Annual Report 1995

GENETIC RESOURCES UNIT

November 1995

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PROJECT AREA: GENETIC DIVERSITY

Genetic Resources Unit. Annual Report 1995

The Genetic Resources Unit of CIAT (GRU) passed two Reviews during 1995:

- the Fourth External Programme and Management Review of CIAT (EPMR) in early 1995,

- the Internally Commissioned External Review (ICER) on CGIAR Genebanks Operations, 3-6 August 1995.

With the Signing of the Trusteeship Agreement between the CGIAR and FAO in October 1994, and with the coming of the Fourth Technical Conference of FAO on Plant Genetic Resources of Leipzig in June 1996, the collections maintained in trust by CIAT GRU are becoming recognized internationally as part of the basis of food security and sustainable agriculture worldwide, and as part of a wider effort of conservation of agrobiodiversity. Given the importance of CIAT collections and acknowledgements of additional responsibilities, the GRU had to upgrade its activities to the standards established by FAO/IPGRI for international base collections.

By March 1995, the GRU completed internal diagnostic documents on the status and maintenance of the three commodity germplasm collections, which served as basis for the ICER Review on CGIAR Genebanks Operations. By October 1995, the GRU operations scheme was reviewed and a short and medium term plan for upgrading was established.

On the other hand, by October 1995, CIAT adhered to the implementation of the CGIAR's System-wide Information Network for Genetic Resources (SINGER) and prepared plans for its input into the SINGER that would make germplasm collection data available worldwide through emailing.

Project UG01. Title: Conservation of Agrobiodiversity of *Phaseolus* **Beans** 4600

Purpose: To assemble, conserve and characterize the **Phaseolus** beans germplasm by using established management methodologies and techniques, as well as to develop strategic research to build up a more comprehensive, useful and readily available collection.

Outputs and Activities:

UG01.1 Assembling. A *Phaseolus* collection assembled, including germplasm of the five cultivated species and their wild relatives.

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(i) Status of the *Phaseolus* beans collection. In 1995, the *Phaseolus* germplasm collection grew to 27,918 available accessions. The percentages of the collection for the five cultivated species and their respective wild ancestral forms are as follows: *P. vulgaris* with 25,045 accessions (89.7%), *P. lunatus* with 1,548 accessions (5.5%), the complex *P. coccineus* and *P. polyanthus* with 890 accessions (3.2%), *P. acutifolius* with 271 accessions (1.1%). On the other hand, the truly wild non-cultivated species, 19 species, represents 0.6% of the collection. (Table 1.)

(ii) Acquisition: Given the present size of the collection, and following the criteria established in the last years, the acquisition of *Phaseolus* beans is a more selective process by which only worthwhile germplasm is considered for introduction into the CIAT collection. Thus, a total of 307 materials from five countries, i.e. Mexico, Guatemala, Honduras, Colombia, Argentina, were accepted for introduction during 1995. All these accessions were the result of different collecting missions, and corresponded almost exclusively to landraces (74%) and wild forms (22%) of *P. vulgaris, P. lunatus, P. coccineus, and P. polyanthus.* A smaller percentage (3%) of truly wild non cultivated species were also introduced (Table 2). Special mentioning merits the germplasm from Guatemala and Colombia, due to their importance for providing possible clues in the evolutionary domestication process; intensive studies are underway to analyze this germplasm.

UG01.2 Conservation: To produce adequate seed quality and quantity to meet FAO/IPGRI standards for short-term and long-term conservation internal responsibilities, as well as to face new worldwide challenges such as restoration and safe duplications.

(i) Increase. A total of 1,322 accessions were processed in the meshhouses at Popayan (1,800 masl.) as part of the process of increase and introduction of new germplasm into the collection. In many instances, this process demands to plant an accession several times, before it is incorporated in the bank, in order to obtain the minimum quantity of seeds required for the subsequent multiplication in Tenerife; as a result of this increase process, 483 accessions were assigned with a new G number and incorporated into the bank. Following recommendations of the External Panel Review, plans are underway to expand the meshhouse area needed for the inial increase of the cultivated species and their wild ancestral forms, with special emphasis on the complex *P. coccineus-P. polyanthus* due to their outcrossing nature. Two big meshhouses were already built, and there other two planned for 1996.

(ii) Multiplication. Two phases are carried out simultaneously for this purpose. One phase is carried out under field conditions at Tenerife (2,000 masl.), for materials that were previously increased under meshhouse conditions in Popayan, and requiring further multiplication to obtain the desired seed quantity for short and long term storage. A total of 1,353 accessions were multiplied in this location, mostly of *P. vulgaris*. Another phase is carried out under meshhouse conditions at Popayan (1,800 masl.) for materials having low number of seed stock, therefore, requiring special treatment to insure seed production. A total of 90 accessions were processed for this purpose. As a result of the External Panel

Review recommendations, the planting area of the isolated field at Tenerife has been already duplicated in order to augment the number of accessions multiplied by year, as well as to increase the number of seeds per accessions, as an attempt to fill several present and future compromises, i.e. short-long term storage, viability monitoring, safe duplication and restoration to NARS.

(iii) Core Collection. The goal established two years ago to "clean" of diseases the core collection continued during 1995. With the new set of 252 additional accessions which were cleaned this year, about 80% of the core collection of *P. vulgaris* (1,400 accessions) is already clean and available for international distribution. All the core accessions already cleaned have been stored under short term storage, as a separate set from the main collection. A biochemical- molecular characterization of this core is underway and its advances are reported in another section.

(iv) Long term conservation: At present there are a total of 6,579 accessions of *Phaseolus* beans already placed under long term storage, representing about 24% of the total collection. A new set of almost 1,000 accessions, of the germplasm multiplied at Tenerife, will soon be incorporated into this type of storage.

UG01.3 Documentation: Documentation of the *Phaseolus* collection as an essential tool for improving data management and basic foundation for analysis of the genetic variability available.

After completing the transference of all data to the new application under ORACLE software, additional improvements are underway such as the incorporation of images into the database and the agreement to put the database available to the international community via INTERNET; the latter commitment will be implemented through the SINGER project in a period of two years.

UG01.4 Distribution: Make germplasm and related information freely available to scientists in national and international institutions.

(i) Seed distribution. The GRU distributed 5,656 accessions of *Phaseolus* beans. The Bean Program was the major requester with 5,431 (96%), whereas 225 (4%) accessions were distributed internationally to 17 countries (Tables 3 and 4). The highest percentage of the requests corresponded to *P. vulgaris* (81%), followed by *P. lunatus* (11%), *P. acutifolius* (5%), and *P. coccineus* (2%); the remaining corresponded to mixtures. The international distribution of germplasm is now a slower process due to changes made by the Colombian government regarding the tests needed for issuing phytosanitary certificates.

(ii) Phytosanitary seed testing. A total of 3,009 *Phaseolus* beans samples, intended for international distribution from both the Bean Program and the Genetic Resources Unit, were checked by the Seed Testing Lab. for virus, bacteria and fungi. The general results

showed a slight improvement in the percentage of clean materials (68%) compared with 1994 (61%). (Table 5). However, there are still rather appreciable percentages of the fungus *Macrophomina* affecting materials from the Bean Program. Likewise, the percentages of positives for BCMV are rather high for materials from the Bean Program, as well as for the Genetic Resources Unit. Also, an increase in the percentages of Bean Southern Mosaic Virus (BSMV) positives was detected in 1995 (Table 6). Concerning the Genetic Resources Unit, a new approach is underway to improve the efficiency of cleaning of the germplasm which includes several sequential steps of phytosanitary checking at the greenhouse initial increase, and then in the greenhouse afterwards.

Perspectives:

As a result of the major review events mentioned in the introduction, an upgrading plan has been already initiated for the *Phaseolus* beans germplasm collection; this plan has will be accomplished in the short and medium term (2-6 years). The first steps have been the increase of meshhouse space at Popayan for the initial increase and, also, the expansion of the field planting area at Tenerife for the subsequent multiplication and cleaning of the germplasm. The next step, which is expected to be accomplished during 1995, will be the establishment of a routine monitoring of seed viability; an intensive training of the genebank support personnel has been initiated on this subject. Likewise, in order to meet the compromise acquired under the SINGER project for putting the data of the germplasm collections on INTERNET, an action plan for 1996 has also been drafted which includes not only the transference of the information to the SINGER project but, also, a complete review, updating, standardization and validation of the present existing data.

Project UG02: TROPICAL FORAGES GERMPLASM 94610

Project Purpose

Routine management and strategic research for forage genetic resources, to improve efficiency in germplasm management, and enhance germplasm utilization by increasing knowledge on a wide range of tropical forage species.

Rationale

The main advances in germplasm improvement in tropical forages have been through exploiting the natural plant genetic diversity among and within species. This is still an important area for germplasm improvement, and acquisition, characterization, and conservation of a comprehensive germplasm collection of wild legume and grass species with forage potential will remain important activities. In addition, several species maintained in the collection proved to be useful for soil cover and erosion control, and thus provide an additional service of soil improvement. The collection of wild tropical legumes and grasses maintained at CIAT is one of the largest worldwide, which represents a wealth of plant diversity from the main centers of biodiversity: tropical America and South East Asia for tropical legumes, and Africa for grass genera.

Present Status of the Tropical Forages Germplasm Bank

The tropical forages germplasm (TFG) collection consists of more than 180 genera with more than 750 wild, undomesticated species of possible forage potential. About 90% of the collection belongs to the legume family and 10% to grasses (Table 7). More than 50% of this collection belong to the leguminous genera *Stylosanthes, Desmodium, Centrosema, Zornia* and *Aeschynomene*, in order of importance (Table 8).

At present, there is sufficient seed of 15,927 accessions available for distribution. The main needs with respect to the germplasm collection are to ensure its integrity against the risk of loss through adequate duplication of seed of high quality, and to rationalize activities with other tropical forage genetic resource centers. In addition, there is a need to strengthen research in seed physiology to ensure optimum conservation at best cost opportunities.

UG02.1 Acquisition and Introduction

The TFP's general research strategy is to exploit the natural genetic variability of undomesticated species, especially of legumes. In recent years, acquisition of new germplasm was strategically focused on filling in geographic and genetic gaps, and in response to international requests to specific needs. In 1995, new germplasm of *Arachis pintoi* (eight accessions) was obtained from Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia (CENARGEN), Brazil,and other 44 accessions of legumes which originated predominantly from the germplasm collected in Thailand and Vietnam and recently released from phytosanitary follow-up (Table 9).

UG02.1.1 Post-entry phytosanitary follow-up

The tropical forage germplasm received by CIAT has to undergo a post-entry phytosanitary follow-up by the Instituto Colombiano Agropecuario (ICA), in collaboration with the TFP's Phytopathology Section, before initial seed multiplication can be undertaken. Plants are grown in a special glasshouse until flowering and are visually checked at different growth stages. If any peculiarity appears, additional laboratory tests are carried out, especially with the assistance of CIAT's Virology Research Unit (VRU).

The VRU has taken over the responsibility to index all germplasm accessions of all the tropical forages species being introduce into Colombia by CIAT . In 1995 the VRU indexes 157 individual plants by serology, electron microscopy and gel electrophoresis of viral nucleic acids. The different virus encountered in *Arachis, Calopogonium, Stylosanthes, Brachiaria* and *Paspalum*, are being characterized to develop reliable diagnostic techniques to facilitate the international exchange of tropical forages germplasm.

In 1995 33 accessions of legume and grass present in the phytosanitary follow-up were released (Table 10).

The post-entry phytosanitary follow-up has become a severe bottle-neck for clearing a backlog of accessions which have not yet been increased. Phytosanitary and seed health testing procedures had been compiled in 1993 (Kelemu, 1993). In 1995 a working group was formed to assist ICA in establishing the procedures for the introduction of tropical forage germplasm to Colombia. This may include making use of the ICA quarantine facility at Mosquera, Bogota, if it can be demonstrated that tropical materials will grow in this temperate environment.

UG02.1.2 Proposed activities for 1996

- Establish proper procedures for post-entry phytosanitary follow-up in collaboration with ICA, including provision of additional glasshouse space.
- Process backlog (priority list with TFP assistance)
- Continue to index tropical forage germplasm for diseases (VRU).

UG02.2 Conservation

UG02.2.1 Status of the Collection

The collection of wild tropical legumes and grasses maintained at CIAT is one of the largest worldwide, and represents a wealth of plant diversity from the humid and subhumid tropics, in particular, legumes from tropical America and South East Asia and grasses from Africa. Acquisition commenced in 1971. The collection now consists of approximately 21,000 conserved accessions of 186 genera and 752 species with forage potential of which 90% are legumes and 10% grasses.

The actual collection status for the 18 "key" species is shown in Table 11. More than 87% of the germplasm of them has been multiplied and is available for distribution.

UG02.2.2 Initial multiplication

After acquisition and release from post-entry phytosanitary follow-up, germplasm is multiplied. Initial multiplication was carried out in CIAT's greenhouses and fields at Palmira, Quilichao and Popayan (Table 12). So far 77% of CIAT's Tropical Forages Collection (15,927 accessions) has been increased and has enough seed for distribution.

On the basis of seed stored both in short and long term conditions 50% has more than 5,000 seeds, and thus meets the preferred FAO/IPGRI standards of more than 2,000 seeds stored. However about 31% was increased more than ten years ago. The remaining accessions of original seed which has not been multiplied yet is classified as backlog. Even though the backlog was greatly reduced this year there are still 4,708 accessions to under go initial multiplication, being the main proportion in the key genera *Desmodium* and *Stylosanthes*. About 41% the backlog is originally from Brazil and Colombia. In addition to this backlog there is the seed that has been stored for long time that need regeneration since viability decreases with time.

Besides the seed bank there are also collections of some valuable original materials, particularly grasses, which have problems to produce good quality seed (Table 13). Nevertheless, it is expensive, difficult and risky to keep these collections in field conditions an thus the possibility of keeping some on these materials in vitro needs to be explored.

UG02.2.3 Long-term storage

One third of the legume germplasm is now conserved in the base collection under long-term storage conditions, and ten percent of the grasses are now stored in the base collection (Table 14).

There is a need to know which factors influence seed quality and thus determine the time span until regeneration is necessary. This may vary widely between species. However, more resources are required to allow initiation of adequate research on the physical and chemical factors affecting viability and longevity of seeds in storage to make conservation more effective.

UG02.2.4 Reproductive biology

The seed coat colour work for the study of outcrossing in *Centrosema plumieri, C. acutifolium* and *C. macrocarpum* continued this year.

UG02.2.5 Proposed activities for 1996

Needs for up-grading the CIAT GRU were documented during internal and external reviews of genebank operations. The main needs were determined in the area of conservation: processing of backlog accessions, multiplication, safety duplication, seed viability and seed health testing, long-term storage, and research into seed physiology.

- To make any progress in the up-grading of the CIAT genetic resources activities, conservation needs to be emphasized, especially processing of backlog accessions, multiplication, safety duplication, seed viability and seed health testing, long-term storage, and research into seed physiology. However, the up-grading can only be achieved if resources are available.

- Continue determination of mode of reproduction in *Brachiaria* germplasm accessions and start a study of outcrossing of sexual accessions of *Brachiaria*.

UG02.3 Characterization

UG02.3.1 Morphological characterization of *Arachis* germplasm of *Caulorrhizae* section

The morphological study carried out in collaboration with a Universidad Nacional de Colombia thesis student included 29 accessions of *Arachis pintoi*, recently introduced to CIAT-Palmira. Thirty-two IBPGR (1990) preliminary descriptors were used for *Arachis*, but taking into account those that proved most discriminating in the work of Maass et al. (1993) and Bermúdez (1994). Regarding morphological characterization, the most homogeneous quantitative characters among accessions were diameter of the standard petal and length of the basal segment of the fruit, with coefficients of variation of 9.45% and 8.88%, respectively. Those that varied most were petiole length and isthmus length, with coefficients of variation of 77.18% and 35.48%.

UG02.3.2 Biochemical characterization of Arachis germplasm of *Caulorrhizae* section

The biochemical characterization of isoenzymes was carried out with the collaboration of the same student of the morphological characterization on 52 accessions of *A. pintoi* and the only two accessions of *Arachis repens* to flower under CIAT-Palmira conditions. The methodology used was established by Ramírez et al. (1987), and modified for *A. pintoi* by Maass et al. (1993). Peg tip tissue was used for isoenzyme extraction. Six isoenzymes were studied: αß-esterases (αß-EST), α-esterase (α-EST), ß-esterase (ß-EST), αß-acid phosphatase (ACP), glutamate oxaloacetate transaminase (GOT), diaphorase (DIA), and peroxidase (PRX).
For SDS-PAGE electrophoresis of total seed proteins, the methodology used for extracting bean seed proteins was that of Hussain et al. (1988), modified for *A. pintoi*. One seed (increased seed) for each of 12 accessions of *A. pintoi* and the two accessions of *A. repens* was used in the monodimentional electrophoresis.
Partial results do not show correlations between groupings of biochemical polymorphism and the geographical distribution of the target accessions. The isoenzymes that show greater polymorphism, in descending order, are the esterases, DIA, GOT, ACP, and PRX.
No biochemical differentiation was established between the two species of Caulorrhizae. The qualitative descriptors (seta on the border of the apical leaflet and color of the standard petal) were constant in all accessions.

Stylosanthes guianensis

The results of PAGE-electrophoresis of four isozymes (α-EST, ß-EST, αß-ACP, and DIA) and of native seed proteins indicated that this germplasm collection presents broad intraspecific

polymorphism with these biochemical genetic markers. The characterization of 561 accessions yielded 523 different banding patterns, when the banding patterns of the tested markers were combined (93% discrimination obtained). Furthermore, 499 of these patterns are unique, indicating that the accessions presenting these patterns differ genetically.

Therefore the *Stylosanthes guianensis* collection at CIAT probably contains a relatively small number of genetic duplicates. These 561 accessions, a randomly representative sample, account for over 56% of the 1,000 accessions of this species held at CIAT.
These preliminary results indicate that the *S. guianensis* collection has broad intraspecific variability regarding these markers. Furthermore, the PAGE-electrophoretic patterns of the isozyme αß-ACP and of native seed proteins of *S. guianensis* show patterns in their geographical distribution.
Band 3 of αß-ACP was found in germplasm from Central American and Andean countries, but not in germplasm from Brazil and Argentina.

Regarding native seed proteins, germplasm from Central America, Colombia, and Venezuela had band 14, but bands 13 and 15 were absent; however, germplasm from Brazil, Argentina, and Peru had bands 13 and 15, but band 14 was absent.

In conclusion, these biochemical markers form two groups of electrophoretic patterns. This study is a contribution to the identification of the spatial patterns (geographical distribution) of genetic diversity (biochemical polymorphism) of *S. guianensis* in the Americas. The correlation of polymorphism with geographical areas will also enhance the understanding of the geographic origin and movement of germplasm by complementing studies on passport data, morphology, cytogenetics, and molecular DNA.

Panicum maximum

Identification of accessions of *Panicum maximum* is carried out with the collaboration of a Universidad Nacional de Colombia student. Accessions CIAT 6799 and 6944 of *P. maximum* were outstanding in agronomic and grazing trials carried out at Carimagua in the Llanos Orientales of Colombia. However, it was observed that the morphological characteristics of vegetative material of these accessions brought from Carimagua differed morphologically from that of the original maintained at Quilichao. Two hypotheses were raised: these materials, until then considered as apomictic, could have some percentage of sexuality; or mechanical confusion could have occurred.

Biochemical (isozymes), cytological (embryo-sac), and morphological analyses are being conducted to establish the identity of these two accessions and of other reassembling accessions (CIAT 6177, 6144, and 6977). Vegetative material from all planting sites at Quilichao and Carimagua (provenances) was established in the greenhouse at Palmira. A series of 40 morphological descriptors has been established for *P. maximum*. There is large intra-accession morphological variation for several provenances. Three tissues (root, leaf lamina and sheath) and 6 enzymes, α-EST, ß-EST, ACP (αß-acid phosphatase), GOT, DIA, and PRX (peroxidase),

were tested in order to standardize the extraction procedure of native proteins (isozymes) for this species. The best band quality was obtained with leaf lamina and α- and ß-EST isozymes. Preliminary results also show intra-accession variation by isozymes.

UG02.3.3 Taxonomy

The identification of 60 accessions was received this year, including 51 grass specimens identified by S.A. Renvoize, Royal Botanical Garden, Kew, England.

UG02.3.5 Proposed activities for 1996

Continue characterization of key species with emphasis on new germplasm of *Arachis* and *Cratylia*.

Participate in the biochemical characterization of *Desmodium heterocarpon* subsp. *ovalifolium* germplasm accessions within the GTZ-collaborative project.

UG02.4 Data Management and Documentation

UG02.4.1 Data management

Data base is operational for germplasm management. It fulfills the requirements of users who need specific information on combination of parameters for specific accessions.

UG02.4.2 Passport data

Data of germplasm which originated from Africa and that of Brazil is being revised for the publication of the catalogs. The remaining catalog to be produce is for germplasm acquired from other countries different from which there is already catalogs published.

UG02.4.3 SINGER

The CGIAR's System-wide Information Network for Genetic Resources (SINGER) is being developed to provide a mechanism for the management and use of genetic resources data across the System. CIAT participated in a SINGER planning meeting held in October 1995 in Mexico. Agreement was achieved among participants about the objectives for SINGER, who the users will be, and on which data shall be fed into the system. CIAT has been developing a mechanism for revising germplasm passport and distribution data that will be delivered to SINGER.

UG02.4.3 Herbarium

640 herbarium specimens were added during 1994-95. The reference herbarium now has a total of 16,313 specimens with 12,112 or 50% covering the registered accessions. 120 of the 168 genera and 551 of the 831 species registered in the collection are represented in the herbarium. Computerized labels were developed for the herbarium specimens (Table 15).

The herbarium received visitors from several countries who carried out taxonomic and other botanical studies.

Colombian grass species at the Royal Botanic Gardens, Kew, UK.

To enhance the knowledge of native grass genera with forage potential, a list was made of Colombian species of the subfamilies Panicoideae and Chloridoideae held by the Herbarium of the Royal Botanic Gardens, Kew, England. These subfamilies include the perennial grasses of tropical origin that have evolved in grassland-herbivore ecosystems, especially in Africa. Almost all useful tropical forage species belong to these two subfamilies. The information on each sample consists of locality, altitude, number and collector, date of collection, and field notes. The R.B.G. database includes 329 species originating from Colombia, with more than 1,400 herbarium specimens.

Eighty percent of the species belong to the subfamily Panicoideae, reflecting its broad degree of speciation in Colombia. Within these two subfamilies at Kew, there are type specimens of 12 species, i.e., as the first botanical record of the species, including species from genera: *Ichnanthus*, *Paspalum*, *Axonopus*, *Digitaria*, *Arundinella*, *Andropogon*, *Schizachyrium*, and *Hyparrhenia* which all belong to the subfamily Panicoideae.

The database contains records of samples collected since the late 1700's. The geographical areas with the highest number of species are Atlantic coastal region, inter-Andean valleys, and the regions of Orinoquia and Amazonia. Forty-six percent of the species were collected from altitudes between 1,000 and 2,000 masl.

UGO2.4.4 Proposed activities for 1996

Participate actively in the data revision and standardization in the SINGER project.

Complete passport data revision for Africa and remainder of the germplasm from other Countries.

Edit and publish germplasm catalog of Brazil, Africa and Other Countries.

Prepare a list of grasses from the tribes Chloridoideae and Panicoideae with information available from Colombian herbaria.

UG02.5 Distribution

The Tropical Forages germplasm bank distributed more than 1,000 germplasm samples requested this year by CIAT and collaborating institutions (Table 16).

Project UG03: In vitro CASSAVA (Manihot) GERMPLASM 9561

Project Purpose
Establish management methodologies and techniques, as well as strategic research to assemble, conserve and characterize the in vitro germplasm collection of cultivated and wild species of *Manihot* for making it a more comprehensive, useful and readily available collection.

Status of the In Vitro Cassava Germplasm

As of October 1995, the in vitro germplasm holds a total of 6,069 accessions consisting of 5,567 clones of cultivated *Manihot esculenta*, 351 accessions of 30 wild species, and 147 genotypes from a cross for genetic mapping (Table 17). On the other hand, a total of 4,583 accessions of *M. esculenta* including the genetic stock plus CIAT hybrids are held in the field bank. The field bank for the wild species is held under similar conditions than those for the cultivated, consisting to date of 275 genotypes for 34 species.

UG 03.1 Acquisition of *M. esculenta*

During 1995, 84 new accessions of *M. esculenta* were introduced to the cassava in vitro bank. Among those, 50 clones were received from Argentina as remainder of what was collected in the 1993 -IPGRI-INTA-CIAT Project, six other as improved Brazilian clones resistant to witche's broom, three from Brazil and Colombia which were only at the field collection plus 25 other elite clones produced by the Cassava Breeding Program. It is important to note that 42 other clones from Vietnam (30), Salvador (8) and Bolivia (4) were also received but because of phytosanitary restrictions or lack of minimal passport information, they could not be introduced into the bank yet. Efforts have been made to obtain this relevant information on name, origin of material, place of collection; this information, constitutes a basic criteria for introducing clones into the bank. A new attempt to receive the Vietnam clones is undergoing.

UG 03.2 Collecting and acquisition of *Manihot* species

Manihot spp. of Brazil and Uruguay
During this year, from the Costa Allem's fourth expedition collection of wild strains of cassava in Brazil, project supported by CENARGEN/EMBRAPA and IPGRI, a new shipment of wild species was received at CIAT. Seed of 19 species from Brazilian centers of diversity were shared with CIAT this year. In addition to those, seeds of *M. pilosa* from Brazil and possibly *M. grahami* from Brazil and Uruguay were also introduced (Table 18). Seeds of five species directly produced from open pollinated plots at CENARGEN were also received (Table 19).

Thus, a few seeds for nine new species *M. diamantinensis*, *M. fruticulosa*, *M. janiphoides*, *M. januarensis*, *M. juscelinensis*, *M. maranhensis*, *M. mossamedensis*, *M. nana*, and *M. pilosa* were received this year. All these were added to the seed inventory, kept under cold room conditions and after quarantine regulation be completed, they will be available for use and evaluation.

UG 03.3 Conservation

UG 03.3.1 Expansion of conservation facilities

During last annual review, the need of additional conservation space for the in vitro cassava collection was discussed and documented. The actual storage (32 m^2) holds a maximum of 6,720 clones with five tubes per clones in 25 mm tube size. To date, a total of 6,069 clones counting the wild strains conserved, not including additional tubes used for testing alternative media, and extra operational space for monitoring the tubes before locating them in their final place, stressed the need of additional space. On the other hand although the cassava germplasm at the collection represents about 60 to 70% of the existent diversity (Hershey 1991 in IPGRI, 1994) and with an average introduction of a about 140 clones by year (EPMR 1994, internal document), supported the decision for asking additional space. In April, the expansion of the laboratory through capital equipment for upgrading the GRU collections was finally approved. In October, after discussing several options, the work started. The expansion of the laboratory will add 15 m^2 to the conservation area, 4 m^2 to the induction room and some redistribution of the areas within the laboratory. Work is expected to be completed by January 1996.

UG 03.3.2 Cryopreservation of cassava shoot tips in liquid nitrogen

Since 1988 the BRU has initiated research toward the postulation of a viable methodology to establish an in vitro cassava base collection in liquid nitrogen (IVBG) at CIAT. To date results are satisfactory but still additional and continuous research efforts need to be made to accomplish the purpose.

Several factors contribute to successfully cryopreserve cassava shoot tips, being critical: tissue desiccation, preculture and cryoprotection with Sorbitol and DMSO. There is actually a basic protocol developed to recover viable cassava plants from frozen shoot tips in liquid nitrogen, using cultivar MCOL 22 as a model.

This year the BRU have concentrated efforts on the composition of the post-freezing media for obtaining viable tissue and plant regeneration. In addition, they have also initiated experiments to develop an encapsulation technique of shoot tips as subject for cryopreservation; also, they are initiating work to extend cryopreservation to cassava cultivars that normally showed a low response to the current protocol.

Progress report:

I. Post-freezing (reculture) recovery conditions: The effect of cytokinins concentration in post-freezing cryopreserved shoot tips is observed in Table 20. In general, shoot regeneration increased with increasing Kinetin (KIN) and/or 2iP (Isopentil Adenine) concentration, while for BAP (Benzyl Amino Purine) the situation was the opposite. A low concentration (i.e.

0.04mg/l) of BAP yielded a high shoot regeneration., however, as BAP concentration increased a detrimental effect on shoot recovery was observed. No shoots were generally regenerated when recovery media was supplemented with Adenine (A) or Thidiazuron (TDZ).

The general gradient of response to cytokinins in the recovery medium of cryopreserved cassava shoot tips would be: KIN>2iP>BAP>TDZ>A.

II. Shoot tip encapsulation: Encapsulation of shoot tips in a soft matrix such as sodium alginate is expected to provide a practical and rapid way of cryopreservation in liquid nitrogen. The osmotic effect of sucrose and its related conditioning affect on encapsulated shoot tips needs to be assessed during the preculture phase as these are prepared for freezing.

The effect of sucrose concentration and time of treatment has been tested on the viability and shoot regeneration from beads (alginate-encapsulated shoot tips) without freezing (Table 21). Sucrose at 0.5M provided the best condition for viability and shoot formation throughout 1-7 days preculture. Shoot formation decreased drastically with sucrose concentration, and slightly with preculture time at 0.5M.

Sequential treatment with variable sucrose concentrations, and duration of treatment, can also exert differential response in terms of shoot regeneration. Treatment with 0.5M sucrose for one day, followed by 0.75M for two days, clearly allowed higher shoot recovery without freezing than more extended sequential sucrose treatments or single sucrose concentration treatments (Table 22).

Outlook:
I. GRU will join in 1996 to extend the current cryopreservation protocol to a wider range of cassava genotypes. We will initiate the screening of varietal response with the core collection.
II. Identify recalcitrant (i.e. non-responding) cassava genotypes and carry out experiments designed to adjust the protocol to these materials.
III. Continue experiments using encapsulated shoot tips for freezing under the slow and rapid freezing protocols.
IV. A collaborative IPGRI-CIAT project will start operations during next year.

UG 03.3.3 Conservation of *Manihot* species

As stated before (GRU Annual Review 1994), one of the main problems trying to micropropagate wild species is the lack or poor rooting ability of some of them to artificial media. During 1995 a thesis research that had as a general objective to improve the existing protocols for micropropagation was concluded.

From a total of 351 genotypes corresponding to 30 species held at the in vitro bank, one hundred sixty genotypes for 25 wild species were selected in the study. Apical shoots and nodal cuttings were explanted in five different media and four evaluations, every two weeks, were

made for six variables. The response variables estimated were: No. alive leaflets, No. dead leaflets, No. of apical shoots, Stem longitude, Root presence (%), Callus presence (%).
For all the species and at the fourth evaluation, the rooting response was between 40.7 and 73.0 (Table 23), similar results were obtained by Baca 1991 after evaluating 32 species in five culture media. On the contrary, callus presence at the fourth evaluation was observed in a range of 1.7 and 15.5% for all the species. Considering that the presence of callus is a undesirable characteristic in micropropagation, these results in comparison to a previous research were highly acceptable.

There is a general tendency to form groups of species to a specific culture media. For practical purposes, the species at the bank have been classified as having High, Intermedia and/or Low rooting ability. Some species having high rooting ability (>90% for any media) are; *M. esculenta* subs *flabellifolia*, *M. pentaphylla* and *M. fruticulosa*, of Intermedia (90 - 40%), *M. aesculifolia*, *M. carthaginensis* and *M. glaziovii*, of Low rooting ability (< 40%), *M. anomala* and *M. quinquepartita* (Table 24).

UG 03.4 Characterization and duplicate identification

During this year, 49 more clones of *M. esculenta* were analyzed with the α β esterase system, completing 4,861 clones analyzed so far. Those clones corresponded to 43 hybrids, 4 Colombian clones, 1 Brazil and 1 Costa Rica clones. This information has been used for characterizing and supporting criteria for detecting duplicated in the collection.

Last December, a thesis was completed that has as objective identify the duplicates within the cassava collection (Jímenez, A. 1994). This work corresponded to a secondary step on the process of identifying duplicates in the collection. 2,800 clones were planted at the field and morphologically characterized; grouping analysis using eight descriptive variables plus 12 isozyme bands was used to form the final grouping of clones. From the cluster analysis, 2,056 clones formed individual groups (had unique pattern), and 744 clones formed 280 groups from two to six clones. Since at least one clone within a group with two or more clones is the representant of the group, we can assume that the level of duplication for the collection is about 17%. Several clones from those 280 groups have been processed through fingerprinting after M-13 cloning and information has served as the base to eliminate clones from the field. This year 52 confirmed clones at this level were eliminated from the field but a replication of three tubes are conserved in the in vitro collection (Table 25).

UG 03.5 Germplasm distribution

During 1995, 23 requests for distribution of germplasm in the in vitro form were processed. Those corresponded mostly to NARs in developing countries although some to Universities in developed countries, all partners in research (Table 26).

UG 03.6 Indexation of clones

During this year, the VRU submitted a total of 112 clones indexed for viral diseases. To support the indexation of clones in the in vitro collection, an assistant position was filled. The assistant will try to cope with the existing backlog of clones indexing. Supported by the VRU, the assistant will partially try to implement the PCR based technique for CVMV, needed for detect this problem in Brazilian and Argentinean germplasm while applying routine detection tests for the others well known pathogens.

UG 03.7 Network activities

After the inaugural meeting of the Manihot Genetic Resources Network held at CIAT in August 1992 and for the initiation of activities to be accomplished within the Network objectives, two different activities were programmed during this year.

A Latinoamerican workshop on genetic resources of cassava germplasm was held at Cruz das Almas, Brazil, in October 23 through 27. The workshop was financially supported by the Institutional Relation Office at CIAT, by EMBRAPA CENARGEN and IPGRI. The meeting gathers curators responsible for the biggest germplasm banks at Brazil and six from other Latinoamerican countries. All cassava germplasm collection management aspects on collection, characterization, duplication and documentation among others were discussed and a consensus outlined by the end of the meeting.

The other workshop supporting the Network was the Taxonomy of Manihot workshop. This meeting was held at CIAT during Nov 7 through 10. The consultancy of Dr. Costa Allem, presently carrying out a botanical revision of the genus was associated with several other lectures and practical activities. The objectives were to know the genus from the taxonomical and conservation stand point as for the identification of personnel interested in further cooperation.

UG04. Coordination: To coordinate GRU efforts for the proper conservation and study of the commodity germplasm collections to make them available and usable by users from now onwards.

Particular emphasis was put on the preparation of elements for the two Reviews, EPMR and ICER, and implementation of the latter with internal system review, planning and budget. In view of upgrading, field operations at the GRU have been reorganized around "field" and "clean" areas. The cassava *in vitro* lab has been reorganized and expanded with areas reserved for the cassava *in vitro* collection, the subculturing and the media preparation. Steps have been taken to start monitoring viability of GRU seed collections from 1996 onwards systematically on a single accession basis.

Research during 1995 has been conducted with BRU and Commodity programmes on the organization of gene pools of common bean, lima bean and cassava. Study of phylogeny

aspects in common bean has been initiated, to help the breeding programmes to use more appropriate sets of germplasm in wide crossing. Patterns of genetic diversity along space macrogradients in a few selected forage species are being studied, and possible explanations are being tested with the help of GIS.

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International course on biotechnology for conservation of agrobiodiversity. Coordinated by CIAT-BRU. Palmira, Colombia, September 11- October 6, 1995.

Hands-on training for staff from Colombia, Peru, Ecuador, Cuba, Belize and Brazil, on *in vitro* propagation of cassava clones.

International Diploma Course on Herbarium Techniques for CIAT GRU staff organized by Royal Botanic Gardens, Kew, United Kingdom, 4 June- 27 July 1995.

Course on seed physiology for CIAT GRU staff organized by Prosemillas, CIAT, November 1995.

International Activities:

International Workshop on Training and Research between Spain and Latin America for Conservation and Use of Plant Genetic Resources. Coordinated by IPGRI, CATIE, Costa Rica, June 27-30, 1995.

International Workshop on Taxonomy and Conservation of *Manihot* genetic resources. Coordinated by CIAT, Palmira, Colombia, November 7-10, 1995.

International Workshop on Field Genebank Management: Problems and Potential Solutions, University of Puerto Rico, Mayaguez, Puerto Rico, 12-18 November, 1995

Germplasm exploration in central and western Guatemala for *Manihot* and *Phaseolus* germplasm in January 1995, with the Universidad de San Carlos de Guatemala.

Postdoctoral fellowship with CIAT BRU and GRU: research on common bean domestication in Guatemala with the help of molecular markers, with the Universidad de San Carlos de Guatemala, in November 1995.

Table 1. Status of the *Phaseolus* bean collection available at the Genetic Resources Unit, CIAT, as of October 1995.

Species	Accessions available for distribution (no.)		
	Wild	Cultivated	Total[a]
Domesticated			
P. vulgaris	817	24,228	25,045
P. lunatus	122	1,444	1,548
P. coccineus	69	529	598
P. polyanthus	2	290	292
P. acutifolius	145	126	271
Wild species[b]			
P. angustissimus	*P. augusti*		
P. glabellus	*P. filiformis*		
P .grayanus	*P. glaucocarpus*		
P. macrolepis	*P. leptostachyus*		
P. microcarpus	*P. micranthus*		
P. neglectus	*P. minimiflorus*		
P.pachyrrhizoides	*P. oligospermus*		
P. pluriflorus	*P. pedicellatus*		
P. polystachyus	*P. xanthotrichus*		
P. tuerckheimii			
			164
Total			27,435

a. In both cultivated and wild forms, subdivisions of the original accessions have been made when genetic mixtures have appeared.

b. Forms classified as "weedy" or "regressive" are included within the term "wild" of the domesticated species.

Table 2. Sources of *Phaseolus* germplasm acquired during 1995.

Country	Species					Σ
	P. vulgaris	*P. lunatus*	*P. coccineus*	*P. polyanthus*	Wild spp.	
Mexico	14	-	3	-	-	17
Guatemala	10	7	13	3	6	39
Honduras	-	8	-	-	3	11
Colombia	161	25	26	26	-	238
Argentina	-	-	-	-	2	2
Total	185	40	42	29	11	307

Table 3. International distribution of *Phaseolus* seed samples by the GRU , 1995.

Region	Country	Requests (no.)	Accessions (no.)
North America	United States	2	2
Central America	Mexico,	1	3
South America	Argentina, Chile, Colombia	6	42
Caribbean	Puerto Rico	1	3
Europe	Italy, France, Belgium, Spain, Greece, Bulgaria, Netherlands	9	76
Africa	Burkina Fasso, Zimbabwe	2	28
Asia and Oceania	India, Turkey	3	68
Total	17 Countries	24	225

Table 4. Distribution of *Phaseolus* seed samples by GRU to CIAT's Bean Program. 1995

Section	Requests (no.)	Accessions (no.)
Germplasm characterizat.	14	107
Andean Gene Pool	3	4
Mesoamerican Gene Pool	10	4,368
Phytopathology	6	61
Entomology	16	775
Physiology	6	48
Others	4	68
Total	59	5,431

Table 5. Number of samples analyzed at the Seed Health Testing Laboratory in 1995, and percentage of clean samples.

Program Section Requester	No. samples (% clean)
Bean Germplasm Characterization	627(60)[1]
Mesoamearica Bean Genetics	162(45)
Andean Bean Genetics	241(78)
Bean Genotype x Environment	1034(83)
Bean Pathology	461(75)
Bean Phytonutrition	130(69)
Genetic Resources Unit	354(69)
Total	3009(68.4)

[1] Numbers in parenthesis are percentage of clean samples

Table 6. Percentage of infected samples with Fungi, Bacteria, and Viruses found in seed testing of *Phaseolus* germplasm for international distribution in 1995.

RESEARCH PROGRAM	FUNGI[1]		BACTERIA[2]		VIRUS[3]	
	Macp.	*Rhiz.*	*Xant.*	*Pse.*	Poty[4] BCMV	BSMV
Bean Germplasm Characterization.	25.8	4.1	0.6	0.0	10.4	3.7
Mesoamearica Bean Genetics	1.9	3.7	0.0	0.0	39.5	19.8
Andean Bean Genetics	16.3	0.0	3.3	0.0	0.8	1.2
Bean Genotype x Environment	8.0	1.74	0.0	0.0	8.5	3.8
Bean Pathology	6.5	0.2	0.0	0.0	40.1	1.3
Bean Phytonutrition	2.3	16.9	0.0	0.0	23.8	10.8
Genetic Resources Unit	0.3	0.0	0.8	0.0	23.2	2.3

[1] *Macrophomina* y *Rhizoctonia* [2] *Xanthomonas* y *Pseudomonas* [3] BSMV y BCMV
[4] Tests only on susceptible accessions.

Table 7. Tropical forages germplasm conserved at CIAT (as of 31.10.1995).

Taxon	Legumes			Grasses	Total
	W	H-W	H		
Genera	51	22	69	44	186
Species	130	56	414	152	752
Accessions	1,293	3,686	13,659	1,995	20,634

W = Woody;
H = Herbaceous;
H-W = Comprising a range of woody and herbaceous materials.

Table 8. Collection status of the tropical forages germplasm maintained at CIAT (as of 31.10.1995).

Genus	Accessions registered (no.)	Accessions conserved (no.)	Accessions multiplied (no.)	Backlog accessions (no.)	Accessions in base collection (no.)
Legumes					
Aeschynomene	1,036	998	658	340	293
Arachis	178	75	68	7	30
Calopogonium	581	536	410	126	122
Centrosema	2,596	2,451	2,231	220	1,051
Desmodium	3,246	2,904	1,921	983	746
Galactia	668	571	560	11	378
Leucaena	216	199	177	22	151
Macroptilium	659	615	608	7	466
Pueraria	288	258	238	20	116
Rhynchosia	510	444	228	216	33
Stylosanthes	4,036	3,609	2,876	733	1,109
Vigna	838	741	654	87	338
Zornia	1,091	1,027	896	131	78
Other	4,903	4,210	2,956	1,254	1,467
Total legumes	20,846	18,683	14,481	4,157	6,378
Grasses					
Andropogon	149	91	89	2	-
Brachiaria	1,121	654	563	91	135
Hyparrhenia	117	53	40	13	4
Panicum	848	598	512	86	35
Paspalum	154	105	71	34	24
Other	691	494	171	323	1
Total grasses	3,080	1,995	1,446	549	199
Other families	3	1	-	2	-
Grand total	23,929	20,634	15,927	4,708	6,577
Percent of total (%)		100%	77.18	22.81	31.87

Table 9. Acquisition of Tropical Forage Germplasm in 1995.

Genus	Collection	Exchange	Total 1995
	Occasional		
Legumes			
Arachis	-	8	8
Desmodium	14	-	14
Cajanus	3	-	3
Stylosanthes	2	-	2
Other Legumes	25	3	28
Total Legumes	44	11	55

Tabla 10. Accessions in Post Phytosanitary Follow up 1995.

Genus	Accessions
Legumes	
Arachis	12
Abrus	1
Aeschynomene	1
Centrosema	1
Desmodium	3
Flemingia	1
Macotyloma	1
Mucuna	1
Pueraria	1
Total Legumes	22
Grasses	
Andropogon	1
Brachiaria	2
Digitaria	2
Melinis	1
Paspalum	5
Total Grasses	11
Grand Total	33

Table 11. Collection status of species from the legume genera *Centrosema, Desmodium, Pueraria, Stylosanthes*, and *Zornia*, and the grasses *Andropogon, Brachiaria, Hyparrhenia*, and *Panicum* for which CIAT has the international mandate (no. of accessions as of 31.10.1995).

Species	Registered	Conserved	Multiplied	Base collection	Backlog
Legumes					
C. brasilianum	281	272	271	200	1
C. macrocarpum	395	381	364	299	17
C. pubescens	926	905	782	311	123
D. heterophyllum	111	97	89	5	8
D. heterocarpon ssp. *ovalifolium*	156	149	147	89	2
P. phaseoloides	248	232	214	113	18
S. capitata	331	305	305	283	-
S. guianensis	1587	1414	1068	570	346
S. macrocephala	132	130	130	127	-
Z. glabra	26	26	26	6	-
Total legumes	4193	3911	3396	2003	515
Grasses					
A. gayanus	130	85	84	-	1
B. brizantha	510	321	291	82	30
B. decumbens	90	59	58	11	1
B. dictyoneura	18	10	10	-	-
B. humidicola	115	72	57	6	15
B. ruziziensis	91	45	38	5	7
H. rufa	17	10	10	1	-
P. maximum	770	558	498	31	60
Total grasses	1741	1160	1046	136	114
Grand total	5934	5071	4442	2193	629
Percent of total (%)		100%	87.6%	42.2%	12.4%

Table 12. Number of tropical forage accessions which underwent initial multiplication and rejuvenation by the CIAT GRU, excluding field collections, in 1995.

Genus	Palmira		Quilichao	Popayán
	Greenhouse	Field		
Legumes				
Aeschynomene	6	-	-	-
Arachis	20	100	-	-
Cajanus	5	-	2	-
Calliandra	-	1	3	-
Calopogonium	1	5	1	-
Canavalia	1	-	-	-
Centrosema	6	20	15	25
Chamaecrista	4	2	24	-
Clitoria	3	3	2	-
Crotalaria	3	1	-	-
Desmodium	105	4	50	13
Dioclea	1	2	1	-
Eriosema	3	1	-	-
Galactia	3	-	1	-
Gliricidia	-	-	1	-
Indigofera	3	-	1	-
Mucuna	11	1	1	-
Rhynchosia	2	7	1	-
Stylosanthes	32	-	31	-
Tephrosia	2	2	-	-
Teramnus	2	1	-	-
Vigna	5	12	-	-
Zornia	8	-	-	-
Other	32	7	39	3
Total legumes	258	169	173	41
Grasses				
Andropogon	2	-	-	-
Brachiaria	4	-	-	83
Hyparrhenia	-	-	-	-
Melinis	1	-	-	-
Panicum	254	-	-	-
Paspalum	-	-	41	-
Other	3	-	1	2
Total grasses	264	-	42	4
Grand total	522	451	166	30

Table 13. Genera represented in the field collections at CIAT stations at Palmira, Quilichao, and Popayán (no. of accessions as of 31.10.1995).

Genus	Palmira	Quilichao	Popayán
Legumes			
Arachis	84	-	-
Shrub			
Leucaena	163	-	-
Other Shrub legumes	144	-	-
Total legumes	391		
Grasses			
Andropogon	75	-	-
Brachiaria	-	435	162
Hyparrhenia	-	40	-
Panicum	-	500	112
Other grasses	76	-	-
Total grasses	151	975	274
Grand Total	542	975	274

Table 14. Inventory of tropical forage germplasm in the base collection conserved under long-term storage (no. of accessions), in 1995, at CIAT.

Genus	Increase 1995	Inventory 31.10.95
Legumes		
Aeschynomene	24	293
Arachis	4	30
Calopogonium	5	112
Canavalia	-	112
Centrosema	28	1,051
Chamaecrista	1	236
Clitoria	2	34
Crotalaria	6	145
Dendrolobium	4	33
Desmanthus	-	117
Desmodium	56	746
Flemingia	2	57
Galactia	6	378
Leucaena	37	151
Macroptilium	2	466
Neonotonia	11	45
Phyllodium	2	101
Pueraria	57	116
Rhynchosia	4	33
Stylosanthes	34	1,109
Tadehagi	-	94
Teramnus	4	94
Uraria	-	106
Vigna	5	338
Zornia	8	78
Otros (36 genus)	24	270
Total Legumes	327	6,378
Grasses		
Brachiaria	38	135
Digitaria	1	1
Hyparrhenia	4	4
Panicum	28	35
Paspalum	24	24
Total grasses	95	199
Grand total	422	6,577

Table 15. Inventory of herbarium (no. of specimens) and proportion of germplasm accessions documented by herbarium material (%) at CIAT (as of 31.10.1995).

Family, subfamily	Genera[1,2]	Species[1,2]	Specimens/ accessions	Percent (%)[3]	Total specimens[4]
Legumes					
Faboideae	70 (86)	382 (546)	10,414	52.5%	14,234
Mimosoideae	12 (18)	34 (62)	229	43.6%	285
Caesalpinioi-deae	7 (10)	30 (38)	316	79.8%	384
Total legumes	89 (114)	446 (646)	10,959	52.6%	14,903
Grasses					
Chloridoideae	8 (14)	17 (27)	43	22.2%	64
Panicoideae	23 (40)	88 (158)	1,110	38.7%	1,346
Total grasses	31 (54)	105 (185)	1,153	37.4%	1,410
Grand total	120 (168)	551 (831)	12,112	50.6%	16,313
Percent (%)	71.4%	66.3%	50.6%		

1. Genus and species documented at herbarium without brackets.

2. Genus and species registered at germplasm bank in brackets.

3. Proportion of registered germplasm accessions (23,926), documented by herbarium specimens; some accessions, although represented by herbarium specimens, are not any more available in the seed bank.

4. Total of specimens: some accessions which have or have had doubt in taxonomic identification are held in more than one specimen to be sent to the specialist.

Table 16. Distribution of tropical forage germplasm (no. of samples) by CIAT's Genetic Resources Unit, in 1995.

Genus	CIAT Headquarters	Other institutions	Total
Legumes			
Aeschynomene	1	85	86
Arachis	77	54	131
Cajanus	72	18	90
Calopogonium	4	5	9
Centrosema	34	38	72
Chamaecrista	4	1	5
Codariocalyx	4	10	14
Cratylia	7	4	11
Desmodium	29	16	45
Flemingia	8	5	13
Galactia	27	-	27
Leucaena	15	9	24
Macroptilium	2	10	12
Pueraria	6	14	20
Rhynchosia	1	2	3
Stylosanthes	135	9	144
Teramnus	11	-	11
Vigna	55	10	65
Zornia	-	5	5
Other (29 genus)	93	106	195
Total legumes	585	401	978
Grasses			
Andropogon	-	3	3
Brachiaria	95	143	238
Hyparrhenia	3	-	3
Panicum	5	7	12
Pennisetum	-	3	3
Other (7 genus)	13	23	36
Total grasses	116	179	295
Grand total	701	576	1273

Table 17. Number of cassava accessions maintained as germplasm at CIAT, October 1995.

SOURCE	CIAT CODE	No. OF ACCESSIONS IN VITRO BANK	No. OF ACCESSIONS FIELD BANK
CULTIVATED			
Argentina	MARG	121	15
Bolivia	MBOL	3	3
Brazil	MBRA	1,340	793
Colombia	MCOL	2,003	1,751
China	MCHN	2	2
Costa Rica	MCR	148	130
Cuba	MCUB	77	70
D.Rep.	MDOM	5	5
Ecuador	MECU	117	102
Fiji	MFJI	6	6
Guatemala	MGUA	91	78
Indonesia	MIND	51	51
Malaysia	MMAL	67	66
Mexico	MMEX	102	90
Nigeria	MNGA	19	17
Panama	MPAN	43	39
Paraguay	MPAR	231	176
Peru	MPER	405	390
Philippines	MPHI	6	6
Puerto Rico	MPTR	15	15
Thailand	PTAI	31	7
United S.	MUSA	10	9
Venezuela	MVEN	249	215
CIAT/ICA Hybrids		425	401
GENETIC STOCK		147	146
SUBTOTAL		5,714	4,583
WILD SPECIES			
30spp in vitro,34 spp field		351	275
3 undefined spp.		4	-
TOTAL		6,069	4,858

Table 18. Collected seeds from wild species of *Manihot* received during 1995 (Species with asterisk new to CIAT)

Species	Collector No.	Accession No.	State	Collection site		CIAT accession No.	Seeds received
M. anomala	4568	BRA-107727	Minas Gerais	1616S	4342W	M anm 006	50
M. caerulescens	4495	BRA-107751	Bahia	1112S	4030W	M cae 015	40
"	4496	BRA-107760	Bahia	1112S	4030W	M cae 016	15
"	4510	BRA-107778	Bahia	1232S	4122W	M cae 017	20
M. carthaginensis	4318	BRA-107808	Mato Grosso	1912S	5739W	M cth 018	31
*M. diamantinensis**	4505	BRA-107867	Bahia	1132S	4114W	Mdia001	1 5
M. dichotoma	4520	BRA-107905	Bahia	1408S	4016W	M dch 002	50
"	4525	BRA-107913	Bahia	1423S	4022W	M dch 003	40
M. epruinosa	4491	BRA-107972	Pernambuco	0833S	3653W	M epr 012	12
"	4560	BRA-108006	Minas Gerais	1543S	4316W	M epr 013	40
"	4582	BRA-108031	Minas Gerais	1513S	4413W	M epr 014	39
M. flemingiana	4204	BRA-103748	Tocantins	0959S	4834W	M fga 004	20
*M. fruticulosa**	4332	BRA-108120	Minas Gerais	1933S	4700W	Mfru 001	14
"	4347	BRA-108146	Goias	1610S	4830W	M fru 002	21
M. grahamii ?	-	-	Soriano (URY)	-	-	M grh 004	97
"	-	-	BRA (?)	-	-	M grh 005	-
*M. janiphoidea**	4564	BRA-108235	Minas Gerais	1538S	4258W	M jap 001	100
"	4569	BRA-108243	Minas Gerais	1627S	4345W	M jap 002	30
*M. jansenioides**	4583	BRA-108278	Minas Gerais	1513S	4413W	M jan 001	80
*M. juscelinoana**	4541	BRA-108294	Minas Gerais	1837S	4339W	M jus 001	5
M. mdroxicensis	4514	BRA-108359	Bahia	1230S	4118W	M mcn 002	20
"	4084	BRA-102610	Bahia	1342S	4119W	M mcn 003	5
*M. maranhensis**	4464	BRA-108367	Maranhao	0403S	4611W	M mar 001	7
*M. neusaimadaensis**	4086	BRA-102636	Goias	1512S	4711W	M nas 001	10
*M. nana**	4338	BRA-108448	Goias	1748S	4745W	M nan 001	5
*M. pilosa**	4532	BRA-108529	Minas Gerais	2009S	4259W	M pil 001	13
"	4533	BRA-108537	Minas Gerais	2009S	4259W	M pil 002	30
"	4538	BRA-108561	Minas Gerais	2113S	4335W	M pil 003	100
"	4539	BRA-108570	Minas Gerais	2145S	4341W	M pil 004	150
"	-	-	BRA ?	-	-	M pil 005	?
M. quinquepartita	4224	BRA-103870	Tocantins	0646S	4752W	Mqpt 020	10
"	4225	BRA-103888	Tocantins	0644S	4750W	M qpt 021	20
"	4242	BRA-103934	Maranhao	0634S	4722W	M qpt 022	10
"	4250	BRA-104001	Maranhao	0613S	4720W	M qpt 023	20
"	4253	BRA-104027	Maranhao	0557S	4724 W	M qpt 024	10
"	4255	BRA-104035	Maranhao	0442S	4746 W	M qpt 025	10
"	4257	BRA-104043	Para	0420S	4743 W	M qpt 026	20
M. sparsifolia	4097	BRA-102687	Goias	1430S	4858W	M spr 004	5
M. tripartita	4414	BRA-108855	Goias	1437S	4910W	M tpa 008	30

Table 19. Seeds from open pollinated plots at CENARGEN/EMBRAPA received during 1995

Species	Originally collector No.	Year of harvest	Production place	CIAT Seed lot ID	Seeds received
M. anomala	3402	94	CENARGEN /EMBRAPA	OW 128	4
M. flabellifolia Pohl	3533	94	CENARGEN /EMBRAPA	OW 129	6
M. glaziovii	3220	94	CENARGEN /EMBRAPA	OW 130	4
M. irwinii	3443	94	CENARGEN /EMBRAPA	OW 131	10
M. pilosa	3389	94	CENARGEN /EMBRAPA	OW 132	10

Table 20. Effect of Cytokinins on plant recovery from cassava shoot tips cryopreserved in L.N. (cultivar MCOL 22)

CYTOKININ	CONCENTRATION					
	0.04 mg/l		0.3 mg/l		0.5 mg/l	
	% VIAB.	% SHOOT	% VIAB.	% SHOOT.	% VIAB.	% SHOOT
2iP	63	0	96	44	100	75
BAP	84.6	57.7	88	44	19	0
KIN	54.2	4	100	59	100	80
A	0	0	52	0	24	0
TDZ	64	24	87.5	0	100	0

Table 21. Effect of Sucrose concentration and preculture time on shoot tip recovery from alginate beads without freezing.

SUCROSE CONCENTRATION	DAYS OF PRECULTURE							
	1		3		5		7	
	VIAB. %	SHOOT %	VIAB. %	SHOOT %	VIAB. %	SHOOT %	VIAB. %	SHOOT %
0.5 M	100	100	100	83	100	70	100	90
0.75	100	10	100	10	100	0	100	0
1M	100	0	100	0	100	0	90	0

Table 22. Effect of Sucrose concentration and treatment time on viability and shoot recovery from encapsulated shoot tips without freezing (cultivar MCOL 22)

TREATMENT	% VIABILITY	% SHOOTS
0.5-0.75-1M/1 day each	100	60
0.5/1day-0.75M/2 days	100	80
0.5M/3 days	100	62
0.75M/3 days	100	0

Table 23. Culture media effect for 25 Manihot spp during four evaluations

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

* Media separation through Duncan** Analysis of Cadmod

Means with different letter are statistically different according to Duncan

Table 24. *Manihot* spp. rooting percentage 60 days after planting[1]. CIAT November 1995.

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

[1] Compared to 100% rooting of cultivated species in 17N

[2] No. genotypes in the in vitro bank to Nov. 1995

[3] Percentage based on 2-3 tubes per genotype per treatment (1 replication). Does not include root quality.

[4] Culture Media:

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

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

$12A_3$:1MS, 3% Sucrose, 0.2 mg/lt Kinetin, Inositol, Thiamine, 1 g/lt A.C., 0.48 mg/lt $CuSO_4$, 7 gr/lt agar

WPM_1:1/2WPM, 2% Sucrose, Thiamine, Inositol, Ac. Nicotinico, Piridoxina, Glycine, Arginine, K_2SO_4, $Ca(NO_3)_2.4H_2O$,1 g/lt A.C., 1.01mg/lt IBA, 7 g/lt agar

WPM_2:1/3WPM1, 2% Sucrose, Thiamine, Inositol, Ac. Nicotinico, Piridoxina, Glycine, Arginine, K_2SO_4, $Ca(NO_3)_2.4H_2O$, 1 g/lt A.C., 1.01mg/lt IBA, 7 g/lt agar

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

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

Table 25: Clones forming groups analyzed with DNA M-13 marker

GROUP[1]	GENOTYPES			
569	ARG 11 *	ARG 12 *-		
759	BRA 55 *	BRA 56 *-		
525	BRA 60 *	BRA 61 *-		
450	BRA 62 *-	BRA 69 *		
422	BRA 99 *	BRA 102 *-	BRA 107 *-	BRA 164 *-
450	BRA 118 *	BRA 186 *-		
38	BRA 201 *-	BRA 890 *		
644	BRA 340 *-	BRA 342 *		
47	BRA 376 *	BRA 378 *-	BRA 435	BRA 418 *-
808	COL 768	COL 912A*	COL 927 *-	COL 1962 *-
847	COL 220 *-	COL 240 *	COL 281 *-	
1464	COL 376 *	COL 380 *-	COL 588A *-	COL 727 *-
1454	COL 661 *	COL 663 *-		
401	COL 1667 *	COL 1671 *-		
710	CR 5 *	CR 9 *-		
4	CR 17	CR 48	CR 49 *	CR 51 *-
187	CUB 23 *	CUB 30 *-		
647	CUB 27 *	CUB 37 *-	CUB 64 *-	CUB 66 *-
1714	ECU 13	ECU 19 *-	ECU 21 *	ECU 28
561	PAN 19 *	PAN 103 *-		
523	PAN 100 *	PAN 102 *-		
503	PAR 15 *	PAR 16 *-	PAR 29 *-	PAR 149 *-
85	PAR 32 *	PAR 182 *-	PAR 183 *-	
968	PAR 135	PAR 136	PAR 155 *	PAR 166 *-
401	PER 182 *-	PER 210 *		
306	PER 187 *	PER 190 *-	PER 214 *-	
1246	PER 281 *	PER 291 *-		
409	PER 282	PER 295 *-	PER 328 *	
393	VEN 8 *-	VEN 142 *		
1723	VEN 9 *	VEN 87 *-		
1508	VEN 138 *-	VEN 147 *		
1526	VEN 153	VEN 155	VEN 156 *	VEN 157 *-
1526	VEN 187 *-	VEN 198 *		
1328	BRA 32 *-	PER 197 *		
768	BRA 127 *-	VEN 25 *		
1525	COL 1624 *	VEN 72 *-	VEN 73 *-	VEN 184 *-
626	VEN 330 *-	CUB 74 *		

[1]Groups according to Jimenez, A. 1994 *Clones tested and confirmed as being similar within a group - clones removed from field during 1995 (TAB-GRP)

Table 26. Distribution of *Manihot* Germplasm (*in vitro*).CIAT Oct 1995

GERMPLASM RECIPIENT	NUMBER OF ACCESSIONS	NUMBER OF REQUEST
Centre Staff in Host Country	194* (1367) **	2
Centre Staff in Other Countries	-	-
Other IARC's	-	-
NARS in Developing Countries	195 (1068)	9
NARS in Developed Countries	126 (536)	10
Private Sector in Developing Countries	9 (45)	1
Private Sector in Developed Countries	-	-
Others	5 (10)	1
TOTAL OF MATERIAL SENT	529(3026)	-
TOTAL OF REQUEST	-	23
TOTAL OF DIFFERENT ACC. SENT BY YEAR	446	

* Include 70 accessions from M. spp germplasm (20 wild species and 2 undefined spp).
** Numbers in parenthesis are the numbers of samples sent (these could include repeated accessions).

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