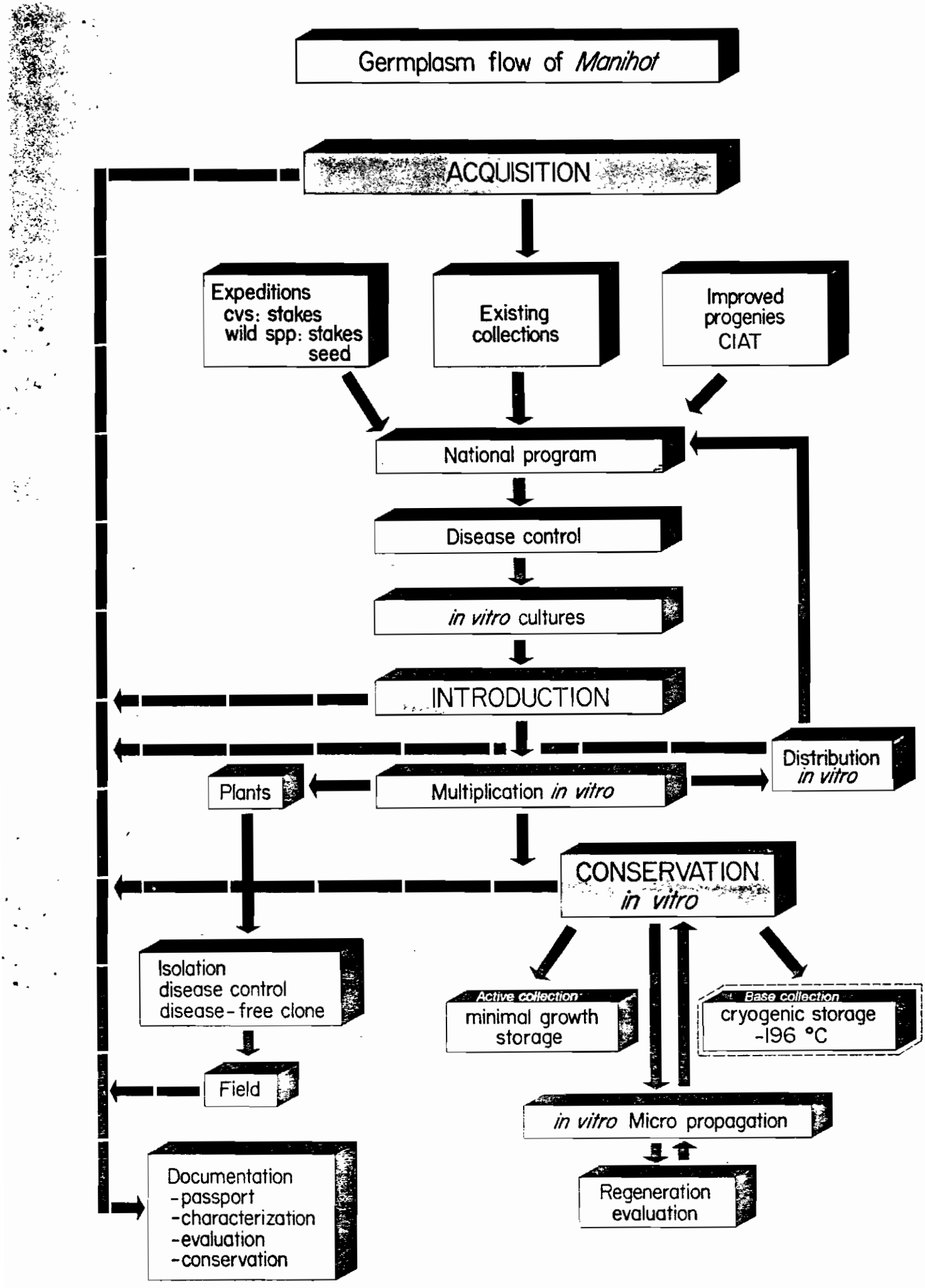
UTILIZATION OF TISSUE CULTURE TECHNIQUES FOR THE CONSERVATION AND INTERNATIONAL EXCHANGE OF CASSAVA (Manihot esculenta) GERMPLASM

W. M. Roca, J. Narvaez, J. Rodríguez, R. Reyes, G. Mafla, J. Beltrán, J. Roa, H. Ramirez, A. Rodríguez, 1984.

GENETIC RESOURCES UNIT
APARTADO AEREO 6713
CIAT, CALI, COLOMBIA

22p. Engl., Illus.


Germplasm flow of *Manihot*
ACQUISITION
Expeditions
cvs: stakes
wild spp: stakes
seed
Existing collections
Improved progenies CIAT
National program
Disease control
in vitro cultures
INTRODUCTION
Distribution *in vitro*
Plants
Multiplication *in vitro*
CONSERVATION *in vitro*
Isolation
disease control
disease-free clone
Active collection
minimal growth storage
Base collection
cryogenic storage
-196 °C
Field
in vitro Micro propagation
Regeneration evaluation
Documentation
-passport
-characterization
-evaluation
-conservation

MICRO PROPAGATION OF CASSAVA BY MEANS OF MERISTEM- TIP CULTURE

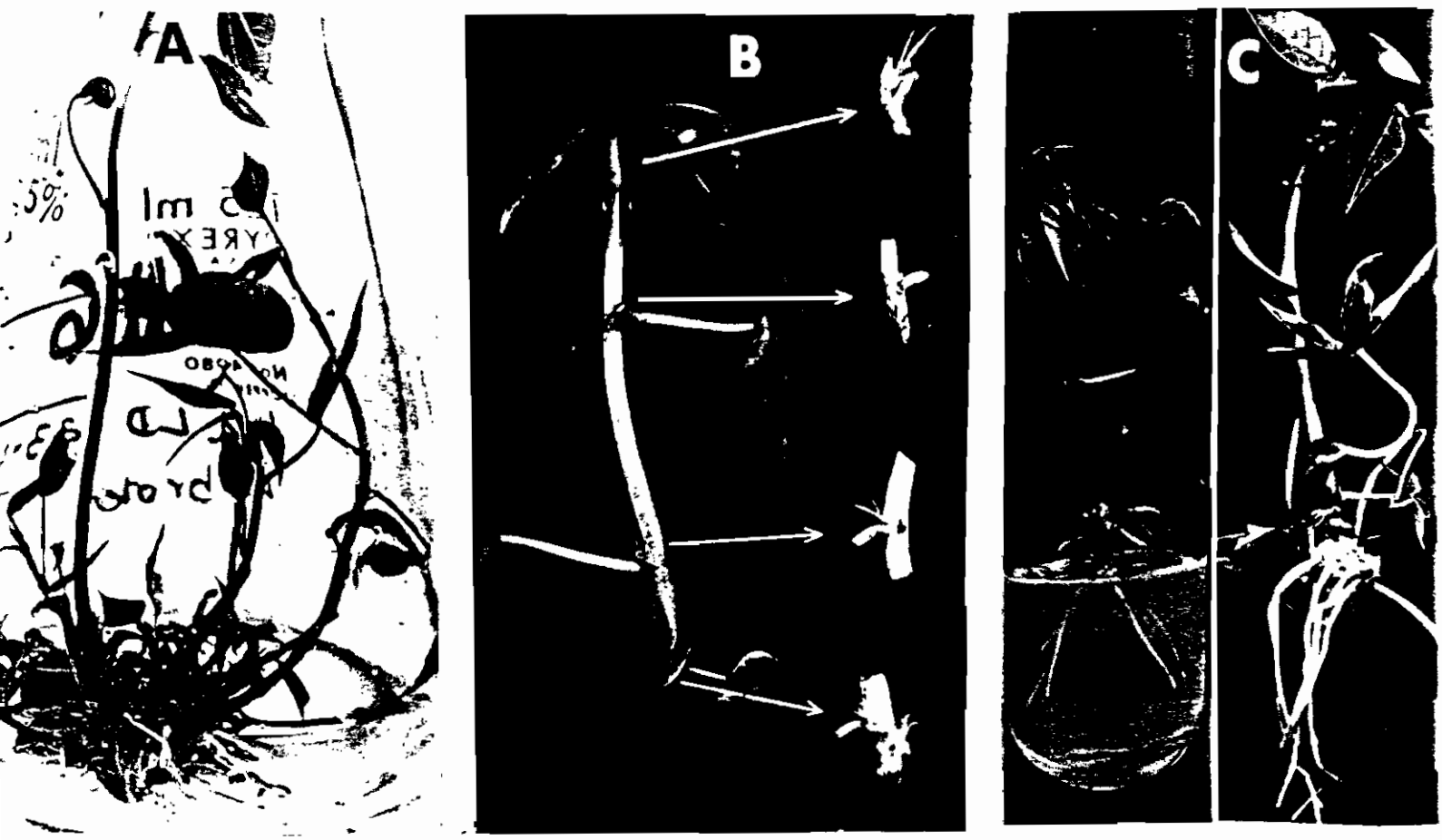


FIGURE 1.

A. Multiple shoot culture derived from meristem-tips

B. Nodal micro-cuttings obtained from each shoot, as at A

C. Complete plantlet developed from nodal micro-cuttings, as at B: left, after 3 weeks; right, ready for potting.

Over 2.000 cassava varieties have been propagated through this technique .

TABLE 1. POTENTIAL PROPAGATION RATES* OF CASSAVA THROUGH IN VITRO METHODS AND COMPARISON WITH CONVENTIONAL TECHNIQUES

	Field	Greenhouse	In vitro nodal culture		In vitro multiple shoot culture	
Months	Commercial Cuttings	Single node Propagation	Nodes/month 2	4	Shoots produced/month 20	40
6	-	$4\text{-}10 \times 10^1$	8×10^0	5×10^2	8×10^6	5×10^8

FIGURE 5B. In vitro storage facility at CIAT (Tissue Culture Lab).

Dimensions: 5 x 6 x 2.5 m
Capacity: 6,000 cassava clones, with 5 replicates each

A. View from outside.

B.and C. Inside views.

TABLE 4. NUMBER OF CLONES, STORAGE DURATION, AND FREQUENCY OF TRANSFERS OF CASSAVA GERMPLASM STORED IN VITRO

Number of clones*	Storage duration (months)	$\bar{X}$ number of transfer
1,106	0 - 12	0.0
145	13 - 18	0.6
287	19 - 24	1.0

FIGURE 2. Poot yield of frag-skin free clones (right) and infected (left). Frog skin is known to be caused by a virus complex. In vitro propagated clones are indexed by: serological (agar and Elisa), grofting and electrophoretic techniques.

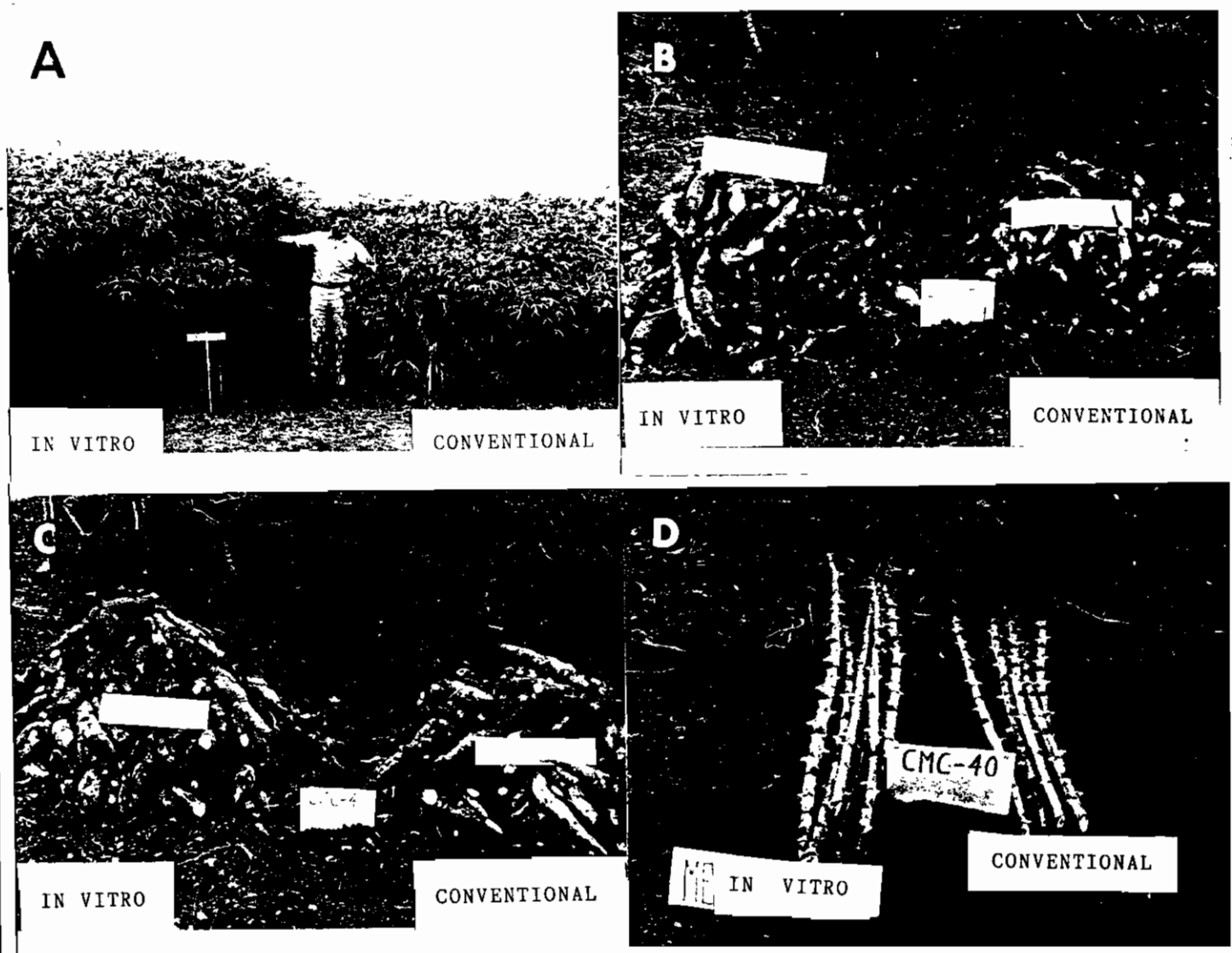


FIGURE 3. Rehabilitation of local cassava cultivars by means of meristem-tip culture.

A. Increase in vigour of cv "Llanera" (left) in second year following *in vitro* propagation, as compared to a conventionally propagated crop (right).

B. Root yield increase due to *in vitro* propagation of cv "Llanera" (yield of 20 plants).

C. Same as B, for cv. "CMC-40"

D. Increase in size and width of stems due to *in vitro* propagation, cv. "CMC-40".

INTERNATIONAL EXCHANGE OF CASSAVA GERMPLASM USING IN VITRO TECHNIQUES

FIGURE 4. A. Cassava cultures prepared for shipment from CIAT to National Programs.

B. Package of cultures ready for shipment.

C. Clones recovered from in vitro cultures transfered from Brasil to CIAT-

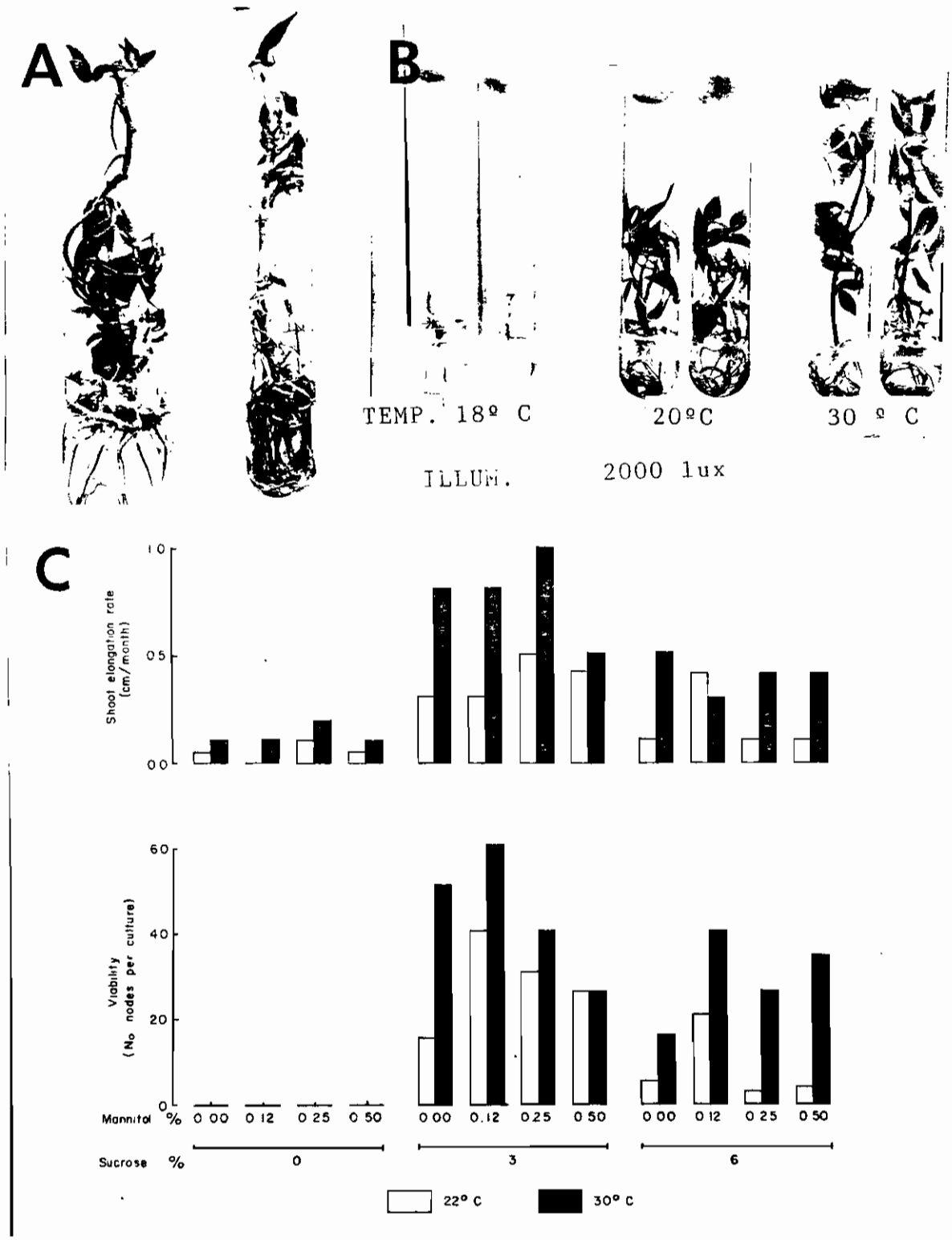


FIGURE 5A A. Left: A cassava culture after 18 months storage at 20°-22° C Right: As at A, but maintained at 28°- 30° C

B. Effect of storage temperature on growth and viability of cassava cultures. Illumination: 2000 lux.

C. Effect of mannitol and sucrose concentration on growth and viability of cassava cultures stored at 22° C and 30° C.

TABLE 2. DISTRIBUTION OF CASSAVA CLONES FROM CIAT TO NATIONAL PROGRAMS USING IN VITRO TECHNIQUES

Region	No of countries	Number of clones *						
		1979	1980	1981	1982	1983	1984	TOTAL
America South	4	20	9	14	-	22	6	71
Central & Caribbean	6	-	-	33	26	23	200	282
North	2	-	30	32	19	18	10	109
Asia	6	34	16	10	34	34	9	137
Africa	1	-	5	-	24	-	22	51
Oceania	1	-	-	8	-	-	9	17
Other	4	-	7	1	2	6	-	16
TOTAL/YEAR		54	67	98	105	103	256	
GRAND TOTAL (August,1984)					683			

* 5-10 Test tubes per clone.

TABLE 3. TRANSFER OF LATIN AMERICAN CASSAVA COLLECTIONS TO CIAT USING IN VITRO TECHNIQUES

Procedence of Germplasm	No cvs. Transfered each year from Nat. Programs					
	1979	1980	1981	1982	1983	1984
CIPA, Chiclayo, PERU	127	-	-	-	26	-
CIPA, Tarapoto, PERU	-	-	-	-	80	-
CNPMF, Bahía, BRASIL	-	130	110	-	-	-
CENARGEN, Brasilia, BRASIL	-	-	45	-	-	-
EUPAE, Manaus, BRASIL	-	-	-	72	12	-
IPAGRO, R.G. do Sul BRASIL	-	-	-	73	9	-
EMGOAPA, Goias, BRASIL	-	-	-	121	-	-
CPATU, Pará, BRASIL	-	-	-	74	-	-
EMEPA, Paraiba, BRASIL	-	-	-	3	-	-
Indian Res., Amaz,BRASIL	-	-	-	9	-	-
Sta. Catarina, BRASIL	-	-	-	-	172	-
CPAC, Brasil, BRASIL	-	-	-	-	56	-
IAN, Caacupé, PARAGUAY	-	-	-	-	154	-
Corrientes, ARGENTINA	-	-	-	-	21	-
CATIE, Turrialba, COSTA R.	-	-	-	-	-	135
IDIAP, Rio Hato, PANAMA	-	-	-	-	-	23
TOTAL/YEAR	127	130	155	352	530	158

GRAND TOTAL (August,1984) 1.452

EVALUATION OF PHENOTYPIC STABILITY OF CASSAVA CLONES STORED IN VITRO

Five cassava clones; M. Mex 20, M. Col 650, M. Col 2197, CM - 305-38 and CM 323 -375 were retrieved from minimal growth culture conditions after 42,12,18,30 and 30 months of storage, respectively. Following micro-propagation by nodal cuttings, the clones were transplanted to the field along with two types of control plants: meristem-culture propagated plants and conventionally propagated plants through stem cuttings.
Phenotype stability was evaluated on the bases of morphological, agronomic and biochemical criterion.

MORPHOLOGY. Parameters evaluated: color of apical leaf, pubescence of apical leaf, shape of leaf lobes, color of petiole, petiole lenght, color of stem epidermis, color of stem cortex, color of root epidermis, color of root cortex and color of root parenchyma. As shown in Table 5, out of the 10 morphological characteres and the 5 cassava clones, significant variations were found in four characteres and only in the cv. M. Mex 20. Clearly, the deeper green color of leaves as well as the 30% larger petioles and leaves are due to passing the plants through an *in vitro* stage prior to either storage or simple micro-propagation. However, *in vitro* storage, but not simple meristem culture, was the cause of root epidermis and cortex variation in color.
All other morphological descriptors evaluated remained unaltered in the other four clones.

AGRONOMY . The following agronomic parameters were evaluated at harvest: plant height, height of stem at first branching, number of branches, yield of fresh roots, and fresh weight of tops.

Again only clone M. Mex 20 showed variation in plant height, 55% taller due to in vitro passing; and the height at which the first branching occured. Storage-derived and meristem cultured plants were 85% and 62% taller respectively than stem cutting propagated plants. While yield of fresh roots was significantly higher in in vitro processed plants of clones M.Mex 20 and M.Col 650, it was quite low in the other three clones. However, the harvest index in all five clones increased as one passes from stem cutting propagation to meristem culture and to in vitro storage. This shows that in vitro processing resulted in more efficient distribution of growth (Table 5).

BIOCHEMISTRY. Extracts of five different plant tissues from stake sprouts were run through starch gel electrophoresis using five buffer systems. Gel slices from each tissue and buffer system were stained for 16 isozymes.

Out of these trials, 12 isozymes, two buffer systems and two tissues (stem nodes and root tips) were chosen for phenotypic evaluation. (Table 6).

Variations in the banding patterns of different cassava cultivars (Fig. 6A, B,C,) were evident for several enzymes. However gels from different plants of the same clone showed no differences at all. This demostrates the validity of the technique for phenotyping. Gels of plants retrieved from in vitro storage, meristem-tip propagated plants and stem cutting propagated plants from each of the 5 clones and for each of the 12 isozyme showed no differences in electrophoretic patterns (Figs. 7I and 7II) .

Adjustment of electrophoretic analysis to in vitro cultures would save time, space and costs of evaluation in large germplasm collections such as CIAT's . Fig. 8A and B- shows enzyme patterns of stake sprouts and in vitro cultures Clearly, the enzyme concentration in the tissue extract fron in vitro cultures is lower than from sprouts; and no qualitative differences ocurred. Work to expand this approach to other genotypes and more enzymes is underway.

CONCLUSIONS. With the exception of variation in the color of root epidermis and cortex, no changes have resulted solely from in vitro storage in any of the morphological, agronomic and biochemical characters evaluated. The morphological changes observed in the clone M.Mex 20 can be attributed to the elimination or dilution of the causal agent for the frog skin disease, achieved by passing the plants through in vitro culture. Here, alterations in morphology were accompainced by increase in vigor and yield. Clone M.Col 650 also showed yield increase but not morphological changes. Interesting, all clones showed more efficient distribution of growth as a consequence of in vitro culture.

Evaluation of these materials in a second growth cycle should confirm their phenotypic stability.

LIQUID NITROGEN STORAGE

Following retrieval from nitrogen, 200 cultures of 7 cassava cultivars have been transfered to CIAT from the Plant Biotechnology Institute, Saskatoon Canada.

After micro-propagation by nodal cuttings, plants have been moved to the field along with their stem cutting counterparts. Phenotypic stability will be evaluated using approaches similar to the ones described above.

TABLE 5. MORPHOLOGICAL AND AGRONOMIC CHARACTERS SHOWING VARIATION IN PLANTS REGENERATED FROM IN VITRO STORAGE AS COMPARED TO PLANTS PROPAGATED BY MERISTEM CULTURE AND STEM CUTTINGS*

Parameter	Cultivar	Stored material	Merist.cult. propagation	Stem cutting propag.
1. Color apical leaf	M.Mex 20	darkgreen	darkgreen	light green
2. Petiole length (cm)	M.Mex 20	25.8	25.7	19.9
3. Color root epidermis	M.Mex 20	light brown	white	white
4. Color root cortex	M.Mex 20	yellow	white	white
5. Plant height (m)	M.Mex 20	2.8	2.8	1.8
6. Height 1st. branching (m)	M.Mex 20	2.4	2.1	1.3
7. Yield fresh root (ton/ha)	M.Mex 20	29,000	30,000	14,000
	M.Col 650	23,000	19,000	17,000
	M.Col 2197	7,000	6,000	21,000
	CM 305-38	23,000	24,000	38,000
	CM 323-375	11,000	18,000	26,000
8. Harvest index	M.Mex 20	0.5	0.4	0.4
	M.Col 650	0.7	0.6	0.3
	M.Col 2197	0.6	0.5	0.5
	CM 305-38	0.8	0.7	0.7
	CM 325-375	0.6	0.6	0.5
9. Frog skin disease	M.Mex 20	-	-	+
	M.Col 650	-	-	+
	M.Col 2197	-	-	-
	CM 305-38	-	-	-
	CM 325-375	-	-	-

* Out of 10 morphological and 6 agronomic parameters evaluated.

TABLE 6. ISOZYMES AND BUFFER SYSTEMS FOR EVALUATION OF CASSAVA CLONES STORED IN VITRO

ISOZYMES	BUFFER SYSTEM	
	STD	PC
Acid phosphatase (AcP)	+	
Alcohol dehydrogenase (ADH)	+	
Glutamate-oxaloacetate-transaminase (GOT)	+	
Glutamate dehydrogenase (GDH)		+
Isocitric dehydrogenase (IDH)		+
Malate dehydrogenase (MDH)		+
Malic enzyme (ME)	+	
Peroxidase (Prx)	+	
Phosphogluco-isomerase (PGI)	+	
Phosphogluco-mutase (PGM)		+
6-phosphogluconate dehydrogenase (6-PGDH)		+
Shikimic dehydrogenase (SKDH)		+

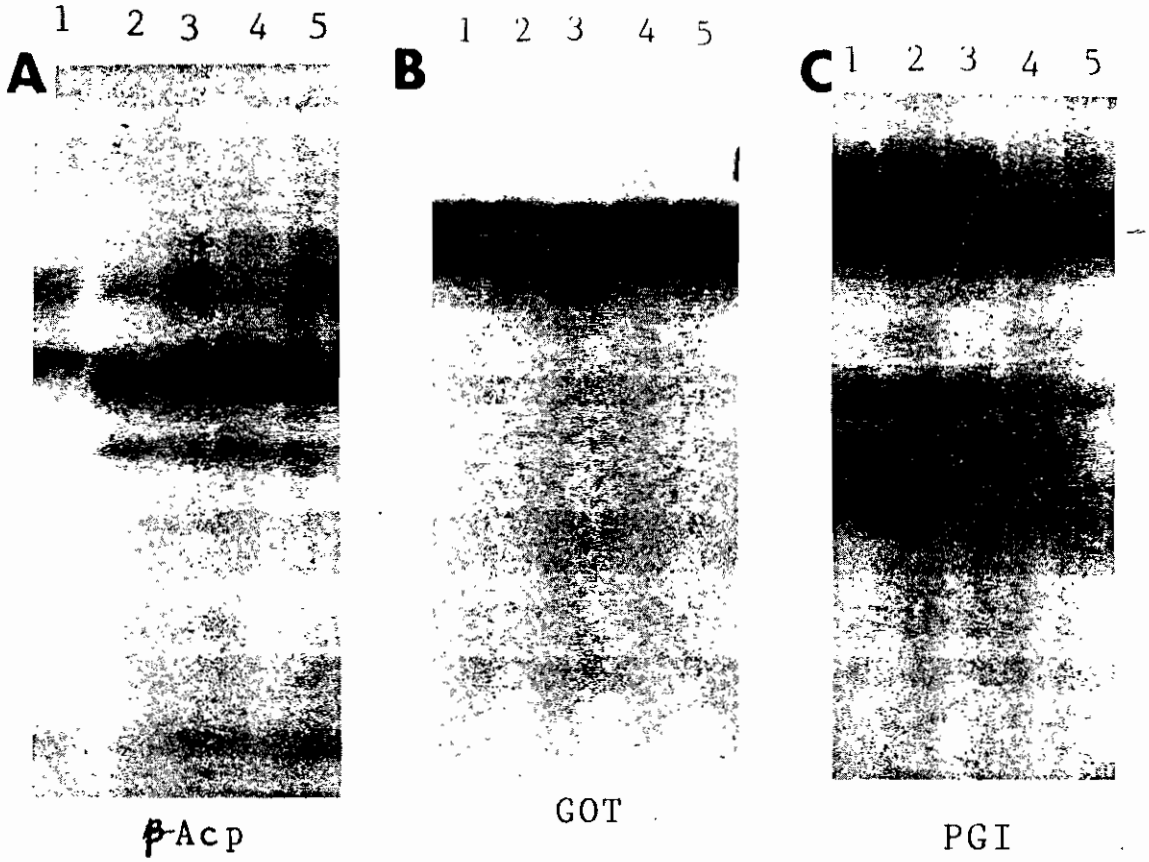


Figure 6. Genotype finger printing of cassava clones by starch electrophoresis.

1, 2, 3, 4, 5: cassava clones

A. Acid phosphatase

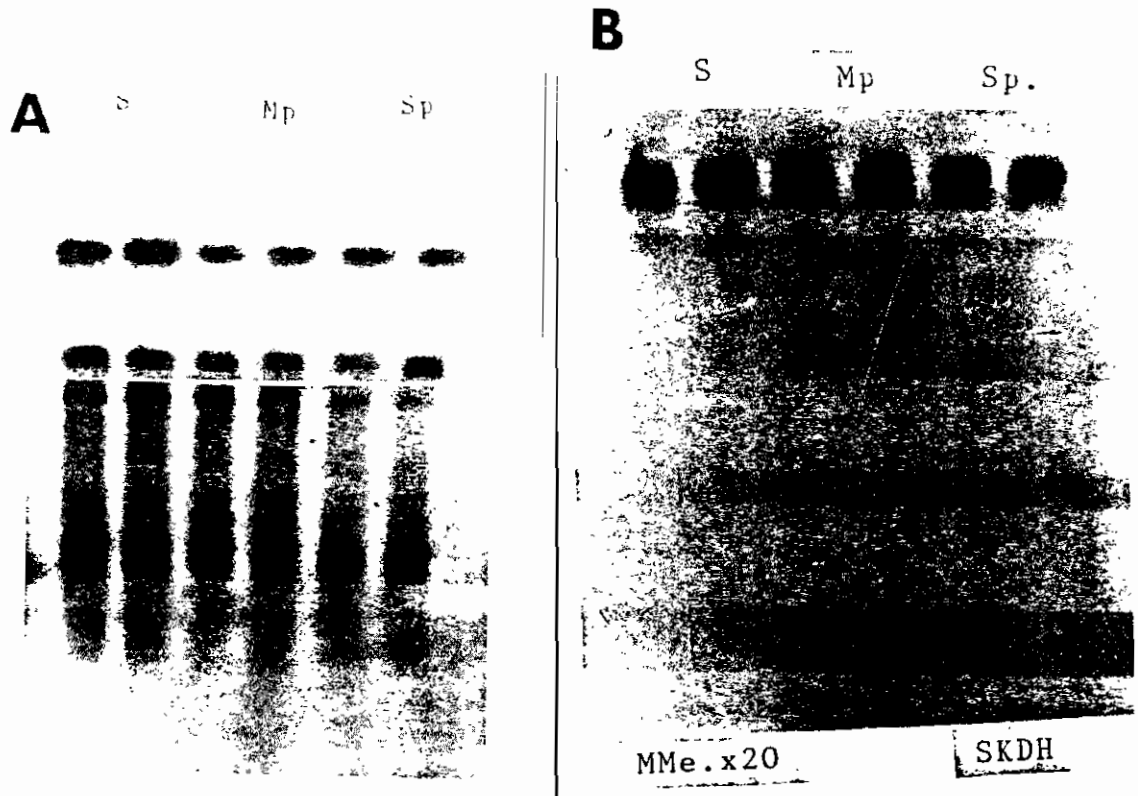
B. Glutamate-oxaloacetate-transaminase

C. Phosphogluco - isomerase

FIGURE 7I. Isozyme patterns of cassava clones stored *in vitro* (S), micro-propagated by meristem culture (Mp) and propagated by stem cuttings (Sp).

cv. M Mex 20 A. peroxidase;
B. shikimic dehydrogenase

cv. M Col 650 A. glutamate-oxaloacetate transaminate ;
B. malic enzyme



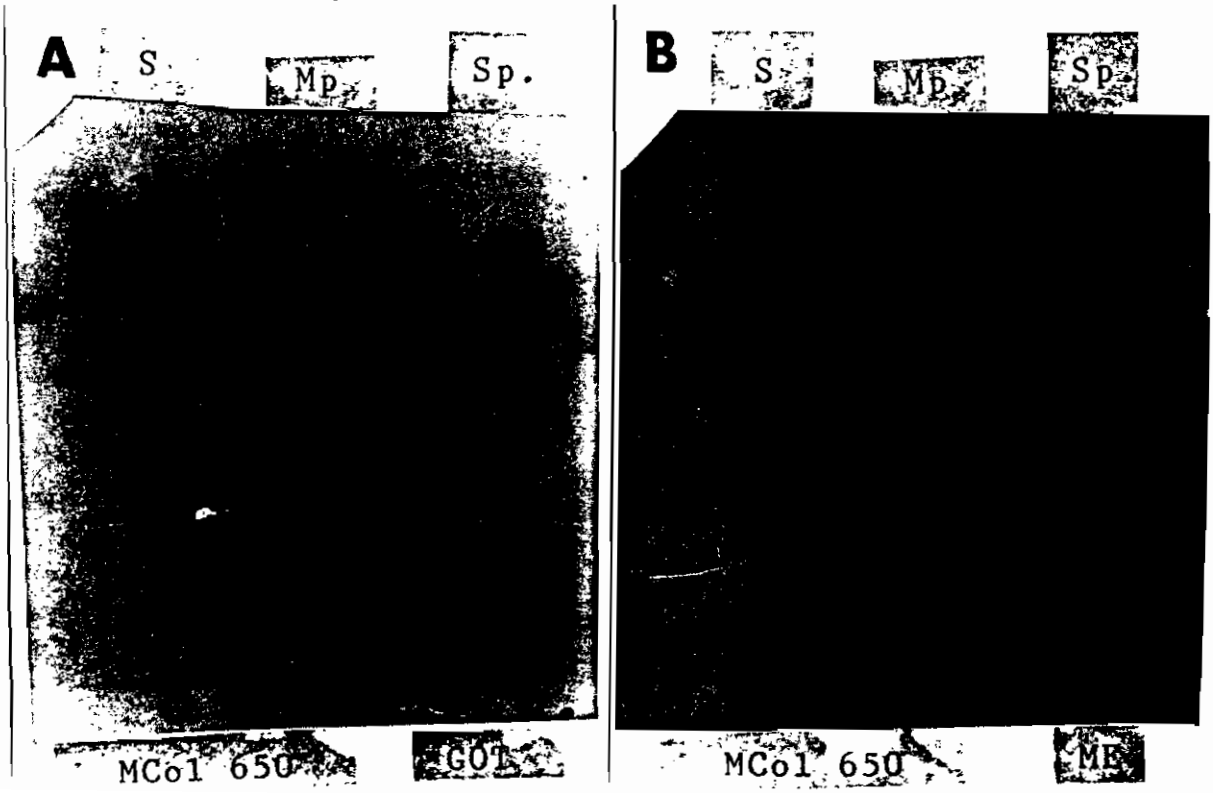


FIGURE 7II. Continuation

cv. CM 323-375: A. Acid phosphatase

B. Alcohol dehydrogenase

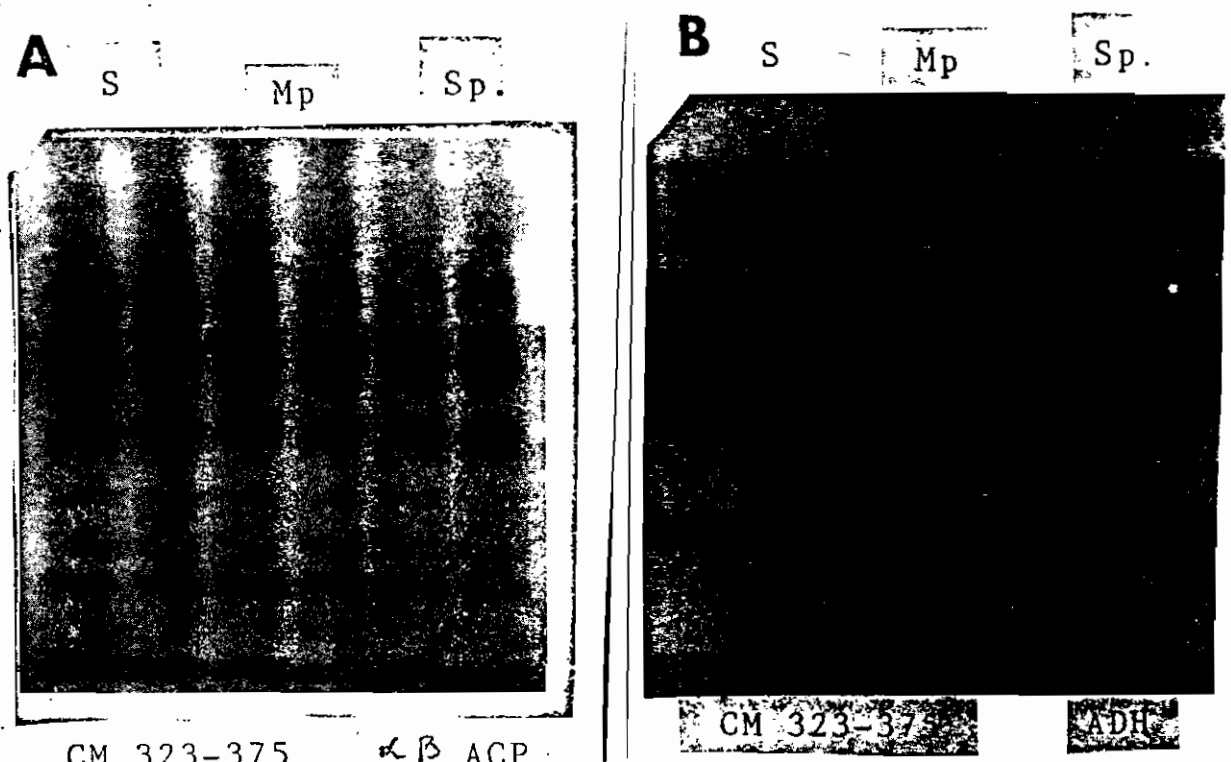


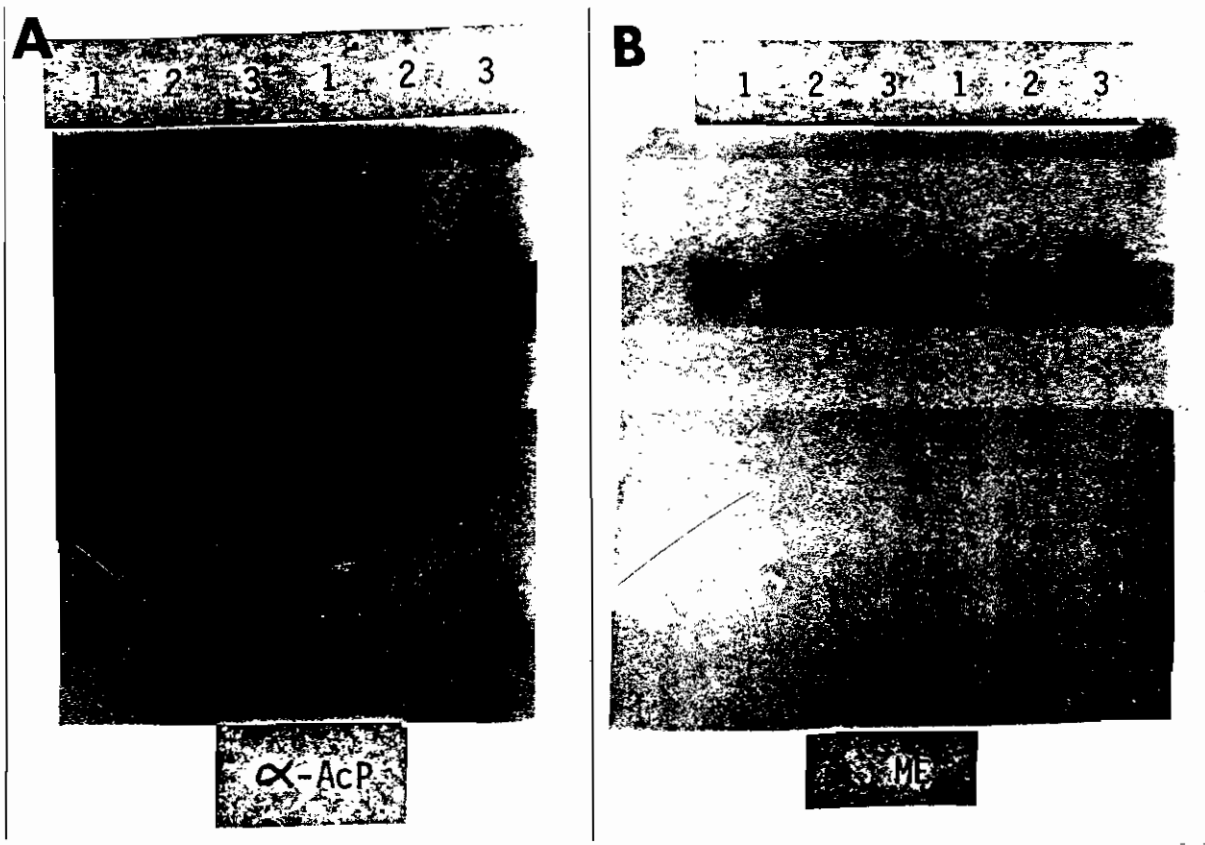
FIGURE 8. Isozyme patterns of cassava sprouts (from mature stakes)and *in vitro* cultures of cv. M. Col 22, and comparison with *in vitro* cultures of *Manihot aesculifolia*

A. Acid phosphatase patterns:

1. *M. aesculifolia* cultures
2. M., Col 22 cultures
3. M. Col 22 stake sprouts.

B. Malic enzyme patterns:

1. M. Col 22 stake sprouts
2. M. Col 22 cultures
3. *M. aesculifolia* cultures



IN VITRO METHODS FOR THE CONSERVATION AND INTERNATIONAL EXCHANGE OF WILD MANIHOT SPP

It is recognized that approximately 100 wild Manihot spp. are distributed in the major centers of diversity, i.e. from Mexico to Paraguay and Northern Argentina.

The utilization of wild Manihot germplasm in the genetic improvement of cassava is restricted because of problems encountered in collection and propagation activities.

Main limitations at the collection sites are scarcity of seeds and/or stem cuttings; and poor rooting and sprouting of cuttings or very low seed germination are restrictions commonly found at the germplasm center. On the other hand, current meristem tip culture techniques used with cassava do not function as well with wild Manihot species.

Research has been carried out to develop embryo and shoot- tip culture techniques for micro propagation of wild Manihot spp. Using a modified MS medium supplemented with AG, thiourea and sucrose, embryo germination and plant development was achieved in 10 wild Manihot spp.

Figs 9I and 9II shows various stages in embryo germination, seedling growth and potting.

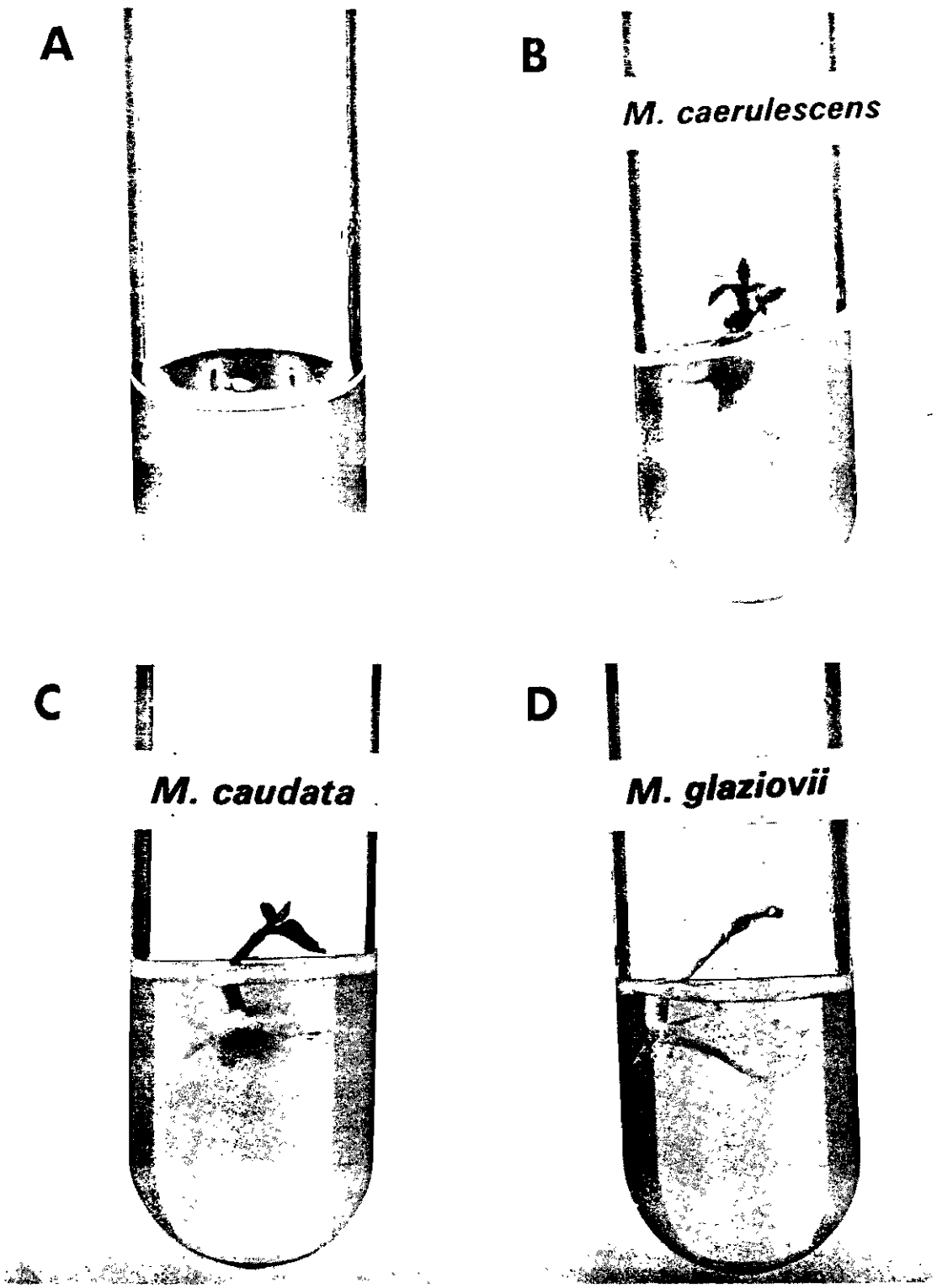


FIGURE 91. Embryo culture of wild Manihot spp.

A. Excised embryo in culture.

B,C,D. in vitro embryo germination of wild species.

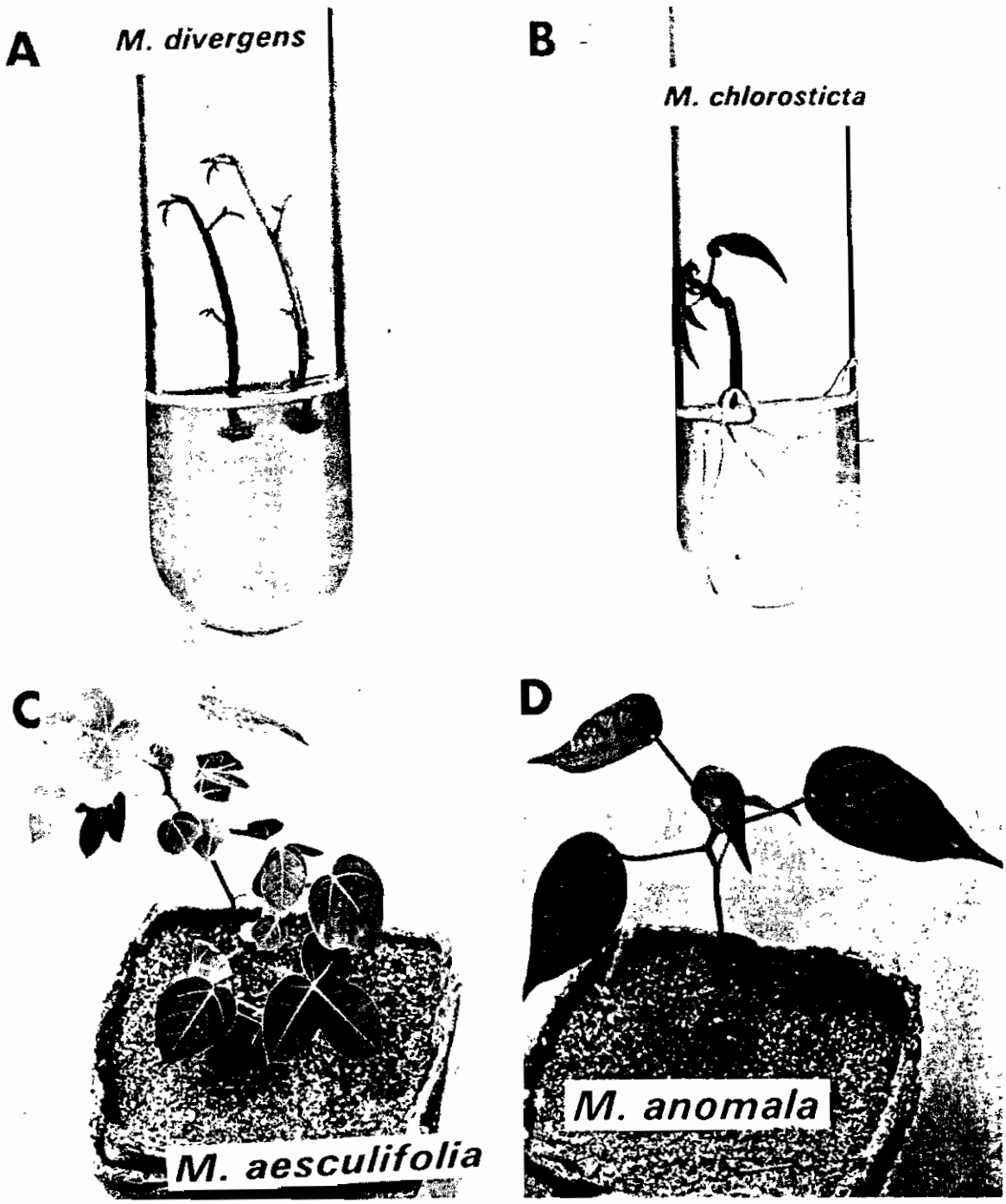


FIGURE 9II. Same as Fig. 9I

A. and B. in vitro embryo germination

C. and D. potting of embryo-derived plants.