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CIAT Project on Saving Biodiversity SB-01

Genetic Resources Unit
Report on Activities

CIAT

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CIAT Project on Saving Biodiversity SB-01
Report on Activities of SB-01 Project for 1997

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1. Executive Summary

This reports presents some of the major changes introduced during 1997 as follow-up to ICER'95, achievements in the maintenance and service to the FAO collections, and progresses in research and training. Highlights included: The training of staff, the implementation of methods and routine checking for viability of stored accessions, and the establishment of new procedures for germplasm increase and regeneration. Over 2,600 accessions were multiplied and characterized, over 11,000 samples were distributed, and over 1,500 samples were tested against seedborne diseases in 1997.

As follow up to ICER'95 and also as an implementation of the Global Plan of Action of FAO, modernization of GRU was vigorously pursued in 1997 with the building of a lab for seed viability monitoring, the building of a new drying room, the fixing of a new drying device for beans and tropical forages, and the building of two mesh-houses for germplasm regeneration.

In the research part, significant progress has been made in finding media for *in vitro* conservation of wild *Manihot* species, slow growth of cassava *in vitro* and protocols for the conservation of cassava shoot tips in liquid nitrogen. A procedure for zygotic embryo culture has also been established for the safe transfer of cassava germplasm. Efforts have been made to relate ELISA with infective virus presence. Better understanding has been gained in secondary gene pools of both common and Lima bean, in the taxonomy of *Phaseolus*, while patterns of diversity have been further explored in tepary, *Centrosema* and *Stylosanthes*.

Five research thesis have been supervised, and input provided into four courses in the region, while more than 20 hands-on training opportunities have been provided.

2. Introduction

This report presents activities carried out for the maintenance and service to the FAO Designate Collection, that is the germplasm collections held in trust at CIAT under the auspices of FAO, along the agreement signed in October 1994.

The recommendations of the ICER'95 on gene bank operations and the international standards set in 1994 have a profound bearing on such activities, and also have helped us to guide a modernization and an Upgrading Plan, both initiated in 1996, and conducted under financial constraints.

As conservation is expensive, and as it should be oriented towards the germplasm worth conserving in view of present and future utilization, it is important to ensure that such germplasm is identified. In addition, methodologies should constantly be improved, above all in terms of their liability and cost-effectiveness. These two points will orient a very significant part of the conducted research, and knowledge and improvements gained on this side shall almost automatically reflect on the activities for the maintenance and service to the international collections.

3. Project Objectives

SB-01 objectives are:
+ to make FAO collections fully meeting international standards
+ to make FAO collections and their pertinent information fully available
+ to make FAO collections genetically and socially relevant
+ to contribute to the formation of human resources in conservation methodologies in the region
+ to provide scientific input in *in situ* conservation of farmers' landraces and wild relatives.

The immediate project objectives for this year are directly related to the implementation of recommendations of ICER'95.

4. Staffing and Staff developments

The following table indicates staff distribution across major project activities during 1997; two staff from the Biotechnology Research Unit also bring expertise to the project.

Table 1 - Staff distribution in SB-01 as per September 1997

Staff	Conservation	Production	Electrop.	SHL	Service	Total
Professionals	4	3	1	3	1	12
Support	8	11	1	1	1	22
Total	12	14	2	4	2	34

During 1997 thanks to the CIAT Fund for Human Resources Development one member of staff has gone for her MSc. at the University of Reading, United Kingdom; three members of staff took work licenses for training in English outside Colombia. Courses in English and statistics were also provided in Colombia. Team building practices were explained to all GRU staff in 1997.

5. Report on Activities and Progresses

5.1. Germplasm Conservation Activities

5.1.1. Conservation of *Phaseolus* and Forages Species

Status of collections at present is as follows (Table 2).

Table 2. Status of the Phaseolus beans and of the tropical forages germplasm collections conserved at GRU-CIAT (1997)

Germplasm collection	Genera	Species	Origin No. countries	Total No. access
Phaseolus beans	1			
P. vulgaris		1	91	25,454
Other cultivated spp.		4	53	3,002
Wild spp.		22	12	167
Subtotal	1	27	97	28,623[1]
Tropical Forages				
Legumes	102	614	69	18,559
Grasses	48	165	41	1,916
Subtotal	150	779	74	20,475
Manihot				
M. esculenta	1	1	24	5,537
Wild spp	1	30	*	330
Subtotal	1	31	24*	5,867
Total	152	837	195	54,965

1 Subdivisions are made when gentic mixtures appeared

The re-engineering of the different procedures, together with the training of staff, has continued during 1997, with view of performance improvements and better use of scarce resources, together with new equipments.

Predrying: relates to placing into the drying room or device set at low relative humidity (RH) and relatively cool temperature (22^0C, 35% RH) the collected pods in the case of Legumes or inflorescences in the case of Poaceae to facilitate threshing and to lower the moisture content (MC) of the seed to a more secure level of moisture.

Highlight for this step in 1997 includes the acquisition and fixing of a novel drying device with large capacity for bean and legume germplasm.

Threshing and Precleaning:
This area still needs some fixing and is proposed for improvement in 1998.

First Drying: Seed is then taken to the drying room, set at 20^0C, 22% RH. Seeds remain there at least for two weeks and equilibrate between 4 and 9% MC. No systematic moisture content is taken for each sample, but we monitor the drying procedure.

Highlight for this step in 1997 includes the fixing of a new drying room with large capacity (70 m^3) for all seed germplasm (drying room 2). This was done thanks to a grant from the CIAT Capital Fund.

Temporary Storage: this step is still a bottleneck given the fact that we are running out of space in cold-stores under short-term.

Viability assessment: For both groups of germplasm this refers to the germination test, including vigor determination for all the samples. For Poaceae, because of common problems with seed production as immaturity and dormancy, besides germination, a test for viability based on TZC Tetrazolium is performed on many of the entries. Some procedures are illustrated in Table 3.

All entries at the end of multiplication are tested for viability. On the other hand, monitoring of entries with more than 15 years of storage and enough seed is also performed. This helps to identify problems with entries already stored and calls for regeneration.

Highlight for this step in 1997 includes the building of a novel seed monitoring laboratory with large capacity for bean and forage germplasm. This was done thanks to a grant from the CIAT Capital Fund.

Table 3. Methods of germination for some forage species at GRU.

LEGUMINOSAE	PRE-TREATMENT					SUBSTRATE			CONDITIONS		
	0.3% $KN0_3$	SULFURIC ACID			MECHANIC	G.ROLLS	PETRI DISH	SAND	LAB.CND.	GERMINATOR	GREENHOUSE
		10'	15'	20'						20/35°C	
Arachis						X		X	X	X	X
D00ioclea				X	X	X	X		X	X	
Centrosema					X	X	X		X	X	
Galactia					X	X	X		X	X	
Desmodium			X			X	X		X	X	
Stylosanthes		X				X	X		X	X	
Cratylina					X	X	X		X	X	
Canavalia					X	X	X		X	X	
Leucaena				X	X	X	X		X	X	
PROCEAE											
Panicum								X			X
Brachiaria	X			X		X		X		X	X
Hyparrenia								X			X
Andropogum								X			X
Paspalum	X						X	X		X	X
Chloris	X							X		X	X
Pennisetum								X			X

Final drying: Before packing in the categories for conservation, seed is again subjected to drying to adjust the moisture of the sample to the safe moisture for conservation. Moisture test at this stage is performed on sample basis.

Highlight for this step in 1997 includes the fixing of new shelves for improved drying in the drying room 1 (obtained through a grant from Italy in 1990).

Packing and Final Storage:
While capacity is OK for long-term storage (30% occupancy), the capacity in short-term conservation has gone to 110%. This is a point for improvement during 1998.

5.1.2. Viability assessment for *Phaseolus* and Forages

In the past four years, germination tests have been perfomed on 3,735 samples of *Phaseolus* and 680 of forages (Table 4). This table tells us two lessons:

i) The low number of entries analyzed in comparison with the total number of entries under our responsibility, reflect that this activity was behind the numbers of materials multiplied for many years.

ii) There are significant numbers of accessions with low viability urging for regeneration.

While the first lesson indicates that the decision to build a special facility for the specific monitoring of seed viability was sound, the second lesson indicates that accessions have been poorly prepared for storage and/ or stored for years under unappropriate levels of MC. The upgrading in the drying facilities was thus also a sound decision.

Fortunately now, with the recent facility for seed testing built this year and responding to recommendations of the 1995 ICER, this activity has been established as routine at the Unit.

Table 4. Germination tests for *Phaseolus* and forages during the last 4 years

	Phaseolus Germination (%)					**Forages Germination (%)**				
	0	1-50	51-84	85-100	TOTAL	0	1-50	51-84	85-100	TOTAL
1994	-	-	-	-	-	1	5	101	91	198
1995	-	-	-	-	-	0	14	33	239	286
1996	96	404	756	571	1,827	0	0	0	0	0
1997	2	89	401	1,416	1,908	0	0	7	189	196

Projected program
From now onwards, viability testing will have to be attacked on two fronts:
1) assessing viability of entries with enough seed that are ending multiplication or regeneration that particular year,and 2) monitoring of already stored samples.

With the operational capacity in place, in the case of *Phaseolus*, it is expected to catch up with testing of the two year backlog with the production sections and then simultaneously process between 2,000 and 2,500 samples a year. For the second front, it is expected to monitor randomly selected samples -with enough seed- on the basis of time of storage for every year of storage. This information will probably identify what year of production to be emphasized for regeneration purposes or just identify individual entries that may present viability problems. Entries with 85% germination and enough seed will immediately be stored in the long term storage.
As well as for *Phaseolus*, forages entries from both fronts are expected to be tested. In the first group, 500 samples are expected to be processed by year, while entries already stored and with enough seed will be tested.

5.1.3. Conservation of Manihot esculenta

The genetic resources of cassava consist of local or introduced landraces, improved cultivars, some genetic stocks and related wild species maintained most of them both as in vitro and as field gene bank. As September 1997, the in vitro bank at CIAT held a total of 5,684 cassava clones, and 30 *Manihot* species. The *Manihot* species are maintained as genotypes, totalling 330 clones.

A problem with mites has been experienced but overcome this year, after a period of two-month hard work. This emphasizes once more the need for speeding up research on finding suitable procedures for duplicating the cassava collections (see part on Research).

5.2. Germplasm Production Activities

Background

Under the new operational context of the GRU, which was initiated in the second semester of 1996, the Germplasm Production Section was assigned with all activities concerning the increase and/or multiplication of the collections, mainly of *Phaseolus* beans and of tropical forages, conserved in the germplasm bank. The key objective in the short-medium term is to upgrade the germplasm collections to FAO standards (1994) and to meet international responsibilities recently emphasized at the Leipzig Conference of 1996. These activities include:

+ Acquisition and initial increase, under post-quarantine greenhouse/meshouse, of germplasm intended for introduction to the genebank. Further multiplication of germplasm recently introduced either in mesh-houses or field conditions at the different ecological sites that the GRU has selected for this purpose.
+ Multiplication / regeneration, either for seed rejuvenation purpose or for increasing seed stocks to FAO standards, of germplasm already stored in the bank.
+ Basic morpho-agronomic characterization of beans and tropical forages.

A. Initial increase / Post-quarantine introduction

1) Initial increase / post-quarantine introduction

The objective is to introduce germplasm which really adds new genetic variability not present in the collections. Under current quarantine/post-quarantine protocols, in part given the unknown phytosanitary conditions prevailing at original site, the process of introduction is rather slow, taking between 2-3 years until an accession can formally be included into the genebank (Table 5).

Table 5. Germplasm of *Phaseolus* beans and tropical forages processed for introduction[1] to the GRU bank in 1997

	***Phaseolus* Beans**	**Tropical Forages (No. acc.)**		**Total**
Year	(No. acc)	Legumes	Grasses	(No. acc)
1997	230	52	6	288

[1]Post-quarantine greenhouse/meshhouse, Palmira

B. Multiplication / Regeneration

When dealing with germplasm collections of such wide range of genera, species and origin as those of the CIAT collections, the criteria for establishing proper methods and procedures of multiplication must consider several biological factors, as well as the practical feasibility of the said procedures. Among the main biological factors which need to be taken into account are: the breeding system, floral morphology, pollination mechanisms as well as growing requirements and multiplication rates. However, a germplasm bank holding hundreds of species, whose standards of multiplication may also differ, need to develop multiplication and/or regeneration procedures, which are cost effective in relation to the degree of conservation achieved (long-term storage) and the production of sufficient seed to meet likely demands. Concerning multiplication/regeneration of the *Phaseolus* beans and of the tropical forages collection, there were substantial modifications of procedures during the period 1996-1997.

1) Phaseolus beans

For agronomical and pathological reasons, the shift from Popayan towards Tenerife was completed, with the planting of 2.5 additional Ha, in order to increase the number of materials regenerated annually (Table 6).

Table 6. Phaseolus Beans accessions proccessed for multiplication under greenhouse – meshouse and field conditions (GRU, 1997)

Year	Greenhouse[1]	Field[2]	Core[3] 'cleaning'	Total
1997	373	2,093		2,456

[1] Palmira, Popayan
[2] Mostly at Tenerife
[3] Greenhouse Palmira

2) Tropical forages

At present, the GRU has three locations for multiplication of tropical forages : Palmira (1,000 m.a.s.l.) with 3.5 hectares, Quilichao (1,000 m.a.s.l) with 3.1 hectares and Popayan (1,800 m.a.s.l.) with 1.0 hectare. An attempt, although at minor scale, has been made to utilize Tenerife as an additional site for increase of materials having difficulties to produce seed in the other locations. The wide spectrum of genera and species of the tropical species, plus their inherent nature of being mostly wild species, make the multiplication and/or regeneration activity a somewhat delicate process. Several research works with some of the key tropical forages genera, indicated that the rate of allogamy or outcrossing appeared higher than it was assumed, therefore, the traditional process of multiplication of forages accessions was reviewed and modified in several aspects:

i) Number of plants per accession: Traditionally, three to five plants were sowed per accession and the seeds harvested of all plants were mixed whatever quantity each plant produced. This procedure was efficient for planting large number of materials at each location, however, the maintenance of the original genetic composition of individual accessions was in doubt, given a possible genetic drift.

Both the low number of individuals representing the original accession and, the potential unequal genetic

representation of the seeds harvested, due to differences in reproduction rates among plants were the main reasons for such doubt. In order to avoid the above constraints, the number of plants per accession was changed to 100 as the standard
number. For the cases in which the original accession does not have such number of seeds available, then the highest number of seeds available is planted.

ii) Control over drift: Seeds from individual plants are harvested and mixed in equal proportion from each plant. A total of 7,000, 15,000 or 35,000 seeds are expected to be harvested per accession, depending upon seed size, medium to large, small and very small, accordingly.

iii) Distance between plants: Fifty to eighty cms were the distances used in the previous system, however, such distance was changed to 1.50 meters. This distance gives plants a better chance for full growth development and it is also a safe measure against the possibility of plants intertwingling among them. In addition, the distance between rows was standarized to 3 meters, in order to allow mechanization of field operations such as irrigation, weeding, control of pests and diseases.

iv) Planting only one accession of a given species in a location: To avoid the potential risk of species outcrossing in the field, it was decide to have only one accession per species in a field location. In the past methodology, entire sets of accessions of one species were planted in the same field location.

v) Combining bush or prostrate types with climbing types: One row of a bush or prostrate accessions is follow by a row of climbing accessions. This set up avoid the potential mixing of plants and also facilitates harvesting.

The above changes ensure the production of seed quantities and at the same time maintains the genetic integrity of the original accessions. However, a drawback of these changes is the reduction of the number of accessions which can be processed per field location and per year. Likewise, these changes are being applied to the multiplication under greenhouse or meshhouse conditions, where 100 plants per accession are also planted. Accessions that for several reasons cannot be taken to the field are left until they produce seeds, nevertheless reducing notably the space available. The solution for this limitation is to increase the area available for field and/or meshhouse multiplication, expecting consequently the availability of the resources needed for the maintenance of the additional area.

During 1997, a total of 828 accessions were processed for multiplication and/or regeneration. Legumes accounted for 30% of the total, while grasses were the rest 70% (Table 7). The highest proportion of the grasses is due to the processing of two big collections corresponding to *Brachiaria* and *Panicum*, which were moved from Quilichao to Popayan and Palmira respectively. By mid 1995 a positive finding was the suitability of Popayan for increasing seed of most species of *Brachiaria*. This genus had seed production problems at Quilichao, however, after a preliminary test under Popayan conditions, it was found out that almost 100% of the accession of *Brachiaria* set seed successfully; thus, the whole collection, about 600 accessions, is now established at Popayan. Because of problems with smut, the collection of *Panicum* in the field in Quilichao had to be discontinued, and moved to Palmira, in mesh-houses. As for the implementation of the changes explained above, both Palmira and Quilichao are now under this new scheme.

Table 7. Tropical forages accessions proccessed for multiplication under greenhouse-meshhouse
And field conditions (GRU, 1994-1997)

	Legumes		Grasses		
Year	**Greenhouse[1]**	**Field[2]**	**Greenhouse[3]**	**Field[4]**	**Total**
1997	143	106	11	568	828
Subtotal	249		579		828

[1] Palmira
[2] Palmira, Quilichao, Popayan
[3] Living collection CIAT
[4] Living collection Popayan

C. Characterization

The ultimate goal of a genebank is the conservation of the germplasm collection of any species, however, in order to make the germplasm collection useful to researchers, such purpose has to be complemented with the concerning descriptive information about what is being conserved; this descriptive information process is called characterization. The characterization process begins just when the germplasm is collected, continues with the introduction, then it goes further to morphological, biochemical, molecular descriptions and finally, it can go through particular evaluation responses to biotic and abiotic factors. In short, characterization attempts to describe the phenotype and the genotype of an individual accession and, also, to provide an estimate of the global genetic variability of the said species and/or the genus.

The CIAT's germplasm collections of *Phaseolus* beans and of tropical forages have been worked out at different levels of characterization since they were initially assembled. These levels changed in time according to the development and improvement of concepts and techniques.

1) *Phaseolus* beans

The germplasm collection of *Phaseolus* beans has been characterized at plant level mostly for morpho-agronomic traits based on IPGRI descriptors (1982); nevertheless, special genepools have been also characterized at cell level, either for biochemical and/or molecular traits. As mentioned before, this characterization starts just when the materials are introduced, when a description of the original seeds is done in detail. Thereafter, both at the initial post-quarantine increase and multiplication stages, a basic morphoagronomic characterization is always carried out. The selected traits used for this basic characterization provide a useful information description about the phenotype of an accession. As for the case of *P. vulgaris* and *P. lunatus,* this characterization provides hints on the ecological adaptation of the germplasm.

However, when a huge number of accessions of one single species is planted per season, as it is the CIAT case, the morphoagronomic characterization has to be useful and practical. This means to avoid data overload of repetitive values for those descriptors which are inherent to the species; besides, it permits to make a more efficient use of personnel and operational resources.

In fact, since the early formation of the collection, long lists of descriptors were attempted for characterization of the *Phaseolus* beans, however, after several years of continuous changes and modifications a final set of basic descriptors were selected for the general characterization of the *Phaseolus* beans germplasm collection. These descriptors have appeared as the more polymorphic and variable of the genus, due perhaps to the fact that they were under high selection pressure in the domestication process. Table 8 shows the list of descriptors used for the basic morphoagronomic characterization of *Phaseolus* beans.

In 1997, about 1,800 accessions of *Phaseolus* beans were characterized for basic morphoagronomic descriptors, taking advantage of the processes of introduction and multiplication/regeneration. Most of this work corresponded to *P. vulgaris* (93%), while the rest 7% was done for other cultivated *Phaseolus* species mainly *P. coccineus*, *P. polyanthus* and *P. lunatus*. These data have been constantly used by bean

Table 8. Descriptors used for basic characterization of *Phaseolus* beans (GRU, 1997)

Seed Characterization	Growth habit	Flower	Pod
Primary color	1. Bush determinate	Color	Type
Secundary color	2. Bush indeterminate	Days to flowering	
Tertiary color	3. Prostrate indeterminate		
100 seed weight (size)	4. Climber indeterminate		
Brilliance	5. Climber determinate		
Shape			

researchers as a tool in the selection of germplasm for adaptation trials, search of parental materials for breeding projects, special evaluations to biotic or abiotic stresses, and also as a complement to the criteria in the assembling core collections such as cultivated and wild *P. vulgaris*, and of *P. coccineus*. Parallel to the morphological characterization work, several focal projects on additional biochemical characterization, i.e. proteins and/or isozymes, have been carried out on selected accessions of *Phaseolus* germplasm.

2) Tropical forages

The morphological characterization of tropical forages presents several alternatives, given the large number of genera and species gathered at the CIAT germplasm collection. The establishment of a single list of morphological descriptors applicable to all the species of this collection is not feasible and, rather, each species may have its own special list. However, an attempt has been made to characterize those species or genera which have been selected as of priority to CIAT commodity projects involved with the use of tropical forages germplasm. The lists of descriptors published by IBPGR (1985), with slight modifications, have been taken as the basis for the morphological characterization of such species. In addition to the above work on key species, a routinary characterization at plantlet stage, using 19 descriptors, has been done for all the species processed

in the post-quarantine introduction increase. This activity is used as a complement to the taxonomical identification, either at the genera or species level. At present, there are 2,356 accessions characterized at plantlet stage, covering 66 genera and 213 species.

During 1997, given the planting of *Brachiaria* collection in Popayan, care is being taken to complete morpho-agronomic characterization, and particularly data such as: flowering time, ground cover, height. Notes on the breeding system i.e. cleistogamy, allogamy or apomixis, are also taken.

Documentation

A major highlight in 1997 has been the continuation of the implementation of the Systemwide initiative for the proper documentation of plant germplasm and the possibility to access it through InterNet, that is the contribution to the SINGER project. This project included a thorough updating, revision and validation of all passport and germplasm transferring data for the three germplasm collections. At present, the CIAT germplasm collections of *Phaseolus* beans, Tropical Forages and *Manihot* cassava (54,965 accessions) can be consulted via Internet for the following information.

Accession information : passport data
Taxonomy information : taxonomy classification and species name
Collector information : name of collector(s) and related institution
Safety information : sites of duplicates
Map : mapping of collecting site
Characterization : (for future input)
Reference : (concerning literature, for future input)

E. Reference Herbarium

With the Biologist in charge of the herbarium away for her MSc. at the University of Reading, U.K., as part of the CIAT policy for professional development, activities were reduced during the second half of 1997. Prior to her departure, a list of Colombian species of the subtribes Panicoideae and Chlorodoideae was extracted from the collection held at the Herbarium of the Royal Botanic Garden, Kew, England, was compiled, in view of the coming stay in England and a future review of economic species of grasses of the northern Andes. Given the interest of other CIAT forage projects, voucher specimens for more than 650 accessions of *Desmodium* were sent to specialists for identification and species confirmation.

5.3. Germplasm Health Control Activities - Seed Health Status Determination

Introduction

The seed health status determination is one of the most important activities to maintain germplasm phytosanitary standards in a germplam bank for conservation and distribution of accessions.

The seed health laboratory (SHL) was originally established by CIAT in 1983, in response to the FAO-sponsored meeting, "Consultation on germplasm exchange and phytosanitary activities of international centers". SHL's main responsibility is to test the seed health status of germplasm (beans, tropical pastures and recently cassava) intended for international export and also to contribute to maintain the phytosanitary standards of the germplasm collections in CIAT.

Materials and Methods

SHL checks only seed samples to intercept seedborne pathogens of quarantine importance (Fungi, Bacteria and Viruses) (Tables 9 and 10). To detect those pathogens SHL uses the methodologies recommended by CIAT's pathologist and virologists. When a recipient country request additional statements, the SHL executes additional tests whenever possible to comply the specific quarantine regulations of the recipient country. The SHL usually checks seeds against seedborne pathogens that are recorded in the seed production areas (Table 10).

Table 9. Bean seedborne pathogens tested by the SHL for seed health in relation to bean seed producing areas.

PATHOGENS	LOCATION
FUNGI	
Alternaria tenuis	Palmira, Vijes, Tenerife
Cercospora canescens	Palmira, Vijes, Tenerife
Colletotrichum lindemuthianum	Popayán, Palmira, Vijes, Tenerife
Fusarium oxysporum f.sp *phaseoli*	Popayán, Tenerife
Macrophomina phaseoli	Palmira, Vijes, Tenerife
Phaeoisariopsis griseola	Popayán, Tenerife
Phoma exigua var *diversispora*	Popayán, Tenerife
Rhizoctonia solani	Palmira, Vijes, Popayán, Tenerife
Sclerotium rolfsii	Palmira, Vijes
Thanapephorus cucumeris	Palmira, Vijes, Popayán, Tenerife
BACTERIA	
Xanthomonas campestris pv *phaseoli*	Palmira, Vijes, Tenerife
Pseudomonas syringae pv *phaseoli*	Popayán, Tenerife
VIRUS	
BCMV	Palmira, Vijes, Popayán, Tenerife
SBMV	Palmira,Vijes, Popayán, Tenerife

Table 10. Seedborne pathogens of quarantine importance in some tropical pastures species.

SPECIE	PATHOGENS
A. LEGUMES	
Stylosanthes guianensis	**Colletotrichum gloesporioides**
Centrosema spp	*Colletotrichum gloesporioides*
	Pseudomonas fluorescens
	Centrosema mosaic Virus
Arachis pintoi	Peanut mottle Virus (PMV)
Leucaena sp.	*Colletotrichum* spp *Pseudomonas fluorescens* biotipo II
Flemingia sp.	*Colletotrichum* spp
Pueraria sp.	*Colletetrichum* spp
Sesbania spp	*Colletotrichum* spp
Calliandra spp.	*Colletotrichum* spp
B. GRAMINAE	
Andropogon sp	*Dreschlera* spp
Panicum sp	*Tilletia*

In relation to fungi two incubation methods are used : blotter test and agar plate. The basic principle in these methods is to provide a high level of relative humidity and optimum light and temperature conductive for fungal fructifications development. After plating the samples are incubated under near ultraviolet light (NUV) and fluorescent light with a 12 hr cycle light and dark at 20-27^{o} C. After eight days of incubation the seeds are examined using a stereobinocular microscopy. The fungi are identified by morphology of fructifications.

Seedborne bacteria *Xanthomonas campestris* pv *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp, in tropical pastures are considered. Its detection is realized using dilution and plating in semiselective culture media (MXP to *Xanthomonas* and King B to *Pseudomonas)*. To detect *Curtobacterium flaccumfasciens pv. flaccumfasciens* in tropical pastures (*Zornia spp)* YDCA, growing test under high temperature (36-37^{o} C) and Gram stain are used.

Examination of bean seed samples to check BCMV is using ELISA test with monoclonal Potyvirus group antibodies. To detect BSMV in beans and CCMV and CSxV in cassava ELISA tests with polyclonal antibodies is used .

Results

A total of 1,369 of bean (*Phaseolus vulgaris* L.) seed samples were tested during 1997. A total of 65% of those samples did not have pathogens of quarantine significance mentioned here as "clean samples" (Table 11). Additionally variability in the percentage of "clean samples" in each year was observed (figures are given since 1994 to visualize trends). In general there was a range of "clean samples" between 59 and 70%. The 30 - 40% accessions were discarded to international exchange. It is a high percentage of discarded accessions because the seeds are produced under tropical field conditions where is very difficult to produce seeds completely free of seed-borne pathogens.

Table 11. Number of seed samples analyzed at the seed health testing laboratory 1994- 1997 and percentage of clean samples.

Research Program	**Number of samples (Percentage clean)**			
	1994	**1995**	**1996**	**1997**
Bean Germplasm Characterization	82(85)	627(60)	557(62)	848(56)
Mesoamerica Bean Genetics	69(35)	162(45)	195(55)	-
Andean Bean Genetics	462(41)	241(78)	398(68)	-
Bean Genetics and environment	267(53)	1034(83)	86(79)	-
Bean Enthomology	85(20)	-	-	-
Bean Pathology	-	461(75)	544(55)	-
Bean Phytonutrition	49(70)	130(69)	2(100)	81(74)
Genetic R. Unit (Beans Gmbank)	617(75)	354(69)	3940(51)	440(60)
Tropical Pastures	13(100)	71(85)	49(90)	11(45)
TOTAL	1644	3080	5771	1380
Average of clean samples (%)	60	71	70	59

In affected materials the fungi *Macrophomina phaseoli* (11.2%) and *Rhizoctonia solani* (2.1%) were more frequent (Table 12). *Colletotrichum lindemuthianum*, *Phomopsis* sp. and *Phoma* sp. were observed in low percentages (0.1%). Virus as BCMV (17.4 %) and SBMV (7.0%) and the bacteria *Xanthomonas campestris* pv *phaseoli* (0.45%) were the other pathogens detected in the seeds (Table 13). It is very important to mention that the percentages of BCMV and SBMV are increasing, showing maybe difficulties in vector (Aphids and Beetles) control programs in the field where the seeds are produced.

Analysis realized for 144 seed samples of the following tropical forages: legumes genera *Abizia, Aeschynoneme, Arachis, Centrosema, Cratylia, Desmodiun, Dioclea, Erytrina, Flemingia, Gliricidia, Leucaena, Mucuna, Pueraria, Stylosanthes, Vigna* and *Zornia*, and the genera of Gramineae *Andropogon, Brachiaria y Panicum,* showed low percentages of fungi. *Colletotrichum gloeosporiodes, Phomopsis* sp. and *Macrophomina phaseoli* were observed in legumes, whereas *Dreschlera* sp., *Phoma* sp. and *Tilletia* sp. were observed in Gramineae. Some seed samples of *Arachis pintoi* showed light reactions in ELISA test to Potyvirus. The percentage of "clean samples" were between 45-100%.

Table 12. Percentage of *Phaseolus vulgaris* seed samples infected with fungi after seed testing (1994-1997).

Research Program	**Fungi**							
	Macrophomina				**Rhizoctonia**			
	1994	**1995**	**1996**	**1997**	**1994**	**1995**	**1996**	**1997**
Bean Germp. Characterization	12.2	25.8	12.0	1.6	0.0	4.1	0.5	0.4
Mesoamerica Bean Genetics	25.7	1.9	14.3	-	2.9	3.7	0.0	-
Andean Bean Genetics	51.3	16.3	20.1	-	6.5	0.0	1.8	-
Bean Genetics and environment	33.7	8.0	2.3	-	6.4	1.7	0.0	-
Bean Enthomology	10.0	-	-	-	5.0	-	-	-
Bean Pathology	-	6.5	1.6	-	-	0.2	1.1	-
Bean Phytonutrition	51.4	2.3	0.0	2.5	0.0	16.9	0.0	2.5
Genetic Resources Unit	5.2	0.3	3.7	0.0	1.1	0.0	0.4	0. 45
Average	27.1	8.7	7.7	1.4	3.1	3.8	0.5	1.1

Table 13. Percentage of *Phaseolus vulgaris* seed samples infected with bacteria and virus after seed testing (1994- 1997).

Research Program	Virus								Bacteria			
	SBMV				BCMV				*X. campestris pv. phaseoli*			
	1994	1995	1996	1997	1994	1995	1996	1997	1994	1995	1996	1997
Bean Germp. Characterization	1.2	3.7	10.7	7.9	3.0	10.4	24.7	32.8	0.0	0.6	0.0	0.6
Mesoamerica Bean Genetics	0.0	19.8	13.1	-	9.1	39.5	18.0	-	0.0	0.0	0.6	-
Andean Bean Genetics	10.3	1.2	13.0	-	13.9	0.8	7.3	-	5.0	3.3	0.5	-
Bean Genetics and environment	7.9	3.8	0.0	-	7.1	8.5	7.9	-	0.0	0.0	1.1	-
Bean Enthomology	0.0	-	-	-	0.0	-	-	-	0.0	-	0.0	-
Bean Pathology	-	1.3	7.9	-	-	40.1	43.0	-	-	0.0	0.0	-
Bean Phytonutrition	0.0	10.8	0.0	7.4	0.0	23.8	0.0	6.2	0.0	0.0	0.0	0.0
Genetic Resources Unit	6.0	2.3	6.7	16.4	12.2	23.2	31.4	30.0	0.0	0.0	0.2	0.0
Average	3.6	6.1	7.3	10.6	6.5	21.0	19.0	23.0	0.71	0`.60	0.30	0.2

In cassava, based on the results of 1200 tissue samples of sexual seed mother plants analyzed, the virus CCMV and CSXV were detected in low percentages (1.4 % CCMV and 2.0 CsXV). There are no evidence of seed transmission of these two potex virus in cassava.

5.4. Research Activities

Research in SB-01 is mainly oriented at the definition of target of conservation efforts, and the improvement of conservation methods. The first part aims at defining which species, gene pools and specific genes are worth conserving in view of current and future programmes of crop improvement. It also links with targetted collecting of germplasm, or the recommendation of specific populations to be included in protected areas. The second part of the research effort aims at improving methods and techniques used at the GRU, both in terms of liability and cost efficiency. On the other hand, it also aims at providing scientific input on *in situ* conservation of landraces and wild relatives of crops. Finally, there are reserch activities specifically targetted at the promotion of poorly valued sets of germplasm.

5.4.1. What to conserve?

Different pieces of research initiated previously by several investigators, some being with GRU and/ or BRU, others being with the commodity programmes (today changed into projects), have been continued in 1997. Highlights include:

Activity - 1 Identification of species of the secondary gene pool of common bean.

D.G. Debouck, S. P. Singh, W. Roca

Introduction

Interspecific hybridization for bean breeding although attempted as early as 1855 has not produced much stable progenies and elite commercial varieties (Hucl and Scoles, 1985). One reason could be that not all closely related species have been crossed with common bean but relatively distant species, thus the interest of increasing germplasm availability of the secondary gene pool of *P. vulgaris*.

Previous field work (Debouck et al., 1989) in Costa Rica has revealed the presence of a wild species with epigeal germination and floral morphological traits characteristic of outcrossing *Phaseolus* species such as *P. coccineus, P. polyanthus*. A cpDNA analysis (Schmit et al. 1993) has shown that this wild species shares cpDNA polymorphisms in common with *P. vulgaris* and *P. polyanthus*, not with *P. coccineus*. This species, called *P. costaricensis*, could thus belong to the secondary gene pool of common bean.

Results

Initial evaluation trials against the foliar fungi complex prevailing at high altitudes (anthracnose, ascochyta blight) and against BGMV has shown that *P. costaricensis* has some interesting potential. On the other hand, it has been crossed successfully with common bean, namely a non-carrier of lethal, gene-pool specific genes. F2 and back-cross materials are presently under study.

Prospects

The new species seems restricted to the mountainous ranges of eastern Costa Rica and western Panama. While its range has been fairly explored in Costa Rica, it is known in Panama only from herbarium specimens. Contacts have been made with the network PROFRIJOL in order to carry out germplasm explorations in the latter.

The reciprocal cross shall be attempted once *P. costaricensis* resumes blooming, as well as crosses with *P. polyanthus*. Further evaluation of all available accessions of *P. costaricensis* shall be undertaken. In a second phase more crosses shall be made with accessions of *P. vulgaris* belonging to the different races.

Collaborators

U. Gembloux, Belgium: J.-P. Baudoin
USDA, ARS, NSSL, Fort Collins: G. Freytag
Network PROFRIJOL

Outputs

Freytag G.F. & D.G. Debouck. 1996. *Phaseolus costaricensis*, a new wild bean species (Phaseolinae, Leguminosae) from Costa Rica and Panama, Central America. Novon 6(2): 157-163.

Singh S.P., Debouck D.G. & W. Roca. 1997. Successful interspecific hybridization between *Phaseolus vulgaris* L. and *P. costaricensis* Freytag & Debouck. Annu. Rept. Bean Improvement Coop. 40: 40-41.

Literature Cited

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Hucl, P. and Scoles, G. J. 1985. Interspecific hybridization in the common bean: a review. HortScience 20 (3): 352-357.

Schmit, V., du Jardin, P., Baudoin, J.P. and Debouck, D.G. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. 87 (4): 506-516.

Activity - 2 Taxonomic studies in South American wild species of *Phaseolus* related to the Lima Bean.

Ana Lucia Caicedo, J. Tohme, D.G. Debouck

Introduction

Two gene pools with different wild ancestral forms have been demonstrated for *Phaseolus lunatus* L. (Gutiérrez Salgado et al., 1995), one gene pool having originated in the northwestern Andes. A group of wild species with morphological similarities with the wild Andean Lima bean exist in the Andes south of Colombia. Two species, *P. augusti* growing from Ecuador to Argentina and *P. pachyrrhizoides* from Peru only, seem to be valid species (Brako and Zarucchi, 1993), although sometimes difficult to separate. We use a new technique to assess genetic diversity - AFLPs - that combines restriction enzymes with PCR and which look promising in beans (Tohme et al., 1996) on a diverse sample of different taxa related to Lima bean.

Results

The technique was particularly powerful in revealing good level of polymorphisms and repeatability between all taxa. The grouping by UPGMA separates well the small seeded wild Lima beans from the ones with larger seeds. It does not allow to clearly separate *P. augusti* from *P. pachyrrhizoides*; instead, accessions from these two species form a continuum with little differences among them, and could be grouped according to geographic origin.

Prospects

There might thus be only two wild South American species forming the secondary gene pool of Lima bean, *P. pachyrrhizoides* and *P. mollis*, the latter being an endemic from the Galapagos. Colombian materials of Lima bean look particularly interesting, since a possible transition between the two gene pools could be looked for there.

Outputs

Caicedo, A.L. 1996. Caracterización molecular de especies silvestres suramericanas de *Phaseolus* por medio de AFLPs. Tesis Biologia. Universidad de los Andes, Bogotá, Colombia. 146 p.

Literature Cited

Brako, L. and Zarucchi, J.L. 1993. Catalogue of the flowering plants and gymnosperms of Peru. Missouri Botanical Garden, St. Louis, Missouri, USA, 1286p.

Gutiérrez Salgado, A., Gepts, P. and Debouck, D.G. 1995. Evidence for two gene pools of the Lima bean, *Phaseolus lunatus*, in the Americas. Genet. Resources & Crop Evol. 42 (1): 15-28.

Tohme, J., González, D.O., Beebe, S. and Duque, M.C. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36 (4): 1375-1384.

Activity - 3 Molecular phylogeny of the genus *Phaseolus sensu stricto* of North and Central America.

J. Tohme, D.G. Debouck

Introduction

With recent progress made on gene maps (Nodari et al., 1993) and comparative mapping, and transformation and *in vitro* culturing on the other hand (Mejía-Jiménez et al., 1994), bean breeding is soon at the crossroad, with unexpected possibilities for widecrossing coming through. Conversely, it becomes urgent to make additional progress on genetic resources, particularly in terms of representativeness of collections and understanding of phylogenetic relationships. A review of the genus is presently under way at GRU, with clarification provided on several taxa (e.g. *P. costaricensis*); in several cases molecular genetics has provided critical information for taxonomical decisions. We plan to use different kinds of markers (seed proteins, AFLPs on nuclear, mt and cpDNA, and sequencing of specific and polymorphic gene sequences).

Results

Techniques have been established with polymorphisms revealed with all of them. We purposedly included highly contrasting taxa, so that polymorphism if any could be observed. Confirmation through these different markers has been obtained about the taxonomic position of: *P. glabellus* as a taxon outside *P. coccineus, P. costaricensis* as close relative to *P. vulgaris, P. lunatus* as quite distant from *P. vulgaris* but not as distant as *P. glabellus*, and also the validity of many wild species (*P. chiapasanus, P. minimiflorus, P. microcarpus*). There would be justification for the recognition of 2-3 sections.

Prospects

We have powerful tools to help us define natural groups within *Phaseolus*, and so to better reflect natural affinities, and practically possibilities of widecrossing, instead of making crosses at random as it has been done majorily so far. What we learn with this methodology could also be extended to other problems of plant systematics and phylogeny of neotropical plants (e.g. *Capsicum* peppers, passion fruits, etc), and so to widen the experience of our partners.

Collaborators

Laboratory of Molecular Systematics
Smithsonian Institution, Washington

Literature Cited

Mejía-Jiménez, A., Muñoz, C., Jacobsen, H.J., Roca, W.M. and Singh, S.P. 1994. Interspecific hybridization between common bean and tepary bean: increased hybrid embryo growth, fertility, and efficiency of hybridization through recurrent and congruity backcrossing. Theor. Appl. Genet. 88 (2): 324-331.

Nodari, R.O., Tsai, S.M., Gilbertson, R.L. and Gepts, P. 1993. Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. Theor. Appl. Genet. 85 (5):513-520.

Activity - 4 Patterns of genetic diversity in *Centrosema macrocarpum* across its geographic range.

Juan Manuel Osorno G., César Ocampo, D.G. Debouck

Introduction

Centrosema macrocarpum has proven to be a promising forage legume for the tropics (Schultze-Kraft and Clements, 1995). CIAT collection is of 389 accessions from the Neotropics, mainly Brazil, Colombia and Venezuela. We wanted to gain more evidence about diversity present in the collections, namely in view of recomendations of several authors to collect more germplasm of this species. Second, we were interested in testing validity of variety *andinum* recognized within this species, with the help of biochemical markers (allozymes have proven to be useful: Penteado et al., 1996).

Results

Nine enzyme systems reveal polymorphism. For GOT, 6-PGDH, α-β EST and DIAP, specific alleles separate populations of the geographic range north of the Amazon or south of it. Enzyme diversity indicate a high rate of outcrossing, with perhaps geographic variation for it. Patterns in native seed proteins indicate a relationship between populations from northern South America and Central America. No major differences in patterns were found either with native seed proteins nor with allozymes between var. *andinum* and the rest of accessions. Additional morphological observations raise doubts about the validity of such taxon.

Output

Osorno González, J.M. 1997. Estudio de la diversidad genética y distribución natural de una colección de *Centrosema macrocarpum* Bentham (Fabaceae, Papilionoideae) mediante técnicas de electrophoresis. Tesis Ing. Agr. Universidad Nacional de Colombia, Palmira, Colombia. 176 p.

Literature Cited

Penteado, M.I. d.O., Sáenz de Miera, L.E. and Pérez de la Vega, M. 1996. Genetic resources of *Centrosema* spp.: genetic changes associated to the handling of an active collection. Genetic Resources & Crop Evolution 43 (1): 85-90.

Schultze-Kraft, R. and Clements, R.J. 1995. - *Centrosema* spp. (Leguminosae-Papilionoideae). *In:* "Evolution of crop plants - Second edition", Simmonds, N.W. and Smartt, J. (eds.), Longman Scientific & Technical Limited, London, United Kingdom, pp. 255-258.

Activity - 5 Patterns of genetic diversity across space gradients in *Stylosanthes guianensis*

César Ocampo, Alba Marina Torres, Brigitte Maass

Introduction

Stylosanthes guianensis is a forage legume of worldwide importance, widely distributed in the American tropics (Skerman et al., 1988). Being still largely an untamed plant, no much variability is expressed in phenotypes. In order to assess the genetic diversity collected so far, and to identify areas for further collecting, patterns of genetic diversity across its range were tested with the help of electrophoresis techniques (PAGE for 4 isozymes and native seed storage proteins).

Results

The results of PAGE-electrophoresis of four enzymes (α-EST, β-EST, $\alpha\beta$-ACP and DIA) and native seed proteins indicated that the collection held at CIAT contains a reasonable level of infraespecific variability. The characterization of 527 accessions yielded 306 different banding patterns; when the banding patterns of the tested markers were combined, a 58% discrimination was obtained. Furthermore, 240 patterns are unique; thus the accessions presenting such patterns are genetically different from each other. There are obvious differences, namely:

- between the two main botanical varieties, var. *vulgaris* and var. *pauciflora,*
- between Brazil and Colombia,
- between Brazil and the rest of tropical America,
- between Central America and Colombia, and the rest of South America.

Polymorphism of these markers shows that tropical areas of Brazil possess the greatest diversity; Brazil also has the greatest interspecific diversity of the genus *Stylosanthes*. Central and northeastern Brazil could be proposed as the center of origin and diversification of this species. Variety *pauciflora* is more diverse than variety *vulgaris*; interestingly, high levels of resistance to anthracnose are present in the former. Several protein polymorphisms and those of ACP suggest some correlation with geographic origin and original climate type.

Prospects

One should test further the correlation between enzymatic electromorph and original climate type; if true such correlation opens the new prospects for germplasm evaluation.

Outputs

Maass BL, Torres AM, & Ocampo CH. 1997. Biochemical evidence of geographic and and taxonomic patterns of variation in *Stylosanthes guianensis* (Aublet) Swartz. Under review.

Jones P, Clavijo LA, Ocampo CH, Sawkins M. 1997. Diversidad Genética en *Stylosanthes*: una aplicación GIS. Poster presented at the 6th International Congress of Latinamericanist Geographers, 20-23 July 1997, Arequipa, Peru.

Literature Cited

Skerman, P.J., Cameron, D.G. and Riveros, F. 1988. The tropical pasture legumes. *In:* "Tropical forage legumes", Skerman, P.J., Cameron, D.G. and Riveros, F. (eds.), Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 194-487.

Activity - 6 Analysis of variability found in phaseolin types in *Phaseolus vulgaris*

C. Ocampo, Orlando Toro, D.G. Debouck

Introduction

Phaseolin, the major seed storage protein of common bean (Osborn, 1988), has proved to be an excellent - cheap and polymorphic - marker in evolutionary studies (Gepts, 1988). Thanks to the parallel diversity found in wild forms and sympatric traditional landraces, several and independent domestication events have been demonstrated to take place in Mesoamerica and the Central and southern Andes (Gepts et al., 1986). Additionally, zones of particular diversity were found in Colombia (Gepts and Bliss, 1986), and in southern Ecuador and northern Peru (Debouck et al., 1993). Such results have been recently confirmed with the help of AFLP markers (Tohme et al., 1996).

Given the usefulness and practicability of such marker, it is appropriate and timely for CIAT to establish reference collections and to document the diversity found so far in phaseolin types. The description of phaseolin type is also becoming a routine descriptor in bean germplasm characterization, namely for the definition of gene pools and races (Singh et al., 1991). It was therefore useful to fully document patterns found in one di-SDS-PAGE and 2-di-IEF-SDS-PAGE electrophoresis.

Results

Although this globulin has narrow range of molecular weights (45-52 kD) and isoelectric points, a total of 61 banding patterns has been found so far, 29 being present in Mesoamerican materials and 32 in the Andean region, be wild or cultivated. In Mesoamerican materials all 29 patterns are present in wild forms, while only four exist in cultivated forms so far. A contrasting situation prevails in the Andes where 15 patterns have been found in cultivated forms (11 with no counterpart in the wild forms so far), and 17 types exclusive of wild forms.

Prospects

With these preliminary results, frequencies are not representative of the real situation, although reductions of genetic diversity as a result of domestication are possible (and have been noted already). With full characterization of each phaseolin type it will be possible to document better such bottleneck effects, and accordingly improve the representativeness of our collections. Although these data must be considered carefully, it seems that founder effects vary between major gene pools and/or regions of bean domestication. The original genetic basis might thus be different from one group to another, with different consequences on potentialities in breeding.

Throughout this study, we have experienced trouble in keeping germplasm of 'Mu' variant, which seems perhaps linked to some lethal characters. It is unknown whether such germplasm is coming from inter gene pool crosses where such viability problems have been reported (Singh and Gutiérrez, 1984).

Literature Cited

Debouck, D.G., Toro, O., Paredes, O.M., Johnson, W.C. and Gepts, P. 1993. Genetic diversity and ecological distribution of *Phaseolus vulgaris* (Fabaceae) in northwestern South America. Econ. Bot. 47 (4): 408-423.

Gepts, P. 1988. Phaseolin as an evolutionary marker. *In:* "Genetic resources of *Phaseolus* beans", Gepts, P. (ed.), Kluwer Academic Publishers, Dordrecht, Holland, pp. 215-241.

Gepts, P. and Bliss, F.A. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. Econ. Bot. 40 (4): 469-478.

Gepts, P., Osborn, T.C., Rashka, K. and Bliss, F.A. 1986. Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris* L.): evidence for multiple centers of domestication. Econ. Bot. 40 (4): 451-468.

Osborn, T.C. 1988. Genetic control of bean seed protein. CRC Crit. Rev. Plant Sci. 7 (2): 93-116.

Singh, S.P., Gepts, P. L. and Debouck, D.G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 45 (3): 379-396.

Singh, S.P. and Gutiérrez, A. 1984. Geographical distribution of the DL1 and DL2 genes causing hybrid dwarfism in *Phaseolus vulgaris* L., their association with seed size, and their significance to breeding. Euphytica 33: 337-345.

Tohme, J., González, D.O., Beebe, S. and Duque, M.C. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36 (4): 1375-1384.

Activity – 7 Patrerns of genetic diversity in *Phaseolus acutifolius* in Central America and southwestern
USA.

Claudia Patricia Florez R., O. Toro, C. Ocampo, D.G. Debouck

Introduction
Tepary bean has long been known as a domesticated bean species with particular adaptation to dry areas. Contradictory information exists in the literature with respect of place of domestication. Manshardt and Waines (1983) indicated that Sonora, Sinaloa, Durango and Jalisco, all in Mexico, could be the places of origin of the tepary bean. Another study indicates Sonora and Sinaloa as possible locations for domestication (Schinkel and Gepts, 1988). For Garvin and Weeden (1994), the Mexican states of Jalisco and Sinaloa are the putative places of domestication of tepary.

A total of 270 accessions of the world collection held at CIAT has been evaluated by means of SDS-PAGE of seed storage proteins and isozyme electrophoresis on 10 systems (AAT, ACO, ADH, ACP, DIA, MDH, PGI, PGM, PRX and RBCO).

Results
Twenty-four patterns were found in globulins of wild forms, while only two patterns were found in the cultivated form, evidencing a strong founder effect upon domestication. The XI and XIX globulin patterns typical of cultivated genotypes have no counterpart in the wild materials; it is thus difficult to infer the region(s) where tepary has been domesticated. The enzymatic evidence shows however a close similarity between two wild accessions from Jalisco and the cultivated ones. Another place could not be discarded however, since a single cultivated accession not analyzed previously displayed different proteinic and enzymatic patterns.

Prospects
There is little probability that the world tepary collection could be increased, as this crop has experienced severe erosion throughout its historic range. Most of its potential for further breeding and evolutionary studies lies in the wild forms, which have not been uniformly collected.

Outputs
Florez Ramos, C.P. 1996. Patrones de diversidad genetica y domesticacion en el frijol tepari (*Phaseolus acutifolius* Asa Gray). Tesis Ing. Agr. Universidad Nacional de Colombia, Palmira, Colombia. 167p. *Thesis awarded as the best thesis for the year at the University*.

Literature Cited
Garvin, D.F. and Weeden, N.F. 1994. Isozyme evidence supporting a single geographic origin for domesticated tepary bean. Crop Sci. 34: 1390-1395.

Manshardt, R.M. and Waines, J.G. 1983. Isozyme variation and the origin of domesticated tepary beans (*Phaseolus acutifolius* Gray). Annu. Rept. Bean Improvement Coop. 26 18-19.

Schinkel, C. and Gepts, P. 1988. Phaseolin diversity in the tepary bean, *Phaseolus acutifolius* A. Gray. Plant Breeding 101: 292-301.

Activity - 8 Patterns of genetic diversity in *Phaseolus lunatus* in Colombia and Andean South America.

Irene Perea Arango, O. Toro, C. Ocampo, D.G. Debouck

Introduction

Two gene pools have been shown in cultivated Lima bean on the basis of biochemical (Gutiérrez Salgado et al., 1995) and molecular (Nienhuis et al., 1995) markers. We also know that these two gene pools have arisen from two different kinds of wild ancestral forms, differing in minor morphological traits (Debouck et al., 1987). We have seen that these two gene pools come into contact in Colombia (Gutiérrez Salgado et al., 1995), and that wild Lima beans exist in the Colombian Andes (Toro Ch. et al., 1993). Using novel materials (280 accessions) and biochemical markers, we tried to gain additional evidence about patterns of diversity and (maybe) domestication in Colombia.

Results

In cultivated Colombian forms, five Mesoamerican and four Andean patterns were found. Wild forms from the Northern Coast and Caldas were all 'M1', while wilds from Boyaca were 'M1' and 'M12'. Weedy forms from the same region were 'M7', 'M12', 'A12' and hybrid. Using enzymes (13 polymorphic loci), no novel alleles apart from the ones reported previously were found in wilds. However, as it has been shown elsewhere on Costa Rican wild forms (Maquet et al., 1996), some enzyme (MDH, ME) electromorphs common among Andean materials were also found in Colombian materials with Mesoamerican proteinic patterns. This would indicate as it is the case for some Costa Rican materials that Colombia too would be contact zone between the two major gene pools.

Prospects

These preliminary results reinforce hypothesis presented elsewhere about the situation of wild common bean in Colombia (Debouck, 1996; Tohme et al., 1996) as place of contact, introgression, but maybe also early origin before the formation of gene pools. They should be expanded with other molecular markers; collecting of additional wild forms particularly from southwestern Colombia is needed.

Output

Perea Arango, I. 1997. Contribución al estudio de la diversidad del frijol lima (*Phaseolus lunatus* L., Fabaceae) en Colombia. Universidad de Antioquia, Medellín, Colombia. 140 p.

Literature Cited

Debouck, D.G. 1996. Colombian common and Lima beans: views on their origin and evolutionary significance. Revista CORPOICA 1 (1): 7-15.

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Maquet, A., Zoro Bi, I., Rocha, O.J. and Baudoin, J.P. 1996. Case studies on breeding systems and its consequences for germplasm conservation. 1. Isoenzyme diversity in wild Lima bean populations

in central Costa Rica. Genet. Resources & Crop Evol. 43 (4): 309-318.

Nienhuis, J., Tivang, J., Skroch, P. and dos Santos, J.B. 1995. Genetic relationships among cultivars and landraces of lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J. Amer. Soc. Hort. Sci. 120 (2): 300-306.

Tohme, J., González, D.O., Beebe, S. and Duque, M.C. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36 (4): 1375-1384.

Toro Ch., O., Lareo, L. and Debouck, D.G. 1993. Observations on a noteworthy wild lima bean, *Phaseolus lunatus* L., from Colombia. Annu. Rpt. Bean Improvement Coop. 36 53-54.

5.4.2. How to conserve better?

This part of SB-01 research deals specifically with the improvement, i.e. in terms of liability and cost-effectiveness, of conservation methods.

Activity - 9 Conservation of wild *Manihot* in vitro.

Helena Velasquez, G. Mafla

Introduction

Due to their distinct biological and ecological nature or provenance, the logistics of the conservation of *Manihot* species require conditions different from those appropriate to cassava. One of the main problems in trying to conserve and micropropagate wild species is the lack or poor rooting ability of some of them on artificial media (GRU, 1994). During 1995 a thesis research had as a general objective the improvement of the existing protocols for micropropagation. Another independent repetition in time has been conducted in 1996-1997.

Methods

From a total of 351 genotypes corresponding to 30 species held in the in vitro bank, one hundred sixty eight genotypes for 25 wild species were selected for this study. Apical shoots and nodal cuttings were explanted in five different media and four evaluations, every two weeks, were done for six variables. The estimated variables were: No. alive leaflets, No. dead leaflets, No. of apical shoots, Stem length, Root presence (%) and Callus presence (%) (Table 1).

Results

For all variables, the response about rooting was affected by the interaction of species and culture media, hence we could not establish which media were the best for all the species, but could estimate which media could be convenient for each species.

For all species at the fourth evaluation, the rooting response was between 40.7 and 73.0. After selecting the best media by species, we could agree that in general, $12A_1$ reported the higher rooting percentage for most of the species (Table 1). On the contrary, callus presence at the fourth evaluation was observed in a range of 1.7 and 15.5% for all the species. Considering that the presence of callus is a undesirable characteristic in micropropagation, these results in comparison to a previous research were highly acceptable. Media WPM_1 reported lower values of callus for most of the species. Previous studies reported 44.9% to 66.7% on rooting and from 62% to 90% on callus presence (Baca, 1991).

In general, as showed in Table 2, there is a tendency to recommend more than one media for one species. Also, independently from the type of media, for practical purposes, all the species at the in vitro bank have been classified as having High, Intermedia and/or Low rooting ability. Some species having high rooting ability (>90% for any media) are: *M. esculenta* subsp *flabellifolia*, *M. pentaphylla* and *M. fruticulosa;* Intermediate (90 - 40%), *M. aesculifolia*, *M. carthaginensis* and *M. glaziovii;* and of Low rooting ability (< 40%), *M. anomala* and *M. quinquepartita*.

Further research has to be conducted to overcome rooting response and micropropagation problems for those species such as *M. anomala* and *M. quinquepartita* yet not responding.

Output

Velasquez, E. 1995. Mejoramiento de las Tecnicas de Micropropagacion In vitro para las especies silvestres de *Manihot* spp (Euphorbiacea). Tesis Genetica. Universidad del Valle, Cali, Colombia. 120 p.

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Anonymus 1994. Unidad de Recursos Geneticos. CIAT Annual Report. 54 p.

Baca, A.1991. Evaluacion y mejoramiento de las tecnicas para micropropagar y conservar germoplasma de especies silvestres de yuca (*Manihot* sp). Tesis Genetica. Universidad del Valle, Cali, Colombia. 77 p.

Table 1.Culture media effect for 25 *Manihot* spp for each evaluation

		* Media Value				** Value in %	
EVALUATION	Culture Media	No. Alive leaflets	No. Dead leaflets	No.Apical shoots	Stem Long.	Root Presence	Callus Presence
1	$12A_1$	0.54	0.01	0.68	0.45	19.9	0.0
	$12A_2$	0.51	0.01	0.64	0.38	17.0	0.0
	$12A_3$	0.60	0.01	0.74	0.49	11.9	0.3
	WPM_1	0.45	0.02	0.61	0.37	10.8	0.3
	WPM_2	0.40	0.00	0.58	0.33	10.8	0.2
2	$12A_1$	1.10	0.02	0.84	0.92	62.6	4.8
	$12A_2$	0.99	0.02	0.71	0.65	51.4	5.2
	$12A_3$	1.2 0	0.02	0.86	0.99	37.1	5.2
	WPM_1	0.76	0.03	0.63	0.55	41.1	1.5
	WPM_2	0.72	0.03	0.66	0.51	40.3	0.2
3	$12A_1$	1.35	0.09	0.86	1.49	70.4	7.8
	$12A_2$	1.14	0.11	0.72	1.01	57.6	8.4
	$12A_3$	1.45	0.09	0.89	1.66	46.3	9.5
	WPM_1	0.93	0.08	0.64	0.83	45.0	2.3
	WPM_2	0.90	0.07	0.65	0.73	47.3	3.4
4	$12A_1$	1.61	0.18	0.89	2.46	73.0	14.4
	$12A_2$	1.34	0.19	0.73	1.64	59.3	15.5
	$12A_3$	1.79	0.18	0.93	2.74	40.7	14.9
	WPM_1	1.1	0.12	0.63	1.20	42.5	3.4
	WPM_2	1.1	0.11	0.66	1.18	45.2	1.7

Table 2. *Manihot* spp. rooting percentage for all the species at the GRU in vitro bank. (60 days after planting[1])

Species	No. Genotypes In vitro[2]	Rooting %[3]	Culture Media[4]
M. aesculifolia (Maes)	4	71.4/50.0	$12A_2/12A_3$
M. alutacea (Malt)	8	56.2/61.9	$12A_1/WPM_2$
M. anomala (Manm)	3	33.3/33.3	$12A_2/WPM_1$
M. brachyloba (Mblo)	1	46.0	J
M. caerulescens (Mcae)	24	72.0/69.2	$12A_1/12A_2$
M. carthaginensis (Mcth)	102	77.4/73.9	$12A_1/12A_2$
M. cecropiaefolia (Mcec)	6	90.9/69.2	$12A_1/12A_2$
M. chlorosticta (Mchl)	7	100.0	All
M. epruinosa (Mepr)	1	66.7/66.7	$12A_1/WPM_1$
M. filamentosa (Mfmt)	5	76.9/91.6	$12A_1/12A_2$
M. esculenta-flabellifolia (Mfla)	29	100.0	All
M. fruticulosa (Mfru)	1	100.0/100.0	$12A_1/12A_2$
M. glaziovii (Mgla)	5	50.0/37.5	$12A_1/12A_3$
M. guaranitica (Mgut)	37	45.9	$12A_1$
M. hastatiloba (Mhas)	4	77.8/60.0	$12A_1/12A_2$
M. irwinii (Mirw)	2	100/80	$WPM_2/12A_2$
M. jacobinensis (Mjac)	16	90.9/83.3	$12A_1/12A_2$
M. longipetiolata (Mlon)	7	88.9/62.5	$12A_1/WPM_1$
M. orbicularis (Morb)	10	62.5	$12A_1$
M. peltata (Mpel)	1	100.0/66.7	$12A_1/12A_2$
M. pentaphylla (Mpnt)	1	100.00/100.0	$12A_1/WPM_2$
M. pilosa (Mpil)	2	100.0/100.0	$12A_1/12A_2$
M. pseudoglaziovii (Mpse)	1	100.0	All
M. purpureo-costata (Mpur)	1	100.0/100.0	$12A_1/12A_2$
M. quinquepartita (Mqpt)	1	< 25	-
M. rubricaulis (Mrub)	20	84.4/70.3	$12A_1/12A_2$
M. sparsifolia (Mspr)	3	50.0/44.4	$12A_1/12A_3$
M. triphylla (Mtph)	16	78.9/66.7	$12A_1/12A_2$
M. tristis (Mtst)	29	100.0	All
M. violacea (Mvio)	4	100.0/88.9	$12A_1/WPM_2$
TOTAL 30 spp.	351		

[1] Compared to 100% rooting of cultivated species in 17N.[2] No. genotypes in the in vitro bank to Nov. 1995

[3] Percentage based on 2-3 tubes per genotype per treatment. Does not include root quality.

[4] Culture Media:

$12A_1$:1/2MS, 3% Sucrose, 0.2 mg/lt Kinetin, 100 mg/lt Inositol, 1 mg/lt Thiamine, 1 g/lt A.C., 0.48 mg/lt $CuSO_4$, 7g/lt Agar

$12A_2$:1/3MS, 3% Sucrose, 0.2 mg/lt Kinetin, 100 mg/lt Inositol, 1 mg/lt Thiamine, 1 g/lt A.C., 0.48 mg/lt $CuSO_4$, 7g/lt Agar

$12A_3$:1MS, 3% Sucrose, 0.2 mg/lt Kinetin, 100 mg/lt Inositol, 1 mg/lt Thiamine, 1 g/lt A.C., 0.48 mg/lt $CuS0_4$, 7 gr/lt Agar

WPM_1:1/2WPM, 2% Sucrose, 1 mg/lt Thiamine, 100 mg/lt Inositol, 0.5 mg/lt Ac. Nicotinico, 0.5 mg/lt Piridoxina, 2.0 mg/lt Glycine,200 mg/lt Arginine, 900 mg/lt K_2S0_4, 452.5 mg/lt $Ca(N0_3)_2.4H_20$,1 g/lt A.C., 1.01mg/lt IBA, 7 g/lt Agar

WPM_2:1/3WPM_1, 2% Sucrose, 1 mg/lt Thiamine, 100 mg/lt Inositol, 0.5 mg/lt Ac. Nicotinico, 0.5 mg/lt Piridoxina, 2.0 mg/lt Glycine,200 mg/lt Arginine, 900 mg/lt K_2S0_4, 452.5 mg/lt $Ca(N0_3)_2.4H_20$,1 g/lt A.C., 1.01mg/lt IBA, 7 g/lt Agar

17N: 1/3MS, 2% Sucrose, 1 mg/lt Thiamine, 100 mg/lt Inositol, 0.01 mg/lt NAA, 0.01 mg/lt GA_3, 25 mg/lt (10-52-10), 7g/lt Aga

J:1/2MS, 4% Sucrose, 0.2 mg/lt Kinetin, 100 mg/lt Inositol, 1 mg/lt Thiamine, 100mg/lt Arginine, 1.5mg/lt IBA, 1g/lt C.A, 7g/lt Agar.

Activity - 10 Contribution to the knowledge of seed dormancy in Euphorbiaceae for some species of the genus *Manihot.*

C. Guevara , J.A. Ospina

Introduction

Cassava is commonly propagated by the means of stakes but the sexual seed is an important vehicle in the genetic improvement of the species. Seed either freshly harvested or after variable periods of storage, presents low germination, due to the dormancy condition common to this family. Taking in consideration age (time of storage), the main objective of this study was to test the effect of temperature, mechanic scarification, gibberelic acid (GA_3), ethylene, red light and/or their combinations on eight seed populations. The treatments selected to break dormancy were tested on seed populations of 2 clones of *M. esculenta*, 4 of *Manihot* related species (wild) and one of *Ricinus communis.*

Since dormancy is a common characteristic in several seed species, this information could be of value for managing seed testing within a seed bank as the one held at CIAT.

Materiales and Methods

Seed source

Eight seed populations of *Manihot* and one of *Ricinus* were tested. Because availability and similarities with *Manihot* seed, *Ricinus communis*. was added to the study.

Table 1. Seed populations

GENUS	**TYPE**	**SPECIE**	**POPULATION (Maternal source)**
Manihot	Cultivated	*M. esculenta*	SG 427-87*
			NGA 5**
	Wild	*M. pseudoglaziovii*	PSE (B)
			PSE (G)
		M. chlorosticta	CHL 002
			CHL 003
		M. aesculifolia	AES 002
		M. carthaginensis	CTH
Ricinus	Wild	*Ricinus* sp.	

* SG 427-87 = Open pollimated population of the cassava clone MCol 2060

** NGA 5 = TMS 005. (IITA clone) improved cultivar from controlled cross with *M. glaziovii*

Seed harvesting and conditionning
To help on harvest and to protect the fruits from insect damage, developing capsules were covered with a net bag around the pedicel. Field maturity is reached about 90 days after fertilization, when the fleshy pericarp and the endocarp dry up and show the endocarp. Once capsules dehisced within the bag, seed were taken to the lab for cleaning, drying and storage until enough seed was collected to run the tests.

For drying, seed were placed during 10 days in the dry room (T=25°C, HR=22%), where they reached an equilibrium moisture content between 4 to 6% (fresh weight basis). Seed were then placed in sealed plastic bags and stored under cold conditions (T=5-7°C, HR=35%). Before packing and after immersion in water, seed were separated into its heavy or light fraction; seed for all tests were those that sank in water.

Storage effect
The dormancy value for each population was measured after 0, 9 and 14 months of storage.

Treatments to break dormancy
Eleven treatments were tested but only ten (1-10) were properly replicated. Table 3 describes them. Germination rolls within perforated plastic bags were used for all the cases. A final germination count was made at the end of the fourth week including those normally germinated seeds as well as those firm seeds. Firm seed (those that embibed water but did not germinated and suspected of being dormant) was by the tetrazolium test (TZC) evaluated to confirm its viability (AOSA, 1970).

The response variables measured were : Total and comparative germination, dormancy, viability (by the means of TZC). The method to calculate dormancy was the one proposed by Diulgherof (1991). Data were statistically analized by the use of ANOVA and because interaction, means of population within a treatment were separated by Duncan at a level of 0.05 of significance.

Results
For duration of study (14 months), we can state that the reaction of sexual seed of *Manihot* corresponds to the conventional behaviour of orthodox seed. Within 4 to 6 % seed moisture content, seed can be stored under cold room conditions while retaining its viability. Since other Euphorbiaceae seed species such as *Hevea* sp. are considered recalcitrant (Hong, T. D. et al. 1996), this contributes to clarify common ideas about *Manihot* seed characteristics.

Storage effect
As expected, the time of conservation produced a differential effect on the releasing of the dormancy condition of the seed populations studied. *M. carthaginensis, M. pseudoglazi*ovii and the cultivated selection SG 427-87, remained unmodified (100% dormancy) through out the time of conservation (14 months), while *Ricinus* at the 9th month, had retained partially the condition (60% dormancy). Intermediate results were obtained for the other seed populations (Table 2.)

According to the results the populations can be classified from the deepest to the lightest dormancy level like this; CTH, PSE(B), PSE(G), SG 427-87 > NGA 5 > CHL 002, CHL 003 > AES 002 >*Ricinus* sp.

For those populations that did not respond to storage (a clear example is SG 427-87), the effect of those key treatments (temperature and/or scarification involved) was very definite, while for the others (including *Ricinus*) there was a confounding effect of age with the treatments used to break dormancy.

Table 2. Dormancy (%) of material (controls) through time

POPULATION	0 month	9 months	14 months
AES 002	100	100	58
CTH	100	100	100
CHL 002	100	100	80
CHL 003	100	100	79
PSE (B)	100	100	100
PSE (G)	100	100	100
SG 427-87	100	100	100
NGA 5	100	100	89
Ricinus sp.	100	60	0

Treatment effects

Due to the diverse array of responses and as indicated by the ANOVA for all the measurement variables, it can be concluded that there must be very specialized mechanisms that control germination and dormancy over seeds of *Manihot* and *Ricinus*.While some treatments were suitable to break dormancy on some populations, they did not work well for others. As example, 35/20^{o}C alternate temperature resulted in 5% of dormancy for SG 427-87 while 95% for the other cultivated population NGA 5 (Table 3).

Most of the treatments with some level of success (< 30% dormancy) involved temperatures in the range of 30 - 40^{o}C. This corresponds to previous results and common practice handling to get germination as recommended by the cassava program. For them, alternate temperatures of 36/25 ^{o}C result in about 10% dormancy for most of the *Manihot esculenta* seed and up to 60^{o}C as thermotherapy treatment for bacterial diseases with still good germination results.

The treatment that involved GA_3, as presoaking solution or as substrate did not yield good result for seeds of *Manihot* but did for *Ricinus*. It was possible that the concentration tested were not suitable or as suggested by other authors, the presence of an additional cytokinin be required (Khan, 1971). Future work with those phytohormones is recommended, involving the definition of the proper combination and suitable physiological doses. Preliminary results with light treatments also suggest additional work.

These results suggest that ideally -by population- at least one treatment for release dormancy should be identified. As a practical approach, a not too specific treatment can also be recommended for a population or group of populations and expecting to provide an acceptable range of germination. Additional work on *M. carthaginensis* should be continued, one of the genotypes with the lowest response to any treatment evaluated.

Conclusion

Being dried to 4-6% and after 14 months of storage in cold, sealed conditions, *Manihot* seed of the populations studied, retained its viability confirming its characteristic as orthodox. Time of storage affected dormancy distinctly across the seed populations. This effect has to be considered any time efficiency of dormancy treatments is measured.

The nature of dormancy on *Manihot* is suspected of being complex. While some populations respond to scarification alone, others were more favored for alternate temperature or to combined effects. Although seed embibes water normally, germination do not occur until a not yet well defined factor triggers germination to occur. Since the best treatments corresponded to the combined effect of scarification, temperature, red light and ethylene, it can be concluded that in some cases there must be a physical and in others a physiological mechanism that controls germination and dormancy of the seed populations tested. In the case of *Ricinus sp.*, since the only breakage of the seed coat and/or the removal of the caruncle induced germination, an exogenous type of dormancy is suggested for this species. Preliminary results with GA_3, ethylene and light treatments require additional work.

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Table 3. Dormancy (%) in seed of some *Manihot* and *Ricinus* for several treatments

Population	Dormancy %									
	1	2	3	4	5	6	7	8	9	10
AES 002	56.7 c	0.0 d	96.7 a	96.6 b	100.0 a	88.4 a	79.0 a	26.7 b	70.0 a	28.4 b
CTH	93.4 a	82.6 a	98.4 a	100.0 a	100.0 a	100.0 a	83.4 a	73.4 a	46.4 b	28.4 b
CHL 002	100.0 a	70.0 b	100.0 a	96.7 a	95.0 a	94.8 a	23.4 c	3.4 c	17.6 c	18.4 c
CHL 003	100.0 a	43.4 c	100.0 a	100.0 a	100.0 a	100.0 a	59.7 b	29.7 b	65.0 a	53.4 a
PSE (B)	98.4 a	73.7 b	100.0 a	100.0 a	98.4 a	100.0 a	65.0 b	14.2 b	20.0 c	13.4 c
PSE (G)	96.7 a	23.4 c	98.3 a	100.0 a	98.4 a	100.0 a	71.7 a	25.0 b	70.0 a	31.7 b
SG 427-87	0.0 d	0.0 d	100.0 a	100.0 a	100.0 a	96.7 a	10.1 c	3.4 c	16.8 c	0.0 d
NGA 5	94.5 a	33.4 c	100.0 a	100.0 a	100.0 a	100.0 a	51.9 b	0.0 c	44.5 b	2.0 d
Ricinus	78.4 b	98.3 a	4.5 b	0.0 c	43.4 b	75.0 b	20.0 c	0.0 c	0.0 c	0.0 d

Columns sharing the same letter, do not differ at P≤0.05 of significance according to Duncan test

TREATMENT	DESCRIPTION
I. Allternate temperature (35/20°C)	8/16 hr, with photoperiod in germination chamber
2. Constant temperature (35°C)	Germination chamber
3. GA_3 200 ppm	Replacing water for wetting substrate , lab conditions
4. GA_3 500 ppm	Replacing water for wetting substrate, lab conditions
5. GA_3 500 ppm	As pretreatment and lab conditions
6. Red light (650 nm)	Within a modified chamber with light source (Gro lux/WS, 20W). Seed was embibed previously before exposing to the light. Time of exposure 1 h.
7. Scarification + embibition (24h)	With sand paper, by the micropilar region
8. Scarification + emb. + red light	Same as above and 1 hr light exposure, dark conditions and lab temperature
9. Scarification + emb. + red light (35° C)	Same as above and controlled germination chamber
10. Scarification + emb.	With sand paper, by the micropilar region, warm water (35° C), lab. Cnd.
11. Ethylene	Within dessicators holding splitted apples. Seed was placed on petri dishes.

Activity – 11 Zygotic embryo culture of *manihot esculenta* Crantz: a practical approach for the safe international movement of cassava seed stocks.

C. Guevara , J.A. Ospina , G. Mafla and V. Verdier

Introduction

As indicated by the Technical Guidelines for the Safe Movement of Germplasm (FAO/IPGRI, 1991), cassava germplasm can be moved as true (or botanical) seed, pathogen tested in vitro material, or as cuttings from pathogen tested in vitro material that has been grown under containment. Because many cassava diseases are not seedborne, cassava-producing countries requiring target progenies for selection trials, had received for many years segregating populations in the form of botanical seed produced by the Cassava Program at CIAT.

Between 1995 and 1997, segregating populations (BC1-TMS, IITA, i.e. backcrosses to a Nigerian clone TMS 30572) for the Project "Molecular mapping of genes conferring resistance to cassava mosaic disease in African germplasm ACMV" were produced at CIAT. For gene mapping purposes, both CIAT and the International Institute of Tropical Agriculture (IITA) in Nigeria would require genetically identical individuals in both locations. Being open pollinated, cassava seed is always genetically variable, and because Nigerian regulations prohibit the import of stakes or in vitro plantlets from vegetative tissue, establishing genetically identical populations in both environments was difficult. The Genetic Resources Unit at CIAT tested two methods of establishing in vitro germinated seed: (1) germinating the entire cassava seed directly on culture media, and (2) excising and culturing both mature and immature embryos. The latter method proved to be more effective.

Immature embryos were included to increase the number of genotypes (to represent the diversity of a segregating population large number of seeds or cultured zygotic embryos are required) as well as to meet a schedule for population establishing at IITA (as in any breeding effort for perennial plants). After embryo culturing and growth, established seedlings were propagated prior to be shipped to IITA, and by this way copies of the same genotypes were secured at both stations.

In vitro culture of mature embryos is a promising technique that has been used for cassava and other *Manihot* species (Biggs et al. 1986; Chávez et al. 1988; Marín et al. 1990). It has been used to overcome problems of seed dormancy or low seed viability in several other species (Collins and Grosser 1984). CIAT has done some preliminary work with immature cassava embryos suggesting that 40 days after pollination is the time for maturity; counting on this physiological status, this report (CIAT 1988) indicates that the earliest embryos that can be harvested.

Materials and Methods

Seed source, seed harvesting and processing

A half-sib backcross mapping population was generated by crossing in both directions a set of five F1 plants to their female parent, TMS 30572, an improved Nigerian variety that is widely grown in West Africa and that carries resistance for ACMV. The male parent of the F1 plants (CM 7857) is CM 2177-2, an improved variety that is adapted to the Caribbean coast of Colombia. An initial set of 400 pollinations, provided mature fruits three months after manual pollination. A second round of 964 pollinations, involving two F_1 families, provided immature seed being harvested about forty days after pollination. For both cases, bags were placed around pollinated flowers to protect the fruits and on mature fruits to ease seed collecting after dehiscence. For mature fruits, seeds were brought to the laboratory for further drying and conditioning as described by Ospina (1996). After immersion in water, the seed lot was separated into two density fractions, light and heavy -depending if they floated or sank in water-, with only the heavy fraction being processed. For immature fruits, to facilitate opening of the carpels, one batch of fruits was air-dried at 20°C and 22% RH for 2 days. Another batch was not air- dried, scrapping the fleshy pericarp and mesocarp from the capsules with a scalpel until the hard inner endocarp was exposed. The carpels were then opened using pliers and the seed removed. Because of scarcity of seed stock, and in contrast to mature seeds, for immature dried seeds, both seed fractions -heavy and light- were used.

Embryo excision, culture and growth. Under aseptic conditions, surface sterilization of seeds was done with 70% alcohol for 1 min, followed by 0.5% sodium hypochlorite for 6 min and three rinses with sterile water. Next, with the use of sterile pliers the seed was splitted open along the longitudinal axis and with scalpel and blade, the embryonic axes were removed. The excised embryonic axes were explanted on 17N medium: 1/3 MS ; 0.054 μM NAA; 0.029 μM GA3; 2.964 μM thiamine-HCl; 554.938 μM inositol 100 mg/l; 2 % sucrose; 0.7% agar and 25 mg/l of a commercial fertilizer containing N 10, P 52, K 10 (Roca, 1984). The explanted embryo axes were placed in a germinator under alternate temperature 35°C for 16 h and 25°C for 8 h during 10 days. For the first 5 days the embryos were incubated in darkness to promote the protrusion of the radical system. For the next 5 days the embryos received 12 h of light to encourage the formation of the first leaves. After this period of incubation, the germinated embryos were transferred to the growth chamber, set at standard conditions: 27°C, 12 h of light (1000 lux of light intensity).

Micropropagation of seedlings. After 30-40 days of incubation in the growth chamber, and responding to genotypic effect, most of the seedlings were big enough to be micropropagated. Apical shoots or cuttings at the level of the cotyledonary node were then explanted on 4E medium: MS; 0.108 μM NAA; 0.177 μM BAP; 0.145 μM GA3; 2.964 μM thiamine-HCl; 554.938 μM inositol 100 mg/l; 2 % sucrose; 0.7% agar and a pH of 5.7-5.8 according to the protocol developed by Roca et al (1989). After the cuttings were taken, the seedlings were prepared for distribution previous testing for the presence of CBB on a well representative sample of the population.

Cassava bacterial blight testing. Cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *manihotis* (Xam) is a seed-borne disease of importance for seed transfer between countries (Elango and Lozano 1980). As stated in the Import Permit issued by Nigeria, and to support the Phytosanitary Certificate issued by the Colombian Quarantine Office through the Instituto Colombiano Agropecuario (ICA), a random sample from 36 seedlings were tested with the polymerase chain reaction (PCR) technique and dot-blot test as described by Verdier et al. (1996). Healthy tissues (leaves and stems), infected tissue and sterile distilled water were used as controls. An extract from each sample was also plated on LPG medium to count the number of bacterial colonies.

Shipment. After completing the requirements set out by ICA for international exchange: Nigerian import permit and Colombian fitosanitary certificate, 276 in vitro seedlings were prepared for shipping to Nigeria. A box containing the tubes, related documentation and mailing label was handcarried to safely assure the transfer.

Results

Mature and immature embryos establishment

Table 1 summarizes the processes for both mature and immature seed: from embryo excision, through explanting and germination to normal in vitro seedling establishment. With mature seed, 86% of seedling establishment was achieved. With immature seeds, drying, done to promote seed release affected embryo establishment to such an extent that only a 36% seedling recovery was obtained. In contrast, 100% recovery was obtained when immature seeds were processed without previous drying.

These results can be explained because in general immature seeds with moisture contents more than 60% can be very sensitive to damage from drying (Hong et al. 1996). Another reason for the low rate of establishment may have been that, for this group of seed, both the heavy or light fractions were processed indistinctly, thus, for the light fraction the establishment values may have been skewed.

Whether mature or immature, the period from explanting to seedling establishment took about 60 days on 17N medium. In vitro seedlings differ from in vitro cuttings in that they develop a primary root, where as cuttings develop adventitious roots. In contrast to cuttings, seedlings also have a cotyledonary node and develop a hypocotyl. This criteria helps to identify provenance of an in vitro plantlet. As expected, and since each seed is a genetically different genotype, variability in growth and vigor was observed between families and within families. Differences were noticeable in length of shoot and hypocotyl and number of nodes.

CBB testing. The PCR test showed no presence of *Xanthomonas axonopodis* pv. *manihotis* (Xam), the causal agent of CBB. None of the 36 BC1 tissues samples used showed amplification with the CBB diagnostic primers while the positive control (infected tissues) showed amplification of the expected fragment 900 bp. The dot-blot analyses for all samples also showed no signs after

hybridization with the specific probe and the control (infected tissue presented a positive reaction). Xam was not either detected in the samples after 48 h of incubation on LPG medium. The fact that no CBB was found in any of the assays means that the seedlings tested were CBB free at the outset and provided an insight that CBB is not a disease at the CIAT station where the crosses were produced, making them safe for quarantine transferring.

Shipment. Once the requirements were completed, 276 in vitro seedlings were personally taken to IITA, Ibadan, in May 1997. To keep the same number of explants, individuals at CIAT and for further use in RFLP genotyping and mapping resistance to CBB, before shipment the seedlings had been subculture by cutting just above the level of the cotyledonary node.

Conclusions

Instead of shipping segregating populations as botanical seed as is usually done and in order to have copies of the same genotype in more than one station, seed stocks can now be sent as in vitro cultured seedlings provenance from zygotic embryos.

In vitro cultured embryos has several advantages:

- Large number of individuals from a segregating population can be processed with a high rate of seedling establishment.
- Clonal copies of the individuals to be distributed can be attained by cutting the seedling above the level of the cotyledonary node while the original seedling is transferred.
- Seedlings tested negative for a seedborne disease like CBB, are likely to be a recommended approach for the safe international transfer of seed stocks.

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Table 1. In vitro culture and germination of zygotic embryos from mature and immature seeds

MATURE SEED				
Crosses	No. of embryos			No. seedlings Established in vitro
	Explanted	Contaminated	NonGerminated	
TMS 30572 x CM 7857-4	22	0	4	18
TMS 30572 x CM 7857-77	25	0	2	23
TMS 30572 x CM 7857-10	40	0	8	32
TMS 30572 x CM 7857-51	20	0	0	20
TMS 30572 x CM 7857-115	50	0	7	43
Total	157	0	21	136
IMMATURE AIR-DRIED SEEDS (20 C; 20% RH two days)				
Crosses	No. of embryos			No. seedlings Established invitro
	Explanted	Contaminated	NonGerminated	
TMS 30572 x CM 7857-4	10	0	0	10
TMS 30572 x CM 7857-77	234	6	117	111
CM 7857-4 x TMS 30572	51	11	29	11
CM 7857-77 x TMS 30572	143	18	95	30
Total	438	35	241	162
IMMATURE NON-AIR-DRIED SEEDS				
Crosses	No. of embryos			No. seedlings Established invitro
	Explanted	Contaminated	NonGerminated	
TMS 30572 x CM 7857-4	90	0	0	90
TMS 30572 x CM 7857-77	56	0	0	56
CM 7857-4 x TMS 30572	29	0	0	29
CM 7857-77 x TMS 30572	77	0	0	77
Total	252	0	0	252

Activity - 12 In vitro growth control in cassava, using osmotic regulators and ethylene inhibitors

Graciela Mafla, Julio César Roa, and Claudia L. Guevara

Introduction

The traditional method of in vitro tissue culture of cassava (*Manihot esculenta* Crantz) (Roca et al. 1989) permits the management of 6,060 varieties, with an average conservation time of 12 to 14 months. To reduce the laborious task of subculturing and to minimize the risks this entails for genetic stability especially if frequent, we need to minimize the growth rate and to increase the time of in vitro conservation. The world collection of cassava germplasm, a highly representative sample of this crop's genetic diversity, is held at CIAT. However, for safety reasons, it must be duplicated and held in another institute. To achieve this objective, the culture medium currently in use is being investigated for its potential to prolong conservation time up to, say, 24 months.

A central objective in conserving germplasm is to prevent materials from deteriorating. Different systems such as reducing temperatures, incorporating growth inhibitors and osmotic compounds, and decreasing the availability of nutrients have been used to slow the growth of a culture in vitro (Roca et al. 1989).

Water deficit in plants, created by osmotic stress, can affect a series of metabolic processes and thus reduce or stop growth. Substances most used as osmotic regulators for in vitro conservation are sucrose, mannitol, and sorbitol. Sucrose, is a disaccharide, and constitutes most of the soluble carbohydrate reserves of higher plants, and is accumulated as a product of photosynthesis. Mannitol and sorbitol are polyols (sugar alcohols), which, by their nature, are highly hydroxylic and can replace water in the cytoplasm's polysaccharides. They thus help maintain the normal operation of enzymes and cellular membranes when the level of water decreases through osmotic pressure (Schobert 1977). Sucrose can act as an osmotic agent: concentrations of sucrose lower than 1% and more than 3% have proven highly prejudicial to the viability of in vitro cassava cultures. Maximum viability has been obtained with 2% or 3% of sucrose at 22 °C. When mannitol is used in the absence of sucrose, viability is severely reduced; it should therefore be added to the culture medium with sucrose and at a molarity of no more than 0.1 *M* (CIAT 1986).

Another factor is ethylene, which exercises a dramatic effect on several aspects of plant growth. In vitro plants release a wide variety of substances into the atmosphere of the flask, including ethylene. Although the protected conditions under which the tissues grow prevent microbial contamination and retard dehydration, they also cause a large accumulation of released compounds that continue acting on the tissues (Gould and Murashige 1985). Ethylene plays a significant role in accelerating growth. The quantity of ethylene produced in tissue culture depends on the environmental conditions under which the culture lives (Huster et al. 1979).

The present study analyzes the impact that the following factors have on the in vitro growth rate: osmotic regulators (sucrose, mannitol, and sorbitol), ethylene inhibitors (salicylic acid and silver nitrate), and abscisic acid.

Materials and Methods

Plant material was selected after analyzing subculture information in the database of the cassava in vitro bank. The database began almost 17 years ago, when conservation of this germplasm started. The core collection (630 varieties), a sample of the world collection, was classified into three groups according to the time prior to subculturing: less than 8 months; between 8 and 16 months, and more than 16 months. Two varieties were selected from each category: respectively, M COL 2056 and CM 2177-2; M ARG 2 and M BRA 337; M NGA 16 and M VEN 329 A. To increase the number of plants originating from the cassava in vitro bank and thus have explants for the different treatments, we micropropagated apical shoots and nodes enough according to the method developed at CIAT (Roca et al. 1984).

Basal culture mediums 8S (MS, 0.088 μM BAP, 0.29 μM GA_3, 0.054 μM NAA, 2.96 μM thiamine-HCl, 554.93 μM M-inositol, 2% sucrose, 0.7% agar) was used as control (Roca et al. 1984). Treatments were additions to 8S at different concentrations of sucrose, mannitol, sorbitol, abscisic acid, salicylic acid, and silver nitrate according to theTables 1A and 1B. The different media were placed in test tubes of 25 x 150 mm size. The tubes were covered with aluminum foil and autoclaved at 115 lb/sq. inch for 12 min to be sterilized .

The standard explant used in all trials comprised nodes, originating from the middle part of the in vitro plant. The explants were placed in the conservation room at a temperature between 23 and 24 °C, with a 12-h photoperiod and lighting at 1000 lux. A randomized complete block design was used to assign treatments to experimental units. The design comprised six varieties x four replications x 36 treatments including controls and three evaluations (two, every month and one, at the 6^{th} month). Variables for evaluation used were length of stem and roots; number of green leaves, dead leaves, apical shoots, nodes, roots; and presence of aerial roots. The results were analyzed by analysis of variance (ANOVA) and the treatments were compared, using the Duncan test at a level 0.05 of significance.

Table 1A. Final molarity of each osmotic regulator.

Treatment	Sucrose (M)	Mannitol or sorbitol (M)	Final molarity
1	0.02	0.08	0.10
2	0.03	0.07	0.10
3	0.04	0.06	0.10
4	0.05	0.05	0.10
5	0.05	0.10	0.15
6	0.075	0.075	0.15
7	0.08	0.12	0.20
8	0.10	0.10	0.20
9 (control, 8S)	0.07	-	-

Table 1B. Concentrations used for abscisic acid and two ethylene inhibitors.

Treatment	Abscisic acid (μM)	Salicylic acid (μM)	Silver nitrate (μM)
1	0.37	1.00	23.54
2	1.89	5.00	47.08
3	3.78	10.00	58.85
4	7.56	15.00	70.62
5	9.45	20.00	82.40
6 (control, 8S)			

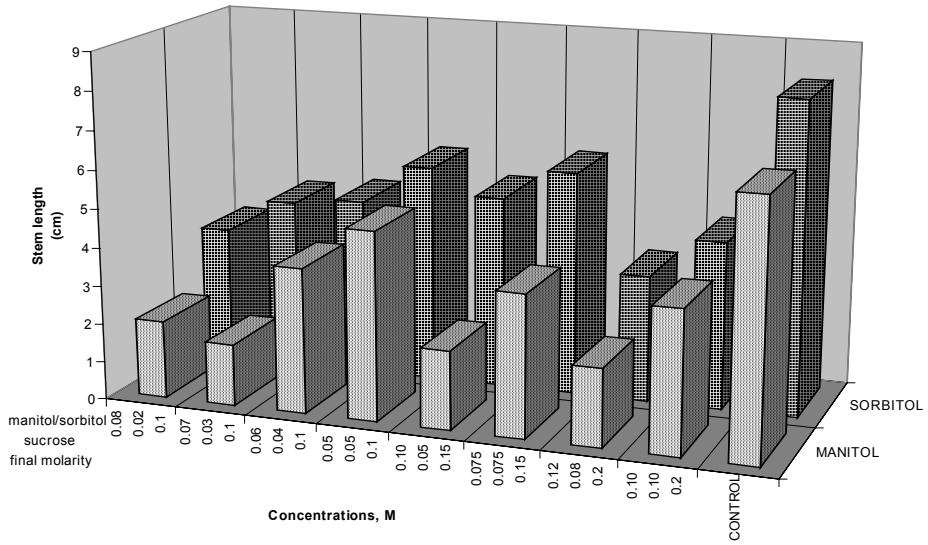
Results

Effects of Osmotic Regulation

To determine the efficiency of sucrose, mannitol, and sorbitol as osmotic regulators, eight combinations of concentrations (16 treatments) were evaluated during 6 months. Results showed that indistinctly with manitol or sorbitol the combination led to a marked decrease in growth. Overall, the effect of sucrose with mannitol was greater than with sorbitol, reducing stem length by as much as three times that of the control (Figure 1).

Probably the reducing effect of sucrose between the lower ranges studied (0.02 and 0.05M) is independent from the concentration of manitol or sorbitol used and is probably due to the lack of substrate for oxidation reactions. On the contrary, the effect of sucrose in higher concentrations (between 0.05 and 0.1M) and with levels for manitol between 0.075 and 0.1M can be due to the osmotical effect of both components.

Figure 1. Effects of mannitol and sorbitol on the in vitro growth of six varieties of cassava.



An increase in solutes produces a more negative osmotic potential on the media and, consequently, greater stress in the cell. If this potential is greater than that of the cell, then the cell will loose its turgor, thus affecting cellular expansion and hence its size. The control of turgor is a process that is highly sensitive to stress, and any loss decreases both growth and stem elongation (Hale and Orcutt 1987).

Fortunately, so far such reduction in growth during the first 6 months of conservation did not affect the viability of the culture in terms of leaves retained and potential for multiplication (number of both apical shoots and nodes) (Table 2). The highest concentrations of both sorbitol and mannitol showed increased root length and an increased number of aerial roots, compared with the lowest

concentrations.

This suggests a possible mechanism for reducing water stress: by decreasing the transpiration rate, or increasing the rate of water absorption, and thus inducing radicular growth.

In terms of reducing the growth rate of in vitro cassava, some combinations of mannitol (1,2,5 y 7) and sorbitol (7, 8) surpass the control. However, for such concentrations to be truly advantageous, they must also permit long-term viability at a level much higher than that provided by the control. We hope to extend the conservation time of short-cycle varieties (M COL 2056 and CM 2177-2) to 16 months and that of long-cycle varieties (M NGA 16 and M VEN 329 A) to 24 months. In this experiment we used higher molarities that than the ones before reported and with little apparent negative effect. These molarites can be used, but only and when a balance exists between the concentration of sucrose and the mannitol and sorbitol added.

Table 2. In vitro growth of six varieties of cassava (*Manihot esculenta*) under different osmotic conditions.

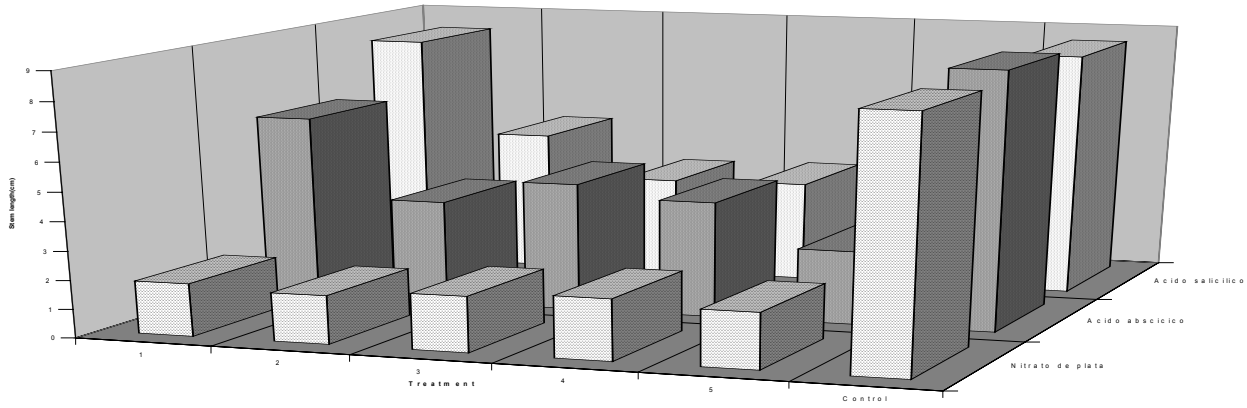
Concentration	Stem length (cm)	Green leaves (no.)	Dead leaves (no.)	Apexes (no.)	Nodes (no.)	Roots (no.)	Root length (cm)	Aerial roots (no.)
Mannitol								
Control	6.61	4.75	2.75	1.52	2.16	5.27	3.83	1.22
Treatment 1	2.02	2.94	1.05	0.97	0.10	1.05	1.46	0.40
2	1.60	1.41	0.97D	0.69	0.33	0.88	1.34	0.11
3	3.77	4.13	1.58	1.08	1.22	2.30	2.84	1.08
4	4.90	4.22	2.05	1.08	1.69	3.88	3.26	1.25
5	2.05	2.33	1.30	1.02	0.22	2.08	2.76	1.11
6	3.69	3.05	1.88	0.94	1.00	4.41	3.68	3.36
7	2.02	1.48	1.05	0.80	0.42	2.68	3.12	1.91
8	3.70	2.77	1.97A	0.97	0.91	3.77	3.66	4.00
Sorbitol								
Control	8.10	4.88	3.27	1.63	2.77	5.97	4.22	2.22
Treatment 1	3.50	3.88	1.55	1.00	0.83	2.66	2.71	0.47
2	4.40	3.97	1.41	.091	1.33	2.66	3.29	0.66
3	4.59	3.83	1.16	1.02	1.47	3.00	3.06	1.02
4	5.65	4.44	2.08	1.11	1.52	4.30	3.45	1.58
5	5.00	3.08	1.55	0.83	1.77	3.61	3.16	1.83
6	5.81	2.91	1.80	0.86	1.88	3.75	3.50	4.02
7	3.30	2.55	1.19	0.86	0.94	3.77	3.40	2.72
8	4.35	2.94	1.38	0.88	1.19	4.30	3.86	5.19

Ethylene Inhibitors

The generation of ethylene by plants grown in test tubes has been reported previously . The species, environment, growth stage, type of cells and tissues, concentration level of ethylene, and interaction with other hormones are factors that influence the effects ethylene has on the plant. The gas can promote elongation, growth, and development (Huster et al. 1979).

We were able to demonstrate that the effect ethylene has on growth can be reduced by adding silver nitrate and salicylic acid. A decrease in stem length, compared with that of the control, could be observed in evaluations of the two compounds and of most of the concentrations studied. Only salicylic acid in lower concentrations was similar to the control (Figure 2).

Figure 2. Effect of ethylene inhibitors and abscisic acid on invitro growth of six varieties of cassava.



Since ACC (L-aminocyclopropane L-carboxylic acid) was discovered as the key to the biosynthesis of ethylene, the mechanism by which this gas is regulated is now better understood. Cobalt ions and anaerobiosis impede the conversion of ACC to ethylene, whereas silver ions inhibit the action of ethylene.Those ions affect many physiological responses and are most effective in detaining leaf senescence (Aharoni and Lieberman 1979; Beyer 1976). Other relevant properties are specificity, persistence, and lack of phytotoxicity. SA blocks the action of the ethylene-forming enzyme, although the internal block of ACC may also be another possibility (Leslie and Romani 1986) . SA can affect the levels of CO_2 in the leaf and induce stomatal closure. Also it interfers with oxidative phosphorylation, stimulating the occurrence of subcellular respiration. The high concentrations of salicylic acid (SA) that we used increased stem length and decreased the length and number of roots. The data also showed that, equally with silver nitrate, SA is an effective, nontoxic inhibitor of ethylene synthesis.

Table 3. In vitro growth of six varieties of cassava under different concentrations of abscisic acid and ethylene inhibitors.

Treatment		Stem length (cm)	Green leaves (no.)	Dead leaves (no.)	Apexes (no.)	Nodes (no.)	Roots (no.)	Root length (cm.)	Aerial roots (no.)
Abscisic acid									
Control		8.90	5.44	2.88	1.22	3.41	5.36	4.01	2.38
Conbination	1	6.40	4.41	1.69	1.08	1.97	3.77	2.96	1.36
	2	3.63	2.94	1.00	0.91	0.94	2.25	1.82	0.75
	3	4.47	2.91	1.52	1.02	0.88	3.00	1.99	0.72
	4	4.05	2.69	0.86	0.91	0.83	3.80	1.90	1.47
	5	2.57	2.50	0.83	0.91	0.55	2.66	1.83	0.52
Salicylic acid									
Control		8.51	5.11	2.55	1.13	2.86	4.61	3.95	2.47
Combination	1	8.30	5.08	1.97	1.19	2.69	4.44	3.72	2.36
	2	4.96	3.25	0.97	0.72	1.63	3.30	2.80	1.30
	3	3.46	2.91	1.33	0.94	0.86	2.47	2.47	0.30
	4	3.53	2.36	1.75	0.94	0.77	2.94	2.96	1.25
	5	2.29	2.38	1.27	0.80	0.36	1.44	1.33	0.52
Silver nitrate									
Control		8.54	5.63	2.58	1.30	3.27	5.13	4.31	2.36
Combination	1	1.83	2.75	0.30	1.00	0.02	2.69	1.50	0.02
	2	1.68	2.58	0.27	0.86	0.11	2.30	1.37	0.25
	3	1.92	3.50	0.27	1.05	0.05	3.47	1.69	0.05
	4	2.10	2.80	0.50	1.00	0.16	2.55	1.33	0.13
	5	1.93	3.22	0.38	1.00	0.11	2.47	1.20	0.11

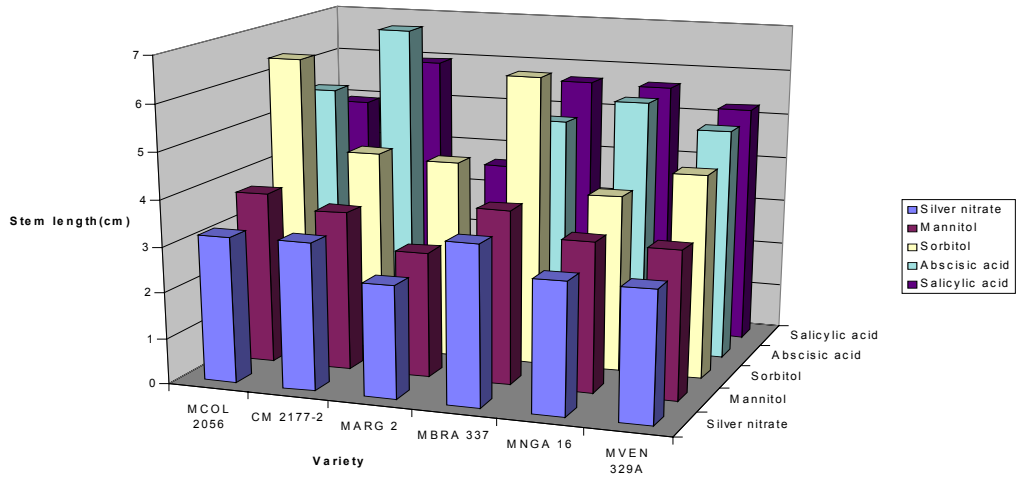
Abscisic Acid

The presence of ABA can be considered as inducing osmotical stress and as an important factor that affects root growth and plant metabolism under water deficit. When a high concentration of abscisic acid (ABA; 9.45 μM)(combination 5) was added to the medium, stem length was reduced from 8.90 cm to 2.57 cm (Table 3). This is another clear effect of osmotical pressure of the media to the plant. Numerous interactions probably exist between growth rate, ABA content, and turgor of pressure. In corn, a decrease in water potential is correlated with reduced growth rate (Ribaut and Pilet 1991).

Varietal Response

The overall response in stem length of the six varieties, for all the evaluated compounds are shown in Figure 3. The treatments with silver nitrate and mannitol presented uniform response for all of them better than with the other compounds. Although the selected varieties corresponded to short, medium and long storage ability, the uniformity of response for all of them reflects the level of confidence we may have when infering these results above the whole collection stored in vitro.

Figure 3. In vitro growth of six varieties of cassava with different compounds.



Prospects

1. To continue with those evaluations that are pending (at 8, 10, 12 months, etc.) to verify the results so far obtained in the first 6 months of this trial.

2. Once the most promising treatments are defined, evaluations will be carried out on genetic stability, using an appropiate molecular marker, and monitoring on certain enzymes, such as peroxidase, transaminase, and carbonic anhydrase, that are linked to behavior under water stress and to photosynthetic processes. Studies of the CO_2 compensation point will also be carried out.

3. To confirm the viability of the cultures submitted to these treatments (micropropagation ability).

4. To evaluate a wider range of varieties with those treatments that provided superior results on growth limitation.

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Activity – 13 Cryopreservation of Cassava Shoot Tips.

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Introduction

We have developed a basic cryopreservation protocol based in chemical cryoprotection and dehydration cooling to recover viable cassava plants from shoot tips in liquid nitrogen (L.N.) (Escobar, et al 1997). Several factors have been tested to improve the response of recalcitrant varieties as like pre-treatment in 4E medium (Roca, 1984) for 3 days, lower osmotic concentration, use of different concentration of cytokinins in the recovery media; those resulted in increased survival after freezing (BRU-Annual Report, 1995).

Moreover, we have developed a rapid freezing protocol which performed similarly to the programmed freezing protocol (Escobar, 1996). We are working to develop an encapsulation-dehydration technique which is expected to significantly improve the classical protocol and possibly use a large scale methodology.

Material and Methods

Shoot tips were used as explants for initiating the cryo protocol. Genetic stability is likely to be maintained when continued organized growth of the meristem in culture follows culture initiation (Ford-Lloyd, 1986). We selected several cultivars for testing genotypic effects, and used MCol 22 as a control cultivar. The material was maintained under *in vitro* conditions in a 4E medium. Table 1 shows the procedure variations of three cyropreservation methods tested at CIAT on cassava.

Table 1. Methods of cassava cryopreservation.

	Methodology		
Steps	**(1) Programmed**	**Rapid**	**(2) Encapsulation-dehydration**
Explants	Shoot tips	Shoot tips	Shoot tips
Pre-treatment	4E/3 days	4E/3 days	4E/3 days
Pre-culture	(3) C4/3 days	C4/3 days	0.75M/3d
			0.5-0.75M / 1,2 d
			0.5-0.75-1M / 1,1,1 d
Cryoprotection	+	+	-
Drying	2 h	2 h	16 h
% W.C.	?	?	15-20
Freezing rate	Program 4.	Rapid	Rapid
Thawing	37 C	37 C	37 C
Recovery conditions	R1,R2,$4E_{ss}$	R1,R2,$4E_{ss}$	R4
	1 month	1 month	1 week

(1) Modification of the basic protocol.
(2) In the Encapsulation-dehydration technique sucrose is used as media.
(3) M6 or M7 is used for recalcitrant varieties (BRU-Annual Report, 1992) because these have a lower osmotic concentration than C4; in the classical protocol, sorbitol, sucrose and DMSO are used in all media.

Results

1. The response of cassava after freezing was influenced by the cytokinin: 2iP and Kin gave better shoot formation than BAP at 0.5 mg/l concentration. A (Adenine) and TDZ (Thidiazuron) did not favor shoot formation, and when BAP concentration was increased a drastic detrimental effect on shoot recovery was evident (Table 2).

Table 2: Effect of cytokinin on plant (shoot) recovery from cassava shoot tips cryopreserved in L.N. (cv MCol 22).

Concent.	**0.04 mg/l**		**0.3 mg/l**		**0.5 mg/l**	
Cytokinins	**%Viability**	**% Shoots**	**%Viability**	**% Shoot**	**Viability**	**% Shoot**
2iP	63	0	96	44	100	75
BAP	84.6	57.7	88	44	19	0
KIN	54.2	4	100	59	100	80
A	0	0	52	0	24	0
TDZ	64	24	87.5	0	100	0

2. A new cryopreservation technique is based on vitrification (the transition of water directly from the liquid phase into an amorphous phase or glass, whilst avoiding the formation of crystalline ice: Towill, 1996). Four procedures are based on vitrification: Encapsulation-dehydration, Desiccation, Pregrowth-desiccation and "Vitrification" per se.

Results with ultra-rapid freezing were not different from the programmed protocol (Table 3). Since ultra rapid freezing does not require the use of controlled freezer, it could be more expeditious for introduction of large cassava collections into LN.

Table 3. Effect of cooling rate on cryopreservation of shoot tips of different cassava varieties.

Variety	**Freezing Rate**	**% Viability**	**% Shoots**
MCol 304	Program	63.6	18.1
	Rapid	100.0	54.5
MCol 1389	Program	90.9	9.1
	Rapid	90.0	10.0
MCol 1468	Program	53.8	0.0
	Rapid	84.6	0.0
MPar 71	Program	84.6	76.9
	Rapid	92.8	71.4
MCol 22	Program	76.5	49.75
	Rapid	80.25	55.5

Prospects

Using beads (shoot tips encapsulated in analginate) is possible to dehydrate tissue to a point where water content (W.C.) (based on fresh weight) is not lost in response to freezing. For developing cassava beads, we tested different dehydration times and obtained better results between 12-16 hours which gave a 15-20% water content (Fig. 1) .

Sucrose may decrease W.C. of cells and increase dry weight (Uragami *et al*, 1990). Cassava has shown a sensitive behavior to direct exposure to high sucrose level (Escobar *et al*, 1997). We have learned that cassava shoot tips pre-treated in a sequential media with variable sucrose concentrations and treatment duration shows a differential response in term of shoot regeneration (BRU-Annual Report, 1995); thus, 0.5-0.75M sucrose for 1-2 days respectively and 0.5-0.75-1M sucrose for 1-1-1 days each resulted in better response after freezing when cultivars were dehydrated for 12-16 hours. Eight hours treatment did not give a consistent response maybe because there is more residual W.C. (25-30%) than with the 12-16 hours treatment (Fig. 2). Benson *et al* (1992) reports that cassava shoot tips 30-35% W.C. as optimal, but our work does not show any response at this W.C.

Grafica Roosevelt.

Prospects

(i) Standardize the technique that reduces varietal effects after freezing (ii) Develop the logistical aspects of the process for implementation in CIAT; (iii) Implement direct immersion and Encapsulation-dehydration techniques to improve the efficiency of establishing the *In vitro* Base Gene bank at CIAT; (iv) DNA analysis of different cassava conservation approaches (cryopreservation, *in vitro* and field) to determine stability after freezing.

Literature Cited

Benson E E, Chabrillange N and F Engelman. 1992. Mise au point de méthodes de cryopresérvation de méristèmes pour la conservation à long terme des ressources génétiques du manioc (*Manihot* spp.). ORSTOM

BRU-Annual Report, 1992

BRU-Annual Report 1995

Escobar R H, G Mafla and W. M. Roca. 1997. A methodology for recovering cassava plants from shoot tips maintained in liquid nitrogen. Plant Cell Reports 16:474-478

Escobar R H and W M Roca. Cryopreservation of cassava shoot tips throught rapid freezing. in : III1 International Scientific Meeting Cassava Biotechnology Network (CBN). August 26-30 Kampala-Uganda.

Ford-Lloyd B and Jackson M. E. 1986. Plant Genetic Resources: An introduccion to their conservation and use. Arnold Publishers. U.K.

Roca W M. 1984. Cassava. Sharp W R, Evans DA, Amirato RV, Yamada Y (eds). In: Handbook of plant cell culture:Crops species. Vol2. MacMilliam Publ, New York, pp 269-301

Towill L E. 1996. Vitrification as a method to cryopreserve shoot tips. Trigiano R and Gray A (eds). In: Plant tissue culture concepts and laboratory exercises. CRC. Chapter 34 , pp. 297-304

Uragami A, Sakai A and Nagaï M. 1990. Cryopreservation of dryied axillary buds from plantlets of *Asparagus officinalis* L. grown *in vitro*. Plant Cell Reports. 9:328-331

Activity 14 - Adaptation of Seed Health Test Techniques to detect Pathogens in Seeds.

Elisa Test in Seeds and its relation to BCMV pathogenicity

N. Bravo, B. Pineda y R. Hidalgo

Introduction

ELISA test is a diagnostic technique that produces a color reaction when the viral protein is present in samples but it doesn't give information about the pathogenicity of the viral nucleic acids which really produce the infection. In dry seeds it is necessary to obtain more information about the relation between the ELISA results and the presence of infective virus in it.

Materials and Methods

A study to establish the correlation between ELISA test in *Phaseolus vulgaris* L. seeds and the bioactivity of Bean common mosaic virus (BCMV) was carried out. Seed samples of six accessions of the *P. vulgaris* core collection from the CIAT germplasm bank previously analized for ELISA test and classified in ranks of color reactions were used . An monoclonal antiserum (Potyvirus, Agdia Inc) was used following the Adgia Inc protocols. The seed extracts that showed positives ranks of color reaction were mechanically inoculated on plants of *P. vulgaris* var Dubbele Witte (susceptible to all BCMV strains). Additionally seeds from the same source that was used in ELISA tests were planted under greenhouse conditions. Presence or absence of BCMV symptoms was recorded.

Results

A direct relation between ELISA results in high color reaction rankings and virus infectivity was found, and between accessions that showed infective extracts and seed transmission because only *Phaseolus* materials which had infective extracts, seed transmission happened too. Light reactions didn't show infective extracts neither plants with symptoms.

Its very important to clarify that if it is to classify aspositive or negative materials about BCMV presence the results of this experiment are meaningful only under the present conditions.

Literature Cited

Bravo, N., B. Pineda y R. Hidalgo.1994. Determinación de la patogenicidad del virus del mosaico común del frijol (BCMV) en extractos de semilla de Phaseolus vulgaris . In: ASCOLFI 20 Años. Memorias XV Congreso. Medellín , Agosto 31- Sep 2 /94

Bravo, N., B. Pineda y R. Hidalgo. 1996. La prueba ELISA en semillas de frijol y su relación con la bioactividad del virus del mosaico común del frijol (BCMV). Fitopatología Colombiana 20(2): 37-43

Activity 15 - Adaptation of the Pathogenicity TEST of *Xanthomonas ampestris pv Phaseoli* for use in Seed Health Diagnostic.

M. del S. Balcazar and B. Pineda L.

Introduction

In laboratory, for testing diagnostic to *Xanthomonas campestris* pv *phaseoli,* are used dilution techniques, plating in semi-selective (MXP) and serology for seed health testing. However it is necessary to have other alternative tools such as the pathogenicity tests when there aren't specific antisera for serology.

Pathogenicity tests are biologic tools very useful to determine the relation between the presence of a bacteria in a plant or a seed, and the disease that it produces. Not all the microorganisms or bacteria on seeds produce diseases, only those bacteria that inoculated on an susceptible host under favorable environmental conditions can reproduce typical symptoms.

Materials and Methods

Before the pathogenicity test bean seeds of the susceptible variety BAT 41 were planted in ikopor pots with sterilized soil. Plants obtained were maintained under a growing chamber condition (20-27^{o}C, photoperiod 12 h light and 12 h. dark). Fifteen days later the plants were inoculated by cutting with scissors wetted in a bacterial suspension of $5x10^{7}$ cels / ml. As a positive control was used a suspension of *X. c*. pv. p*haseoli* from bean pathology program. The absolute control was distillated water.

Inoculated plants and controls were covered with a plastic bag and maintained under the growing chamber conditions . The first symptoms were observed in plants inoculated with isolates from seeds and positive controls 12 days after inoculation (20 days after sowing seeds). In reisolates the presence of *X. c.* pv *phaseoli* was confirmed

Results

The pathogenicity test is a diagnostic tool but requires a long time to obtain the information. We think that its application in the SHL as an alternative when there aren't antiserum to serology tests is not a problem.

Literature Cited

Schuster, M.L., and Coyne D. P. 1981. Biology, Epidemiology, Genetic and Breeding for Resistance to Bacterial Pathogens of *Phaseolus vulgaris* L Horticulturel Reviews . Vol 3

Saetltler, A. W. 1994. Bacteriosis común . In : CIAT. 1994. Problemas de producción de frijol en los trópicos. 2ed. Pastor Corrales, M. y Schwartz, H. F. (eds). Cali , Colombia . p 303-329.

Activity - 16 Studies of gene flow in wild-weed-crop complexes and implications for *in situ* conservation.

Steve Beebe, J. Tohme, D.G. Debouck

Introduction

American early agricultures are often horti-cultures where plants are sown, managed, harvested, stored and selected on individual plant basis, with major phenotypical effects in plants. Aspects of plant husbandry and conservation much reflect cultural originality and differences. From such practices applied for thousands of years (a likely duration for American agriculture: Smith, 1997) in the American tropics and subtropics, one would expect the genetic diversity to be virtually null. And it is indeed the case with many modern cultivars of several American crops (e.g. common bean: Sonnante et al., 1994; tomato: Miller and Tanksley, 1990). But in primitive landraces there is often some remnants of genetic diversity; we were interested in exploring the reasons and mechanisms for such remaining diversity.

Results

Using the common bean model we notice that the so-called primitive agricultures with this crop often come in contact with the distribution of the wild ancestral form. We also note that this model species is still an open biological system with frequent crosses between all forms resulting into a hybrid swarm, namely in the Colombian and Peruvian Andes. With the help of biochemical markers, we have gained additional evidence about the presence of such gene flows, apparently operating in both ways from the wild to the cultivated form and *vice versa*.

Prospects

These preliminary results should be expanded with additional markers also on materials from other regions in order to quantify the flow and its time and space variations. A sociological study should document farmers' reactions to the flow. From both perspectives, one could draw conclusions about the biological and social relevance for the future of a crop, and consequently about the need for an *in situ* conservation of wild-weed-crop complexes.

Output

Beebe, S., Toro Ch., O., González, A.V., Chácon, M.I. and Debouck, D.G. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Resources & Crop Evol. 44 (1): 73-91.

Literature Cited

Miller, J.C. and Tanksley, S.D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet. 80 (4): 437-448.

Smith, B.D. 1997. The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. Science 276: 932-934.

Sonnante, G., Stockton, T., Nodari, R.O., Becerra Velásquez, V.L. and Gepts, P. 1994. Evolution of genetic diversity during the domestication of common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 89: 629-635.

5.4.3. How to enhance genetic resources?

Plant breeding is often considered as the sole possibility to add value to genetic resources. Indeed most of the germplasm collections assembled by the CGIAR were the basis for the breeding work that lead to the Green Revolution. There are however other perspectives such as the use of landraces *per se* in which an increased genetic diversity is made available from the gene bank to farmers. Here follow examples by which a wider use of landrace collections kept in the GRU by farmers is being sought.

Activity - 17 Introduction and adaptation for small farmers production, of lima beans, *Phaseolus lunatus*, to the Eastern plains of Colombia.

R. Hidalgo, Universidad de Los Llanos

Introduction

The Eastern Plains of Colombia cover an extensive area between the slopes of the Andean range and the border of the Amazonas jungle; climate is hot tropical and humid. This region is of a rather recent colonization and its population is mostly of farmer immigrants from the populated Andean areas; major crops such as rice, cotton, oil palm have bean introduced for large scale production. A high proportion of the immigrants are consumers of common bean, *Phaseolus vulgaris*, hence, this crop has been tried for adaptation to these ecological conditions, unfortunately, without success. Root rot, foliar fungal and bacterial diseases are the limiting constraints for production of the common bean grown under these conditions. Although the common bean is the most important and widely dispersed crop species of the genus *Phaseolus* (Pachico, 1989), it is not well adapted to hot, humid conditions (Beebe and Pastor C. 1991). Nevertheless, among the five cultivated species of the genus, there is a particular germplasm group of lima bean which was domesticated and adapted to tropical areas of Central America and the Caribbean, besides, it has the same nutritive value of the common bean (Baudoin, 1991). Thus, this species has a great adaptation potential to areas with similar ecological conditions such as those of the Eastern Plains of Colombia. The GRU has assembled a germplasm collection of this species which at present has about 1,700 accessions.

Materials and Methods

1. In 1995, a trial nursery for evaluating adaptation of several species was carried out at the experimental station of the University of Los Llanos (Universidad de Los Llanos) (altitude: 420 m.a.s.l.). Main climatological characteristics are: mean temperature 27^{o}C, annual rainfall 3,200 m.m. and average relative humidity 80%. This trial included common bean, *Phaseolus vulgaris*, lima bean, *Phaseolus lunatus* and cowpea, *Vigna unguiculata* .

2. A nursery of 100 accessions of lima bean of tropical origin, selected from the world germplasm collection held at CIAT genebank, was assembled. A thesis work was carried out at the same experimental station, in order to evaluate the potential of the species, as well as to select promising accessions for either further breeding objectives and/or immediate adoption by farmers of the region.

Results

1. The preliminary nursery trial including common bean, cowpea and lima bean, demonstrated that common bean was a species highly susceptible to diseases under this hot and humid climate and, consequently, the yield results were very poor. On the other hand, cowpea and lima bean were well adapted and tolerated very well the disease pressure. The lima bean appeared almost as immune to all the fungi and bacteria attacking the common bean. Although cowpea appeared also as having good levels of resistance, lima bean is more appealing to consumers because of its resemblance to common bean grain types; furthermore, the cowpea under storage is very susceptible to insects.

2. The nursery of lima bean confirmed the excellent adaptation of this species to the ecological conditions of the Eastern Plains of Colombia. The great majority of the accessions showed both vegetative and reproductive adaptation with high levels of disease resistance and/or tolerance. A small subset of promising accessions was selected, which will be incorporated into a regional project to promote its cultivation. This project will be coordinated by the University of Los Llanos.

Outputs

1. Thesis work: Rodriguez, S., Bustamante, D. 1996. "Estudios de adaptación de 16 materiales genéticos de Frijol (*Phaseolus vulgaris*) a condiciones del Piedemonte Llanero". Villavicencio. Colombia.

2. Thesis work: Silva, N., Ortiz, C. 1997. "Evaluación preliminar de 114 materiales geneticos de frijol lima (*Phaseolus lunatus*), preseleccionados del banco mundial de germoplasma del CIAT, en condiciones agroecologicas de la granja de la Universidad de los Llanos". Villavicencio, Colombia.

3. A set of about 20 accessions of lima bean *Phaseolus lunatus*, selected as potential materials for further breeding work and/or adoption studies by small farmers.

Literature Cited

Baudoin, J. P. 1991. Systèmes culturaux, valeur alimentaire et utilisation dans le monde. In: La culture et l'amélioration de la légumineuse alimentaire *Phaseolus lunatus* L. en zones tropicales. Extraits de Bulletin des Recherches Agronomiques de Gembloux. 1989. 24(3). 263-296 p.

Beebe, S.E. and Pastor, C., M. 1991. Breeding for disease resistance. In: Schoonhoven, A. van; Voysest, O. (eds.). Common beans: research for crop improvement. Wallingford, UK, C.A.B. International; Cali, Colombia, Centro Internacional de Agricultura Tropical, 561-617 p.

Pachico, D. 1989. Trends in world common bean production. In: Schwartz, H.F.; Pastor Corrales, M.A. (eds.). Bean production problems in the tropics. Cali, Colombia, Centro Internacional de Agricultura Tropical, 1-8 p.

Activity – 18 Introduction and adaptation of popping beans, nuñas, to the Central Eastern Highlands of Colombia.

R. Hidalgo, INIAG, Universidad Tecnológica de Tunja, Colombia

Introduction

The Incas and their ancestors depended on boiled beans, for much of their nourishment. However, many of these people lived at high altitudes, where scarcity of fuel wood makes it difficult to cook ordinary dry beans. To overcome this limitant, they developed another form of seed preparation for consumption: toasting (National Research Council 1989). In fact, certain bean landraces varieties called nuñas (a quechua indigenuous name), or popping beans, burst when subjected to heat. These landraces were selected by the Incas in the highlands of Peru and Bolivia in the preceramic era (Debouck, 1989), perhaps due to the difficulty to cook beans by boiling. These beans are toasted during 5-10 minutes in a hot frying pan, usually coated with vegetable or animal fat; the resulting product is soft and floury. They may become a widely available, nutritious, tasty and fuel conserving food. Nuñas also posses a wide genetic variability (Tohme et al., 1995), which may be of value for breeding programs.

The Technological University of Tunja (Universidad Tecnológica de Tunja) is looking for alternative crops useful for small farmers of the highlands of Cundinamarca and Boyaca (Central States of Colombia). Nuñas beans are considered of special potential for these regions due to their climatic conditions which resemble those of Ecuador, Peru and Bolivia where these beans are cultivated (altitude higher than 2,500 m.a.s.l. and temperature below 15^{o}C). On the other hand, the Genetic Resources Unit has collected and assembled a unique germplasm collection of these beans, which is being evaluated for both their popping quality and their genetic variability.

Materials and Methods

A thesis work is underway at the Experimental Station of the University of Tunja (2,700 m.a.s.l. 13^{o}C), to explore the agronomical adaptation of this germplasm, as well as to evaluate the stability of the popping quality of the seed. The seed bursting property varies due to the genetics of the trait and also due to ecological changes. CIAT provided a set of 30 nuñas beans accessions from the world germplasm collection of *Phaseolus* beans held at CIAT genebank. These materials were selected for their particular good popping quality.

Outputs

The GRU has both an identified set of about 300 nuña beans accessions and a smaller subset of more than 30 accessions considered as of high popping quality. These materials are ready to be used by any project looking for alternative crops in highlands. Also, a thesis work at the University of Tunja with final results will be known by mid 1998.

Literature Cited

Debouck, D. G. 1989. Early beans (*Phaseolus vulgaris* L. and *P. lunatus* L.). Domesticated for their aesthetic value?. Annual Report Bean Improvement Cooperative. 32:62-63.

National Research Council. 1989. Lost crops of the Incas: little known plants of the Andes with promise for worldwide cultivation. National Academy Press. Washington, D.C. USA. p 113-179 p.

Tohme, J., O. Toro, J. Vargas and D. Debouck. 1995. Variability in Andean Nuña Common Beans (*Phaseolus vulgaris* fabaceae). Economic Botany. 49(1):78-95.

Activity - 19 Re-assembling the common bean landraces germplasm collection of the Southern Andes of Colombia.

R. Hidalgo, Universidad de Nariño, Colombia

Introduction

The Nariño region is located at the southern limit of Colombia with Ecuador and its geography corresponds mostly to the Andean highands type, although it also has a small hot, humid tropical area on the Pacific Coast. The Nariño state is characterized by having varied agricultural systems ranging from traditional small farm to large scale intensive agricultural production. Traditional small farm system predominates in the Andean region, where beans of the genus *Phaseolus*, have had a long history as an important edible crop. Because of the Inca influence a great number of bean landraces were introduced and adopted from Peru and Ecuador in precolombian times.

In the last three decades, such traditional systems suffered a drastic change due to the introduction of cereals, i.e. wheat and barley, displacing many of the bean landraces from the small farmers. However, recent global economic changes are now pulling out the cereal production from this region and, therefore, the governmental institutions are looking for a reinforcement of the previous traditional crops such as beans. Towards this aim a first step is to assemble the Nariño germplasm collection of beans, starting with the germplasm collected by CIAT in the mid 80's and conserved it the genebank and the germplasm dispersed in several institutions of that state.

Materials and Methods

1. A combination of the germplasm of bean landraces still existing in the University of Nariño, plus the accessions conserved at CIAT will form the basic working collection for further studies on evaluation to pests and diseases constraints, as well as to re-adoption studies by farmers.
2. Assessment of the above collection regarding its geographical distribution, in order to identify gaps what were traditional bean producing zones. With the latter information, further explorations will be carried out in an attempt to recover the still existing variability.

Outputs

A set of 164 accessions of common bean landraces from CIAT genebank in the project of the University of Nariño for the initiation of the evaluation studies.

5.5. Formation of Human Resources

As it can be seen in annex, a substantial part of the activities were also devoted to the formation of human resources, most in the form of supervision of research thesis and hands-on training.

5.6. Distribution of Germplasm

The following tables sum up the distribution of germplasm during 1997. One should note that in spite of reductions of commodity research in the centre, germplasm continues to be requested in fairly large amounts and kinds.

Table 14. Distribution of *Manihot* in vitro Germplasm (1997).

	Number of Accessions (No. of Samples)
	1997
Centre Staff in Host Contry	138(844)
Centre Staff in Other Countries	-
Other IARC's	-
NARS in Developing Countries	39(178)
NARS in Developed Countries	7(40)
Private Sector in Developing Countries	4(20)
Private Sector in Developed Countries	3(14)
Others (Includes Universities)	28(133)
TOTAL	219(1,229)
Total of requests	26

Table 15. Distribution of *Phaseolus* Germplasm (1997).

	Number of Accessions (No. of Requests)
	1997
Centre Staff in Host Contry	6,319(59)
Centre Staff in Other Countries	20(2)
Other IARC's	-
NARS in Developing Countries	2,698(10)
NARS in Developed Countries	29(3)
Private Sector in Developing Countries	-
Private Sector in Developed Countries	4(1)
Others (Includes Universities)	1,411(12)
TOTAL	10,481(87)

6. Prospects

With the operation of the new facility, seed viability monitoring for accessions stored in the past 24 years will tell us a lot about conditions of storage and conditions at harvest; we will also know the exact extent of the regeneration effort, apart from the needs because of germplasm distribution. It is hoped to conclude an agreement with ICA in 1998 that will allow us to process backlogs. Existing facilities shall be used to the fullest extent given multiplication needs. Infrastructure improvements shall be concentrated on upgrading of cold-stores and fixing of cleaning areas.

The SINGER project and overall the new systems in place for viability testing, accession multiplication and processsing, have significantly increased flows of germplasm and flows of activities. Such flows should be monitored, namely to keep our teams on focus, and to trace the use of our resources, the most valuable being time. Therefore, the full review of our documentation system shall be an important point for progress during 1998.

Research in conservation methods shall focus on improved protocols for seed drying and viability monitoring. Slow-growth in vitro has shown promise and should be pursued. Cryconservation of cassava shoot tips should now be progressively extended to the cassava core collection. Some protocols for the cryoconservation of tropical seed shall also be tested, as a step towards the value testing of such conservation methodology.

Research about conservation target shall continue with progresses on the *Phaseolus* monograph, together with its molecular taxonomy. A few experiments on gene flow in view of *in situ* conservation and implications for the management of transgenics shall also be conducted.

7. Annexes

7.1. PROJECT STAFF, Genetic Resources Unit

1. Conservation Group:

C. L. Guevara, Ph.D.	Specialist Germplasm Conservation
G. Mafla, Biologist	Research Assistant (In vitro Cassava)
H. Velasquez, Biologist	Technician (In vitro Cassava)
J. C. Roa, Biologist	Expert (In vitro Cassava)

2. Production Group:

R. Hidalgo, M.Sc.	Specialist Germplasm Production
O. Toro, Tech.	Expert (Bean Germplasm)
A. M. Torres, Biologist	Research Assistant *
I. R. Moreno, Ing.Agr.	Research Assistant
A. Ciprián, Tech.	Technician (Tropical Forages)

3. Service:

D. Debouck, Ph.D.	Head, Genetic Resources Unit
B. Pineda, M.Sc.	Research Associate (Seed Health Testing)
S. Balcázar, Bacteriologist	Lab. Technician (Seed Health Testing)
A. Valderrama, Biologist	Research Assistant (Seed Health Testing)
C. Ocampo, Biologist	Research Assistant (Electrophoresis Lab.)
S. Albarracín	Bilingual Secretary

4. Pathology

E. Alvarez, Ph.D.	Pathologist

5. Biotechnology Research Unit

W. Roca, Ph.D.	Head, Biotechnology Research Unit
R. Escobar, Biologist	Research Assistant (Biotechnology)

* On Sabbatical Leave, University of Reading, U.K .

7.2. List of publications by Project Staff 1997

A. In refereed journals:

1. Beebe, S., Toro Ch., O. González, A.V., Chácon, M.I. & D.G. Debouck. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Resources & Crop Evol. 44(1): 73-91

2. Escobar R.H., Mafla, G., Roca, W.M. 1997. A methodology for recovering cassava plants from shoot tips maintained in liquid nitrogen. Plant Cell Reports. 16: 474-478.

B. In non-refereed journals:

1. Singh S.P., Debouck D.G. & W. Roca. 1997. Successful interspecific hybridization between *Phaseolus vulgaris* L. and *P. costaricensis* Freytag & Debouck. Annu. Rept. Bean Improvement Coop. 40: 40-41.

C. In books:

1. Bonierbale, M., Guevara, C., Dixon, AGO., Ng. N.Q., Asiedu, R. and Ng. S.Y.O. Ng. 1997. Cassava, Chapter 1, in Biodiversity *in* Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centres (Dominic Fuccillo, Linda Sears and Paul Stapleton, editors). Cambridge University Press. Cambridge. UK, pages 1-20.

2. Hidalgo, R., Beebe, S. 1997. Beans, Chapter 12, in Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centres (Dominic Puccillo, Linda Sears and Paul Stapleton, editors). Cambridge University Press. Cambridge. UK, pages 141-158.

D. In proceedings:

1. Pineda, B. y M. del S. Balcazar. 1997. Estatus de sanidad de semilla de frijol en análisis de laboratorio. In: ASCOLFI Memorias XVIII Congreso, Palmira, Ciat, Julio 30 Agosto 2 /97. p 60.

7.3. List of Thesis Research supervised by Project Staff

1. Giraldo, M. 1997. Efecto del genotipo de yuca (*Manihot esculenta* Crantz) a la crioconservación en nitrógeno líquido. Tesis Biólogo. Universidad del Valle, Cali, Colombia. 70 p.

2. Klein, Bettina. 1997. Caracterización bioquímica de la colección de *Desmodium heterocarpum* var. o*valifolium* conservada en el CIAT. Tesis Bióloga. Hohenheim University, Stuttgart, Germany. 110 p.

3. Osorno, J. M. 1997. Estudio de la diversidad genética y distribución natural en una colección de *Centrosema macrocarpum* Benth (Fabaceae:Papilionoideae) mediante técnicas de electroforesis. Tesis Ing. Agr. Universidad Nacional de Colombia, Palmira, Colombia. 172 p.

4. Perea, I. 1997. Contribución al estudio de la diversidad del frijol Lima (*Phaseolus lunatus* L.) en Colombia. Tesis Bióloga. Universidad de Antioquia, Medellín, Colombia. 140 p.

5. Reyna, G. 1997. Efecto de la congelación rápida de ápices de yuca (*Manihot esculenta* Crantz) *Euphorbiaceae* a la crioconservación en nitrógeno líquido. Tesis Biólogo. Universidad del Valle, Cali, Colombia, 100 p.

7.4. List of Conferences and Scientific Communications presented by Project Staff

Debouck, Daniel G.

1. Palmira, Colombia, 30 July 1997, invited opening lecture for the 18th Congress of Colombian Phytopathology Association: "Biodiversidad y Evolución del Fríjol y de sus Patógenos".

2. Mexico, D.F., Mexico, 22 May 1997, invited opening lecture for the Comisión del Medio Ambiente del Senado de la República: "Convenio de Diversidad Biológica: repartir beneficios o asumir responsabilidades".

3. Caracas, Venezuela, 29 January 1997, invited opening lecture for the Sistema Económico Latinoamericano Workshop on Biodiversity: "Conservación y uso sostenible de los recursos genéticos".

7.5. List of National and International Courses with input from Project Staff

Debouck, D.G.

1. Curso Intensivo en Técnicas de Genética Molecular para el Inventario y la Caracterización de la Biodiversidad, Instituto von Humboldt - Smithsonian Institution - CIAT, 5 de Marzo de 1997, Palmira, Colombia.

2. Curso Internacional de Recursos Fitogenéticos, INIA-JICA, Tercer Curso: Conservación de Recursos Fitogenéticos, 6-12 de Marzo de 1997, Centro Regional de Investigaciones, La Platina, Santiago, Chile.

3. Curso de Postgrado, "Colección, conservación, y caraterización de los recursos genéticos de la Amazonia", 20-25 de Octubre de 1997, Universidad Nacional de Ucayali, Pucallpa, Perú.

Pineda, Benjamín

4. Curso de capacitación en semillas., Secretaria de Agricultura de Antioquia, Medellín, Colombia, Marzo 1997.

7.6. List of Trainees trained by Project Staff

Biotechnology Lab

1. De los Angeles Torres, María. Instituto de Investigaciones Fundamentales en Agricultura Tropical. La Habana, Cuba. 1997.

2. De Meerleer, Marianne. Hogeschool Gent, Bélgica. 1997.

Electrophoresis Lab

3. Márquez, Mónica. CENICAFE, Chinchina, Colombia. Julio - Agosto 1997.

4. Massie, Anabel. Universidad Nacional Salta (Escuela de Agronomía), Salta, Argentina. Abril 1997

5. Fernández, Juan Fernando. Instituto Von Humboldt-Universidad of Missouri (USA). Julio-Agosto 1997.

In vitro Lab

6. Elias, Marianne. Centro Nacional de la Investigación Científica, CNRS, Montpellier, Francia, 25-27 agosto 1997.

7. Espejo, Nohora. Jardín Botánico José Celestino Mutis. Bogotá, Colombia. 1997

8. Jiménez, José H. Escuela Superior Politécnica. Riobamba, Ecuador. 1997.

9. Osorio, Diego. Compañía In vitro plantas, Medellin, Colombia, Marzo 20 de 1997.

10. Samaniego, Edison, O. Escuela Superior Politécnica. Riobamba, Ecuador. 1997.

11. Sarmiento, Jorge. Jardín Botánico José Celestino Mutis. Bogotá, Colombia. 1997

Seed Health Lab

12. Jiménez, José H. Escuela Superior Politécnica. Riobamba, Ecuador. 1997.

13. Massie, Anabel. Universidad Nacional Salta (Escuela de Agronomía), Salta, Argentina. Abril 1997

14. Osorio, Diego. Compañía In vitro plantas, Medellin, Colombia, Marzo 20 de 1997.

15. Samaniego, Edison, O. Escuela Superior Politécnica. Riobamba, Ecuador. 1997.

16. Xioming, Wang. Institute of Crop Germplasm Resources. Chinese Academy of Agricultural Sciences. Beinjing, China. 1997.

Beans Germplasm Bank

17. Amaya, María del Socorro. Servicio Nacional de Aprendizaje SENA. Popayán, Colombia. 1997

18. Arango, Martha P. Servicio Nacional de Aprendizaje SENA. Popayán, Colombia. 1997

19. Cardozo, Carlos Ivan. Universidad Nacional de Colombia, Palmira, Colombia. 1997.

20. Garcia, Mario A. Universidad Nacional de Colombia, Palmira, Colombia 1997

21. Jiménez, José H. Escuela Superior Politécnica. Riobamba, Ecuador. 1997.

22. Juarez, Fanny. Universidad Nacional de Salta. Salta, Argentina. 1997

23. Katto, Clara Inés. Universidad Nacional de Colombia, Palmira, Colombia. 1997.

24. Ortiz, Carlos. Universidad de Los Llanos. Villavicencio, Colombia. 1997

25. Samaniego, Edison, O. Escuela Superior Politécnica, Riobamba, Ecuador. 1997.

26. Silva, Nubia. Universidad de Los Llanos. Villavicencio, Colombia. 1997

27. Zelener, Noga. Instituto Nacional de Tecnología Agropecuaría. Buenos Aires, Argentina. 1997

7.7. International Posters

1. Jones, P., Clavijo, L.A., Ocampo, C.H., Sawkins, M.C. 1997. Diversidad Genética en Stylosanthes: una Aplicación de GIS. Meeting of the Society of American Geographers, 20-23 July 1997, Arequipa, Perú.