The Improvement and Testing of *Musa*: a Global Partnership

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INIBAP's Mandate

The International Network for the Improvement of Banana and Plantain (INIBAP) was established in 1984 and has its headquarters in Montpellier, France. INIBAP is a nonprofit organization whose aim is to increase the production of banana and plantain on smallholdings by:

- initiating, encouraging, supporting, conducting, and coordinating research aimed at improving the production of banana and plantain;
- strengthening regional and national programs concerned with improved and disease-free banana and plantain genetic material;
- facilitating the interchange of healthy germplasm and assisting in the establishment and analysis of regional and global trials of new and improved cultivars;
- promoting the gathering and exchange of documentation and information; and
- supporting the training of research workers and technicians.

Planning for the creation of INIBAP began in 1981 in Ibadan with a resolution passed at a conference of the International Association for Research on Plantain and Bananas. In May 1994, INIBAP was brought under the governance and administration of the International Plant Genetic Resources Institute (IPGRI) to enhance opportunities for serving the interest of small-scale banana and plantain producers.

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***Cover illustration*:** Symptoms of black leaf streak/black Sigatoka disease on a leaf of a highly susceptible 'Cavendish' cultivar growing on Aitutaki Island, Cook Islands (photo: DR Jones, INIBAP).

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Proceedings of the First Global Conference of
the International *Musa* Testing Program
held at FHIA, Honduras
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Edited by
DR Jones

INTERNATIONAL NETWORK FOR THE
IMPROVEMENT OF BANANA AND PLANTAIN

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The opinions in the publication are those of the authors and not necessarily those of INIBAP.

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Editorial note

Some references have been submitted without complete publishing data. They may thus lack the full names of journals and/or the place of publication and the publisher. Should readers have difficulty in identifying particular references, staff at INIBAP will be glad to assist.

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Introduction

INIBAP and the International *Musa* Testing Program

N Mateo

The Global *Musa* Evaluation Program, supported by UNDP and normally referred to as the International *Musa* Testing Program (IMTP) is a truly worldwide, collaborative endeavor. The purpose of this initiative is defined as follows:

"The primary objective of IMTP is to effect coordinated development and evaluation of new germplasm in an effort to produce resistant Musa *cultivars which meet local requirements and with which small-scale farmers can replace existing banana and plantain cultivars susceptible to Sigatoka diseases and Fusarium wilt. An objective of IMTP is to increase the capacity of national organizations to carry out appropriate research on local-consumption banana and plantain and ultimately to embark on locally adapted programs for genetic improvement of banana and plantain."*

The results of the initial phase of IMTP, and the proposed organization and scope of Phase II, will be discussed during this Conference. It is, however, relevant to highlight some essential characteristics of IMTP. First, IMTP was a direct result of requests from national programs (NARS) to have disease-resistant and highly productive *Musa* germplasm made available. Secondly, the excellent opportunity of using FHIA hybrids to fulfill this need. Thirdly, UNDP's vision and resolution to support this global collaborative endeavor.

In the initial phase of IMTP, only the FHIA breeding program was able to contribute advanced germplasm for global evaluation for resistance to black leaf streak/black Sigatoka disease. The results of this initial phase are very impressive: three highly productive FHIA hybrids resistant to black leaf streak/black Sigatoka have been released and are currently available worldwide. Since their release in October 1993, INIBAP has distributed these hybrids to over 30 countries.

On the basis of the global availability of FHIA hybrids, as well as additional releases of improved germplasm from EMBRAPA in Brazil, IITA in West Africa, BANBOARD in Jamaica, TBRI in Taiwan, and TNAU in India, it is possible to state that the common assertion **"all cultivated bananas and plantains come from natural germplasm**" is no longer valid. This indeed is a milestone in the history of *Musa* research.

During the next phase of the IMTP, a more ambitious and exciting approach is envisaged. Advanced breeding materials from four research organizations will be tested worldwide, not only against their reaction to black leaf streak/black Sigatoka disease, but also their reaction to Sigatoka/yellow Sigatoka disease and Fusarium wilt. The willingness

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of national, regional, and international institutions to cover the expenses related to the testing sites clearly indicates that enhanced germplasm is in considerable demand.

IMTP plays a key role in the global need for enhanced *Musa* germplasm. The need (requests from NARS), the opportunities (development and availability of good germplasm) and the vision and willingness to carry out the testing, are all prerequisites for the success of this program.

INIBAP strongly believes that once other improved and natural germplasm is made available to the *Musa* community, there will be a growing need to encourage further testing through national evaluation programs (NEPs). The concept is simple: given the diversity of ecological and socioeconomic circumstances in *Musa*-producing regions, the development of only one or two "super bananas" or "super plantains" does not make much sense. Small-scale growers require a "basketfull of alternatives (a wide variety of first-class, resistant germplasm)" which they can evaluate on their farms and from which they can make appropriate selections. The adoption of suitable material to their particular needs will then follow. In this context, the more material released from the IMTP trials, the more options will be available to the NARS and farmers.

IMTP fits very well into INIBAP's mandate and activities as described in the Medium Term Plan, 1994-98, which is summarized in Appendix l below.

In this Appendix, the specific areas where IMTP fits into INIBAP's Medium Term Plan (MTP) are indicated with an asterisk (*). In practice, this means that IMTP is more than just an evaluation of germplasm and includes other significant elements. Details of these elements are provided in the paper by DR Jones on IMTP Phase II.

INIBAP looks forward to close and enhanced partnership with NARS and regional and international institutes to make IMTP the vehicle by which farmers may have access to a wide variety of disease-resistant and productive banana and plantain germplasm.

Appendix 1: INIBAP's Program Components

The overall program structure of INIBAP can be thought of as a simple matrix of thematic and regional elements. The Germplasm and InfoDoc/Communications Programs are organized globally, but include key activities in the regions. Other regional research components, besides those that interact closely with the Global Program, are handled and monitored by the RACs (Regional Advisory Committees) which are coordinated by an INIBAP staff member.

Germplasm program

The objective of this program is to ensure that the world has an effective system of biological research on *Musa* capable of meeting current and new challenges in future years. It can be conveniently divided into the following categories.

Musa germplasm conservation and exchange

The agreements reached at the International Workshop on Conservation and Documentation of *Musa* Germplasm, which was organized jointly by INIBAP and IPGRI

in 1989, have set an international and national framework of *Musa* germplasm conservation efforts. The workshop identified a critical role to be played by INIBAP, which has worked on the implementation of the workshop recommendation. Within the period of this MTP, the following activities will be carried out in respect of further implementation of the *Musa* Germplasm Conservation Network, although some modifications are expected as a result of future interaction with key partners, as follows.

Collecting*. Three major missions are projected over the MTP: Viet Nam, Indonesia, and the islands off the coast of East Africa.

Characterization. Collections of East African highland banana cultivars in Uganda and Papua New Guinea cultivars in Queensland, Australia, will be fully characterized. Projects will also be initiated in other collections, such as the field gene banks in Viet Nam.

Conservation. Approximately 1053 accessions are maintained in vitro at the INIBAP Transit Center (142 wild species, 225 diploid cultivars, 562 triploid cultivars, 54 tetraploid, 4 enset, and 103 unclassified). Medium-term conservation systems now in place may be converted to cryopreservation before the end of the MTP. INIBAP will continue to implement the concept of the *Musa* Germplasm Conservation Network agreed in 1989 by curators of leading collections. This includes the standardization and categorizing of germplasm information contained in about 86 in-vivo and in-vitro collections around the world using the MGIS (*Musa* Germplasm Information System) initiative supported by IDRC. New accessions will be placed in the in-vitro base collection at the Transit Center and most of them should be fully identified, characterized, and virus-indexed.

Duplication. The INIBAP Transit Center (ITC) in-vitro collection is to be fully duplicated for safety reasons. The process has started with one-third of accessions going to Asia. The rest will be going to Africa and Latin America. Cryopreservation, if feasible, will also be implemented for safe duplication in partner institutions.

Virus indexing*. It is expected that, during the MTP, the INIBAP Virus Indexing Centers at CIRAD, Montpellier, France, and QDPI, Australia, will run at their maximum capacity of about 115 accessions per annum. A third center at TBRI, Taiwan will be constituted during the MTP period.

Safe exchange of germplasm*. Every year, over 300 accessions are distributed worldwide. Demand for germplasm is expected to increase as more advanced hybrids become available from breeding programs. INIBAP follows, in essence, the FAO/IBPGR Guidelines for the Safe International Exchange of *Musa* Germplasm. These guidelines will be updated during the period of the MTP.

Breeding and the Breeders' Network

This major task will enhance and promote collaboration and a division of labor among the world's key breeding programs. The research requirements of the most important *Musa* types cannot be met by a single breeding program; therefore, collaborative agreements and complementation are essential. INIBAP expects to raise complementary funding to support more interaction between breeding programs. The first meeting of the Network

will take place in May 1994[1], and, therefore, it is not possible to specify in detail INIBAP's roles and contributions, nor those of the potential members of the Network.

Testing and evaluation of germplasm*

This activity is mostly carried out through the IMTP (International *Musa* Testing Program) supported by UNDP. In the current second phase of this project, four breeding programs have provided advanced germplasm to INIBAP to be tested in approximately 30 sites around the world for black leaf streak/black Sigatoka, Sigatoka/yellow Sigatoka, and Fusarium wilt resistance.

National Evaluation Programs (NEPs)*

Once natural and improved germplasm with desirable traits become more widely available, there will be a need for further adaptive research and evaluation by NARS. INIBAP recognizes that, even though it will not be able to support this effort from core resources, NEPs are an essential component of the research process that requires encouragement and coordination. NEPs conducted by NARS will be the key to adoption by farmers of alternative planting materials that may fit their particular ecological and socioeconomic circumstances. In this context, availability of in-vitro facilities, planting material production mechanisms, and the role of the private sector, will likely become important issues.

Strategic research

INIBAP will continue to be involved in the following broad areas of research, to a great extent through the research program at KUL, Belgium, but also with various other partners:

- Transformation of banana and plantain
- Cell suspension technology
- Cryopreservation
- Somaclonal variation
- Banana genome mapping
- Pathogen variability*
- Virus diagnosis*
- Laboratory/glasshouse/growth-cabinet disease screening methodologies*
- Elucidation of banana bunchy top disease agents.

Intelligence gathering (strategic information)

INIBAP, jointly with key partners and clients, will monitor worldwide the severity, evolution, and impact of the major *Musa* diseases and pests. This information is essential for priority setting and the development of appropriate research strategies.

InfoDoc/Communications Program

The overall objective of this Program is to make quality data available to *Musa* researchers in every part of the world, using the latest advances in data storage and

[1]See: INIBAP. 1994. Banana and Plantain Breeding: Priorities and Strategies: proceedings of the first meeting of the *Musa* Breeders' Network held in La Lima, Honduras, 2-3 May 1994. Montpellier, France: INIBAP. 55 pp.

transmission. It, too, will have a strong regional dimension, including the development of regional databases. *Musa* research is still a relatively narrow field, with a limited number of practitioners and, therefore, the INIBAP InfoDoc/Communications Program should be able to function as the institutional memory of the global research effort.

The specific components of the Program are the following:

1. Development and updating of trilingual *Musa* databases and related tools.
2. Development and updating of production and consumption figures by key *Musa* types.
3. Production of database outputs, such as directories, specialized bibliographies, etc.
4. Operation of InfoDoc question-and-answer services.
5. Training on the InfoDoc system.
6. Editing and publication of specialized *Musa* publications, including *Musarama* and INFO*MUSA*.
7. Setting-up/follow-up of the regionalization process.
8. Preparation and editing of project proposals and reports.
9. Conduct public awareness and fund-raising campaigns jointly with IPGRI.
10. Organization of scientific and technical seminars/workshops.

It is anticipated that the Program will also have a very active role in the MGIS initiative.

Regional research and training*

In all regions, a few strong *Musa* research programs (whether national, regional, or international) coexist with relatively weak programs which are in the earlier stages of development. INIBAP's prime concern will be for the emerging new programs, but it is able to offer services of interest even to the strongest program. INIBAP is systematically trying to bring together all research programs in a given region—national, regional, and international—with a view to fostering concerted efforts and collaborative activities. Since it does not have its own physical research facilities, INIBAP can be seen by all programs as a catalyst, not a competitor.

The basic objectives of the regional activities, in line with the operational goals for INIBAP, will be the following:

a) to provide the products and services of the Germplasm and Info/Doc Communications Programs and enhance their impact at the national level;

b) to promote research and training efforts to deal with region-specific problems and opportunities; and

c) to strengthen the ability of NARS to conduct research on bananas and plantains as well as *Musa* germplasm conservation efforts.

This set of recommended activities results from inputs from RACs, NARS, and other INIBAP partners.

International *Musa* Testing Program Phase I

DR Jones

Introduction

In 1989, UNDP funded an International *Musa* Testing Program (IMTP) with the overall goal of evaluating new banana and plantain hybrids for resistance to black leaf streak/black Sigatoka (*Mycosphaerella fijiensis)* and to help stimulate the breeding and identification of black leaf streak/black Sigatoka-resistant germplasm.

IMTP began in 1990, and cooperative partnerships were established with NARSs and IARCs to evaluate germplasm under different ecological conditions and in areas where pathogenic variants of *M. fijiensis* may exist. All germplasm originated from the FHIA breeding program in Honduras.

By the end of 1991, seven FHIA hybrids (Table 1) plus a range of wild species and cultivars chosen as reaction standards (reference clones) were being tested around the world. During 1992, data on hybrid reaction to black leaf streak/black Sigatoka were collected and collated for analysis. INIBAP organized an experts' meeting in Buga, Colombia, from 28 September to 2 October 1992, to discuss the information from the six selection sites in Honduras (FHIA), Costa Rica (CORBANA), Colombia (ICA), Nigeria (IITA), Cameroon (CRBP) and Burundi (IRAZ). The results were compiled and form the basis of a document that was presented to an intergovernment delegation in Honduras early in 1993. These results are described in this report, together with other useful information that was obtained during the course of the project.

Technical Guidelines

The trial consisted of planting, under specific standardized conditions, 10 plants of each reference cultivar and 10 plants of each hybrid to be tested (Table 1). The reference clones were selected by international *Musa* scientists at a workshop on Sigatoka diseases held at San José, Costa Rica, in April 1989 (Fullerton, Stover 1990). Their perceived reactions are indicated on Table 1.

In the trial, each line of the reference clones and/or hybrids was separated by a line of Grande Naine (or another susceptible cultivar in the Cavendish subgroup) to be sure

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Table 1. Germplasm included in IMTP Phase I.

FHIA hybrid	Genome	Related type	Parentage
FHIA-01 (SH 3481)	AAAB	Pome	Prata Anã (dwarf Prata) x SH 3142
FHIA-02 (SH 3486)	AAAA	Cavendish	Williams (Cavendish) x SH 3393
FHIA-03 (SH 3565)	AABB	ABB group	SH 3386[1] x SH 3320
FHIA-04 (SH 3653)	AAAB	Plantain	AVP 67 (French plantain) x SH 3437
FHIA-05 (SH 3706)	AAAB	Plantain	AVP 67 (French plantain) x SH 3437
FHIA-06 (SH 3583)	AAAB	Maia Maoli	Maqueño x SH 3437
FHIA-07 (SH 3584)	AAAB	Maia Maoli	Maqueño x SH 3437

Reference clones	Genome	Type	Perceived reaction to BLS/BS[2]
Tuu Gia	AA	edible cultivar	HR
Musa acuminata ssp. *burmannicoides* (Calcutta IR 124)	AA	wild species	HR
Musa acuminata ssp. *malaccensis* (Pahang IR 296)	AA	wild species	HR
Pisang Lilin	AA	edible cultivar	HR
Pisang Berlin	AA	edible cultivar	R
Pisang Mas	AA	edible cultivar	R
Musa balbisiana (Tani)	BB	wild species	R
SF 215/NBA 14	AA	edible cultivar	S
Niyarma Yik	AA	edible cultivar	HS

[1]SH 3386 = (Gaddatu x BB) x SH 2471.
[2]HR: Highly Resistant.R: Resistant.S: Susceptible.HS: Highly Susceptible. BLS/BS = black leaf streak/black Sigatoka.

that the inoculum of *M. fijiensis* was the same for all the plants. The experiment also had guard rows of Grande Naine around the outside (Fig.1). All plants of reference clones and hybrids were observed for reaction to *M. fijiensis* from 3 months after planting to shooting time (emergence of bunch stalk).

Each week, all the plants with an unfolded leaf at Brun's stage B (see Annex 1) were marked. Each leaf was given a number and the date noted. Infection was assumed to have occurred on this leaf at this time. The number of days elapsing between stage B and the appearance of initial fleck symptoms or Fouré's stage 1 (Fouré 1986) was noted and called the incubation time (IT). The number of days elapsing between the appearance of initial fleck symptoms and mature lesions or Fouré's stage 6 (Fouré 1986) was noted and called the evolution time (ET). The number of days elapsing between the appearance of the unfolded leaf at Brun's stage B and the appearance of mature lesions was noted and called the disease development time (DDT).

x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	5	5	5	5	5	5	5	5	5	5	x	16	16	16	16	16	16	16	16	16	16	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	12	12	12	12	12	12	12	12	12	12	x	9	9	9	9	9	9	9	9	9	9	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	3	3	3	3	3	3	3	3	3	3	x	13	13	13	13	13	13	13	13	13	13	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	10	10	10	10	10	10	10	10	10	10	x	1	1	1	1	1	1	1	1	1	1	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	14	14	14	14	14	14	14	14	14	14	x	11	11	11	11	11	11	11	11	11	11	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	4	4	4	4	4	4	4	4	4	4	x	2	2	2	2	2	2	2	2	2	2	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	8	8	8	8	8	8	8	8	8	8	x	15	15	15	15	15	15	15	15	15	15	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
x	6	6	6	6	6	6	6	6	6	6	x	7	7	7	7	7	7	7	7	7	7	x
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Key:

x = guard rows (plants of a Cavendish cultivar used as inoculum generators)
1 = FHIA-01
2 = FHIA-02
3 = FHIA-03
4 = FHIA-04
5 = FHIA-05
6 = FHIA-06
7 = FHIA-07
8 = Tuu Gia
9 = *Musa acuminata* ssp. *burmannicoides* (Calcutta IR 124)
10 = *Musa acuminata* ssp. *malaccensis* (Pahang IR 296)
11 = Pisang Lilin
12 = Pisang Berlin
13 = Pisang Mas
14 = *Musa balbisiana* (Tani)
15 = SF 215/NBA 14
16 = Niyarma Yik

Figure 1. Trial showing position of test hybrids/reference clones and guard rows (inoculum generators).

The average IT, ET, and DDT was calculated for all 10 plants/reference clones and hybrids. At shooting time and harvesting time, the youngest leaf with mature lesions or spots (YLS) was also noted. It was suggested that basic agronomic data of hybrids could also be collected (Table 2).

Results

The detailed results of IMTP Phase I have been compiled in a final project document (Jones, Tezenas du Montcel 1994) and will not be repeated here. However, average DDTs

Table 2. Agronomic data that could be collected.

	3 months	6 months	9 months	12 months	18 months
Height (cm)	_____	_____	_____	_____	_____
Girth (cm) at 100 cm above ground		_____	_____	_____	_____
Suckering habit:	Free _____	Intermediate _____		Inhibited _____	
Production parameters (days):		P - S _____	S - H _____	P - H _____	
AWB (kg) _____	N° of hands _____		N° of fingers _____		
Length of finger (cm) _____	Weight of finger (g) _____		Finger _____		
Taste of the fruit:	Sweet _____	Acid _____	Stodgy to be cooked _____		
Fertility: seeds	Yes _____	No _____			
pollen	Yes _____	No _____			

Key: P = planting. S = shooting (bunch emergence). H = harvest.

are presented for the reference clones (Table 3) and the FHIA hybrids (Table 4). The final results showing the reactions of reference clones and FHIA hybrids are shown in Table 5.

Statistical Analysis

Drs Ahmad Rafie (FHIA) and Xavier Perrier (CIRAD-FLHOR) undertook the statistical analysis of all the data supplied by officers-in-charge of trial sites in all countries. This analysis showed that there was significant interaction between the genotypes and countries where they were evaluated. It was suggested that this difference in response was caused either by climatic differences or differences due to pathogenic variants of *M. fijiensis*. The former is considered more likely.

FHIA-01 and FHIA-02 were always superior in their performance against black leaf streak/black Sigatoka in comparison with other hybrids. One major conclusion of the analysis was that only the DDT need be measured to evaluate the reaction of germplasm to black leaf streak/black Sigatoka in future trials.

Table 3. The average disease development time (DDT) in days of the reference clones.

	Tuu Gia	*M.a. malaccensis*	*M.a. burmanni-coides*	*M. balbi-siana*	Pisang Lilin	SF 215	Pisang Mas	Pisang Berlin	Niyarma Yik
Colombia	125	104	-	88	87	77	72	72	59
Honduras	-	-	-	-	-	70	63	81	48
Costa Rica	68	59	68	57	42	32	33	36	30
Cameroon	-	-	-	-	-	-	59	68	37
Nigeria	-	69	-	53	97	43	43	64	36
Burundi	-	-	-	136	-	77	76	-	78

Table 4. The average disease development time (DDT) in days of the FHIA hybrids.

	FHIA-01	FHIA-02	FHIA-03	FHIA-04	FHIA-05	FHIA-06	FHIA-07
Colombia	122	110	80	89	75	84	76
Honduras	81	90	68	60	49	51	53
Costa Rica	65	63	43	46	32	40	33
Cameroon	95	95	67	66	45	76	48
Nigeria	78	79	52	56	40	52	39
Burundi	134	148	105	-	94	-	-

Table 5. Combined results of IMTP Phase I.

FHIA hybrid	Main use	Reaction to BLS/BS[2]
FHIA-01[1]	Dessert	HR
FHIA-02[1]	Dessert	HR
FHIA-03[1]	Cooking	R
FHIA-04	Cooking	R
FHIA-05	Cooking	HS
FHIA-06	Cooking	S
FHIA-07	Cooking	HS

Reference clone	Reaction to BLS/BS[2]
Tuu Gia	ER
Musa acuminata ssp. *burmannicoides*[3]	ER
Musa acuminata ssp. *malaccensis*	ER
Pisang Lilin[3]	HR
Pisang Berlin[3]	R
Pisang Mas	S
Musa balbisiana	R
SF 215/NBA 14	S
Niyarma Yik[3]	HS

[1]Recommended for global distribution.
[2]ER: Extremely Resistant (hypersensitive-like response). HR: Highly Resistant.
R: Resistant. S: Susceptible. HS: Highly Susceptible. BLS/BS = black leaf streak/black Sigatoka.
[3]Selected as standards for future IMTP trials.

Discussion and Conclusions

Host reaction

* The DDT is a good guide to the reaction of *Musa* germplasm to *M. fijiensis*. The longer the DDT, the more resistant is the accession.

* The DDT varied according to the selection site. Countries in order of ranking from the longest average DDT on all hybrids to the shortest were as follows: Burundi > Colombia > Cameroon > Honduras > Nigeria > Costa Rica.

These differences in DDT could be explained by environmental factors which influence inoculum pressure and disease development. Costa Rica seems the most favorable for *M. fijiensis.* Cooler temperatures at the high-altitude sites in Burundi and Colombia slow pathogen growth and sporulation.

* Two distinct types of reaction may occur in *Musa* accessions challenged by *M. fijiensis*:

– A hypersensitive reaction or extremely resistant response (ER: the disease is blocked at an early stage and mature lesions do not develop).

– A lesion development reaction (LDR: the symptoms of the disease evolve from small chlorotic/yellow spots to mature necrotic lesions). Clones exhibiting this type of reaction can vary in their response to black leaf streak/black Sigatoka from highly susceptible (very short DDT) to highly resistant (very long DDT).

Reference clones

* *M. acuminata* spp. *burmannicoides* (Calcutta IR 124) reacted in an ER manner to *M. fijiensis* in Colombia, Honduras, Nigeria, and Cameroon, but in an LDR manner in Costa Rica. Tuu Gia reacted in an ER manner in Honduras, Cameroon, Nigeria, and Burundi, but in an LDR manner in Colombia and Costa Rica. *M. a.* ssp. *malaccensis* (IR 296 Pahang) reacted in an ER manner in Cameroon and Burundi, but in an LDR manner in Colombia, Costa Rica, and Nigeria. All the other reference clones had an LDR type of response at all sites.

* The variation in reaction type of *M. a.* ssp. *burmannicoides*, Tuu Gia, and *M. a. malaccensis* between sites may reflect the effects of altitude, inoculum pressure, differing interpretations of host responses by observers, or all three.

– It is known that plantains and some FHIA hybrids become more susceptible to *M. musicola* at altitude. It is possible that some resistance mechanisms to *M. fijiensis* could similarly be affected.

– High inoculum pressures resulting from very favorable environmental conditions could lead to mass infections which allow lesion development in tissue which might otherwise restrict pathogen growth.

– The ER response in certain clones results in necrotic spots larger than those normally associated with a hypersensitive reaction. This may be due to the level of pathogen development before host cell death. It is possible that sporulation may occur in

the centers of some lesions formed in this way. This type of response could have been classified by some observers as a mature lesion.

* At the INIBAP workshop on Sigatoka diseases (Fullerton, Stover 1990), the reactions of reference clones to black leaf streak/black Sigatoka were tentatively defined (Table 1). Some of these reactions now have to be modified in light of the results of IMTP Phase I (Table 5). Four of the nine clones have been selected as suitable for representing a range of reactions to black leaf streak/black Sigatoka in IMTP Phase II.

FHIA hybrids

* All the FHIA hybrids had an LDR type of behavior. Among the seven FHIA hybrids evaluated, four of them had a good level of resistance before flowering to black leaf streak/black Sigatoka. They were: FHIA-01; FHIA-02; FHIA-03; FHIA-04.

These four hybrids proved to be resistant at five selection sites. However, FHIA-04, unlike FHIA-01, FHIA-02 or FHIA-03, lost its resistance after shooting. FHIA-05, FHIA-06, and FHIA-07 were susceptible to black leaf streak/black Sigatoka.

Recommendations

It was recommended that FHIA-01, FHIA-02, and FHIA-03 be released for distribution.

FHIA-01

FHIA-01, which has a sub-acid or apple flavor, could be of great value to farmers in India, Brazil, and Australia where palates favor this taste. Not only is it highly resistant to black leaf streak/black Sigatoka, but work in Australia has also shown that FHIA-01 is resistant to Fusarium wilt (races 1 and 4) and is apparently resistant to the burrowing nematode *Radopholus similis*. It is a strong plant and supports large bunches without propping. Research at FHIA indicates that the fruit has a good postharvest green life and fingers do not detach prematurely from the crown when ripe. The detached hand or cluster is also resistant to crown rot, a postharvest disease caused by one or several fungi in combination which invade cut crown tissue.

Pesticide application on FHIA-01 is expected to be minimal because of its resistant qualities. It is, therefore, ideally suited to smallholdings where growers cannot afford to purchase chemicals. Coupled with its robustness, FHIA-01 will be the first productive dessert banana that can be cultivated successfully by subsistence farmers. FHIA-01 may also have an impact in East Africa if acceptable when cooked green.

FHIA-02

FHIA-02 has not been investigated as thoroughly as FHIA-01, but it is known to have a shorter postharvest green life which excludes it from any export trade. However, it could well have attributes that would make it competitive for local markets and needs further study. Its flavor is sweeter (very similar to Cavendish banana) than FHIA-01. Recent

work suggests that this hybrid is susceptible to Fusarium wilt in Honduras, which may limit its potential in many areas. However, it would be expected to perform well in South Pacific countries where Fusarium wilt is not found.

FHIA-03

FHIA-03 is a drought-resistant banana that survives well under seasonal dry conditions and is expected to become important in Africa. The pseudostem of FHIA-03 is fibrous, like *Musa textilis*, and this quality makes it resistant to falling in high wind. Although primarily a cooking banana, it has a good dessert flavor when ripe (soft and fruity).

Improved Experimental Protocol for IMTP Phase II

A first meeting called "Training course on the IMTP technical guidelines to evaluate hybrids against *M. fijiensis*" was organized by INIBAP at Turrialba, Costa Rica, in June 1991. During this course the IMTP technical guidelines were discussed in depth and some amendments were suggested.

A second meeting of collaborators was held in Buga, Colombia on 28 September - 2 October 1992, to discuss the preliminary results of IMTP. At this meeting, the technical guidelines were again discussed and a number of different amendments were proposed and argued. The results of these deliberations were considered when technical guidelines for IMTP Phase II were prepared.

Postscript

FHIA-04, FHIA-05, FHIA-06, and FHIA-07 were found to be infected with banana streak virus (BSV) in tests undertaken by Dr Marie-Line Caruana at INIBAP's Virus Indexing Center at CIRAD-FLHOR in 1993-94. BSV was most likely present on plants at the FHIA banana breeding station in Honduras during the development of the hybrids and was infecting the material taken by INIBAP for shoot-tip culture.

The indexing results explain claims by officers-in-charge of trial sites that these clones were exhibiting virus symptoms. It was speculated at the time that infections may have been from local sources and were possibly caused by cucumber mosaic virus. However, as a precaution, INIBAP recommended that all plants of accessions with symptoms should be destroyed.

When FHIA hybrid germplasm was acquired by INIBAP for IMTP Phase I, BSV had not been recorded in the Americas. Because banana bunchy top virus and banana bract mosaic virus were not found in the Western Hemisphere, it was wrongly concluded that the FHIA material was of a high health status and did not need to be virus-tested before multiplication and distribution.

All future germplasm from IMTP will be indexed at one or more of INIBAP's Virus Indexing Centers before distribution to collaborators and clients.

Acknowledgments

IMTP Phase I was a truly collaborative effort which involved many individuals and institutions around the world. INIBAP would like to thank FHIA for providing hybrids for trial in IMTP Phase I. All participants in Colombia, Costa Rica, Honduras, Nigeria, Cameroon, and Burundi are also thanked for making this pioneering exercise a success. In particular, INIBAP would like to acknowledge the key role played by the following individuals who took direct responsibility for managing the trials and recording data at the sites:

Dr Sylvio Belalcazar	ICA, Colombia
Dr Franklin Rosales	FHIA, Honduras
Ing Douglas Marin	CORBANA, Costa Rica
Dr Eric Fouré	CRBP, Cameroon
Dr Dirk Vuylsteke	IITA, Nigeria
Mr Ferdinand Ngezahayo	IRAZ, Burundi

INIBAP is grateful to Drs Ahmad Rafie of FHIA and Xavier Perrier of CIRAD for their significant contribution to the statistical analysis of results of IMTP Phase I, and modifications of design for IMTP Phase II.

INIBAP is also grateful to UNDP for providing funds which made this important link between banana breeders and banana growers possible, and to the World Bank as executing agents for the project.

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Distribution of Recommended FHIA Hybrids

DR Jones

INIBAP's main objective is to increase the productivity of the smallholder. This is the man or woman who grows banana and plantain around the house to feed the family or who cultivates a small plot to supply fruit to local or export markets. It is important that these smallholders receive the superior germplasm developed by breeding programs. IMTP is a link between the breeding programs and NARS and, in addition to organizing the testing of new hybrids, it must also be concerned with the distribution of material to those who might benefit the most. To this end, INIBAP has been actively engaged in the distribution of FHIA-01, FHIA-02, and FHIA-03 to those NARS requesting material, and has also been stimulating national evaluation programs (NEPs).

Countries where FHIA material has been supplied or will be supplied in the very near future as virus-indexed tissue cultures by INIBAP are listed in Table 1. FHIA have also been involved in distributing their own germplasm and recipient countries are also included in Table 1.

Table 1. Countries and organizations[1] receiving FHIA hybrids recommended from IMTP Phase I.

Germplasm from INIBAP	Germplasm from FHIA[2]
Africa/Indian Ocean	
Burundi (IRAZ)	Ghana (Crops Research
Cameroon (CRBP)	Institute - CSIR)
Congo (University Marien N'Gouabi)	South Africa (BPIU; Leeways
Côte d'Ivoire (MAAR through IITA)	Laboratory[3])
Gabon (CIAM through CRBP)	Uganda (NARO)
Guinea (DERIL)	
Kenya (KARI)	
Malawi (Ministry of Agriculture through IITA)	
Mauritius (Food and Agriculture Council)	
Nigeria (IITA)	
Seychelles (MAF)	
South Africa (BPIU)	

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(Table 1, continued)

Germplasm from INIBAP	Germplasm from FHIA[2]
Tanzania (TND)	
Uganda (NARO)	
Zanzibar (MALNR through IITA)	
Zimbabwe (University of Zimbabwe)	
Asia/Pacific	
China (SCAU)	Australia (QDPI)
Fiji (SPC)	Indonesia (Chiquita)
India (ICAR-IIHR)	Tonga (MAFF through QDPI)
Indonesia (AARD-CRIH)	Western Samoa (MAFFM through QDPI)
Malaysia (MARDI)	
New Caledonia (CIRAD-FLHOR)	
Pakistan (NARC)	
Philippines (BPI)	
Taiwan (TBRI)	
Tahiti (MAE)	
Thailand (HRI)	
Vietnam (INSA)	
Western Samoa (MAFFM)	
Americas	
Barbados (CARDI)	Bolivia
Bolivia (PROINPA-IBTA)	Colombia (BIOTECOL[3])
Brazil (EMBRAPA-CNPMF)	Costa Rica (ECOS del AGRO)
Colombia (ICA)	Cuba[4] (MINAG)
Costa Rica (CORBANA)	Ecuador (INIAP)
Dominican Republic (FDA)	El Salvador (University of El Salvador)
Mexico (CICY)	Grenada (WINBAN)
Panama (Fundación Natura)	Honduras (17 Organizations & Companies)
USA (USDA - ARS in Puerto Rico)	Jamaica
Venezuela (FONIAP-CENIAP; Zulia University)	Mexico (University of Chapingo; Agricola Pampitas)
	Nicaragua (IRENA-NORAD)
	St. Lucia (WINBAN)
	USA (AGRISTAR[3]; University of the Virgin Islands)
Europe/Middle East	
England (University of Reading)	Austria (IAEA)
Spain (CITA, Canary Islands)	England (University of Reading)
	Israel (Volcani Center)
	Spain (CULTESA[3], Canary Islands)

[1] See list of acronyms and abbreviations on page 287 – [2] Information supplied by Dr. Franklin Rosales, FHIA
[3] Tissue Culture Company – [4] It is estimated that 100,000 plants of FHIA-01 and 1,500,000 plants of FHIA-03 will have been produced in Cuba by the end of 1994 (P Rowe, pers. comm.).

International *Musa* Testing Program Phase II

DR Jones

Introduction

In April 1991, plant pathologists, nematologists, and virologists from the Asia/Pacific region met in Brisbane, Australia, under the auspices of INIBAP-ASPNET, to discuss the major *Musa* disease problems of the region. Although Sigatoka diseases were high on the list of concerns, especially in the Pacific, Fusarium wilt ranked as the number one problem in most countries (Valmayor 1991).

This disease seriously affects the cultivation of popular cultivars in Asia such as Silk (syn. Pisang Rastali, Rasthali, Latundan, Pisang Raja Sereh), Pisang Awak (syn. Kluai Namwa, Chuoi Tay, Katali), Pisang Berangan (syn. Pisang Barangan, Chuoi Bom, Lakatan), Gros Michel (syn. Pisang Embun, Pisang Ambon Putih, Ambon, and Kluai Dok Mai) and was also a problem on Cavendish types grown for both export and local production. On a global level, Fusarium wilt was recognized as a factor limiting the production of Bluggoe and other popular banana cultivars in Africa and the Americas.

Conference participants agreed that an IMTP protocol for field-screening germplasm against Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f.sp. *cubense*, needed to be developed so that hybrids from the breeding programs could be evaluated for resistance. During his visit to Australia at the time of the conference, Dr Ramon Valmayor, INIBAP's ASPNET Coordinator, obtained funding from the Australian International Development Assistance Bureau (AIDAB) that would enable a preliminary analysis of the genetic variability of *F. oxysporum* f.sp. *cubense* isolates from the region to be undertaken by Mr Ken Pegg (QDPI) and colleagues from the University of Queensland and the Queensland University of Technology (QUT).

Submission to UNDP

In September 1991, the first proposal for IMTP Phase II was submitted to the United Nations Development Program (UNDP) for funding as a "Global Program to Develop a Coordinated *Musa* Germplasm Evaluation System of Newly Improved Accessions". This

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was an ambitious 5-year project costing US$7.8 million which included eight major funding components:

1. Global coordination of the *Musa* germplasm evaluation system.
2. Early evaluation of *Musa* germplasm at breeding programs before global testing.
3. Research for improved early screening methods using small plants.
4. Sanitation, true-to-type verification, and distribution of *Musa* germplasm for global testing.
5. Global evaluation trials screening *Musa* germplasm against black leaf streak/black Sigatoka and Fusarium wilt.
6. Training in disease screening methodology.
7. Annual global conference.
8. Collection and maintenance of relevant *Musa* germplasm.

After a favorable review, the project was approved by the Standing Committee on Program Matters of the UNDP Governing Council at a meeting in Geneva in May 1992 with a total budget of US$4.8 for 3 years. A meeting between UNDP, INIBAP, and the World Bank in July 1992 agreed to the necessity for a number of modifications. INIBAP reworked the budget and submitted a final plan of work to UNDP in September 1992. This included the salaries of an IMTP Leader to coordinate the project globally, and an IMTP Officer for the Latin America/Caribbean region. On the advice of UNDP, funds requested by INIBAP for local evaluation sites were dropped, monies for early evaluation of *Musa* germplasm were converted to a grant of US$250,000 to FHIA, and the training component was strengthened. Also, the need for a Project Steering Committee that would meet in conjunction with the annual global conference was recognized. Consequently, the need for a full-time Latin America/Caribbean IMTP Officer was thought inappropriate, as INIBAP's Regional Coordinator could perform some of these duties part-time and the IMTP Leader could do others. Compensation for the time INIBAP's Regional Coordinators in all regions would spend on IMTP-related duties was requested from UNDP, but rejected on the grounds that this would be INIBAP's contribution to the project. However, at the time of writing, INIBAP was negotiating with UNDP on the possible funding of 30% of Germplasm Officer who would be undertaking IMTP related activities as part of his duties.

Fusarium Wilt Experts' Meeting

As a first step towards developing a global protocol for the field screening of *Musa* germplasm to determine reaction to *F. oxysporum* f.sp. *cubense*, INIBAP organized a meeting of Fusarium wilt experts in Taiwan in December 1992. This meeting coincided with a joint Taiwan Banana Research Institute and INIBAP-ASPNET international symposium on recent developments in banana cultivation technology (Valmayor et al. 1993).

The meeting was attended by Mr Zilton Cordeiro (EMBRAPA-CNPMF, Brazil), Mr Zaag de Beer (Banana Board, South Africa), Dr Julio Hernandez (CITA, Canary Islands, Spain), Dr Shin-Chuan Hwang (TBRI, Taiwan), Dr Anacleto Pedrosa (Del

Monte, Philippines), Mr Ken Pegg (QDPI, Australia), Dr Randy Ploetz (University of Florida, USA), Mr Wilberforce Tushemereirwe (Kawanda Agricultural Research Institute, Uganda) and observers from Taiwan, Australia, South Africa, and the Philippines, who also contributed to the discussions. The decisions taken at this important meeting form the basis of the final Fusarium wilt technical guidelines document presented in Annex 2 of these proceedings.

Project Coordination

In January 1993, Dr David Jones, Scientific Research Coordinator with INIBAP, was appointed IMTP Leader in charge of global coordination. Dr Jones' responsibilities were to administer the IMTP network from the supervision of training activities to the organization of experts' meetings and analysis of data. He was also to act as secretary to the IMTP Steering Committee.

Germplasm for Evaluation

INIBAP approached the main *Musa* breeding programs to nominate germplasm for inclusion in IMTP Phase II. The criterion for selection was that enough initial screening had been undertaken to suggest that the material had resistance to the disease which it was to be screened against, and that it also had good agronomic potential. Seven breeding programs responded to INIBAP's request and sent 19 tissue cultures of their superior hybrids/mutations/somaclonal variant selections to INIBAP's Transit Center. In addition to clones from breeding programs, it was thought appropriate also to include some natural germplasm (landraces) and *Musa* species for evaluation. This would provide valuable information on the resistance of this material (some of which is used in genetic improvement programs) to pathogenic variants, which would ultimately benefit breeders and research workers. Standard reference clones with known reaction to the diseases were also included as controls.

[A list of the germplasm finally selected for IMTP following discussions at the IMTP Global Conference and the release of virus indexing results is presented in Annexes 1 and 2. During the IMTP Global Conference, breeders nominated germplasm for the next phase of IMTP. This is to be acquired by INIBAP for the Transit Center in the near future and then virus-indexed as soon as possible. It is envisaged that germplasm will be available for Phase III very soon after Phase II is completed.]

Sanitation

Work began immediately to acquire those accessions nominated by the breeding programs for inclusion in IMTP Phase II that were not already held in tissue culture at the INIBAP Transit Center at KUL, Belgium. Accessions were dispatched to INIBAP

Virus Indexing Centers for quarantine and testing. All hybrids, cultivars and *Musa* species to be evaluated in IMTP Phase II began to be screened for virus diseases in 1993.

Banana streak virus (BSV) had been detected in FHIA hybrids (FHIA-04, FHIA-05, FHIA-06, and FHIA-07) evaluated in IMTP Phase I. The same seedborne virus was later found to be also present in the IITA and CIRAD-FLHOR breeding programs. Because of concern over this issue and its effect on IMTP, Dr David Jones began negotiations with Dr Ben Lockhart of the Department of Plant Pathology, University of Minnesota, to formulate a project to develop a sensitive diagnostic test for BSV. This test was seen as important to improve screening procedures at INIBAP's Virus Indexing Centers and would also benefit breeding programs. At present, the most reliable means of detecting BSV is to quarantine plants for up to 12 months at temperatures that will lead to the expression of disease symptoms (25-28°C), and observe regularly. Symptoms may not develop until after 5-6 months after planting or even later. At the time of writing, a contract between INIBAP and the University of Minnesota has just been signed that will allow Dr Lockhart to proceed with the BSV project. UNDP agreed to support this work financially from IMTP Phase II funds because of its importance to the safe movement of *Musa* germplasm, a key component in IMTP. It is hoped that work on the development of a diagnostic test for banana bract mosaic virus (BBMV) will also be supported by IMTP.

Training

The training component of IMTP was also considered. Initially, this would concentrate on instruction for site officers on the correct procedures for evaluating *Musa* germplasm in the trials as laid down in the technical guidelines (see Annexes 1 and 2). However, specialized training was envisaged later in *Musa* germplasm management, involving courses in taxonomy, utilization of genetic resources, and breeding strategies. Exchanges of scientists between breeding programs and courses on virus indexing protocols are also thought to be important.

Germplasm Conservation

INIBAP headquarters and the ASPNET regional office determined priorities for germplasm collecting, characterization, and conservation under IMTP Phase II. Localities seen as high-priority areas were Viet Nam, Indonesia (especially Irian Jaya), and islands off the coast of East Africa (Zanzibar, Madagascar, Comores).

Dr Ramon Valmayor opened negotiations with the Government of Vietnam in association with the International Plant Genetic Resources Institute (IPGRI) and an agreement with the National Institute of Agricultural Sciences (INSA) was signed in October 1993. The first collecting missions by INSA are now under way, aided by standardized data forms developed by INIBAP. Accessions of wild species and cultivars collected will be maintained in two field gene banks in Viet Nam, and also in vitro in Viet

Nam and the INIBAP Transit Center. Material will be available to breeding programs, research scientists, and NARS for evaluation purposes.

In June 1993, Dr David Jones visited Zanzibar, Tanzania, to open negotiations with the Ministry of Agriculture, Livestock and Natural Resources to determine if a collecting mission was feasible. Although the germplasm in Zanzibar and Pemba is not as diverse as was originally thought, some cultivars were determined to be of value and worthy of conservation (Jones 1993).

Early Screening

Another main component of IMTP Phase II is research to develop methods for disease screening *Musa* germplasm in the glasshouse or laboratory/environmental cabinet. If small plants, in-vitro plantlets or plant parts, such as detached leaves, could be reliably used in tests to determine reaction to Sigatoka and Fusarium wilt diseases, this would eliminate the need for field trials which are costly and time-consuming. Such tests would benefit unconventional breeding programs where large numbers of somaclonal variants, induced mutations or transformed germplasm need to be screened quickly and accurately. It has been shown that it may be possible to use toxins from Sigatoka pathogens in screening tests (Molina, Krausz 1989), but more work needs to be undertaken to confirm this. The inoculation of small plants with conidia from Sigatoka pathogens grown in vitro, followed by incubation in environmental cabinets or glasshouses, may also prove useful (Mourichon et al. 1987). However, the development of similar tests for Fusarium wilt may prove more difficult. Projects submitted to INIBAP are now being considered for funding.

Test Sites

No funds were allocated by UNDP in IMTP Phase II for the establishment and maintenance of trial sites. However, most participating NARS/Institutes believed that IMTP has much relevance for their national research programs and, consequently, were prepared to finance trial sites at their own expense. Thirty-three test sites have been identified in 19 countries (Table 1). These sites were selected on the following criteria:

– The scientific expertise available locally to undertake the trials.

– The relevance of the target disease in local *Musa* production.

– The possible local pathogenic variability of the fungus causing the target disease.

– The local climatic conditions that may influence the host/pathogen interaction.

The results of work undertaken by Mr Ken Pegg (QDPI), Ms Natalie Moore (University of Queensland) and Ms Suzanne Sorensen (Queensland University of Technology) in the analysis of genetic variability of isolates of *F. oxysporum* f.sp. *cubense* from Southeast Asia influenced decisions on Fusarium wilt site locations in this region. Much variability was found in Malaysia and Indonesia, and it was considered

Table 1. IMTP Phase II trial sites.

Country	Site	Officer-in-charge	Organization[1]
Sigatoka/yellow Sigatoka			
Cameroon	Dschang	Dr Eric Fouré	CRBP
Colombia	El Agrado, Quindio	Dr Sylvio Belalcázar	CORPOICA
Cuba	Alquizar, Havana Province	Dr Luis Perez Vincente	INISAV
India	Podavur, Tamil Nadu State	Dr Harishchandra Singh	ICAR/NRC
	Kannara, Kerala State	"	ICAR/KAU
St Lucia	Union	Dr Henry Fagan	WINBAN
Thailand	Sawi, Chumphon Province	Mr Det Wattanachaiyingcharoen	HRI
Black leaf streak/black Sigatoka			
Cameroon	Nyombe	Dr Eric Fouré	CRBP
Costa Rica	La Rita, Guápiles	Dr Ronald Romero Calderón	CORBANA
Cuba	Camaguey Province	Dr Luis Perez Vincente	INISAV
Honduras	La Lima, Cortés	Dr Franklin Rosales	FHIA
Nigeria	Onne, Port Harcourt	Dr Rodomiro Ortiz	IITA
Philippines	Davao, Mindanao	Ms Lydia Magnaye	BPI
Tonga[2]	Vaini, Tongatapu	Ms Paelata Nai	MAF
Uganda[3]	Kawanda, Kampala	Mr Wilberforce Tushemereirwe	NARO
Fusarium wilt			
Australia[2]	Wamuran, Queensland	Mr Ken Pegg	QDPI
Brazil	Cruz das Almas, Bahia	Dr Aristoteles Pires de Matos	EMBRAPA-CNPMF
Cuba	Santiago de Las Vegas, Havana Province	Dr Luis Perez Vincente	INISAV
Honduras	La Lima, Cortés	Dr Franklin Rosales	FHIA
India	Podavur, Tamil Nadu State	Dr Harishchandra Singh	ICAR/NRC
	Kovvur, Andhra Pradesh State	"	ICAR/APAU
	Bangalore, Karnataka State	"	ICAR/IIHR
Indonesia	Segunung, Java	Ir Agus Muharam	AARD
	Solok, Sumatra	Ir Sahlan	AARD
	Jeneponto, Sulawesi	Ir Lukman Hutagalung	AARD
Malaysia	Serdang, Selangor	Ms Siti Hawa Jamalludin	MARDI
	Kulai, Johor	Ms Siti Hawa Jamalludin	MARDI
Philippines	Davao, Mindanao	Ms Lydia Magnaye	BPI
South Africa	Hazyview, Eastern Transvaal	Mr Zaag de Beer	BPIU

(Table 1, continued)

Country	Site	Officer-in-charge	Organization[1]
Spain	Valle de Guerra, Tenerife, Canary Islands	Dr Julio Hernandez Hernandez	CITA
Taiwan	Chiuju, Pingtung	Dr Chin-Yan Tang	TBRI
Thailand	Phichit, Phichit Province	Mr Det Wattanachaiyingcharoen	HRI
Uganda[3]	Rubare, Bushenyi District	Mr Wilberforce Tushemereirwe	NARO

[1]See list of acronyms and abbreviations on page 287
2 Testing to be funded by ACIAR
3 Testing to be funded by ODA

important to situate more trials in these countries. Fortunately, collaborators in this area were willing to financially support more than one site. A trial site has also been situated at over 1400 m in southwestern Uganda where East African Highland cultivars are being affected.

One innovation in IMTP Phase II is the inclusion of sites to gauge the reaction of the germplasm to Sigatoka/yellow Sigatoka. Not all material resistant to black leaf streak/black Sigatoka is resistant to Sigatoka/yellow Sigatoka, including some improved diploids used in breeding programs. Sigatoka/yellow Sigatoka is still the most important leaf spot at altitudes above 1200-1400 m in the tropics (Jones, Mourichon 1993), in cool, subtropical growing areas and in countries not yet reached by black leaf streak/black Sigatoka.

INIBAP considers it essential to determine the resistance of new hybrids to Sigatoka/yellow Sigatoka. Two sites are to be at sea level in the Caribbean on the island of St Lucia and in Cuba, three will be in Asia in India and Thailand, and another two will be at altitude in Colombia and Cameroon. Altitude has an effect on the reaction of some germplasm to Sigatoka/yellow Sigatoka. Plantains are known to be resistant to the disease at sea level, but susceptible at elevations higher than 500 m in the Latin American/Caribbean region (Stover, Simmonds 1987). Recently, FHIA hybrids found resistant to black leaf streak/black Sigatoka and to Sigatoka/yellow Sigatoka at sea level have been found to be susceptible to Sigatoka/yellow Sigatoka at 1320 m in Colombia (S. Belalcázar, pers. comm.).

Breeders' Visits to Trial Sites

Provision has been made in the IMTP Phase II budget for breeders to visit trial sites to inspect germplasm being evaluated. It is anticipated that groups of breeders will travel together with the IMTP Leader during the course of the evaluation.

Steering Committee

On the first day of the IMTP Global Conference, a Steering Committee was nominated to advise on all pertinent issues related to IMTP Phase II. It is expected that those nominated would serve for at least 2 years with the possibility of an extension for another year. The terms of reference for the Steering Committee are outlined in Table 2. Officers of the first Steering Committee are as follows:

Chairman: Dr Franklin Rosales (FHIA)
Secretary: Dr David Jones (INIBAP)
Members: Dr Dirk Vuylsteke (IITA)
Dr Aristoteles Pires de Matos (EMBRAPA-CNPMF)
Ms Lydia Magnaye (BPI)
Ms Siti Hawa Jamaluddin (MARDI)
Mr Wilberforce Tushemereirwe (NARO)

Table 2. IMTP Steering Committee.

Composition and terms of reference

1. Three banana/plantain breeders (3).
2. Three plant pathologists, preferably from each of the three major producing regions (3).
3. INIBAP's IMTP Leader (1), who acts as Executive Secretary.

Selection and tenure

Selection to take place during the IMTP Global Conference. Tenure of 2 years (reselection possible for one extra term) with the exception of the IMTP Leader, who is a permanent member.

Meetings of the Steering Committee

Meetings will normally be held on an annual basis, and at the same time as the IMTP Global Conference.

Responsibilities

1. Advise on the design of the IMTP annual work-plans and the implementation of the IMTP research activities. Make recommendations to INIBAP on germplasm movement, technical guidelines, training needs, strategic research, breeders' visits, collecting missions, and other IMTP components.
2. Review progress of the program on a yearly basis, and report to the plenary session of the IMTP Global Conference.
3. Advise on the organization of the IMTP Global Conference and in the preparation of major IMTP outputs (reports, documents, training materials, etc.).
4. Advise the IMTP Leader on IMTP activities and developments in the regions.

Technical Guidelines and Acknowledgments

The technical guidelines that are to be used by trial sites officers to implement all stages of IMTP Phase II evaluation trials are presented as Annexes 1 and 2 of these proceedings. These were finalized after discussions undertaken with Sigatoka and Fusarium wilt experts who attended the IMTP Global Conference.

Particular thanks must go to the following: Drs Dirk Vuylsteke and Rodomiro Ortiz (IITA) for advice on the key agronomic characters of the germplasm that should be assessed; Dr Friedhelm Gauhl and Cornelia Pasberg-Gauhl (IITA) for providing the Sigatoka scoring assessment method (Gauhl et al. 1993) and for help in identifying important environmental factors to be recorded in Sigatoka trials; Dr Ronald Romero Calderón (CORBANA) and Luis Perez Vincente (INISAV) for their contribution in the formulation of a Sigatoka infection index; Mr Zaag de Beer (BPIU), Dr Aristoteles Pires de Matos (EMBRAPA-CNPMF), Mr Ken Pegg (QDPI), Dr Randy Ploetz (University of Florida), Dr Mauricio Rivera (FHIA), Mr Ken Shepherd (EMBRAPA-CNPMF), and Mr Wilberforce Tushemereirwe (NARO) for their significant input into discussions relating to the rating systems to be used in the Fusarium wilt trials; Drs Ahmad Rafie and Franklin Rosales (FHIA) for helping to devise field layouts and data collecting strategies that would result in statistically valid information being recorded; Ir Ines Van den houwe for contributing instructions on the care of tissue culture after deflasking; all the delegates to the IMTP Phase I experts' meeting held in Buga, Colombia, 28 September to 2 October 1992 (Jones, Tezenas du Montcel 1994).

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Part 1

Target Diseases of IMTP Phase II

Sigatoka Diseases

Leaf Spot Diseases of Banana and Plantain caused by *Mycosphaerella musicola* and *M. fijiensis*

E Fouré

Introduction

Banana and plantain leaf spots caused by *Mycosphaerella musicola*, the agent of Sigatoka/yellow Sigatoka and by *M. fijiensis*, the agent of black leaf streak/black Sigatoka, can be considered as the most serious diseases of *Musa* from the economic standpoint.

Leaf attacks lead to necrosis, a decrease in photosynthetic tissue, and a loss of gross harvest yield. Also the disease accelerates fruit maturation rates which lead to premature ripening and result in loss in net exportable yield. *M. fijiensis* also causes serious damage to leaves of plantain, a staple food in many countries in West Africa.

Black leaf streak/black Sigatoka was recognized for the first time in Fiji in 1963. Since then, it has been found elsewhere and has steadily replaced Sigatoka/yellow Sigatoka in producing countries. It is now widespread in Southeast Asia, the Pacific, Central America and Africa (Fig. 1).

Black leaf streak/black Sigatoka appeared in Africa more recently than in the other continents. It was identified in 1980 after a commercial plantain estate was established in Gabon. Neighboring countries (the Congo, Equatorial Guinea, Cameroon) soon faced the same problem. All the banana-producing countries in West Africa are now threatened by *M. fijiensis*, which differs from *M. musicola* in certain biological features and also in pathogenic activity.

Because of the threat posed by the spread of *M. fijiensis* in Africa, it was urgent to develop techniques to improve control of the disease in commercial export banana plantations and, above all, to maintain local food production. Black leaf streak/black Sigatoka control in smallholdings is still difficult because of farming structures (village garden plots and intercropping) and the cost of agrochemicals.

The same also applies to *M. musicola*, the agent of Sigatoka/ yellow Sigatoka disease, which, in highland areas of Africa, causes considerable damage, not only to Cavendish and Pome cultivars, but also to plantain, which is a resistant host at lower altitudes. This

CRBP, BP 832, Douala, Cameroon

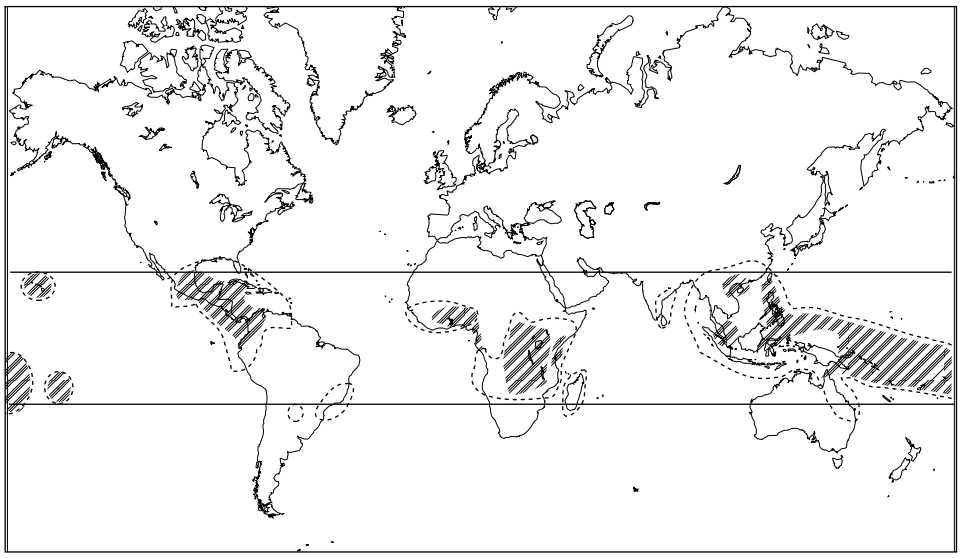

Figure 1. Geographical distribution of black leaf streak/black Sigatoka (*Mycosphaerella fijiensis*, hatched areas) and Sigatoka/yellow Sigatoka disease (*M. musicola*, dotted lines).

leaf spot disease thus poses a threat to food crops in regions that are still free of black leaf streak/black Sigatoka (Fouré, Lescot 1988; Mouliom Pefoura, Mourichon 1990; Mourichon, Fullerton 1990). The implementation of control strategies has, therefore, been based on several approaches:

– studies of the different phases of the development cycle of leaf spot diseases and the various factors affecting their duration;

– cultural control in smallholdings;

– chemical control strategies in commercial plantations based on forecasting methods;

– genetic control.

Epidemiology

Climatic conditions undoubtedly affect the development of leaf spot diseases. Precise links have been established between certain climatic parameters and the development of the two diseases.

Studies of the different phases of the development cycles of leaf spot disease (Fig. 2a, b) and of the various factors influencing the duration of these phases, provide a better understanding of the dynamics of the diseases in banana-production zones and the potential for outbreaks in plantations. The results are of significant help in the improvement of planned control measures.

The first research in Africa on the biological and epidemiological characteristics of black leaf streak/black Sigatoka was undertaken in Gabon (Fouré et al. 1984; Fouré

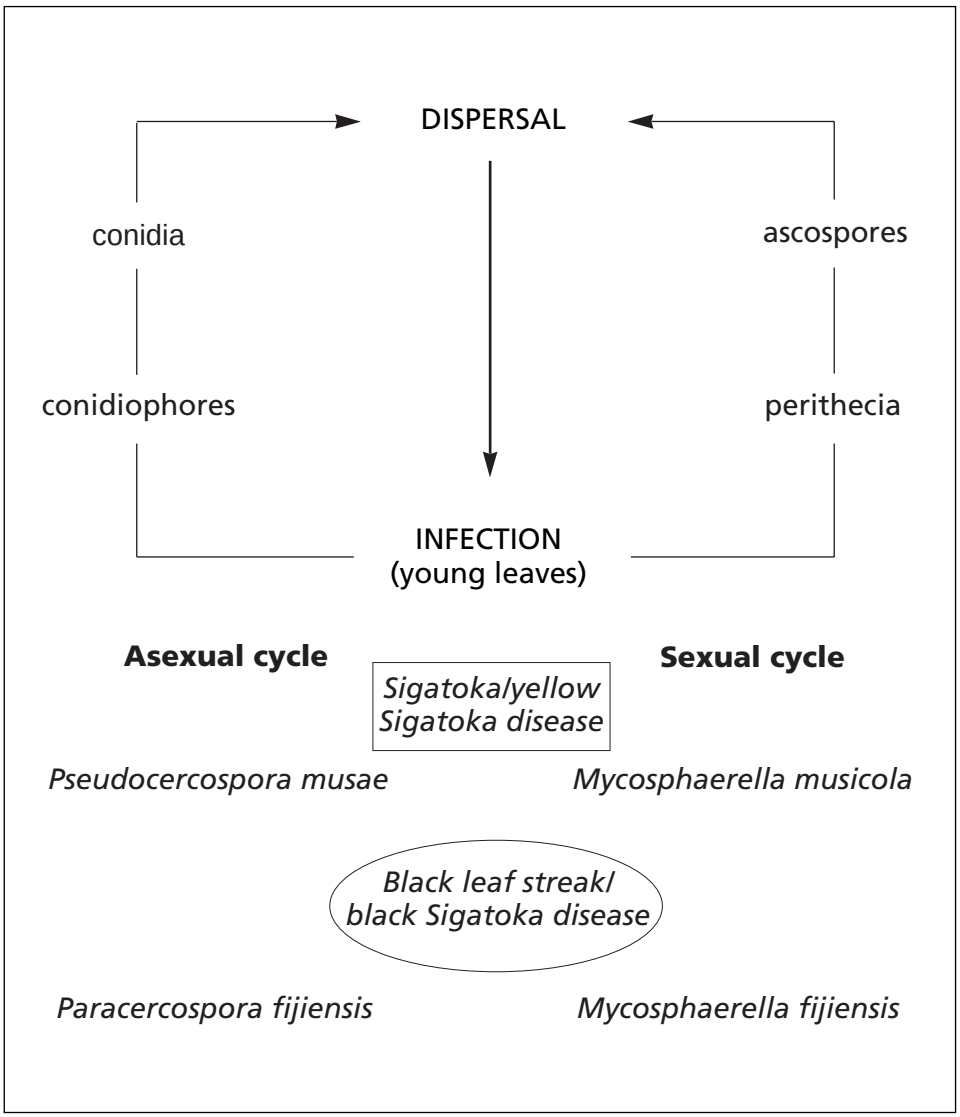

Figure 2a. Sexual and asexual cycles of Sigatoka/yellow Sigatoka disease and black leaf streak/black Sigatoka disease.

1985). A number of parameters related to symptomatology, morphological characteristics of the conidium and ascospore phases, sporulation intensity and study of the lateral transport of ascospores were used to study the phases of incubation and of development of the diseases on banana plants of various genomic groups.

Substantial climate-related variations have been recorded in the black leaf streak/ black Sigatoka development cycle. In very favorable periods for the disease, the extremely high density of stage-1 streaks (Fouré 1987) on the leaves invariably causes

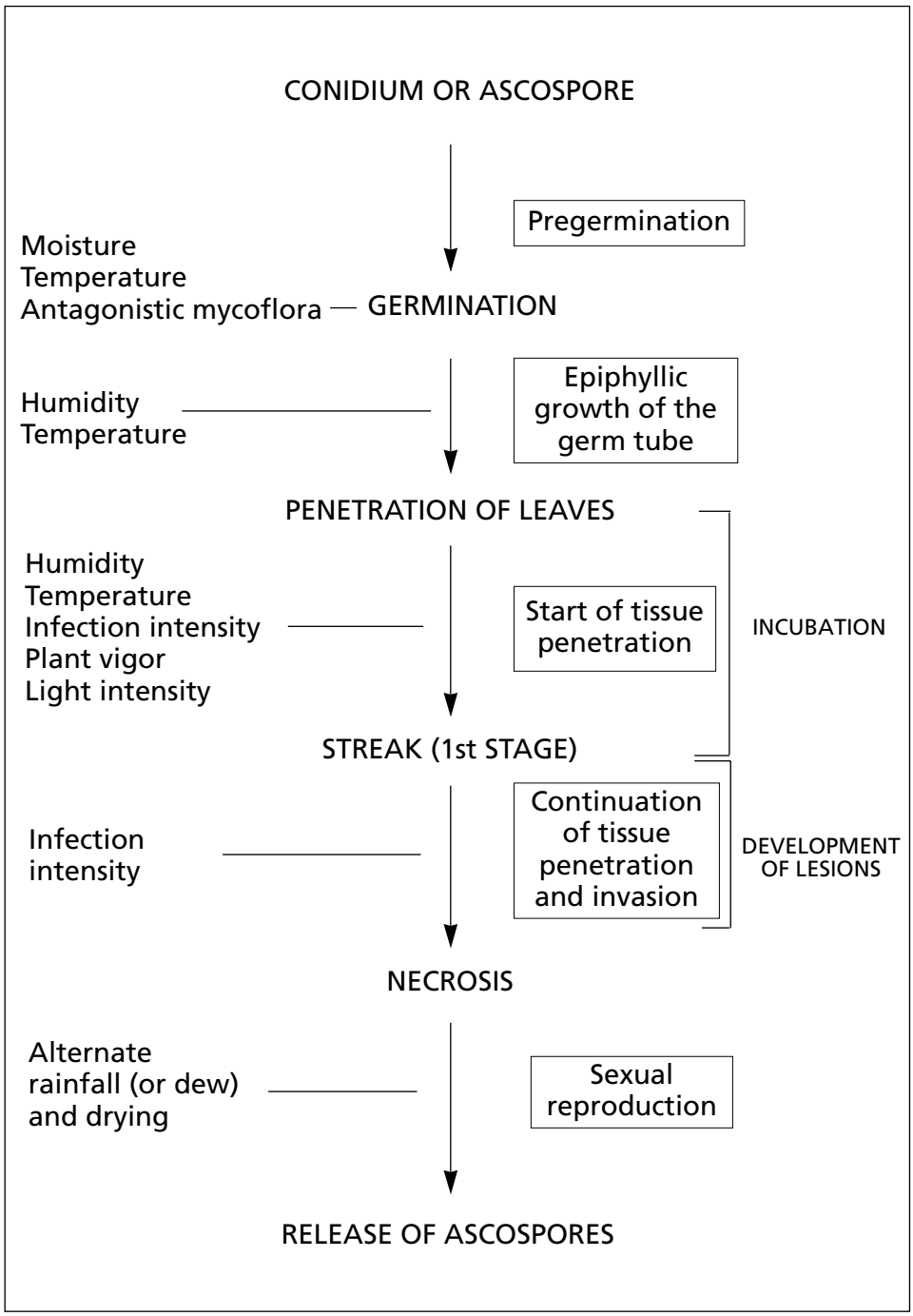

Figure 2b. Summary of the developmental stages of leaf spot diseases and the main factors that affect the duration of these stages.

the rapid appearance of necrotic patches and blackening of the affected leaf without the appearance of discrete lesions.

Isolated stage-5 or 6 lesions are seen only when there is a small quantity of inoculum and when climatic conditions are not particularly favorable for the rapid spread of the disease. Black leaf streak/black Sigatoka can develop in a susceptible variety according to the two cycles shown diagrammatically in Figure 3.

Close correlations have been established between certain climatic parameters and development of the disease (Fouré, Moreau 1992, Lhomme 1990, Jimenez et al. 1994). The closest correlations were found with evaporation and precipitation. Rainfall affects the development of the disease over a period ranging from 1 to 4 weeks after infection (Fouré, Moreau 1992). The favorable climatic periods for black leaf streak/black Sigatoka are thus those of high precipitation and low evaporation.

Reference should also be made to various publications on *M. musicola* and *M. fijiensis* that have contributed to a better knowledge of the biology and epidemiology of the two leaf spot diseases (Brun 1963; Meredith et al. 1973; Stover 1965, 1968, 1970, 1983; Quinon 1972; Lehmann-Danzinger 1986; Gauhl 1989; Sandoval Ramirez 1988).

Control Methods

Chemical control

In commercial plantations, the intensive use of fungicides (as many as 50 spraying operations per year in certain areas) considerably increases production costs and leads to substantial problems of pollution of the environment.

The use of forecasting methods as part of integrated management of leaf spot diseases of banana is aimed at reducing the number of treatments while maintaining disease control. Continuous area-by-area analysis of two categories of data can be used to monitor development of the diseases:

– **biological descriptors,** consisting of an appraisal of the development of the disease in various ecological zones in the growing area by observation of foliar symptoms;

– **climatic descriptors** (evaporation and temperature), determining the duration of effectiveness of spray applications from climatic information.

In Guadeloupe, the climatic warning method is sufficient for the control of *M. musicola*, although the presence of centers of infection or 'hot spots' in some areas necessitates the permanent maintenance of an observation network to monitor evolution of the pathogen and to ensure satisfactory functioning of the climatic warning system (Ganry, Meyer 1972a,b; Bureau, Ganry 1987).

These strategies, combined with the use of systemic fungicides, have enabled a remarkable reduction to be made in the number of spraying operations in comparison with control based on a regular spray schedule. In Guadeloupe, an average of six spraying operations are carried out annually to control Sigatoka/yellow Sigatoka disease. In Cameroon, where a biological warning system is used, spraying to control black leaf

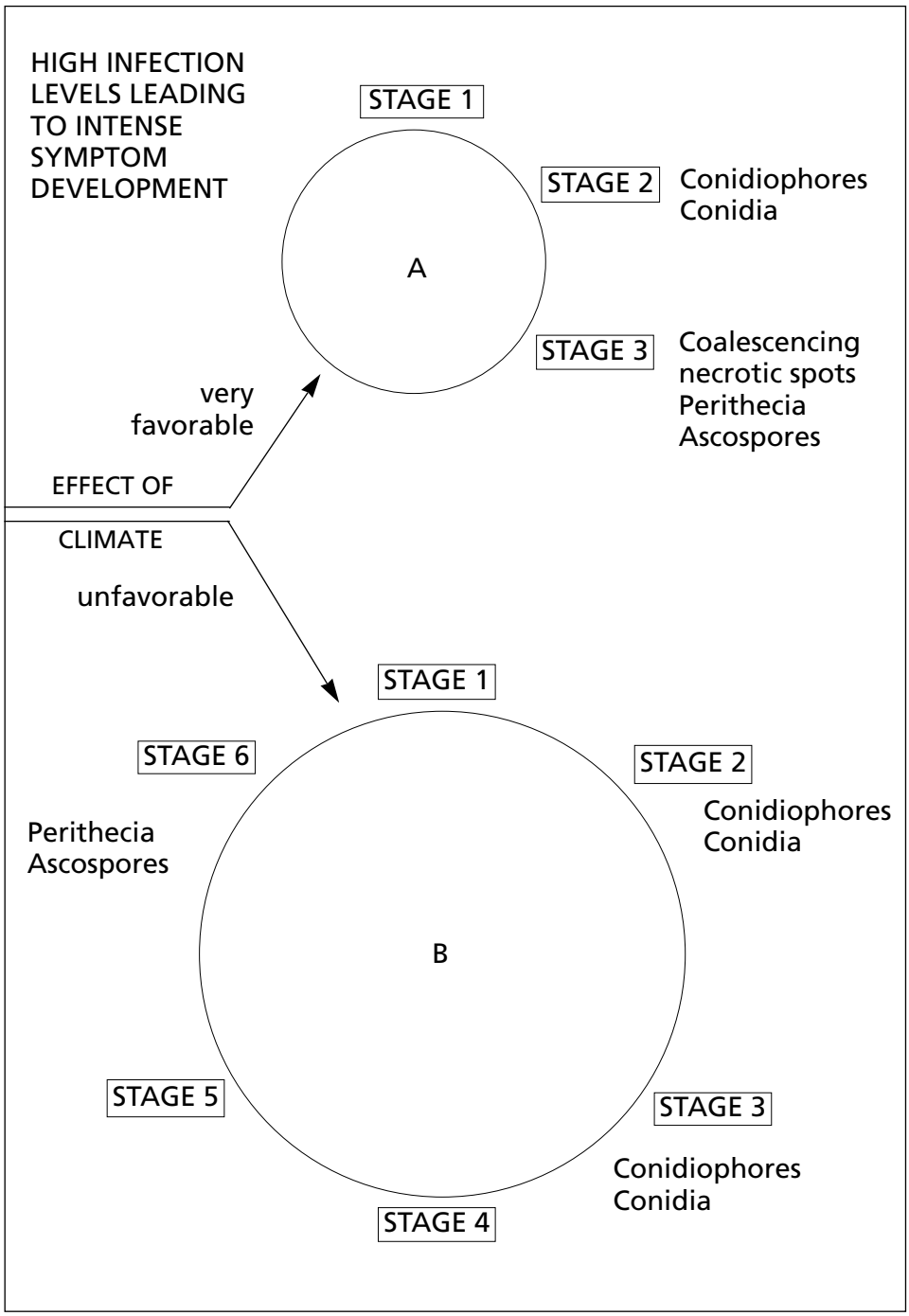

Figure 3. Possible patterns of the development of black leaf streak/black Sigatoka disease on a susceptible variety.

In Cameroon, where a biological warning system is used, spraying to control black leaf streak disease/black Sigatoka disease is carried out 12-15 times a year (Fouré 1988).

The overall aim of warning systems in commercial plantations is to reconcile economically profitable production with satisfactory disease management while at the same time reducing the pollution of the environment. The recent appearance and spread of triazole-resistant *M. fijiensis* strains in the main Central American production zones means that choice of control strategies must be more carefully planned than ever before. Alternating or mixing fungicides and periodic laboratory monitoring of the possible appearance of resistant phenotypes are ways of dealing with this problem.

Varietal susceptibility and host-pathogen interactions

It is clear that explanations for the various types of host reactions to infection should be sought late in the infection cycle after the stomatal penetration phase. Research has also clearly shown the following features.

a) The degree of resistance of *Musa* to Sigatoka diseases is not as closely correlated with the presence of the B genome in the genotype as was once thought (Laville 1983). Resistance is not so straightforward, marked resistance being present in diploids and triploids carrying only the A genome as well as germplasm with a high B genome component (Tezenas du Montcel 1990).

b) There is no correlation between the duration of the incubation period prior to the appearance of symptoms and susceptibility or resistance (Fouré 1987).

c) There is either no sporulation or only slight sporulation in resistant varieties (Fouré 1985).

Characterization of the different types of host-pathogen interaction

The experimental methods used for characterization have been described in detail in previous publications (Fouré et al. 1984; Fouré 1985). The following parameters were used:

– incubation time of the disease (infection to stage 1);

– development time of lesions from stage 1 to stage 6 or necrosis due to merging of earlier stages;

– intensity of asexual and sexual sporulation.

The methodology developed in Cameroon has made it possible to identify and describe different types of interactions (Fouré et al. 1990).

Phenotype 1

This is the expression of marked resistance to black leaf streak/black Sigatoka. To date, all banana cultivars in this category display an identical or very similar behavior to that of Yangambi Km5 (AAA 'Ibota'). The main features are:

– blockage of the development of symptoms at stage 1 or stage 2;

– no asexual sporulation;

– no sexual sporulation.

In the light of knowledge obtained from other host-pathogen combinations, the behavior of *Musa* species and cultivars exhibiting this phenotype is qualitative and it appears to indicate that resistance is close to hypersensitivity. The hypersensitive response, in most cases, is assumed to be controlled by a single or a small number of host-resistance genes and is termed 'vertical' resistance.

This type of resistance is fairly attractive to breeders, but is more easily overcome by mutations in pathogen populations. Its effectiveness may, therefore, be short-lived.

Phenotype 2

This is characterized by a partial resistance to black leaf streak/black Sigatoka. Resistant banana cultivars in this category display behavior comparable, for example, to Fougamou (ABB 'Pisang Awak'). The main features are as follows:

– normal, but slow, development from stage 1 to a necrotic stage (stage 6, or the merging of early stages);

– asexual and sexual sporulation;

– plants have large numbers of functional leaves at harvest.

In most other crops, this quantitative behavior is controlled by many genes giving the plant durable or 'horizontal' resistance.

Phenotype 3

This is characterized by marked susceptibility to the disease:

– normal, but rapid, development from stage 1 to a necrotic stage (stage 6, or the merging of early stages);

– sporulation (asexual or sexual) of the fungus may be considerable if the weather conditions are favorable for the development of the disease;

– there are few functional leaves on plants at harvest.

It can be assumed that both Phenotype 2 and 3 are of the same quantitative response that probably involves several genes. It can, therefore, be considered that there are in fact only two distinct types of behavior:

– phenotype 1;

– phenotype 2 with different levels of behavior ranging from considerable partial resistance to degrees of susceptibility in which the disease is fully expressed.

Ongoing research on the genetic determinism of resistance

Formal genetic studies are essential for studying the determinism of resistance to black leaf streak/black Sigatoka. Such work has not been possible to date since the appraisal of levels and types of resistance has mainly concerned cultivars bearing sterile parthenocarpic fruit and belonging to the AAA, AAB, ABB, and AA genomic groups.

Recent information on the responses of different wild—and hence fertile—diploid AA clones now makes it possible to envisage research using these species as parents to

study inheritance of the resistance factors and the type of genetic control involved (monogenic, oligogenic, or polygenic).

Experimental crosses have been made in Guadeloupe (CIRAD-FLHOR) between three apparently homozygous *Musa acuminata* clones. One exhibits a phenotype 1 reaction to black leaf streak/black Sigatoka, another the phenotype 2 reaction and the third the phenotype 3 reaction.

The F_1 progenies obtained are in the process of evaluation for their response to black leaf streak/black Sigatoka in Cameroon (CRBP). Very little segregation is expected at this level because of the homozygozity of parent plants. F_1 individuals have been retained for selfing and segregation for resistance that will be measured in the F_2. This work will lead to determination of the number and nature of the genes involved in resistance and will be linked to molecular studies (RFLP analysis and allied techniques).

The results will also lead to molecular appraisal and identification of allele variability in genes conferring resistance to black leaf streak/black Sigatoka in banana germplasm.

This project will help breeders to implement strategies in which different sources of resistance to black leaf streak/black Sigatoka are utilized. It will also lead to identification of regions on the banana genome that code for resistance. These loci may be isolated in the future and incorporated in plasmids for genetic engineering. Transformation must be carried out with full knowledge of the types of genes transferred to avoid risk of a breakdown in resistance.

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Mycosphaerella fijiensis: Diversity and Possibilities for the Early Screening of Germplasm for Resistance

X Mourichon

Genetic Diversity within *Mycosphaerella fijiensis*

Introduction

Sigatoka leaf spot diseases of banana are caused by two related pathogenic ascomycete fungi: *Mycosphaerella fijiensis* Morelet, the cause of black leaf streak/black Sigatoka disease, and *Mycosphaerella musicola* Leach ex Mulder, the cause of Sigatoka/yellow Sigatoka disease. *M. fijiensis* is characterized by its stronger pathogenicity on a broader range of hosts, making it the most destructive leaf disease of banana.

Research on black leaf streak/black Sigatoka is of the highest urgency in order to develop effective strategies for preserving banana production. Integrated solutions are needed, including selection of resistant clones and rational chemical control. Because of this worldwide problem, it is necessary to strengthen such a program in close connection with genetic improvement.

However, one of the current problems frequently mentioned by banana breeders is the lack of information on the nature of pathogen populations. Knowledge is essential if genetic improvement is to be conducted in a satisfactory way. If no information on the variability of pathogen populations is available, breeding for resistance is difficult.

Thus, it appeared to be essential to draw up an inventory of parasite populations and, above all, to evaluate their intraspecific diversity in the various banana areas, by using both pathotypic and selectively neutral markers.

Objectives of research

a) To investigate the genetic diversity within *M. fijiensis* and characterization of populations with molecular markers (selectively neutral markers).

b) To quantify the extent of pathogenic variability by using a differential set of *Musa* varieties (pathotypic markers).

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c) To select a set of "pathotypes" that represent the full diversity of the pathogen populations in terms of "virulence" and "aggressiveness".

d) To elaborate strategies for efficient resistance gene management, permitting durable black leaf streak/black Sigatoka resistance.

Previous and related research

Genetic diversity and population structure

Work has been carried out on genetic diversity at the CIRAD-FLHOR Plant Pathology Laboratory in Montpellier, France, where it is possible—as it is not in a producing country—to establish a live collection of *M. fijiensis* isolates. More than 500 single spore isolates representing pathogenic populations in the different production zones (southeast Asia, Pacific, Africa, and Latin America) are now available for analysis.

The first genetic analysis of *M. fijiensis* was initiated using DNA restriction fragment length polymorphisms (RFLPs). These genetic markers have been used to study inter- and intraspecific variation in many fungal phytopathogens (Bruns et al. 1991; Michelmore, Hulbert 1987).

Technical and methodological aspects of the application of these techniques to *M. fijiensis* have been determined. A genomic library of a reference strain has been constructed (cosmid library) and low-copy nuclear DNA probes isolated. Fifty-seven isolates of *M. fijiensis* from different geographical origins were assayed for RFLPs (Carlier et al. 1993a,b; Carlier et al. 1994). The highest level of diversity in this pathogen was found in Southeast Asia, the region where it probably originated. In other epidemic zones, isolates formed genetically homogeneous groups specific to each region: Africa, the Pacific Islands, and Latin America.

The comparatively lower variability detected in Africa, the Pacific and Latin America could be interpreted as a limited introduction of a small number of individuals in each of these regions. The genetic distances between each of these groups were relatively large, although the Pacific Islands and Latin American groups seemed related. Since black leaf streak/black Sigatoka was first observed in the Pacific Islands (Mourichon, Fullerton 1990), all or most of the introductions in Latin America may be derived from individuals from this region. In other cases, one of the factors responsible for the genetic differentiation between populations could be independent introductions of different genotypes in each region from Southeast Asia (Carlier et al. 1994).

It is clear that RFLPs are useful tools for detecting variation in *M. fijiensis* and for testing hypotheses about its origin and spread in banana-producing regions. RFLPs can provide more information on the geographical distribution of genetic variability of this pathogen.

Pathogenic variability

Several isolates of *M. fijiensis* from various countries and localities have already been tested for pathogenicity on young plants of a standard set of *Musa* cultivars and species. The first results, obtained in New Zealand (Fullerton, Olsen 1993), indicated that the individual isolates had consistent, but different patterns of pathogenicity on the host set.

Some cultivars and species were consistently susceptible to all isolates. Others were resistant to some isolates, but susceptible to others (differential reactions). Some isolates from the Pacific Islands and Papua New Guinea were pathogenic on cultivars and species commonly used as sources of resistance in breeding programs (Calcutta 4, Paka, Pisang Lilin).

The strains tested to date represent only a small proportion of those available. It is likely that an even greater diversity will be demonstrated as more strains are evaluated. This work is in progress at CIRAD, Montpellier.

It is suggested that the genetic diversity is a result, to a great extent, of the sexual stage and, above all, the clearly demonstrated heterothallic nature of *M. fijiensis*. Under specific conditions, the perfect stage of *M. fijiensis* can be produced in vitro (Mourichon, Zapater 1990), permitting the study of mechanisms of the genetic variability of pathogenicity.

Outcomes

The first step in breeding for resistance is the identification and characterization of the resistant germplasm in a breeding program. It is important that the most useful resistance genes or gene combinations be identified. Knowledge of pathogen variation and its distribution allows for more accurate assessment of resistant germplasm.

a) A useful outcome of population structure analysis is that it will provide tools for assessing levels of resistance. Germplasm and breeding lines could be inoculated with selected pathogen strains that contain high pathogen diversity. It has already been demonstrated that *Musa* germplasm, which reacts favorably in the laboratory, does well in farmers' fields. This approach is based on the assumption that the pathogen populations used in screening tests are representative of those in farmers' fields. If the structures of the pathogen population at both the screening sites and in farmers' fields are understood, varieties can be tested at appropriate locations with a minimum number of isolates that represent the full diversity of the pathogen population.

b) Understanding the structure of the pathogen population will contribute to the improvement of disease management by allowing resistance genes and plant genotypes to be identified and characterized relative to the spectrum of pathogen diversity (in terms of virulence and aggressiveness). It will also provide a knowledge base directly relevant to the design of strategies for cultivars deployment incorporating durable black leaf streak/black Sigatoka resistance.

Development of Early Screening Methods for determining Reaction to Black Leaf Streak/Black Sigatoka Disease

Introduction

Field evaluation under conditions of natural infection was for a long time the only method available for selecting banana cultivars with resistance to black leaf streak/black

Sigatoka. This method does not require prior knowledge of plant population systems and host-parasite interactions. Field selection is, therefore, uncertain and lengthy, especially in the case of breeding for quantitative characteristics with a polygenic background.

Although field screening methods are relatively simple, they are limited by environmental factors (climate, soil) that affect the expression of resistance. Field evaluation should, therefore, be validated by comparisons with reference cultivars in multilocation trials. Moreover, a large number of observations is required due to the uneven inoculum pressure and the irregular development of infection.

Field performance of an accession remains the ultimate reference when host-plant resistance is evaluated. However, random conditions and high cost make screening procedures under natural field conditions unsuitable as a rapid and reliable test for identifying resistant genotypes. A major goal of plant pathologists is, therefore, to increase the efficiency of breeding for disease resistance by developing early selection methods for preliminary screening.

Research objectives at CIRAD

A research program aims at increasing the efficiency of the overall breeding process for durable resistance to black leaf streak/black Sigatoka disease through three complementary approaches:

– the improvement of artificial inoculation techniques under controlled conditions;
– the development of biochemical markers-assisted selection;
– the use of in-vitro screening techniques with *M. fijiensis* toxins.

Previous and related research

Artificial inoculation under controlled conditions represents the first step towards a simplification of selection procedures. Observations of artificially inoculated and acclimatized microplantlets are recorded over a single cycle of infection in which symptom development must conform to mature plant behavior under natural multiple infection cycles (Beveraggi et al. 1992; Mourichon et al. 1987). Previous research has already described appropriate methods for producing inoculum (conidial suspensions), for carrying out inoculations (Mourichon et al. 1987) and for evaluating the intensity of symptoms.

As has been previously shown (Fouré et al. 1990), three kinds of behavior characterize all wild and cultivated varieties (AA, AAA, AAB, ABB).

a) High resistance (HR), as shown by Yangambi Km5 and some wild and cultivated AAs. This response is characterized by a natural blocking of the disease in the early infection stages.

b) Partial resistance (PR), characterized by slow disease development from the appearance of symptoms up to necrosis. Several levels of partial resistance may be observed.

c) Susceptibility (S), which is characterized by rapid disease development.

Host-parasite interaction studies have been undertaken in the laboratory by using an experimental host-parasite system which included representative cultivars of each group: HR, PR, and S (Beveraggi et al. 1992; Mourichon et al. 1987; Mourichon et al. 1988; Mourichon et al. 1989).

Analysis of healthy and inoculated tissues, as well as biochemical and cytological studies (photonic, electronic and fluorescence microscopy) display two distinct interactions.

a) The PR character seems to be linked with a constitutive phenolic component and with the presence of specialized polyphenol-storing cells in parenchyma. Once the material is released into the intercellular spaces, it comes into contact with the fungal hyphae and limits their spread into the parenchyma. It has been demonstrated that this polyphenolic material may not have a crucial role in the early infection stages, but it has a definite effect once necrosis begins.

b) The HR character of Yangambi Km5 is related to a hypersensitive reaction and could be associated with the increase of activity of hydrolytic enzymes.

In-vitro screening techniques using toxins have been successfully applied for some host-pathogen interactions in which the metabolites produced by the pathogen were primary or secondary determinants of pathogenicity. Phytotoxic compounds were found in culture filtrates of *M. fijiensis* (Upadhyay et al. 1989), but their importance in the infection process has yet to be demonstrated.

The role of the toxic metabolites of *M. fijiensis* in pathogenicity and their utilization in selecting germplasm resistant to black leaf streak/black Sigatoka disease has been studied at the Faculté des Sciences Agronomiques de Gembloux (Lepoivre, Acuna 1989; Lepoivre et al. 1992). Susceptible, partially resistant, and highly resistant cultivars were evaluated for their reaction to toxins of *M. fijiensis*. Bioassays were developed to quantify the toxic effect of metabolites obtained from *M. fijiensis* culture filtrates using detached leaves and mesophyll cell suspensions. This initial study at Gembloux indicated that toxins are probably not involved in symptom initiation, but could serve as secondary determinants of infection that contribute to lesion expansion.

Research findings at CIRAD

Artificial inoculation under controlled conditions

Inoculation procedures must be able to assess qualitative (highly resistant cultivars) and quantitative resistance (partially resistant cultivars) in using a set of "pathotypes" that represent the full diversity of the pathogen populations in terms of "virulence" and "aggressiveness".

Inoculation procedures to assess qualitative or vertical resistance are simple and do not require rigorous control of inoculum and incubation conditions. On the other hand, for quantitative (horizontal) resistance, inoculum level, host age, and incubation and postinoculation, conditions must be strictly controlled.

Artificial inoculation should not be so severe as to overestimate the capacity of an agent to cause disease or to underestimate the resistance of the host.

Further complementary research is needed to study all these factors in order to assess qualitative and quantitative differences in susceptibility, quality of inoculum, physiological age of acclimatized plantlets, and environmental conditions prior to and, especially, after inoculation.

Biological markers of resistance

Preliminary findings about the biochemical mechanisms involved in disease development of highly and partially resistant cultivars show the urgent need for more information. This will permit better management of banana breeding strategies, particularly for durable resistance to black leaf streak/black Sigatoka.

The significance of highly resistant genotypes has to be analyzed in terms of the genetic determinant of this resistance (usually mono- or oligogenic) and in terms of pathogen specificity.

Partial or horizontal resistance is often multigenic, emphasizing the necessity for marker-assisted selection. Therefore, further studies are needed to determine the degree of involvement of preformed polyphenol storing cells. The findings would indicate the significance of this component and the need to identify markers (preformed compounds) related to PR.

In-vitro screening techniques using *M. fijiensis* toxins

The results from Gembloux indicating that toxins are not the primary determinants in the host-pathogen interaction are in agreement with an electron microscopic study conducted at CIRAD which showed that *M. fijiensis* develops between the spongy mesophyll cells in the boundary zone of the lesion. This type of biotrophic development does not suggest the involvement of toxin(s) in initiating infection (Beveraggi et al. 1992). Slow lesion development in cultivars which display horizontal resistance is correlated with lower sensitivity to *M. fijiensis* culture filtrates.

The preliminary conclusions about the pathological significance of the toxins of *M. fijiensis* as screening agents need to be validated by:

– widening the number of reference cultivars showing partial resistance, evaluation of the relationship between the level of the resistance to the level of infection (under natural field inoculation), and the sensitivity of germplasm to the toxic compound produced in vitro by *M. fijiensis*;

– comparing the behavior of intact plants towards the toxins (detached leaf assay) and the sensitivity of banana tissue expressed in vitro (callus and cell suspensions).

The results can be used to develop an appropriate screening technique. The toxins can be applied to the detached leaves of plantlets or tissue cultures in vitro (callus, cell suspensions, protoplasts) if it is confirmed that toxin tolerance will be expressed in the regenerated plants.

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Fusarium Wilt

Fusarium Wilt and IMTP Phase II

RC Ploetz

Introduction

Fusarium wilt (Panama disease) is a lethal disorder of banana. The causal soilborne fungus, *Fusarium oxysporum* f.sp. *cubense* (*Foc*), colonizes and occludes the xylem of susceptible cultivars to cause a terminal wilt. Chemical control measures are not effective against the disease and soils, once infested with the pathogen, cannot be planted with susceptible clones for up to 30 years.

Many important banana cultivars and most of the world's significant banana-production areas are affected by Fusarium wilt (Stover, Simmonds 1987). Despite its widespread distribution, however, the disease is best known for its impact on a relatively small segment of world production, the export trades (Stover 1962). The trades began in about 1870 with essentially one cultivar, Gros Michel (AAA) From 1890 to the early 1960s, about 40,000 ha of Gros Michel were destroyed by Fusarium wilt, causing economic loss for exporters and economic hardship in areas responsible for and dependent upon a viable banana industry. By the end of the Gros Michel era, the trades were forced to convert to cultivars of the Cavendish subgroup (AAA).

Although the Cavendish cultivars still perform well in most export situations, Fusarium wilt remains a production constraint on other clones. The purpose of this paper is to review the history and epidemiology of Fusarium wilt and to examine recent studies on pathogen variability that help interpret the historical data. Approaches and realistic goals for the Fusarium wilt portion of IMTP Phase II are summarized in light of these results.

Disease History and Epidemiology

Early events

Fusarium wilt was first reported in Australia on Sugar (AAB 'Silk') (Bancroft 1876). However, most of the early research on the disease concerned Gros Michel in the export trades. It was observed in export plantations as early as 1890 in Costa Rica and Panama and, shortly thereafter, in other areas in tropical America and Africa (Stover 1962).

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Although much of what is written below describes the export situations, these observations are also relevant to other cultivars and IMTP Phase II.

The reports in Costa Rica and Panama forecast Gros Michel's ruin in the trades. Considering the destructive capabilities of Fusarium wilt and the high susceptibility of Gros Michel, it is surprising that this required 70 years. Several aspects of the early history in tropical America were puzzling. First, the disease was noticed a long time after susceptible cultivars were first introduced to many areas (Stover 1962). For example, it was reported on Gros Michel in Jamaica 66 years after the cultivar was taken to the island, and in Martinique after 100 or more years. Even longer lapses of time were noted in other areas for nonexported cultivars such as Silk and Bluggoe (ABB). It is probable, though hardly provable, that these extended intervals reflected rare introductions of the pathogen that took many years to become evident (VCG data, presented below, support the hypothesis of rare introductions to the new world from Asia). It is also likely that the heterogenous, backyard nature of early plantings had an effect.

Monocultures are powerful driving forces in epidemics. The presumed lack of monocultures in the early days probably retarded recognition of Fusarium wilt in the above situations (the death of a few affected plants in mixed or intercropped plantings would be less obvious than the death of large numbers of the same cultivar in a plantation), whereas their use by the export trades undoubtedly sealed the fate of Gros Michel.

Soon after the export trades were established, Fusarium wilt was recognized throughout tropical production areas. Still, the incidence, severity, and progression of the disease on Gros Michel was quite variable. Production was sustained for 20-30 years in many areas, but for a far shorter time in others (Stover 1962). In Surinam, major plantations were started in 1904, but had to be abandoned by 1912, whereas production stopped after 12 years in the Quepos area in Costa Rica. When the re-establishment of production was attempted in infested areas after flood fallow, this too resulted in only brief periods of productivity. For example, Gros Michel was produced for only 9 years (1950-59) in the Almirante II area in Panama.

Why Gros Michel was quickly decimated in some areas and not in others is not entirely clear, but several reasons could be proposed. If the assumption of rare introductions of *Foc* to the new world areas is valid, the original distribution of the pathogen within and among plantations must have been very sporadic. This would have affected disease incidence and development, and could have been a major cause for the inconsistencies noted above. Also, some soils are known to suppress the development of Fusarium wilt (Marois 1990; Toussoun 1975). The so-called "long-life" soils allow production to continue in spite of the presence of the pathogen, and may be more widely spread than is currently appreciated (e.g. Shepherd et al. 1987; Stover 1990). Finally, the soilborne nature of the disease surely played a role in the longevity of the Gros Michel-based trades. Compared with airborne diseases such as black leaf streak/black Sigatoka, Fusarium wilt moves more slowly within and among plantations (long-distance spread is almost exclusively restricted to the movement of infested rhizomes by man). Thus, even

after *Foc* was introduced into a plantation, it may have been many years before damage progressed to where it was perceived as a problem.

Recent events

Recent developments have redefined some of the old assumptions about Fusarium wilt while confirming others. These events also provide new perspectives on the disease and pathogen in different locations and on different hosts.

Race 4 has been the most visible of the new developments due to its impact on cultivars used by the export trades and the fact that the affected areas are in developed countries that have resources and scientific personnel to investigate this problem. The appearance of race 4 raises questions about the resistance of Cavendish cultivars and rekindles concerns that the export trades will lose another trade standard to Fusarium wilt.

Less well publicized are situations in East Africa and Asia. In East Africa, Fusarium wilt has affected production of introduced cultivars for only about 40 years and the traditional highland cultivars (AAA) for perhaps not more than 10 years (Ploetz et al. 1990). Since the short East African history is fairly well documented, it provides a rare opportunity to evaluate temporal development of Fusarium wilt in a newly affected area. In contrast, the Asian situation involves endemic populations of *Foc* that have probably coevolved with their banana hosts for millennia. The disease's history cannot be studied in this region, but evaluations of the affected clones and pathogen variability here are quite useful.

Race 4

Although far more Cavendish is grown by export trades in the tropics than in the subtropics, export production of these clones nonetheless occurs in the latter areas. Some fruit is exported from the Canary Islands to Spain, from the Madeira Islands to Portugal, and from Taiwan to Japan. Production of Cavendish cultivars for domestic consumption is more common in the subtropics and it occurs in at least 15 countries (Stover, Simmonds 1987).

Traditionally, the distribution of race 4 was thought to be limited to the subtropics in the eastern hemisphere (Ploetz 1992). As the number of reports on Cavendish clones increases, however, a re-evaluation of this judgment would seem warranted (see below).

Su et al. (1977) described race 4 as a distinct variant of *Foc* in Taiwan, some 50 years after damage to Dwarf Cavendish (AAA 'Cavendish') was noted in the Canary Islands (Ashby 1926). Ashby's report from Tenerife was followed by reports in 1940 on Dwarf Cavendish in South Africa (Natal) (Stover 1962), in 1953 on Williams (AAA 'Cavendish') in Australia (Queensland) (Purss 1953), and in 1967 on Giant Cavendish (AAA 'Cavendish') in Taiwan (Su et al. 1977). Losses now also occur in the Madeira archipelago and on La Palma, La Gomera, El Hierro, and Gran Canaria in the Canary Islands (Hernandez et al. 1993); Transvaal in South Africa (Ploetz et al. 1990); and New South Wales and Western Australia (Ploetz et al. 1990; Shivas et al. 1994). Nonconfirmed reports of Fusarium wilt on Cavendish clones also exist from the western hemisphere in Brazil (JA Ventura, pers. comm.).

The most significant damage to Cavendish cultivars in the subtropics now occurs in Australia (southern Queensland and northern New South Wales), South Africa (Transvaal), and Taiwan (Ploetz et al. 1990). In each of these areas, major research efforts are under way on pathogen characterization and the identification of clones that could replace the standard Cavendish cultivars.

Predisposition of some sort apparently contributes to the race 4 outbreaks in the subtropics. Either relatively cool temperatures during winter months or high water tables (Taiwan) seem to be required for Fusarium wilt to develop on the Cavendish cultivars in these areas. Workers in Australia have recently investigated the influence of winter temperatures on Williams and Dwarf Parfitt (AAA 'Cavendish'), cultivars that are, respectively, susceptible and resistant to race 4 (Moore et al. 1993; N Moore, pers. comm.). Their results indicate that Williams suffers chlorophyll degradation and reduced photosynthetic competency during the winter, which are responses that also occur, but to a lesser extent, in Dwarf Parfitt. Although it is not known whether these responses are responsible for the susceptibility or resistance of these cultivars, it is possible that gel and tylose formation in the xylem, host reactions that are required for resistance (Beckman 1990) and which are presumably dependent upon photosynthesis, are compromised in Williams during cold weather. A more complete understanding of the relationship between reduced photosynthesis during cold weather and the development of Fusarium wilt in the subtropics awaits further work.

More than 30 years after the Cavendish clones were used to replace Gros Michel, they continue to be the mainstays of the tropical export trades. In general, damage on the Cavendish clones in the tropics mimics that in the subtropics in that it occurs where some sort of predisposition is found (Ploetz et al. 1990). For example, pockets of damage in Guadeloupe and Jamaica occur above 700 m in elevation in lowlying or poorly drained soils, and on Mindanao in the Philippines where suboptimal edaphic conditions exist.

When the performance of Cavendish cultivars in the tropics is evaluated, it is useful to note where the trades have traditionally been located. Until recently, export production has occurred in areas in which banana is not endemic. Since these areas are now known to be represented by a narrow segment of the world *Foc* population (see below), a relevant question might be: Would Cavendish plantations succumb in areas in which *Foc* diversity is greatest; i.e. where *Musa acuminata* evolved and the AAA dessert clones originated? Based on recent events, monocultures of the Cavendish clones might not fare as well in Southeast Asia as they have in tropical America.

East Africa

Fusarium wilt first appeared in East Africa in the early 1950s. After its initial identification in Kenya, northern Tanzania, and Uganda, the disease spread to most of the remaining countries in the region (Anonymous 1954; Jameson 1953; Ploetz et al. 1990, 1992; Ploetz 1992; Sebasigari, Stover 1988; Wallace 1952). Fusarium wilt now also occurs in Burundi, Malawi, Rwanda, Zaire, and the Pemba and Zanzibar islands of Tanzania. Nonconfirmed reports indicate that the disease might also be found in southern Tanzania and Zambia (Ploetz et al. 1992) (Fig. 1).

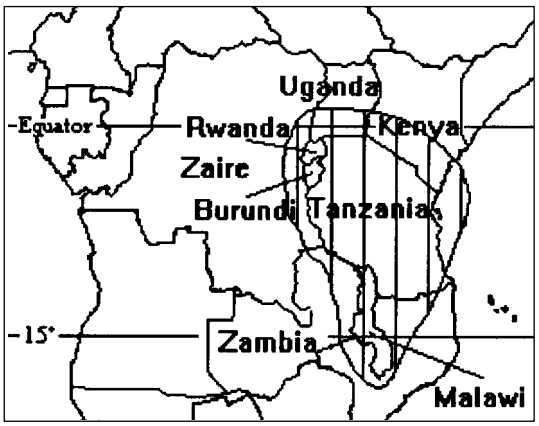

Figure 1. Current distribution of Fusarium wilt in East Africa.

In most cases, the affected cultivars were recently introduced to East Africa, probably after the second world war: These include: Gros Michel; Sukari Ndizi (AB 'Ney Poovan'); Silk and Pome (AAB); Bluggoe and Pisang Awak (ABB). In general, the introduced cultivars are valued for their tolerance of drought, poorer soils, the banana borer (*Cosmopolites sordidus*), nematodes (*Pratylenchus goodeyi* and *Radopholus similis*), and good taste or beer-making qualities (Sebasigari, Stover 1988). In addition, the exotic clones require less intensive management than the highland cultivars (Tushemereirwe 1993). For these reasons, the exotics have begun replacing the traditional highland cultivars in many areas.

Until recently, Fusarium wilt had not been observed on the highland cultivars. Limited damage now occurs in Uganda and Zaire above 1400 m in elevation (Ploetz et al. 1990; Tushemereiwre 1993; Tushemereiwre, Ploetz 1993). Incidence in affected fields is usually <5%. Symptoms include abnormally thin pseudostems and incomplete filling of fingers. Atypically, minimal or no foliar chlorosis and wilting occurs on affected plants, and vascular discoloration rarely progresses into the pseudostem. Since these appear to be recent outbreaks, the potential for future damage is unclear.

Asia

The first reports of Fusarium wilt in Asia came from India in 1911, Java in 1916, and several other countries in the 1920s (Stover 1962). Fusarium wilt is widespread in the region and affects many preferred and commercially important cultivars (bin Doon 1991; Chandra 1991; Hwang 1991; Muharam, Subijanto 1991; Pegg et al. 1993; Roperos, Magnaye 1991; Singburaudom 1991; Valmayor 1990). Although the disease is found in many of the areas in which *M. acuminata* and *M. balbisiana* originated (Simmonds 1962), some of the endemic areas are not affected (Figs 2,3). How New Guinea (Papua New Guinea and Irian Jaya), southeastern Indonesia, and the Bismarck archipelago have remained free of Fusarium wilt is somewhat puzzling, considering the disease's long history in neighboring areas and the presence of susceptible wild species (e.g. *M. acuminata* ssp. *banksii*). Presumably, the geographic and cultural isolation of these islands has hindered the movement of *Foc*.

Unfortunately, information on Fusarium wilt in Asia is somewhat limited. Research on banana diseases is generally unavailable from some of the countries (e.g. Cambodia, Laos, Myanmar, and Viet Nam). Also, information that exists in some of the other areas

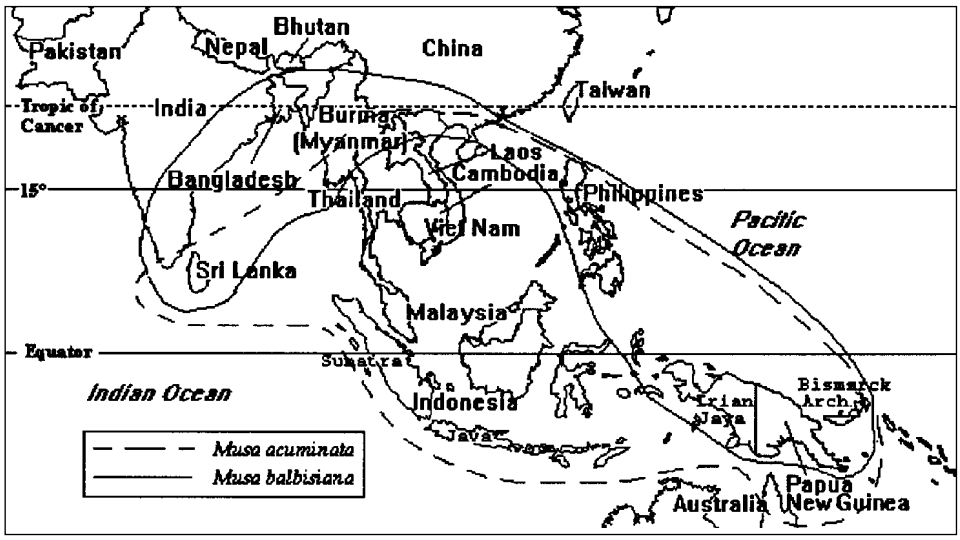

Figure 2. Geographic distribution of *Musa acuminata* and *M. balbisiana*. Note that substantial overlap exists in the species' ranges. It is in these regions that hybrids between the two species presumably arose (taken from Simmonds 1962).

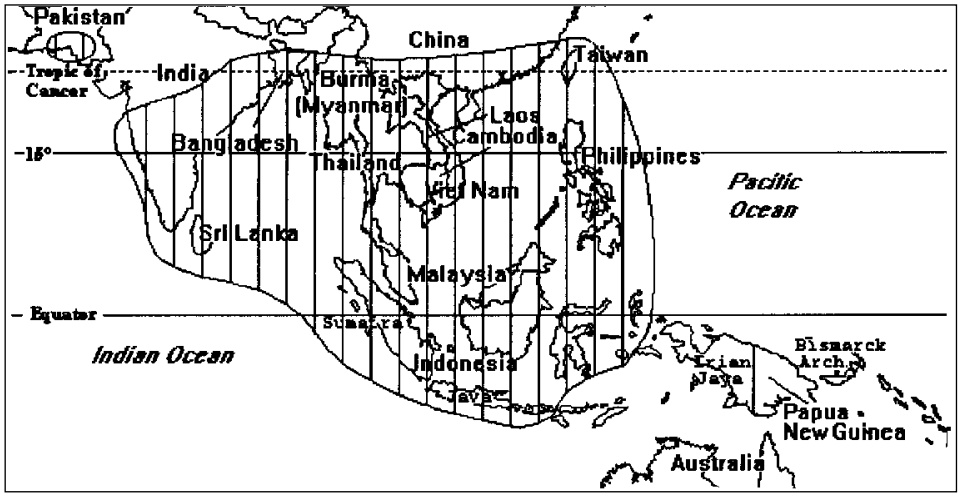

Figure 3. Current distribution of Fusarium wilt in Southeast Asia and the Indian subcontinent.

may be anecdotal or confused by the plethora of cultivar synonyms or the esoteric (parochial bias recognized) cultivars that are affected.

The diversity of affected clones is greatest in an area centered in Malaysia, Sumatra, and Java (bin Doon 1991; Muharam, Subijanto 1991; Pegg et al. 1993). AA and AAA dessert (e.g. Pisang Barangan, Pisang Ambon Putih [Gros Michel]), AAB dessert (e.g. Pisang Rastali [Silk]), and ABB cooking (Pisang Awak, Pisang Kepok [Saba], and Pisang

Siem [Pisang Apu]) cultivars succumb here, as well as others with unclear affinities (e.g. Pisang Batan). In addition, two other cultivars that normally resist the disease, Pisang Mas (AA 'Sucrier') and Grande Naine (AAA 'Cavendish'), have been recently affected in commercial plantations. Since the above cultivars are among the most important in Asia, Fusarium wilt exerts a considerable impact on banana production.

To service Oriental and Middle East markets, Cavendish plantations have increased in Asia. Within the last 2 years, damage to plantations of the Grande Naine and Valery (AAA 'Cavendish') has occurred in Malaysia and Sumatra (KG Pegg, pers.comm.; Ploetz, unpublished). Although it is still too early to attach much significance to these reports, it is interesting to note that *Foc* isolates from the Sumatra location are in a race 4 VCG, 01213 (see below). Obviously, future attention to these outbreaks is warranted.

Pathogen Variability

Races

Three races of *Foc* affect banana. Race 1 was responsible for the epidemics on Gros Michel in export plantations in tropical America, Africa, and elsewhere, and race 2 damages Bluggoe and other, less-widely grown, clones. Race 4 is virulent on race 1 and race 2 suscepts, in addition to most of the Cavendish cultivars.

Pathogenic variation exists within each of the races described above. It is well recognized that the term "race" in *Foc* does not imply a defined, genetic relationship with the host, as occurs with other pathosystems (e.g. *Puccinia*: wheat). Races of *Foc* are simply groups of strains that are pathogenic on the differential cultivars (Stover, Buddenhagen 1986). Other races of *Foc* might emerge if cultivars other than the current differential set (i.e. Gros Michel, Bluggoe, Cavendish) were used (Ploetz et al. 1990).

Pathogenicity tests are needed to investigate unusual situations in East Africa and Southeast Asia. In East Africa, strains of the pathogen in the same VCG are attacking Pisang Awak and Bluggoe, race 1 and race 2 suscepts, respectively, in addition to Ney Poovan, a cultivar that is reported to be resistant to Fusarium wilt elsewhere (Ploetz et al. 1990, 1992; Ploetz 1990; Stover, Simmonds 1987). Thus, a new "race" may be involved. In turn, the anomalous situations in Malaysia and Sumatra on Cavendish cultivars that were mentioned above suggest a broader ecological niche for race 4 than was previously suspected. Although pathogenicity tests have generally not been conducted, the diversity of affected clones in Southeast Asia also suggests that pathogenic variability is extensive in the region. Much could be learned about the banana:*Foc* pathosystem via studies in this area of the world.

What further work in this area will reveal regarding the pathogenic range of this fungus remains to be seen. With previous experience as a guide, however, it is probable that cultivars that were considered resistant will eventually succumb in geographic areas or agroecosystems (especially plantation monocultures) in which they were previously not grown.

Vegetative compatibility

That species of fungi are amalgams of genetically and geographically separated populations has been appreciated relatively recently. With this appreciation has come a recognition of the importance of populations, as opposed to individuals, when one considers the biology, ecology, and pathology of these fungi: species can be diverse and evolving aggregates instead of the rigid and mostly uniform units that were previously envisioned.

A trait that is often used to identify genetically isolated populations of fungal plant pathogens is vegetative or somatic compatibility (Leslie 1993). Vegetative compatibility defines clonally derived populations of individuals. For many fungi, particularly those without a sexual cycle, vegetative compatibility groups (VCGs) are the relevant evolutionary units within the species. Isolates in a VCG are often in the same race and usually share similar geographic ranges and physiological characters.

The first reports on vegetative compatibility in *Foc* were published about 7 years ago (Correll, Leslie 1987; Ploetz, Correll 1988). These and subsequent studies have delineated two ancestral populations of *Foc*, VCG 0120 and VCG 0124-0125, that are represented throughout world banana-production areas. The existence of two progenitor VCGs was proposed early in the work on *Foc* VCGs based on pathogenic and geographic diversity that was evident in the world *Foc* collection in Homestead (Ploetz 1990). Whereas other VCGs are represented seldomly in more than two areas, and never in more than a single race, VCGs 0120 and 0124-0125 are found in numerous countries and are both pathogenically variable (e.g. Table 1). These results also indicate that VCG diversity in *Foc* is unusually high for a forma speciales of *F. oxysporum.* Other forma speciales of this species usually contain no more than three or four VCGs (e.g. Bosland, Williams 1987; Correll et al. 1986; Larkin et al. 1990), but there are at least 20 VCGs in *Foc* (N Moore, pers. comm.; Pegg et al. 1993; Ploetz 1993) (Table 1).

VCG diversity in *Foc* is most pronounced in Asia, home of *M. acuminata* and *M. balbisiana* and the observed pathogenic diversity described above (Pegg et al. 1993; Ploetz 1993). In fact, in only a few situations are there VCGs outside Asia that are not also found in the region. In the Americas and Africa, relatively few VCGs have been described that are unique to these regions. These results alone suggest that *Foc* probably did not originate in these areas and that minimal evolution of *Foc*, at least for vegetative compatibility, has occurred outside Asia.

Although data are lacking for some of the areas, VCG diversity in Asia appears to be distributed in discrete areas and is highest in Southeast Asia where the greatest number of *M. acuminata* subspecies are found. Based on the VCG data, and other characteristics of the isolates they studied, Pegg et al. (1993) suggested that the ancestral VCGs 0120 and 0124-0125 evolved independently in the centers of diversity for *M. acuminata* and *M. balbisiana*, respectively, and that less widely distributed VCGs probably arose from one of the progenitor VCGs by mutation, a hypothesis that had been proposed earlier (Ploetz 1990).

Genetic studies illustrate the uniformity of isolates in the various *Foc* VCGs and allow comparisons within and among VCGs to be made (Boehm et al. 1994; Koenig et al. 1993;

Table 1. Summary of VCG results for *Fusarium oxysporum* f.sp. *cubense*.

VCGs	Locations
0120	Australia, Brazil, Canary Islands, Costa Rica, Guadeloupe, Honduras, Indonesia, Jamaica, Malaysia, South Africa
0121	Taiwan
0122	Philippines
0123	Malaysia, Philippines, Taiwan, Thailand
0124-0125	Australia, Brazil, Burundi, China, Cuba, Florida, Honduras, India, Jamaica, Malawi, Nicaragua, Tanzania, Thailand, Uganda, Zaire
0126	Honduras, Philippines
0128[1]	Australia, Comores Islands
0129	Australia
01210	Cuba, Florida
01211	Australia
01212	Tanzania
01213	Indonesia, Malaysia, Taiwan
01214	Malawi
01215	Costa Rica, Indonesia
01216-01220[2]	

[1]VCG 0127, originally reported by Ploetz and Correll (1988), is no longer considered a valid VCG of *Foc* and is not listed here.

[2]At least five new VCGs have become evident in recent work on isolates from Southeast Asia (N Moore, pers. comm.).

Sorensen 1994). These studies corroborate the previously proposed ancestral lines for *Foc*. Group I and Group II (not to be confused with VCGs) that are now recognized are comprised, respectively, of isolates in VCGs 0120 and 0124-0125 and their presumed progeny VCGs. For example, randomly amplified polymorphic DNA (RAPD) and electrophoretic karyotype (EK) data point towards close relationships among VCGs 0120, 0121, and 0129, all of which are Group I VCGs and contain race 4 isolates, but distant relationships between these VCGs and those in Group II. Additionally, the genetic data and the growing collection of isolates from Asia now allow the above hypothesis on the origins of some of the VCGs to be tested. Genetic diversity that is evident in worldwide populations of *Foc* again suggests that this pathogen arose in Asia.

The practical implication of this work is to verify that a relatively narrow genetic spectrum of *Foc* is distributed outside Asia, and that most or all of the *Foc* VCGs probably arose from either 0120 or 0124-0125. Only VCG 0123 presents some problems in that the genetic data are confused as to whether it is derived from a 0120 or 0124-0125

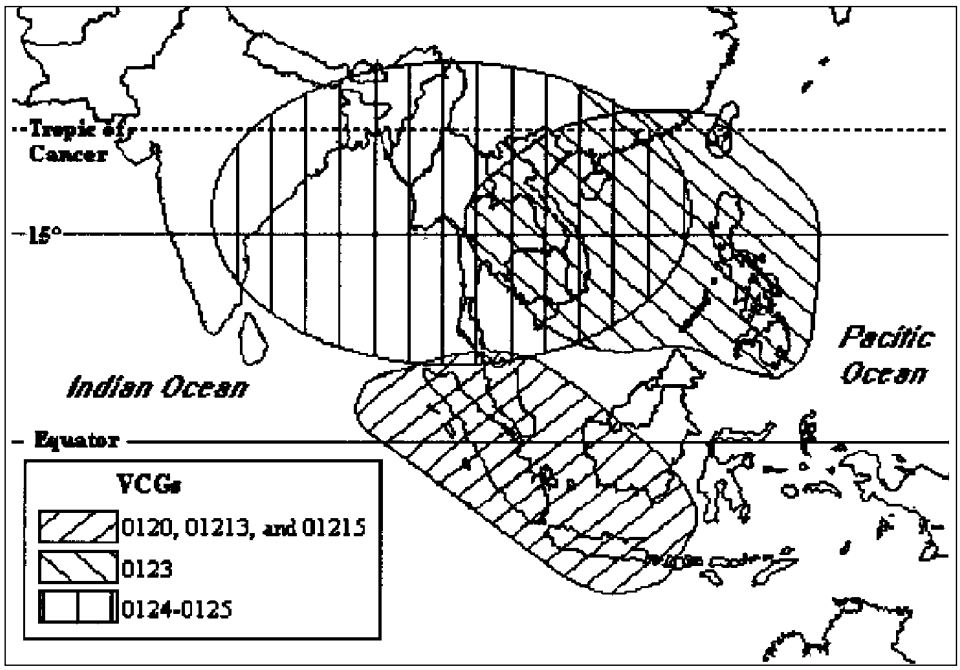

Figure 4. Distribution of the most prevalent *Foc* VCGs in Asia.

(i.e. in Group I or Group II). Although its overlapping geographic distribution with 0124-0125 (see Fig. 4) might suggest a relationship to Group II, more information is needed.

Looking Ahead to IMTP Phase II

To summarize, diversity in *Foc* is relatively narrow in the Americas and Africa. This is probably due to the relatively recent and, apparently rare, introductions of the pathogen to these areas from Asia. *Foc* is most diverse in Southeast Asia, especially where *Foc* and *M. acuminata* are presumed to have coevolved. Vakili (1965) noted correlations between the native ranges of various subspecies of *M. acuminata* and the presence of Fusarium wilt. Resistant subspecies (e.g. *burmannica*, *malaccensis*, *microcarpa*, and *siamea*) evolved in areas in which the disease was endemic, whereas the susceptible subspecies *banksii* arose in an area that was free of the disease (i.e. Papua New Guinea). Presumably, resistance developed in *M. acuminata* subspecies due to selection pressure exerted by the pathogen with which it coevolved. In turn, pressure was exerted on *Foc* populations for new pathotypes by the resistant host germplasm that was selected.

With regard to IMTP Phase II, it is clear that fewer screening sites may be needed in order to determine the performance of IMTP germplasm in the Americas and Africa, than in Southeast Asia. Significant effort should be made to conduct work in the centers of origin for *Foc* and banana, since it is in these locations that the most significant

information on host resistance and pathogen diversity could be obtained. It is also clear from this information that continued vigilance with quarantine restrictions needs to be observed; certainly, under no circumstance should international movement of germplasm occur in any form but as tissue culture plantlets. Obviously, the movement of *Foc* variants that are now becoming evident in Southeast Asia to other production regions could be disastrous.

Finally, in light of the soilborne nature of Fusarium wilt, it is important to reiterate the differences between this disease and an airborne disease such as black leaf streak/black Sigatoka. Even in well-prepared field sites, it is probable that the distribution and relative levels of *Foc* inoculum will be variable, and that the relative levels and ultimate detection of different pathotypes will also differ over time and according to the genotypes that are present in the field (e.g. Brake et al. 1990). Since these inconsistencies will probably cause some erroneous "resistant" ratings to occur, especially if these experiments are not of a sufficient duration, the screenings should be conducted for as long as possible.

With regard to black leaf streak/black Sigatoka, Fullerton and Olsen (1993) reported that Paka (AA) and T8 (AAAA) succumbed to an initially rare population of *Mycosphaerella fijiensis* only after 9 years in the same site on Rarotonga in the Cook Islands. They used these results to illustrate why a single year was probably inadequate for black leaf streak/black Sigatoka screenings during IMTP Phase I. For Fusarium wilt, an analogous interval for the detection of a rare pathotype might be in the order of 20 or more years. Although screening trials of such a length will, of course, be impossible in IMTP Phase II, it is important to realize that a single season may be insufficient to obtain all but some rudimentary data on the performance of the screening genotypes in many locations.

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Variability in Populations of *Fusarium oxysporum* f.sp. *cubense* from the Asia/Pacific Region

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Introduction

Asia is the center of origin of edible bananas. Cultivars with *Musa acuminata* characters (AA, AAA) originated primarily in the equatorial regions of Indonesia and Malaysia, whereas cultivars with both *M. acuminata* and *M. balbisiana* expression (AB, AAB, ABB) have their major center of origin in the more northern, monsoonal regions of Asia, such as India (Simmonds 1962). In 1965, Vakili (1965) considered Fusarium wilt to be endemic in Myanmar (Burma), Thailand, Indochina, and the Malayan Peninsula and suggested that the *M. acuminata* subspecies *burmannica*, *malaccensis*, *microcarpa* and *siamea* coevolved with *Fusarium oxysporum* f.sp. *cubense* (*Foc*). It is now widely acknowledged that *Foc* originated in Asia and was subsequently dispersed from this region in planting material (Buddenhagen 1990, Simmonds 1962, 1966). In Asia, banana is probably several thousand years old (Simmonds 1962). Some 2000 years ago, man carried planting material eastwards from Asia to the Pacific Islands and westwards to Africa. Several clones were then taken to the Americas from West Africa.

Fusarium wilt has long been a problem in the Australian banana industry. It is interesting to note that the first world recording of this disease was made in southeast Queensland in 1874 (Bancroft 1876). Surprisingly *Foc* has not been found in Papua New Guinea and the South Pacific Islands (Jones 1988, Pegg et al. 1993) where numerous susceptible banana cultivars exist. This suggests that the pathogen does not readily arise from nonpathogenic or other parasitic populations of *F. oxysporum*. The only report of Fusarium wilt occurring in the Islands of the Pacific was from Hawaii in 1904 (Stover 1990) when it was recorded in Gros Michel plants introduced from Nicaragua in 1903. Fusarium wilt was recently recorded in Pisang Rubus (ABB) at Manokwari, Irian Jaya. The pathogen was apparently introduced to this area with infected planting material as a result of recent transmigration from Java to Irian Jaya.

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In-Vitro Research: Australia

Several different techniques have been used to study biodiversity in *Foc* populations from Australia. These include vegetative compatibility analysis, the production of volatile compounds on starch substrate, and the molecular technique of RAPD-PCR.

Vegetative compatibility

Ploetz and Correll (1987) reported vegetative compatibility groups (VCGs) among a worldwide collection of *Foc*. Sixteen VCGs have now been identified (RC Ploetz, pers. comm.). Vegetative compatibility, which is genetically controlled, has been used to divide Australian isolates of *Foc* into six VCGs (Brake et al. 1990, Moore et al. 1993). Within Australian populations of *Foc* there is good correlation between VCG and race. The studies by Brake et al. (1990) and Moore et al. (1993) reported that race 1 isolates of *Foc* belonged to VCGs 0124, 0124/5, 0125 and were widespread in Lady Finger (AAB 'Pome') plantations in Queensland and New South Wales. Race 2 isolates were placed in VCG 0128 and were recorded from two plantings of Bluggoe and other ABB cooking banana cultivars in northern Queensland. Race 4 isolates belonged to VCGs 0120, 0129 and 01211 and were recorded in Williams (AAA 'Cavendish') and Lady Finger plantations in southern Queensland and northern New South Wales. Isolates belonging to a new VCG were recently found attacking Williams growing in waterlogged soils at Carnarvon in Western Australia (Pegg et al. 1994).

Volatile production

The production of volatile compounds from isolates of *Foc* cultured on starch substrates has been used to differentiate strains of this pathogen. Brandes (1919) reported that isolates of *Foc* grown on steamed rice and different liquid media either produced or did not produce a characteristic benzolic odor. Isolates from Panama, Costa Rica, and Jamaica produced 'aromatic aldehydes', whereas those from Cuba did not produce these compounds and were named *Fusarium cubense* var *inodoratum* (Brandes 1919). It was later reported by Stover (1959) that isolates of *Foc* from the rhizomes of diseased Gros Michel bananas could be divided into two major clonal groups: designated as 'odoratum' and 'inodoratum' due to the presence or absence of a pronounced odor when cultures were grown on peptone dextrose agar or steamed rice for 4-8 days. Stover (1962) later used gas chromatography to demonstrate that the volatile substances produced by 'odoratum' strains were distinctly different from those produced by 'inodoratum' strains.

Stover's (1962) technique has been used in conjunction with gas chromatography analysis to differentiate between Australian isolates of *Foc* of known VCG and pathotype (Moore et al. 1991). Absolute correlation between the production of volatile substances, VCG and pathogenicity was found for the 57 Australian isolates tested; isolates in race 1 (VCGs 0124, 0124/5, 0125) and race 2 (VCG 0128) did not produce a detectable odor and gave gas chromatogram profiles with no peaks, whilst isolates in race 4 (VCGs 0120, 0129 and 01211) produced easily detectable volatile odors which were qualified by characteristic gas chromatogram profiles. Moore et al. (1991) reported that the volatile

compounds produced were so distinctive that independent researchers had no difficulty in classifying cultures as 'odoratum' or 'inodoratum'. Furthermore, a detectable difference in the nature of the volatile odors of isolates in VCGs 0120 and 01211 from that of isolates in VCG 0129 was qualified by gas chromatography (Moore et al. 1991). Isolates in VCG 0120 and 01211 produced a 'sweet' benzolic odor and gave gas chromatogram profiles with six significant peaks, whereas isolates in VCG 0129 produced a 'nutty' benzolic odor and gave gas chromatogram profiles with only one significant peak (Moore et al. 1991). The single peak in the chromatograms of Australian isolates of *Foc* in VCG 0129 had the same retention time (3.1–3.2 min) as one of the six significant peaks in the chromatograms of Australian isolates in VCGs 0120 and 01211 (Moore et al. 1991).

RAPD-PCR

VCG characterization has provided a focus for the genetic characterization of isolates of *Foc* using molecular techniques such as RAPD-PCR, which permits greater discrimination between isolates. Although groupings made on the basis of vegetative compatibility reflect the genetic variability of the fungus, VCG analysis does not provide sufficient information on the extent of genetic variation within each VCG or the genetic similarity between different VCGs. Results from RAPD-PCR analyses of Australian isolates of *Foc* have validated the use of vegetative compatibility to assess the genetic structure of populations of this pathogen (Sorensen 1993, Sorensen et al. 1993).

Australian isolates could be divided into distinct groups based on their DNA banding patterns, which were consistent with groupings made on the basis of VCG analysis (Brake et al. 1990, Moore et al. 1993) and volatile production (Moore et al. 1991). Phylogenetic analysis of RAPD-PCR results of Australian populations of *Foc* (Sorensen 1993) suggest that strains of *Foc* in the race 4 VCGs 0120, 0129 and 01211 are very closely related and that isolates in the race 2 VCG 0128 are closely related to those strains in the race 1 VCGs 0124, 0124/5, 0125.

The RAPD-PCR technique was valuable for comparing isolates from the unique VCG present at Carnarvon in Western Australia (Pegg et al. 1994) with known race 1 and race 4 isolates. Comparison of the RAPD-PCR banding patterns both visually and by phenetic analysis indicated that the population of *Foc* at Carnarvon was more closely related to race 1 populations than to race 4 populations, and it is therefore predicted that this population is unlikely to cause widespread losses in Cavendish cultivars at Carnarvon.

In-Vitro Research: Asia

In this study, vegetative compatibility analysis, volatile production, and RAPD-PCR were used to characterize isolates of *Foc* from Asian countries which included China, India, Indonesia, Malaysia, the Philippines, and Thailand. One isolate from Irian Jaya, a region previously considered to be free from Fusarium wilt (Jones 1988; Pegg et al. 1993), was also included. Table 1 presents synonyms used for some of the common banana cultivars in the Asian region.

Table 1. Synonyms for banana cultivars in Asia (from Jamaluddin 1986; Valmayor et al. 1981; JW Daniells, pers. comm.).

Indonesia	Malaysia	Philippines	Thailand
Pisang Mas (AA)	Pisang Mas	Amas	Kluai Khai
Pisang Ambon Putih (AAA)	Pisang Embun	Ambon	Kluai Hom Dok Mai
Pisang Siem (ABB)	Pisang Awak	Katali	Kluai Namwa
Pisang Barangan (AA/AAA)	Pisang Berangan	Lakatan	-
Pisang Raja Sereh (AAB)	Pisang Rastali	Latundan	-
Pisang Raja (AAB)	Pisang Raja	Radja	-
Pisang Kepok (BBB)	Pisang Nipah	Saba	Kluai Hin

Vegetative compatibility

Of the 109 isolates of *Foc* from Asia characterized using vegetative compatibility analysis, 66 belonged in known VCGs (Table 2). Four new VCGs were identified among 27 isolates from Indonesia and Malaysia which could not be placed in any of the previously described VCGs (Table 3) and 16 isolates were not compatible with any known VCGs or among themselves (Table 4). Isolates from Indonesia, Malaysia, and the Philippines belonged in eight different VCGs, and most of the isolates of unknown VCG were also from these countries. By contrast, isolates from India and Thailand gave a very uniform VCG profile, with the majority of isolates belonging to the putative race 1 VCGs 0123, 0124 and 0125 (Table 2). Only one isolate was received from China, and this also belonged in VCG 0124/5 (Table 2).

Volatile production

Of the 119 Asian isolates tested for volatile production, 47 isolates were classified as 'odoratum' types and 72 belonged to the 'inodoratum' group. Of the 'odoratum' isolates, 30 belonged to previously described VCGs, 14 to the yet unnumbered Indo 25 and Mal 11 VCGs, and three were of unknown VCG. Of the 'inodoratum' isolates, 46 belonged to the known VCGs 0123, 0124 and 0125 and 13 belonged to the yet unnumbered Indo 5 and Mal 6 VCGs. Thirteen of the 'inodoratum' isolates were of unknown VCG.

Two types of chromatogram profile were generated by the 'odoratum' isolates. Isolates in VCGs 0120, 0122, 01213 and the Indo 25 and Mal 11 VCGs produced 'sweet' benzolic odors which gave chromatogram profiles with six significant peaks. One isolate from Indonesia (Indo 16) and two isolates from the Philippines (Phil 1 and 18), which were of unknown VCG, also produced a 'sweet' benzolic odor and gave chromatogram profiles with six significant peaks. These profiles were very similar to those produced by Australian race 4 isolates in VCGs 0120 and 01211. The second type of profile had two peaks and was produced by isolates in VCG 0126 from Indonesia and

(continued on p. 78)

Table 2. Vegetative compatibility among strains of *Fusarium oxysporum* f.sp. *cubense* from Asia (1991-94).

Isolate[1]	Cultivar	Origin
	VCG 0120	
Indo 14	Pisang Ambon Putih (AAA)	West Java, Indonesia
Indo 20^2, 22^2, 23^2, 24	Unknown cultivars	Jatesari, East Java, Indonesia
	VCG 0122	
Phil 10	Grande Naine (AAA)	Twin Rivers Plantation, Tagum, Davao del Norte, Philippines
	VCG 0123	
Mal 5	Pisang Awak (ABB)	Perak, Malaysia
Phil 3	Latundan (AAB)	Tagum, Davao, Philippines
Phil 8	"	Solana, Misamis, Oriental, Philippines
Phil 13	"	Bayugan, Agusan del Sur, Philippines
Phil 16	Latundan (AAB)	Abuyog, Leyte, Philippines
Phil 17	Abaca	Visca, Baybay, Leyte, Philippines
Phil 19	Latundan (AAB)	La Opinion, Camarines Sur, Philippines
Thai 1-2, 2-1, 2-2, 2-3	Kluai Namwa (ABB)	Ban Sakainam, Petchaboon, Thailand
Thai 3-1	"	Pakchong, Nakorn Rachasima, Thailand
Thai 4-2	"	Suwan Farm, Pak Chong, Thailand
Thai 10	"	Kampangsam, Thailand
Thai 19-1, 19-2	"	Central Plain, Thailand
Thai 20-2	"	Thasas, Chumporn, Thailand
	VCG 0124	
India 4	Poovan (AB)	locality unknown, India
India 8	Gros Michel (AAA)	locality unknown, India
India 10	Bareli Chinia (AAB)	Hajipur, India
India 11	Raja Vazhai (AAB)	Hajipur, India
India 12	Chinia (AAB)	Hajipur, India
India 13	Malbhog (AAB)	Hajipur, India
Thai 6-1	Kluai Namwa (ABB)	Ban Pa Kew, Kanjanaburi, Thailand

(Table 2, continued)

Isolate[1]	Cultivar	Origin
Thai 9-1, 9-2	"	Thongphapoom, Kanjanaburi, Thailand
Thai 12-2, 14, 15-1, 15-2	"	Kanjanaburi Province, Thailand
	Isolates cross-compatible with VCG 0124 and 0125	
China 1	Chinese Fen Jaio (ABB)	Chung Shan, Guangdong, China
India 5	Gros Michel (AAA)	locality unknown, India
India 6	Rasthali (AAB)	locality unknown, India
India 9	Rasabale (Rasthali) (AAB)	Mysore, Karnataka, India
Mal 25[1], 25[2]	Pisang Awak (ABB)	Batu 11, road from Padang Besar to Kangor, Perlis, Malaysia
Thai 8-1	Kluai Namwa (ABB)	Thaiyok, Kanjanaburi, Thailand
Thai 12-1, 13	"	Kanjanaburi Province, Thailand
Thai 17	"	Central Plain, Thailand
	VCG 0125	
India 1, 2	Mysore (AAB)	locality unknown, India
India 3	Rasthali (AAB)	locality unknown, India
Mal 24	Pisang Awak (ABB)	Kampong Pauh, Arau, Perlis, Malaysia
Thai 7-1, 7-2	Kluai Namwa (ABB)	Ban Thungna, Kanjanaburi, Thailand
	VCG 0126	
Indo 33	Pisang Manuring (ABB/BBB)	Sulawesi, Indonesia
Indo 38	Pisang Rubus (?)[3] (ABB)	Manokwari, Irian Jaya
Phil 6	Latundan (AAB)	San Carlos, Valencia, Bukindon, Philippines
Phil 7	Cardaba (ABB/BBB)	Villanueva, Misamis, Oriental, Philippines
	VCG 01213	
Indo 26/RP[1]	Pisang Kepok (ABB/BBB)	Sumatra, Indonesia
Indo 29/RP	Pisang Batan (?)[3]	Sumatra, Indonesia
Indo 30/RP	Pisang Susu (AAA)	Sumatra, Indonesia
Indo 31/RP	Pisang Lilik Tandom (?)[3]	North Sumatra, Indonesia
Indo 32/RP	Pisang Barangan (AA/AAA)	Sumatra, Indonesia
Indo 34	Pisang Barangan (AA/AAA)	Cibinong, Java, Indonesia

(Table 2, continued)

Isolate	Cultivar	Origin
Mal 15, 17	Pisang Mas (AA)	Kuala Kangsar, Perak, Malaysia
Mal 18	Pisang Raja (AAB)	MARDI, Selangor, Malaysia
Mal 20	Pisang Berangan (AA/AAA)	MARDI, Selangor, Malaysia

[1]Accession numbers from Department of Primary Industries, Plant Pathology Laboratories, Indooroopilly, Queensland. RP numbers donated by RC Ploetz from an international collection of *Foc* held at the University of Florida, Homestead, USA. [2]Isolate cross-compatible with VCG 01215. [3]Genomic constitution unknown.

Table 3. New vegetative compatibility groups identified among isolates of *Fusarium oxysporum* f. sp. *cubense* from Asia (1991-94).

Isolate[1]	Cultivar	Origin
	Indo 5 Group	
Indo 5, 7, 12, 13,18	Pisang Siem (ABB)	West Java, Indonesia
Indo 27/RP	Pisang Raja Sereh (AAB)	locality unknown, Indonesia
Indo 28/RP	Pisang Kepok (ABB/BBB)	North Sumatra, Indonesia
	Indo 25 Group	
Indo 25/RP	Pisang Ambon (AAA)	locality unknown, Indonesia
Indo 35	Pisang Raja Sereh (AAB)	Cibinong, Java, Indonesia
Indo 36	Pisang Raja Garing (?)[2]	Cibinong, Java, Indonesia
Indo 37	Pisang Ambon Putih (AAA)	Cibinong, Java, Indonesia
	Mal 6 Group	
23775	Pisang Rastali (AAB)	MARDI, Selangor, Malaysia
Mal 6	"	Kg. Taboh, Naning, Negri Sembilan, Malaysia
Mal 7	"	Kg. Kota, Sitiawan, Perak, Malaysia
Mal 8	"	Kg. Rimba, Repah, Negri Sembilan, Malaysia
Mal 10	"	Kota, Negri Sembilan, Malaysia
Mal 23	Pisang Kebatu (ABB)	Jelabu Sembilan, Negri Sembilan, Malaysia
	Mal 11 Group	
Mal 1	Pisang Raja (AAB)	Bendang Siam, Perak, Malaysia
Mal 2[1], 2[2]	"	MARDI, Selangor, Malaysia
Mal 11	Pisang Mas (AA)	Batu 2, Jalan Chaah, Johor, Malaysia
Mal 14	Pisang Raja (AAB)	Bendang Siam, Perak, Malaysia

(Table 3, continued)

Isolate	Cultivar	Origin
Mal 21	Pisang Berangan (AA/AAA)	Johor Tropical Products Holdings, Johor, Malaysia
Mal 22	Grande Naine (AAA)	Johor Tropical Products Holdings, Johor, Malaysia
Mal 26[3]	Williams (AAA)	MARDI, Selangor, Malaysia
Mal 27	Pisang Rastali (AAB)	MARDI, Selangor, Malaysia
Mal 28[3]	Grande Naine (AAA)	MARDI, Selangor, Malaysia

[1]Accession numbers from Department of Primary Industries, Plant Pathology Laboratories, Indooroopilly, Queensland. RP numbers donated by RC Ploetz from an international collection of *Foc* held at the University of Florida, Homestead, USA. [2]Genomic constitution unknown. [3]Isolate cross-compatible with VCG 01213.

Table 4. Isolates of *Fusarium oxysporum* f.sp. *cubense* from Asia which do not belong in known VCGs and are not compatible among themselves (1991-94).

Isolate[1]	Cultivar	Origin
India 7	Poovan (AAB)	locality unknown, India
Indo 9	Pisang Angleng (AA)	West Java
Indo 10	Pisang Jimbluck (?)[2]	West Java
Indo 16	Pisang Siem (ABB)	West Java
Mal 3	Pisang Awak (ABB)	Ko'bah, Kedah, Malaysia
Mal 4	"	Kota Sarang, Semut, Kedah, Malaysia
Mal 9	"	Batu 13, Yong Peng, Johor, Malaysia
Phil 1	Latundan (AAB)	Ula, Davao City, Philippines
Phil 4	"	Panabo, Davao del Norte, Philippines
Phil 11	"	Guianga, Davao City, Philippines
Phil 18	"	Iriga City, Camarines Sur, Philippines
Phil 20	Abaca	Legaspi City, Albay, Philippines
Phil 21	Latundan (AAB)	Pagbilao, Quezon, Philippines
Phil 22	Katali (ABB)	UPLB College, Laguna, Philippines
Phil 23	Siusok (ABB)	UPLB College, Laguna, Philippines
Thai 1-1	Kluai Namwa (ABB)	Khao Kho, Petchaboon, Thailand

[1]Accession numbers from Department of Primary Industries, Plant Pathology Laboratories, Indooroopilly, Queensland. [2]Genomic constitution unknown.

the Philippines which also produced a 'sweet' benzolic odor in culture. The chromatograms of all Asian 'odoratum' isolates also had a peak with a retention time of approximately 3.2 min.

Generally all isolates within a VCG responded uniformly with respect to volatile production, i.e. isolates in the same VCG belonged in the same volatile group. The only exceptions were isolates in VCG 0126. Isolates in this VCG from Indonesia and the Philippines produced volatile compounds and were classified as 'odoratum' types. However, in a previous study (NY Moore, pers. comm.), five isolates from cultivars Highgate (AAA 'Gros Michel') and Maqueño (AAB 'Maia Maoli/Popoulou') from Honduras, which were placed in VCG 0126 by Ploetz (1990), did not produce volatile compounds and were classified as 'inodoratum' types. Ploetz (1990) found that isolates in VCG 0126 were quite distinct from isolates in other VCGs based on several phenotypic traits and preliminary results from RFLP analysis. Using RAPD-PCR analysis, Sorensen et al. (1994) grouped isolates in VCG 0126 with the race 4 VCGs 0120, 0121, 0122, 01210, 01211 and 01212. Further characterization of isolates is required to make clear the relationship of strains within this VCG.

Table 5 presents vegetative compatibility groups recorded from Australia and Asia, divided according to volatile production.

RAPD-PCR

Genetic variation within Asian isolates of *Foc* was assessed using RAPD-PCR. The isolates were subdivided into either group 1 or group 2 based on their RAPD-PCR banding patterns. Isolates from VCGs 0120, 0121, 0122, 0126, 01213, and the yet unnumbered Indo 25 and Mal 11 VCGs, belonged in group 1, and isolates from VCGs 0123, 0124, 0124/5, 0125, and the yet unnumbered Indo 5 and Mal 6 VCGs, belonged in group 2 (Table 6). Within the Asian collection, many isolates of *Foc* did not belong to a known VCG; however, it was possible to classify these isolates based on their RAPD-PCR banding patterns. Groups 1 and 2 both contained isolates from unknown VCGs. There was good correlation between RAPD-PCR group and race; all race 4 isolates belonged in group 1 and all race 1 isolates belonged in group 2. Isolates of unknown race were present in each of the groups.

All race 4 isolates from Asia produced a similar RAPD-PCR banding pattern to that of race 4 isolates from Australia, the Canary Islands, South Africa, and to putative race 4 isolates from Honduras. The average genetic similarity between the race 4 VCGs (0120, 0121, 0122, 0129, 01211) was 85%. This indicates a close relationship between the race 4 isolates examined, independent of geographic origin or VCG.

The race 1 isolates examined from Asia also produced a similar banding pattern to that of race 1 isolates from Australia and Honduras. However, there was greater diversity (72% similarity) between the race 1 VCGs (0123, 0124, 0124/5 and 0125) than there was between the race 4 VCGs. All isolates of VCGs 0124, 0124/5 and 0125 produced a similar banding pattern (genetic similarity of 80%) and clustered independently of VCG.

Table 5. Volatile characteristics of vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense* from Asia and Australia.

Country	Odoratum	Inodoratum
China		0124/5
India		0124, 0124/5, 0125, Unknown[1]
Thailand		0123, 0124, 0124/5, 0125, Unknown
Indonesia	0120, 01213, 01215, Indo 5, Unknown	Indo 25, Unknown
Malaysia	01213, Mal 11	0123, 0124, 0124/5, 0125, Mal 6, Unknown
Philippines	0122, 0126, Unknown	0123, Unknown
Taiwan	0120[2], 0121[2], 01213[2]	0123[2]
Australia	0120, 0129, 01211	0124, 0124/5, 0125, 0128, Carnarvon

[1]Including isolates of unknown VCG.

[2]Isolates identified and assigned to VCG by RC Ploetz, University of Florida, Homestead, USA.

Table 6. Broad groupings within *Foc* by various methods of characterization.

Method of characterization	Groupings	
Vegetative compatibility (Ploetz 1990; Brake et al. 1990; Moore et al. 1993; Pegg et al. 1993; Moore 1994)	VCGs 0120, 0121, 0122, 0126, 0129, 01211, 01213, 01215, Indo 25, Mal 11	VCGs 0123, 0124, 0125, 0128, 01210, 01212, 01214, Indo 5, Mal 6, Carnarvon
Volatile production (Stover 1962; Moore et al. 1991; Pegg et al. 1993; Moore 1994)	'Odoratum'	'Inodoratum'
RAPD-PCR (Sorensen 1993; Sorensen et al. 1994)	RAPD-PCR Group 1	RAPD-PCR Group 2
Electrophoretic karyotyping (Boehm et al. 1994)	EK Type II	EK Type I
Pectic enzyme analysis (Pegg et al. 1994)	'slow moving' Pectic zymogram group	'fast moving' Pectic zymogram group
Current race classification (1994)	Race 4	Races 1 and 2

Discussion

The range of techniques used in this study to assess biodiversity in Asian isolates of *Foc* provided good information on the geographic origins of *Foc* populations as well as evidence for the coevolution of this pathogen with its banana hosts.

It is possible that *Foc* originated twice, rather than once, in Asia before being spread throughout the world. A biphyletic origin for this pathogen is supported by studies based on volatile production, vegetative compatibility, RAPD-PCR analysis, pectic enzyme analysis, and electrophoretic karyotyping, which divide isolates of *Foc* into two broad groups that correlate with race (Table 6).

Simmonds (1962) proposed that *M. acuminata* evolved in the equatorial regions of Indonesia and Malaysia whilst *M. balbisiana* evolved in the India-Myanmar region to the north. Asian isolates of *Foc* from putative race 4 VCGs were predominantly recovered from cultivars with *M. acuminata* genomes, in the more equatorial regions of Asia such as Indonesia, Malaysia, and the Philippines; whereas isolates in the putative race 1 VCGs were predominantly recovered from cultivars with partial *M. balbisiana* genomes, in countries such as China, India, and Thailand to the north of Asia. It is therefore proposed that strains of *Foc* with race 4 virulence coevolved with the *M. acuminata* clones in the Indo-Malaysian region, and that strains of *Foc* with race 1 virulence coevolved with *M. balbisiana* clones in the north of Asia. Ploetz (1990) has suggested that the race 4 VCG 0120 and the race 1 VCGs 0124 and 0125 are the progenitors of other VCGs of *Foc*, since they are geographically widespread and represent different races.

In view of the tremendous diversity of *Musa* varieties throughout Asia, and the age of this pathosystem, it is not unexpected that Asian populations of *Foc* possess a great deal of genetic diversity. Further studies of populations of *Foc* in Asia are required to determine how much more diversity is present in that region. Collections of *Foc* are required from Myanmar, Viet Nam, and Cambodia, and additional isolates are required from Indonesia and Malaysia in particular. A comprehensive assessment of Asian populations of *Foc* would allow an evolutionary history of this pathogen to be constructed, and from this it will be easier to predict the behavior of this pathogen and formulate appropriate control measures. During collecting missions in Asia it may be possible to locate and collect *Musa* germplasm with resistance to pathogen populations in particular areas.

In this study, isolates were received from wilt-affected Cavendish cultivars in Malaysia (Johor and Selangor) and the Philippines. Fusarium wilt has previously only been considered to be a major threat to Cavendish cultivars in subtropical regions of the world (Ploetz 1990) where it is believed that cold-induced stress compromises resistance to race 4 in Cavendish plants. Cavendish cultivars have been attacked for many years in localized areas in the Philippines, but the disease has not been observed to spread (Stover 1990). Fusarium wilt is causing increasing losses in an export plantation of Grande Naine in Johor where 17 ha have already been destroyed (DR Jones, pers. comm.). Ploetz (pers. comm.) has recently identified VCG 01213 from wilted plants of Valery (AAA 'Cavendish') in Del Monte holdings in Sumatra, where major losses have

also been incurred. If there prove to be no predisposing factors involved in these outbreaks, it would seem that these populations of *Foc*, so far identified as VCG 01213 and the yet unnumbered Mal 11 VCG, are more virulent than other race 4 populations and may pose a serious threat to Cavendish production in the tropics, where most of the world export trades are located. More importantly, perhaps, are the losses that these populations of *Foc* are causing in locally consumed cultivars such as Pisang Mas (AA 'Sucrier') and Pisang Berangan (AA/AAA 'Lakatan').

Genetic and molecular techniques have subdivided *Foc* into small homogeneous groups. Determining how these groups differ in virulence provides a challenge for future research. This will be extremely difficult as no techniques have yet been developed for directly or indirectly assessing pathogenicity in *Foc*. Also in many countries quarantine restrictions present the pathogenicity testing of nonendemic strains. However, the diversity of *Foc* must be fully assessed so that pathogen populations against which new genotypes are being selected are well understood. Failure to do so will continue to frustrate attempts to breed or select universally resistant cultivars.

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Part 2

Germplasm Transfer

Risks Involved in the Transfer of Banana and Plantain Germplasm

DR Jones

Introduction

One of the most important aspects of IMTP Phase II is to ensure that collaborators receive healthy germplasm to initiate trials. In IMTP Phase I, FHIA hybrids were not screened because, when they were acquired from the breeding program, the dangers of virus diseases in Central America were not fully realized. At this time, the only *Musa* virus that had been recorded in Honduras was cucumber mosaic virus (CMV), and it was thought that the FHIA germplasm would be of a high health status. However, this was later found to be incorrect. Following reports of unusual virus-like symptoms in four of the seven hybrids on trial in most of the collaborating countries, INIBAP thought it prudent to determine if the infections were of local origin or not. Plantlets derived from mother stock that was used to supply material for IMTP Phase I were sent to INIBAP's Virus Indexing Center (VIC) at CIRAD-FLHOR, Montpellier, for indexing. In the meantime, INIBAP advised officers-in-charge of trial sites to destroy all plants in the affected lines as a precaution.

The results from Montpellier showed that FHIA-04, FHIA-05, FHIA-06 and FHIA-07 were affected by banana streak virus (BSV). It was fortunate that these hybrids were susceptible to black leaf streak/black Sigatoka and, therefore, not proposed for further distribution and evaluation.

This episode illustrates the need for extreme caution when it comes to germplasm movement and the acquisition of material for propagation and dissemination. BSV was most likely present on plants at the FHIA banana breeding station and had gone unrecognized and undiagnosed. BSV is strongly suspected as being seedborne because breeding experiments with Mysore in Trinidad (Wardlaw 1961) and Brazil showed that symptoms were expressed in progeny. Therefore, the virus may have been infecting the breeding lines used to generate the FHIA hybrids.

INIBAP is virus-indexing all the germplasm that has been nominated for inclusion in IMTP Phase II. Tests have shown that hybrids from three of the world's leading conventional breeding programs are infected with BSV. These results have led to a recognition

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that BSV is a serious problem that could undermine the efforts of breeders if not controlled. INIBAP is encouraging breeding programs to take some responsibility for the health status of their products. It is no advantage to breed superior hybrids that are meant to increase yields if they contain disease that will ultimately lower yields.

Indexing by INIBAP has also revealed that some clones being used to develop embryogenic cell suspension and protoplast culture techniques also contain virus. This discovery was significant as it resulted in the cancellation of plans to disseminate plants derived from these cultures for evaluation purposes.

Germplasm Movement

The safe movement of *Musa* germplasm was reviewed by Stover (1977). In this paper, the danger of disease and pest transfer on traditional planting material such as corm pieces and suckers was outlined. Also, the advantages of using tissue-cultured plantlets was emphasized for the first time.

In 1988, FAO, IBPGR, and INIBAP organized a meeting of *Musa* virologists and quarantine experts to define protocols for the international transfer of *Musa* genetic resources. The recommendations of this meeting were published as the first in a series of booklets produced by FAO and IBPGR on the safe movement of germplasm (Frison, Putter 1989). In essence, the advice given was that all movement should be by tissue culture which would eliminate the risks of transfer of pests and fungal, bacterial, and nematode diseases. The argument was, that if these organisms were present, the culture medium would become contaminated and cultures destroyed. The only risk of disease from in-vitro material was considered to be from viruses. The virus diseases described were banana bunchy top, infectious chlorosis or banana mosaic, banana streak, and banana bract mosaic.

Since 1988, our knowledge of *Musa* virus diseases has increased substantially, and the advantages of shoot-tip culture as the medium for *Musa* movement have been further promoted (Vuylsteke et al. 1990). A history of the major events in the area of the safe movement of germplasm has recently been reviewed by Jones and Tezenas du Montcel (1993).

Viruses Affecting *Musa*

Banana bunchy top virus (BBTV)

BBTV is probably the most important virus affecting *Musa* and the subject of a comprehensive review (Dale 1987). Banana bunchy top disease was first recognized in Fiji in 1889 and then reported from Taiwan in 1890 and Egypt in 1901. In 1913, symptoms were seen in Australia and subsequent work by CJP Magee established that the causal agent was a virus carried by the aphid vector *Pentalonia nigronervosa*.

The present distribution of the disease is shown in Figure 1. The international spread of bunchy top has been by the transfer of infected suckers.

The characteristic symptoms caused by BBTV are well known (Plate 1). If infection occurs at an early stage in development, the plants are dwarfed and no bunches are produced. Bunchy top has recently been recognized in Pakistan where it is decimating the banana industry based on the dwarf cultivar Basrai (AAA 'Cavendish') in the south of Sindh province. Between 1990-91 and 1991-92, government statistics reveal that the area of land under banana cultivation fell by 55%. Whole banana-growing districts are going out of production, and this is directly attributable to BBTV. It is feared that the industry will decline further as more and more conventional planting material becomes infected. Authorities are beginning a program of control based on the provision of virus-tested, tissue-cultured planting material to the growers and the destruction of affected banana plants.

Although some cultivars may escape infection when inoculum pressure is light, possibly because of anatomical characters that reduce the chance of transmission or discourage the aphid vector, there is no evidence of physiologically-based genetic resistance to BBTV. However, as part of an INIBAP-sponsored research project on BBTV at the University of Gembloux in Belgium, representative clones of all the major *Musa* groups will be tested for their reaction to BBTV.

The transmission of BBTV in micropropagated cultures was first described by Drew et al. (1989). Infected plantlets were indistinguishable from uninfected control plantlets in culture. When plantlets were deflasked after 12 months in culture and allowed to grow, all plants developed severe symptoms of BBTV within 1 month and all had died after 4 months. When the same experiment was repeated after 16 months in culture, about 75% of plants eventually showed typical BBTV symptoms, although it took up to 6 months for

Figure 1. Distribution map indicating countries where symptoms of banana bunchy top disease have been observed.

Plate 1. Basrai (Dwarf Cavendish) in the Sindh province of Pakistan with symptoms of banana bunchy top disease. Note the 'rosette' or 'bunchy top' appearance of the plant caused by the production of progressively shorter, narrower, and more erect leaves.

all plants to develop symptoms. The other 25% of plants grew normally and appeared healthy. These were later proven to be uninfected (Drew et al. 1992).

BBTV has been eliminated from Lakatan (AA/AAA) by heat-treating shoot-tip cultures for extended periods (Ramos, Zamora 1990). Subsequently, a report was made that the uneven distribution and low concentrations of BBTV after exposure of proliferating tissue cultures to heat leads to BBTV-free primordial cells, which in turn develop into healthy plants (Wu, Su 1991). In 1993, proliferating tissue cultures of four accessions infected with BBTV and held at the INIBAP Transit Center since 1986 under slow growth conditions were sent to the University of Gembloux for the regeneration of plantlets for BBTV studies. All plantlets grew normally and indexed negative for BBTV (C Anceaux, pers. comm.). Therefore, it seems that extended storage and subculturing of BBTV-infected tissue, even in the absence of heat therapy, may normally lead to the loss of BBTV from primordial cells. However, there still needs to be extreme caution in the dissemination of micropropagated plants, especially from areas where BBTV is known to occur.

The threat of latent strains of BBTV carried in tissue culture led to the development of diagnostic tests for the detection of the virus. Originally, BBTV was believed to be caused by the luteovirus (Dale et al. 1986; Iskra et al. 1989; Wu, Su 1990a). Subsequently, monoclonal antibodies against BBTV were produced (Wu, Su 1990b) and one commercialized by General Biologicals Corporation, Taiwan. However, Harding et al. (1991) and Dietzgen and Thomas (1991) purified 18-20 nm isometric virus-like particles from infected plants, and these particles contained ssDNA. A cDNA probe and monoclonal

antibodies were developed in Australia that detected the DNA-virus associated with banana bunchy top disease. These were shown to be equally sensitive in comparative tests at the CIRAD laboratories in Montpellier organized by INIBAP in 1990. The monoclonal antibody test was subsequently commercialized by Agdia Inc., USA, after it was shown to detect BBTV from a number of locations (Dietzgen, Thomas 1991).

Su, using the monoclonal antibody test developed in Taiwan for the detection of BBTV, has claimed to have found BBTV in plants exhibiting mild symptoms or in symptomless plants in Malaysia, Thailand, and South Africa in 1991 and 1992 (Su et al. 1993). This has caused a considerable amount of controversy, as plants with typical symptoms of banana bunchy top have not been described in these countries. Su also claims that BBTV has been detected in garland flower (*Hedychium coronarium*) and canna (*Canna indica*) which may act as alternative hosts (Su et al. 1993). The situation is confused as banana plants found to be positive by Su's antibody test in South Africa have reacted negatively to the test commercialized by Agdia®(G Pietersen, pers. comm.). There is a possibility that false positive reactions may account for some of the confusion and this may be associated with the composition of the extraction buffer.

The latest information suggests that BBTV exists as two distinct populations. Research at the Queensland University of Technology has shown that an isolate of the ssDNA component of BBTV from Queensland differs in nucleotide sequence from those of isolates from New South Wales and Burundi by less that 1%. However, this same Queensland isolate differs from isolates from the Philippines and Taiwan by more than 10%. More work is in progress to further correlate sequence variability with geographical distribution (Karen et al. 1993).

One question that needs to be answered is whether there is a RNA component of BBTV in addition to the DNA component. There are two possibilities. The first is that the RNA is of host origin and is caused by a stress reaction in the plant. The second is that the RNA component is a virus that mediates in the transmission of the DNA component (J Dale, pers. comm.). Work is in progress at the University of Gembloux, CIRAD-FLHOR, and QUT to help resolve this issue.

Cucumber mosaic virus (CMV)

CMV has a worldwide distribution and is found in most *Musa*-growing areas. It causes a disease known as banana mosaic, infectious chlorosis or heart rot. Incidence varies according to the locality. In some countries, such as Australia, CMV is only rarely found in *Musa*. In other countries, such as Colombia, CMV is a major problem. CMV is a common pathogen of cucurbits that are cultivated either near banana plants or under their canopies in many countries. Transmission from infected cucurbits to banana occurs by means of aphid vectors, of which *Aphis gossypii* is the most important. The highest incidence of CMV in *Musa* occurs where banana and plantain grow in plots where *Commelina diffusa* and other CMV-infected weed hosts are prolific. Where planting material is derived from in-vitro propagated sources, levels of CMV can be extremely high. This is thought to be due to the lush, low-lying foliage of tissue-cultured planting material being particularly attractive to the aphid vectors of CMV.

Plate 2. A leaf of Robusta (Giant Cavendish) distorted by cucumber mosaic virus in Coimbatore, India.

The effect of CMV on the growth of plants varies. When banana is infected at an early stage, the plant becomes stunted and, if a bunch develops, yields are very significantly reduced. When plants are infected at a later stage of development, symptoms range from mild chlorotic mosaics to severe leaf distortion (Plate 2). Necrosis of the cigar leaf and pseudostem have also been reported. In Morocco, CMV causes a severe heart rot problem in banana grown under polyethylene. There are also thought to be variations in symptoms resulting from strain differences. Latent infections of CMV are known to occur.

CMV is carried through in-vitro culture of *Musa*, and has also been shown to be seedborne (Gold 1972; Stover 1972). The heat treatment of rhizomes followed by meristem culture has been claimed to eliminate CMV (Berg, Bustamante 1974).

Banana streak virus (BSV)

Less than 2 years ago, BSV was thought to be found in only a few countries in Africa and the Middle East. Today, its distribution is known to be much more widespread (Fig. 2). In some areas it appears to be spreading and causing significant damage and in other locations it is a minor problem being found only in some plants of one or two cultivars with no evidence of spread except by propagation.

BSV has been reviewed recently by Jones and Lockhart (1993). Foliar symptoms resemble those caused by CMV, especially in the early stages (Plate 3). However, necrotic

Figure 2. Distribution map indicating countries where symptoms of banana streak disease have been observed. The disease is reported to be spreading or important or both in countries shaded black (based on information from ML Iskra-Caruana, DR Jones, BEL Lockhart, and JE Thomas).

streaks can later develop which are characteristic of BSV (Plate 4). Another characteristic of BSV is the periodicity of symptom expression. Plants may not exhibit streak symptoms on all leaves and, for several months at a time, emerging leaves may be symptomless or show only slight symptoms. For this reason, plants in quarantine should be grown for extended periods and examined regularly for symptoms. In INIBAP's Virus Indexing Centers some plants have developed symptoms only after 5 months under optimum environmental conditions for disease expression. Under suboptimal conditions, latent infections have been reported.

It is highly likely that, in the past, many *Musa* pathologists have mistaken BSV for CMV. Photographs reputed to show symptoms of CMV in some publications look identical to those of BSV (Wardlaw 1961; Stover 1972). Stover (1972) and Stover and Simmonds (1987) reported that the incidence of CMV in Horn plantain (AAB) in Honduras was extremely high, symptoms being expressed more in cooler weather. However, recent surveys have indicated that these symptoms are most likely caused by BSV, which is widespread in Horn plantain in Honduras (M Rivera, B Lockhart, pers. comm.).

When viewed under the electron microscope (28-30 nm), particles of CMV and BSV are distinctly different. Whereas CMV is isometric, BSV has a bacilliform shape (30 x 130-150 nm). BSV is a member of the badnavirus group and is serologically related to sugarcane bacilliform virus (ScBV) (Lockhart, Autrey 1988) which also produces streak symptoms in experimentally inoculated banana plants (Lockhart, Autrey 1991). ScBV is widespread and has been found to be transmitted by the pink sugarcane mealybug

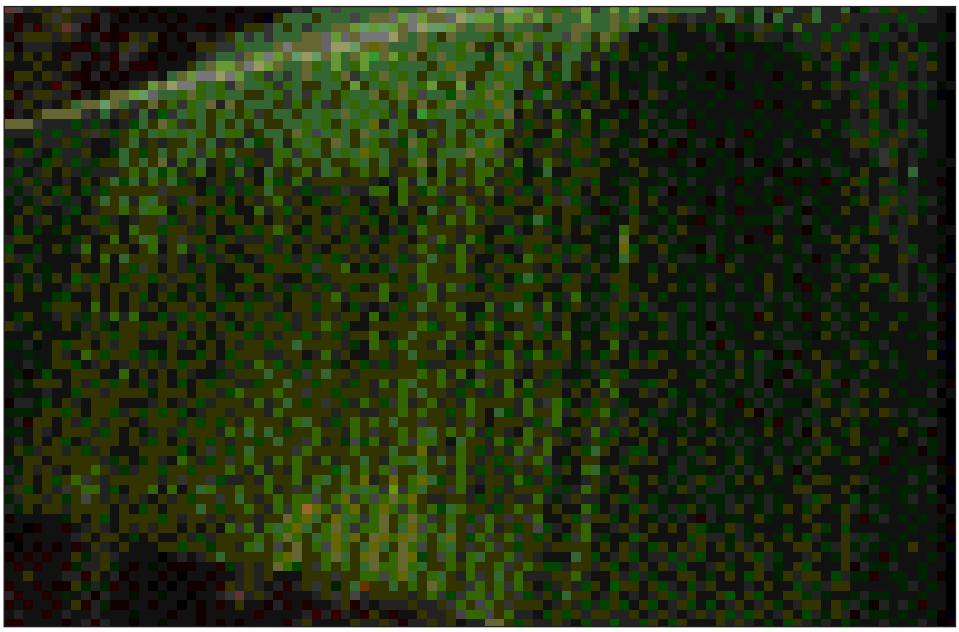

Plate 3. Symptoms of banana streak disease which resemble those caused by cucumber mosaic virus on Karibale (AAB) near Bangalore, India.

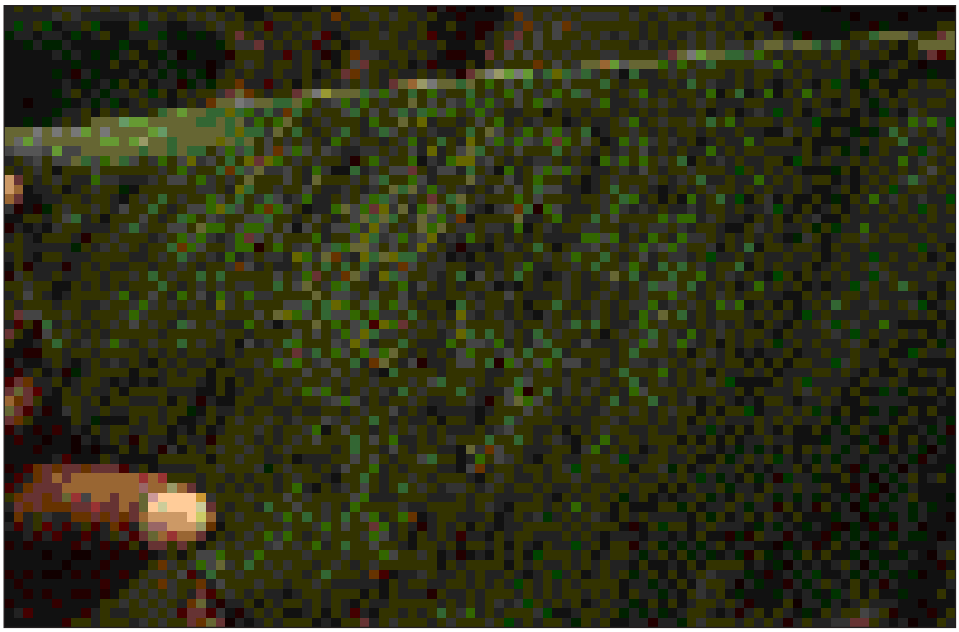

Plate 4. Necrotic streak symptoms caused by banana streak virus on Karibale (AAB) near Bangalore, India.

(*Saccharicoccus sacchari*) from sugarcane to banana and by the citrus mealybug (*Planococcus citri*) from banana to banana. In many tropical areas, sugarcane is grown in close proximity to banana and it is highly likely that transmission from the former to the latter occurs. Further local spread between banana plants would be by mealybug vector and long-distance spread by movement of infected planting material.

In Tamil Nadu state in India, where banana is grown in rotation with rice and sugarcane, BSV is very common, especially in Mysore (AAB). Almost all clones of this cultivar carry BSV, and it was originally thought that the disease symptoms were of genetic origin (Wardlaw 1961). However, a clone of Mysore without BSV symptoms has been identified in Malaysia and Guadeloupe (Pisang Ceylan). INIBAP holds Pisang Ceylan in the Transit Center, and the accession has not shown any symptoms after 12 months of growth in quarantine and it has also indexed negative for BSV in tests undertaken by Dr Ben Lockhart. Another Mysore clone (Thap Maeo) in the EMBRAPA-CNPMF collection in Brazil also seems to be free of BSV symptoms (K Shepherd, pers. comm.). This accession has been acquired by INIBAP and will be virus-indexed in the near future.

The replication method of badnaviruses encourages a high level of mutation and a number of serologically distinct, naturally occurring isolates of BSV have been identified. It would be useful to know if all strains are vector-transmitted, and whether there is any vector-strain specificity, which could explain why BSV spreads in some areas and not in others. Badnavirus-host interaction results in the formation of proteinaceous filamentous chains (tubules) which are visible under the electron microscope and BSV is no exception. Usually, the higher the concentration of these tubules, which are 16 nm in diameter and variable in length, the fewer the number of BSV particles (B Lockhart, pers. comm.).

Diseased plants have reduced growth and vigor and produce smaller bunches. In Brazil, bunches from the Mysore clone without symptoms of BSV (Thap Maeo) have been estimated to be approximately 40% heavier than those from Mysore clones with symptoms. An internal necrosis of the pseudostem, which leads to plant top dieback, is associated with BSV in some countries such as Nigeria (F Gauhl, pers. comm.) and Rwanda (Lockhart 1994).

BSV has been reported on commercial Cavendish plantations in Ecuador and Côte d'Ivoire. In Ecuador, symptoms were swollen veins and indiscrete blotches on leaves and necrotic streaks on fruit (B Lockhart, pers. comm.). In Côte d'Ivoire, yield losses over two cycles varied between 7% in plants with mild symptoms, to 90% or more in plants with very severe symptoms (Lassoudière 1974). It is possible that yield loss relates to BSV concentration in the plant at the time the bunch is produced and develops (B Lockhart, pers. comm.). Fluctuating virus concentrations are also the likely cause of symptom periodicity.

As part of IMTP, INIBAP has commissioned Dr Ben Lockhart of the University of Minnesota to develop a sensitive diagnostic test for BSV that will enable the presence of the pathogen to be detected in *Musa* before symptom expression. Such a test would be important at INIBAP's VICs to help ensure the safe movement of *Musa* germplasm, for use at breeding programs with BSV problems, and for studies on the epidemiology of BSV.

Banana bract mosaic virus (BBMV)

Banana bract mosaic disease was first recognized in the Philippines in 1988 as something different from banana mosaic caused by CMV (Frison, Putter 1989). However, the distinctive symptoms of the disease had been noted in 1979 (Magnaye, Espino 1990) and it was most likely present much earlier. In 1988, after symptoms had been described, two banana workers reported seeing banana cultivars with similar bract mosaics at Coimbatore in southern India (E De Langhe, K Shepherd, pers. comm.). A visit by the author to India in 1992 confirmed that symptoms identical to those recognized in the Philippines as banana bract mosaic were present on *Musa* around Bangalore and Coimbatore (INIBAP 1993). Filamentous virus particles were seen in specimens from infected plants (ML Iskra-Caruana, pers. comm.).

Banana bract mosaic disease is caused by a flexuous, filamentous virus particle which is most likely a member of the potyvirus group. Transmission has been reported using the aphid vector *Rhopalosiphum maidis*, *Aphis gossypii* (Magnaye, Espino 1990) and *Pentalonia nigronervosa* (ML Iskra-Caruana, pers. comm.). Infections are characterized by spindle-shaped streaks and stripes on pseudostems (following the removal of dead leaf sheaths) and obvious mosaics in bracts (Plate 5). Streaks may also be seen on petioles (Plate 6), bunch stalks, and sometimes leaves (Plate 7). Leaf veins may also become prominent (see Plate 7). Yield losses in the popular Saba and Cardaba (ABB/BBB) cooking banana cultivars on Mindanao Island have been reported as high as 40% (Magnaye 1994). During a visit by the author to the Philippines in 1988, many banana cultivars were seen with symptoms caused by BBMV in *Musa* field genebanks at Los Baños in Luzon, and Davao in Mindanao. There have also been reports of the disease affecting Cavendish cultivars in commercial plantations. In 1988, banana bract mosaic reached epidemic proportions around General Santos City on Mindanao Island and 25,000 mats were destroyed. It is obvious that BBMV is the cause

Plate 5. Symptoms of banana bract mosaic disease on the bracts of Karpuravalli (ABB 'Pisang Awak') in southern India.

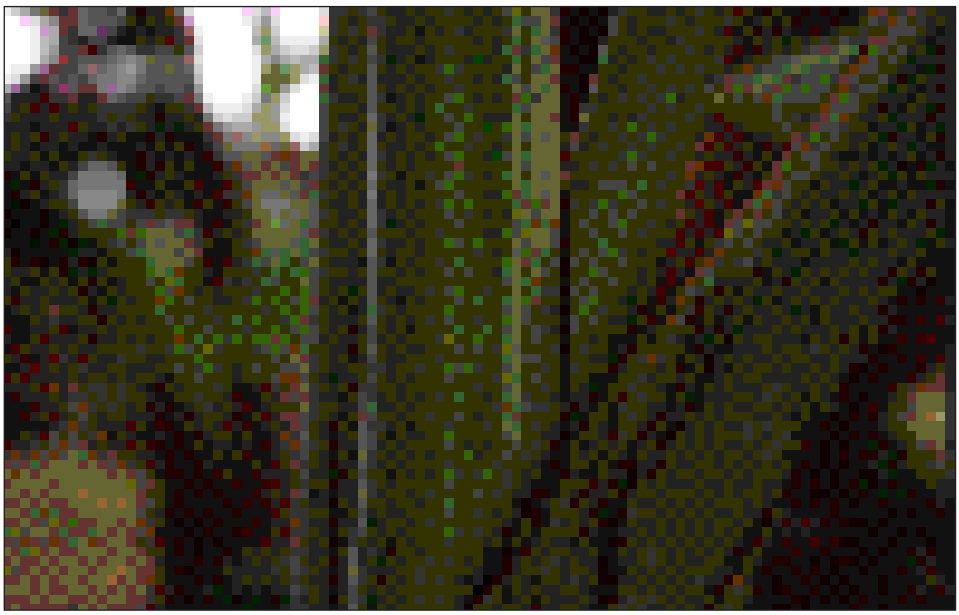

Plate 6. Chlorotic streak symptoms caused by banana bract mosaic virus on the petiole of Galamay Señora (AAB 'Pisang Kelat') in the Philippines.

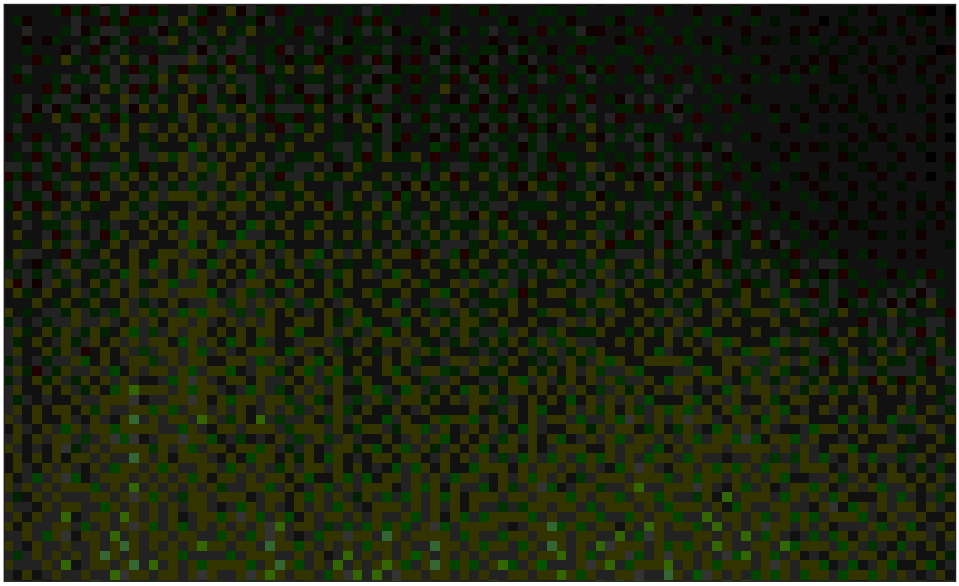

Plate 7. Subtle chlorotic streak and raised vein symptoms of banana bract mosaic disease in the leaf of Cardaba (ABB/BBB) in the Philippines.

of a very serious disease of *Musa*. Great care must be taken to ensure that BBMV does not spread beyond its present areas of distribution.

Other Viruses

Other viruses may exist that affect *Musa*. Samples of leaf tissue from IITA have recently been found to contain isometric virus particles that did not react to CMV antisera and appeared morphologically different from CMV under the electron microscope (B Lockhart, pers. comm.). They have also been miscellaneous reports of filamentous particles in *Musa* from Africa (ML Iskra-Caruna, B Lockhart, pers. comm.) and the Americas (Rivera et al. 1993, ML Iskra-Caruana, pers. comm.). Recently, a long, flexuous rod-shaped particle similar to a closterovirus has been found in Pisang Seribu (AAB) in Florida (B Lockhart, pers. comm.). The importance of these viruses is as yet unknown.

Discussion

The importance of ensuring germplasm is healthy can never be overestimated. Superior genetic material disseminated worldwide that is meant to alleviate food shortages and improve the living standards of smallholders in developing countries should be free of disease. Countries with less sophisticated quarantine systems than are found in developed countries rely, to a large extent, on genetic resource centers and breeding programs to supply clean material. Unfortunately, this is not always the case (Jones 1987).

INIBAP continues to encourage organizations that are disseminating *Musa* germplasm to take appropriate precautions to quarantine and virus test their material. BSV is of particular concern because it is present in a number of breeding programs. It has been argued that BSV is most likely widely distributed given its apparent link with ScBV in sugarcane. However, there is no evidence to suggest that BSV is present in all *Musa*-producing countries. Many different serologically distinct strains of BSV exist that may vary in their effect on yield and on their potential for spread by mealybugs. Some strains seem to do little damage and do not appear to spread; others are lethal to *Musa* and are spreading. *Musa* workers must be cautious that severe strains are not introduced to countries with mild strains. In any case, even if BSV did not occur as different strains and was present in all banana- and plantain-growing countries, it would not be good practice to disseminate contaminated germplasm for trials and possible use as mother stock for in-vitro multiplication.

INIBAP uses the technical guidelines for the safe movement of *Musa* germplasm developed by FAO/IBPGR/INIBAP (Frison, Putter 1989) as a basis for *Musa* dissemination and, despite some criticism of the time-consuming process involved, INIBAP is moving germplasm safely. Quarantine restrictions are a cause of major frustration to *Musa* breeders who want to see the results of their labors distributed as quickly as possible to those who need improved germplasm. Obviously, there is a need to speed up the quarantine screening process, given the necessity to quickly disseminate germplasm for IMTP and other purposes, while keeping risks as low as possible. INIBAP is giving high

priority to the development of more sensitive tests for *Musa* viruses that, it is hoped, will result in quicker protocols for virus screening. Until then, everyone needs to be prudent.

Acknowledgments

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Screening Banana and Plantain Germplasm at INIBAP's Virus Indexing Centers

DR Jones[1], ML Iskra-Caruana[2]

Introduction

The coordination of international exchanges of wild *Musa* species, *Musa* cultivars, and *Musa* hybrids is one of INIBAP's most important functions. In order to prevent the introduction of pests and diseases with germplasm, INIBAP has devised a *Musa* Germplasm Exchange System (MGES) based on an International Transit Center in a non-*Musa* production zone and two Virus Indexing Centers (VICs). MGES was initiated after a meeting organized by FAO/IBPGR/INIBAP on the safe movement of *Musa* germplasm held in the Philippines in 1988 and was based on the recommendations of the participants (Frison, Putter 1989). These recommendations were subsequently modified after a workshop on banana bunchy top disease hosted by INIBAP/CIRAD-IRFA (now CIRAD-FLHOR) in Montpellier, France, in 1990, and they are due to be updated again in the near future.

Role of INIBAP's Transit Center

INIBAP's Transit Center at the Laboratory of Tropical Crop Husbandry, Katholieke Universiteit Leuven, Belgium (officer-in-charge: Ir Ines Van den houwe) receives accessions from existing field collections, in-vitro collections, germplasm collection missions, and breeding programs. Introduction may be in the form of suckers or tissue cultures. If in the form of suckers, tissue cultures are established from shoot-tips. This eliminates threats from insects, soil pests such as nematodes, and fungal and bacterial diseases. Virus diseases are not necessarily eliminated and accessions must be screened for these pathogens.

A single subculture is initiated from one bud of a proliferating bud mass to obtain a clone. This line is used to provide a culture which is further multiplied to supply the Transit Center with 20 tubes of material for medium-term storage under low light and

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cool temperatures, and also to produce plantlets for virus indexing and screening. Five rooted plantlets of each accession are sent to the VICs.

Screening Germplasm for Virus Diseases

INIBAP has two fully functional VICs. Both are attached to Virology Research Units with expertise with *Musa* viruses. One is located at CIRAD-FLHOR, Montpellier, France, in a non-*Musa* production zone (officer-in-charge: Dr Marie-Line Iskra-Caruana) and can process 75 accessions a year. The other is with QDPI in Brisbane, Queensland, Australia, in a subtropical *Musa* production zone (officer-in-charge: Dr John E Thomas) and can handle approximately 40 accessions a year. A third VIC is planned for TBRI, Taiwan, which should become operational in 1995.

When five tissue-cultured plantlets of each accession arrive at a VIC, they are removed from their polyethylene containers as soon as possible and slowly acclimatized to indexing glasshouse conditions. Four of the five plants are screened and one held in reserve. It is believed that these four plantlets are a representative sample of the material held at the Transit Center. If the clone in the Transit Center is infected with virus, then there is a very strong possibility that at least one, but most likely more, of the plantlets will also be infected. Screening lasts until at least 10 fully functional leaves have been produced by the plant, which normally takes about 9-12 months. Plants are held as far as possible at optimal temperatures (25-28°C) for the growth of banana and plantain. This is achieved by the use of evaporative cooling fans during summer in the indexing glasshouses in Brisbane and Montpellier and heaters during winter in the facility at Montpellier. Temperatures lower than the optimum range will slow plant growth considerably and lengthen the period of screening. Very high and very low temperatures may inhibit virus replication and suppress symptom expression.

The protocol for screening depends on the geographical origin of each accession. If the material originates from a country where BBTV is known to be present, it is indexed at both VICs as a precaution. If it comes from an area adjacent to where BBTV is known to occur, it is indexed at one VIC only. Germplasm from the Americas does not need to be indexed for BBTV because the virus has not been found in the Americas. However, an exception to this procedure was made with germplasm collected in Papua New Guinea (PNG) by IBPGR/QDPI in 1988-89. Indexing for BBTV was waived because disease surveys conducted by QDPI and the PNG Department of Agriculture and Livestock provided very strong evidence that BBTV was not present in PNG at the time of collection. Also, the germplasm was quarantined for 9-12 months in Australia and inspected at regular intervals by a QDPI plant pathologist. INIBAP considered the PNG accessions to be of a high health status.

Plants undergoing screening at VICs are inspected regularly for virus symptoms. All plants with symptoms are destroyed, but an attempt is always made to identify the disease. Symptoms caused by BBTV are usually obvious (Plate 1), but confirmation is obtained using a commercial ELISA test kit (Agdia®) that was developed by Dr John Thomas (QDPI). As additional tools, a cDNA probe for BBTV, developed by Dr James Dale (QUT), is

Plate 1. Symptoms of banana bunchy top disease in young Cavendish plants at CIRAD-FLHOR, Montpellier.

available to both VICs, and VIC-QDPI has developed a polymerase chain reaction technique to enhance detection. Some expertise has now also been gained at VICs in the recognition of BSV and BBMV infections in the initial stages of expression (Plates 2,3). If virus-like symptoms are observed, a partially purified sap preparation is examined under the electron microscope for virus particles. ISEM is also undertaken if BSV is suspected, as antisera to several strains have been supplied to the VICs by Dr B E Lockhart. Virus particles of BSV are bacilliform in shape and those of BBMV are flexuous rods (Plates 4,5). Both are conspicuous in partially purified sap preparations viewed under the electron microscope. If isometric particles are observed (Plate 6), an ELISA test is undertaken to confirm the presence of CMV using antisera that detect a wide range of CMV strains.

Plate 2. Early symptoms of banana streak disease in a leaf of a plantain hybrid in the CIRAD-VIC glasshouse, Montpellier.

It is highly likely that most infected plants would express symptoms whilst growing in the VIC glasshouse for the 9-12-month indexing period. However, symptomless infections of CMV (Stover 1972) and BSV can occur and it has been argued that other *Musa* viruses could also be latent. For this reason, INIBAP-VICs routinely screen all plants for latent infections of CMV using the ELISA test and for latent infections of BSV and BBMV by electron microscopy of partially purified preparations. Accessions from BBTV-affected regions are also routinely indexed using the Agdia® test. It has been shown that concentrations of BBTV are greatest in the petioles of the youngest leaves (Thomas 1991) and these are used to prepare samples for ELISA testing. For CMV, samples for testing are either prepared from pooling lamina specimens from randomly selected leaves, as the concentration of this virus is believed to vary in different parts of the plant, or from leaves whose petioles were used for BBTV testing. Indexing for symptomless infections of BBTV and CMV takes place 3 months after planting and at the end of the indexing period.

Plate 3. Early symptoms of banana bract mosaic disease in the leaf of Talip (AA) from the Philippines in quarantine with CIRAD-FLHOR, Montpellier.

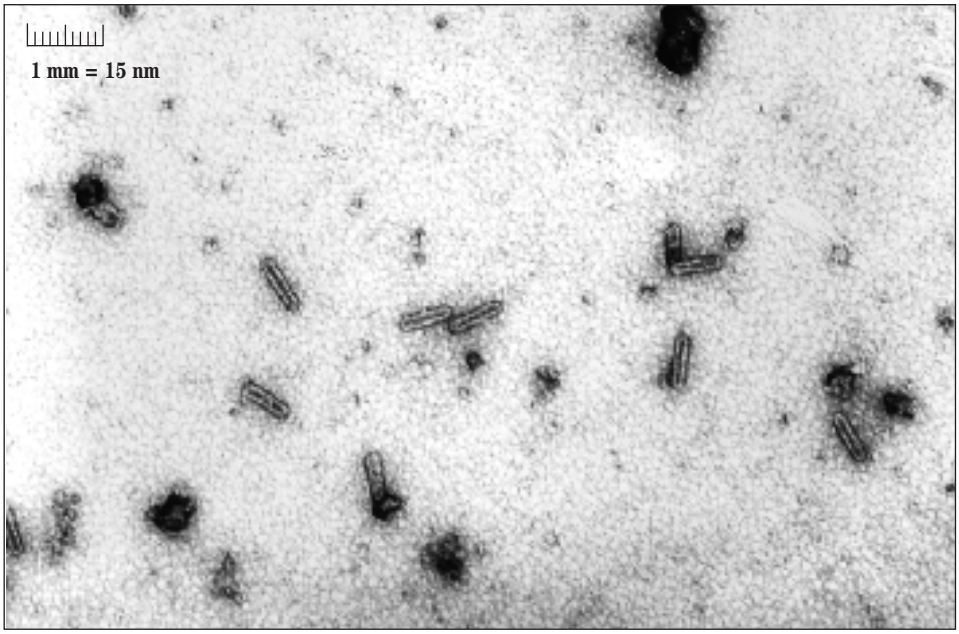

Plate 4. Electronmicrograph showing bacilliform particles of banana streak virus.

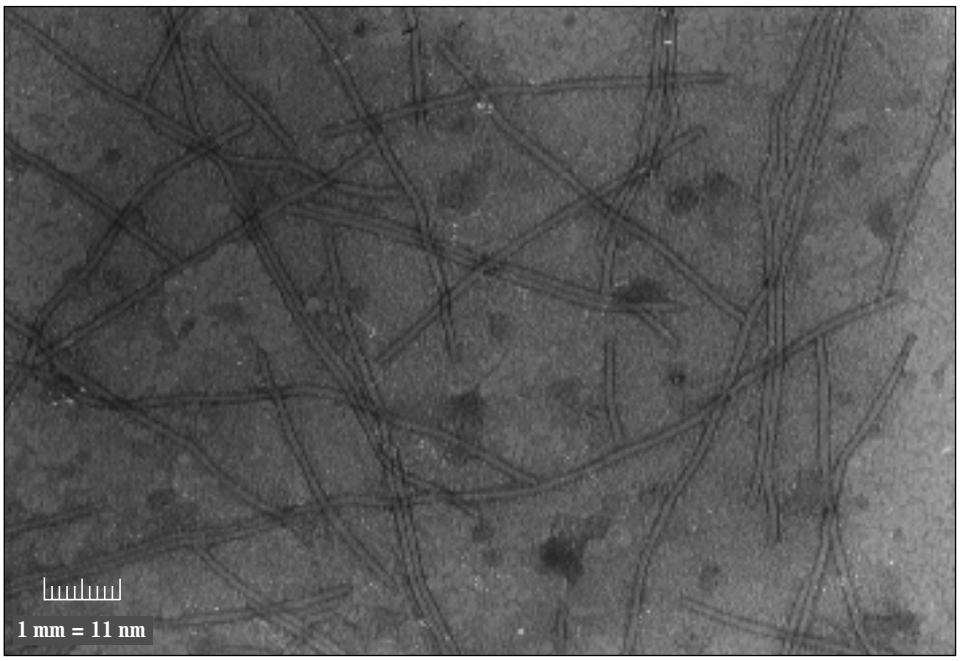

Plate 5. Electronmicrograph showing filamentous particles of banana bract mosaic virus.

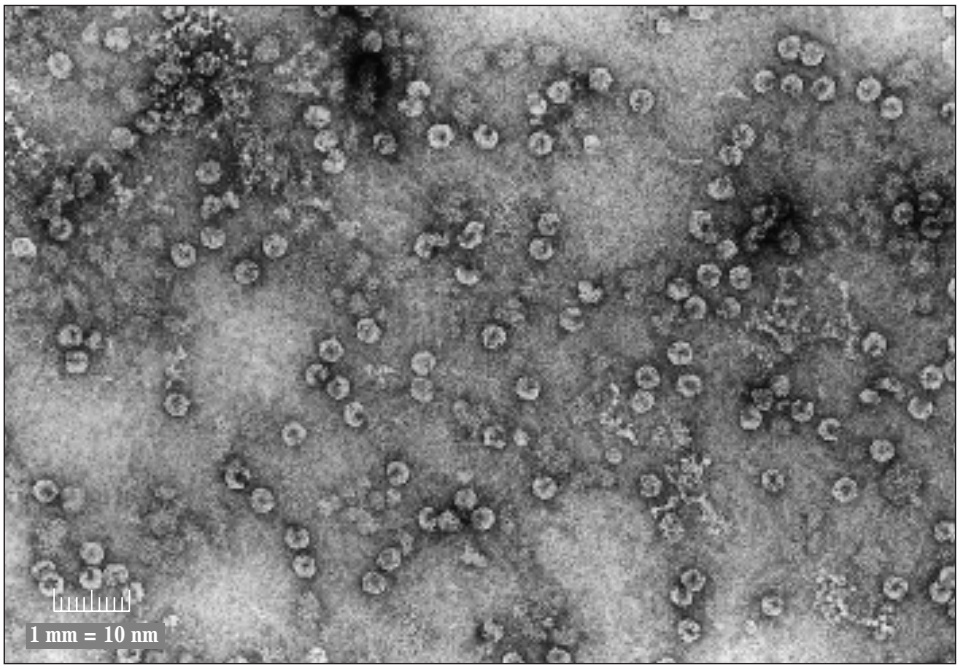

Plate 6. Electronmicrograph showing isometric particles of cucumber mosaic virus.

When, after screening, all four test plants of an accession are given a clean bill of health by the VIC or VICs, INIBAP's Scientific Research Coordinator authorizes the Transit Center to place the clone on the list of available accessions. However, if one or more of the test plants is found to be diseased, stock cultures of that accession at the Transit Center are either destroyed or held pending the possible development of a therapeutic treatment in the future.

Statistics

The VIC at CIRAD-FLHOR has quarantined and indexed a total of 123 accessions and the VIC at QDPI a further 100 accessions. In addition, 257 PNG accessions collected by IBPGR/QDPI and held at the ITC have been quarantined and indexed for latent infections of CMV to INIBAP standards by QDPI for Australian plant quarantine authorities. Of the screened accessions, 42 have been found to carry virus or viruses. BSV was detected in 32 of these, CMV in 10, unidentified isometric particles in 3, and unidentified filamentous particles in 5.

Germplasm from INIBAP's Transit Center

INIBAP's Transit Center is now holding about 1000 accessions and, of these, over 400 have been screened for virus. A further 400 are available for distribution as they are considered to be of high health status based on a knowledge of their country of origin, history of maintenance in field gene banks, and disease screening. However, these accessions are sent to individuals, institutes, and NARSs on the condition that, without being indexed at a VIC, INIBAP cannot fully guarantee their health status. The status of these accessions is being reviewed in light of the recent findings, which suggest that BSV can be carried at low concentrations in some germplasm without expressing any apparent symptoms.

Germplasm from INIBAP's Transit Center is provided free-of-charge in limited quantities (usually five cultures/accession) for agronomic and scientific use. The material can be sent as proliferating tissue for further in-vitro multiplication or as rooted plantlets ready for establishing in pots. Each shipment is accompanied by a phytosanitary certificate issued by the Belgian Ministry of Agriculture, a commercial invoice, a reception report to be completed by the receiver, and, if necessary, an import permit.

Requests for germplasm from INIBAP's Transit Center should be sent to the INIBAP-LACNET Regional Coordinator in Costa Rica, the INIBAP-ASPNET Regional Coordinator in the Philippines, or the Germplasm Officer at INIBAP headquarters.

Discussion

The protocols used at INIBAP's VICs are the best available today for screening *Musa* germplasm for virus disease. Nevertheless, they are a compromise between the necessity

to move improved germplasm quickly and the need to be cautious to prevent the spread of serious diseases. It could be argued that *Musa* needs to be held in quarantine for more than one cycle to be absolutely sure that a virus disease is not present or that the test plantlets supplied to the VICs from the Transit Center could by chance be all derived from primordial cells that have avoided contamination by a virus that infects the rest of the culture. However, if procedures were developed to avoid any risk whatsoever, then germplasm movement would be delayed for an unacceptable period of time. On the other hand, there are tremendous pressures for germplasm to be moved too hastily by individuals and organizations who do not fully appreciate the dangers involved and are motivated by other priorities. INIBAP has used a system based on the FAO/IBPGR Technical Guidelines for the Safe Movement of *Musa* Germplasm (Frison, Putter 1989) to allow material to flow fairly quickly, yet be screened sufficiently to satisfy the requirements for effective quarantine.

There will always be differing opinions and views on the subject of quarantine, depending on the outlook and perspective of the individual. INIBAP is certain that its *Musa* transfer policy will evolve along with the advances that will inevitably be made in the areas of virus diagnostics and virus-*Musa* interactions. In the near future, FAO and INIBAP/IPGRI will call *Musa* virologists and quarantine experts to a meeting to discuss modifications to the Technical Guidelines for the Safe Movement of *Musa* Germplasm, the international code of conduct that all organizations should use as a basis for *Musa* exchange.

Acknowledgments

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Further information

More detailed information on *Musa* indexing procedures and protocols employed by INIBAP's VICs can be obtained from Dr J E Thomas (fax: +61 7 371 0866) or Dr M L Iskra-Caruana (fax: +33 67 61 55 81). Copies of the Technical Guidelines for the Safe Movement of *Musa* Germplasm are available from IPGRI headquarters in Rome (Fax: +39 6 575 0309).

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Musa Germplasm Distribution from the INIBAP Transit Center

I Van den houwe[1], DR Jones[2]

Introduction

The INIBAP Transit Center (ITC), located at the Catholic University of Leuven (KUL), Belgium, holds in trust the largest in-vitro *Musa* collection in the world. Besides its role as a gene bank, the ITC is also involved in the international transfer of banana genetic resources, via the *Musa* Germplasm Exchange System (MGES), to plant breeders and plant researchers throughout the world. The ITC also plays a key role in the International *Musa* Testing Program (IMTP), providing selected germplasm to ecologically different testing sites. As part of the activities of the *Musa* Germplasm Information System (MGIS), the ITC is to furnish a standard set of *Musa* germplasm accessions to institutes interested in participating in taxonomic studies.

ITC started its activities in 1985 with a core collection of 17 accessions. Its collection now contains 1056 accessions (April 1994), representing the large genetic diversity within the genus *Musa*. The accessions were acquired from curators of other existing collections in the world, breeding programs, NARSs, research workers, botanical gardens, and collecting missions in 38 different locations.

Medium-term Storage

During the early 1980s, tissue-culture techniques for rapid clonal propagation and storage under limited growth conditions were investigated at the KUL Laboratory of Tropical Crop Husbandry (Banerjee, De Langhe 1985). From this work, standard protocols were developed which are outlined below.

Proliferating tissue cultures are maintained on a Murashige and Skoog (1962) mineral salt mixture, supplemented with 10 µM (2.25 $mg.L^{-1}$) N6-benzylaminopurine (BA), 1 µM (0.175 $mg.L^{-1)}$ indole-3-acetic acid (IAA), Murashige and Skoog vitamins, 30 $g.L^{-1}$ sucrose, 10 $mg.L^{-1}$ ascorbic acid and 2 $g.L^{-1}$ gelrite. The pH is adjusted to 5.8 before autoclaving for 20 min at 120°C. A relatively high level of cytokinin is used to

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reduce the dominance of the apical meristem with the result that adventitious shoots and buds arise from the explant.

Under normal growth conditions (28 ± 2°C and 5000 lux), proliferating tissues of shoot tips need to be subcultured every 6-8 weeks. At the ITC, slow growth is achieved by storing cultures at a temperature of 15 ± 1°C and a light intensity of 2000 lux. Temperatures below 14°C cause damage and subsequently provoke serious losses.

Under slow growth conditions, accessions are subcultured only once per year on average (De Smet, Van den houwe 1991). Some accessions, however, can be stored up to a maximum of nearly 615 days, while others need to be subcultured every 60 days (De Smet et al., submitted for publication). These large differences in storage capacity are related to genomic composition: for example, East African highland banana cultivars (AAA) and AAB banana types (other than plantains) can be stored significantly longer than all other genotypes. Also, in general, parthenocarpic bananas can be stored for longer periods than wild bananas. In particular, storage time for wild *Musa balbisiana* accessions is significantly shorter than for any other genotype (De Smet et al., submitted for publication).

Germplasm Distribution

Since 1985, the ITC has distributed accessions held in the collection to interested research institutes and plant breeders. So far, the ITC has exported more than 2500 accessions, which means that, on average, one accession is supplied per working day. The number of accessions supplied has increased, especially since 1993 (Table 1). To date, nearly 65 institutes from all continents have benefited from this activity. To minimize the spread of economically important pests and diseases, all operations involved in the shipment of germplasm follow, in essence, the FAO/IBPGR/INIBAP Technical Guidelines

Table 1. Export of *Musa* germplasm from the INIBAP Transit Center for the period 1985-93.

	1985	1986	1987	1988	1989	1990	1991	1992	1993	Total	%
Latin America and Caribbean	0	0	13	127	88	111	68	61	99	567	24
West and Central Africa	20	27	154	105	109	54	79	0	24	572	24
East Africa	0	0	0	105	69	42	21	48	66	351	15
Asia and Pacific	12	0	3	5	48	68	50	83	75	344	14
Europe	0	0	10	14	34	122	103	104	179	566	24
TOTAL	32	27	180	356	348	397	321	296	443	2400	100

for the Safe Movement of *Musa* Germplasm (Frison, Putter 1989). This document provides several recommendations for the transfer of *Musa* germplasm.

Proliferating tissue cultures (Fig.1)

If the person ordering germplasm has access to an in-vitro laboratory and micropropagation is possible, samples of proliferating tissue cultures are provided. The ITC selects proliferating cultures from the clone stored under medium-term storage conditions and subcultures it on a new proliferation medium if necessary. After approximately 5 weeks of growth under normal growth conditions, the cultures are trimmed and transferred into plastic culture vessels containing 15 mL of proliferation medium. Cultures are then grown under normal growth conditions for 2 weeks. Seven proliferating cultures are prepared per accession and the five best-performing cultures are selected for dispatch. Each individual culture vessel bears a label with the ITC code and the accession name, and is packed in shock-absorbing watertight material. Cultures are dispatched from the ITC about 2 months after the order is received from INIBAP headquarters (Table 2).

Rooted plantlets (Fig.1)

If in-vitro facilities are not available to the person ordering the germplasm, the ITC supplies rooted plantlets. The time needed to fulfill an order for rooted plantlets is about 4 months on average (Table 2). The most suitable germplasm for this purpose is identifiable in culture as a cluster of 5-10 shoots on proliferation-inducing medium. This material can be easily multiplied to a high number of cultures and the shoots can be easily separated from each other for regeneration into individual plantlets. On a regeneration medium, these shoots will still produce a few buds or tiny shoots at their bases, but these can be removed during subculture.

Many cultures grow differently and this seems to be dependent on genotype. For example, East African highland banana cultivars and wild *Musa acuminata* species form a single shoot or a cluster of a few shoots in vitro. Hence their regeneration is fast, but their proliferation slow. The degree of proliferation increases when the portion of the B-genome in the genotypic constitution increases. ABB and BB accessions, therefore, multiply very fast, forming clusters of meristems covered with small leaves. However, their regeneration to single plantlets is very time-consuming. Experience has shown that three to six subcultures on a regeneration medium are required to obtain individual rooted shoots of ABB and BB clones. This takes about 6-8 months. The blackening of the culture, which is related to a high level of proliferation, is in addition a hindering factor for the regeneration of plantlets. ABB and BB accessions show considerably more oxidation of polyphenolic compounds than accessions belonging to other genotypes.

Cultures, even within one genotype, range from one shoot to a cluster of shoots. This is probably due to the random selection of the explants (apical meristems and adventitious buds) during subculturing. This heterogeneous growth response thus prolongs the time to supply an adequate amount of homogeneously growing plantlets.

After selection of proliferating cultures under medium-term storage conditions, the accessions are either subcultured once or a few times on a regeneration medium. This

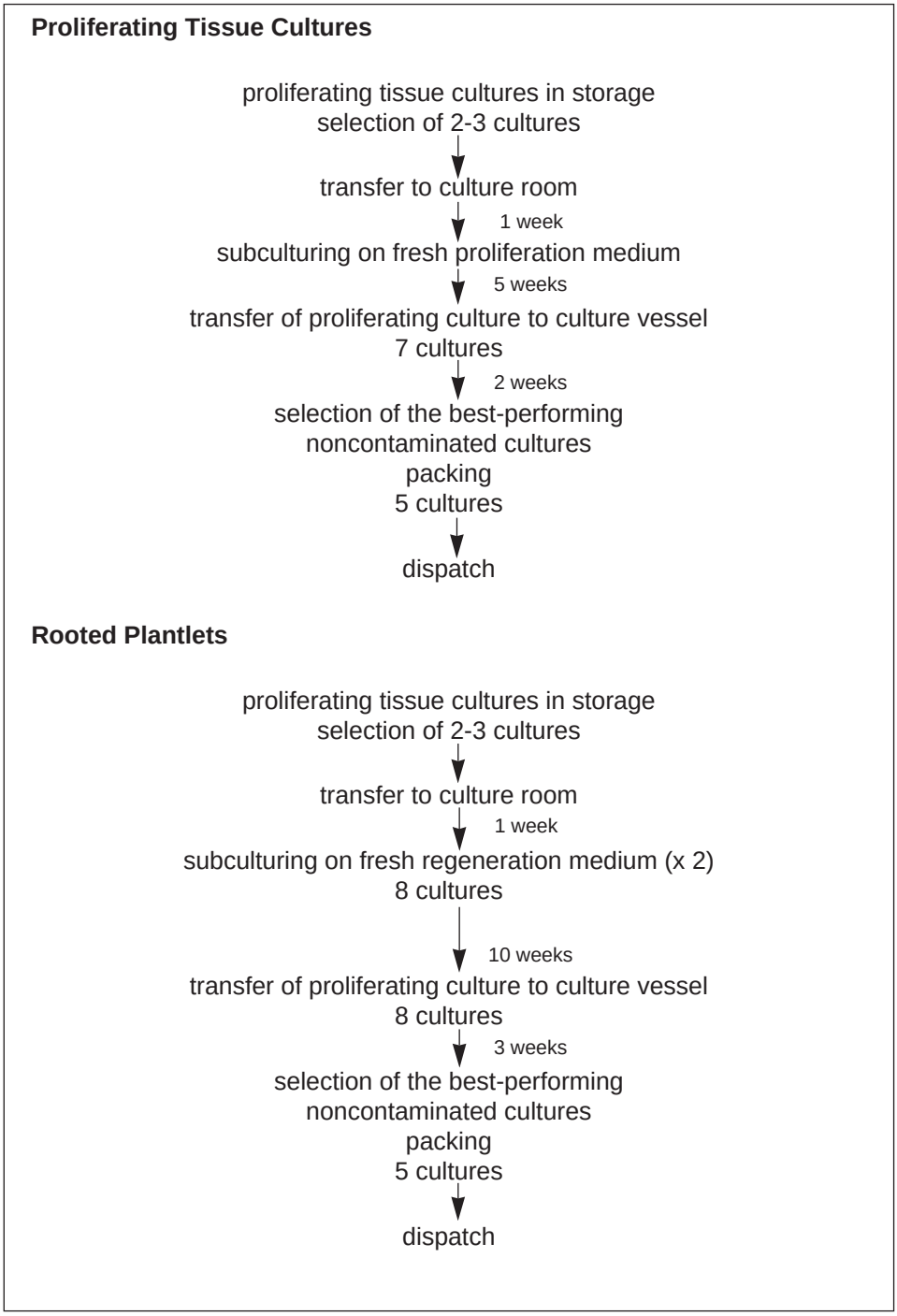

Figure 1. Preparation of *Musa* germplasm for distribution.

Table 2. Duration in months between receiving a request for germplasm and dispatch.

Proliferating tissue cultures				
Year	Type of vial	Average	Maximum	Minimum
before 1992	Plastic culture vessel	2.2	6.8	0.5
1992	Plastic culture vessel	2.2	6.5	0.5
1993	Plastic culture vessel	2.1	4.5	0.5
Rooted plantlets				
Year	Type of vial	Average	Maximum	Minimum
before 1992	Glass test-tube	3.2	6.8	0.5
1992	Cultu sak®	3.8	12	0.5
1992	Cultu sak®	3.9	10	0.5

regeneration medium differs from the proliferation medium in cytokinin content, which is reduced to 1 µM. Eight regenerated shoots are transferred into sterile Cultu saks® filled with 10 mL of rooting medium (i.e. a Murashige and Skoog [1962]) mineral salt mixture at half strength, supplemented with 1 µM (0.203 $mg.L^{-1}$) of the stronger auxin indole butyric acid (IBA) to induce rooting, Murashige and Skoog vitamins, 30 $g.L^{-1}$ sucrose, 10 $mg.L^{-1}$ ascorbic acid, and 2 $g.L^{-1}$ gelrite. Plantlets about 4 cm tall and with three to four leaves are most suitable for transferring to Cultu saks®. For 3 weeks they are kept in the culture room in order to grow and develop roots. The five best-performing plantlets are carefully selected for dispatch. Each individual bag has a label bearing the ITC code and the accession name.

Early in 1992, the ITC switched from using glass tubes to Cultu saks® for the dispatch of rooted plantlets. They are a valuable alternative to glass tubes because they have the positive attributes of being airtight and watertight. They are flexible and able to withstand shock during transportation. In addition, they protect the culture from contaminants, but allow gas exchange. Upon arrival, plantlets that are 5-10 cm tall and have a well developed root system can be planted out in soil in a nursery. If the plantlets are smaller, or if transplanting is not immediately possible, it is advisable to keep the plantlets in the Cultu saks® in an upright position under sufficient light, but not direct sunlight, at temperatures between 20 and 30°C. Experience has shown that such cultures grow and can be kept for at least 8 weeks under these conditions.

Shipments of *Musa* germplasm from the ITC to clients are always accompanied by a letter and a packing list. A questionnaire on the condition in which the material arrived at its destination (receiving report) is also enclosed. This is completed by the receiver and returned to the ITC. A phytosanitary certificate, issued by the plant quarantine service of the Belgian Ministry of Agriculture, accompanies the exported material together with a commercial invoice for countries outside the European Union. From some recipient countries, an import permit is required before material can be sent.

Recently, shipments of rooted plantlets have also been accompanied by recommendations on how to handle these young in-vitro plants after deflasking.

All germplasm is shipped by courier and reaches its destination within 1 week after dispatch from the ITC.

Distribution of Germplasm for IMTP

As a part of the IMTP Phase I, the ITC produced and distributed about 1500 rooted plantlets to six ecologically different testing sites: CORBANA (Costa Rica), CRBP (Cameroon), FHIA (Honduras), ICA (Colombia), IITA (Nigeria), and IRAZ (Burundi).

Seven hybrids from FHIA were selected for evaluation for resistance to black leaf streak/black Sigatoka disease and nine standard host-range accessions were also tested. The distribution of germplasm started in December 1990 and, by the end of 1991, all testing sites had received the entire set of accessions. The germplasm was sent as 15 rooted plantlets per accession which were individually packed in glass test-tubes.

Many accessions selected for IMTP Phase II were only received from donor institutes in 1993. Before distribution to the different testing sites proceeds, all accessions involved will be indexed at an INIBAP Virus Indexing Center (VIC). Between 24 February 1993 and 7 October 1993, five plantlets of the accessions involved were regenerated and sent to the VICs in either France (VIC-CIRAD) or Australia (VIC-QDPI).

In 1993, the ITC started the multiplication of proliferating cultures of the relevant accessions for IMTP Phase II. These stock cultures are stored under reduced growth conditions awaiting final virus indexing results. When the results are known, the ITC will start doubling the desired number of stock cultures.

Proliferating tissue cultures will be sent to those collaborators who have facilities for in-vitro culture and who can produce their own plantlets (Table 3). The shipments will begin late in 1994.

The ITC will also deliver about 9 240 sterile rooted plantlets, individually packed in Cultu saks®. For the Sigatoka trial, 11 accessions are involved and 35 plantlets of each will be dispatched to every site. There will be 6 test sites. The Fusarium wilt trial is larger as 21 accessions and 11 testing sites need plantlets (Table 3). Thirty plantlets of each accession will be sent to each site.

The ITC is to furnish stock cultures to a private tissue culture laboratory. This laboratory will produce and pack sterile rooted plantlets and deliver them to the ITC. They will be checked for contamination, labelled, and dispatched to the test sites. The first shipments of plantlets for IMTP Phase II are planned for early 1995.

All interested parties will be informed of the date and details of the shipment and in some cases the receiving institutes will be requested to provide the ITC with an import permit 3 months before the planned date of dispatch. Collaborators have indicated to the ITC when it would not be appropriate to receive plantlets because of adverse planting conditions.

Table 3. IMTP Phase II collaborators and their requirements for either proliferating tissue cultures or plantlets.

Sigatoka sites	
Proliferating tissue cultures:	Cameroon (CRBP[1]) Costa Rica (CORBANA) Cuba (INISAV) Honduras (FHIA) India (ICAR) Nigeria (IITA)
Plantlets:	Colombia (ICA) Philippines (BPI) St. Lucia (WINBAN) Thailand (HRI) Tonga (MAFF) Uganda (NARO)
Fusarium wilt sites	
Proliferating tissue cultures:	Cuba (INISAV) Honduras (FHIA) India (ICAR) South Africa (BPIU)
Plantlets:	Australia (QDPI) Brazil (CNPMF-EMBRAPA) Canary Islands (CITA) Indonesia (AARD) Malaysia (MARDI) Philippines (BPI) Taiwan (TBRI) Thailand (HRI) Uganda (NARO)

[1] see list of acronyms and abbreviations on page 287

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Part 3

Conventional Breeding for Disease Resistance

Musa Breeding at FHIA

P Rowe, F Rosales

Introduction

The most destructive worldwide disease of plantain and banana is black leaf streak/black Sigatoka leaf spot. Chemical control of this disease has always been prohibitively expensive for the farmers who produce plantains and cooking bananas for domestic consumption, with the result that production has become lower and prices higher in several countries already handicapped by severe limitations of both food availability and purchasing power. For example, there are reports that plantains, which were formerly the cheapest food in Zaire, are now becoming a luxury item because of reduced yields. With the development of pathogen resistance to the systemic fungicides, the difficulty and expense of controlling this disease have now even placed the continued cultivation of the export banana in jeopardy in many areas.

Viewed retrospectively, the best thing that ever happened in regard to genetic improvement of *Musa* was the destruction of Gros Michel by Fusarium wilt. This catastrophe prompted the establishment of an exhaustive breeding program by the United Fruit Company in 1959 when black leaf streak/black Sigatoka was still unknown. Since the only practical solution to the current devastation by black leaf streak/black Sigatoka is the development of resistant varieties, the wisdom and vision of the executives of this export company and the donors (IDRC, FAO, UNDP/INIBAP, WINBAN, USAID, NRI, and the governments of Honduras and Ecuador) who have supported the program under FHIA, are readily evident. The activities and accomplishments in this program, before and after it was donated to FHIA in 1984, are detailed in a recent publication (Rowe, Rosales 1993b).

This paper describes the three hybrids resistant to black leaf streak/black Sigatoka—the FHIA-01 dessert banana, the FHIA-21 plantain, and the FHIA-03 cooking banana—which have been developed in this program and which appear to have immediate potential for greatly increasing domestic food production. Objectives in further breeding of disease-resistant dessert banana, plantain, and cooking banana are also discussed.

FHIA, Apartado Aéreo 2067, La Lima, Honduras

Breeding Objectives

The objectives are to develop productive dwarf dessert banana, plantain, and cooking banana hybrids with resistance to black leaf streak/ black Sigatoka, the burrowing nematode and race 4 of Fusarium wilt. Since 90% of the world production of these crops is for domestic consumption, the top priority is to safeguard these staple tropical foods. However, the export banana is vital to the economic welfare of many countries and a tremendous potential exists for introducing plantains as a new food in export markets, especially if a productive plantain with the flavor of Maqueño (AAB 'Maia Maoli') can be developed. Thus, development of disease-resistant hybrids for export is also an important objective.

Methods

Genetic improvements for all types of banana are dependent on the development of agronomically superior diploids with disease resistance. These bred diploids are then crossed onto seed-fertile triploids of dessert banana, plantain, and cooking banana for the synthesis of tetraploid hybrids. Selected tetraploids are evaluated for commercial qualities, including resistance to diseases.

In the initial stages of the program, the Highgate dwarf mutant (AAA 'Gros Michel') was the only seed-fertile triploid used as the fixed female parent to produce tetraploid hybrids. This approach was based on the findings in the Trinidad and Jamaican programs (Shepherd 1968) that were extremely valuable in providing the guidelines for the breeding activities.

Later, it was found that the Dwarf Prata (AAB 'Pome' from Brazil) is also useful as a triploid parental line in breeding dessert banana (Rowe 1990). The discovery that French plantain (AAB 'Plantain') and Maqueño are seed-fertile and produce tetraploid progenies with improved bunch qualities provided opportunities for breeding disease-resistant plantain (Rowe 1987). Cross-pollinations onto Gaddatu (ABB 'Gubao') have now shown that this clone is an excellent parental line in breeding new cooking bananas (Rowe, Rosales 1993a).

Significant Results

Dessert banana hybrids

The FHIA-01 tetraploid hybrid (AAAB), which was derived by crossing the burrowing-resistant SH-3142 diploid (AA) onto Dwarf Prata, is the most significant accomplishment to date in breeding dessert banana in the FHIA program. The desirable qualities of this hybrid (Fig.1) have already been described (Rowe, Rosales 1993c) and only brief further discussions are presented here.

Figure 1. A plant and bunch of the FHIA-01 tetraploid hybrid showing fruit development in the absence of any chemical measures to control black leaf streak/black Sigatoka.

In Australia it has found that the FHIA-01 hybrid is resistant to race 4 of Fusarium wilt which attacks the Cavendish export cultivars (K Pegg, pers. comm.). This finding is extremely valuable since genetic resistance is the only control measure for this disease. FHIA-01 is the one known hybrid with export qualities which could be grown if race 4 were to become prevalent in the Central and South American producing areas.

FHIA-01 is also the only currently known hybrid which could be grown without control of black leaf streak/black Sigatoka. While the Cavendish clones are more productive than FHIA-01 if black leaf streak/black Sigatoka is controlled, this hybrid provides an alternative in the event that Cavendish must be replaced by a cultivar resistant to black leaf streak/black Sigatoka in the near future. Relative bunch sizes of FHIA-01 and Williams (AAA 'Cavendish') when grown in the same plot with no control of black leaf streak/black Sigatoka are shown in Figure 2.

It is anticipated that the immediate usefulness of FHIA-01 is in areas with marginal growing conditions. Since it is not only resistant to the two most destructive diseases, but is also tolerant of cold temperatures and unfavorable rainfall and soil fertility conditions, it could provide bananas for vast areas in Africa, Asia, and Latin America that have not previously had ready access to dessert bananas because of the susceptibility of the natural cultivars to diseases.

Gros Michel is still grown for local consumption in the mountainous regions of Honduras and Colombia where race 1 of Fusarium wilt is not present. One problem with Gros Michel is that, when a hanging harvested bunch begins to ripen naturally, the whole bunch ripens at once. In contrast, the oldest hand of FHIA-01 bunches ripens first and

Figure 2. Relative bunch sizes of FHIA-01 (left) and Williams (AAA 'Cavendish') when grown in the same plot with no control of black leaf streak/black Sigatoka.

the rest of the hands ripen sequentially over a period of several days under natural conditions. When the fruit of FHIA-01 is gassed to induce ripening, the fruit ripens uniformly. However, this sequential natural ripening trait extends the period over which the fruit from a particular bunch is available, and this is an important consideration when taking into account that most of the world's bananas are consumed domestically.

The primary drawback of FHIA-01 fruit for export is that it is softer than Cavendish when ripe. While this relative softness is not a deterrent to its being grown for domestic use, exporters and marketers would have to implement special handling practices for FHIA-01—e.g. selling it green in the supermarkets. In this case, the consumer would allow the fruit to ripen naturally at home (in the same manner that is commonly practiced with tomatoes) before consumption. However, a preferred alternative would be to have a banana with the good qualities of FHIA-01 in terms of disease resistance, but that would also have the desired fruit firmness when ripe. This is an objective in the ongoing breeding schemes.

Plantain hybrids

Several tetraploid plantain hybrids have been selected in the FHIA program, but the most promising from those that have received subsequent evaluation is FHIA-21 (AAAB). This hybrid was derived from AVP-67 (French plantain) x SH-3142 and has the plantain-type shape, pulp color, and flavor. Informal surveys have shown a preference of FHIA-21 over Horn plantain (AAB 'Plantain'), both when fried green and baked ripe. The ripe fruit of this hybrid has an even more pronounced plantain aroma than that of Horn plantain.

The outstanding agronomic features of FHIA-21 are that it is resistant to black leaf streak/black Sigatoka and it produces a large bunch. However, dehanding is necessary for promoting development of the thick and long fingers preferred in the export market. Representative first-crop FHIA-21 bunches dehanded to 5, 6, and 7 hands, as compared with non-dehanded FHIA-21 and Cuerno (Horn plantain) in a trial at the FHIA plantain experimental farm, are shown in Figure 3.

The FHIA plantain station is located in a major plantain-producing area and has the handicap of poor drainage (high water table) which is typical of the region. However, a preliminary observation of FHIA-21 and Cuerno under good soil and drainage conditions in the field of a farmer in a different area gave the same relative results. When planted at the same time and grown together in the same plot, the FHIA-21 bunches (dehanded to 4 or 5 hands) weighed more than twice as much as the bunches of Cuerno (Fig. 4).

No black leaf streak/black Sigatoka control measures were used in the FHIA experimental farm, but fungicides were applied to Cuerno in the farmer's field. While postharvest studies are still pending to determine the export potential of FHIA-21, the indications are that this hybrid would be valuable for immediate cultivation for domestic consumption.

Another possible alternative for the traditional Horn plantain in certain areas is the FHIA-01 hybrid which is a stronger plant and produces a larger bunch than FHIA-21. While plantains have an aroma that results in their being preferred over other types of cooking bananas when fried or baked, this aroma is not as evident when plantains are

Figure 3. Representative first-crop bunches of FHIA-21 dehanded to 5, 6, and 7 hands and not dehanded, as compared with Cuerno, in a trial at the FHIA plantain experimental farm. The 5-hand FHIA-21 bunch weighed 19.0 kg and the Cuerno bunch weighed 7.0 kg.

Figure 4. Two 10.0-kg bunches of Cuerno (left) and two 25.0 kg bunches of FHIA-21 (dehanded to 4 and 5 hands) that are typical of relative bunch sizes in the plant crop of this variety and hybrid when planted at the same time in the same plot. The bunches shown in Figure 3 were grown in a marginal area (poor drainage) and the bunches shown above are from a plantain farmer's field which had good drainage.

boiled green. Thus, cooking bananas without the aroma of plantain would probably be acceptable (in the areas where boiling plantains is common) if they were otherwise palatable in regard to flavor and texture. The problem, until now, has been that no alternative disease-resistant bananas with desirable eating qualities when boiled have been available.

When FHIA-01 is boiled green, it has a very attractive white exterior and golden interior color, and an excellent texture and flavor. Other desirable features of FHIA-01 are that it has a long green life after harvest and the fruit does not disintegrate when boiled. The fruit is also delicious when baked ripe.

The photographs of FHIA-01 shown in Figures 1 and 2 are of plants and bunches which show the potential of this hybrid under relatively good conditions, but still with no control of black leaf streak/black Sigatoka. A plant more representative of expectations when grown under the variable conditions of small holders is shown in Figure 5. Even though this plant did not receive proper agronomic practices, it produced a 33-kg bunch which was supported without propping. The bunch of this FHIA-01 plant, compared with an exceptional bunch of plantain (14 kg), is shown in Figure 6. In Africa, comparisons between FHIA-01 and plantain have been made at IITA's Onne station in Nigeria which has poor soils and high black leaf streak/black Sigatoka pressure. The average bunch weight of FHIA-01 was 15.1 kg as compared with 3.9 kg for plantain. In the same trial, the average bunch weight of Valery (AAA 'Cavendish') was 7.2 kg (D. Vuylsteke, pers. comm.).

These comparisons indicate that planting FHIA-01 as a substitute for plantain in West and Central African countries would immediately more than double the yield presently obtained from plantains. This dual-purpose hybrid would not only provide greatly increased quantities of a staple food when boiled green, but also would supply a tasty ripe fruit in areas where the previously available dessert banana cultivars could not be grown because of their susceptibility to diseases.

Cooking banana hybrids

The most widely grown cooking banana is Bluggoe (ABB) (Fig.7). This hardy clone is tolerant of marginal growing conditions where other banana and plantain types cannot be grown. However, Bluggoe is susceptible to Moko disease and race 2 of Fusarium wilt, and these two diseases are now greatly reducing the availability of this traditional food in many areas.

The FHIA-03 hybrid (AABB) resistant to black leaf streak/black Sigatoka, which has Gaddatu in its pedigree, is even more hardy than Bluggoe and is more productive. The flavor of FHIA-03 has been favorably received in Choluteca and Mosquitia in Honduras where this hybrid has shown tolerance of drought and poor soils, respectively. The very strong semidwarf plant supports bunches with weights up to 50 kg without propping (Fig. 8). In Grenada, where Bluggoe is a staple food and Moko is a serious problem, FHIA-03 appears to have a high level of resistance to this disease. Plants of this hybrid have now been provided by IITA to Malawi, where race 2 of Fusarium wilt has eliminated large areas of Bluggoe.

A different type of cooking banana is adapted to the higher altitudes of East Africa and is a staple food for 20 million people. All the essentially identical East African

Figure 5. A plant of FHIA-01 showing anticipated bunch size if this hybrid were grown under the variable conditions of plantain farmers in areas where boiled green plantains are a staple food. This hardy banana hybrid has an excellent flavor, texture, and color when boiled green and is the first disease-resistant hybrid developed that has exceptional fruit qualities, both as a green boiling banana and as a ripe dessert banana.

Figure 6. The 33-kg bunch of the FHIA-01 plant shown in Figure 5 (left) compared with a 14-kg bunch of plantain. These relative comparisons indicate that planting FHIA-01 as a substitute for plantain in certain areas would more than double the yield currently obtained.

highland varieties are very susceptible to black leaf streak/black Sigatoka, and this disease is now present in all countries in that region. The flavor and texture of boiled FHIA-03 are practically identical to those of the East African highland cooking bananas, and this hybrid could also possibly be an acceptable disease-resistant substitute for these AAA clones. FHIA-03 has been sent to Burundi by INIBAP and to Uganda for evaluation of its performance at higher altitudes. It has been reported that farmers in Burundi are taking it from the experimental area for planting in their own farms.

Fifteen meristem-cultured FHIA-03 plants were taken to Cuba in 1991, and these were rapidly multiplied to 15,000 plants for comprehensive evaluation. After the plant growth had been observed and the green and ripe fruit had been tasted, it was planned immediately to expand the area planted to 130 ha.

Relative bunch sizes of Bluggoe and Nyamwihogora (AAA, East African highland cooking banana), as compared with FHIA-03 in the FHIA experimental plots, are shown in Figure 9. In spite of its huge plant pseudostem diameter and bunch size, FHIA-03 is relatively fast in regard to time to shooting and time between fruiting cycles. Its one known weakness is that it tends to ripen quickly after harvest. For home consumption, this ripening could be prevented by removing the older hands from the bunch as they are needed while leaving the younger hands on the unharvested bunch. This procedure is easily accomplished with a short ladder since the plant is semidwarf and strong. It has been shown that this partial harvesting not only preserves the green life of the fruit, but also permits the remaining hands to continue enlarging in size.

Figure 7. The hardy Bluggoe cooking banana (ABB) which is the most widely cultivated clone in marginal areas where other banana and plantain types cannot be grown. However, this natural cultivar is susceptible to Moko and race 2 of Fusarium wilt. These two diseases have now eliminated Bluggoe in many of the areas where it was traditionally cultivated.

Figure 8. Plant and bunch features of the very vigorous semidwarf FHIA-03 hybrid cooking banana, which was derived from crosses onto Gaddatu (ABB 'Gubao').

Figure 9. Relative bunch sizes of Bluggoe (left) and a typical East African highland cooking banana (right) compared with FHIA-03 in the FHIA experimental plots. The bunch weight of FHIA-03 is 46 kg.

As is the case with FHIA-01, it is also anticipated that FHIA-03 would be useful as a productive alternative to plantain in areas where plantains are primarily eaten when boiled green.

Limitations and Conclusions

As discussed above, the FHIA-01, FHIA-21, and FHIA-03 hybrids resistant to black leaf streak/black Sigatoka appear to have approximately double the yield of the natural cultivars (of dessert banana, plantain, and cooking banana) for which they are suggested as potential replacements. A continued concerted effort to introduce and evaluate these hybrids in the known areas where there are critical needs for hybrids resistant to black leaf streak/black Sigatoka that are productive alternatives to the traditional cultivars, should now be of highest priority.

At the same time, breeding must continue in order to solve other needs: genetic diversity for black leaf streak/black Sigatoka resistance to safeguard against the breakdown of one particular source of resistance; dwarfness in plantain; greater variability in types of cooking banana to meet specific preferences; a Maqueño-type plantain with faster ratooning speeds for promotion as a new export crop; disease resistance in export banana (especially to black leaf streak/black Sigatoka and the burrowing nematode); and resistance to other nematodes and the weevil borer in plantain.

The FHIA program has the advanced breeding lines already in hand for making the indicated cross-pollinations to develop hybrids which will contribute towards solving most of these needs. Development of these hybrids is currently under way and the breeding approaches being used are described in the recent publications by the program (Rowe, Rosales 1993a, b, c).

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Plantain Breeding at IITA

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Plantain and Banana in Africa

Plantain and banana are important food crops in the humid forest and mid-altitude zones of sub-Saharan Africa, providing more than 25% of the carbohydrates for approximately 70 million people in the region (Vuylsteke et al. 1992). In addition to being a staple food for rural and urban consumers, plantain and banana provide an important source of rural income, particularly for the small holders who produce them in compound or home gardens (Dorosh 1988; Nweke et al. 1988). The gross value of annual production exceeds that of several other main food crops, such as maize, rice, cassava, and sweet potato (Vuylsteke et al. 1993b).

The bulk of cultivated *Musa* are triploids ($2n = 3x = 33$). Almost completely sterile, they develop fruit by parthenocarpy. The most important cultivars vary in their genomic constitution, which is generally as follows: dessert banana (AAA), East African highland banana (AAA), plantain (AAB), and cooking banana (ABB). The genome of cultivated clones is derived from the diploid wild species *M. acuminata* Colla. and *M. balbisiana* Colla., which contributed the A and B genomes, respectively (Simmonds 1962).

Although Southeast Asia is considered to be the center of origin of *Musa*, a remarkable diversity of plantain and banana exists in sub-Saharan Africa (De Langhe 1961; Ortiz et al. 1993c; Swennen, Vuylsteke 1987). Each of the different types is grown in a distinct subregion. Thus, the AAB plantain cultivars are predominant in the humid lowlands of West and Central Africa, while AAA cooking and beer banana types are prevelent in the East African highlands (Swennen, Vuylsteke 1991a). The former region harbors the world's greatest variability of plantain and is thus considered a secondary center of plantain diversification. Similarly, East Africa is considered a secondary center of diversity for banana of the *Musa* AAA group. This secondary diversification is the result of somatic mutations and human selection during the long history of cultivation of the crop in the region (De Langhe 1964).

Sub-Saharan Africa produces about 35% of the world bananas and plantains, about 70 million t. a^{-1}. Pest and disease pressure on the crop has been increasing over the past

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15 years due to intensification of production (Wilson 1988). Also, rising population pressure on the land has led to altered farming practices, among which shortening fallow periods are most conspicuous. These rapid agricultural changes provide the context for IITA's work in this area, particularly that aimed at developing appropriate and ecologically sustainable technology for resource, crop, and pest management in *Musa* farming systems (Vuylsteke et al. 1993c).

Production Constraints

Black leaf streak/black Sigatoka leaf spot disease, caused by *Mycosphaerella fijiensis* Morelet, is considered to be the most serious constraint to plantain and banana production in sub-Saharan Africa (Swennen et al. 1989). The disease was accidentally introduced to this continent some two decades ago and spread rapidly, first in Central and West Africa, and later in East Africa (Wilson, Buddenhagen 1986). The pathogen can cause severe leaf necrosis, reducing yields by 30-50% (Mobambo et al. 1993). All plantain cultivars are susceptible to black leaf streak/black Sigatoka (Hahn et al. 1989), as are, at least, some of the most widely grown banana cultivars in East Africa (Vuylsteke, Swennen 1988). Other major diseases, mainly in East Africa, are Fusarium wilt and banana bunchy top virus (Swennen, De Langhe 1989).

An important insect pest of plantain and banana in Africa is the banana weevil (*Cosmopolitus sordidus* Germar), whose larvae bore tunnels in the corm, thus weakening the plant. Plantain and the East African highland banana are susceptible to the insect, though its effects have not yet been quantified (Vuylsteke et al. 1993c).

Another formidable obstacle to plantain cultivation in West and Central Africa is the rapid yield decline that occurs after a year or two when the crop is grown in open field plantations (Wilson 1987). In contrast, plantations of dessert banana remain productive for many years, as does plantain within home or village compound gardens, where it benefits from regular application of organic matter in the form of household refuse (Nweke et al. 1988). The causes of this problem are complex. In East Africa, declining yields are undoubtedly related to reduced soil fertility and mineral deficiencies in the poorer soils. Yield decline under open field conditions can be arrested somewhat through mulching (Wilson et al. 1987). In addition to soil fertility problems, damage caused by the banana weevil and nematodes generally worsens with time (Swennen et al. 1988). Also, plants lodge easily under field conditions as a result of factors linked to the plant's basic physiology. These include poor root development, slow ratooning, and "high mat" (a tendency of the plant base to grow out of the soil). These problems can probably be ameliorated through plant breeding (Ortiz, Vuylsteke 1994a).

Postharvest losses of plantain are a serious deterrent to expanded production in some African countries (Wilson 1987; 1988). Oversupply during the main production season is the primary cause, but losses can also be attributed to poor methods of harvesting, transporting, and storing the fruit (Ferris et al. 1993a,b; Thompson et al. 1992).

Musa Breeding and Genetics at IITA

Research on plantain at IITA began in 1973. In 1979, the center of plantain research was transferred to the Onne station, in the more suitable ecological niche of the humid forest zone. IITA included plantain and banana among its mandate crops in 1987 and the Plantain and Banana Improvement Program (PBIP) was created in 1991 as a full part of the Crop Improvement Division of IITA (Vuylsteke et al. 1993c).

IITA was encouraged by several African governments to launch an urgent campaign to control black leaf streak/black Sigatoka. IITA scientists were impelled by the need to save the plantain for the millions of smallholders who depend on it for subsistence and livelihood. In 1987, a *Musa* genetic improvement program was initiated at IITA which aimed to incorporate durable host-plant resistance to black leaf streak/black Sigatoka leaf spot disease into plantain (Swennen, Vuylsteke 1991b). The Institute was aware, however, that the development of resistance to streak virus in maize (Efron et al. 1989), which is a much simpler plant to cross, took 10 years. IITA had expected at least another 10 years to develop improved plantain germplasm resistant to black leaf streak/black Sigatoka. Therefore, plantain scientists at Onne Station assessed the potential of Sigatoka-resistant cooking banana cultivars (ABB group) as a possible substitute crop (short-term approach) (Hahn et al. 1990), and at the same time they started a long-term endeavor: breeding plantain through a combination of conventional and innovative cross-breeding techniques (Vuylsteke et al. 1994a).

Adoption of the resistant cooking banana cultivars, which were tested in West Africa in collaboration with NARS and INIBAP, has so far been limited, because of West Africans' lack of familiarity with them and their clear preference for plantain (Vuylsteke et al. 1992).

High-Yielding Plantain Resistant to Black Leaf Streak/Black Sigatoka

The problems in developing improved *Musa* germplasm have been attributed to difficulties associated with banana breeding at the genetic and practical levels. Genetic improvement has been severely hampered by lack of genetic variability and low levels of female fertility within the germplasm collections. Genetically speaking, plantain is a triploid, having three sets of chromosomes instead of the two carried by many crop plants (which are diploids). Moreover, space (6 m^2 per plant) and time (1.5 years from seed to seed) requirements also impede breeding progress. These problems were alleviated by screening the germplasm for fertility (Swennen, Vuylsteke 1988; 1990), ploidy manipulations (Vuylsteke et al. 1993f) and intensive application of tissue culture techniques (Swennen et al. 1992; Vuylsteke, Swennen 1993). By the end of 1989, the future of plantain was already beginning to look bright—-there had been a breakthrough in the crossing of susceptible plantain cultivars with resistant diploid banana clones (Swennen, Vuylsteke 1993). Patience, vision, and core commitment by IITA management have also fostered success.

The development of tropical *Musa* plantain hybrids (TMPx)

Thirty sources of black leaf streak/black Sigatoka resistance were identified among the more than 400 *Musa* accessions screened in the field gene bank (Swennen, Vuylsteke 1991a). Most of the resistant accessions are diploid *M. acuminata* (AA), both wild and cultivated types, and ABB cooking banana cultivars.

The 113 different plantain cultivars in the Onne collection were screened for female fertility by hand-pollinating these with a wild banana (Swennen, Vuylsteke 1993). Twenty-nine French and eight False Horn plantain cultivars were identified as female-fertile because they produced viable true seed. This is the highest number of seed-fertile plantain clones reported so far. Also, seed production rates in plantain at the Onne station seem to be higher than previously expected (Swennen et al. 1991). Average seed-set ranged from less than 1 to more than 20 seeds per bunch, depending on the cultivar (Jenny et al. 1993; Vuylsteke et al. 1993f). The relatively high female fertility of plantain at Onne was one of the key elements in breeding advancement.

The initial breeding approach involved producing primary tetraploid progenies via 3*x* x 2*x* crosses, in which the triploid female plantain (AAB) produced 2n (= 3*x*) eggs (Vuylsteke et al. 1993d). The bulk of the pollination effort was on two plantain cultivars (Obino l'Ewai from Nigeria and Bobby Tannap from Cameroon) of which 3750 plants (2.25 ha) were rapidly propagated by in-vitro culture techniques. The main pollen parent was a wild relative, *M. acuminata* ssp. *burmannicoides* clone Calcutta 4, which is highly resistant to black leaf streak/black Sigatoka (Swennen, Vuylsteke 1993). Its resistance was readily inherited in its offspring, but not its poor bunch characters (Vuylsteke et al. 1993f).

More than 250 hybrids were field-established in early evaluation trials in 1989-90. From these, 20 tetraploids were selected for in-vitro multiplication and further field evaluation in preliminary yield trials and in comparison with their plantain parents. Selection criteria were partial resistance to black leaf streak/black Sigatoka and good agronomic and fruit quality characters (Vuylsteke et al. 1993g). Genotype response to black leaf streak/black Sigatoka infection was measured by recording the number of the youngest leaf spotted (YLS), counting down from the first (top) unfurled leaf, on plants at flowering, being the time at which leaf production stops. Increasing YLS values indicate the presence of more healthy leaves on the plant and, hence, greater resistance to the fungus (Craenen 1994). After intensive testing from 1989 to 1992 at Onne station in the humid forest zone near Port Harcourt, Nigeria, a total of 14 TMPx genotypes were further selected for distribution to agricultural research programs. On average, it took 1000 seeds, produced from hand-pollination of 200 plants (0.12 ha), to obtain one selected tetraploid hybrid per year.

The TMPx clones in Table 1 are identified by their original cross/progeny number. All the TMPx have higher levels of black leaf streak/black Sigatoka resistance than their susceptible plantain parents, as determined by an evaluation of the youngest leaf spotted. Selected TMPx may show a gain of up to six leaves without black leaf streak/black Sigatoka spotting when compared with the fungicide-treated parents (Table 1). Host response to black leaf streak/black Sigatoka in the partially resistant TMPx germplasm is based on

Table 1. Agronomic evaluation of 14 selected TMPx clones at Onne, Nigeria, from 1990 to 1992[1] (after Vuylsteke et al. 1993g).

Genotype	Parents[2]	YLS #	PH cm	HTSh cm	DFF days	BW kg	H #	F #
TMPx 548-4	OL x C4	10.0^{**}	330^{**}	280^{**}	126^{**}	16.7^{**}	6.5^{**}	103^{**}
TMPx 548-9	OL x C4	11.0^{**}	290^{**}	310^{**}	131^{**}	16.5^{**}	6.0^{**}	83^{**}
TMPx 582-4	BT x C4	11.0^{**}	300^{**}	270^{**}	135^{**}	14.3^{ns}	7.5^{ns}	124^{**}
TMPx 1621-1	OL x C4	7.5^{ns}	340^{**}	280^{**}	120^{**}	13.8^{**}	6.5^{**}	90^{ns}
TMPx 1658-4	OL x PL	9.3^{**}	320^{**}	185^{ns}	134^{**}	21.5^{**}	7.5^{ns}	123^{**}
TMPx 2637-49	OL x C4	11.6^{**}	360^{*}	225^{**}	127^{**}	16.6^{**}	6.4^{**}	95^{ns}
TMPx 2796-5	BT x PL	10.0^{**}	335^{ns}	205^{**}	123^{**}	21.3^{**}	6.5^{**}	101^{ns}
TMPx 4479-1	BT x C4	9.0^{**}	295^{**}	290^{**}	114^{**}	13.2^{ns}	6.0^{**}	88^{**}
TMPx 4698-1	OL x C4	10.5^{**}	345^{**}	275^{**}	130^{**}	20.0^{**}	8.5^{**}	124^{**}
TMPx 4744-1	OL x C4	10.0^{**}	360^{*}	235^{**}	125^{**}	11.5^{*}	6.0^{**}	89^{ns}
TMPx 5511-2	OL x C4	10.0^{**}	345^{**}	165^{*}	118^{**}	17.8^{**}	6.0^{**}	87^{ns}
TMPx 5706-1	OL x C4	7.5^{ns}	340^{**}	250^{**}	133^{**}	13.6^{**}	6.8^{ns}	96^{**}
TMPx 6930-1	OL x C4	10.8^{**}	330^{**}	240^{**}	138^{**}	17.5^{**}	7.5^{ns}	121^{**}
TMPx 7002-1	OL x C4	13.0^{**}	340^{**}	270^{**}	120^{**}	17.5^{**}	6.0^{**}	115^{**}
Bobby Tannap[3]		7.0	340	171	92	14.0	7.8	100
		±0.2[4]	± 2	± 3	± 0	± 0.2	±0.1	± 1
Obino l'Ewai [3]		7.0	370	188	92	12.4	7.2	91
		± 0.2	± 2	± 5	± 0	± 0.2	±0.1	± 1

[1]YLS = youngest leaf spotted, PH = plant height, HTSh = height of tallest sucker at harvest, DFF = days to fruit-filling, BW = bunch weight, H = number of hands, F = number of fingers.

[2]OL = Obino l'Ewai; BT = Bobby Tannap; C4 = Calcutta 4; PL = Pisang Lilin.

[3]Fungicide-treated plots.

[4]Mean ± standard error (n=200).

^{ns,*,**} = nonsignificant or significant at $P = 0.05$ or 0.01, respectively, for multiple comparison within columns of TMPx genotypes with the respective plantain parent. Critical values of Student's "t" distribution were adjusted by Sidak's multiplicative inequality based on $a'=1-(1-a)^{1/c}$, in which a' is the level of significance required for statistical testing and c is the number of comparisons between the hybrids and the plantain parents (11 and 3 for OL and BT, respectively).

slower or delayed disease development. The ensuing reduction of leaf spot damage results in a larger healthy leaf area during fruit-filling time and, thus, larger and heavier fruit. Black leaf streak/black Sigatoka resistance results from the interaction between a major recessive gene and two modifiers with additive effects (Ortiz, Vuylsteke 1994c).

The tetraploid hybrids show close phenotypic resemblance to their respective female plantain parents. All hybrids display parthenocarpic fruit development and all but one, TMPx 4698-1 (Fig.1), have erect fruit orientation like their plantain parents. Most hybrids have a pendulous bunch orientation, except TMPx 582-4 and TMPx 4744-1, which have a subhorizontal bunch. Neutral flowers are deciduous in all hybrids, except TMPx 4744-1, which has semipersistent neutral flowers. TMPx 548-9 (Fig. 2), 582-4, 4479-1,

Figure 1. Black leaf streak/black Sigatoka-resistant tetraploid hybrid TMPx 4698-1 (middle) derived from a cross of the susceptible triploid plantain cultivar Obino l'Ewai (left) with the highly resistant wild diploid banana Calcutta 4 (right).

Figure 2. Black leaf streak/black Sigatoka-resistant tetraploid hybrid TMPx 548-9 (middle) derived from a cross of the susceptible triploid plantain cultivar Obino l'Ewai (left) with the highly resistant wild diploid banana Calcutta 4 (right).

4698-1, 4744-1, 5511-2, 6930-1, and 7002-1 exhibit male bud imbrication. The TMPx germplasm develops one or two suckers freely while further suckering is inhibited. Regulated suckering is a highly desirable trait for perennial plantain production. In contrast to the plantain parents, which are female-fertile but male-sterile, the hybrids are female- and male-fertile and can be used as parents to produce secondary triploid hybrids by 4*x* x 2*x*, and vice versa, crosses.

The agronomic performance of TMPx clones, along with that of the fungicide-treated plantain parents, is shown in Table 1. TMPx germplasm is shorter in plant stature and has a taller sucker (follower) at harvest, both of which are desirable traits. Most of the TMPx clones yield more than their fungicide-treated plantain parents. Most hybrids have fewer hands, but they generally have a higher number of fruit per bunch, which is an important component of yield.

TMPx 548-9 (Fig.2), also known as PITA-2, is an exceptional hybrid. This clone has a cylindrical pendulous bunch and large parthenocarpic fruit with yellow-orange pulp. The fruit is heavier and thicker than the fruit of its plantain parent Obino l'Ewai. The performance of TMPx 548-9 was compared with that of its plantain parent in an independent replicated clonal evaluation trial at Onne (Mobambo et al. 1993). The susceptible plantain parent was maintained under fungicide and nonfungicide conditions. TMPx 548-9 flowered 56 to 97 days earlier than Obino l'Ewai, with or without fungicide. Yield of the TMPx 548-9 plant crop (33.5 $t.ha^{-1}$) was 43% higher than that of the fungicide-treated plot of Obino l'Ewai (23.5 $t.ha^{-1}$) and 100% higher than in the nontreated plot of Obino l'Ewai (15.7 $t.ha^{-1}$). It is inferred that black leaf streak/black Sigatoka resistance is not the sole component of higher yield in TMPx 548-9. High yield could also be partly due to the manifestation of heterosis in this tetraploid hybrid (Vuylsteke et al. 1993d).

Plantain-derived diploids with black leaf streak/black Sigatoka resistance (TMP2x)

Diploid, triploid, and tetraploid progenies were obtained from the 3*x* x 2*x* crosses (Vuylsteke et al. 1993f). In plantain-derived F_1 populations, diploids represented about 80%, but there was a significant difference between plantain cultivars in the proportions of different ploidies. This suggests a difference in the rate of 2n egg production between cultivars.

While tetraploid hybrids are of immediate interest as potential new cultivars, the plantain-derived diploids (TMP2x) play an increasingly important role in germplasm enhancement at the 2*x* level and as a source of plantain alleles (Vuylsteke, Ortiz 1993). Since 1991, crosses between tetraploid hybrids and diploids were performed to produce secondary triploids. The latter approach was pursued mainly to reintroduce male sterility, as such avoiding seed-set in the hybrids (Vuylsteke et al. 1993e).

All the TMP2x have higher levels of black leaf streak/black Sigatoka resistance than their susceptible plantain parents, as determined by an evaluation of YLS. Host response to black leaf streak/black Sigatoka in the partially resistant TMP2x germplasm is based

on slower or delayed disease development. Less leaf spot damage ensues. Such host response seems to provide more durable resistance.

Two exceptional Bungaoisan-derived hybrids, TMP2x 2348-6 and TMP2x 2348-7, were initially selected due to their high bunch weight and large parthenocarpic fruits (Table 2). In addition, these two fertile TMP2x have short to medium plant size and low apical dominance, i.e. improved suckering behavior.

Other promising diploid hybrids derived from Bobby Tannap and Obino l'Ewai were evaluated in preliminary yield trials in both plant and ratoon crops. Growth and yield characteristics of the average diploid population, the selected TMP2x and of the male parent Calcutta 4 are listed in Table 3.

All selected TMP2x do not show the high apical dominance of plantain. Moreover, TMP2 x 597-2, 1199-6, 2625-20, 2829-62, and 9722-1 develop only one or two suckers freely while further suckering is inhibited. Such regulated suckering is a highly desirable, dominant trait for perennial plantain production. In contrast to their plantain parents, which are male-sterile, all the diploid hybrids, except TMP2x 9722-1, are male-fertile. Figure 3 shows the bunch of TMP2x 1518-4 along with those of its plantain (Bobby Tannap) and wild banana (Calcutta 4) parents. Another two clones, TMP2x 1297-3 and TMP2x 1605-1, were also selected in the period 1989-1991. TMP2x 1297-3 and 1605-1, resistant to black leaf streak/black Sigatoka, have low apical dominance and high-yielding subhorizontal (to almost pendulous) bunches with parthenocarpic fruit. TMP2x 1605-1, a diploid derived from the giant plantain Ntanga-2, is male-sterile but female-fertile. TMP2x 1297-3 is a diploid derived from the French Reversion somaclonal variant of Agbagba. TMP2x 1297-3 offers the opportunity to breed in the previously inaccessible False Horn plantain gene pool. For example, TMP2x 1297-3 was crossed with its full-sib triploid TMPx 1112-1 to produce tetraploid and diploid segregating offspring in which directional selection for large fruit size was applied. This breeding scheme aims

Table 2. Agronomic evaluation of four selected TMP2x clones and average plantain-derived diploid population performance in early evaluation trials at Onne, Nigeria, in 1989/1990[1] (after Vuylsteke and Ortiz, pers. comm.).

Clone	YLS #	PH cm	HTSh cm	BW kg	H #	F #	FW g	FL cm	FC cm	DFF days
TMP2x 2348-6	10	2.7	2.9	13.9	8	126	98	15	10	139
TMP2x 2348-7	9	2.2	2.9	15.2	7	117	130	15	10	165
TMP2x 597-2	9	3.6	1.9	7.8	7	108	72	18	10	118
TMP2x 1448-1	9	3.5	3.2	6.0	8	161	33	11	9	181
2x population[2]	8	2.5	2.5	4.3	6	92	46	11	10	115
	±0.2	±0.1	±0.1	±0.7	±0.2	±6	±6	±1	±1	±6

[1]YLS = youngest leaf spotted, PH = plant height, HTSh = height of tallest sucker at harvest, BW = bunch weight, H = number of hands, F = number of fingers, FW = fruit weight, FL = fruit length, FC = fruit circumference, DFF = days to fruit-filling.

[2]Mean ± standard error.

Table 3. Agronomic evaluation of 10 selected TMP2x clones in comparison with the performance of their diploid male parent Calcutta 4 and the average plantain-derived diploid population in preliminary yield trials at Onne, Nigeria, 1991-93 (plant and ratoon crops)[1] (after Vuylsteke and Ortiz, pers. comm.).

Clone	YLS	PH	HTSh	BW	H	F	FW	FL	FC	DFF
	#	m	m	kg	#	#	g	cm	cm	days
TMP2x 597-2	10	3.6	3.5	7.4	6	94	79	15	10	125
TMP2x 1199-6	10	3.1	2.5	8.3	7	125	65	16	9	115
TMP2x 1448-1	9	3.1	3.3	5.8	7	122	50	13	8	125
TMP2x 1518-4	10	3.4	2.6	5.6	9	146	54	13	6	103
TMP2x 1549-5	10	3.6	2.8	5.8	10	187	35	13	7	117
TMP2x 1657-4	9	3.1	2.7	7.9	8	155	48	13	8	125
TMP2x 2625-20	12	2.6	2.6	7.9	8	112	69	14	10	149
TMP2x 2829-62	11	3.4	2.8	5.5	8	137	35	14	7	123
TMP2x 4600-12	12	2.9	2.5	6.1	7	107	55	11	8	143
TMP2x 9722-1	12[3]	3.0	2.8	5.7	9	142	39	14	7	87
2x population[2]	8	2.6	2.2	3.0	6	95	32	11	7	117
	±0.2	±0.2	±0.1	±0.2	±0.1	±4	±2	±0.3	±0.2	±3
Calcutta 4[3]	-[3]	2.2	2.8	0.8	7	113	6	5	3	104
		±0.2	±0.2	±0.3	±0.2	±15	±2	±1	±1	±4

[1]YLS = youngest leaf spotted, PH = plant height, HTSh = height of tallest sucker at harvest, BW = bunch weight, H = number of hands, F = number of fingers, FW = fruit weight, FL = fruit length, FC = fruit circumference, DFF = days to fruit-filling.

[2]Mean ± standard error.

[3]Highly resistant to black leaf streak/black Sigatoka; leaf spots not readily observed.

to develop improved *Musa* germplasm with fruit size and quality like the preferred False Horn plantains.

Most of the improved plantain-derived diploids were crossed with primary TMPx tetraploids to assess their breeding value. Several secondary triploids, combining short to medium plant stature, high levels of resistance to black leaf streak/black Sigatoka, low apical dominance or regulated suckering, pendulous bunches with parthenocarpic fruit, and acceptable yields, were identified in the segregating populations. This was expected because promising tetraploid hybrids (TMPx) were earlier selected even when using a wild nonselected male parent such as Calcutta 4. Segregation for plant size was expected because all hybrids are carriers of the recessive dwarfism gene. Moreover, TMP2x should produce offspring with more plantain-like characteristics than progenies derived from Calcutta 4, because TMP2x have 50% plantain genes in their genome. This may be important for improving fruit quality in the plantain breeding population.

Registration of improved germplasm

Registration of improved germplasm, combining black leaf streak/ black Sigatoka resistance, high yield, and other useful attributes, is actively pursued in order to place

Figure 3. Black leaf streak/black Sigatoka-resistant plantain-derived diploid hybrid TMP2x 1518-4 (center), obtained from crossing the 3*x*-susceptible Bobby Tannap plantain (left) with the highly resistant wild 2*x* Calcutta 4 banana (right).

this new technology in the public domain and to provide landmarks of technological advancement. Fourteen improved tropical *Musa* plantain hybrids (4*x*), now referred to as TMPx, were registered in the journal HortScience (Vuylsteke et al. 1993g). Registration of some plantain-derived diploid hybrids is in preparation.

Gaining Insight into the *Musa* Genome

Genetic information is required to develop scientific breeding strategies. However, few genetic studies have been undertaken in *Musa*, despite the importance of the crop. Moreover, others have claimed that "formal genetic studies of nearly or quite sterile triploids are impossible", illustrating the absence of inheritance studies in plantain and banana during the past 40 years. As a consequence, very few genetic markers were available in *Musa* before 1992.

Several characteristics of the crop make genetic analysis of *Musa* difficult. The low rate of hybrid progenies recovered after interploidy/interspecific crosses, resulting in small sample sizes, is the major obstacle to genetic analysis. Nevertheless, the production of testcross segregating populations, obtained from triploid (heterozygous parents) x diploid (homozygous recessive parent) crosses, and of diploid plantain-banana hybrids have made genetic analysis in *Musa* possible (Ortiz 1992). Genetic analyses in the TMP2x were simplified due to disomic inheritance. As such, the plantain and banana genomes, which were inaccessible until recently, were investigated.

Widening plantain variability through segregation in the plantain genome

Variation in growth and yield parameters, qualitative morphological traits, and black leaf streak/black Sigatoka reaction was observed within the same tetraploid family obtained from crosses of plantain with Calcutta 4, a true-breeding line (Vuylsteke et al. 1993f). This observation was surprising, as it was expected to have an entirely uniform progeny from the combination of unreduced female gametes and the male gametes of a homozygous species. Therefore, the occurrence of genetic segregation in the triploid plantain genome during the modified megasporogenesis leading to 2n egg formation was inferred (Ortiz, Vuylsteke 1994c). Hence, the production of megaspores and embryo sacs with the maternal chromosome number does not necessarily imply that these carry the intact maternal genotype (Vuylsteke et al. 1993f).

This inference challenged the commonly accepted premise about the $3x$ x $2x$ breeding approach, in which the $3x$ female genome is apparently fixed with recombination only possible from the $2x$ male parent. Due to the occurrence of segregation and recombination in the $3x$ female plantain genome, much more variability can be recovered from crosses on plantains.

Genetics of traits

The genetics of resistance to black leaf streak/black Sigatoka (Ortiz, Vuylsteke 1994c) and to banana weevil, dwarfism (Ortiz, Vuylsteke 1993b), albinism (Ortiz, Vuylsteke 1994b), apical dominance and suckering behavior (Ortiz, Vuylsteke 1994a), fruit parthenocarpy (Ortiz, Vuylsteke 1992), bunch orientation (PBIP 1993), pseudostem waxiness (PBIP 1993), male and female sterility (PBIP 1993), bunch weight, its yield components and other agronomic quantitative traits (Ortiz, Vuylsteke 1993b) were elucidated.

Continuous variation has been considered as a feature of quantitative polygenic traits. However, there are several traits, e.g. plant height, for which 'major genes' can be grouped into classes, but within each class there is continuous variation. Several traits showing continuous distribution in plantain and banana are mainly affected by major genes (Ortiz et al. 1994a). For example, major genes controlling dwarfism, black leaf streak/black Sigatoka resistance and apical dominance are inherited as recessive genes in the plantain germplasm (Ortiz, Vuylsteke 1993b; 1994a,c).

Trisomic inheritance and genome differentiation

It is generally accepted that plantain is a triploid derived from interspecific crosses between the diploid species *M. acuminata* and *M. balbisiana*, which contributed the A and B genomes, respectively. Consequently, the AAB genome designation was given to plantain due to its interspecific origin and based on a putative differentiation between the A and B genomes. Genome differentiation was investigated with the aid of genetic-marker segregation in diploid populations derived from $3x$ (plantain) x $2x$ (wild banana) crosses. Most of the markers analyzed fitted a trisomic rather than a disomic ratio (Ortiz, Vuylsteke 1994d). Hence, plantain has a trisomic pattern of inheritance because each

linkage group occurs three times instead of twice. Furthermore, there was no preferential pairing between the homologous chromosomes of the A genome, but random distribution of the paired chromosomes to the cell poles during anaphase I of the first meiotic division. This suggests that there is no genome differentiation between *M. acuminata* and *M. balbisiana* and, therefore, the AAB genomic designation for plantain should be discontinued or replaced with a more specific genetic characterization when necessary. This was further supported by analysis of male and female fertility in diploid hybrids (PBIP 1993). Pollen production and stainability, as well as seed-set after interdiploid crosses, were the criteria to determine whether the hybrids were sterile or fertile. Under the hypothesis of genome differentiation, AB hybrids should be sterile. Thus, if this hypothesis were correct, 1/3 of the diploid hybrids should be both male- and female-sterile. The high percentage of fertile hybrids, however, suggests that this hypothesis was not true.

Genetic analysis and choice of selection methods for population improvement

There were significant differences between different plantain populations for the fruit-filling period, bunch weight, and fruit parameters (PBIP 1993). This suggests that selection for fruit size in plantain could be possible. Significant differences within populations were also observed for bunch weight and its components. Therefore, improvement within populations will be effective to select genotypes with increased bunch weight. However, the difference between clones of the same population may be the result of ploidy levels. Significant contrasts between ploidy levels in each family were found for bunch weight and fruit size (Vandenhout 1993). On average, tetraploids yielded more than diploids. Tetraploid hybrids have high-yielding bunches with large fruit, but have the same number of hands and fingers as their full-sib diploids. This suggests that tetraploidy is the optimum ploidy level for maximum expression of bunch and fruit weight in plantain-banana hybrids. Tetraploidy enables the expression of higher order interactions (tri- and tetrallelic), which is important for maximizing yield in vegetatively propagated polyploid crops (Peloquin, Ortiz 1992). Results suggested that selection between families would be the most effective method to improve plant height, bunch weight, and fruit weight and its components. Individual selection within ploidy level for each family would also be effective for almost all traits.

Phenotypic recurrent selection schemes for traits with additive gene action, recurrent selection with progeny testing for traits with nonadditive gene action, and reciprocal recurrent selection for traits with both additive and nonadditive gene action are employed to improve the plantain gene pool at the diploid level.

Looking ahead: breeding in other *Musa* gene pools

The next breeding target of IITA is the amelioration of the East African highland beer and cooking banana (Vuylsteke et al. 1993b). This seems to be possible because several seed-fertile cultivars have been identified, multiplied in vitro and field-established in

large pollination blocks at Onne. It is important to note that TMB x 612-74, a hybrid resistant to black leaf streak/black Sigatoka derived from Bluggoe, a cultivar very popular in Malawi, has already been selected (Fig. 4). It has high bunch weight (>17 kg/bunch) and big fruit (>200 g) at Onne (Vuylsteke et al. 1993e).

Equal Partnership with NARS in Research:

Clonal phenotype, which corresponds to a specific genotype, can vary from year to year in the same location and/or from location to location in the same year in the same agroecological zone. This phenomenon, which influences genotype ranking in different environments, is known as genotype-by-environment (GxE) interaction. Breeding programs aim to identify genotypes that have high and stable yield in a range of environments across a targeted region.

Multilocational trials are required to assess yield stability (Ortiz 1993a; Ortiz et al. 1994b) and durability of black leaf streak/black Sigatoka resistance (Vuylsteke et al. 1993a) of the plantain-banana hybrids across environments . Multilocational evaluation trials (METs) and advanced *Musa* yield trials (AMYTs) were set up with local partners in Africa and tropical America (Table 4). IITA and NARS contributed equally by dedicating their own resources towards the success of this joint testing activity. IITA provides planting materials, field designs, data analysis, as well as individual and group training, and research guides (Gauhl et al. 1993; Swennen, Ortiz 1994). Trials are monitored with

Figure 4. Black leaf streak/black Sigatoka-resistant tetraploid hybrid TMBx 612-74 (middle) derived from a cross between the triploid susceptible cooking banana cultivar Bluggoe (left) with the highly resistant wild diploid banana Calcutta 4 (right).

frequent visits. NARS run the trial with their own budgets. This demonstrates commitment of both partners in the implementation of the flow of materials and technology transfer. This option for equal partnership in research redefines the function of CGIAR's breeders in relation to scientists in national programs, who must mobilize the local expertise available within NARS (Ortiz 1993b).

The results from the Humid Forest Stations of IITA at Onne (Nigeria) and M'Balmayo (Cameroon) showed that TMPx 1658-4 (Fig. 5) had high and stable yielding clones in this agroecology (Ortiz et al. 1994a). Moreover, this clone and its half-sib TMPx 2796-5 (Fig. 6) were the highest yielding hybrids at the IITA station in the derived savanna or transition zone, which had a dry season 3 months longer than at the Onne station. Plantain hybrids often outyielded not only their susceptible plantain parent, but also other ABB cooking banana cultivars with less susceptibility to black leaf streak/black Sigatoka (Ortiz, Vuylsteke 1993a; Ortiz et al. 1994a).

The black leaf streak/black Sigatoka reaction of 20 genotypes across West and Central Africa was recorded (Ortiz et al. 1993b) and results are shown in Table 5. Plantain and Valery (AAA 'Cavendish') are equally susceptible to black leaf streak/black Sigatoka. They had about 50% of their standing leaves showing final stages of disease development

Table 4. Institutions involved in multilocational evaluation trials (METs) and advanced *Musa* yield trials (AMYTs).

METs	
Nigeria:	NIHORT-Ibadan, NRCRI-Umudike, BSDP-Oquo Ibono, ADP-Akure, A&RD-Calabar, TTS/FDA-Ugwuogba-Oji River, Pamol-Ibadan, Shell-Warri, Shell-Bori, NAOC-Green Rivers Project, and IITA Stations at Ibadan and Onne
Benin:	UNIBEN-Benin City
Cameroon:	CRBP-Nyombe and IITA Station at M'Balmayo
Uganda:	ESARC(IITA)-Namulonge
Ghana:	CRI-Assin Fosu
Dominican Republic:	Fundacion de Desarrollo Agropecuario, Inc.-Sto Domingo
Cuba:	INIVIT-Villa Clara, Sto Domingo
Australia:	QDPI-South Johnstone
AMYTs	
Nigeria:	NPQS-Ibadan and ADPs at Umuahia and Owerri
Côte d'Ivoire:	IDEFOR-Abidjan
Ghana:	University of Ghana-Kade
Burundi:	IRAZ-Rugombo
Uganda:	UNBRP-Kawanda
Tanzania (Zanzibar):	Ministry of Agriculture-Kizimbani
Malawi:	Ministry of Agriculture/Agricultural Experiment Stations at Songwe and Mkondezi
Kenya:	ICIPE-Ungoye

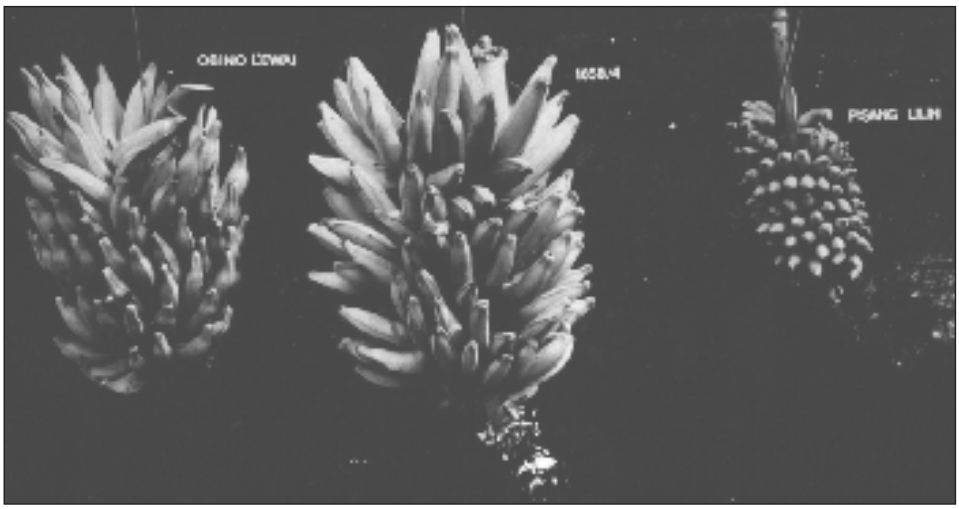


Figure 5. Black leaf streak/black Sigatoka-resistant tetraploid hybrid TMPx 1658-4 (middle) obtained by crossing the susceptible triploid plantain cultivar Obino l'Ewai (left) with the highly resistant cultivated diploid banana Pisang Lilin (right).

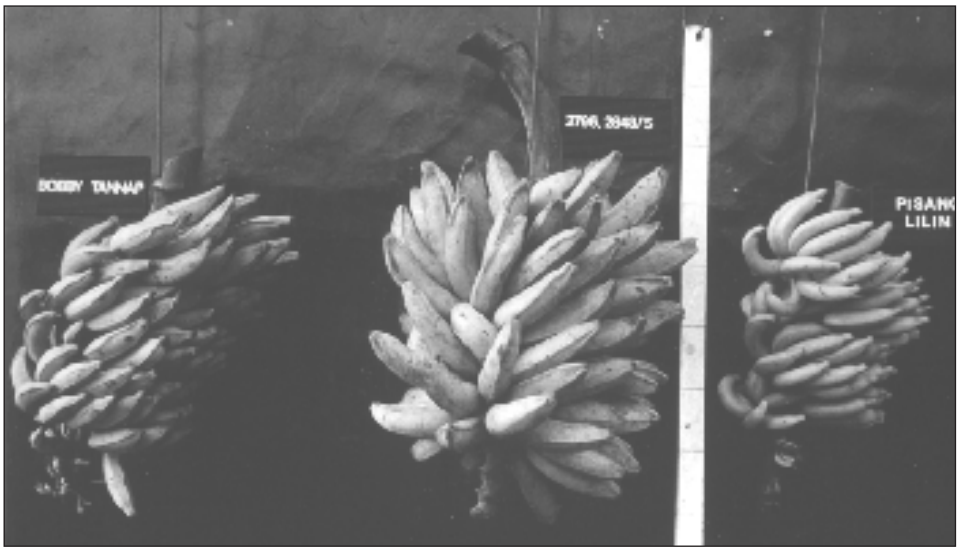


Figure 6. Black leaf streak/black Sigatoka-resistant tetraploid hybrid TMPx 2796-5 (middle) obtained by crossing the susceptible triploid plantain cultivar Bobby Tannap (left) with the highly resistant cultivated diploid banana Pisang Lilin (right).

whereas ABB cooking banana cultivars, such as Bluggoe and Cardaba, initially considered as potential substitutes for plantain, are less susceptible to black leaf streak/black Sigatoka. Although there were significant differences in host response to black leaf streak/black Sigatoka among the hybrids, they show partial resistance to the disease, i.e. slow disease development in their leaves. None of the TMPx hybrids have the high level of

resistance (considered as hypersensitivity) of Calcutta 4. However, this type of resistance is more prone to breakdown. A more durable resistance in the TMPx germplasm might be expected because its host-plant response to black leaf streak/black Sigatoka slows the progress of an epidemic without inhibiting its initiation.

Regression analyses, following the linear model $BSR_{ij} = \mu + \beta_i I_j + \delta_{ij}$, were used to describe the genotypic response to black leaf streak/black Sigatoka (BSR_{ij}) of the i^{th} clone in the respective j^{th} environment (Ortiz et al. 1993a). In this equation, μ is the clonal mean over all the environments, β_i is the regression coefficient which measures the response of the i^{th} clone to varying environments, δ_{ij} is the deviation from the regression of the i^{th} clone at the j^{th} environment, and I is the environmental index. Calcutta 4, Pisan Lilin, and TMBx 612-74 were not included in the analysis because they did not show any leaf with black leaf streak/black Sigatoka-induced necrotic spots in specific environments (Table 5). A practical way to select stable genotypes resistant to black leaf streak/black Sigatoka is illustrated in Figure 7.

The vertical lines are one $LSD_{0.05}$ above and below YLS grand mean and at the grand mean, whereas the horizontal line is drawn parallel to the X axis from the slope β=1. Thus, eight spaces were defined. Cultivars and experimental hybrids in the right lower part of the graph should be considered as having stable black leaf streak/black Sigatoka resistance. Indeed, TMPx 5511-2 had a $\beta = 0.40$, which was not significantly different from zero. This means that the environment and the genotype-by-environment interaction did not affect the expression of black leaf streak/black Sigatoka resistance in this clone. The TMPx clones had different β values which indicate that selection for stable black leaf streak/black Sigatoka resistance may be possible in this population. On average, the Obino l'Ewai derived hybrids had higher black leaf streak/black Sigatoka resistance stability than those derived from Bobby Tannap. This was not surprising because the susceptible black leaf streak/black Sigatoka response of Obino l'Ewai is more stable than that of Bobby Tannap.

Independent analyses of variance combined over environments in each group of materials were carried out to compare the improved TMPx germplasm versus their susceptible parents (Obino l'Ewai and Bobby Tannap) and other reference cultivars. Thus, estimates of variance components were obtained, and coefficient of variability (CV) and broad-sense heritability (ratio of genetic variance to total phenotypic variance) were calculated for the hybrids and the susceptible cultivars (Table 6).

Broad-sense heritability estimates show clearly that the improved germplasm has on average a more stable as well as more heritable variation for black leaf streak/black Sigatoka response than the natural susceptible germplasm. Thus, a faster progress through selection for black leaf streak/black Sigatoka resistance may be expected in the TMPx population than in the natural germplasm. The CVs indicated that on average the recorded YLS was smaller, but more variable, in the natural germplasm than in the improved TMPx germplasm.

In general, the improved germplasm has a more stable response to black leaf streak/black Sigatoka than the natural *Musa* germplasm.

Table 5. Percentage of leaves spotted (ILS[1]) with black leaf streak/black Sigatoka in multilocational trials in West and Central African locations (rainy season of 1993) (after Ortiz et al. 1993b).

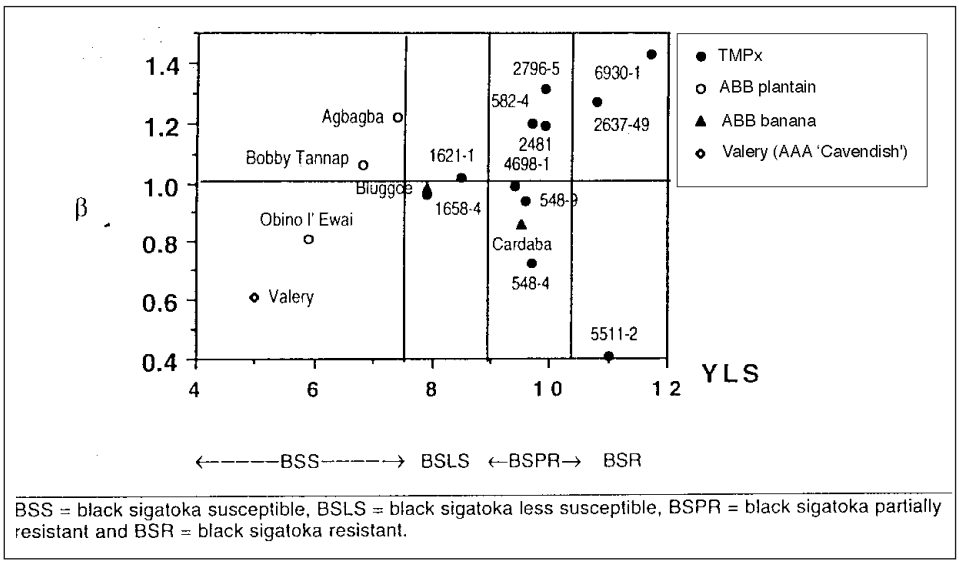
Clone	Ghana	Cameroon	Nigeria							Clonal
	Assin-Fosu	M'Balmayo	Onne	Umudike	Calabar	Bori	Obrikom Akure	Ibadan	X+SE[2]	
548-4	27	12	20	14	38	0	10	25	16	18±4 de
548-9	20	24	14	12	31	11	8	23	28	19±3 de
582-4	25	28	33	23	24	29	5	28	22	24±3 de
1621-1	30	20	42	19	40	17	19	28	33	28±3 d
1658-4	39	37	34	26	32	29	9	36	19	29±3 d
2481	24	5	14	14	14	2	0	19	17	12±3e
2637-49	30	0	19	31	21	12	0	14	10	15±4e
2796-5	21	15	15	7	18	9	8	22	9	14±2e
4698-1	34	9	20	0	19	12	10	17	12	15±3e
5511-2	41	4	19	16	18	0	6	25	16	16±3e
6930-1	24	0	17	26	22	0	12	12	3	13±3e
612-74	25	0	13[3]	25	28	0	22	0	4	13±4e
Agbagba	69	59	62	33	45	59	45	44	52	52±4 ab
B. Tannap	54	52	49	36	42	64	41	44	51	48±3 bc
Obino l'Ewai	61	60	58	n/a	45	53	50	45	n/a	53±3 ab
Valery	64	70	58	49	67	55	n/a	49	60	59±3a
Bluggoe	44	49	32	43	38	53	26	32	40	40±3c
Cardaba	44	52	29	44	36	28	40	60	44	42±3c
Pisang Lilin	n/a	17	13	9	12	11	10	17	10	12±1e
Calcutta 4	0	0	0	0	0	0	n/a	n/a	0	0±0f
Environmental means	36	26	28	22	30	22	18	28	24	
+ SE	+4a	+4b	+4bc	+4bc	+4b	+4bc	+4c	+4b	+4bc	

Means followed by the same letter within the column of clone means and the row of environmental means are not significantly different according to Student-Neuman-Keul's multiple range test at a = 0.05.

[1]Based on youngest leaf spotted (YLS) and number of standing leaves (NSL), ILS = 100 X [NSL-(YLS-1)]/NSL.

[2]Standard error = $s/n^{1/2}$, where s is the standard deviation of the sample and n is the number of observations.

[3]Leaf spots not readily observed.



BSS = black sigatoka susceptible, BSLS = black sigatoka less susceptible, BSPR = black sigatoka partially resistant and BSR = black sigatoka resistant.

Figure 7. The relation between black Sigatoka/black leaf streak response, as measured by youngest leaf spotted (YLS), and stability (ß) (after Ortiz et al. 1993a).

Table 6. Genetic variance(σ^2_G), genetic-by-environmental variance(σ^2_{GE}), phenotypic variance (σ^2_P), broad-sense heritability (H^2), youngest leaf spotted (YLS, mean ± standard error) and coefficient of variability (CV, %) in improved hybrid (TMPx) and natural susceptible germplasm.

Germplasm	σ^2_G	σ^2_{GE}	σ^2_p	H^2	YLS	CV
TMPx	0.983	1.189	2.172	0.45	9.8 ±0.3	11.1
3*x* susceptible *Musa*	0.773	1.902	2.675	0.29	6.3 ±0.5	21.9

Chemical and Host-Plant Resistance Strategies to Control Black Leaf Streak/Black Sigatoka

Though it is possible to control the disease with fungicides, these are too expensive for the smallholders who grow most of the crop in Africa. In addition, fungicide applications are hazardous to health in the village homesteads. Moreover, as was pointed out recently "fungicides are the most abundant pollutants in water downstream from banana plantations, where they can kill fish or prevent them from reproducing. Anything to reduce pesticide use in the tropics is strongly to be welcomed" (New Scientist, 17 April 1993, p.9). Therefore, resistance breeding was considered by IITA as the most

appropriate component intervention to control black leaf streak/Sigatoka leaf spot (Vuylsteke et al. 1993c).

Potential farmer gains were quantified by using two different black leaf streak/black Sigatoka control strategies: fungicide treatments and improved hybrids resistant to black leaf streak/black Sigatoka. Results of preliminary evaluation of hybrids along with their susceptible plantain parent under fungicide and nonfungicide protection were used to compare both control strategies (PBIP 1993). For a proper assessment of gains, the potential yields ($t.ha^{-1}.a^{-1}$) of TMPx black leaf streak/black Sigatoka-resistant plantain hybrids and those obtained by their plantain parent Obino l'Ewai with and without fungicide treatment were determined. The potential yield of untreated Obino l'Ewai was used as the reference point (Fig.8). The best hybrid, TMPx 548-9, had a 225% increase in yield over untreated Obino l'Ewai, whereas fungicide control increased yields of Obino l'Ewai by only about 70% in comparison with untreated Obino l'Ewai. Moreover, farmers require an investment of at least US$750 $ha^{-1}.a^{-1}$ to provide chemical protection against black leaf streak/black Sigatoka disease, while few suckers of the hybrids may be provided free-of-charge or at a minimum cost by NARS for further multiplication.

A rough estimate was made of the advantages of host-plant resistance over chemical protection to black leaf streak/black Sigatoka leaf spot disease taking into consideration the cost/benefit ratio at rural markets in the area near Onne Station. In normal periods, 6 kg of plantain costs about N 30 (30 N = 1 US$ in December 1993), while in periods of scarcity, a 6 kg bunch costs up to N 50. During periods of scarcity, a farmer's gains by increasing production due to chemical protection might be as high as US$1100. This may

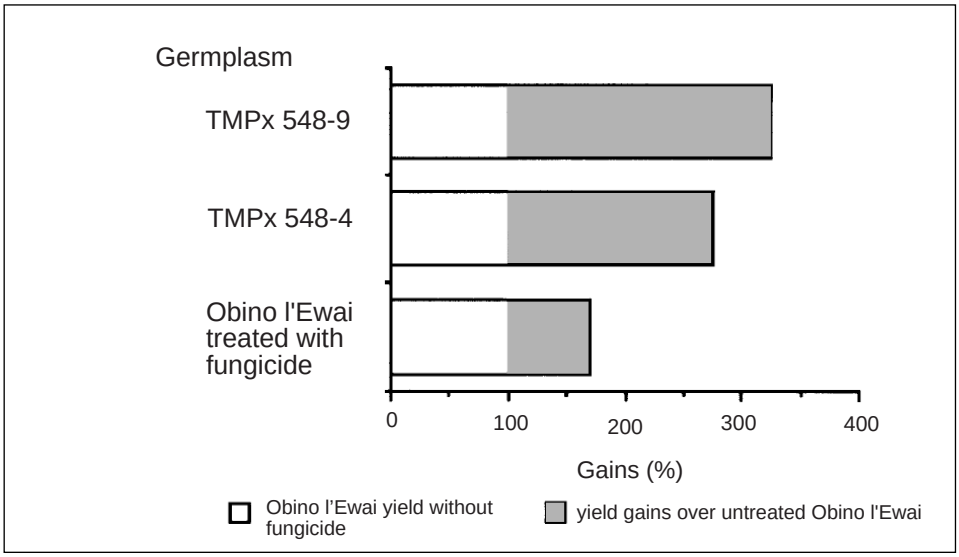


Figure 8. Yield gains from fungicide application vs host-plant resistance to control black leaf streak/black Sigatoka. Obino l'Ewai without fungicide treatment was the reference point (9.7 $t.ha^{-1}.a^{-1}$ = 100%) to determine gains in potential yield with respect to fungicide treatment and to Obino l'Ewai derived tetraploid hybrids TMPx 548-4 and TMPx 548-9 (after PBIP 1993).

decrease to US$370 in normal periods when plantains are abundant in the rural markets. With the TMPx black leaf streak/black Sigatoka-resistant germplasm, the gains may be about US$6050 in periods of scarcity and US$3630 in normal periods. Therefore, host-plant resistance through the utilization of favorable genes could have a comparative advantage over fungicides of 10:1 in normal periods of supply and 5.5:1 in periods of scarcity.

A Holistic Approach for Sustainable and Perennial *Musa* Production

The main areas of PBIP research (breeding and genetics, biotechnology, postharvest studies, and agronomy) are independent yet interrelated because of a holistic approach that has been adopted to develop sustainable plantain/banana production and utilization systems (Vuylsteke et al. 1993b). Single-component interventions do not provide complete solutions to the different constraints affecting *Musa* production in sub-Saharan Africa. Indeed, PBIP's breeding philosophy summarizes our long-term goal: "to develop not only disease- and pest-resistant materials, but also better cultivars for the smallholders who produce plantain and banana in compound or home gardens and large field plantations". Better cultivars must have high and stable yields, adaptation to target areas, resistance to/tolerance of biotic and abiotic stresses, and acceptable quality.

Genotype-by-cropping system interaction

The main components of the biological production system, plant and environment, are normally discussed independently. However, plant-environment interactions must be considered in the development of sustainable systems. Therefore, improved technological packages, i.e. better cultivars in combination with improved crop husbandry practices (Ruhigwa et al. 1993; Wilson, Swennen 1987), must be refined by adaptive testing in target areas with NARS partnership. Integration of cropping systems and crop improvement technologies will achieve maximum interaction between these components.

Early research on plantain agronomy at IITA (1973-87) focused on crop husbandry techniques for long-term productivity (Vuylsteke et al. 1993b,c). The essential role of soil organic matter for sustainable production of plantain was established. Innovative production systems, such as alley cropping with multispecies hedgerows were developed (PBIP 1992). The aim was to provide the adequate environment in which an improved genotype, adapted to the target agroecology, will sustain its yield without degrading the resource base. Currently physiological investigations aim to determine leaf stomata conductance and leaf transpiration and their correlation with potential drought tolerance (Ekanayake et al. 1994a,b).

Based on considerable knowledge accumulated during 10 years of agronomy and physiology research, IITA published a manual on plantain cultivation in West Africa (Swennen 1990), which has been distributed among national programs, participants in IITA training, and farmers.

Postharvest research

There are many postharvest quality attributes related to fruit palatability and durability that have to be satisfied before any improved varieties are adopted by farmers (Eggleston et al. 1992a,b; Ferris 1993a). The project has undertaken the appraisal of consumer preferences and postharvest properties of new varieties to ensure their acceptability and successful introduction (Ferris 1993b). Research has focused on the consumer acceptability of new, black leaf streak/black Sigatoka-resistant hybrids by investigating fruit palatability (flavor, texture, response to cooking procedures) and durability (shelf life, ripening, handling, and storage aspects) (Ferris et al. 1994). For example, fully matured fruits of Obino l'Ewai and TMPx 548-9 were evaluated by a taste panel of trained Nigerian assessors for a preliminary consumer acceptability test at Onne (Vuylsteke et al. 1993g). Green and yellow peel-colored fruits were fried to prepare chips and a local Nigerian dish 'dodo', respectively. Taste and texture were similar regardless of peel color, but the fruit color of TMPx 548-9 was less preferred. Nevertheless, TMPx 548-9 was rated as "good".

In-vitro culture techniques and biotechnology for *Musa* germplasm enhancement

Efforts to propagate, conserve, and breed cultivated *Musa* are fraught with many obstacles (slow propagation, low reproductive fertility, lack of genetic variability) specific to the biology of this vegetatively-propagated crop (Asiedu et al. 1992). Hence, plant tissue culture and molecular genetic methods are increasingly being used as techniques for the handling and improvement of *Musa* germplasm (Vuylsteke, Swennen 1992).

At IITA, shoot-tip culture is routinely used for the propagation, exchange, and conservation of *Musa* germplasm (Vuylsteke 1989). For example, micropropagation has been pivotal in the rapid deployment of the breeding program by supplying large numbers of plants of female and male parents for the crossing blocks and of promising new hybrids for the evaluation trials. Also, more than 300 new *Musa* accessions were introduced as shoot-tip cultures during the past 8 years (Vuylsteke et al. 1990a). Embryo culture and rescue techniques (Vuylsteke et al. 1990b) are still applied to increase the germination rate of the true seed produced during breeding. More than 10,000 seeds obtained from plantain crosses are handled in the tissue-culture laboratory at Onne each year (PBIP 1993).

Technical assistance in the development of tissue-culture laboratories has been provided to national research institutions in Nigeria (Vuylsteke, Swennen 1989). This is another area in which PBIP envisage a sustainable institutional development. Tissue culture (TC) laboratories will play an important role for distribution and multiplication of clean and improved planting materials of selected genotypes. Main limitations in NARS laboratories are chemicals and power supplies. To overcome this constraint, support of the international and regional donor community is sought jointly. It is expected that these TC facilities will be used not only for *Musa*, but also for other crops

that benefit from in-vitro culture techniques. This approach may enhance the sustainability of the system. It is also envisaged that, in the near future, requests for planting materials sent to IITA could be handled by these TC labs. By selling improved in-vitro propagules of different crops, NARS could recover their operational costs. This may increase the chance of success and survival of the TC labs that will be set up with the support of international and regional donors.

More advanced studies in cell and molecular biology are currently under way. Somaclonal variation in plantains derived from shoot-tip culture has been described in detail by IITA scientists (Vuylsteke, Swennen 1990; Vuylsteke et al. 1988; 1991; 1994b). Work on the genetic stability of plants regenerated from cell suspensions (Dheda et al. 1991; 1992) and from cryopreserved cultures is currently ongoing in collaboration with the Catholic University of Leuven (KUL), Belgium (De Smet 1993). In the area of new molecular techniques, the use of molecular markers (RFLPs, VNTRs, RAPDs) for phylogenetic studies (Jarret et al. 1993), construction of a linkage map, marker-assisted selection in breeding, and mapping Sigatoka resistance loci is being investigated in collaboration with the USDA/ARS at Griffin, Georgia. DNA-fingerprinting techniques are also being used to detect somaclonal variants and elucidate the origin of somaclonal variation in collaboration with the University of Birminghan, England and KUL.

Exchange of Information with NARS

Limited access to basic information is still perceived as being a major problem of plantain and banana researchers in Africa (Wilson 1989). Hence, in early 1993, IITA began publishing *Mus*Africa, a plantain and banana newsletter for Africa. The newsletter is sent to collaborators and *Musa* scientists as a means of maintaining links with NARS and to provide rapid transfer of information and technology. Also, partners in the multilocational testing have a forum in the newsletter for exchange of information and ideas as well as for description of improved germplasm. This offers the opportunity to develop networking. A key element for success of this network is the development of horizontal relationships between the network partners with PBIP scientists as catalytic agents.

Potential Impact of TMPx Germplasm in African Agriculture

Based on information provided by the Technical Advisory Committee of the Consultative Group on International Agricultural Research (CGIAR), the potential impact of the TMPx germplasm in the African rural economy was calculated. The annual gross value of *Musa* production in Africa is about US$2.8 billion. Only 1% of the total production goes to the export trade. Therefore, the 225% yield increase that may be possible by using TMPx germplasm might have a potential impact of US$6.2 billion.

The total investment of IITA in plantain breeding in the development of TMPx germplasm has consisted of 2 core scientists (breeder and tissue culturist) for a 5-year period (1987-1992). Five years is about the time from an initial cross to the registration of a hybrid. IITA costs for international staff, local staff support, travel, supplies and other expenses, was about US$2 million for the 5-year period. The ratio of potential impact to investment, therefore, was established as US$6.2 billion/US$2 million. In other words, for US$1 invested by IITA in the development of 14 improved TMPx hybrids with black leaf streak/black Sigatoka resistance, the African economy might have a yearly gain of about US$3100.

In conclusion, genetic improvement of plantain and banana, by a modified but conventional cross-breeding approach, is an important component in the scientific path towards sustainable production of *Musa* cultivars. The impact of this research depends on the incorporation of TMPx germplasm as an integral component of *Musa* production systems. This requires that NARS scientists, working closely with international agricultural research centers (IARCs), transfer this improved germplasm along with other sustainable technologies to farmers of developing countries. In this regard, the International *Musa* Testing Program (IMTP), under the auspices of INIBAP, must provide the opportunity for the evaluation of improved germplasm developed by different programs (CIRAD, EMBRAPA, FHIA, IITA, TBRI, etc.), which should be freely available for extensive testing, local selection, and cultivar release by NARS scientists.

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Breeding Prata and Maçã Cultivars for Brazil

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Introduction

Interest in banana genetic improvement in Brazil dates back to the 1970s. But, beyond the assembly of a national collection of cultivars at EMBRAPA-CNPMF in Bahia, little had been done prior to 1982 to import genetic variability. That year, however, marked the first international collecting trip, to India, the Philippines, Papua New Guinea, and Hawaii, to be followed by others in 1983 and 1985.

The breeding program proper was initiated at the end of 1982, although few fertile AA diploids were then at hand and flowering, and those that were restricted to single accessions of five different subspecies of *Musa acuminata*, earlier donated by the Jamaican program. From that time on, the great emphasis of breeding research has been on the AAB group and, more specifically, on the Prata subgroup, including the larger-fruited mutant Pacovan. There has also been interest in Prata Anã, a semidwarf form of yet unknown origin, but which has eating characteristics close to those of Prata. Nonetheless, it is apparently distinct in certain botanical characters.

Fundamentally, the Prata type is a very tall, unproductive banana, bearing relatively small bunches and fruit even in low-density plantings, and not producing frequently enough to compensate for these shortcomings. Pacovan has advantages in fruit size and is thought to respond better to irrigation; on the other hand there are doubts about its adaptability to the cooler climatic zones of Brazil. Because of its shorter stature, Prata Anã can be planted at much higher densities and it is cold-tolerant, but it, too, is slow between crops and has smallish fruit.

These defects could probably, and should, be remedied by breeding. But the principal objective in the Brazilian program has always been disease resistance with Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*), Sigatoka/yellow Sigatoka (*Mycosphaerella musicola*) and black leaf streak/black Sigatoka (*M. fijiensis*) as priorities. The Prata subgroup and Prata Anã are moderately susceptible to the first disease, and highly or very highly susceptible to the latter two.

Tests for resistance to Fusarium wilt are being reported in Brazilian journals (Cordeiro et al. 1991, 1993b); some information on resistance to Sigatoka/yellow Sigatoka has also

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been published (Shepherd 1990). Evaluations of black leaf streak/black Sigatoka reactions of the CNPMF material have necessarily been entirely dependent on the much appreciated collaboration of CATIE, with INIBAP's sponsorship.

Most of this paper is taken up with an appraisal of the hybrid-generating properties of 'Prata' triploids, with the performance of different diploids and of the tetraploid progenies they generated against these diseases. There is also mention of the difficulties encountered in attempted breeding with Maçã (AAB 'Silk') and news of a more optimistic recent development with this banana type, with its major problem of extreme susceptibility to Fusarium wilt.

Since a diversity of hybrids will be mentioned in the tables and in their appropriate sections, it is helpful to explain how these have been designated at EMBRAPA-CNPMF since the start of the program. The system was imposed to facilitate the identification of parentages. Triploid female parents have been given a double letter coding and all diploids used in crosses (and a few pollen-fertile triploid sources) have received two-digit code numbers. Any combination of two letters followed by two digits then represents a specific triploid-diploid cross, while the junction of two pairs of digits indicates a diploid hybrid family. Individual genotype numbers are appended after a hyphen. The following is a check listing of codings which appear in this work.

Triploids:	PC, JV, SM & ST - common Prata (different accessions); PV - Pacovan; PA - Prata Anã; MC - Maçã; YB - Yangambi No.2;
Wild diploids:	02 - *Musa acuminata* ssp. *zebrina* Buitenzorg; 03 - ssp. *burmannica* Calcutta; 04 - ssp. *banksii* Madang; 05 - ssp. *malaccensis* Pahang; 41 = 0304 - Calcutta x Madang;
Diploid cultivars:	12 - Lidi/Lilin; 15 - Madu; 23 - S/N 2 (a PNG diploid which arrived at CNPMF without a label);
Imported hybrid:	42 - M-53.

Tetraploid Plant Production from the Prata Subgroup

An overall survey of results obtained from the use of certain male parents is given in Table 1. No distinction has been attempted between different types or accessions of the Prata subgroup, except that (Pacha) Nadan has been excluded as virtually totally female-sterile. From this table, it will readily be seen that four pollen parents have been most used until very recent times, the wild strain Calcutta (03), the cultivar Lidi (12), the wild hybrid 0304 (41), and the Jamaican selection M-53 (42).

Of these four, Lidi accounted for the smallest number of tetraploid hybrids produced, but the relatively poor out-turn may have been partly fortuitous. For example, although seed-sets on the whole have been very satisfactory, they have never been even nearly constant from one period to another. In particular, great surges in seed set have occurred with pollinations in some summer seasons (Table 2), but not in all and not always in the corresponding month or months. It is also true that germination management went through one or two unfortunate phases.

Table 1. Pollinations of the Prata subgroup at EMBRAPA-CNPMF: success rates from the principal male parents 1982-92.

Male parent[1]	Fruits	Good seeds	Seeds per 100 fruits	Tetraploids obtained[2]
Wild type				
Calcutta (03)	29 942	3 348	11.2	291
Buitenzorg (02)	6 946	259	3.7	42
Madang (04)	5 544	380	6.8	71
Pahang (05)	762	204	26.8	24
Cultivar				
Lidi (12)	30 419	772	2.5	56
Madu (15)	7 723	68	0.9	4
S/N 2 (23)	2 284	5	0.2	0
Wild F_1				
0304 (41)	22 591	1 170	5.2	175[3]
Parthenocarpic hybrid				
M-53 (42)	97 812	1 874	1.9	279[3]

[1]In parentheses are appended the code numbers applied to these diploids at CNPMF, to simplify the recognition of hybrids.
[2]"Tetraploids" here include some plants that were found to be either inconstant in chromosome number or to have one or two chromosomes more or less than 44.
[3]Germination technique had been improved in these later crosses.

Table 2. Some instances of sudden surges in female fertility of the Prata subgroup at EMBRAPA-CNPMF, all in the hot, dry summers.

Period of pollination	Male parent[1]	Fruits	Good seeds	Bad seeds	Good seeds/ 100 fruits
12/82-01/83	Calcutta (03)	1030	264	127	25.6
02/84-04/84	Wild type	1190	1652	49	138.8[2]
	Lidi (12)	2273	265	50	11.7
12/84	Lidi (12)	1088	116	7	10.7
02/87	0304 (41)	1831	708	25	38.7
	M-53 (42)	1995	563	30	28.2

[1]In parentheses are appended the code numbers applied to these diploids at CNPMF.
[2]The standing record for a Prata bunch at CNPMF came in this period, when a bunch with only 49 fruits yielded 287 good seeds.

Comparisons between pollen can be wholly reliable only when these are applied more or less alternately to the bunches which open in a common period, as rarely happened within or between classes of male parent. Exceptionally, a difference between

0304 and M-53 has been so confirmed (see Table 2), but of a much lower order than Table 1 suggests. Nevertheless, there appear to exist great differences in pollen effectiveness, a feature which is generally noted in the pollination of parthenocarpic banana clones. There is no evidence that these differences are linked to differential male fertility. All the wild forms of Table 1 are known to be homozygous for chromosome structural changes and are fully male-fertile, for example. Also, 0304 must be heterozygous for two translocations in its chromosomes, a factor well-known to limit gametic fertility, while M-53 from its origin cannot be heterozygous for more than one.

An alternative explanation has been advanced and supported by experimental data (Shepherd et al. 1987a). This is that such differences are related to differential pollen tube growth rates. Given the generally low rates of pollen tube penetration in ovaries of parthenocarpic forms (Shepherd et al. 1987b), it would not be surprising if the quicker tubes were the more successful ones.

Although first employed in Jamaica in 1965 as a routine practice in a breeding program, embryo culture was not adopted in Brazil from the beginning, and then had its "uncertain times". By 1986, after a conversion from a Knudson-based medium to a modified MS, and other modifications in procedure, the method began to work well. The period selected for Table 3, therefore, best reflects the full potential viability of seeds from the Prata subgroup. Clearly, the germination of sound seeds should present no problem, although some periods of excessive contamination have ensued.

A striking feature of hybrid production from the Prata subgroup has been the effect of season of pollination on the relative frequencies of different hybrid types (Table 4). Pollinations in the hot, dry summer period have consistently yielded elevated numbers of high polyploid products. Those in the intervening, cooler and more humid seasons have been less consistent from year to year, but on average have contributed a much higher rate of tetraploid recovery, along with a marked rise in numbers of diploids and low aneuploids. Even so, the efficiency of tetraploid production, in relation to numbers of

Table 3. Condensed results of embryo culture of the Prata subgroup at EMBRAPA-CNPMF: seeds from pollinations in the period May 1986 to April 1987.

Endosperm	Embryo	Seeds	Contaminated	Germinated[1]
Normal	Normal	983	6	857(87)
	Abnormal[2]	662	6	434(66)
	All	1645	12	1291(78)
Reduced	All	39	2	15
Absent	All	9	0	1
Subtotal		1693	14	1313(77)
Various	Absent	128		
TOTAL		1821	14	1313(72)

[1]In parentheses are germination percentages.

[2]"Abnormal" might be deformed or deficient in the haustorium, the most common failing, or in the meristematic stem, or might even be generally deformed.

Table 4. Seasonal variation in frequencies of different hybrid types from the Prata subgroup at EMBRAPA-CNPMF: pollinations in the period December 1982 to November 1990.

Season of pollination	Germinations	DFG[1]	Types of hybrid[2]			
			±2x	±4x	HP	X
December-April	2415	406(17)	41(2)	534(22)	1426(59)	8
May-November	852	176(21)	110(13)	349(41)	211(25)	6

[1]DFG - deaths, feeble, and "gross" (abnormal plants found only in cultures performed in 1987-88).

[2]±2x - diploids and hyperdiploids with counts of 23 or more; ±4x - includes some plants that were found to be inconstant in chromosome number or had one or two more or less than 44; HP - higher polyploids; X - counts ranging from 33 to 53 and not approximating closely to 4x; in parentheses are percentages of total plants.

flowers pollinated, is always greatest as a consequence of one of the seed output surges demonstrated in Table 2.

Tetraploid Production from Prata Anã

Seed production from Prata Anã has been consistently difficult, even with the most effective pollen parents (Table 5). Despite a major effort to exploit the cross with M-53 from 1989 to 1992, the seeds recovered amounted to only about one per 1000 flowers pollinated.

The output of tetraploid hybrids has been consequently quite low, although the germination rate in embryo culture has been good (Table 6). High-polyploid hybrids have been uncommon, but diploids and low aneuploids have been more frequent.

Table 5. Pollinations of Prata Anã at EMBRAPA-CNPMF: success rates from the principal male parents, 1982-92.

Male parent[1]	Fruits	Good seeds	Seeds per 100 fruits	Tetraploids obtained
Wild type				
Calcutta (03)	7 314	68	0.93	12
Buitenzorg (02)	2 524	13	0.52	1
Madang (04)	3 818	12	0.31	1
Cultivar				
Lidi (12)	17 851	37	0.21	5
Madu (15)	2 934	0		
Wild F_1				
0304 (41)	14 003	23	0.16	4
Parthenocarpic hybrid				
M-53 (42)	136 666	138	0.10	24

[1]In parentheses are appended the code numbers applied to these diploids at CNPMF, to simplify the recognition of hybrids.

Table 6. Condensed results of embryo culture in Prata Anã at EMBRAPA-CNPMF: seeds from pollinations in the period 1985 to 1992.

Endosperm	Embryo	Seeds	Contaminated	Germinated[1]
Normal	Normal	103	3	70(68)
	Abnormal	65	2	40(62)
Reduced	All	9	1	4
Absent	All	3	1	0
	Subtotal	180	7	114(63)
Various	Absent	10		
TOTAL		190	7	114(60)

[1]In parentheses are germination percentages.

This difficulty of tetraploid production is particularly unfortunate, since Prata Anã otherwise offered the most direct means of securing semidwarf tetraploid "Pratas".

Modes of Genotype Selection

Normally, the first selection stage has been based on one plant per genotype and on the first bunch produced, although an occasional embryo yields two or more shoots. Rigid criteria cannot be safely applied at this stage; it is sufficient that a plant should be vigorous, resistant to Sigatoka/yellow Sigatoka, and bear at least a moderate bunch. Sometimes a genotype is rejected also for unusually weak or slow suckering. Such a mild degree of selection was thought even more important when plants were being assessed at different seasons and in varying levels of soil fertility, while available space did not allow the use of many control plants of triploid parents.

The first clonal check on selected genotypes has been based on a varied number of plants of each, aiming at a minimum of five. This policy took account of limited space again, and also of possible time-saving, in the prompt roguing of the poorer types and the earlier identification of the most promising. Thereafter, expansion would depend on suckering rates, since the Brazilian team does not yet have much confidence in multiplication of tetraploids in the laboratory. It has been after the first clonal results that tests have been undertaken for resistance to Fusarium wilt. For more advanced agronomic testing, the aim is to enrol the aid of other institutions in diverse parts of Brazil, each accommodating a replicate of a national trial on a standardized protocol, including local varieties as controls.

Testing for Resistance to Fusarium Wilt

The methodology adopted was partly suggested by that formerly used by the program in Jamaica; with Brazilian adaptations, it has been outlined by Cordeiro et al. (1993a). The

test area was originally infested by a planting of the highly susceptible Maçã banana, in which disease attack finally became universal. Subsequent planting of test materials has been on a totally random scheme of single-plant replicates, at 2 x 2 m, with up to 10 replicates and with susceptible and resistant controls in similar or greater numbers. Since conditions in the test plot have not always been favorable for the expression of above-ground symptoms, evaluation in trials concluded up to 1991 was based solely on rhizome infections (scores 0-5 on the scale below), while a score of 6 was added for the latest test, very recently concluded, for plants visibly diseased. Rhizome symptoms were assessed as:

0 – rhizome totally clean;
1 – isolated infection points;
2 – infection up to 1/3 of the vascular ring;
3 – infection of between 1/3 and 2/3 of the ring;
4 – more than 2/3 of the ring infected;
5 – generalized infection of the rhizome.

The first major test was planted in 1988 and completed in 1989, when the great majority of plants had been examined soon after shooting bunches; a few were assessed while still in a vegetative phase. The aim then was to secure as much information as possible on the behavior of parents and hybrid families, rather than concentrate on individual tetraploid genotypes. Most of the data in Table 7 are from this trial, others from a subsequent one which appears to have been even more efficient. Results from both have been published by Cordeiro et al. (1991, 1993b).

Of the wild male parents, the best resistance source was shown to be Madang (04), *M. acuminata* ssp. *banksii*, although this plant has other serious defects in its hybrids, including a pronounced susceptibility to Sigatoka/yellow Sigatoka. The other wild forms, Buitenzorg (02) and Calcutta (03), evidently segregated for resistance, as did Lidi (12), but in at least the two latter cases in a more or less continuous fashion. For these crosses, Table 7 includes clones with resistant (mean score <1), susceptible (mean >3.5), and intermediate reactions. It is presumed that the uninfected plant of PC03-211 was a rare escape, as the later repeat test tends to confirm. In this clone, as well as in ST12-04, above-ground symptoms had been seen in some plants. None of the more resistant genotypes in Table 7, including PC12-05, are now regarded as showing any agronomic merit.

The second large test, from 1989 to 1991, included a further range of diploids and triploids, five tetraploid hybrids of some agronomic promise and a beginning of the testing of hybrids with 42. Although with few plants of each, the latter indicated a chance of an abrupt segregation, as the two examples of Table 7 show. The one heavily infected plant of PV42-58 was most likely an admixture from another selection; six further plants in tests in 1992/93 remained healthy.

Most important was the discrimination achieved among the five more critical hybrids (Table 8). JV03-15 was clearly as susceptible as the triploid parents, for all practical purposes; PV03-76 and PA12-03 were not much better, while PV03-44 and PA03-22 were moderately resistant.

Table 7. Resistance to Fusarium wilt of parents and selected hybrids in tests at EMBRAPA-CNPMF (A: 1988/89 and B: 1989/91).

Cultivar/hybrid	Test	Plants tested	Score frequencies[1]						Mean score
			0	1	2	3	4	5	
Prata subgroup	A	80	3	1	1	6	6	63	4.5
	B	30				1		29	4.9
Prata Anã	A	3			1			2	-
	B	19			1		1	17	4.8
Lidi	A	10	7	2	1				0.4
M-53	B	9	9						0
PV02-34[2]	A	4	2	1		1			(1.0)
PV02-24	A	4						4	(5.0)
SM03-101[2]	A	6	3	1	2				0.8
PC03-232[2]	A	7	2		5				1.4
PC03-199	A	6	2	1	2			1	1.7
PC03-143	A	7	1	1		1	2	2	3.1
SM03-68	A	6				4		2	3.7
	B	5						5	5.0
PC03-211	A	7	1					6	4.3
	B	5						5	5.0
PC04-01[2]	A	6	6						0
other x04	A	10	10						0
PC12-05[2]	A	9	8	1					0.1
PV12-03	A	9	1		2	4	2		2.7
ST12-04[2]	A	9					1	8	4.9
	B	5						5	5.0
SM42-36[2]	B	4						4	(5.0)
PV42-58	B	5	4					1	0?

[1]Scores given are on rhizomes only and record degrees of horizontal infection of the vascular ring, from 0 = uninfected to 5 = more than two-thirds diseased.
[2]PV indicates Pacovan while PC, SM and ST are accessions of common Prata; 02 is Buitenzorg, 03 is Calcutta, 04 is Madang, 12 is Lidi, and 42 is M-53.

Resistance to Sigatoka/Yellow Sigatoka

As stated above, some general results have been previously published (Shepherd 1990). Tetraploid hybrids from the use of Calcutta were all quite resistant, but not uniformly so, depending much also on current climatic conditions. More marked segregation was evident in the few hybrids with the male parents Pahang and Lidi.

Table 8. Resistance to Fusarium wilt of parent triploids and of five selected tetraploids in a test at EMBRAPA-CNPMF, 1989 to 1991.

Cultivar/hybrid	Test	Plants tested	Score frequencies[1]						Mean score
			0	1	2	3	4	5	
Triploids									
Prata subgroup		30					1	29	5.0
Prata Anã		19			1		1	17	4.8
Tetraploids[2]									
PV03-44		10	3	3	3	1			1.2
PV03-76		10	2			1	1	6	3.7
JV03-15		10				2		8	4.6
PA03-22	10		4	3	2		1		1.1
PA12-03	10		1		1	1	2	5	3.8

[1]Scores given are on rhizomes only and record degrees of horizontal infection of the vascular ring, from 0 = uninfected to 5 = more than two-thirds diseased.

[2]PV indicates Pacovan, JV is an accession of common Prata, and PA is Prata Anã; 03 is Calcutta, and 12 is Lidi.

No Lidi hybrid could be classified as highly resistant; possibly the best and the worst were both crosses of Prata Anã, respectively PA12-03 and PA12-05. More recent observation confirmed that many Lidi hybrids tend to accumulate lesions rapidly in older plants, although these may remain predominantly at an immature stage. Many M-53 hybrids have shown a very strong resistance, such that lesions are hard to find on even the oldest leaves, if at all.

In general, it seems that the genetic control of resistance is complex, with the joint operation of genes of major and minor effect, not necessarily all dominants. Whatever and however many the genes involved, the introduction of Sigatoka/yellow Sigatoka resistance could scarcely be any serious barrier in the breeding of Prata-type tetraploids.

Resistance to Black Leaf Streak/Black Sigatoka

While still limited in extent, reports from Costa Rica on two small series of assessments have been useful both as to the longer-term prospects of some tetraploids and as a partial guide to future breeding (Galindo et al. 1992; Escalant 1993). In the first test series, Pacovan and Prata Anã were both as susceptible as had been feared. Among diploids, M-53 proved to be rather less resistant than the control clone Lidi and much less so than the Calcutta hybrids 0304 and 0305, particularly in the increased level of attack after shooting. Three tetraploid hybrids of Pacovan x Calcutta also displayed leaf loss after shooting, but were at least adequately resistant in a general appraisal.

The second report brought a surprise and a disappointment. The good news was that at least some tetraploid hybrids with M-53 as male parent could yet be classified as resistant; the bad was that the Lidi cross PA12-03 did not reach a similar level of performance.

Agronomic Merits of the Tetraploids Produced

In the first place, it has to be continually emphazised that, in the greater part of the immense tropical area of production of Prata-type banana in Brazil, resistance to Sigatoka/yellow Sigatoka is in itself a major production gain, as pointed out by Cordeiro (1990). The other aspect to be repeated is that Prata itself is an unproductive cultivar even in the absence of this disease. Therefore, in relative terms, a hybrid may offer superior attributes without necessarily being an outstanding performer. This philosophy was never lost in the selection of the five best genotypes in the earlier clonal tests.

Among general defects, the first waves of hybrids did not increase the numbers of fruit per bunch. Further, the two "outstanding" PV03 hybrids initially had fruit size equal to that of the Pacovan mutant, but recent production has rarely reached this standard. Eating quality of the Calcutta-based hybrids also leaves something to be desired. Nevertheless, the triple resistance of PV03-44 makes it a potential substitute for Prata in some parts of Brazil and it has received favorable attention in the state of Espirito Santo, particularly, where Fusarium wilt is a serious problem in Prata.

The case of the Lidi hybrid PA12-03 is a special one in that, despite its very limited resistance to Fusarium wilt in the conditions of CNPMF, and its relative susceptibility to black leaf streak/black Sigatoka, it is resistant to Sigatoka/yellow Sigatoka, it has exceptional cropping speed for this type of banana, it has a very acceptable Prata flavor and, at least in the more favorable part of the year, it bears large fruit. Therefore, as the best "Prata" banana available for the moment, not necessarily as a long-term survivor, it is now being released as fast as conventional suckers can be produced, under the name of Pioneira.

Final assessment of many M-53 hybrids has been held back by over a year of very subnormal rainfall at CNPMF, together with a lack of irrigation capacity. Surely, one or more selections from these crossings will unite effective resistance to all three priority diseases (PV42-53, perhaps), together with large fruit of excellent quality, although they are generally tall plants and not quick-producers.

Breeding Maçã Bananas

Maçã must be among the most susceptible of all banana cultivars to what has long been described as race 1 of Fusarium wilt; in its reaction it is distinctly worse than the Gros Michel subgroup. On the other hand, it has always been the most popular table banana in Brazil, now by its scarcity commanding the highest prices on national markets. For

this reason, the EMBRAPA-CNPMF program cannot omit to pursue every possibility of producing a hybrid with at least very similar eating characteristics.

The beginning of this story is a sad one; the CNPMF experience agreed with that of Cheesman and Dodds (1942), that while Maçã produces seeds after pollination with AA diploids, germination is difficult and no tetraploid has been found among the few hybrids obtained (Table 9).

In 1985, however, on a visit to the then CIRAD-IRFA (now CIRAD-FLHOR) collection in the French Antilles, it was observed that Figue Pomme included at least two different forms, and that the accession Yangambi No.2 was not the varietal type. It did not, for instance, display the latter's tendency to arching of the leaves. At CNPMF, it has also appeared to be somewhat less susceptible to Fusarium wilt, although far from resistant. In recent times this has permitted the establishment of a pollination block, rather than plants in isolation from one another. Most important of all, early trial pollinations yielded three tetraploid hybrids.

As also shown in Table 9, this accession went into more intensive pollination in 1992, with pollen from M-53, and the outcome has been good. Seeds are produced in satisfactory numbers on average, although in an erratic fashion (132 of the good seeds came from only two bunches). Germination rates in culture have also varied much between seed lots, but are adequate overall (Table 10). The most important fact is that

Table 9. Pollinations of Maçã (MC) and of Yangambi No.2 (YB) at EMBRAPA-CNPMF: success rates in seed and tetraploid production.

Parent		Fruits	Good seeds	Seeds	Tetraploids
Female	Male			per 100 fruits	obtained[2]
MC	Various[1]	7 859	103	1.84	0
YB	Various[2]	865	29	3.35	3
	M-53[3]	5 628	208	3.70	42

[1]No pollinations since 1989.

[2]First explorative pollinations up to 1991.

[3]Intensive effort in 1992.

Table 10. Condensed results of embryo culture in Yangambi No.2 at EMBRAPA-CNPMF: seeds from pollinations in 1992.

Endosperm	Embryo	Seeds	Contaminated	Germinated[1]
Normal	Normal	75	8	38(51)
	Abnormal	90	4	43(48)
Reduced	All	6	0	2
	Subtotal	171	12	83(49)
Various	Absent	26		
TOTAL		197	12	83(42)

[1]In parentheses are germination percentages.

about half of the plants germinated have been tetraploids, so that the CNPMF team will soon have genotypes in the field from which to select.

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Musa Breeding at CIRAD-FLHOR

F Bakry, JP Horry

CIRAD-FLHOR is involved in banana improvement against diseases in two ways: (a) the rapid modification of germplasm in the Cavendish subgroup using such nonconventional methods as transformation with tissue culture; and (b) the creation of new clones of other triploid banana types through a conventional cross-breeding scheme using diploid clones.

Nonconventional Methods

This program, which is a collaborative effort between CIRAD-FLHOR, CATIE, the University of Orsay, and KUL, is focused on banana cultivars of the Cavendish subgroup because of their high sterility and economic importance.

The first step is to look for resistance to viruses: with priority given to CMV, then to BBMV, BBTV, and BSV in that order. Strategies for engineering weevil borer and nematode resistance may also be developed.

Various tools are required to develop these methods:

1. The availability of the resistance genes in a construction suitable for transformation (gene + good promoters). This construction is already available for CMV.

2. Techniques of genetic transformation: biolistics (particle gun) applied to cell suspensions or embryogenic callus; electroporation applied to protoplasts.

3. Efficient systems for plant regeneration from the treated material.

Thus, CIRAD-FLHOR is looking for the development of biolistic transformation methods and for regeneration systems by somatic embryogenesis from cell suspensions and callus. Electroporation applied to protoplast culture is being developed by our partners in the University of Orsay, France (Megia et al. 1992), within a joint European Commission project.

Somatic embryogenesis

During a first phase, calluses were obtained from immature male flowers after 4-10 months of culture on the same medium (Escalant et al. 1993). These calluses, when subcultured on an appropriate solid medium, developed embryogenic structures able to generate plantlets.

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In a second phase, secondary embryogenesis was notably enhanced by temporary immersion of the material in containers into a liquid medium. Changing the composition of the medium in the containers leads to the formation of plantlets. Using this system, 20,000 plants could be obtained per container per month (Escalant et al. 1994).

Those calluses initiated from flowers are also used for the establishment of cell suspensions in liquid medium. With Grande Naine (AAA 'Cavendish'), this suspension contains 8 million embryogenic clumps per liter, which doubles every 3 weeks (Côte et al. 1994). The material consists of clumps of a few cells and has the advantage, compared with an organized structure such as an embryo, of lowering the risk of obtaining chimaera after transformation.

Initially, regeneration of Grande Naine and French Sombre (AAB 'Plantain') has been achieved using this method. At CATIE, primary calluses have already been obtained from Gros Michel (AAA 'Gros Michel') and the hybrids FHIA-01 (AAAB SH 3481) and EMBRAPA 403 (AAAB JV.03.15).

At CATIE, field trials are being carried out on Grande Naine derived from secondary embryogenesis in order to test the conformity of the regenerated material. 'Plantain' types from cell suspensions are being evaluated in Cameroon, and Matavia (ABB 'Bluggoe') protoclones, regenerated from protoplast culture by our partners in the University of Orsay, are being observed in Guadeloupe. Matavia plants, the most advanced, do not currently show somatic variation.

If these conformity tests do not display high variability, it is planned to develop these techniques of somatic embryogenesis for massive propagation of commercial types in order to lower the costs of planting for farmers.

Transformation

Stable transformants of banana have been obtained recently using a combination of cell suspensions and biolistics. Transitory expressions of marker genes have been obtained in cell suspensions of *Musa acuminata* ssp. *malaccensis* at CIRAD-FLHOR in Montpellier, France (F Côte, pers. comm.). The expression of the GUS gene has recently been observed in cell suspensions by the CIRAD-FLHOR team 1 month after gene transfer by particle bombardment. Regeneration of plantlets from these suspensions is in progress to verify possible integration of the marker genes.

Conventional Breeding for the Creation of New Banana Clones

This work is focused on CIRAD-FLHOR, Guadeloupe, in close collaboration with CIRAD, Montpellier, France (PJL Lagoda, F Careel) and CRBP, Cameroon (E Fouré, C Jenny, E Auboiron).

CIRAD-FLHOR has developed an original strategy based on the creation of triploids using natural and improved diploid germplasm (Bakry et al. 1990; Horry et al. 1993). The

chromosome stock of these diploids is doubled by a colchicine treatment in order to induce the formation of tetraploids.

A second cross between this tetraploid and another diploid leads to the synthesis of triploid individuals. This approach has several advantages:

– The final product, being triploid, is highly sterile.

– The synthesis is flexible due to the high diversity available in the diploid germplasm. In this scheme, the genetic variability is given by three parents. At any time, new criteria of selection may be integrated in order to respond to new objectives or new strains of fungi.

– The use of a doubled diploid leads to the retention, in the triploid, of the genetic structure of the diploid (Bingham 1980).

Evaluation of the genetic diversity in wild species and diploid cultivars

Our breeding scheme relies on a good knowledge of diploid germplasm.

Wild species and cultivars are morphologically characterized in Guadeloupe and Cameroon; and black leaf streak/black Sigatoka resistance is evaluated in Cameroon (Fouré 1993). At the same time, the genetic diversity is studied at the molecular level using mainly RFLP markers in Montpellier (Carreel et al. 1994a). More than 150 diploid cultivars have been classified through checking their relationships with wild types on the one hand, and triploid cultivars on the other. Moreover, genetic distance among the diploids and the heterozygosity level for each one have been established.

It has been proved that part of the genomic composition of some diploid cultivars from Papua New Guinea has been derived from *M. schizocarpa*.

The evidence of a strong bias towards maternal transmission of chloroplast DNA and paternal transmission of mitochondrial DNA in *M. acuminata* has been demonstrated (Fauré et al. 1994). These uniparental modes of transmission and the comparatively lower rate of mutation of cytoplasmic genomes make the study of their genetic variability, an interesting tool to investigate banana intergroup and subspecies relationships.

Following the observation that both cytoplasmic data sets (mitochondrial and chloroplastic) are complementary, information on the different origins of cultivars may be inferred (Carreel et al. 1994b):

– Diploid cultivars with no recognized morphological structure may be divided into eight groups based on chloroplastic and mitochondrial patterns. Different *M. acuminata* subspecies or *M. schizocarpa* are classified in each group.

– The various AAB/ABB subgroups can be related to diploids. It has been found, for example, that the B genome has:

  – a maternal origin in 'Pisang Rajah' and 'Pisang Kelat'; and

  – a paternal origin in 'Bluggoe', 'Ney Mannan', and 'Pelipita'.

It has been shown that:

– both cytoplasmic genomes present in 'Pisang Awak' (ABB), 'Monthan' (ABB), and 'Peyan' (ABB) are derived from *M. balbisiana*; and

– no cytoplasmic genome present in 'Plantain' (AAB), 'Popoulou' (AAB), and 'Laknao' (AAB) is derived from *M. balbisiana*. Moreover, the A components of these AAB subgroups are derived from wild *M. acuminata* ssp. *banksii*.

This study will be compared with nuclear patterns in order to elucidate the genetic structure and to infer a possible phylogeny between diploid varieties (raw material for genetic improvement) and triploid cultivars (currently cultivated clones). The results will be used in a strategy to find better parents for given types of banana.

Improvement of diploid varieties

The best parents are being chosen in respect of the different kinds of triploids to be created (dessert or cooking banana).

The selection is being made to take into account their agronomic and genetic characters, and their resistance to diseases, especially black leaf streak/black Sigatoka. The work already carried out aims at determining the behavior of the diploid cultivars in crosses. These results so far demonstrate the necessity to determine good specific combining abilities between various parents.

Several diploid hybrids, selected in Guadeloupe for their agronomic and organoleptic characters, were sent to CRBP in Cameroon for an evaluation of their resistance to black leaf streak/black Sigatoka. Most of them showed good partial resistance.

Creation of triploid varieties (Fig. 1)

This last step relies on the 'tetraploidization' of mono- or interspecific cultivars in order to obtain auto- or allotetraploid cultivars. Ten autotetraploid clones and 3 allotetraploid clones have already been obtained. The first crosses between diploids and these tetraploids began in 1992. A study of 750 plants showed that these crosses between AAw and AAAAcv gave mainly triploid plants (Lemaire 1993).

By mid 1994, more than 400 AAA and 150 AAB hybrids obtained by these crosses have been planted for field evaluation and selection in Guadeloupe.

A first varietal creation was obtained through a cross between a wild *M. balbisiana* accession and an autotetraploid AAAA which produced IRFA 909. IRFA 909 is a synthetic AAB with 34 chromosomes. This plant, 2.60 m tall, is very vigorous without any inhibition of suckers. The pendulous bunch has 9 hands and its weight is 14 kg in the first cycle. The second flowering occurs 5 months after the first one. The fruit has similarities to Silk or Prata fruit. The taste is sweet-acid initially and becomes sweeter at complete maturity. The skin of the fruit is very thick, and the female sterility is quasi-absolute.

As both parents are resistant to black leaf streak/black Sigatoka, Sigatoka/yellow Sigatoka, and Fusarium wilt, IRFA 909 is expected to be resistant to these diseases. This clone will be tested very soon in Montpellier in early evaluation trials against various strains of *Mycosphaerella fijiensis*, and also in the field in Cameroon.

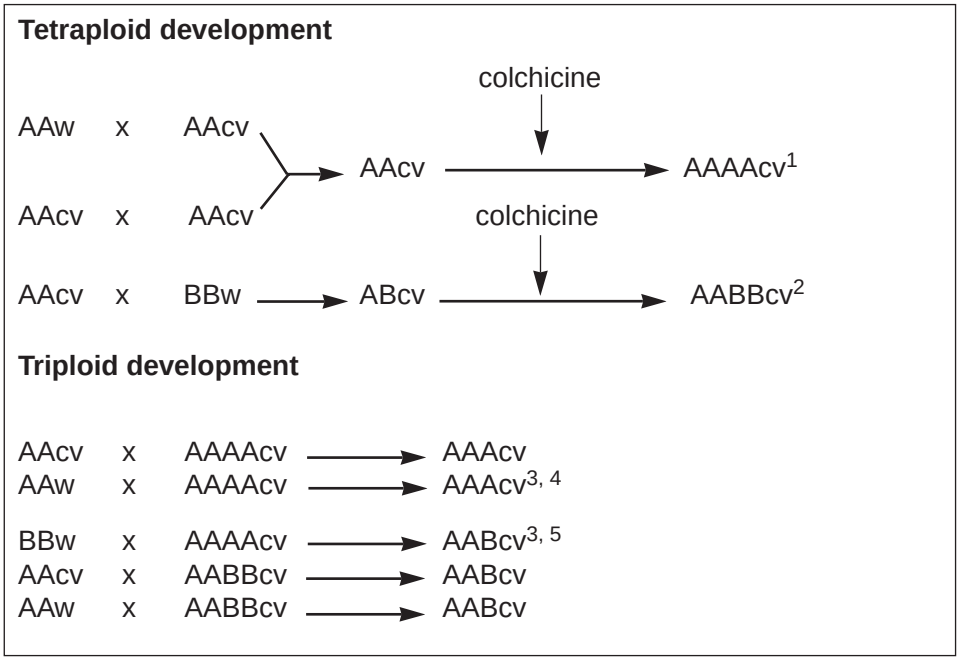

Figure 1. Banana improvement strategy through hybridization at CIRAD-FLHOR.

Key : cv = cultivar; w = wild species; [1] = ten clones available in 1994; [2] = three clones available in 1994; [3] = 550 individuals undergoing field evaluation in 1994 from there two crosses; [4] = 98% of the progeny are triploid; [5] = IRFA 909 was selected from this cross.

Various other hybrids are in preparation using the same method, and CIRAD-FLHOR will have other dessert or cooking banana clones available for testing during this year.

Looking Ahead

In the future, CIRAD-FLHOR aims at further enhancing its scheme for triploid synthesis. In this respect, a molecular marker-based linkage map has been established (Fauré et al. 1993). Seventy-seven markers, which were arranged in 15 linkage groups, were used to build this map. Another map is already in preparation which will be more saturated, including about 250 markers.

By comparing variations from one plant to another in the field with their corresponding patterns in RFLP, CIRAD-FLHOR will be able to locate the regions on this map that code for disease resistance, agronomic characters, and then for multiple allelic configuration.

Thus, it has already been found that resistance to black leaf streak/black Sigatoka is coded in at least two independent regions on the genome. It is hoped to produce the

same kind of results with other characters, such as height, the number of hands or fruit, etc.

The results will be mainly used for two purposes.

1. Early-stage selection in test-tubes or the glasshouse. Having in hand the biomolecular identity of each hybrid, it will be possible to obtain much data on the genotype of the plant prior to planting. Thus, all plants that have unfavorable characters can be eliminated before planting. This will represent important progress for breeders.

2. Transformation work. Useful banana genes will be isolated, integrated in plasmids with good promoters, and used in the transformation of other banana cultivars.

The CIRAD-FLHOR program will also be improved by the future application of androgenesis to produce pure lines. The use of pure diploid lines will enhance the efficiency of the initial crosses (Fig. 1). The advantages of this method are the following.

At the homozygote level:

– Clonal characters will only reflect the additive value of traits, which is highly inheritable;

– diploid fertility will be higher by elimination of structural heterozygosity.

At the F_1 level:

– The mean additive values of characters will be increased by the heterosis effect;

– All individuals that originate from one cross are theoretically identical. Thus, the number of progeny that needs to be studied will be significantly lower.

This method is currently being established in Guadeloupe (Bakry, Horry 1992) and androgenesis has been achieved using the Pahang clone of *M. acuminata* ssp. *malaccensis* (F Kerbellec, pers. comm.). Androgenetic callus has been obtained from 40% of pollen, and over 50% of shoot regeneration from these calluses was obtained. DNA content, quantified by flow cytometry, showed that the regenerated plants are haploid, diploid (single duplication), and tetraploid (double duplication).

Acknowledgments

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Breeding Plantain-Type Hybrids at CRBP

C Jenny, E Auboiron, A Beveraggi

Introduction

With an annual production of 72 million t (FAO 1992), bananas and plantains are among the major fruit crops of the world. Although cooking bananas predominate, plantain stands as one of the most important food crops in Africa. Moreover, the recent fall in coffee and cocoa prices has led to a new interest in plantain cultivation, particularly since the development of farming systems for this *Musa* type.

This situation largely accounts for the increase in land clearing in Africa, worsened by the need in bush-fallowing to frequently change plots when agronomic constraints, pests, and diseases become too severe. In order to slow down this process, genetic improvement has a role to play through placing new cultivars resistant to major diseases at the farmers disposal. Improved cultivars would enhance sedentary cultivation, reduce pesticide use, and contribute to the preservation of the environment.

Two wild species are the origin of most cultivated bananas. These are *Musa acuminata* (A) and *Musa balbisiana* (B). If these wild bananas are diploids ($2n = 2x = 22$), cultivated banana clones can be diploids, often triploids ($2n = 3x = 33$) and occasionally tetraploids ($2n = 4x = 44$). Numerous subgroups exist in the AAB triploid group related to their characteristics, use, and geographic distribution. The plantain subgroup of the AAB group has undergone considerable secondary geographic spread in Africa, linked to high phenotypic diversification. The study of this diversity, through the use of molecular markers (Horry 1989), has shown it is not linked to any great genetic heterogeneity. All have black leaf streak/black Sigatoka disease susceptibility (Fouré et al. 1990). Natural diversity being nonexistent in this subgroup, it was essential to build up a genetic improvement program to produce plantain-like hybrids, having both a good level of resistance to disease (especially black leaf streak/black Sigatoka), and adequate fruit quality.

Some triploid varieties show residual female fertility, and are capable of producing nonreduced female gametes ($2n = 3x = 33$). This is the case for plantain cultivars and Pacific cooking banana cultivars (AAB 'Maia Maoli/Popoulou'). It is also well known that by crossing such a triploid variety with a diploid, it is possible to obtain $4x$ tetraploid

CRBP (Centre Régional Bananiers et Plantains), BP 832, Douala, Cameroon

hybrids. This result has already been used with success by FHIA, IITA, and CIRAD-FLHOR and others. Thus, the medium-term objective of the CRBP program is the creation of tetraploid hybrids by interploid crosses. Proposed crosses are consequently based on the conservation of the triploid female parent genome, which permits the preservation of agronomic characters and fruit qualities, and adding to this the haploid genome from the male diploid parent with genes for resistance to disease. In the progeny, only tetraploid hybrids are conserved, being the only hybrids that retain the full genome complement from the plantain parent.

If, at the start, this program was neither new nor original (Tezenas du Montcel 1993, Vuylsteke et al. 1993), CRBP has made some specific improvements. First, CRBP has at its disposal one of the largest field collections of plantain cultivars, and is, therefore, able to screen the entire variability in this subgroup. In addition, CRBP proposes to carefully control the transmission of the characters derived from the male parent. Thus, CRBP will use AA diploid clones as homozygous as possible. If necessary, an inbreeding program will be established in order to increase the homozygosity of these male parents. CRBP has also the advantage of an excellent environment at Njombé in Cameroon, in which all the ecological requirements of plantain are found in respect of climate and soil. Black leaf streak/black Sigatoka is present at Nyombé, and hybrids can be screened for resistance using Fouré's scoring procedures (Fouré et al. 1984). Other environments are available in Cameroon, such as the Western Highlands, where Sigatoka /yellow Sigatoka is present. Also, it is now possible to screen plants against weevil borer (*Cosmopolites sordidus*) and nematodes (especially *Radopholus similis* and *Pratylenchus goodeyi*). We present here some of the first results obtained from this program of research at CRBP.

Materials and Methods

Female fertility screening using Calcutta 4

Crossing during the first year concentrated on screening plantain and Maia Maoli/Popoulou cultivars for female fertility using *Musa acuminata* ssp. *burmannicoides* (Calcutta 4). This is a highly male-fertile clone (at least 90% pollen viability determined by the Alexander [1969] method), strongly homozygous, and presenting a phenotype "highly resistant" to black leaf streak/black Sigatoka (Fouré et al. 1990). Results have been recorded in terms of seed production, but the quality and the viability of the embryos obtained were also assessed. Later, the hybrids produced were observed in the field.

Breeding using M53 and M53Hn

The CRBP program uses mainly the M53 hybrid, a synthetic diploid bred in Jamaica, as the male parent. The genealogy and the main features of M53 are described in Figure 1. However, because M53 is highly heterozygous, it is not compatible with the envisaged

breeding program. In order to minimize this disadvantage, an inbreeding program for M53 is being undertaken. Successive self-pollinations are being made to increase the overall rate of homozygosity (Fig. 2). Intermediate hybrids are called M53Hn, where n is

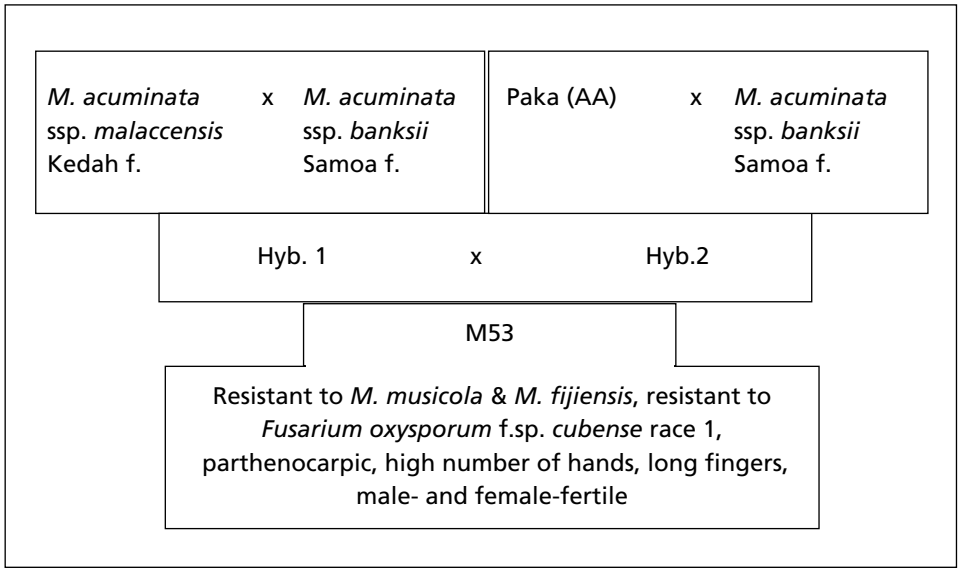

Figure 1. Genealogy of M53 and its main features.

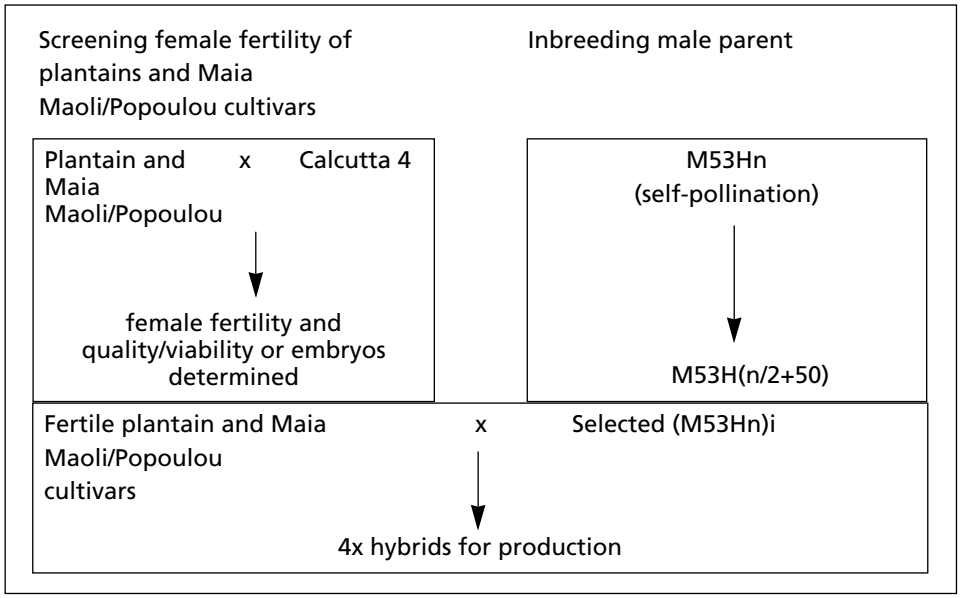

Figure 2. The conventional 3*x*/2*x* hybridization program at CRBP: breeding plantain-type hybrids for field use.

the mean rate of homozygosity. Thus, an important component of the program is the progressive improvement of the male parent. At present, only M53H50s (50% homozygous clones) have been selected and used. A new generation of M53H50s has been achieved on a larger scale, in order to get a better selection.

Embryo rescue

The number of hybrids produced is not directly linked to the number of seeds produced. In fact, a considerable number of harvested seeds are completely empty, or contain no albumen, and have either no embryo or an abnormal one. The embryos found are then extracted from the seeds and cultivated in vitro. This method permits the germination of all true embryos, and even of some embryos that will never grow as normal plantlets under natural conditions.

Phase 1: germination. Embryos are placed in vitro in Petri dishes on an MS (Murashige, Skoog 1962) medium with Morel vitamins, 30 $g.L^{-1}$ saccharose, 2 $g.L^{-1}$ phytagel, 2 $mg.L^{-1}$ BAP and 1 $mg.L^{-1}$ AIA (pH 5.8).They are then incubated in the dark at 28°C. If no germination is observed within 3 months, embryos are eliminated.

Phase 2: growth. Reactive embryos are placed on a growth medium as soon as the size of the plantlet allows transfer. The growth medium is composed of MS solution, Morel vitamins, 0.25 $g.L^{-1}$ active charcoal and 2 $g.L^{-1}$ phytagel (pH 5.8). Plantlets develop at 28°C under light for 12hd $^{-1}$.

Phase 3: acclimatization. This requires two phases of 1 month's duration each. In the first phase, plantlets are put in small pots in a 1:1 medium of soil and coffee husks and grown under mist. Light intensity is progressively increased, and misting is slowly decreased. In the second phase, acclimatization takes place in a shaded glasshouse, in 2.5 L bags of the same medium. Plants are then ready for the field.

Chromosome counting

Chromosome counting was undertaken using a protocol established by Shepherd (see Bakry, Horry 1992). Root-tips are taken from plants in the field before 08:00 a.m. during the period of highest mitotic division and immediately placed in hydroxyquinoline solution to stop mitosis. After 7 h, the root-tips are immersed in a solution of acetic acid, ethyl alcohol and water (4:1:5) for 12 h and then flattened between a slide and a cover-slip in orcein. Observation is made under x250 magnification.

Results and Discussion

Female fertility screening using Calcutta 4

AFF is defined as apparent female fertility (= number of seeds produced, irrespective of their quality). The screening of female fertility has been undertaken using *M. acuminata* ssp. *burmannicoides* (Calcutta 4). Table 1 summarizes the average results for the two subgroups tested. As very few crosses were undertaken with Maia

Maoli/Popoulou cultivars, discussion will be limited to the results of crosses with plantain cultivars.

The AFF for plantain is higher than the AFF for plantain observed at CIRAD-FLHOR, Guadeloupe. For 11 cultivars tested in the same way, the average AFF is 10.4 seeds/bunch at CRBP and 6.0 seeds/bunch at CIRAD-FLHOR.

Table 1. Results of successful crosses using Calcutta 4 and M53 as the male parents.

Male parent:	Calcutta 4			M53		
Female parent :	Plantain[1]	Maia Maoli/ Popoulou[2]	Total	Plantain	Maia Maoli/ Popoulou	Total
Number of hybridizations	46	6	52	14	2	16
Number of harvested seeds	361	104	465	20	0	20
Mean number of seeds/hybridization (AFF)	7.85	17.33	8.94	1.43	–	1.25
Number of embryos obtained	108	39	147	6	–	6
Number of embryos/ hybridization (IFF)	2.35	6.50	2.83	0.43	–	0.38
Mean number of embryos/seed	0.30	0.38	0.32	0.30	–	0.30
Number of living embryos	9	14	23	3	–	3
Survival rate in vitro (living/total embryos)	0.08	0.36	0.16	0.50	–	0.50
Mean survival rate (living embryos/seed)	0.02	0.13	0.05	0.15	–	0.15
Number survivors/ hybridization (DFF)	0.20	2.33	0.44	0.21	–	0.19
Number of hybrid-izations to produce one hybrid in the field	5.11	0.43	2.26	4.67	–	5.33

[1]Plantain - mainly French plantain, but also some False Horn plantain whose AFF=0.

[2]Maia Maoli/Popoulou - AFF=0 for Popoulou (CMR), but AFF was high for Poingo.

An earlier study showed that the AFF in Njombé was also higher than the AFF recorded at IITA (Onne, Nigeria), mainly due to differences in soil fertility (Jenny et al. 1993). For 46 crosses analyzed at CRBP, the average AFF was 7.85 (Table 1). The results obtained with individual cultivars are presented in Table 2.

Table 2. Different levels of female fertility observed in the plantain subgroup using Calcutta 4 as the male parent.

Female parent	Number of bunches harvested	Average seed and embryo set					
		AFF	Good	Embryos	True	IFF	DFF
French Rouge 03	1	37	33	6	6	6	1
Mbai	1	26	24	13	12	13	1
Amoung	1	22	13	5	4	5	1
Rose d'Ekona	2	18.5	13	7	4.5	6	0.5
Ibou Dikondo	1	16	11	6	3	6	1
Okel	2	13.5	7	4	3.5	4	1.5
Ekona n°1	1	13	10	1	0	1	0
French Sombre (Noir de Loum)	6	12.3	8.8	2.5	1.3	2.5	0
Red Ogoni	1	12	10	0	0	0	0
Ndom Rouge	1	12	10	4	2	4	0
Oyong	1	11	9	3	1	3	1
Bobby Tannap (Fokamezo)	3	10.3	10	6.3	6.3	6	0
French Clair (Obino l'Ewai)	5	9.6	5.4	1.8	1	1.8	0.2
Ovang	2	9	9	7	7	7	0
Nyombé 1	1	5	4	2	1	2	0
French Rouge	1	2	1	1	1	1	0
Zip Ekon	1	2	0	0	0	0	0
Elat noir / Fouem / Madimadi / Mbotoko vert/ Mebae Me Ngomo / Meko'O / Mulolou / Ndoun / Ngok Egome / Njock Kon / Ntanga 6 / Okele / Plantain n°2 / Red Yade		0	0	0	0	0	0

Seed quality: AFF = apparent female fertility = number of seeds produced. Good = nonspoiled, nonfloating seeds. Embryos = number of embryos, whatever their quality . True = number of complete embryos + full reserves. IFF = intermediate female fertility = number of embryos placed in vitro. DFF = definitive female fertility = number of hybrids planted in the field.

Seed quality is evaluated during shelling. First, floating and spoiled seeds are eliminated (lack of albumen). Selected seeds are opened, and the presence of an embryo is noted. A seed is said to be "true" if reserves are complete and the embryo looks normal. As there are often few embryos per hybridization, all embryos, true or not, are placed in vitro: this is the second parameter (IFF = intermediate female fertility = number of embryos placed in vitro for each cross). The average IFF observed at Njombé for crosses using Calcutta 4 is 2.35 embryos/bunch.

The best embryos germinate or proliferate in clusters after 2-3 weeks. However, some embryos just swell while others do not develop at all, or die. Leaf emission is very slow and leaf growth is reduced with some developing embryos while others do not produce any true organs. DFF (definitive female fertility) is defined as the number of hybrids actually planted (irrespective of their chromosomic structure). Using Calcutta 4 at CRBP, an average of 0.20 hybrids have been produced from each cross (Table 1).

Seasonality of seed production

Crosses using Calcutta 4 are still continuing because some cultivars have not yet been tested, but also because some seed production seasonality has become apparent. Hybridizations of French Sombre and French Clair (AAB 'Plantain') have been undertaken for a full year. Results suggest that the highest seed production period is when crosses are carried out between May and November during the rainy season. The results are presented in Figure 3. In absolute terms, i.e. looking at the number of seeds produced per bunch, the optimum period lies between August and November (Fig. 4).

Figure 3. Monthly variation in seed set following successful crosses between French Sombre and French Clair (female parents), and Calcutta 4 (male parent).

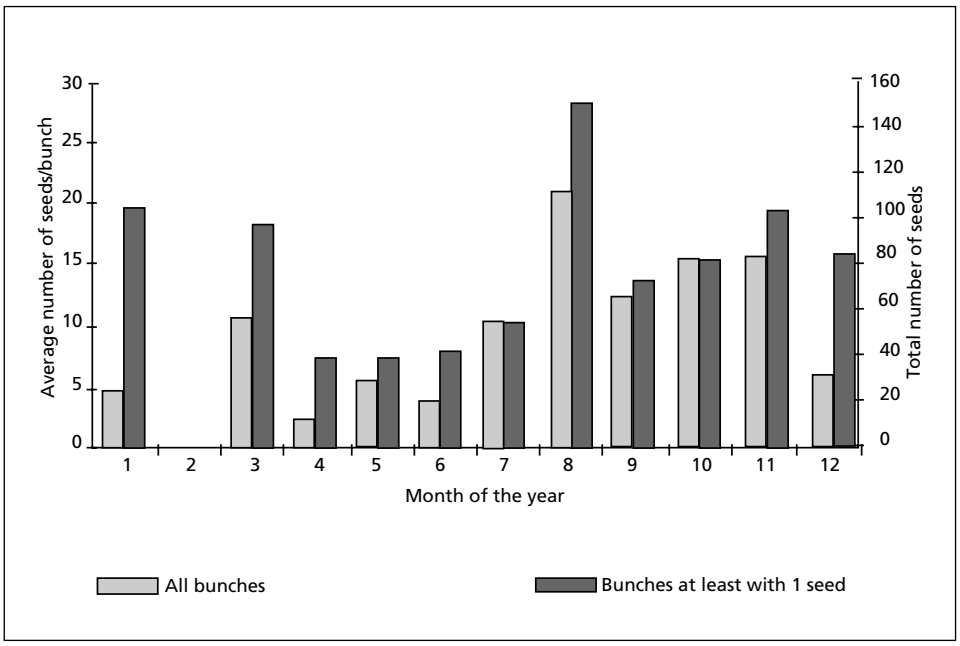

Figure 4. Monthly variation in the number of seeds produced in successful crosses between French Sombre and French Clair (female parents), and Calcutta 4 (male parent).

Breeding using M53 and M53H50 hybrids

Crosses using M53 began in January 1993. Hybridization using selected M53H50 commenced as soon as male flowers were available. Table 1 summarizes the average results for hybridization with M53.The overall AFF for M53 recorded on 16 analyzed bunches is clearly lower than with Calcutta 4. However,as regards the crosses currently analyzed in the laboratory, the survival rate of embryos in vitro is higher for crosses with M53. Also, the average surviving percentage of embryos in relation to the number of seeds produced is 15% with M53, as compared to 2% with Calcutta 4.

If one compares the general results obtained with Calcutta 4 and M53, we have equivalent DFFs for both male parents (0.20 survivor/hybridization for Calcutta 4 and 0.21 for M53). The low AFF of M53 is offset by a high in-vitro survival rate of about 50%, while only 8% of Calcutta 4 hybrids survive. Results are still lower with M53H50 (Table 3). With all M53H50, the maximum AFF was only 0.70 (mean of 33 bunches recorded) and, up to April 1994, no embryo had survived.

The most important result that was obtained with the M53H50 population concerned resistance to black leaf streak/black Sigatoka. Two hundred and fifty F_1s from the self-pollination of M53 were observed in the field. As M53 shows a good partially resistant phenotype against black leaf streak/black Sigatoka, 40% of the F_1s were also partially resistant, with degrees of resistance ranging around M53's level. However, the other 60%

Table 3. Fertility of some M53H50s: completed hybridizations with AAB-plantain cultivars as female parent.

			Seeds		Embryos		Survivors	
Male parent	BS	Hyb	N°	AFF	N°	IFF	N°	DFF
M53H50 - 005	PR (10.2)	3	4	1.33	0	0.00	0	0.00
M53H50 - 025	PR (9.3)	3	1	0.33	0	0.00	0	0.00
M53H50 - 050	PR (10.1)	3	0	0.00	0	0.00	0	0.00
M53H50 - 092	HR	4	11	2.75	1	0.25	0	0.00
M53H50 - 164	HR	11	5	0.45	3	0.27	0	0.00
M53H50 - 198	PR (7.9)	3	0	0.00	0	0.00	0	0.00
M53H50 - 227	HR	3	2	0.67	0	0.00	0	0.00
M53H50 - 241	HR	3	0	0.00	0	0.00	0	0.00
Total		33	23		4		0	
Means				0.70		0.12		0.00

BS: Black leaf streak/black Sigatoka reaction (HR = highly resistant, PR = partially resistant [Fouré et al. 1990] with the average youngest leaf with necrosis in parenthesis). All observations were made during the dry season, when disease development is slow, and these data must therefore be assessed with some caution.

Reference: Grande Naine: susceptible (6.5), M53: PR (10.9)

Hyb = number of hybridizations.

AFF, IFF & DFF = apparent, intermediate and definitive female fertility obtained with these male parents on all tested plantain cultivars.

expressed a “highly resistant” phenotype (similar to that of Calcutta 4), which was not expected at all because it was believed to be linked to a completely different set of genes corresponding to another type of resistance (vertical resistance). Generally speaking, observation of M53H50 shows a loss of vigor for these hybrids in comparison with parental M53. In addition, large differences are noted regarding the male fertility level of these plants:

– some flowers are abnormal (M53H50-216: no stamens, but several styles partially aborted; M53H50-241: three stamens only, free tepal aborted, perigon cut);

– quantities of pollen and associated viability are variable (presence of pollen noted from “absent”, i.e. M53H50-241 and -226, to “abundant”, i.e. M53H50-164 and -198); Alexander’s test revealed viability from 40% (M53H50-198) to 90% (M53H50-048, -154, and -227).

Among the 15 selected M53H50s, -005 and -092 seem to be the best agronomically. As for seed production and development of embryos, acceptable AFFs have been obtained with M53H50-005, -092, -164, and -227. These observations may raise some doubts with the inbreeding scheme. However, an additional large scale self-pollination of M53, under way in 1994, should permit the selection of better-performing male parents.

Field observations

The first hybrids were planted in the field in July 1993 and flowering began in February 1994, most likely on diploids. The evaluation of the hybrids for reaction to black leaf streak/black Sigatoka has begun. To date, all hybrids evaluated are derived from crosses using the "highly resistant" male parent Calcutta 4. Resistant hybrids show a "partially resistant" response (Table 4).

Table 4. First evaluation of 3*x*/2*x* hybrids produced at CRBP.

Crosses	N°	Hybrid code	Ploidy level	Black leaf streak/black Sigatoka resistance
Mbai x Calcutta 4	1	ANA 001	triploid	PR (11.5)
French Rouge 3 x Calcutta 4	1	ANA 019	NE	S
Oyong x Calcutta 4	1	ANA 020	diploid	S
Obino l'Ewai x Calcutta 4	1	ANA 031	NE	NE
Amoung x Calcutta 4	1	ANA 015	NE	PR (10.0)
Ibou Dikondo x Calcutta 4	1	ANA 016	NE	PR (10.1)
Okel x Calcutta 4	1	ANA 014	NE	PR (8.9)
Kelong Mekintu x M53	1	ANA 033	NE	NE
Fokamezo x M53	1	ANA 034	NE	NE
Kelong Mekintu x M53	1	ANA 035	NE	NE
French Clair x M53	5	ANA 036, 037, 038, 039 & 042	NE	NE
Rose d'Ekona x Calcutta 4	1	ANA 041	NE	NE
Poingo x Calcutta 4	14	ANA 003	diploid	PR (10.0)
		ANA 004	triploid	PR (10.2)
		ANA 005	NE	PR (9.1)
		ANA 006 007, 008	NE	S
		ANA 009	NE	PR (9.7)
		ANA 010 & 011	NE	S
		ANA 026	triploid	NE
		ANA 027, 028, 029 & 030	NE	NE

Note 1: Fokamezo = Kelong Mekintu and Obino l'Ewai = French Clair.

Note 2: S = susceptible; PR = partially resistant; NE = not evaluated. The number of the youngest leaf with necrosis is shown in parenthesis. Reference: Grande Naine: S (6.5); M53 : PR (10.9)

Ploidy level : diploid (20 to 24 chromosomes); triploid (30 to 36 chromosomes)

All female parents are AAB-plantain cultivars, except Poingo which is an AAB-Maia Maoli/Popoulou cultivar.

Conclusion

Technical results

The conventional $3x/2x$ hybridization technique is now proven and female-fertile plantain parents have been identified. The first hybrids obtained using Calcutta 4 have potential interest. The use of M53 as a male parent seems possible despite its poor AFF, as its DFF is equivalent to Calcutta 4.

M53 is an interesting AA hybrid, as much for its agronomic qualities as for its resistant reaction to black leaf streak/black Sigatoka. However, its heterozygosity is a problem that must be overcome before the transmission of characters can be accomplished in a controlled manner.

The inbreeding scheme used to improve male parents must be very selective. In fact, heterosis constitutes a major part of M53 vigor, and it is necessary to keep as much of the parental strength as possible in selected progeny. On the other hand, black leaf streak/black Sigatoka resistance does not seem to be much related to heterosis. Thus, selection of improved male parents must be undertaken using a large population.

As androgenesis is already employed at CIRAD-FLHOR, it seems likely that this technique could also be successfully applied to M53 in order to quickly obtain pure-lines. An androgenetic callus of M53 has already been produced in Guadeloupe.

Qualitative results

It is now known that transmissibility of black leaf streak/black Sigatoka resistance is easily achieved with Calcutta 4. Research with M53 is in progress. The most important result with Calcutta 4 hybrids relates to their black leaf streak/black Sigatoka resistance. All hybrids produced are “susceptible” or “partially resistant” even though Calcutta 4 expresses a “highly resistant” phenotype. This supports the idea that the mechanisms of black leaf streak/black Sigatoka resistance inheritance may be somewhat more complicated than commonly believed.

Future developments

The systematic counting of chromosomes to identify triploid hybrids, and its application at the glasshouse stage, will help to reduce selection work in the field. In order to increase the efficiency of the hybridization program, it would also be interesting to know whether or not there is a correlation between the season, seed production and seed quality, including the chromosomic structure of embryos.

Since January 1994, the fruit of various cultivars has been studied in order to obtain technical parameters for quality. When this work is finalized, the fruits of all hybrids produced will be compared routinely with known standards.

An effort will be made to select more suitable male parents through inbreeding schemes, but also through inspection and evaluation of new varieties. In the future, it is

possible that other types of resistance (for instance to *Fusarium oxysporum* f.sp. *cubense* and *Radopholus similis*) could be included in the breeding program.

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Musa Improvement in India

S Sathiamoorthy

Introduction

The agro-ecological situations in India are so diverse that banana is grown in coastal regions, deltaic areas, and inland to altitudes of up to 1500 m. The systems of banana cultivation also differ within regions and subregions. They are broadly classified as: (a) the gardenplot system; (b) the wetland system (deltaic areas only); and (c) the perennial system.

In garden plots, banana is annually replanted in rotation with vegetables. Ratooning is occasionally practiced.

In the upper levels of the deltaic regions of Tiruchirappalli and Periyar districts of Tamil Nadu, banana is grown in wetlands in rotation with rice. In addition to planting annually (single crop), a two-ratoon system is also practiced.

In the perennial system, there is no annual replanting. Old plantations of over 75 years do exist in 'padugai' lands (high-level silt deposited along river banks) on the plains in Thanjavur district of Tamil Nadu. Banana cultivars, predominantly Virupakshi (AAB 'Pome'), are grown perennially without annual replanting in hills at altitudes ranging from 800 to 1500 m, either as a sole crop or as a shade crop for coffee. In the perennial system (in both plains and hills) banana is grown without irrigation under rainfed conditions.

Irrespective of where they are grown, Cavendish clones and French plantain are not generally ratooned.

Cultivars

India has a number of commercial banana cultivars ranging in diversity from delicate edible diploids such as Matti (AA) and Sanna Chenkadali (AA), which can be nurtured only in sheltered and humid environments, to such hardy hybrid triploids as Monthan (ABB) and Ney Vannan (ABB), which can tolerate seasonally arid monsoon climates prevailing in most parts of the country. Nendran (AAB 'French Plantain') is the food fruit of the southern state of Kerala. There is a wide range of commercial cultivars of diverse genome and ploidy. The most important commercial cultivar is Poovan (AAB 'Mysore'),

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Table 1. Important cultivars of dessert banana in India.

Genome	Cultivar	Subgroup
AA	Matti (crocodile finger banana)	
	Namarai	
	Sanna Chenkadalai	
AB	Ney Poovan	
	Kunnan (7 types)	
AAA	Robusta and Dwarf Cavendish	Cavendish
	Chenkadali	Red
	Manoranjitham	
	Chakkarakeli	
AAB	Poovan	Mysore
	Rasthali	Silk
	Virupakshi	Pome
	Pachanadan	Pome
ABB	Karpooravalli	Pisang Awak

followed by Robusta and Dwarf Cavendish (AAA 'Cavendish'). In three areas of Maharashtra and Gujarat states, Dwarf Cavendish is the predominant cultivar. Tamil Nadu state is the largest banana-growing region in India. Important cultivars in India are tabulated in Tables 1 and 2.

Regional and subregional consumer preferences in India vary considerably, and these determine the choice of the cultivars grown. A detailed account of the clonal situation in India has been reported by Bhakthavatsalu and Sathiamoorthy (1979). Tamil Nadu Agricultural University (TNAU) has 243 accessions which, after critical analysis, were found to comprise 48 distinct types and 23 mutants, totalling 71 distinct cultivars. There are only 11 diploids of AA genome and all the AB diploid cultivars are of no breeding significance because they are nonpolleniferous.

Cultivar Evaluation

Morphology

Detailed investigations of the morphological characters and taxonomical status of banana were previously published by Venkataramani (1949) and Jacob (1952). The results of metroglyph analysis of several southern Indian banana cultivars revealed that most are closer to *M. balbisiana* than *M. acuminata* (Raman et al. 1968). Genetic

Table 2. Important cultivars or plantain and cooking banana in India.

Genome	Cultivar	Synonyms; remarks
AAB	Nendran	French plantain
plantain	Moongil	Horn plantain
	Myndoli	Giant plantain
	Valathen	Wine plantain
ABB	Nalla Bontha	Bluggoe
cooking	Thella Bontha	Silver Bluggoe
banana	Nalla Bontha Bathees	Small fruited Bluggoe
	Monthan	Kanchkela
	Booditha Bontha Bathees	Small fruited Monthan
	Kuribontha, Chakkia	—
	Ney Vannan	—

analysis of seven characters in dessert and cooking banana cultivars was reported by Sree Rangasamy et al. (1980) at TNAU. Nayer et al. (1980) reported wide variations in 13 morphological characters based on phenotypic and genetic coefficient of variation, heritability, and genetic advancement. Valsalakumari et al. (1985) carried out extensive genetic divergence studies and grouped 62 banana cultivars into eight clusters.

Cytology

A survey of Indian cultivars, focused on their cytotaxonomical status and breeding potential, was carried out by Bhakthavatsalu and Sathiamoorthy (1973). A detailed study of microsporogenesis in 14 cultivars of different ploidy levels was made by Sathiamoorthy (1979, 1987). He observed irregular meiosis even in first and second divisions. A wide variation in tetrad shape and the number of microcytes per tetrad was also observed. Chromosome association during pollen meiosis, mainly at metaphase I of the first division, was studied in 16 clones. All kinds of chromosomal aberrations were reported. Raman (1973) and Sathiamoorthy (1987) observed that the average association of univalents is higher in triploids than in diploids. In general, all the banana cultivars exhibit some degree of chromosomal malfunctioning during microsporogenesis. Precocity in separation, loose pairing, many bridges at anaphase, variable interchanges, occasional pairing failure, and unequal distribution of chromosomes at I and II divisions are commonly observed. On the whole, the genomic differences of the clones are not observed in their cytological behavior (Table 3).

Table 3. Chromosome associations in some banana clones (Sathiamoorthy 1987).

		Chromosome association at metaphase I			
Clone	Genome	IV	III	II	I
Diploids					
M. acuminata ssp. *burmannica*	AA	-	-	10	2
M. balbisiana clone Sawai	BB	-	-	10	2
M. laterita (sec. Rhodochlamys)	-	-	-	11	-
Anaikomban	AA	-	-	9	4
Matti	AA	1	-	8	2
Namarai	AA	-	-	7	3
Pisang Lilin	AA	-	-	9	4
Tongat	AA	1	-	6	6
Paka	AA	-	-	8	6
Triploids					
Robusta	AAA	1	5	5	4
Dwarf Cavendish	AAA	-	6	5	5
Wather	AAA	-	5	8	2
Nendran	AAB	-	5	6	6
Monthan	ABB	1	4	6	5
Tetraploids					
Kluai Teparot	ABBB	5	1	7	6
Bareli Chinia x Pisang Lilin	AABB	6	-	8	4

Pollen studies

The quantity of pollen produced per anther, variation in pollen size, viability by stainability, and germinability in vitro were assessed in 34 *Musa* accessions, including three wild species (Sathiamoorthy, Rao 1980; Sathiamoorthy 1987) (Table 4).

The successful use of triploids as a pollen parent has led to the study of microspore ploidy levels of banana clones of various ploidy and genome. Using Darlington's ratio of spore ploidy level, Sathiamoorthy (1987) assessed the ploidy level of pollen grains based on size. It is interesting that many triploids as well as tetraploids produced haploid pollen grains (Table 5).

In TNAU breeding programs, Robusta (AAA), Nendran (AAB), and Pisang Seribu (AAB) have already been used successfully as a male parent with highly female-fertile wild species, as well as with diploid cultivars. This offers good scope for introducing into the breeding program many useful horticultural traits not present in diploids.

Table 4. Mean pollen output of diploids, triploids, and tetraploids (Sathiamoorthy 1987).

Clone	Genome	Pollen output	
		per anther	per flower
M. balbisiana	BB	47,142	235,710
M. acuminata	AA	40,119	200,595
M. laterita	-	38,540	192,700
Ambalakadali	AA	12,224	61,120
Anaikomban	AA	9,367	46,835
Eraichivazhai	AA	13,065	65,325
Matti	AA	4,523	22,615
Namarai	AA	3,592	17,960
Nivedyakadali	AA	3,541	17,705
Pisang Lilin	AA	15,710	78,550
Tongat	AA	12,540	62,700
Paka	AA	2,522	12,610
Robusta	AAA	5,000	25,000
Dwarf Cavendish	AAA	5,050	25,250
Gros Michel	AAA	10,500	52,500
Highgate	AAA	11,375	56,875
Wather	AAA	10,500	52,500
Red	AAA	7,500	37,500
Theinkadali	AAA	7,750	38,750
Rasthali	AAB	2,750	13,750
Thiruvananthapuram	AAB	5,750	28,750
Nendran	AAB	4,500	22,500
Virupakshi	AAB	5,000	25,000
Krishnavazhai	AAB	4,750	23,750
Kalibow	AAB	5,000	25,000
Monthan	ABB	4,500	22,500
Peyan	ABB	3,750	18,750
Ney Vannan	ABB	5,750	28,750
Burkel	ABB	4,500	22,500
Bodles Altafort	AAAA	24,050	120,250
Kluai Teparot	ABBB	10,575	52,875
Ney Vannan x *M. balbisiana* clone Sawai	ABBB	31,450	157,250
Bareli Chinia x Pisang Lilin	AABB	18,375	91,875

Table 5. Percentage haploid, diploid, and tetraploid spores produced by banana clones (based on Darlington's ratio) (Sathiamoorthy 1987).

Clones	Haploid	Diploid	Tetraploid
Diploids (wild)			
M. balbisiana	69.8	-	-
M. acuminata	61.7	-	-
Diploid cultivars			
Ambalakadali	31.7	13.3	-
Anaikomban	52.0	4.0	-
Eraichivazhai	61.0	14.1	-
Matti	25.0	21.0	-
Namarai	47.8	26.2	-
Nivedyakadali	37.6	5.8	-
Pisang Lilin	42.5	-	-
Tongat	22.1	1.9	-
Paka	32.0	3.3	-
Triploids			
Robusta (AAA)	12.0	15.8	0.2
Dwarf Cavendish (AAA)	10.2	15.8	4.0
Gros Michel (AAA)	12.0	20.0	-
Highgate (AAA)	17.7	14.7	-
Red (AAA)	7.3	11.8	2.7
Wather (AAA)	19.7	12.1	-
Theinkadali (AAA)	19.7	12.1	-
Rasthali (AAB)	-	16.7	-
Thiruvananthapuram (AAB)	10.0	12.0	-
Virupakshi (AAB)	-	20.0	5.0
Krishnavazhai (AAB)	-	-	13.3
Kalibow (AAB)	20.0	51.4	-
Nendran (AAB)	14.5	15.2	-
Monthan (ABB)	24.5	3.5	-
Kanchkela (ABB)	26.5	5.2	-
Peyan (ABB)	4.0	12.0	4.5
Burkel (ABB)	15.5	8.5	-
Ney Vannan (ABB)	28.0	4.4	-
Tetraploids			
Bodles Altafort (AAAA)	-	30.5	-
Bareli Chinia x Pisang Lilin (AABB)	1.7	11.9	-
Kluai Teparot (ABBB)	9.5	20.0	-
Ney Vannan x *M. balbisiana* (ABBB)	8.3	28.3	-

Breeding Objectives and Problems

Banana breeding was started in India at the Central Banana Research Station, Aduthurai (Tamil Nadu), in 1949. There was no specific breeding objective and crosses made then were helpful only in understanding genetics and breeding behavior of banana. Many basic data were accumulated, and the results of breeding work in Jamaica and Honduras paved the way for formulating purposeful breeding strategies in the current breeding program at Tamil Nadu Agricultural University.

The cultivation of banana in India is threatened by several pests and diseases from time to time. Because of the polyclonal situation, the problems are also many and cultivar-specific. The diseases and pests of the cultivars of commerce are shown in Table 6.

Table 6. Major pests and diseases of banana and plantain in India.

Malady	Cultivars affected
Virus diseases	
Banana bunchy top virus	All banana and plantain clones
Cucumber mosaic virus	Cavendish clones, Poovan, and Red
Banana streak virus	Matti, Cavendish clones, Poovan, and Virupakshi
Banana bract mosaic virus	Cavendish clones, Pome clones, Poovan, Red, and many ABB clones including Monthan and Karpooravalli
Bacterial diseases	
Heart rot (*Erwinia caratovora*)	Cavendish clones and Nendran
Fungal diseases	
Sigatoka/yellow Sigatoka	Cavendish clones, Red, Rasthali, Poovan, Nendran, and Pome clones
Fusarium wilt	Rasthali, Karpooravalli, Red, Pome clones, and ABB cooking banana clones
Pests	
Nematodes	Cavendish clones, Red, Rasthali, Poovan, Nendran, and Pome clones
Weevil borer	Nendran, Red, and Cavendish clones
New unknown maladies	
'Neer vazhai'	Nendran
'Kottai vazhai' (seediness)	Poovan

The major problems encountered by the farmers in order of priority are Sigatoka/yellow Sigatoka, Fusarium wilt, nematodes, banana bunchy top virus (BBTV), banana bract mosaic virus (BBMV), and infectious chlorosis caused by cucumber mosaic virus (CMV); but their significance varies from region to region. BBTV is a major problem in perennial banana groves, as exemplified by the fact that Virupakshi, previously grown in hilly areas over 10,000 ha, has been reduced to a mere 200 ha within 5 years. The breeding objectives, therefore, differ according to the location, cultivars, and growing systems.

Past achievements in breeding

Although banana breeding was started during 1949, due to lack of knowledge on cytogenetics of banana, the male parents employed were all wild species—*M. balbisiana* and *M. laterita* (Rhodochlamys)—and the female parents were Poovan, Rasthali, Peyan (ABB), Thote (ABB), Peykunnan (ABB 'Pisang Awak'), Ney Vannan (ABB), etc. The progenies were all mixtures of diploids, tetraploids and some aneuploids. One of the tetraploid progenies of the cross Ney Vannan x *M. balbisiana* clone Sawai showed some promise. It was a cooking banana with better bunch grade and more fruit than the female parent. However, it was slow-growing, and highly susceptible to Fusarium wilt (Anon. 1968).

Since 1971, extensive interdiploid crosses were made to develop new diploids at TNAU, Coimbatore, with the primary objective of imparting resistance to nematodes, Sigatoka/yellow Sigatoka disease, and Fusarium wilt. Crosses were also made using pollen from Robusta with a tetraploid (AABB) as female parent. The resultant hybrid was an AB diploid with dwarf stature. Though it is parthenocarpic, it is seed-fertile and male-sterile. The characters of different diploid hybrids (and three triploids hybrids) are given in Table 7.

The hybrids were also screened for resistance to nematodes and Fusarium wilt (race 1 and 2) in infested soils maintained for the purpose and in pot-culture studies. The major nematodes causing considerable yield loss are *Radopholus similis, Helicotylenchus multicinctus, Pratylenchus coffeae* and, more recently, *Meloidogyne incognita.* The hybrids were screened for reaction to Sigatoka/yellow Sigatoka by interplanting with the most susceptible Cavendish clones. Hybrids of potential breeding value are tabulated in Table 8.

Improvement of Pome group

Banana cultivars of the Pome group are generally grown at high altitudes where they develop their much-favored and characteristic flavor and taste. An attempt was made to improve a Pome cultivar called Kallar Laden (AAB). One of the AB hybrids from a cross between Kallar Laden x *M. balbisiana* clone Sawai was used as the female parent in a cross with a diploid cultivar Kadali (AA) to develop a triploid hybrid (H 135) which was released later as CO 1 banana (Azhakiamanavalan et al. 1985) (Table 9).

Table 7. Growth, yield, and quality characters of parthenocarpic hybrids (phase II evaluation) (Sathiamoorthy 1987).

Parents	Hybrid no.	Ploidy	Pseudostem		Suckers per mat	Bunch weight (kg)	Hands	Fruits per bunch	Fruit length (cm)	Fruit girth (cm)	Total soluble solids (%)
			Height (cm)	Girth (cm)							
Matti x *M. acuminata*	H 21	2 x	302	75	15.0	10.5	7.1	179	13.6	9.7	20.0
Matti x Anaikomban	H 59	2 x	280	70	6.1	19.3	13.2	286	13.8	9.0	26.3
Matti x Anaikomban	H 61	3 x	249	51	12.2	12.8	7.6	135	13.8	17.3	29.5
Matti x Anaikomban	H 65	2 x	355	74	9.3	17.8	11.3	141	18.1	13.3	19.5
Matti x Pisang Lilin	H 69	2 x	239	67	8.1	5.2	6.4	139	10.0	7.8	23.0
Matti x Pisang Lilin	H 74	3 x	350	81	8.0	16.3	14.0	366	14.0	7.8	19.7
Matti x Pisang Lilin	H 76	2 x	297	67	9.4	7.8	7.3	131	11.0	7.6	20.8
Matti x Pisang Lilin	H 81	2 x	333	72	9.1	11.7	7.9	109	12.8	7.9	19.6
Matti x Namarai	H 82	2 x	301	69	6.7	8.3	8.0	107	13.3	9.6	20.5
Matti x Namarai	H 84	2 x	427	80	19.3	12.7	11.7	290	11.6	7.1	21.2
Matti x Namarai	H 85	2 x	210	57	5.1	5.8	6.4	69	10.6	10.1	20.3
Matti x Namarai	H 86	2 x	317	78	6.3	9.3	8.1	165	15.3	8.2	23.1
Matti x Namarai	H 89	2 x	301	70	18.4	12.3	11.2	198	14.6	7.3	24.0
Matti x Tongat	H 90	2 x	361	72	6.2	11.8	8.3	122	14.2	8.9	19.9
Matti x Tongat	H 94	2 x	303	78	13.6	7.4	6.1	97	12.7	6.9	22.7
Matti x Tongat	H 95	3 x	279	74	6.9	14.6	10.8	208	14.3	10.6	28.5
Matti x Tongat	H 96	2 x	297	78	15.6	15.3	12.1	167	14.9	10.7	27.1
Matti x Tongat	H 103	2 x	303	69	10.0	16.7	10.4	179	13.8	11.9	28.7
Matti x Tongat	H 105	2 x	278	62	8.0	9.3	7.3	128	12.4	8.5	19.9
Matti x Tongat	H 106	2 x	320	79	12.1	13.3	10.9	172	13.1	9.0	21.3
Matti x Tongat	H 107	2 x	185	49	4.2	6.5	5.8	79	9.6	10.1	20.8
Matti x Tongat	H 109	2 x	311	76	17.6	20.7	14.3	296	15.4	8.9	23.7
Matti x Tongat	H 110	2 x	150	49	10.7	5.9	6.3	74	9.7	8.8	26.5
Bareli Chinia x Pisang Lilin x Robusta	H 201	2 x (AB)	148	48	10.0	5.3	6.0	90	6.5	6.0	24.2
Kluai Teparot x Wather	H 12	4 x (ABBB)	327	84	4.7	12.8	5.3	64	10.1	10.4	17.4
Kluai Teparot x Nendran	H 2	2 x	295	77	5.2	3.4	2.6	18	12.3	11.8	18.0

Table 8. Hybrids of potential breeding value (Sathiamoorthy 1987).

Parents	Hybrid	Genome	Dwarfness	Bunch weight and grade	Fruit length	Resistance		Fertility	
						Sigatoka leaf spot	Burrowing nematode	Male	Female
Diploids									
Matti x *M. acuminata* ssp. *burmannica*	H 21	AA	x[1]	x	✔[2]	✔	✔	✔	✔
Matti x Anaikomban	H 59	AA	x	✔	✔	✔	✔	✔	✔
Matti x Anaikomban	H 65	AA	x	✔	✔	✔	✔	✔	✔
Matti x Namarai	H 84	AA	x	✔	x	x	✔	✔	✔
Matti x Namarai	H 89	AA	x	✔	✔	✔	x	✔	✔
Matti x Tongat	H 96	AA	x	✔	✔	x	x	✔	✔
Matti x Tongat	H 103	AA	x	✔	✔	✔	x	✔	✔
Matti x Tongat	H 106	AA	x	✔	✔	x	x	✔	✔
Matti x Tongat	H 109	AA	x	✔	✔	✔	✔	✔	✔
Matti x Tongat	H 110	AA	✔	x	x	x	x	✔	✔
Bareli Chinia x Pisang Lilin x Robusta	H 201	AB	✔	x	x	✔	✔	✔	✔
Triploids									
Matti x Anaikomban	H 61	AAA	x	✔	✔	x	x	✔	✔
Matti x Pisang Lilin	H 74	AAA	x	✔	✔	x	✔	✔	✔
Matti x Tongat	H 95[3]	AAA	x	✔	✔	✔	✔	✔	✔
Matti x Tongat	H 107	AAA	Semitall	✔	x	✔	✔	✔	✔

[1]x = unfavorable. ✔ = favorable. [3]Bright orange flesh.

Table 9. Important traits of CO 1 and Virupakshi banana (Azhakiamanavalavan et al. 1985).

Characters	CO 1	Virupakshi
Plant height at flowering (cm)	270.00	285.30
Girth (cm)	61.50	62.60
Weight of bunch (kg)	10.57	10.00
Number of hands/bunch	7.00	7.00
Number of fruits/bunch	87.00	77.00
Fruit weight (g)	160.50	137.00
Weight of peel (g)	53.00	47.00
Weight of pulp (g)	107.50	92.00
Total soluble solids (%)	22.60	21.00
Sugars (%)	18.18	17.53
Reducing sugars (%)	9.61	8.92
Nonreducing sugars (%)	8.57	8.61
Acidity (%)	0.57	0.56

Two hybrids, H_1 (Agniswar [AAB 'Pome'] x Pisang Lilin) and H_2 (Vannan [AAB 'Pome'] x Pisang Lilin) are now being tested in plains and higher altitudes. Both are triploids (AAB) with characters akin to the female parent with long fingers. The characters of the hybrids and their parents are compared in Table 10.

Table 10. Vegetative and bunch characters of hybrids and their parents.

Hybrid	Days to harvest	Ht at flowering (cm)	Girth at flowering (cm)	No. of leaves at flowering	No. of suckers produced	Bunch weight (kg)	No. of hands	No. of fingers	Total soluble solids (%)	Acidity (%)
(Agniswar x Pisang Lilin) H_1	322	264	61	14.3	4.3	12.4	8.3	113	27.45	0.295
(Ney Vannan x Pisang Lilin) H_2	329	288	59	13.4	4.2	14.4	9.8	179	25.66	0.253
Agniswar	368	298	62	13.4	4.4	6.9	6.2	105	26.74	0.284
Ney Vannan	356	305	58	12.6	4.0	7.6	7.5	108	25.13	0.287
Pisang Lilin	242	108	32	8.9	4.6	4.7	4.5	60	27.62	0.248

Inter-section crosses (Eumusa x Rhodochlamys)

M. laterita (of section Rhodochlamys) was used as the male parent in crosses with *M. acuminata* ssp. *burmannica.* In all the progeny, the rhizomatous and spreading habit of *M. laterita* and the bract color and biseriate floral arrangement of *M. acuminata* were dominant, confirming the genetical homozygosity of the parental species. None of the hybrids was parthenocarpic. On an average, they produced 80 suckers/mat in a period of 10 months. Of the three progenies of the cross Monthan x *M. laterita,* two were diploids and one was an aneuploid (2n = 24) suggesting a chromosomal segregation of 20/13 in megasporogenesis in Monthan, and that such ovules survived. Raman (1973) also recorded aneuploids in progenies of Peyan x *M. laterita* and Peyan x *M. balbisiana.* ABB cultivars, therefore, are capable of producing viable aneuploid ovules.

Current Breeding Objectives and Programs

Synthesis of diploids

New diploids are being bred through interdiploid crosses. Triploids, particularly Cavendish clones, French plantain, and Red are being employed as male parents. Some of their diploid hybrid seedlings are under testing.

Improvement of banana

With available diploid forms, breeding work is in progress to improve Red and Karpooravalli by introducing dwarfness and resistance to Fusarium wilt, Sigatoka/yellow Sigatoka disease, and nematodes. Improvement of Red deserves special attention since a diploid banana cultivar with red fruit, Sanna Chenkadali (AA), which has resistance to nematodes, Fusarium wilt, and Sigatoka/yellow Sigatoka disease, offers itself as an excellent gene source. A survey of Cavendish-growing regions has led to the identification of a high-yielding Giant Cavendish mutant. It yields bunches weighing 55 kg with 17 hands comprising 342 fingers. This is under multilocation testing, before being distributed to the farmers.

Improvement of plantain and cooking banana

The seed-set is poor in Nendran (AAB), and the taste of the hybrids differs conspicuously. The consumers are well aware of characteristic taste and flavor of Nendran fruit, and so any improvement of this cultivar should not interfere with its basic edible qualities. It is therefore necessary that suitable diploid male parents be developed first, with resistance to Sigatoka/yellow Sigatoka disease and nematodes. Dwarf plantains would be advantageous.

Among the ABB cooking banana types, Monthan is the foremost cultivar. A variant with smaller fruit called Kuribontha is popular in certain regions of southern and northern India. Both are susceptible to Fusarium wilt, but tolerant of nematodes and resistant to Sigatoka/yellow Sigatoka. The improvement of cooking banana aims at

developing dwarf Fusarium wilt-resistant Monthan. Dwarf AB diploids such as H 201 can serve as good female and male parents. The high-yielding cooking banana cultivar Pacha Bontha Batheesa (ABB), with fruit size, shape, and edible cooking qualities almost the same as Monthan, produces bunches weighing 40 kg with 16 hands and 200 fruits; it can serve as a female parent for the improvement of this group. The small-fruited Ney Vannan needs to be improved by increasing its resistance to Fusarium wilt (race 2) and reducing its height.

Mutation Breeding

Recently, mutation breeding has begun with in-vitro plantlets of Red banana, Giant Cavendish, Poovan, Virupakshi, Nendran, Monthan, and Karpooravalli.

Improvement of *Musa* through biotechnological approaches has yet to gain momentum in India, though sporadic attempts are being made in different centers. Collaborative research with advanced centers in other countries would be more rewarding. Thus, *Musa* breeding in India involves many cultivars, and the breeding objectives vary accordingly. Considering the vast varietal wealth of bananas and widely varying agro-ecological conditions for banana growing and availability of suitable resource persons, the outlook for *Musa* breeding in India appears to be reasonably optimistic.

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Part 4

Mutation Breeding for Disease Resistance

Musa Improvement in Cuba

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Introduction

In 1898, Cuba had a banana production area of 31,401 ha. In 1935, the country was one of the biggest exporters in the Caribbean region, shipping 121,359 $t.a^{-1}$ mainly fruit of the Gros Michel cultivar. From that time on, and due to the appearance of Fusarium wilt, banana production started to decline. By 1950, all export activities stopped, and the foreign companies moved on to other countries with better soil, a better climate, and fewer disease problems.

Banana production remained, but only for domestic consumption using rudimentary technology and thus accelerating the decline in production which, by 1971, had dropped to 73,500 $t.a^{-1}$. However, starting in 1971, the land under banana increased and, by 1987-1988, production had reached 270,000 $t.a^{-1}$ on an area of 74,496 ha (37,068 ha owned by the State and 37,928 ha by individuals) with the following clonal composition: AAB: 59.5%; ABB: 1.5%; and AAA: 39.0%.

Starting in 1988, a new development phase of cultivation was initiated with the widespread use of localized irrigation and in-vitro propagated planting material, which resulted in the production of 495,700 t of banana by 1991.

With the appearance of black leaf streak/black Sigatoka disease in 1990, the country's disease problems increased and clones from the AAB plantain group, which were the most preferred for human consumption, were no longer cultivated. They had been replaced by those of the ABB group, such as Burro CEMSA, Pelipita, and now FHIA-03 (AABB), which has great potential.

Conventional Improvement Methods

Introduction

The introduction of planting material has been the basic source of new production varieties. The Instituto Nacional de Investigaciones en Viandas Tropicales (INIVIT), has a germplasm collection with more than 200 accessions already characterized and organized in a database. These accessions are now being evaluated. Banana and plantain hybrid introductions from FHIA, Honduras, have great relevance to the improvement program.

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Selection and hybridization

An outstanding achievement has been the selection of a Bluggoe (ABB) clone, named Burro CEMSA, which is resistant to Fusarium wilt, a disease which is widespread in the country.

INIVIT is developing a hybridization breeding program and has already obtained its first hybrid plants.

Biotechnology Tools

Large-scale propagation

In order to respond to the accelerated production development plan in the country, extensive and large-scale propagation programs began in 1988. They were based on research results obtained at the Universidad Central de las Villas, which set up a 'biofactory' network able to produce more than 50 million plantlets a year. This permitted the mass production of clean planting material of traditional varieties, as well as the rapid introduction into cultivation of Grande Naine and Parecido al Rey dessert banana cultivars, and the ABB cooking banana cultivars Burro CEMSA and Pelipita.

Biotechnological breeding techniques

Somaclonal breeding for disease resistance. As a result of the research conducted at the Taiwan Banana Research Institute (Hwang 1991, Hwang, Ko 1988) which produced somaclones resistant to Fusarium wilt and the advances in the use of in-vitro mutations in banana and plantain reported by Novak et al. (1994), a program focused on finding banana somaclones tolerant of, or resistant to, disease has been initiated. This involves work with the clones Grande Naine (AAA 'Cavendish'), Parecido al Rey (AAA 'Cavendish'), Manzano (AAB 'Pome') and Gros Michel (AAA 'Gros Michel'), to improve their resistance to *Fusarium oxysporum* f.sp. *cubense*, races 1 and 2. Four Manzano and two Gros Michel somaclones, which have remained symptomless for 2 years in infested soil, have been selected and multiplied in vitro in order to conduct clonal and genetic characterization research and to recheck their resistance. Some of the selected plants have shown either beneficial or detrimental modifications to agronomic characters compared with the original clones.

Mutation breeding for disease resistance. The mutagenic agent employed was Cobalt 60, with radiation doses of 15 and 40 Gy. This treatment was applied to small plants in order to improve resistance to black leaf streak/black Sigatoka disease. Populations of more than 35,000 irradiated plants of two clones (Grande Naine and Parecido al Rey), have been grown in the field under natural inoculum levels. The level of variability in these populations induced by irradiation was above 25%, but the first 21,000 plants evaluated did not show additional disease resistance levels based on the criteria of the number of healthy leaves and fingers that fill at harvest. An additional 10,000 irradiated Grande Naine plants are now being evaluated.

Toxins have been used as in-vitro selection agents following the findings of Molina and Krausz (1989). Isolates of *M. fijiensis* have been detected with growth, development, and toxin-production differences. A nonphytotoxic culture medium for *M. fijiensis* has been developed. The first results, after crude, filtered, and concentrated toxin solutions had been applied to the leaf surface at levels of not less than 20 μ, demonstrated a differential reaction for Pelipita and Grande Naine clones.

In experiments with callus tissue, to which crude and filtered toxin solutions were added to the growth medium at different concentrations, no significant differences in reaction were found for Grande Naine, Pelipita, Yangambi Km5, and FHIA-03 clones, possibly due to high levels of phenolic compounds.

Populations of Manzano (11,000 plants) and Gros Michel (5,600 plants) were obtained by irradiating meristem apices growing in vitro in an attempt to produce clones resistant to Fusarium wilt. Plants were inoculated with conidia of *Fusarium oxysporum* f.sp. *cubense* at a concentration of approximately 3 x 10^4 spore/mL by injecting the pseudostem. The plants showing no symptoms (8.16% of Manzano and 3.01% of Gros Michel) were transplanted to highly-infested soil for further selection.

Research has recently been started into how to obtain toxins from cultures of *F. oxysporum* f.sp. *cubense* and into their use for in-vitro selection of plantlets and calluses with resistance. The first results of these studies show that the reaction of plantlets to the presence of crude, filtered, and concentrated toxin solutions is directly proportional to increases in concentration of the toxin. Differences in toxin production in fungus isolates were found, permitting the determination of highly aggressive strains. No relation was found as regards plant injury between undamaged roots and roots damaged before the inoculation. Through the use of polyacrilamide gel electrophoresis, the esterase isozyme system differentiates between race 1 and 2 of *F. oxysporum* f.sp. *cubense*.

Somaclonal breeding for increased yield. Because vitro-plantlets have been cultured since 1988 on a large scale, with the appearance, albeit infrequently, of somaclonal variants, selection studies have been undertaken which have led to the collection of several plants with superior agronomic characters. These include larger bunch size, greater number of hands, larger finger size and, in some Grande Naine and Parecido al Rey clones, reduced height. These are now under trial.

INIVIT has selected, via somaclonal variation, the superior somaclone SH 3436-9, which is being distributed throughout the country. This variant is also considered to have more resistance to Sigatoka disease than the original FHIA hybrid SH 3436 and is being included in IMTP Phase II.

Somatic embryogenesis. From the in-vitro initiation from male flower sections, it was possible to obtain callus formation of *Musa acuminata* (AA), Grande Naine, Parecido al Rey, Liborito (AAB 'Plantain'), Pelipita, and Burro CEMSA. These were used to obtain cell suspensions after a period of 30 days, and somatic embryos, although at low frequencies, 40 days later. Recently, work has led to the production of embryos from callus and cell suspensions following the method proposed by collaborators at CATIE. This has permitted remarkable technical advances and the production of the first Grande Naine plants. A joint project between several countries of the region is under

way, aiming to improve this technique as the basis for a mass propagation system, and also for genetic engineering purposes.

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In-Vitro Mutation Techniques for *Musa*

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Introduction

Banana and plantain (genus *Musa*) are among the most important food crops in tropical and subtropical countries. Together, they constitute the fourth most important global food commodity after rice, wheat, and maize in tonnes of gross production value. About 90% of the total banana and plantain world production (approximately 70 million $t.a^{-1}$) is consumed in the producing countries and only 10% is exported. The banana industry represents a major source of income and employment for people in tropical countries. However, the production of *Musa* is seriously and increasingly threatened by several diseases caused by pathogenic fungi, bacteria, viruses, and nematodes. Since most of the accepted banana and plantain clones are vegetatively propagated, generally triploid and hence almost sterile, bearing parthenocarpic fruits, their improvement through sexual cross-breeding is extremely difficult. Mutation breeding offers a unique alternative approach for genetic improvement of such vegetatively propagated crops.

Mutation Breeding

The induction of mutations is a means of creating genetic variability which is the baseline for subsequent selection of improved traits. Broertjes and Van Harten (1978), Kao (1979), and Krikorian and Cronauer (1984) reviewed mutation breeding methods in banana. Suckers were irradiated prior to excision and to in-vitro culture. Donini and Micke (1984) proposed shoot-tip culture as a tool for mutation induction in vegetatively propagated crops. Menendez (1973) and Yang and Lee (1981), on the other hand, applied chemical mutagens on seeds and seedlings of banana. However, the isolation of the induced mutations from these explants was not very successful due to development of chimeric structures resulting from the treatment of multicellular structures, such as suckers or meristems. Mutation is a single-cell event and mutation induction in the cell

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complex leads to chimerism. Hence, methods have to be established that avoid chimerism by treatment of plant structures of single-cell origin or, through mutagen treatment procedures that result in the induction of large mutated sectors in multi-cellular structures. Mutated sectors can be increased by repeated selective subcloning either into periclinal chimeras or solid homohistont plants. Chimerism limits the timely success of in-vivo mutation breeding in banana because it takes months to produce new shoots.

Mutation breeding in vitro

In-vitro micropropagation techniques offer new opportunities for mutation induction, and can be employed at various steps of the breeding process. Plants regenerated through in-vitro propagation may have the following origin: (a) shoot tips with a pair of leaf primordia (1-2 mm in size); (b) meristem (meristematic dome); (c) adventitious buds; (d) somatic cell embryos. For mutation breeding, somatic embryos or adventitious shoots that originate from one or a few cell initials are of importance. In-vitro techniques can also rescue homohistont mutant tissue from a sectoral chimera by repeated subculture. Furthermore, in-vitro techniques allow preliminary screening for direct or indirect selection of salinity tolerance, drought tolerance, pathotoxin tolerance, and tolerance of herbicides (Ingram, MacDonald 1986).

Mutagen sensitivity of shoot tips

Mutation breeding studies for genetic improvement of banana and plantain were initiated in this Laboratory in 1985 as a part of the joint FAO/IAEA Program in Agriculture. These investigations were intended to develop in-vitro techniques for efficient induction of mutations to facilitate selection by avoiding or dissolving chimeras, and for clonal propagation of selected mutants (Novak, Micke 1988). Shoot apices with two pairs of leaf primordia (1-2 mm in size) were used for mutagenic treatment. Prior to mutation induction, a large number of in-vitro plantlets were micropropagated to secure enough uniform material for mutagenesis.

Different *Musa* cultivars exhibited significant differences in radiosensitivity. These differences depended on the ploidy level and the hybrid constitution of the genome A (*M. acuminata*) and B (*M. balbisiana*). Diploid clones were most sensitive to gamma irradiation while the tetraploids expressed the lowest level of irradiation damage (Novak et al. 1990). In their studies on efficient chemomutagenesis Omar et al. (1989) reported the indispensable use of DMSO (dimethylsulphoxide) as a carrier agent for uptake of EMS (ethylmethane sulphonate). The optimum concentration and the required time of incubation for mutagenic treatment was also found to be dependent on the ploidy level and genome constitution, which should be assessed for mutation breeding in a prior chemosensitivity test.

A radiation dose that does not exceed a 50% reduction in survival or plant regeneration was recommended as a suitable mutagenic treatment for *Musa* to avoid multiple background mutations.

Mutagen sensitivity of adventitious buds

Anatomical studies showed two kinds of bud proliferation systems operating during the micropropagation of banana and plantain: one at the axillary position of the explant and the other on the surface of the corm tissue as an adventitious bud (Novak et al. 1986; Hamilton 1965; Ma, Shii 1974). Adventitious buds either originate from a single cell or from a few epidermal or hypodermal cells (Broertjes 1982). Mutation is a single-cell event and solid homohistont tissue is derived from a mutant cell initial that carries a particular mutation. Direct organogenesis of adventitious buds on culture explants offers a unique potential to facilitate dissolving chimeras or to produce homohistont plants. Preliminary experiments on radiosensitivity of adventitious buds were undertaken in this Laboratory. They showed that cells of adventitious origin are more sensitive than shoot tips. Use of feeder cells during mutagenic treatment is recommended to avoid excessive cell damage and warrant improved regeneration.

Somatic embryogenesis

Somatic embryos can be used on a larger scale for mutation induction than shoot tips since:

– the embryos develop synchronously and germinate directly into the plantlets;

– somatic embryogenesis provides a valuable tool for overcoming the intrinsic problems of chimerism;

– indirect somatic embryogenesis may be a useful method to enhance the genetic variation via somaclonal variation.

The technique of somatic embryogenesis and the regeneration of plants for dessert and cooking banana have already been reported by Novak et al. (1989). Proembryogenetic calli are initiated from the basal part of a leaf sheath and rhizome tissue on modified (Shenk, Hildebrandt 1972) medium containing 30 µm 3,6 dichloro-2-metho benzoic acid (Dicamba). Further development is promoted in liquid modified Murashige and Skoog (1962) medium containing zeatin, which is essential for differentiating the bipolar structure of fully developed somatic embryos. Conversion of somatic embryos into plantlets takes place in a double-layer medium system. Explants isolated from in-vitro plantlets exhibited a higher embryogenic response compared with in-vivo explants. Generally, somatic embryos have a stronger tendency to form roots without any further development of the shoot which ultimately limits the total percentage of germination. This system allows 0.1-0.5% of somatic embryos to germinate into plants. Several hundred plants from the Laboratory originating from somatic embryos of *Musa* genotypes (SH 3362 [AA-FHIA synthetic diploid], Grande Naine [AAA 'Cavendish'], Saba [AAB/BBB 'Saba'], Agbagba [AAB 'Plantain']), are now being evaluated in the field.

A preliminary experiment showed that leaf and corm explants retain their morphogenic abilities in embryogenic cell suspension, at least up to a gamma dose of 25 Gy (Novak et al. 1989).

Plant variants from in-vitro mutagen treatment of shoot meristems

After mutagenic treatment, considerable morphological variation is observed among regenerated plants. The frequency of phenotypical variation, i.e. morphological (plant stature, leaf shape), physiological (sucker growth and multiplication, flowering time, fruit ripening), and agronomic characters (bunch quality) ranges from 3 to 40% of the tested M_1V_4 plants, depending on genotype and irradiation dose (Novak et al. 1990).

Polyploidy induction

In-vitro techniques have been successfully used for inducing polyploidy in the improved diploid clone SH 3362 when used as a parent in cross-breeding programs as the donor of resistance gene(s) to race 4 of *Fusarium oxysporum* f.sp. *cubense*. Shoot-tip cultures were treated with oryzalin (15, 30, and 60 µM) or colchicine (2.5, 5, and 10 µM) with 2% of DMSO for 7 days. After three vegetative propagation cycles, the plantlets were regenerated and screened for stomata size and density. At the same time the ploidy level of the plantlets was analyzed with a flow cytometer.

In-vitro selection

To facilitate handling of large numbers of in vitro plantlets, it is important to develop a preliminary screening strategy based on such traits as salinity tolerance, herbicide tolerance, pathotoxin tolerance, and temperature adaptation (Ingram, MacDonald 1986). In-vitro screening for susceptibility/tolerance of different *Musa* cultivars to pathogenic fungi and their toxins is being investigated in this Laboratory. In one study, shoot tips of susceptible and tolerant clones of banana were exposed to different concentrations of metabolites produced in the culture filtrate of the fungus *Fusarium oxysporum* f.sp. *cubense* and to fusaric acid (Morpurgo et al. 1994). No correlation between in-vivo and in-vitro response was obtained. The result suggested that neither the crude culture filtrate nor a nonhost-specific toxin was usable for reaction to Fusarium wilt.

Peroxidase, a multipurpose enzyme, is involved in the defense mechanism of plants to disease (Seevers et al. 1971; Graham, Graham 1991). To discriminate between susceptibility and tolerance, peroxidase activity in *Musa* was used as a selection index to gauge reaction to Fusarium wilt (Morpurgo et al. 1994). The results were in good agreement with the host-pathogen interaction: early enzymatic activity increased in the incompatible host pathogen but not in the compatible interaction.

Molecular Genetic Markers

DNA oligonucleotide and DNA amplification fingerprinting techniques have been used for the identification of cultivars and breeding materials of banana and plantain (Kaemmer et al. 1992). Both the oligonucleotide and the DNA amplification fingerprinting (a variant of random amplified polymorphic DNA [RAPD] techniques), do not

reveal any differences between tissues of one and the same plant and among individuals of the same clone. A unique fingerprint has been found for each *Musa* clone. Both fingerprinting techniques permit the detection of bands characteristic for the A and B genome and also permit discrimination between triploids (AAA) (e.g. Gros Michel, Cavendish). The ^{60}Co-induced mutant GN-60A is clearly differentiable from the original cultivar, Grande Naine, using this technique.

Conclusion

Banana and plantain cultivars are postulated to be heterozygous. Mutagenic treatment may uncover the recessive alleles, by mutating or deleting the corresponding dominant alleles, but a high frequency of desirable mutations may not be manifested due to the dominant alleles in the heterozygous triploid background. It is difficult to induce desired mutations in *Musa* without altering the genetic background. Moreover, a separation of desired from undesired mutations appears practically impossible as this apomictic species does not permit recombination. Hence a large population has to be treated and screened to guarantee the manifestation of desired mutations and avoid multiple background mutations.

There is an urgent need to develop in-vitro screening techniques to facilitate the handling of large mutagenized populations for accelerating the breeding process and to make it more economic. To enhance the probability of success of selection, the use of biochemical markers and/or physiological indicators appears necessary, since many of the economically desired characters are not expressed under tissue-culture conditions. More systematic studies are needed to compare somaclonal and induced mutations.

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Complementary Approaches to Cross-Breeding and Mutation Breeding for *Musa* Improvement

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Introduction

In *Musa*, genetic improvement through cross-breeding is extremely difficult, since cultivars are vegetatively propagated, generally triploid, and hence sterile, bearing parthenocarpic fruits. Whenever crossing is possible, the variability generated is so great that there is little chance of selecting for improved types among seedling progeny. On the other hand, mutation induction in vegetatively propagated plants can change one or more characters of an outstanding cultivar without altering the remaining genotype (Broertjes 1972: as cited by Sigurbjörnsson 1977). The large number of mutant varieties generated over the past 20-30 years demonstrates the effectiveness of the method. However, limitations do exist in mutation breeding: for instance, most induced mutations are of a recessive nature and, due to the fact that a specific mutation is a unicellular event, chimera formation usually occurs after mutagenic treatment of a multicellular apex.

This paper describes: (a) the results of mutation breeding as applied to the improvement of leading cultivars of *Musa*; (b) how mutation breeding might assist cross-breeding programs; and (c) how the FAO/IAEA program supports agricultural research in developing countries.

Preliminary Results of Mutation Breeding used to Improve Leading Cultivars of *Musa*

Plant breeding essentially consists of three phases: generation of genetic variation, selection of useful genotypes, and comparative tests to demonstrate the superiority of the selected genotypes for specific agronomic traits. Inducing mutations by radiation or

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chemical mutagens contribute primarily to increased genetic variation. The subsequent selection and testing procedures used in mutation breeding and conventional breeding programs are essentially the same. However, in mutation breeding, large population sizes and efficient screening techniques are essential criteria for success.

Plant Breeding Unit, IAEA Laboratories, Seibersdorf, Austria

Over the last 10 years, this Plant Breeding Unit has developed unconventional breeding methods in *Musa*. In February 1985 the cultivar Grande Naine (AAA 'Cavendish') was provided to the IAEA Laboratories, Seibersdorf, by FHIA. Shoot meristems were micropropagated and used in mutagenic experiments. After in-vitro mutagenesis, plantlets were regenerated to the M_1V_4 stage and planted in the Seibersdorf glasshouse. One early-flowering plant was noticeable among a population of Grande Naine plants regenerated from shoot tips irradiated with a 60 Gy dose of gamma radiation. This plant grew vigorously and began flowering after 9 months, in comparison with 15 months in the nonirradiated control.

Potentially useful doses for mutation induction were assessed in a prior test (0-100 Gy); 50% of the plants survived approximately 35 Gy. In other words, the GN-60A clone survived a high radiation dose which may explain why it is such a useful variant.

Research and development work in this Unit on different mutagenic agents has generated new plant material for field-testing.

Thus, the GN-60A clone has been micropropagated and its vegetative progeny sent to Honduras, Australia, South Africa, and Malaysia for field-testing under plantation conditions. New breeding technologies must also be validated in collaborative plant breeding institutions before they can be distributed to counterparts in IAEA- and FAO-supported projects.

Fundación Hondureña de Investigación Agrícola (FHIA), La Lima, Honduras

In Honduras, the Technical Services and Research Branch of the United Brands Company has reported that about 20% of the GN-60A plants are superior for one or more characters compared with the control cultivar Grande Naine. There are plants with early-flowering traits, cylindrical fruit bunches, superior organoleptic qualities and bunch size (bunches of 55 kg in contrast to 32 kg of the average Grande Naine). Generally, plants of the mutant clone are shorter than the original cultivar, an advantage in areas subject to high winds. We are now compiling the evaluation of 700 plants after seven harvests to study the stability of the improved characters.

Recently, FHIA, Honduras, reported that they have twice found a seed in the pollinated bunches of one of the GN-60A plants. These two seeds did not germinate, but it looks promising as a prospective seed-fertile Cavendish type. The *Musa* breeding team in FHIA is now multiplying this plant for further pollinations.

Queensland Department of Primary Industries (QDPI), Australia

In Australia, QDPI has reported that GN-60A has shown faster cycling (343 days between planting and bunch harvest) than Grande Naine controls (382 days), but not remarkably faster cycling than Williams (363 days), the Australian industry's Cavendish standard. A larger field trial to generate more information was established in 1993.

Malaysian Research and Development Institute (MARDI), Kuala Lumpur, Malaysia

In Malaysia, MARDI has reported that inflorescence emergence (spiking) occurred 24 weeks (168 days) after field-planting and 55% of the plants flowered at 26 weeks (182 days). Plants were harvested 11 weeks later. The same earliness was observed in vegetatively propagated progeny of selected individuals. The plants selected for earliness also showed high-yielding capacity (average weight of bunch was 26 kg/plant in the second harvest), short stature, good bunch characters and flavor. Under similar management and field conditions, Williams yielded an average of 23 kg/plant at a planting density of 1900 plants/ha.

These early-flowering plants were micropropagated for planting on a commercial scale.

Banana Growers' Association of South Africa, Nelspruit, South Africa

In 1993, plants of GN-60A were also sent to the Banana Plant Improvement Unit (BPIU), South Africa. These plants were planted in mid-December 1993, and GN-60A will be compared with other Cavendish clones (Grande Naine (CA), Grande Naine (Israeli), Williams, Chinese Cavendish, and a selected clone from Taiwan).

In other breeding institutions in Africa, Latin America, and Asia, the GN-60A clone has received the same positive overall response when compared with the control cultivar Grande Naine. Nevertheless, a larger standardized multilocation trial is necessary to confirm the stability of this putative mutant clone. It is also essential that the selection of the putative mutant clone and its safe movement be coordinated with INIBAP.

The use of other mutagenic agents on local *Musa* clones

The mutation breeding technology developed by the FAO/IAEA Program employing different mutagenic agents (gamma rays, fast neutrons, EMS) is being used on valuable and local well-adapted clones of *Musa* in national programs in Colombia, Costa Rica, Panama, Cuba, Brazil, Nigeria, Sudan, Pakistan, Malaysia, and Australia.

Very encouraging results through in-vitro mutation breeding have been obtained by the National University, Heredia, Costa Rica. In fact, they have selected 13 clones of Grande Naine with high tolerance of black leaf streak/black Sigatoka. This has been achieved by using 0.5% v/v EMS for 2 h at 30°C (Navarro et al. 1994). This work is part of a Technical Cooperation Project supported by IAEA.

Putative mutant clones were produced from other cultivars of banana and plantain for field-testing: Williams Parecido al Rey (AAA 'Cavendish'); Agbagba (AAB 'Plantain'); Pisang Mas (AA 'Sucrier'); Pisang Rastali (AAB 'Silk'); Burro CEMSA (ABB 'Bluggoe').

How can Mutation Breeding assist Cross-Breeding Programs?

Mutation induction in a diploid used as a parent in cross-breeding

Diploid clones of both wild and edible banana are essential starting material for banana breeding. The first steps in conventional cross-breeding programs are hybridization and selection of recombinants at the diploid level to obtain agronomically advanced diploids with a different source of resistance (FHIA 1993).

In mutation breeding programs, it is generally recommended to use the leading variety as the starting material. Mutation breeding applied in diploids gives better results than in triploids because of the reduced genomic size, and also because induced mutations are generally recessives (Table 1). Mutation induction in heterozygous triploids limits the probability of obtaining homozygous recessives and thus the manifestation of improved traits.

Mutation breeding has been more successful in improving agronomic characters than in increasing disease resistance. However, since disease resistance is mostly conditioned by dominant genes that have an extremely low probability of being induced by mutagenic treatment, mutation breeding has not shown many positive results in improving disease resistance in other crops, and so may not be the best breeding strategy in *Musa*, either. An alternative approach has been used by Smith et al. (1990) in QDPI, Australia, who have based part of their mutation breeding program on the irradiation of the FHIA synthetic AA diploid SH 3362 (which is highly resistant to *Fusarium oxysporum* f.sp. *cubense* race 4). It is hoped that this subsequent mutation breeding procedure may result in the development of material with improved agronomic characters to complement the disease resistance. Thus, cross-breeding and mutation-breeding programs are both important in *Musa* improvement; greater emphasis on a complementary approach may be a more successful road to follow.

Polyploidy induction

In-vitro techniques have been used for inducing polyploidy in the improved diploid clone SH 3362 used in cross-breeding programs as the donor of resistance gene(s) to race 4 of *Fusarium oxysporum* f.sp. *cubense*. In the Agency's Laboratories, shoot-tip cultures were treated with oryzalin (15, 30, and 60 µM) or colcichine (2.5, 5, and 10 mM) with 2% DMSO (dimethylsulfoxide) for 7 days. After three vegetative propagation cycles the plantlets were regenerated and screened for stomata size and density. At the same time the ploidy level of the plantlets was analyzed with a flow cytometer.

Table 1. Effect of mutagenic treatment at a locus level.

Original genotype	Modified genotype	Type of changes	Possible phenotypic effect	Occurrence probability
A A	A -	deletion	none or slight	high
A A	A a	recessive mutation	none or slight	very low
A a	A -	deletion	none or slight	high
A a	a -	deletion	very pronounced	high
A a	a a	recessive mutation	pronounced	very low
A a	A A	dominant mutation	none or slight	extremely low
a a	a -	deletion	none or slight	high
a a	A a	dominant mutation	pronounced	extremely low

Van Duren (pers. comm.) showed that oryzalin was more effective than colchicine in polyploidy induction. Culture of shoot tips treated with oryzalin resulted in a high frequency of solid tetraploids (25%). Although mixoploids sometimes persisted in three cycles of vegetative propagation, nonchimeric tetraploids were easily identified through flow cytometry. Stomata size and density estimation did not identify chimeras and could not substitute flow cytometric analysis for assessing ploidy level.

Measurement of ploidy levels among plants of breeding populations after crossing, or the monitoring of polyploidy induction in tissue culture, are the most important applications of flow cytometry for *Musa* improvement programs. The method is sufficiently simple and robust to allow thousands of plants to be analyzed routinely and quickly. Flow cytometry is particularly useful in early detection of genomic irregularities (aneuploidy, mixoploidy) among populations of plants regenerated from tissue culture.

The solid tetraploid plants are further propagated in vitro and their ploidy level confirmed before shipment to collaborative institutes for field-testing. These unique materials may be used for production of synthetic triploids after crossing $4x$ x $2x$, or the reciprocal (Hamill et al. 1992).

The early elimination of polyploid somaclones would increase uniformity and the agronomic quality of planting material.

Technology Transfer

Being one of the few organizations with its own laboratories to support technology transfer, the IAEA is able to provide research and technical assistance to developing countries on request.

The major mandate of the Agency's Laboratories is to develop and transfer nuclear and related techniques and know-how to scientists in developing member states to allow them to address problems facing agricultural production. This process involves not only research, development and training but also the provision of technical services (e.g. seed irradiation, analytical support, etc.) to field projects.

The Plant Breeding Unit of the IAEA Laboratories, Seibersdorf, has developed advanced breeding methods in *Musa*, using mutation breeding techniques combined with in-vitro tissue culture and genetic engineering approaches. This has been achieved through collaborative research projects with national institutes and universities and with collaborators in FAO/IAEA Coordinated Research Programs and Technical Cooperation Projects.

Three forms of training are provided to plant breeders from member states: (a) FAO/IAEA interregional and regional training courses, to which specialist lecturers/ demonstrators contribute; (b) in-service fellowship 'techniques' training; and (c) scientific visits.

Conclusion

Cross-breeding and mutation breeding techniques combined with tissue culture and genetic engineering are central to the genetic improvement of *Musa*.

Cooperation and collaboration between national and international groups involved with banana and plantain improvement are mandatory for effective exchange of research information, optimum utilization of research resources, and for efficient transfer of technology to all banana- and plantain-producing countries.

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Musa Mutation Breeding in Taiwan

CY Tang, SC Hwang

Introduction

In Taiwan, banana production is mainly for export. Because of the subtropical climate and the market demands, the banana production system in Taiwan is different from those in other banana exporting countries. The important features of this system are: (a) smallholders: most of the banana orchards are small with an average size of 0.3 ha; (b) seasonal: most of the bananas are harvested between March and June due to the better fruit quality and the demands of the export markets; and (c) annual cropping: most of the farmers in the southern region replant their banana fields every year.

The main reasons for this system are to ensure that harvesting time will fall in the export season, and to avoid damage by strong wind during the typhoon season in summer (Tang, Liu 1993). In addition to the suckers—the conventional planting material used by most banana growers—about 1 million tissue-culture plantlets are produced every year for the annual planting of banana orchards. These unique features of the production system dictate the breeding methodology as well as the objectives of the improvement program. Mutation breeding by selection among somaclonal variants is the major approach for banana improvement in Taiwan. By these methods, somaclonal variants with resistance to race 4 of Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*), shorter plant stature, early flowering, or higher bunch weight were selected. The following reports the progress of banana mutation breeding in Taiwan.

Early Work on Mutation Breeding

The most important banana cultivar in Taiwan is Pei-Chiao (AAA 'Cavendish') which is a Giant Cavendish clone. It was introduced from south China about 250 years ago. It is tall and has high yield, good flavor, and broad adaptation. In 1919, a natural mutant called Hsien-Jen-Chiao was discovered in central Taiwan where banana bunchy top virus (BBTV) was causing severe losses. Hsien-Jen-Chiao is well adapted in slope areas and was claimed to be BBTV-resistant. However, the resistance is no longer observed in this cultivar.

The earliest report on induced mutation in banana appeared in 1971. This work was done by Shii using ethylmethane sulfonate (EMS) (TBRI 1975). Between 1972 and 1975

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several attempts were made to irradiate banana suckers using gamma-rays (Yang 1980). A systematic search for effective mutagens was carried out in 1975-77. Shoot apexes isolated from suckers under aseptic conditions were put on semisolid medium in test-tubes and were subjected to mutagens, such as gamma ray (^{60}Co), thermal neutron (^{32}P), EMS, and diethyl sulphate (DES). Treated explants were then placed in a culture room for regeneration and detection of mutations. It was found that gamma-ray irradiation with a dosage of 25 Gy was most effective in the induction of mutations (Kao 1979). A total of 377 explants were treated in two experiments; the survival rates varied from 8.5 to 18.1%. Mutations were observed in different stages of plant development. Albino plants were seen in some test-tubes and pots. Under field conditions, mutations with prominent morphological changes, such as short plant stature, early appearance of suckers, pseudostem with pale green color, and mid-rib with red coloration, etc., were observed.

In 1979, another experiment was carried out by Yang. He applied 0.01-1% EMS, DES, colchicine, or sodium azide to either suckers or the young shoot of tissue-culture plantlets. No mutation was observed in treated suckers, but nine mutations showing abnormal leaf shape and one with shorter plant stature were found among 264 treated plantlets (Yang 1980). These experiments indicated that gamma-ray and chemical treatment, especially EMS, were effective in induced morphological mutations from meristematic tissue or young plantlets produced by tissue-culture technique.

Improvement of Banana Cultivars by Somaclonal Variants

A mass propagation program for production of disease-free banana plantlets using meristem culture has been established since 1983 in Taiwan (Hwang et al. 1984). Somaclonal variation was commonly observed among banana plantlets produced by tissue culture methods (Hwang 1986; Vuylsteke et al. 1991; Daniells, Smith 1993). From a survey, Hwang (1986) reported that the percentages of off-types were 0.37% in 30,000 young plantlets and 2.43% in 46,260 matured plants in the field. The variants include those with variegated leaves, abnormal leaf shape, shorter plant stature, changes in pseudostem color, and bunch shape, etc. Most of these variants were genetically stable.

The occurrence of somaclonal variants opened the door to an alternative method for the improvement of banana cultivars.

Selection for resistance to race 4 of Fusarium wilt

Race 4 of Fusarium wilt, which attacks Cavendish and other types of banana, first appeared in Taiwan in 1967 (Su et al. 1980). After 10 years, the disease was widespread and became the most important constraint in banana production on the island. In 1984, a screening program for disease resistance was started by growing tissue-culture plantlets in diseased fields highly infested with the pathogen. By this procedure,

10 resistant clones from Pei-Chiao were selected (Hwang, Ko 1988). Although these resistant clones carried inferior agronomic traits, variants with improved agronomic traits were found among their progenies produced by tissue culture (Hwang, Ko 1989). Among them, GCTCV-215-1 and GCTCV-105-1 are promising for commercial production.

GCTCV-215-1 was selected in 1988 (Hwang 1991). It showed a moderate level of wilt resistance with a disease incidence ranging from 4.8 to 17.3% (Table 1). Compared with Pei-Chiao, GCTCV-215-1 was slightly taller, had a slender pseudostem and longer growing cycle (Table 2). The bunch weight of GCTCV-215-1 was lighter than that of Pei-Chiao by about 10% (Table 3) in disease-free conditions. However, the bunch shape and degreening during ripening are more uniform than the local cultivar. GCTCV-215-1 was officially released as Tai-Chiao No.1 in 1992. A total of 3.5 million plantlets of Tai-Chiao No.1 have been distributed to farmers since 1990. It is estimated that about 1200 ha of banana orchards are currently planted with this cultivar.

GCTCV-105-1 was selected in 1991 from the clone GCTCV-105 which was highly resistant, but low in yield. It had good agronomic characters with plant height about 249-270 cm. Compared with Pei-Chiao, leaves of GCTCV-105-1 were shorter and more erect. Bunches was more compact and hands had shorter fingers. A comparison of bunch characters between GCTCV-105-1 and Pei-Chiao is shown in Table 4.

Because of the higher level of resistance to race 4 of Fusarium wilt and shorter fingers (which are preferred by consumers), an intensive field trial on GCTCV-105-1 is being undertaken for further investigation.

Table 1. Disease incidence in GCTCV-215-1 and Pei-Chiao.

A. In disease nursery

Year	Cultivar	Sucker		TC plantlet	
		Number of plants	Disease incidence (%)	Number of plants	Disease incidence (%)
1989/90	GCTCV-215-1	427	5.2	1881	17.2
	Pei-Chiao	560	77.8	1647	74.6
1990/91	GCTCV-215-1	1080	10.8	1538	17.3
	Pei-Chiao	436	57.3	673	70.6

B. In commercial plantings[1]

Year	Cultivar	Number of orchards surveyed	Mean disease incidence(%)
1990/91	GCTCV-215-1	50	4.8
	Pei-Chiao	23	39.1
1991/92	GCTCV-215-1	256	6.4
	Pei-Chiao	57	33.6
1992/93	GCTCV-215-1	235	8.6
	Pei-Chiao	45	45.5

[1]Orchard size varied from 0.2 to 1.0 ha.

Table 2. A comparison of agronomic characters between GCTCV-215-1 and Pei-Chiao.[1]

Cultivar	Plant height (cm)	Girth (cm)	Leaf ratio (length/ width)	No. of hands per bunch	No. of fingers per bunch	Finger length (cm)
GCTCV-215-1	288	66.8	2.59	8.0	136.0	17.7
Pei-Chiao	270	70.5	2.37	7.9	136.8	18.5

[1]Data are averages of 200 plants for each cultivar.

Table 3. A comparison of bunch weight between GCTCV-215-1 and Pei-Chiao.

	Bunch weight (kg)					
Cultivar	March	April	May	June	July	Average[1]
GCTCV-215-1	18.4	22.6	25.5	26.5	27.2	24.0a[2]
Pei-Chiao	21.4	25.8	29.1	29.0	-	26.3b

[1]Data are means of three locations.

[2]Data followed by the same letter are not significantly different (P= 0.05).

Table 4. A comparison of bunch characters between GCTCV-105-1 and Pei-Chiao.

Month harvested	Cultivar	Number of hands	Number of fingers/ hand	Finger length[1] (cm)	Bunch weight (kg)
April	GCTCV-105-1	8.0a[2]	17.9a	21.3a	23.3a
	Pei-Chiao	7.3a	16.3b	25.8b	25.6b
May	GCTCV-105-1	7.7a	16.3b	22.8a	24.9a
	Pei-Chiao	7.0a	17.4b	28.9b	27.0b

[1]Average of the length of two middle fingers of the third hand from the top.
[2]Data followed by the same letter are not significantly different (P=0.05).

Selection for dwarfness

Reduction of plant height is important in the banana production system in Taiwan for two reasons: to reduce yield loss caused by typhoons and to increase the efficiency in farm management. A semidwarf Cavendish cultivar, which was introduced from Barbados, was evaluated and released to farmers in 1993 (Tang, Chu 1993). Dwarf plant type is common among somaclonal variants. Hwang (1986) reported that 1.3% of plants propagated through tissue culture were dwarf. They usually have stout pseudostems, shorter leaves and petioles. Choking was common when shooting occurred in low temperature. In 1991-92, 16 clones were selected for dwarfness from fields established by tissue-culture plantlets of Pei-Chiao. Seven of them were classified as semidwarf (2.0-2.5 m) while the other nine were dwarf (2 m). They were propagated by tissue culture and were evaluated in the 1993-94 season. The dwarf type was truly inherited, and thus all plants derived from

the nine dwarf clones were also dwarf. However, only one out of seven semidwarf clones retained the semidwarf characters in the progeny. The other six clones produced tall plants (>2.5 m). Environmental effect on the expression of plant height should be considered during the selection process.

The resistant clone, GCTCV-215-1, is slightly taller and more slender in its pseudostem. It is, therefore, more vulnerable to lodging caused by strong wind, especially after flowering. In 1992, a semidwarf mutant (TC1-229), derived from GCTCV-215-1, was found in a farmer's field. About 300 plants propagated from this clone were grown in 1993 for evaluation. Preliminary results indicate that the semidwarf plant type is truly inherited. The plant height of TC1-229 was about 227 cm, which was 50-60 cm shorter than GCTCV-215-1. It maintained a high level of resistance to race 4 of Fusarium wilt. Further evaluation of this clone is in progress.

Selection for earliness

One drawback of the resistant clone GCTCV-215-1 is the longer growing cycle. It takes about 13 months from planting to harvest, about 1 month longer than, Pei-Chiao. This is considered a major defect because banana is planted as an annual crop in order to adjust the harvesting time for the export markets from March to June. The market price of banana during the export season is about twice that of the off-season. In 1991-92, a search for early flowering clones was undertaken in GCTCV-215-1 fields which were established using tissue-culture plantlets. A total of 39 clones that showed distinct earliness in flowering were selected and propagated by tissue-culture.

In 1993, a preliminary evaluation trial was conducted. Each entry consisted of 16 plants in two replications. The number of days from planting to 50% flowering of plants in each plot were recorded for comparison. The preliminary results are shown in Figure 1. The number of days to 50% flowering ranged from 217 to 274. Four of them (10.3%) were classified very early because they flowered in less than 230 days, and 8 clones (20.6%) flowered between 230-239 days, significantly earlier than the other clones. Further evaluation will be conducted to confirm the genetic nature of earliness of these clones. One interesting finding from this experiment was the loss of resistance in some clones to race 4 of Fusarium wilt during the process of selection for earliness. Twenty-seven out of 39 clones (Fig.1) had lost their resistance to the disease. Among the resistant clones, only two were in the early groups while the others were in the late groups. Differences in the resistance level provided evidence for genetic diversity among these clones. It also indicates that selection for clones with earliness and disease resistance is possible.

Improvement of bunch weight

The bunch weight of banana is determined by the number of hands, and the number and size of fingers. Increase in the number or the size of these components will lead to an increase in yield. All the Fusarium wilt-resistant clones reported by Hwang and Ko in 1988 were inferior in agronomic traits, including low in bunch weight. Among the tissue-

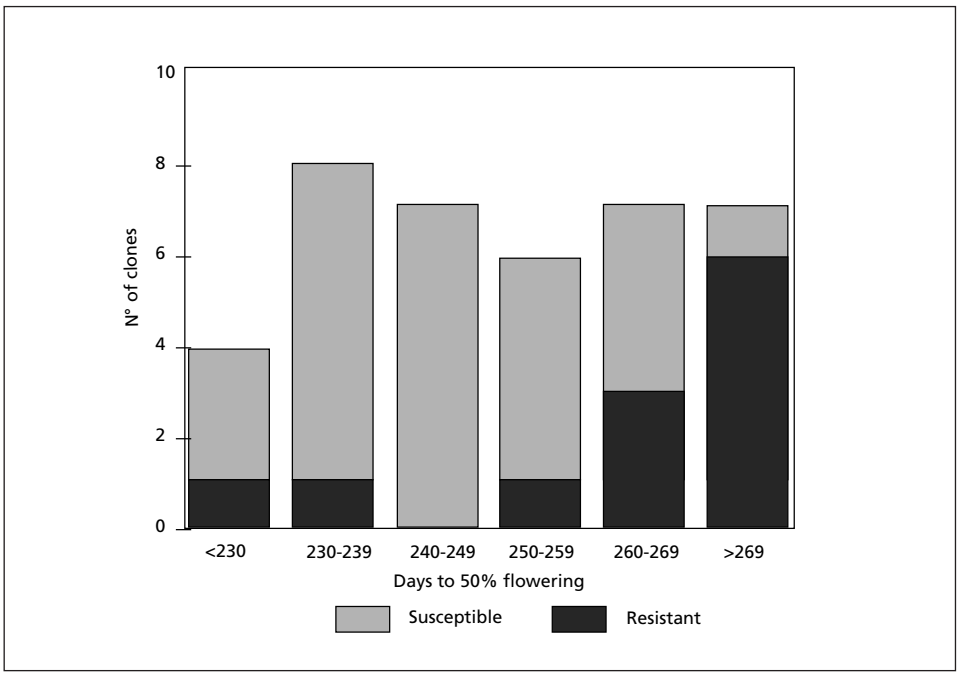

Figure 1. Frequencies of flowering days among 39 clones selected for earliness.

culture propagated progeny of these resistant clones, variants with improved agronomic traits including bunch weight were found (Hwang, Ko 1989). Table 5 shows the bunch weight and disease incidence (%) of several resistant clones. Though these data were obtained from different experiments, they indicate that bunch weight of banana is genetically controlled and can be improved by selection among somaclonal variants. Again, the resistance to disease may be lost during the selection process for higher yield, as shown in GCTCV-40 (Table 5).

Table 5. The bunch weight and disease incidence of various clones in different selection cycles.[1]

	Bunch weight (kg)			Disease incidence (%)		
Cultivar	C0[2]	C1	C2	C0	C1	C2
GCTCV-40	-	17.0	24.0	-	0.3	44.4
GCTCV-44	-	18.6	25.0	-	0.0	4.5
GCTCV-53	-	13.8	21.5	-	3.9	6.2
GCTCV-119	-	17.2	26.5	-	2.2	4.8
Pei-Chiao[3]	25.9	-	-	63.3	-	-

[1]Data were compiled from different experiments.
[2]C0, C1, and C2 are cycle 0, cycle 1, and cycle 2.
[3]Pei-Chiao is the parental clone of the others.

In 1990, a new Fusarium wilt-resistant clone (GCTCV-216) was obtained in a farmer's field. From a preliminary study, the average plant height of this clone was 339 cm and the circumference of the pseudostem was 82 cm. It was tall and stout. The growing cycle was 13 months and the average bunch weight was 34.7 kg with 10-11 hands , which was about 10 kg more than that of its parent Pei-Chiao. The potential of this clone for commercial production is being studied.

Changes in fruit quality

Fruit quality of banana is the key to the success of a new cultivar for commercial planting. Table 6 shows information on the fruit quality of some resistant clones derived from Pei-Chiao. When Pei-Chiao is used as the standard for comparison, variation among different resistant clones in fruit quality, including flavor and

Table 6. Fruit quality of various clones resistant to race 4 of Fusarium wilt.

Clones	Resistance level	Taste[1]	Ripening disorder[2]	Remarks on texture	Remarks on finger characteristics
GCTCV -40	High	+	-	Soft	Short
-44	High	+	-	Soft	Long & slender
-46	Moderate	++	++		Short
-53	Moderate	+	+++		Short
-62	Moderate	+	-		Fewer
-104	High	++	-		Fewer
-105	High	+++	++	Granular	Short
-119	High	+++	+++	Granular	
-201	Moderate	++	++		
-215	Moderate	++	+		Slender
Pei-Chiao	Susceptible	++	++		

[1]+, ++, +++ indicates that the taste is fine, good, and very good, respectively.

[2]Uneven ripening occurred between April-May. - : no problem; + : light; ++ : moderate; +++ : serious.

degreening, was obvious. Some of them, such as GCTCV-44 and GCTCV-215-1, were similar to Pei-Chiao in terms of taste and flavor. GCTCV-215-1 was better in uniform degreening. Higher degrees in sweetness and granular texture in clones GCTCV-119 and GCTCV-105 are distinctly different from those of Pei-Chiao. The level of acceptance of such changed eating quality by consumers is still under investigation. GCTCV-53 was the worst in terms of fruit quality. It was lower in sugar content, less aromatic and higher in the frequency of uneven degreening. Precautions on the changes in fruit quality should be taken in the process of mutation breeding.

Discussion

Somaclonal variation was reported in many crops (Larkin, Scowcroft 1981). It was also commonly found in different genotypes of *Musa* including AAA and AAB groups (Hwang 1986; Vuylsteke et al. 1991; Reuveni, Israeli 1990; Daniells, Smith 1993). Various hypotheses were proposed to explain the origin of such variation (Larkin, Scowcroft 1981; Vuylsteke et al. 1991). From the study of *Musa* mutation breeding in Taiwan, it was found that somatic variation is an effective means for cultivar improvement in banana. This approach is particularly useful in banana for the following reasons.

1. Improvement in *Musa* using traditional hybridization procedure is complicated and difficult. Selection for beneficial mutants from somaclonal variation is relatively straightforward.

2. Banana is a vegetatively propagated crop. Any stable genetic changes can be fixed and multiplied for evaluation and utilization.

3. Accumulation of beneficial genes step-by-step is made possible by recurrent selection among tissue-culture progenies. In Taiwan, the micropropagation system of banana cultivars was established in 1983. An average of 1 million plantlets are supplied to banana growers every year because of the unique feature of the banana farming system in Taiwan. The efficient propagation technique and availability of a large number of tissue-culture plantlets facilitate mutation breeding for the improvement of banana cultivars. Either through application of selection pressure for a particular trait, such as selection for resistance to Fusarium wilt, or through cooperation with farmers growing such cultivars as TC1-229 (a dwarf mutant of GCTCV-215-1 as reported in this paper), useful mutations were selected.

Although the procedure of mutation breeding is relatively simple, several precautions should be taken:

a) Because of the low frequency of mutation, a large sample size is required for selection of useful mutants. Any mutants which appear 'useless' at the beginning should not be discarded. The selection of improved variants from 'useless' mutants is possible.

b) Tissue-culture plantlets should be grown under uniform field conditions. This is important in increasing the efficiency of selection for traits such as earliness and semi-dwarf stature, which are affected profoundly by differences in the microenvironment.

c) The pleiotropic effect of mutated genes is common. Changes in fruit quality or other agronomic traits may also occur with beneficial mutation.

Selection for beneficial mutants from somaclonal variation has been demonstrated to be an efficient breeding method in *Musa*. By growing the new *Fusarium wilt* resistant cultivars, Taiwan banana growers have now benefited from the results of mutation breeding.

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Mutation Breeding of Banana in Malaysia

SH Jamaluddin

Introduction

Banana is the second most widely cultivated fruit in Malaysia covering 36,000 ha in 1991, next only to durian (62,000 ha). In terms of export earnings from fruit, banana ranks sixth after pineapple, durian, star fruit, papaya and water melon, in that order, contributing about 9.3% or RM13.3 million in real value (Anon. 1992). Banana cultivation in Malaysia comprises smallholdings of 0.4-0.8 ha of mixed clones and caters mainly for the needs of the domestic market. In recent years, however, the scenario for banana cultivation has changed significantly with the involvement of large commercial banana farms in the industry. This has led to more systematic and sophisticated techniques of farm management, harvesting, and packaging being introduced. The main cultivars grown on a large scale are Grande Naine, Williams and others in the AAA 'Cavendish' subgroup. Fruits of these types are mainly for export to Singapore, Hong Kong, Japan, the UK, and the Middle East. Pisang Berangan (AAB 'Lakatan') is also grown in plantations, but the fruit is largely for the domestic market.

Local clones, such as Pisang Mas (AA 'Sucrier')and Pisang Rastali (AAB 'Silk'), are also very popular. However, their cultivation is somewhat limited because of their lower yields and comparatively higher susceptibility to diseases. The cooking types—Pisang Awak (ABB), Pisang Abu (ABB), Pisang Nangka (AAA), Pisang Raja (AAB), and Pisang Tandok (AAB)—are other cultivars popularly grown by smallholders. Regional consumer preferences and clonal adaptability dictate the abundance and cultivation of one clone rather than another. With greater current interest in monoculture and its significant increase in hectarage, challenges to produce high-yielding and resistant banana clones are real and urgent. The main disease problems in Malaysia are Fusarium wilt and Sigatoka leaf spot, which contribute to considerable yield and economic losses.

Conventional breeding of banana is complex and time-consuming. However, with recent advances in techniques of biotechnology and induced mutagenesis, improvement of banana is becoming a reality. This paper describes the techniques adopted in Malaysia, the variants generated, and their potential in banana improvement in Malaysia.

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In-Vitro and Mutation Breeding Techniques

The techniques described served as a means to generate variants for subsequent field evaluation, screening, and selection.

Somatic embryogenesis

Somatic embryogenesis comprises several steps which include callus initiation, cell suspension, maturation of somatic embryos, and, finally, plant regeneration (Jamaluddin, Novak 1992). The explant sources were corm tissues and basal leaf sheaths from in-vitro cultured plants, which were transversely sliced into thin pieces. Plant regenerations were successfully achieved for two popular dessert clones, Pisang Mas and Pisang Rastali.

The plants are currently undergoing early observation in the field aimed at finding variants with superior agronomic traits and disease resistance. In previous work, Hwang et al. (1993) have successfully generated a variant with disease resistance and superior agronomic characters through tissue culture. Plants regenerated from somatic embryogenesis have been observed by many workers to achieve higher variation than would plants regenerated from meristem and shoot-tip culture. There is, therefore, a good chance that this technique will produce some promising variants.

Induced mutagenesis

Both physical and chemical mutagens were used for mutation induction. Shoot tips comprising a meristematic dome surrounded by one or two leaf primordia were used as explants, and the clones treated were Pisang Rastali, Grande Naine, Williams, and Pisang Berangan.

Physical mutagens

The mutagens used were fast neutrons and gamma rays. For the preliminary radiosensitivity tests to determine LD50, 30 shoot tips per dose were used. For gamma irradiation, 6 doses of 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, and 60 Gy were administered, and the effect of gamma irradiation on the explants was determined by recording their fresh weights over a period of 6-8 weeks. LD50 is that dose which would give 50% reduction in growth of the explant in reference to the nonirradiated control, and this dose is assumed to give adequate mutation frequency (Novak et. al. 1986). For fast neutrons, the doses administered were 1.0 Gy, 1.5 Gy, 2.0 Gy, 2.5 Gy and 3.0 Gy, following similar experimental procedures as for gamma irradiation.

Gamma irradiation. For gamma irradiation the optimal dose for Pisang Rastali is 30 Gy, Pisang Berangan is 25 Gy and Grande Naine 60 Gy.

Results showed that almost 30% of the Pisang Rastali plants derived from induced mutation were variants (Table 1). These cover the characters affecting the leaf, stem, and bunch traits, plant stature, and time to flowering. Some characters were detected when the plants were at the nursery stage, while others were found at maturity. Grass-like variants remained stunted and succumbed more often to disease before flowering.

Variants with loose basal pseudostem sheaths became apparent only when the plants were field-planted. Most of these plants did not succeed in producing a bunch. Two distinct variations from the normal for bunch traits were a significant gap within the bunch and fused fingers. The gap did not affect fruit quality, although existence of the gap did reduce bunch weight because there are fewer hands. The occurrence of fused fingers, however, would reduce the saleability of the fruits.

For plant stature, severe stuntedness is an undesirable trait. Such variation usually persisted after field establishment and bunching was rare, plants finally succumbing to disease. Moderately stunted variants may grow more slowly than normal, and come to flowering at a much lower height. This may be regarded as dwarfness in stature, a positive trait in terms of ease of management. However, the other traits that are usually associated with dwarfness are smaller bunches and fewer hands per bunch.

Table 1. Types and frequency of variation from gamma-irradiated Pisang Rastali.

	Types of variation	Frequency (%)
Leaf characters:		
	Variegation	1.3
	Narrow, grass-like (poor vigor)	1.0
	Drooping petioles	0.8
	Unequal lamina size	0.6
	Wrinkled	0.5
	Erect petioles	0.5
Pseudostem characters:		
	Greenish red to red pigmentation	5.3
	Totally green	2.0
	Streaking/striped	2.0
	Loose basal sheaths	0.3
Bunch characters:		
	Fused fingers (>50%)	0.5
	Gap within the bunch	0.2
Plant stature		
	Stunted (poor vigor)	5.0
	Dwarfness	4.0
Flowering		
	Earliness to flowering	3.0
Total		27.0

Earliness to flowering is another attractive variant that was recorded for about 3.0% of the population. The variants came to shooting as early as 6.0-6.5 months, compared with 7-8 months for the control. Shooting to harvesting time, however, remained the same for both irradiated and nonirradiated plants (50–60 days).

For disease resistance, screening carried out by recording such disease symptoms as stem splitting, leaf yellowing, and plant death indicated that there were some variants which showed some degree of resistance to Fusarium wilt. The appearance of the disease symptoms for these variants was delayed when compared with the controls. However, these 'potential' variants need to be subjected to further intensive screening for confirmation of their resistance status before recommendation for use by farmers.

For Grande Naine, the results have been reported by Tan et al. (1993), indicating a potential variant named Fatom-1 has been identified. Potential traits include earliness to flowering (24 weeks), high-yielding capacity (average of 26 kg/bunch), short stature, and good bunch characteristics and good fruit flavor. Intensive disease screening is continuing.

Pisang Berangan is still in its early vegetative stage, and data recording is still in progress.

Fast neutrons. For fast neutrons the optimal dose determined for Pisang Rastali was 2.0 Gy and for Williams 3.0 Gy. Pisang Rastali has just been planted in the field. Observations for 6-month-old fast-neutron irradiated Williams showed 6.0% were stunted, 4.5% had abnormal stem color, and 3.3% had leaf abnormalities. About 39.0% of the plants showed varying degrees of infection with Fusarium wilt, ranging from leaf yellowing to plant death.

Chemical mutagen

Only Pisang Rastali was treated with the chemical mutagen, ethylmethane sulphonate (EMS). The shoot tips were immersed in the filter-sterilized mutagen at two concentrations: 0.2% (24.67 mM) and 0.4% (48.34 mM) for 2 and 4 h at 28°C, following closely the technique used by Omar et al. (1989). At least 30 shoot tips were used for each treatment. Immediately after the treatment, the shoot tips were thoroughly rinsed with sterile water and transferred onto solid MS medium, supplemented with BA, for further shoot growth and multiplication. The suitable concentration (LD50) was similarly determined by recording fresh weight over time.

The optimal concentration determined was immersion in 0.4% EMS for 4 h. The induced plants have just been planted in the field after undergoing four to six cycles of micropropagation and will be evaluated in the second half of 1994.

Conclusion

The results obtained so far are too early to confirm the true value of the variants generated from in-vitro and mutation-induction techniques. However, potential traits such as earliness to flowering, reduced plant stature, and increased yield have emerged

for Grande Naine (Fatom-1) and Pisang Rastali. The stability of these potential traits and the true status of their perceived resistance to Fusarium wilt need to be confirmed with more intensive screening. The techniques serve, however, as valuable alternatives and complement conventional banana breeding.

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Mutation Breeding for Banana Improvement in Australia

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The Australian Banana Industry

The Australian banana industry occupies an area of 12,000 ha and is estimated to be worth A$250 million per year. All of the fruit grown is consumed in Australia and bananas have become the major individual fresh-fruit line in the market. Only the commercial industry is of economic and social importance in Australia. With the possible exception of the aboriginal settlements in far northern Australia, backyard plantings are more for ornamentation than as a source of food. Their importance lies in the fact that they act as a reservoir and breeding ground for pests and diseases.

The cultivars Williams, Mons Mari and Grande Naine (AAA 'Cavendish') account for 90% of the production, while Lady Finger or Pacha Naadan (AAB 'Pome'), is the only non-Cavendish cultivar with significant commercial production.

The major production is located in coastal areas of the north and south of Queensland and northern New South Wales. Minor production areas are at Carnarvon and the Ord River in Western Australia. The major environmental differences are the higher rainfall conditions in northern Queensland, the cold winter and chilling problems in southern Queensland and northern New South Wales, and the low rainfall and high temperatures of Western Australia. These environmental differences and local edaphic factors influence the growing conditions and agronomic practices in use. Also the distribution and relative importance of specific diseases varies greatly between growing areas.

In 1981, black leaf streak/black Sigatoka (*Mycosphaerella fijiensis*) was recorded in Australia for the first time on islands in the Torres Strait and in the Bamaga area on the tip of Cape York Peninsula. The presence of black leaf streak/black Sigatoka in this region is recognized as a major threat to the Queensland industry. There is a risk, with more tourists visiting the area and improved road access from the south, that the disease may spread to the major production areas in north Queensland in the future.

Fusarium wilt, on the other hand, has long been a problem to the Australian banana industry. The disease, which was first described in Queensland in 1874, is caused by the

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soilborne fungus *Fusarium oxysporum* f.sp. *cubense (Foc)*. Race 1 has continued to destroy susceptible Lady Finger plantations since early this century, and the total area under production has been maintained only by planting in new areas. There is now a shortage of suitable noninfested land.

Since the 1950s, Fusarium wilt has been avoided by many growers by replanting infested land with Cavendish cultivars resistant to race 1. These cultivars are now the mainstay of the industry.

Fusarium wilt in Cavendish cultivars was first recorded in southern Queensland in the early 1950s but only a few plants were affected. In the late 1970s and early 1980s, the incidence of this new race of Fusarium wilt (race 4) has increased alarmingly, and today the disease occurs, sometimes severely, in 42 plantations in the southern Queensland area (Pegg, Langdon 1987). The inability to control the disease by chemical or cultural practices and the lack of a commercial cultivar resistant to race 4 that would replace Cavendish means that research into this area is a priority in Australia's banana research program.

In-Vitro Mutation Breeding

Introduction

Cavendish are sterile, parthenocarpic triploids and their use in conventional breeding programs, despite repeated attempts to use them as female parents, has largely been unrewarding.

Due to the difficulty and costs associated with conventional banana breeding, alternative strategies for genetic improvement have been actively pursued in Australia. One such strategy involves mutation induction. Mutation induction has become an established tool in plant breeding with hundreds of improved varieties having been released to growers from many different crop species (Micke et al. 1987). The main advantage of mutation induction in a vegetatively propagated crop, such as banana, is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique part of the genotype. This is not yet possible using conventional approaches to banana improvement. A summary of the results of an in-vitro mutation breeding program for the development of Fusarium wilt race 4-resistant Cavendish cultivars, suitable for the subtropical conditions of southeast Queensland and northern New South Wales, is presented in this paper.

Materials and methods

Conditions for the initiation and growth of micropropagated bananas have been described by Hamill et al. (1993) and Smith and Drew (1990). Briefly, Williams, New Guinea Cavendish, and Dwarf Parfitt (AAA 'Cavendish') were initiated on a Murashige and Skoog (1962) basal medium supplemented with 10 µM benzylaminopurine, 2% sucrose, and 0.8% Difco-Bacto agar. This medium also supported rapid shoot

multiplication. Cultures were incubated at 28°C with a 16-h photoperiod. Cool white fluorescent tubes provided a light intensity at the culture surface of 80 $\mu E.m^{-2}.s^{-1}$. Before acclimatization in the glasshouse, plants were subcultured to a hormone-free medium for root initiation and the development of vigorous, single shoots. Plants were established in the field when they reached a height of 20 cm in 2.5-L planter bags.

The irradiation procedure involved the transfer of explants, containing the shoot tip and insheathing leaf bases obtained from in-vitro grown plantlets, to Petri dishes which contained a multiplication medium. Cultures were incubated for 7 days before exposure to gamma radiation from a ^{60}Co source. The doses used were 0, 10, 20, 30, 40, and 50 Gy. Dosage was calculated using a Fricke dosimeter (O'Donnell, Sangster 1970). Plants were then subcultured for two to three cycles before disease-resistance screening. Smith et al. (1990) described the Fusarium wilt race 4 screening experiments which involved: (1) the use of *Foc* culture filtrates in vitro, (2) root-dip inoculations, and (3) direct planting in a *Foc*-infested field site at Wamuran (latitude 27°S) in southeastern Queensland. Any survivors from the first two screening trials were also tested in the field. The site at Wamuran was a commercial block of Cavendish that first became infested with *Foc* race 4 during 1976. The pathogen eventually spread and, by 1984, the block was no longer commercially viable. The site was leased by QDPI for development as a race 4 screening site and nursery. Levels of inoculum were maintained and increased by redistributing infected material on site. The *Foc* population was intensively sampled and characterized by VCG and volatile analysis. These tests confirmed that the isolates belong in the 'odoratum' group and were VCGs 0120, 0129, and 01211 (Brake et al. 1990; Moore et al. 1991).

Plants that survived for one or two harvests without developing any symptoms of Fusarium wilt were divided into suckers and bits and replanted in infested sites. This was usually repeated for a second and third cycle to overcome the possibility of 'escapes' and to verify that unique clones may have arisen following micropropagation and irradiation.

Results and discussion

Determination of resistance to Fusarium wilt race 4 in the *Musa* gene pool

In conjunction with the mutation breeding trials, a range of *Musa* species, cultivars, and accessions have been evaluated for resistance to race 4 at our screening site at Wamuran. These plants were obtained from local and overseas germplasm collections and breeding programs. Currently 368 accessions have been screened and, of these, only seven accessions have shown resistance to Fusarium wilt race 4 (Table 1). They included two wild species, *Musa ornata* and *M. velutina*; two superior breeding diploids and one tetraploid from the FHIA breeding program, SH 3362, SH 3142 and FHIA-01; and Dwarf Parfitt, an extra-dwarf Cavendish clone. Mysore (AAB 'Mysore') was susceptible when young but tolerated race 4 as the stool matured. It is also interesting to note that the presence of banana streak virus has been confirmed in Mysore in Australia, and it is not known what effect this may have on this variety's reaction to Fusarium wilt.

Table 1. *Musa* species and cultivars considered to show resistance to Fusarium wilt race 4 (*Fusarium oxysporum* f.sp. *cubense*).[1]

Species/cultivar	Genotype	Origin	Comments
FHIA-01	AAAB	Tetraploid from FHIA breeding program	Dessert fruit with good commercial prospects
SH 3362, SH 3142	AA	Improved diploids from FHIA breeding program	Important as male, diploid parents, but of no immediate commercial use
Mysore	AAB	Imported into Queensland from Jamaica and Honduras, originally from Indian subcontinent	Dessert fruit, moderate-yielding, mature plant resistance
Dwarf Parfitt	AAA	Local selection from New South Wales	Extra dwarf Cavendish. 1 m at bunch emergence. No commercial value
Musa ornata, *M. velutina*		Local selections from New South Wales, originally from Indian subcontinent	Ornamental *Musa* species, colorful bracts

[1]Based on observations from 368 accessions grown on heavily-infested soils at Wamuran in southeastern Queensland.

Though all of these plants showed good levels of resistance, they were not immune to Fusarium wilt. Under extreme conditions of cold and drought, as can be experienced in the subtropics, even some plants from this group can become infected and show symptoms of the disease. In fact, immunity is not a realistic goal to aim for in a Fusarium wilt breeding program in banana because, regardless of the pathotype or cultivar used, the fungus is able to penetrate and become established in the vascular system of the root (Pegg, Langdon 1987). Resistance can break down under periods when the plants are stressed and the pathogen is active. However, if the level of resistance observed from these accessions can be expressed in a suitable commercial cultivar, then the possibility of serious epidemics of the disease may be considerably reduced. We believe Fusarium wilt can be effectively managed with resistant cultivars.

Effect of gamma irradiation on in-vitro growth of Cavendish cultivars

The LD50 for micropropagated Williams was approximately 40 Gy; however, shoot multiplication and general vigor of plantlets was poor (Fig.1). The optimal dosage range was 20 Gy and was used for the other Cavendish cultivars in this program. At this dosage visual changes were apparent and plant survival, at 73%, was sufficiently high to make the technique practical on a larger scale. The radiosensitivity of the Cavendish cultivars used in these experiments compares well with that found by Novak et al. (1990) for Grande Naine.

Reaction of gamma-irradiated Cavendish cultivars to Fusarium wilt race 4

Two strategies were adopted for the in-vitro mutation breeding program. The first approach involved irradiating the Cavendish cultivars, Williams and New Guinea Cavendish, susceptible to race 4, to improve their resistance but retain their favorable agronomic characters. The second approach involved irradiating the agronomically inferior Cavendish cultivar, Dwarf Parfitt, to improve performance but retain resistance. Over 20,000 plants have been evaluated in the field and only the putative mutants derived from Dwarf Parfitt show the most promise as a Cavendish replacement resistant to race 4 under the subtropical conditions existing in Queensland and New South Wales.

The first strategy of irradiating superior, but disease-susceptible, Cavendish cultivars has yielded a few promising lines, but a markedly improved level of resistance has not been observed. Planting material collected from the surviving plants, including the few that survived culture filtrate and root dip-inoculation experiments, has been replanted for a second, third, and now for a fourth cycle of evaluation. Of the few remaining plants, all are characterized by a dwarf, robust stature (average height of 1.9 m at bunching). Unfortunately, these plants are also prone to 'choking', a phenomenon in which the bunch fails to emerge fully from the crown of the plant, and which is influenced by adverse environmental conditions.

The second strategy was that of irradiating Dwarf Parfitt, an extra-dwarf Cavendish banana that has shown a high level of resistance to race 4. Unfortunately, Dwarf Parfitt are very small, with an average height of 1.0 m at bunching, and are extremely prone to 'choking'. Following irradiation of approximately 500 explants at a dose of 20 Gy, 35 M_1V_3 plants were recovered that possessed improved characters. Plants were larger, they bunched earlier, yield was considerably increased, and severe choking was eliminated (Table 2). One of the better-yielding selections from this group was established in tissue culture and has been released to the INIBAP Transit Center as Giant Parfitt.

After completing the plant crop harvest, bits and suckers from each stool were removed and replanted in an adjacent, infested site, together with planting material from Williams and Dwarf Parfitt. Planting material was taken only from those stools with no internal symptoms of Fusarium wilt. Of the original 35 M_1V_3 plants, therefore, 8 were infected and 27 were taken through to the M_1V_4 cycle. Unfortunately, Giant Parfitt was one of those that showed internal discoloration. It was concluded at the time that the plant's ability to prevent invasion was compromised by the earlier act of digging suckers for establishing tissue cultures. This caused root damage and led to fungal invasion.

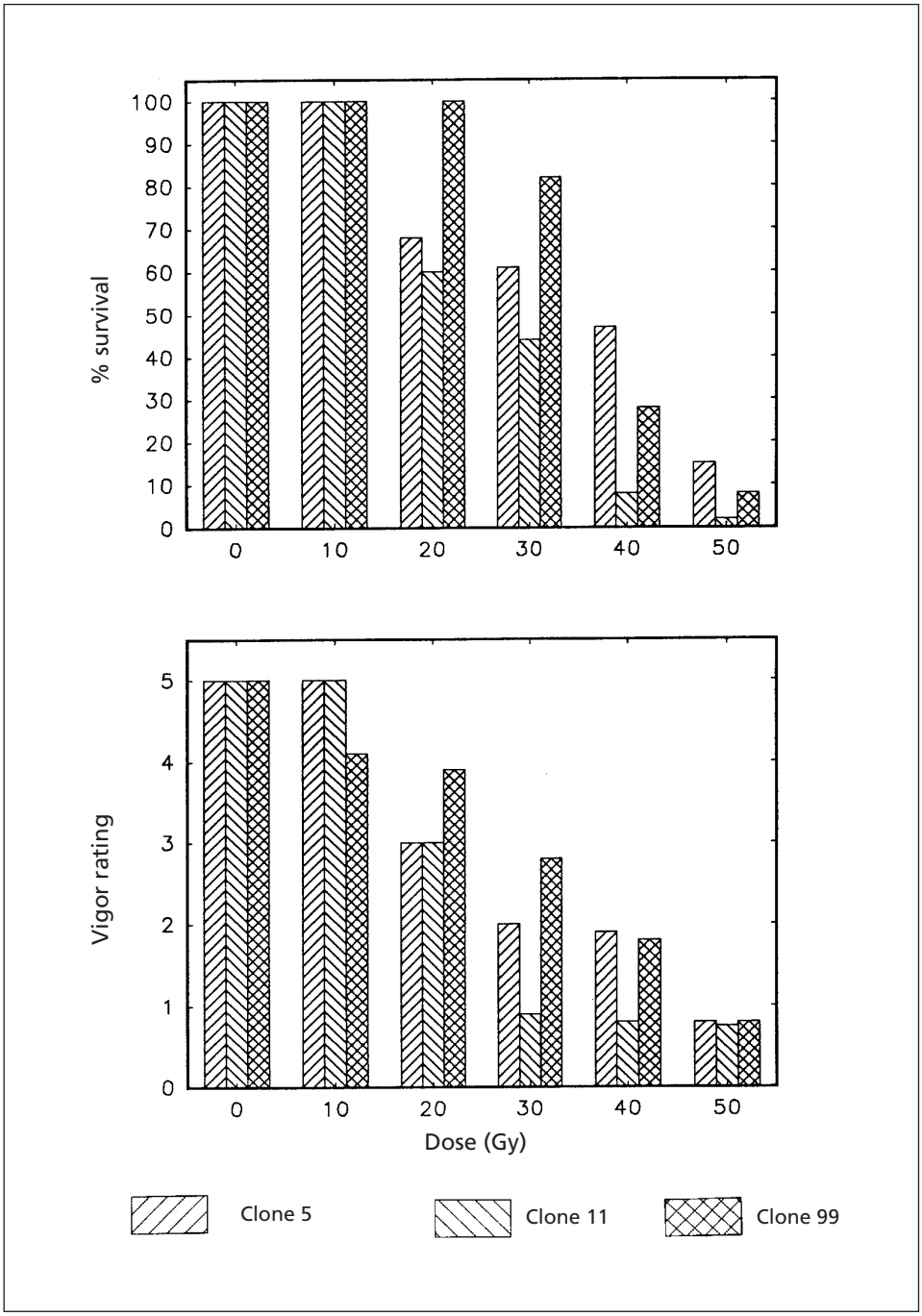

Figure 1. Effect of gamma irradiation on in-vitro survival and vigor of selected Williams clones. The vigor of surviving plantlets was rated on a scale of 1 to 5: from (1) little or no growth, chlorosis and necrosis of leaves, to (5) excellent growth and vigorous, green shoots.

Table 2. Plant crop data of Dwarf Parfitt and its putative mutants in southeastern Queensland.[1]

	Dwarf Parfitt	M_1V_3 putative mutants
Height at bunching (cm)	104.0	182.0
Bunch weight (kg)	8.2	24.2
No. of hands/bunch	9.0	10.3
Planting to harvest (months)	19.8	16.3

[1]Values are the averages from approximately 20 plants. All of the Williams in this block succumbed to Fusarium wilt before meaningful yield data could be collected.

Table 3. Disease assessment ratings of Giant Parfitt and Williams in southeastern Queensland.[1]

	External	Internal	% infected
Williams	0.9	2.7	100%
Giant Parfitt	0.5	1.4	70%

[1]Plants destructively harvested 7 months after planting and values are the averages from 12 plants. External ratings are on a scale of 0-3 (from 0 = no symptoms to 3 = death of the plant). Internal ratings are on a scale of 0-5 (from 0 = no vascular discoloration in the corm to 5 = totally infected).

Subsequent studies have confirmed that the plants have a higher level of resistance to race 4 than Williams, but it was not as great as previously observed (Table 3).

Plant crop data of the M_1V_4 generation revealed large differences in yield and susceptibility to Fusarium wilt between different mutant lines (Table 4). It also suggested that there was still scope for selecting a Cavendish resistant to race 4 with yields approaching that of Williams. For instance, DPM-2, 22, 25, 15, and 16 suggest tolerance is possible without a large loss in productivity. These plants have been established in culture in order to multiply them for further tests and evaluation.

Conclusion

Of the material so far produced from QDPI's in-vitro mutation breeding program, the putative mutants derived from Dwarf Parfitt continue to show the most promise as a Cavendish resistant to race 4. We are still at an early stage in the evaluation of this material, and we wish to determine the genetic stability of this material and make further comments on the nature of the resistance. It is interesting that Fusarium wilt of Cavendish is most prevalent in the subtropical regions of the world (Ploetz et al. 1990) where periods of cold and drought may place plants under stress. However, during the past 2 years serious damage to plantations of Grande Naine, Williams, and Valery (AAA 'Cavendish') has occurred in tropical Malaysia and Sumatra (KG Pegg, pers. comm.), suggesting the presence of a tropical race 4.

Moore et al. (1993) have suggested that the sensitivity of cultivars to stress induced by cold temperatures, and the associated disruption of photoassimilation mechanisms,

Table 4. Plant crop data for Williams, Dwarf Parfitt, and gamma-irradiated mutants (DPM).[1]

Cultivar/line	No.	% *Foc*	Bunch wt[2] (kg)	No. hands[2]	Finger length[2,3] 3rd hand (cm)
Williams	21	33%	34.3	11.3	23.0
Dwarf Parfitt	16	6%	12.0	9.2	13.8
DPM-1	12	33%	33.9	12.0	21.9
DPM-2	7	0%	32.8	10.9	23.2
DPM-3	13	15%	29.5	11.6	20.4
DPM-4	14	29%	28.5	11.3	21.5
DPM-5	11	55%	32.2	12.0	21.5
DPM-6	9	67%	(32.6)	(11.0)	(21.5)
DPM-7	8	50%	29.1	10.3	21.0
DPM-8	5	80%	(38.0)	(13.0)	(20.5)
DPM-9	11	27%	20.4	8.2	21.3
DPM-10	10	10%	25.1	10.3	21.1
DPM-11	10	60%	29.9	10.5	22.3
DPM-12	9	33%	29.5	10.5	21.3
DPM-13	8	25%	30.8	12.0	22.3
DPM-14	7	14%	22.7	10.8	18.9
DPM-15	12	25%	33.4	10.9	22.7
DPM-16	5	20%	34.2	11.0	22.8
DPM-17	4	50%	32.2	11.0	22.8
DPM-18	12	17%	27.6	10.2	22.3
DPM-19	8	13%	30.8	11.0	21.7
DPM-20	7	29%	31.4	11.0	21.7
DPM-21	14	43%	26.6	10.4	21.0
DPM-22	7	0%	33.1	11.8	22.9
DPM-23	10	40%	28.7	11.6	20.8
DPM-24	9	11%	29.5	10.4	21.8
DPM-25	10	20%	34.8	11.2	21.4
DPM-26	1	100%	-	-	-
DPM-27	11	27%	29.9	10.9	22.3

[1]Data in parentheses are from one plant only.
[2]Data are the mean of plants that had no symptoms of Fusarium wilt.
[3]Data are from the third finger from the outer whorl of the third hand from the proximal end.

contributes to susceptibility to race 4 in the subtropics. Host-defense mechanisms (i.e. formation of gels, tyloses, and phenolic infusions), which block invasion by the pathogen (Beckman 1990), are primarily driven by photoassimilates, either from storage or current photosynthesis.

Therefore, a decrease in the carbon-assimilation capacity of a banana plant may reduce its ability to block invasion by the pathogen.

In support of this hypothesis, Moore et al. (1993) have shown that Dwarf Parfitt (resistant to *Foc* race 4) maintained higher chlorophyll concentrations and a higher rate of CO_2 assimilation than Williams (susceptible to *Foc* race 4) during the winter months. Furthermore, Dwarf Parfitt maintained its leaf area during winter while that of Williams significantly declined. These features resulted in a higher threshold of CO_2 assimilation efficiency in Dwarf Parfitt than in Williams during the latter part of winter and early spring. Similarly, Dwarf Parfitt was able to maintain a higher chlorophyll florescence induction (F_v / F_m ratio), indicating that it was less affected by cold temperatures during winter and able to make a more rapid recovery in spring, when mean temperatures increased to more favorable levels for growth.

By developing a greater understanding of the physiological mechanisms of resistance, there is an opportunity that a reliable screening technique can be developed and applied, either in culture or in the nursery, to screen the large numbers of plants produced from mutation or conventional breeding programs. The lack of a suitable small plant-screening technique continues to be one of the major obstacles in effectively breeding for Fusarium wilt resistance.

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Mutation Breeding of Banana in South Africa

ZC de Beer, AA Visser

The Banana Industry in South Africa

The cultivar Dwarf Cavendish (AAA 'Cavendish') was introduced into Natal, South Africa, during the 1920s. Planting of banana in the Transvaal followed shortly afterwards in the 1930s (Robinson 1993). During the following 50 years only Dwarf Cavendish was cultivated, as it was considered the cultivar most suited to subtropical climatic conditions. Williams (AAA 'Cavendish') was introduced in 1977. The popularity of Williams increased and it has now become the predominant commercial cultivar. The cultivars Chinese Cavendish, Grande Naine, and Valery (AAA 'Cavendish') were evaluated experimentally (1983-88) and released in 1989 (Robinson 1993). Grande Naine and Chinese Cavendish seem to be very popular, although they do not yet occupy 10% of the production area.

Currently 14,067 ha of banana are cultivated, with a total production of 210,500 t in 1992/1993 (Banana Board Annual Report 1991/1992). However, due to the drought conditions experienced countrywide, the production declined to 140,000 t for the 1993/1994 season.

Banana Plant Improvement

The ideal commercial banana cultivar for the subtropical climatic conditions of South Africa was described by Robinson (1993) as follows:

– The pseudostem should be short and sturdy: this provides stability against strong winds and permits easier bunch management.

– A high leaf emergence could lead to a shorter vegetative cycle time. The cycle time tends to be excessively long as no leaf development takes place during the cold winters.

– There should be an absence of, or a reduced tendency towards, the winter-induced problems of choke throat and November dump.

– High bunch quality, comprising a long fruit stalk, a cylindrical, nontapering bunch, long fingers and high bunch mass.

BAGSA Banana Plant Improvement Unit, Private Bag X11208, Nelspruit 1200, Republic of South Africa

Besides these horticultural demands, resistance to or a degree of tolerance of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) race 4 and to a lesser extent nematodes, is required as well.

Fusarium wilt was first reported in Natal and has since affected the Hazyview/Burgershall area (Ploetz et al. 1990), which together comprises nearly half the total area under banana production. Letaba, Levulbu, Onderberg, and Sabie areas remain unaffected.

It was the dramatic increase of Fusarium wilt in the Hazyview/Burgershall area that led to the founding of the Banana Plant Improvement Unit (BPIU) in 1988 by the Banana Board.

Banana Plant Improvement Unit/Banana Growers' Association of South Africa

Research at the BPIU is committed to the development and selection of banana cultivars which are resistant to or show a degree of tolerance of Fusarium wilt and/or nematode infection, and which have equal or superior horticultural qualities.

Contributions in the funding of the research projects were made by the Banana Board. In 1993 the Banana Board was disbanded and the Banana Growers' Association of South Africa (BGASA) was founded in October 1993 as the representing body for the South African banana industry. BGASA subsequently took over the funding of the BPIU.

Breeding of Banana in South Africa

Improvement of the commercially grown banana cultivars is extremely difficult, since they are sterile and mostly parthenocarpic polyploids (Novak et al. 1989). A different breeding strategy for banana plant improvement was decided upon, implementing various biotechnological approaches. These techniques cannot replace conventional breeding, but only complement and support the *Musa* improvement system (Afza et al. 1993).

Somaclonal variation

The increased genetic variation among in-vitro regenerated plants has been termed somaclonal variation. This variation could be a source of useful new variability for banana improvement. Cultivars Dwarf Cavendish, Williams, and Sodwana (AAA 'Cavendish'), as well as somaclonal variants of Pei-Chiao (AAA 'Cavendish') from Taiwan (e.g. GCTCV-44, -53, -119, and -215) were propagated and then screened by a drastic in-vivo selection process for resistance to Fusarium wilt.

Mutation induction by gamma irradiation

The induction of mutations through gamma irradiation was aimed at altering one or a few characteristics of a generally acceptable cultivar, but otherwise retaining the

original genotype (Broertjes, Van Harten 1988; Novak et al. 1989). Dwarf Cavendish, Williams, Chinese Cavendish, Grande Naine, and Sodwana (a local selection), were submitted to gamma radiation (60 Gy). The irradiated tissue-culture plants were rooted and evaluated in *Foc*-infested soil for their tolerance/resistance to Fusarium wilt and/or horticultural superiority. Most of the treated clones proved to be either susceptible to *Foc*, or stunted and low-yielding. However, one of the treated Sodwana clones (HBBJM) survived, and produced an acceptable bunch. The progenies of this clone were propagated in vitro, and were planted for evaluation in *Foc*-infested soil the following season (see 'Evaluation–Field selection').

Single-cell technology

Somatic embryogenesis and plant regeneration from cell suspensions could be used as effective tools for the genetic improvement through mutation induction. The leaf bases and top layers of newly formed growth tips (Novak et al. 1989; Vuylsteke, Swennen 1993) of Dwarf Cavendish, Williams, and Taiwan clone GCTCV-44 were placed on a solid medium enriched with 2,4-D for 2-3 months without subculturing. The embryonic globules which developed were used to establish embryogenic suspension. New somatic embryos and embryonic cells were obtained. Germination of the somatic embryos and subsequent regeneration, however, remains to be accomplished.

Evaluation

All induced variants, together with possible 'escape' plants from infected areas that could possibly be resistant or might show a degree of tolerance, as well as all imported cultivars, are evaluated for resistance against Fusarium wilt and horticultural superiority.

In-vivo selection

In-vitro propagated plants, together with induced variants, 'escape' plants, and newly acquired cultivars, are firstly evaluated in vivo. Three-month-old tissue-culture plants were consistently used. An inoculum of *Foc* grown on a sand/maize mixture or millet seed was used to infest the soil. After 4 weeks, the banana plantlets were then externally and internally evaluated for disease symptoms. Surviving clones were propagated and screened again. Only 1-2% of the plants tested survived. To date, 290 clones have been selected.

Identification of banana and plantain cultivars with random amplified polymorphic DNA (RAPD) markers

Identification of *Musa* cultivars is based upon various combinations of morphological, phenological, and floral criteria (IBPGR 1984). Close genetic relationships among cultivars and frequent somatic changes, as well as changes due to the environment, are major obstacles for the correct identification of a clone (Kaemmer et al. 1992).

Recent developments in molecular technology made it possible to detect and exploit the naturally occurring variation within the DNA sequence (Martin et al. 1991; Quiros et al. 1991). Random DNA sequences are amplified by the polymerase chain reaction (PRC)

with arbitrary primers. Differences in the specific amplified products would indicate a variation in DNA sequence as visualized through electrophoresis.

Twenty-two accessions of *Musa* comprising AA, AAA, AAAB, AAB, and ABB genotypes were characterized. Leaf tissue of two to eight plants per genotype were sampled. One gram of each leaf was used to extract the DNA of the different genotypes which were then analyzed for RAPD markers. Sixty 10-mer primers from Operon Technologies are used for the PCR amplification. So far only 20 primers have been evaluated.

Differences in marker patterns were obtained using one primer with SH 3142, SH 3362 (AA FHIA breeding diploids), FHIA-01 (AAAB FHIA hybrid), Pacific plantain (AAB 'Maia Maoli'), Mangaro Torotea (AAB 'Plantain'), Njock Kon (AAB 'Plantain'), Horn plantain (AAB 'Plantain'), Ducasse (ABB 'Pisang Awak'), Pelipita (ABB 'Pelipia'), and Bluggoe (ABB 'Bluggoe').

For most of the primers evaluated, similar banding patterns were obtained with the Cavendish genotypes. However, it was possible to distinguish between Grande Naine (Israeli), Grande Naine (Central America), and Grande Naine (Novaria) with three of the primers. Banding profiles of Sodwana differed with several primers, while, Poyo Dwarf Cavendish, Williams, GCTCV-53 and GCTCV-215 produced different banding patterns, each with a different primer. This indicated the limited amount of genetic diversity among the Cavendish genotypes.

Differences in marker patterns were detected using one primer between normal Williams plants, dwarfed plants and normal-looking Williams plants producing a mutated bunch.

RAPDs could thus provide a quick and reliable method for the identification of not only *Musa* cultivars, but induced variants as well.

Field selection

Possible resistant clones were planted at high density in naturally infested soils in the Hazyview/Burgershall area. The plants were inspected monthly for disease symptoms and horticultural characters. After flowering the best clones will be selected, propagated, and planted again for further evaluation.

Selections made from previous field trials, together with PK1 and PK3 (GCTCV-53 variants), as well as the irradiated Sodwana HBBJM, an in-vivo selected somaclonal variant of Sodwana (HR), and the Taiwanese GCTCV-215/1 were planted in *Foc*-infected soil during

Table 1. Percentage of diseased plants at 14-15 months after planting in soil naturally infested with *Foc*.

Clones	Number of plants established	% diseased
GCTCV-215/1	1458	7.61
HBBJM	1542	1.75
PK1	245	2.04
PK3	595	1.68
HR	536	42.50

December 1992 and January 1993. After 14-15 months only 2.04% of PK1 and 1.68% of PK3 succumbed to the disease, whereas 7.61% of the GCTVC-215/1 was infected and 1.75% of HBBJM. A disappointing 42.5% of the HR Sodwana selection had succumbed (Table 1).

Conclusion

The most important function of banana breeding as quoted by Rowe and Richardson (1975) is the development of genetically diverse cultivars for long-term protection against endemic disease.

The production of disease-resistant clones to replace the popular cultivars could possibly be achieved when a combination of traditional and mutation breeding methods as well as the available biotechnology is applied. Such a strategy, however, calls for extensive international collaboration to enhance the probability of increasing the desired cultivars.

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Part 5

Training

Training Activities in Latin America and the Caribbean

R Jaramillo

Introduction

In 1992, the panel of the First CGIAR External Program and Management Review of INIBAP stated that "human resources will ultimately be a decisive factor in any collaborative research activity, be it national, regional, or international. Training is fundamental if the Regional Network is going to assist NARS in developing sustainable institutional capacity for research on *Musa*. For NARS to develop that capacity, the panel believed that three criteria must be met:

a) training is undertaken or facilitated at all levels: technical, managerial, and professional;

b) both short- and long-term training needs are met; and

c) universities and research institutions in the region and abroad are deliberately involved by INIBAP in training and *Musa* research, as well as in developing a capacity to offer advanced degrees on *Musa.*"

Similar thoughts were expressed 5 years before by the members of the INIBAP-LAC Regional Advisory Committee (RAC).

Since its initiation in 1987, the RAC defined training as one of the main tasks of the Network as a whole, which means both INIBAP and its partners.

At that time, after an analysis of the first survey conducted by UPEB in 1985 on the number of researchers working on banana and plantain in the region (in which the lack of qualified human resources necessary to create, adapt, and transfer technology in those crops was detected), it was recommended that an interdisciplinary advisory group should be formed to identify training needs. These might include workshops, in-service training, short courses, and support for setting up postgraduate courses at major universities or regional centers.

The first RAC established as training priorities the following topics: breeding, tissue culture, germplasm management and evaluation, taxonomy of *Musa*, banana and plantain production techniques, and information/documentation management.

In 1989, the RAC recommended that most of the training activities should be carried out in the region, in close collaboration with network partners (NARS, univers-

INIBAP-LAC, CATIE, Turrialba, c/o Apdo 4824-1000, San José, Costa Rica

ities, regional centers), to meet clearly defined needs. It was further recommended that links between developing and developed countries be established through bilateral training in special research skills.

As it was noted that training opportunities were very limited in some countries, due to the lack of an established program of research on banana and plantain, and taking into account that the most advanced research programs on *Musa* in the region had active scientific communities that were rich sources of training expertise, the group recommended that exchanges or visits of scientists in partner institutions, and in-service training of qualified personnel, should be organized. Due to its multiplier effect, training of trainers should also receive priority.

Survey of Human Resources for Research on Banana and Plantain

During the current global economic crisis, which permits governments to cut budgets for NARS and has also led to decreasing allocations of funds from donors to the CGIAR system, which affects all IARCs, especially INIBAP, the challenge for INIBAP-LAC and its partners has been to analyze accurately the differing needs across the countries in order to channel training resources effectively.

Following the RAC recommendations, and taking into account the survey conducted by UPEB on researchers working on banana and plantain, the first step taken was to make an analysis of the human resources available in the region.

Despite the fact that the INIBAP Directory of *Musa* scientists represents around 60% of the total researchers (not all questionnaires distributed were answered), the document gives an idea of the distribution of specialized human resources working on *Musa* by countries and subregions (see Table 1).

In general, the number of researchers does not correlate with the socioeconomic importance of banana and plantain in the region and the problems affecting them. Moreover, more than 60% of the researchers are concentrated in the most advanced programs located in Brazil, Colombia, Costa Rica, Cuba, and Honduras.

In some countries, banana and plantain cultivation is a very important part of the economy, either for local consumption or for export, but in which the research activities on *Musa* are very weak or nonexistent. More than half of the total researchers have a higher academic degree, and fewer than one-fifth have PhD degrees. Nevertheless, research conducted at the most advanced programs is considered to be of a very high quality, with relevance both for the region and worldwide.

Additionally, the survey showed that most of the researchers in LAC are concentrated in two fields: agronomy/crop management and crop protection. At the time of the survey, very few researchers were devoted to tissue culture/biotechnology and physiology, as can be observed in Table 2. Such information was an indication to both NARS and INIBAP concerning in which areas training should be emphasized in the coming years.

Based on these realities, both the Network partners and INIBAP itself assumed the challenge to take every opportunity to link research activities with training, using the

Table 1. Distribution by countries of researchers working on banana and plantain in Latin America and the Caribbean[1] (1991).

Country	Academic degree			Total
	BS/IA/Lic.	MS/MSc	PhD	
Belize			1	1
Brazil	7	26	6	39
Colombia	11	11	4	26
Costa Rica	15	8	6	29
Cuba	19		4	23
Dominican Republic	1			1
Ecuador	10	6	1	17
France (Martinique/ Guadeloupe)	4	1	2	7
Guatemala	3	4	1	8
Honduras	5	1	6	12
Jamaica	1			1
Mexico	4	7	2	13
Nicaragua	2	1		3
Panama	2	2		4
Puerto Rico	1	3	4	8
St Lucia		1	2	3
Venezuela	10	4	2	16
TOTAL	95	75	41	211

[1]Figures presented in this table have been extracted from the "Directory of Researchers working on Banana and Plantain", published by INIBAP and UPEB, which includes information provided by *Musa* collaborating scientists and technicians through survey questionnaires distributed by both institutions.

Table 2. Distribution by topics of researchers working on banana and plantain in Latin America and the Caribbean[1] (1991).

Topic	Number	%
Agronomy/crop management	67	32
Crop protection	64	30
Genetics, breeding, botany, taxonomy, germplasm collection	24	11
Soil science (fertilizers, chemistry, mineral nutrition, drainage, irrigation, soil survey, etc.)	23	11
Agro-industry (processing)	16	8
Tissue culture/biotechnology	8	4
Physiology (including postharvest physiology)	7	3
Socioeconomics	2	1
TOTAL	211	100

[1]Source: "Directory of Researchers working on Banana and Plantain", published by INIBAP and UPEB.

local expertise at NARS, universities, and regional centers, as well as in overseas universities and international centers.

Both the Network participants and INIBAP undertook to develop a new awareness of the existing needs for, and the importance of, training.

Progress on Training during 1991-93: Institutional Case Studies

INIBAP-LAC is in process of collecting results of the efforts made on training by network partners during the last 3 years. Such progress can be illustrated through activities developed by the institutions listed below.

CATIE (Centro Agronómico Tropical de Investigación y Enseñanza)

This Regional Center, based in Costa Rica, has developed an outstanding capacity to train personnel in tissue culture techniques through short courses or at the Master of Science level. The Biotechnology Unit has been in charge of the training activities. This Unit has been receiving technical support from CIRAD, which outposted three scientists at the Unit.

Plantain is one of the most important research topics in biotechnology at CATIE, and its findings in cell suspension, somatic embryogenesis, and cryopreservation have been presented in several international meetings on *Musa*.

During the past 3 years, 32 persons from 15 countries were trained in the basic principles of the tissue culture techniques for the micropropagation of banana and plantain. Additionally, 8 postgraduate students conducted their research on topics related to *Musa* biotechnology (see Tables 3 and 4).

Three postgraduate students of the Integrated Pest Management Program conducted research studies on banana and plantain nematodes and black leaf streak/black Sigatoka. One student of the Sustainable Agriculture Program also developed an expert system on plantain.

There exists very close collaboration between CATIE and INIBAP in assessing training priorities and needs in the region. INIBAP has supported both individual and group training at CATIE and the Regional Coordinator has been acting as an external examiner for all postgraduate theses related to *Musa*.

Funds to support these activities came from different donors (USAID, CIRAD [French Cooperation], UNESCO, IDRC, INIBAP, UNDP, and country institutions).

Demand for training is increasing, especially from those countries that have a very weak research capacity in *Musa*.

UPEB (Unión de Países Exportadores de Banano)

This intergovernmental organization, based in Panama, has the most advanced information and documentation center on *Musa* in the region, and has been coordinating the work of the INIBAP Regional Info/Doc Network on Banana and Plantain.

Table 3. Number of trainees on *Musa* topics at CATIE during 1991-93[1].

Topic	Course	Year 1991	1992	1993	Total
Tissue culture	Short course	4	17[2]	11[3]	32
	MSc	4	2	2	8
Phytopathology	MSc	1		1	4
Nematology	MSc		1		
Expert systems	MSc		1		

[1] Source: Biotechnology Unit, CATIE, 1994.

[2] Course organized jointly by CATIE and INIBAP.

[3] Short training courses on tissue culture techniques in tropical crops including *Musa*.

Table 4. Participants from LAC countries in training activities on *Musa* topics at CATIE during 1991-93.

Country	Year[1] 1991 SC	1991 MSc	1992 SC	1992 MSc	1993 SC	1993 MSc
Bolivia			1			
Brazil			1			
Colombia			1			
Costa Rica	2		3	1	1	1
Dominican Republic			2	1	2	
Ecuador			1			
El Salvador					2	
Guatemala		1	1		1	
Guyana			1			
Honduras			1	1		
Jamaica			2			
Mexico		1	1	1	3	
Nicaragua	2	1	1		1	
Panama		1	1			1
Venezuela					3	
TOTAL	4	4	17	4	13	2

[1]SC: Short courses. MSc: Master of Science.

Besides its close partnership with INIBAP, it has served as a model for the regionalization process of the INIBAP Information and Documentation System. UPEB translates and publishes the Spanish version of the INIBAP newsletters (*Musarama* and INFO*MUSA*). The Regional Information/Documentation Network was set up in 1990,

during a workshop held in Costa Rica, which was coorganized by UPEB, CORBANA, and INIBAP and supported by IDRC and CTA. Two specific objectives of the Network are related to training:

– To provide training in the methodologies which are to be used by national centers within the framework of the Regional Network.

– To train users, so they can use the Network services adequately.

Databases were installed in cooperating countries and, during 1991-92, 41 specialists in Info/Doc sciences from 12 countries were trained in operating the system (see Table 5). Additionally, several national centers (Brazil, Colombia, Costa Rica, Cuba, Nicaragua, Panama), in collaboration with UPEB, organized user-training workshops that were attended by 420 users, mostly researchers, agronomists, advanced students, and some growers. That is the reason why there exists an enormous demand for documents in the region, and why INIBAP newsletters are in high demand.

Supporting funds for training activities in Info/Doc come mainly from IDRC, CTA and interested organizations of the region. Training for users is a permanent request from all banana- and plantain-producing countries.

CORBANA (Corporación Bananera Nacional), Costa Rica

This semiautonomous organization, founded in 1974, has the vital role of guiding the banana industry in Costa Rica.

CORBANA has established a research department supported from taxes from the export of bananas. Currently, the main areas of research are: crop protection (mainly phytopathology and nematology), soils, crop husbandry, tissue culture (since 1992), and crop diversification. Its staff comprise 18 researchers (3 PhDs, 4 MSs, 11 specialized agronomists) and 35 technicians. Taking into account its comparative advantage in crop husbandry, and the interest demonstrated by growers to develop better capacity in their farm management, CORBANA has established a 3-month course on this topic for agronomists, technicians, and growers. In 1991, 22 people were trained, 23 in 1992, and 43 in 1993.

Additionally, between 1991 and 1993, 40 persons from different institutes received short training courses (at least 1 week) on phytopathology, nematology, mineral nutrition, and plantain production. Attendance for field days organized by CORBANA grew from 150 participants in 1992 to 750 participants in 1993.

In 1991, CORBANA initiated a training program for its scientific staff through different arrangements. Two researchers are currently conducting postgraduate studies in a US university: one at PhD level in phytopathology and the other at MS level in postharvest studies.

This program is a long-term investment for this institution and is based on the successful results obtained by the research department over a decade.

ICA (Instituto Colombiano Agropecuario)

This institute has a well-known plantain program, which has been working in innovative plantain production systems, especially at high altitude. Since 1984, the program has

Table 5. Training and technical cooperation on information and documentation through the Regional Info/Doc Node (UPEB) and INIBAP[1], 1990-92.

	Year					
Country	1990[2]		1991		1992	
Brazil	1		2	(40)[3]		
Colombia	2	(100)	2	(150)	2	
Costa Rica	4		4			(18)
Cuba	3			(30)		
Guatemala	1				4	
Honduras	1		1		2	
Mexico	1		1			
Nicaragua	1					(12)
Panama					2	(70)
St Lucia (WINBAN)	1					
Trinidad/Tobago	1					
Venezuela	1		4			
TOTAL	17	(100)	14	(220)	10	(100)

[1] Sources: UPEB and INIBAP, 1994.

[2] Regional Workshop organized by INIBAP.

[3] Figures in parentheses refer to the persons who attended workshops on the use of the Info/Doc System, organized by UPEB and national institutions.

been receiving support from IDRC, the Coffee Growers' Committee, and INIBAP through different projects.

The scientific team is composed of 3 PhDs, 6 MSs, and 2 specialized agronomists. Two of the MS graduates are on study leave. Half of the staff is working part-time on plantains. Besides publication of scientific papers and the staff's participation in all regional meetings on banana and plantain, the Plantain Program published a book in 1992 in which research results obtained from 1984 to 1991 were included.

Colombia is the main plantain producer and consumer in the region. Annual production is 2.5 million tonnes, and the average annual per capita consumption is around 80 kg.

In 1992 ICA took the responsibility to transfer new technologies generated by the Program throughout the country.

As can be observed in Table 6, 1620 people participated in the different training activities arranged by the technical team. A quarter of the participants were agronomists working in different areas of the country, and one-tenth of the trainees were students from various schools of agronomy, as part of their studies in tropical crops. One-third of the trainees were growers. The plantain technical team has received several awards for its enormous effort in transferring its scientific findings into practical applications.

On the other hand, the staff has been receiving support from different sources for updating specific skills, such as through technical visits to the *Musa* programs developed

Table 6. Activities on transfer of technology organized by the ICA's Plantain Program, 1992-93.

Activity	Number	Participants					
		Agronomists	Agronomists/ growers	Agronomists/ students	Students	Growers/ agronomists/ students	Total
Short courses	18	343	373	40	50		806
Lectures	5	8	250		35		293
Field trips	7	6	28	29	81		144
Symposium	3	77				300	377
TOTAL	33	434	651	69	166	300	1620

by CIRAD-FLHOR in Guadeloupe, Montpellier, and Cameroon, sponsored by the Ministère des Affaires Etrangères, France; molecular markers applied to *Pseudomonas solanacearum* in Texas A&M University (USA), sponsored by IDRC, and postgraduate courses in USA (tissue culture and genetics) and Germany (plant nutrition), supported by ICA itself.

ICA's authorities are aware that the training program should be strengthened in order to create a new generation of scientists who will replace those who retire in the next decade.

Postgraduate Studies in *Musa*

The Regional Advisory Committee and INIBAP are very interested in detecting outstanding researchers and in stimulating them to join the regional *Musa* scientific community. They also seek to create possibilities for young researchers who are working on banana and plantain to obtain placements for postgraduate studies.

Table 7 shows the number of highly qualified researchers from some countries who joined the *Musa* community during the past 3 years and those who are conducting postgraduate studies in *Musa* topics.

EMBRAPA, in Brazil, has a well established program to train its researchers either in the same country or abroad. This institution has made an enormous effort progressively to build self-sufficiency to match the country's scientific needs, in order to develop its agriculture, especially in banana which is the fruit second in importance after orange.

ICA and CORPOICA in Colombia have been linking main programs and projects to develop human resources with special research skills. The National Plantain Program has succeeded in attracting trained scientists in different fields, and is looking for assistance to train a new wave of young scientists.

As the second-ranking banana exporter, Costa Rica is in the process of forming a highly qualified group of scientists in *Musa* research. This country has received long-

Table 7. Researchers who obtained a PhD degree during 1992-93 and are actually working on banana and plantain programs, and PhD candidates (1994-96) who are conducting studies on *Musa* topics and were linked with the INIBAP programs.

	Year					
Country	1991	1992	1993	1994	1995	1996
Brazil	1	1	2			2
Colombia					1	1
Costa Rica				2	1	1
Honduras			2			
Puerto Rico						1

term support from the Ministère des Affaires Etrangères, France to train three *Musa* scientists to PhD level.

As was already mentioned, CORBANA and the National Council on Science and Technology (CONICIT), are supporting postgraduate studies of two researchers in USA.

FHIA recently hired two PhD graduates to strengthen its Banana Breeding Program.

These examples demonstrate the interest of several institutions in the region to train and to incorporate new professionals, and to structure and extend their research programs. This is a recognition that training is vital for attaining a higher level of technical and scientific knowledge, and for enhancing the strength of existing programs.

Training in LAC through IMTP Phase I

The idea of evaluating promising germplasm was intrinsic to INIBAP's creation. The Network initiators foresaw the impact that new materials would have as production alternatives to farmers who have seen their crop yields diminishing as a result of devastating diseases.

To organize the IMTP in LAC was a challenge because there was no historical background concerning this issue. It was a learning-by-doing process.

INIBAP-LAC organized three regional workshops and a global meeting, in accordance with needs which emerged during the execution of the UNDP-supported project.

As can be observed in Table 8, the first training activity was a workshop for the teams involved (ICA, CORBANA, and FHIA) in the field-evaluation of germplasm for reaction to black leaf streak/black Sigatoka, and to discuss the correct procedures and methodology according to IMTP's technical rules and protocols for its assessment. Several modifications were suggested to improve the protocol. Two other organizations (EMBRAPA and CATIE) were invited to contribute to the discussions.

This first gathering was crucial for the project's success, as all technical teams collaborated with each other, even to the extent of establishing the databases by which to analyze the enormous amount of information collected.

The second activity was organized to take into account the lack of knowledge in *Musa* germplasm exchange and management. The regional course was held at CATIE and trainees learned about the principles of *Musa* taxonomy, utilization of genetic resources, breeding programs (mostly focused on tissue-culture techniques and plantlet acclimatization procedures). The people trained came from NARS, universities, and organizations interested in genetic resources. This course paved the way for future requests for selected hybrids for the establishment of National Evaluation Programs.

The purpose of the global meeting of experts on *Mycosphaerella fijiensis* was: to review the data collected by each technical team in Africa and LAC, to propose amendments to the technical rules, and to nominate materials for inclusion in IMTP Phase II. This meeting can be considered as the starting point, not only for the IMTP's Phase II, but also for those who may want to enrich their knowledge regarding the epidemiology of *Mycosphaerella* spp.

Table 8. Participants from Latin America/Caribbean in courses, workshops, and meetings linked with IMTP Phase I, supported by UNDP, 1991-93.

Country/ Organization	Workshop on IMTP-BLS/BS[1] 06/91	Regional course on Germplasm Management (tissue culture) 06/92	IMTP-BLS/BS Experts' Meeting 10/92	First Regional Meeting on IMTP 03/93
Bolivia		1		
Brazil	1	1	1	1
Colombia	4	1	7	2
Costa Rica	2	3	2	2
Cuba			1	2
Dominican Republic		2		2
Ecuador		1		2
Guadeloupe		1		1
Guatemala		1		
Honduras	3	2	3	6
Jamaica		2		1
Mexico		2		2
Nicaragua		1		
Panama		1		1
Puerto Rico				1
Venezuela				2
CATIE	3	7	1	1
UPEB				1
WINBAN				1
TOTAL	13	26	15	28

[1]BLS/BS = black leaf streak/black Sigatoka.

As a culmination of IMTP's Phase I, INIBAP organized a regional meeting to make public, for the first time, the results of one of the most successful Network programs and, maybe, the one which has created the greatest expectations, both at regional and global levels. Participants came from the main region's banana-and plantain-producing countries and regional organizations, representing 19 countries. Such a wide geographical coverage made easier the interaction between decision-makers and executors of national research plans and scientists specializing in banana and plantain research. This meeting was a tangible example of the Network's development regarding collaborative research.

Training Priorities in LAC for IMTP Phase II

In view of the results of IMTP's Phase I and the Third Regional Advisory Committee's recommendations, the following training activities should be considered as priorities.

1. To organize a regional workshop (1 week) for the technical teams to be involved in the field-evaluation of germplasm (Brazil, Cuba, Colombia, Costa Rica, Honduras) for reaction to Sigatoka diseases and Fusarium wilt, and to discuss procedures and methods based on IMTP's technical rules and protocols for assessment. Pathologists from other producing countries/organizations should be invited (CATIE, WINBAN, Ecuador, Jamaica, Venezuela).

2. To organize a regional course (1-2 weeks) on *Musa* germplasm exchange and management, which includes principles in taxonomy, genetics, breeding quarantine rules, indexing, tissue-culture techniques, and plantlet endurance.

3. To arrange the participation of regional experts at the global meetings.

4. To organize the exchange of scientists carrying out parallel work in partner institutions.

5. To transfer specific skills and research capabilities in specialized centers to phytopathologists in the region working on Fusarium wilt, and to virologists working on CMV and BSV.

Annexes

Annex 1:

Technical Guidelines for IMTP Phase II: Sigatoka Sites

Clones to be Evaluated for Reaction to Black Leaf Streak/Black Sigatoka and Sigatoka/Yellow Sigatoka

The germplasm to be supplied to test sites for evaluation is presented in Table 1 below.

Table 1. Germaplasm for evaluation against Sigatoka diseases

FHIA (Honduras)			
1. SH 3444 (AAAA)	FHIA-23	Highgate x SH 3362	ITC 1265
CNPMF/EMBRAPA (Brazil)			
2. PV 03-44 (AAAB)	EMB 402	Pacovan x Calcutta 4	ITC 1262
3. PA 03-22 (AAAB)	EMB 404	Prata Anã x Calcutta 4	ITC 1261
INIVIT/INIFAT (Cuba)			
4. SH 3436-9 (AAAA)		Somaclonal variant of SH 3436 (Highgate x SH 3142)	ITC 1283
Natural germplasm			
5. Yangambi Km5 (AAA)		Ibota	ITC 1123
6. Saba (ABB/BBB)		Saba	ITC 1138
7. Pisang Ceylan (AAB)		Mysore	ITC 0650
Reference/standard clones			
8. Calcutta 4 (AAw)	Highly resistant/hypersensitive response		ITC 0249
9. Pisang Lilin (AA)	Highly resistant		ITC 0001
10. Pisang Berlin (AA)	Susceptible		ITC 0611
11. Niyarma Yik (AA)	Highly susceptible		ITC 0269
12. Local cultivar	To be selected by officer-in-charge of each test site as an appropriate local standard to compare reactions		

Supply of Planting Material and Trial Establishment

All test and reference plants will be supplied as tissue cultures. The deflasking and care of young plantlets is described below. They should be allowed to grow in pots until the 6-9 broad-leaf stage. Care must be taken to ensure plants do not become pot-bound.

Planting should occur in shallow furrows or basins. These should be filled with soil once the plant is established. For successful establishment, plants would need to be monitored to ensure soil in the root zone was moist. Ideally, an irrigation system should be installed. Fertilizers according to local requirements or based on the results of soil analysis tests should be added to the soil to boost growth in the early stages. Measures should be taken during establishment to prevent losses from weevil borers.

After establishment (2-3 months from planting), the normal management practices of the region should be adopted. These should be uniformly applied to the trial and recorded.

Handling of in-vitro rooted plantlets

In-vitro rooted plantlets are distributed as sterile cultures in watertight Cultu saks®.

Plantlets that are 5-10 cm tall and have well-developed roots are ready for planting in pots. If plantlets are smaller or if transplanting is not immediately possible, it is advisable to place the plantlets in the Cultu saks® in an upright position under sufficient light (not direct sunlight), at a temperature between 20 and 30°C. Under these conditions plantlets can be kept for a few weeks.

To transplant rooted plantlets to soil requires some care. Please proceed as follows or use your own proven method.

– Cut open each Cultu saks® chamber at one vertical edge.

– Carefully remove the plantlet from the Cultu saks® by holding the base gently with blunt-end forceps. Place the plantlet on your palm.

– Remove culture medium adhering to the roots and leaves by placing the plantlet in a container of water (bucket) and shaking gently. Do not damage the stem nor the root system.

– Transplant the plantlet to plastic pots or bags (15-cm diameter) filled with a 30:70 peat sand mix of which the upper 2 to 3 cm are fine (sifted). The upper roots should be covered by 2-3 cm soil.

– After transplanting, water the plantlets immediately.

– Keep the plants under high humidity. A simple humidity chamber can be constructed by enclosing a wooden frame in strong transparent plastic. The humidity chamber (about 40-60 cm height) is placed over the pots in a shaded area where temperature is kept at 25-32°C. The humidity inside the chamber is maintained by spraying water regularly to saturate the air. The heat building-up inside the chamber is minimized by leaving a 2-3 cm opening at the base to allow air to circulate.

– During the first week after transplanting, mist the humidity chamber twice a day to saturate the atmosphere. This is very important because low relative humidity at this stage will easily destroy the plantlets. Water the plants once a day with tap water.

– One week after transplanting, spray the humidity chamber and the plants only once a day.

– One month after transplanting, remove the plants from the humidity chamber.

– Keep the plants in a nursery under shade until about 30 cm tall (2-3 months) before establishment in the field.

– Transplant the plants into the field during the wet season, but not later than 6 weeks before the onset of the dry season.

(Protocol for handling in-vitro rooted plantlets provided by Ir I Van den houwe, ITC)

Field Plan

Enough plants of each of the 12 test accessions and reference clones will be provided to allow the trial to be planted as in Figure 1. Note that there are five plants in each plot

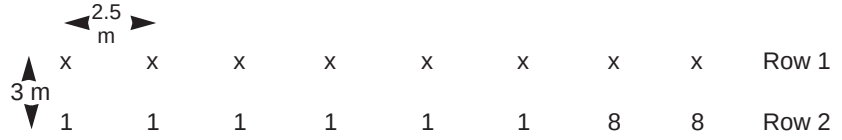
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
1*	1	1	1	1	1	8	8	8	8	8	11	11	11	11	11	6	6	6	6	6	4	4	4	4	4	3	3	3	3	3	3* Rep I
2*	2	2	2	2	2	7	7	7	7	7	5	5	5	5	5	10	10	10	10	10	9	9	9	9	9	12	12	12	12	12	12*
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
4*	4	4	4	4	4	9	9	9	9	9	3	3	3	3	3	6	6	6	6	6	7	7	7	7	7	5	5	5	5	5	5* Rep II
10*	10	10	10	10	10	11	11	11	11	11	12	12	12	12	12	1	1	1	1	1	2	2	2	2	2	8	8	8	8	8	8*
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
11*	11	11	11	11	11	1	1	1	1	1	10	10	10	10	10	9	9	9	9	9	7	7	7	7	7	6	6	6	6	6	6* Rep III
8*	8	8	8	8	8	12	12	12	12	12	2	2	2	2	2	5	5	5	5	5	4	4	4	4	4	3	3	3	3	3	3*
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
5*	5	5	5	5	5	7	7	7	7	7	2	2	2	2	2	11	11	11	11	11	3	3	3	3	3	6	6	6	6	6	6* Rep IV
12*	12	12	12	12	12	8	8	8	8	8	1	1	1	1	1	2	2	2	2	2	8	8	8	8	8	5	5	5	5	5	5*
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
12*	12	12	12	12	12	2	2	2	2	2	8	8	8	8	8	7	7	7	7	7	6	6	6	6	6	9	9	9	9	9	9* Rep V
11*	11	11	11	11	11	5	5	5	5	5	1	1	1	1	1	4	4	4	4	4	3	3	3	3	3	10	10	10	10	10	10*
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

Total area = 77.5 m (along rows) x 45.0 m (down rows) = 3488 m^2 = 0.35 ha.
x = plants of a Cavendish clone (Grande Naine, Williams, Valery, etc. but not Dwarf Cavendish).
1,2,.. = clones 1-12 as indicated in Table 1.
* These plants of test clones at the end of rows should not be used to collect data.

Figure 1. Sigatoka screening site layout.

and that plots are randomised in each of the five blocks. Plants for Cavendish guard rows/inoculum generators will need to be provided by the collaborator at each site.

There should be 2.5 m between plants in each row and 3 m between rows (as below).



The layout of blocks in the field should aim to minimize expected variations (e.g. soil changes, such as pH: (see Fig.2).

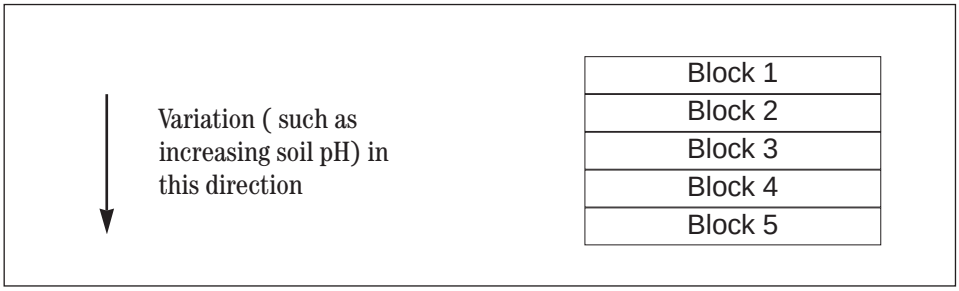


Figure 2. Block layout at Sigatoka screening site (suggested by A Rafie, FHIA).

Data to be Collected

The data to be collected are summarized numerically in Figure 3. Detailed guidelines follow under the same numbers.

1. Date of planting
   – Record the date the trial was planted.

Planting

1 –Date of planting

Growing Phase (3 months after planting to shooting)

2 –Disease development time
3 –Youngest leaf spotted
4 –Leaf emission rate
5 –Disease severity (6 months after planting)

Shooting

6 –Disease severity
7 –Plant height at bunch emergence (shooting)
8 –Time from planting to bunch emergence (shooting)

Shooting to Harvest Phase

9 –Youngest leaf spotted

Harvest

10 – Disease severity
11 – Time from planting to harvest
12 – Height of 'follower' sucker at harvest
13 – Weight of bunch at harvest
14 – Number of hands in bunch at harvest
15 – Number of fingers in bunch at harvest
16 – Average weight of each finger at harvest

Environmental Factors

17 – Environmental data

Agronomic Factors

18 – Management practices

Disease Factors

19 – Sigatoka disease

Figure 3. Summary of data to be collected during Sigatoka screening trials.

2. Disease development time (DDT)

– Readings should begin 3 months after planting.

– Every test plant, except the extra plants at the ends of rows (see Fig.1), should be used for data collection.

– Plants should be inspected once a week. Plants with cigar leaves near Brun's Stage B (see **Appendix A**) should be selected and marked (indelible black felt-tip pen, colored ribbon, tags) with the date it was estimated that the leaf was at Brun's Stage B.

– These leaves should be inspected once a week until 10 or more discrete, mature, necrotic lesions or one large necrotic area with 10 or more light-colored dry centers are visible. This date should be recorded. The time of appearance of mature lesions should be estimated if it occurs between inspections. The DDT in days can then be worked out for this leaf.

A mature lesion of black leaf streak/black Sigatoka is defined as Stage 6 by Fouré **(Appendix B)**.

A mature lesion of Sigatoka/yellow Sigatoka is defined as Stage 6 or the 'third spot stage' by Meredith **(Appendix C)**.

– This process should be repeated every week. That is, every week plants with cigar leaves at Brun's Stage B are selected for observations. Observations to check for 10 mature lesions on these leaves should also be made once a week.

3,9. Youngest leaf spotted (YLS)

– This is the number of the first fully unfurled leaf with 10 or more discrete, mature, necrotic lesions or one large necrotic area with 10 light-colored dry centers, counting leaves down from the top of the plant.

– A mature lesion of black leaf streak/black Sigatoka is defined as Stage 6 by Fouré **(Appendix B)**.

– A mature lesion of Sigatoka/yellow Sigatoka is defined as Stage 6 or the 'third spot stage' by Meredith **(Appendix C)**.

– This information should be recorded for each leaf that has been used to assess DDT (see above). After shooting, when leaves cease to be produced, the YLS value should be recorded weekly until harvest.

4. Leaf emission rate (LER)

– This should be calculated regularly (at least monthly) for each test and reference plant beginning 3 months after planting and finishing at bunch emergence (shooting).

– LER can be worked out from DDT readings. Count the number of leaves produced between marked leaves at Brun's Stage B **(Appendix A)** on each plant and divide by the time between observations.

– LER can be expressed as 'number of leaves produced per week' and may be fewer than 1.

A format for recording and calculating DDT, YLS and LER is presented in Appendix D (Table 2).

5,6,10. Disease severity

– Leaves should be scored for disease levels using the Gauhl's modification of Stover's system (see **Appendix E**, Fig. 5).

– Percentage of leaf area killed by the Sigatoka pathogen expressed as disease grades should be recorded for each leaf on each test plant (see **Appendix E**, Table 3) .

– This information should be recorded:

a) 6 months after planting;

b) at bunch emergence (shooting);

c) at harvest.

– Only upright leaves should be recorded.

– After Sigatoka severity has been recorded, the infection index for each test plant should be calculated as shown in **Appendix E**.

7. Plant height at bunch emergence (shooting)

– This is the distance in meters from the ground to the angle made between the bunch stalk and bunch cover leaf.

8. Time from planting to bunch emergence (shooting)

– This can be calculated in days derived from the dates of planting and bunch emergence.

11. Time from planting to harvest

– This can be calculated in days derived from the date of planting and harvest.

12. Height of 'follower' sucker at harvest

– This is the distance in meters from the ground to the junction between the youngest and next youngest leaf of the 'follower' sucker at the time the bunch is harvested from the mother plant. All other suckers except the 'follower' sucker should be rogued as they appear.

13. Weight of bunch at harvest

– This is the weight in kilograms of the bunch immediately following harvest.

– At harvest, cut the bunch stalk (peduncle) **above** the first hand at the scar of the last bract and immediately **below** the last hand.

14. Number of hands in bunch at harvest

– Cut the hands from each bunch following weighing, and record the number of hands.

15. Number of fingers in bunch at harvest

– Count and record the number of individual fruit in all hands cut from each bunch.

16. Average weight of each finger at harvest

– Weigh all the hands from each bunch and divide by the number of fingers counted in 15.

A format for recording agronomic data (see 1, 7, 8 and 11-16) is presented in Appendix F (Table 4).

17. Environmental data

– Environmental data should be collected from the nearest weather station to the trial. Where trials are conducted in the grounds of collaborating institutes, this should not be a problem, as many centers have recording equipment.

– Daily fluctuations in temperature and in humidity should be monitored using a thermohydrograph. Rainfall (collected in a rain gauge and measured in mm) could be calculated for the week if daily readings cannot be taken.

– Readings should begin at planting and continue until harvest.

– The soil of the test site should be analyzed.

– A climatic map on the long-term climatic trend should be provided, if possible, to give an overview of the annual fluctuations of temperature and rainfall.

– A format for recording environmental data is presented in **Appendix G** (Table 5)

18. Management practices

– The trial should be managed according to the agronomic practices of the NARS or the institute.

– All management practices should be applied uniformly over the whole trial site.

– Details of fertilizer application, nematode/weevil control measures and irrigation/drainage management should be recorded.

– Obviously, leaf spot should not be controlled.

19. Sigatoka disease ratio

– Leaf samples should be collected from each accession 6 months after planting and examined to determine the ratio of black leaf streak/black Sigatoka and Sigatoka/yellow Sigatoka lesions.

– In most locations, it is expected that only one pathogen will be present, but both are possible at some sites where black leaf streak/black Sigatoka and Sigatoka/yellow Sigatoka could be coexisting.

– The ratio of black leaf streak/black Sigatoka to Sigatoka/yellow Sigatoka should be expressed as a percentage.

Appendix A: Stages of an Unfolding Banana Leaf (after Brun)

The young unfolded leaf is coiled into a double spiral. The right half of the leaf is situated in the hollow of the central petiole, while the left half of the leaf covers both the petiole and the right side.

The time lapse for the unfolding of a leaf varies. Under favorable climatic conditions, it takes about 7 days, but can take up to 15-20 days under poor conditions (drought, malnutrition, etc.).

In order to understand the unfolding process, it is important to recall that the formation of the leaf takes place within the pseudostem before shooting. The new leaf is tightly coiled, whitish, and particularly fragile.

The shooting of the leaf results in an extraordinarily rapid growth of the leaf sheath (4 m in 10 days for Gros Michel). The young leaf slips into the petiolar canal of the preceding leaf and the development of a leaf corresponds to two successive phenomena, that of 'growth' and that of 'unfolding'.

To facilitate the description of the unfolding process, the latter has been divided into five successive stages. These stages are defined arbitrarily, since the process is in reality a continual one. The first two stages can be considered as corresponding to the 'growth' phase, the third stage represents the end of the growth and the beginning of the unfolding process, and the fourth and fifth concern the unfolding itself.

These different stages have been defined as follows :

Stage A: The 'cigar', about 10 cm in length, is still joined to the preceding leaf.

Stage B: The 'cigar' is bigger, but has not yet reached its full length.

Stage C: The 'cigar' is completely free. It reaches its full length and the diameter of its apex has considerably increased following the loosening of the spiral.

Stage D: The left-hand side has already unfolded, and spreading takes place at the extreme apex.

Stage E: The upper part of the leaf has unfolded and the base is in an open cornet shape.

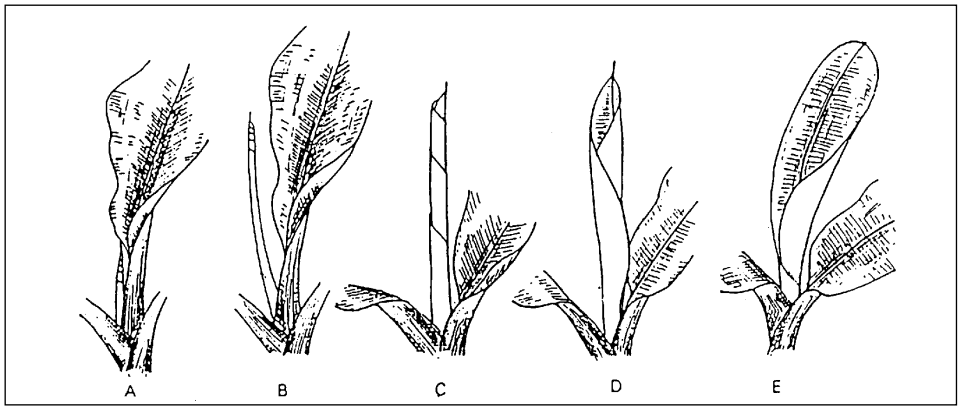


Figure 4. Stages of an unfolding leaf.

Appendix B: Symptoms of Black Leaf Streak/Black Sigatoka (after Fouré)

Stage 1 is the first external symptom of the disease. It appears as a small spot whose whitish or yellow color resembles the first stage of Sigatoka/yellow Sigatoka disease. These symptoms are not visible in transmitted light and can be observed only on the underside of the leaf.

Stage 2 (Plate 1) appears as a stripe, generally brown in color and visible on the underside of the leaf; later the symptom also appears on the upper part of the limb as a stripe, the yellow color of which resembles the stripe at Stage 1 of Sigatoka/yellow Sigatoka. The color of this stripe will change progressively to brown and later to black on the upper side of the leaf, but will retain the brown color on the underside.

Stage 3 differs from the previous one by its dimensions. The stripe becomes longer, is enlarged and, in certain conditions (weak inoculum and unfavorable climatic conditions), can reach 2 or 3 cm.

Stage 4 appears on the underside as a brown spot and on the upper side as a black spot.

Stage 5 is when the elliptical spot is totally black and has spread to the underside of the leaf. It is surrounded by a yellow halo with the center beginning to flatten out.

Stage 6 (see Plate 1) is when the center of the spot dries out, turns clear gray, and is surrounded by a well-defined black ring, which is, in turn, surrounded by a bright yellow halo. These spots remain visible after the leaf has dried out because the ring persists.

Plate 1. Range of symptoms of black leaf streak/black Sigatoka disease from streaks (Fouré's Stage 2: close to fingers on left) to mature lesions (Fouré's Stage 6: close to fingers on right).

Appendix C: Symptoms of Sigatoka/Yellow Sigatoka (after Meredith)

1. Initial streak stage. The spot is only just visible to the naked eye as a minute yellowish-green speck (about 1.0 x 5.0 mm).

2. Second streak stage. The initial streak increases in size, notably in length rather than in breadth, still remaining yellowish-green.

3. Third streak stage. The streak begins to broaden slightly as well as increase in length and it begins to turn rusty red, usually near its center.

4. First spot stage. The streak turns dark brown and at the same time, or within 24 hours, a light brown water-soaked halo forms around the spot when the leaf is turgid. This halo is especially well seen if the spot is held up against the light in the early morning. The spot increases considerably in size during this stage. The streak has now reached the stage when it is clearly recognizable as a spot.

5. Second spot stage. The dark brown part of the spot shrinks, appears sunken, and the water-soaked halo turns a darker brown.

6. Third spot stage (see Plate 2)**.** The spot is fully developed, the sunken central area having turned gray and the halo usually dark brown or black, forming a well-defined ring round the spot. Such a spot remains well-defined even when the leaf is dead, the dark ring round the spot remaining quite distinct.

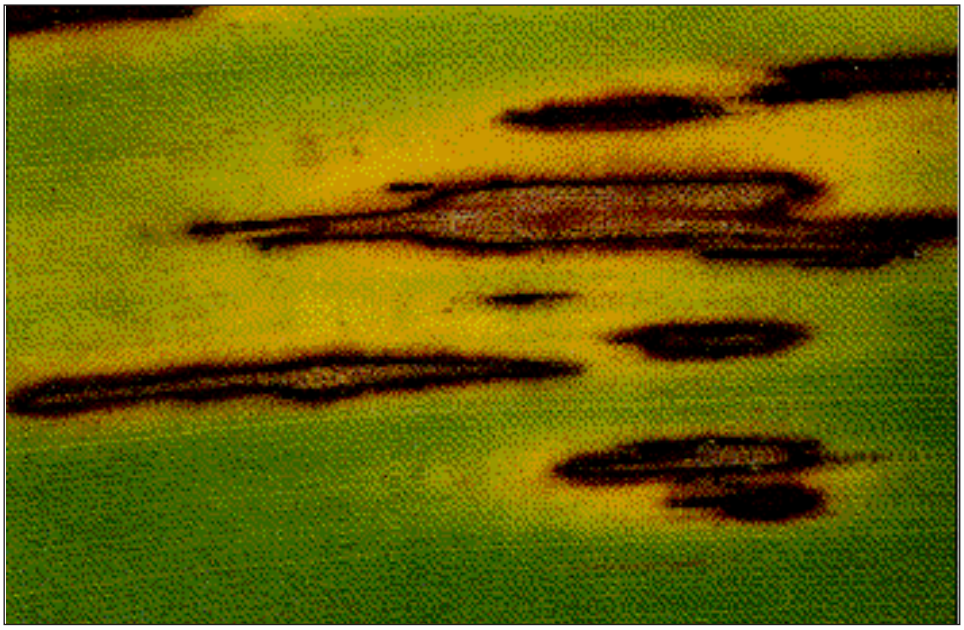


Plate 2. Spot stages of Sigatoka/yellow sigatoka disease showing some mature lesions with gray centers (Meredith's third spot stage).

Appendix D: Disease and leaf emergence data to be collected for each test plant

Table 2. Format for recording and calculating DDT, YLS and LER

1	2	3	4	5	6
Date leaf marked at Brun's Stage B	Date 10 or more mature lesions are visible on marked leaf	Disease development time (DDT) in days	Youngest leaf spotted (YLS) at date in column 2	New leaves produced between column 1 readings	Average leaf emission rate (LER)/week

*Indicates lesions did not develop beyond Fouré's stage 2 (for insertion by user).

Appendix E: Sigatoka severity scoring and calculations

The Gauhls' modification of Stover's Sigatoka severity scoring system is shown in Figure 5.

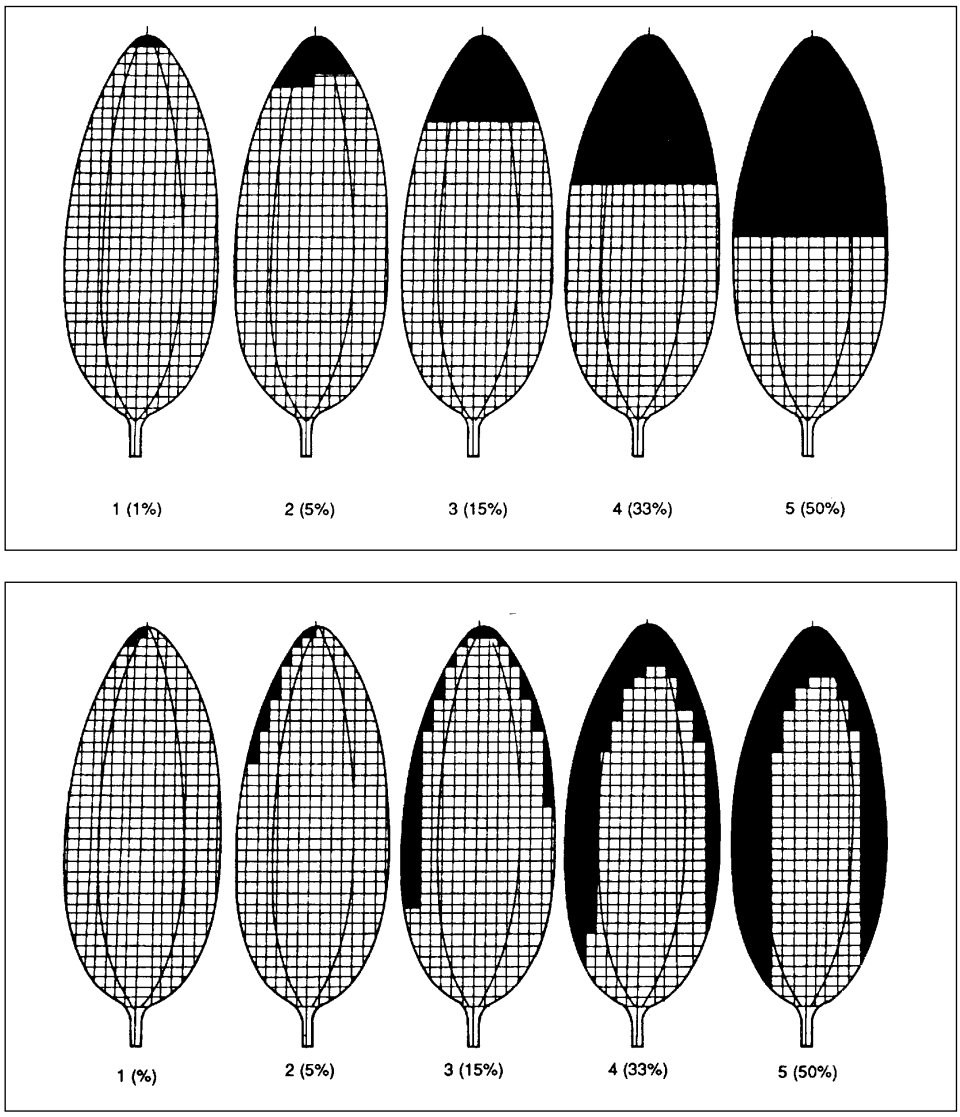


Figure 5. Two examples of maximum percentage of leaf area spotted in disease grades 1 - 5 (adapted from Stover 1971, see Gauhl F, Pasberg-Gauhl C, Vuylsteke D, Ortiz R. 1993. Multilocational evaluation of black Sigatoka resistance in banana and plantain. IITA Research Guide 47, IITA, Ibadan, Nigeria, 59 pp).

Table 3. Sigatoka severity scoring to be completed for upright leaves on each test plant at the growth stages indicated (scores have been included as an example).

Leaf no. / Growth stage	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
6 months	0	0	0	1	2	2	2	3	5	6	6	6	–	–
Bunch emergence	0	0	0	1	2	2	–	4	5	6	6	6	–	–
Harvest	0	1	2	5	5	6	6	–	–	–	–	–	–	–

Key:

*	=	youngest completely unfurled leaf
0	=	no symptoms
1	=	Less than 1% of lamina with symptoms (only streaks and/or up to 10 spots)
2	=	1 to 5% of lamina with symptoms
3	=	6 to 15% of lamina with symptoms
4	=	16 to 33% of lamina with symptoms
5	=	34 to 50% of lamina with symptoms
6	=	51 to 100% of lamina with symptoms
–	=	missing leaf or dead leaf hanging down the pseudostem (when a leaf is missing or dead and hanging down the pseudostem, it should not be included in the infection index calculations).

Calculation of infection index

Calculate the infection index for each plant in each replication at each growth stage:

$$\text{Infection index} = \frac{\Sigma\, nb}{(N-1)\,T} \times 100$$

Where n = number of leaves in each grade
b = grade
N = number of grades used in the scale (7)
T = total number of leaves scored

Example: at bunch emergence as in Table 3.

$$\text{Infection index} = \frac{3\,(0) + 1\,(1) + 2\,(2) + 1\,(4) + 1\,(5) + 3\,(6)}{(7-1)\,11} \times 100$$

$$= \frac{1 + 4 + 4 + 5 + 18}{6 \times 11} \times 100 = \frac{32}{66} \times 100$$

$$= 48.5$$

(Infection index calculation courtesy of Dr R Romero, CORBANA)

Appendix F: Agronomic data to be collected for each test plant

Table 4. A format for collecting agronomic data.*

Planting date:.....
Bunch emergence (shooting) date:.....
Plant height at bunch emergence[1]:.....
Number of days from planting to shooting:.....
Harvest date:.....
Number of days from planting to harvest:.....
Height of 'follower' sucker at harvest[2]:.....
Weight of bunch at harvest[3]:.....
Number of hands in bunch[4]:.....
Number of fingers in bunch[5]:.....
Average weight of each finger[6]:.....

* Key agronomic data suggested by Drs D Vuylsteke and R Ortiz, IITA.

[1]This is the distance in meters from the ground to the angle made between the bunch stalk and bunch cover leaf.

[2]This is the distance in meters from the ground to the junction between the youngest and next youngest leaf of the 'follower' sucker at the time the bunch is harvested from the mother plant.

[3]This is the weight in kilograms of the bunch immediately following harvest. (At harvest, cut the bunch stalk [peduncle] **above** the first hand at the scar of the last bract and immediately **below** the last hand.)

[4]Cut the hands from the bunch and record the number of hands.

[5]Record the number of individual fruit in all of the cut hands.

[6]Divide the collective weight of the hands by the number of fingers in the hands.

Appendix G: Environmental data to be collected at each site from planting to harvest.

Table 5. A format for recording environmental data

Week	1	2	3	4	5
Rainfall					
Highest temp. Lowest temp. Average temp.					
Highest humidity Lowest humidity Average humidity					
No. of days rain recorded No. of hours average humidity reached 90% or above					

Annex 2:

Technical Guidelines for IMTP Phase II: Fusarium Wilt Sites

Protocol for the Field Screening of *Musa* Germplasm for Reaction to Fusarium Wilt

1. A site should be selected that has a severe natural infestation of Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*). The site should be in an appropriate location bearing in mind convenience of access for day-to-day operations and reaction assessment. The site should be located where vandalism is unlikely to occur. Edaphic factors such as soil type and soil pH should be determined. The soil should also be analyzed for mineral content. The cropping history of the site should be documented.

2. If possible, inoculum levels of *Fusarium oxysporum* f.sp. *cubense* should be further increased by growing a crop of appropriate susceptible cultivars on the site, cutting-up diseased pseudostems/corms and plowing the debris into the soil.

3. The site should be deeply plowed and ripped before planting the trial to evenly distribute the inoculum. It should be tested for nematodes and some treatment may be necessary to reduce populations if severe infestations are found.

4. The test and reference plants will be supplied as tissue cultures. The deflasking and care of young plantlets is described in **Appendix A**. They should be allowed to grow in pots until the 6-9 broad-leaf stage (approx. 300 mm tall). Care must be taken to ensure plants do not become pot-bound.

5. Planting should occur in shallow furrows or basins. These should be filled with soil once the plant is established. For successful establishment, plants would need to be monitored to ensure soil in the root zone was moist. Ideally, an irrigation system should be installed. Fertilizer according to local requirements or based on the results of soil analysis should be added to the soil to boost growth in the early stages. Measures should be taken during establishment to prevent losses from weevil borers.

6. After establishment (2-3 months from planting), the normal management practices of the region should be adopted. These should be uniformly applied to the trial and recorded. Only the 'follower' sucker should be allowed to develop. Other suckers should be removed.

7. Plants should be planted on a 3 x 2 m grid. Twenty plants of each test accession and reference clone (see **Table 1**) should be planted randomly (see **Table 2**). Guard rows are not necessary in this type of trial.

8. Environmental data should be collected during the course of the trial (see **Table 3**).

Table 1: List of Clones to be Evaluated for Reaction to Fusarium Wilt

FHIA (Honduras)			
1. SH 3481 (AAAB)	FHIA-01	Prata Anã x SH 3142	ITC 0504
2. SH 3565 (AABB)	FHIA-03	SH 3386 x SH 3320	ITC 0506
3. SH 3649 (AAAA)	FHIA-17	Highgate x SH 3362	ITC 1264
4. SH 3444 (AAAA)	FHIA-23	Highgate x SH 3362	ITC 1265
CNPMF/EMBRAPA (Brazil)			
5. PV 03-44 (AAAB)	EMB 402	Pacovan x Calcutta 4	ITC 1262
6. PA 03-22 (AAAB)	EMB 404	Prata Anã x Calcutta 4	ITC 1261
TBRI (Taiwan)			
7. GCTCV 119 (AAA)		Giant Cavendish variant	ITC 1282
8. GCTCV 215 (AAA)		Giant Cavendish variant	ITC 1271
INIVIT/INIFAT (Cuba)			
9. Burro CEMSA (ABB)		Bluggoe (res. *Foc* race 2)	ITC 1259
Natural germplasm			
10. Pisang Mas (AA)			ITC 0653
11. Saba (BBB/ABB)			ITC 1138
12. Pisang Nangka (AAB)			ITC 1062
13. Cultivar Rose (AA)		IRFA 907/IDN 110	ITC 0712
14. Yangambi Km5 (AAA)			ITC 1123
15. Pisang Jari Buaya (AA)			ITC 0312
16. Pisang Lilin (AA)			ITC 0001
17. Calcutta IR 124 (AAw) *Musa acuminata* ssp. *burmannicoides*			ITC 0249
Reference/standard clones			
18. Gros Michel (AAA)		(Race 1 suscept.)	ITC 1122
19. Bluggoe (ABB)		(Race 2 suscept.)	ITC 0643
20. Williams (AAA)	Cavendish	(Race 4 suscept.)	ITC 0570
21. Pisang Ceylan (AAB)	Mysore (adult plant resistance)		ITC 0650
22. Local cultivar	To be selected by officer-in-charge of each test site as an appropriate local standard		

Table 2. Randomization of 22 test accessions with 20 replications.

1	7	3	11	2	14	9	17	21	5	20	12	1	19	16	18	8	15	4	13	10	6
5	11	8	12	15	16	7	3	2	13	18	9	6	20	4	21	10	22	19	17	1	14
7	1	12	8	5	4	6	15	13	1	11	16	3	10	17	19	2	20	21	18	14	9
4	11	6	13	14	3	15	8	5	10	17	18	19	12	7	20	22	22	9	21	16	2
6	7	10	1	15	9	16	17	20	19	2	22	8	5	14	13	3	12	21	18	11	4
2	8	12	6	5	13	17	1	18	21	3	7	20	10	15	4	11	16	22	19	14	9
3	7	12	9	13	2	11	6	14	15	8	18	5	1	20	22	21	19	10	4	17	16
7	10	11	5	14	15	16	3	12	17	4	6	13	9	19	21	22	22	2	20	18	8
4	8	13	14	10	1	17	9	18	11	2	19	21	6	3	20	7	16	15	5	12	1
5	9	11	13	2	7	16	10	6	17	8	14	3	18	19	12	4	15	1	21	22	20
8	2	10	12	6	14	1	15	3	7	4	13	16	9	18	5	20	22	21	19	17	11
3	12	6	14	5	15	13	9	2	17	11	18	20	1	21	22	19	10	4	7	16	8
4	11	8	13	16	17	6	18	3	15	7	19	5	20	22	21	14	1	12	9	10	2
5	12	7	3	14	2	15	9	18	16	11	20	1	10	21	6	13	22	8	19	17	4
6	1	9	11	13	7	3	10	14	12	4	5	2	8	15	17	19	20	22	21	16	18
4	7	10	3	8	15	16	17	18	2	6	19	20	5	21	22	9	14	1	13	12	11
7	9	13	5	10	17	4	18	3	19	11	15	8	20	22	12	21	2	22	16	14	6
8	10	12	1	16	2	6	19	5	20	21	9	13	3	11	7	4	14	15	22	18	17
2	6	11	13	14	5	15	10	17	8	20	22	21	19	12	1	7	18	16	4	9	3
15	9	12	7	13	8	16	18	20	1	6	19	22	21	4	11	17	15	10	14	5	2

See Appendix C for names of accessions corresponding to numbers 1-22.
Trial size = 42 x 57 m = 2394 m^2 = 0.24 ha.

Table 3. Format for collecting environmental data at each site from planting to harvest.

Week	1	2	3	4	5
Rainfall					
Highest temp. Lowest temp. Average temp.					
No. of days rain recorded					

9. Disease severity (external symptoms) should be noted for all plants every month from 3 months after planting until harvest, using the following system.

Yellowing of foliage	(1)	no yellowing
	(2)	slight yellowing
	(3)	extensive yellowing
Splitting of pseudostem base	(1)	no splitting
	(2)	mild splitting
	(3)	severe splitting
Vascular discoloration in leaf bases (as revealed after outer leaf sheaths are pulled down)	(1)	no discoloration
	(2)	mild discoloration
	(3)	intense discoloration
Changes in new leaves (irregular pale margins, narrowing, burning plus ripping of lamina and becoming more erect)	(1)	symptoms absent
	(2)	symptoms present
Shortened internodes (which may be difficult to detect in dwarfs and semidwarfs)	(1)	no shortening
	(2)	some shortening
	(3)	very obvious shortening
Wilting	(1)	no wilting
	(2)	some wilting
	(3)	severe wilting
Petiole buckling	(1)	no buckling
	(2)	some buckling
	(3)	severe buckling

Monthly readings from 3 months after planting until harvest will give the rate of disease development. External symptoms can develop between flowering and harvest.

10. Agronomic characters, such as plant height, bunch weight, and number of hands, etc. (as outlined in **Table 4**) should also be recorded for all plants at harvest.

11. A reading of internal symptoms should also be taken on all plants at harvest. Internal symptoms will be determined by slicing off the lower portion of the corm horizontally until the transverse cut is about 1/4 of the way up the corm. The cut area should then be examined for discoloration caused by Fusarium wilt using the following scale (see **Plates 1-6**):

1. Corm completely clean, no vascular discoloration
2. Isolated points of discoloration in vascular tissue
3. Discoloration of up to 1/3 of vascular tissue
4. Discoloration of between 1/3 and 2/3 of vascular tissue

Table 4. Format for collecting agronomic data.*

Planting date:.....
Bunch emergence (shooting) date:.....
Plant height at bunch emergence[1]:.....
No. of days from planting to shooting:.....
Harvest date:.....
No. of days from planting to harvest:.....
Height of 'follower' sucker at harvest[2]:.....
Weight of bunch at harvest[3]:.....
Number of hands in bunch[4]:.....
Number of fingers in bunch[5]:.....
Average weight of each finger[6]:.....

* Key agronomic data suggested by Drs D Vuylsteke and R Ortiz, IITA.

[1]This is the distance in meters from the ground to the angle made between the bunch stalk and bunch cover leaf.

[2]This is the distance in meters from the ground to the junction between the youngest and next youngest leaf of the 'follower' sucker at the time the bunch is harvested from the mother plant.

[3]This is the weight in kilograms of the bunch immediately following harvest. (At harvest, cut the bunch stalk [peduncle] **above** the first hand at the scar of the last bract and immediately **below** the last hand.)

[4]Cut the hands from the bunch and record the number of hands.

[5]Record the number of individual fruit in all of the cut hands.

[6]Divide the collective weight of the hands by the number of fingers in the hands.

5. Discoloration greater than 2/3 of vascular tissue

6. Total discoloration of vascular tissue and/or discoloration of leaf bases. A vertical cut through the discolored areas of the corm will reveal if the discoloration extends to the leaf bases.

12. Samples of infected pseudostem or corm tissue from all diseased plants should be prepared as in Figure 1 and sent to a recognized laboratory (see below) for analysis. If all 20 plants of an accession become infected, samples should be taken from 4-5 randomly selected plants for analysis. If only a few plants of an accession become infected, samples from all diseased plants should be sent for analysis. *Fusarium oxysporum* f.sp. *cubense* should be isolated from affected tissue on arrival at the laboratory. Isolates should be characterized using VCG analysis to give an indication of the pathogen population. Ideally, isolates should be further characterized using RAPD-PCR techniques.

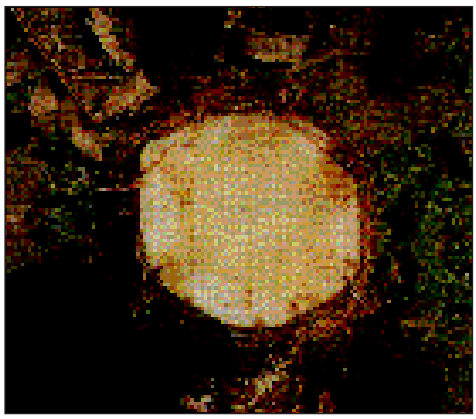
Collaborators for analysis of *Fusarium oxysporum* f.sp. *cubense* isolates are:

Asia/Pacific Region:
Mr Ken G Pegg
Plant Protection Unit
Department of Primary
Industries, 80 Meiers Road
Indooroopilly Q4068, Australia
(Fax: +61 7 371 0866)

Americas/African Region:
Dr Randy C Ploetz
IFAS-TREC, University of Florida
18905 SW 280th Street
Homestead, Florida 33031,
USA
(Fax: +1 305 246 7003)

Plates 1-6: Scale for rating the internal corm symptoms caused by Fusarium wilt (photographs courtesy of Mr Z Cordeiro, EMBRAPA-CNPMF).

1. Corm completely clean, no vascular discoloration.



2. Isolated points of discoloration in vascular tissue.

3. Discoloration of up to 1/3 of vascular tissue.

4. Discoloration of between 1/3 and 2/3 of vascular tissue.

5. Discoloration greater than 2/3 of vascular tissue.

6. Total discoloration of vascular tissue.

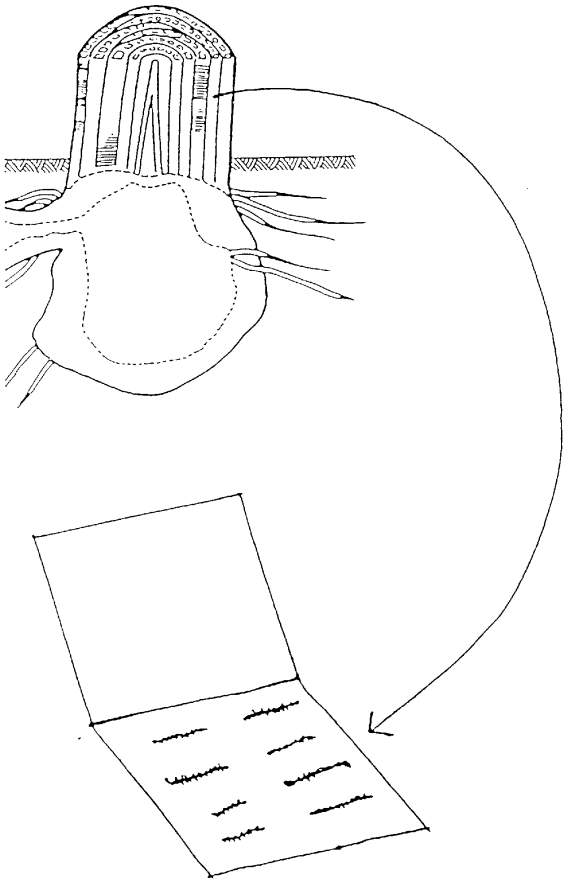


Figure 1. Steps in the preparation of *Fusarium oxysporum* f. sp. *cubense* (courtesy of K. Pegg, QDPI)

Appendix A: Handling of In-Vitro Rooted Plantlets

In-vitro rooted plantlets are distributed as sterile cultures, in watertight Cultu saks®.

Plantlets that are 5-10 cm tall and have well-developed roots are ready for planting in pots. If plantlets are smaller or if transplanting is not immediately possible, it is advisable to place the plantlets in the Cultu saks® in an upright position under sufficient light (not direct sunlight), at a temperature between 20 and 30°C. Under these conditions plantlets can be kept for a few weeks.

To transplant rooted plantlets to soil requires some care. Please proceed as follows, or use your own proven method.

– Cut open each Cultu saks® chamber at one vertical edge.

– Carefully remove the plantlet from the Cultu saks® by holding the base gently with blunt-end forceps. Place the plantlet on your palm.

– Remove culture medium adhering to the roots and leaves by placing the plantlet in a container of water (bucket) and shaking gently. Do not damage the stem nor the root system.

– Transplant the plantlet to plastic pots or bags (15 cm diameter) filled with a 30:70 peat sand mix of which the upper 2 to 3 cm are fine (sifted). The upper roots should be covered by 2-3 cm soil.

– After transplanting, water the plantlets immediately.

– Keep the plants under high humidity. A simple humidity chamber can be constructed by enclosing a wooden frame in strong transparent plastic. The humidity chamber (about 40-60 cm height) is placed over the pots in a shaded area where temperature is kept at 25-32°C. The humidity inside the chamber is maintained by spraying water regularly to saturate the air. The heat building up inside the chamber is minimized by leaving a 2-3 cm opening at the base to allow air to circulate.

– During the first week after transplanting, mist the humidity chamber twice a day to saturate the atmosphere. This is very important because low relative humidity at this stage will easily destroy the plantlets. Water the plants once a day with tap water.

– One week after transplanting, spray the humidity chamber and the plants only once a day.

– One month after transplanting, remove the plants from the humidity chamber.

– Keep the plants in a nursery under shade until about 30 cm tall (2-3 months) before establishment in the field.

– Transplant the plants into the field during the wet season, but not later than 6 weeks before the onset of the dry season.

(Protocol provided by Ir I Van den houwe, ITC)

Annex 3:

Acronyms and Abbreviations

AARD	Agency for Agricultural Research and Development (Indonesia)
A&RD	Agricultural and Rural Development Division
ACIAR	Australian Centre for International Agricultural Research
AFF	apparent female fertility
AIDAB	Australian International Development Assistance Bureau
AMYT	advanced *Musa* yield trial
APAU	Andhra Pradesh Agricultural University (India)
ARS	Agricultural Research Service (USDA)
ASPNET	Asian and Pacific Network (INIBAP)
BANBOARD	Banana Board of Jamaica
BA	N6-benzylaminopurine
BBMV	banana bract mosaic virus
BBTV	banana bunchy top virus
BGASA	Banana Growers' Association of South Africa
BLS	black leaf streak
BPI	Bureau of Plant Industry (Philippines)
BPIU	Banana Plant Improvement Unit (South Africa)
BS	black Sigatoka
BSV	banana streak virus
CARDI	Caribbean Agricultural Research and Development Institute
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza (Costa Rica)
cDNA	complementary DNA
CEMSA	Centro de Mejoramiento de Semillas Aganicus (Cuba)
CENIAP	Centro Nacional de Investigaciones Agropecuarias (FONIAP, Venezuela)
CIAM	Centre d'introduction, d'adaptation et de multiplication de matériel végétal, vivrier, fruitier et maraicher
CICY	Centro de Investigaciones Científicas de yucatán (Mexico)
CIRAD	Centre de coopération internationale en recherche agronomique pour le développement (France)
CITA	Centro de Investigación y Tecnología Agraria (Canary Islands, Spain)
CGIAR	Consultative Group on International Agricultural Research

CMV	cucumber mosaic virus
CNPMF	Centro Nacional de Pesquisa de Mandioca e Fruticultura (EMBRAPA)
^{60}Co	cobalt 60
CONICIT	Consejo Nacional de Ciencia y Tecnología (Costa Rica)
CORBANA	Corporación Bananera Nacional (Costa Rica)
CORPOICA	Corporación Colombiana de Investigación Agropecuaria
CRBP	Centre régional bananiers et plantains (Cameroon)
CRIH	Central Research Institute for Horticulture (AARD)
CSIR	Council for Scientific and Industrial Research (Ghana)
CTA	Technical Centre for Agricultural and Rural Co-operation (The Netherlands)
cv	cultivar
CV	coefficient of variability
DDT	disease development time
DERIL	Développement rural intégré dans la province de Labé (Guinea)
DES	diethyl sulphate
DFF	definitive female fertility (CRBP) or days to fruit filling (IITA)
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
EK	electrophoretic karyotype
ELISA	enzyme-linked immunosorbent assay
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EMS	ethylmethane sulphonate
ER	extremely resistant
ESARC	East and Southern Africa Regional Center (IITA)
ET	evolution time
EU	European Union
F	number of fingers
FAO	Food and Agriculture Organization (UN)
FC	fruit circumference
FDA	Fondación de Desarrollo Agropecuario (Dominican Republic)
FHIA	Fundación Hondureña de Investigación Agrícola (Honduras)
FL	fruit length
FLHOR	Département des productions fruitières et horticoles (CIRAD)
Foc	*Fusarium oxysporum* f.sp. *cubense*
FONIAP	Fondo Nacional de Investigaciones Agropecuarias (Venezuela)
FUNDAGRO	Fundación para el Desarrollo Agropecuario (Ecuador)
FUSAGRI	Fundación Servicios para el Agricultor (Venezuela)
FW	fruit weight

GCTCV	Giant Cavendish tissue culture variant
H	number of hands
HR	highly resistant, high resistance
HRI	Horticultural Research Institute (Thailand)
HS	highly susceptible
HTSh	height of tallest sucker at harvest
IAA	indole-3-acetic acid
IAEA	International Atomic Energy Agency
IARC	international agricultural research center
IBA	indole butyric acid
IBP	Instituto de Biotecnología de las Plantas (Colombia)
IBPGR	International Board for Plant Genetic Resources (now IPGRI)
IBTA	Instituto Boliviano de Tecnología Agropecuaria (PROINPA)
ICA	Instituto Colombiano Agropecuario
ICAR	Indian Council of Agricultural Research3
ICIPE	International Center for Insect Physiology and Ecology
IDEFOR	Institut des forêts (Côte d'Ivoire)
IDRC	International Development Research Centre (Canada)
IFF	intermediate female fertility
IIHR	Indian Institute of Horticultural Research
IITA	International Institute of Tropical Agriculture
ILS	percentage of leaves spotted
IMTP	International *Musa* Testing Program
INIAP	Instituto Nacional de Investigaciones Agropecuarias (Ecuador)
INIBAP	International Network for the Improvement of Banana and Plantain
INIFAT	Instituto Nacional de Investigación Fundamental en Agricultura Tropical (Cuba)
INISAV	Instituto de Investigaciones de Sanidad Vegetal (Cuba)
INIVIT	Instituto Nacional de Investigaciones en Viandas Tropicales (Cuba)
INSA	National Institute of Agricultural Science (Viet Nam)
IPGRI	International Plant Genetic Resources Institute
IRAZ	Institut de recherches agronomique et zootechnique de la Communauté economique des pays des grands lacs (Burundi)
ISEM	immunosorbent electron microscopy
IRENA	Instituto Nicaragüense de Recursos Naturales y del Ambiente
IRFA	Institut de recherche sur les fruits et agrumes (now FLHOR)
IT	incubation time
ITC	INIBAP Transit Center
KARI	Kenyan Agricultural Research Institute
KAU	Kerala Agricultural University (India)

KUL	Katholieke Universiteit Leuven (Belgium)
LAC	Latin America and the Caribbean
LACNET	Latin America and Caribbean Network (INIBAP)
LD50	Lethal dose required to kill 50% of a population
LDR	lesion development reaction
LER	leaf emission rate
LS	less susceptible
MAAR	Ministry of Agriculture and Animal Resources (Côte d'Ivoire)
MAE	Ministère de l'agriculture et de l'élevage (Tahiti)
MAF	Ministry of Agriculture and Fisheries (Seychelles)
MAFF	Ministry of Agriculture, Fisheries and Forestry (Tonga)
MAFFM	Ministry of Agriculture, Forest and Fisheries and Meteorology (Western Samoa)
MALNR	Ministry of Agriculture, Livestock and Natural Resources (Zanzibar, Tanzania)
MARDI	Malaysian Agricultural Research and Development Institute
MET	multilocational evaluation trial
MGES	*Musa* Germplasm Exchange System
MGIS	*Musa* Germplasm Information System
MINAG	Ministry of Agriculture (Cuba)
MS	Murashige and Skoog (culture medium)
MTP	Medium Term Plan
NAOC	Nigeria Agip Oil Co. Ltd.
NARC	National Agricultural Research Center (Pakistan)
NARO	National Agricultural Research Organization (Uganda)
NARS	national agricultural research systems
NEP	national evaluation program
NIHORT	National Horticultural Research Institute (Nigeria)
NORAD	Norwegian Agency for Development and Cooperation
NPQS	Nigerian Plant Quarantine Service
NRC	National Research Center (for *Musa*, India)
NRCRI	National Root Crops Research Institute (Nigeria)
NRI	Natural Resources Institute (UK)
NSL	number of standing leaves
ODA	Overseas Development Administration (UK)
OPS	Office for Project Services (UNDP)
PBIP	Plantain and Banana Improvement Program (IITA)
PCR	polymerase chain reaction

PH	plant height
PNG	Papua New Guinea
PR	partial resistance
PROINPA	Proyecto de Investigación de la Papa (Bolivia)
PYT	preliminary yield trial
QDPI	Queensland Department of Primary Industries (Australia)
QTL	quantitative trait loci
QUT	Queensland University of Technology (Australia)
R	resistant
RACs	Regional Advisory Committees (INIBAP)
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
S	susceptible, susceptibility
SC	short course
SCAU	South China Agricultural University
ScBV	sugarcane bacilliform virus
SPC	South Pacific Commission
ssDNA	single-stranded DNA
syn.	synonym
TBRI	Taiwan Banana Research Institute
TC	tissue culture
TMPx	Tetraploid hybrid derived from plantain (IITA)
TMP2x	plantain-derived diploid (IITA)
TNAU	Tamil Nadu Agricultural University (India)
TND	Tanzania Natural Development Ltd.
UNDP	United Nations Development Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNPRB	Uganda National Banana Research Program
UPEB	Unión de Países Exportadores de Banano (Panama)
US	United States
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
VCG	vegetative compatibility group
VIC	Virus Indexing Center (INIBAP)
VNTR	variable number tandem repeat

w	wild species of *Musa*
WINBAN	Windward Islands Banana Growers' Association
YLS	youngest leaf spotted

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