CIAT Project on Saving Biodiversity SB-01
Report on Achievements and Progresses SB-01 Project for 1998

Table of Contents

1. Introduction

This report presents achievements, progresses and activities carried out for the maintenance and service to the FAO Designate collections held in trust in CIAT during 1998. It also presents progresses made for the filling of objectives of SB-01 CIAT Project on Agrobiodiversity.

The recommendations of two recent reviews ICER'95 and ICER'97 continue to have profound bearing on such activities, particularly for the Upgrading Plan that the GRU has initiated in 1996 to adjust the FAO Designate Collections to the International Standards for Genebanks (1994).

2. Project work breakdown structure and Objectives

The objectives of Project SB-01 as per the Mid-term Strategic Plan of CIAT (Figure 1) are to:
+ make the FAO Designate collections fully meeting the international standards for genebanks
+ make the FAO Designate collections and their pertinent information fully available
+ make the FAO Designate collections genetically and socially relevant
+ contribute to the formation of human resources in conservation methodologies in the region
+ provide scientific input in *in situ* conservation of farmers' landraces and wild relatives

Each objective area can be better visualized as an inter-related sub-project; there is thus a log-frame for each sub-project that is presented separately in Annex 1.

3. Project Logical Frame

Please refer to Annex 1.

4. Highlights

+ silver nitrate extends subculturing time *in vitro* up to six more months
+ encapsulation/ dehydration technique improves cryoconservation of cassava
+ cryoconservation protocols work for passion fruit seed
+ immunofluorescence quickly detects common bacterial blight in bean seeds
+ molecular evidence shows an Andean origin and a secondary gene pool of Lima bean

Please also refer to Annex 2.

5. Progress Report

5.1. Sub-Project 1: Objective: to make the FAO Designate Collections fully meeting the international standards for genebanks

Final Output: FAO Designate Collections complying with international standards for genebanks

The activities include:
+ the processing of backlogs of original materials, obtained either from explorations or from donations
+ the multiplication/ regeneration of materials already introduced in CIAT collections in the past (and declared to FAO as Designate Collections)
+ research on protocols to limit risks of genetic contamination, drift and genetic erosion
+ research on reliable and cost-effective conservation technologies
+ upgrading of GRU facilities

Output 1.1: germplasm received at CIAT introduced into the process

Activity 1.1: Processing backlogs through quarantine and first multiplication

This group of activities refers to the first multiplication of materials (Figure 2), obtained either from germplasm explorations or donations by NARS.

	Beans	**Forages**	**Total**
Awaiting processing	14,236	3,170	17,406
Processed in 1998	2,000 (203+1,797) (14%)	704 (22%)	2,704

A total of 203 bean accessions have been introduced, while an additional group of 1,797 accessions includes materials introduced in previous years and for which a minimum amount of seed for field multiplication is being sought (Activity contributing to **Output 1.2**). The milestone was 1,000 accessions in 1998 (203 + 704 = 906). Given the length of the juvenile phase in some forage materials, only a fraction has been completely multiplied already. During this process, 184 forage accessions did not germinate and could be considered as 'lost accessions' due to poor viability at collecting and/ or poor storage since.

Output 1.3: FAO Designate Collections regenerated (thus satisfying criteria of amounts and viability)

Activity 1.3: Regeneration of FAO Designate Collections

This group of activities specifically refers to the regeneration (= field or glass-house multiplication) of accessions already multiplied in the past, and for which amounts of seed are low because of generous past distribution, and/ or for which viability is low. It is not relevant for the cassava collection maintained *in vitro*.

	Beans	**Forages**	**Total**
Awaiting regeneration	20,770	7,835	28,605
Processed in 1998	2,101 (10.1%)	761 (9.7%)	2,862 (10%)

The discontinuing of operations at the Tenerife substation in June 1998 because of severe security problems prevents us to reach the target of 4,000 accessions as per the 1998 work plan. The milestone for 1998 was 6,000 accessions as indicated to the Programme Committee in November 1996 (4,000 beans + 2,000 forages). During this process, 158 forage accessions could be considered as 'lost accessions' as they did not germinate due to poor storage in the past.

The regeneration process also includes two other groups of activities: viability testing and final packing (**Output 1.4.** and Activity 1.4). The following tables indicate flows of materials during 1998.

Viability Testing for *Phaseolus* and Forages during 1998

	PHASEOLUS			**FORAGES**		
	Germination %	*P.vulgaris*	*P. lunatus*	Germination %	LEGUMINOSAE # accessions (# species)	POACEAE # accessions (# species
Already stored materials	1-50	-	-	1-50	102	2
	51-84	-	-	51-84	166	-
	85-100	-	-	85-100	167	1
TOTAL		0	0		435 (70)	3 (3)
Recently multiplied materials	1-50	10	0	1-50	-	-
	51-84	94	21	51-84	13	-
	85-100	1,609	42	85-100	107	2
TOTAL		1,713	63		120 (28)	2(2)

The milestone for 1998 was 8,000 accessions (6,000, those processed through regeneration and 2,000, those of already stored materials).

Final storage and packing of accessions of *Phaseolus* processed during 1998

	P. vulgaris		*P. lunatus*	*P. acutifolius*
	TEN 1995 B	TEN 1996 A	TEN95 B	PAL 96 B
LONG TERM (Base + Duplicates + Repatriation) + SHORT TERM (Distribution-Monitoring)	707	910	50	133
SHORT TERM only (Distribution-Monitoring)	202	82	52	12
TOTAL	934[*1]	993[*2]	103[*3]	159[*4]

[*1] (25 plots did not yield any seed)
[*2] (1 plots did not yield any seed)
[*3] (1 plots did not yield any seed)
[*4] (14 plots did not yield any seed)

Final storage and packing of accessions of Forages processed during 1998

	Accessions previously multiplied with enough seed for packing		Accessions recently multiplied with enough seed for packing	
	LEGUMES	POACEAE	LEGUMES	POACEAE
LONG TERM (Base + Duplicates + Repatriation) + SHORT TERM (Distribution-Monitoring)	167	1	107	2
SHORT TERM only (Distribution-Monitoring)	268	2	13	2
TOTAL	435	3	120	4

The milestone for 1998 was 6,000 accesssions (that is the same number as the one regenerated; 4,000 beans and 2,000 forages).

Output 1.5: Alternate liable and affordable conservation methodologies developed

Activity 1.5.1: Research on protocols to limit risks of genetic contamination, drift and genetic erosion

This activity is carried out in cooperation with the National Plant Genetic Resources Programme of CORPOICA, Colombia, and is part of the thesis research of a biology student of Universidad del Valle, Colombia.

1.5.1. Management practices to limit genetic drift and erosion in wild bean accessions

Felix A. Guzman, Orlando Toro, César H. Ocampo and D. G. Debouck

Introduction

El frijol común, especie de mayor consumo humano de este género, es anual, su sistema de reproducción predominante es la autopolinización con menos del 5% de cruzamiento externo (Gepts and Debouck, 1991). Una de las principales funciones de los bancos de germoplasma es mantener la variabilidad genética de las especies, para evitar pérdida de la diversidad genética por erosión o deriva genética, por urbanización, por destrucción del hábitat silvestre y por tecnificación de la agricultura (Frankel and Brown, 1984). Sin embargo, los procedimientos de multiplicación de semillas realizados en los bancos de germoplasma pueden disminuir la variabilidad genética del material que se desea conservar (Roos, 1988). Con este estudio se propone evaluar en tres localidades (Popayán, Tenerife y CIAT-Palmira), durante dos generaciones, la producción de semillas en tres poblaciones de frijol común silvestre conservadas en el banco de germoplasma del CIAT, originarias de Guatemala, Norte del Perú y Noroeste Argentino y por medio de marcadores bioquímicos (enzimas del metabolismo-alelos raros y faseolinas) y moleculares de DNA (AFLPs), se analizara su diversidad genética y se estudiará la deriva genética como causa de cambios en el material conservado.

Results

Como una primera etapa, se determinó las faseolinas (patrón y su frecuencias) en las tres poblaciones silvestres a usar en el estudio, asi se encontró para la población de Guatemala, la faseolina M16 en 60 individuos analizados, en la población del Norte del Perú se encontró la faseolina I, también en 60 individuos analizados y en la población del Noroste Argentino, analizando también 60 individuos encontramos las faseolinas T, C, J4 y H1. También con estas poblaciones, ya se ha logrado encontrar buena actividad con 10 sistemas isoenzimáticos (aunque posteriormente se hará con al menos 20 sistemas isoenzimáticos), algunos de ellos ya han mostrado loci polimorficos a nivel intrapoblacional. Estos 10 sistemas isoenzimáticos que han mostrado buena actividad son: EST, GOT, ACP, PRX, DIA , PGM, 6-PGDH, IDH, MDH, y PGI.

Prospects

Con los alelos raros de los loci isoenzimáticos y polimorficos, se cuantificará y se modelizará el flujo génico en estas poblaciones y sus descendencias, ya que el estudio de la deriva genética debe hacerse a través de muchas generaciones, sin embargo el tiempo de duración de este trabajo sólo permite estudiar dos generaciones, por lo tanto se justifica el uso de una modelización computarizada ya que ésta permite simular un buen número de generaciones para obtener información adicional, haciendo que las conclusiones de este estudio tengan mayor validez. Con estas herramientas se pretende obtener conocimiento que permita desarrollar estrategias de manejo que aseguren el mantenimiento de la diversidad genética, inicialmente colectada, durante maniobras sucesivas de regeneración en el banco de germoplasma.

Literature cited.

Frankel, O. H. and Brown, A. H. 1984. "Plant genetics resources today: a critical appraisal" In: "Crop Genetic resources: conservation and evaluation", J. H. W. Holden; J. T. Williams (eds.), International Board for Plant Genetic resources (IBPGR). United Kingdom, pp. 249-257.

Gepts, P., and Debouck, D. G. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.) In: "Common beans: research for crop improvement", van Schoonhoven, A. and Voysest, O. (eds.), Commonwealth Agricultural Bureaux International, Wallingford, United Kingdom, pp. 7-53.

Roos, E. E., 1988. *Phaseolus* seed storage methodologies. In: "Genetic Resources of *Phaseolus* Beans", P. Gepts (ed.), Kluwer Academic Publishers, Dordrecht, Holland, pp.31-49.

(same overall output 1.5)

Activity 1.5.2: Research on reliable and cost-effective conservation technologies

Three research activities are under way for improving the liability and cost-effectiveness of our conservation methodologies:

+ slow growth procedures for cassava maintained in vitro
+ cryoconservation of cassava shoot tips
+ cryoconservation of seed material

1.5.2. Effect of silver nitrate on growth of cassava (*Manihot esculenta*) in vitro.

Graciela Mafla

Introducción

La conservación in vitro a un plazo más largo, minimizando la tasa de crecimiento e incrementando el periodo de conservación, es necesaria ya que permite reducir los subcultivos además de que minimiza el riesgo sobre la estabilidad genética de los clones almacenados con esta técnica. Según los avances del trabajo anterior (Mafla, et al.1997) con reguladores osmóticos e inhibidores de etileno (Etapa I), revelaron como el nitrato de plata ($AgNO_3$), inhibidor de etileno, produjo unos efectos benéficos sobre el lento crecimiento invitro de la yuca. En el presente trabajo (Etapa II) se seleccionaron dos de las concentraciones de $AgNO_3$ (las cuales mostraron una mejor respuesta en la Etapa I) y se evaluaron un mayor número de variedades para observar el efecto que éste compuesto tiene sobre el germoplasma invitro de yuca.

Materiales y Métodos

Se realizó una selección de 44 variedades *invitro* correspondientes al Core Collection, cuyos promedios de conservación oscilan entre 8-18 meses (Tabla 1). El medio basal 8S (MS, 0.088$_U$M BAP, 0.29 $_U$M GA_3, 0.054 $_U$M NAA, 2.96 $_U$M thiamine-Hcl, 554.93 $_U$M M-inositol, 2% Sucrosa, 0.7% agar) fué utilizado como control (Roca et al. 1984). Los tratamientos consistieron en la adición al 8S de 58.85 $_U$M y 70.62 $_U$M de $AgNO_3$. Los diferentes medios se sirvieron en tubos de ensayo de 25x150mm cubiertos con papel aluminio y autoclavados a 115 lb/seg por 12 minutos para ser esterilizados.

Se utilizaron los nudos como tipo de explante para éste ensayo, estos se tomaron de la parte media de la planta invitro. Un diseño completamente al azar fué utilizado, 44 variedades x 4 replicaciones x 3 tratamientos incluyendo los controles. Las condiciones del cuarto de conservación permanecieron estables(23-24 ^{0}C, 12 –h fotoperiodo, 1000 lux).

Se presentarán los resultados de la evaluación realizada después de 18 meses (Etapa I) y 6 meses de conservación (Etapa II) y se hará en términos de longitud de tallo.

Tabla 1. Comportamiento in vitro de las variedades seleccionadas del Core Collection.

Código	Variedad	Promedio de Conservación	No. Subcultivos	Fecha entrada a Conservación in vitro
V1	ARG 2	8.50	11	1984
V2	BRA 337	10.28	12	1983
V3	COL 2056	7.54	8	1988
V4	NGA 16	16.73	5	1988
V5	VEN 329A	18.45		1994
V6	CM 2177-2	7.53	9	1986
V7	ARG 11	11.00	9	1984
V8	BRA 894	11.63	7	1988
V9	BRA 69	13.69	11	1981
V10	PAR 110	14.64	7	1986
V11	NGA 5	18.19	5	1988
V12	PRT 19	11.54	11	1983
V13	BRA 885	12.48	7	1988
V14	BRA 12	12.87	12	1978
V15	MAL 2	9.32	13	1981
V16	NGA 1	13.13	8	1987
V17	COL 1468	9.76	14	1981
V18	BRA 900	13.53	6	1988
V19	BRA 383	10.80	11	1983
V20	MEX 59	14.58	8	1982
V21	ECU 82	9.16	13	1981
V22	CUB 74	10.30	13	1981
V23	COL 22	10.03	16	1979
V24	MMC 1	8.76	13	1982
V25	CG 11410-	12.07	6	1989
V26	CM 523-7	9.76	13	1980
V27	NGA 2	12.19	8	1987
V28	CM 3306-4	13.30	7	1987
V29	PAN 51	10.64	12	1982
V30	COL 1505	9.19	12	1980
V31	COL 1939	10.87	8	1988
V32	COL 6082-	12.16	3	1994
V33	BRA 931	10.31	3	1993
V34	COL 2215	10.47	10	1985
V35	BRA 632	11.72	10	1984
V36	IND 33	11.52	9	1986
V37	BRA 881	12.92	7	1988
V38	BRA 605	10.42	12	1984
V39	ECU 41	12.77	6	1989
V40	BRA 886	12.22	7	1988
V41	SM 484-2	11.84	4	1992
V42	TAI 1	11.35	11	1981
V 43	BOL 3	12.07	5	1990
V44	CM 4063-6	11.38	4	1992

Resultados

La selección de las dos concentraciones de $AgNO_3$ (58.85 $_{U}$M y 70.62 $_{U}$M) utilizadas en éste ensayo fueron retomadas del trabajo previo sobre reguladores osmóticos e inhibidores del etileno con seis variedades tambien seleccionadas del Core Collection.Esta selección se hizo cuando el experimento llevaba doce meses de conservación y en ese momento presentaban las mejores caracteristicas.

A los 18 meses de conservación (Etapa I) se puede observar como las seis variedades mantienen una tasa de crecimiento menor que el control en las cinco concentraciones evaluadas , siendo los mejores resultados en las concentraciones de 70.62 $_{U}$M y 82.40 $_{U}$M (Figura 1). Se ha observado

con $AgNO_3$ una menor defoliación con relación al control y además una proliferación de yemas debido al rompimiento de la dominancia apical. Con relación al porcentaje de supervivencia se encontró que fué mayor del 80% con cuatro de las cinco concentraciones evaluadas y menor del 20% en el control, esto significa que se ha logrado incrementar el periodo de conservación especialmente en aquellas variedades de menos de 8 meses (Figure 2).

Figure 1. Effect of silver nitrate oninvitro growth of six varieties of cassava after 18 months of conservation.

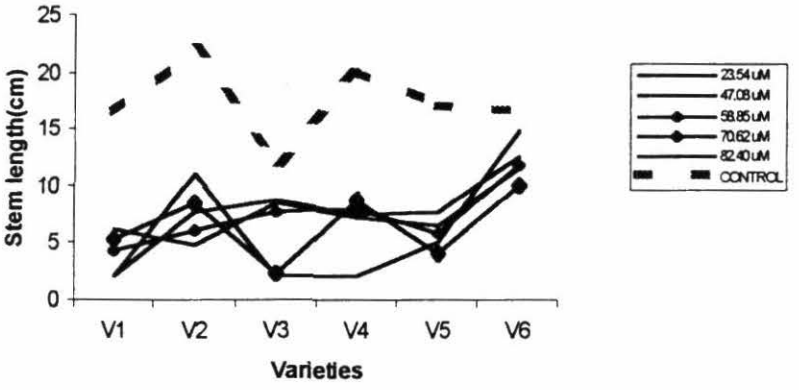
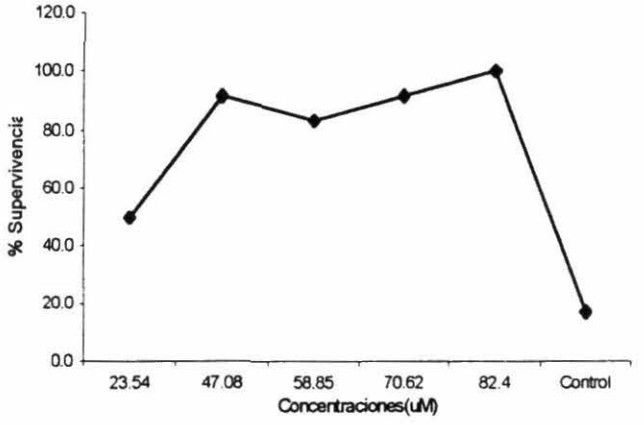
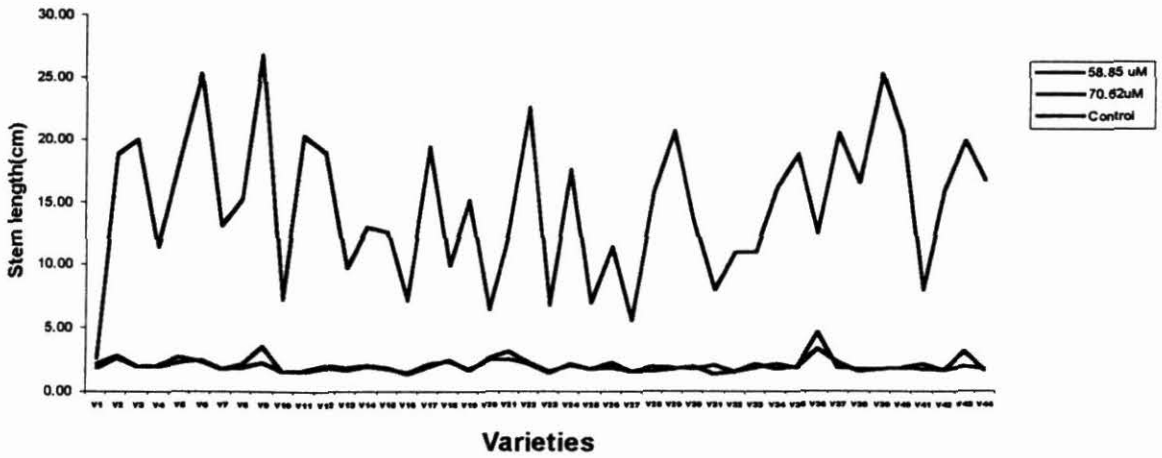


Figure 2. Porcentaje de supervivencia de variedades de yuca a los 18 meses de conservación con Nitrato de plata.



La adición de $AgNO_3$ (58.85 uM y 70.62 uM) al medio de cultivo presentó un efecto sobre el crecimiento in vitro de la yuca en las 44 variedades estudiadas (Etapa II), como se puede observar al sexto mes de conservación (Figura 3). Los valores de crecimiento basados en la longitud del tallo en presencia de $AgNO_3$ (1.48- 4.55 cm) fue muy reducido en comparación al control el cual presenta una alta elongación (2.63- 26.75 cm) con relación al inhibidor del etileno. Con estos resultados preliminares nos podemos dar cuenta como el $AgNO_3$ juega un papel importante en la reducción del crecimiento y probablemente no afecta la interacción con el genotipo.

Figure 3 . Effect of silver nitrate on invitro growth of cassava after 6 months of conservation.



Perspectivas

Confirmar la viabilidad de los cultivos sometidos a estos tratamientos (capacidad de micropropagación).

Evaluar la estabilidad genética utilizando marcadores moleculares.

Literatura citada

Roca WM; Rodriguez JA; Mafla G; Roa JC. 1984. Procedures for recovering cassava clones distributed in vitro. CIAT, Cali, Colombia. 8p.

Mafla G; Roa JC; Guevara C. 1997. In vitro growth control in cassava, using osmotic regulators and ethylene inhibitors. CIAT, Cali, Colombia. 65-74.

1.5.3. Cryopreservation of cassava shoot tips: encapsulation-dehydration technique.

Roosevelt H. Escobar, M. Paola Rangel, Willy M. Roca

Background

Cassava shoot tips were successfully cryopreserved using an encapsulation/dehydration technique. The technique (Palacio, 1997) allows direct placing of shoots into liquid nitrogen, preventing the use of expensive programmable freezing equipment and opening the possibility of large-scale conservation methodology at CIAT (Escobar *et al.* 1998). In order to optimize the factors controlling the rates of plant recovery after freezing, cytokinins, mixtures of gelling agents and supplementation of beads were tested.

Results

Tables 1,2 and 3 sum up results obtained in 1997-98.

Table 1: Effect of growth regulators on viability and shoot recovery after freezing of cassava encapsulated-dehydrated shoot tips.

Cultivar	Growth regulator (mg/l)	% Viability	% Shoot recovery
M Col 1468	BKZ (0.33 each)	90	25
	K1	71.4	4.8
	2iPKZ (0.17 each)	90	45
	2iPKZ (0.33 each)	80	20
	2iPKZ (0.5 each)	90	20
M Bra 507	BKZ (0.33 each)	100	35
	K1	80	40
	2iPKZ (0.17 each)	95	65
	2iPKZ (0.33 each)	95	50
	2iPKZ (0.5 each)	90	10
M Ven 232	BKZ (0.33 each)	84.2	0
	K1	90	65
	2iPKZ (0.17 each)	88.8	5.6
	2iPKZ (0.33 each)	89.5	10.5
	2iPKZ (0.5 each)	89.5	0
M Col 22	BKZ (0.33 each)	100	45
	K1	100	47.4
	2iPKZ (0.17 each)	95.4	72.8
	2iPKZ (0.33 each)	93.7	68
	2iPKZ (0.5 each)	100	52.6

B, K, Z and 2iP are all growth regulators

Plant regeneration rates could be increased through the inclusion of benzyladenine (B), kinetine (K), zeatin (Z) and 2iP. We previously found that the type and concentration of cytokinin could improve shoot response after freezing (BRU-Annual Report 1995). K1 and 2iPKZ (at 0.17-0.33 mg/l each) had the best effect on shoot recovery after freezing (Table 1). Two recalcitrant cultivars, MVen 232 and MCol 1468, improved their response with this treatment. Viability after freezing is more consistent with the encapsulation-dehydration than with programmed freezing; the average viability value per cultivar was more than 80%. This gives us the opportunity to recover more shoots per treatment. We have observed that when using beads with growth regulator response of frozen shoot improved.

Table 2. Effect of agar brand and consistency of recovery medium on viability and plant recovery from frozen shoot tips.

Cultivar	Consistency of medium	Agar relation ®Duchefa:®Phytagel	% Viability	% Shoot recovery
M Col 1468	Solid (0.45%)	3:1	89.4	58
	Semisolid (0.35%)	3:1	88.9	50
	Control (0.35%)	1:0	100	56.2
M Bra 507	Solid (0.45%)	3:1	100	66.7
	Semisolid (0.35%)	3:1	100	38.9
	Control (0.35%)	1:0	100	52.6

Some agars contain inhibitory substances which may prevent morphogenesis in certain cultures, rate of growth can be slow, toxic exudates from explants do not diffuse away quickly. Hyperhydracity could be avoided using mixtures of Gel-rite and Agar. We have found that a relation 3:1 with the K1 medium shows improves shoot recovery from frozen cassava shoot tips (Table 2).

Table 3. Effect of bead supplementation on the response of cassava shoot tips after freezing in L.N.

Cultivar	Bead supplementation	% Viability	% Shoot recovery
MCol 1468	4E	83.3	50
	K1	80	70
	BKZ (0.33 each)	100	90
	Without	81.8	63.6
MBra 507	4E	90	50
	K1	63.6	45.4
	BKZ (0.33 each)	54.5	9.1
	Without	66.6	41.7
MVen 232	4E	100	81.8
	K1	90.9	90.9
	BKZ (0.33 each)	91.6	83.3
	Without	66.7	66.7
MCol 22	4E	91.7	75
	K1	75	66.7
	BKZ (0.33 each)	75	50
	Without	66.7	50

It seems that beads are not so permeable to media components at the beginning of culture; tissue could starve. Supplementation of beads with media components could support the initial growth till shoot emerges. We have found that medium K1 is more effective especially after freezing MVen 232 and MCol 1468 (table 3).

Conclusion and future plans

We found that adjusting the shoot recovery steps (media and conditions) the percentage of shoot recovery after freezing can be increased. Encapsulation/dehydration could be a simple way to introduce cassava collection liquid nitrogen. We will test this improved methodology on a subset of the cassava core collection.

References

BRU-Annual Report. CIAT. 1995

Escobar R.H, Palacio J.D., Rangel M.P and W. M. Roca.1998. Crioconservación de ápices de yuca mediante encapsulación-deshidratación. *In:* III Latin American meeting on plant biotechnology REDBIO'98 La Habana-Cuba June 1-5.

Palacio J.D. 1997. Crioconservación de ápices de yuca (*Manihot* esculenta Cranz) utilizando la técnica de encapsulación-deshidratación. Tesis CIAT.

1.5.4. Exploration of techniques towards the cryo-preservation of seed materials of tropical origin

Claudia L. Guevara , John Alexander Ospina

Background

In Nov 1997, COLCIENCIAS approved a scholarship for a cooperative project towards the definition or fine tuning of cryopreservation protocols for seed germplasm of tropical origin.

Since the experimental approaches could be highly related to the seed type, the materials were selected as follows. For the orthodox type: *Phaseolus vulgaris, P. coccineus, P. filiformis, Brachiaria decumbens* and *Arachis pintoi*. For intermediate type: *Passiflora molissima, P. india, P. ligularis* and *P. edulis*; within the recalcitrant type, *Erythrina edulis*.

The reasons to pursue this work were, among others:
Build up local and regional strength in this conservation technique while attempting to produce models for any other seed species.
Once a proper protocol has been identified, conservation at -196 °C constitutes the ultimate approach for attaining the maximum seed longevity. Although it has not been fully demonstrated that temperatures of -40 °C, -70 °C or -196 °C are significant better than -20 °C in terms of physiological preservation, estimates suggest the colder the storage the longer the potential storage time. (Stanwood, 1985; Stanwood and Sowa, 1995). Nevertheless, the potential advantage of liquid nitrogen (LN_2) as storage medium would be preferred over mechanical refrigeration systems based on that all metabolism would be essentially stopped virtually eliminating the regeneration cost of valuable entries within a seed species.
Also, as not much direct measurements can be actually done to compare the relative benefits of two already defined safe seed conservation methods, namely at -20 °C vs -196 °C, much can be done to identify the factors involved on the definition of safe protocols.
This year, the major emphasis was on the group of Passiflorae mostly for the continued interest of CIRAD-IPGRI on this group of species.

Approaches

Orthodox seed that can be dried without damaging and generally store well, appears to be adaptable to cryopreservation with some limitations (Stanwood and Bass, 1981). For those type of species, the moisture level is easily and reversibly manipulated by the RH (Vertucci and Farrant, 1995). In addition to this, control of other factors that produce limitants like shattering is also seeked. For recalcitrants, since those species can not tolerate significant degree of desiccation, the approach will be the drying and isolation of axes (Dumet *et al.*, 1998; Normah *et al.*, 1994). Thus, desinfecting and invitro culture conditions for regrowth has to be defined in advance.
For intermediate, the approach, similar to the orthodox, it will be to identificate the optimum range for attaining cryoexposure.

Results

Activities during this first year of the project, consisted in getting familiar with the morphology types of the seed in consideration; germination requirements; degree of dormancy present in the samples; definition of patterns of viability with 2,3,5- Triphenyl-2H-tetrazolium chloride TTC and definition of drying environments and time of exposure: air dry containers with silica gel, drying rooms.

Prospects

With the information produced, precisely defined cryo-protocol for the four *Passiflora* species will be produced. It will be fine tuning of the technique for the orthodox species and initiation the research with zygotic embryos for *Erythrina edulis*, uniformizing seed sizes and maturity stages and previous definition of in vitro culture conditions.

References

Dumet, D., P. Berjak, F. Engelmann, 1998. Cryopreservation of zygotic and somatic embryos of tropical species producing recalcitrant or intermediate seeds. *In:* M. K. Razdan (ed.) Conservation of plant genetic resources in vitro., vol. 1.

Normah, M.N., B.M. Reed, X. Yu, 1994 Seed storage and cryoexposure behavior in hazelnut (*Corylus avellana* L.). Cryo-letters. **15:** 315-322.

Stanwood, P.C., 1985 Cryopreservation of seed germplasm for genetic conservation. *In:* K. K. Kartha (ed.) Cryopreservation of Plant Cells and Organs. CRC Press, Boca Raton, FL. pp. 199-226.

Stanwood, P.C., L.N. Bass, 1981 Seed germplasm preservation using liquid nitrogen. Seed Sci. Technol. **9:** 423-437.

Stanwood, P.C., S. Sowa, 1995 Evaluation of onion (*Allium cepa*) seed after 10 years of storage at 5, -18, and –196°C. Crop Sci. **35:** 852-856.

Vertucci, C.W., J.M. Farrant, 1995 Acquisition and loss of desiccation tolerance. *In:* J. Kigel and G. Galili (eds.) Seed development and germination. Marcel Dekker, Inc., New York. pp. 237-271.

Outputs 1.6: Improved facilities for germplasm conservation, improved performances, money saved

Activity 1.6: Upgrading of CIAT facilities

In 1998 improvements to GRU facilities include:
+ the fusion of two old cold stores into one and the fixing of new insulation boards
+ the conversion of one room into a packing room with lower air moisture
+ the conversion, at the edge separating the main building from the field area, of one room into a cleaning room in order to have only cleaned seed germplasm in the main building
+ better facilities for the ICA Plant Quarantine Office in CIAT

The fixing of the cold stores has had a delayed start, but is now progressing as planned. The work for the packing room is finished; a better air drying system has been listed in the 1999 request to the Capital Fund. The cleaning room has not yet been started. The work for the facilities for the ICA Plant Quarantine is completed. If requests to the Capital Fund are fully approved in 1999, the upgrading of facilities should be completed within the same year.

5.2. Sub-Project 2: Objective: to make the FAO Designate Collections and their pertinent information fully available

For an international center like CIAT, one germplasm that has not been checked for seed borne diseases is not available. The following group of activities also links closely with the filling of Objective 1: appropriate plant health controls are part of international standards of genebanks.

Outputs 2.1: safe germplasm ready for distribution, protocols for disease indexing improved

Activity 2.1.1: Germplasm health control

Seed health assessment is one of the most important activities to maintain germplasm phytosanitary standards in a germplasm bank for conservation and distribution of accessions. When germplasm is exchanged internationally, there is a risk of accidental introduction of plant pathogens along with seeds or vegetative plant parts. In order to manage this risk the Seed Health Laboratory (SHL) applies indexing procedures to ensure that distributed materials are free of pathogens of quarantine importance.

Materials and Methods

SHL use internationally accepted methodologies after recommendations by CIAT's pathologists and virologists to intercept seed-borne pathogens as fungi, bacteria and viruses according with those pathogens recorded in seed production areas (Annual Report 1997). In seed health testing four main groups of activities are carried out: 1) sample reception and registration, 2) preparation of working samples, 3) preparatory work to put the working samples into final format for testing, and 4) analysis. Testing for fungi includes two incubation methods: blotter test and agar test plate under high levels of humidity and optimum light and temperature conditions. The final step is the examination of incubated seeds on blotters or agar.

Seed borne bacteria (*Xanthomonas campestris* pv *phaseoli* and *Pseudomonas syringae* pv *phaseolicola* in beans, and *Pseudomonas* spp, in tropical pastures) are tested using dilution and plating on semiselective culture media such as MXP or King B, in addition to immunoprecipitation test with specific antisera or pathogenicity tests. Testing *Curtobacterium flaccumfasciens* pv. *flaccumfasciens* in tropical pastures (*Zornia* spp) is achieved by subculturing on YDCA, by Gram staining and incubation under high temperature (36-37° C). Testing for seed borne viruses includes serological methods such as ELISA, using monoclonal or polyclonal antisera and/ or seedling-symptom test.

Results

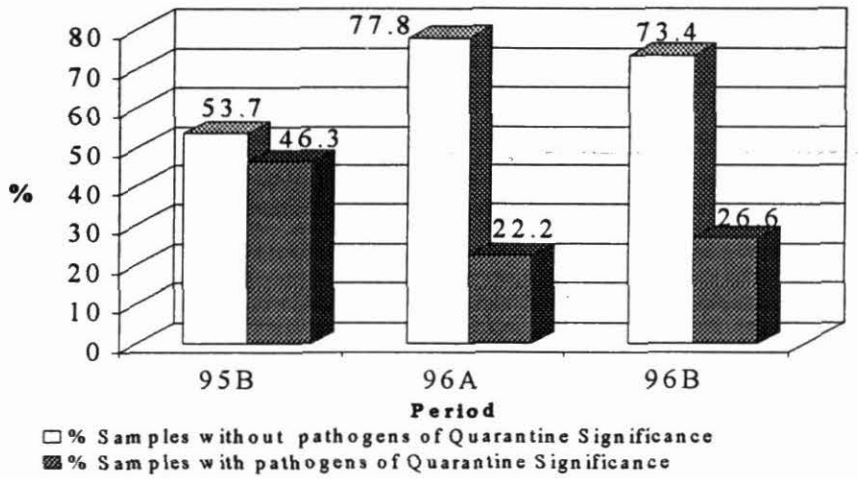
A total of 433 of bean seed samples from germplam characterization project were tested during 1998. A total of 75.3 % of those samples did not have pathogens of quarantine significance. In affected materials the fungi *Macrophomina phaseoli* (2.1%) and *Rhizoctonia solani* (1.2 %) were more frequent. Some viruses such as BCMV (15.9 %) and SBMV (5.3%) were also detected. For tropical forage legumes as *Arachis pintoi* 10 samples were tested and only one showed the fungi *Rhizoctonia solani.*

In 142 seed samples of *Phaseolus lunatus* from GRU, 47 accesions did not show pathogens of quarantine importance. The affected materials showed *Macrophomina phaseoli* (40.4 %), *Rhizoctonia solani* (3%) , of *Phoma exigua* (7 %), and *Phomopsis sp* (0.7%).

Analysis realized for 1,089 seed samples of *Phaseolus vulgaris* from GRU showed 74.1% of samples without pathogens of quarantine significance. In affected materials the fungal infections were very low (0.9%), although it was possible detect in some samples the presence of *Colletotrichum lindemuthianum, Rhizoctonia solani* and *Phoma exigua.* No seed borne infection by *Xanthomonas campestris* pv *phaseoli* was detected, but indeed by *Pseudomonas syringae* pv *phaseolicola* (0.1%); BSMV (7.1 %) and BCMV (17.4%) were the viruses detected in seeds.

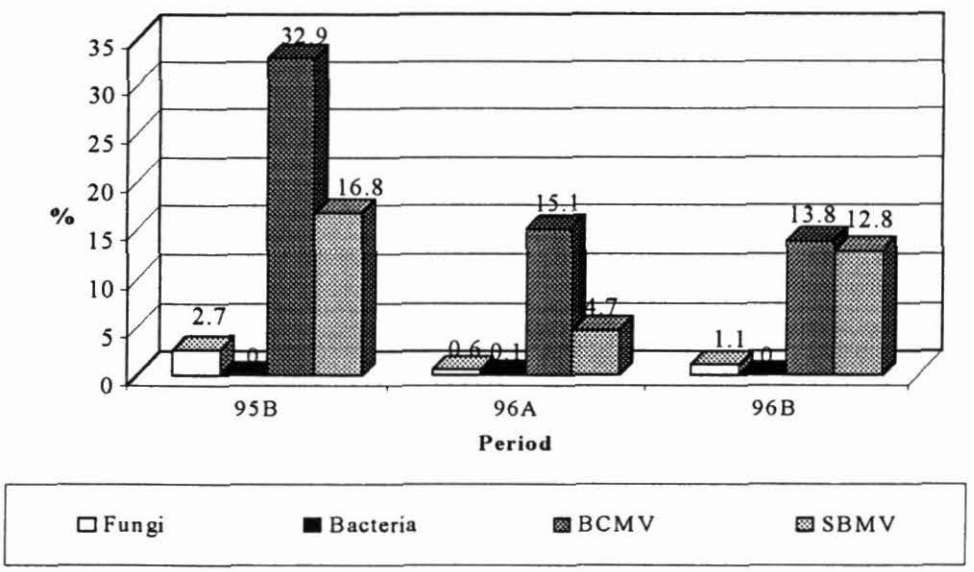
Seed samples produced during 95B, 96A, and 96B semester were obtained from Tenerife multiplication plots for analysis. Its health status, analyzing each period, showed 53.7% (95B), 77.8% (96A) and 73.4% (96B) samples without pathogens of quarantine importance (Figure 1).

Figure 1. Seed health status of *Phaseolus vulgaris* germplasm from Tenerife during three production periods.



In affected materials fungal and bacterial infections were low in all periods while viral infections were relatively high, particularly BCMV reaching percentages as 32.9% during 95B semester. Looking at the general trend of health status, one can tentatively conclude that seeds recently produced have better status (Figure2).

Figure 2. Percentage of *Phaseolus vulgaris* seed samples from Tenerife infected with pathogens in three production periods.



References

CIAT. 1997.Annual Report 1997, CIAT project on saving Biodiversity SB-01, Genetic Resources Unit Report on Activities, p. 14-18.

(same overall output 2.1)

Activity 2.1.2: Disease indexing of cassava germplasm

La determinación (indización) y erradicación de virus a los materiales almacenados en el Banco de Germoplasma de *Manihot esculenta* Crantz, promueve el mantenimiento de una colección fitosanitariamente limpia de virus, facilitando el intercambio internacional y regional de acuerdo con los reglamentos mundiales fitosanitarios para intercambio de material vegetal.

Materials and methods

Los materiales de yuca evaluados en el laboratorio de Sanidad de Semillas, provienen del Banco de Germoplasma *in vitro* y de campo de *Manihot esculenta* Crantz. La detección de virus se realiza mediante la aplicación de técnicas inmunoenzimáticas (ELISA) para los virus de Mosaico Común (CCMV) y Virus X (CsXV), técnicas moleculares (PCR) para el Virus del Mosaico de la Nervadura (CVMV) y técnicas biológicas (injerto) para la detección del agente causante de la enfermedad de Cuero de Sapo.

El proceso para determinar la limpieza y disponibilidad de un material para intercambio internacional y regional, involucra una serie de pasos como, recolección de hojas para la realización de las pruebas de ELISA, extracción de ADN para la realización del PCR aplicación de termoterapia y extracción de ápices, siembra en medio adecuado (17N) para el enraizamiento y posterior siembra en invernadero para engrosamiento de estaca, elaboración de injertos de estaca no lignificada con enraizamiento en agua, mantenimiento de los injertos en un cuarto de crecimiento con las condiciones de temperatura y fotoperíodo adecuadas para la manifestación de síntomas en el clon indicador (MCol 2063). Los materiales que han resultado positivos para alguna prueba, son de nuevo colocados en termoterapia y extracción de meristemos para su posterior evaluación viral.

Results

Actualmente contamos con 602 materiales disponibles fitosanitariamente para el intercambio internacional y regional. Un total de 2,210 materiales de yuca fueron evaluados por ELISA durante 1998. El 3.53.91% de los materiales resultaron positivos para CCMV y el 11,541% para CsXV. El 25.5% de los materiales con datos positivos, fueron sometidos de nuevo a la prueba de ELISA después de termoterapia y extracción de ápices. El 94.12% de éste grupo, presentaron resultados negativos. Se efectuó prueba de PCR para 173 materiales del Brasil, que arrojó un 80.9% de materiales con resultado positivo. Este alto número de materiales con resultado positivo ha motivado la realización de nuevas pruebas para corroborar los resultados, proceso que se esta llevando a cabo sin resultados definitivos aún.

Prospectos

Los materiales procesados por el Laboratorio de Cultivo de Téjidos, son luego sembrados en invernadero. En este momento se han entregado 425 materiales para engrosar estaca y efectuar posteriormente las pruebas de injerto y PCR, con el objeto de continuar ofreciendo clones de *Manihot esculenta* satisfactorios fitosanitariamente para intercambio internacional y regional.

Table Pathogen testing and Indexation Status of Germplasm at CIAT, Sept. 1998.

No. of ACCESSIONS							
CULTIVATED	in vitro Bank	Available for distribution to 1997	1998 Processing toward available distribution				
			Termotherapy in vitro	INDEXATION			
				ELISA		PCR	GRAFTING
				CCMV	CsXV	CVMV	CFSD
Argentina	118	7	84	15	15		
Bolivia	7	2	1	2	2		
Brazil	1,340	96	617	496	496	173	
Colombia	2,003	102	1,250	1126	1054		
China	2	2	-	0	0		
Costa Rica	148	15	16	21	21		
Cuba	77	18	4	3	3		
Dominican Republic	5	0	-	5	5		
Ecuador	117	19	70	71	71		
Fiji	6	2	4	1	1		
Guatemala	91	10	8	8	8		
Indonesia	51	9	34	0	0		
Malaysia	67	13	9	7	7		
Mexico	102	15	33	36	36		
Nigeria	19	3	13	0	0		
Panama	43	7	13	12	12		
Paraguay	231	35	25	29	29		
Peru	405	53	136	141	141		
Philippines	6	3	-	0	0		
Puerto Rico	15	5	7	7	7		
Thailand	31	4	20	6	6		
United States	10	4	1	1	1		
Venezuela	249	26	134	152	152		
Vietnam	9	0	6	0	0		
CIAT/ICA Hybrids	385	152	-	65	65		
CROSSING FOR G. MAPPING	147			6	6		
SUBTOTAL	5,684	602	2,485	2,210	2,138	173	0
WILD SPECIES							
30 spp in vitro	330						
3 Undefined spp	3						
TOTAL	6,017	602	2,485	2,210	2,138	173	0

Output 2.2: Germplasm, Passport and Characterization data available to users

Activity 2.2.1: Reform to GRU Databases

So far, the GRU has operated several databases under different formats (dBase III, dBase IV, ORACLE). Following recommendations of ICER'95 and ICER'97, it was decided to fuse all databases into one (CIAT is responsible eventually for a single collection of germplasm towards the FAO Commission), using the ORACLE format. Such fusion would allow: i) a much better monitoring of flows along all operations and across commodities, ii) consequently to i) a much better tracking of problems and monitoring of expenses along the process, and iii) the preparation of standard reports to FAO, countries, etc. Given the unresolved staff imbalance mentioned by the ICERs, this activity was started with a student (with specialty in computer systems) under the joint supervision of the CIAT Information Unit and GRU. GRU Staff has started the full revision of all current descriptors about germplasm and about flows in view of uniformity across the 3 groups of commodities. The novelty is that the reform of the GRU system as it will appear on the computer screens fully matches the flow of operations (Figure 2). Status: we are at the steps "Introduction", "First Multiplication", and "Distribution". This activity has suffered some delays from the late contracting of the student and limited time from Information Unit Staff.

Activity 2.2.2: Characterization of Germplasm Accessions

Using the IBPGR standard descriptor lists and revised ones, the characterization went on for beans (3,898 accessions) and for forages (1,105 accessions). Part of that information was prepared for its transfer into the server for SINGER.

The following table indicates numbers for which characterization data (along IBPGR/IPGRI Descriptor lists) have been recorded. Flowering time, growth habit, plant height, time to maturity, pests and diseases are particularly recorded.

Materials	**Palmira**	**Quilichao**	**Tenerife**	**Popayan**
Field				
Forages				
Gramineae				560
Leguminoseae	250	283	12	
Beans	1,160	---	2,101	637
TOTAL	1,410	283	2,113	1,197

Activity 2.2.3: Distribution of germplasm from the FAO Designate Collections.

Distribution of germplasm during 1998

	PHASEOLUS		FORAGES		MANIHOT (in vitro)		
	Number of accessions (No. of requests)		Number of accessions (No. of requests)		Number of accessions No. of samples (No. of requests)		
CIAT Staff in Colombia	2,931	(69)	(200	18	278	703	(18)
CIAT Staff in other countries							-
Other CGIAR Centres							-
NARS in developing countries	3,534	(24)	(89)	(5	53	370	(7)
NARS in developed countries	119	(10)		)	4	16	(1)
			(33)	(1)			
Private sector in developing countries	-		(20)	(1	6	30	(2)
Private sector in developed countries	9	(1)	-	) -	1	5	(1)
Others (includes universities)	1,900	(24)	(176)	(8)	24	162	(2)
TOTAL	8,493	(128)	518 (33)		366	1,286	(31)

Output 2.3. National collections restored to NARS

Activity 2.3: restoration of national collections to national genebanks

The GRU has currently two formal requests of restoration: one from Ecuador and the other from Chile. Since 1996, the national collections of these two countries are under multiplication; specifically in 1998 1,160 accessions from Chile were multiplied. Once enough seed shall be produced, the restoration sample will be shipped.

Output 2.4. FAO Designate Collections safe duplicated

Activity 2.4: Safe duplication of FAO Designate Collections in different genebanks

The GRU has two on-going agreements about safe-duplicating its entire seed collections, with CATIE (Costa Rica) and CENARGEN (Brazil). A visit was paid to CATIE in April 1998 to check about status of current duplicate and willingness of CATIE to continue with the agreement. Conversations were held with Brazil. The bottleneck is operational resources to resume and sustain the effort (64,000 accessions of seed materials to be shipped, or 6,400 in 10 years, or

12,800 in 5 years). As detailed in 1.5.3 above, research is going on to improve cryoconservation protocols that would allow the safe duplication in liquid nitrogen in at least two different places (earlier contacts with CIP indicated the interest of CIP in this scheme under terms of reciprocity).

Output 2.5. Refined Core Collections

Activity 2.5: Further characterization of germplasm for improved core collections

No specific activities for the further refinement of existing core collections were planned in 1998. Such core collections were defined on the basis of existing available reserve collections, not on the entire collections received at CIAT. Additional progress shall be possible once current backlogs have been solved out.

Output 2.6: Improved disease indexing techniques

Activity 2.6: Production of antisera to detect *Xanthomonas campestris* pv *phaseoli* in bean seeds using immunofluorescent staining

María S. Balcazar, Benjamín Pineda L.

Introduction

Common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli* is a major seed borne disease of beans, *Phaseolus vulgaris* L., and its causal agent is considered as a pathogen of quarantine significance. The detection of the bacteria in seeds is done by using semiselective culture media such as MXP (Claflin et al., 1987), serology, pathogenicity test and other procedures which have a relative sensitivity and are normally time consuming. Indirect immunofluorescent staining is also used to detect *X. canpestris* pv *phaseoli* with successful results (Malin et al., 1983).

We present here advances on antisera production in order to adapt the immunofluorescent staining procedure to identify *X. capestris* pv *phaseoli* in bean germplasm as seed.

Materials and methods

Antisera obtention: Bacterial isolate (Xcp 123 lyophilized) used to produce antisera was obtained from CIAT Bean Pathology Laboratory. Initially it was reactived on yeast extract calciun carbonate agar (YDCA) and then subcultured one more time in YDCA. After forming colony units a bacterial suspension containing $5x10^8$ c.f.u/ml was prepared and inoculated on 10 days-old BAT 41 bean seedlings following the procedure described elsewhere (Annual Report 1997), and reisolated from developing lesions. With the colonies reisolated in YDCA a bacterial suspension in saline solution (Sodium chloride 0. 85%) was prepared; it showed 0.5 of absorbance at 660-nm wave length. The solution was then centrifuged at 12,000g for 10 minutes; the resulting pellet was washed three times with phosphate-buffered saline (PBS , 0.1M Potasium phosphate buffer, pH 7.2) . The pellet was resuspended in sterilized saline solution that was centrifugated and the obtained pellet washed again and finally resuspended in 3.0 ml saline solution, adjusting bacterial concentration at $5x10^8$. c. f. u. /ml. Cells (0.2 ml of bacterial suspension) were injected into marginal ear veins of two New Zealand rabbits. Repeated injections using antigen (0.2ml) as

prepared along above indications were made at weekly intervals. One week after 5th injection blood was extracted and the antisera agglutination titer determined.

Fluorescent antibody staining procedure: This part of the project is in progress using the technique described in Malin et al., 1983.

Results

At least 30 ml of specific antisera were collected with a good titer (1:2560). This product is ready for gamma globuline purification and conjugation with fluorescein isothiocianate (FITC) to apply the detection procedure in the SHL.

References

CIAT. 1997. Annual Report, p 14-18

Claflin , L. E., Vidaver, A.K. and Saser , M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli.* Phytopathology 77(5): 730-734

Malin, E. M., Roth, D.A., and Belden, E. L. 1983. Indirect immunofluorescent staining for detection and identification of *Xanthomonas campestris* pv *phaseoli* in naturally infected bean seed. Plant Disease 67:645-647.

5.3. Sub-Project 3: Objective: to make the FAO Designate collections genetically and socially relevant

Output 3.1. Designate Collections better characterized

Better understanding has been obtained this year on genetic diversity of tepary and Lima beans and related taxa, and on the number of phaseolin variants existing in common bean.

3.1.1. Patterns of domestication and genetic diversity in *Phaseolus acutifolius* in Central America and Southwestern USA

C. P. Florez., O. Toro, C. H. 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. 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 Sinaloa, 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 (Flores, 1996; Garvin and Weeden, 1994).

Results

Los resultados obtenidos este año, dan evidencias que la colección actualmente disponible en el CIAT de frijol tepari silvestre y cultivado, el alelo S (slow allele) del locus ACO-2 sólo se encuentra exclusivamente en las poblaciones silvestres de Sinaloa (G40103) y Jalisco (G40106), lo cual confirma la hipótesis de Garvin and Weeden (1994), sobre un único origen geográfico del frijol tepari cultivado. Sin embargo, con los resultados que obtuvimos este año, podemos profundizar un poco más sobre la domesticación del frijol tepari.

En primer lugar, Schinkel and Gepts (1988), habian reportado la globulina XI (presente sólo en el tepari cultivado) en tres poblaciones silvestres, la G40103 (Sinaloa), la G40071 (Arizona) y la G40054 (Sonora). Al trabajar con el primer incremento de toda las semilla original disponible en el banco del CIAT de estas tres poblaciones encontramos que la globulina XI no se haya, en cambio se encuentran unas globulinas diferenetes a la XI tanto en la electroforesis SDS-PAGE en 1D como en 2D, a excepción de la población de Sinaloa que presenta una globulina idéntica a la globulina XI en 1D, pero diferente en 2D (tabla 1).

Garvin and Weeden (1994) habian reportado el alelo lento del locus ACO-2 (exclusivo de los cultivados) sólo en las poblaciones de Sinaloa (G40103) y de Jalisco (G40106), ahora encontramos que el alelo ACO de los cultivados se encuentra sólo en los incrementos de toda la semilla original disponible en el banco del CIAT de estas dos poblaciones silvestres y que además la población de Jalisco presenta una sóla globulina bien diferente (patrón XIV) a la XI tanto en electroforesis SDS-PAGE tanto en ID como en 2D, con lo cual Jalisco queda descartado como origen geográfico de domesticación. En cambio para la población de Sinaloa se encuentra que esta presente la globulina XVII, la cual es idéntica a la XI en 1D y diferente en un sólo polipéptido en la de 2D. Además al haber evaluado ya en forma sistemática tanto para la enzima ACO como para globulinas toda la colección de tepari disponible en el CIAT y sólo encontrar el alelo S del ACO-2 (exclusivo de los cultivados) en estas dos poblaciones silvestres y la Globulina XI (exclusiva de los cultivados) en ninguna población silvestre ,a excepción de la población de Sinaloa, en la cual su globulina es casi idéntica a la XI, e incluso las otras fracciones de la proteina total de semilla (Pha y fracción de 22-29 Kda) son idénticas a la XI. Por lo tanto hasta ahora en las poblaciones de semilla silvestre de frijol tepari disponibles en el banco del CIAT, esta población de Sinaloa (la G40103), seria el más opcionado (Sinaloa) para ser el centro de origen del frijol tepari cultivado. Además este sitio ya habia sido propuesto como uno de los centros de origen más opcionados por los tres grupos de investigadores (en epocas y con hipotesis diferentes) que más han trabajado sobre la domesticación del frijol tepari (Mansshardt and Waines, 1983; Schinkel and Gepts, 1988; Garvin and Weeden, 1994), (tabla 1).

Por último, Florez (1996) plantea un segundo evento de domesticación para el frijol tepari, basandose en que encontró que la población cultivada, la G40084, de Durango presenta una

globulina única y bien diferente en 1D y 2D a la globulina XI (el patrón XIX), el cual no se encuentra en ningún *silvestre y que* además que el análisis cluster de las 10 isoenzimas que evaluo, muestra que esta población es bien diferente al resto del material cultivado y silvestre disponible en el momento en el banco del CIAT. Sin embargo, es de observar que esta población también presenta el alelo S de ACO de los cultivados, a pesar de ser muy diferente de ellos por las otras isoenzimas. Basado en lo anterior, este año evaluamos el primer incremento de toda la semilla original disponible de las poblaciones silvestres que han sido recolectadas en Durango (la G40083) ó cerca de este Estado (Chihuahua, la G40082) y encontramos que presentan la globulina VI, la cual es muy similar a la XIX en 1D y bien diferente en 2D. Adicionalmente, al hacer una evaluación sistemática de toda la semilla de incremento que tiene la población G40084 (no hay semilla original disponible y la de incremento fue obtenida a partir de un lote de 9 semillas original), encontramos sólo el patrón XIX (tabla 1).

En conclusión, con los resultados obtenidos este año, sumados a *los obtenidos* a los grupos de investigadores anteriormente citados, se puede anunciar que con las técnicas bioquímicas (isoenzimas y proteinas de semilla en electroforesis1D y 2D) ya se ha evaluado en forma sistemática la colección mundial de frijol tepari tanto silvestre como cultivada disponible en el momento en el CIAT y para conocer con mayor exactitud y sin lugar a dudas, la domesticación del frijol tepari cultivado (dudas que aún deja la mejor hipótesis hasta el momento, como es la de Garvin and Weeden, 1994) se deben llevar a cabo dos estrategias, la primera se deben hacer nuevas colectas del tepari silvestre (el cual no ha sido uniformemente colectado en su área de distribución natural) y segundo se debe recurrir a marcadores más poderosos, como los moleculares de AND (especificamente los AFLPs), ya que los bioquímicos por si sólos no serian suficientes. Sin embargo, al tener ya completos los datos del locus ACO-2 y de las globulinas para *la colección mundial* de tepari disponible en el momento, nos da la evidencia bioquímica de que Sinaloa, hasta ahora es el centro de origen más probable para el tepari cultivado y que además se observa un hecho común con respecto a las dos globulinas presentes en los cultivados (la XI y la menos común, la XIX), es que no se han encontrado en su contraparte silvestre, pero si se han encontrado unas globulinas casi idénticas (la XVII y la VI) a ellas en las poblaciones silvestres más opcionadas como centros de origen de los cultivados (Sinaloa para la globulina XI y Durango, Chihuahua para la globulina VI) , lo cual podria plantear el interrogante si estas globulinas de los cultivados no han mutado (divergencia evolutiva de los cultivados en el tiempo) a partir de estas globulinas silvestres (asi, la XI a partir de la XVII y la XIX a partir de la VI), este interrogante podria ser dilucidado haciendo un estudio comparativo con los marcadores de los AFLPs de las poblaciones silvestres y cultivadas que presentan estas variantes proteicas.

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.

References

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

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.*

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.

Tabla 1. Globulinas y locus ACO-2 analizadas en el primer incremento de la semilla original de las poblaciones silvestres, que son los probables ancestros del frijol tepari cultivado.

Población No. G	No. Sem. Orig. Anali. (1° increme)	Estado Biológico	Origen	Provincia	Globulina	Locus ACO
40103	150	Silvestre	Mexico	Sinaloa	XVII	Slow (S)
40106	50	Silvestre	Mexico	Jalisco	XIV	Slow(S)
40054	22	Silvestre	Mexico	Sonora	III, XXIV, XXVI	Fast (F)
40071	15	Silvestre	USA	Arizona	XXVI	Fast (F)
40082	1	Silvestre	Mexico	Chihuahua	VI	Fast (F)
40083	2	Silvestre	Mexico	Durango	VI	Fast (F)
40084	30	Cultivado	Mexico	Durango	XIX	Slow (S)
40171	9	Silvestre	Mexico	Durango	II	Fast (F)
40177	20 (F1 y F2)	Weedy	USA	Arizona	IV	H, F, S
40208	8	Silvestre	USA	Arizona	XXI, XXVII	Fast (F)
40210	6	Silvestre	USA	Arizona	II, VIII	Fast (F)
40280	47	Weedy	Mexico	Sonora	II	Fast (F)

3.1.2. Molecular evidence for an Andean origin and a secondary gene pool for the Lima bean (*Phaseolus lunatus* L.) using chloroplast DNA variation

B. Fofana, J.P. Baudoin, X. Vekemans, D.G. Debouck, P. du Jardin

Faculté des Sciences Agronomiques de Gembloux, Belgium; Laboratoire de Génétique et d'Ecologie végétales, Université Libre de Bruxelles, Brussels, Belgium; GRU/CIAT, Cali, Colombia

Chloroplast DNA (cpDNA) diversity has been examined using PCR-RFLP and RFLP methodologies for phylogenetic studies in the genus *Phaseolus*. Twenty-two species of which four of the five cultivated species (*P. lunatus* L., the Lima bean; *P. vulgaris* L., the common bean; *P. coccineus* L., the runner bean and *P. polyanthus* Greenman, the year-bean) represented by 86 accessions were included in the study. Six PCR primers designed from chloroplast DNA and a total chloroplast DNA probe were used for generating markers. Phylogenetic reconstruction using both Wagner parsimony and the neighbor-joining method was applied to the restriction fragment data obtained from each of the molecular approaches. *P. vulgaris* L. was shown to separate with several species of largely Mesoamerican distribution, including *P. coccineus* L. and

P. polyanthus Greenman, whereas *P. lunatus* L. forms a complex with three Andean species (*P. pachyrrhizoides* Harms, *P. augusti* Harms and *P. bolivianus* Piper) co-separating with a set of companion species with a Mesoamerican distribution. Andean forms of the Lima bean are found to be more closely related to the three Andean wild species than its Mesoamerican forms. An Andean origin of the Lima bean and a double derivative process during the evolution of *P. lunatus* are suggested. The three Andean species are proposed to constitute the secondary gene pool of *P. lunatus*, while its companion allies of Mesoamerican distribution can be considered as members of its tertiary gene pool. On the basis of these data, an overview on the evolution of the genus *Phaseolus* is also discussed.

3.1.3. **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).

References

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.

Output 3.2. Novel materials acquired or collected

Activity 3.2. Germplasm exploration carried out in Costa Rica

This exploration results in the disclosing of 29 wild populations for six species. Nine more populations were found for *P. costaricensis*, 10 for wild *P. lunatus*, one for *P. oligospermus*, one for *P. tuerckheimii*, four for wild *P. vulgaris* and four for *P. xanthotrichus*. Ninety-three herbarium voucher specimens were collected for 19 populations of the six species (left at CR). These results confirm the presence of wild *P. vulgaris* on both slopes of the central valley of

Costa Rica, namely in the life zones bh-MB and bmh-P, and of *P. costaricensis* in the life zone bmh-MB. These life zones of limited range in Costa Rica have been heavily cut down, thus fully justifying the germplasm collection. For both species the range of distribution has been almost completely sampled. *P. costaricensis* is likely absent in Cordillera de Tilarán, and the probability to find it in the Coto Brus mountain is low. A paper has been prepared (photographs pending).

Output 3.3. Genetic erosion monitored and documented

Merging the results of explorations carried out in 1987 and 1998, genetic erosion affecting populations of wild bean species in Costa Rica has been documented. The results are presented in a research paper (photographs pending!).

Output 3.4. Unique genes better sampled and characterized

No activity has been planned in 1998 for this output as the progress on backlogs (i.e. novel accessions and characterization of recently multiplied materials) is still beyond schedule to identify with BRU and IP projects specific genes in the entire collections, and ensure their representativity in different genetical backgrounds.

5.4. Sub-Project 4: Objective: to contribute to the formation of human resources in conservation sciences and techniques in the region

Annexes 7.3-6 show results of training activities, and outputs as thesis, courses, etc.

5.5. Sub-Project5: Objective: to provide scientific input in *in situ* conservation of farmers' landraces and wild relatives

Output 5.2. Contribution to protected areas in Latin America

Activity 5.1. Demography studies through computer simulation of the wild Lima bean in the Central Valley of Costa Rica

This was done in cooperation with IPGRI RegOff Americas and the University of Gembloux, Belgium, through the supervision of a doctorate dissertation see 7.3).

Activity 5.2. Mapping of wild populations in relation to protected areas

Using results of germplasm explorations of 1987 and 1998 in Costa Rica, the distribution of each wild bean species was computed against existing protected areas, and indications were provided on where to locate/ expand protected areas. A paper has been written (photographs pending!).

Output 5.3. Practices on on-farm conservation documented

Contacts were made during 1998 in order to find out a suitable place to develop additional studies on on-farm management of genetic resources. One possibility could be materialized as project proposal in Colombia and/ or in Ecuador.

5.6. Regional developments

FAO Regional Conference for the implementation of the Global Plan of Action of FAO
This activity that was not included in the 1998 work plan. It however allowed good exposure for the SB-01 Project and GRU, as 31 countries of Latin America and the Caribbean attended the FAO Regional Conference. It also offered opportunities to continue the strengthening of cooperation links with the Regional Office for the Americas of IPGRI, namely a training activity for the Caribbean region planned for mid 1999.

Audit for the National Programme of Genetic Resources of CORPOICA of Colombia, 17-26 June 1998. This activity was not included in the work plan of 1998, but allowed the strengthening of work relationships the national programme of Colombia.

6. Annexes

6.1. SB-01 Project and Genetic Resources Unit Staff

1. Conservation Group:

C. L. Guevara, Ph.D.	Specialist, Germplasm Conservation
R. Escobar, Biologist	Research Assistant (Cryobiology)
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 * * (Tropical Forages)
I. R. Moreno, Ing.Agr.	Research Assistant (Tropical Forages)
A. Ciprián, Tech.	Technician (Tropical Forages)

3. Service:

D. G. 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

* Left January, 1998
** On Sabbatical Leave, University of Reading, U.K .

6.2. List of publications by Project Staff in 1998.

A. In refereed journals:

1. Acosta-Gallegos, J. A., Quintero, C., Vargas, J., Toro, O., Tohme, J. & Cardona, C. 1998. A new variant of arcelin in wild common bean, *Phaseolus vulgaris* L., from southern Mexico. Genet. Resources & Crop Evol. 45: 235-242.

2. Fofana, B., Baudoin, J. P., Vekemans, X., Debouck, D. G. & du Jardin, P. 1998. Molecular evidence for an Andean origin and a secondary gene pool for the Lima bean (*Phaseolus lunatus* L.) using chloroplast DNA variation. Theor. Appl. Genet. 97: *in press*.

B. In non-refereed journals:

1. Singh, S. P., Debouck, D. G., & Roca, W. 1998. Interspecific hybridization between *Phaseolus vulgaris* L. and *P. parvifolius* Freytag. Annu. Rept. Bean Improvement Coop. 41: 7-8.

2. Singh, S. P., Debouck, D. G.& Roca, W. 1998. Broadening the genetic base of common bean cultivars. Annu. Rept. Bean Improvement Coop. 41: 39-40.

C. In books:

1. Goncalves Fukuda, Wania & Guevara, C.L. 1998. Descriptores morfológicos y agronómicos para caracterización de Mandioca (*Manihot esculenta* Crantz). Documentos - CNPMF No. 78 ISSN 0101-5171 - JUNHO/1998. EMBRAPA Cruz das Almas, Bahia, Brasil, 23 a 28 de Octubre de 1998. Pp. 7-33.

D. In proceedings:

1. Debouck, D. G., Araya, R., Camacho, F., Sánchez, P.& González, W. 1998. Exploración de germoplasma para el género *Phaseolus* (Fabaceae, Phaseolinae) en Costa Rica. In: XLI Reunión Annual PCCMCA (Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos y Animales), Reunión Mesoamericana de Agronomía, Nicaragua, del 20-23 de Abril de 1998. p 87.

2. Mafla, G. , Roa, J. C. & Guevara, C. L. 1998. Control del crecimiento in vitro de la yuca con el uso de reguladores osmóticos e inhibidores de etileno. In: I Seminario de Investigación en Biología Celular y Molecular, Universidad del Cauca, Departamento de Biología, Popayán, 20-1 de Mayo 1998.

6.3. List of thesis research supervised by Project Staff in 1998

1. Degreef, Jérôme. 1998. Développement d'un modèle démographique et applications à la conservation *in situ* de populations sauvages de haricot de Lima (*Phaseolus lunatus* L.) dans la Vallée Centrale du Costa Rica. Communauté Francaise de Belgique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Unite de Phytotechnie des Regions Intertropicales, Thèse de Doctorat, 144 p.

6.4. List of conferences and scientific communications presented by Project Staff in 1998

Debouck, D. G.

1. Avances en investigación en diversidad genética de algunos cultivos alimenticios neotropicales. CATIE, Turrialba, Costa Rica, 21 April 1998.

2. Recursos fitogenéticos amazónicos: metodologías para conservación y aprovechamiento frente a los desafíos del sector agropecuario. Villavicencio, Colombia, 7 May 1998.

3. Aprovechamiento de la biodiversidad: nuevas perspectivas en cruzamientos amplios. Palmira, Colombia, 28 May 1998.

6.5. List of national and international courses with input from Project Staff in 1998

Molecular markers for assessing agrobiodiversity. CIAT, Instituto Alexander von Humboldt of Colombia, Smithsonian Institute of USA, 9-20 March 1998.

6.6. List of trainees trained by Project Staff in 1998

Electrophoresis Lab

1. Garcia, Mario A. Estudio de la diversidad genética de *Capsicum* en Colombia. Tesis Doctorado. Universidad Nacional de Colombia, Palmira.

2. Gutiérrez, Martha Cecilia, Investigadora, becaria de COLCIENCIAS. Determinación de híbridos en forrajes tropicales. Tesis Doctorado. Universidad Nacional de Colombia, Palmira.

3. Guzmán, Felix Alberto. Estructura genética de poblaciones silvestres de frijol común (*Phaseoleus vulgaris* L.) y simulación de deriva genética.Tesis pregrado Biología, Universidad del Valle, Colombia.

4. Segura, Sergio Damian. Isozyme variation in five species of *Passiflora* subgenus *Tacsonia* and *Passiflora manicata.* Tesis Doctorado. Ecole Nationale Supérieure Agronomique, Montpellier, France.

5. Solarte, Ingrid Paola. Relaciones filogenéticas en un grupo de especies silvestres del género *Phaseolus* establecidas mediante estudios palinológicos y electroforéticos. Tesis pregrado Biología, Universidad del Valle.

In vitro Lab

1. Aviles, Gustavo, Asociación de Comites Comarcales, León , Nicaragua, Abril 2, 1998.

2. Blanco, Omar, Asociación de Comites Comarcales, León , Nicaragua, Abril 2, 1998.

3. Franco, Tito, IPGRI, RegOff Americas, 16-17 Octubre, 1997

4. Hoogendijk, Michiel, IPGRI, RegOff Americas, 16-17 Octubre, 1997

5. Rey, Leonardo, CORPOICA, Ibagué, 25 Septiembre, 1998.

Viability Lab

1. Franco, Tito, IPGRI, RegOff Americas, 16-17 Octubre, 1997

2. Hoogendijk, Michiel, IPGRI, RegOff Americas, 16-17 Octubre, 1997

6.7. International Posters

1. J. A. Ospina [1] , C. L. Guevara[1], L. H. Caicedo[1] and V. Barney[2] 1998. Effects of Moisture content on Passiflora Seed in Liquid Nitrogen.. JIRCAS/IPGRI Joint International Workshop, 20-23 Oct, 1998. Tsukuba, Japan.

[1] CIAT, Genetic Resources Unit, Centro Internacional de Agricultura Tropical, , AA 6713, Cali, Colombia [2] CIRAD-IPGRI, Centre de Coopération Internationale en Recherche Agronomique pour le Développement-International Plant Genetic Resources Institute, IPGRI/CIAT, AA 6713, Cali, Colombia

6.8. Visitors

The professional staff of the Genetic Resources Unit attended the visit of 52 institutions of different places and countries, for a total of 350 people during the period from January to September, 1998.

7. *Donors*

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

Ministerio de Agricultura y Desarrollo Rural, Colombia

PRONATTA, Colombia

Central American Network for Bean Production PROFRIJOL

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

Annex 1. SB-01 Project Log-Frame

Project: Saving Biodiversity, Genetic Resources Conservation and Characterization
Manager: Daniel G. Debouck

Sub-Project # 1: the International Standards

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

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

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

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

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

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

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

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

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

Annex 2. Project SB-01. Highlights for 1998.

Sub-Project # 1: the International Standards

Outputs	Targets for 1998	Achievements	Reasons for Diverging
Output 1.1 Backlogs of introduced materials processed	1,000 accessions	906 accessions	Agreement ICA-CIAT delayed and yet not funded Quarantine glass-house space in different altitudes
Output 1.2 Backlogs of materials pending on multiplication multiplied	2,000 accessions	1,797 accessions	Space of mesh-houses Manpower
Output 1.3 Materials pending on regeneration regenerated (incl. In vitro)	6,000 accessions 4,200 (in vitro)	2,862 accessions 4,200 (in vitro)	Security Manpower Distant field plots
Output 1.4 Materials processed into final packing	8,000 (viability) 6,000 (packing)	1,898 2,751	Staff Staff
Output 1.5 Improved conservation techniques	See Research proposals	On time	

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

Outputs	Targets for 1998	Achievements	Reasons for Diverging
Output 2.1 FAO Designate Collections cleaned against seed borne diseases (incl. In vitro)	2,862 accessions (6,000)	1,674 accessions	Staff Equipment delayed
Output 2.2 Germplasm, passport and characterization data available to users	3,000 accessions Reform GRU databases	5,003 accessions 1/3 progress	Staff (incl. Information Unit) Student started in June
Output 2.3 National collections restored to NARS	Duplicates back to Ecuador and Chile	In process	Mesh-house space Manpower
Output 2.4 FAO Designate Collections safe duplicated (incl. In vitro)	Contacts with CATIE and CENARGEN resumed	Visit to CATIE and discussion with CENARGEN	
Output 2.5 Refined core collections	Not planned in 1998	---	Not enough progress on backlogs
Output 2.6 Improved disease indexing techniques	See Research Proposals	On time	

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

Outputs	Targets for 1998	Achievements	Reasons for Diverging
Ouput 3.1. Designate Collections better characterized	See Research Proposals	One paper published, the other submitted	
Output 3.2 Novel materials acquired or collected	One exploration	Exploration done; paper in preparation	
Output 3.3 Genetic erosion monitored and documented	Twenty populations monitored	Forty populations monitored; paper in preparation	
Output 3.4 Unique genes better sampled and characterized	Not planned in 1998	---	Not enough progress on backlogs

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

Outputs	Targets for 1998	Achievements	Reasons for Diverging
Output 4.1 NARS human resources trained	4 Trainees 1 Course	Done (see 7.3, 7.6)	
Output 4.2 Conferences in national/ international fora	2 Conferences	Done (see 7.4)	
Output 4.3 Public awareness products	1 article in the press 1 news on TV	Done	
Output 4.4 Education and training materials	1 education material	Done (see 7.2.C)	

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

Outputs	Targets for 1998	Achievements	Reasons for Diverging
Ouput 5.1. Project proposals prepared	2 Concept Notes for distribution	4 Concept Notes prepared	
Output 5.2 Contribution made towards protected areas in Latin America	Survey about wild relatives for Costa Rica	Paper in preparation	
Output 5.3 Practices on on-farm conservation documented	Contacts for Project Proposals	Done	

Fig. No. 1

SB-01: Integrated Conservation of Neotropical Plant Genetic Resources

Project Goal

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

Project Purpose

To integrate ex situ and in situ conservation.

Subproject 1:	**Subproject 2:**	**Subproject 3:**	**Subproject 4:**	**Subproject 5:**
To make the collection-designate meet international standards (i.e. viability, quantity and plant health aspects).	To make the collection-design available to users (i.e. farmers breeders and agronomists).	To make the collection-designate fully relevant to the purposes of conservation.	To contribute through training to capacity building in conservation sciences and techniques in the region.	To develop in situ methodologies for farmer landraces and wild relatives.

Outputs

- Improved conservation techniques.	- Sets of germplasm restored to NARS.	- Novel materials acquired or collected.	- NARS human resources trained.	- Project proposal on in situ Conservation prepared.
- 3,000 accessions fully regenerated.	- Refined core collections including novel materials.	- Genetic erosion monitored and documented.	- Public awareness products developed toward the establishment of	- CIAT contribution made 5 genetic reserves in LA.
- Status collection-designate improved against vulnerability.	- Data (passport,characterization and Evaluation made available to users.	- Improved core collections in terms representatives.	- Workshops and technical courses carried out.	- Landrace diversity in traditional farming systems monitored.
- Alternate liable and affordable conservation methodologies developed.	- Available spectrum of agrobiodiversity widened.		- Communications presented in inter-National symposia.	

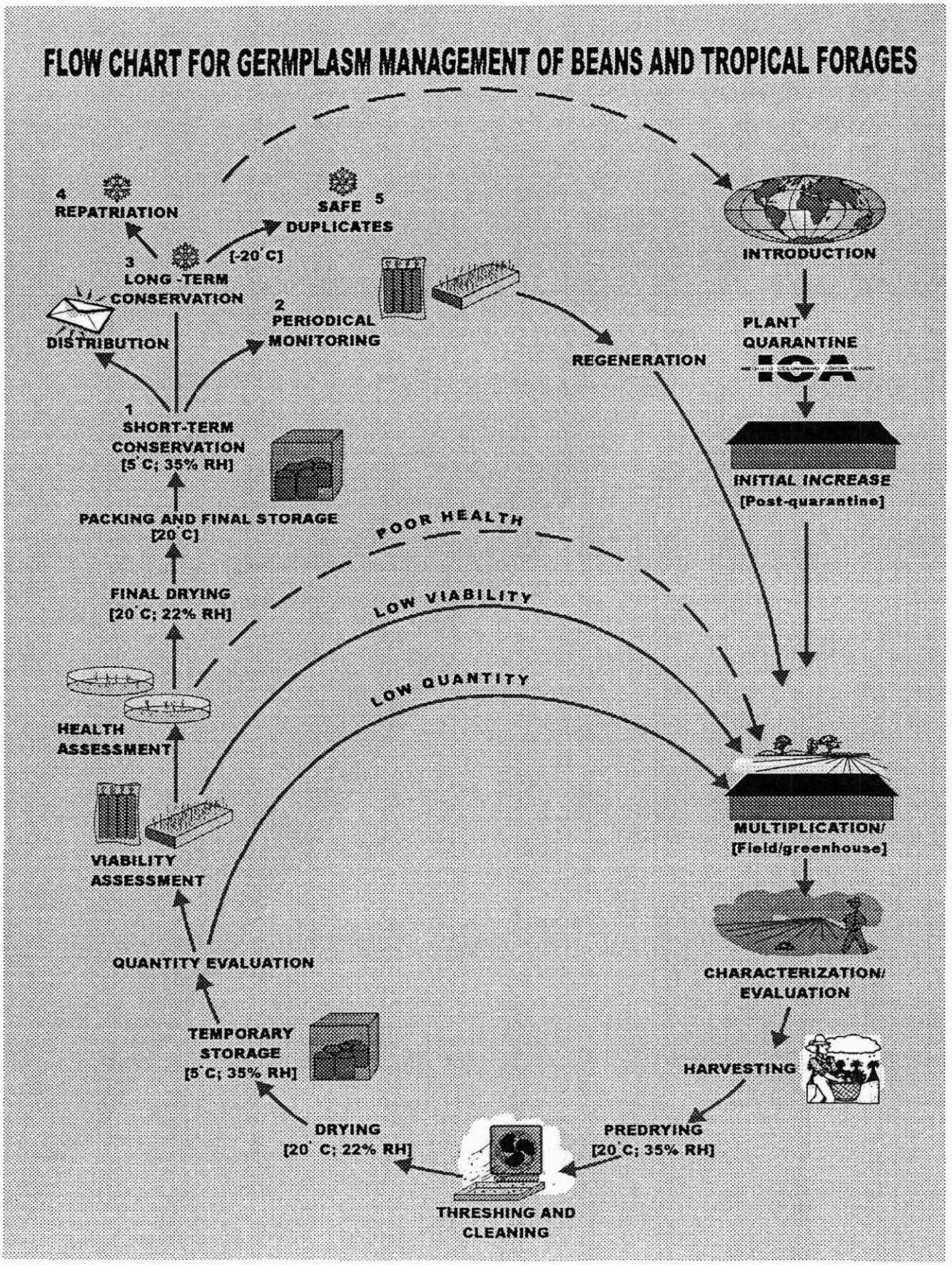

FLOW CHART FOR GERMPLASM MANAGEMENT OF BEANS AND TROPICAL FORAGES
4 REPATRIATION
SAFE 5 DUPLICATES
[-20°C]
3 LONG-TERM CONSERVATION
DISTRIBUTION
2 PERIODICAL MONITORING
INTRODUCTION
PLANT QUARANTINE
ICA
REGENERATION
1 SHORT-TERM CONSERVATION [5°C; 35% RH]
INITIAL INCREASE [Post-quarantine]
PACKING AND FINAL STORAGE [20°C]
POOR HEALTH
FINAL DRYING [20°C; 22% RH]
LOW VIABILITY
LOW QUANTITY
HEALTH ASSESSMENT
MULTIPLICATION/ [Field/greenhouse]
VIABILITY ASSESSMENT
QUANTITY EVALUATION
CHARACTERIZATION/ EVALUATION
TEMPORARY STORAGE [5°C; 35% RH]
HARVESTING
DRYING [20°C; 22% RH]
PREDRYING [20°C; 35% RH]
THRESHING AND CLEANING

Fig. No. 2