During 1980 the Tissue Culture section focused on the utilization of meristem culture methods for disease eradication, germplasm conservation and international exchange. Research also continued to refine techniques now used as well as to develop new applications.

Cassava Meristem Culture

The successful regeneration of cassava plants from meristem cultures depends on the interaction of the genotype with the chemical composition of the culture medium (CIAT Ann. Rept. 1978). Under meristem culturing, cassava varieties responded differently in forming shoots and roots simultaneously in a given medium (Fig. 1); most varieties tended to differentiate shoots more consistently than roots. An improved two-step system was devised that permits the regeneration of complete plants regardless of the cassava variety.

The technique is shown in the lower portion of Figure 1. Within 4-5 weeks, every meristem gives rise to three plantlets; thereafter each culture can be multiplied again through nodal cultures by a factor of 5, monthly. Furthermore, every shoot as differentiated on multiple-shoot cultures (CIAT Cassava Progr. 1979 Ann. Rept.) can be multiplied by nodal cultures as well. Over 600 cassava varieties have been processed successfully in this manner.

Disease Eradication

Frog skin disease. The method for cleaning up cassava materials infected with the frog skin disease consists of culturing 0.5-0.6 mm meristem tips excised from stake sprouts grown 3-4 weeks at 40°C (day) and 35°C (night). The effectiveness of the technique was proven by propagating stakes from meristem-derived plants for consecutive cycles in the field. In experiments carried out with 13 cultivars, eight resulted 100% disease-free while five had 82 to 90% disease-free plants at the end of the first cycle; however, all cultivars resulted 100% disease-free after the second cycle of propagation.

Following the success in controlling frog skin disease using the thermotherapy-meristem culture methodology, routine work was begun in 1980 to clean cassava materials from diverse origins. About 300 cultivars were treated, recovered from meristem cultures, potted and handed over to the Cassava Program. Materials included both some infected with frog skin disease, others without apparent infections and collected materials brought to CIAT either as stakes or in meristem cultures.

Grafting studies. The frog skin disease is 100% transmitted downwards through a graft union (CIAT Ann. Rept. 1977). Understanding the extent of its upward transmission could be relevant to studies on the mode of translocation of the causal agent and its dissemination. Using disease-free plants derived from meristem cultures along with their diseased counterparts, reciprocal grafts (i.e., diseased scions onto healthy stocks and *vice versa)* were made with four cassava varieties.

All roots of normal grafts and those of the reciprocal ones (healthy scions onto diseased stocks) showed disease symptoms 3-4 months after grafting. Stem cuttings were made from various heights along the scion of each reciprocal graft, rooted and grown in the greenhouse for three months.

The greatest relative degree of symptoms and the largest number of roots with symptoms were on stem cuttings closest to the graft union; fewest symptoms were observed on cuttings originating at the top of the shoot (Table 1). Surprisingly, when cultivar M Col 721 was the disease-free scion, no symptoms were detected in any plants from its stem cuttings, even though disease symptoms were present when the same clone acted as the stock. This may suggest that structural factors are preventing translocations of the disease upwardly, although it may move downwards freely.

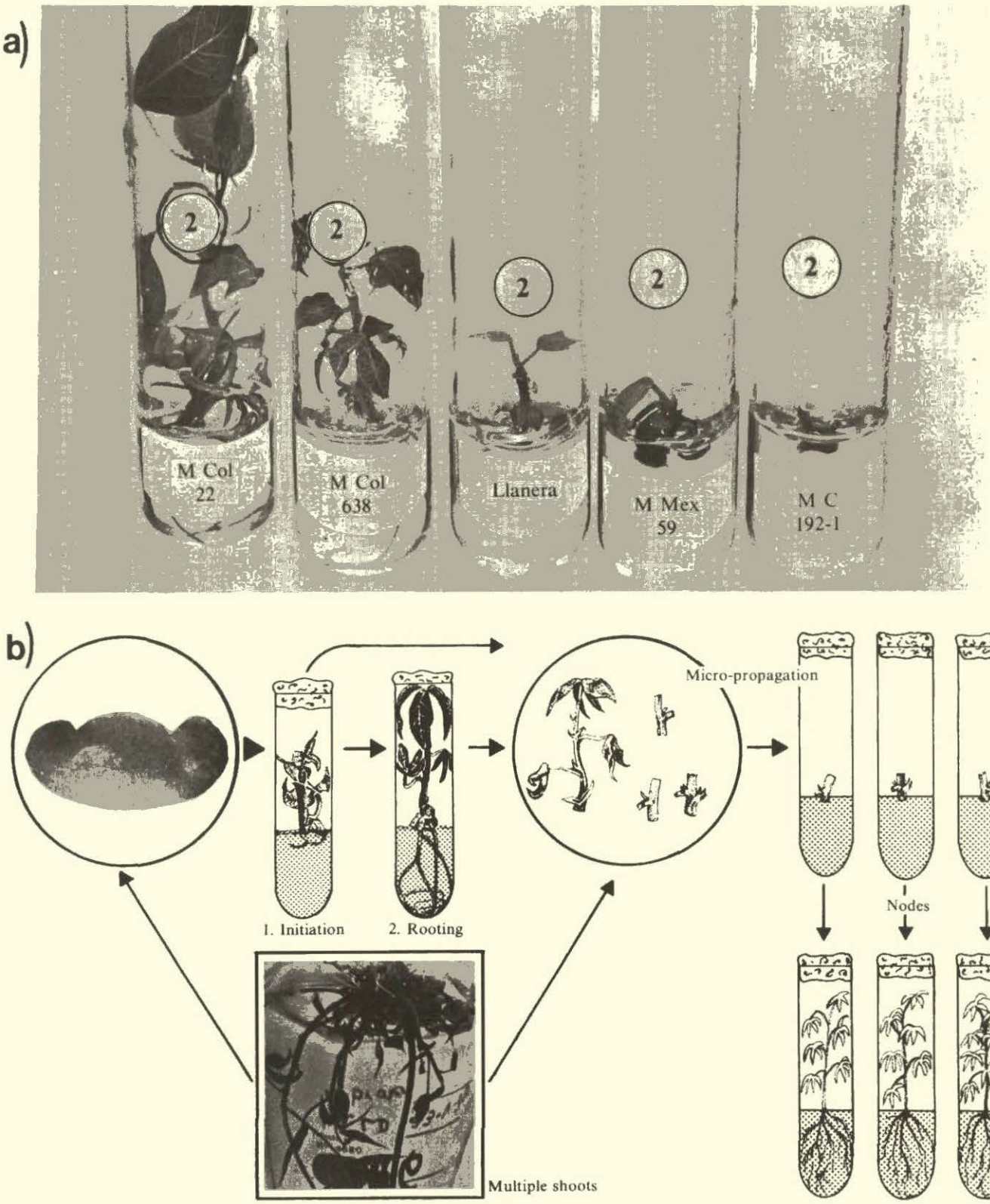

Figure 1. a) *Simultaneous development of shoots and roots in a single medium is influenced by the genotype.*
b) *Two-step technique to regenerate plants from meristem cultures regardless of the cassava variety.*

Table I. Transmission of the frog skin disease through reciprocal graft unions of various cassava varieties.

Graft [1]		Downward transmission	Upward transmission		
			Stem cut position [2]	Relative symptoms	No. roots with symptoms [3]
Scion	Stock				
M Col 67(+)	M Col 67(-)	100%			
M Col 67(-)	M Col 67(+)		1	+	2/6
			2	++	2/4
			3	+++	8/8
			4	++++	10/10
M Col 67(+)	M Col 33(-)	100%			
M Col 33(-)	M Col 67(+)		1	+	3/6
			2	+	4/8
			3	++	10/10
			4	+++	8/8
M Col 67(+)	M Col 721(-)	100%			
M Col 721(-)	M Col 67(+)		1	-	0/6
			2	-	0/5
			3	-	0/10
			4	-	0/8
M Col 329(+)	M Col 721(-)	100%			
M Col 721(-)	M Col 329(+)		1	-	0/6
			2	-	0/8
			3	-	0/10
			4	-	0/12

[1] (+) diseased plants; (-) meristem culture-derived, disease-free plants.
[2] Stem cuttings obtained above the graft union from plants showing root symptoms: 1=top shoot; 4=shoot closest to graft union.
[3] No. of roots with symptoms/ Total no. of roots thicker than 0.5 cm.

Germplasm Conservation

Conditions have been devised to maintain meristem-derived cassava cultures *in vitro* for protracted periods. Objectives are to slow the growth rate of cultures to a minimum while increasing viability to a maximum. The work has demonstrated the importance of storage temperature and illumination, as well as the composition of the culture medium.

Shoot growth rate of meristem cultures was directly related to storage temperature, within the relative limits of 15°-35°C and depending upon the materials. Growth temperatures below 18°C were detrimental to most varieties, especially after 3-5 months of exposure.

The amount of rooting during storage is also important. Rapid rooting shortened the transfer period of the cultures due to deterioration following the oxidation of phenolic-type exudates to the medium from the old roots. Low temperature, which retards shoot growth, tended to promote rooting; high illumination (about 4000 lux) also favored rooting. When illumination was lowered and composition of the culture medium altered by adding a high level of cytokinin and a low concentration of sucrose, not only rooting was delayed, but cultures remained nearly 100% viable so that the transfer period was extended to at least two years.

Therefore, conditions established for the storage of meristem-derived cassava cultures *in vitro* are:

Temperature:	20°-22°C
Illumination:	1000-1500 lux
Photoperiod:	12 hours

Culture medium: Mineral salts of Murashige-Skoog (full strength, with inositol and thiamine-HCL)
low sucrose
high cytokinin
high gibberellin
agar

Type of culture: Nodal cultures and multiple-shoot cultures in 18 x 150 mm test tubes

Transfer period: At least two years.

In order to establish CIAT's cassava germplasm bank *in vitro*, a room (3 m wide x 4 m long x 2.5 m high) was set up this year with the above conditions. The room can store more than 14,000 test tubes of cultures (Fig. 2).

Cassava materials from different sources are being transformed routinely into meristem cultures for storage. Priority for storage is given to materials cleaned of frog skin disease, to clones from the germplasn bank and advanced hybrid lines, and to materials recently introduced to CIAT.

Up to November 1980, materials have been maintained for over two years without replenishing the culture medium; others have been kept for 15-18 months and most varieties maintained for up to one year. Throughout storage, the cultures produce new axillary buds. The average number of axillary buds per variety is directly related to the capability of plant regeneration upon retrieval from storage; thus, it is a measure of the culture's viability. On the average, cassava varieties stored have produced at least three axillary buds during storage periods of 3-24 months, and the variability of response between different varieties is low enough (0.12-0.17 C.V.) so as to provide confidence in the application of the technique.

In order to test whether varietal characteristics of materials have remained stable during long-term storage, cultures have been retrieved every six and 12 months, propagated and transplanted to the field for comparison with stake-propagated materials. In terms of the general growth and morphology of tops and roots, plants stored *in vitro* remained true to type. However, meristem-derived materials showed less variation in yield between plants than did stake-derived ones. This work is continuing.

Figure 2. *Light- and temperature-controlled storage room for cassava meristem-derived cultures* **in vitro**, *in CIAT's Genetic Resources Unit. The room has a capacity of more than 14,000 test tubes, each containing a single plant.*

International Exchange of Germplasm

During 1980, 61 cassava varieties were shipped from CIAT to eight countries as aseptic meristem cultures.

As a follow-up to a training course on cassava meristem culture, 18 varieties were delivered on a trial basis to five Southeast Asian countries. Preliminary results showed that successful use of the method depended mainly on two factors — the arrival conditions of the cultures, which in turn depend on the lenght of transit, and on the effectiveness of handling the cultures after arrival. Handling success of 45-70% was obtained in the first shipment, prepared and hand-carried by the trainees themselves; later shipments to Thailand and Malaysia were handled with greater success (85-95%), as estimated from information provided from trainees (Table 2). Although some cultures arrived showing deterioration due to etiolation and phenolic-type browning in protracted darkness — from air mail shipments of 25-30 days — these gave rise to plants after proper handling.

Another 43 cassava varieties sent in a total of nine shipments to Brazil, Costa Rica and the United States all arrived in good condition after both hand-carried and air-mailed trips of 3-12 days.

In order to minimize the detrimental effects of long trips on cultures, work is being conducted for conditioning the materials to stand long periods of darkness without irreversible damage, as well as to implement rapid propagation methods of recovered materials after their distribution from CIAT. A multiplication technique that utilizes single leaf-bud cuttings, developed in the Philippines, is being adapted to conditions at CIAT, to rapidly build up materials from importation of a few cultures (see discussion in Agronomy section).

Because of their disease-free condition, meristem cultures can be utilized not only to distribute cassava materials from CIAT to other countries, but to enrich CIAT's germplasm bank with new introductions. This method of germplasm transfer provides adequate safeguards to minimize the risks of disease dissemination existing when cassava stakes are moved.

The transfer to Colombia of cassava collections from Peru and Brazil has been prevented previously due to the presence of coffee rust in those countries. Under arrangements made with the Colombian plant health authorities, nearly 200 cassava varieties, as aseptic meristem cultures, have been introduced to CIAT in December 1979 and October 1980, from Peru and Brazil, respectively (Fig. 3).

Table 2. Distribution of cassava materials as meristem cultures from CIAT to various locations in Southeast Asia.

Destination	Method of shipment	No. tubes/ No. varieties	Trip duration (days)	Condition of cultures upon arrival	Tubes handled successfully after arrival (%) [1]	
					recovery	propagation
Thailand	1. hand-carried [2]	16/8	10	good	65	75
	2. air-mailed	8/4	30	etiolated, brown	85	85
	3. hand-carried	4/2	8	good	90	-
Malaysia	1. hand-carried [2]	18/9	7	good	70	70
	2. air-mailed to Kew					
	Gardens, UK; Kew	10/2	10	good		
	Gardens to Malaysia	10/2	15	etiolated	95	-
Philippines	1. hand-carried [2]	8/8	8	good	45	80
	2. air-mailed	4/2	25	etiolated, brown	50	-
Sri Lanka	1. hand-carried [2]	9/9	8	pale green	60	-
Indonesia	1. hand-carried [2]	8/8	10	pale green	70	-

[1] Estimated on the basis of information received from trainees.
[2] Cultures prepared and carried by trainees attending course at CIAT, November 1979.

Figure 3. *A) More than 130 varieties of cassava were brought to CIAT from Brazil in this single package (45 x 25 x 22 cm). B) Plants derived from meristem cultures brought from Peru are maintained under quarantine in this greenhouse before incorporation into CIAT's germplasm bank.*

Other Tissue Culture Systems in Cassava

Anther culture. The microspores, being the direct product of meiosis, are haploid cells which under appropriate conditions can be shifted to perform an entirely new role, namely, the development of tissues and plants instead of the male gametes. The number of species which have yielded to androgenesis is considerable; however, no results have been obtained with cassava as yet. Under a training scholarship, work has been initiated at CIAT in anther culture of cassava. So far, a strong varietal influence was observed in the development of macroscopic structures (i.e., callus cells) in anther cultures as a response to the factors of the medium. Work is underway to ascertain the conditions conducive to organogenesis in anther-derived callus cultures. Although the techniques are still in their infancy, the incorporation of haploids into specific cassava breeding programs could be useful since genes in these materials segregate in gametic rather than zygotic proportions and could be linked to somatic fusion and operate in conjunction with conventional breeding. A possible practical use of haploids may be the production of hybrid cassava seed.

Protoplast cell cultures[1]. The ability to regenerate plants from individual cells now exists for a limited number of species. This new techonology can assist in the identification, assembly, recombination and selection of novel forms of genetic variability.

With the support of a grant from the Rockefeller Foundation to Dr. J. Shepard, work was done at Kansas State University, Kansas, U.S.A., by Drs. E. Shahin and D. Bidney which involves the enzymatic isolation of large numbers of cassava leaf cells without their walls, called protoplasts, followed by the incubation of these protoplasts in a medium of complex formulation. The protoplasts will undergo a series of cell divisions to form multicellular colonies and unorganized callus tissue. By the implementation of several more media, shoot development has occasionally been observed. Work is currently underway to define the proper conditions for routine single cell regeneration techniques.

Similar work, though on a limited scale, was also initiated by Dr. R. Litz at the Agricultural and Research Center, University of Florida, Homestead, U.S.A.

Using callus cultures induced on various type of tissues, Drs. G. G. Henshaw and J. Stamp are working under a grant from the British ODM, at the University of Birmingham, England. Here, attention is paid to those cultural conditions which favor the development of meristematic "nodules" in the callus mass. Further work will place emphasis on the use of embryonic tissues and sequential culture regimes.

CIAT has provided selected materials for use in the studies referred to above.

Freeze preservation[1]. Research continued at Saskatoon, Canada (Dr. K. Kartha) and Birmingham, England (Drs. G.G. Henshaw and J. O'Hara) to store cassava meristems in liquid nitrogen (-196°C). In both laboratories, meristem cultures subjected to an array of freeze-thaw protocols have showed low survival with callus formation, but no organogenesis has been obtained from the callus cells as yet.

[1] This work, although not carried out at CIAT, is reported here because it directly relates to tissue culture work done at CIAT. The Center collaborates with these efforts by providing selected materials and by discussing with the authors of this work the orientation and methodology of the research.

Errata

Page	Columm	Element	Printed:	Should be:
6	1	Figure 2	M Col 59	M Mex 59
6	2	Figure 3	M Col 59	M Mex 59
6	2	Figure 3	LSD (P<0.05)	LSD (P<0.05)
7	1	Figure 4	M Col 59	M Mex 59
60	2	Second para., line 8	more to growth	more top growth
61	2	Line 1	and K contents	and K concentrations
20	1	Figure 1	I - Tolerant III - Tolerant V - Tolerant	I - Intermediate-resistant III - Intermediate-resistant V - Intermediate-resistant
62	1	Figure 3	Stems □	Stems △
64	1	Figure 5	5.0 4.0 % N	5.0 4.0 % N 3.0
66	1	Figure 8	Figure 44	Figure 8
93	2	Footnote	*Left during 1979.	*Left during 1980.