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Genetic Resources Unit

During 1978 the Genetic Resources Unit has become established in its remodeled building, so that at the end of the year the three cold rooms, the five laboratories, the herbarium and the two threshing areas were all fully utilized by staff of the Unit. A year ago no germplasm was actually housed in the Unit; by the end of 1978 over 26,000 accessions of *Phaseolus* bean (four species), tropical forage legumes and grasses (some 23 genera), and cassava were being stored in the Unit.

During the year the Genetic Resources Unit changed from being a collector of other *Phaseolus* collections to become an active collector of unique *Phaseolus* germplasm in central Mexico, one of its centers of diversity. A one-month study of herbarium specimens in Mexico was followed by a three-month field collection trip done in cooperation with the Instituto Nacional de Investigaciones Agrícolas (INIA). To mid-November, 120 seed samples covering 14 *Phaseolus* species, plus 165 plant samples, mostly weedy (*silvestris*) types were collected in eight Mexican states (from Durango south to Michoacan). Materials were also collected under contract in Peru, Spain and Portugal.

The CIAT *Phaseolus* bean germplasm has increased from some 13,000 accessions

in 1977 to over 21,000 at present. More importantly, this germplasm is now actively being evaluated for genetic and agronomic characters and those materials contaminated with bacterial and/or viral diseases are being multiplied under careful control, and in close cooperation with the Bean Program, to produce seeds free of such contamination. Computerized data file systems have been developed to facilitate the entry, updating and retrieval of information gained in field and laboratory evaluations of germplasm.

Responsibility for the tropical forages germplasm held at CIAT (some 4500 accessions) has recently been transferred to the Genetic Resources Unit, where certain stages of evaluation and multiplication and all storage will take place. Some activities will be shared with the Beef Program, especially field evaluation at CIAT-Quilichao and Carimagua. A flexible data management system able to accommodate all evaluation inputs is being developed cooperatively with the Beef Program.

Since April the plant physiologist has been able to test and set up methods that permit the rapid development of cassava plantlets from meristem tissue cultures. The time required between the taking of the small tissue sample and its development into a small plant ready for transfer

into the field is only eight weeks. In contrast, other techniques are being tested that will permit storing the cassava meristem plantlets in test tubes for at least a year free of disease and other field problems. Towards the end of 1978 action was taken to organize selected national centers into a small network that will allow distribution of cassava germplasm as tissue cultures through national quarantine inspection.

Phaseolus Germplasm

Acquisition and Seed Increase

Nearly 7500 *Phaseolus* accessions were acquired from other sources since January 1978 so that the size of the present CIAT collection is more than 21,000 entries (Table 1). Most of these new materials are *Phaseolus vulgaris* sent here from the USDA Regional Plant Introduction Station at Pullman, Washington to complete our collection of all 8554 *Phaseolus* materials with P. I. numbers. Also included in the new materials are the Norvel collection from Mexico, the collection from the Institute of Horticultural Plant Breeding at Wageningen, the Netherlands,

and that of the Instituto de Ciencias y Tecnología Agrícola (ICTA) in Guatemala.

In addition to obtaining materials from various germplasm banks, CIAT has been involved with collecting *Phaseolus* material in Peru (233 samples of *P. vulgaris* and 39 of *Phaseolus lunatus*) as well as in Spain and Portugal (411 samples of *P. vulgaris* and 13 *Phaseolus coccineus*). Collection for more *Phaseolus* germplasm in Mexico has also been initiated this year in conjunction with the Instituto Nacional de Investigaciones Agrícolas (INIA), utilizing an FAO associate expert and partial funding by the International Board for Plant Genetic Resources (IBPGR). In this field collection, attention is being given to wild *Phaseolus* species, since these may be useful in the interspecific crossing program done in cooperation with the Faculté des Sciences Agronomiques, Gembloux, Belgium.

Of the 21,000 *Phaseolus* samples received, more than 13,000 have been seed increased. This represents a major effort which started in 1970, as each accession has to be checked for disease and genetic

Table 1.

Phaseolus samples on hand, accessions increased and accessions evaluated in the Genetic Resources Unit, as of November 1978.

Species	No. of accessions	No. seed increased	No. evaluated
<i>P. vulgaris</i>	19,910	12,600	9500
<i>P. lunatus</i>	1010	310	-
<i>P. coccineus</i>	430	150	-
<i>P. acutifolius</i>	70	60	60
Other <i>Phaseolus</i> ¹	100	50	-

¹ Includes samples of 8 wild species.

uniformity. Efforts in 1978 were concentrated on cleaning up a major part of the collection for major seed-borne diseases, such as bacterial blight, common bean mosaic virus and anthracnose. This aspect has been so important that materials harvested in the field have been stored as distinct seed lots labelled as to presence or absence of disease. More than 3500 accessions have been grown at CIAT-Palmira, Popayán and CIAT-Quilichao in April and May 1978. Another 5300 were planted in October and November at CIAT-Palmira and at Popayán.

Agronomic Evaluation of Collection

Since 1970 more than 9500 accessions of *P. vulgaris* have been evaluated at CIAT

for some of the 32 taxonomic and agronomic characters considered most important for this genus (Table 2). However, since not all of these accessions have been evaluated for all 32 characters, efforts were made in 1978 to collect more information. As in previous years, this was done by growing an accession in a 6-m row at CIAT-Palmira at the onset of the rainy season. More than 500 accessions were evaluated between April and June and another 230 are being evaluated at the end of the year. An important development in 1978 was the identification of 200 lines of *P. lunatus* which are uniform for seed characters. These materials are now being evaluated at CIAT-Palmira.

From the 9500 accessions of *P. vulgaris*

Table 2.

Characters evaluated at CIAT-Palmira for the *Phaseolus vulgaris* germplasm collection.

Character	No. of accessions evaluated	Character	No. of accessions evaluated
Days to emergence	4406	Pod per plant	7238
Hypocotyl length	4386	Branches with pods	4331
Hypocotyl color*†	8960	Branch angle	4362
Leaflet length	4401	Seeds per pod	4337
Leaflet width	4401	Seed shape	7229
Canopy height	6521	Major seed color*	7827
Nodes at flowering*	4373	Secondary seed color*	1889
Nodes at maturity*	3457	Seed brilliance*	7230
Days to flowering*	4401	Seed weight*	8457
Duration of flowering	4386	Yield per plant	4292
Flower color	8978	Total dry matter	4311
Photoperiod sensitivity	691	Rust	2172
Growth habit*	8997	Anthracnose	456
Plant height*	6968	Common bean mosaic virus	823
Stem thickness	4399	Bacterial blight	2762
Racemes per plant	7913	<i>Empoasca</i>	5038

† Characters with asterisks are those recommended by the *Phaseolus* Germplasm Advisory Committee of the International Board for Plant Genetic Resources, in July 1978.

evaluated, some 600 sets of duplicates (involving 1400 accessions) have been identified based on name and origin. This figure is expected to increase as more information on the materials gradually becomes available.

Seed Storage

During early 1978, standard methods recommended by the International Seed Testing Association (ISTA) were set up for the Unit. Seed germination and vigor as measures of seed viability are now determined routinely for *Phaseolus* bean accessions using rolled papers maintained at 100% relative humidity for seven days at 20°-30°C. Over 1750 accessions now have been tested for germinability; of 1113 general accessions tested, 69.0% had 90% or higher germination rate. In contrast, when 461 lots of clean seed were tested 90.5% showed a germinability level that high. While percent germination and percent vigor (estimated as number of significantly larger seedlings in each germination test) are only weakly correlated in the general accessions, this positive correlation was increased for the clean seeds to $r = 0.597$. This implies that when environmental factors, especially diseases, are removed, plant vigor may indeed be shown to be a genetic character.

A simple method for seed drying based on desiccation at room temperature was developed. Numerous small lots of seed (typically less than 1 kg for *Phaseolus* bean) are placed in desiccator cabinets over silica gel for seven days. During this period seed moisture content drops to below 7%. Such dried seeds, when packed into moisture-proof laminated packs, maintain this low moisture content regardless of ambient relative humidity. It is estimated that *Phaseolus* bean germplasm kept at -10°C and 7% content will retain 90% of its initial viability for a period of 300 years.

Germplasm Distribution

A total of 14,800 samples of *Phaseolus* germplasm (mostly *P. vulgaris*) were distributed in 1978. The distribution of materials outside CIAT was as follows: South America (391 samples), North America (190), Central America (6000), Asia (200), Africa (856), Europe (376) and Far East (346). Within CIAT nearly 6500 accessions have been distributed to the Bean Program, especially to the breeders.

Data Management and Cataloging

Management of germplasm data is an integral function of the Genetic Resources Unit. Sources of germplasm data include those from collection or introduction, maintenance, evaluation and distribution. For *Phaseolus* germplasm, a number of computer files to manage such data have been developed in conjunction with the Biometrics Section. Similar work has been initiated for the tropical forages germplasm with the collaboration of the Beef Program.

Some of the germplasm information generated should be made available to users at large by publishing a catalog. A revised version of the "promising" materials (803 selections of *P. vulgaris*) catalog has been completed, with revisions on seed characters and growth habit and updating of all field evaluation data. Similar work on the tropical forages germplasm in CIAT has been completed, to make available a catalog with computerized information on accession numbers, genus, species, source, and origin or collection site.

Multivariate Analysis

Multivariate analysis has been explored as an objective method to study genetic diversity in *Phaseolus* germplasm and also

to investigate the interrelationships of variables currently under evaluation. To test this method, a new set of data has been generated to provide reliable information having wider genetic and environmental bases. Replicated evaluation trials were done at CIAT-Palmira, CIAT-Quilichao, and Popayan using both wide spacing (30 cm) and narrow spacing (8 cm) between plants. Ten accessions were randomly selected from *P. vulgaris*, *P. lunatus* and *Phaseolus acutifolius*, with some selection made for habit groups within each species. The characters recorded include the first 27 agronomic traits listed in Table 2. Means for the relatively more stable characters were calculated across locations, spacings and replications, and these were used for multivariate analysis.

Cluster analysis showed that genetic diversity could be easily demonstrated by the groupings of similar materials (e.g., species and growth habits) based on the above variables. Some overlapping of habit groups within each species occurred, but this was overcome by normalizing all variables, i.e. putting them on a scale of 0-9. The results of this study could be extended into two areas where some indication of genetic diversity is now needed. The first involves identifying the genetic diversity of the advanced progenies coming from the bean breeders and the second is in the study of similarities and differences in the germplasm bank accessions of *P. vulgaris*.

Using *P. vulgaris* germplasm data from the Promising Materials Catalog, last year 75% of the total genetic variability could be accounted for by four principal components. Using the new data, only three components or factors are needed to represent 83% of the total variability. Factor 1 carried 40% of the total variability; it included the characters growth habit,

plant height, nodes at flowering, racemes per plant, nodes at maturity, seeds per pod and 100-seed weight. Factor 2 accounted for 29% of total variability and included leaflet length, leaflet width, stem thickness, dry matter yield and grain yield. Factor 3, describing only 14% of total variability, involved characters like length of hypocotyl, days to flower, pods per plant, racemes with pods and duration of flowering. Such results imply that selection of a few key characters, with one or more from each factor, would provide a basis for future evaluation over a wide range of environments. At present, growth habit, yield, and days to flower have been selected as the minimum characters for evaluating more than 6000 accessions of *P. vulgaris* grown out between the La Selva, Obonuco and Popayan locations.

Cassava Tissue Culture

Potential of the Method

The exchange of cassava germplasm with other countries is basic to CIAT's role in the improvement of this tropical crop. However, many countries have created strict quarantine barriers that prevent the distribution of vegetative materials because of the hazards of disseminating pests and diseases. Similarly, conventional vegetative field cultivation often exposes these valuable materials to pests and diseases. Tissue culture methods can be used for the vegetative propagation of cassava and such risks significantly reduced or eliminated. The potentially high propagation rates that can be achieved with tissue cultures, coupled with their freedom from microorganisms, small space requirements and relatively simple handling procedures, make it feasible to utilize these tissue culture systems for the maintenance and international exchange of cassava germplasm.

Research was initiated this year in the Genetic Resources Unit, in cooperation with the Cassava Program, aimed at developing cassava meristem culture methods for: (1) conservation of cassava genetic resources in clonal form for long periods of time and free of diseases; (2) international transfer of valuable cassava germplasm in disease-free conditions; and, (3) rapid multiplication of materials. The cycle from meristem to plants in the field, passing through the test tube and greenhouse stages, has been completed during the year.

Development of Meristem Culture Methodologies

In various experiments, meristem and shoot tips of 10 cassava varieties were subjected to an array of concentrations of cytokinins, gibberellins and auxins as supplements, either singly or in combinations, to the mineral salt and vitamin medium of Murashing-Skoog (with 2% sucrose). The differentiation of a 0.4-0.6 mm meristem tissue into a complete plant (Fig. 1, A-C) was highly dependent upon the appropriate regime of growth regulators and, to a certain extent, upon the cassava variety. Low levels of benzyl aminopurine (BAP) combined with low gibberellic acid (GA) concentrations were suitable for the differentiation of shoots in 90% of the varieties, but rooting was generally inhibited. Addition of 0.1 mg/liter naphthalene acetic acid (NAA) to the culture medium favored shoot and root differentiation in three of the varieties when acting together with GA and in 80% of the material when combined with BAP and GA. Slight increases in the concentration of BAP favored shoot growth, but reduced rooting; however, the exposure of cultures to low temperature (20°C) tended to overcome the inhibition of rooting due to BAP. On the other hand, transfer of

rootless shoots to 0.1 mg/liter of both GA and NAA, with 3% sucrose and at 28-30°C, strongly promoted rooting in 90% of the material.

Thus, the reponse of different cassava varieties to meristem culturing seems to be mainly related to their rooting capability. Based on the types of responses observed at this stage, two different media have been designed: one contains the three hormones at the lowest concentrations and the second contains only GA (0.05 mg/liter) and BAP (0.02 mg/liter). For all practical purposes, the latter should induce shoots in most materials, while the former was designed to promote both shoots and roots simultaneously.

In separate experiments nodal segments, which comprised an axillary bud and its subtending little leaf, were cut from the shoots that developed *in vitro* and were cultured to form "nodal cultures". A medium that contained 0.01 mg/liter BAP, 0.1 mg/liter NAA and 0.2 mg/liter GA plus 3% sucrose supported rapid development of the nodal cultures into complete plants in 90% of the varieties. Depending upon the number of well-developed axillary buds, three to six plants could be grown from each shoot within a month.

Three varieties were used to obtain higher multiplication rates in meristem

Figure 1. Sequential development of cassava plants from meristem culture: (A) A dissected shoot apex showing the dome-shaped apical meristem flanked by two primordial leaves (X40). (B) Differentiation of shoot and root from a meristem after three weeks of culture (X1.2). (C) A five-week-old plant derived from meristem culture (X1.0). (D) A plant derived from meristem tissue, after potting at eight weeks (X0.5).

Effect of temperature on the growth of cassava shoot tips after four months in culture. (E) Incubated at 30°C day and 25°C night temperatures (C0.8). (F) Incubated at 20°C (X0.8).

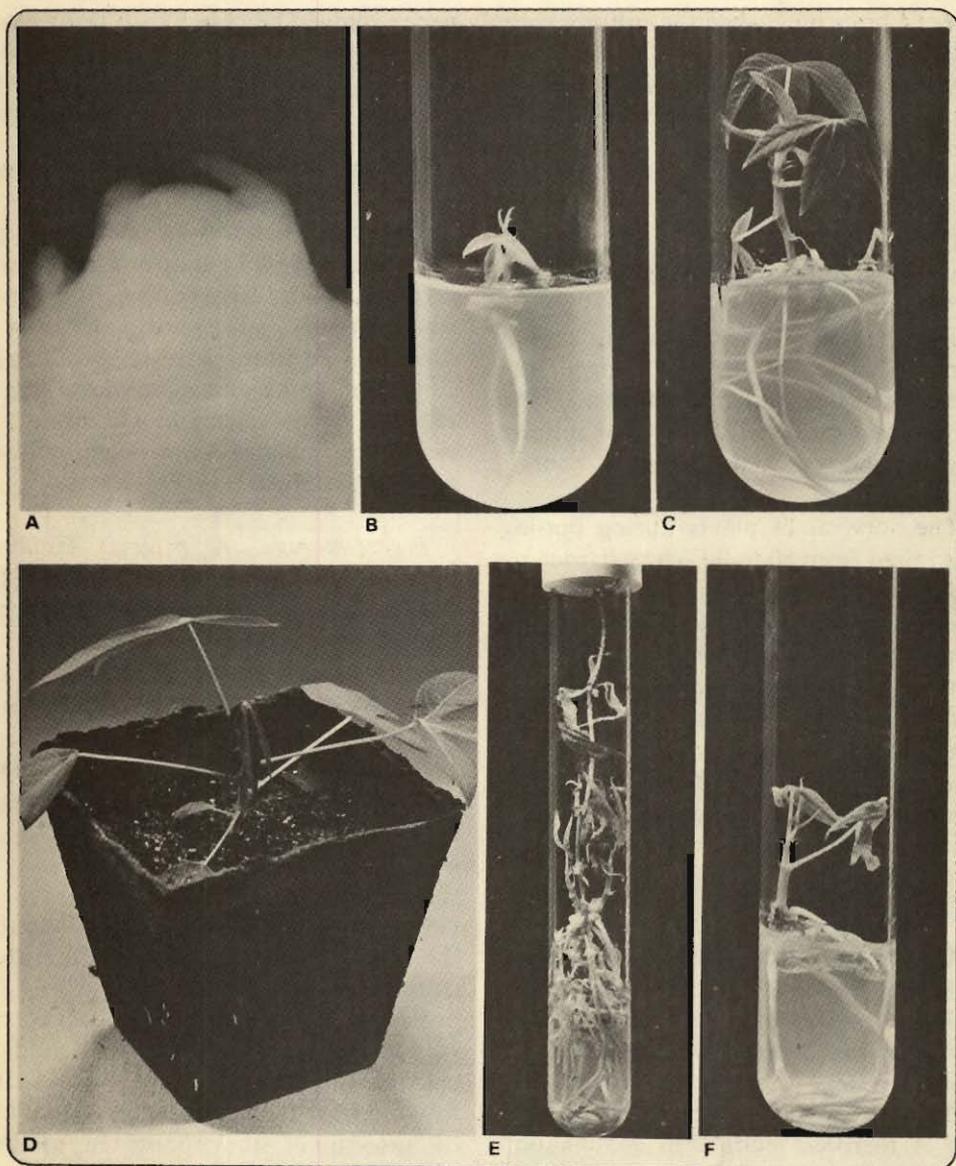


Figure 1.

cultures. Under the influence of high levels of BAP (0.05-0.2 mg/liter), multiple shoot cultures developed from a single isolated shoot apex in two of the varieties. Up to 20 shoots could be produced due to higher rates of branching when the cultures were rotated in liquid media. Chemical and physical means will be sought which could enhance the formation of multiple axillary and/or adventitious shoots in meristem cultures.

During the incubation of the cultures, the temperature was controlled at 28°-30°C during the day and at 24°-25°C during the night. Illumination was kept at about 1000 lux at the beginning of the incubation, then raised to about 2000 lux for the next three to five weeks of culture; photoperiod was controlled at 14 hours.

The survival of plants during potting was raised from 50 to 90% by a hardening treatment which consists of increasing the illumination to 5000 lux and loosening test tube caps one week prior to potting. A potting substrate of vermiculite, fine sand and gravel (1:1:1 v/v) was used. After one week in the laboratory, the pots were transferred to the greenhouse for further growth (Fig. 1,D) and gradual exposure to ambient conditions before field transplanting.

Disease Eradication

The use as planting material of cassava stem cuttings infected with the frog skin disease results in highly significant yield losses (CIAT Annual Report, 1977). The use of meristem culture in combination with thermotherapy for the eradication of the frog skin disease from infected varieties has been initiated. Temperatures may exist at which pathogen host combinations should be grown to obtain maximum inactivation of the pathogen. Hence, both

the intensity and the duration of the thermotherapy applied prior to or during meristem culture are being investigated. Also, the effect of the size of the meristem tissue on the eradication of the disease is under study. The objective of this work is to develop improved techniques to routinely clean up valuable materials (Fig. 2).

Conservation of Genetic Resources

Meristem cultures could be very valuable for the long-term conservation of germplasm since large collections could be stored in small spaces, with the risks of disease contamination practically eliminated and maintenance costs greatly reduced. Two systems of the storage of cassava germplasm as meristem cultures are being studied.

Freeze-storage. A project has been initiated, in cooperation with CIAT, in the Prairie Regional Laboratory, Saskatoon, Canada to investigate the feasibility of preserving cassava shoot apices at the temperature of liquid N (-196°C). Rates of freezing and thawing, as well as the use of cryoprotective and hardening treatments, will be evaluated for their effects on the survival of the meristematic cells.

Minimum growth storage. Work is under way at CIAT to develop methods for the maintenance of cassava meristem cultures at a minimum growth rate for protracted periods of time. Nodal cultures of two cassava varieties were used as starting explants for storage. Results after four months of storage indicate: (1) the rate of growth of the shoots in cultures maintained at 20°C can be reduced from 5.5 cm/month (at 30°C day and 25°C night) to 0.5 cm/month (Fig. 1, E and F); (2) growth could be further reduced, with 100% survival of the cultures, to the rate of 0.2 cm/month if the sucrose level of the

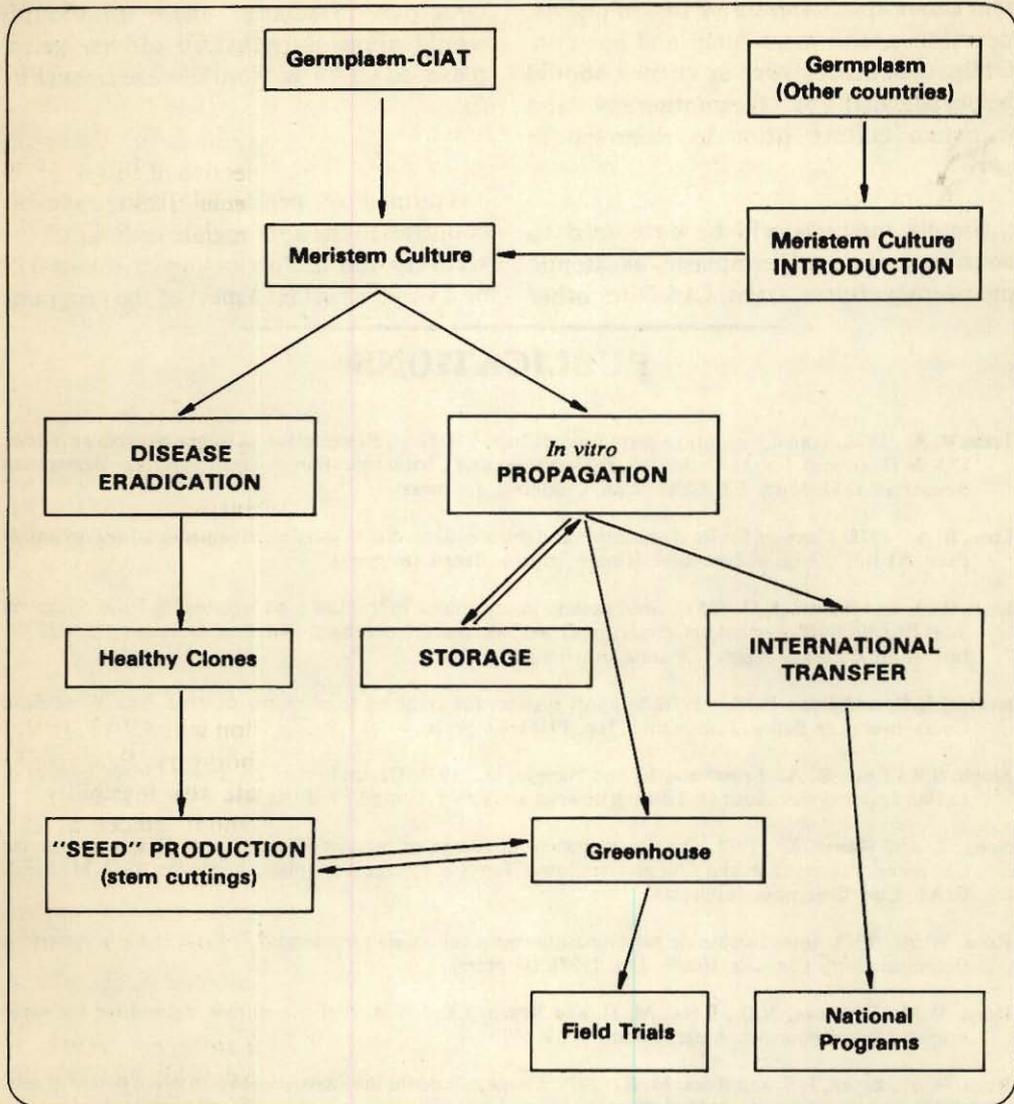


Figure 2. Outline of the various steps in the application of meristem tissue culture to cassava.

culture medium is increased to 5%, but higher sucrose levels tend to reduce the survival of the cultures; (3) the addition of low concentrations of BAP to the culture medium slows down the growth even further without reductions in survival; (4) cultures maintained at 15°C became gradually chlorotic and senescent after one month of storage.

Further research should provide definite information on optimal temperatures, kind and size of explants for storage, propagation rates and genotype stability of the materials recovered from storage.

International Transfer of Germplasm

Tissue cultures initiated from 0.4-0.6

mm shoot apices should be free of insects, nematodes, and most fungi and bacteria. Obligate parasites, such as viruses, should be eradicated by thermotherapy and meristem culture prior to shipment *in vitro*.

Simple methods will be developed to distribute cassava germplasm as aseptic meristem cultures from CIAT to other

countries. Similarly, these procedures would allow introduction of new germplasm to CIAT without disease risks (Fig. 2).

Training of personnel from recipient countries in the appropriate techniques for recovery and multiplication of stocks will be a very important aspect of the program.

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