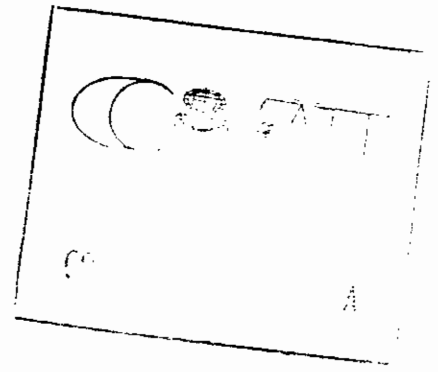


GENETIC RESOURCES UNIT
ANNUAL REPORT 1982

PERSONNEL OF THE GENETIC RESOURCES UNIT

Dr. William M. Roca - Physiologist - (Acting Head)

Germán Alvarez - Research Associate - Forage Germplasm
Rigoberto Hidalgo - Research Associate - Bean Germplasm
*Thierry Vanderborght - FAO Associate Expert - Bean Germplasm

Javier Narváez - Research Assistant - Tissue Culture
Jorge Rodríguez - Research Assistant - Tissue Culture
Hember Rubiano - Research Assistant - Bean Germplasm

Javier Beltrán - Tech I - Tissue Culture
Graciela Mafla - Tech I - Tissue Culture
Julio Roa - Tech I - Tissue Culture
Orlando Toro - Tech I - Bean Germplasm

José Wagner Echeverry - Tech II - Tissue Culture
Julieta Ortiz - Tech II - Bean Germplasm

Luis Garzón - Tech III - Bean Germplasm
Piedad Florez - Tech III - Tissue Culture
Daniel Morales - Tech III - Bean Germplasm
Marlene Valenciano - Tech III - Tissue Culture
Lorenzo Zambrano - Tech III - Forage Germplasm

Helga Dierolf - Secretary

ASSOCIATED PERSONNEL:

Dr. Michael Holle - IBPGR Regional Representative for Latin America - Located at the GRU.

---

* Left in 1982.

C O N T E N T S

	Page
HIGHLIGHTS	1
PHASEOLUS BEANS GERMPLASM	6
Status of the *Phaseolus* Collection	6
Seed increase or Multiplication	11
Evaluation	13
Storage	22
Seed and/or Information Distribution	23
Perpectives	24
TROPICAL PASTURE GERMPLASM	26
Collection Maintenance	26
Other Activities	30
CASSAVA TISSUE CULTURE	35
Recovery of Healthy Clones	35
Germplasm Conservation	46
Cryogenics	57
Perspective	57
Routine Germplasm Storage	59
International Exchange of Clonal Material	61
RICE ANTHER CULTURE	65
Production of Homocygous Lines	65
Variability	66
Applications	66
TISSUE CULTURE OF *STYLOSANTHES* SPP.	69
Anther Culture	69
Cell and Callus Culture	71
Regeneration Potential	73
Variability	73
Perspectives	75

Draft for CIAT 1982 Annual
Report prepared by W. M.
Roca, R. Hidalgo and G.
Alvarez

GENETIC RESOURCES UNIT

Highlights

During 1982, the Genetic Resources Unit (GRU) continued to collaborate with each of CIAT's commodity programs in the acquisition, introduction, multiplication, evaluation, conservation and distribution of germplasm. In addition, research on specific subjects was conducted in order to both, improve current methodologies and to open new avenues of application.

The following are the highlights of the GRU's 1982 activities:

Phaseolus Bean Germplasm

1. Resulting from germplasm acquisitions in the last five years, the bean collection has reached over 32,500 accessions, the majority of which are *P. vulgaris*, but the other 4 *Phaseolus* and 10 non-cultivated species are also included. In 1982, collection expeditions continued, in collaboration with the IBPGR, in Mexico, Brasil and Peru; 2. The major donnor of *P. vulgaris*, as well as of the other

cultivated Phaseolus species, were North America, Europe, Central and South America; 3. Based on the phytosanitary knowledge of the donnor country, greenhouse and field facilities, a procedure to increase new germplasm acquisitions was adopted in collaboration with the Bean Program and ICA; 4. Using 27 "minimum" descriptors, P. vulgaris accessions have been characterized. It was found that the predominant seed colors in the collection are white, the reds, blacks and cream; the cream-beige group was majoritary amongst the materials with mixed colored seed; 5. The study has also showed that duration of flowering, number of nodes at maturity and pods per plant presented the highest variation, whereas seeds per pod and days to flowering had low variation. Seed character evaluations in the other cultivated Phaseolus suggested a similar availability as in the common bean; 6. Preliminary results of a project on inter-specific hybridization with the University of Gembloux, indicated that, although most of the wild forms of P. vulgaris (337 in CIAT's collection), do not provide enough sources for plant architecture, they seem quite rich as disease and pest resistance sources. Seed increase of P. coccineus has demonstrated a high morphoagronomic variability intra- and inter-accessions; 7. The bean"working" (short-term) collection stored at the GRU has increased to nearly 17,000 accessions and the long-term collection now comprises over 2,500 accessions; 8. In the last five years, more that 66,000 seed samples have been distributed to both

National Programs and to CIAT scientists.

Tropical Pastures Germplasm

1. In 1982, over 5,500 accessions, comprising 46 species, were introduced to the GRU for maintenance, and the pasture collection increased to 5,600 accessions; 2. Materials from more than 640 accessions of 85 species were planted in the field for seed increase, and 230 accessions of the genus *Aeschynomene* in the glasshouse. Otherwise not possible, seed set in *Desmodium* and *S. guianensis* accessions was achieved in the Popayán plot; 3. Germination tests were carried out in 16 grass genera; 4. Germplasm samples were distributed to CIAT scientists (over 1,000 seed samples)and to 6 countries; 5. Over 1,660 accesion samples of 30 genera have been collected, prepared and/or processed as for herbarium samples, this year; 6. Updating of the pastures germplasm catalog has been initiated in collaboration with the Data Services Unit; 7. In collaboration with the TPP, the adoption of electrophoretic "finger-printing" methods was initiated.

Tissue Culture

Cassava. 1. During 1982, healthy clones were recovered from more than 270 varieties infected with the frog skin disease; 2. Cleaning-up studies showed that rapid growth of

meristem-tip explants increased the rate of cleaning and that small explants obtained from sprouts grown at high temperature yielded more than 95% healthy plants from varieties infected with two viral diseases; 3. Variation in leaf size occurred in the entire population of plants, in one variety, regenerated by meristem culture; 4. This year, the in vitro gene bank of cassava reached more than 1,340 varieties. Materials have been in storage for up to 54 months with about one sub-culture every 18 to 24 months; 5. "Minimal growth" storage studies indicated that reduction in the rate of growth and increase in culture viability could be achieved with low temperature, osmotic stress, inclusion of activated charcoal and low nitrogen level in the culture medium during storage; 6. Plants have been regenerated from cassava meristems stored in liquid nitrogen at Saskatoon, Canada; 7. In 1982, a total of 80 varieties have been distributed in vitro to 9 countries, and in collaboration with IBPGR, nearly 350 varieties have been transfered from Brasil to CIAT using meristem cultures.

Rice. 1. Field evaluation of anther culture derived diploid plants has demonstrated complete homogenity within lines and high heterogeneity between lines, hence the homocygous condition of the plants was proved; 2. Work was initiated to produce homocygous lines by anther culture of F_1 hybrid crosses between tolerante and susceptible varieties

to aluminum toxicity; 3. Screening for callus induction in 24 varieties suggested that most varieties with high tissue culture capability were tolerant to aluminum toxicity, as well.

Stylosanthes spp. 1. Work started this year on anther and callus culture of *S. guianensis* and *S. capitata*, as an aid to crop improvement; 2. The appropriate size of the flower bud, for anther culture of both species was determined and it corresponded to microspores in the tetrod to the uni-nucleate stage; 3. Callus induction from anther culture could be influenced by the medium, but was primarily a response to the genotype; 4. The regeneration capacity from anther culture as well as from leaf tissue callus was quite high in *Stylosanthes*. Within 3 months, nearly 1,800 plants could potentially be regenerated from an anther or from a leaf segment; 5. Variations in growth habit and leaf size were observed amongst the plants regenerated from anther culture.

PHASEOLUS BEANS GERMPLASM

Status of the Phaseolus Collection

After five years of the establishment of the Genetic Resources Unit, the world *Phaseolus* collection comprises a total of 32,588 accessions from about 47 countries. Part of this germplasm has been the result of expeditions funded by CIAT and IBPGR since 1978 to Mexico, Iberian Peninsula, Perú, Brasil and several African countries. Expeditions have been reinforced with the appointment of an IBPGR officer for Latin America in CIAT.

The recent taxonomy of the genus *Phaseolus* includes 4 cultivated species plus around 35 wild or non-cultivated species. CIAT's collection includes the four cultivated species and their corresponding wild ancestors plus 10 wild non-cultivated species. (Table 1). Among the cultivated species the common bean *Phaseolus vulgaris* represents 89% of the collection, while *Phaseolus lunatus*, *Phaseolus coccineus* and *Phaseolus acutifolius* add up to 11% of the collection; the non-cultivated or wild species are only 0.3% of this germplasm.

Additionally, the bank keeps 429 accessions of bean of other genera, mainly *Vigna*.

Table 1. Status of the *phaseolus* bean collection held at CIAT (Octubre, 1982)

Cultivated Species	No. of Accessions	%
P. vulgaris	28,542	
P. vulgaris ancestral form	332	88.7
P. lunatus	2,282	
P. lunatus ancestral form	62	7.2
P. coccineus subsp. *coccineus*	710	
P. coccineus subsp. *polyanthus*	314	3.3
P. coccineus ancestral forms	58	
P. acutifolius	89	
P. acutifolius ancestral form	59	0.5
Non-cultivated Species:		
P. anisotrichus, *P. filiformis*, *P. galactoides*, *P. microcarpus*, *P. metcalfei*, *P. pedicellatus*, *P. polystachius*, *P. parvulus*, *P. ritensis*, *P. wrightii*	84	0.3
TOTAL	32,532	100.0
Other Genera		
Vigna, *Psophocarpus* *Macorptilium*, others	429	

Source and Origin of the Germplasm

With the access to new computer facilities, a study about the sources and/or the real or previous origin of the germplasm was initiated. This is an attempt to group the collection by common geographical areas or origin this is an aim to group the accessions and/or to help further detection of duplicate groups of germplasm.

Phaseolus vulgaris. The source of the germplasm of this species is very diverse, in fact, 47 countries representing the five continents have sent either their entire national collections or partial samples from their research programs. Comparing the source with the origin of the germplasm (Table 2), a noticeable change in the rank for the different zones can be observed. Thus, North America (33%), Europe (22%), Central America (18%) and all South America appear as the major donors of common bean germplasm. However, by tracing the donor's previous source or the real origin of the materials, Central America appears as the greater contributor (31%), followed by all South America (17%); this result reinforces the theory on the American origin of beans. On the other hand, it also may be suggesting a high probability of germplasm duplication.

One outstanding result concerns the lack of previous

Table 2. Comparison of the source vs. the origin of the germplasm of *P. vulgaris*.

Zone	Source		Origin[1]	
	No. of Accessions	%	No. of Accessions	%
North America	9,568	33.1	1,104	3.8
Central America	5,217	18.1	9,020	31.2
Caribbean	84	0.3	77	0.2
South America Andean countries	3,692	12.8	3,899	13.5
South America Non-Andean	1,3161	4.7	1,413	4.9
Europe	6,463	22.4	3,436	11.9
Africa	2,065	7.1	1,657	5.7
Middle East	-	-	2,016	7.0
Asia-Oceania	424	1.5	1,067	3.7
Unknown	-	-	5,185	18.0
TOTAL	28,874	100.0	28,874	100.0

1. Refers to previous donor's source or real origin

information on 18% of the germplasm. This situation together with the insufficient data about collection sites on the rest, makes it very difficult to trace individual duplications. An attempt to search for more information is underway. The aim is to group the germplasm by common regions of origin; this will be complemented by an agronomic and seed type characterization, color and habit being two basic grouping characters. With this methodology it is expected to cluster germplasm introductions which may have a high percentage of similarity; samples from these "packages of similar germplasm" will be more useful to scientists, specially breeders; it will also facilitate the management of the collection.

Other Cultivated Species of *Phaseolus*. The situation for the other cultivated species is similar to that of the common bean, while North America (33%) and Europe (21%) are the major donors of the germplasm of these species (Table 3), Central America (35%) and South America (22%) appear as the greater original providers of such germplasm. Likewise, a high percentage of germplasm has no information on the previous source of the CIAT's donor. The lack or the insufficient information is the main problem faced when germplasm is introduced into the collection.

Table 3. Comparison of the Source vs. the Origin of the Germplasm of *P. lunatus*, *P. coccineus*, *P. acutifolius*

Zone	Source (%)	Origin (%)
North America	35.6	4.3
Central America	18.6	36.2
Caribbean	0.4	0.9
South America - Andean	4.7	9.0
South America - Non Andean	2.7	12.6
Europe	11.5	2.6
Africa	26.2	4.1
Middle East	-	0.5
Asia - Oceania	0.3	1.3
Unknown	1	28.5
TOTAL	100.0	100.0

Seed Increase or Multiplication

Due to the size of the collection and the needs of the Bean Team, a major emphasis is placed on the increase of new germplasm when received. However, due to the very small seed samples received, seed quality problems and legal quarantine requirements, a methodology to increase new germplasm has

been established in agreement with the quarantine services of ICA. This methodology consists of three steps as follows:

Quarantine

ICA classifies the materials according to the risk of the country source and then decides which germplasm can be directly increased in CIAT's greenhouses and which must be increased in ICA's facilities. Last release of 5,000 accessions to be grown in CIAT's greenhouse was authorized late 1981.

CIAT's Greenhouse

The germplasm released by ICA is grown in the greenhouse. Due to the fact that most of the introduced materials are old or in bad shape, they need to be pregerminated. This is done with a previous scarification by a cut on the seedcoat and a further desinfestation with 8% sodium hypochlorite; seeds are then placed on petri dishes in a germinator under temperature, light and humidity control. The seeds that germinate are transplanted to pots in the greenhouse, where we do a phytosanitary follow-up and take preliminary data. The percentage of success in the pregermination varies greatly, but depending on the seed source, losses can range between 10-16%.

The above process plus the adequate management of the greenhouse make this step laborsome and rather slow, which combined with the reduced greenhouse - meshhouse space available, allows to process only 700 accessions per planting with a production range of 50-100 seeds per accession or 1,400 accessions per year.

Isolated Field

The seed produced in the greenhouse is taken to an isolated field, This field is located in Dagua (26°C and 500 m. a.s.l.) a xerophitic, dry area with low rainfall, the incidence of bacteria and fungi is very low; however, the phytosanitary follow-up is sustained. Three meter rows per accession is used which produces in general more than 100 grams of seeds; likewise, preliminary morphoagronomic data is registered.

Evaluation

The objective of the evaluation is to "characterize" or individualize the accessions of the germplasm. To evaluate the germplasm 27 "minimum" morphoagronomic characteristics are used for *Phaseolus vulgaris*. Almost 1,000 accessions were evaluated in 1981. The evaluation of the other cultivated species has not been formally started, only a partial evaluation regarding pests and diseases has been carried out on *P. coccineus*

and *P. acutifolius*.

Seed Description

Regarding the common bean, the most important characteristic used to classify commercial bean is the seed type. A study has been initiated to classify the germplasm according to seed types. The first part of this study refers to color grouping (Figure 1) in which it can be observed that the predominant seed colors in the germplasm of the common bean are white (22.5%), all the reds (21.1%), black (20.2%) and cream (18.0%). Regarding the presence of seeds having more than one seedcoat color, it can be seen that the cream-beige group is the one presenting the highest percentage of this type of germplasm; the other colors that are combined are always less than 35% of their germplasm combined with more than one color; black color is the one which shows the lowest percentage of seeds combined (2.6%).

Agronomical Characteristics

Phaseolus vulgaris. Additionally to the search of the origin of the collection to group similar germplasm, a comparative study of the main agronomical characteristics is being carried out. It will be useful to establish which evaluation characteristics are more reliable when considering

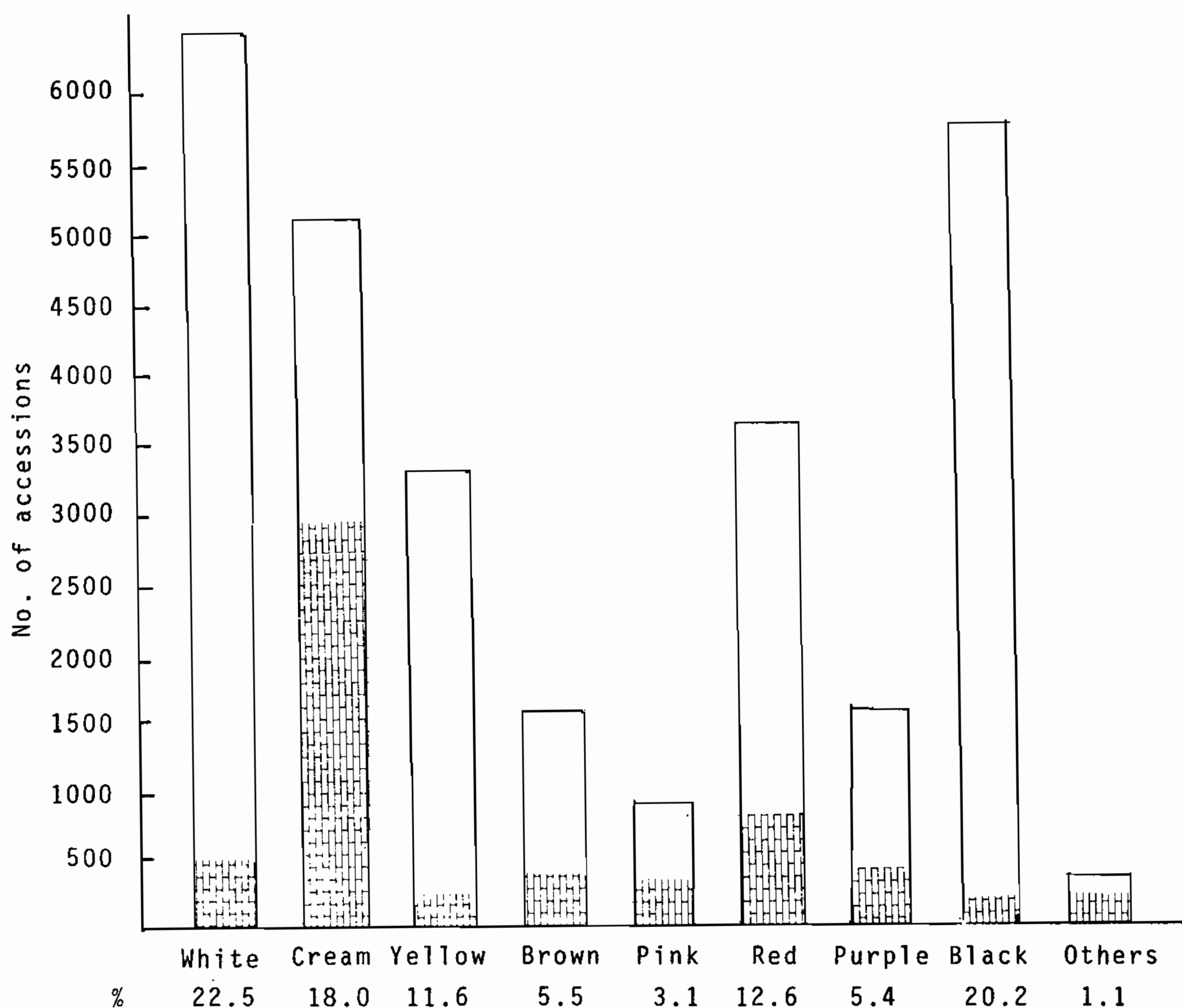


FIGURE 1. SEED COLOR DISTRIBUTION OF THE GERMPLASM OF *P. VULGARIS* AND THEIR PROPORTION OF PURE COLOR vs. COMBINED COLORS (Dark Area). TOTAL ACCESSIONS: 28,874.

the clustering criteria; besides, it can be used as a tool to pick inconsistencies in the data or to select outstanding or exotic germplasm. Results are shown in Table 4, which includes 6 of the most important agronomical characters out of the 27 "minimum" descriptors used for evaluation; the data applies to Palmira conditions.

Although this is a general comparison of the germplasm evaluated including the four growth habits, it can be observed that duration of flowering (c.v. 30.6%), nodes on the main stem at maturity (c.v. 36.1%) and pods per plant (c.v. 57.5%) are the characters presenting a remarkable variation which indicates an ample natural variability available to breeders for selection. On the other hand, seeds per pod (c.v. 22.0%) and days to flowering (c.v. 13.2%) are the characters presenting less variation which may be indicating difficulty for selection of outstanding germplasm (Table 4); however, the coefficient of variation for all the variables is still considerably high. In summary, as a means of comparing the variability and by taking the lowest variation, days to flowering (c.v. 13.2%), as the reference point for the other traits, it can be infered that the variability factor, for duration of flowering is 2.3, for nodes to flowering is 2.0, for nodes to maturity is 2.7, for seed per pod is 1.7 and for pods per plant is 4.4 times more variable than days to flowering.

More detailed conclusions are being elaborated for each one of the growth habits including all the variables used for evaluation.

Table 4. Variability of some morphoagronomic traits used for the evaluation of the *P. vulgaris* Germplasm[1]. Palmira.

Characteristic	Mean	Standard Deviation	C.V.(%)	Sample Size
Days to Flowering	36.0	4.7	13.2	9,003
Duration of Flowering (days)	23.3	7.1	30.6	8,958
Nodes to Flowering	12.6	3.4	26.8	8,192
Nodes to Maturity	15.3	5.5	36.1	7,772
Pods per plant	16.6	10.1	57.7	9,644
Seeds per pod	4.9	1.1	22.0	8,727

1. Includes all the four growth habits.

Other cultivated species of *Phaseolus*. A preliminary seed description on the other cultivated species shows that the availability of colors is similar to that of the common bean (Table 5). However, whites, cream-beige, and reds are the

Table 5. Color distribution (%) of the germplasm of *P. lunatus*, *P. coccineus* and *P. acutifolius*

	Species		
Seed Color	P. lunatus[1] (%)	P. coccineus[1] (%)	P. acutifolius[1] (%)
White	43.7	12.2	50.3
Cream-Beige	13.0	14.9	6.5
Yellow	2.0	10.4	5.9
Brown-Maroon	1.0	1.5	7.8
Pink	2.7	8.1	-
Red	15.4	18.9	-
Purple	3.9	7.2	0.6
Black	14.8	20.0	7.8
Others	3.4	6.8	20.9
TOTAL	100.0	100.0	100.0

1. Percentage of the available germplasm

predominant colors for *P. lunatus*, while the whites are for the majority of *P. acutifolius*; this species does not show red colors. For *P. coccineus* (a cross pollinated species), the predominant is the one which is recorded, since individual accessions show different colores.

Interspecific hybridization (CIAT-University of Gembloux Project). The project aims to increase the variability of common bean through interspecific hybridization with its wild relatives and other species of the genus specially *Phaseolus coccineus*. The seed increase and evaluation of wild *Phaseolus* has been finished and the main conclusions are:

To date the CIAT collection includes 337 wild *Phaseolus vulgaris* accessions.

The results of the agronomical and morphological evaluations indicate that most of the wild forms do not seem to be an interesting source for plant architecture characters for the improvement of common bean *Phaseolus vulgaris*.

The results of the evaluation for diseases and pests resistances indicate that the wild *P. vulgaris* forms seem to be especially interesting for the stored grain insects resistances. Some accessions presented a very outstanding level of resistance never found in the cultivated species (Table 6).

Table 6. Evaluation of disease and pest resistance of the wild *P. vulgaris* germplasm.

Disease or pest	Number of accessions evaluated	Number of accessions reported as:		
		Susceptible	Intermediate	resistant
Bean Golden Mosaic Virus	136	134	2	0
Common Bacterial Blight (*Xanthomonas phaseoli*)	249	235	14	0
Ascochyta leaf spot	276	246	30	0
Leafhopper (*Empoasca kraemeri*)	235	235	0	0
Zabrotes subfasciatus	234	193	32	9
Acanthoscelides obtectus	227	23	58	146

The origin of common bean has been a much debated question for many years. Two hypotheses have been presented by different authors either that Mexico or South America is a single center of origin or that those two locations are two independend centers of domestication. The very little number of South American wild forms in the CIAT germplasm did not allow to confirm those hypotheses. More collection activities in the area of origin of wild *P. vulgaris* from Mexico

to Argentina with emphasis on the latter would allow to study the relationship between the Mexican and the South American wild form.

A preliminary study of variability was carried out in order to find discriminant characters to eliminate duplicates and to separate the different _P. vulgaris_ wild forms. The most discriminant characters are: white variegation along the primary leaf vein, the bracteole size and shape, the number of veins per bracteole, the seed color and pattern, the peduncle length and the number of floral insertions on the raceme, the number of seeds per pod and the 100 seed weight.

Respecting the increase of _P. coccineus_, as reported, last year, a methodology for this cross pollinated species was already established by using meshcages with 13 plants and manual pollination; during 1981-82, 150 accessions were planted at Popayán and 48 accessions were seed increased according to that methodology. During the seed increase, agronomical and morphological characters were observed in order to: a) characterize the accessions, b) define variability of accessions, c) establish a list of characters that appear to be essential for preliminary evaluation. The preliminary results show a very high morphoagronomic variability intra end inter accessions. A list of 39 descriptors for evaluation was proposed as a result of this evaluation.

Storage

One of the prevailing responsabilities of the Genetic Resources Unit is germplasm conservation. To meet this objective, two types of storage have been established: long and short term.

Short term is the active or "working" collection with the following conditions: 5-8°C cold room temperature, 12%-14% seed moisture content, 60-65% relative humidity, and packed in plastic jars (800 grs.). To date there are about 17,000 accessions (mostly of P. vulgaris) which have filled the capacity of the cold room.This situation implies to envisage a future gradual change of container type or a shelf re-arrangement of the cold room.

Long term conditions to preserve the collection has been set up with the following characteristics: -2° to -6°C cold room temperature. 5%-8% seed moisture content, 60-65% relative humidity and packed in sealed laminated foil bags. To date there are more than 2,500 accessions of P. vulgaris stored under these conditions. However, equipment problems as well as cold room isolation difficulties have delayed the continuance for storing germplasm at a faster rate.

Seed and/or Information Distribution

The foremost responsability of the germplasm management is to make it readily available to institutions and scientists. This task has been entirely achieved since the creation of the GRU. In fact, during the last five years a total of 66,668 samples have been distributed. Of this total, 21,707 samples have been sent to institutions and scientists of the national programs (Table 7). On the other hand, 45,067 samples have been distributed to the CIAT Bean Team and other programs within CIAT (Table 8). These numbers give a clear idea about the distribution of the germplasm.

Table 7. Germplasm seed service to National Institutions and scientists outside CIAT, during 1977-82.

Zones	Total Requests	Total Accessions
North America	32	562
Central America	36	14,318
Caribbean	8	1,048
South America(Andean)	54	1,488
South America(Non-Andean)	37	1,091
Europe	51	1,125
Africa	17	1,473
Asia-Oceania	27	682
TOTAL	262	21,787

Table 8. Germplasm seed service to CIAT Bean Team during 1977-82.

Disciplines	Total Requests	Total Accessions
Breeding I	63	5,921
Breeding II	38	18,622
Entomology	79	12,080
Phytoplathology	47	2,916
Physiology	11	747
Agronomy	43	3,508
Microbiology	12	1,034
Tropical Pastures	5	17
Special Studies	4	53
Cultural Practices	3	155
Cassava	2	4
Seed Unit	2	10
T O T A L	309	45,067

Perspectives

A more intensive effort will be placed on the following:

a) Increase of new germplasm since up to date only 53% of the common bean germplasm has been increased and partially evaluated. This would imply to reduce the number of accessions and/or the number of variables. It

will also require of more greenhouse - meshhouse space, as well as of more laboratory facilities (i.e. germinators). A parallel policy with ICA should be elaborated to speed the release of germplasm.

b) Detailed studies on the germplasm that we already have. The objective will be the clustering of similar germplasm based on origin, seed types and agronomical characteristics. A "finger-printing" technique could be of great help for having a more reliable characterization. This type of studies will be useful not only for knowing the collection, but also for planning expeditions in search of germplasm that is lacking in the collection.

c) Increase of germplasm non-adapted to Palmira. This will imply to augment labor and land in Popayán, more dedicated to common bean.

d) To collect wild types mainly of _P. vulgaris_ and _P. coccineus_ in South and Central America.

e) Evaluation of the storage facilities to solve future needs of space and of seed processing (i.e. viability).

TROPICAL PASTURE GERMPLASM

During 1982, the Tropical Pasture Section emphasized the collaboration with the Germplasm Section of the Tropical Pastures Program, in order to achieve a better planification of activities.

Collection Maintenance

The volume of germplasm maintained in storage has increased to 5,600 accessions with the materials transfered from the TPP (Table 9). More than 80% of the accessions were transfered from the TPP-Germplasm Section. In the future most of the TPP germplasm will be kept by the GRU only, contributing with the TPP in the maintenance of the whole collection.

Seed Increase

In general the strategy for seed increase continued to put emphasis on the materials difficult to set seed and prioritized by the TPP. Seed increase was again carried out in three locations:

Palmira - Field. This still constitutes the main legume seed multiplication plot. As shown in Table 10, material from 641 accessions belonging to 85 species of 25 different genera

Table 9. Materials introduced to the GRU germplasm bank in 1982.

G e n u s	No. of species	No. of accessions	Source
Stylosanthes	7	219	TPP-Germplasm
Centrosema	8	81	"
Desmodium	7	41	"
Calopogonium	3	7	"
Galactia	2	16	"
Zornia	5	70	"
Aeschynomene	4	66	"
Cassia	1	3	"
Brachiaria	2	2	"
Andropogon	1	1	"
Panicum	1	1	"
Panicum	1	84	TPP-Breeding
Brachiaria	4	12	"
TOTAL	46	553	

were planted. In close collaboration with the TPP-Germplasm the planting of the _Aeschynomene_ and _Pueraria_ collections was done. Out of the former genus, 350 accessions have been assigned for seed increase and other purposes (e.g. taxonomy); and from the latter genus, 80 accessions are to be planted late November, 1982, including new materials brought recently by the TPP-Germplasm from a collection trip in South East Asia.

Table 10. Pasture seed increase by the Genetic Resources Unit in 1982

Genus	No.of species			No.of accessions			Seed production(grs.)			
	N-2	G.H.	Pop.	N-2	G.H.	Pop.	N-2	G.H.	Pop.	Total
Aeschynomene	16	13	-	350	230	-	4095.7	412.7	-	4508.4
Canavalia	1	-	-	6	-	-	1391.2	-	-	1391.2
Centrosema	6	-	1	37	-	1	246.5	-	-	246.5
Clitoria	1	-	-	3	-	-	211.4	-	-	211.4
Calopogonium	2	-	-	2	-	-	50.4	-	-	50.4
Desmanthus	1	2	-	1	17	-	89.8	3.4	-	93.2
Desmodium	12	9	3	34	29	15	867.2	120.8	232.4	1220.4
Dioclea	1	1	-	7	1	-	110.8	0.8	-	111.6
Alysicarpus	1	-	-	1	-	-	2.0	-	-	2.0
Acacia	1	-	-	1	-	-	17.2	-	-	17.2
Cassia	1	-	-	1	-	-	1.9	-	-	1.9
Crotalaria	1	-	-	1	-	-	0.5	-	-	0.5
Galactia	2	1	-	8	2	-	98.1	18.9	-	134.3
Leucaena	1	-	-	1	-	-	157.7	-	-	157.7
Macroptilium	2	2	-	2	2	-	149.2	21.0	-	170.2
Mimosa	1	-	-	2	-	-	20.3	-	-	20.3
Periandra	1	-	-	3	-	-	34.6	-	-	34.6
Poiretia	1	-	-	2	-	-	2.2	-	-	2.2
Pueraria	4	-	-	80	-	-	*	-	-	-
Rhyncosia	7	1	-	16	4	-	724.6	44.3	-	768.9
Stylosanthes	15	-	1	52	17	1	1046.6	103.5	2.2	1152.3
Tephrosia	1	-	-	1	-	-	109.8	-	-	109.8
Teramnus	2	-	-	4	-	-	242.3	-	-	242.3
Vigna	1	-	-	6	-	-	14.2	-	-	14.2
Zornia	3	1	-	20	1	-	58.5	9.7	-	68.2
					TOTAL		9760.0	735.1	234.6	10729.7

* To be planted in November, 1982. N-2= Field/Palmira.; G.H= Glasshouse; Pop= Popayán

Palmira - glasshouse/meshhouse. A large part (230 accessions) of the *Aeschynomene* collection used for seed increase and taxonomic purposes was kept in the glasshouse. Besides, 73 accessions from 17 species of 8 genera were maintained and increased.

The *Arachis* live collection (21 accessions) still remains in the meshhouse, awaiting to be sent to Brasil. In addition, 43 accessions of grasses from the former "vitrina" are maintained for vegetative multiplication. A portion of it will be sent to the ICA-Obonuco, Pasture Section, and the other will be kept alive because of its difficulty to set seed.

Popayán. Seventeen accessions, mainly from the genus *Desmodium*, are maintained in this location. Several *D. heterocarpon* have produced enough seed in Popayán whereas in other places they were not productive. *Stylosanthes guianensis*, CIAT 1876, which usually did not set seed under CIAT conditions, has been able to do so under Popayán conditions.

It was possible to harvest, from the plantings in the three locations, a total of 10,729 grams of seed.

Seed Germination

Germination tests continue to be conducted, with strong

emphasis to the grasses. In this year, 193 samples of accessions belonging to 16 grass genera were tested; from them, 84% were *Panicum*. The *Panicum* collection is rapidly increasing therefore, the storage characteristics, e.g. seed viability and latency, for this genus need to be determined.

Seed Distribution

The GRU is assisting the TPP-Germplasm with the distribution of seed samples to other TPP sections as well as to collaborators outside CIAT.

Within CIAT, 1,025 seed samples were distributed, belonging to 31 different genera. Seeds were sent to Argentina, USA, Ethiopia, Peru, Brasil and Australia, with 12, 8, 1, 4, 2 and 30 seed samples, respectively.

Other Activities

"Vitrina"

Until about the middle of this year, a live collection of grasses was grown in the field in order to maintain accessions that do not set seeds, and as a demonstration plot. The latter purpose was not fulfilled, since many materials were nor representative of the TPP main stream of germplasm, and the former

was in doubt due to the proximity of the plots to each other, thus a high percentage of mixture ocurred.

It was thus agreed, to transfer and re-arrange the materials in another plot. Seven grasses from the genera Andropogon and Brachiaria, and 11 legumes from Centrosema, Desmodium, Zornia, Stylosanthes and Pueraria were chosen and directly planted in the field. It is expected that a truly representative demonstration plot of the TPP germplasm will be obtained..

Herbarium

Generally, the genera included in the forage collection suffer from the lack of complete taxonomic determination. An important task requires to be accomplished, as soon as possible, to clarify the taxonomy of all the species involved in the collection. Information on the taxonomy of the accessions of some genera has already been received, e.g. Aeschynomene and Rhynchosia; but others (e.g. Zornia) still remain with many unidentified species.

Efforts to overcome this problem have been carried out during this year. Over 1,260 accession samples, comprising 30 genera, mainly from the TPP-Germplasm and the GRU greenhouse and Quilichao TPP-Germplasm plots, have been collected

and processed. Currently, a collection of 407 accession samples of *Zornia* is being prepared for study by specialists.

Data Management

Information on the quantities of seed per accession, as well as complementary data on origin, sender, receiver, herbarium, etc., have been compiled in a specially designed book for that purpose. Although it is a time consuming job, it has demonstrated a higher acuracy and the book system can handle up to 20,000 accession numbers. The use of a microcomputer would greatly help in this task.

Catalog. Basic information of the accessions has been compiled in a catalog, managed through the computer of the Data Service Unit. Corrections and updating are being handled at present.

Training

As part of the TPP-Germplasm and GRU collaboration a worker was transfered for 3 months to the program. This training has helped to improve handling of the materials placed in the legume seed multiplication plot and in the glass house.

Since June, 1982 the Research Associate of the section

has been in a much closer relation with the TPP-Germplasm. Efforts have been made to accelerate the up-dating of the Forages Collection Catalog.

Future Activities

In the near future this section will continue with the supportive work for the TPP-Germplasm, in the areas of seed increase, storage maintenance and seed sample distribution. Besides, work will be carried out on the systematic measurement of seed germination and seed moisture content, as well as seed viability in order to assay the storage conditions, and eventually, to modify them if necessary.

For these activities, certain technical facilities such as seed germinator, seed moisture tester, and some adjustable temperature chambers, will be needed. It would then be possible to standardize methodologies to evaluate seed storage. Also, additional personnel, with technical level, able to handle data, and any other responsable work will be necessary.

It also would be desirable to bring students to develop special projects, as thesis. This would expand our knowledge on other methods of storage and would give us criteria as to handling of seeds.

The use of electrophoretic "finger-printing" as a taxonomical aid, and of *in vitro* storage of grass species with very low or nil seed production, would also require study.

CASSAVA TISSUE CULTURE

During 1982 work focused on the use of meristem-tip culture techniques to a) recover healthy clones from systemically infected varieties; b) build-up a gene bank *in vitro*; and c) exchange germplasm with national programs. In addition, research to improve and refine current methodologies, and to open new areas of application, continued.

Recovery of Healthy Clones

As it was shown previously, heat therapy enhanced the effectiveness of meristem culture in cleaning-up cassava materials from the frog skin disease (CIAT Annual Report 1981). The differential effect of the high temperature on virus multiplication and movement and on the promotion of shoot growth probably account for such results. Hence, the size of the meristem explant used for culture should play a crucial role in the rate of cleaning.

Research was carried out on the interaction of temperature, rate of growth and size of the meristem explant, in the recovery of clean plants from frog skin and mosaic infected clones. In addition, chemotherapy was tested as a means to cleaning up from frog skin disease.

Thermotherapy

Effect of thermotherapy to shoot-tips *in vitro*. Large shoot-tip explants (1.0 to 1.2 mm in size) were isolated from greenhouse grown (25°to 28°C) stakes of two varieties infected with the frog skin disease, and cultured under three temperature regimes: 25°C D/N*, 30°C D/N and 40°C/35°C D/N. Throughout a three-week period, and at each temperature treatment,the culture's shoot elongation was monitored and plants were grown, potted and transplanted to the field for evaluation.

Irrespective of temperature, the highest peaks of *in vitro* shoot elongation ocurred between the 10th to the 16th day of treatment, being those peaks: 0.3-0.5, 0.4-0.7, and 0.5-2.0 mm/day for the 25°C, 30°C and 40°/35°C regimes, respectively. Field evaluation at 4 to 5 months of age, resulted in all plants remaining infected throughout the 25°C treatment. However, at 30°C,the highest rates of frog skin symptom-free plants coincided with the highest peaks of *in vitro* shoot elongation. At the 40°C/35°C regime, cleaning rates gradually increased from 40% at the 12th day to 100% at the 21st day, with no relation to elongation rates.

---

* D/N: day/night temperatures, respectively.

It appears that high elongation rates of the cultures may enhance cleaning only at intermediate temperature and that the increase in cleaning rate due to the higher culture temperature over rides any elongation effect *per se*.

Effect of kind of explant used for rooting. Under the conditions described above, and prior to potting and field transplanting, every differentiated shoot was taken either as a whole or only the stem tip was cut and transplanted into another medium for rooting. The use of stem tips, instead of entire shoots, resulted in 20% to 30% increase in cleaning rates at both the 30°C and the 40°C/35°C regimes.

Effect of thermotherapy to sprouting stakes. Meristem explants of three sizes were isolated from buds of frog skin infected stakes which were sprouted under two temperature conditions.

The rate of cleaning was inversely related to the size of the meristem explant. All plants remained infected when large explants were obtained for culture from stakes sprouted at the lower temperature. However, at the higher temperature, the rates of cleaning achieved greatly increased even when the explants used were medium and large in size (Table 11).

The results indicate the requirements of thermotherapy

Table 11. Interaction of the sprouting temperature of stakes with the size of meristem explants used for culture in the rate of cleaning frog skin infected clones (cv. M. Col 33)

Treatment[a]			Plants with symptoms[c]				
Sprouting Temp.(0°C)	Meristem[b] size(mm)	Symptom-free plants (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
26	0.4	79	14	7	0	0	0
	0.8	16	17	18	33	16	0
	1.2	0	0	0	36	64	0
40/35	0.4	96	4	0	0	0	0
	0.8	65	35	0	0	0	0
	1.2	85	15	0	0	0	0

a. Duration of treatment: 2 weeks

b. Small (0.4 mm) explant comprised 1-2 primordia
Medium (0.8 mm) explant comprised 4 primordia
Large (1.2 mm) explant comprised 6-8 primordia

c. Symptom degree: 1 = mild; 5 = very severe.

Data averaged over 5-6 plants per treatment.

to the stakes during sprouting and of small meristem explants to achieve high cleaning rates. Thermotherapy of 3 to 4 weeks must enhance such rates further.

Cleaning plants from double viral infections. Stakes obtained from frog skin diseased plants of the cv. M. Col 33 were sprouted in the greenhouse, at a mean temperature of 26°C, and under heat therapy, at 40°C/35°C D/N.

Mild leaf mosaic symptoms appeared in the plants differentiated on large shoot-tip explants from the greenhouse-sprouted stakes, the proportion being higher when the explants were cultured at 30°C than at 40°C/35°C (Table 12). Interestingly, the mosaic symptoms were not visible in the original plants in the greenhouse nor in the field, but they showed up through grafting done in collaboration with the cassava Pathologists. However, when the same material was subjected to heat therapy during sprouting, and both small and large meristem explants were used, all plants resulted free of both frog skin and mosaic, even if the explants were cultured at 30°C (Table 12).

Chemotherapy

Amongst many chemotherapeutical substances, virazole or ribavirin seems to interfere directly with the synthesis of a wide range of RNA and DNA viruses. Water solutions of virazole

Table 12. Effect of temperature treatments, during stake sprouting and/or during *in vitro* culture of meristem explants, on the recovery of clean stocks from plants infected with both frog skin and mosaic diseases (cv. M. Col 33).

Treatments[a]			Symptom-free plants (%)[b]	
Sprouting temp.(°C)	Meristem size(mm)	*In vitro* temp.(°C)	Frog skin disease	Mosaic disease
26	-	-	0	0
	1.2	30	0	5
		40/35	82	20
40/35	0.5	30	100	100
		40/35	100	100
	1.2	30	100	100
		40/35	100	100

a. Duration of treatment: 3 weeks

b. Frog skin disease evaluated in 4-5 month old plants; 5-6 plants per treatment.

Mosaic disease evaluated by grafting with clean stakes of cv. Secundina (Collaboration of U. Jayashinge and B. Pineda).

(0 through 40 ppm) were applied to both, rooted stem cuttings in sterile substrate and to large shoot-tip explants in vitro, obtained from the cv. M. Col 33 plants with the frog skin disease.

After 4 weeks of treatment, virazole concentrations above 20 ppm resulted fitotoxic to the stem cuttings and similar levels retarded the growth of the shoot-tip cultures. All plants grown from the stem cuttings remained with the disease symptoms. However, the shoot-tip cultures treated with 20-40 ppm virazole yielded plants completely free of symptoms only if, prior to potting, the stem tip of each culture was cut and used for rooting in a virazole-free medium, following 40-80 days of treatment.

Due to the promise of virazole as a chemotherapeutic agent for the frog skin disease, more studies are underway to enhance the cleaning process through meristem culture.

Regeneration of Varietal Decline

Gradual decline or degeneration of local cultivars may result from the accumulation of latent diseases or from physiological breakdown on time. Preliminary results, of a thesis research with the cvs. M. Col 1438 (Llanera) and M. Col 1468 (CMC-40) are presented here.

Hot water therapy to stakes. Fresh stakes, obtained from plants apparently free of diseases, were immersed in water baths at 40°C and 50°C during 5 to 30 minutes, and then planted in the field for evaluation.

The best treatment, i.e. 40°C for 5 to 20 minutes, increased yield of roots in 20% and the production of planting material in 25% to 30% of the cv. CMC-40; but only 2% increase in root yield and 10% in "seed" production was obtained with the cv. Llanera.

Hot air therapy of stakes during sprouting followed by meristem culture. Meristem tips were isolated for culture from stakes of the two cvs., sprouted either in the greenhouse or under heat therapy, at 40°C/35°C D/N during 4 weeks. The resultant plants were planted in the field along with non-treated stakes for evaluation.

At the third month in the field, differences in leaf lobe size of the cv. Llanera were quite obvious between all the plants grown from stakes and the entire population of plants, i.e. 100 in total, obtained by meristem culture, both with and without prior thermotherapy (Figure 2). Leaf length to width ratios were, in average, 75% higher in the meristem plants than in the stake plants. Furthermore, the narrow lobed leaves were also 10% to 15% thicker than the control (stake plants)

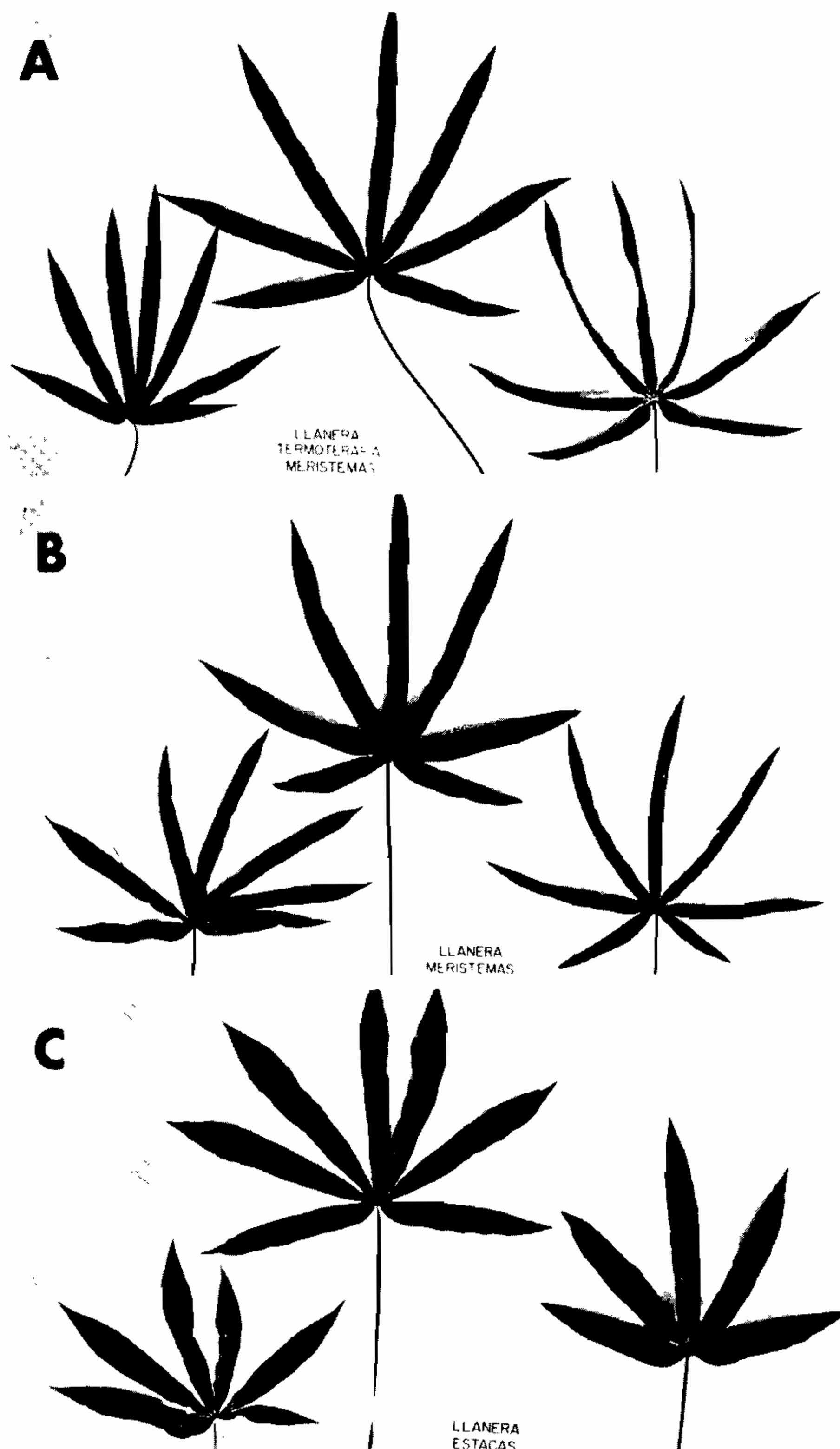


Figure 2. Change in leaf lobe size of the cv. Llanera regenerated from meristem culture. Left to right: lower, middle and upper leaves from 4 month old field-grown plants. A. From meristem culture after heat therapy of stakes during sprouting; B. From meristem culture without thermotherapy; C. From stakes without heat therapy nor meristem culture. Magn. A, B, and C = X 0.2.

due to a 50% increase in width of the spongy mesophyll only. But, the total area of the meristem plant leaves was about 60% smaller than the stake plant leaves. In contrast to Llanera, the leaf length to width ratios of the cv. CMC-40 remained almost unchanged in both meristem and stake plants.

Because the changes observed in leaf size have remained throughout the growth of the plants and failed to reverse to the "normal" broader lobe in reciprocal grafts, the explanation of its possible causality awaits evaluations of root yield, "seed" production, etc. in the following reproductive generations.

Routine Varietal Cleaning-up Process

Work continued to assist the Cassava Program in generating clean stocks from diseased varieties (Table 13). Local varieties from 10 cassava growing regions of Colombia have been selected for cleaning. Healthy clones of those varieties will be handed to the Cassava Program for inclusion as controls in the regional trials.

In collaboration with the Cassava Pathologists, disease detection tests will be applied to the materials prior to processing by the thermotherapy-meristem culture protocol, so as to speed up the process.

Table 13. Sources, diseases and amounts of materials cleaned-up by meristem culture in 1982.[a]

Source	Diseases	No. of varieties[b]
M. Col varieties	frog skin, CBB	124
M. Bra varieties	preventive cleaning	123
M. Per varieties	frog skin, CBB	8
M. Ecu, M. Mex, etc. varieties	frog skin	8
Others		10
	TOTAL	273

a. Three-five stakes per variety were exposed to heat therapy (40°C/35°C, D/N) during sprouting prior to meristem culture.

b. Handed to the Cassava Program.

Germplasm Conservation

Two approaches have been followed for the studies on the conservation of cassava clones *in vitro*: a) through modification of the physical or chemical environment of the cultures, so as to provide "minimal growth"; and b) through storage at ultra-low temperature or "cryogenesis".

Minimal Growth Conditions for Storage

As shown ealier, temperature and illumination interacts with the composition of the culture medium in reducing the culture's rate of growth so as to extend the transfer period (CIAT, Annual Report 1980). However, the extent to which those treatments affect the viability of the cultures was not clearly defined. To test various physical and chemical factors, as they influence the rate of growth and the viability of cassava clones stored *in vitro*, several experiments have been set up. Single node cuttings, from meristem-derived plantlets, were used as explants for the storage experiments.

Growth rate determinations were based on shoot elongation, taking into account that very low rates often are detrimental and very high ones tend to shorten the transfer period. As a measure of viability, the number of shoots formed per culture and the number of axillary buds per shoot

represent the capability for plant regeneration through the culture of node cuttings. Results obtained from the initial 5-6 month of treatment are shown in the Tables 14, 15, 16, and 17, and in Figure 3.

Effect of storage temperature. With the roots initiated on the node cuttings, the cultures were stored under three temperature regimes, with three levels of illumination (Table 14).

The rate of growth of the cultures was directly related to storage temperature, without any effect due to the illumination level. Viability tended to increase, with slightly more shoots per culture, at the medium temperature regime; and at 28°C, the formation of axillary buds doubled and further increased with illumination. Withdrawal of GA and NAA from the medium resulted in 100% increase of axillary buds per shoot, but only at 28°C. Except at the medium illumination level, root growth was always higher than shoot growth.

Storage at medium temperature, with the medium level of illumination, provided conditions for slow enough growth accompanied by good viability.

Effect of osmotic stress. In order to provide high osmotic pressure the culture media for storage was supplemented

Table 14. Effect of temperature on the growth rate and the viability of cassava cultures stored *in vitro* at three illumination levels.[a]

Treatment			Viability[b]	
Illumination (lux)	Temp. (°C)	Elongation of shoots (cm/month)	Shoots per culture (No.)	Axillary buds per shoot (No.)
500	18	0.2	1.0	6.7
	23	0.5	2.0	6.5
	28	1.6	1.3	13.0
1000	18	0.5	1.1	7.3
	23	0.6	1.4	7.0
	28	1.6	1.1	15.5
2000	18	0.4	1.2	6.5
	23	0.5	1.6	7.7
	28	1.5	1.2	17.0

a. Data averaged over 6 cultures per treatment.

b. Represent the culture's potential capability for micro-propagation.

Culture medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA with 3% sucrose and 0.8% agar, dispensed in 25 x 150 mm test tubes.

with three levels of sucrose in combinations with several concentrations of mannitol. While mannitol is not metabolized by the cultures and thus acts as a powerful osmoticum, sucrose though metabolized can exert osmotic action only at high concentration. Media osmotic concentration can affect the growth of cultures through a reduction of water and nutrient uptake by the tissues. The rooted cultures were maintained at two temperatures (Figure 3).

At both temperatures, all mannitol concentrations resulted highly detrimental to culture survival when sucrose was not present in the medium. At the lower temperature, viability increased with 0.25% and 0.12% mannitol levels, only if sucrose was present at 3% and 6%, respectively. With increasing storage temperature, the surviving levels of mannitol were also higher. The extreme stress condition given by low temperature, high sucrose and high mannitol resulted completely damaging to culture survival (Figure 3). Single shoot cultures predominate at the lower temperature, at all mannitol concentrations, increasing to 1.3-1.7 shoots per culture at the higher temperature, with the medium sucrose level. Similar relationships held in the formation of axillary buds.

Thus, in order to provide reduced growth and high viability simultaneously, low concentrations of mannitol and sucrose should be used under low temperature storage. At a

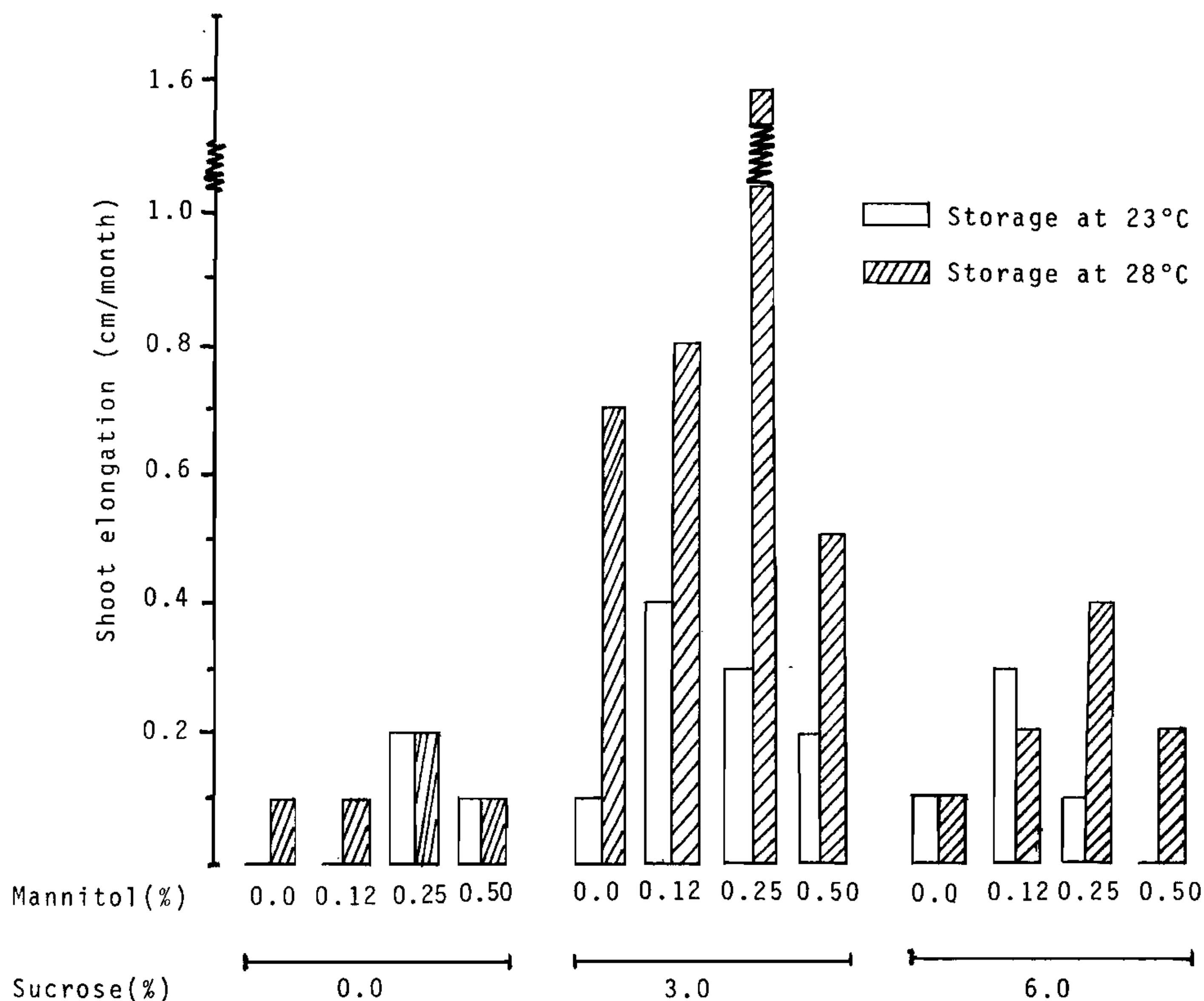


Figure 3. Effect of osmotic stress, provided by mannitol and sucrose in the culture medium, on the rate of growth of cassava stored *in vitro* at two temperatures.

-- Data averaged over 6 cultures per treatment.

-- Medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA, with 0.8% agar, dispensed in 25 x 150 mm test tubes.

higher temperature, increasing levels of both osmotica would be required.

Effects of medium and vessel volume. The volume of medium and the availability of growth space for the cultures should influence the rate of growth and the viability of cultures during storage through various mechanisms, especially by dillution of toxic exudates (e.g. phenolic compounds) from the tissues and sufficient oxigenation for growth. Node cuttings were first rooted, then subjected to two storage temperatures in two types of vessels so as to provide different medium and growth space volumes (Table 15).

At either temperature, the culture's growth rate in the larger vessels was twice as high as in the test tubes; however, their viability, in terms of the number of shoots formed per culture, also doubled (Table 15). The improvement in micropropagation potential due to an increase in growth space could make it possible to maintain high viability, with protracted transfer periods, at storage temperatures lower than 23°C, if large vessels are utilized. Furthermore, both root and shoot appearance were healthier in the larger than in the smaller vessels.

Effect of activated charcoal. Earlier observations pointed out the detrimental effect on culture survival of phenolic

Table 15. Effect of medium and vessel volume on the rate of growth and the viability of cassava cultures stored *in vitro* at two temperatures.[a]

Treatment			Viability[c]	
Temperature (°C)	Volume of vessel[b]	Elongation of shoots (cm/month)	Shoots per culture (No.)	Axillary buds per shoot (No.)
23	small	1.1	1.4	12.7
	large	2.0	2.8	11.2
28	small	1.0	3.6	9.7
	large	2.5	6.1	9.3

a. Data averaged over six cultures per treatment.

b. Small vessel: 25 x 150 mm test tubes with 10 ml of media.
Large vessel: 55 x 140 mm bottles with 70 ml of media.

c. Represent the culture's potential for micro-propagation.

Medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA, 0.8% agar.

exudates by the culture roots to the medium,after the 3rd to 4th month of storage at 20° - 24°C (CIAT, Annual Report 1980). Although dependent mainly on the variety, deterioration due to phenolic oxidation could be reduced by absorbance of such compounds with activated charcoal.

Preliminary results showed, as expected,that charcoal in the medium reduced greatly root browning and deterioration, and leaf fall to about half at low temperature (Table 16).

Surprisingly however, charcoal reduced the rate of growth of the cultures, along with increased viability in terms of shoots formed per culture at both low and high temperature. In this respect, the two varieties tested showed similar tendency, although one exhibited quite high rates of growth (Table 16). It should be possible to either increase the concentration of charcoal or lower the storage temperature in order to reduce growth rates even further.

Effect of growth inhibitors. Growth reduction during storage should be accomplished through the addition to the medium of plant growth regulators that retard or inhibit growth.

A trial on the use of abscisic acid resulted in very strong detrimental effect of the cultures even at the lowest

Table 16. Effect of adding activated charcoal to the culture medium on the rate of growth and viability of cassava stored *in vitro* at two temperatures.

Treatment				Viability	
Temp. (°C)	A.C.[a] (%)	Elongation of shoots (cm/month)	Leaf fall (%)	Shoots per culture (No.)	Axillary buds per shoot (No.)
cv. M. Col 22					
23	0.0	0.8	80	2.0	7.5
	0.5	0.4	40	6.0	5.3
28	0.0	1.5	50	2.0	6.0
	0.5	1.0	40	3.0	8.7
cv. M. Col 1467					
23	0.0	2.1	70	1.0	15.0
	0.5	1.7	60	2.0	16.5
28	0.0	5.1	60	3.0	8.0
	0.5	2.4	50	4.0	4.0

a. Activated charcoal added to the medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA with 3% sucrose and 0.8% agar. Vessels: 25 x 150 mm test tubes.

concentration (i.e. 2.5 mg/l) tested. It is suggested that cassava may be very sensitive to abscisic acid. Hence even lower concentrations should be tried in the presence of sugars which may enhance viability.

Effect of low mineral nutrition. Reduction of growth rate of the cultures could also be accomplished by lowering the level of certain mineral elements of the culture medium. Nitrogen, because of its definite effect on vegetative growth, was first studied. Rooted node cuttings were stored at two temperature regimes in media containing reduced amounts of total nitrogen (Table 17). At either temperature, the culture's growth rate was directly related to the concentration of nitrogen in the medium, becoming detrimental at the lowest concentrations. At 23°C, 40 mM of total nitrogen resulted in a growth rate which provided high viability; and at 28°C, the optimal nitrogen concentration was 10 to 20 mM (Table 17).

Further study is needed on the interaction of nitrogen with other major elements of the culture medium in order to devise a minimal medium.

The judicious utilization of the various physicochemical factors studied should aid to devise the conditions for "minimal growth" storage of cassava with high viability and genetic stability.

Table 17. Effect of medium nitrogen content on the rate of growth and the viability of cassava cultures stored *in vitro* at two temperatures.[a]

Treatment			Viability[c]	
Temp. (°C)	Total N[b] (mM)	Elongation of shoots (cm/month)	Shoots per culture (No.)	Axillary buds per shoot (No.)
23	0	0.1	1.0	3.3
	10	0.1	1.0	5.0
	20	0.2	1.7	4.3
	40	0.4	2.0	6.7
	60	0.4	1.0	12.3
28	0	0.2	1.0	5.3
	10	0.4	1.0	10.3
	20	0.4	1.3	8.2
	40	0.6	2.0	8.3
	60	0.8	2.3	11.9

a. Data averaged over 5-6 cultures per treatment.

b. NO_3 + NH_4 nitrogen in the culture medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA with 3% sucrose and 0.8% agar, dispensed in 25 x 150 mm test tubes.

c. Represents the culture's potential for micro-propagation.

Cryogenics

The growth rate of the cultures could not only get retarded as shown above, but completely suppressed if the tissues are appropriately stored at ultra-low temperature. At the temperature of liquid nitrogen (i.e. -196°C) all the cell metabolic activities enter in a state of suspended animation and thus storage becomes potentially indefinite and the possibilities of genetic change practically eliminated.

Until about a year ago, cryopreservation work on cassava meristems was unsuccessful. Recently however, results from Dr. K. Kartha's studies at Saskatoon, Canada are quite encouraging. The "droplet-freezing" method, as developed at Saskatoon, has increased survival up to 80%, but only a maximum of 20% of the frozen meristems gave rise to plants after retrieval from liquid nitrogen (Figures 4A and 4B). While work to improve the current results goes on, CIAT will continue to collaborate with Dr. Kartha in the provision of materials and in testing clones recovered from cryopreservation.

Perspective

Given the recent advances in both the minimal growth and cryogenics of cassava, a dual clonal germplasm conservation system can be envisaged for the near future: a) the base

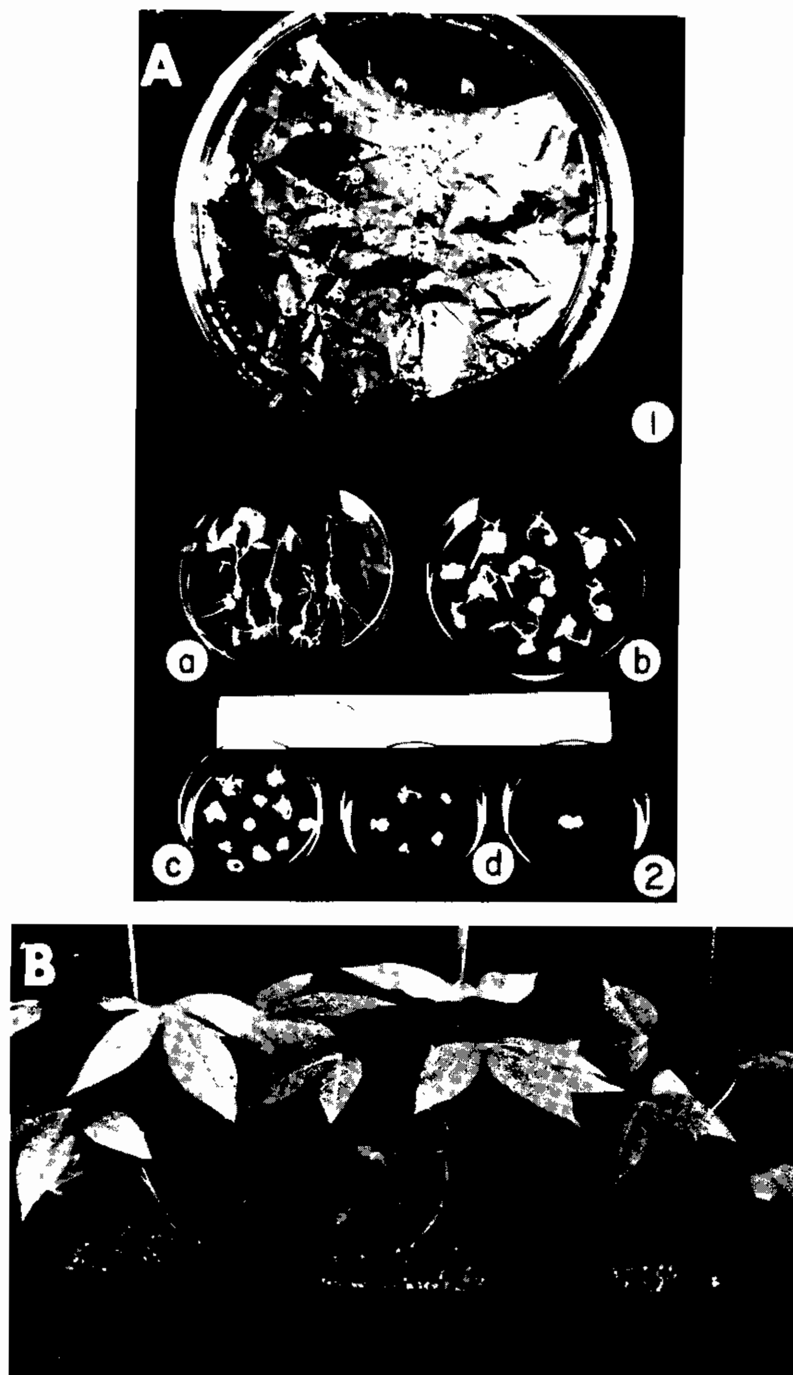


Figure 4. Cryopreservation of cassava meristems.

A: 1. Meristem-tips arranged for freezing on aluminum foil; 2. Variability in the responses of meristems after retrieval from storage in liquid nitrogen: a and b show plant regeneration; c and d show leaf differentiation and callus formation, respectively.
B: Potted cassava plants regenerated from meristems retrieved from storage in liquid nitrogen (-196°C). (Photographs A and B supplied by Dr. K. K. Kartha, P. R. L., Saskatoon, Canada).

gene bank, for long-term storage, using liquid nitrogen; and b) the active gene bank, for short-term storage, using minimal growth conditions.

Both storage methods are complementary to each other. While retrieval from the long-term bank would be very sporadic, turn-over in the active collection would be much higher since it will provide materials for rapid micro-propagation and use for international exchange and regional trials.

Routine Germplasm Storage

Although the conservation methodologies by means of "minimal growth" still require some refinement, a routine work was initiated in 1981 to gradually transfer the field cassava collection into *in vitro* culture form for storage. Rooted node cutting segments, obtained from meristem-grown plantlets, have been utilized as explants for storage.

From a total of 1,346 varieties, more than 50% entered storage in 1982 and the remaining have been under storage for up to 36 months and only a few during 54 months.

The frequency of sub-culture increased with storage time, from about 0.5 in 12 months to 2.5 subcultures in 36 months (Table 18).

Table 18. Source, amounts, storage-time intervals and frequency of sub-culture of cassava materials conserved *in vitro* under minimal growth conditions.[a]

Source of materials	Amount of varieties (No.)	Storage-time interval (months)	Amount of varieties (No.)	Sub-culture ($\bar{X}$ No.)
M. Bra	599	0- 6	704	0
M. Col	531			
M. Per	61	6-12	315	0.6
CIAT hybrids	50			
M. Ven	30	12-24	255	1.2
M. Mex	18			
M. Cub	14	24-36	64	2.5
M. Ecu	12			
Others	31	36-54	8	3.4
TOTAL	1.346[b]	TOTAL	1.346	

a. Medium: MS + 0.02 mg/l BA + 0.1 mg/l GA + 0.01 mg/l NAA, with 3% sucrose and 0.8% agar, dispensed in 25 x 150 mm test tubes.

Conditions: Temperature = 23° - 24°C, illumination = 2000 lux, photoperiod = 12 hrs.

b. Until November, 1982.

International Exchange of Clonal Material

Work continued this year to utilize *in vitro* methods for the exchange of selected materials (advanced hybrid lines and varieties) with the National Programs of several countries.

The *in vitro* system, even though it provides adequate safeguards to minimize the risk of disease dissemination, is only effective if handled by well trained personnel in the receiving institution. Training in the basic methodologies to recover and propagate plants from the imported cultures has been carried out for the personnel of most countries. This strategy has elicited the establishment of minimum facilities and results of the work carried out in several national programs have been quite successful (Figure 5).

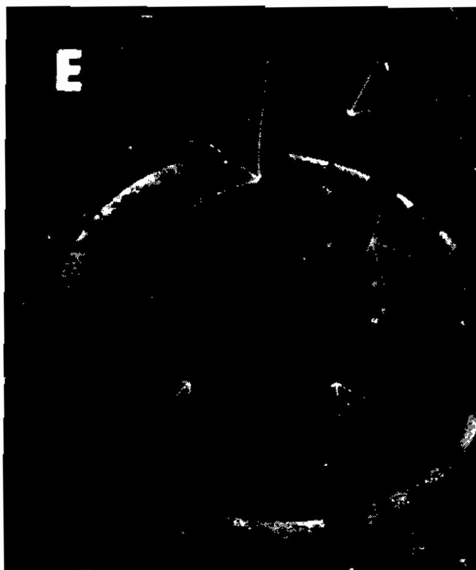
Distributions from CIAT

In 1982 a total of 80 cassava varieties have been distributed *in vitro* to 9 countries (Table 19). Node cuttings, obtained from meristem cultures, prepared in turn from stakes sprouted under heat therapy, were rooted and grown under high illumination prior to packing in polystyrene containers. In many instances, the node cuttings were obtained from the collection stored *in vitro*. It is expected that all future shipments will be prepared from the *in vitro* gene bank.

Figure 5. Distribution of CIAT cassava materials *in vitro*.

A, B and C: micropropagation, potting and leaf-bud rapid multiplication, respectively in South Africa.
D: Field evaluation of CIAT germplasm introduced *in vitro* to Indonesia.
E: Potted plants recovered from *in vitro* cultures imported to the Philippines.
F: Field evaluation of CIAT lines sent *in vitro* to Costa Rica.
(Source of photographs: A, B and C supplied by Ms. T. Trench, A.A.C.C.R., Mtunzini, South Africa; D by Ms. N. Zuraida, I.F.C., Bogor, Indonesia; E by Dr. R. Barba, U.P.L.B., Philippines; F by Dr. L. Müller and Mr. H. Serrano, CATIE, Costa Rica).

D


E

F

Table 19. Cassava material distributed in 1982 as *in vitro* cultures from CIAT to other countries.

Country	Institution	Amount of varieties (No.)
México	INIA- Zacatepec	10
Nicaragua	IICA- MIDINRA	7
Malaysia	MARDI	9
Philippines	Univ. Los Baños	6
Barbados	Min. Agric.	3
China	South China Inst. Bot.	6
Israel	Agric. Res. Org.	2
South Africa	Center Cassava Res.	12
	C.E.D.	12
U.S.A.	Univ. Florida	10
	Dekalb Agri. Research	3
	TOTAL	80

Every shipment, mostly sent via air mail, was accompanied by a descriptive list of the materials, and a phytosanitary certificate. A "phytosanitary statement" has been prepared for inclusion in the package; it describes all treatments and indexing procedures carried out on the materials in order to increase the probability of being free of disease and pest.

Introductions to CIAT

Arrangements made with CENARGEN (Brasil) and ICA (Colombia) have permitted in 1980-81 to carry out the transfer of 270 cassava varieties as meristem cultures from the collection held at the CNPMF (Cruz das Almas - Bahía).

This year, with the collaboration of IBPGR, nearly 350 cassava cultivars have been brought to CIAT as meristem cultures. Most of these materials were held as collections in EMGOAPA (Goias), EUPAE (Manaus), Rio Grande do Sul and CPATU (Pará), and some from Paraiba and Mato Grosso. A few stakes per variety were grown at CENARGEN where the meristem culture for transfer was undertaken.

RICE ANTHER CULTURE

Production of Homocygous lines

Once the basic technique to induce plants from the male gametes by culturing anthers was established (GRU Annual Report, 1981), the genetic consequences of anther culture, i.e. that homocygous diploid plants are produced directly from the haploid meiotic segregants, needed evaluation.

Anthers were obtained from field-grown plants of three F_2 crosses and one variety. Chromosome counts on root tips of the regenerated plants showed, in average, 60% diploids and the remaining all haploids (x = 12).

To elucidate the question whether the diploid plants resulted from spontaneous chromosome doubling (through endomitosis or endoreduplication) during the *in vitro* stage or originated from somatic cells of the anther, every anther-culture diploid line was grown in the field and segregations in the plant's features were monitored. In contrast to the F_2 donor plants, each anther-derived line remained completely homogenous during its growth and development and throughout three sexual generations; hence, the lack of segregation proved their homocygocity. However, differences in plant height and precocity were quite obvious among lines, thus

corroborating their distinct genetic origin from the pollen microspores.

Variability

With strong genotype dependance, certain abnormalities were observed in a few of the regenerated plants. Albinism was nearly 80% in the cv. Bluebonnet-50, but very low or nil in the other genotypes. Two diploid homocygous lines of the same cv. developed a spikelet sterility problem, very similar to the so called "straight head". This abnormality passed through three seed generations, which suggests a mutation origin.

Applications

An obvious practical use of anther culture is the production of homocygous diploid lines from F_1 hybrids for evaluation by the breeder.

If a trait is to be incorporated into a given line or cultivar, only one individual would need to be identified which has obtained the entire chromosome complement from one of the parents, except for the chromosome carrying the gene whose introduction is desired. In the case of a trait controlled by recessive genes, it would quickly express in the

doubled haploids.

Aluminum toxicity is one important problem of upland rice in Latin America, and tolerance seems under the control of recessive genes.

In collaboration with the Rice Program, a thesis research on anther culture for the selection of lines with tolerance to aluminum toxicity has been initiated. Out of 24 varieties, supplied by the upland rice breeding section, 10 tolerant, 6 intermediate and 8 susceptible varieties were selected by means of the Relative Root Length technique.

As shown before (GRU Annual Report, 1981) the success in anther culture greatly depends on the variety. The same 24 varieties were screened for their responsiveness to anther culture in order to select varieties with high tissue culture capability, both within the tolerant and the susceptible materials, and used as parents for F_1 reciprocal crosses. These hybrids will be the donors of anthers for the study.

Interestingly, most varieties which had high frequency of callus induction were at the same time tolerant to aluminum toxicity and, conversely, the most susceptible ones had low capability for callus induction (Table 20).

Screening organogenic capacity of the 24 varieties is underway; and F_1 reciprocal crosses of tolerant with susceptible varieties have been grown in the field for the extraction of anthers.

Table 20. Capability for anther culture of 24 rice varieties tolerant, intermediate and susceptible to aluminum toxicity.

Frequency of callus induction (%)[a]	Varietal response to aluminum toxicity[b]			
	Tolerant (No.)	Intermediate (No.)	Susceptible (No.)	Total (No.)
20-50 (high)	8/10	2/6	1/8	11/24
10-15 (medium)	1/10	1/6	2/8	4/24
<10 (low)	1/10	3/6	5/8	9/24

a. % of 400-500 anthers plated per variety.
b. Evaluated by the Realtive Root Length Technique.

TISSUE CULTURE OF *STYLOSANTHES* SPP.

Cell and tissue culture methods in *Stylosanthes* spp. could be utilized to: a) induce plants from the meiotic segregant cells, by means of anther/pollen culture, to directly produce homocygous diploid plants; b) treat cells, preferable haploid, with physical or chemical stresses and select strains tolerant to a specific stress; c) obtain interspecific somatic hybrids through protoplast fusion.

In order to al least attempt any of those possibilities, it is of paramount importance to establish methodologies for the regeneration of plants from the anther microspores and from the somatic cells of other tissues.

A Thesis research project (S. Jaramillo) was initiated this year to study the potential of anther and cell/callus cultures for the regeneration of *S. guianensis* and *S. capitata* plants.

Anther Culture

The ability of the microspore to follow a sporophytic rather than a gametophytic development during anther culture depends on a high degree on the stage of the microspore's ontogeny following meiosis. Such stage seems optimal at

approximately the first microspore mitosis, i.e. early to late uninucleate microspore.

Flower buds of varying sizes were obtained from two F_1 hybrids and two ecotypes of S. guianensis and two other ecotypes of S. capitata and used to study the relationship between bud size and microspore development. From nearly 8,000 and over 3,000 microspores observed in S. guianensis and S. capitata, respectively, most flower buds of 1.7 to 2.8 mm in size contained microspores at the tetrad to uninucleate stages of development in both species (Figures 6A and B). However, the buds of S. capitata were slightly larger than S. guianensis for the same microspore stages (Table 21).

Callus induction on anther culture was influenced by the genotype and to a lesser extent by the culture medium. The inclusion of cytokinins in the medium promoted callus in most genotypes. F_1 hybrids of lines with high (50%) and low (30%) callus induction produced higher frequency (over 65%) of callus induction (Figure 6C).

Upon transfer of the calli to low hormone media, shoot and leaf primordia began to differentiate on their surface (Figure 6D).

Table 21. Frequency of occurrence of the microspore developmental stages in flower buds of two sizes of *S. guianensis* and *S. capitata*.

	S. guianensis		*S. capitata*	
Bud size (mm):	1.0-1.7	1.8-2.8	1.0-1.7	1.8-2.8
Microspores (No.)[a]	352	7,554	454	2,634
Microspore stages				
Mother cells(%):	30	0	50	0
Tetrad to uninucleate(%):	70	62	50	80
Binucleate (%):	0	38	0	20

a. Total number of microspores observed at each bud size.

Cell and Callus Culture

Callus was induced on leaf segments obtained from greenhouse-grown *S. guianensis* plants. Organs differentiated on the callus under conditions similar to anther-callus culture. These results corroborated the findings of L. Mroginski in Saskatoon, Canada.

Using either, anther-derived or leaf-derived calli, the

Figure 6. Anther and cell/callus culture of *Stylosanthes* spp.

A. Microspore tetrad just liberated from callose walls, following meiosis.

B. Microspore at the middle to late uninucleate stage, as for anther culture.

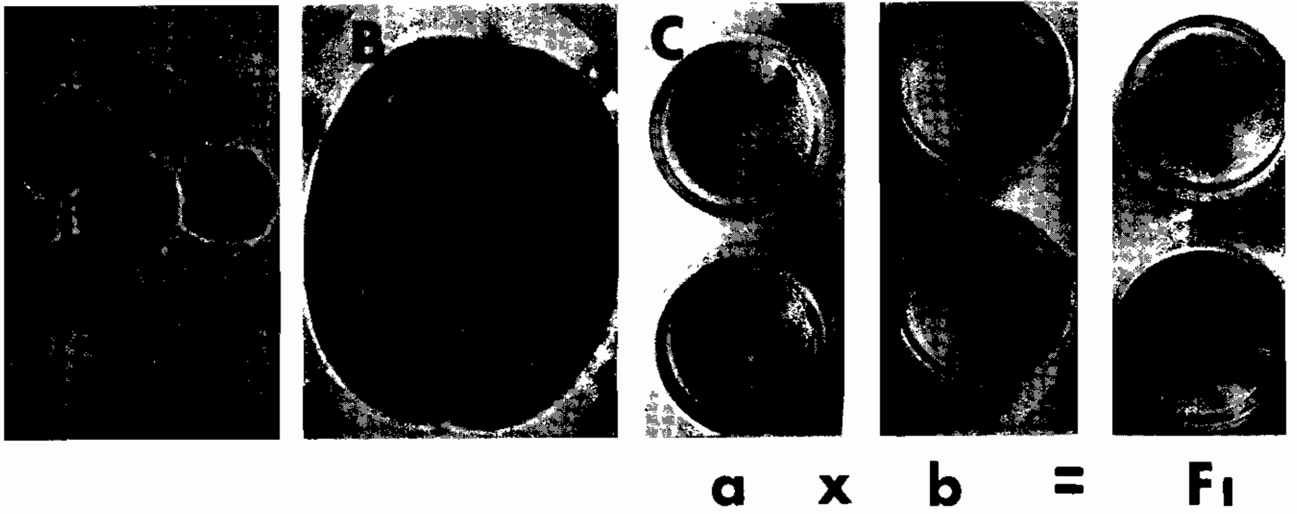
C. Effect of hybrid vigour on the induction of callus on anthers of *S. guianensis*: a = 1063 (line with high induction); b = 1020 (line with low induction).

D. Re-differentiation on callus from anther or leaf tissue (arrows).

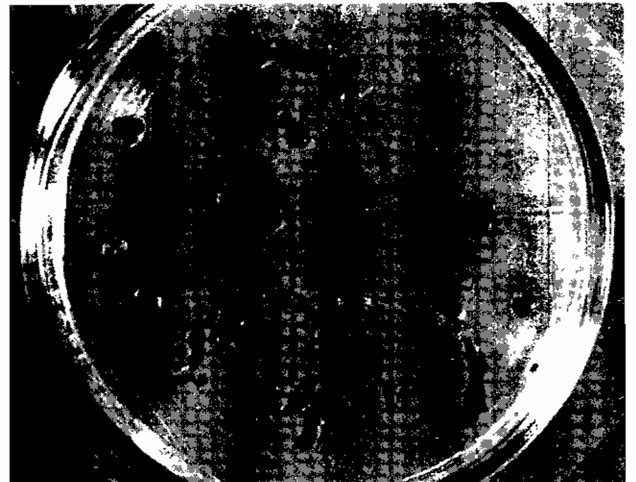
E. Organogenesis on fragmented callus as at D.

F. Further shoot proliferation.

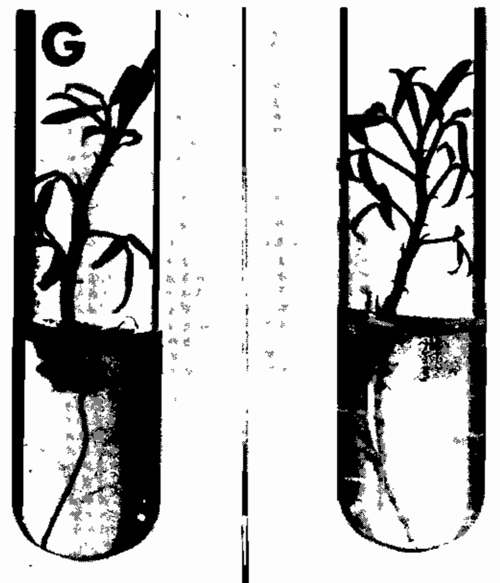
G. Rooting and growth of plants from anther or leaf-tissue callus of *S. guianensis* and *S. capitata*.


B
C
a x b = F1

D



F


G

organogenic callus was dissected into small fragments which quickly formed single (Figure 6A) or multiple (Figure 6F) shoots. Rooting occurred readily on single shoots (Figure 6G).

Regeneration Potential

In comparison with most species in which the differentiation of plants from cell/callus cultures is possible, Stylosanthes has shown a very high potential. A callus mass, induced on either anthers or leaf segments, can be framented into 16 parts; each part can differentiate 10 meristematic nodules, which in turn give rise to 3-5 shoots accompanied by a basal organogenic callus. Proliferation of an organogenic callus can result in 3-4 shoots, which when cut and rooted give rise to complete plants (Figure 7). The whole cycle can yield nearly 1,800 plants in a period no longer than 3 months.

Variability

If the callus grown from the anther has originated through mitosis induced on a microspore, it is reasonable to expect a degree of variability, e.g. vigor, size, growth habit, etc., among the regenerated plants.

Out of 202 plants of the F_1 cross 1021 x 2160 of S. guianensis, 5 plants with bushy growth habit, 32 with erect

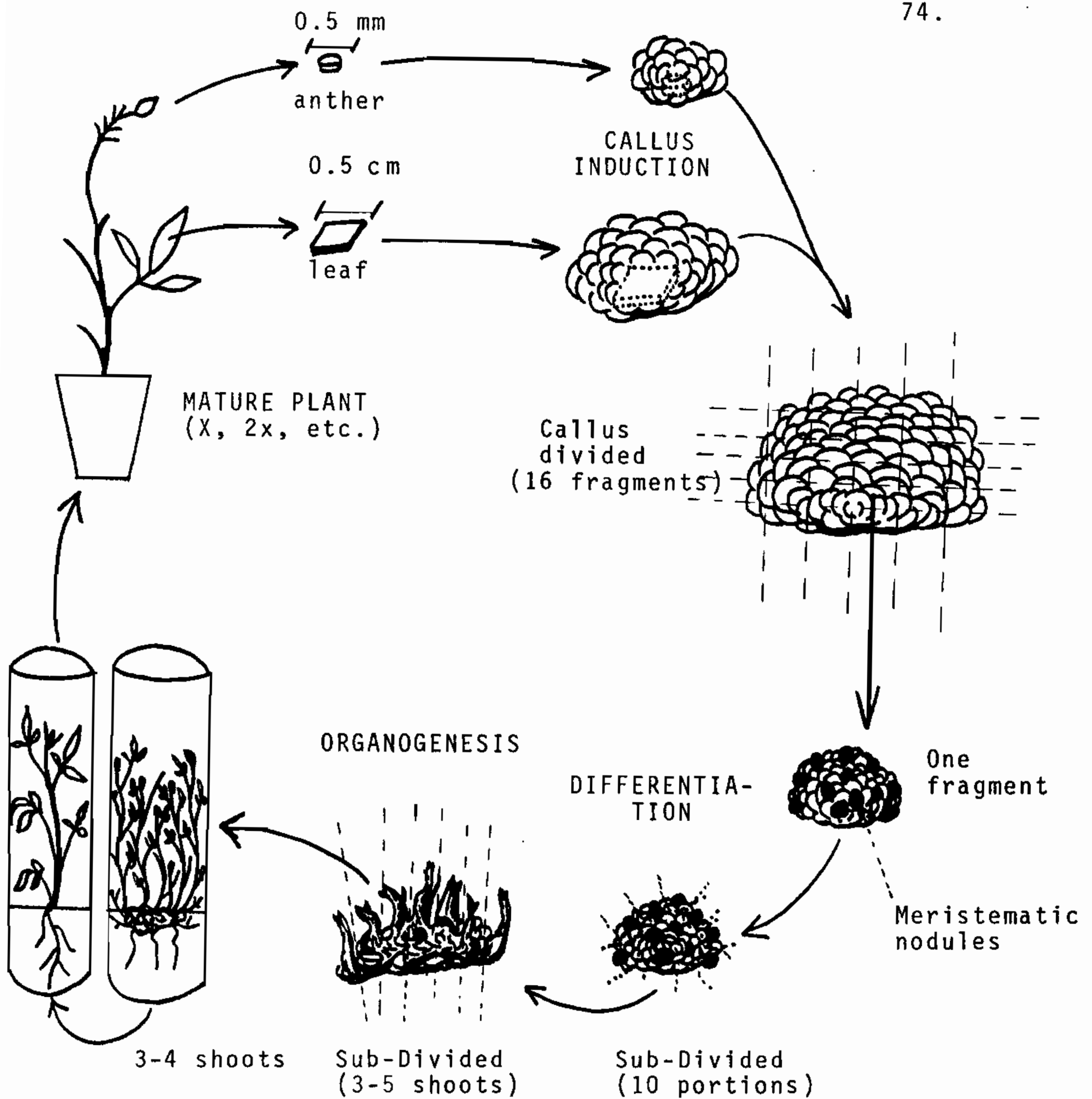


Figure 7. Schematic representation of organogenesis and plant proliferation on cell/callus cultures induced from anthers and/or leaf segments in Stylosanthes spp.

stems and 5 with small leaves have so far been observed after one month of growth in the greenhouse. Initial work on chromosome counting of root tips cells showed a range of chromosome complements from 14 to 23 (2x = 20). Further cytological work, as well as field evaluations of homogeinity within the anther-derived lines should provide additional data on the origin of the regenerated plants.

Perpectives

The tissue culture capabilities of *Stylosanthes* spp. open possibilities for tissue culture manipulation which should pave the way to practical utilization as an aid in the genetic improvement of the species. Anther culture could provide a means to quick homocigocity, hence pure lines for evaluation by the breeder could be produced;inter-specific hybridization may be attempted by crossing di-haploid *S. capitata* with diploid *S. guianensis* or through protoplast fusion; the selection of tolerance at the cellular level to diseases or physiological stresses could be tried.