against *Drechslera* spp, *Phoma* spp, *Curvularia* spp., *Cladosporium* spp., and other genera of fungi was very low, but it was possible to observe some control effect when the sprays were initiated on booting stage (Table 38). Concluding, Propiconazole spraying before flowering initiation can be effective to prevent *Sphacelia* infections.

Table 38. Genera of fungi detected on seed samples and percentage of *Brachiaria brizantha* accesions affected by them, after Propiconazole spraying under field conditions, beginning at four growth stages.

Growth stage (beginning)	Genera of fungi													
	Sphacelia spp.	*Drechslera* spp.	*Phoma* spp.	*Curvularia* spp.	*Acremonium* spp.	*Colletotrichum* spp.	*Fusarium* spp.	*Nigrospora* spp.	*Alternaria* spp.	*Cladosporium* spp.	*Epiccocum* spp.	*Aspergillus* spp.	*Rhizopus* spp.	*Chaetomium* spp.
Booting	0	66.7	50.0	50.0	16.7		50.0		16.7		50.0		16.7	
Early flowering	20.0	100	100	80.0	50.0		60.0		30.0	10.0	40.0	40.0	10.0	
Full flowering	17.0	97.6	95.1	87.8	70.7	4.9	85.4	24.4	24.4	21.9	41.5	24.4	29.3	17.1
Maturity	50.0	100	50.0	75.0	100	100	75.0	25.0	25.0	25.0	50.0			
Full flowering (without fungicide spraying)	9.1	100	100	72.7	81.8		81.8			9.1	45.4	45.4	54.5	18.2

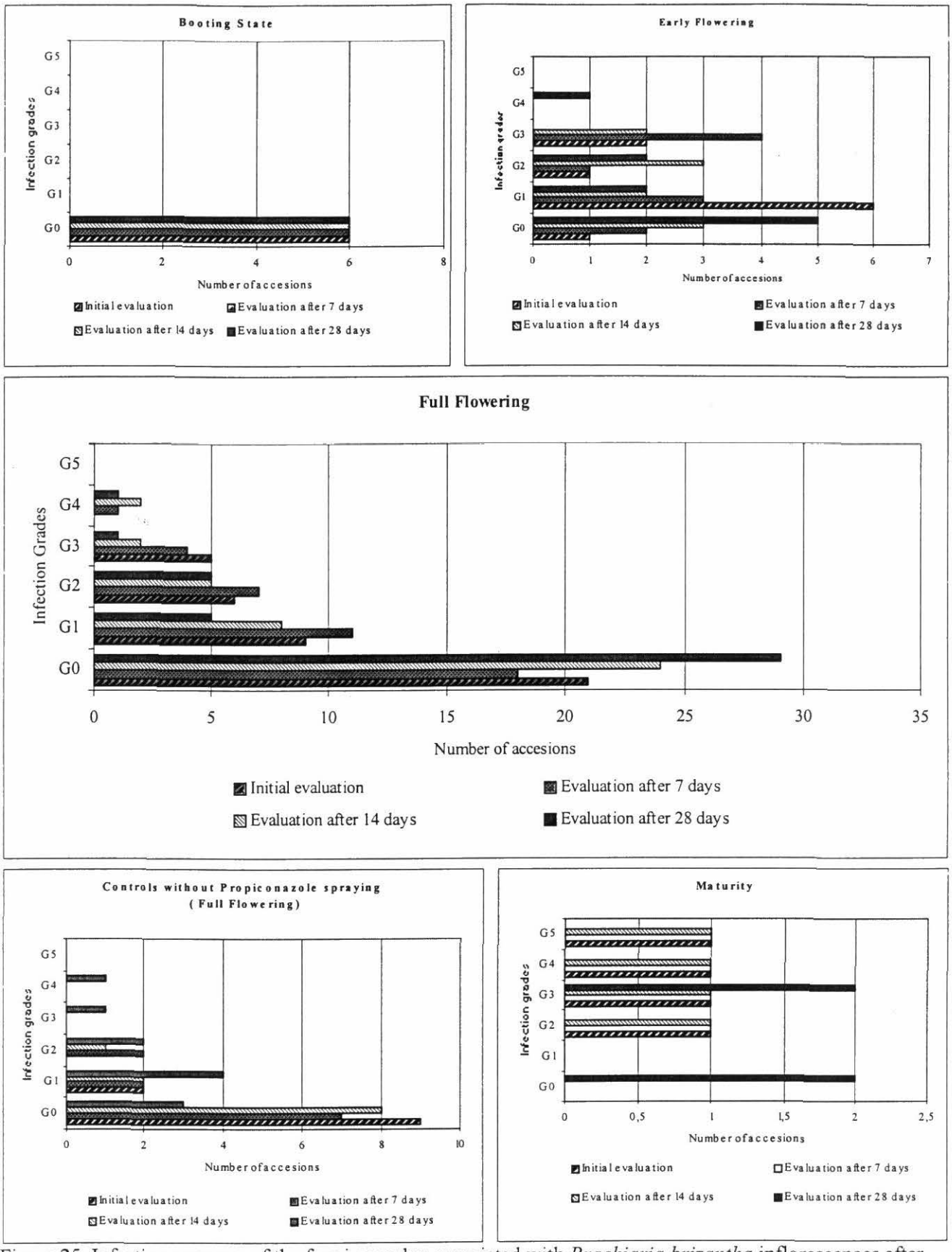

Figure 25. Infection progress of the fungi complex associated with *Brachiaria brizantha* inflorescences after Propiconazole spraying at different plant growth stages.

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Activity 2.6.2. Evaluation of three fungicides to control seed-borne fungi (*Sphacelia* sp., *Drechslera* spp., *Phoma* sp., *Cerebella* sp.) affecting the quality of seed production of *Brachiaria brizantha,* accession 16322.

Introduction

The regeneration of germplasm under field conditions includes an inherent risk of reinfections by pathogens as fungi that affect the production and quality of the seeds. Infections by fungi could diminish the viability during the conservation and impede the safe international distribution when these pathogens are of quarantine importance.

In some previous studies carried out under field conditions in the station Santa Rosa (Popayan) we found a fungous complex the principal components of which were fungi such as *Sphacelia* sp., *Drechslera* spp., *Phoma* spp., *Epicoccum* sp. (*Cerebella* sp.). They were affecting the production of quality seed of *Brachiaria* spp. (García & Pineda, 2000). Evaluations carried out at Germplasm Health Laboratory (GHL) using some fungicides under *in vitro* conditions against the same fungi isolated from *Brachiaria brizantha* seeds produced at Santa Rosa showed that fungicides as Propiconazole and other chemicals had control effect on them (CIAT, 2001). In other studies carried out recently the efficacy under field conditions of the Propiconazole to control the fungous complex in 72 *Brachiaria brizantha* accessions was evaluated at several growth stages. The results about control show high variability, possibly caused by the diversity of accessions expressed by echelon flowering and production of seed inside accessions, beside difficulties in the evaluations. In order to have better control over that variability, a study in which only one accession would be utilized, was undertaken with the purpose of clarify the evaluation

difficulties and establish the efficacy of the Propiconazole and additional fungicides as Mancozeb (Manzate 200) and Metalaxyl + Mancozeb (Ridomil Gold).

Materials and Methods

At the station Santa Rosa during 2002A semester a research under field conditions was carried out. In order to test the efficacy against the fungi found upon *Brachiaria* inflorescences three fungicides, each one with three evaluation doses were selected (Table 39). Sprays of fungicides were made on labeled 6x3 m plots of the *Brachiaria brizantha* accession 16322 starting it at booting state. Four fungicide sprays were made every two weeks during two months starting on March 12, 2002 using an Agro Laura Sprayer. A distribution completely at random of ten treatments (nine with fungicides and one without chemicals) was utilized.

Table 39. Selected fungicides to evaluate their effect on fungi affecting seed quality production of *Brachiaria brizantha,* accession 16322

Fungicide	Commercial name	Concentration	Formulation	Dose kg or L/ha (Comercial product)
Mancozeb	Manzate 200 WP	80%	Wet Powder	5.0
				6.1
				7.2
Metalaxyl + Mancozeb	Ridomil Gold MZ 68 WP	4% Methalaxyl + 64% Mancozeb	Wet Powder	2.0
				2.5
				3.0
Propiconazole	Tilt 250 EC	25%	Emulsion Concentrated	0.38
				0.61
				0.77

Evaluation of the progress of the fungi infection after fungicide sprays was scored using the scale with seven severity grades of symptoms described in activity 2.6.1. To get the information a wood frame 50x50 cm was thrown away three times totally at random in each one of the plots.

Two weeks after the last spray the first harvest of seeds in each plot was made, cutting off mature inflorescences, which were placed in polypropylene bags permitting a certain grade of ventilation to avoid the rot of seeds. Two weeks after the first harvest the second harvest was made and inflorescences were handled as for the first harvest. Four days after each harvest bags with inflorescences were shaken to remove the seeds, which were then conditionned following GRU established procedures.

In order to establish the seed health status samples of 100 seeds of each accession were analyzed using two methods: seed-washing test and incubation in blotter (Neergard, 1977; Agarwal & Sinclair, 1987). Presence of fungi was observed through the stereoscope and light microscope and the identification of genera was made comparing descriptions and pictures found in specialized literature (Barnnet & Hunter, 1998; Zillinsky, 1983; Ahmed & Ravinder Reddy, 1993).

Results

Final evaluation of fungal infection at harvest time showed low infection grades (below G3) in all treatments including the control without fungicide spraying (Table 40), as there were no differences between the treatments. The analysis of the seed health status indicates low percentages of infection in samples from the first harvest and a notable increment in the percentage of some fungi on seeds from the second harvest number II (Table 41). This may be due to infections after the last fungicide spray because harvest number II was picked up approximately one month after last fungicide spraying.

Table 40. Harvest time average grades of fungi infection complex affecting *Brachiaria brizantha* inflorescences after fungicide spraying

Treatment	Dose (kg. or L/ha)	Grades of fungi infection (Average)	
		I*	II
Manzate 200 WP	5.00	2.33**	3.00
Manzate 200 WP	6.10	2.00	2.33
Manzate 200 WP	7.20	2.00	1.67
Ridomil Gold MZ 68	2.00	2.33	2.33
Ridomil Gold MZ 68	2.50	2.00	2.33
Ridomil Gold MZ 68	3.00	2.00	2.67
Tilt 250 EC	0.38	2.00	3.00
Tilt 250 EC	0.61	2.00	3.00
Tilt 250 EC	0.77	2.00	2.00
Control without fungicide	--	2.00	2.67

* Harvest number ** Infection grades expressed on ranged scale 0 – 5.

One can note that the early harvest produces seeds with low percentage of fungi infection while the late harvest, after fungicide spraying, show high percentage of infection by *Drechslera* spp. and moderate ones with *Phoma* spp., *Curvularia* spp., *Fusarium* spp *Epicoccum* spp. and *Aspergillus* spp. (Table 41).

Table 41. Percentage of fungi detected on Brachiaria seeds harvested after fungicide treatments

Treatment	Dosis (kg.; L/ha)	*Sphacelia sp.*		*Drechslera* spp.		*Phoma* spp.		*Curvularia* spp.		*Cladosporium* spp.		*Fusarium* spp.		*Epicoccum* spp.		*Nigrospora* spp.		*Alternaria* spp.		*Chaetomium* spp.		*Pithomyces* spp.		*Trichotecium* spp.		*Aspergillus* spp.		*Rhizopus* spp.	
		I*	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Manzate 200 WP	5.0	0(+)	0	0	54	0	13	0	9	0	2	0	37	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	2
	6.1	1	0	0	64	1	4	0	12	0	2	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0	3	0	0
	7.2	(+)0	0	4	63	0	7	0	11	0	0	0	4	0	6	0	0	0	5	0	0	0	0	0	0	0	3	0	0
Ridomil Gold MZ 68	2.0	0	0	0	69	0	3	1	5	0	0	0	23	0	0	0	3	0	0	0	8	0	2	0	0	0	3	0	0
	2.5	0	0	0	46	0	4	2	9	0	4	0	6	0	2	0	0	0	0	0	0	0	0	0	2	0	7	0	0
	3.0	0	0	0	63	0	7	0	16	0	0	0	3	0	5	0	0	0	0	0	2	0	0	0	0	0	5	0	0
Tilt 250 EC	0.38	(+)0	0	0	71	0	5	1	8	0	0	0	37	0	5	0	0	0	1	0	0	0	0	0	0	3	1	0	0
	0.61	0	0	0	69	0	12	0	0	0	0	0	48	0	2	0	0	0	1	0	0	0	0	0	0	0	2	0	0
	0.77	0	0	0	88	0	4	0	13	0	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	1	5	0	0
Control without fungic.		0	0	0	53	0	7	1	4	0	0	0	3	0	3	0	1	0	0	0	0		0	0	0	0	5	0	0

* Harvest number (+) Presence of *Sphacelia* spp. determined by washing test method

About the effect of fungicide sprays, under this trial conditions, it was difficult determine if there was an effect or none on disease control, considering the low levels of infection of the plots during this research. Another reason may be the changes occurred in the environmental conditions (rain and humidity) on pre-harvest time that facilitated the fungi attack when the residual effect of fungicides was expired. It is important to bear in mind the effect of post harvest handling of seeds because the final humidity of inflorescences during conditionning procedures also can facilitate fungal infections.

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Activity 2.6.3. Preliminary tests to establish the presence of the Coryneform Gram-positive Bacteria *Curtobacterium flaccumfaciens* pv *flaccunfaciens* in seeds of *Zornia* spp.

Introduction

The plant pathogenic coryneform bacteria cause a variety of diseases, specially wilts. This group of bacteria includes some species of quarantine importance such as *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Dowson. Seeds transmit the bacteria and they are the most important source of infection (Dinesen, 1979).

In Colombia during 1980 and 1981 at CIAT Experimental Station of Santander de Quilichao (Cauca) some wilted plants of *Zornia* spp were noted, from which a bacteria was isolated and then identified as *Corynebacterium flaccumfaciens* (Torres et al., 1981). Since the bacteria is considered as a pathogen of quarantine importance in the GHL, seed samples have been tested initially without getting isolates of this kind of Gram-positive microorganism. Recently in seed samples of *P. acutifolius, Crotalaria* spp. and *Brachiaria brizantha*, we obtained colonies of some pigmented Gram-Positive bacteria which were used in a preliminary study (CIAT, 2001). Since the bacteria was originally isolated from *Zornia* spp. harvested in Santander de Quilichao it was necessary to verify by experiment if the microorganism was in seeds of the target species harvested from that source. The results are presented in this report.

Materials and Methods

During the research seed samples of 37 *Zornia* spp. accessions from two sources were selected (Table 42). Initially the seed samples of each accession were incubated in sterile saline solution (0.85% NaCl) overnight. Using GHL routine tests to intercept seed borne after dilutions aliquots of seed leachates were plated on Petri dishes with culture media as Nutrient Agar (NA, DIFCO) and Nutrient-broth yeast extract agar (NBYA). Petri dishes were incubated during 48 hours at 27^{0}C. Suspicious fluid, creaminess pigmented, slightly convex with regular border colonies was selected and using spreads of bacteria on clean slides were stained with Gram stain. Colonies of Gram-positive isolates were subcultured, and incubated during two days under higher temperature (36-37^{o}C). Later in order to identify the bacteria, using their biochemical properties, suspensions of 15x10 ^{8} CFU/mL were injected inside Vitek special plates (bioMérieux Vitec, INC, Hazelwood MO63042) containing culture media with color indicator reactives. After 36 hour incubation plates were read in a VITEK reader (Vitec, INC) and we analyzed the obtained information. In order to complete the bacteria identification some complementary tests were performed using a Gram-Positive ID Kit (Becton Dickinson BBL Crystal^{TM}, Nippon Becton Dickinson Company Ltd.) containing 29 enzymatic and biochemical substrates. In addition a biological test using hypersensibility reactions on *Mirabilis jalapa* after bacterial infiltration was used.

Results

Only samples of four accessions showed bacterial isolates with Gram positive reactions and growths at 37 ^{0}C. They were: *Zornia brasiliensis* 8025 harvested from Palmira 1983A, Z. *brasiliensis* 8849 from Palmira 1983A, *Zornia glabra* 286 from Santander de Quilichao 1982A, and *Z. glabra* 14263 from Palmira 200A. The other 33 seed samples did not show any Gram-positve isolates.

The characteristics of the four isolates are summarized in Table 42. All isolates were catalasa positive and oxidades negative, as well as the reference bacteria *Curtobacterium flaccumfaciens* and *C. michiganenese*. About mobility only isolate from Zornia 8849 showed reaction as *C. flaccumfaciens*. About the other characters there was low affinity.

Preliminary results of hypersensibility biological test on *Mirabilis jalapa* showed positive reactions (Figure 26) with the four isolates but not in agreement with the observations of the Danish Government Institute of Seed Pathology where the reactions were obtained 2-4 days after inoculation on two months age plants. It is very important to repeat this research by testing different environmental conditions and inoculum concentrations as well as including pathogenicity tests on Zornia 8025, Zornia 8849, Zornia 14263 and Zornia 286 accessions.

The results of this preliminary research were not conclusive about the presence of *C. flaccunfaciens* pv *flaccunfaciens*, a bacteria of quarantine importance. The sole firm conclusion is that the four Gram positive isolates obtained from *Zornia* seeds were Coryneform plant bacteria (Vidaver & Davis, 1988), but not the target quarantine species.

Table 42. Characteristics of four Gram positive bacteria isolated from samples of *Zornia* spp. seeds

Characters	Isolates number (Zornia Accession)				Characters of reference Bacteria	
	8025	8849	14263	286	*C. flaccumfaciens*	*C. michiganense*
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-
Motility	V	+	-	-	+	-
Acids production:						
Ribose	-	-	-	-	+	-
Cellobiose	-	-	-	-	+	V
Melezitose	-	-	-	-	+	-
Inuline	-	-	-	-	-	-
Mannitol	-	-	+	-	+	V
Sorbitol	-	-	-	-	-	-
Hydrolysis:						
(Sucrose)	-	-	-	-	-	V
Arabinose	-	+	+	-	-	
Lactose	-	-	-	-	-	
Maltoside	-	+	+	+	V	
Maltotriose	-	+	+	-	V	
Trehalose	-	-	+	-		
Celobiose	-	+	+	+		
Glycerol	-	-	+	-		
Esculine	-	+	+	-		
α-metilglucoside	-	-	+	-		
Hypersensibility reaction on *Mirabilis jalapa*	¿?	¿?	¿?	¿?	+	

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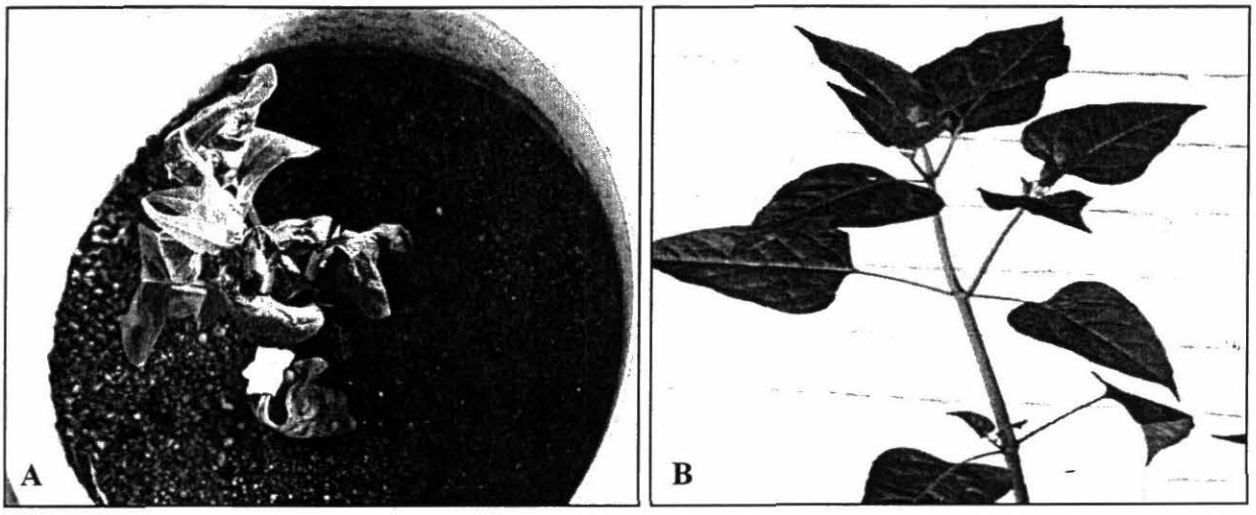

Figure 26. **A.** *Mirabilis jalapa* inoculated with a Gram positive bacteria isolated from seeds of *Zornia brasilensis* acession 286; **B**. *M. jalapa* inoculated with sterilized water.

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

Output 3.1: Designate Collections better characterized

Activity 3.1.1. Characterization of different phaseolin types in wild forms and landraces of common bean (*Phaseolus vulgaris* L.).

Phaseolin is the major seed storage protein of *Phaseolus vulgaris* L. It is a globulin, soluble only at higher salt concentration, accounting for 35 to 46 % of total seed nitrogen. The legume globulins contain low levels of the sulfur-containing amino acids, methionine and cysteine. These deficiencies are detrimental to monogastric animals (including humans) that have to rely on a supply of these amino acids in their diet. Through a collaborative activity with one Bean Project (bean improvement) and The Universidad Nacional de Colombia, we are trying to obtain the extraction, purification and detection of the different phaseolin protein types existing in wild forms and landraces, in order to study the nutritive value of these seed protein variants. We are using 22 different phaseolin types in different genetic backgrounds (Table 43). The first step is the extraction and purification of the different phaseolin types, and next its analysis by 1di-SDS-PAGE electrophoresis.

Table 43. Materials used in studies of nutrition value of phaseolin variants.

Accession	Phaseolin types
G12857	A, Ca, H_2
S33738	H_1
G19895	J_1, J_3
G23592	J_2, J_4
G24408	L
G50869	M11
G23439	M13
G12881	M7
G12878	M9
G23771	Pa
G24674	Simple 1
G11027	Simple 2
G50898	Simple 3
G12851	Sd
G23419	T
G23442A	To
DOR 390 (cultivated, recurrent parent))	S
Offsprings of backcross between DOR 390 (phaseolin S) and G23585 (phaseolin I)	I

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Activity 3.1.2. Characterization of complexes of *Phaseolus vulgaris* L and gene flow in Colombia

Introduction

Seed storage proteins have been studied intensively because they are useful genetic markers in five main areas: (1) diversity genetic, (2) evolution and domestication of crops, (3) gene flow among plant populations, (4) genome relationships, and (5) as a tool in plant breeding. These proteins are characterized by a high level of polymorphism, limited environmental influence on their electrophoretic pattern, a simple genetic control, a complex molecular basis for genetic diversity, and homologies between storage proteins that extend across taxa. With these works we are reporting the utility that displays this marker for the study of the genetic resources of *Phaseolus vulgaris* L. beans.

Results and Discussion

Using one-dimensional SDS-PAGE technique for seed storage proteins, we have analyzed twelve Colombian populations, which have been multiplied and conserved in the *Phaseolus* germplasm bank held at CIAT. For this study, we only took recently multiplied seed. The original seed of these populations was collected and classified as cultivated materials. However, during the first generation of seed multiplication, we observed segregation in seed size and colors indicating possible wild-weedy-crop complexes (Table 44). The analysis of seed proteins indicates several types of Mesoamerican and Andean phaseolins in these complexes. These results go along those obtained by Beebe and co-workers (1997) on Colombian materials, and could be indicative of high level of gene flow in Colombia.

Table 44. Phaseolin types found in the wild-weedy-crop complexes in Colombian populations of common bean.

CIAT No.	Biological status	Departament	County	No. of sampled seed	Phaseolin types
G50988	Wild Weedy Crop Escaped	Boyaca	Guayata	27 13 11 3	S, C, H_I S, C C, H_I, T S, C
G50630	Crop	Antioquia	Andes	45	S, C, T, H_I
G50632	Crop	Antioquia	Andes	54	S, CH, C, T
G50646	Crop Weedy	Antioquia	Andes	12 4	T, C, CH T, C
G50785	Crop Wild Weedy Escaped	Antioquia	Andes	39 34 60 16	S, CH, C, T, H_I S, CH, C, T S, CH, C, T, H_I C, T, H_I
G50859	Cro	Cauca	Timbio	106	S, B, C, T, H_I
G51132	Weedy	Cauca	Timbio	2	B
G50861	Crop	Cauca	Timbio	1	S
G50879	Crop Wild Weedy	Caldas	Salamina	73	B, C, T, H_I B, C B, C, H_I
G51009	Crop	Antioquia	SantaFé de	6	B

			Antioquia		
G51046	Crop	Cauca	Timbio	3	T
G50983	Wild	Cundinamarca	Choachi	4	S, Mu ?
	Weedy			30	S, C, Mu ?
	Crop			3	S, C
	Escaped			6	S, C

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Contributors: O. Toro, C.H. Ocampo

Activity 3.1.3. Characterization of the genetic diversity and redundancy in an avocado (*Persea americana* Mill.) collection using molecular markers (AFLPs) (collaborative work with CORPOICA funded by Ministerio de Agricultura y Desarrollo Rural, Colombia).

Introduction

The cultivated avocado (*Persea americana* Mill.) is a sub-tropical fruit tree, diploid with 2n = 24 chromosomes and a moderate genome size of 883 Mb (8.83 x 10^8 bp). Genome analysis and breeding of avocado is quite difficult mainly because of the size of the trees, a long juvenile phase, and a lack of satisfactory genetic knowledge. Three horticultural or ecological races of avocado have traditionally been recognized, namely the Mexican, Guatemalan, and West Indian races. These races are separated on the basis of morphological, physiological, and horticultural traits, and are adapted to different climates and ecological conditions (Fiedler et al., 1998). In the last few years, several types of molecular markers have been used for the genome analysis of avocado, such as isozymes, RFLPs ((chloroplast and ribosomal DNA), DNA fingerprints (DFP), SSR and RAPDs. Nevertheless, until now no study of genetic diversity of avocado using AFLPs has been reported (Sharon et al., 1998). Amplified fragment length polymorphism (AFLP) markers are a recently developed system that combines the specificity of restriction enzyme analysis with the relative technical simplicity of the polymerase chain reaction (PCR). AFLPs have been used to fingerprinting accessions, for genetic linkage mapping, and for genetic diversity analysis. The advantages are that they are easy to use, sample a large number of loci per reaction, and are reproducible between laboratories. They do have the same disadvantage as randomly amplified polymorphic DNA markers that they are inherited in a dominant manner (Vos et al., 1995). Through this study we wanted to know the level redundancy (possible genetic duplicates) and extent of genetic diversity present in an avocado collection maintained by CORPOICA in Palmira.

Results and Discussion

Leaf tissue was collected for DNA extraction from 61 accessions of avocado. Accessions represent many landraces from Colombia, México, Guatemala and Trinidad y Tobago. We have standardized the procedure for DNA extraction of avocado. That extraction proves to be difficult because of the high content and activity of polyphenoloxidases (that causes the extract to acquire a gray or brown coloration) that interfere with the DNA extraction. The standardization has been based on the protocol modified by Dellaporta for the extraction of DNA for rice microsatellites.

Our modifications for avocado consist basically in using polyvinilpirrolidone (Pvp-40) to eliminate polyphenoloxidases and to avoid the degradation of DNA. In addition we did a washing with phenol and three washings with a mixture of chloroform: isoamylic alcohol, consecutively. Once the DNA was washed, we precipitated it with isopropanol, pelleted it and washed it with ethanol to finally dissolve it with TE (10 mM Tris-HCl /1mM EDTA pH 8.0). These washings were essential to make the digestion, and later the DNA amplification. In a later stage we will follow the method of AFLP Analysis System I with minor modifications.

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Contributors: I. Sánchez (CORPOICA); C.H. Ocampo (Genetic Resources Unit, CIAT); G. Gallego, J. Tohme (Biotechnology Research Unit, CIAT).

Activity 3.1.4. Isozyme characterization of the genetic diversity and phylogenetic relationships in the genera *Vasconcellea* and *Carica* (*Caricaceae*) of Colombia and Ecuador (collaborative work with IPGRI RegOff Americas funded by FUNDEAGRO)

Introduction

Colombia and Ecuador are countries with a great diversity of species of the family *Caricaceae* (Badillo, 1993). The genus *Carica* is the most important one with the fourth tropical fruit at world wide level: *Carica papaya* L. (FAO, 1996). The genus *Vasconcellea* is second in economic importance with the mountain papayas (National Research Council, 1989). Like the other genera in this family, the species of both genera are diploid with 2n = 18 and predominantly dioecious (Badillo, 1993). Both genera have their origin in tropical America where they provide economically important edible fruits and latex (Badillo, 2000). Another potential of the species of *Vasconcellea* is as source of resistance genes to diseases such as the virus of the annular spot, the main problem in the production of common papaya (Magdalita, 1997). Studies of genetic diversity based on molecular and biochemical markers have been few and with samples reduced to less than two plants per species (Jobin et al., 1997). We thus attempt to advance the isozyme characterization of these species in Colombia and Ecuador, in order to know the genetic distances among them.

Materials and Methods

A total of 167 plants was evaluated representing 66 accessions of papaya (*C. Papaya)* and seven species of highland papayas (*V. cundinamarcensis, V. goudotiana, V. sphaerocarpa, V. cauliflora, V. crassipetala, V. stipulata, V* x *heilbornii*) (Table 45). These accessions were taken from the collections of the University of Caldas, Corpoica (La Selva, Colombia), and the Technical University of Ambato (Ecuador). Three samples represent material collected in situ in Colombia. Out of the accessions maintained in Corpoica, one of *C. papaya* is coming from

Cuzco, Peru. Fourteen isozyme systems were evaluated using young leaves like weave source for the extraction. The methodology developed by Ramirez (1987) was used for the extraction, electrophoresis and histochemical staining of enzymes, with the following modifications in the electrophoresis: 250 V, 60 mA for polyacrylamide gel electrophoresis, and 160 V, 45 mA for starch gel electrophoresis. The fixation and storage of gels was made according to the methodology developed by Hussain et al. (1988). The biochemical data (enzyme bands) were interpreted as dominant markers and were compiled in a data matrix on the basis of presence (1) or absence (0) of selected bands. The bands of each isozyme system were identified according to their order of migration cathode-anode. The index of similarity of Jaccard was used to compute the distances. The resulting matrix was analyzed as group of the closest neighbor (neighbor joining). The calculations were processed with the Winstat program, version 97.

Table 45. Geographic origin, germplasm bank and number of accessions characterized of the species of the genera *Vasconcellea and Carica*.

Genus	Collection	Origin geographic		No. of accessions
		Country	Province	
Vasconcellea sphaerocarpa	La Selva [1]	Colombia	Antioquia	5
		Colombia	Tolima	1
Vasconcellea crassipetala	**In situ**	Colombia	Caldas	1
Vasconcellea cauliflora	La Selva	Colombia	Antioquia	2
		Colombia	Valle	1
Vasconcellea cundinamarcensis	La Selva	Colombia	Antioquia	9
	U. de C.[2]	Colombia	Cundinamarca	8
	U. de C.	Colombia	Boyaca	7
	U. de C.	Colombia	Tolima	3
	U. de C.	Colombia	Nariño	2
	In Situ	Colombia	Quindio	3
	U. de C.	Colombia	Cauca	1
	U. T. A.[3]	Ecuador	Chimborazo	1
	U. T. A.	Ecuador	Tungurahua	1
Vasconcellea goudotiana	U de C	Colombia	Cundinamarca	3
	U de C	Colombia	Caldas	1
	U de C	Colombia	Huila	2
	U de C	Colombia	Tolima	1
	U de C	Colombia	Cauca	1
	La Selva	Colombia	Risaralda	1
	La Selva	Colombia	Quindio	1
	La Selva	Colombia	Valle	1
	La Selva	Colombia	Antioquia	2
Vasconcellea stipulata	U. T. A.	Ecuador	Chimborazo	1
	U. T. A.	Ecuador	Bolivar	1
Vasconcellea x heibornii	U. T. A.	Ecuador	Chimborazo	1
	U. T. A.	Ecuador	Tungurahua	1
Carica papaya	La Selva	Colombia	Antioquia	3
		Perú	Cuzco	1

[1] Germplasm bank conserved ex-situ in La Selva, Colombia
[2] Germplasm bank conserved ex-situ in the Universidad de Caldas, , in Manizales, Colombia
[3] Germplasm bank conserved ex-situ in the Universidad Técnica de Ambato

Results and Discussion

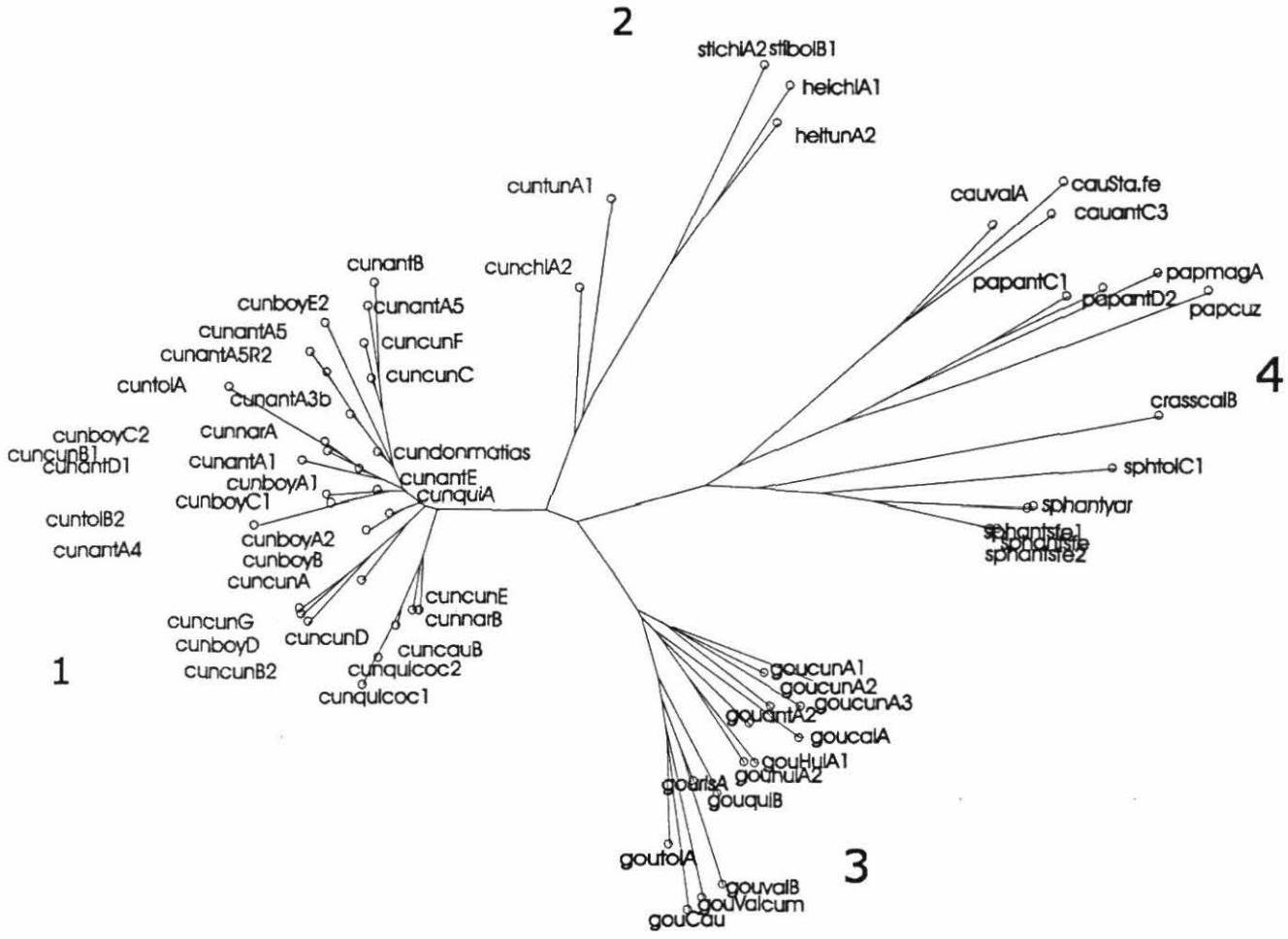
The first result was the standardization of protocols for eight of the fourteen isozyme systems tested, which showed clear staining and revealed polymorphism (Table 46). We have found a high isozyme diversity. The eight isozyme systems display in their majority bands or combinations specific to a species or a group. At the intraspecific level, polymorphism is very variable according to the species. Cluster analysis separated Colombian accessions of *V. goudotiana* and *V. cundinamarcensis*, the former showing geographic differentiation within Colombia, the latter exhibiting differentiation only at the country level (Figure 27). The little differentiation observed between accessions of different regions from Colombia can partly be explained by the strict allogamy of the plant (dioecious), which favors a strong intra-population diversity and limits the diversity between populations (Schoen & Brown, 1991). The Ecuadorian accessions formed a distinct group, with *V x heilbornii* closer to *V. stipulata* than to *V. cundinamarcensis*. *V. sphaerocarpa*, *V. cauliflora*, *V. crassipetala*, while *C. papaya* formed a distant group. The distance between *C. papaya* and *Vasconcellea* species is not larger than between some *Vasconcellea* species, a result that contrasts with the high intergeneric differentiation reported in studies with DNA markers (Aradhya et al., 1999).

Table 46. Isozyme systems resolved from the young leaves tissues of the the genera *Vasconcellea and Carica*, which showed suitable resolution and revealed polymorphism.

Enzyme	Abbreviation	number (E. C.)[1]	System	Buffer	Polymorphism
Aß-Esterases	Aß-EST	3.1.1	PAGE[2]	TB[4]	High
Diaphorase	DIA	1.6.4.1	SGE[3]	**HC**[5]	Medium
Aspartate aminotransferase	GOT or AAT	2.6.1.1	PAGE	TB	Medium
Aß-Acid phosphatase	Aß-ACP	3.1.3.2	PAGE	TB	High
Shikimate dehydrogenase	SKDH	1.1.1.25	SGE	HC	Medium
6-phosphogluconate dehydrogenase	6-PGDH	1.1.1.43	SGE	HC	High
Phosphoglucomutase	PGM	5.4.2.2	SGE	HC	High

[1]Enzyme Commission (E.C.) number
[2]Polyacrylamide gel electrophoresis
[3]Starch gel electrophoresis
[4]Tris-borate buffer system
[5]Histidine-citrate buffer system

Figure 27. Classification tree (Neighbor Joining, distance of Jaccard) obtained with the data of the isozyme analysis. The calculations were processed with the Winstat program, version 97.
cun=*V. cundinamarcensis*, sti=*V. stipulata*, hei=*V.* x *heilbornii*, gou=*V. goudotiana*, pap=*C. papaya*. cau=*V. cauliflora*, crass=*V. crassipetala*, sph=*V. sphaerocarpa*.
cun=Cundinamarca, cal=Caldas, nar=Nariño, cau=Cauca, boy=Boyacá, qui=Quindio, tol=Tolima, ant=Antioquia, ris=Risaralda, val=Valle, sta fe=Santa fe de Antioquia, mag=Magdalena, Hui=Huila, cuz=Cuzco(Perú) chi=Chimborazo (Ecuador), tun=Tungurahua (Ecuador) bol=Bolivar (Ecuador); Letters = A-g: locality; R = 1-6 Repetition



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Activity 3.1.5. Standardization of isozyme methods in native mycorrhizal fungi germplasm collected in maize producing zones of Colombia

Introduction

The use of biofertilizers (mycorrhiza) in agriculture and forestry is an efficient system to increase yield and quality of the harvests, while reducing costs and protecting the natural resources basis (Corpoica, 1998). In order to take advantage of the maximum potential from the mycorrhizal fungi in the agricultural production we need to use efficiently the diversity of this genetic resource, starting with its collection and study. The objective of this work was the standardization of protocols of isozyme systems for mycorrhiza for the characterization of its diversity.

Results and Discussion

Biological material

Isozyme systems were evaluated using native spores of mycorrhizal fungi associated with maize in three maize producing sites of Colombia: Tibaitatá (Cundinamarca), Turipaná (Córdoba) and La Libertad (Meta). The spores of these fungi were previously characterized for their morphological traits in order to identify taxonomically the morphotypes present in these collections; three genera were found: *Glomus, Scutellospora* and *Acaulospora* (Table 47).

Tabla 47. Geographic origin, size and number of spores of the genera (mycorrhizal fungi) used for the isozyme standardization.

Genus	geographic origin	approximated size of the spores	Number of used spores
Gigaspora	Meta	350 µm	30
Acaulospora	Meta	120 µm	200
Scutellospora	Córdoba	220 µm	80
Glomus	Córdoba	110 µm	250
Glomus	Cundinamarca	150 µm	150
Glomus	Cundinamerca	180 µm	200

Enzyme eletrophoresis

We could standardize protocols for four of the ten isozyme systems tested, with suitable resolution and revealed polymorphism. The four selected enzymes were: Aß-Esterases (Aß-EST, E. C. 3.1.1), Diaphorase (DIA, E.C. 1.6.4.1), Aspartate aminotransferase (AAT, E. C. 2.6.1.1) and malate dehydrogenase (MDH, E. C. 1.1.1.37). For the standardization of these methods on native spores, our work has been based on that developed by Ramirez et al (1987) and modified for mycorrhizal fungi by Mejia et al. (1996). The methodology for isozyme extraction, running and staining was modified for the native spores as follows: about 250 spores were macerated with a mini-pestle (Micro Tissue Grinder, Wheaton 357848 0.2 ml) until all tissue is disrupted. The buffer used for extraction is the TRIS-HCl 0.05 M, pH 8.3, in a proportion of 1:10 (volume:volume). The samples were centrifuged for 20 min. at 14 000 rpm in an Eppendorf microcentrifuge. The supernatant (25 µL) was used to conduct the electrophoresis on polyacrylamide of 4 % in concentration and 12 % in separation. The electrophoretic separation was initiated with 16 mA, 90 V, 1-2 W, and finished with 25 mA, 150 V, 12 W, which lasted about three hours. The gels were stained, fixed, and stored according to the methodology compiled by CIAT (1988). In conclusion, the methodology has been improved and adapted for the work with native spores of the mycorrhizal fungi.

Data analysis

The bands were labelled sequentially, with those migrating closest to the anodal end being designated as number 1 (Koenig and Gepts, 1989). The most common band was designated as 100 and all other bands were measured in millimeters from the standard. In each gel, the genera *Scutellospora* and *Glomus* were included as standard controls (Figure 27).

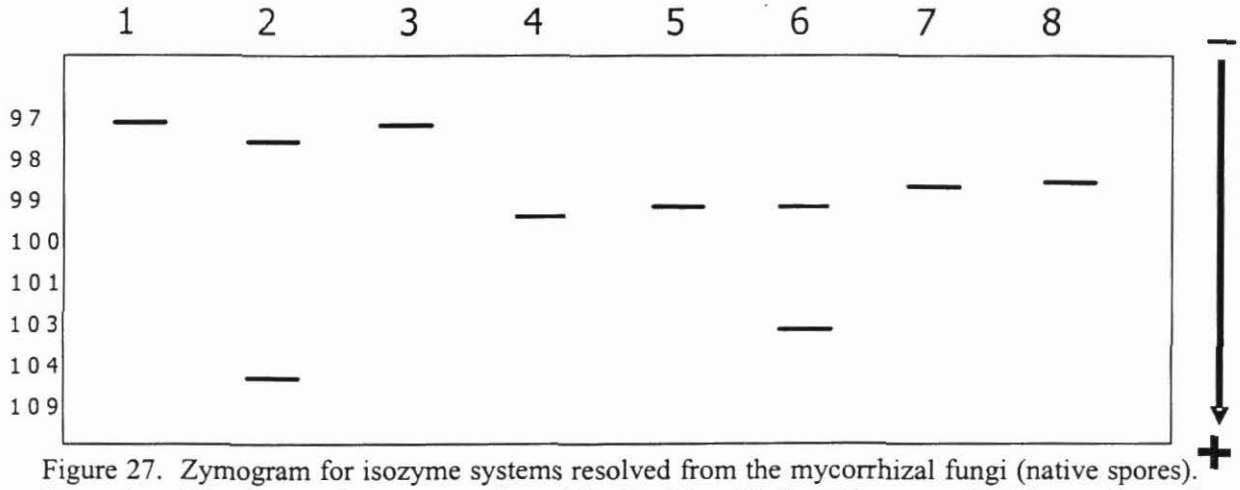

Figure 27. Zymogram for isozyme systems resolved from the mycorrhizal fungi (native spores). Lanes 1 and 2 relate to the genera collected in Cordoba, (1) *Scutellospora*, (2) *Glomus*; lanes 4 and 5 relate to the genera of Meta, (4) *Gigaspora*, (5) *Acaulospora*; the lanes 6 and 7 relate to the fungi from Cudinamarca (6) *Glomus* and (7) *Glomus*; and lanes 3 and 8 refer to the checks (3) *Scutellospora* and (8) *Glomus*

The electromorphs (bands) obtained for the six enzyme systems were used as binary descriptors. Individuals were scored for presence or absence of each electromorph. The individuals were grouped by zymotype. If two individuals differed by at least one electromorph, they were considered as two different zymotypes. A pair-wise similarity matrix was calculated using the simple matching coefficient. This similarity matrix was employed to construct a dendrogram by

the unweighted pair group method with arithmetical averages (UPGMA), using the SAHN-clustering and TREE program from the NTSYS-pc, version 2.02i . The dendrogram obtained from the isozyme profile analysis (Figure 27) shows that at the 0.80 similarity level all samples are different (Figure 28).

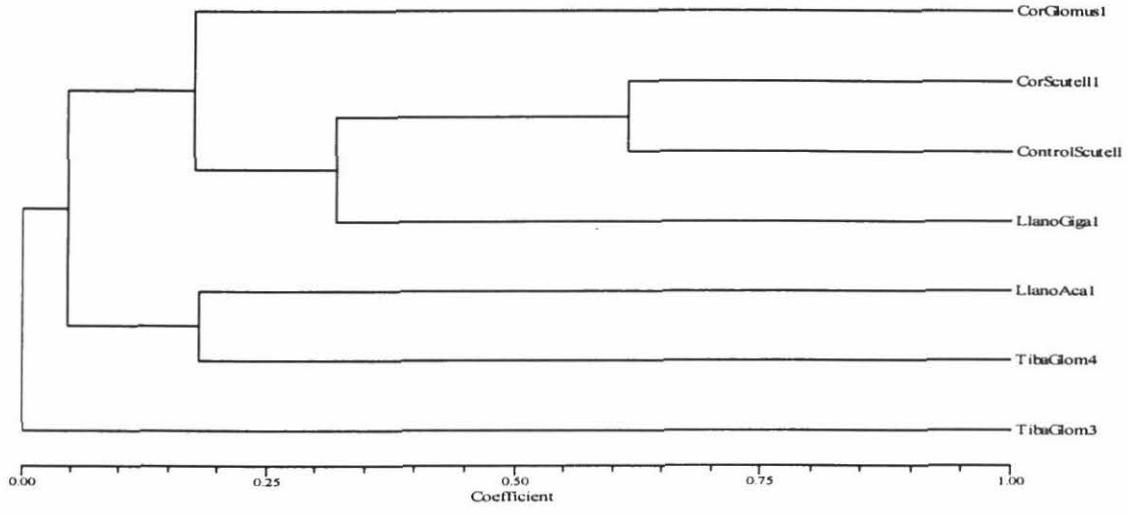

Figure 28. Dendrogram derived from a UPGMA cluster analysis, using the Dice similarity index based on isozyme banding patterns. All samples were resolved at the 0.80 similarity level.

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Contributors: C.C. Rojas Marulanda, M. Ramírez (Programa Nacional de Recursos Biofísicos, CORPOICA); C.H. Ocampo (Genetic Resources Unit, CIAT).

Output 3.4 Unique genes better sampled and characterized

Activity 3.4.1. Identification de QTLs associated with the accumulation of iron and zinc, and the mapping of gene candidates like phaseolin in common bean *Phaseolus vulgaris* L.

Introduction

More than a third of the world population suffers from deficiency in iron and zinc, where the most affected sectors are women in reproductive age and children. Since bean is an important nutritive element, protein source, calories, minerals and vitamins, it is a candidate for the improvement of the nutritional quality of human populations. Accessions of the bean core collection held in CIAT, which differ in their ability to accumulate iron and zinc, were selected for the construction of a genetic linkage map. In order to determine the location of loci responsible for the quantitative characteristics associated with the mineral accumulation, we used molecular markers (microsatellites, RAPDs, etc.) and a biochemical marker (phaseolin). We use one biochemical technique (1di SDS-PAGE) for the detection of the phaseolin protein polymorphism. The bean mapping population is comprised of F_1 plants from an intraspecific cross between the two Andean parents (G21242, phaseolin C and G21078, phaseolin T).

Results and Discussion

Three electrophoretic types of phaseolin were found among the progeny. The "T" phaseolin was present at the highest frecuency (83%), followed by the "S" type with 11%, pattern that would not be expected in a crossing of Andean x Andean, since it is a typical Mesoamerican phaseolin. Also we observed the "C" type with 4.44 %. Considering this result, phenomena like introgression could have happened on the part of the Mesoamerican genepool towards the Andean one by process of domestication of Mesoamerican bean within the Andean region. Paredes and Gepts (1995) in addition suggest that the C phaseolin type could come from the recombination between the T and S phaseolin patterns. This would indicate that previous crossover between wild genepools could have existed. With this evidence, the location of the phaseolin on chromosome 7 was possible; this finding agrees with the results reported by Kami et al. (1995), who locate it on the same chromosome.

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Contributors: C. Astudillo Reyes, M. Blair (Bean Project, CIAT); C.H. Ocampo (Genetic Resources Unit, CIAT).

Subproject 4: the International Cooperation and Capacity Building

Output 4.1. NARS human resources trained

Activity 4.1.1. Lecturing in specialized courses

The Staff of the Genetic Resources Unit participated in the course of the MSc. degree in Neotropical Genetic Resources given by Universidad Nacional de Colombia. Lectures were presented and practical demonstrations were given in aspects of *ex situ, in situ* and *in vitro* conservation, germplasm health and viability.

Contributors: G. Mafla, B. Pineda, D.G. Debouck

The Staff of the Genetic Resources Unit participated in the course on ex situ conservation of genetic resources jointly carried out with IPGRI RegOff Americas for 16 professionals of the Instituto Colombiano Agropecuario, with lectures and practical demonstrations.

Contributors: M. Baena (IPGRI RegOff Americas); A. Ciprian, O. Toro, G. Mafla, B. Pineda, M.S. Balcazar, N.C. Flor, C. Ocampo, D.M. Montero, D.G. Debouck

Detail on input in other courses and individual personalized training is given in 6.

Output 4.2. Conferences in national/ international fora

A total of eleven conferences and scientific presentations were presented in international and national for a (see list in 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 topic of plant genetic resources was selected, and initial contacts were made with communication specialists at Epcot, CIP (the other partner at Epcot), and IPGRI (in order to link with the campaign on the Global Conservation Trust).

Contributors: C. Llano, D.G. Debouck (CIAT GRU); 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

Activity 5.1.1. Monitoring gene flow between wild relatives and landraces in common bean in Costa Rica (collaborative project with the Universidad de Costa Rica and the University of Hannover funded by BMZ of Germany). IU495D

Introduction

The present study aims to establish the movement of genes in the complex (wild/weed/cultivated) of the crop model *Phaseolus vulgaris L.* as a result of cross pollinations. Beside outcrossing studies in two locations in Costa Rica carried out by the Costa Rican partner, we study possible cases of gene flow on accessions collected in 1987 and 1998 in the Central Valley of Costa Rica. We focus on intermediate forms in order to (1) find evidence of gene flow from cultivated materials into the wild, and (2) quantify its importance.

Materials and Methods

Seeds were collected in 1987 and 1998 from 6 populations of wild and weedy forms identified by the localities: Zarcero, Quircot, Tabarca, Aserrí, Jérico and Chaguites (Araya et al. 2001). For the morpho-agronomic evaluation, we study pod dehiscence, "agouti" testa (the wild phenotype being dominant in F1 and segregating materials), and seed size (quantitatively inherited). In addition, we use biochemical (phaseolin and allozyme) and molecular markers (microsatellites and cpDNA) to determine gene transfer. The determination of cpDNA is important to quantify the phenomenon of 'cytoplasm capture', where the cytoplasm is taken out of the maternal parent after a series of backcross with the pollen donor parent. One hundred eighty seven original seeds between wild, intermediate and cultivated have been analyzed. The weedy seeds were selected depending on their agronomic characteristics, resulting in a total of 95 seeds possibly being the result of gene flow.

Results

Allozymes

Isozymes have been useful to determinate genetic similarity among materials. A preliminary study with diaphorase has shown a specific polymorphic pattern for Mesoamerican and Andean gene pools, composed of five rapid alleles and six slow alleles, respectively. We have analyzed the following enzyme systems: DIA, Me, Mdh, Skdh, on the wild and weedy materials (130 accessions). The patterns obtained for the diaphorase enzyme were analyzed using *UPGMA* method for the average ligament to the genetic distances. However, the distinctions among gene pools were not clear showing at the beginning that these materials own a 80% genetic similarity with the Mesoamerican and Andean controls. These preliminary results need to be evaluated with the other enzyme systems mentioned above.

Phaseolin

The analysis of the phaseolin marker (controlled by nuclear genes) provides quick and reliable indications for the determination of origin and possible cases of introgression. In the analysis of phaseolin of the cultivated material from the study area the predominant phaseolin was "S". However we have obtained four different patterns of phaseolin such as CH, T, H, (S+I), which means a possible introduction of Andean cultivated materials into the Central Valley of Costa Rica.

The 25 wild materials (Table 49) show phaseolin S, a pattern of phaseolin usually found in cultivated materials of Mesoamerica; the presence of this phaseolin might indicate genetic flow since Costa Rican wild forms usually have 'M' phaseolins.

Table 49. Type, location, number of individuals and their phaseolin.

Wild forms - Location	Number of individuals	Phaseolin type	100 seed weight g
Chaguites	10	SIMPLE 4	6.8
Zarcero	3	S	14.3
Aserrì	11	M1	5.2
Tabarca	6	M6	9.7
	2	M1	
Jerico	14	S	5.2
	3	SIMPLE 4	
Quircot	13	S	6.9
	2	SIMPLE 4	

Weedy forms - Location	Number of individuals	Phaseolin type	100 seed weight g
Chaguites	22	SIMPLE 4	5
Tabarca	11	M6	12.7
	2	M1	
Jerico	29	S	5.3
	3	SIMPLE 4	
Quircot	24	S	12.2
	4	SIMPLE 4	
Cultivated forms - Location	Number of individuals	Phaseolin type	100 seed weight g
Quircot	41	S	19.8
Quircot	1	CH	15
Quircot	1	T	25.1
Quircot	1	Het.	20
Quircot	1	Het.	18.4

Since phaseolin type 'CH' and 'Het' (for heterozygotic pattern) are uncommon in cultivated types, we checked pattern in the next generation.

Cultivated forms – Location	Phaseolin type 1st Generation	Number of individuals 2nd Generation	Phaseolin type 2nd Generation
Quircot	CH	1	CH
		1	CH
Quircot	Het.	1	Het.
		1	Het.

Microsatellite Markers

For the microsatellite marker, we had first to standardize the PCR conditions for labeled primers with phosphoramidite 6-FAM, HEX,TET and electrophoresis conditions, first with in polyacrylamide electrophoresis (6%) and staining silver staining.

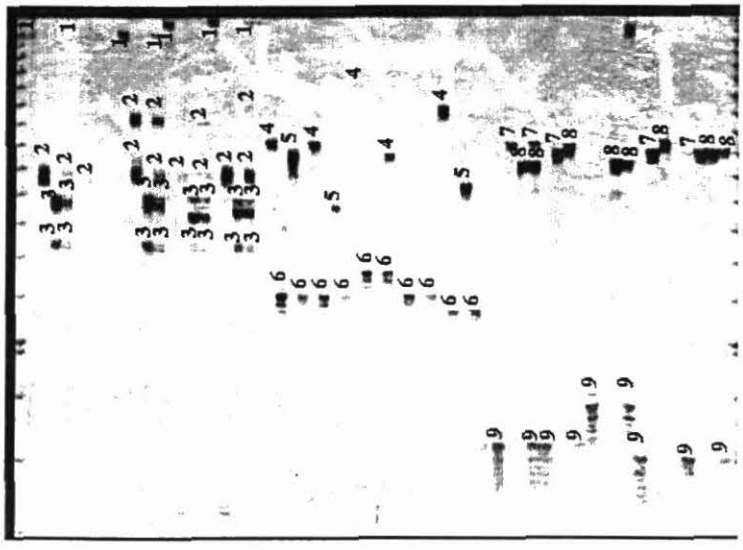

Figure 29. Different primer combinations of primers used in this study. Gel of Polyacrylamide 6% with silver staining. 1:GATS91; 2:BM188; 3:BM205; 4:BM187; 5:BM183; 6:BM189;7: BM140; 8:BM175; 9:BM172.

In the first step we used accessions from different geographic origins in order to ascertain the discriminant value of the microsatellites (Table 50), with the following primer combinations BM205, BM188 and GATS91. We also established the conditions of electrophoresis, fluorescence of primers, and analysis of the microsatellites by GeneScan, Genotyper and Ntsys softwares.

Table 50. List of materials of *Phaseolus vulgaris* and their status.

FI Number	Origin	G Number	Status
FI 5749	CRI	G51062B	Wild
FI 5750	CRI	G50897	Wild
FI 5755	CRI	G50898A	Wild
FI 5759	CRI	G23418	Wild
FI 5762	CRI	G50898	Wild
FI 5767	CRI	G51062A	Weedy
FI 5769	CRI	G23417B	Weedy
FI 5772	CRI	G23417	Weedy
FI 5776	CRI	G51062C	Weedy
FI 5778	CRI	G51062A	Wild
FI 5785	CRI	G23416	Wild
FI 5787	CRI	G18970	Weedy
FI 5789	CRI	G18970	Cultivated
FI 5792	CRI	G16267	Cultivated
FI 5798	CRI	G24138A	Cultivated
FI 5802	CRI	G24140	Cultivated
FI 3884	GTM	G50718	Wild
FI 4455	ARG	G19897	Wild
FI 1148	PER	G12857	Wild
FI 1178	ECD	G23723	Wild
FI 1923	MEX	G12949	Wild
FI 2121	COL	G24408	Wild
FI 934	ARG	G23592	Wild

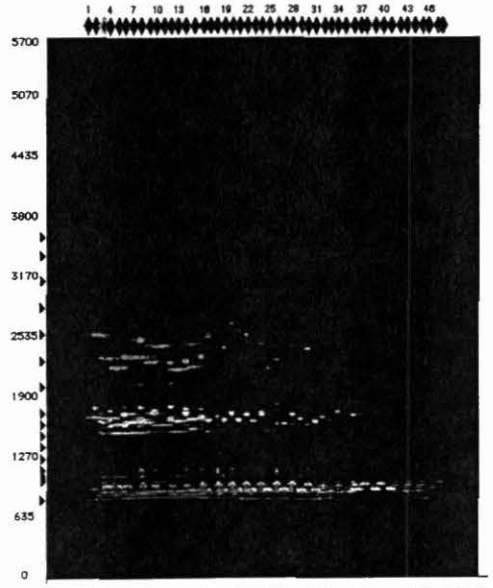

Figure 50. Microsatellites material shown in Table 50. Blue:GATS91; Green: BM205; Yellow: BM188.

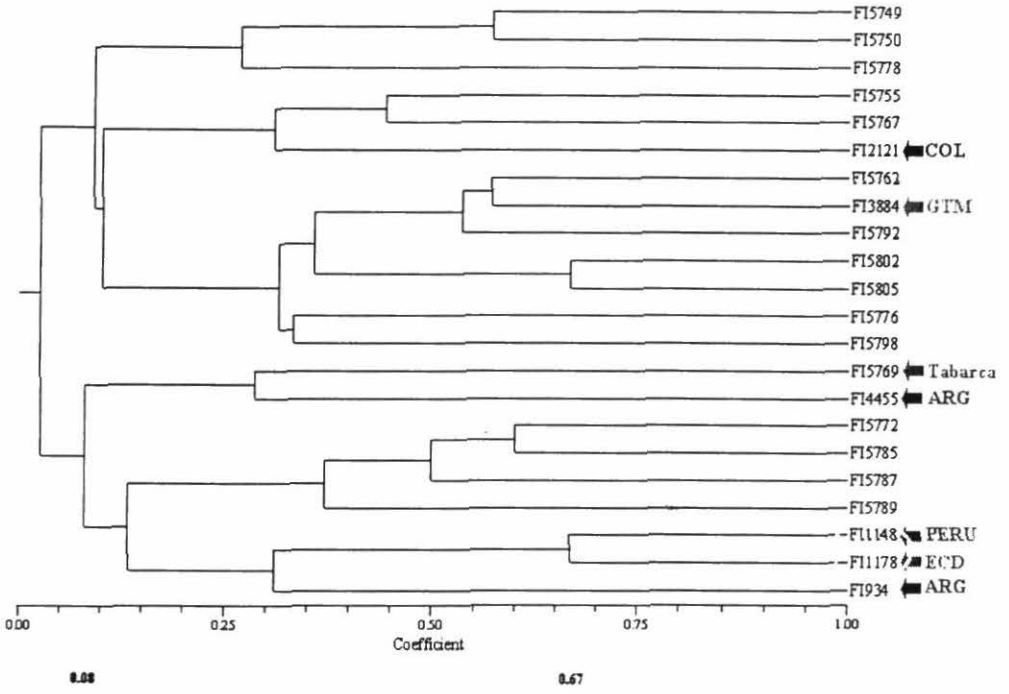

Figure 51. Grouping of *P. vulgaris* accessions based on microsatellites data using UPGMA.Genetic distance is according to Nei.

The materials analyzed with microsatellites were grouped by UPGMA's method using the genetic distance of Dice, obtaining separated clusters for Mesoamerican and Andean gene pool with 7 percentage of similitude. The control materials of Andean gene pool were FI1148, FI1178, and FI934. They split with a similitude coefficient of 0.31. The materials FI5769 (weedy form with a 100 seed weight of 11g), FI 5772 (weedy, 9.7g), FI5785 (wild, 6g), FI5787 (weedy, 10g) and FI5789 (cultivated, 30g) might be the result of an introgression process with Andean materials.

The accession FI 2121 from Colombia showed Mesoamerican characteristics, the structure of Colombian populations seemed to be random introgression of local wild beans with beans of other groups (Tohme et al. 1996).

Chloroplast DNA marker

According to Chacón Sánchez (2001) Costa Rica owns a specific haplotype called H, while haplotype G belongs to Guatemala, Honduras, Colombia, and El Salvador. Therefore we desire to establish the haplotypes of the different materials, starting with a verification of the haplotypes present in the wild, using the Single Nucleotide Polymrphism (SNPs) methodology as their separation by PCR products proved to be particularly difficult. The primers used were selected to obtain the haplotypes of cpDNA for *Phaseolus vulgaris L.* (Table 51). Two chloroplast fragments *rps14-psaB* spacer, *ndhA* intron were sequenced (Table 51), and we found one SNPs in each fragment; the next step is to design the corresponding primers and to determine the accession haplotypes.

Table 51. Chloroplast regions sequenced in this study to find out SNPs

DNA Region	Distinctive character	Amount of SNPs	Size bp
*Rps*14-*psa*B spacer	Haplotype H	1	600
*ndh*A intron	Haplotype H Haplotype G	1	1500

References

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

Chacón Sánchez, M.I. 2001. Chloroplast DNA polymorphisms and the evolution and domestication of the common bean (*Phaseolus vulgaris L.*) . Department of Agricultural Botany. University of Reading, United Kingdom. PhD Thesis.

Tohme, J, D.O. Gonzalez, S. Beebe & M.C. Duque. 1996. AFLP Analysis of gene pools of a wild bean core collection. Crop Science 36: 1375-1384.

Contributors: R.I. González Torres (Universidad Nacional de Colombia, Bogotá); E. Gaitán, J. Tohme (Biotechnology Research Unit); O. Toro, C. Ocampo, D.G. Debouck (Genetic Resources Unit).

6. Annexes

6.1. List of publications by Project Staff in 2002

A. In refereed journals:

Bayuelo-Jímenez, J.S., D.G. Debouck & Lynch J.P. 2002. Salinity tolerance in *Phaseolus* species during early vegetative growth. Crop Science 42 (6): 2184-2192.

B. In non-refereed journals:

Chacón, M.I., B. Pickersgill & D.G. Debouck. 2002. Cases of past cytoplasmic introgression of nuclear genes in common bean (*Phaseolus vulgaris* L.). Annu. Rept. Bean Improvement Coop. (USA) 45: 230-231.

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

Muñoz, L.C., M.W. Blair & D.G. Debouck. 2002. Genetic diversity of the CIAT tepary bean (*Phaseolus acutifolius* A. Gray) collection measured with amplified fragment length polymorphism markers. Annu. Rept. Bean Improvement Coop. (USA) 45: 234-235.

Ocampo, C.H., Martín, J.P., Ortiz, J.M., Sánchez-Yélamo, M.D., Toro, O. & D.G. Debouck. 2002. Possible origins of common bean (*Phaseolus vulgaris* L.) cultivated in Spain in relation to the wild genetic pools of the Americas. Annu. Rept. Bean Improvement Coop. (USA) 45: 236-237.

C. In books:

Alvarez, E, B, Belloti, A., Calvert, L., Arias, B. , Cadavid L F., Pineda, B., Llano, G. & Cuervo, M. 2002. Guía práctica para el manejo de las enfermedades, las plagas y las deficiencias nutricionales de la yuca. Imágenes Gráficas S.A. Cali. 120p.

D. In proceedings:

Chacón, M.I., B. Pickersgill & D.G. Debouck. 2002, Polimorfismo del ADN del cloroplasto y la domesticación del fríjol común (*Phaseolus vulgaris* L). *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 10-18 Octubre de 2002. Pp. 10.

Debouck, D.G. Overview and present situation of the International Undertaking. III Symposium on Genetic Resources of Latin America and the Caribbean, Londrina, Paraná, Brazil, 19-22 November 2001. Pp. 27-29.

Mafla, G., Roa, J.C. & Debouck, D.G. 2002. Conservación in vitro y utilización del germoplasma del género Manihot. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp. 212

Mafla, G., Roa, J.C., Flor, N.C. & Debouck, D.G. 2002. Efecto del ancymidol y el nitrato de plata sobre el crecimiento de lulo (*Solanum quitoense* Lam.) y tomate de árbol (*Solanum betaceum* Sendt.) conservados in vitro. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp. 103

Muñoz L.C. , M. W. Blair & D.G. Debouck. 2002. Observaciones sobre el estatuto de parientes silvestres del frijol tepari, *Phaseolus acutifolius* Asa Gray. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp. 9.

Pineda L, B.,Balcazar, Maria del S. & Flor, N. C. 2002. Microflora asociada a germoplasma de frijol, pasturas tropicales y yuca. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp 60.

Pineda, B., Rivera. A.L., Balcazar, Maria del S., Ramírez J. L. & Debouck, D. Efectividad de la aplicación de Propiconazole en el control del complejo fungoso (*Sphacelia* spp, *Drechslera* spp. *Phoma* sp. , *Cerebella* sp.) en inflorescencias de *Brachiaria brizantha. In* : Memorias XXIII Congreso Ascolfi. Julio 3-6 de 2002. Bogotá. Pp 96.

Salcedo J. M. Protocolos de conservación de semillas de solanum betaceum *(Solanaceae, Solaneae) y Urochloa spp. (Poaceae, Paniceae) In* Memorias: VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp.152

Torres, A.M., Debouck D.G., A. Ciprian & O. Toro. 2002 . Conservación de recursos genéticos de forages tropicales y frijol: Un servicio para la investigación de la agrobiodiversidad en la región. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 10-18 Octubre de 2002. Pp. 90.

Torres, A.M. & D.G. Debouck . 2002. El Herbario CIAT como colección de referencia para la autentificación, caracterización, y conservación de germoplasma tropical. *In:* Memorias VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002. Pp 92.

6.2. List of thesis research supervised by Project Staff in 2002

Menéndez Sevillano, M.C. Estudio y conservación del germoplasma silvestre y primitivo de *Phaseolus vulgaris* L. en el Noroeste de Argentina. Tesis doctoral. Universidad de Santiago de Compostela, Pontevedra, Spain, 190p.

6.3. List of conferences and scientific communications presented by Project Staff in 2002

Debouck, D.G. Overview and present situation of the International Undertaking. III Symposium on Genetic Resources of Latin America and the Caribbean, Londrina, Brazil, 20 November 2001.

Debouck, D.G. Bioprospección y recursos genéticos – observaciones sobre metodologías. Taller nacional de bioprospección, Universidad Nacional de Colombia, Ministerio del Medio Ambiente, Bogotá, 18 April 2002.

Debouck, D.G. Observaciones sobre metodologías de bioprospección y colecta de recursos genéticos – cómo buscar? Instituto de Investigaciones en Diversidad Biológica 'Alexander von Humboldt', Bogotá, 24 April 2002.

Debouck, D.G. Avances sobre el Tratado internacional sobre recursos fitogenéticos para alimentación y agricultura. Centro Internacional de Agricultura Tropical, Palmira, 8 May 2002.

Debouck, D.G. Gene flow studies in common bean in the Americas. Puntarenas, Costa Rica, 16 May 2002.

Debouck, D.G. Navy beans for space odysseys – views about their taxonomy, evolution, and conservation. Missouri Botanical Garden, St. Louis, Missouri, USA, 4 June 2002.

Debouck, D.G. Concepto de especies y domesticación de plantas, Taller sobre Bioseguridad de Plantas Transgénicas, CIAT, Palmira, Colombia, 20-21 September 2002.

Debouck, D.G. Observations on yellow-seeded beans. 9th Session of the FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy, 16 October 2002.

Debouck, D.G. Overview on the International Treaty on Plant Genetic Resources for Food and Agriculture, Katholieke Universitiet Leuven, Belgium, October 18 2002.

Torres, A.M. The conservation of forages and beans at the Genetic Resources Unit of CIAT. Universidad Nacional, Córdoba, Argentina, Marzo 2002.

Torres, A.M. Cladistics: a tool for systematics. Universidad del Valle, Cali, Colombia. Abril 2002.

6. 4. List of international and national courses with input from Project Staff in 2002

Master of Science in Neotropical Genetic Resources' degree given by the Universidad Nacional de Colombia, Palmira, April-October, 2002.

Course on *ex situ* conservation of genetic resources of plants. IPGRI-ICA, Palmira, 10-12 September, 2002.

I Taller Regional de Propagación Rápida (*in vitro*) y Transformación Genética. CIAT, Palmira, Febrero 25 a March 1, 2002

Curso intensivo de capacitación en sistemas modernos de producción y procesamiento de yuca en Colombia. CIAT, Palmira, June 25-28 de 2002

Curso manejo integrado de plagas y enfermedades. CIAT- Escuela Politécnica del Ejército, Ecuador. CIAT, Palmira, September 2- 13 de 2002.

6.5. List of trainees trained by Project Staff in 2002

In Conservation

Osorio, Victoria Eugenia. Training in *ex situ* conservation of seeds, CORPOICA-Macagual, 17-21, June, 2002.

Muñoz, Jacqueline. Training in *ex situ* conservation of sedes, CIAT, 18-28 Julio, 2002.

In vitro Lab

Kerpel, Ido. Training in procedures for recovering cassava clones distributed *in vitro*. Double harvest, Haití, 22-24 Octubre 2001.

Proaño, Karina. Training in conservation and management of *in vitro* cassava germplasm. Escuela Politécnica del ejercito, Ecuador. 26-30 Agosto 2002

In Germplasm Health Lab

Sandra Milena García. Cassava Patology. CIAT. Training in Cassava virus indexing techniques (Elisa Test and grafting).

Yudy Zamira Moreno. Cassava Patology. CIAT . Training in Cassava virus indexing techniques (Elisa Test and grafting).

Javier Beltrán. Universidad de Sucre. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting).

Juan Manuel Díaz. Universidad de Sucre. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting).

Dani Theodoro Junghans. EMBRAPA – BRASIL. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting).

Participantes Curso Biotechnology of Cassava. CIAT – CORPOICA. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting). Abril 30-2002.

Andrés Caballero. CORPOICA, Regional Santa Marta. Grafting Cassava technique.
Miguel Martínez. CORPOICA, Regional Montería. Grafting Cassava technique.

Karina Proaño. Escuela Politécnica del Ecuador, ESPE. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting).

Participantes Curso Conservación Ex situ de Recursos Fitogenéticos. MADR, ICA, IPGRI and CIAT. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting) Septiembre 2002.

Participantes Curso Manejo Integrado de Plagas. Escuela Politécnica del Ecuador, ESPE. Training in Cassava virus indexing techniques (Elisa Test, PCR Technique and grafting). Septiembre 2002.

N. C. Flor was the trainer of the people mentioned above.

Electrophoresis Lab

Carlos Alexander Montoya & Mónica Viviana Arbelaez. Universidad Nacional de Colombia, Palmira, Colombia. 03-21 December 2001 and 14 January – 27 March 2002.

Claudia Cristina Rojas Marulanda. Programa Nacional de Recurosos Biofísicos de la Corporación Colombiana de Investigaciones Agropecuarias (CORPOICA). Tibaitata, Colombia. 13 Noviembre – 21 December 2001 and 18 Febreruary – 27 March 2002.

6.6. Posters

Debouck D.G., A.M. Torres, A. Ciprian & O. Toro. 2002. Conservación de recursos genéticos de forages tropicales y frijol: Un servicio para la investigación de la agrobiodiversidad en la region (Colombia). VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 10-18 Octubre de 2002.

Flor, N.C. , Pineda B. & G. Mafla. 2001. CIAT cassava in vitro collection cleaned against "seed borne "diseases of quarantine importance. Fifth International Scientific Meeting of the Cassava Biotechnology Network, St. Louis, Missouri, USA. 4-9 November 2001.

Mafla. G., J.R. Roa & D.G. Debouck. 2001 Observations about the distribution of cassava germplasm from an international collection. Fifth International Scientific Meeting of the Cassava Biotechnology Network, St. Louis, Missouri, USA. 4-9 November 2001.

Chacón, M.I., B. Pickersgill & D.G. Debouck. 2002, Polimorfismo del ADN del cloroplasto y la domesticación del fríjol común (*Phaseolus vulgaris* L). VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 10-18 Octubre de 2002..

Mafla, G., Roa, J.C. & D.G. Debouck. 2002. Conservación in vitro y utilización del germoplasma del género Manihot. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Mafla, G., Roa, J.C., Flor, N.C. & D.G. Debouck. 2002. Efecto del ancymidol y el nitrato de plata sobre el crecimiento de lulo (*Solanum quitoense* Lam.) y tomate de árbol (*Solanum betaceum* Sendt.) conservados in vitro. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Muñoz L.C., M. W. Blair & D.G. Debouck. 2002. Observaciones sobre el estatuto de parientes silvestres del frijol tepari, *Phaseolus acutifolius* Asa Gray. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Pineda L, B.,Balcazar, Maria del S. and N.C. Flor. 2002. Microflora asociada a germoplasma de frijol, pasturas tropicales y yuca. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Salcedo J.M. 2002. Protocolos de conservación de semillas de solanum betaceum *(Solanaceae, Solaneae) y Urochloa spp. (Poaceae, Paniceae)* VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Torres, A.M. & D.G. Debouck. 2002. El Herbario CIAT como colección de referencia para la autentificación, caracterización, y conservación de germoplasma tropical. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 13-18 Octubre de 2002.

Torres, A.M., Debouck D.G., A. Ciprian & O. Toro. 2002. Conservación de recursos genéticos de forages tropicales y frijol: un servicio para la investigación de la agrobiodiversidad en la región. VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica, Cartagena, 10-18 Octubre de 2002.

6.7 Visitors

The Professional Staff of the Genetic Resources Unit attended the visit of 452 people from 67 different government bodies, institutions, companies, etc. A total of 315 people from 14 institutions visited the In Vitro Lab (universities, schools and farmers)

6.8 Donors

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

Ministerio de Agricultura y Desarrollo Rural, República de Colombia

Systemwide Programme on Information for Plant Genetic Resources (SINGER), CGIAR

Proyecto FONTAGRO "Genetic Resources of Carica Papayas", with IPGRI RegOff Americas

FLOW CHART FOR GERMPLASM MANAGEMENT OF BEANS AND TROPICAL FORAGES