TISSUE CULTURE OF PLANTAIN (Musa spp.) FOR IMPROVING YIELD POTENTIAL

FINAL REPORT OF ACTIVITIES

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INDEX

I INTRODUCTION 1

II OBJECTIVES 10

III WORK ACCOMPLISHED 11

A. RESEARCH ACTIVITIES 11

1. Increasing the efficiency of *in vitro* propagation of plantains 11

a) Procedure for obtaining primary explants 12

i Disinfection 15
ii Importance of final explant size 19

b) Optimum media formulations 21

i Semisolid media 21
ii Liquid media 29
iii Use of antioxidants 30

c) Testing the response of different varieties 31

d) Application to tolerant cultivars of plantains .. 31

e) Process of acclimatation 38

2. Field experimentation and evaluation 40

a) Establishment of plantations with *in vitro* plants . 40

b) Comparison of explant types 43

c) Fruit characteristics 48

d) Resistance testing for Black Sigatoka 51

3. Organoleptic evaluation of tolerant varieties 54

4. Distribution of tolerant material 56

5. *In vitro* germplasm collection 57

a) Establishment of a collection 61

b) Maintenance 61

i Explant size 65
ii Zero growth temperature 65
iii Use of high osmotic concentration 65

c) Problem of somaclonal variation 69

i Use of apical bud 72
ii Use of axillary buds 73

6. *In vitro* screening for Black Sigatoka 73

a) Culture of the fungus and preparation of inoculum 75

b) Results of inoculations with mycelium 76

c) Other methods of inoculation 79

7. Somaclonal variation 81

a) Protoplast isolation from leaf mesophyll 81

b) Callus formation 81

8. Literature cited 84

B. OTHER ACTIVITIES 86

1. Cooperation 86

a) Local 86

b) Regional 86

c) World wide 88

2. Training 90

a) In service training 90

b) Courses 91

c) Students 92

3. Participation in Congresses 92

4. Publications 93

5. Information Center 94

6. Laboratory facilities 95

C. CONCLUSIONES AND RECOMENDATIONS 96

D. APPENDIX
Computerized listing of references on *Musa* tissue culture. 100

I. INTRODUCTION

Nowadays bananas are a very common fruit, not only in tropical countries but all over the world. Thanks to advanced production methods and improved world trade, the banana fruit has conquered a large share of the fruit market, with all-year-round availability. Among the world tropical crops bananas rank high, right after the most common staple food crops: cereals and tubers (Figure 1).

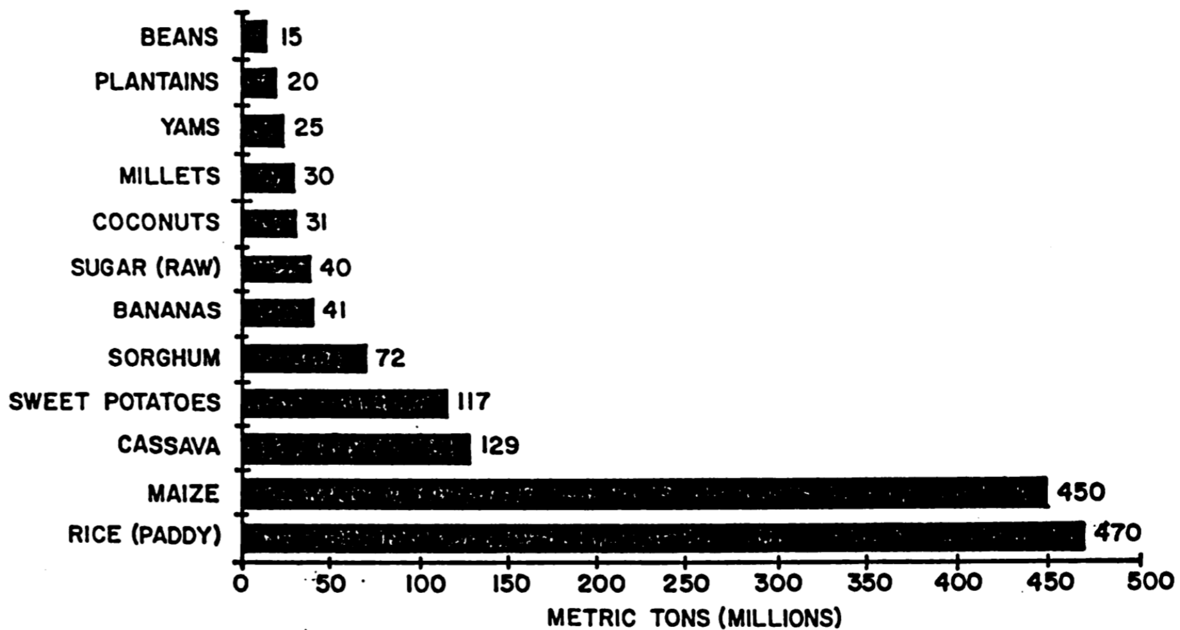

Figure 1. World production of tropical crops 1984 (FAO 1984).

In parts of Asia bananas and plantains (cooking bananas) have probably been used as food for aslong as civilization existed. In Europe, and especially in the Americas, bananas were almost unknow until the beginning of last century. The first plants were introduced in America around 1500. During the first half of the 19th century some small plantations were developed and small and sporadic shipments of the fruit were made, especially to the U.S.A. However, gradually more plantations were started in different countries and regular exportation started, so around 1865 from Colombia, 1870 from Jamaica, followed by the end of that decade by Costa Rica and Panama.

With the installation of the Central American railway in different countries of the isthmus, toward the end of the 19th century, the improved transportation system to the ports of embarcation allowed banana trade to florish. Although at the beginning most banana plantations were small, and production fluctuated considerably due to numerous problems encountered, this changed gradually after the foundation of the United Fruit Company, in 1899, from amalgamation of the Boston Fruit Company and Keith's interests. Banana growing became typically large plantation farming, limited as an extensive culture principally to the alluvial plains near the Atlantic coast. The monopoly thus created on an international scale was broken only recently. Although large international companies are still by far the largest producers, gradually smaller local companies and private enterprises, frequently grouped in cooperatives, are becoming more and more important in internatinal trade. The most important banana exporting countries in America are Guatemala, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Cuba and Jamaica. It should be pointed out, however, that most of the banana prodution (over 60%) is consumed locally thus providing an important part of the diet.

Whereas the common dessert banana is usually produced in large-scale operations, plantains (cooking bananas) are being grown almost exclusively by small farmers. The fruits, rich in starch, are commonly cooked or boiled when still green for use in stews, soups, etc. and mostly fried when fully ripe, when part of the starch has been hydrolyzed to sugars. Plantains represent in many parts of the tropics (including Africa and Asia) an important share of the staple diet. They also provide frequently an additional income to small farmers, who cultivate them around their houses, when sold on the local market.

Only recently plantains have gained some importance as an export crop, since there exists a growing demand in Europe and especially in the Southern United States, where the influence of the hispanic population is strong. If this trend continues plantains may become an important export comodity. This

development, however, is not smooth and easy.

In the past several diseases and pests have plagued production of bananas and plantains. A good example of the damage a single pathogen can do to an extensively grown monoculture, without any genetic variability due to the vegetative propagation, is the socalled Panama Disease or Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense*. Fusarium is a widespread and very common microorganism, attacking many plant species. Around the beginning of this century, evidently this fungus adapted itself to the banana plant. The first severe attack was reported in 1907 in the Chiriqui province of Panama. It spread very fast throughout the Western Hemisphere. 'Gros Michel', the most important variety grown, proved very susceptible to the pathogen. Within a very short time banana growing was practically wiped out in many parts. However, the importance of Panama disease, against which exist no effective means for control due to the soilborne nature, has declined during the last decades, when 'Cavendish' types started to replace 'Gros Michel'. This change also required development of new packing and export procedures, shifting from complete bunches of 'Gros Michel' to dissection of the bunch and packing the hands into boxes for 'Cavendish' because of easy scarring.

Moko Disease or Bacterical Wilt (caused by *Pseudomonas solanacearum*) represents also a threat to banana production, especially in the Western Hemisphere. Stringent plant quarantine and phytosanitary means help to prevent the spreading of this disease in large-scale plantations. Since plantains are mostly grown in small holdings, Moko represents but a minor problem.

Far more serious is the group of diseases called leaf spots (Sigatoka) which have profoundly altered banana production, since their control has implied the modification of the methods of cultivation. The Sigatoka leaf spots involve three very closely related pathogens which can be distinguished by the characteristics of the conidiophores and conidia, although symptoms are very similar.

The original Sigatoka disease, now called Yellow Sigatoka, is caused by

Mycosphaerella musicola. It was first identified in Java in 1902 and produced the first epidemic in the Sigatoka Valley of Fiji in 1910. It was reported from the Caribbean area in 1934 and rapidly spread throughout the banana growing areas of the Western Hemisphere. Black Sigatoka, as it was first called in Central America, is caused by *M. fijiensis* var *difformis*. It was discovered in Honduras in 1972 and is by far the most virulent and devastative one of the three. In fact, since its appearance and spread it has greatly replaced the Yellow Sigatoka in the same areas.

Following the first Black Sigatoka epidemic in 1973 in Honduras, the pathogen appeared in Belize in 1975. It reached the Molagua Valley of Guatemala in early 1977. In 1979 spotting appeared in the Santa Clara area and Central Plateau of Costa Rica, initially infecting some 3000 ha of plantains. By 1982 Black Sigatoka had spread from southern Mexico to the banana zone of the Atlantic and Pacific coasts of Panama, continuing thereafter its expansion by invading northern Colombia.

Chemical control of the *Mycosphaerella* pathogens affecting bananas and especially plantains, is becoming more complex and extremely costly. Whereas for Yellow Sigatoka spraying intervals may vary from 3 to 4 weeks, for Black Sigatoka these have to be reduced to only a few days under favorable conditions for the spread of the disease. The serious nature of Black Leaf Streak and Sigatoka makes it impossible to grow good quality bananas for export without an intensive spray regime, but the high cost of control makes it uneconomic unless a very effective degree of management is involved, with attention to all other pest and disease problems as well as cultural aspects. Spraying will also have to be initiated on plantains if yield and quality are to be maintained. The increased costs can be met only by maximizing production per hectare on first class soils. Marginal levels of production will no longer be economic. Another complicating factor is the development of resistance of the microorganism for the systemic fungicides used, such as benzimidazoles and dithiocarbamates.

A small farmer usually cannot afford to maintain his few plants diseasefree by applying similar technology as in large plantations. Additionally,

Figure 2
Commercial plantain plantation ('Horn plantain') severely affected by Black Sigatoka; note the lack of healthy leaves on fruiting plant in foreground

Figure 3
Immature bunch of fruits of a susceptible plantain variety after attack by Black Sigatoka

Figure 4
Individual old plantain plant (ABB) tolerant to Black Sigatoka (same location as Fig. 5)

Figure 5
Remains of a large susceptible plant after severe attack by Black Sigatoka.

almost all the presently grown commercial plantain varieties in Central America are susceptible to Black Sigatoka. The devastating effects have been well demostrated in Costa Rica where practically the entire plantain population was wiped out in large areas. The economic loss was considerable, especially in small commercial plantations (Figures 2,3). Similar socioeconomic effects have been reported from Asia and especially Africa where plantains also form an important part of the staple diet, principally of the rural population. These factors indicate that the only way out for the small farmer is to replace the lost plants with resistant or at least tolerant varieties (Figures 4,5).

Cultivated bananas and plantains (*Musa* x *paradisiaca* L.) consist principally of triploid varieties, with some diploid and tetraploid ones. The extensive work of Simmonds and Shepherd (1956) suggests that edible bananas evolved in the Old World by several mechanisms from two wild species *Musa acuminata* Colla (*M. cavendishii* Lam ex Paxt.) and *M. balbisiana* Colla. *M. acuminata*, which has a primary center of diversity in the Malysia-Indonesia region, carries the socalled A genome. Plants occur as diploids in both wild (seeded) and parthenocarpic forms. The edible diploid forms (AA) probably arose by the establishment of varying degrees of parthenocarpy and female sterility. The edible (seedless) triploids (AAA) developed through occurrence of triploidy within *M. acuminata* itself. This presumably arose by fusion of a normal haploid male gamete with an egg cell containing a doubled or unreduced chromosome complement. Human selection and clonal (vegetative) multiplication ensured the persistence of the most desirable of these clones. Even so, somatic mutation could have occurred and plants with favored qualities again would have been selected and perpetuated.

M. balbisiana, of the Indian subcontinent-Malayan region, carries the socalled B genome and occurs only as seeded diploids (BB). When *M. acuminata*, whether diploid (AA) or triploid (AAA), came into contact with *M. balbisiana* there arose various new forms with the hybrid genomic constitution. The edible diploid forms of these chance hybridizations are seen as originating through the out-crossing of edible diploids of *M. acuminata* to seeded forms

of *M. acuminata* and *M. balbisiana*, followed by human selection among the edible (parthenocarpic) progeny of these hybridization. The whole new range of edible triploids arose by haploid pollination of diploid egg cells, e.g., egg cells AA x male gamete B = AAB; egg cells AB x male gamete A=AAB; egg cells AB x male gamete B=ABB. (No edible diploid or triploid *M. balbisiana* are known.). The edible export or dessert bananas (AAA) are highly susceptible to Black Sigatoka, whereas AAB plantains have some resistance and ABB plantains (frequently referred to as cooking bananas) the highest level of resistance of the edible varieties (Fig. 4).

Other diseases, like Bunchy Top, caused by a virus, so far have not yet been introduced in the American Continent.

With the appearance of race 4 of *Fusarium oxysporum* f. sp. *cubense*, which also attacks the plants of the Cavendish group, banana growing seems to be threatened.

Although this pathogen, first discovered in 1967 in Taiwan, has so far been limited to cooler regions of banana production (Canary Islands, South Africa, Taiwan) it also has been reported from the Philippines. Due to the soil-borne nature, its control is almost impossible, requiring resistant plant materials, the same as in the case of the burrowing nematode (*Radopholus similis*) which also attacks Cavendish varieties.

Conventional plant breeding which had its initiation in 1922 in the Imperial College of Tropical Agriculture, Trinidad, so far has not yet produced any new variety with the same fruit characteristics and quality as 'Gros Michel' or 'Cavendish' (Rowe 1985). Breeding is handicapped by the seedliness, a requisite for consumption.

De Langhe (1969) has presented at length both the possibilities and difficulties for improvement of banana. He emphasizes that "When all is said and done, the improvement of bananas amounts to a continual fight against fertility, a property, which is, however, the essential instrument of the improvement itself".

Intents have been made to cross the triploid 'Gros Michel' ('Cavendish' does not produce any seed) whith diploids, which resulted in tetraploids,

showing a certain degree of resistance to some diseases. However, real progress in banana breeding is only possible (Rowe 1985) when starting with new parent plants, emphasizing thus the need for large germplasm collections. Since the mutation rate of fungi can be quite fast, it is doubtful, if by conventional breeding methods new resistant varieties can be produced at the same rate as the pathogens adapt themselves and attack the new planting materials. The example in Taiwan, where resistant plants to race 4 of Fusarium wilt were found only among tissue-cultured plants, indicates that only newer and more modern methods like somaclonal variation caused by tissue culture processes, may play an important role in the future of banana and plantain growing.

II. OBJECTIVES

The original objectives of the project were the following: "Since in plantains genetic variability is practically non-existent due to the constant clonal reproduction in the field, and especially in the Americas, and limited to natural mutations, it seems desirable to divide the objectives of this research project into two different aspects:

1. Rapid vegetative clonal propagation.
   The objectives of this first step were to develop a methodology which would permit large number of plantlets to be obtained from one original explant. Athough there had been some work done on bananas (see complete listing of literature references under "Tissue culture of banana"), when the project was started nothing was known about tissue culture of plantains. Since different clones or cultivars of the same species frequently differ in their requirements regarding medium composition, hormone concentration and environmental conditions, a thorough investigation was needed to be carried out to ascertain the general applicability of the methodology developed.

2. Increasing genetic variability
   The objectve of this second step was to produce a large number of plantlets which differ somewhat in their genetic constitution. This second objective was to be worked out after completion of step one".

Later on an addendum was included by AID/ROCAP stating:

"In addition to the specific objectives, CATIE will:

3. Seek to establish contacts and collaborative arrangements with other scientists and institutions, regionally and globally, engaged in tissue culture research in order to accelerate the research and to minimize unnecessary duplication

4. Store all information obtainded from other sources, as well as the generated by the research they conduct, in the CATIE computer information system for easy retrieval and distribution as appropriate".

III. WORK ACCOMPLISHED

A. RESEARCH ACTIVITIES

The research carried out under this project can be divided into several activities.

1. Increasing the efficiency of *in vitro* propagation of plantains

Since all edible *Musa* spp. have no seeds, vegetative propagation is the only way to obtain planting material. This process is slow, especially at the beginning of the introduction of a new variety. Only after a relatively large number of adult plants are available, enough planting material can be provided. An adult multiple pseudostem plant can produce as an average six to eight suckers (daughters or followers) per year (Figure 7). Special treatments, like eliminating the apical bud of large pseudostems can increase the number up to twenty (Barker 1959) or even 150 (Hamilton 1965). Thus, for introducing a new cultivar, especially in larger areas, many decades are needed.

Tissue culture offers an ideal means to obtain a large number of plantlets within a relatively short period. When this project started, this innovative approach had not yet been tested with *Musa* spp. Only a few publications were available on tissue culture of *Musa* spp. (Berg & Bustamante 1974, Ma & Shii 1972, 1974, Mohan Ram & Steward 1964).

None of these publications delt with the possibilities of using tissue culture for rapid clonal propagation, or investigated differences in requirements regarding the composition of the nutrient medium for different varieties, germplasm combinations or even species. It is well known for many plants that there exist great differences even among cultivars or varieties of the same species regarding response to tissue culture. Therefore it was necessary to plan the research in several steps: a)Procedure for obtaining explants; b)Optimum media formulations and environmental conditions for high rate of reproduction; c)Testing the responses of different varieties and germplasm combinations; d)Adaptations of the methodology to special resistant or tolerant cultivars for rapid mass propagation; e)Process of acclimatation

and transfer to the field.

a) Procedure for obtaining primary explants

The corm of a *Musa* plant is rather bulky and grows completely underground, with the pseudostems emerging from the soil. The desired explant in *Musa* is the apical bud, either of the main pseudostem (before initiation of flowering) or preferably of strong suckers (Figure 6). It consists of the true meristem in form of an apical dome and one or several leaf primordia in different developing stages.

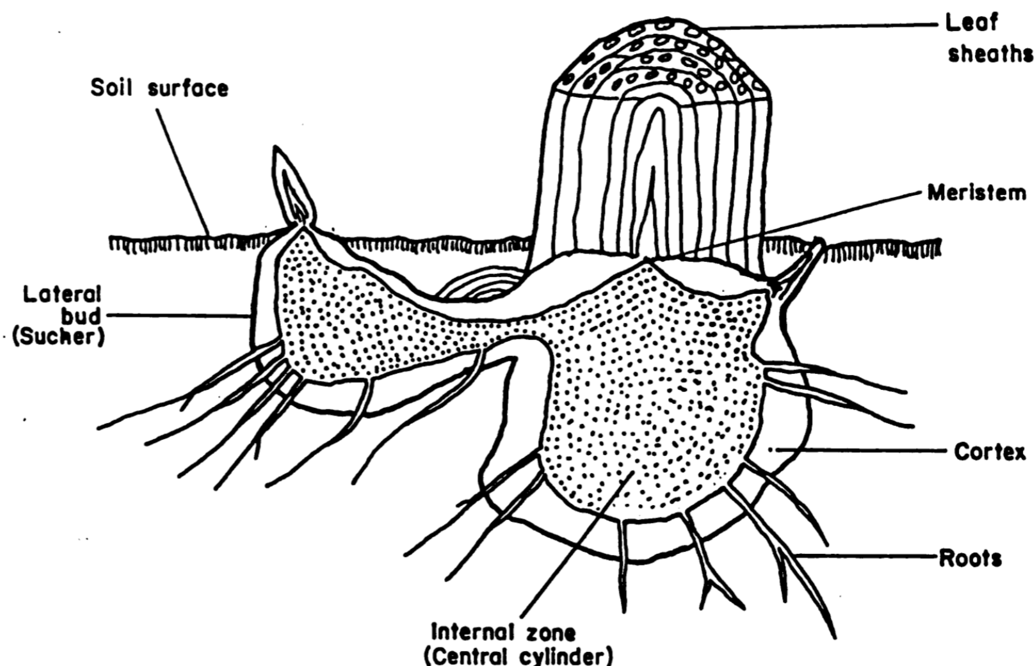

Figure 6. Schematic representation of a tranversal section of a typical corm of *Musa*.

Figure 7
Young shoots (suckers) arising from the corm of a mother plant at left

Figure 8
Young suckers after separation from the corm of the mother plant, which serve as source of primary explants

Figure 9
Primary explants (natural size) after removing outer parts, showing leaf bases (top) and part of the corm (bottom)

In order to excise the explant, a sucker (Figure 7) is removed from the base of the corm (Figure 8). After rinsing in water to remove most of the adhering soil, the material is subsequently trimmed by removing the excess parts of the corm tissue together with successive leaf sheaths until the diameter at the base is approximately 1 to 3 cm (Figure 9).

i. Disinfection

Although, if proper care is taken, the trimmed apical bud is free of microorganisms (except if invaded by systemic pathogens), it was found very difficult to keep the material completely sterile due to the ease of contamination while handling it. Therefore it proved necessary to use a disinfectant solution for surface sterilization prior to extraction of the explant proper.

Several compounds were tested, such as calcium hypochlorite solutions (0.1 to 0.2% w/v), sodium hypochlorite in form of commercial bleach "Ajax" (0.3 to 0.6% active ingredient), merthiolate (1% in ethanol 70%), Savlon* (0.1 to 1% aqueous solution), mercury bichloride (0.1 to 0.2% aqueous solution), and ethyl alcohol 70%. Since the surface contamination is rather low and not due to natural conditions, it was found that all compounds were practically equally effective, especially if a small amount of a surfactant was added to reduce surface tension, such as Tween 20.

However, there existed certain differences regarding toxicity. Since hypochlorite compounds disintegrate completely on contact with organic substances, after a short time no trace is left on the explant. The calcium ion as such is less alcaline as compared to sodium, so calcium hypochlorite proves to be better for delicate objects. In the case of *Musa* no differences could be found. The mercury salts and compounds are very effective sterilants but require many rinses to assure removal of most adhering traces. Savlon (active chemical ingredient: cetrimide) has a very low phytotoxicity.

*Trade mark of a disinfectant by ICI (Imperial Chemical Industries) Macclesfield,Inglaterra.

Figure 10
Final shaping of explants (natural size) after sterilization

Figure 11
Inoculation of primary explant (apical bud) into agar-based nutrient medium

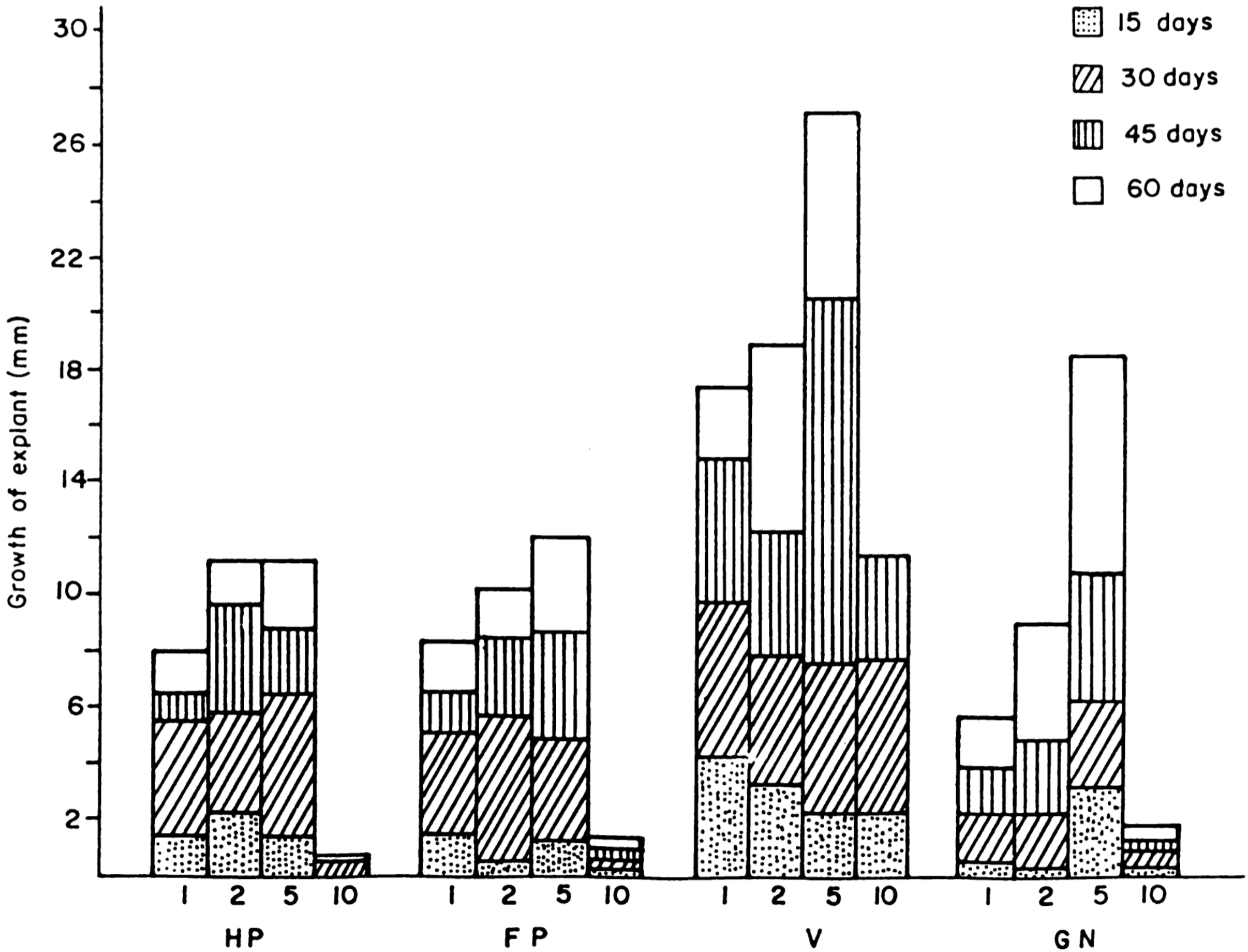

Figure 12. Growthrate of explants of four varieties of *Musa* (HP = Horn plantain, FP = French plantain, V = Valery, GN = Grande naine) in relation to explant size after several time intervals

Table 1. Initial and final weight (after 60 days of incubation) of explants of different sizes (1,2,5 and 10 mm) of four *Musa* varieties

Cultivar	Initial size (mm)	Initial weight (mg)	Final weight (mg)	Relative growth rate (RGR)
Horn plantain	1	3	32	9,7
	2	9	56	5,2
	5	14	133	8,6
	10	62	159	1,6
French plantain	1	3	30	9,0
	2	9	55	5,1
	5	15	125	7,3
	10	60	152	1,5
Valery	1	3	49	15,3
	2	8	105	12,1
	5	15	127	7,5
	10	60	145	1,4
Grande Naine	1	3	33	10,0
	2	10	99	8,9
	5	15	125	7,3
	10	61	160	1,6

It proved to be excellent for disinfection and the plant material can remain for several hours in a dilute solution without any discernable damage to living tissue.

After disinfection, always working under a laminar flow hood, the material was rinsed several times with sterile distilled water and further dissected until the explant consisted of the meristem surrounded by the bases of several of the smallest leaves (Figure 10).

ii. Importance of final explant size

Since in the avaible literature no mention was made as to the idial size of the explant in *Musa*, an experiment was initiated to determine the most adequate size and its correlation to efficiency of regeneration of complete plantlets. For this purpose four varieties were selected, two bananas (AAA) 'Valery' and 'Grande Naine' and two plantains (AAB) 'Horn plantain' and 'French plantain'.

After excission and sterilization of the materials, as described above, the explants were reduced in size to the desired dimensions: 1,2,5 and 10 mm total height and also diameter at base. These dimensions correspond to an initial weight of approximately 3,9,15 and 60 mg. The cultures were incubated on a modified MS medium (BAP 0,5 mg/l) for a total of 60 days under normal culture conditions (16/8 h photoperiodism, fluorescent lighting with 2000 lux at shelf level, 27$\pm$2°C) in a growth chamber. The relative growth rate (RGR) was estimated using the formula:

$$\text{RGR} = \frac{\text{(final fresh weight)} - \text{(initial fresh weight)}}{\text{(initial fresh weight)}}$$

As can be seen in Table 1, there existed considerable differences in response to initial size. As such, the smallest explant (1 mm) had the highest relative growth rate, whereas the largest one had a very low relative growth rate. This is also clearly marked in height of growth (Figure 12).

Table 2. Degree of oxidation as related to the size of explant in four varieties of *Musa* after 60 days

Size of explant (mm)	Horn plantain	French plantain	Valery	Grande naine
1	S	S	L	L
2	L	S	L	L
5	L	L	L	L
10	F	F	F	F

S = no oxidation
L = slight oxidation
F = strong oxidation

Table 3. Percentage of survival rate of explants of different size of four varieties of *Musa* as related to loss by contamination

	Size of explant				
Variety	1 mm	2 mm	5 mm	10 mm	Average
Valery	90	65	70	55	70
Grande naine	95	85	100	40	80
Horn plantain	95	100	80	70	86
French plantain	100	100	100	90	98

There also existed differences regarding varieties, with the bananas growing faster than the plantains. As such, the largest explant of plantains showed little or no growth during the initial 15 days of culture.

The results indicate that an explant size with a base diameter of approximately five millimeters is best suited for initiation of fast growth, indipendent of the variety.

Other effects, such as phenolic oxidation and survival rate due to contamination losses are also related to initial size of explant.

It is apparent (Table 2) that the larger the initial explant size, the stronger the phenolic oxidation will be.

Regarding survival rate due to secondary contamination, Table 3,it is clear that the smaller the explant size, the higher the survival rate and less contamination is apparent.

As a final conclusion from these results, considering all factors involved, an explant size of some 5 mm (diameter at base) is best suited for initiation of *Musa* tissue cultures.

b) Optimum media formulations

i. Semisolid media

When the research on *Musa* tissue cultue was initiated, with emphasis on plantains, little useful information could be found in the literature, especially regarding fast clonal propagation.

Initially the medium formulation proposed by Vessey & Riviera (1981) was adopted, with slight modifications (1 mg/L indole-3-acetic acid [IAA] and 3 mg/L N^6-benzyladenine [BAP]).

Primary explants could be easily cultivated during the initial stages. However, overall growth was very slow, especially in plantains.

A second medium, of De Guzman *et al.* (1980), also slightly modified (5 mg/L BAP), was best for the development of plantlets. When growing explants were cultured from 6 to 8 weeks on this medium, good development and formation of adventitious buds was obtained.

Table 4. Medium of Murashige y Skoog (MS) (modified)

Components	Amount per liter			
	weight		molarity	
NH_4NO_3	1650	mg	20.6	mmol
KNO_3	1900	mg	18.8	mmol
$CaCl_2.2H_2O$	440	mg	2.99	mmol
$MgSO_4.7H_2O$	370	mg	1.50	mmol
KH_2PO_4	170	mg	1.25	mmol
H_3BO_3	6.2	mg	100	umol
$MnSO_4.4H_2O$	22.3	mg	100	umol
$ZnSO_4.7H_2O$	8.6	mg	29.9	umol
KI	0.83	mg	5.00	umol
$Na_2MoO_4.2H_2O$	0.25	mg	1.03	umol
$CuSO_4.5H_2O$	0.025	mg	0.100	umol
$CoCl_2.6H_2O$	0.025	mg	0.105	umol
$FeSO_4.7H_2O$	27.8	mg	100	umol
$Na_2EDTA.2H_2O$	37.4	mg	100	umol
Sucrose	30000	mg	87.6	mmol
Thiamine-HCl	0.1	mg	0.296	umol
Inositol	100	mg	555	umol
Nicotinic acid	0.5	mg	4.06	umol
Pyridoxine-HCl	0.5	mg	2.43	umol
Glycine	2	mg	26.6	umol

Source: MURASHIGE, T. & SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497. 1962.

Table 5. MEDIA FORMULATIONS FOR PLANTAIN TISSUE CULTURE (AMOUNTS PER LITER OF MEDIUM)

Medium components	Medium 1 Culture initiation (primary explant)	Medium 2 Multiplication (adventitious buds)	Medium 3 Growth and root formation (plantlet formation)
Inorganic commercial salt mixture MS*	4.36 g	4.36 g	4.36 mg
Commercial sucrose	30 000 mg	30 000 mg	30 000 mg
Difco Bacto agar	7 000 mg	7 000 mg	7 000 mg
Geigy Sequestrene 330** (or Fe-NaEDTA)	10 mg	10 mg	10 mg
Nicotinamide	0.5 mg	0.5 mg	0.5 mg
Pyridoxine HCl	0.5 mg	0.5 mg	0.5 mg
Thiamine HCl	0.1 mg	0.1 mg	0.1 mg
m-Inositol	100 mg	100 mg	100 mg
Glycine	2 mg	2 mg	2 mg
BAP (N^6-benzylaminopurine)	1 mg	3-5 mg***	- mg

* KC Biological, Inc.
** Additional soluble iron
*** According to varieties

One of the most commonly employed formulations (Table 4) is the one of Murashige & Skoog (1962). It has proven to be adaptable to most plants for vegetative multiplication (George & Sherrington, 1984). In order to find out its suitability for *Musa* tissue culture a large number of tests were carried out, with modifications, such as variation of the sucrose and vitamin concentrations, total salt concentration (1, 1/2, 1/3, 1/4 MS), addition of chemically defined subtances (amino acids, other vitamins, charcoal) or undefined ingredients (coconut water, yeast and casein hydrolysates at various concentrations), and the usual growth regulators a) auxins: indoleacetic acid (IAA), indole-butyric acid (IBA), naphthaleneacetic acid (NAA), 2,4 dichlorophenoxy acetic acid (2,4-D), paraquat; b) cytokinins: N^6-benzylaminopurine (BAP), kinetin (K), 2-IP (N^6-isopentenylaminopurine), and combinations thereof.

It gradually become evident that the genus *Musa* adapts itself to quite a variety of media formulations, showing more or less growth, none or abundant adventitious budding and root formation.

As such, the basic MS medium is well suited, requiring only slight modifications (Table 5). It can be seen that the establishment of the primary explant occurs best with the addition of a small amount (1 mg/L) of BAP. If multiple adventitious bud formation is desired, the established primary explants should be transferred to a medium with a somewhat higher BAP concentration (up to 5 mg/L, depending on the variety). Rooting will be greatly depressed under these conditions, thus making it necessary to transfer the separated buds to a medium without BAP (see Figure 14). It was found that most cultivars, if left for longer periods of time on a medium with low BAP concentration (0.5-1 mg/L) will eventually produce some adventitious buds and also gradually develop some roots.

For fast clonal propagation it is, however, advisable to make the two changes regarding BAP concentration in order to speed up the multiplication and regeneration processes, once a primary explant has been established (Figure 13). The process can be repeated over and over, thus giving rise to a large number of plantlets.

Figure 13
Initiation of culture of apical primary explants of plantains in agar-based medium

Figure 14
Development of single explant showing complete regeneration (shoot and root formation)

In all cases the pH of the media was adjusted to 5.7 ±.1. Several types of culture vessels were used. For single plantlet formation test tubes or vials, for multiplication Gerber baby food jars fitted with plastic Magenta* caps, or Magenta A7 vessels (Figure 14). The medium, after distribution, was autoclaved at 121°C for 15 minutes. After inoculation (Figure 11) all cultures were incubated in growth rooms at 26 or 28°C ±2°C for optimum growth rate. Temperatures higher (and also lower) proved less adventageous for several reasons. The illumination was provided initially by daylight fluorescent tubes with an intensity of approximately 2000 lux at shelf level. Later on these were substituted by GE Plant Gro and Sho tubes, which emit a spectrum similar to the action sypectra curve for photosynthesis. Growth was actually accelerated under this type of illumination and leaves remained much greener.

In many cultures a slight to medium yellowing (chlorosis) was observed, especially affecting the youngest leaves. Applications of different salt solutions of minor elements confirmed the existance of iron deficiency. Although it is known (Singh& Krikorian, 1980) that there exists some problem with Fe-Na-EDTA in the MS formulation, the total amount of Fe provided by the recommended dosis is usually deficient. Thus supplementary iron was added as Sequestrene 330 (Geigy) or as Fe-Na-EDTA. An additional amount of 10 mg/L of either of these compounds proved satisfactory to eliminate the chlorosis.

Initially all media formulations were prepared from stock solutions. However, since frequently larger amounts of plantlets needed to be produced, several commercial MS powdered salt formulations were tried. The MS salt base produced by KC**, worked well and permitted to speed up operations considerably.

As agar the usual Difco Bacto agar was used. However, other makes were tried***. Since all agar products are relatively expensive, trials were ca-

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* Magenta Corporation, Chicago
** KC Biological, Inc. Lenexa, KA
*** Sigma Chemical Co. St. Louis, MO.

Table 6. Average values of several parameters of plantlets of *Musa* AAA (cv. Grande Naine) cultivated with different gelling agents and BAP concentrations.

BAP concentration and gelling agent amounts /liter of medium	Height (cm)	Number of leaves	Weight (g)	Number of roots	Number of adventitious buds
BAP 0 Bacto Agar 5 g	5.23	6.3	3.58	9.1	0.3
BAP 0 Gelrite 1.5 g	5.33	6.0	4.34	8.8	0.22
BAP 0 Agar Sigma 5 g	5.85	6.0	3.35	7.2	0
BAP 1 mg Bacto Agar 5 g	5.37	6.5	8.99	10.0	0.8
BAP 1 mg Gelrite 1,5 g	6.8	6.4	3.57	8.33	0.33
BAP 1 mg Agar Sigma 5 g	6.45	6.12	6.5	6.73	0.12
BAP 2 mg Bacto Agar 5 g	4.2	6.3	2.0	6.8	0
BAP 2 mg Gelrite 1.5 g	5.56	6.3	3.5	7.9	0.7
BAP 2 mg Agar Sigma 5 g	5.57	7.25	4.5	8.3	0.25
BAP 4 mg Bacto Agar 5 g	4.65	6.9	13.01	10.6	5.2
BAP 4 mg Gelrite 1,5 g	4.75	6.6	13.07	10.3	5.1
BAP 4 mg Agar Sigma 5 g	5.2	7.0	12.08	8.7	5.0
BAP 8 mg Bacto Agar 5 g	5.3	6.4	5.48	7.2	1.0
BAP 8 mg Gelrite 1.5 g	3.20	4.5	3.92	5.8	1.5
BAP 8 mg Agar Sigma 5 g	3.27	7.0	2.75	4.4	1.0

Figure 15
Culture vessel with four explants showing lateral adventitious shoot formation

Figure 16
Developing plantain explants; left: initial development; center: initial phase of regeneration; right: multiple adventitious bud formation

rried out to find cheaper substitutes. As can be seen in Table 6, no significant differences can be found when comparing the three usual gelling agents: Difco Bacto Agar, Sigma Agar and Gelrite*. If a much larger sample than in this trial is studied, there exists a tendency for faster growth and longer and whiter, more vigerous roots when explants are cultivated on Gelrite. Besides the much lower cost, another advantage of this product is the transparency of the medium which permits to detect much more easily bacterial contamination in early stages.

ii. Liquid media

It is well known that liquid media offer certain advantages over semisolid ones. This is mostly due to the larger contact area between the nutrients of the medium and the entire surface of the explant, instead of the small area between explant and medium surface. Therefore many formulations of liquid media were tested in order to compare the speed of growth, multiplication rate, and regeneration of plantlets.

Since liquid media require constant agitation to assure adequate supply of oxygen, all available types of agitating apparatus were utilized, such as orbital shakers with Erlenmeyer flasks, rotary drums with test tubes, roller apparatus with different sizes of roller bottles. It was quite evident that in liquid shaken culture growth was accelerated. However, the leaves were more slender and delicate and the arising adventitious buds were, due to the lack of directional gravitational force, orientated star-like in all directions, showing also some curvature (Figure 16). This fact constitutes a major difficulty when separating the buds for further subculture. Also in the tropics culture vessels with larger volumes, such as roller bottles, are hard to maintain sterile when effecting transfer or subcultures. For these reasons it seemed adventageous to use only semisolid media, even for mass propagation, since the protocols are relatively simple as compared to those of other investigators (Krikorian & Cronauer, 1984), where a liquid medium is used for culture initiation, requiring several successive transfers to different media formulations.

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*Merck, Sharp and Dohme, San Diego, CA.

iii. Use of antioxidants

Musa plants contain in their tissues (vegetative and generative) large amounts of mucilage which contains phenolic and polyphenolic compounds. In contact with air these are oxidized to dark brown or blackish substances. Therefore it can be observed that explants turn brown and later on black shortly after isolation. These oxidized compounds usually have antiseptic properties and when produced in larger quantities inhibit growth of the explants, and tissues usually die. Growth inhibition is most severe in species that naturally contain high levels of tannins or other hydroxy-phenols, which are oxidized by copper-containing enzymes, such as polyphenol oxidases and tyrosinases.

In order to reduce browing, leaching of the still reduced compounds from the explant has been used as a pretreatment, In the case of *Musa* this proved not effective due to the large amounts of mucilage. Modifying the redox potential was more promising. Brief immersion in an aqueous solution of cystein hydrochloride (25-100 mg/L) or a combination of ascorbic and citric acids (50-100 mg/L each) applied right after the rinse in sterile distilled water, reduced explant browning and later on medium discoloration considerably. However, there exist differences regarding the degree of oxidation, depending on varieties and source of explant (active apical meristem or dormant lateral bud of corm). In some cases it proved to be necessary to include cystein-HCl in the medium for the primary explant (20-100 mg/L). Activated charcoal, as frequenly used, did not provide better protection, even in higher quantities (5-10 g/L). Similarly, the use of polyvinylpyrrolidone (PVP, M.W. 44 000) did not give any important improvements.

One way out, as recommended by several investestigations, is frequent changes of medium during the initial culturing of primary explants. This, however, results in much increased labor.

It should be noted that the problem of oxidation is actually only important during the establishment of a primary culture. As soon as subcultures are started from adventitious buds, practically no more browning occurs, probably due to the reduced synthesis of phenolics during *in vitro* culture.

Although in some cases severe browning and blackening of the primary explant occurs, if these compounds do not strongly diffuse into the medium, usually the negative effects result only in a delay of growth of the explant. It can be observed that after some time the blackened corm tissue breaks open to give rise to a whitish new corm growth, from which adventitious buds can differentiate.

c) Testing the response of different varieties

It is well known that different species of a genus, as well as varieties of one species or even clones require different media composition for optimum growth and development. Since practically no information existed as to such differences, a small *in vitro* germplasm collection of *Musa* was established, containing the two diploid species AA, BB and the most common combinations, especially triploids AAA, AAB and ABB. When testing explants derived from these, certain differences became evident, mostly related to growth rate and ease of adventitious budding. However, these differences were not important enough as to prevent the use of a single medium for maintaining all types in *in vitro* conditions. *Musa* thus represents rather an exceptional case, which greatly facilitates the tissue culture methods for rapid propagation.

a) Application to tolerant cultivars of plantains

As pointed out previously, the only possible way for the small farmer, who cannot invest in modern technology, is to grow less susceptible, tolerant or, ideally, resistant varieties of plantains. Although some resistant varieties exist in many areas, they are of much inferior quality than the commercial ones and not acceptable to local population for human consumption.

Since the time factor was critical, efforts were made to obtain and work with some varieties at least tolerant to Black Sigatoka. As it turned out, two cultivars existed in the germplasm collection at La Lima, Honduras*, 'Pelipita 14055' and 'Saba 5A', the fruits of which are of sufficient quality for consumption.

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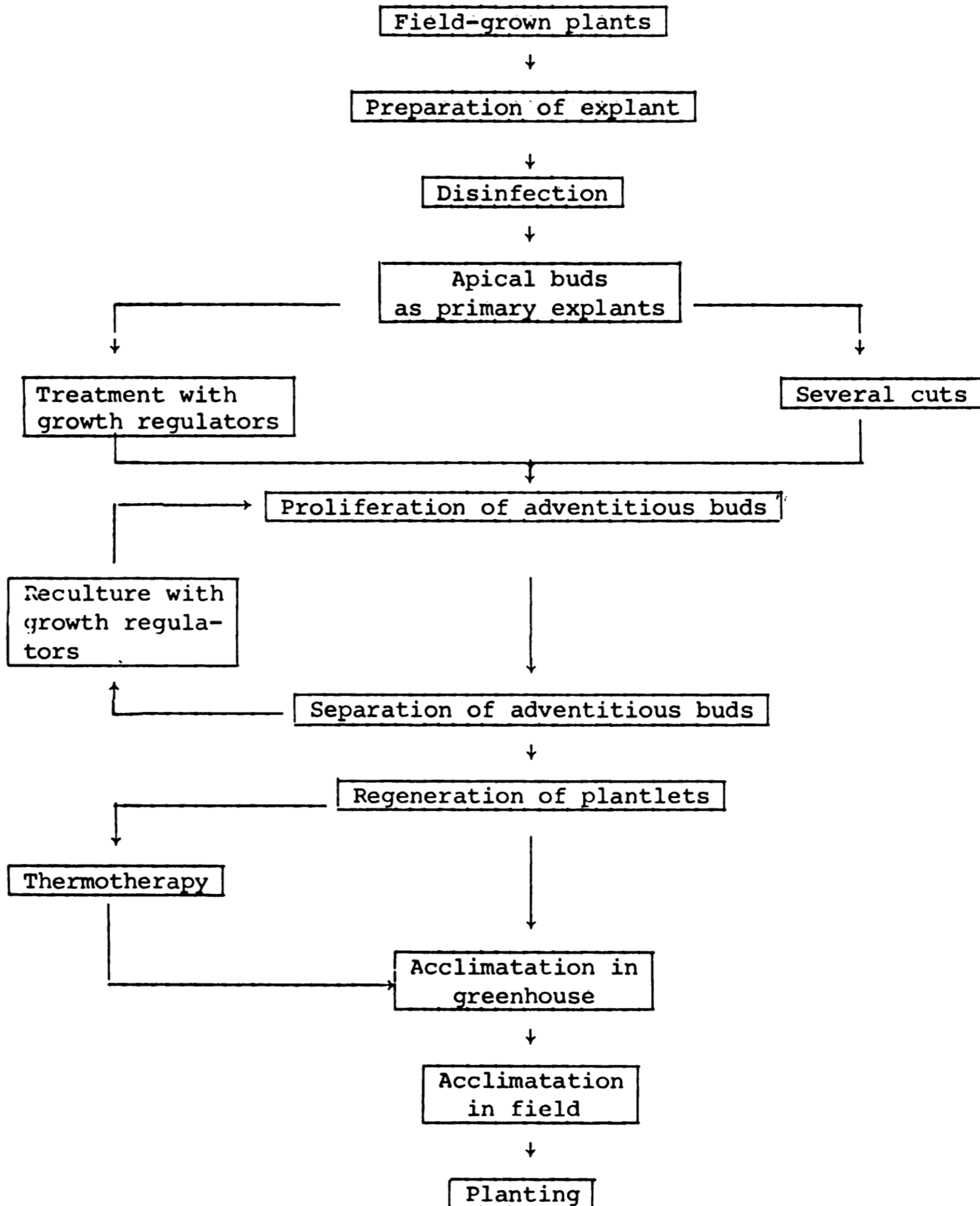
*Formerly United Fruit Company, now FHIA, Fundación Hondureña de Investigaciones Agrícolas

Figure 17. Diagram of the main steps involved in clonal propagation of *Musa* spp.



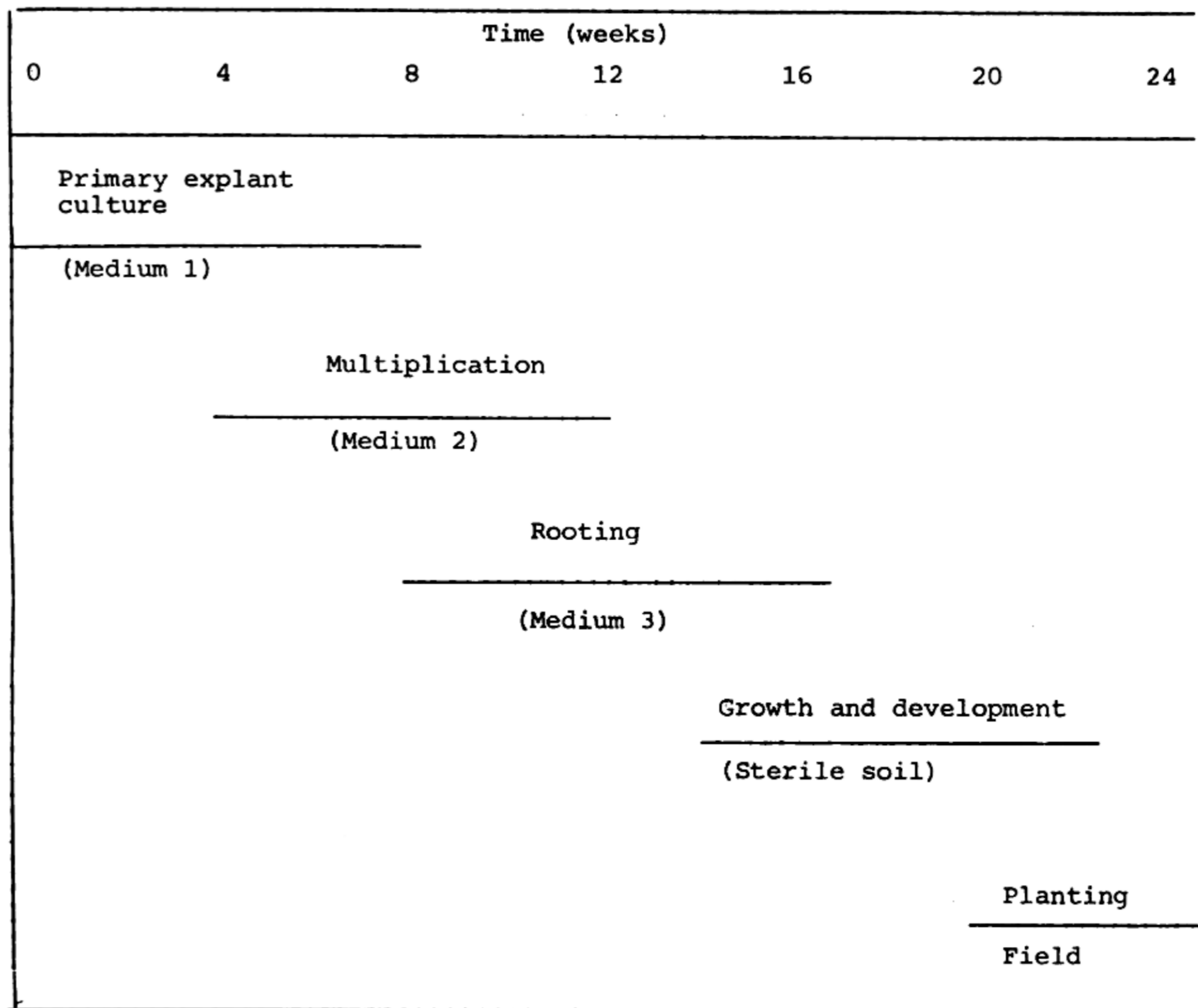


Figure 18. Sequential timing of *in vitro* plantain tissue culture, from initiation of culture with primary explant to field planting

Initially two *in vitro* cultures were obtained of each cultivar and submitted to propagation. To achieve this, several methods were tried. Vessey & Rivera (1981) had recommended to make a series of vertical cuts across the apical part of the explant (apex) before initiation of culture. In this way it was supposedly possible to obtain from one explant multiple buds.

However, this method proved not satisfactory for propagating 'Pelipita' and 'Saba' since the cuts provoked a considerable browning of the wounded tissue, that could not be avoided by the use of antioxidants. In fact, the concentration of the oxidized compounds was so great that even frequent subculturing at short intervals could not avoid loss of plants and extreme retardation of growth.

More effective was the use of cytokinins. The optimum concentration for both varieties proved to be 4 mg of BAP/L. When placing explants on such medium, within 4 to 6 weeks after subculture, about 15 to 30 adventitious buds developed on each explant (Figures 15,16). Theoretically in less than one year more than 10^6 plantlets could be produced from one single primary explant (See Figure 17). The adventitious buds were then separated and upon subculturing produced again a similar number of buds in a similar time interval.

These results show clearly the great potential of tissue culture in *Musa* as an innovative method for rapid multiplication of certain materials as compared to the cumbersome and extremely slow way when suckers are used from one initial mother plant (Barker 1959, Hamilton 1965).

Addition of activated charcoal (0.1 to 1.0% w/v) to the media did not stimulate root growth and actually retarded shoot growth. This behavior is quite different from most other plants where beneficial effects of charcoal are reported, especially if the explants are very susceptible to rapid browning.

On the other hand it was noted that for regeneration it is advisable to use only secondary explants that have no adventitious buds developing, since these can retard or even suppress regeneration.

'Saba' as well as 'Pelipita' develop rapidly (in less than 10 days) a vigorous and extensive root system, when placed on regeneration medium with very low BAP content or lacking hormones completely.

Figure 15
Regenerated plantain plantlet after planting into sterile soil for acclimatation

Figure 20
Plants in misting chamber after finishing the process of acclimatation

Figure 21
Regenerated and acclimated plantain plant ready for transplanting to the field

These results show that varying only one component of the medium, the cytokinin BAP, growth, multiplication, and regeneration can be accomplished. The multiplication phase, if repeated several times, can provide within a few months an extremely large amount of planting material (Figures 16,17).

e) Process of acclimatation

Usually plants grown *in vitro* need a rather sophisticated scheme of acclimatation in order to place them under field conditions, especially in the tropics. However, in the case of *Musa* circumstances are quite different since acclimatation is exceptionally easy. When the root system is well developed, plantlets are removed from the culture vessel and planted into a well textured soil (Figure 19) that has been treated with methylbromide or steam sterilized (not absolutely necessary). It is not even important to remove traces of the left-over medium which still adhere to the roots. As containers can serve pots or black polyethylene bags. Even in the absence of humidity and temperature control, survival rate is usually above 90%, if the recently transplanted material is kept under shade and at a constant but moderate soil moisture content.

Under greenhouse conditions a large number of plants can also be acclimated with only misting and some pest control. In this case, survival rate is practically 100% (Figure 20).

It is recommendable to let plants grow and produce several new leaves and reach a total height of some 50 cm before taking them to the field (Figure 21).

Planting can be done as with suckers following routine procedures. Initially the continued provision of partial shade in the field, using cut palm fronds or branches of adjacent trees, proved beneficial and effectively inhibited chlorophyll bleaching and drying of the larger leaves. However, even without shading, new leaf emergence was observed within 20 days of planting and subsequent growth was vigorous. The survival rate in the field was, in fact, higher among *in vitro* grown plants when compared to suckers, a fact probably due to the well developed root system. The entire time interval from initiation of culture (primary explant) to the field establishment requires approximately five to six months (Figure 18).

Table 7. Mean monthly height and diameter at base of four varieties of *Musa*, Bribrí, Costa Rica. March-July. 1984.

Variety	Height (cm)					Diameter (cm)				
	March	April	May	June	July	March	April	May	June	July
Grande naine Rhizome	91 (59)*	102 (43)	121 (44)	152 (43)	172 (31)	26 (20)	30 (26)	32 (33)	36 (41)	42 (29)
Horn plantain Rhizome	123 (70)	156 (58)	192 (58)	246 (58)	292 (45)	26 (58)	32 (53)	37 (57)	45 (58)	51 (45)
Pelipita *in vitro*	113 (75)	146 (71)	181 (70)	232 (71)	293 (71)	28 (51)	32 (63)	38 (70)	46 (71)	54 (71)
Saba Rhizome	76 (44)	107 (40)	163 (43)	222 (39)	289 (35)	22 (11)	26 (26)	32 (42)	41 (39)	48 (35)
Saba *in vitro*	98 (74)	140 (65)	190 (65)	249 (65)	307 (50)	25 (31)	30 (55)	37 (65)	47 (65)	50 (50)

*Numbers in parenthesis refer to total number of plants analyzed.

It should be mentioned that experimental transplanting from culture vessels directly to the field still resulted in a high survival rate (80-90%), although growth was much slower due to die-back of the non-acclimated leaves.

2. Field Experimentation and Evaluations

In order to evaluate the possibility to substitute the usual two commercial varieties 'Horn plantain' and 'French plantain' by 'Pelipita' and 'Saba', several experiments were carried out.

a) Establishment of plantations with *in vitro* plants

Since no information existed as to the possibility of using tissue-culture grown plants for field planting, comparative tests were being conducted with planting materials derived in the conventional way by using parts of the corm in form of lateral buds (see Figure 6) or suckers (daughters). Field experiments were established in four sites, two in Costa Rica (Bribrí and San Carlos) in cooperation with the Ministry of Agriculture, and one each in Nicaragua and Panama. Besides comparison of approximate cost, the survival rate, phenology and yields were studied.

The experimental design was randomized blocks. All areas were surrounded by susceptible (and heavily infected) bananas ('Grande Naine'), a fact that guaranteed abundant inoculum. *In situ* hardened *in vitro* plants were used in case of 'Pelipita' and 'Saba'. However, in the latter variety also corms (suckers) were used for comparison, similar to the way 'Grande Naine' and 'Horn plantain' were planted.

In Table 7 the comparative results for the four initial months are presented. As becomes evident from the data, that all plants had a good start. Interesting to note is the fact that *in vitro* grown plants wich have much less stored reserves as compared to a sucker, were somewhat superior to conventionally planted material. This has repeatedly been reported in other species. An explanation could be that *in vitro* plants are initially free of all pathogens and pests, permitting a faster growth. In fact, some *in vitro* grown plants started to fruit already after 5 months, about half the time normally required. This is surprising since the sucker (part of the corm)

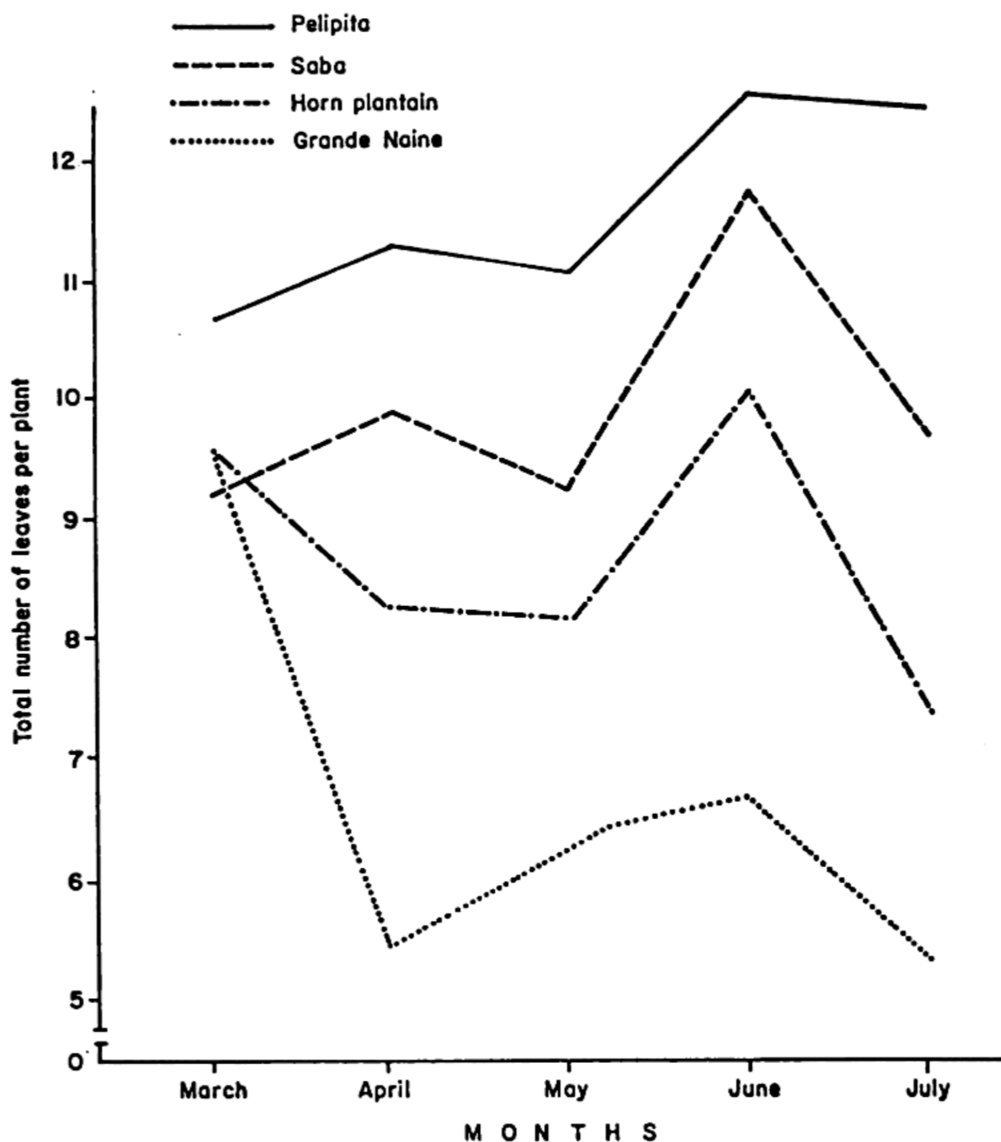


Figure 22. Total number of leaves per plant in three varieties of plantains (Horn plantain, Pelipita, Saba) and one banana (Grande Naine) during five months growth in the first year after planting. Bribri, Costa Rica.

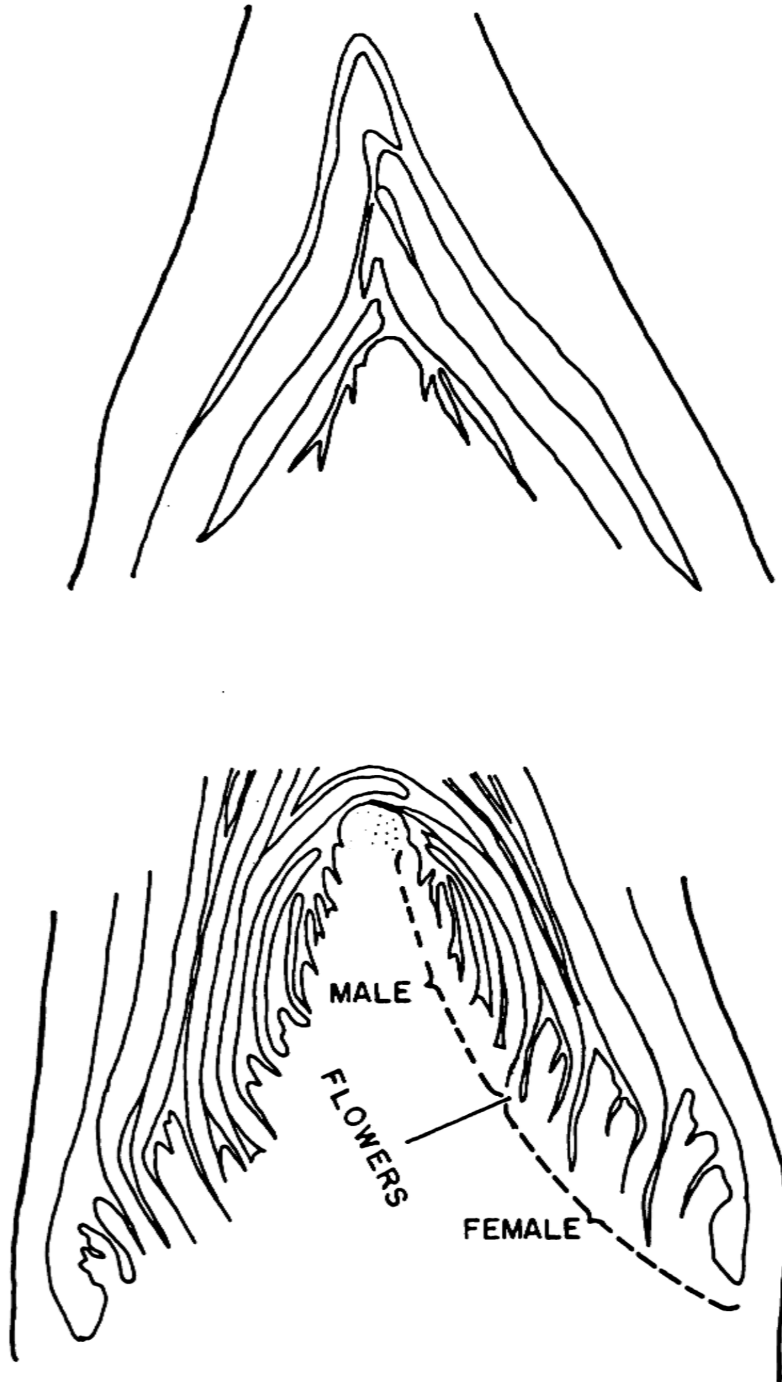


Figure 23. Schematic longitudinal section through the apical part of a developing *Musa* inflorescence. Above: before differentiation of flowers; below: after differentiation of male and female flowers. Explant dotted.

usually used for initiation of plantations, is several times the size of the *in vitro* grown plant and has much more reserve materials stored.

The total number of leaves was quite superior in 'Pelipita' and 'Saba' (Figure 22). This fact could explain why these varieties (besides being tolerant) have a higher productivity due to the larger photosynthetically active area (high leaf area index).

The results clearly show that *in vitro* propagation of bananas and plantains for large scale initiation of plantations is perfectly feasable.

Regarding the cost many factors have to be taken into consideration. On the production side: especially the cost of labor and reagents, and volume of plants produced. Since transportation cost is much less than in case of suckers, preliminary estimations compare very favorably for *in vitro* production of plantlets.

b) Comparison of explant types

The development of the inflorescence of *Musa* can be divided into two phases: The first flowers, at the basal part of the shaft, are female, followed by male flowers formed under continuos growth of the distal (staminate) portion of the axis. Whereas the female flowers remain attached in clusters to the axis and develop into mature parthenocarpic fruits, the male flowers are shed successively by abscission from the base of the abortive ovaries after exposure for about one day, that is, after the bract which covers each cluster of flowers has fallen off. Thus only the tightly covered flowers in the youngest (terminal) portion of the axis still remain in form of a 'bell' or 'navel' (Figure 23), leaving distally a bare axis up to the female flowers.

It has been known that the still much undifferentiated apical part of the male inflorescence (see Figure 25) can be used as explant material. Under certain conditions the terminal part reverts to vegetative tissue, giving finally rise to complete plantlets.

In order to fully analyze the possibility of using the male inflorescence as explant material, a cooperative experiment with Dr. A.D. Krikorian, Department of Biochemistry, State University of New York at Stony Brook, was

started. The original material came from mother plants in Venezuela and consisted of two varieties similar to the 'Horn plantain': 'Harton verde' and 'Harton negro'. The derived plantlets were raised in Stony Brook and transported to Costa Rica for field planting.

Three types of propagules were used:

1. Plantlets derived from vegetative buds of the corm of 'Harton verde' and 'Harton negro' (34 plantlets).
2. Plantlets derived from male inflorescenees of 'Harton verde' and 'Harton negro' (34 plantlets).
3. Suckers from corms of field-grown 'Horn plantain'.

Table 8. Field evaluation of two explant types, apical bud and male inflorescence, of two varieties of plantains, 'Harton verde' and 'Harton negro' as compared to the common variety 'Horn plantain'.

	'Harton negro'		'Harton verde'		'Horn plantain' (check)
	Bud	Inflorescence	Bud	Inflorescence	Sucker
Heigh after 9 months cm	380	375	300	280	405
Diameter at base after 9 months cm	55	45	52	53	60
Days to flowering	270	275	269	279	270
Days to fruiting	360	360	358	375	355
Hands per bunch	7.6	8.1	7.1	6.8	8.2
Fingers per hand	4.3	4.1	4.2	3.7	4.3
Weight of raquis (kg)	0.79	0.63	0.82	0.72	0.84
Weight per hand (kg)	1.55	1.02	1.70	1.47	0.62
Total weight bunch (kg)	12.9	12.2	12.7	11.8	13.1

Table 9. Characteristics of the bunch of three plantain cultivars ('Saba A_2', 'Pelipita 14055' and 'Horn plantain') Turrialba, 1984

Variables		Saba		Pelipita		Horn plantain	
		Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Number of hands per bunch		6.80	0.45	7.8	0.45	8.6	0.89
Weigh of hand (g)		6 994	140.11	2 538.0	221.08	1 613	394.18
Number of fingers per hand		13.0	0.85	12.7	0.47	4.71	0.43
Lengh of central finger of each hand (cm)	ext.	15.2	0.55	15.4	0.74	23.9	0.65
	int.	11.2	0.29	11.9	0.37	17.4	1.65
*Calibration of central finger of each hand in $\frac{1}{32}$ of an inch	front	44	1.61	51	1.58	54	2.16
	side	62	0.32	61	1.29	57	2.61
Diameter of rachis at the height of each hand (cm)	1	15.1	0.74	19.4	1.19	14.4	0.42
	2	13.8	0.45	17.4	0.82	12.4	0.42
	3	12.2	0.57	15.8	0.91	10.5	0.71
	4	11.7	0.45	14.6	0.82	9.7	0.84
	5	10.8	0.57	14.0	1.00	8.5	0.49
	6	10.2	0.57	13.0	0.61	7.8	0.84
	7	10.0	0.0	12.7	0.45	7.4	0.99
	8			12.5	0.58	6.6	0.89
	9					6.4	1.01
	10					5.5	0.00
Distance between insertion of hand on rachis (cm)	1 y 2	6.5	1.15	7.8	0.79	6.1	1.13
	2 y 3	6.1	0.30	5.9	1.15	4.6	0.42
	3 y 4	5.8	0.27	6.1	0.50	4.8	0.27
	4 y 5	4.7	0.44	5.1	0.69	4.1	0.55
	5 y 6	3.6	0.44	4.5	0.78	3.6	0.74
	6 y 7	2.8	0.24	3.5	1.08	2.5	0.29
	7 y 8			2.9	0.91	2.4	0.51
	8 y 9					2.0	0.00
	9 y 10					1.4	0.00
Nodal protuberance at second hand (cm)	lengh	6.5	0.2	8.6	0.34	7.7	0.83
	width	1.5	0.0	1.5	0.18	1.9	0.22
	thickness	2.1	0.2	2.4	0.15	2.7	0.47
Length of rachis (cm)		107.0	4.82	142.0	7.97	63.0	5.79
Weight of rachis (g)		2 100.0	256.12	3 536.0	519.45	956.0	116.10
Weight bunch (kg)		16.69	1.84	22.91	1.81	14.16	16.65
Measurement of central finger of second hand	volume (ml)	185.00	16.98	218.00	12.25	309.00	24.52
	weight pulp (g)	75.00	6.18	104.00	5.65	187.00	22.10
	weight peel (g)	76.00	5.91	95.00	8.65	108.00	11.66
	total weight (g)	152.00	12.02	199.00	12.80	295.00	29.84

Figure 24
Unripe fruit bunch of 'Horn plantain'; note the length of the fingers

Figure 25
Tissue-culture derived 'Saba'
plantation during fruiting

Figure 26
Fruit bunch of tissue-culture derived 'Saba'
plant

The evaluation included, besides the usual parameters (Table 8) of growth (diameter and height of the pseudostem, fruit characteristics, weight and phenology) the evaluation of degree of susceptibility to Black Sigatoka. When comparing data obtained, no significant differences could be observed.

All plants proved highly susceptible to Black Sigatoka. This, as such, is not surprising since the sample was too small to expect any manifestation of somaclonal variation.

This experiment proves that both types of primary explants can be used to establish new plantations.

c) Fruit characteristics.

Since one of the objetives was to find resistant varieties in order to replace the very susceptible commercial ones: 'Horn plantain' and also 'French plantain', it was essential to carry out field experimentation in order to determine morphological fruit characteristics on a comparative scale for 'Pelipita' and 'Saba'.

Evidently the differences in taste and flavor are not the only factors for a possible rejection as a staple food, since shape and size of the fruit also play an important role.

One of the reasons could be that plantain varieties (called locally "guineos") with similar characteristics as 'Pelipita' and 'Saba' are being used mostly for feeding hogs.

The most important differences and distinctive characteristics are the size and shape of the fingers. In the usual commercial plantain ('Horn plantain') they are long, slender and curved (Figure 24) whereas in 'Pelipita' and 'Saba' (Figure 26) they are much shorter and more rounded, almost straight.

As can be seen in Table 8 'Horn plantain' had more hands per raceme (bunch). However, the much higher number of individual fruits (fingers) per hand was much greater in 'Saba' and especially 'Pelipita',

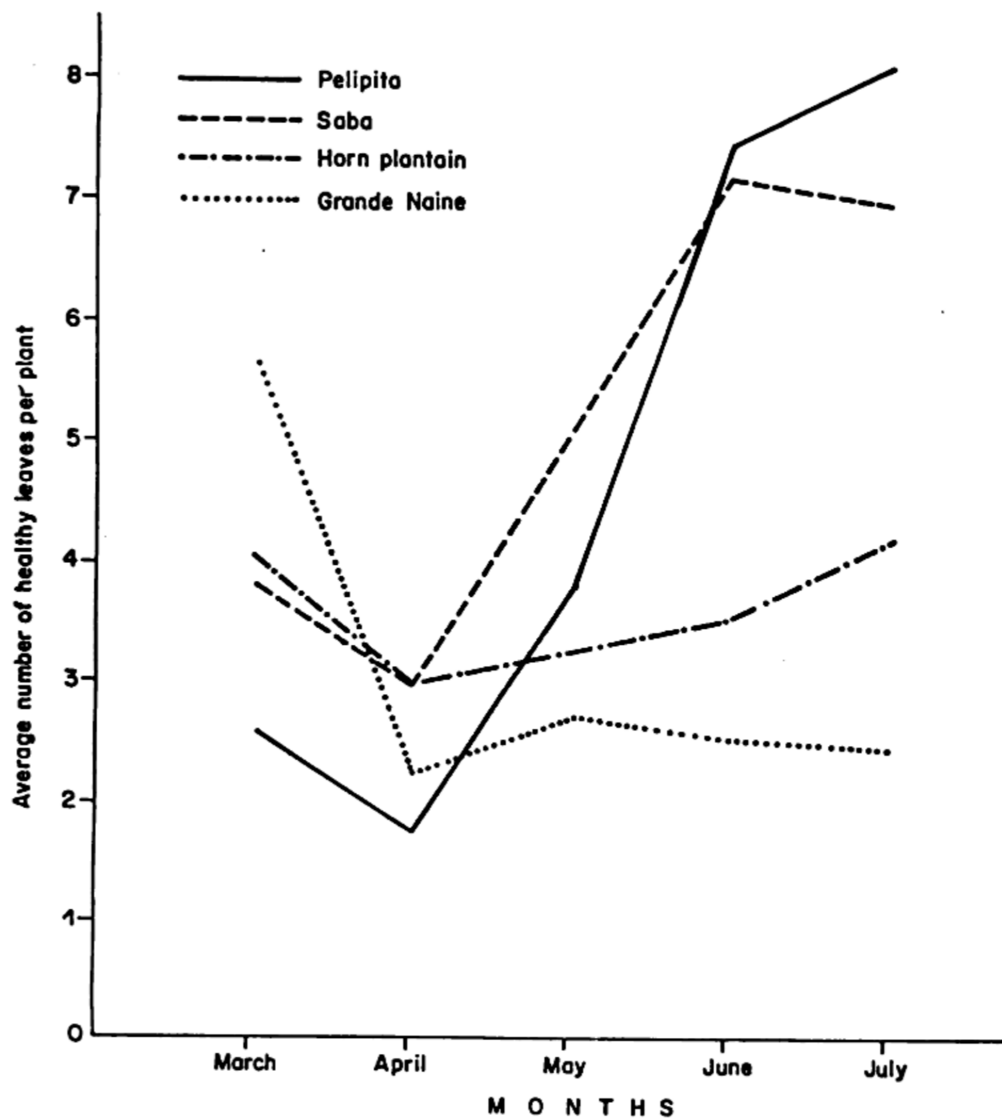


Figure 26. Average number of healthy leaves per plant in three varieties of plantains (Horn plantain, Pelipita, Saba) and one banana (Grande Naine) after attack by Black Sigatka during five months of growth. Bribri, Costa Rica.

Table 10. Comparison of Black Sigatoka incidence
Bribrí, Costa Rica, June 1984

Average of 15 plants

Variety	Total leaves per plant	Youngest leaf with visible infection	Leaves infected per plant	Healthy leaves per plant	Leaves infected	Plants infected
	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$	%	%
Grande Naine	5.4	3.4	2.9	2.5	61.2	100
Horn Plant-tain	7.4	5.2	3.1	3.6	64.3	100
Pelipita	12.6	9.2	4.3	8.2	34.3	100
Saba	18.8	8.3	2.6	7.1	26.8	100

resulting in a higher weight of the total raceme when compared with 'Horn plantain'. Regarding the individual fruits, the most distinctive characteristic is the length of a finger. Based on total pulp per raceme yield values of 'Pelipita' exceed those of 'Horn plantain' (7%) and 'Saba' (28%).

In general the average yields of 'Pelipita' and also 'Saba' compare very favorably with those of the most common commercial variety. Therefore, if yield is the most important factor, besides tolerance to Black Sigatoka, substitution is not only very feasable but also highly recommendable. However, any attempt to introduce a new food source, be it a new variety of a traditional crop or a novel crop, acceptance depends upon a number of factors, including personal preference.

d) Resistance testing for Black Sigatoka

Black Sigatoka, the disease caused by *Mycosphaerella fijiensis* var. *difformis*, is by far the most important restrain to plantain growing in Central America and part of the Caribbean region, to which the fungus has already been introduced since its discovery in 1972 in Honduras. Therefore field tests were carried out to evaluate the degree of resistance of 'Pelipita' and 'Saba'.

A previous partial characterization of the cultivars Pelipita and Saba had shown that both are relatively resistant to Black Sigatoka. 'Pelipita' also resists Bacterial Wilt (Moko) and races 1 and 2 of Panama Disease. 'Saba' has shown resistance to Panama Disease but is slightly susceptible to Bacterial Wilt. Both show resistance to the burrowing nematode (*Radopholus similis*) the major root pathogen in cultivated *Musa* spp.

A comparison of data obtained in the Atlantic region (southeastern part) of Costa Rica, Table 10, indicates that all four varieties showed signs of infection. This can be explained by the fact that 'Pelipita' and 'Saba' are not resistant in its true sense, but should be classified as only tolerant. As can be seen the percentage of infected leaves (the older ones) is significantly lower in these two varieties. At the same time more young and healthy leaves are available in the tolerant cultivars (Figure 26). It is

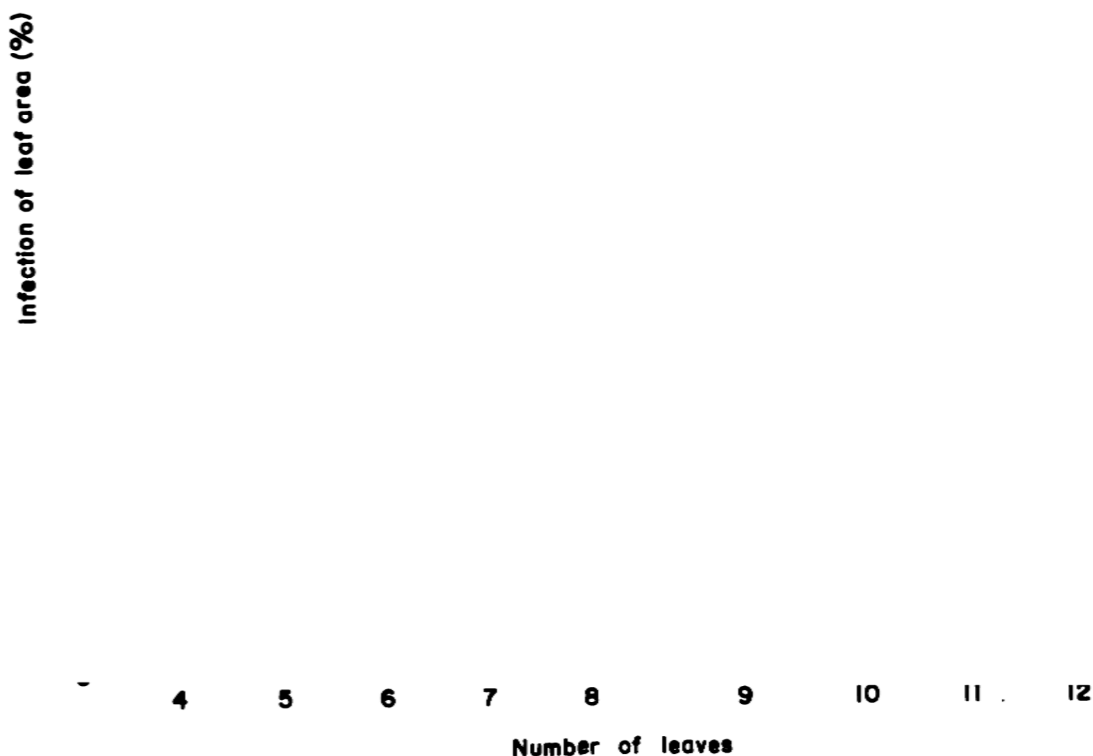


Figure 27. Total infected leaf area by Black Sigatoka per plant in leaf succession in three varieties of plantains (Horn Plantain, Pelipita, Saba) and one banana (Grande Naine) at the end of the first year after planting; the relative scale used is based on Stover 1971.

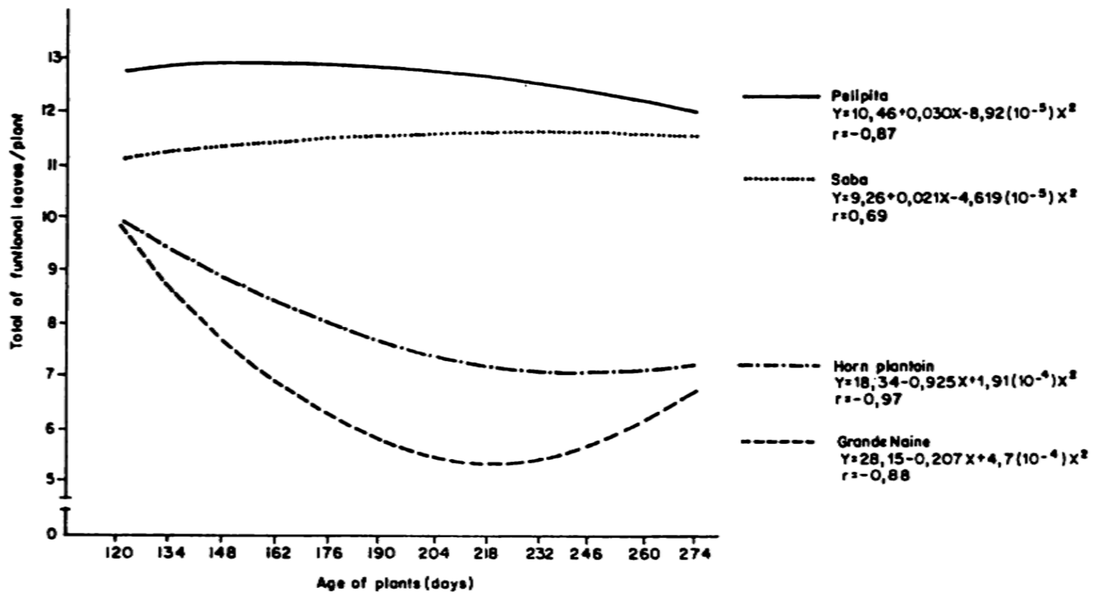


Figure 28A. Regression curves for the total number of functional leaves per plant of three varieties of plantains (Horn plantain, Pelipita, Saba) and one banana (Grande Naine) during early growth of the plants. San Carlos, Costa Rica.

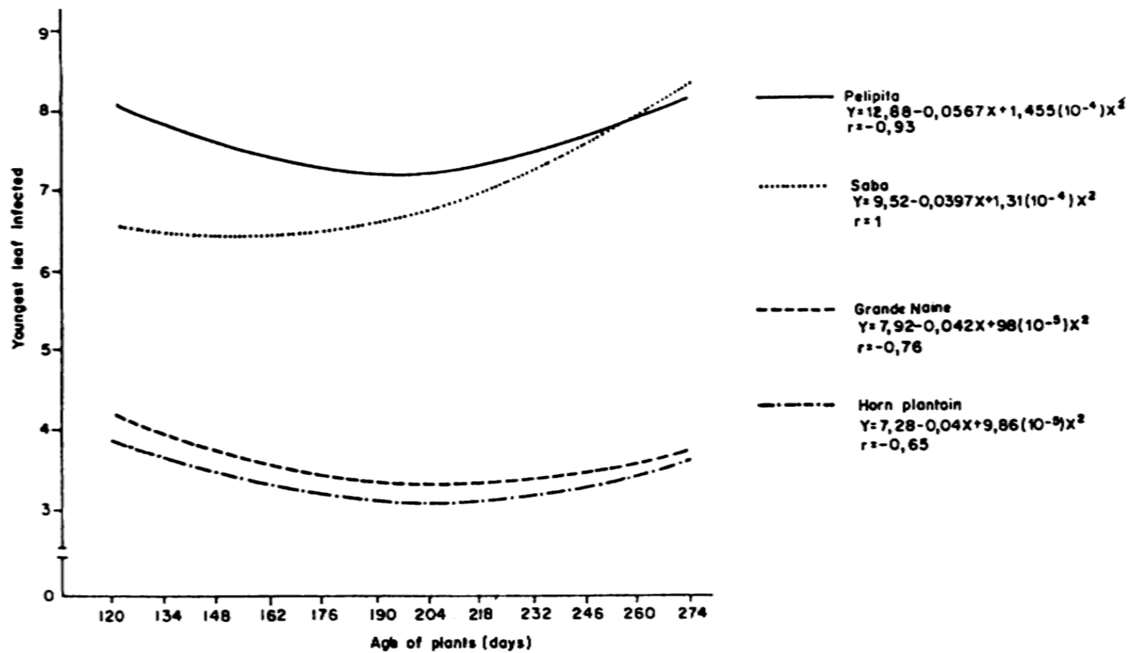


Figure 28B. Regression curves for the youngest leaf infected by Black Sigatoka in three varieties of plantains (Horn plantain, Pelipita, Saba) and one banana (Grande Naine) during early growth of the plants. San Carlos, Costa Rica.

well known that a healthy *Musa* spp. plant has on the average, when fully grown, from 10 to 15 photosynthetically active leaves. New leaves are produced at a rate of one per 6 to 10 days, with an average of one per week, if temperature is optimum. Since at least 6 to 7 photosynthetically active leaves are needed to produce and mature fruit, the infection in the oldest leaves of 'Pelipita' and 'Saba' therefore does not have an important effect on productivity.

In another experiment carried out in the Atlantic plain (northeastern part) of Costa Rica similar results were obtained when observing the plants during the first year after planting. When estimating the percentage of infection in leaves using a relative scale, it showed (Figure 27) that the banana 'Grande Naine' and also the commercial plantain 'Horn plantain' had already infections in the very youngest leaves whereas 'Saba' and especially 'Pelipita' showed sings of the presence of the fungus only in the older leaves. When calculating the regression for both healthy and diseased leaves a clear tendency for the two groups, highly susceptible and tolerant, becomes immediately evident (Figure 28).

Other similar experiments were established in Nicaragua (Nueva Guinea) and Panama (Progreso: David and Barví) on a cooperative base with the respective ministries of agriculture or their specific research organization. Results have shown that the degree of tolerance of both 'Pelipita' and 'Saba' did not vary very much under these different climatic conditions.

3. Organoleptic evaluation of tolerant varieties

A partial organoleptic evaluation had been carried out previously in Honduras. The texture of 'Saba' and 'Pelipita' is more farinaceous than in the case of the 'Horn plantain'. The first two are also sweeter and 'Saba' has a distinct higher acidity. 'Saba' is a favorite cooking banana in the Philippines and is preferred over 'Pelipita' in Honduras when cooked green, though no difference in preference existed when the fruits of the two varieties were ripe.

Table 11A. Average points of evaluation of three cultivars of plantains for sensorial characteristics after boiling in salt water

Sensorial characteristics	Cultivar 'Horn plantain	'Pelipita'	'Saba'
Appearance	51	69	25
Color	52	73	25
Intensity of odor	49	64	37
Characteristic odor (aroma)	51	60	47
Intensity of flavor	44	55	31
Characteristic flavor	58	54	33
Texture (hardness)	49	51	17
Texture (general)	59	62	53

Table 11B. Average points of evaluation of three cultivars of plantains for sensorial characteristics after frying

Sensorial characteristics	Cultivar 'Horn plantain	'Pelipita'	Saba
Appearance	59	66	42
Color	67	72	42
Intensity of odor	51	54	33
Characteristic odor (aroma)	64	63	53
Intensity of flavor	43	48	30
Characteristic flavor	45	44	48
Texture (hardness)	44	58	17
Texture (general)	45	48	53

A more detailed evaluation was carried out by a panel in cooperation with the Center of Investigation for Food Technology of the University of Costa Rica (Calderón). The plantains tested were 'Pelipita' and 'Saba' in comparison with the usual commercial cultivar 'Horn plantain'. The principal parameters tested and the results obtained appear in Table 11 . Two tests were carried out after preparation of the fruits following the two traditional ways of cooking: still green boiled in salt water (Table 11 A) and ripe fried in pan (Table 11 B). Relative scales assigning points to each procedure were used with 0 points for the most negative and 100 points for the most positive result. As can be seen 'Pelipita' is preferred over 'Saba', even when boiled, contrary to the results in Honduras. 'Pelipita' proved to be superior or at least equal in most parameters when compared to the usual 'Horn plantain', as well cooked as fried.

4. Distribution of tolerant material

Once the methodology for rapid clonal propagation was established, it was possible to produce larger amounts of planting material. This has been distributed according to requests received. One big advantage of tissue culture is that when there exists a demand, according to the number of cultures requested, planting material can be made available from the stock in about 3 to 5 months (Figure 18). At the same time the aseptic condition of the material assures that it is free of any pests and pathogens, except for possible virus diseases. Since serious virosis have not yet been reported in the Western Hemisphere, usually no restrictions exist for distribution of *in vitro* material.

Because it was known from the beginning of the project that 'Pelipita' and 'Saba' were supposedly resistant to Black Sigatoka there was much demand for these two cultivars. Although the field evaluation has shown that only partial resistance or tolerance exists, the infections of the oldest, photosynthetically already inactive leaves, does not impede excellent yields. Therefore the decision was made to release both cultivars as well for experimental purposes as for actual plantings. Among the recipients figure

ministries of agriculture, universities, experiment stations, medium and small farmers. So far this material has been taken to the following countries

Austria	Nicaragua
Belgium	Nigeria
Brazil	Panama
Colombia	Puerto Rico
Costa Rica	Taiwan
Dominican Republic	Trinidad and Tobago
Ecuador	United States
France	Venezuela
Germany (West)	Virgin Islands
Guadeloupe	
Guatemala	
Holland	
Honduras	
Mexico	

Due to space and personal limitations of the laboratory, all demands for large quantities of 'Pelipita' and 'Saba' could not be handled (some requests were for 200 000 plants!). Smaller quantities (up to several thousand plantlets) were produced and given to the appropriate agencies for cooperative experimentation, especially in Costa Rica, Panama and Nicaragua.

Besides 'Saba' and 'Pelipita' several other clonal materials have been requested and made available, including complete germplasm collections.

5. *In vitro* germplasm collection

The germplasm of the cultivated bananas and plantains is derived from two wild seeded species: *Musa acuminata* and *Musa balbisiana*. In the past classification of the varieties was confusing and difficult. This situation was eased when Simmonds and Shephard (1956) suggested to use the term genome (haploid chromosome set) to designate the origin and as a key for simplified classification. Thus the genome derived from *M. acuminata* (11 chromosomes) is designated as A and the one from *M. balbisiana* (11 chromosomes) as B. The edible dessert bananas have only genome A whereas plantains and cooking

bananas have a variable combination of genome A with genome B.

Germplasm of *Musa* spp.

Wild species

Musa acuminata
AA
Genome
(A)

Musa balbisiana
BB
Genome
(B)

Cultivated forms

AA	AAA	AAAA	AB	AAB	AAAB	ABB	AABB	ABBB
Examples								
Lady finger	Gros Michel Cavendish	Artificial Hybrids	Ney pouvan kunnan	Horn plantain French Plantain	Kudu Kudu	Pelipita Saba Bluggoe	kalamagol	Pisang batu Tiparot

There exist few germplasm collections of *Musa* in the world, and in the Western Hemisphere only three are of major importance, namely the ones in Honduras, Jamaica and Brazil. One of the reasons is that large field collections have become difficult and very costly to manage and maintain, especially after some of the more severe diseases and pests have started to invade a collection. The constant use of protective measures, such as fungicides and pesticides, is a must, since collections usually also include very susceptible materials. Also the space required and the qualified labor, more and more difficult to get, contribute to the high cost.

In vitro germplasm collections have already become important in Latin America as an alternative means of preservation, especially in vegetatively propagated plants (potatoes in CIP, International Potato Center, Peru, and Cassava in CIAT, International Center for Tropical Agriculture, Colombia). So far no *in vitro* collection of *Musa* germplasm has been started, except for the various varieties usually grown for experimentation in tissue culture laboratories which work with this genus. Since bananas, as well as plantains, are an important crop in the tropics and plantations consist usually of but a few varieties, all derived vegetatively from a mother plant, germplasm preservation is especially important and a necessity for further crop improvement.

Figure 29. *In vitro* Germplasm Conservation of *Musa* spp.

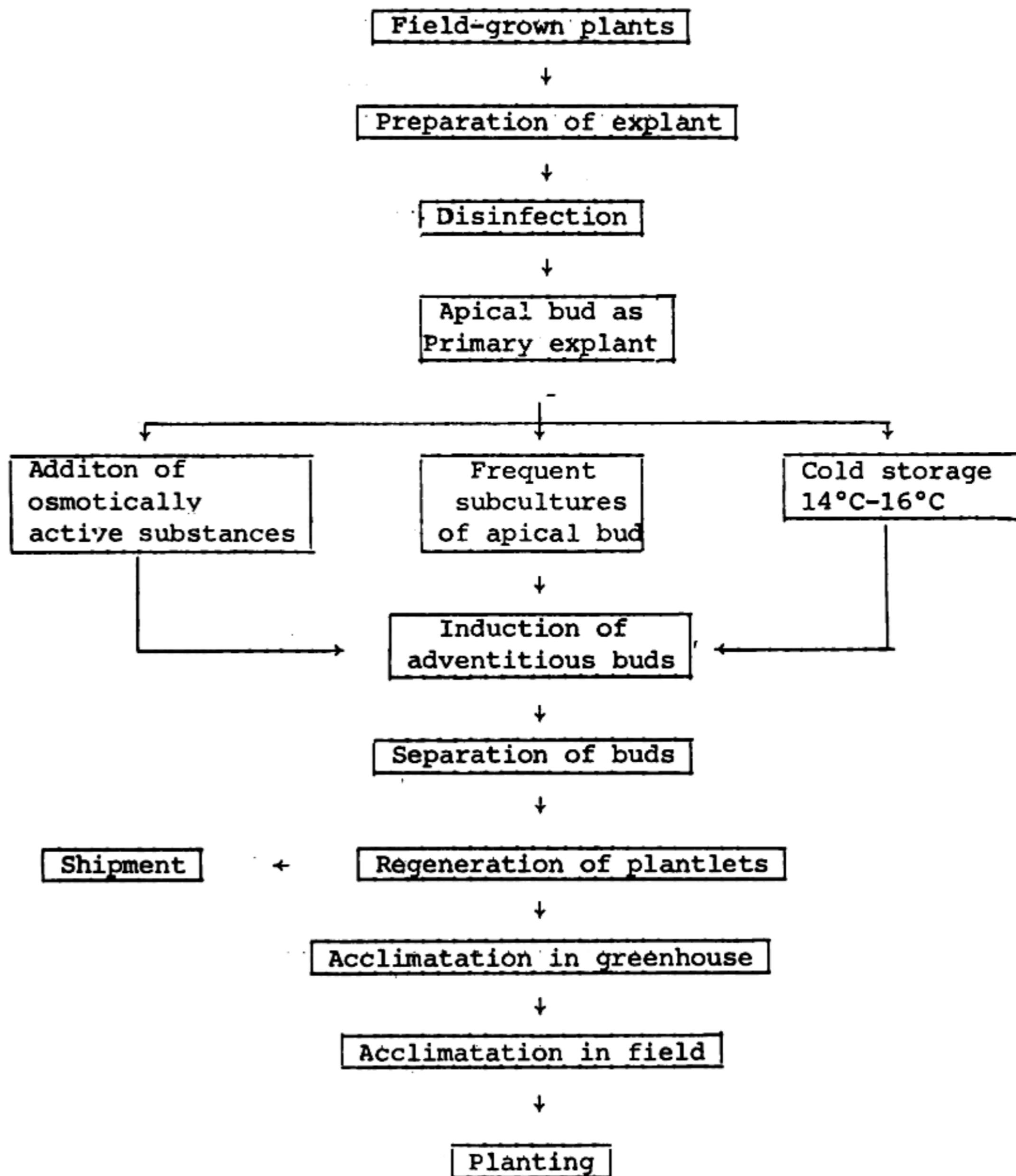


Table 12. List of Germplasm *in vitro* of *Musa* spp.

No.	Germplasm	Genome
1.	*Musa balbisiana*	BB
2.	*Musa acuminata*	AA
3.	*Musa acuminata* ssp *truncata*	AA
4.	" " " *malaccensis*	AA
5.	" " " *burmannica*	AA
6.	*Musa textilis*	
7.	*Musa velutina*	
8.	*Musa ornata*	
9.	*Musa beccaru*	
10.	*Musa coccinea*	
11.	Diploid II-239 Tongat	AA
12.	Diploid V-34 T jau Lagada	AA
13.	Diploid II-33 Guyod	AA
14.	Diploid II-217 Pisang Madu	AA
15.	Diploid III-106 Pisang jari buaya	AA
16.	Diploid AVP-35 Lidi	AA
17.	Diploid III-133 Sinwobogi	AA
18.	Banano blanco	AAA
19.	Banano enano	AAA
20.	Banano rojo	AAA
21.	Burro CEMSA	?
22.	Caribe morado	AAA
23.	Cavendish	AAA
24.	CEMSA 1735	?
25.	Congo	AAA
26.	Coco	AAA
27.	Curraré alto	AAB
28.	Curraré enano	AAB
29.	Chato	ABB
30.	Dátil	AA
31.	Dominico	AAB
32.	Dwarf cavendish	AAA
33.	French plantain	AAB
34.	Guineo cuadrado	ABB
35.	Guineo negro	?
36.	Guineo rojo	ABB
37.	Grande naine	AAA
38.	Gros Michel	AAA
39.	H. Williams	AAA
40.	Hartón negro	AAB
41.	Hartón verde	AAB
42.	Horse plantain	AAB
43.	Jardinero	?
44.	Lacatán	AAA
45.	Lady finger	AB
46.	Laknaw	AAB
47.	Maioa	?
48.	Manzano	AAB
49.	Mestiso	?
50.	Mysore	AAB
51.	Pelipita	ABB
52.	Pelipita nica	ABB
53.	Pitogo	?
54.	Pizangkerban	AAB
55.	Plátano 300	AAB
56.	Plátano cuerno	AAB
57.	Saba	ABB
58.	Siguatepeque	ABB
59.	Silk	AAA
60.	Valery	AAA

Since no useful information existed regarding *in vitro* preservation of germplasm of *Musa*, several experiments were conducted regarding establishment and maintenance of a small collection (Figure 29).

a) Establishment of a collection

Starting on a small scale, gradually the list of acquisitions has been incremented to some 60 species and varieties. The diversity of the genome allowed to test experimentally differences in the genome, since it is well known in many other plants that there exist, even within varieties of the same species, important differences regarding requirements for media composition, growth conditions, etc.

The procedure for introducing new materials into a collection is the same as for initiating any aseptic culture (Figure 29). However, when dealing with an array of very different types, differences can be expected. As it turned out, some varieties showed very strong oxidation of the primary explants whereas others did not turn brown at all. Antioxidants and frequent media changes could overcome this problem in all cases. When subculturing any adventitious buds produced on a primary explant that showed strong oxidation, the secondary explants had little or no browning, and subsequent subcultures were completely free of this phenomenon. Another difference that became apparent, was the variable formation of adventitious buds. Whereas some varieties had abundant budding in medium 2 with low cytikinin content, others required a higher concentration of BAP for abundant adventitious bud formation. However, all species and varieties indicated in Table 12 could be established on medium 1.

b) Maintenance

Since *in vitro* germplasm collections do require constant care in order to assure survival and maintenance of the material in optimum conditions, ways must be found to reduce safely the amount of labor involved. Therefore a series of tests were initiated in order to obtain some basic information on storage of *in vitro* growing *Musa* plants. Such information included: best explant size, zero growth temperature and growth inhibiting or reducing

Table 13. Various parameters associated with *in vitro* conservation of five cultivars of *Musa* at different temperatures after a six months incubation period. All values are averages of 20 plants and are expressed in percentage.

CULTIVARS	14°C					16°C					18°C					20°C				
	C	O	W	S	R	C	O	W	S	R	C	O	W	S	R	C	O	W	S	R
SABA (ABB)	5	5	15	85	90	-	5	10	80	95	5	5	5	100	100	10	-	5	90	100
PELIPITA (ABB)	5	10	20	75	95	5	-	15	80	95	-	-	5	90	100	-	-	5	85	100
HORN PLANTAIN (AAB)	10	10	20	90	85	-	5	15	75	95	-	5	5	90	100	5	5	5	95	100
CARIBE MORADO (AAA)	-	10	35	75	70	5	-	20	75	100	-	5	15	80	85	-	5	20	85	100
GROS MICHEL (AAA)	-	5	25	60	100	10	-	15	65	85	-	-	5	90	100	-	-	5	95	100

C: CONTAMINATION
O: OXIDATION
W: WILTING
S: SURVIVAL
R: REGENERATION

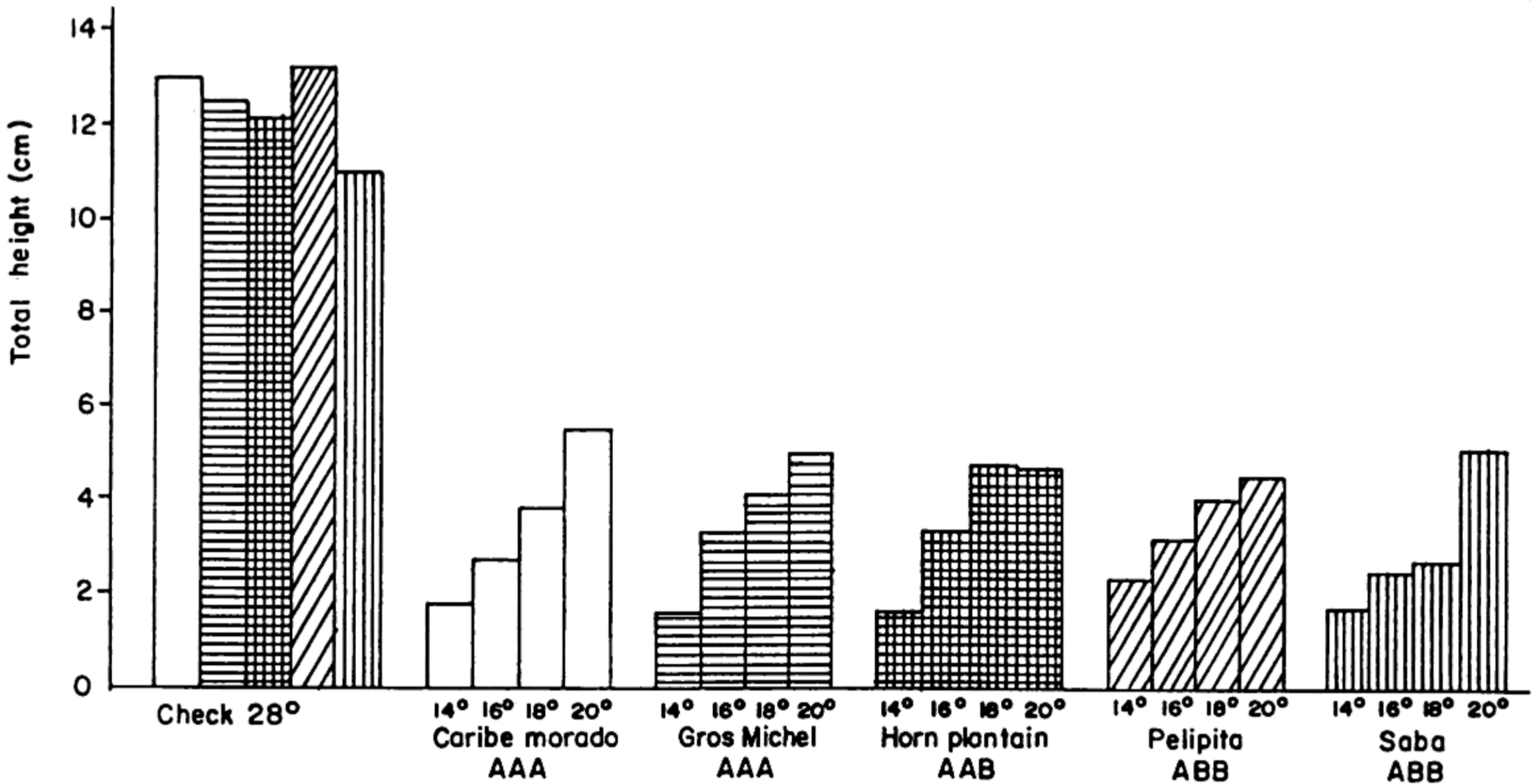


Figure 30. Effect of four different temperatures (0°C) on the longitudinal growth of five varieties of *Musa* after six months of incubation.

Figure 31. Weight of plantlets of five varieties of *Musa* after five months incubation at different temperatures

substances.

i Explant size

Regarding the most adequate size of explant, the results have already been presented (See under: Importance of final explant size, p. 19).

ii. Zero growth temperature

It is well known that temperature is an important factor regarding growth rate. The relation follows the law of optimum, with the three cardinal points: minimum, optimum and maximum temperatures. The temperatures for these three points depend on the species. If the temperature is close to the minimun required, growth is extemely slow, however, plants will usually survive, and when reaching the minimum temperature growth comes to halt, the socalled zero growth temperature has been reached.

In order to establish the zero growth temperature for different types of germplasm combinations, five varieties of *Musa* were selected. As can be seen in Figure 30 the different genomes behaved very similarly under different temperatures. The actual growth rate was slightly higher for the AAA genome. This is, to some extent, surprising, since under field conditions plantains are usually grown at higher elevations than bananas.

As can be expected the growth rate decreased gradually when lowering temperature, reaching at 14°C almost the zero growth condition (Figure 31). When extrapolating it can be assumed that zero growth occurs between 12°C and 14°C. However, at 14°C growth is sufficiently suppressed for reducing the number of subcultures in a germplasm collection.

The survival and regeneration rates were quite acceptable (Table 13) at 14°C, although oxidation was more apparent. From these data it seems that a slightly higher temperature (15° to 16°C) may, therefore, be more desirable.

iii. Use of high osmotic concentrations

There exist several substances which can reduce the growthrate considerably, such as CCC (cycocel), ALAR, maleic hydrazide, etc.

Preliminary experiments with these substances at different concentra-

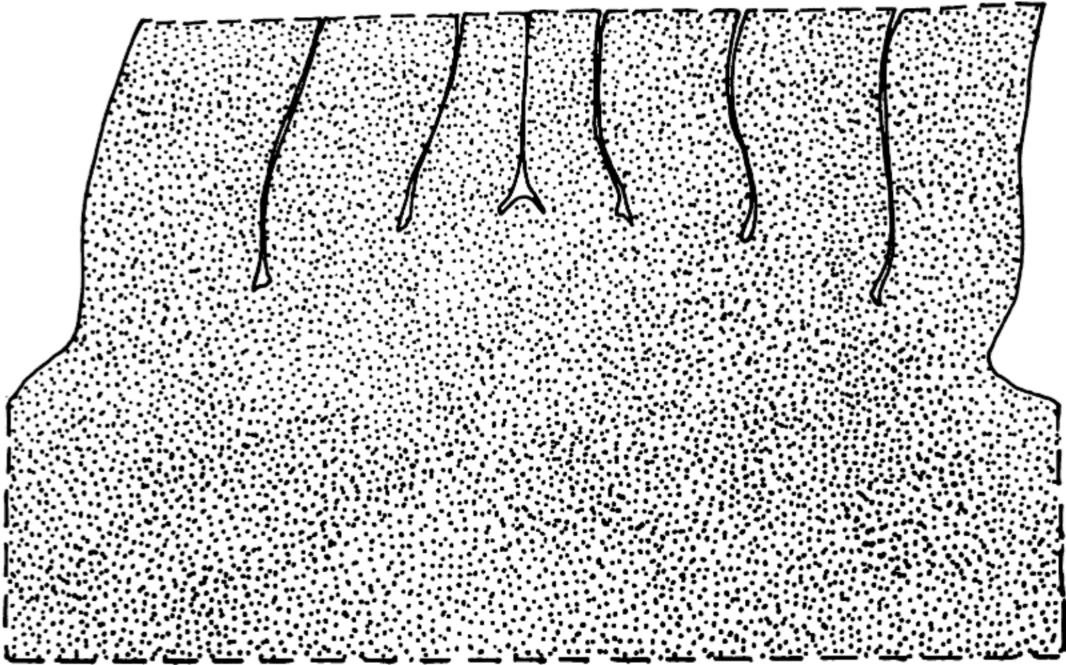


Figure 32. Typical primary explant with the apical meristem surrounded by several leaf primordia, the lower portion is formed by part of the corm.

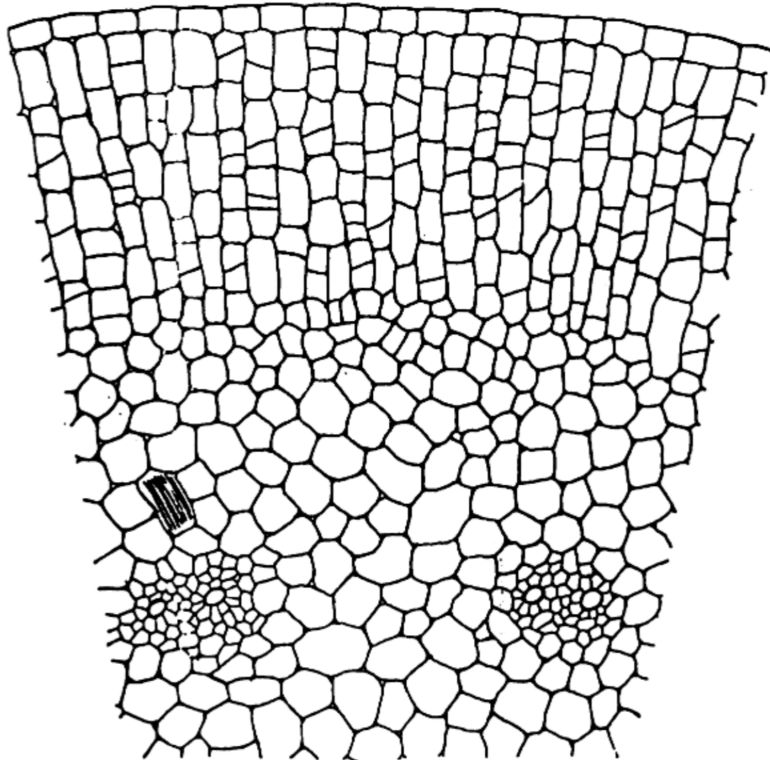


Figure 33. Epidermis and subepidermical layers formed on surface of exposed corm part after a short period of in *in vitro* culture.

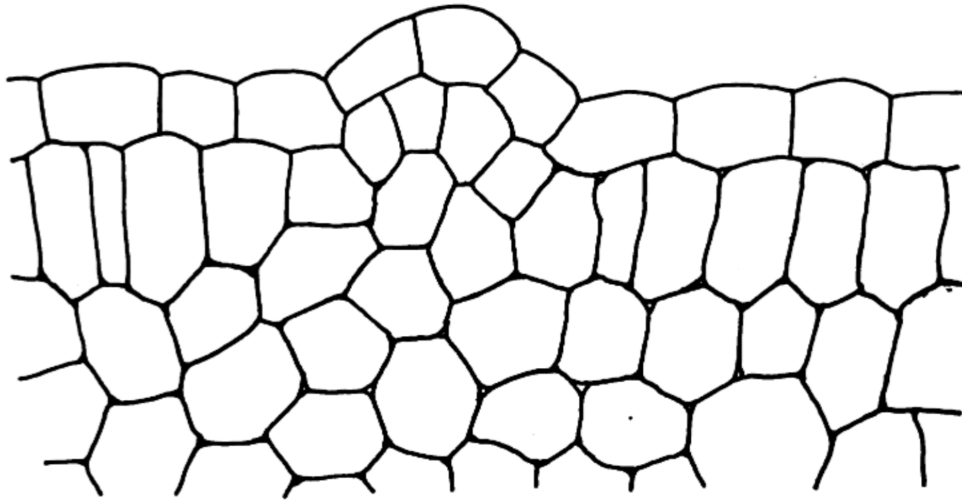


Figure 34. Initiation of an addventitious bud on the surface of the section belonging to the corm of the primary explant.

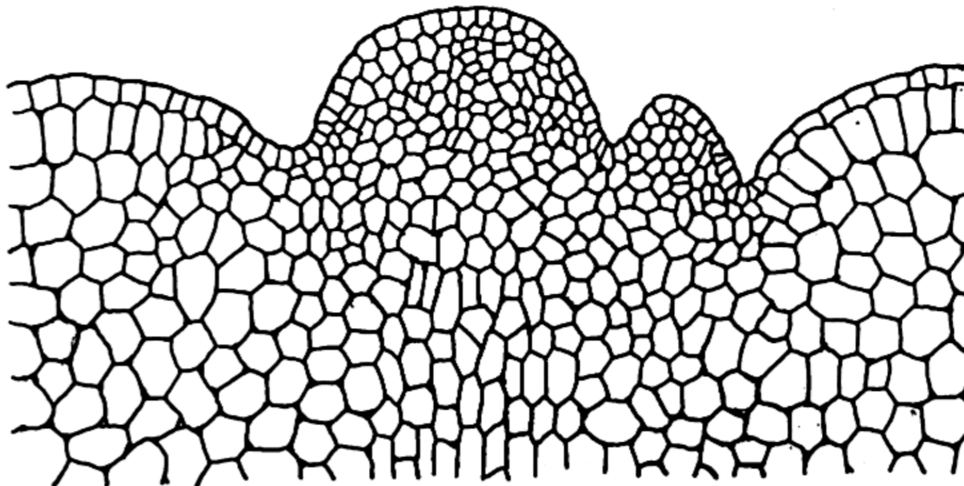


Figure 35. Development of an adventitious bud and initiation of a leaf primordium.

Figure 36. Advanced adventitious bud formed on the surface of the section of the corm of a primary explant.

Figure 37. Schematic longitudinal section through the elongated axis of a *Musa* plantlet after *in vitro* treatment with abscisic acid.

tions did not show any promising results. Therefore it was decided to induce changes of the osmotic potential of the medium by addtion of certain carbohydrates; among these the sugar alcohol mannitol proved best. It has a very low penetration rate, is not toxic, and cannot be metabolized under normal conditions.

The treatments consisted in preparation of media with the adition of varying amounts of mannitol (0%, 2%, 4% and 6%). The increase in mannitol concentration causes a corresponding reduction of the growth rate. When surpassing 6% mannitol, the explants did not grow and practically all died. A concentration of 4% mannitol, together with 6% sucrose, gave maximum reduction without causing at the same time death of the explant due to water stress (Figure 41). Attempts of regeneration after 6 months were completely successful in all treatments in which the explant survived.

A combination of high osmotic potential with low temperature can thus extend the time interval between subcultures to up to two years.

c) Problem of somaclonal variation

Another important aspect is the fact that *Musa* spp. has a rather high somatic mutation rate when propagated by adventitious buds. In some instances, as for example Jamaica, the off-type percentage of tissue culture derived plants in a plantations has been as high as 35%. This figure, however, refers only to morphological, visible changes.

Although a high somaclonal variation rate may be very desirable when looking for mutants with certain specific new characteristics, in the case of germplasm preservation it is a nuisance. If the usual procedure is employed, that is propagation by use of adventitious buds, a high variation rate can be expected. If it happens that the secondary explant used for further propagation is already mutated, then all materials produced will be off-type.

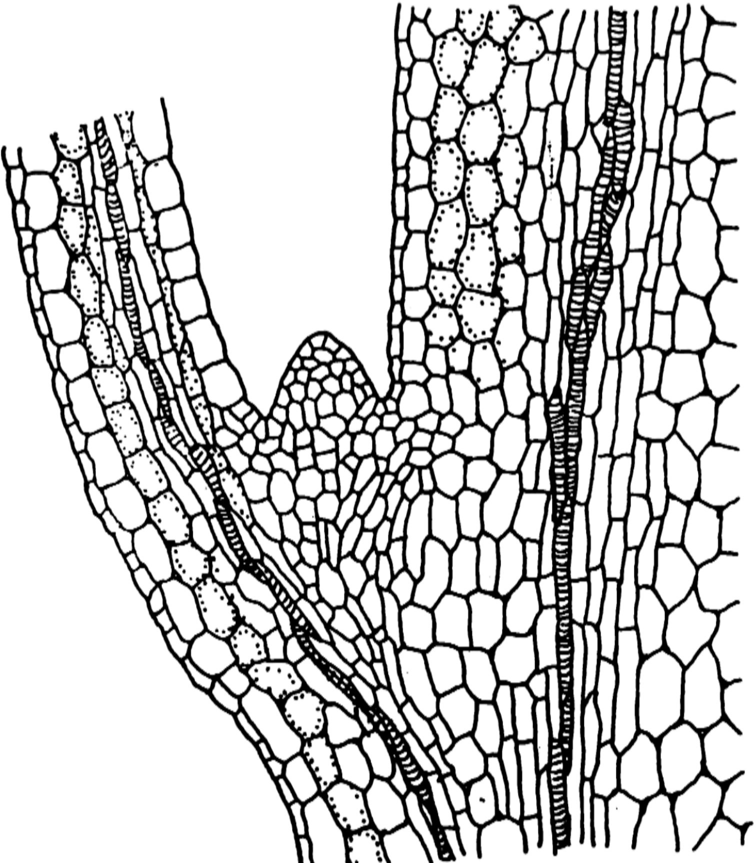


Figure 38. Axillary bud consisting of primary meristem of *Musa* plantlet after *in vitro* treatment with abscisic acid.

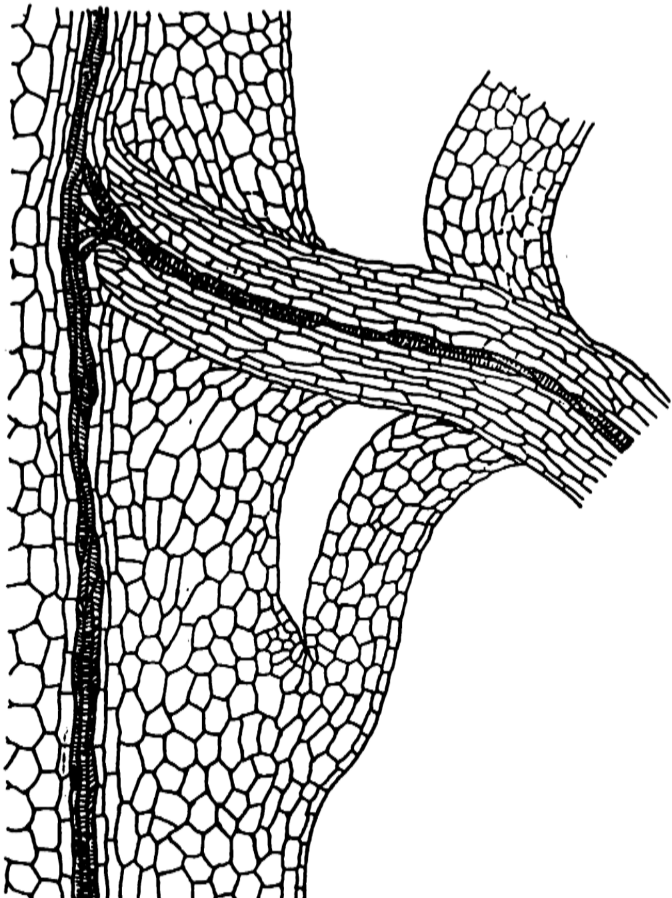


Figure 39. Root originating in the central cylinder above a node, perforating the leaf base in a plantlet treated *in vitro* with abscisic acid.

Figure 40.
Effect of abscisic acid (ABA) on morphogenesis of plantain explants; right: check, left explants in medium containing 5 mg/L ABA, showing elongation of internodes and root formation on nodes.

Figure 41.
Effect of different osmotic treatments on growth of explants of plantain; from left to right: 0% mannitol and 3% sucrose (check); 1% mannitol and 3% sucrose; 2% mannitol and 3% sucrose; 4% mannitol and 6% sucrose; 6% mannitol and 9% sucrose.

There exist many causes and explanations as to the reason for somaclonal variations. So far, however, nothing is known in *Musa* about the factors that induce changes and the type of changes produced. Although high BAP concentrations can be one of the causes, in *Musa* cultures the amounts used of this growth regulator are rather low. The most probable cause could be the adventitious origin of the secondary explants.

If one considers the normal propagation of *Musa* by suckers (Figure 6) no variations can be detected in huge numbers of plants produced. It is not clear from the literature if the meristem of suckers is of primary or secondary nature. In case of secondary explants in culture, all newly formed meristems are of secondary nature, since neither in nature nor in tissue culture the axillary buds at the base of each leaf primordium develop.

A preliminary anatomical study revealed that a typical explant (Figure 32) consisting of the apical meristem, several leaf primordia and part of the corm tissue, will produce a new epidermis and then suber-like subepidermal tissue on all exposed surfaces of the corm (Figure 33).

The adventitious buds arise by division of several epidermal and subepidermal cells (Figures 34,35,36) thus a multicellular origin is most probable. If some cells are variants, the derived plants ought to be true chimeras.

In order to avoid any possible somaclonal variation two possibilities have been tested.

i. Use of apical bud

If the primary explant is continuously cultivated in medium 1, few or no adventitious buds are being formed. By eliminating any such structures, maintaining always the primary meristem growing, no genetic or epigenetic alterations should be expected, even after many subcultures (Figure 30). However, any multiplication to obtain several plants after permanence in the germplasm collection, would require field planting of the plantlet derived from the meristem of the original explant and then use of conventional propagation by suckers.

ii. Use of axillary buds.

Each leaf axil has in its center an axillary bud consisting of reminants of primary meristem. These buds, which do not develop in tissue culture, are too rudimentary to be excised from an explant after a certain time in culture.

When treating primary explants, after establishing the culture, by adding 5 mg ABA (abscisic acid) to the medium, surprisingly an elongation of the axis occurs (Figure 40), with every internode becoming several millimeters long (Figure 37). Each leaf axil contains a bud with primary meristem (Figure 38) and above this bud a root is formed, which perforates the corresponding leaf base (Figure 39). The enlargement and formation of the internodes allows then to separate each node for individual culture. Thereafter the axillary bud could be stimulated to grow. So far not enough time has been available to study the conditions for overcoming partial inhibition of bud growth. If this could be accomplished, several plants could be obtained, all from primary meristem.

6. *In vitro* screening for Black Sigatoka

In order to select somaclonal variants that are resistant or tolerant to Black Sigatoka, a fast and efficient screening method is needed since a large number of plants is involved.

The usual way to test for it is to grow the plant in the field in presence of ample inoculum provided by diseased plants. This method gives the most accurate results, since all phases of the development of the plant can be evaluated. However, this method becomes impractical as soon as large numbers of plants are involved, due to the very high cost of establishing a plantation and, after one year, eliminating practically all plants that do not show any resistance. At the same time the infection rate depends on several factors (Foure, 1982), such as endogenous (growth, nutrional status, etc.) and environmental ones (climate, presence of other diseases and pests, soil, etc.).

A faster method was developed by Dr. R.H. Stover (1971) by growing the plants to be tested in large containers in a greenhouse for some 6 to 9

showing individual lesions.

Figure 43.
Severely affected leaf with large necrotic areas due to Black Sigatoka damage

months. This, again, requires much space and investment.

Since some work has been published regarding *in vitro* testing for disease resistance in other plants, it was decided to try to develp an *in vitro* testing procedure for Black Sigatoka in *Musa*.

For this plantlets of several different species and cultivars (genomes) were used: *M. acuminata* (AA), 'Gros Michel' (AAA), 'Valery' (AAA), 'Caribe morado' (AAA), 'Horn plantain' (AAB), 'Chato' (ABB), 'Saba' (ABB), and 'Pelita' (ABB).

a) Culture of the fungus and preparation of inoculum

In order to be sure to obtain a good representation of all possible races of the fungus, severly infected leaves (Figures 42,43) of plantains and bananas were collected in field plantations near the Atlantic coast of Costa Rica.

In order to obtain the initial inoculum in form of ascospores the methodology developed by Stover (1976) was adopted. In Central America the most common fungi which discharge ascospores from Sigatoka diseased leaf tissue are *Mycosphaerella minima*, *Leptosphaeria* sp., *Micronectriella* sp., *Mycosphaerella musae*, *Mycosphaerella musicola* and *Mycosphaerella fijiensis*.

Segments of previously dried infected leaves were attached with moistened filter paper to the inside of the cover of Petri dishes that contained simple agar medium. After several hours or days the ascospores were discharged and under a microscope the *M. fijienses* spores selected.

These spores were originally cultivated on modified Mycophil agar*. When transfered to a stationary liquid medium the growth rate was much increased. In Mycophil medium the ascorpores of *Mycosphaerella fijiensis* produce two types of mycelium: a) dark grey or brown gray with irregular edge, type DGB (Dark-Grey-Brown) and b) light grey pinkish, type PGP (Pale-Grey-Pink). The cultures of DGB produced more conidia, but cultures are not as stable as those of PGP.

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*Fisher Scientific Co., New York.

So far 42 cultures were started of *M. fijiensis*. The distribution of myceliumtypes can be seen in Table 13.

Table 14. Differences in the morphology and growth of *M. fijiensis* on modified Mycophil agar (26°C±1°C, photoperiod $^{16}/_{8}$ h).

	TOTAL	TYPE DGB	TYPE PGP
Isolated spores	42	19	23
Diameter of cultures (mm) (Average after 14 days)	4.8 (n*=31)	4.4 (n=14)	5.1 (n=17)

*n= Number of cultures observed.

The cultures grow very slowly, on the average 4.8 mm in 14 days. Subcultures on a fresh medium were carried out after 4-5 weeks. After the first change, all cultures grew. However, after the second subculture 6 cultures of type DGG and 2 cultures of type PGP stopped growing, as well on semisolid as in liquid medium.

For inoculation two cultures of type DGB were used, which, after 58 days of initial culture, showed good phytopathogenicity. However, after 104 days of continuous growth in the same medium, one of them lost completely the pathogenicity. These results indicate the necessity to start new culture from single spores every 2 months and maintain a continuous program for testing for pathogenicity in order to assure reproducible results.

b) Results of inoculation with mycelium

When inoculating the upper and lower leaf surfaces in detached leaves, these showed yellowing symptons after two weeks. In most cases the mycelium grew on top of the leaf surfaces, with some penetration. Under the microscope it could be observed that the hyphae penetrated through stomates; They

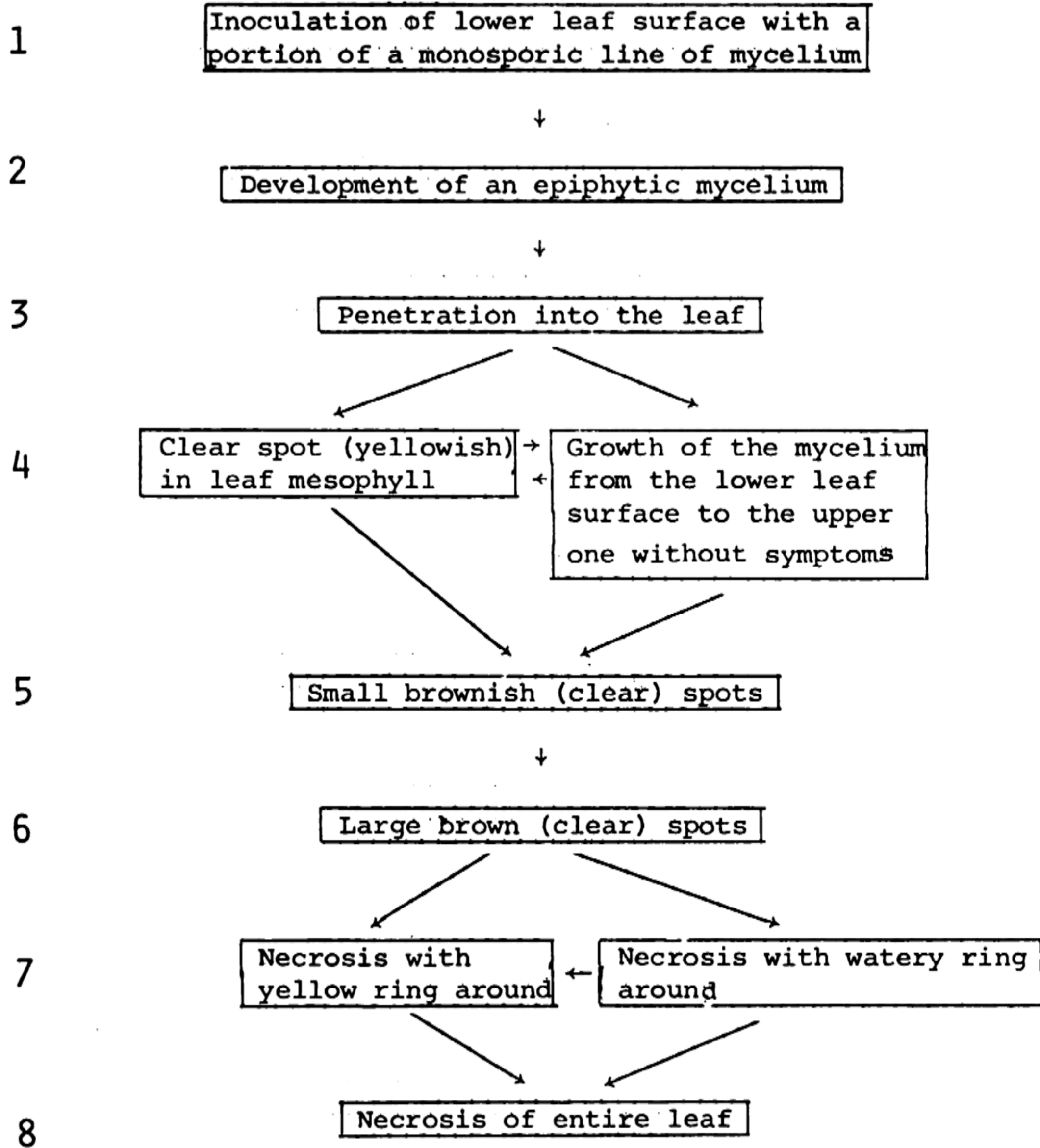


Figure 45. Soheme for development of Black Sigatoka infection in *Musa* plantlets *in vitro*.

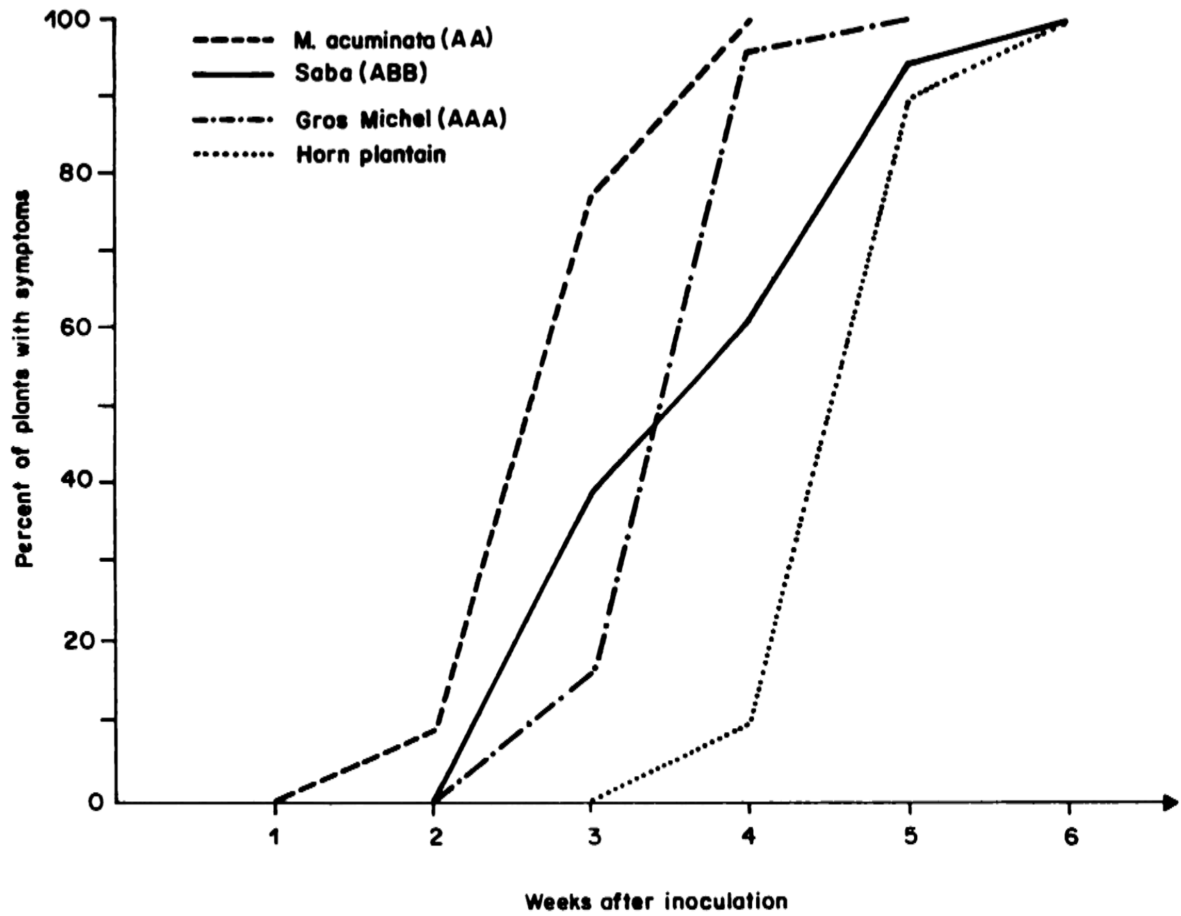


Figure 46. Percentage of plants with symptoms after inoculation with conidia of *M. fijiensis in vitro* (28°C±2°C, photoperiod 16/8h).

formed conidiophores alone or with conidia protruding from other stomates. There were no apparent differences regarding upper or lower leaf surface.

As a next step young *in vitro* plantlets were inoculated (Figure 45). It could be noted that in this case the symptoms developed faster when the mycelium was placed on the lower leaf surface. The differences between susceptible and tolerant varieties, as known from field testing, were, however, not very apparent. This may be due to the strong inoculum and a possible toxin contained in the medium around the mycelium.

In order to establish differences in pathogenicity between monosporic culture lines a test was carried out, using 17 different cultures. After some three to four weeks almost all inoculated plants of 'Horn plantain' were severely attacked. Thus, evidently no differences among monosporic lines exist.

In another experiment 25 plantlets of all previously mentioned cultivars were tested (Figure 46). It can be seen that there existed differences with respect to the speed with which symptoms developed, but in regard to infection, only a slight delay could be detected in 'Saba', a tolerant form; however, 'Horn plantain' was even more delayed, being a susceptible cultivar.

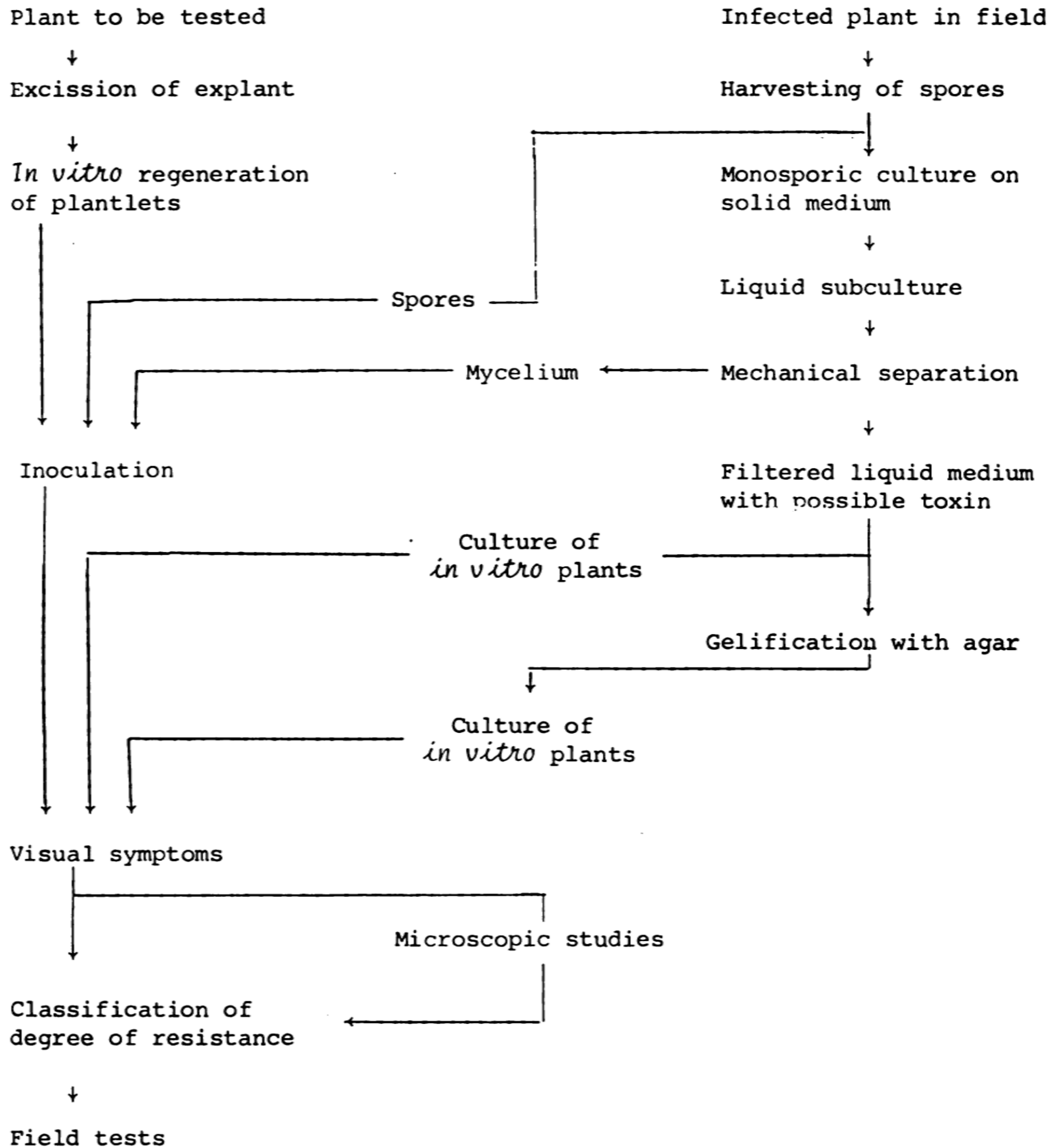
The results of this method of inoculation indicate that symptoms develop rather fast in a few weeks time, a fact that makes this method ideal for screening large populations, however, the severe attack by life, growing mycelium placed on the very tender leaves does not permit distinguishing degrees of resistance.

c) Other methods of inoculation

The imperfect form of M. *fijiensis* is condidered to be a *Cerospora*. It is well known (Durbin 1981) that most speciest of *Cercospora* produce a toxin, cercosporin. Although no such substance has so far been reported from Black Sigatoka, the rapid decline of an infected plant, together with the increase in severeness of an attack in full sunlight, indicate that the same or a similar substance exists.

Preliminary trials were carried out to extract and somewhat purify a

Figure 47. Scheme for the verification of possible resistance toward Black Sigatoka by inoculation of *in vitro* plantlets of *Musa* spp.



possible toxin, using a liquid culture of the fungus. When the extract was added to the medium for cultivating *Musa* plantlets, the reactions were again far too strong to be able to distinguish degrees of resistance among cultivars.Figure 47 shows a general diagram describing the possible ways for developing an *in vitro* test plan.

7. Somaclonal variation

One of the important contributions of tissue culture to agriculture is the possibility to isolate variations of the genome from vegetative tissue. The most logic approach to obtain these somaclonal variants is by initiating the regeneration process from a single cell, thus avoiding the formation of chimeras. As well protoplasts as cell cultures are ideally suited for this purpose. As starting materials for these cultures usually leaf mesophyll and friable callus are employed.

a) Protoplast isolation from leaf mesophyll

Several experiments were carried out using different enzymes, a pectinase and a cellulase, successively (two step method) or in combination (one step method) with leaves from *in vitro* grown plants as substrate. Although it was possible to obtain protoplasts, the yield was not sufficient to surpass the critical concentration for cultivation. When trying to use leaves of field-grown plants, all protoplast cultures were contaminated due to the high infection rate (endophytes), even in leaves that appear to be completely healthy.

In no instance could any division in protoplasts be observed, neither regeneration of cell walls.

b) Callus formation

Tissues of plantains and *Musa* in general, do not readily form callus *in vitro*. This is rather surprising because in almost all other plants the contrary is true. However, a series of studies to evaluate means to induce callus and subsequently organogenesis in cultured *M. balbisiana* zygotic embryos yielded significant results. Culturing these embryos, after removing them from the seeds, on a modified MS medium containing 100 mg/L 2,4-D and

0.1% (w/v) activated charcoal at 25°C in total darkness, resulted in callus proliferation and the subsequent recovery of whole plants from this callus. Other growth regulators, such as picloram, NAA, 2,4,5-T or IAA were not as effective. Zygotic embryos of *M. acuminata* failed to respond in a similar manner. A follow-up study was performed using vegetative tissues from both *M. balbisiana* and *M. acuminata*. Callus induction was achieved from leaf blade tissue of both species on MS medium supplemented with 2,4-D (100 mg/L + 0.1% w/v activated charcoal). Entire regenerated plants have only been recovered from callus of *M. acuminata*. Attempts to induce organ formation from callus that originated from vegetative tissues of *M. balbisiana* still continue. It is believed that the success with these two diploid *Musa* species, which are the progenitors of the edible triploid plantains and bananas, will be applicable to the latter.

Various tissues of 'Pelipita' including pseudostem tissue, root tissue, ovules, shoot-tips, immature fruit slices, male inflorescences, and axillary buds, all derived from field-grown material, were cultured on semi-solid media containing a concentration range of various auxins alone or in combination with cytokinins. In initial studies callus formation on a limited extend was achieved only with male inflorescences in liquid culture. Subsequent transfer of this callus to semisolid media resulted in the accumulation of phenolic compounds and later death of the tissue. Attempts to induce proliferation of this callus have had success only when maintaining it on filter paper supports (bridges) in liquid media. A low percentage of male inflorescences initiated some callus. Organ formation from this callus, however, was not achieved.

Initial experiments to induce callus from leaf tissue taken from plants in the field were generally unsuccessful regardless of media composition or culture environment. Only 5% of the cultured leaf explants responded. However, by utilizing leaf base tissue from *in vitro* maintained stock plants and considering the results obtained with *M. acuminata* and *M. balbisiana*, callus actually has been successfully induced in leaf tissue of plantain, when

cultures were placed on MS medium containing 2,4-D (20 mg/L) at 25°C, in the dark. The addition of activated charcoal (0.1% w/v) reduces considerably the problems associated with the oxidation of phenolic compounds. A concentrated effort to recover whole plants from this callus has not yet been conducted.

It was possible to obtain cell cultures from some of the more friable calluses by agitation in liquid medium. These cells could be maintained alive for a considerable time. However, only very few divisions could be observed. Almost all the cells were large, elongated or curved and highly vacuolized. It is well known from the literature that this type of cell is not responsive to culture, since usually only those cells divide which are small and densly packed with cytoplasm.

Considering the difficulty in obtaining friable callus and divisions in cell cultures it seems, in the case of *Musa*, much more important to concentrate on the extremely high rate of somaclonal variation encountered in plants derived from adventitious buds.

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B. OTHER ACTIVITIES

Besides the research several other activities, related to the work in the laboratory, were carried out. Many of these are still in progress and will continue in the future.

1. Cooperation

One of the additional objectives was to establish contacts and collaborative arrangements with other institutions and scientists.

a) Local

Besides other personnel of CATIE several investigators and students of the University of Costa Rica (UCR) and the Technological Institute were involved in cooperative projects in various areas, such as organoleptic evaluation of fruit quality of the tolerant varieties compared to the usual susceptible one, and field evaluations of infection rates in comparative experiments.

The Costa Rican Ministry of Agriculture cooperated in two field experiments, testing the growth rates of *in vitro* plants as compared to normally propagated plants, fruit characteristics and resistance evaluation.

Similarly ASBANA (Asociación Bananera Nacional) permitted use of their land to install several experiments for evaluation of different parameters.

Exchange of several materials was carried out with the Standard Fruit Company which maintains a large tissue culture laboratory in the southwestern part of Costa Rica. There exist plans to supply in the near future tissue culture plants of 'Pelipita' to the company for field evaluation in Ecuador.

b) Regional

Close cooperation was established with the tissue culture laboratory of IDIAP (Instituto de Investigaciones Agropecuárias de Panamá), headed by M.Sc. S. Pons.

Several mutual visits took place in order to discuss problems regarding both projects. A complete copy of the collections of germplasm of CATIE was

made available to Mrs. Pons, the same as larger quantities of certain varieties. An assistant received specialized training in CATIE's laboratory on the anatomy of *Musa* flowers, fertilization and embryo development, using histological techniques.

Field experiments with tissue culture-grown plants were established in two locations in Panama, in cooperation with IDIAP, in order to evaluate the tolerant varieties Pelipita and Saba.

In Nicaragua joined field experimentation with the Ministry of Agriculture was initiated to test the two tolerant varieties, supplied as tissue culture plants. At the present preparations are being initiated to supply a large number of 'Pelipita' plantlets in order to introduce this variety and replace the usual susceptible one ('Horn plantain').

FHIA (Fundación Hondureña de Investigación Agrícola), formerly United Fruit Company, at La Lima, had supplied the initial cultures of 'Pelipita' and 'Saba'. Contacts were maintained continuously with this organization, resulting in further germplasm exchange, with CATIE personnel offering consultation services for their *Musa* tissue culture laboratory and methodology for *in vitro* germination of hybrid seeds.

During a consultation trip to Jamaica, in 1986, closer cooperation between CATIE, the tissue culture laboratory of the local Ministry of Agriculture, and the Jamaican Banana Board was established. At the present plans are being elaborated to make use of the off-types observed in the plantations of the Eastern Banana Estates, resulting from *in vitro* propagation of 'Grande Naine', in order to verify the genetic stability of such mutants. The advanced diploids and tetraploids produced by the Banana Board breeding program (Dr. R. Gonsalves) at Bodles will be evaluated by CATIE for resistance to Black Sigatoka and Moko.

A course on banana tissue culture was offered in the Dominican Republic by CATIE personnel.

Close cooperation regarding reference collection on *Musa* tissue culture has been in progress with the Documentation Center of the UPEB (Unión de

Países Exportadores de Banano) in Panama.

c) World wide

At the time of initiation of the work very few laboratories in the world were concerned with tissue culture of *Musa*, all working with the dessert banana.

One of the most important ones is the tissue culture laboratory at the State University of New York (SUNY) at Stony Brook, N.Y., headed by Dr. A.D. Krikorian.

Closer cooperative work was initiated by exchange of information on *Musa* tissue culture methods, literature, plant material, and visits for consultation. Materials that have been developed in the SUNY Laboratory were sent to CATIE for further development and field experimentation (work still in progress).

Close collaboration was also started with personnel of the plant pathology department of the University of Göttingen/Germany. As a result a doctorate student worked for close to two years on the pathological aspects of Black Sigatoka infection, specifically cultivation of the pathogen and *in vitro* testing methodology for resistance evaluation. Part of this work is still in progress in Göttingen with cultures initiated in CATIE.

Preliminary contacts have been established with EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) in Brazil, regarding exchange of plant material, training of personnel of EMBRAPA and testing their new varieties for resistance to Black Sigatoka.

Exchange of methodologies, literature and plant material has been initiated with IITA (International Institute of Tropical Agriculture) in Nigeria. This collaboration will be strengthened in the near future.

With the IAEA (International Atomic Energy Agency) in Viena exchange of information and plant material was initated. Dr. F. Novak of the Seibersdorf Laboratory (IAEA-FAO) visited CATIE several times. As a result a specialized FAO (Food and Agricultural Organization) team visited CATIE to make two video movies, one on banana cultivation in general and one on tissue culture of

Musa. These films will be distributed all over the world as visual aids for teaching. At the present preliminary discussions are on the way regarding establishment of a regional network on *Musa* tissue culture, with CATIE as coordinating institution, sponsered by FAO/IAEA. Plans are also in progress to send an assistant of CATIE to Viena in 1987 for special training in *Musa* mutation breeding.

Contacts have been established with Dr. O. Reuveni of the Agricultural Research Organization, the Volcani Center, in Israel, regarding closer future cooperation.

Plant material has been exchanged with the Faculteit der Landbouwwetenschappen of the Katholieke Universiteit, Leuven, Belgium. It is planned to have an assistant of CATIE sent to this faculty for training in tissue culture of *Musa* in 1987.

Similarly plant material has been exchanged, the same as literature and methodologies, with Dr. C. Teisson of the Laboratoire de Cultures des Tissus of GERDAT (Centre de Recherches de Montpellier) in Montpellier, France. Mutual visits permitted personal contacts and discussions on common problems of *Musa* tissue culture. It is planned to send in 1987 an assistant of CATIE to GERDAT for training in *Musa* tissue culture, specifically callus formation and cell culture.

Discussions are in progress with the director of INIBAP (International Network for Improvement of Banana and Plantain), Dr. E. De Langhe, in order to study the possibility of establishing a word-wide germplasm collection of *Musa in vitro* at CATIE.

With ICRO (International Cell Research Organization) of the UNESCO, Paris, an international course on Plant Tissue Culture Methods and their Applications in Agriculture was offered in CATIE in 1985.

Many other useful contacts have been established, principally as a result of attending the Word Congresses on Plant Cell and Tissue Culture in 1982 and 1986, as well as the active participation in the AID International Biotechnology Network, with seat in Fort Collins, Colorado.

2. Training

During the duration of the project, emphasis was placed on training personnel in tissue culture techniques in *Musa*.

a) In service training

I senior scientist, Dr. R. Jarret who was hired as a post doctorate fellow by the project, spent one year in the tissue culture laboratory of CATIE, specializing in *in vitro* techniques in *Musa*. After his resignation to accept a position in the US, Dr. Jarret continued to work with *Musa*.

. Several laboratory assistants received intensive training, so as to be able to carry out any phase of research in *Musa* tissue culture.

JORGE A. SANDOVAL F., (CATIE), who carried out most of the routine work and is dedicated full time to *Musa* tissue culture.

SEBASTIAN SALAZAR (CATIE).

ROBERTO FERNANDEZ (CATIE).

Additionally the following persons received in service training for different periods:

José Manuel Quiros	Linda Vista, S.A.	C.R.
Jorge Ramírez	American Flowers	C.R.
Cornelia Passberg Gaul	University of Göttingen	Germany
Hans Lenselink	Agricultural University of Wageningen, Holland	
Vilma Jiménez B.	University of Costa Rica, San José	
Mario Soto O.	CATIE.	
Ana. P. Rojas	University of Costa Rica, San José	
María I. Mora	Mexico	
Laura Paris M.	INIAP (Instituto de Investigaciones Agropecuarias) Guayaquil, Ecuador	
Franklin Rosales	FHIA (Fundación Hondureña de Investigación Agrícola) Honduras	
Rosario Zabaneh C.	CENTA (Centro de Tecnología Agrícola, El Salvador	

Oscar Rolando Argueta R.	FHIA (Fundación Hondureña de Investigación Agrícola) Honduras
Juan C. Sánchez V.	Escuela de Ciencias Ambientales, National University of Costa Rica, Heredia
Luz Marina Reyes C.	ICA (Instituto Colombia Agropecuario) Colombia

Several other people, including graduate students, spent short periods in the laboratory to observe the methodologies employed.

b) Courses

The most important course initiated in the laboratory in 1986 is the graduate course (M.Sc. level) on: Introduction to techniques of plant tissue culture.

This course was taken by 20 graduate students. It will be given annually in the future as part of the curriculum for the M.Sc. degree.

A special short course was offered in May 1985 in collaboration with ICRO (International Cell Research Organization) and the Kellogg Foundation. Several renowned authorities were invited as lecturers. In total 25 participants took this course, coming from many different parts of Latin America.

An intensive training course was offered in 1985 in the laboratory of IDIAP, Panama, with participation of the staff of the tissue culture laboratory and several students and professors of the University of Panama.

A short course on tissue culture techniques in *Musa* was dictated in the tissue culture laboratory of the Ministry of Agriculture of the Dominican Republic.

The senior scientist participated as lecturer in several short courses (regional) offered in CATIE, on germplasm preservation and two courses on the technology of tissue culture in coffee.

c) Students

Two undergraduate students of the University of Costa Rica did their research for their thesis in *Musa* tissue culture, the same as several graduate students. Some of this research is still in progress:

SANDOVAL, J. Estudios morfológico y anatómico de formaciones de yemas adventicias en el cultivo *in vitro* de *Musa* M.Sc. Thesis. CATIE.

MORA, I. Utilización del efecto osmótico e inhibidores del crecimiento en la conservación *in vitro* de germoplasma de *Musa*. M.Sc. Thesis. CATIE.

NUÑEZ, C. Determinación de la temperature de 'cero crecimiento' en *Musa* (AA, AAA, BB, AAB, ABB) bajo condiciones *in vitro*. Su importancia en la conservación de germoplasma. Undergraduate thesis, UCR-CATIE.

SANDOVAL, J. Determinación del tamaño adecuado del explante en cuatro cultivares de *Musa*. Undergraduate thesis, UCR-CATIE.

3. Participation in congresses

The personnel of the tissue culture laboratory participated actively in several congresses and symposium, national and international.

The first congress on tissue culture attended was the Fifth World Congress of Plant Tissue and Cell Culture, in Tokyo, Japan in 1982. This participation proved of utmost importance to the research, since many important contacts could be made, involving later on cooperative arrangements.

Other important events in which papers regarding results of the research were presented, were:

1. 1985. First Annual Conference of the International Plant Biotechnology Network, Fort Collins. Colorado.
2. 1985. Simposio: Biotecnología en las Américas; Aplicaciones en la Agricultura Tropical. San José, Costa Rica. CONICIT-INTERCIENCIA.
3. 1985. Sétima Reunión de la Asociación para la Cooperación en Investigaciones Bananeras en el Caribe y en América Tropical (ACORBAT). San José, Costa Rica.
4. 1986. Reunión Regional INIBAP (International Network for Improvement of Bananas and Plantains) CATIE. Costa Rica.

5. 1986. IV Congreso Internacional sobre Agro-fisiología de Banano y Plátano ASBANA. San José. Costa Rica.

6. 1986. VIth International Congress of Plant Tissue and Cell Culture, Minneapolis, Minnesota.

7. 1986. II Congreso Panamericano de Ingeniería Agronómica y de Alimentos. Guatemala.

8. 1986. FIAGA 86. Feria Internacional de Agricultura, Ganadería, Pesca y Alimentación. Atlapa-Panama.

4. Publications

The following publications are based on the results obtainded during the research:

1. NUÑEZ, C.; SANDOVAL, T.; MULLER, L. 1986. Determinación de la temperatura de 'cero crecimiento' en *Musa* (AA, AAA, BB, AAB, ABB) bajo condiciones *in vitro* y su importancia en la conservación de germoplasma In: Resumenes del IV Congreso Internacional sobre Agro-Fisiología de Banano y Plátano. ASBANA. San José, Costa Rica. s.p.

2. MORA, I.; SANDOVAL, J.; MULLER, L. 1986. Utilización del efecto osmótico en la conservación *in vitro* de *Musa*. In: Resumenes del IV Congreso Internacional sobre Agro-fisiología de Banano y Plátano. ASBANA. San José, Costa Rica. s.p.

3. MULLER, L.; SANDOVAL, J. 1986. *In vitro* germplasm conservation of *Musa* spp. In: Abstracts, VIth International Congress of Plant Tissue and Cell Culture. University of Minnesota. Minneapolis, Minnesota. p. 426.

4. MULLER, L. 1986. Clonal propagation of plantains *Musa* spp. In: The International Plant Biotechnology Network, Tissue Culture for Crops Project. Newsletter N°6. p. 11.

5. SANDOVAL, J. 1985. Determinación del tamaño adecuado de explante para la propagación *in vitro* en cuatro cultivares de *Musa* spp. Thesis Ing.Agr. University of Costa Rica. 50 p.

6. SANDOVAL, J. 1985. Micropropagación de musáceas. ASBANA (Costa Rica) 9(24): 21-23.

7. SANDOVAL, J.; MULLER, L. 1985. Influencia del tamaño de explante en la propagación *in vitro* de cuatro cultivares de *Musa*. In: Resumenes de la VII Reunión de la Asociación para la Cooperación en la Investigación Bananera del Caribe y la América Tropical (ACORBAT). San José, Costa Rica. p. 35.

8. MULLER, L. AND KRIKORIAN, A.D. 1985. Glosario de los términos más frecuentemente empleados en al cultivo de tejidos. Preliminary edition, CATIE, Turrialba, mimeographed 1985.

9. JARRET, R.L.; RODRIGUEZ, W. AND FERNANDEZ, R. 1984. Biotechnology in Costa Rica: Tissue culture propagation and dissemination of disease resistant plantain germplasm. CATIE, Turrialba. 20 p. Mimeographed.

10. JARRET, R.L.; RODRIGUEZ, W. AND FERNANDEZ, R. 1985. Evaluation, culture, propagation and dissemination of 'Saba' and 'Pelipita' plantains in Costa Rica. Scientia Hort. 25: 137-147.

5. Information Center

There exists a much felt need for the availability of literature on tissue culture. Since only in recent years a few specific journals were started, most of the literature is widely spread in many different journals the world over, sometimes in such where one would not even expect it. It is therefore very cumbersome to try to get references on a certain topic in this field, especially since libraries in Latin American countries are usually not well equipped and prices of journals and books lately increase very rapidly, causing problems in financing specific collections.

Therefore the dicision was taken, when the project was initiated, to collect as many references and literature on plant tissue culture as possible. No publications on plantain tissue culture could be located, and in the case of bananas, only very few had been published. In order to develop in plant tissue culture new methodologies, it is essential to rely to some extent on previous work carried out by renown specialists.

When the project was started there existed a collection of some 2000 references on all aspects of plant protoplast, cell, tissue and organ culture. With the help of a part-time documentalist, this collection was increased to almost 18 000 references, which constitute about 80% of all literature available on this subject. These references exist at the present in form of a card index arranged by authors, and part of it is already organized by key words.

It is expected, within a reasonable time, to computerize the information in order to be able to prepare specific bibliographies on request and also later on publish the information in form of a book.

6. Laboratory facilities

With the funds available from the project it was possible, besides getting all the reagents, glassware, etc. needed for an efficient operation, to purchase several important items, such as a small automatic autoclave, double-still, laminar flow hood, inverted microscope, electronic balances, three lighted incubators, etc. All these apparatus helped to improve considerably the efficiency of operation of the laboratory by complementing existing equipment and that purchased by other funding sources. After installation in a new laboratory building, especially designed for this kind of work, a very modern plant tissue culture facility could be established (one of the finest in Latin America) to serve as a basis and center for future work in this field of biotechnology on a regional or even continental basis.

C. CONCLUSIONS AND RECOMMENDATIONS

When considering the use of tissue culture for improving yield potential of plantains, after the official termination of the research project, it becomes evident from the results obtained, that this is perfectly possible. After developing a method for fast clonal propagation, thousands of plantlets of 'Saba' and 'Pelipita' were produced in a relatively short time, a fact impossible by conventional means. These plantain varieties contribute in two ways to improve very much yield potential: a)Especially 'Pelipita' is much higher yielding than the conventional 'Horn plantain' and also 'French plantain', b)Because they are tolerant to Black Sigatoka, there occurs no yield reduction in fungus infected areas. Since they are also tolerant or resistant to other pests and pathogens, a high yield potential is garanteed in most areas.

The very ample distribution of the tissue culture propagated tolerant varieties in areas where the usual commercial varieties were wiped out by Black Sigatoka, helped to convince people of their value. The ever increasing requests for very large numbers of plantlets indicate clearly, especially since organoleptic evaluations have shown their palatability, that these new varieties are gaining ground fast and that people are gradually changing their prejudice against varieties with thick, short fingers. Another contributing fact is the price. In Costa Rica, during the last years, prices for 'Horn plantains' have risen some 1000%, causing a change from a staple food item to a luxury item.

It is expected that the distribution of the two tolerant varieties will be accelerated by the fact that several other laboratories in the region have started to propagate them, following the ontlines elaborated by CATIE and the *in vitro* materials placed at their disposition.

After installation of new growth chambers in the near future, the laboratory of CATIE will be able to comply at least with part of the large orders. This will be important since so far no larger closed areas have been planted with tissue culture produced plants, making it therefore important to

investigate the rate of possible somaclonal variations in mass-propagated plantains plants.

When considering the two original specific objectives of the research it can be said that the first one has been fully accomplished. The methodology elaborated is simple and has been amply diffused by publications, personal contacts, and consultation. The existing plans for a Regional Network on *Musa* tissue culture, sponsered by FAO-IAEA, with CATIE's laboratory serving as coordinating agent, should prove of utmost importance.

The research activities permitted to establish the bases for the second specific objective. Actually somaclonal variation in *Musa* is rather complicated. So far it was not yet possible to understand the cause of it, so as to be able to make use of it and to direct research towards obtaining a higher rate. From tissue culture work in banana it is evident that rates may vary from below 1% to above 40%, visual symptoms only, in the same variety. Thus, to some extent, the high rates sometimes encountered may have their cause in the laboratory procedures employed.

The complete realization of the second specific objective was handicapped because of several reasons. In the first place protoplast and cell cultures in plantains are difficult to establish and no regeneration is possible with the present knowledge on requirements for such. Thus only adventitious buds can be used. The most important handicap, however was the fact that neither land nor funding could be found to establish large-scale plantings of 'Horn plantain' with tissue culture plants. Due to Black Sigatoka, no new plantations are being installed, on the contrary, old ones are either abandoned or eliminated. For new plantings 'Pelipita' is prefered due to the tolerance.

However, at the present discussions with cooperatives and companies in Costa Rica are in progress to find ways for planting larger numbers of 'Horn plantain', using tissue culture grown plants, in part treated with mutagens (EMS, MMS) or high hormone doses (BAP, 2,4-D), in order to increase chances of variants. Initially emphasis will have to be on perfecting soon the *in vitro* screening methods. This would permit to reduce greatly the number

of plants taken to the field. Tentative contacts have been made with several investigators and institutions in order to establish some type of collaboration.

Although no resistant or tolerant mutant of 'Horn plantain' or 'French plantain' was produced, the work on somaclonal variation will be continued and in fact very much strengthened in the near future, including also dessert bananas. Thus there will be continuation of the work initiated by the grant, building on the bases worked out during the research.

Another important achievement is the basic knowledge gained regarding *in vitro* germplasm conservation of *Musa*. Due to the fast propagation of so many serious diseases and pests, live collections are being seriously threatened, endangering conventional breeding work in progress. However, much research will be needed to guarantee that *in vitro* preservation is feasable without endangering the genetic stability of the stored materials by somaclonal variation. The International Network for improvement of Bananas and plantains (INIBAP) seriously considers to concentrate valuable germplasm material from all parts of the world in an *in vitro* collection to be located in CATIE.

Another very important aspect as a result of the grant is the laboratory as such. The funds permitted to purchase much needed equipment to make the installations efficient and permit research in several areas. These installations have also served already in the past to train people, by providing in service training and formal graduate training. In the future the education aspects will be stressed even more by offering annually special intensive courses of 3 months duration in cooperation with AID-IPNet.

In conclusion it can be stated that the overall objective has been achieved. There will be continuation of the research initiated by the grant, with emphasis on solving rapidly the problems that have arisen which, at the same time would complete the initial objectives fully.

It can be said that the first additional objectives, to establish collaborative arrangements with other scientists and institutions working in

tissue culture of *Musa* has been accomplished. Especially during international congresses and meetings (IAPTC, IPNet, ACORBAT) it was possible to become acquainted and also discuss common problems with practically all persons working world-wide in *Musa* tissue culture.

The second additional objective, the reference collection, has been improved, containing now about 80% of the published literature on plant protoplast, cell, tissue, and organ culture. Complete computarization should permit soon to offer, especially for laboratories in third world countries, which have problems in obtaining specific literature, bibliographies on all subjects. In the past many investigators outside CATIE, even from other countries, have come to use the facilities the reference and photocopy collections offer.

APPENDIX

CATIE-LABORATORIO CULTIVO DE TEJIDOS. TURRIALBA, COSTA RICA
FECHA: 12/11/86
DESCRIPTOR: CULTIVO IN VITRO DE MUSA.

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*No disponibles en la Biblioteca del Lab. Cultivo de Tejidos. (CATIE).