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The **International Network for the Improvement of Banana and Plantain (INIBAP)** (Avenue du val Montferrand, B.P. 5035, 34032 Montpellier Cédex, France) was established in 1985 to foster additional research on these important food crops. Its headquarters are in Montpellier, France. Regional networks are being established in Western and Eastern Africa, Latin America and the Caribbean, and the Asia/Pacific region.

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# Banana and Plantain Breeding Strategies

**Proceedings of an International  
Workshop held at Cairns, Australia  
13-17 October 1986**

*Editors:* G.J. Persley and E.A. De Langhe

*Workshop Steering Committee:*

Edmund De Langhe, INIBAP (Chairman)

Brian Cull, QDPI

Ramiro Jaramillo, Costa Rica

Harry Stover, Honduras

Ramon Valmayor, Philippines

Trevor Williams, IBPGR

George Wilson, IITA

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## Foreword

Bananas and plantains (*Musa* spp.) are one of the few tropical crops which have not been bred successfully, and all presently cultivated varieties are natural selections. The spread of Black Sigatoka disease in Latin America and Africa over the past decade, and its severity on plantains which were previously unaffected by Yellow Sigatoka disease, has given added impetus to research programs concerned with breeding bananas and plantains for disease resistance. The creation of the International Network for the Improvement of Banana and Plantain (INIBAP) is a reflection of that impetus.

The purposes of this workshop were to identify the major constraints to banana and plantain production in different geographical regions, outline the desired targets of improvement programs for each of these regions, and describe research strategies which would lead to the successful breeding of bananas and plantains with the desired characteristics, particularly resistance to the major diseases.

The workshop was cosponsored by the International Network for the Improvement of Banana and Plantain (INIBAP), the Queensland Department of Primary Industries (QDPI), and the Australian Centre for International Agricultural Research (ACIAR).

The participants included scientists involved in banana and plantain improvement in West and Eastern Africa, Latin America and the Caribbean, Asia, Australia and the Pacific Islands. In addition, there were other key scientists involved in laboratory-based research on bananas and plantains.

The papers presented at the workshop represent an important record of the present status of research on bananas and plantains worldwide. The papers and the workshop recommendations will provide clear guidelines for future research directions, including genetic improvement for disease resistance and the possible adaptation of new technologies to these crops, especially in the areas of in vitro culture and pathogen detection. INIBAP, ACIAR and QDPI trust that the volume will provide a useful guide to the present status of banana and plantain cultivation worldwide, the problems facing growers of these important food and cash crops, and the research strategies required to overcome these problems. The communication of ideas, formulation of research targets, and coordination of research efforts are major tasks of INIBAP, and we believe this workshop made a substantial contribution to these goals.

ACIAR and INIBAP would like to thank Dr G. Alexander, Director-General of the Queensland Department of Primary Industries for enabling QDPI to host the workshop in Cairns. Dr G. Behncken QDPI Director of Horticulture and other staff of the Horticulture Branch, particularly Mr B. Watson, Mr G. Daniels, Mr P. O'Farrell and Mr B. Cull were most helpful in making local arrangements for the workshop and organising a field tour to the North Queensland banana-growing district, and we thank them for their efforts.

We are grateful to the Australian Development Assistance Bureau, the International Development Research Centre, the United States Agency for International Development, and the United States Department of Agriculture who also sponsored delegates to the workshop.

Ms Lucille Lurette, IDRC/INIBAP administrative officer, was most helpful in the organisation of the workshop and we thank her for her efforts.

We are also grateful to Mr Reg MacIntyre and Mr Paul Ferrar for their valuable work in the preparation of the Proceedings.

**J.R. McWilliam**  
*Director ACIAR*

**E. De Langhe**  
*Director INIBAP*

## Summary of Discussions and Recommendations

THIS workshop was sponsored by the Australian Centre for International Agricultural Research (ACIAR), the International Network for the Improvement of Banana and Plantain (INIBAP) and the Queensland Department of Primary Industries (QDPI).

The purposes of the workshop were to describe the targets for improvement programs in different geographical regions; review the state-of-the-art in the breeding of bananas and plantains; and identify research opportunities, with emphasis on breeding methodologies and disease control.

The workshop was attended by 40 scientists from 16 countries. They considered 28 papers which described regional plant improvement needs, current breeding programs, pests and diseases, tissue culture, germplasm collection, conservation and taxonomy.

The problems limiting the cultivation of bananas and plantains in Africa, Latin America and the Caribbean, Asia, Australia and the Pacific Islands were outlined. The current activities of the four major breeding programs in Honduras, Brazil, Guadeloupe and Jamaica were also described, together with their future plans for the improvement of edible *Musa*. The prospects of using some of the new techniques of genetic engineering of plants to incorporate disease resistance genes into *Musa* spp. were discussed.

The status of diseases and pests on bananas and plantains was assessed. The most important change in the past decade has been the appearance and spread of more virulent forms of Sigatoka leaf spot (Black Sigatoka/Black Leaf Streak) in Latin America and Africa. The new forms are not only more virulent on their traditional banana hosts, but they also attack plantains, which are not attacked by Yellow Sigatoka, the previously dominant form of Sigatoka disease. Black Sigatoka has replaced Yellow Sigatoka wherever it has spread.

The second important change has been the appearance of race(s) of the *Fusarium* wilt pathogen on Cavendish bananas, which were previously considered to be resistant. This change has been reported from widely separate locations in the subtropics (Australia, South Africa and Taiwan) and from one location in the tropics (Mindanao Island in the southern Philippines).

Movement of bananas and plantains is restrained by the need to avoid the inadvertent introduction of Bunchy-Top disease into areas where it does not occur (such as Latin America). There is at present no reliable method for the indexing of material to certify it as being free of the bunchy-top pathogen. The few laboratories working on this subject are using different approaches to develop a reliable indexing method, and the relative merits of these were discussed.

Tissue culture techniques are used commercially for the rapid propagation of banana clonal material for cultivation in several countries, as well as experimentally for generating variation. Somaclonal variation has been studied extensively in Taiwan and the Philippines as a technique for generating Cavendish types with resistance to Race 4 of the *Fusarium* wilt pathogen.

The taxonomy of *Musa* is still a source of some confusion. Detailed taxonomic studies have been conducted on *Musa* in Thailand, and the Philippines and West and Eastern Africa. As well as classical morphological taxonomy, more modern techniques involving chemotaxonomy may clarify the taxonomic position of various *Musa* types.

The substantive issues raised in the discussion and the major research opportunities

identified are summarised below, in the four areas of: 1) Regional Needs; 2) Breeding Strategies; 3) Diseases and Pests; and 4) Germplasm.

### Regional Needs

All plant improvement programs have the overall goal of increased productivity. The specific *Musa* improvement targets, however, are all oriented towards disease and/or pest resistance and may dominate the breeding program. These targets differ from one region to another and are listed below.

Latin America/ Caribbean	West Africa	Eastern Africa	Asia*	Oceania**
<i>Production Problems</i>				
Nematodes	Sigatoka	Weevil borer	Agronomic characters	Sigatoka
Sigatoka Moko	Nematodes Agronomic characters	Nematodes Bunchy-top	Sigatoka Bunchy-top	<i>Fusarium</i> Nematodes
<i>Fusarium</i>	Weevil borer Bunchy-top	<i>Fusarium</i> Salt tolerance	<i>Fusarium</i> Nematodes	Weevil borer Cold tolerance Bunchy-top
<i>Market</i>				
Export Domestic	Domestic Export	Domestic Export	Domestic Export	Domestic Export
<i>Fruit type</i>				
Dessert Plantain	Plantain Dessert	Cooking Beer Dessert	Cooking Dessert Plantain	Dessert Cooking

\* Information from India and China not included.

\*\* Australia, Pacific Islands.

### Breeding Strategies

#### Breeding Targets

The main targets in the breeding of bananas and plantains are resistance to the following diseases and pests: (1) The Sigatoka complex (*Mycosphaerella* spp.); (2) Races of the *Fusarium* wilt pathogen (*Fusarium oxysporum* f.sp *cubense*); (3) Nematodes; (4) Weevil borers; (5) Moko disease; and (6) Bunchy-top.

The relative importance of these targets varies amongst geographic regions. It also depends upon which type of bananas (or plantains) is most widely cultivated in an area. The priority breeding targets by cultivar group are:

	Export dessert bananas	Local bananas (Silk/Pome)	Plantains	Highland triploid bananas	Cooking bananas (ABB/BBB)
Sigatoka	***	**	***	*	*
<i>Fusarium</i>	***	***	*	*	*
Nematodes	***	***	**	***	*
Weevil borer	*	*	***	***	*
Moko	*	*	*	*	*
Bunchy-top	*	*	*	*	*

Priority: \*\*\* high; \*\* medium; \* low

Occurrence of *Fusarium* spp. on Highland bananas in Eastern Africa is suspected but not confirmed.

#### Breeding Methodologies

The consensus of the meeting was that there was a need to strengthen and diversify the breeding of bananas and plantains. The following approaches were recommended:

- (1) Continued development of superior tetraploid plants;

- (2) Development of improved diploid and tetraploid plants utilising genetic recombination (including the B genomes) by either intercrossing or self-pollination;
- (3) Creation of triploid plants (for example, by crossing superior diploid and tetraploid plants; making diploid crosses; or by using the technique of colchicine doubling to create material for diploid × tetraploid crosses for the production of triploid progeny);
- (4) Utilisation of the techniques of in vitro culture to supplement the conventional breeding programs.

### **Supporting Studies for Breeding Methodologies**

The above approaches to the breeding of bananas and plantains need to be complemented by other research activities in order to provide basic information to underpin the breeding programs. The major activities recommended are:

- (1) Expansion of the gene pool collections;
- (2) Basic biological studies on host/pathogen interactions as a prerequisite for genetic manipulation techniques and for improved disease screening;
- (3) Improved tissue culture techniques to facilitate the manipulation of the banana plant for utilisation in micropropagation;
- (4) Mutation breeding to provide useful genetic variation;
- (5) Basic molecular biology of *Musa* spp. to develop the knowledge required for the application of biotechnology.
- (6) Understanding the sexual cycle in banana to clarify the mechanisms of male and female sterility, pollen potency and in vitro fertilisation techniques;
- (7) Evaluation of B-genomic material and *Musa schizocarpa*;
- (8) Further studies to identify parameters of productivity and harvest index such as dwarfism; and
- (9) Comparative studies of yield potential of ploidy levels with similar genomic composition.

### **Banana Micropropagation**

#### CLONAL MULTIPLICATION

Banana micropropagation is important for the rapid multiplication of elite, disease-free material for field planting, for the dissemination of material around the world, and for germplasm maintenance.

One of the most serious problems in using this technique is the incidence of 'off-types' in micropropagated material. This incidence appears to vary considerably between different cultivars and between different laboratories where culture media and techniques differ. Reports at the meeting ranged from 3 to 25% off-types being produced in material being micropropagated for clonal fidelity.

The problem of off-types needs to be considered carefully. There are obvious advantages in the use of micropropagation techniques, but until the incidence of off-types (somaclonal variants) can be regulated, the technique will continue to draw criticism. The production of dwarf off-types, particularly, needs attention. A system for early detection is urgently needed. Many of the obvious leaf variants, etc. can be rogued at the culture or nursery level. Biochemical markers (fingerprints) may need development.

Banana bunchy-top also poses a threat to dissemination of banana cultures as there is evidence that it can be transmitted during successive subcultures in an almost symptom-free condition. A BTYV detection/screening technique able to be used with in vitro material is urgently needed.

#### SOMACLONAL VARIATION

Banana micropropagation is also being used to generate somaclonal variants for use in disease screening schemes. In Taiwan this technique has been used alone and in conjunction with in vitro mutation breeding to generate material for screening for disease resistance to *Fusarium* wilt. This approach should also be feasible in screening

for other diseases and pests as well as in various agronomic features. Mutation breeding would apparently increase the frequency of producing variants for screening.

The theory behind these breeding approaches is that one of a few genes can be altered while maintaining desirable agronomic traits. This is not true of conventional breeding approaches. In practice, however, some undesirable agronomic features are produced, and the variants need to be screened for yield and other agronomic features as well as disease resistance. The trait must also be stable and 'fixed' into the genome so it will not be lost in subsequent generations. An extensive somaclonal variation program is being conducted in Taiwan, to search for resistance to Race 4 of the *Fusarium* wilt pathogen.

In order for micropropagation techniques to be useful, there is a need to develop suitable glasshouse and if possible in vitro screening techniques that can be correlated with field tolerance or resistance. Field testing and evaluation will always be necessary for the final assessment. Susceptible clones/variants/mutants should be eliminated in the early stages and field work can concentrate on the more promising material. It would be a great advantage in the utilisation of micropropagation techniques in breeding to understand more fully the pathogen/host interactions with regard to infection and susceptibility/resistance reactions.

#### BANANA CELL AND CALLUS CULTURE

Rapid progress is being made in the genetic engineering of several plant species. Potentially useful genes such as herbicide tolerance can be inserted in suitable vectors and transferred into a limited number of plant species. Important genes for banana improvement will be incorporated via plant tissue culture, unless other vector systems become available.

In order to be able to fully utilise the products of genetic engineering for *Musa* spp. it is important to support continued research into the development of techniques for the reliable regeneration of large numbers of plants from cell callus cultures, and in the culture of protoplasts.

#### OTHER ALTERNATIVE BREEDING TECHNIQUES

Breeders and pathologists have been using embryo culture techniques already in their improvement programs. Developing a suitable colchicine treatment for bananas in vitro may facilitate the production of tetraploids from improved diploids for triploid breeding programs.

Anther pollen culture will be heavily dependent on developing suitable regeneration techniques from cell and callus cultures. If progress is made there, haploids can then be produced and doubled for the synthesis of homozygous breeding lines. Techniques such as this will find importance in *Musa* breeding programs in the future.

### **Germplasm Distribution and Evaluation**

The following evaluations of banana and plantain clones are recommended:

- (1) Multisite evaluation of diploids for yields, agronomic characteristics and disease resistance;
- (2) Evaluation of available triploid and tetraploid germplasm in different locations for yield, agronomic characteristics and disease resistance; and
- (3) Evaluation of diploids in representative locations to assess host/pathogen interactions in different environments.

### **International Cooperation**

The proprietary rights of clonally propagated crops such as bananas and plantains cannot be adequately protected. Therefore it is recommended that banana and plantain breeding programs should receive additional public (both governmental and international agency) funds for research, particularly to assist the welfare of small producers for whom these are important food and cash crops.

If breeding programs in different countries are willing to contribute selected material available for distribution and evaluation in various countries, the material

should be suitably named so as to be easily identified with the breeding program from where it originated (as is the case with sugarcane varieties distributed worldwide).

There is a need for continued and expanded information exchange amongst banana workers. It is recommended that INIBAP encourage the dissemination of information about banana/plantain research, in association with other interested organisations.

### **Diseases and Pests**

Susceptibility of many cultivars to a few extremely serious diseases and pests, and the continuing spread of 'new' diseases into areas not previously infected, are the major factors affecting yield and production costs in bananas and plantains worldwide. The major diseases and pests are:

- (1) Black Sigatoka and Black Leaf Streak, caused by *Mycosphaerella fijiensis*;
- (2) *Fusarium* wilt caused by various races of *Fusarium oxysporum* f.sp. *cubense*;
- (3) Root and rhizome rot caused by the nematode *Radopholus similis*, with a borer (*Cosmopolites*) also implicated in certain areas;
- (4) Bunchy-top, probably caused by a virus; and
- (5) Moko disease, caused by *Pseudomonas solanacearum*.

Priority research areas for diseases and pests of bananas and plantains are:

#### *Sigatoka diseases*

- (1) Sources of resistance and genetics;
- (2) Host/pathogen interactions in different environments;
- (3) Screening procedures (in vitro, and for seedlings);
- (4) Clarification of relationships amongst pathogens (causing Black Sigatoka, Black Leaf Streak, Yellow Sigatoka).

#### *Fusarium wilt*

- (1) Breeding for resistance to Race 4;
- (2) Genetics of resistance;
- (3) Screening procedures (in vitro and for seedlings);
- (4) Race clarification;
- (5) Differential varieties for clarification of races and their distribution.

#### *Nematodes*

- (1) Breeding and genetics;
- (2) Screening procedures;
- (3) Chemical control;
- (4) Pathogenesis (including associations between nematodes, borers and fungi);
- (5) Role in plantain decline.

#### *Bunchy-Top disease*

- (1) Rapid detection procedures;
- (2) Characterisation of pathogen;
- (3) Host range;
- (4) Resistance.

#### *Moko disease*

- (1) Sources of resistance.

The substantive issues raised in the discussion and the recommendations for future research are outlined below.

#### *Sigatoka Diseases*

Black Sigatoka (*Mycosphaerella fijiensis*) is thought to have originated in the area of Papua New Guinea and the Solomon Islands. The presence of many cultivars in this area with resistance to Black Sigatoka is an indication of the coevolution of the host and pathogen in this area. It would be a fruitful location for research on the genetics of the host and the pathogen.

The areas requiring further research are:

- (1) *Breeding and Genetics*. Sources of resistance to *Mycosphaerella musicola* (Yellow Sigatoka) and *Mycosphaerella fijiensis* (Black Sigatoka/Black Leaf

Streak) have been identified. This resistance now has to be inserted into varieties with other desirable agronomic characters. Research is also required on how resistance to the Sigatoka pathogens is inherited. Multigenic resistance would be preferable to single gene resistance with its inherent dangers of breakdown.

- (2) *Host/pathogen interactions* in different environments also need to be studied, using a set of differential varieties, evaluated according to standard rating procedures.
- (3) *Disease screening procedures*: Specific screening procedures need to be developed for rapid mass screening of seedlings and plantlets produced in vitro. A set of standard varieties is also required to give relative ratings over time.
- (4) *Clarification of relationships amongst the pathogens*: The three *Mycosphaerellae* pathogens (*M. musicola*, *M. fijiensis* and *M. fijiensis* var. *difformis*) appear to be closely related. A method of producing perithecia in a closed system is needed to determine interrelationships and possible development of new 'races.' Biochemical procedures should also be tested for determining the affinity of different isolates.

The information on the relationships between the three pathogens is important for breeding programs, since it would simplify the breeding targets if resistance to Yellow Sigatoka also inferred resistance to Black Sigatoka (and Black Leaf Streak, if it is a different disease).

#### *Fusarium Wilt*

The areas requiring further research are primarily concerned with emergence of a new race (Race 4) of *Fusarium oxysporum* f. sp. *cubense*, able to attack Cavendish bananas. Race 4 presently occurs mainly at higher latitudes, which are at the margins of *Fusarium* wilt distribution. Race 4 is thus presently more serious in cooler areas such as in Taiwan and southern Queensland, Australia.

What is the origin of the Race 4 populations? What is the capacity of the *Fusarium* wilt organism to change? The answers to these questions may lie in the centre of coevolution of the host and pathogen in Asia.

The areas requiring further research are:

- (1) *Breeding for resistance*: It appears that the new edible banana clones bred to date do not have resistance to Race 4, which is threatening Cavendish variety production in Taiwan, Australia, and South Africa. A few diploids appear to be resistant but a wider range of resistant diploids is needed.
- (2) *Genetics of resistance*: This needs to be investigated and methods found for incorporating resistant genes into new varieties that could replace Cavendish clones.
- (3) *Disease screening procedures*: Several screening procedures are in use but more refinements are needed, especially for screening plants mass-produced in vitro. Test conditions such as temperature and inoculum density need to be standardised so that results from different locations are comparable.
- (4) *Race clarification*: At present, three banana races have been described but their identity and distribution are not clear. It appears that Race 4 isolates from various geographical areas may differ slightly. A collection of races and clones needs to be assembled at one site for analysis by cultural, biochemical and bioassay procedures.
- (5) *Differential varieties*: The identification of differential varieties by genetic studies and resistance inheritance is needed to understand the behaviour and stability (or non-stability) of races of *Fusarium oxysporum* f.sp. *cubense*.

#### *Nematode/Borer Complex*

The nematode *Radopholus similis* is most destructive on some triploid (AAA) varieties. The importance of other nematodes such as *Pratylenchus* spp. needs to be determined. There is an indication that some clones resistant to *Radopholus* spp. are susceptible to *Pratylenchus* spp. *Pratylenchus goodeyi* is spreading in Eastern Africa; *P. goodeyi* and *R. similis* both cause severe root damage on bananas in Tanzania; *P. goodeyi* needs further study before it becomes a more widespread problem in



Africa and elsewhere. There is presently only one major research program on nematodes in the world yet these are the most serious pests affecting bananas and plantains.

The areas requiring further research are:

- (1) *Breeding and genetics*: A narrow base of resistance to *R. similis* has been identified in one diploid group in the Honduran breeding program. However, some resistant diploids developed from this group are susceptible to *Pratylenchus* sp. Little is known about nematode resistance and susceptibility outside this group. There are no accurate reports of the responses of the bred tetraploids to *R. similis*. Nematodes are the most neglected pest or disease from the breeding standpoint.
- (2) *Screening procedures*: *Radopholus similis* reproduces rapidly in vitro. However, no work has been done for mass-screening of seedlings and plants produced in vitro. Screening procedures need to be developed. Also, biotypes and races of the nematode apparently exist but have not been identified with respect to *Musa* responses.
- (3) *Chemical control*: Chemicals used for controlling nematodes can pose a threat to the environment under certain conditions. A better understanding of chemical and non-chemical approaches (biological, genetic, and integrated control) is required.
- (4) *Pathogenesis*: Three pathogenic fungi (*Fusarium solani*, *F. moniliforme* and *Cylindrocarpon* sp.) are always associated with *R. similis* lesions. Rhizomes heavily infected by *Radopholus* are also invaded by the weevil borer *Cosmopolites* sp. in some areas. There have been no published studies on how these different organisms interact and influence resistance or tolerance. With respect to *Cosmopolites*, there are no controlled experimental data indicating population-damage thresholds.
- (5) *Plantain decline*: Nematodes are likely to be a major factor in the 'decline' problem on plantains but their specific role has yet to be clarified. Weevil borers may also be associated with the yield decline.

#### *Bunchy-Top*

Bunchy-top is a serious disease in peasant agriculture in Eastern Africa, Egypt, Asia and the Pacific Islands. A major concern is its possible spread into areas where it does not presently exist. Also, it is a restrictive factor in the international movement of germplasm in vitro, because of the danger of the inadvertent introduction of the disease into new areas (particularly into Latin America). Bunchy-top is thought to be caused by a virus, possibly a luteovirus.

The areas requiring further research are:

- (1) *Detection methods*: Since symptomless carriers are well-known for virus diseases, sensitive, reliable and rapid techniques for indexing and detection, using modern immunological and biochemical techniques, must be developed for bunchy-top.
- (2) *Purification and characterisation of the causal agent*: Some progress has been made in identifying bunchy-top as a luteovirus. Additional work is needed in chemical characterisation and to produce 'pure' material for developing sensitive indexing techniques.
- (3) *Host range, strains, and distribution*: Once detection procedures are defined, host range, strains, and distribution can be studied. The West African 'mild' strain needs study. There is a possibility of non-*Musa* hosts of *Pentalonia* serving as virus reservoirs.
- (4) *Host plant resistance*: Some germplasm has been screened for resistance to Bunchy-top, and this should be continued, especially in Asia.

#### *Moko disease*

Moko disease (*Pseudomonas solanacearum*) can be highly destructive. It is presently confined to the American tropics and Mindanao Island in the southern Philippines. An insect-transmitted strain is spreading in Latin America and the Caribbean. Sources of resistance are required.

## **Germplasm Exploration Conservation, Description and Exchange**

Access to the available germplasm of *Musa* spp. is critical to the success of efforts to improve cultivated bananas and plantains. The participants discussed: (1) what should be the targets for further exploration and collection; (2) the status of current collections; (3) the research needed on conservation methodology; (4) germplasm exchange, and (5) germplasm description.

### **Targets of Exploration**

Extensive collections have been undertaken in Thailand (over 300 accessions), the Philippines (over 80 accessions), Vietnam (approximately 170 accessions), and Papua New Guinea (over 100 accessions). There is also a collection in Malaysia.

The collections in Thailand and the Philippines have been characterised in detail. The report at the workshop on collecting bananas in Vietnam showed that bananas are an important and widely cultivated crop there, grown from the highlands to the Mekong Delta. Wide variations exist in *Musa* spp. in Indo-China.

The recommendations for future research are:

- (1) Further collections should be undertaken for the primary gene pool. This consists of *Musa acuminata*, *M. balbisiana* and other *Musa* spp. The areas in which these species are found are in Papua New Guinea, East Malaysia, Indonesia (Kalimantan), Burma and Indo-China;
- (2) For cultivars, further exploration needs are limited, except in Eastern Africa where exploration has only commenced recently. Some *Musa* types are unique to Eastern Africa. The Popoulu and Maia Maole varieties need to be collected more extensively;
- (3) Exploration should involve a systematic collection of both plant populations and their pathogens. The exploration should be followed by rapid screening (i.e. chemotaxonomic/numerical assessment) to identify specific areas for further detailed field work.

The International Board for Plant Genetic Resources (IBPGR) is actively involved in further exploration and collection of *Musa* spp., and its continued interest was welcomed by the workshop participants.

### **Collections of *Musa* spp.**

- (1) *Field gene banks*: Several IBPGR-designated field gene banks for *Musa* spp. exist. These are in the Philippines, Honduras, Jamaica and Cameroon. Efforts should continue to designate a field gene bank in India. A field gene bank is likely to be established by IRAZ in Eastern Africa. No other major collections need to be considered as IBPGR-designated field gene banks at this stage.
- (2) *Seed gene banks*: A seed gene bank should be established, possibly in Asia. It should be duplicated in two countries, for safety of the material.
- (3) *In vitro storage*: Given the difficulties of long-term storage of clonal material in the field, more effort should be made to store the germplasm in medium term *in vitro*, in suitable locations, using slow-growth techniques.

### **Conservation Methodology**

- (1) The maintenance of germplasm in a field gene bank is costly and often unreliable, especially if much of the material is not used regularly. *In vitro* storage is an attractive alternative. Due to a degree of instability of *in vitro* cultures of *Musa* spp., developmental work is needed on techniques for slow growth and the monitoring of stability *in vitro*.
- (2) Cryopreservation is the desirable long-term target. Research on this topic should be accelerated.

### **Description and Terminology**

- (1) *Description*: There is a need to characterise and evaluate different varieties. Additional characters to be considered are: palatability; biochemical characters (isozymes, flavonoids); *in vitro* characters, including stability, perennial

production (suckering); bunch cycling time; female fertility; pollen viability; gene identification and mapping.

- (2) **Terminology:** The lack of a universally accepted terminology for various types of bananas and plantains causes confusion. It is recommended that the terminology of *Musa* spp. should be clarified, including the production of the list of synonyms covering the generic terms used in different regions. There is also a need for a glossary.

#### **Germplasm Exchange**

- (1) Wild and primitive material should be readily and freely exchanged between countries. However, such exchanges must be safe. The major constraint to safe exchange is bunchy-top disease. Therefore, it is strongly recommended that the development of a reliable indexing method for bunchy-top be given priority.
- (2) Since many countries lack adequate quarantine services to import bananas and plantains and observe the material for visual symptoms of bunchy-top over several months it is recommended that an intermediate centre should screen material for exchange until a reliable indexing method for bunchy-top is developed. INIBAP will coordinate such germplasm exchanges if required.

#### **Conclusion**

Bananas and plantains are a much-neglected crop, given their importance as a basic food crop on three continents, and their contribution as a valuable export crop in many countries. The recent upsurge of Black Sigatoka disease on both bananas and plantains in Latin America and Africa has stimulated more interest in these crops, because of their importance to small producers as a basic food crop.

There are two major diseases, Black Sigatoka and *Fusarium* Wilt, which are costly to control. No new varieties have yet been bred which combine disease resistance with adequate yield and other suitable agronomic characters. Sources of resistance to the Sigatoka-pathogens and some *Fusarium* races are known in both cultivated varieties and bred diploids. The prospects for breeding new varieties with desirable disease resistance are excellent given sufficient support for breeding programs, and appropriate breeding strategies, supported by detailed pathological research.

Bananas and plantains are obviously crops which would benefit from sustained research effort, and the sharing of results from different parts of the world. It was the intention of the workshop to stimulate future cooperation amongst scientists working on bananas and plantains, and draw the attention of research managers and funding agencies to the strategic and applied research opportunities which exist on these important crops.

# Towards an International Strategy for Genetic Improvement in the Genus *Musa*

Edmond De Langhe \*

New ideas, approaches and techniques in breeding *Musa* have emerged recently, motivated by the growing conviction that genetic improvement of bananas and plantains is probably the only answer to the serious threat of diseases and pests in the entire banana- and plantain-growing tropical zone. However, banana breeding has long proved to be difficult, time-consuming and expensive. In the absence of a global strategy, the proliferation of limited programs and projects in various laboratories worldwide may eventually turn out to have been a waste of time and human energy.

The first specific objective of INIBAP is to initiate, encourage, support, conduct and coordinate research aimed at improving the production of bananas and plantains. INIBAP will take every opportunity to accomplish this task. This workshop is a unique occasion for the establishment of an internationally acceptable strategy in the breeding of bananas and plantains.

## Background

The genus *Musa* consists of two groups of plants: (1) the cultivars, which are clones maintained exclusively through vegetative propagation; and (2) the wild plants, which are diploid and which have been classified in species (and subspecies for *M. acuminata*). On the basis of results of many cross-fertilisations and of phenotypical studies, wild relatives of the cultivars were discovered. They have been grouped and described as the two species *Musa acuminata* Colla and *Musa balbisiana* Colla. A limited number of other wild plants, classified as distinct species, may have participated in the genesis of cultivars. (*Musa fehi* Bert. and the related special group of edible bananas, common in parts of the

Pacific, are not taken into consideration here (Dodds 1945).)

The cultivars are characterised by female sterility (from moderate to absolute, according to the cultivar) in combination with parthenocarpy (with quantitative intercultivar differences in its expression) and, for many cultivars, in combination with various levels of male sterility. Different forms of sterility exist and are not sufficiently understood.

The cultivars are either, in order of decreasing numerical importance, triploid, diploid or tetraploid. The triploid cultivars occur almost everywhere because they generally show, in addition to a convenient bunch, the most economical combination of 'bunch-vegetative parts.' The rare tetraploid cultivars are large with relatively small bunches, while most diploid cultivars are rather small with small bunches.

Consequently, breeding in *Musa* should aim at the creation of improved triploid plants (e.g. with resistance to a particular disease, in addition to the quantities of the required cultivar). Tetraploids can be considered as alternatives if triploids are not obtainable.

Sporogenesis in triploid cultivars is very rare and frequently complicated by sterility processes which already exist at the diploid level.

It follows therefore that none of the usual breeding schemes (pedigree selection, cross fertilisation, recurrent selection, population improvement, etc.) can routinely be applied to the triploid cultivars. This is in contrast to most other crops where cultivars (or landraces, populations, lines) can be crossed or selfed, and new combinations in the created germplasm progressively selected. Breeding schemes for genetic improvement of *Musa* cultivars have been, and will be, original, if not unique, complicated and expensive.

## Breeding History

The two most important *Musa* breeding programs in the world (Jamaica, Honduras) were based on the

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\* Director, International Network for the Improvement of Banana and Plantain, Avenue du val Montferrand, B.P. 5035, 34032 Montpellier Cédex, France.

principle of a single (primary) cross whereby: (a) the female parent (Gros Michel or Highgate) is a triploid cultivar with special qualities; (b) the donor is an improved diploid (e.g. with resistance to a banana disease and excellent bunch qualities) possessing a maximal degree of female sterility, but obviously keeping a fair amount of male fertility; (c) among the products, the tetraploids appear to approach best the qualities of the requiring parent, since it is still 'present' with its 33 chromosomes, due to restitution in the female meiosis.

Despite the absence, as yet, of a commercial product from these very expensive schemes, after so many years, it is wrong to consider them as failures, because: (a) the programs were based upon, and accompanied by, taxonomic and cytogenetic research, so considerable genetic knowledge has thus been built up; (b) a range of synthetic diploids with exceptional qualities has been created. Most of these diploids can be used in many programs. The probability for obtaining a valuable product is steadily increasing since new synthetic diploids still exceed former ones in value, accumulating, for example, resistance against several diseases.

Nonetheless, it is possible that these programs concentrated too much on an extremely narrow spectrum (two cultivars) of requiring parents. The programs were correctly focused on tetraploids as the required product. Only tetraploids can be valuable products of a scheme where it is of paramount importance to keep the quality combination of the requiring triploid parent.

Other attempts at breeding *Musa* have also been made elsewhere, but on such a small scale that consistent results are lacking (De Langhe 1969). The programs in Brazil and Guadeloupe are more recent and some time is needed for evaluation (Shepherd and Alves 1984).

Several lessons can be learned from past experiences in banana breeding: (a) the synthetic diploids should urgently be used for other requiring cultivars. A partial shift in this direction has been made since plantains are now included in the schemes; (b) other breeding schemes should be proposed, examined and tried whenever feasible; (c) the pool of existing cultivars (between 300 and 500) should be screened systematically. A brief look at the history of the worldwide spread of cultivars proves that they were probably detected in an incidental way. Hence, the strong feeling that the potential is far from being reached; (d) the spectrum of variability should be broadened considerably. Thousands of different phenotypes exist in many other crops. Modern techniques of genetic manipulation should be integrated.

### Other Facts and Premises

In search of new breeding techniques, one obvious approach would be the recreation of edible triploids. However, the genesis of the triploid cultivars is not understood. Hypotheses have been formulated and several pathways are perhaps possible, but many questions remain.

More fundamental, strategic research is needed to find answers to questions such as: did tetraploids play a key role in the formation of triploids or are they just side-products? What is the exact origin of the various forms of female sterility in edible diploids? What is the origin of the wide variation among the triploid cultivars in restitution during megasporogenesis, and does it play a role in diploids during triploidy-genesis? Is the complex of the two wild relative species well understood? Are the subspecies of *M. acuminata* more than ecotypes? What about homozygosity versus heterozygosity in these wild relatives? Could there be populations in some areas? Do ecoclines exist? Can diploid cultivars clearly be linked, as derivatives, with distinct wild relatives?

In his book *The Evolution of the Bananas*, Simmonds (1962) wrote: 'No direct studies of the breeding systems of the wild bananas have been made . . . Sound taxonomy can be based only on very extensive field work in this species.' Since then, practically nothing has happened.

The results of this research will influence deeply the *Musa* breeding programs. The proof is that progress in the genetics of *Musa*, realised at Trinidad and Jamaica during the 1940s and 1950s, provided the tools for the powerful breeding programs. Therefore, research on genetics should again embrace all presumed wild (or semi-wild) relatives as well as the cultivars and the new breeding products. This research should be carried out in close connection with, or even integrated into, breeding programs.

It is expected that almost all the new breeding schemes will abandon the principle of keeping the genetic integrity of the requiring female parent which was precisely the triploid cultivar till now (Shepherd and Alves 1984; Stover and Buddenhagen 1986). This move will have several consequences: (1) intermediary products with a moderate to fair seed fertility will be created and will open the way to a high genetic variability and thus to the progressive adoption of some conventional breeding techniques; (2) at some point in the scheme, but rather unavoidably near the end, the combination of female sterility and parthenocarpy-potential should be integrated in the progeny, since the final product, the fruits, must be edible just as those of the triploid cultivars; (3) given the many possible genetic combinations inherent in

this approach, various ranges of disease resistance/tolerance to particular diseases may appear, and safe screening techniques before release will be essential.

Abandoning the genetic integrity of the triploid female parent in new breeding schemes means in the genus *Musa*, in the first place, the radical modification in the genomic combinations AAA, AAB, ABB, and perhaps, if they exist, BBB.

The study of genomes has never been conducted. In discussion on the genesis of cultivars, distinction between the subspecies of *M. acuminata* as the original wild relative is rarely made. Yet it has been suggested that the edible AA-diploid 'sucrier' may be an inter-subspecific hybrid between the subspecies *malaccensis* and *burmanica* (Simmonds 1962). No attempt has been made to characterise a AAA-triploid by a more detailed genomic constitution, the assumption being that all three genomes come from the same subspecies. Hence the use in some classifications of the term derivatives (*Banksii* derivatives, *Errans* derivatives, etc.) (Valmayor et al. 1981).

Some subspecies or at least the holotypes are definitely characterised by their resistance to some diseases: *burmanica* is known for its high Black Sigatoka resistance. The presence of the subspecies as a genome could explain the resistance of triploid cultivars.

I suggest that taxonomists and geneticists should consider seriously the possibility of the simultaneous contribution of several subspecies to the genesis of cultivars under the form of genomic combinations, and this at all levels of ploidy. If this could be proved for some triploids, these could in the future be called for example, A1A1A2, A1A1A3, A1A1B (or even A1A2B!). Here lies a fascinating field of investigation for chemotaxonomists.

Attempts at characterising *M. acuminata* subspecies chemotaxonomically have been successful (Jarret 1986). This, together with phenotypical taxonomy and study of the genetic behaviour of the subspecies, could form a firm base for the genomic classification of cultivars.

Distinction between A-genomes (and perhaps B-genomes?) will become particularly relevant in breeding schemes based on hybridisation in various senses, especially in relation to disease (or pest) resistance.

Also, the distinction in edible AA-diploids between pure derivatives in interspecific hybrids may help to clarify the variation in the occasional female restitution at the diploid level.

Most new breeding schemes will produce a wide number of intermediary hybrids. The intermediary hybrids will differ between the programs, depending on the objective and thus on the parents used.

Various and sometimes unexpected combinations of fruit and bunch morphology, of flavours, of stooling habit, will no doubt be created.

This poses the major problem of the evaluation of the potential of newly created material. Since the programs can allow a moderate seed fertility on the provision that sterility can be recuperated later, each of the many possible crosses can be expected to produce a fair number of seedlings and one can easily predict that limits in both field surface and in labour will mean high pressures on the breeder. The temptation to eliminate all material that is not directly useful will be great.

It is suggested that evaluation of new material should be made possible on a worldwide scale, with exchange of in vitro germplasm as the major instrument, in view of a screening of the performance and the significance of intermediary breeding products under various ecological conditions.

Progress in chemotaxonomy of the basic components of the genus *Musa*, strengthened by knowledge coming from the multiple intermediary results of the breeding programs, will progressively pave the way for substantially improved breeding schemes, whereby the nature of the product will be better predicted and the mobilisation of land and labour better planned.

### In Vitro Techniques

The third International Conference held by the International Association for Research on Plantains and other Cooking Bananas (now called IARPCB: International Association for Research on Bananas and Plantains) came to the following resolution in genetic improvement. Laying the groundwork for the major goal of developing disease-resistant, high-yielding varieties specifically, the Conference resolved:

- to foster both conventional and in vitro breeding efforts by work in basic physiology, biochemistry, genetics, pathology and taxonomy;
- to exploit somaclonal variation extensively in the search for disease resistance and other useful characters;
- to expand the selection base by collecting and identifying existing edible germplasm; and
- to seek standardisation of terminology and descriptors of conditions for aseptic culture techniques.

All these resolutions involve directly or indirectly the use of in vitro culture techniques. While they are the expression of great expectations, they were clearly not formulated in the opinion that in vitro breeding should be an alternative to the conventional field breeding.

Despite a major effort for systematic identification of the existing germplasm, and despite the proliferation of intermediary hybrids and of synthetic diploids, the total germplasm generated by the field techniques could still appear in the future to be insufficient. Laboratory techniques dealing with in vitro cultivation, in general, could provide powerful complementary tools for the field breeding.

*Musa* germplasm movement in vitro on a worldwide scale is emerging and it is expected that the movement will accelerate when two bottlenecks are eliminated: (1) the lack, in some regions, of the facilities and trained manpower for in vitro propagation; and (2) the imperfect disease indexing methods. Some diseases, such as bunchy-top, are thought to escape current screening techniques. While the solution for problem (1) is merely a question of organisation and cooperation, problem (2) will require a concerted approach.

Somaclonal variation should not be overestimated as a source of useful mutants. Although rapid in vitro propagation of primary meristems actually created significantly different phenotypes, even in *Musa*, the stability of the phenotypical change should always be questioned. In crops with generative propagation as the norm, the presumed mutants can be rather firmly assessed when the aberrant form reappears clearly during segregation. No safe rule exists for assessing in the field the phenotypical modification in clonally propagated plants, but it is accepted that several vegetative cycles are needed for firm confirmation of genotype modification (Scowcroft 1984). Here again, biochemical methods of identification, if properly used, could be of great help. It may be too optimistic to expect from somaclonal variation major changes such as disease-resistance or seed sterility. On the other hand, since somaclonal mutants are a fact in some crops, the technique of meristem proliferation should be encouraged. But it should also be monitored with the assistance of specialists in different domains such as disease, pest and drought resistance screening, chemotaxonomy, and DNA-hybridisation. Programs based on the technique should consequently be integrated into the international effort of genetic improvement.

Somatic mutants, even when they sprout from the minuscule meristem clumps, may be frequently chimeric, and eradication of the original genotype (e.g. through cell competition) can just be hoped for in each case. Somatic embryogenesis in cell suspensions is a better guarantee for producing genetically modified plants. The results of relevant research in some laboratories is promising but research on somatic embryogenesis should be considerably strengthened in the quantitative sense (Banerjee et al. 1985; Cronauer and Krikorian 1984;

Jarret et al. 1985). The process takes time, and considering the wide genetic variation among cultivars, the method eventually developed with a particular cultivar will probably need to be adapted for others. Somatic embryogenesis should be tried on a wide range of cultivars in several laboratories and the mutual exchange of results should lead to the real breakthrough that is sought.

Triploid cultivars being heterozygotic, it is believed that in *Musa* as well as in other crops androgenesis and, for male sterile clones, gynogenesis in vitro, should eventually lead to dihaploid homozygosity, and hence to a more exact use of donor plants in breeding for distinct characteristics. The large pollen mother cells are indeed attractive material. The task is nevertheless very ambitious since one deals with plants where sporogenesis is so erratic.

On the other hand, it should be underlined that the breeding behaviour of the *M. acuminata* subspecies has not been studied in situ, and that cytogenetic studies were apparently carried out on *M. acuminata* subspecies *malaccensis* only. Heterozygosity may occur in some of the subspecies and the fast creation of homozygotes through androgenesis would indeed be convenient. A number of edible diploids may be interspecific hybrids and if they show a rather regular pollen formation, the androgenesis would be a very practical tool for the isolation of desired characteristics (e.g. parthenocarpy potential, or high female sterility).

Embryo culture in vitro is now already practiced (mature embryos) and may play a significant role in the future, especially at a high frequency of interspecific crosses.

More sophisticated techniques such as protoplast-fusion and genetic manipulation are always fascinating. But, when setting up a sound modern breeding strategy, one should examine these methods carefully in view of: (a) the number of practical issues where the method would be really relevant; (b) the state of affairs with somatic embryogenesis and plantlet development from cells in suspension, which is a necessary step in these advanced techniques; (c) the progress made in the basic knowledge of genetics in *Musa*.

Various other in vitro techniques, such as organoculture and in vitro fertilisation, should be looked at for their potential in relation to *Musa* breeding.

### Physiological Research

The physiology of bananas and plantains is not as well developed as in other crops, and breeders suffer from a lack of knowledge in this area (Swennen and de Langhe 1985). For example,

selection criteria are ill-defined. The following points help to illustrate the research that is needed.

The expression of the parthenocarpy is deeply influenced by phyto regulators but more precise knowledge about sequences and quantitative aspects should help the breeder and the geneticist in looking for the exact sources of parthenocarpy.

The stooling habit varies widely among the cultivars, and from one *acuminata* subspecies to the other. The explanation through variation in apical dominance needs to be substantiated and the breeding criteria adjusted accordingly.

Is it always possible to create a hybrid with a fair pseudostem, a heavy bunch *and* a strong ratoon? What are the limits imposed in this respect, by the various A-genomes from the different *acuminata* subspecies and by the B-genome(s)?

With the exception of the Cavendish group and of the Gros-Michel complex, the biochemical definition of the fruit quality is almost nonexistent. The never-ending confusion in terminology on cooking bananas, plantains, beer bananas, etc. is a direct consequence of this. Nobody can explain, for example, why it is that in Eastern Africa a rather homogeneous group of AAA-cultivars is differentiated in dessert, cooking and brewing cultivars! This fact helps avoid the serious mistake that the 'cooking aspect' should come from the B-genome but, beyond this clarification, it is not understood how such a substantial variation in pulp quality could have been generated: by mutations? by different A-origins?

Crop physiological methodology is seriously lacking, again with the exception of Cavendish and Gros Michel (Ganry 1980; Turner 1985). The production potential per hectare and per year of the cultivars, seen as perennials, cannot be calculated since different components such as L.A.I., N.A.R., leaf angle distribution, effect of leaf curvature (ABB!), dark respiration, conversion efficiency, sink repartition, rooting structure, etc. have not been quantified. Results from such research should provide a number of exact criteria for the breeder.

The physiology of 'host-pathogen-soil' relationship is a vague area in most cases. Resistance/tolerance screening techniques that

should be valid on a worldwide scale can only be set up when genetics and ecology of pathogens, and the host-pathogen interaction, are understood.

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# Needs for Plant Improvement in the Edible *Musa* — An Overview

D.W. Turner \*

THE bananas of the world are under threat from new diseases. The leaf diseases Black Leaf Streak and Black Sigatoka are devastating in places like the Pacific, and especially in subsistence culture, where no control measures are applied. In smallholder and plantation culture the cost of control is up to 30% of gross income (Fullerton, these Proceedings) and resistance to fungicides is developing.

## Priorities

The ranking of priorities must contain a good deal of subjectivity. Even if objective assessments are used (economic loss, area affected, yield reduction) priorities can change according to the method of assessment. The rankings presented in Tables 1-3 need to be interpreted carefully. Some are based on the effects of the diseases and pests on productivity, others on potential effects. Nonetheless it is useful to have some idea of the importance of each.

Looking at Asia, Australia and the Pacific the greatest threat comes from the leaf diseases and bunchy-top, which are present in all three areas, and less so from Panama disease (Table 1). The Sigatoka disease complex affects bananas in many countries within the region but chemical control measures are presently available, although expensive. Bunchy-top has been present for many years and appropriate destruction and quarantine methods can restrict the disease. There is no economic control for the new form of Panama disease, which attacks Cavendish clones in Australia and Taiwan, nor for the long established Race 1 present in Asia and Australia.

Among the pests, scab moth is the most serious, although not the most widespread (Table 2). Its impact is important where the market will not accept blemished fruit. Nematodes and weevil borer are more widely distributed and both need chemicals for adequate control. There is concern about the safety of workers and the perceived negative effects on the

environment caused by these control practices. Alternatives need to be found.

**Table 1.** The incidence of banana diseases in Australia, Asia and the Pacific. Data from Cull, Fullerton and Valmayer (these Proceedings) rated on a scale 1 = very low, 5 = medium and 10 = extremely high.

Disease	Asia	Australia	Pacific
Bunchy-top	0-7 ?	6	8
Mosaic	2 ?	1	-
Moko	7 *		
Panama	10 ?	9	
Yellow Sigatoka	5 ?	8	
Black Sigatoka	5 ?	9+	
Black Leaf Streak	8 ?		10
Leaf Speckle		3	
Postharvest diseases	-	4-5	-

+ = disease absent but perceived as a serious threat.

\* = importance decreasing.

? = ratings not given by author.

- = diseases assumed present but no information given.

**Table 2.** The incidence of pests of bananas in Asia, Australia and the Pacific. Data from Fullerton, Cull and Valmayer (these Proceedings) rated on a scale: 1, very low, 5, medium and 10, extremely high.

Pest	Asia	Australia	Pacific
Nematodes			7
<i>R. similis</i>	6-7 ?	7.5	
Root knot	?	4	
Weevil borer	3-8 ?	6	7
Scab moth	?	8	8
Mites	?	5	-
Thrips	7 ?	7	-
Caterpillars		1	
Mealy bugs	7 ?		
Aphids	7 ?	-	-
Scarring weevil	3-4 ?		

? = ratings not given by author.

- = pests assumed present but no information given.

\* School of Agriculture, University of Western Australia, Nedlands, WA 6009, Australia.

Factors other than resistance to pests and disease are also sought in improvement of bananas and plantains (Table 3). The impression I get is that these may well be side benefits, or things we should look for if we are going to shuffle a few genes around when seeking resistance to pests and diseases.

**Table 3.** Factors of importance in cultivar selection, other than pests and disease. Data from Cull, Fullerton and Valmayor (these Proceedings), rated on a scale 1, very low, 5, medium, 10, extremely high.

Factor	Asia	Australia	Pacific
Wind		9	9
Quality			9
Yield			7
Tolerance to cold		8	
Tolerance to water stress		5	
Cycling period		10	

While there is a good deal of commonality in the problems being faced by the banana industries in the different regions there are differences in approach to these difficulties.

In Australia and the Pacific resistance to Sigatoka leaf diseases, nematodes and Panama disease takes priority over wind resistance, shorter cycling and aspects of fruit quality. In the Pacific emphasis is to be placed on commercial production. This is also true in Australia, by default, since almost no subsistence culture exists. The question arises as to whether the emphasis on commercial production is appropriate, especially in regions where subsistence culture is significant, such as in Asia and the Pacific. By concentrating on commercial production the economic returns of a plant improvement program are easily identifiable. Subsistence culture has historically depended on varieties which are not appropriate to plantation culture (Valmayor, these Proceedings) and may not accept a new, disease-resistant variety, designed for commercial production. The strength of Asia lies in the diversity of varieties present, but this also means a more complex plant improvement program especially if many of the varieties used in subsistence culture succumb to diseases and pests. Nevertheless, the significance of genetic diversity to breeders and to people who grow the different varieties is great and this should be kept in mind.

### Responses to the Problem

The recommendations for Asia, in contrast to those from Australia and the Pacific, emphasise the need to continue collection of edible *Musa* and to sort out synonyms. This seems helpful as then we should all know what variety we are talking about.

There is also a need to classify and describe the collections. While the *acuminata* (A), *balbisiana* (B) genomic classification system of Simmonds and Shepherd (1955) has served us well it has been extended by some to accommodate variation within the genomic groups (Valmayor et al. 1981; Pascua et al. 1984; Shepherd and Ferreira 1984). These changes need clarification so that they can be more widely used. Perhaps the best approach is for financial support to be provided for a taxonomist to have access to all the major collections. The work initiated by Cheesman in the 1920s and continued by others can then be reestablished on a global basis and the present work strengthened and coordinated.

Banana industries in various parts of the world have in earlier times suffered and overcome losses caused by disease. For example, the spread of bunchy-top in Australia in the 1920s was eventually contained by an inspection, destruction and quarantine procedure. The export industry of the Caribbean and Central America responded to the threat of Panama disease by attempting to breed bananas which were resistant to the disease. When resistant varieties were unacceptable to the trade because of postharvest characteristics a change was made to Cavendish clones, some of which were described as the minimum acceptable quality (Simmonds 1966). Despite the considerable progress made over 60 years of banana breeding a clone which is resistant to disease and acceptable to the export trade is not available.

This sobering thought must temper our approach to plant improvement and constrain us to avoid wastage of resources and the repetition of approaches which have proved unfruitful. It should also encourage us to look for alternative ways of approaching genetic improvement in bananas, as recently suggested by Shepherd (1983) and Stover and Buddenhagen (1986).

The problems we face are now global, not confined to one industry in one location. The universal nature of the disease complex is expressed by its occurrence in many locations, its impact on commercial/export as well as subsistence culture, and its impact on the plant, both leaves (Sigatoka complex) and roots (Panama disease and nematodes) are attacked.

The response to the situation must reflect its global nature. Conceptually there is a need to balance disease and pest resistance, productivity and fruit quality in plant improvement programs. Each of these topics needs to be supported by an understanding of the underlying phenomena.

### Resistance to Diseases and Pests

The main issues are the Sigatoka disease complex, Panama disease (Races 1 to 4) and nematodes.

Sources of resistance have been identified but they are few (Stover and Buddenhagen 1986) and the need for multigenic resistance has been stressed (Fullerton, these Proceedings). Strategies for improving the resistance to diseases need to be based on a deeper understanding of host-pathogen relationships. These aspects should, in part, be explored in the field in centres of origin so that the ecology of the plants and their diseases can be more fully understood.

### Productivity

Large bunches with large fruit are impressive but our approach to productivity needs to be broader than this.

One approach is to regard the leaf system as an interceptor of energy, part of which is converted to fruit and other plant parts of use to man. This causes us to question canopy structure, the shape and arrangement of leaves, the distribution of light within the canopy and its conversion to photosynthate. These issues have been extensively explored in other crops over the last 30 years or more. With some modification the generalised model of Monteith (1968) can describe light distribution in a banana leaf canopy (Fig 1; Turner 1981). These models can suggest ways in which the canopy might be improved. Should the arrangement of leaves be altered? For example, should we retain the feature of lamina folding in new varieties? Are there some climates where a more dense or more open canopy might be desirable?

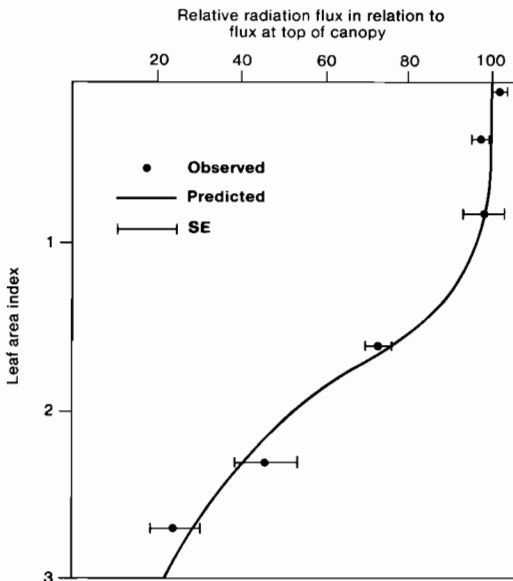


Fig. 1. Observed and predicted relative total radiation interception in ratoon crop bananas using the generalised canopy model based on Monteith (1968).

Questions about the photosynthetic capacity of the leaves can also be raised. Should leaves be photosynthetically more efficient? Can the maximum photosynthetic rate be increased? While many of these questions remain unanswered for the banana we can learn from experiences on other crops. I do not think that large increases in productivity will come from efforts put into manipulation of the canopy (Monteith 1981) as other issues are of higher priority.

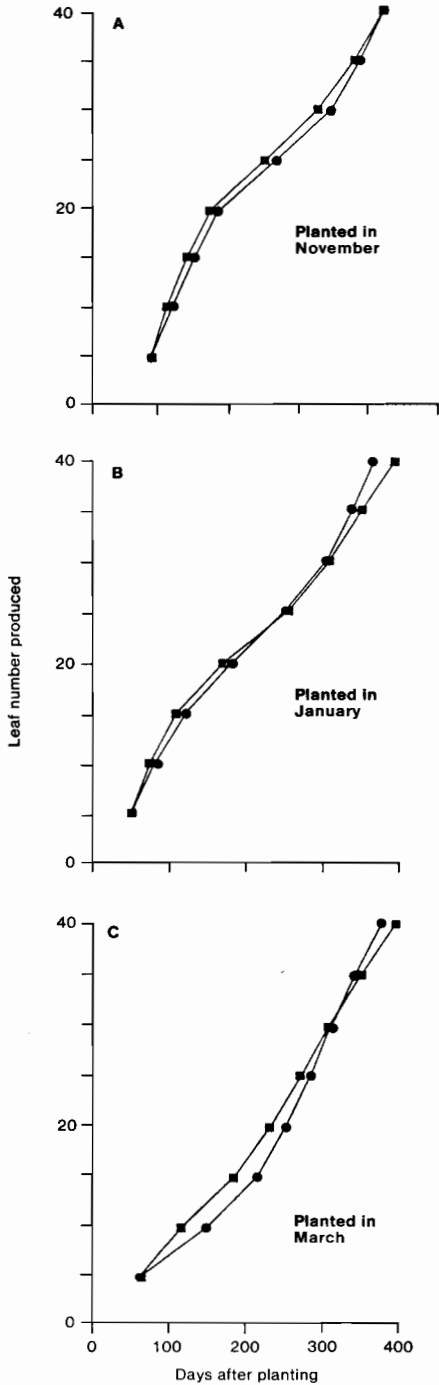
Of greater concern is the number of fruiting sites per hectare which can be increased by greater plant density. The banana is fairly 'plastic' and gains from increased plant density can be eroded by a lengthening of the crop cycle and smaller bunches (Robinson and Nel 1986). In plant improvement we need to ask how many leaves need to be produced before floral initiation occurs? Within the Cavendish clones 30-50 leaves are produced with a large amount of variation within clones. Are all of these necessary? Some bananas produce fewer leaves — less than 20 in *M. velutina*. A further question is how early in the life cycle of the parent can the ratoon crop be initiated and commence to grow? These issues can be explored for various environments by using simple simulation models of the rate of appearance of new leaves (Turner 1981; Mekwatanakarn, pers. comm.). The rate of appearance of leaves over a period of 400 days can be closely simulated (Fig. 2). Using these models our intention is to explore the merits of earlier suckering or a reduction in the number of leaves produced in various environments and to assess the effects of these on the timing of bunch emergence.

Estimating the number of fruit a bunch will have is another aspect of productivity. If we can produce plants which have fewer leaves and begin to grow early in the life of the parent, will the number of fruit on the bunch be reduced? We do not know the limits of the system and we need to establish them quantitatively.

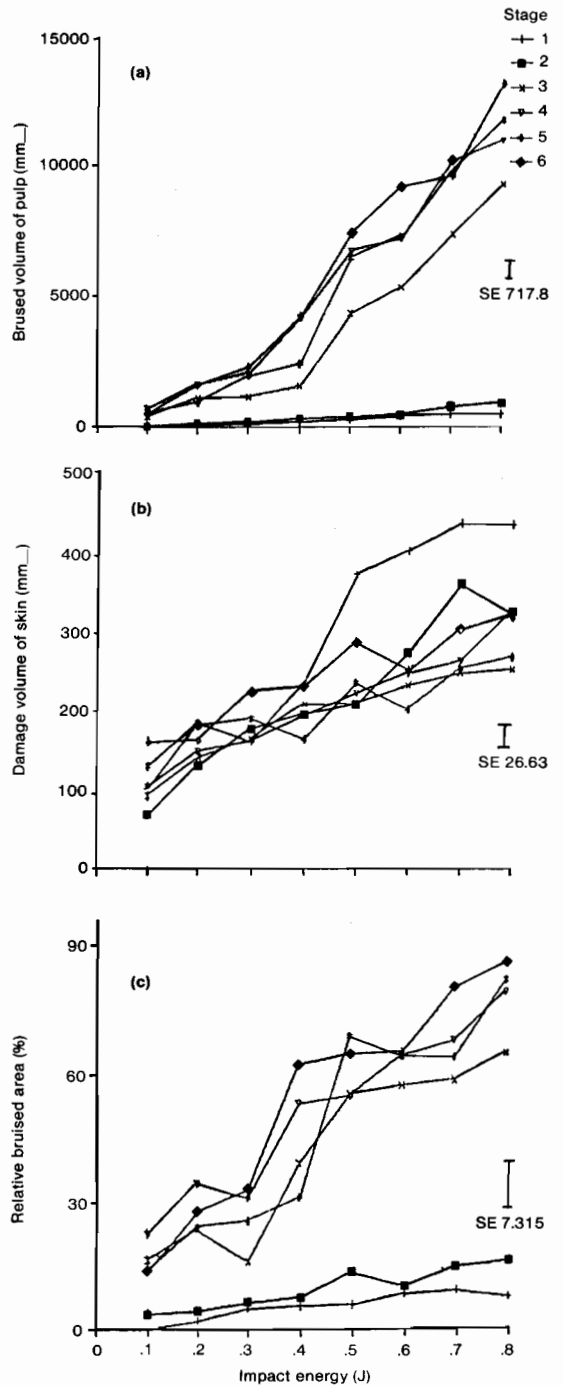
When 'agronomic features' are mentioned in plant improvement programs there is a need to clearly define what is meant by the term. I think we should focus attention on the number of fruiting sites per hectare, expressed as bunches per hectare and fruit per bunch, within a fixed time frame. Then we can assess the significance of changes in fruit size.

### Quality

In previous banana improvement programs the gains made in disease resistance have been more than offset by the deterioration in quality of the fruit, at least as perceived by the export trades. The change from Gros Michel to Cavendish clones meant changing to a banana which was more



**Fig. 2.** The simulation of leaf production on plant crops of Williams bananas grown at Alstonville, NSW, and planted in a) November, b) January and c) March. • = observed; ■ = predicted, using modifications of the apex model of Turner (1981); Mekwatanakarn (pers. comm.).



**Fig. 3.** The change in bruise volume of pulp (a), damage volume of skin (b) and relative bruise area of pulp (c) at six different stages of ripeness of banana subjected to impact energy 0.1 to 0.8 J. Each point represents five replicates. For ripeness stage 1 = hard green, 2 = sprung, 3 = mostly green, 4 = mostly yellow, 6 = overripe.

sensitive to mechanical damage. Handling a fruit with a more sensitive skin needs a greater effort to maintain its appearance.

In apples there is a strong relationship between the energy absorbed by the fruit and the volume of bruised tissue (Schoorl and Holt 1980). This is expressed as the bruise resistance coefficient which varies from one variety to another (Holt and Schoorl 1984). The same principles apply to the banana (Akkaravessapong 1986) but the situation is influenced by the ripening process (Fig. 3). These experiments refer to bruising and yet abrasion is of importance also. We have not yet explored the relationship between the susceptibility of the fruit to bruising and the skin to abrasion. The application of this approach to different varieties of banana will allow us to identify clonal material with skins more resistant to mechanical injury.

The internal fruit quality and postharvest performance of some tetraploid varieties has been explored by New and Marriott (1974). The phenomena of soft texture and short greenlife need to be linked to genotypes so that they can be manipulated in future breeding efforts.

#### Resynthesis — A Synthesis?

The production of a single disease-resistant variety with suitable postharvest quality and high productivity will not have solved the problem, as we will have a monoculture which will be susceptible to the development of new diseases. Internationally, the answer lies in diversity, but a single clone may well suit a particular local need. The resynthesis approach (Stover and Buddenhagen 1986) allows response to a wide range of individual requirements. To work it needs a knowledge of the genetics of the genomes for disease/pest resistance, productivity and quality. These genomes can then be reconstituted to produce the characteristics required by subsistence culture, commercial and export trades. This approach has flexibility but requires a cooperative effort.

The dark problems facing the world's banana industries, be they subsistence culture or export, need to be attacked by a variety of people with a range of skills in genetics, physiology and

agronomy, and the task in front of us is urgent.

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# Status of Bananas and Plantains in West Africa

G.F. Wilson \*

THE genus *Musa* has contributed much to human development and survival in the humid tropics of Africa (Simmonds 1966; De Langhe 1964). Cultivars of the AAA group, mostly beer and cooking bananas, have been major staples in the eastern region, while plantain of the AAB group has been a major carbohydrate source in the western region where more than 60% of the world's plantain is grown and consumed (FAO 1985). Its low labour requirement and relatively high energy output render plantain a suitable staple for areas where labour shortage is usually the main constraint to production (Johnston 1958).

Although one of the main crops of small farmers in the wetter zones, plantain has received little attention from research agencies in the region. This is not because its importance was not recognised, but until lately it had no major production problems that warranted high research priority in countries with limited resources for agricultural research. However, Black Sigatoka or black leaf streak caused by *Mycosphaerella fijiensis* and bunchy-top virus are now posing serious threats. Nematodes and banana borer weevil were always present, but were of limited significance in the traditional cropping systems. However, there are fears that these and other unidentified pests and diseases could become major problems as large-scale intensive production develops in response to high demands and prices. The above threats, along with high food demand, have highlighted the urgent need for more research on plantain. Consequently nearly all governments concerned are interested in developing research programs to find means of increasing plantain production and ensuring adequate food supply.

## The Region

In this report the term West Africa is not restricted to conventional geographic areas, but

covers that area of Africa between 15°N and 15°S latitude and 25°E and 15°W longitude, and includes the western section of Central Africa. The countries concerned are all developing Third World economies that, with few exceptions have problems of rapid population growth and food shortages. Climates range from wet, wet-and-dry, to dry tropics (Norman et al. 1984), but only the wet, and wet-and-dry climates are important for plantains and banana production.

The population of the region is estimated at 175 million, with more than half the number in Nigeria. They are all poor countries with the majority showing negative food production growth rate and high dependence on imported foods.

## Importance

The genus *Musa* contributes to the economy of the region both as a foreign exchange earner and as a staple food. In some countries both aspects are important, but in most only the staple food aspect is relevant.

## Foreign Exchange Earning

Dessert bananas of the Cavendish group are the cultivars of the export trade. They are produced in large plantations with high-level management techniques comparable to those of other major banana-producing areas. At present the export trade is important only to Cameroon, Ivory Coast and Angola (Table 1) where in addition to its foreign exchange earnings, it provides employment for many. Dessert banana production is targeted to the export trade, but because of the high quality standard demanded, a fairly high percentage of the fruits is rejected. These rejects flow readily into the local food supply.

In the decade between 1974 and 1984 production has been declining in the major producing countries and increasing in the minor ones (Table 1). The reason for the decline is not clear, but high production costs and high quality standards have affected many small producers adversely.

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\* International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria.

### Local Staple Food

In nearly all the countries (Table 2) plantain is a staple as well as the raw material for many popular delicacies and snacks. However, while the snacks and delicacies may be widespread throughout a country it is only in the more humid region that it is a major staple. Being highly perishable, plantain does not transport and store well enough to be competitive at distances away from the area of production. Consequently it is mostly consumed in or near the wetter region where it is grown (Guillemot 1976; Melin and Djomo 1972). Trends in Nigeria indicate that good demands exist in the drier areas and increasing quantities are reaching those areas as roads and other transportation facilities improve.

Because plantain is a preferred food and is often used as a delicacy, prices rise sharply in times of scarcity. The growing demand for plantain snacks

and delicacies has been forcing prices up above that affordable by the poor, to whom it is a major carbohydrate source. Though fruits are produced throughout the year, the major harvest comes in the dry season (January–May) when most other starchy staples are unavailable or difficult to harvest. Thus it plays an important role in bridging what is popularly known as the hunger-gap. Where plantains are grown in homestead gardens, they are important food sources at times when food cannot be gathered from distant fields. High energy return per unit of labour (Johnston 1958), gives it an advantage over the other starchy staples where labour is an important production constraint.

Accurate figures on the distribution and use of plantain are not available but rough estimation puts the number of persons in the region who derive more than 25% of their carbohydrates from plantain and banana at about 60 million or 34% of the

**Table 1.** Contribution of banana to foreign exchange earnings in West and West Central Africa (Source: FAO, 1985).

Country	1974			1984		
	Production (t)	Export		Production (t)	Export	
		Tonnes	US\$		Tonnes	US\$
Angola	320 000	65 699	11 439	280 000	NA	NA
Cameroon	94 350	75 481	5 519	65 600	61 985	1 777
Cape Verde	3 500	658	52	5 000	1 200	700
E. Guinea	12 000	3 200	290	18 000	3 016	603
Ghana	8 100	139	7	15 000	NA	NA
Guinea	93 870	51 000	700	115 000	NA	NA
Ivory Coast	207 820	174 660	15 252	140 000	87 892	17 000

**Table 2.** Changes in plantain/banana production in West Africa (tonnes).

Country	Plantain			Banana		
	1974	1984	% change	1974	1984	% change
Angola	-	-		320.0	280.0	-13
Benin				11.5	13.0	13
Cameroon	1 070.5	970.0	-9	94.4	65.6	-30
Cent. Afr. Rep.	57.0	65.0	14	68.0	82.0	21
Congo	33.0	62.0	88	20.1	32.0	59
Eq. Guinea				12.0	18.0	50
Gabon	165.0	170.0	3	7.7	8.0	4
Ghana	1 000.0	650.0	-35	8.1	15.0	85
Guinea	200.0	235.0	18	93.9	115.0	22
Guinea Bissau	23.0	25.0	9			
Ivory Coast	720.0	850.0	18	207.8	140.0	-32
Liberia	26.0	32.5	25	65.0	79.0	22
Nigeria	1 125.0	1 420.0	26	-	-	
Senegal	-	-		2.7	6.0	122
Sierra Leone	16.0	23.8	49	-	-	
Togo	-	-		12.5	15.0	20
Zaire	1 345.4	1 480.0	10	312.4	325.0	4
Total	5 781.4	5 983.3	3	1 179.9	1 147.6	-3

population. Over the past decade production has been increasing in most countries. The exceptions are Cameroon and Ghana (Table 1).

### Cultivars and Types

Plantain and banana are native to Southeast Asia from whence they were introduced to Africa. The time and means of introduction remain unclear (De Langhe 1961, 1964; Simmonds 1966), but the period has been long enough to allow the numerous spontaneous somatic mutations that have resulted in a genetic diversity that is unique to West and Central Africa (Tezenas du Montcel et al. 1983; De Langhe 1961, 1964). However, for reasons unknown, not many of these diversities have been adopted on a large scale. Descriptions of various types of plantain and their uses in Ghana and Nigeria highlighted uses of specific cultivars (Karikari 1971; Ogazi 1980). None of these reports indicated wide adaptation for many cultivars. From all indications only a few cultivars in two types dominate. The most popular types are False Horn and French. The origin of these types is not known but preferences have developed and communities have selected and emphasised different types. So far the only identifiable trend appears linked to the intensity of production. Where plantain is grown in extensive bush-fallow/slash-and-burn systems, the French type, which produces large bunches with many small fingers, and does not ratoon well under West/Central African conditions, is more popular. The more popular cultivars of this type are 'French sombre' and 'French clair.' Attempts to popularise the high-yielding 'Njock Korn' have not had the desired results because of local flavour preferences. Where intensively managed home or compound garden or multistorey systems are practiced, the False Horn type which has small bunches and large fingers is preferred. These are shorter (medium size) plants that ratoon better under intensive management. For example, in Cameroon where plantain is usually grown in bush fallow rotation the French type is most popular, while in Nigeria where compound garden and multistorey systems predominate, False Horn is preferred.

Cultivar names remain a most troublesome and unclear area, contributing to much confusion, discussion and misunderstanding among researchers. In many cases what is described as a cultivar name is often the vernacular name for plantain or banana. For example, the word 'Agbagba' commonly used as a cultivar name for a False Horn type, is the word for plantain in the Yoruba language. Steady progress has been made in describing plantain cultivars in West Africa (Tezenas et al. 1983; De Langhe 1961) but the problem of vernacular names has not been solved,

and the role and potential of minor cultivars are still to be documented.

### Production Systems

Commercial banana production in the region is similar to that in other parts of the world. Thus, there is no need to devote attention to it here. On the other hand, plantain production has many features unique to the region. Small farmers predominate and separation of subsistence from commercial production is difficult. The major systems are as follows:

#### Bush Fallow

The bush fallow or shifting cultivation is a rotation in which short cropping periods alternate with long fallow periods during which soil fertility is restored by the natural vegetation that recolonises the land during the fallow. The length of the fallow period or the time required to restore soil fertility depends on human population density, the inherent richness of the soil, and the type of vegetation. Complex crop mixtures that may include maize, yam, cocoyam, cassava, beans and vegetables as well as plantain are characteristic of the cropping period. When plantain occupies a major position in the complex it is one of the first crops established after land preparation. After the first year when other crops have been harvested, plantain will be ratooned and harvested until they are suppressed by species generated naturally in the bush fallow. In this system plantain yields are low (Table 3), but yield of the other crops makes the system productive and efficient.

#### Multistorey

This system occurs in areas with high human population density and where the rainfall is high enough to support perennial tree crops. Crops in the mixture are arranged in storeys according to canopy level. The mixture includes trees, shrubs and herbs. Oilseeds (e.g. oil palm) occupy the upper storey, then fruits, e.g. African pear (*Dacaroïdes edulis*), African breadfruit (*Pteracarpus africana*), citrus (*Citrus* spp.) and others, the next storey. Plantain comes in at about the third storey below which are cassava, cocoyam, beans and vegetables. The different species are spaced wide and light filters through them to the lower level.

Because plantain can tolerate light reduction of up to 25% without significant yield reduction (Vincete-Chandler et al. 1966), yields are reasonable under these conditions. Management levels are high, plots are kept weed-free and household refuse and farmyard manure may be applied. Mounding and staking are done to prevent toppling. This system is popular in the densely



**Table 3.** Plantain yield in various countries and production systems (source Flinn and Hoyoux 1976).

Country	Yield (t/ha)	Production system	
Zaire	4.0	mixed cropping — culture extensive	
	4.0		mixed varieties
	8.0		variety selection
	15.0	monoculture — mixed varieties	
	20.0		variety selection
	30.0		intensive
	20.0		with mulch
10.0	without mulch		
Eastern Cameroon	7.3	mixed cropping	
East-Central Cameroon	3.5	mixed cropping	
Coastline Cameroon	6.9	mixed cropping	
Western Cameroon	2.4	mixed cropping	
	26.5	pure stand	
Ghana	5.9	mixed cropping	
	20.0	monoculture — pure stand	
Nigeria — ferruginous soils	15.0	monoculture — pure stand	
	30–50	monoculture — village compound garden	

populated area of eastern Nigeria, and seems to evolve naturally in high rainfall areas as the human population density exceeds the carrying capacity of the bush-fallow system.

#### Homestead or Compound Gardens

These are intensively managed small plots in homestead or village compounds. They are usually pure stands except for trees that occupy a higher canopy level. Soil fertility is maintained and weed suppressed by refuse and ash from the household. Productivity and crop longevity are high. Contrary to common belief these are not for subsistence only as sometimes over 80% of the crop is sold (Nweke et al. 1986).

#### Shade or Nurse Crop

In areas where cocoa and coffee are important commercial crops, plantain is an important component of the production. It serves as shade for these crops during the early stages of their development. In the cocoa belts of Ghana, Ivory Coast and Nigeria, plantain production is closely linked to the area planted to new cocoa. However, in parts of Nigeria where cocoa production has declined farmers are adopting one or other of the systems described above for plantain. In some instances plantain production increases as cocoa production decreases with plantain becoming the major income-earning crop.

#### Taungya

Plantain plays a major role in the taungya system.

In this system foresters encourage farmers to establish food crops on lands planted to forest tree seedlings. In caring for the crops the farmers also care for the seedlings. Food crops are phased out when the forest trees are well established. In the wetter regions plantain seems a favourite crop in taungya. Its low requirement allows the farmers to make use of the larger tracts of land opened by the foresters.

#### Pure Stand Fields

Pure stand fields patterned after commercial dessert banana production are fairly recent and are expanding as farmers and government agencies respond to high demand and high prices. They are supposed to be intensively managed with high inputs, but anticipated input levels and management intensity have not been attained. Except for government-supported projects in Gabon and Cameroon commercial farms are usually managed by unqualified managers who rely heavily on local extension services. Unfortunately extension on plantain production is inadequate or non-existent in many countries.

Field size varies from 0.5 to over 500 ha. The larger plots are mostly government-sponsored such as those of SONADECI in Gabon and MEDEVIV in Cameroon. Privately owned fields of 20–100 ha are now common in Nigeria. A trend towards larger fields offers some hope for plantain production in the region in the short run, as these producers are better able to adapt to chemical disease control against Black Sigatoka which threatens plantain and

banana production. While there are obvious advantages to large plantations, the economic background of plantain production in the region suggests that more attention should be paid to smaller (0.5–5.0 ha) commercial pure-stand producers. Promising new technologies aimed at increasing productivity and efficiency of plantain production appear more amenable to small-scale than large-scale production relative to existing constraints.

Table 3 summarises productivity for the various systems. Low yields in mixed cropping do not result from poor plantain performance but are indicative of the proportion of plantain in these plots. Note that intensively managed compound gardens gave the highest yields. Unfortunately no attempts are being made to popularise the compound garden methods.

### Requirements

Except for the new pure stand, the systems described above are characterised by low inputs. Inorganic fertilisers and chemical pesticides are not used, and human labour is the only form of non-solar or indirect solar energy input. These are biological systems that depend on nitrogen fixation, recycling of other nutrients, and biological control of pests and diseases. Low capital status of the farmers precludes introduction of high input technology, and leaves improved biotechnological methods as the only likely means for improving productivity and efficiency. So far the return to human energy input is high, relative to that of other major starchy staples in the region. Therefore, research aimed at improving these systems should focus on biological methods of soil fertility maintenance and restoration, and biological pest and disease control. Positive response to mulch (Fig. 1) suggests the need for more efficient mulching techniques (Wilson et al. 1986; Swennen 1984).

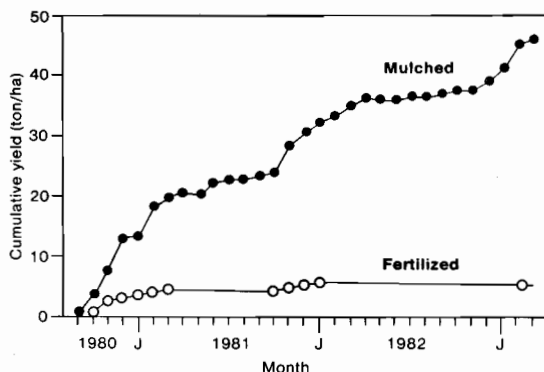


Fig. 1. Cumulative yield of plantain ratoon as affected by mulch over a 3-year period.

For the new pure-stand commercial-type producers, fertiliser and chemical pest control commendations are needed. Plant population densities and production practice for maximum return from the plant crop or alternatively methods for maintaining high ratoon yield should be given high priority in agronomic research. Off-season production for year-round harvest will benefit both producer and consumer.

### Disease

Until the 1970s plantain in this region was regarded as a disease-free crop. None of the common diseases existing then were regarded as economically important. Now, however, Black Sigatoka or black leaf streak caused by *Mycosphaerella fijiensis* var. *diformis* threatens plantain production throughout the region. First reported in Zambia in 1974 and then in Gabon in 1980 it has become a major problem (Frossard 1980). The disease is spreading rapidly and is believed present in all the major areas. Unfortunately, limited resources and personnel have prevented accurate surveys to determine the extent of the disease. Available information puts the estimated disease spread in some of the major producing countries as: Cameroon 80%, Gabon 90%, Ghana 10%, Ivory Coast 10%, and Nigeria 15%. The disease is spreading rapidly as the small farmers are not employing any control measures. Chemical control recommendations are available but the chemicals are usually unavailable and, when available, expensive. Also the small farmers are unable to purchase the equipment necessary for applying the chemicals.

Bunchy-top virus which has caused serious damage to banana and plantain in Asia and Australia is present in Africa, but for reasons unknown has not caused any serious problem here. Though it had been identified on the continent before Black Sigatoka it has not reached the alarming stage of the latter. It is not spreading rapidly and has not caused much damage. But the threat and the fears that changes in the organism, or its vector, may trigger a major catastrophe remain. Resistant cultivars appear the most feasible disease control method for these diseases, but some interim measures are needed until appropriate cultivars are found or developed.

### Pests

Pests of economic importance are the banana borer weevil (*Cosmopolites sordidus*) and nematodes. These pests are widespread but the extent of damage is not known. It is also believed that rapid yield decline of ratoons is linked to these

pests. The trend towards large-scale pure-stand production is increasing the importance of these pests. The new producers have not yet embraced chemical control fully, mainly because the chemicals are expensive and not readily available. Trapping as a method of controlling the weevil is recommended, but so far it has not become popular. The borer weevil seems less important in the native systems (e.g. bush-fallow and compound garden), especially when certain trees or shrubs are part of the crop complex (Kehe 1985). A rough rating would put commercial pure stands at 10 (very high infestation) and bush fallow, multistorey at 6 (medium), and homestead gardens at 3 (low).

Nematodes have been reported in all the major areas, but the severity of infestation and the types of nematodes are known for only a few locations. *Radopholus similis*, one of the most serious nematode pests, does not appear endemic throughout the region (Caveness, pers. comm.).

### **Weeds**

Except for intensively managed compound gardens and multistorey plots, weeds rank high among the problems of producers. Though chemical and manual weed control methods are available, (Ndubizu and Manufor 1984) weedy fields are common sights. Poor weed control is also a major factor influencing yield decline of ratoons.

### **Rapid Yield Decline**

The ability of plantain to ratoon suggests that with the proper techniques, production could be maintained for many years. However, only in intensively managed compound gardens has this been achieved. Explanations for this phenomenon are not available. As stated earlier, rapid yield decline has been important in discouraging large-scale production. The problem seems related to cultivars and types with the giant French types declining faster than the medium False Horn types. Soil organic matter level is also important. Experiments with mulch and fertiliser have indicated that this combination can slow yield decline. But the results have not been consistent enough for definite conclusions (Wilson et al. 1986; Braide and Wilson 1980).

### **Research**

At present fully established and functioning plantain research projects are only in Cameroon and Ivory Coast where the national research organisations work closely with the Institut de recherches sur les fruits et agrumes (IRFA) of France. SONADECI, the organisation responsible

for food production in Gabon has a research component but it is too small to be effective. The National Institute of Horticultural Research of Nigeria regards plantain as a major mandate crop, but shortage of funds has limited its effectiveness. Kade Research Station of the University of Ghana, once the centre for plantain research in that country, has not been very active in the past 6 years. Throughout the region, at universities and research stations, individuals, through personal interest, have been experimenting with plantain. These experiments are mostly academic and have had little influence on plantain in the region.

Until 1985 the International Institute of Tropical Agriculture (IITA) did not include plantain among its mandate crops, but since 1973 it has maintained a special project on plantain research. The project was supported mainly by the Belgium Agency for International Development (AGCD) and the International Fund for Agricultural Development. In 1981 IITA with the support of IFAD brought together plantain research workers in West and Central Africa to discuss plantain production problems, to define objectives and strategies for future research, and to develop a regional research cooperative or network. From this meeting the West African Regional Cooperative for Research on Plantain (WARCORP) was formed. WARCORP consists of scientists from national universities and research stations, IITA, and other research organisations working together to solve the problems of plantain production. It provides the countries with the multidisciplinary research team that they need but could not afford. To date the cooperative has had reasonable success, but the impact has been limited by inadequate funding. It has created awareness of the problems of plantain production and has stimulated interest in plantain research. Requests to participate in its activities, and demands for its services are beyond its present capabilities.

Besides research, WARCORP has supported short-term and higher-degree training and has been active with development programs.

### **Recommendations**

- (1) The countries concerned cannot support individually the research needed. Therefore the West African Regional Cooperative for Research on Plantain (WARCORP) should be mandated and supported to meet the research needs of the region.
- (2) A breeding program targeted at the specific problems of the region should be established.
- (3) A general survey of the region should be taken in order to establish the extent of the problems

and to lay foundations for research and development programs.

- (4) All regional research activities should be closely associated with national research and development programs.
- (5) Training programs aimed at developing scientific and technical skills for national self-sufficiency should be an integral component of the regional project.

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# Regional Needs for Banana and Plantain Improvement in Eastern Africa

John C.M. Ddungu \*

FOR the purpose of this paper the countries included in Eastern Africa are Burundi, Ethiopia, Kenya, Ruanda, Somalia, Tanzania, Uganda and the Kivu region in Zaire.

The banana is the staple food in many parts of these countries except Ethiopia where 'ensete' is used. Systems of production and utilisation vary according to the ecological and socioeconomic conditions of the locality. As far as production is concerned banana is a smallholder crop which is used in both rural and urban areas. It not only provides carbohydrates for the population but is also an important source of income.

Traditionally, at least in some countries, the banana was interplanted with Robusta coffee, vegetables, cocoyams, chillies, tumeric and ginger. The reason behind discouraging interplanting bananas with coffee was that higher yields could be obtained when the two crops were grown separately.

## Past Research

Although the banana is a very important food crop for millions of people, it does not attract international donors for research funding in this region. The national research programs were small because of limited funds and very often were not completed (Awour-Okule and Parish 1969). In Burundi, for example, some work was done on mulching and some variety trials were established. There is a large collection of banana varieties and cultivars at Gitenga in Burundi. In Ethiopia there has been no official program for research on bananas except a few experiments on irrigation at Melka Sodi. In Kenya, variety and spacing trials were conducted on cooking and dessert bananas and nematode surveys showed the seriousness of these pests on the crop. In Ruanda there exists a modest collection of different banana varieties at Rubona. The Ministry of Agriculture in Somalia has recently

set up a research centre on bananas at Gennale to deal with salinity and water management problems. At Maruku research station in Tanzania different fertilisers and organic manures were studied (Tibajuka 1983). Research done in Uganda showed that the banana weevil (*Cosmopolites sordidus*) could be successfully controlled by application of 2.5% dieldrin at the rate of 26 kg/ha but this chemical is no longer used. Fertiliser experiments were also conducted (Ssali 1972). Scientists working in Kivu region of Zaire developed a rapid method of propagating banana suckers.

This brief survey shows that no serious research has been conducted on this crop in the region.

## Present Production Trends and Constraints

Many farmers believe that, in the past, a well-managed banana garden lasted up to 50 years or even more. Presently, a well established banana plantation starts to deteriorate after 4 years in Uganda. The yields are between 8 and 10 t/ha/year.

## Fertility

As one looks at a banana garden of a typical homestead, one sees healthy, tall plants with big bunches immediately behind the house. Further on, plants become shorter and thinner. This suggests that soil fertility declines as the distance increases from the house. Kitchen refuse and sweepings from barns and stables are thrown immediately behind the house thus adding to the fertility of the soil.

## Pests

On closer examination of the banana plants it is not unusual to find that some of them are toppling over. When the corm of the toppling plant is cut open, one finds brown tunnels made by larvae of the banana weevil. The larvae pupate and adult weevils emerge some time later. They in turn lay eggs and the banana garden deteriorates. Production decreases, bunches are small and the fruit under-

\* Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda.

sized. This pest is found in all countries of the region.

Surveys done in most of the countries of the region indicate that nematode infestation on bananas is quite high. *Pratylenchus goodeyi* is prevalent in Tanzania, and in Kenya, *Radopholus similis* in Uganda and in Kenya. It is only in Burundi and Zaire where nematodes are not a serious problem. Banana thrips (*Hercinothrips bicinctus*) cause serious blemishes on banana fingers and lower the market quality of the bunch. These occur in Ruanda, Burundi and Uganda.

#### **Diseases**

Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* was reported in the Kilimanjaro region of Tanzania and has recently been reported in Uganda.

Cigar end rot (*Verticillium theobromae*) is very serious along the borders of Lake Kivu and in some parts of Ruanda. Bunchy-top virus disease was reported in parts of Burundi and in Bukoba, Tanzania.

#### **Socioeconomics**

Population growth is increasing very rapidly in the region. Agricultural land is getting scarce and fragmented. There is need for researchers and governments to address themselves to these problems.

### **Research Needs for Eastern Africa**

With the birth of INIBAP it is hoped that research projects in the region will be adequately funded. One of the objectives of the organisation is to 'initiate, encourage, support, conduct and coordinate research aimed at improving the production of bananas and plantains.' National programs can set up their priorities but it is hoped that INIBAP will also assist in this exercise.

Research work is expensive, requiring skilled people, and specialised equipment. It is therefore being suggested that funds should be given, in the first instance, to collaborative projects that will yield results which will be of mutual benefit to all the countries of the region.

#### **Banana Weevil (*Cosmopolites sordidus*)**

This is a very serious pest in every country of the region. The control methods developed in one place could easily be applied in other parts of the region. Senior entomologists in the region should work with the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, to find ways and means of effectively controlling this pest. It is stated that some banana and plantain varieties are

resistant to weevil attack. What constitutes such resistance should be explored.

Regarding chemical control, application methods should be developed so as to avoid building up of resistance against particular chemicals. Continued screening of insecticides should be carried out to discover new and more effective ingredients to combat the pest.

The area of biological control should also be explored. There may be natural predators and parasites which could control the pest.

There are many well-known cultural methods of controlling the weevil (Namaganda and Ddungu 1985). They include splitting of pseudostems, digging up and chopping old rhizomes and making weevil traps. These should be emphasised to farmers by the extension staff.

Phytosanitary rules regarding movement of suckers from place to place should be strictly enforced. Efforts should be made to always use healthy planting material. With the advent of tissue-cultured planting material, the problem should be circumvented.

#### **The Nematode Complex**

Many different types of nematodes have been recorded on bananas in several countries of the region. Basically nematodes kill rootlets of the plant and thus reduce the absorbing surface. When a banana garden becomes infested with both nematodes and banana weevils plant decline is very rapid. There are on the market chemicals which kill both pests at the same application. Nematologists should screen chemicals for efficacy and how best to apply them economically. Resistance of bananas to nematodes should also be investigated.

#### **Panama Disease**

This is a very destructive disease. It wiped out thousands of hectares in tropical America. It is caused by a soil-borne fungus which is very difficult to control. The only way to overcome it is to use resistant clones. As was mentioned earlier it is present in Kenya, Tanzania and in Uganda.

A team of pathologists, entomologists, soil scientists, and possibly a home economist, should immediately start looking for resistant clones which will be evaluated for palatability, yield potential, tolerance to pests and other diseases and for ability to withstand adverse soil and water conditions. This is a formidable task.

#### **Black Sigatoka Disease**

This is another destructive fungal disease. It was reported in Zambia. Pathologists in Eastern Africa should monitor the disease and try to keep it out if they can, and to be ready for it should an outbreak occur.

## **Fertility Decline**

Most of the agricultural land has been cultivated in the countries under review. If people are to be fed on bananas, yields per unit area of land must be stepped up. Inorganic fertilisers are expensive, and costs continue to rise. Agronomists, soil scientists and economists have the challenge of maintaining the land in good condition for growing bananas. This might involve prevention of soil erosion, water conservation, use of organic manures and crop residues, use of nitrogen fixing plants and soil fertility maintenance practices.

## **Taxonomy, Physiology and Biochemistry**

There are in Uganda alone over 45 different clones which are called different names in different parts of the country. There is need for an in-depth botanical study of each clone, to make comparisons with others noting any similarities or differences, and come up with a nomenclature that will be uniformly used throughout the region. People have suggested chromosome number counts as a basis for the establishment of the desired nomenclature code.

Whatever criterion is used, the important factor is to have uniform names for the same clones. This type of research should be done in close proximity, in collaboration with, the regional germplasm collection at Gitenga, Burundi.

Physiological studies will be necessary to characterise clones suitable for the different agroclimatic zones.

Some basic biochemical studies should also be made on the fruit and its development. It is not known why certain bananas produce beer while others do not. Certain cooking bananas may under certain conditions be used for beer. Others are dual purpose bananas. During the process of ripening some bananas produce ethyl acetate while others do not.

## **Farming Systems Research**

It was pointed out earlier that agricultural land is decreasing not only due to increasing population but also due to urban and industrial development. It is inescapable, therefore, that bananas and plantains will have to be interplanted with other crops. The main objectives of farming systems research will be: (1) to find ways and means of increasing the productivity of banana-based cropping systems so as to ensure that the farmer gets sufficient food for his family and a considerable surplus for sale; and (2) to identify crops which can be interplanted with bananas to generate maximum monetary returns per hectare.

## **Utilisation**

In West Africa, many products such as bread, biscuits, doughnuts, etc., are made from banana and plantain flour. Research to diversify utilisation of bananas is overdue in this region.

## **Training**

In each of the countries of the region there should be trained personnel to staff the research programs. The priority areas should be identified by the country concerned and suitable candidates sent to institutions for training. The fields of training should be specific. Research officers should be trained up to the PhD level. Extension officers should also be trained to high levels so that they master what they have to say to farmers. Existing extension officers working on bananas in the Ministries of Agriculture should periodically be given short refresher courses at recognised institutions of learning.

Provision should be made for research officers and their staff to attend international conferences, workshops and seminars. Provision should also be made to hold regular regional workshops and seminars. There should be sufficient technical and supporting staff in every country.

## **Exchange of Information**

At the moment there is very little information on bananas and plantains available in Ministries of Agriculture. It is hoped that with the formation of INIBAP, journals and reprints of articles will be mailed to workers in this field.

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# Banana and Plantain Production in Latin America and the Caribbean

Ramiro Jaramillo C. \*

THE cultivation of banana and plantain in tropical America and the Caribbean countries has a special importance, not only because they are part of the diet, but also in view of the economic benefits derived from the production activities, through contribution to the gross national product, the establishment of employment sources and the generation of foreign currency and fiscal earnings.

In addition, these crops have a peculiar characteristic: all countries in the western hemisphere consume *Musa* fruits in different amounts. With the exception of Canada, Chile, Uruguay and continental United States, all countries have some areas planted in banana or plantain. These crops are found in the most diverse ecological conditions, but the highest concentration is in the lowlands of the humid tropics.

## Socioeconomic Importance

The importance of these crops differs in each country. In Colombia in 1983, plantain was in first place in agricultural volume and fourth place in value. In Brazil these crops were 14th in cultivated area and second among main fruit products. In Venezuela banana and plantain represents 4.7% of the total value of agricultural production.

## Consumption

The plantain is part of the daily diet in the majority of tropical America and Caribbean countries. Statistics on consumption are not precise, since they vary among regions of the same country. For example, the country of highest consumption is Colombia with 81 kg/person/year. However, in some zones this reaches 200 kg/person/year.

## Value of Plantain Production

The contribution of plantain to the gross national

product in the agricultural sector is difficult to calculate, since in some countries the value of production is estimated based on marketing of the product; the amounts of home consumption are not known, nor do they have estimates of value of its subproducts (pseudostem, leaves, etc.), which are important in animal production.

If we consider home consumption equivalent to 10% of production and a price of US\$85/t (around US\$0.03 per finger) the value of plantain production in the region would surpass US\$600 million.

## Value of Banana Exports

Banana production for export constitutes an important source of foreign exchange for various tropical American and Caribbean countries. In addition, it is an important generator of employment and fiscal earnings.

In employment generation in Ecuador, it has been estimated that activities related to bananas represent close to 30% of the total agricultural employment. This is higher in the Windward Islands (close to 60%) and it fluctuates between 8 and 10% in Central America and Panama.

The earnings for banana exports in Central American countries represent between 21% (Panama) and 28% (Costa Rica, Honduras) of the total earnings from exports in 1981.

In the Windward Islands, banana exports represent more than 60% of the value of total exports (Novoa 1983).

In other important exporting countries where this relationship is lower (8% in Ecuador, 5% in Colombia, 6% in Guatemala), banana production contributes to economic and social development of vast agricultural regions.

The value of exports (f.o.b.) of bananas from member countries of UPEB (Colombia, Costa Rica, Guatemala, Honduras, Nicaragua, Panama, Dominican Republic and Venezuela) during 1983 reached US\$769 million. If the value of exports from Ecuador, French Antilles, and the Windward

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\* CATIE, Apartado 7170, Turrialba, Costa Rica.



Islands is added we obtain a value that surpasses US\$1100 million annually.

Bananas represent considerable earnings for the governments of the exporting countries. This is why the export tax on banana that has been applied in various countries (Costa Rica, Guatemala, Honduras, Nicaragua, and Panama) has meant an additional earning of US\$747 million between 1974 and 1984.

### Production

It is relatively easy to find data about area planted, volume and value of banana and plantain production for export, but it is difficult to obtain figures for internal consumption.

The figures that are presented in Table 1 are approximate. However, it is estimated that the area cultivated with *Musa* in tropical American and Caribbean countries is over 1.5 million ha, of which around 12% is dedicated to planting of banana for exports.

The area planted with AAB plantains ('Horn,' 'French plantain,' 'Harton,' and 'Prata') is over 1 million ha, but there are no figures for area planted with ABB plantains ('Bluggoe,' 'Chato,' 'Topocho,' and 'Pelipita'), which are grown in gardens around homes.

Data on production volume (Table 1) are also

**Table 1.** Area planted and production of bananas and plantains in Latin America and the Caribbean (sources: UPEB 1985; FAO 1986).

Country	Area planted ('000 ha)		Production ('000 t)	
	Group AAB	Group AAA	Group AAB	Group AAA
Brazil	337	60	3345	1460
Colombia	440	23	2248	831*
Costa Rica	12	24	111	980*
Ecuador	50	58	500	1205*
El Salvador	5	—	28	—
French Antilles	2	14	14	292*
Guatemala	9	8	60	375*
Honduras	10	16	130	816*
Jamaica	4	3	29	40 (12)*
Nicaragua	9	3	67	94*
Mexico	14	56	100	1400
Panama	7	14	49	558*
Peru	64	—	731	—
Puerto Rico	4	2	99	91
Dominican Republic	52	2	258	18*
Venezuela	59	52	517	983
Windward Islands		15	—	128*
Total	1078	351	8286	9283

\* Exported bananas.

approximate. With the exception of Brazil, Mexico, Puerto Rico and Venezuela the figures on banana refer to the exported volumes, which are 20–30% lower than the amounts produced, due to losses originated in the norms of quality of exporting companies. So, it is possible to estimate total plantain and banana production in the western hemisphere at close to 20 million t.

### Productivity

The productivity of the AAB plantain is affected by the cropping system used by farmers (subsistence, monocropping or multiple cropping). In Colombia for example, plantain productivity in multiple cropping fluctuates between 4–5 t/ha/year. In monocropping for export the productivity varies between 14–20 t/ha/year.

In relation to banana for export, Central America is the region of highest productivity with yields between 35 and 55 t/ha/year. In the Windward Islands (Dominique, Grenada, Santa Lucia and St. Vincent) where banana for export is intercropped (with coconuts, vegetables, fruit trees, etc.), the productivity fluctuates between 9 and 12 t/ha/year (FAO 1986).

In the French Antilles (Guadeloupe and Martinique), farms larger than 20 ha have an average productivity of 20 t/ha and in farms smaller than 3 ha, productivity reaches 10–12 t/ha/year.

### Cultivars

In spite of the fact that important collections of germplasm and genetic improvement programs are found in Tropical America, the cultivars of banana and plantain for local consumption and export are relatively few.

In the case of the AAB plantain, there is a marked preference for the cultivars 'Horn' ('Harton,' 'Horn') and to a lesser degree for the French plantain ('Dominico'). In some regions of South America's humid tropics (Colombia and Venezuela) the ABB plantains 'Bluggoe,' 'Chato,' 'Topocho' are preferred.

The introduction of 'Pelipita' in tropical America is relatively recent and it is supposed to replace the ABB cultivars susceptible to Moko and Panama disease (race 2).

The AAA bananas that presently are being used for export belong to the Cavendish subgroup ('Giant,' 'Valery,' 'Robusta,' 'Grande Naine'). In the last 10 years there has been a process to change plantations from the 'Valery' or 'Giant Cavendish' clones for 'Grande Naine' which is shorter and less susceptible to damage by winds.

In Panama, for example, of 15 000 ha of banana for export, 64% is planted with 'Valery' and the rest (36%) with 'Grande Naine.'

## Production Systems

### Plantain

The surveys on plantain production in some countries are relatively recent and, with slight variations, similarities in production systems of different countries have been found. These can be illustrated with examples from Colombia and Costa Rica.

In Costa Rica the area in monocropping represents 9% of the farms (480) and 15% of the area (1800 ha). Production is mainly for export and farmers make use of intensive cultural practices with a high utilisation of fertilisers and pesticides (Lemelle et al. 1982).

In the production of plantain intercropped with annual crops (650 ha, 2650 farms) or perennial crops (3550 ha, 2220 farms), the use of agrochemicals is less, as is the intensity of cropping practices. The production is for home consumption and the local market.

In Colombia more than half the planted area (i.e. 228 800 ha) belongs to small farmers who use the plantain as a subsistence crop (Buritica 1984).

Based on data collected in Central America and the Dominican Republic, it is estimated that plantain production in these countries has been in the hands of approximately 40 000 small farmers. Therefore the basic characteristic of plantain production is a low level of technology with the exception of the small exporting sector. Successful export production is due to the extrapolation of management practices of banana production and the use of infrastructure created for banana.

In contrast to banana producers, plantain producers do not constitute an important pressure group, and for this reason they have not been able to influence, in most countries, the decisions on research policy.

### Bananas

In tropical America and the Caribbean there exists a generalised opinion about the advance in the development and application of banana technology. With the change from 'Gros Michel' to the clones of the Cavendish subgroup, in the late 1950s and early 1960s new banana cropping technologies were developed.

Various research teams were established (IFAC-IRFA, Winban, United Fruit Banana Research Board of Jamaica, and more recently, Standard Fruit Del Monte, UPEB, Brazil, Colombia, Costa Rica, Ecuador, and Venezuela) to serve the needs of the different regions.

In the Caribbean the Winban Research and Development Program has been directed at the problems of a large number of growers (more than 18 000) scattered over a wide variety of topographic, edaphic and climatic zones.

In the French Antilles (Guadeloupe and Martinique) IRFA-IFAC developed extraordinary technical and scientific work. In these islands bananas are grown in a great number of small farms.

In Guadeloupe there are some 1100 farms of less than 5 ha, and in Martinique there are 1500 farms of less than 3 ha with production between 10 and 12t/ha.

In Central America and South America, the technological development was due, in large part, to the efforts of the banana companies, which directed resources to research with the purpose of obtaining high productivity and fruit of the highest quality. This development has been most common in the countries where exporting companies grow and purchase bananas (Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Dominican Republic and Panama).

### Agronomic Requirements

It is difficult to establish a complete picture of the specific agronomic requirements for a crop which has such a wide ecological and geographic distribution, and for this reason the considerations are general.

The plantain for local markets is grown from sea level up to 2000 m and in ecological zones as different as the dry tropical forests and the very humid tropical forests. The export plantain is grown generally in areas where the export banana is also grown.

### Water

In tropical America and the Caribbean, banana plantations are found in three rain zones classified according to their need for irrigation (Soto 1986).

The first is formed by the humid plains of the Atlantic zone of Costa Rica and the region of Changuinola in Panama, where rainfall varies between 2500 and 4500 mm/year, distributed throughout the year. Deficits are rare and there is no need for irrigation. On the contrary, drainage systems must be built to evacuate the excess water during the seasons of highest rainfall.

The second zone has an annual rainfall of around 1500 mm, but concentrated in 8-9 months with deficits in the remaining months, which makes irrigation necessary. This is the situation of the plantations of the Aguan Valley in Honduras, the area close to Santa Marta in Colombia, and the plantations near Machala in Ecuador, the French Antilles, Jamaica and the Windward Islands.

In the third zone we find areas with high rainfall (2000-3500 mm/year), but also concentrated in 8-9 months, and so there are water deficits that affect the plantations. Consequently, beside the necessity of constructing efficient drainage systems, irrigation systems are also needed for the months of drought.

Such is the case for the plantations located in Uraba, Colombia, the Valle la Sula in Honduras, and the majority of the plantations in Ecuador.

### Temperature

Banana plantations of Tropical America and the Caribbean are in the optimum growth range for this plant (around 28°C). During January and February, there are moderately low temperatures (winter in the northern hemisphere) that cause a reduction in plant growth, principally in Mexico, Costa Rica, Guatemala, Honduras and Jamaica.

### Wind Effects

The effect of wind is a very important production factor in Mexico, Colombia, Nicaragua, and the Pacific Coast of Panama.

According to Soto (1986) the losses caused by winds can be estimated between 20 and 30%. In the Caribbean, besides the damage caused by winds, hurricanes have struck in the last 10 years ('David' in 1979 and 'Allen' in 1980), that have levelled plantations in the French Antilles and Windward Islands.

### Soils

*Musa* is grown in the most varied soils. Plantains (AAB and ABB) for internal consumption or subsistence are grown in less fertile soils. The clones of the Cavendish subgroup used for export are more demanding and require the most fertile soils for high productivity. The best combination of deep, flat, fertile and well-drained soils are found in the alluvial soils of Central America, Colombia, Ecuador, Jamaica, and the volcanic soils of the French Antilles and the Windward Islands.

The demands of high productivity need the systematic addition of fertilisers. In the aspects related to mineral nutrition, there have been considerable advances. The most important contributions in this field are the results of studies done by IRFA.

### Plantation Management

Management practices are similar in tropical America and the variability of application is due principally to the efficiency and availability of labour in each country or region.

The planting systems most used are in triangles and double rows. The recommended population densities are 1650–1850 production units/ha for Valery and 1850–2000 units/ha for Grande Naine, using one single follower. In most plantations pruning is manual and in some cases 2–4,D is applied.

For weed control mechanical and chemical methods are used. For the latter, herbicides approved by the EPA (Environmental Protection

Agency) are used. Presently the chemical control is based on the application of herbicides, post-emergents (principally paraquat), alone or in combination with other herbicides.

### Bunch Management

The fruit protection operations are combined and involve: bunch covering (polyethylene bags impregnated with insecticide), lower hands removal, male flowers elimination and propping. These practices are well known and numerous papers have been published about them.

### Postharvest Handling

During more than two decades numerous investigations have been carried out on the harvesting, packing, transport, and ripening of fruit. In Central and South America the technology developed by the exporting companies is applied. In the Caribbean the exception is the Windward Islands where harvesting and packing is done in the field by farmers.

### Disease Incidence

The most common diseases affecting banana and plantain in the western hemisphere are Black Sigatoka in Central America, Colombia and Mexico, Yellow Sigatoka in South America and the Caribbean, crown rot and anthracnose, Moko disease and plantain virus (Stover 1978).

Since the detection of Black Sigatoka in Honduras (1972) and its rapid dissemination throughout Central America and northern South America, it has caused great losses to farmers. An example of the decrease of plantain production caused by this disease is illustrated in Panama. In this country Black Sigatoka was detected in 1981 and between 1982 and 1985 the area planted with plantain decreased by 22% (7432 ha in 1982 to 5800 ha in 1985), and 34% of the producers abandoned the activity and production decreased by 47%.

Banana research is now concentrated on the control of Black Sigatoka with chemical products, and control strategies have changed with the introduction of new fungicides. A high level of sophistication has been achieved in the use of aerial equipment (aeroplanes and helicopters), in infrastructure for elaboration of fungicide mixtures and in the use of electronic signals to carry out the operation.

Studies carried out in Costa Rica on total cost of control of Black Sigatoka in banana (more than US\$17.5 million/year), indicate that there is no disease or pest in that country, in any crop, that can compare with the economic effects caused by the disease.

The cost of control of Black Sigatoka in Central

America, Colombia and Mexico, from the time it was detected in each country until 1985, surpasses US\$350 million.

### Nematodes

The nematodes causing most damage in the American region are *Radopholus similis* and *Helicotylenchus multicinctus*. Other species found in high levels, in some zones, are *Rotylenchulus reniformis* (Windward Islands) and *Meloidogyne* sp.

Different pathogenic types of *R. similis* have been detected in the Caribbean and Central America. Some plantations in Honduras, Guatemala and Colombia do not use nematocides. On the other hand, the damages are severe in Costa Rica and Panama.

Nematodes are perhaps the most important pest in Puerto Rico, French Antilles and Windward Islands. Treatment involves field applications of granular nematocides.

### Insect Problems

In the last decade fruit insects have received the greatest emphasis in research in entomology compared with the leaf-eaters and those which attack the pseudostem, corm, and roots.

In the region there are three species of red rust thrips: the *Chaetanophothrips signipennis* (Costa Rica and Panama), *C. clarus* (French Antilles and Windward Islands) and *C. orchidii* that is found in almost all banana-growing zones of tropical America and the Caribbean.

Another insect that causes considerable damage to fruit, in Central America, Colombia and Ecuador is *Colaspis* spp. of which four species have been described: *C. submetallica* (Colombia and Ecuador), *C. blackeae* (Colombia), *C. ostmarki* (Panama and Costa Rica), and *C. gemellata* (Surinam).

The damage caused by these insects can be reduced by means of a polyethylene bag impregnated with insecticide. For *Colaspis* spp. more research is needed on its basic requirements and natural enemies (Ostmark 1978).

*Cosmopolites sordidus* has a great importance in banana in the Caribbean (French Antilles, Puerto Rico, Dominican Republic, Windward Islands) and in plantain in Tropical America. Techniques are known for its control. The pseudostem borers (*Castniomera* sp. and *Castnia* sp.) occasionally cause severe losses, principally in plantain in Central and South America.

Among the most important leaf-eaters

(caterpillars) are: *Cermidia butleri* (from Guatemala to Ecuador) and *Antichloris eriphia* (South America).

Generally a complex of parasites and predators is present that exercises an effective natural control when insecticides are not used.

Some plantations of banana and plantain from Costa Rica to Brazil have been stripped of their leaves by brassolids of the genera *Caligo* and *Opsiphanes*.

Other minor pests are mites, aphids, grasshoppers, scales, mealybugs, leaf caterpillars. However, commercial control measures are available for all pests.

### Cultivar Selection

The most important factors in selecting cultivars are: (a) resistance to *Fusarium oxysporum* f. sp. *cubense* (four races) in bananas; (b) resistance to *Mycosphaerella* spp. pathogens in bananas and plantains; (c) dwarfism, good organoleptic qualities in plantains, and good organoleptic and postharvest qualities in bananas; (d) resistance to *Pseudomonas solanacearum* in bananas and plantains; and (e) resistance to *Radopholus similis* in bananas and *R. similis* and *P. coffeae* in plantains.

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# Cultivation of Bananas and Plantains in Brazil and Needs for Improvement

E.J. Alves, K. Shepherd and J.L.L. Dantas \*

IN Brazil bananas and plantains were probably introduced by the Portuguese and spread along the coast, wherever suitable conditions for their development were found. The first commercial cultivation was established in the early 1900s, located in the lowlands of Rio de Janeiro and São Paulo states. Later cultivation was extended to the other states and regions (Moreira 1969), securing for Brazil the position of the world's number one producer, with a production above 6 million t/year (FAO 1981-83).

Although the crop has great economic and social importance for Brazil, there is no record, up to the 1970s, of a coordinated program of research at national, regional or state level aimed at solving its main problems. Silva et al. (1979) make an exception to this rule only for São Paulo State, where the course of agricultural research was very different from that in other parts of the country. According to these authors, in the period from 1930 to 1970 São Paulo State produced 44 scientific papers on banana, against only 16 produced by the remaining states of the federation.

With the creation of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in 1973, and the establishment of the Programas Nacionais de Pesquisa (PNP), agricultural research in Brazil followed a new course. The principal problems of Brazilian banana culture were identified and priorities were defined for their solution (EMBRAPA 1981). Now, with the creation of INIBAP, we hope to find the necessary support for the solution of these problems.

## Economic Importance

Cultivated from the north to the south, from the coastal belt to the tablelands of the interior, the

banana is the fourteenth most important crop and the second most important fruit crop of Brazil, in terms of area cultivated.

In 1984 the national production reached 469.8 millions of bunches reaped from 395 600 ha. In the same year the northeast and southeast regions accounted for 70% of the production and 66% of the area harvested, the west central region for 11 and 15%, the southern region for 11 and 10%, and the northern region for 8 and 9%. The nine main producing states (Table 1) were responsible for 73.5% of the harvested area and 75.8% of the production (Anon. 1985a). In these states the banana is situated among the 10 chief crops, whether in area harvested, in quantity produced or in value of production. At the national level the area planted increased by 57 000 ha in the period 1974-84, or 16% in the space of 10 years, but has practically stabilised itself in the last years. Regarding the quantity produced, there was an increase of the order of 120 million bunches in the same 10-year period, or 34% (Amaro 1984).

**Table 1.** Areas harvested and production in the main banana-producing states of Brazil in 1984 (Anon 1985a).

State	Area (ha)	Production ('000 bunches)
Bahai (NE)	53 674	74 070
Sao Paulo (SE)	33 364	46 900
Ceará (NE)	28 722	44 990
Minas Gerais (SE)	34 369	36 332
Santa Catarina (S)	23 747	34 724
Goiás (WCent)	37 210	32 490
Rio de Janeiro (SE)	31 880	32 326
Pernambuco (NE)	20 180	31 885
Espírito Santo (SE)	28 054	22 008

Of the total volume produced, exceeding 6 million t/year, 75% is delivered to intermediaries, 11% sold directly to consumers, 10% eaten on the farm, and 1.3% delivered to cooperatives and industrial plants. Less than 1% is exported (FAO 1981-83;

\* Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Centro Nacional de Pesquisa de Mandioca e Fruticultura, Caixa Postal 007, CEP 44.380, Cruz das Almas, Bahia, Brazil.

Blublitz et al. 1981; Alves 1985). In 1984 Brazilian exports of bananas and derivatives earned US\$16.5 million (Anon. 1985b).

Although production costs have risen in recent years (Amaro 1984), properly planted and well run banana fields are generally lucrative. The price received by the growers reached its highest value in the period from August to December (Amaro 1984), making a significant contribution to their net profits.

### Social Importance

Bananas and plantains are crops of very relevant social importance, being adapted to extensive areas of smallholdings. They allow the small producer to enrich his daily diet, to gain extra income and to root himself to the land. A related aspect is that of the high labour input of persons, generally of the smallholder's family, dependent on the cultivation of bananas in Brazil, estimated at more than 2 million people. To this fact may be added the size of the property where bananas are cultivated, which varies from <1 to 1000 ha, but with 90% of the producers falling into the range of <1 to <100 ha (Alves 1985).

Consumed almost invariably in their naturally ripe form, by the most diverse strata of the Brazilian population, bananas form an integral part of the diet of low-income people (Amaro 1984). In the northern region of Amazonia plantains are used as a basic food. They are high in carbohydrates, easily digested and contain useful proportions of vitamins and minerals.

Factors relating to the high consumption of the banana in Brazil (Amaro 1984) are: 1) it is one of the fruit most preferred by consumers, preceded only by the orange; 2) it is available on the market year-round; 3) jointly with avocado it is the most utilised in vitamin preparations; 4) it is present in 90% of the households that make use of fruit salads, in all cities and towns, and at all income levels; and 5) it is regarded by vendors and retailers as among the easiest to sell. These factors are responsible for the high national consumption, 30 kg/capita/year,

considered to be among the highest in the world (Fundação Getulio Vargas 1983).

### Cultivars Used

In Brazil there presently exists no terminological distinction between bananas and plantains; all are bananas. The principal cultivars used are triploids of the AAA and AAB groups, the same as in the rest of the world. Standing out in the AAA group are shorter-statured clones of the Cavendish subgroup, 'Nanica' ('Dwarf Cavendish'), 'Nanicão' and more recently 'Grande Naine.' In the AAB group are found 'Maçã' ('Silk'), cultivated for many years, the recently introduced 'Mysore,' the Prata subgroup, mainly as 'Prata' ('Pome') and 'Pacovan', the unrelated 'Prata Anã' and the plantain subgroup, represented by 'Terra,' 'Maranhão,' 'D'Angolo,' 'Pacova,' etc. (Shepherd et al. 1984).

In the entire national territory, 'Prata' is the most cultivated, followed by the Cavendish clones, 'Maçã,' 'Terra,' and 'Pacovan' (Table 2). 'Maçã' is found in greater frequency in the west-central region, where there exists a long tradition of its cultivation. Plantations of 'Nanica' and 'Nanicão' predominate in São Paulo State (southeast region) and Santa Catarina (south). In the northeast region they are grown in river valleys and generally under irrigation. Small cultivations of plantains are more frequent in the north and northeast regions. 'Pacovan,' a large-fruited mutant of 'Prata,' is becoming widely spread in the northeast (Alves 1985).

According to Champion (1979) historical factors accounted for Brazil receiving, from the time of the Portuguese conquest, banana cultivars originating from India and the East Indies. In consequence, Brazilians have been accustomed by long tradition, especially in the northeast, to the flavours of 'Prata' and 'Maçã' rather than to the particular flavour of Cavendish.

After characterisation and evaluation at the Centro Nacional de Pesquisa de Mandioca e Fruticultura (CNPMP) of EMBRAPA, 'Prata

Table 2. Area cultivated (hectares) of the principal banana cultivars in each Brazilian region (estimates based on Anon 1985a).

Cultivar	Group	Region				
		North	Northeast	Southeast	West-central	South
Prata (Pome)	AAB	26 309	101 671	70 220	5 909	14 305
Pacovan	AAB	—	20 334	—	—	—
Maçã (Silk)	AAB	7 517	5 423	3 830	32 500	—
Plantains	AAB	3 758	5 423	2 553	2 955	—
Nanica + Nanicão (Cavendish)	AAA	—	2 711	51 069	17 727	21 458

Anã,' 'Pacovan' and 'Mysore' have been officially recommended to growers (Alves et al. 1986) and are beginning to be more utilised in the different regions of the country.

### Systems of Cultivation

While in other producing regions of Latin America (Central America, Colombia and Ecuador) a significant percentage of banana production, with a high technological level, is destined for export, production in Brazil is destined almost exclusively to supplying the internal market. Cultivations are generally of a traditional type, with low levels of capitalisation and technology. Many are on sloping terrain, sometimes steeply so, conducive to soil erosion. Other typical faults are improper spacing, lack of pruning, lack of weed control, inadequate use or non-use of fertilisers, lack of water

conservation measures in soils of regions subject to seasonal drought and lack of control of Sigatoka disease (Yellow Sigatoka). Such cultivations are then at a subsistence level or even extractive.

Better managed cultivations are encountered in the state of São Paulo, Santa Catarina, Goias and Minas Gerais, resulting from the application of generated or adapted technology. In recent years, a further stimulus to better practices has been given by the continuing development of irrigation projects, in the northeast region and elsewhere. Conditions are doubtless propitious for more-or-less intensive cultivations (Champion 1979). Under irrigation in the northeast, particularly in the interior of Paraíba State, a productivity of over 70 t/ha/cycle has been obtained with the cultivar 'Nanica' = 'Dwarf Cavendish' (Silva et al. 1978), and four crops have been harvested in 36 months.

**Table 3.** Degree of importance of the principal banana diseases in Brazil.

Cultivar	Degree of importance <sup>a</sup>			
	Panama disease	Yellow Sigatoka	Moko <sup>b</sup> disease	Nematodes
Prata (Pome)	5	10	10	5
Pacovan	5	10	10	5
Prata Anã	5	10	—	5
Maçã (Silk)	10	1	10	5
Mysore	1	1	—	5
Nanica (Cavendish)	1	10	10	10
Nanicão (Cavendish)	1	10	10	10
Terra (Plantain)	1	1	10	5
D'Anglao (Plantain)	1	1	10	5

<sup>a</sup> 10 = very high, 5 = medium, 1 = very low.

<sup>b</sup> Moko only occurs in the northern region.

**Table 4.** Degree of importance of the principal banana pests in Brazil.

Cultivar	Degree of importance <sup>a</sup>					
	<i>Cosmopolites sordidus</i>	Defoliating <sup>b</sup> caterpillars	Pseudostem borers <sup>c</sup>	<i>Opogona sacchari</i>	Thrips	<i>Trigona spinipes</i>
Prata (Pome)	10	5	1	5	1	1
Pacovan	10	5	1	1	1	1
Prata Anã	10	5	1	1	1	1
Maçã (Silk)	10	5	1	1	1	1
Mysore	10	5	1	1	1	1
Nanica (Cavendish)	10	5	1	10 <sup>d</sup>	10 <sup>d</sup>	5
Nanicão (Cavendish)	10	5	1	10 <sup>d</sup>	10 <sup>d</sup>	5
Terra (Plantain)	10	5	1	1	1	5
D'Anglao (Plantain)	10	5	5 <sup>c</sup>	1	1	1

<sup>a</sup> 10 = very high, 5 = medium, 1 = very low.

<sup>b</sup> *Caligo*, *Opsiphanes* and *Antichloris*.

<sup>c</sup> *Castnia licus*, *Eupamelides dedalus* and *E. icarus* in the Amazon region.

<sup>d</sup> In São Paulo State.

**Table 5.** Research and other needs of bananas in Brazil, for the improvement of production standards.

*Cultivars*

- Determination of maximum potential productivity of AAB Group cultivars in different ecosystems.
- Evaluation of short- and medium-statured cultivars of the AAB Group.
- Rapid multiplication of 'Prata Anã,' 'Mysore' and 'Pacovan,' to accelerate their diffusion to growers.
- Acquisition of hybrids of AAB cultivars, resistant to the chief diseases and pests, with better productivity and product quality.
- Evaluation of semi-dwarf Cavendish subgroup cultivars, especially in irrigated areas and valleys of Northeast Brazil.

*Cultivation*

- Use of contour planting or other measures to reduce soil erosion on steep slopes.
- On gentler slopes, the establishment of intervals to facilitate Sigatoka control.
- Attention to drainage on non-mechanised flat lands.
- Drainage networks on mechanisable lands, compatible with the passage of machines and with Sigatoka control.
- In irrigated areas and valleys, soil selection based on physical and chemical characteristics.

*Soil management and conservation*

- Use of mulch and/or green cover in traditional cultivations.

*Mineral nutrition*

- Formulae, time and frequency of application for different cultivars and localities. Determination of NPK absorption curves for Prata, etc.

*Irrigation and drainage*

- Generation of management technology for these factors.

*Sigatoka disease*

- Adjustments needed for mechanised treatment.
- Efficient control by aerial spraying.
- Search for genetic resistance.

*Panama disease*

- Monitoring of pathogen races in each region.
- Search for genetic resistance.

*Moko disease*

- Studies of epidemiology and pathogen survival.
- Search for genetic resistance.

*Nematodes*

- Evaluations of population dynamics.
- Search for genetic resistance and/or biological control.

*Cosmopolites*

- Control measures including biological control.
- Accomplishment of effective control.

*Systems of production*

- Surveys of existing systems in use by small farmers.
- Installation of demonstration plots.

*Postharvest management*

- Diagnosis of problems of fruit for the internal market.
- Development of adequate methods to reduce losses of fruits.
- Definition of systems of management and transport.
- Adoption of modern ripening methods.
- Systems of production and harvests.

**Disease Incidence**

For a country of the continental dimensions of Brazil, the spread of the principal banana disease has occurred in a relatively short time.

Panama disease was verified for the first time in

1930 in the cultivar Maçã, at Piracicaba in São Paulo State, and is presently distributed through the entire national territory. Large commercial cultivations of 'Maçã' were eliminated in the production areas of São Paulo. In the region of the Minas 'triangle' and South Goiás, plantations went



into rapid decline after the introduction of the disease, requiring continuing new planting in new areas.

The introduction of Sigatoka disease (Yellow Sigatoka) was first certified in Amazonas State in 1944, later in Rio de Janeiro State in 1952 and now reaches all the producing regions in the country (Chalfoun and Godinho 1986). In humid conditions the Prata subgroup and 'Prata Anã' may be very severely attacked, suggesting an unusually virulent form of the pathogen. It is feared that the arrival of Black Sigatoka cannot be long delayed, and that this disease will have disastrous consequences for banana production in Brazil.

Race 2 of *Pseudomonas solanacearum*, causal agent of Moko disease, was discovered in Brazil in 1976, in the Federal Territory of Amapa (Tokeshi and Duarte 1976). Today the disease affects also the states of Amazonas, Para and possibly Acre, all in the northern region. It constitutes a serious threat for banana growing in Brazil, in that its incidence might spread from the north into the northeast and southeast regions (Takatsu 1986), responsible as stated earlier for 70% of the national production and 66% of the area harvested.

The burrowing nematode *Radopholus similis*, identified in 1961 in São Paulo State, is now found in various states (Tihohod 1986), sometimes causing severe losses at least in Cavendish cultivars. It appears that only the banana race is present in Brazil, not the citrus one.

A summary of degrees of importance of these pathogens is given in Table 3, in relation to the principal cultivars.

### Incidence of Pests

In a recent review of this subject, Mesquita (1985) reveals that in Brazil there are 78 species of insect which have or include the banana as a host plant. Some are sporadic and regional, and others, although occurring frequently, do so in small populations without causing economic damage. Few require the adoption of control measures and among these is *Cosmopolites sordidus*, which is the principal pest of the national banana culture. The degree of importance of this and other pests in the chief cultivars is shown in Table 4.

### Agronomic Requirements

Some of the common deficiencies of traditional banana plantations have already been noted. Research priorities in this area have been incorporated with those for diseases and pests in Table 5, based on all available information (Instituto Agronomico 1969; Champion 1970, 1979;

Moreira 1975, Bublitz et al. 1981; EMBRAPA 1981; Alves 1984, 1986; Shepherd et al. 1986).

### Principal Research Needs

Among the many subjects of investigation listed in Table 5, as of interest at a national level, the four following themes have been selected as recommendations for more intensive research, which could be of interest not only to Brazil but also to other banana- and plantain-producing countries:

1. Introduction and/or production of new productive cultivars of bananas and plantains resistant to some or all of the principal diseases and pests, with emphasis on Black Sigatoka.

2. Research aimed at biological control of *Cosmopolites sordidus* and possibly of nematodes, with a consequent reduced utilisation of costly and toxic chemicals. In the case of the weevil borer, it is thought that Brazil may be the only country to have developed research in this area.

3. Research on the differential nutritional requirements of diverse cultivars, also related to the stage of development of the plant. This could lead to more rational recommendations for the economic use of inorganic fertilisers.

4. Studies on the postharvest management of fruit of diverse cultivars, including artificial ripening procedures. Serious postharvest losses presently occur in Brazil and perhaps in other zones of production for domestic markets.

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# Banana Improvement Imperatives — The Case for Asia

Ramon V. Valmayor \*

BANANA is the premier fruit of Southeast Asia and is considered of great socioeconomic importance in the countries of the region. Banana is the most important fruit of the Philippines, Thailand, Indonesia and Sri Lanka (Table 1). It ranks second or third in importance among the fruit industries of India, Malaysia and Taiwan. In addition to its primary use as dessert fruit and staple starch, bananas provide various secondary products (i.e. fibres, wrappers, confectionery, vegetables, catsup, wine and vinegar).

As a prime fruit commodity, bananas contribute significantly to the Asian diet and nutrition. It is also an important commodity in domestic trade, thus providing income to numerous small farmers and businessmen. While the banana is grown primarily for local markets, the Philippines and Taiwan are major exporters. Banana is the most important fruit export of the Philippines contributing more than US\$100 million annually. During the last 5 years, the Philippines enjoyed the largest share of the Japanese and Middle East banana markets with an average of 86 and 77% respectively (Segura 1985).

Philippine bananas have a strong position in the Japanese market, the second largest banana-importing country of the world. Japan imported 681 000 t of Philippine bananas in 1982.

## Important Cultivars in Production

The banana export trade of the Philippines and Taiwan is primarily based on Cavendish cultivars. Giant Cavendish has long been the prime export variety of Philippine bananas. Recently, Umalag, a short, local Cavendish cultivar and Grande Naine, an introduced Cavendish variety, have gained popularity due to their shorter stature and subsequent reduction in losses through blowdown

by strong winds. Another recent development is the increasing demand for fancy and exotic local cultivars with luxury fruit appeal to the gift-buying market in Japan. The export of Señorita (very small, very sweet), Lakatan (sweet, aromatic), Latundan

**Table 1.** Area planted to major tropical fruits in Asia.<sup>a</sup>

Country	Fruit	Area (ha)	Rank	%
<i>Southeast Asia</i>				
Philippines	Banana	331 440	(1)	(62)
	Pineapple	60 070		
	Mango	41 280		
	Others	99 250		
Thailand	Banana	208 300	(1)	(28)
	Mango	163 490		
	Pineapple	127 840		
	Others	248 410		
Indonesia	Banana	170 410	(1)	(35)
	Mango	77 390		
	Citrus	66 230		
	Others	176 270		
Malaysia	Rambutan	15 550		
	Banana	14 500	(2)	(19)
	Durian	13 500		
	Others	36 000		
<i>Rest of Tropical Asia</i>				
India	Mango	1 022 220		
	Banana	317 600	(2)	(12)
	Citrus	234 570		
	Others	977 610		
Sri Lanka	Banana	50 460	(1)	(69)
	Mango	9 210		
	Pineapple	4 000		
	Others	8 980		
Republic of China (Taiwan)	Citrus	40 000		
	Mango	15 000		
	Banana	10 000	(3)	(9)
	Other	43 700		

\* Philippine Council for Agriculture and Resources Research and Development, Los Baños, Laguna, Philippines.

<sup>a</sup> Fruit Production and Marketing in Asia and the Pacific. 1985. Asian Productivity Organization (APO). Tokyo.

(sub-acid taste), and Morado (red skin) is approaching half a million 12-kg boxes, which is approximately 10% of total banana export to that country.

The domestic market preference in the Philippines is clearly for indigenous varieties which have long been grown by small producers. Saba, a cooking banana is by far the most important cultivar of the Philippines (Table 2). Although starchy, Saba is not a plantain. It is a robust, triploid *balbisiana* cultivar which thrives well under the most adverse conditions for pure diploid or triploid *acuminata* clones. Saba is drought-resistant, highly resistant to foliar diseases that ravage plantains and the Cavendish group of bananas, and highly tolerant to pests. It is widely grown throughout the Philippines.

**Table 2.** Most important banana cultivars grown for domestic trade in the Philippines (Bureau of Agricultural Economics Statistics, 1984, Manila).

Cultivar	Area (ha)	Production	
		Quantity ('000 t)	Value (P'000)
Saba	122 500	1 552	1 240 810
Latundan	42 860	364	449 070
Lakatan	38 040	474	484 080
Bungulan	29 300	257	235 460
Others	84 910	1 172	1 011 930

Latundan, an AAB clone is the second most widely grown cultivar in the Philippines. Its sub-acid taste is popular among Filipinos. Lakatan and Bungulan are triploid *acuminata* clones and constitute the third and fourth most important cultivars of the Philippine domestic trade. Lakatan is now spelled with a *k* to differentiate it from the Lacatan of tropical America, which was introduced from the Philippines and is actually the Bungulan.

Seventy-five distinct cultivars have been described out of the more than 123 local accessions in the Southeast Asian Banana Germplasm Resource Centre at Davao, Philippines (Valmayor et al. 1981). But most are local curiosities of little economic value. Not more than three dozen varieties ever appear in the village markets, even in the remotest islands. The rest of the cultivars are considered delicacies by ethnic groups and grown in their backyards solely for home consumption.

Banana production in Taiwan is essentially a commercial enterprise. Small landowners form cooperatives which sell the fruit to local and export markets. Although there are more than 60 varieties growing in Chiayi Agricultural Experiment Station, only four cultivars are grown commercially. Hsien-jen-chiao and Pei-chiao, however, are by far the most important and they occupy more than 95% of the area devoted to bananas (Valmayor 1968).

Pisang Ambon Putih, Pisang Raja and Pisang Raja Sereh are the foremost dessert cultivars of banana in Indonesia. At least three strains of Ambon are recognised and grown all over the country. Pisang Raja is considered by the Indonesians as the best banana. Pisang Raja Sereh is synonymous to Latundan and is popular in Java. Pisang Tanduk, Pisang Langka, Pisang Oli and Pisang Kepok are all cooking bananas but only Tanduk and Langka are plantains. Pisang Kepok is a synonym of Saba and is grown in the dry areas of Indonesia. Pisang Oli on the other hand performs better under wet conditions and is commonly cultivated in West Java and Sumatra (Sastrapradja 1984).

The popular dessert cultivars of Malaysia are Pisang Mas, Pisang Rastali, Pisang Embon and Pisang Keling (Jamaluddin 1984). Mas, Rastali and Keling are synonyms of Amas, Latundan, and Inangel of the Philippines. Embon is identical to Ambon of Indonesia. While Pisang Mas is considered the most common dessert cultivar, Pisang Awak, a cooking banana, is the foremost commercial variety in Malaysia. Pisang Awak is synonymous to Katali of the Philippines. The other important cooking cultivars include Pisang Tanduk, Pisang Langka and Pisang Raja. All three are identical to the Indonesian cultivars bearing the same names. Raja and Awak are consumed either fresh or cooked (Valmayor and Pascua 1985).

According to Silayoi and Babprasert (1983) who classified Thailand's banana variety collection of 320 accessions, only 42 are distinct cultivars. And of the 42 cultivars, only four are grown on a commercial scale. The rest are grown in backyards for home consumption or local markets. Kluai Khai, another synonym of Amas and Pisang Mas, is highly esteemed for its sweet fruits. Kluai Hom Thong, which belongs to the Cavendish group, is exported in small quantities to Hong Kong. Kluai Namwa and Kluai Hakmuk are cooking varieties but neither one is a plantain. The former, which is a synonym of Katali and Pisang Awak, is the favourite variety for roasting and the most important commercial cultivar of Thailand.

India has a banana variety collection of 243 accessions in Tamil Nadu. A detailed study of this germplasm assemblage restricted the actual number of clones to 48 varieties and 23 mutants or a total of only 71 distinct cultivars (Sathiamoorthy et al. 1979). India's cultivars of commerce range in diversity from delicate, edible *acuminata* diploids such as the Virupakshi or Lady Finger to the hardy triploids of predominately *balbisiana* genomes such as Monthan and Neyvannan. Both are choice culinary cultivars, the former being a synonym of Matavia or Bluggoe. The foremost Indian dessert cultivar is Poovan also known as Inangel or Pisang

Keling. The other choice clones of commercial value are Rasthali and Nendran. The former is the popular Pisang Rastali of Malaysia, Pisang Raja Sereh of Indonesia and Latundan of the Philippines. Nendran is the only plantain to qualify among the most important cultivars of commerce in India.

Simmonds (1959), in his famous book on bananas, mentioned that the Honderawala, a synonym of Mysore or Inangel of the Philippines and Pisang Keling of Malaysia is the most abundant dessert clone in Sri Lanka. Kolikuttu and Suwandel, which are akin to Latundan or Pisang Rastali and Galamay Senñora or Pisang Kelat Ayer, respectively, are the most highly favoured. Alukehel is the most popular cooking cultivar and is considered identical to Katali, Pisang Awak and Kluai Namwa of Southeast Asia.

When one reads the literature on banana and plantain cultivars in Asia, one can easily be overwhelmed by the endless litany of exotic names. But once the complex synonymy is worked out, suddenly one realises that the various nationalities, each extolling with pride the virtues of their favourite cultivars, are actually talking of one and the same banana.

### **Production and Agronomic Practices**

Banana production in Asia can be categorised basically into three systems: traditional or backyard growers, smallholder commercial growers, and agribusiness plantations (Kobayashi 1985). The backyard growers predominate in Asia. Backyard growers produce bananas primarily for home consumption. The choice of cultivars they grow is dictated by family requirements, i.e. dessert or cooking, quality preferences, climatic conditions, and ease of production. It is in this type of production system where one finds the greatest diversity in cultivars grown as one travels from region to region within a country. Occasional excess produce is sold in local markets. Backyard production does not require cash outlays as labour is supplied entirely by family members. No commercial fertilisers or pesticides are applied; only compost and animal manure are used.

Commercial production by smallholders is a very common practice in all countries of Asia. They proliferate near centres of population where market demand for this fruit is strong and sustained. The choice of varieties to grow is limited by consumers' preferences and suitability of the prevailing agroclimatic conditions of the location. Although this type of production is extensive in nature, it involves cash outlays for land preparation, planting and planting materials, weed control, fertilisers, pesticides, harvesting and marketing. Once the plantation is established and the plants begin to bear

fruit, the farmer starts to recuperate initial investment costs. In general, full reimbursement of investment costs is realised in 2 years.

Large banana plantations that grow fruit for the export markets are also found in Asia. Taiwan and the Philippines have established modern plantations that cater to the exacting requirements of the banana export trade. This is a capital-intensive agribusiness enterprise that requires heavy investment in plantation infrastructure such as service roads and cableways; irrigation and drainage systems; packing houses and support engineering services; transport, storage and port facilities; skilled labour, trained managers and auxiliary services for education, health care and housing for plantation employees. Production practices are applied at optimum levels and yields are high. These large agribusiness operations encourage the growth and establishment of related farming enterprises such as agricultural aviation for pest management; fertiliser and pesticide industries; shipping lines and transport services as well as agricultural machinery and packaging industries.

### **Disease Incidence**

#### **Viral Disease**

Bunchy-top and mosaic diseases are widely spread among the existing banana cultivars and plantains in the Philippines. Bunchy-top is considered as a serious viral disease of the Musaceae. The disease is present in practically all commercial plantations in the Philippines but the extent of infection and production losses have not been determined. The importance of bunchy-top disease was, however, illustrated by the difficulty in providing the essential volume of Lakatan required for regular shipment from Davao to Manila due to the viral infection that affected this banana cultivar (San Juan 1977).

Banana mosaic is considered to be a minor disease in many countries but recent experience in Philippine banana production shows that the disease is widespread among local cultivars such as the cooking bananas, Saba and Cardaba and the fancy Morado. There is a growing concern about the behaviour of mosaic under Philippine conditions considering the favourable climate, the cultivation of susceptible banana varieties, the presence of abaca plantations and other factors (Magnaye 1985).

Recently, Magnaye (1985) reported the reactions of varieties in the Southeast Asian regional banana variety collection located in Davao, Philippines, to bunchy-top and mosaic infection (Table 3).

#### **Moko and Panama Diseases**

The Moko or bacterial wilt caused by *Pseudomonas solanacearum* and fusarium wilt

**Table 3.** Reactions of local banana cultivars to bunchy-top and mosaic diseases under natural infection and/or transmitted experimentally.

	Bunchy-top disease		Mosaic disease	
	Natural infection	Transmitted experimentally	Natural infection	Transmitted experimentally
<i>Local cultivars</i>				
Amas	+	+	+	+
Lakatan	+	+	+	+
Cardaba	+	+	+	+
Mundo	+	+	+	+
Bungulan	+	+	+	+
Latundan	+	+	+	+
Señorita	+	+	+	+
Saba	+	+	+	+
Sulay Baguio	+	+	+	+
Morong Princesa	+	+	(-)	(-)
Radja	+	(-)	+	(-)
Ternate	+	+	(-)	(-)
Turangkog	(-)	(-)	+	(-)
Manang	+	+	(-)	(-)
Pitogo	+	(-)	+	(-)
Inabaca	+	+	+	(-)
Kinamay Dalaga	+	+	+	(-)
Sabang Puti	+	(-)	(-)	(-)
Binalatong	+	(-)	(-)	(-)
Binawe	(-)	(-)	+	+
Canara	(-)	(-)	+	(-)
Pamotion	(-)	(-)	+	(-)
Tudlo Tumbaga	(-)	(-)	+	(-)
Maduranga	(-)	+	+	(-)
Biguihan	(-)	(-)	+	(-)
Dakdakan	(-)	(-)	+	(-)
Pulot	(-)	(-)	+	(-)
Eda-an	+	(-)	(-)	(-)
Batabata	+	+	(-)	(-)
Gubao	(-)	+	(-)	(-)
Inangel	(-)	+	(-)	(-)
Inarnibal	(-)	+	(-)	(-)
Laknau	(-)	+	(-)	(-)
Tudlo Datu	(-)	+	(-)	(-)
Umalag	+	+	+	+
Gao	(-)	(-)	+	(-)
Viente Cohol	(-)	+		
<i>Introduced cultivars</i>				
Pisang Radja Talong	+	(-)	(-)	(-)
P. Talas	+	(-)	(-)	(-)
P. Mundan	+	(-)	(-)	(-)
P. Pulot	(-)	(-)	+	(-)
Kluai Khai Bong	+	(-)	+	(-)
K. Khai	+	(-)	(-)	(-)
K. Thong Khack	+	(-)	(-)	(-)
K. Namwa	(-)	(-)	+	(-)
K. Sa	(-)	(-)	+	(-)
Gros Michel	(-)	(-)	+	(-)

+ = infected with disease.

(-) = disease not observed in field/no transmission test conducted yet.

caused by *Fusarium oxysporum* f. sp. *ubense* are two of the most destructive field diseases of bananas in the Philippines. The Panama disease is also considered a major disease problem in Thailand (Nanthachai 1985) and Indonesia (Kusumo and Sunaryono 1985). It is also reported to be present in Malaysia. On the other hand, among the Asian countries, only the Philippines has encountered a problem with Moko disease.

First observed in 1969 in the Philippines, Moko disease was noted in commercial plantations in Davao del Norte in 1981 specifically on Señorita and Morado cultivars (Roperos and Atabug 1985). Early detection and prompt eradication of the infected mats have contributed to the decreasing trend in Moko disease incidence in commercial plantations although there are still reports of a few sporadic cases of disease outbreak.

Panama disease is an extremely important disease in the Philippines that limits commercial production of susceptible banana cultivars such as Latundan, Pitogo, and Lakatan. Roperos and Atabug (1985) reported that the pathogen totally destroyed 13 ha of Latundan in an area in Davao City within 2 years of planting in 1979. They also noted that when the same *Fusarium*-infested area was planted to Señorita and Morado cultivars, not a single plant was infected with the disease.

It will be recalled that Panama disease devastated the American banana industry owing to the susceptibility of Gros Michel which was then the major cultivar grown in that region. The introduction and cultivation of Cavendish clones resistant to Panama disease has revived the industry. Fortunately for the Central and South American banana industry, the Cavendish clones there have remained resistant to the disease but those in Asia have shown breakdown of resistance. The disease has caused serious crop losses in Taiwan (Sun et al. 1978). According to Buddenhagen (1986), it is not known if the Cavendish resistance-breaking strains in Taiwan, Philippines, Australia and South Africa are of common origin or whether they are of separate origin and therefore may be different in virulence and host range. It is also not known whether many more diverse *Fusarium* strains or races occur in Thailand, Indo-China and other areas in the centre of origin of *Musa*, than the three races so far identified in the areas outside of the host origin.

#### Sigatoka Diseases

Sigatoka diseases, also known as Sigatoka leaf spots, are caused by three closely related fungal pathogens, namely: *Mycosphaerella musicola* (Sigatoka); *M. fijiensis* (black leaf streak) and *M. fijiensis* var. *difformis* (Black Sigatoka). These diseases are widespread in commercial plantations

planted to triploid AAA types of dessert varieties like Giant Cavendish, Dwarf Cavendish, Lacatan, Morado, Gros Michel and Grande Naine.

Surveys carried out as early as 1964 and 1969 showed that black leaf streak pathogen was present in the Philippines, Taiwan, Singapore and Malaysia (Meredith 1970).

In the late 1960s several authors expressed concern about black leaf streak becoming a major threat to banana production in the South Pacific and Southeast Asia if it spreads. It has indeed become a serious disease but so far has been kept under control in commercial plantations with the use of fungicides and other cultural practices such as maintaining optimum plant population, upkeep of drainage canals, removal of sources of inoculum, and fertilisation (San Juan 1977). The control of the disease, however, increased production costs considerably.

The susceptibility of 38 important commercial cultivars to black leaf streak disease was determined by Meredith and Lawrence in 1970. They observed that all the important commercial cultivars such as Giant and Dwarf Cavendish, Robusta, Valery and Gros Michel, Pome and certain plantains and the cooking bananas were highly susceptible; I.C.2, Silk, and three ABB types were moderately susceptible. It is worth noting that among the 38 cultivars tested only Saba, a Philippine cultivar, and an unidentified diploid (AA) were rated as slightly susceptible.

### Pest Incidence

#### Insects

All commercial varieties of banana are prone to insect infestation. In the Philippines, the most important insect pests of bananas are thrips (*Thrips florum*), mealybug (*Dysmicoccus* sp.), aphid (*Pentalonia nigronervosa*), corm weevil (*Cosmopolites sordidus*) and scarring weevil (*Philicoptus iliganus*). Thrips, mealybugs and aphids are common pest problems in all plantations while corm weevil and scarring weevil are restricted to specific plantations (Dawi 1985). In Thailand, Nanthachai (1985) identified corm weevil as a major pest while in Indonesia, Kusumo and Sunaryono (1985) listed scab moth (*Nacoleia octasema*), *Dacus musae*, *Erionota thrax*, and corm weevil as important pests. In India, thrips, lacewing bug (*Stephanitis typica*) and aphids infest banana (PANS Manual No. 1, 1977).

#### Nematodes

Nematodes particularly *Radopholus similis* are major pests of Cavendish banana in Davao, Philippines. *Radopholus similis* is also reported in Sri Lanka, India and Malaysia (PANS Manual No.

1, 1977). Damage to crops is considered significant and controlling the pest in commercial plantations through the use of nematicides is costly.

Surveys conducted in the Philippines on the occurrence and distribution of nematodes showed that the nematodes associated with the Giant Cavendish cultivar belong to eight genera of parasitic nematodes: *Meloidogyne*, *Radopholus*, *Rotylenchulus*, *Rotylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, and *Pratylenchus* (Davide 1985). Except for *Tylenchorhynchus*, all genera found to be associated with Giant Cavendish were also associated with native banana varieties such as Cardaba, Saba, Bungulan, Lakatan, Latundan, Bangan, Inarnibal and Morado (Davide and Gargantiel 1974).

The species were widely distributed, more dominant and more destructive to Cavendish banana: *M. incognita*, *M. arenaria* and *R. similis*. However, *R. similis* was not associated with most local cultivars particularly Lakatan, Latundan and Saba although the other genera were commonly observed.

For the period 1980–84, Davide (1984) determined the reactions of 71 banana cultivars to *R. similis*. Of these, 25 are resistant; 19, moderately resistant; and 27, susceptible. The possibility of controlling *R. similis* through resistance mechanisms therefore exists. Davide also screened 90 banana cultivars against *M. incognita*. Of the 90 cultivars, only 9 showed resistance to *M. incognita* infection; 30 registered moderate or intermediate resistance and 51 gave susceptible reaction. The resistant cultivars are Alaswe, Dakdakan, Inambak, Pastilan, Pugpogan, Maia Maole, Pa-a Dalaga, Sinkor and Viente Cohol.

### Recommendations

The following recommendations are made:

1. Encourage the continuation and completion of a thorough and systematic collection of edible bananas in Indonesia (Irian Jaya, Kalimantan, Sulawesi, Sumatra, etc.), Malaysia (Sabah, Sarawak) and Sri Lanka, and initiate a similar program in Vietnam, Kampuchea and Laos.

2. Classify and characterise national banana germplasm collections (Indonesia, Malaysia, Sri Lanka), identifying synonyms and describing distinct cultivars following a standard format. National variety collections must be maintained as sources of materials for germplasm evaluation projects and international exchange.

3. Assemble all distinct cultivars in the Regional Banana Germplasm Resource Center in Davao, Philippines, and resolve the problem of synonymy at the regional level. A master list of banana and plantain cultivar names and synonyms starting with

the completed projects of Thailand, India and the Philippines must be published to guide banana researchers in all of Asia.

4. Develop facilities and expertise in in vitro maintenance of banana germplasm, since live collections are subject to pest and disease pressures. The tissue culture laboratory will serve as the banana germplasm exchange centre for the region.

5. Evaluate performance of outstanding cultivars obtained through germplasm interchange for productivity, consumer acceptability and reaction to pests and diseases.

6. Screen thoroughly all germplasm materials for resistance to viral diseases, the major problems caused by Moko disease, Panama wilt and Sigatoka leaf spots, as well as the important pests like corm weevil and nematodes.

7. The wealth of banana germplasm resource materials of Southeast Asia is crucial to any global breeding program of this important fruit commodity. As the centre of origin of edible bananas, Southeast Asia also harbours the causal organisms of the major pest and disease problems of bananas and plantains. It is therefore suggested that in considering the site for an international initiative on banana breeding, Southeast Asia must be given prime consideration.

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# Banana Production in Selected Pacific Islands

R.A. Fullerton \*

BANANA production in the Pacific Islands covers a wide spectrum of activities ranging from the utilisation of fruit from self set plants, through small plot subsistence farming and local market supply, to plantations producing quality fruit for export. Historically, export production has been characterised by wide annual fluctuations as crops reacted to the influences of pests, diseases, weather, and varying management methods. Although no data are available on subsistence production it may reasonably be assumed that the 'natural' influences (including diseases) which have affected commercial production would have had an equal, if not greater, effect on subsistence production.

While there are many similarities between the Pacific Islands (physical features, latitude, climate) there are important differences in the scale of production, methods of utilisation, and degree of technology employed in the different countries. For that reason, this paper provides a brief account of production methods and problems for each of the countries (Cook Islands, Niue, Kingdom of Tonga, Western Samoa, Fiji), followed by a general analysis of the major problems in the region.

The banana industries of the Pacific nations are very small by world standards. However, each must be considered in relation to the size of the country in which it is based, its relative importance to the national economy, and to the welfare of the individual growers, many of whom have limited options for commercial farming.

A large number of diseases and pests have been recorded from bananas and plantains in the Pacific (Dingley et al. 1981; Maddison, unpublished data). In this paper, only those known to cause significant economic losses have been considered. Panama disease (*Fusarium oxysporum* f. sp. *cubense*) is not known in most of the Pacific Islands. The organism has been recorded from Fiji, but confirmation of the disease is needed. Morwood (1965) expressed doubt that the 'Panama disease' strain of the

organism occurs in Fiji. The disease was not observed in Fiji during the UNDP/FAO survey of Agricultural Pests and Diseases (Dingley et al. 1981).

Until the early 1960s the principal leaf disease of the region was Yellow Sigatoka disease (*Mycosphaerella musicola*). In 1964, black leaf streak (*Mycosphaerella fijiensis*) was first recorded from Fiji (Leach 1964; Rhodes 1964). Subsequently Mulder and Stover (1976) recorded Black Sigatoka (*Mycosphaerella fijiensis* Morelet var. *difformis*) from Tonga and Western Samoa. Yellow Sigatoka is now seldom seen (Dingley et al. 1981). The black leaf streak/Black Sigatoka complex has emerged as the principal leaf disease of the region. The organisms of the complex exhibit considerable variability and separation of the variety *difformis* from the type variety is difficult. The disease is known throughout the region as black leaf streak and that name has been adopted in this paper.

## Cook Islands

This is a group of 15 islands situated between 8 and 23°S latitude and 156 and 167°W longitude. The northern group of seven islands are coral atolls. Only in the southern group, principally of volcanic origin, is arable farming practiced. The Cook Islands have a population of approximately 17 000 with the majority (10 000) living on the main island of Rarotonga.

Prior to 1978 bananas were produced commercially for export on both Rarotonga and Aitutaki situated 225 km to the north. Since then, production has been confined to Aitutaki. Individual plantations are small (0.5-1.0 ha) with a total area of about 140 ha in production in 1986 (F. Charlie, pers. comm.). The predominant variety grown is a tall Cavendish type. Apart from a small quantity consumed locally, all are exported to New Zealand. A small drying plant utilises export rejects. Production figures since 1973 are shown in Table 1. Banana is one of the principal export crops of the Cook Islands. Comparative values of major export

\* Department of Scientific and Industrial Research, Plant Diseases Division, Private Bag, Auckland, New Zealand.

**Table 1.** Exports of bananas (*tonnes*) from Cook Islands, Tonga and Western Samoa to New Zealand 1973–85 (Sources: Cook Islands Ministry of Agriculture, Agriculture Planning Unit; Tonga Ministry of Agriculture and Fisheries Statistics; Fruit Distributors Ltd, Auckland, N.Z.).

Year	Cook Is.	Tonga	W. Samoa
1973	1741	2860	1000
1974	1212	2830	1316
1975	723 <sup>a</sup>	3060	482 <sup>a</sup>
1976	580 <sup>a</sup>	2770	1346
1977	215 <sup>a</sup>	3230	342 <sup>a</sup>
1978	170 <sup>a</sup>	1420	1440
1979	1105	4308	—
1980	2076	2238	1859
1981	2509	2340	1535
1982	1940 <sup>a</sup>	405 <sup>c</sup>	1139
1983	140 <sup>b</sup>	1083 <sup>b</sup>	1579
1984	1244	2405	387 <sup>a</sup>
1985	1662	2424	22 <sup>a</sup>

<sup>a</sup> Leaf disease.

<sup>b</sup> Drought

<sup>c</sup> Wind/hurricane.

crops in 1984 were copra NZ\$618 000, vegetables NZ\$292 000, banana NZ\$254 000, other fruit (predominately pawpaw) NZ\$512 000. In years of high production the value of bananas can exceed that of copra.

The major agronomic factors affecting production in Aitutaki are black leaf streak, and heavy infestations of burrowing nematode (*Radopholus similis*) and weevil borer (*Cosmopolites sordidus*). Neither bunchy-top virus nor scab moth (*Lamprosema octasema*) are present in the Cook Islands.

Control of black leaf streak is accomplished by fungicide-oil-water sprays applied by means of motorised knapsacks (mist blowers). The fungicides in use are benomyl (as Benlate 50 WP), tridemorph (as Calixin 75% w/v) and propiconazole (as Tilt 250 EC). Ideally sprays are applied fortnightly, extending to 3 weeks during dry periods. Benomyl tolerance has not been found in the Cook Islands. To reduce chances of resistance developing the various fungicides are used in rotation or alternation. All spraying is done by the Ministry of Agriculture.

All plantations are heavily infested with nematodes and weevil borer. Fenamiphos (as Nemacur 10G) is recommended for control of nematode. Stocks are on hand but it is not used because it was considered by growers to be 'too dangerous' after observing deaths amongst chickens foraging in treated plantations. The use of oxamyl (as Vydate L) for these pests is being investigated. Propping is not generally practiced, with the result

that there are frequent, sometimes extreme, losses due to toppling even in moderate winds.

On Rarotonga, a few small plantations are maintained for local market supply. Pest and disease control measures are rarely applied. Production is greatly reduced by black leaf streak and shortages of bananas on the local market are common.

A number of types of plantain occur throughout the group. Many are affected by black leaf streak as well as nematode and borer. Production is low.

Except on the island of Mitiaro, shortages in local supply of bananas or plantains are of little consequence as they are not regarded as staple food items. On Mitiaro, shortages do affect the food supply as well as a small banana-drying industry.

Research on bananas is carried out at Totokoitu Research Station, Rarotonga. Topics include control of black leaf streak and control of postharvest crown moulds. In 1980 and 1981 a number of diploid and tetraploid lines from the Jamaica banana breeding scheme were screened for resistance to black leaf streak. Several resistant lines were identified but none were agronomically suitable for commercial production. A small collection of plantains is maintained at the station.

### Niue

An uplifted 258 km<sup>2</sup> coral island situated at 19°S latitude, 169°W longitude, Niue has a population of less than 3000.

Apart from a small export industry of lime and passionfruit juices, all agriculture is of a subsistence nature. Although the staple starch sources are taro (*Colocasia esculenta*) and cassava (*Manihot esculenta*), bananas are a common component of the farming system and are utilised if they are available. Black leaf streak is prevalent and undoubtedly reduces production from garden plots. Burrowing nematode and weevil borer are endemic. Neither bunchy top nor scab moth are present. No pest or disease control measures are applied.

### Tonga

The Kingdom of Tonga consists of three main groups of islands situated between 15 and 23°S latitude and 173 and 177°W longitude. The main groups are Vava'u in the north, the Ha'apai group in the centre, and Tongatapu and nearby Eua in the south. The population is approximately 92 000 of which 61 000 live on Tongatapu.

In the past bananas were exported to New Zealand from all three island groups. Since 1983 only Tongatapu and Eua export fruit. A limited quantity produced on Ha'apai is utilised on the local market of Tongatapu. Bananas have consistently been a major export crop for Tonga and a principal

source of income for many Tongan landowners. Export figures since 1973 are shown in Table 1. In addition, up to 80 t/month are sold on the local market (Cessford 1986). In March 1986 there were 300 registered growers with a total of 546 ha in production. Most plantations are grown under coconuts. The majority are small (average 1.5 ha) and yields are low (average 10 t/ha). The best plantations yield up to 18 t/ha of export quality fruit (Cessford 1986). Plantation life tends to be short (approximately 3 years). A current redevelopment scheme within the industry, funded as a New Zealand development assistance project, is aiming to increase profitability by increasing productivity and improving the quality of export fruit.

Several factors contribute to the poor performance of Tongan plantations. Competition from the upper storey coconuts combined with failure to apply fertilisers are undoubtedly contributing factors. However diseases and pests have a more acute and significant effect. Without a continuous program for control, black leaf streak will prevent the production of export fruit. Benomyl resistance was detected in Tonga in 1985 (Fullerton, unpublished). Chemicals presently used are tridemorph, bitertanol (as Baycor), propiconazole, and mancozeb applied in alternation or rotation at 2-weekly intervals. Sprays are applied by mist blower by staff of the Tonga Ministry of Agriculture, Fisheries and Forests. Satisfactory control is obtained but the cost is high. Costs for chemicals and oil are in the order of T\$600/ha per year. When labour, fuel and capital costs, are included, the control of black leaf streak may cost up to T\$750/ha/year, or approximately 30% of the gross income for an 'average' plantation.

Bunchy-top is present in Tonga. A survey in 1985 found 0.1–1.7% of plants infected in commercial plantations (Amanaki et al. 1985). The incidence in abandoned plantations can be much higher (10–15%). The principles for containment of the disease are well known (Magee 1967; Walker 1977) but they have not always been applied. The present control program involves an insecticide spray followed by injecting all shoots with glyphosate weedicide. This method is simple, economical and effective. After two rounds of inspection and eradication, the incidence fell to less than 0.1% (Amanaki et al. 1985). The success of control measures within plantations, and the production of disease-free planting material is being limited by an apparently latent form of the disease in Tonga.

Incidence of burrowing nematode and weevil borer is high. Engelberger and Tupou (1983) have estimated that 96% of plantations sustain yield losses of up to 10% as a result of weevil borer. From a survey of nematode infestation it has been estimated that yield increases of between 30 and

60% may be achieved by the use of 2 or 3 applications per year of carbofuran (Steir and Vi 1983). Many growers are reluctant to use insecticide because of the cost. Combined plant losses from bunchy-top infection and topping due to nematodes are, on average, reducing populations of productive plants by 15% (Amanaki et al. 1985).

Banana scab moth is a serious pest. Control is achieved by injecting emerging flowers with trichlorophon. Even when control measures are applied, up to 10% of bunches can still be lost (for export) because of damage caused by scab moth. Plantations have been devastated several times in recent years by cyclones.

Plantains are widely grown in Tonga. They are a traditional component of the 'api (family subsistence plot) along with kape (*Alocasia macrorrhiza*), taro and kumara (*Ipomoea batatas*). Many types of plantain are severely affected by black leaf streak. They also act as reservoirs for bunchy-top virus.

Research on bananas is carried out at the Vaini Research Farm of the Tonga Ministry of Agriculture, Fisheries and Forests. Main topics include surveys of pests and diseases, control of black leaf streak and control of nematodes.

### Western Samoa

Western Samoa consists of two main islands, Upolu and Savai'i, and several smaller islands situated between 13° and 15°S latitude and 168° and 173°W longitude. It has a land area of 2110 km<sup>2</sup> and a population of approximately 151 000. Because basalt rock dominates much of the arable land opportunities for mechanised farming are limited.

In the past, Western Samoa was a consistent supplier of bananas to the New Zealand market. Export data since 1973 are shown in Table 1. In 1986 none are being exported. Until the late 1970s all production was on small, privately owned plantations. In 1977 a government operated plantation of 85 ha was established (with Australian aid assistance) at Tanumalala on Upolu. This plantation was designed to employ modern methods of production and postharvest handling. An aircraft was purchased for aerial spraying for leaf disease. Two large plantations owned by the Western Samoa Trust Estates Corporation (WESTEC) were also able to utilise the aircraft. The subsequent failure of the Western Samoa export industry was almost entirely due to black leaf streak. Resistance to benomyl was detected in 1980 (Fullerton and Tracey 1984). While this was undoubtedly a contributing factor, other logistical problems were probably more important. Reliance on a single aircraft and a part-time pilot, extended periods of wet weather or

low cloud (characteristic of the Western Samoa climate), equipment failures and shortages of chemicals, resulted in frequent disruptions to the Tanumalala and WESTEC control programs. Effective control of black leaf streak was not achieved. Similarly, programs for the large number of small, privately owned plantations could not be maintained by the Western Samoa Ministry of Agriculture spray teams. Bunchy-top, nematode, weevil borer and scab moth all contributed to the decline of the industry.

Although exports have ceased, bananas remain an important component of the economy and the principal source of income for several hundred growers. Green bananas are a staple food in Western Samoa and all production is consumed locally. Plantains are also a favoured food item but supplies are small compared with plantation-grown bananas. The industry is satisfying local demand and making an important economic contribution through import substitution. However, the banana is one of the few crops available which are capable of earning foreign exchange for Western Samoa. Given effective control of pests and diseases, particularly black leaf streak, production could quickly be expanded to allow Western Samoa to reenter the export market.

### **Fiji**

Fiji consists of over 300 islands situated between 15° and 22°S latitude and 177°W and 175°E longitude. The group is dominated by the islands of Viti Levu (10 380 km<sup>2</sup>) and Vanua Levu (5536 km<sup>2</sup>). Other principal islands are Taveuni (435 km<sup>2</sup>) and Kadavu (407 km<sup>2</sup>). Over 100 of the smaller islands are also inhabited. The population exceeds 612 000.

Of the Pacific Islands, Fiji is the most developed agriculturally. Sugar and ginger are the main export crops and the country is nearing self-sufficiency in most commodities. Commercial farming is principally on tenanted smallholdings. A large proportion of the population particularly on the smaller islands engages in subsistence agriculture. In the past, bananas were an important export crop. None have been exported from Fiji since 1974. Both bananas and plantains are widely grown for personal use and local market supply. They are rarely, if ever, managed as plantations. All the principal pests and diseases are present (black leaf streak, bunchy-top, nematode, weevil borer, scab moth). Control measures are not normally applied. Although no information is available on the losses sustained, experience elsewhere suggests they would be substantial.

There is a small collection of bananas and plantains at Koronovia Research Station, Nausori, Viti Levu.

### **Fruit Quality and Market Acceptability**

Prior to the 1960s the Pacific Islands provided all of New Zealand's bananas. Without competition, the 'traditional' fruit defects of sap stain, skin blemishes, and mixed ripening were generally accepted as 'normal.' The inability of the Pacific Islands to meet the growing demand in New Zealand resulted in the first imports from Ecuador in the 1960s. The outstanding quality of the Ecuadorian fruit (large, uniform, blemish free), set new standards in the market and put pressure on Island producers to raise the quality of their fruit. While it is possible to effect a dramatic improvement in quality by correct nutrition, pest and disease control, and improved handling methods, Island grown bananas seldom have the visual impact of fruit from Ecuador or the Philippines. In contrast to the large, straight fruit of cv. Grande Naine grown in Ecuador, Island bananas tend to be smaller and more strongly curved even with good growing conditions; they do however have a reputation for being sweeter. While some differences may be environmentally induced, most are probably due to differences in the genetic potentials of the cultivars.

Until 1985, the Pacific Islands had an assured market for their fruit under a preferential purchasing agreement with New Zealand. That agreement was dissolved in 1985 and the Pacific Islands must now compete on the open market. The Pacific Islands provide only a very small proportion of the New Zealand market requirement (approximately 39 000 t/annum). However the industry is important to the economies of the Pacific nations and the livelihood of their growers. In order to retain this small but vital share of the market, fruit quality must urgently be improved. Island producers must have access to cultivars with improved agronomic characters, particularly higher yield potential and larger, more uniform fruit. The importation of Grande Naine plantlets is being considered by the Kingdom of Tonga.

### **Situation Analysis**

The distribution of principal pests and diseases and their relative importance are shown in Table 2.

Black leaf streak is undoubtedly the most serious problem in the region. It is the major factor affecting commercial production and is responsible for low production from subsistence plots of both banana and susceptible varieties of plantains. Control of black leaf streak is the single largest production cost for bananas in the Pacific. Up to 20 applications of fungicide per year are necessary and any significant break (4-6 weeks) in the program can result in loss of commercial production within 2 months. Few growers have spray equipment

**Table 2.** Production systems and relative importance of pests, diseases and agronomic characters in the Cook Islands, Niue, Tonga and Western Samoa, rated on a scale of 1 (low) to 10 (very high).

Country	Form of production	Black leaf streak	Bunchy-top	Nematode	Weevil borer	Scab moth	Wind	Yield & fruit quality
Cook Is.	Plantation	10	N.P.*	8	8	N.P.	6	8
	Subsistence							
Niue	Subsistence	10	N.P.	5	5	N.P.	3	3
Tonga	Plantation	10	8	8	8	8	6	8
	Subsistence							
W. Samoa	Plantation	10	8	8	8	8	6	8
	Subsistence							
Fiji	Subsistence	10	5	5	5	8	3	3

\* N.P. = not present.

and in general they do not appreciate the need for adhering to a continuous program. Thus it has been necessary for government agencies to assume responsibility for the control of the disease in exporting countries. The scheduling and monitoring of spray gangs to service large numbers of small, isolated plantations on a fortnightly cycle is a major logistic exercise which is not always successful. The relative isolation of the Pacific Islands from sources of supply often leads to shortages or late supply of chemicals, machines and spare parts with the resultant disruption to programs.

The chemical arsenal available for black leaf streak control is limited. The high frequency with which they must be applied carries with it a high risk of resistance developing to some of those chemicals. Benomyl is no longer effective in two of the three producing countries. Of the alternative chemicals in use (mancozeb, tridemorph, bitertanol, propiconazole) neither mancozeb nor tridemorph gives adequate control during the hot, wet summer months. Bitertanol and propiconazole are effective. However both belong to the same group of chemicals (triazoles) and affect the organism in a similar way. Should the organism develop resistance to one chemical then it is also likely to be resistant to the other. The triazole chemicals are considered to be 'at risk' from resistance development and should be used only in rotation, alternation or combination with other, unrelated, chemicals.

The development of cultivars with resistance to the Yellow Sigatoka/black leaf streak/Black Sigatoka diseases would have a major impact on both profitability and production. However caution will be necessary in a breeding program. The fungi involved have already shown themselves to be variable and adaptable to changing conditions. With high populations of the organisms and large numbers of generations per year, resistance based on a single gene may soon be overcome. Breeding programs should concentrate on the use of multigenic resistance imparting tolerance rather

than high level resistance or immunity conferred by a single major gene.

The impact of bunchy-top on production is generally masked by the acutely destructive black leaf streak. When leaf disease is controlled, the effect of bunchy-top becomes apparent. Populations of productive plants are significantly reduced, markedly affecting the profitability of plantations. The insidious nature of the disease does not have the same impact on growers as the more obvious leaf diseases, thus the seriousness of the disease is often underestimated. For this reason, to be effective, any control program must become the responsibility of a government agency. The insecticide + glyphosate method of control used in Tonga is effective in eradicating infected plants. A method to detect the virus in symptomless carrier plants would greatly facilitate the production of disease-free nursery stock.

Burrowing nematode and weevil borer are particularly destructive. Chemical control programs, though effective, present special problems in the Pacific. The chemicals are expensive and growers are reluctant to use them because an immediate effect is not seen. They are also extremely toxic to the operators, a particular disadvantage in an area where operators do not fully appreciate the need for safe handling, and where the climate discourages the use of protective clothing. Many of the islands rely on underground water for household and urban supplies and these countries are becoming increasingly conscious of the dangers associated with the widespread use of soil insecticides. Tolerance to nematodes would be a desirable attribute in new cultivars. It may be possible to ameliorate the problem of weevil borer by a suitable biocontrol program.

The highly visible effects of scab moth and the dramatic effect of the chemical treatment has led most growers to readily adopt a control program. Nevertheless the pest still accounts for significant losses. A biocontrol program may not completely

eliminate the need for chemical control but would facilitate the production of export quality fruit.

None of the other diseases recorded on bananas in the Pacific would warrant special attention at this stage. Many of the other leaf diseases e.g. speckle (*Mycosphaerella musae*), rust (*Uredo musae*) and freckle (*Guignardia musae*) are being kept under control by the black leaf streak program. Their effects in the absence of sprays is not known.

### Recommendations

A banana improvement program for the Pacific region should be aimed primarily at commercial production. Subsistence growers would have access to the improved types if they desired.

First priority must be given to developing effective resistance to the Sigatoka, black leaf streak, Black Sigatoka complex of leaf diseases. Tolerance to nematodes and improved wind resistance would also be desirable attributes. There is a need for improvement in agronomic characters, particularly yield potential and fruit quality.

Although Panama disease is not present in the Pacific, it would be prudent to incorporate resistance to this disease in any cultivars released to the region.

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# Regional Needs for Banana Improvement in Australia

B.W. Cull \*

THE Australian industry is primarily commercially based. An area of some 11 000 ha produces about 200 000 t for a value of A\$80 million/year. The major production is located in coastal areas of northern New South Wales and the south and north of Queensland. Minor production areas are in Western Australia at Carnarvon and the Ord River and near Darwin in the Northern Territory.

The commercial industry has been in a steady state of expansion for the last 20 years. All the fruit is consumed in Australia and it has become the major individual fresh fruit line in the market and therefore a staple food. There is no export of fruit. New Zealand is the only probable, but not likely, export market. Cheaper fruit is available from Central American and South Pacific nations.

Expansion has mainly occurred in the North Queensland area with small new developments in the Ord River and Darwin localities. Land resources for future expansion exist but the market demand is not expected to support any more than a gradual expansion in the future. The Australian market is heavily if not over-supplied with summer fruit of an extremely wide range of species, leaving the winter-spring market period the possible opportunity for expansion.

Backyard bananas are common mainly throughout coastal Queensland. This has an insignificant impact on food production as they are limited by Government legislation to <5 plants/backyard. Their importance lies in the fact they act as a reservoir and breeding ground for pests and disease. Although government legislation exists to control the movement and planting of bananas, and hence the distribution of diseases and pests, this is difficult to police in non-rural areas. There is therefore always a threat of the movement of plants and the spread and build-up of pests and diseases from this backyard source.

The Ord River area of Western Australia, although very small, is expanding and has the land and water potential to become a major banana producer. Expansion will be limited due to competition from other centres closer to markets. It is important to consider both Carnarvon and the Ord River in a plant improvement sense because of the high temperatures and arid conditions experienced.

## Cultivars in Production

The Giant Cavendish banana commands 95% of the production in Australia. The most common named cultivar is Williams, sometimes called William Hybrid. (The term hybrid has no genetic significance.) Mons Marie is a similar, if not the same, clone which has been mixed with Williams and lost its separate identity. The Chinese Cavendish, characterised by retaining the majority of bracts on the rachis, and New Guinea Cavendish are planted on a limited scale.

In recent years there has been a growing interest in new Cavendish clonal material, and Grande Naine, Hsein-Jen-Chiao and Umalag have been imported for evaluation.

Of the non-Cavendish cultivars in production Lady Finger (Pome/Pacha Naadan) an AAB type is the only one with significant commercial production. The main areas of production are southern Queensland and northern New South Wales. Its success as a cultivar depends on the average 57% higher price than Cavendish received per kilogram of fruit. This is related to its slightly more acid flavour, its longer shelf life as a ripe fruit and restricted availability. This price advantage is required as productivity on similar land is in the order of 30–50% of that of Cavendish. It is, however, more cold- and drought-tolerant than Cavendish, making it more suitable for marginal country. Susceptibility to *Fusarium* wilt, race 1, has resulted in a net reduction of area planted over the last 10 years and the long-term future of the cultivar is in jeopardy.

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\* Department of Primary Industries, Maroochy Horticultural Research Station, P.O. Box 5083 SCMC, Nambour, Qld., Australia.



	North Queensland	South Queensland	North NSW	Western Australia	
				Carnarvon	Ord River
<i>Giant Cavendish (Williams)</i>					
Area (ha)	3 020	1 470	320	70	
No. of growers	247	778*	1 998*	144	22
Production (t)	75 075	22 400	72 937	12 903	1 200
<i>Other bananas (mainly Lady Finger)</i>					
Area (ha)	-	490	497	-	-
No. of growers	-	-*	-*	-	-
Production (t)	-	5 096	5 979	-	-

\* No separation of producers of Cavendish and other types is made. A producer who is producing other types will also be likely to produce Cavendish.

Small plantings of horn plantain (Maia Maoli type) have been made in North Queensland and New South Wales. The amount of fruit on the market has not exceeded the curiosity stage hence its long-term market impact cannot be assessed.

Other cultivars found in backyard situations include Sugar (Silk) and Ducasse (Pisang Awak).

#### Form of Production

Only the commercial industry is of economic and social importance in Australia. With the possible exception of the aboriginal settlements in northern Australia backyard plantings are more for ornamentation than as a source of food.

Backyard plants have their economic importance as a problem source of multiplication and movement of diseases and insects.

The North Queensland bananas are grown in the vicinity of 18°S, on flat to rolling land, in wet tropical rainforest conditions around Innisfail-Tully. Further south, between 26°S at Gympie (Queensland) through to Coffs Harbour (30°S) in New South Wales, they are grown above the frost line on hillsides facing from east around through north to northwest. The climate is subtropical with mean monthly maximum and minimum temperatures ranging from 28.4°C to 6.8°C respectively. Rainfall is <2000 mm. Rainfall in North Queensland exceeds 3500 mm while that in southern areas is <2000 mm. In each case rainfall from August to November is very low resulting in high water stress conditions without irrigation.

In the west, the Ord Irrigation area (16°S) is under a tropical monsoonal influence, while Carnarvon at 25°S is a subtropical area in a desert environment. Both western regions are flat land with low-rainfall, irrigated areas, experiencing high temperatures (>40°C) and high evaporative conditions. Mean monthly minima fall to 14.4 and 10.9°C respectively.

All production areas can experience cyclonic and storm damage. Winter chill damage is experienced in varying intensity in all areas except the Ord. The North Queensland area suffers significant chilling only once in 3-5 years, however this can be sudden and severe as the plants do not become gradually preconditioned with the normal advance into winter as found in the south.

#### Agronomic Practices

This section highlights the aspect of commonality and difference with respect to major agronomic production practices which may have a bearing on the plant's performance and hence selection of cultivars. These are outlined in Table 1.

#### Banana Disease Situation

The present disease occurrence and importance to industry situations with respect to the main production areas are outlined in Table 2. In addition, a rating is given of the priority need as expressed by that region for a plant improvement approach to resolve the problem.

*Fusarium* race 4 in Cavendish clones is present in South Queensland and is spreading within the region. Regulation of plant movement and producer awareness has restricted but not halted the movement. Predictions are that it could arise naturally in any banana area with time. The inability to control this disease by chemical or cultural means raises its risk factor very high. Its ability to destroy the Cavendish and Lady Finger industry is obvious. For these reasons it must be considered a high priority in any plant improvement program.

*Fusarium* race 1 is widespread in Australia. Government regulations to prevent its spread have only slowed the rate of spread. Experience with the differential response to cultivars has shown that race 1 in Queensland differs from that in Honduras. Any

**Table 1.** Agronomic requirements of importance to plant selection.

	N. Qld	S. Qld	N. NSW	Carnarvon, WA	Ord R. WA
Land use	Flat & rolling	Hillside	Hillside	Flat	Flat
Spacing	2000-2500 plants/ha	2000 plants/ha	1800 plants/ha	Up to 3300 plants/ha	Up to 3300 plants/ha
Nutrition	High rates of N & K	High rates of N & K	High rates of N & K	Medium rates of N, no K	High rates of N, Medium K
Irrigation usage	High supplementary usage. Sprinkler	Medium to low supplementary usage Sprinkler	Medium to low supplementary usage Sprinkler	Total dependence on irrigation Flood furrow, sprinkler	Total dependence on irrigation Flood furrow, sprinkler
Leaf disease control	Mainly ground some aerial spraying	Mainly ground some aerial spraying	Mainly ground some aerial spraying	Not required	Not required
Weed control	Chemical methods mainly	Chemical	Chemical	Chemical	Chemical
Plant support	Propping & tying	Propping & tying	Propping & tying	Some tying	Some tying & propping
Bunch covering	Yes	Yes	Yes	Yes	Yes
Packaging	Hands in fibre board cartons	Hands in fibre board cartons	Hands in fibre board cartons	Hands in fibre board cartons	Hands in fibre board cartons
Transport to market	Road and rail	Road and rail	Road and rail	Road	Road

new cultivars would need field resistance to this disease.

Black Sigatoka is widespread in the world and has been recorded on the tip of Cape York Peninsula and in the Torres Strait Islands of Australia. Hundreds of kilometres of virtually uninhabited desolate country buffer the commercial production areas of Queensland. With the history of spread of this disease in other parts of the world and the cost of its control, it is recognised as a major threat to Australia.

Root burrowing nematode is a widespread problem in Australia, which necessitates widespread and regular use of nematicides. Total chemical and application cost approximates that which is expended on each of fertilisation and Sigatoka control. Because of the impact on production, cost of chemical and the possible commercial withdrawal of the chemicals, due to environmental or other health issues, the nematode situation is a very significant ongoing problem.

Yellow Sigatoka in its own right requires significant labour and financial outlays to maintain

commercial control. Along with leaf speckle control it does have in Queensland a major influence on spacing and layout to allow for ground spraying. In Western Australia, should it be introduced and become a problem in that environment, then the close planting arrangement, now used to offset high temperature stress, may need to be altered to the detriment of present productivity. A plant improvement program would require a selection approach with respect to all leaf diseases.

Bunchy-top, in the areas in which it occurs, is a continuing cost to the industry through the need for organised manual surveillance and eradication. With such a regulatory system operating, the problem in Southern Queensland and New South Wales seldom becomes a significant loss to an individual. It is possible to maintain economic control of bunchy-top in the Australian situation and therefore ranks lower in priority.

Postharvest fruit diseases do not presently pose a major problem in the industry due to field spraying for other diseases, shed hygiene and fruit treatment. This is one selection aspect requiring close attention

**Table 2.** Commercial importance of diseases. Rating of seen need for plant improvement \*

Disease	N. Qld	S. Qld	N. NSW	Carnarvon, WA	Ord R. WA
Yellow Sigatoka	Very important (8)	Very important (8)	Very important (8)	Not recorded —	Not recorded —
Leaf speckle	Minor (3)	Minor (3)	Minor (3)	Not recorded —	Minor (3)
Cordana leaf spot	Minor (3)	Minor (3)	Minor (2)	Not recorded —	Not recorded —
Black Sigatoka	Not recorded (9)	Not recorded (9)	Not recorded (6)	Not recorded (4)	Not recorded (9)
Fusarium race 1	Present (4)	Widespread (4)	Present (4)	Recorded —	Not recorded —
Fusarium race 4	Not recorded (9)	Medium spread (9)	Recorded once (9)	Not recorded —	Not recorded —
Bunchy-top	Not recorded (5)	Variable spread (5)	Restricted areas (8)	Not recorded —	Not recorded —
Cucumber mosaic virus	Sporadic (1)	Sporadic (1)	Sporadic (1)	Sporadic (2)	Not recorded —
Fruit speckle	Recorded (1)	—	—	Not recorded —	Present (1)
Postharvest fruit diseases	Medium problem (6)	Limited problem (3)	Limited problem (3)	Limited problem (3)	—
Freckle**	Not recorded (2)	Not recorded —	Not recorded —	Not recorded —	Not recorded —
Nematodes <i>Radopholus similis</i>	Very important (7)	Very important (7)	Very important (8)	Important (8)	Important —
<i>Meloidogyne javanica</i>	Present (2)	Present (2)	Present —	Important (8)	Present (5)
<i>Helicotylenchus multicinctus</i>	Present	Present	—	Present	—

\* Ratings are 1 — minimal priority for plant improvement, 10 — highest priority for plant improvement.

\*\* Freckle has been recorded in the Torres Strait Islands to the far north of Queensland.

Yellow Sigatoka — *Mycosphaerella musicola* Fusarium Wilt (Panama) Races 1 & 4 —  
 Leaf Speckle — *Mycosphaerella musae* *Fusarium oxysporum* f. sp. *cubense*  
 Cordana Leaf Spot — *Cordana musae* Fruit Speckle — *Deightoniella torulosa*  
 Black Sigatoka — *Mycosphaerella fijiensis* var. *difformis* Freckle — *Guignardia musae*

to maintain this control capability when developing and selecting new cultivars often selected primarily on field performance.

### Insect Pest Situation

The present insect occurrence and importance to industry situations with respect to the main production areas are outlined in Table 3. In addition a rating is given of the priority need as expressed by that region for a plant improvement approach to resolve the problem.

Appreciating that limited opportunity exists for resolution of insect problems by plant improvement, the pests are discussed in order of industry importance and difficulty of control.

Banana weevil borer is widespread and since resistance to dieldrin has developed it has become

more difficult to manage with less effective, shorter life residual insecticides. Costs related to chemicals and application have escalated. Careful management and disposal of banana trash, old stems and weeds are required to maintain good control of the pest with these newer chemicals. Such care comes at a cost to farmers.

Banana rust thrip has also surfaced as a moderately important pest following the decline in use of dieldrin, which controlled weevil borer and simultaneously controlled rust thrips at no extra cost. The new soil-applied organic phosphates do not give adequate control on their own and bunch spraying with diazinon is required to achieve satisfactory control.

Banana flower thrip is a widespread problem and in some instances, under severe conditions, gives rise

**Table 3.** Commercial importance of insect pests. Rating of seen need for plant improvement\*

	N. Qld	S. Qld	N. NSW	Carnarvon, WA	Ord R. WA
Banana scab moth	Widespread (8)	Not recorded —	Not recorded —	Not recorded —	Not recorded —
Banana fruit caterpillar	Sporadic (1)	Sporadic (3)	—	Not recorded —	Not recorded —
Cluster caterpillar	Sporadic (1)	—	—	Not recorded —	Recorded (1)
Mites	Widespread (6)	Widespread (3)	Widespread (2)	Present (1)	Present (4)
Russet mite	—	—	—	Widespread (3)	—
Banana weevil borer	Widespread (4)	Widespread (4)	Widespread (8)	Not recorded —	Not recorded —
Banana rust thrips	Widespread (7)	Widespread (6)	Widespread (4)	Not recorded —	Not recorded —
Banana flower thrips	Variable spread (6)	Widespread (7)	Widespread (7)	Not recorded —	Not recorded —
Banana aphid	Sporadic (2)	Widespread (2)	Widespread (2)	Not recorded —	Not recorded —
Banana spotting bug	Sporadic (1)	Sporadic (1)	—	Not recorded —	Not recorded —
Red shouldered leaf beetle	Sporadic (1)	Sporadic (1)	Sporadic (1)	Not recorded —	Not recorded —
Banana fruit fly	Widespread (2)	Not present —	Not present —	Not recorded —	Not recorded —

\* Ratings are 1 — minimal priority for plant improvement; 10 — highest priority for plant improvement.

Banana scab moth — *Nacoleia octasema*

Banana fruit caterpillar — *Tiracola plagiata*

Cluster caterpillar — *Spodoptera* spp.

Mites — *Tetranychus Lambi* and *Tetranychus urticae*

Russet mite — *Brevipalpus* sp.

Banana weevil borer — *Cosmopolites sordidus*

Banana rust thrips — *Chaetanaphothrips signipennis*

Banana flower thrips — *Thrips florum*

Banana aphid — *Pentalonia nigronervosa*

Banana spotting bug — *Amblypelta lutescens*

Red shouldered leaf beetle — *Monolepta australis*

Banana fruit fly — *Dacus musae*

to severe fruit scarring referred to as 'corky scab.' The incidence of damage increases under dry conditions. Control of the insect is difficult as it attacks prior to or at bunch emergence.

Banana scab moth is a major problem in North Queensland because it attacks fruit soon after bunch emergence and is particularly troublesome during wet conditions. For both reasons it is difficult to control. The extensive fruit scarring by the insect can result in a significant down-grading of fruit quality and outright rejection. Present chemical and application methods are costly and laborious. No biological control agents are available.

Mites often are an induced problem caused by the removal of natural predators following the use of broad-spectrum insecticides. Mites pose a problem when they attack fruit, leading to skin blemish. Leaf damage is also of concern but its affect on production is difficult to quantify. Management and biological avenues possibly offer viable answers for mite suppression.

### Major Agronomic Requirements for Cavendish Replacement

Any Cavendish replacement would have to approximate if not improve upon Williams with respect to fruit quality, marketing performance and yield. Useful improvements in comparison to Williams would include the following agronomic features ranked in order of importance: (1) shorter cycling period; (2) shorter stature; (3) chilling tolerance; (4) higher performance in close planting; (5) heat tolerance; (6) water stress resistance; (7) more robust stem — less need for support; and (8) bunch drop resistance.

### Other Banana Types

Opportunity exists to expand banana consumption by providing the public with fruit which either satisfies fresh fruit eaters who do not like Cavendish or a new way to use bananas in the daily menu or for entertaining.

Cavendish bananas are too sweet for some

people's taste and a more acid, tangier-flavoured fruit has appeal especially for adults. The Lady Finger banana has appeal in this area but is being lost due to the incursion of *Fusarium* races 1 and 4. A replacement with similar fruit characters to Lady Finger would be very useful especially if it had higher yield.

Variations in flesh colour would give more decorative appeal and use for the fruit. A range of skin and flesh colours occur including red, orange and deep yellow. Similarly very small fruit could have specific use in desserts especially if they were identifiable by flavour or colour.

Cooking forms such as the large plantains could, if well promoted as such, open a sizeable market based on a new dietary niche for banana.

In the selection of other banana types the same or similar disease and insect resistance attributes and agronomic characteristics desired for a Cavendish replacement would hold. This means that any plant improvement program directed to Cavendish replacement could possibly investigate off-types for this purpose. In addition consideration could be given to generating breeding populations based on the main Cavendish replacement breeding material crossed with lines likely to give rise to the above types.

#### **Plant Improvement Requirements**

The growing conditions, agronomic practices and pest and disease pressures vary greatly between growing areas in Australia.

The major environmental differences are the higher rainfall conditions in North 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 influence the agronomic practices in use and also the distribution and relative importance of specific diseases and insect pests.

For these reasons the priorities for plant improvement will vary from locality to locality. An attempt has been made to define long-term priorities based on potential impact on industries and the

likelihood that plant improvement endeavours could resolve the concern under consideration.

#### **Recommended Plant Improvement Priorities**

To meet Australian needs, and taking into consideration the apparent genetic chances of achievement, the following are the priorities to which an international breeding and plant improvement scheme should give emphasis: (1) resistance to *Fusarium* races 1 and 4 (*F. oxysporum* f. sp. *cubense*); (2) resistance to Black Sigatoka (*M. fijiensis* var. *difformis*); (3) resistance to root burrowing nematode (*Radopholus similis*); (4) shorter cycling period while maintaining bunch weight; and (5) cold-chill tolerance. These recommendations apply to both Cavendish replacement and other types of bananas.

Other listed major disease, insect and agronomic factors should be considered as important criteria in a selection program and also when characterising new cultivars.

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# Classification and Breeding of Bananas

N.W. Simmonds \*

BANANAS are of great socioeconomic importance in the moister areas of tropical agriculture. They are soil-conservative, productive, almost non-seasonal and they yield diverse foods from sweet fruits to staple starches as well as numerous useful secondary products, from fibres to wrappings. For all their importance, bananas have been sadly neglected in their food crop role by agricultural research systems. Happily, however, this is now changing with the emergence of INIBAP, and I hope that we are now seeing the beginnings of improved understanding and exploitation of this marvellously attractive and useful group of plants.

## Evolution

### General

The genus *Musa* contains about 30–40 species, all diploids ( $2n = 2x = 14, 18, 20, 22$ ) and all native to Southeast Asia, from India and Thailand to New Guinea and Queensland, Australia. Only two species are of importance for our purpose (*M. acuminata* and *M. balbisiana*) but the genus also contains Manila hemp (abaca, *Musa textilis*). The related genus *Ensete* is of great local economic importance in Ethiopia, where it is the foundation of a unique agriculture.

*Musa acuminata* (AA) and *Musa balbisiana* (BB) are both diploids with  $2n = 22$ . The first (and crucial) step in the evolution of the edible bananas was the development, under human selection, of parthenocarpy and seed-sterility in *M. acuminata*. This gave rise, in Southeast Asia, to the edible diploid cultivars (AA), which survive in some numbers to this day, though economically of little importance. Parthenocarpy is the capacity of the fruits to grow and become full of edible parenchymatous pulp without pollination. Seed sterility is due to cytogenetic factors and is also very important because banana seeds are stony and most

unpleasant to encounter. Edibility, therefore, is parthenocarpy plus seed sterility.

From the AA cultivars, by chromosome restitution at meiosis, there arose the AAA (*acuminata*) triploids, one of the three most important groups. They include both the important export cultivars but many others as well.

Another important step, also taken in Southeast Asia, was the crossing of AA (and perhaps AAA) cultivars with wild *Musa balbisiana* (BB). *Musa balbisiana* is a hardier and more drought-tolerant plant than *M. acuminata*, so the hybrid groups not only extended the range of plant characters and quality features but also helped to extend the geographical range of the bananas out of the wetter tropics into the seasonally drier zone. I know of no good evidence to support the contention that there are also BBB cultivars: i.e. that parthenocarpy also evolved in *M. balbisiana*.

In Southeast Asia, the bananas are probably several thousand years old. About 2000 years ago, they spread in the hands of travellers, eastwards to the remoter Pacific Islands and westwards to Africa (probably via Madagascar). The first European visitors to West Africa found them there and several clones were taken to the New World very soon after discovery. There, the crop spread very rapidly. The present distribution is roughly 30° north and south and bananas are grown wherever there is frost-freedom and enough rain. The history of the crop in Africa still presents many problems and it is not clear why the varieties grown in eastern and western Africa should be so different if they had a common source (Simmonds 1962, 1976).

### Classification

Systematic scoring of characters diagnostic of the two parental species and chromosome counting jointly suffice to diagnose the main cultivated groups. They are designated by genome constitution, thus: AA, AAA, AB, AAB, ABBB (Simmonds and Shepherd 1955; Simmonds 1966; IBPGR 1983). The other groups (AAAB, AABB — Richardson et al. 1965) have not yet been fully

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\* School of Agriculture, University of Edinburgh, Edinburgh, Scotland.

classified but should present no great difficulty. A descriptor list has been published (IBPGR 1983).

Ploidy, of course, is normally established by chromosome counting but the experienced observer can usually diagnose it by eye. It does take experience to do so, however. Diploids are slimmer and have more erect leaves than polyploids. Tetraploids tend to have heavier, drooping leaves, with some breakage at the top of the petiole.

Within each group there are clones which can formally be referred to thus when required: *Musa* AA Group cv. 'Sucrier' and *Musa* ABB Group cv. 'Bluggoe.' There are also many somatic mutants that affect many characters. Sometimes they receive distinct names (as 'Highgate' is a dwarf mutant of 'Gros Michel' and 'Rajapuri' a dwarf mutant of 'Nendra Padaththi'). Occasionally, there are very many mutants, when it may be convenient to refer, for example, to AAB plantain subgroup (informally, the plantain group).

### Characteristics

It is generally believed that triploids are dominant among the cultivated bananas because they have been selected for superiority over diploids in terms of vegetative vigour and yield. This is probably true but has not been formally tested. Tetraploids are about as vigorous and productive as triploids though some workers believe that the former are a little inferior on balance, due to leaf breakage. Tetraploids must be an important feature of any banana breeding program, either as producing clones or as parents, or both.

I said above that *balbisaniana* genomes were important for diverse quality characters as well as for conferring hardiness and disease resistance. Broadly, B genomes give starchiness and acidity to the fruit, characters which are features of cooking quality to many consumers. But it should be noted that starchiness depends greatly on ripeness, so potentially sweet (AAA) bananas are starchy and non-sweet if cooked green (as many people do, in fact, cook them). As to hardiness and disease resistance, *Musa balbisaniana* itself always looks better in drought than any cultivar and it is, in effect, disease-free: I cannot recall ever having seen it infected by Panama disease, leaf spot or nematodes. Cultivars containing B genomes are not so hardy nor so resistant as the wild species itself but they are, on balance, I think, better in both respects than AA or AAA clones, and the ABB group is certainly pretty tough.

I must emphasise a point made above which is rather widely misunderstood. All bananas are starchy foodstuffs if they are cooked green, whatever their potential for sweetness if ripe. Therefore ripening characteristics must not be

allowed to impose any restraints on the food crop breeder.

### Agricultural Importance

The *acuminata* groups, AA, AAA and AAAA, are of very unequal importance. Only one AA clone ('Sucrier') is widespread but others persist in their original haunts in Southeast Asia, from Malaysia to New Guinea, with a few representatives in coastal East Africa (Shepherd 1957). Their principal value lies in breeding potential as male parent breeding stocks (see below). The AAA group is widespread and very important indeed. It provides the two leading export cultivars (and their mutants): 'Gros Michel' and the 'Cavendish subgroup'; also the leading clones used in the great food-crop cultivations of upland East Africa, around Lake Victoria (Shepherd 1957). The latter, though AAA clones, are *treated* as cooking bananas by the grower/consumers. A number of good AAAA clones exist but are yet virtually unexploited; they were bred for export but must have great potential as starchy food crop varieties.

Of the hybrid groups, AB, AAAB, AABB, and ABBB are all scarce and unimportant (food-crop breeding might change that). The AAB and ABB groups, by contrast, are very important indeed. Several AAB clones such as 'Mysore' and 'Silk' are widespread and highly valued as producers of fresh fruit, 'Mysore' being particularly vigorous and productive and the leading variety in India. Another important element in AAB is the plantain subgroup, the most complex assemblage of mutants in the bananas. The plantains are especially abundant in West Africa (and are characterised there by remarkable diversity of mutants) and also in parts of Tropical America. They are unimportant in upland East Africa and uncommon in Southeast Asia. One notes that, though of the same taxonomic group as 'Mysore' and 'Silk,' the plantains are distinctively cooking bananas, so the AAB Group is diverse in a culinary sense.

The ABB clones are fairly numerous, especially in India and parts of Southeast Asia and, being two-thirds *balbisaniana*, are essentially all cooking bananas. 'Bluggoe' and 'Saba' are widespread, locally very important and they are tough, hardy plants. IITA is now multiplying ABB clones in West Africa to replace the plantains severely attacked there by Black Sigatoka disease.

### Breeding

#### Commercial Breeding

Breeding has been carried on for many years in the West Indies (Trinidad and Jamaica) and in Honduras (United Fruit Company). The object was to breed new export bananas. Both programs

produced clones that, it is believed, could have been exported but, in fact, were not.

Both programs have now been wound down, so that there is no longer any active banana breeding in progress but there remains the cytogenetic knowledge, the 'know-how' about banana breeding, to serve as a basis for food-crop breeding (Simmonds 1966; Shepherd 1968; Menendez and Shepherd 1975; Rowe and Richardson 1975; Rowe 1981, 1983).

The breeding plan developed was simple: first, breed good, disease-resistant and pollen-fertile diploids (AA) and then cross them onto the semi-dwarf mutant ('Highgate') of 'Gros Michel.' Very few seeds are obtained (about one per bunch) but those few yield a small proportion of tetraploids ((AAA) + A = AAAA) which are potentially commercial. The best are very good indeed, not too tall, vigorous, productive and disease-resistant, but often of dubious shipping quality. They have considerable local food-production potential, and are now being exploited in Jamaica. The key to commercial breeding lies in effective diploid improvement, hence the crucial importance of good collections of wild *acuminata* and edible diploid cultivars.

Dwarfness is important because tetraploids out of full-sized triploids are generally too big. By good luck, the dwarf mutation of 'Gros Michel' is semi-dominant, so the AAAA progeny are a good, medium size. We may reasonably assume that the same genetic principle will hold elsewhere, so a food-crop breeding program will need dwarf mutants for parents (see below).

New triploids can be made from the cross (A)A × (AA)AA or reciprocal but have so far not proved commercially attractive, though admittedly not yet widely explored. The principle of diploid × tetraploid and reciprocal as a source of triploids is highly relevant to food-crop breeding.

The importance of good collections will be evident (IBPGR 1978). The best are held in Jamaica and Honduras and there are less extensive ones in Brazil, West Africa, Indonesia and the Philippines. The Philippine collection is being built up as a major international centre (IBPGR 1978, 1983). For commercial breeding, the AA bananas, wild and cultivated, are crucial; for food-crop breeding, the range of female clones must be much wider but the synthetic AA male parents remain critical (see below).

### Food-crop breeding

Local tastes in bananas vary widely. People prefer what they are accustomed to and are usually unwilling to change. The object of food-crop breeding must be to produce a wide range of clones, having diverse field and quality characters that can

be adapted to local circumstances. We need not assume that preferences are immutable: under, say, disease stress, new clones may be essential if bananas are to be grown at all. There is plenty of experience to say that tastes can change if they must change. IITA experience with ABB clones as replacements for plantains will teach us something about this matter.

There is little information on food-crop breeding as such but the following points are relevant: (a) some edible bananas are quite sterile but most will give at least a few seeds if pollinated and their progeny are roughly predictable as to genome constitution; (b) the plantains are thought to be sterile but may yet give progeny (Rowe 1981, 1983); (c) given the wide range of fruit qualities required, the possible crosses to be explored are very numerous and the useful outcomes might be either triploid or tetraploid (but probably not diploid); (d) disease-resistant AA male parents remain important in any program (excellent ones already exist) but there will be need also of wider exploration of B genomes; (e) dwarfness will have to be used in many crosses to avoid the production of oversized tetraploids.

The objectives of a food-crop program must be rather widely, perhaps even loosely, stated until enough experience accumulates to permit more precise definition. They will include: (a) yield (though not necessarily on a pure-stand, t/ha basis); (b) aptitude for shade-nurse-intercropping use; (c) diverse fruit qualities, from sweet to acid-starchy, but with emphasis on the starch potential of unripe fruit; (d) diverse disease resistances, though not (to be realistic) all assembled in one clone.

The diseases to be considered include: Panama disease (banana wilt), Moko disease (bacterial wilt), Yellow Sigatoka disease (leaf spot), Black Sigatoka (black leaf streak), burrowing nematodes and bunchy-top virus. We know something about resistances but not yet nearly enough. We must assume, I think, that expensive chemical control measures against diseases will generally be irrelevant for small farmers. At this moment it looks as though peculiar threats are posed by: Black Sigatoka, which has recently become established in West Africa and Central America and is certain to spread; and bunchy-top, widely spread in Southeast Asia, India and West and Central Africa but not in the Americas; its status is ambiguous but it is potentially very damaging. Nematodes cause more insidious but certainly widespread damage.

There is a vital technical adjunct to any international food-crop breeding program, namely, shoot-tip (meristem) culture. The technique is quite simple and already well proven. It will be very useful for multiplication of new clones and extremely useful, even critical, for the international



dissemination of clones through plant quarantine systems. Since the sensible first step in any banana food-crop program must be the wide dissemination of the many useful bananas that are not already generally dispersed, the practical importance of shoot-tip culture can hardly be overemphasised. The IITA program in West Africa emphasises it strongly. A point about shoot-top culture which will need attention is the possibility of somaclonal variation on a scale much larger than would have been expected *a priori*. Several recent studies have revealed this; sometimes it will be a nuisance but the chance of picking up useful mutants (especially dwarfs) will no doubt be kept in view.

### **International Banana and Plantain Improvement**

#### **Breeding Priorities**

I think that the order of priority for exploitation of the various combinations should probably be as follows: (1) AAB, ABB; (2) AAAB, AABB; (3) AAA, AAAA, ABBB. But any such ordering must change as experience accumulates. Some combinations may turn out, by reason of sterility, to be just too difficult to exploit effectively, however attractive they might seem.

#### **Requirements**

Besides the obvious requirements for adequate field and laboratory facilities, expert staff and an excellent collection of plants, the following are also apparent as requirements for the successful development of the breeding program:

- (1) continuing attention to the development of excellent edible diploid AA types as male parents;
- (2) wider exploitation of B genomes, including the synthesis of diverse tetraploids, BBBB;
- (3) better knowledge and exploitation of the yet little known AAAB and AABB tetraploids;
- (4) extensive accumulation of dwarf and semi-dwarf mutants as parents (some will be found, others might be made by somaclonal methods);
- (5) systems of disease screening, some of which will have to be off-station, perhaps far distant; and
- (6) fluent meristem culture techniques and close liaison with quarantine authorities.

#### **Importance of Dwarfs**

I repeatedly refer to semi-dwarfs because I believe they will be of immense value. They have been overwhelmingly adopted by the export trades but will be, I think, of equal value to small farmers because:

- (1) they give high yields at high densities and thus permit exploitation of good patches of land;
- (2) high density permits (but does not necessarily imply) high inputs of other kinds;

(3) weed control is enhanced and sucker pruning reduced;

(4) there is less loss from wind, harvesting is easier and propping bunches against nematode damage is also easier;

(5) broadly, they imply high yields and easy-care at a little extra expense for planting material.

#### **Other Research**

There are several subjects that are hardly central to the breeding program but that would, if better understood, contribute to breeding:

(1) better knowledge of the B genome contribution, specifically: (a) are there or are there not BBB clones and, if there are, where are the progenitor BB types? (b) how many AAAB and AAB clones are there, where do they come from and what use are they?

(2) what is the extent and potential of the new clone group recognised by Shepherd as probably having a contribution from *Musa schizocarpa*?

(3) the field effects of ploidy on productivity evaluated across diverse genome and stature combinations; comparisons would inevitably be unbalanced but enlightening nevertheless;

(4) biomass/partition effects of degrees of dwarfness, evaluated over spacings and again, over genome combinations; and

(5) critical studies of the simpler aspects of ripening biochemistry in relation to nutritional features and genome constitution.

#### **Institutional**

INIBAP will have diverse tasks of an agronomic, socioeconomic, pathological, etc. nature. But the heart of its activities surely lies in the effective exploitation of banana clones for the agricultural good. Some progress will be made with existing clones, so the first step should be the identification, collection, dissemination and evaluation of what is already there but little used, even more or less unused. These tasks must lie essentially with the country organisations, drawn together and helped by the INIBAP regional networks. In the slightly longer term, only new breeding will meet the needs. At first, anyway, it will have to be of an international character constructed to meet the needs of the very diverse participants in INIBAP. Later (probably much later) local breeding programs may develop. The prospects of success seem to me to be excellent because the program starts from scratch, we have a fair base of scientific knowledge and we have fair to good collections in hand. The free and unrestricted movement of genetic material is a *sine qua non*, as also is the development of fluent and efficient methods of passing materials through quarantine.

Thus, structurally, we have the local and regional

activities of INIBAP concerned with the exploitation of genetic material and its major international activity concerned with generating a flow of new varieties for the general good.

### Summary

The cultivated bananas are derived from two wild species native to Southeast Asia: *Musa acuminata* (AA) and *M. balbisiana* (BB). Parthenocarpy and seed-sterility, evolved under human selection, jointly constitute edibility. The edible bananas evolved in Southeast Asia from edible diploid (AA) progenitors and spread throughout the tropics to the moister areas between 30° north and south.

Bananas constitute a hybrid-polyploid complex and are classified by genome constitution thus: AA, AAA, AAAA, AB, AAB, AAAB, AABB, ABBB. Broadly, *balbisiana* (B) genomes confer hardiness, disease resistance and acid-starchy fruit quality.

The scientific bases of commercial banana breeding, aimed at export clones, are fairly well understood, though neither of the two major programs has been successful. Fairly good collections are available and excellent diploid male parents (AA) have been synthesised.

The established scientific principles can be carried over into food-crop breeding programs, with much broader objectives and genetically much more diverse outputs of potentially useful varieties, all polyploid and mostly hybrid. Shoot-tip culture, already a well-established technique, will be of great

value for multiplication and dissemination of clones, both existing ones and newly-bred ones, in due course.

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# Banana Breeding in Honduras

Phillip Rowe \*

HISTORICALLY, bananas and plantains have received limited attention by international agencies concerned with protection of food crops. This has been due primarily to the long period of freedom from those diseases which presented serious threats to production, and to the fact that the transnational banana-exporting companies traditionally had supported research on these crops. Both of these situations which formerly provided security for continued cultivation have recently changed. The varieties grown are presently endangered by the appearance and spread of new diseases, and the transnational company which supported a banana and plantain breeding program for many years has now donated this program to an institution which must seek funding for the continuation of these activities.

The United Fruit Company (now United Brands) anticipated the importance of genetic diversity for assuring the continued production of bananas and began an extensive breeding program in 1959 in Honduras. With the advent of Black Sigatoka, breeding of plantains also became an integral part of this program. Unfortunately, due to its unilateral expense in this endeavour and the economic pressure of a competitive market, the company announced in 1983 that it would no longer be able to continue funding the program.

In recognition of the importance of banana and plantain breeding to the worldwide community of producers and consumers, United Brands unconditionally donated the program in its entirety to the Fundación Hondureña de Investigación Agrícola (FHIA). Financing for 1984 was with Technical Cooperation Project funds from the Food and Agriculture Organization (FAO) of the United Nations. These FAO funds were stipulated for 1 year only, and the present limited funding is provided by the International Development Research Centre (IDRC) of Canada and the governments of Honduras and Ecuador.

The English translation for FHIA is Honduran Foundation for Agricultural Research. The concept of FHIA coincided with the decision by United Brands Company to discontinue the traditional large-scale activities of its Division of Tropical Research. With the donation of these Tropical Research facilities, and the technical and financial support of the U.S. Agency for International Development (USAID) and the Honduran government, FHIA was begun in July 1984 as a private, public service, non-profit foundation dedicated exclusively to agricultural research.

As the organisation responsible for the breeding program, FHIA provides the necessary land, buildings, machinery, technical assistance and administrative services free of charge. However, financing for the major activities of the program must come from other sources. This arrangement is due to the international scope of the breeding program, as compared to the primarily national nature of the other functions of FHIA.

Activities and accomplishments of the program have been described in two comprehensive publications (Rowe and Richardson 1975; Rowe 1984). The germplasm collection consists of more than 450 accessions, and diploid sources of resistance to all the current major diseases (Black Sigatoka, burrowing nematode, and race 4 of Panama disease) were identified in this collection. Only bred diploids which are agronomically superior to the parental accessions are now being used as sources of disease resistance, and the original collection is maintained as a gene pool in the event that it becomes necessary to evaluate this material for diploids resistant to new pathogens or new races of the current pathogens.

As progress has been made in diploid breeding, the diploid breeding lines for subsequent cross-pollinations have become less female-fertile. Thus, like triploids and tetraploids employed as fixed female parental lines in breeding for commercial-type bananas and plantains, most of the advanced diploids require that numerous bunches be pollinated in order to obtain adequate quantities of seeds.

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\* Fundación Hondureña de Investigación Agrícola (FHIA), P.O. Box 2067, San Pedro Sula, Honduras.

Formerly, only a limited number of bunches could be pollinated due to the laborious process of extracting seeds by hand. Now, the quantity of bunches which can be examined is no longer a restriction since the only labour-intensive phase of seed extraction is peeling the fruit. The bunches are placed in rooms made of simple frame structures covered with polyethylene, and applications of 1000 ppm acetylene (generated from calcium carbide and water) for one night to induce uniform ripening. The peeled fruit is then left to ferment one day before most of the pulp is separated from the seeds with a mechanical press. The seeds are easily and quickly removed from the small amount of pulp which remains.

The embryo culture techniques (Cox et al. 1960) which was first used in the Jamaican program (Menendez and Shepherd 1975) has greatly increased the germination rates of the sparse quantities of seeds obtained from many of the most desired cross-pollinations. Greenhouse and nursery facilities are adequate for large quantities of plantlets, and use of steam-sterilised potting soil minimises losses. Approximately 80 ha are available for field plantings.

The staff has recently been strengthened with the addition of a second professional plant breeder. Breeding strategies have now been broadened to investigate the feasibility of colchicine treatment for producing polyploids from diploids as proposed by Stover and Buddenhagen (1986).

The policy of FHIA is to provide the commercial-type banana and plantain hybrids developed, to those countries and institutions which help fund the program. The status of the program with regard to development of new varieties is presented in the following section.

### **Plant Improvement Approaches**

Due to the fixed nature of Highgate as the triploid female parent in cross-pollinations with diploids for production of tetraploids, all genetic improvements in bananas are dependent upon the diploid pollen parents. Indeed, banana breeding has often been defined as diploid breeding. Some recent very encouraging developments in the FHIA program can be more properly appreciated by considering what was involved in producing the diploids which led to these developments.

All the diploids with which the program was begun in 1959 had small bunches. By growing out literally tens of thousands of hybrids from widely diverse crosses between these diploid accessions, the SH-2095 diploid which has outstanding bunch features was selected. This was undoubtedly the most important accomplishment during the first decade of the program. Unfortunately, SH-2095

does not have good pollen, so it could serve only as a female plant in its subsequent use in breeding.

A few thousand bunches of SH-2095 were pollinated with many different diploids in attempts to produce hybrids with bunches like SH-2095, but with good pollen. The best progeny selected from these hybrids was SH-3217. Since it is seed- and pollen-fertile, SH-3217 provided the advanced agronomic qualities in a parental line which could be used both as male and female in further cross-pollinations for diploid improvement.

Early emphasis on breeding for agronomic qualities was necessary due to the inferior agronomic features of the disease-resistant diploids. Once agronomically advanced hybrids were available, the goal became to develop diploids with combinations of agronomic excellence and resistance to the three primary diseases, Black Sigatoka, the burrowing nematode, and race 4 of Panama disease.

The IV-9 subspecies *burmanica* accession was the best source of resistance to Black Sigatoka in the germplasm collection. In fact, this resistance to Black Sigatoka is the only redeeming feature of IV-9 since its less than 2.0 kg non-parthenocarpic bunch is among the poorest of all the wild fully-seeded diploids. In efforts to produce resistant hybrids with improved bunch qualities, many different advanced diploids were crossed with IV-9 and some 2500 hybrids were planted in the field for evaluation. Only one hybrid, SH-2989, which was resistant to Black Sigatoka and had a much better bunch than IV-9 merited selection from this huge population.

With SH-2989 as the new better source of resistance, extensive crosses were begun with this hybrid to further improve bunch qualities. Several hundred bunches of SH-2989 were pollinated and some 500 progenies were obtained for field observation. One hybrid, SH-3437, which has a high level of resistance to Black Sigatoka and bunch weights of up to 35 kg was selected from these segregating populations. The importance of agronomically superior diploids is evident from the fact that SH-3217 (which was described earlier) was the other parent of SH-3437. Presently, SH-3437 is being used very extensively in cross-pollinations both with other diploids to obtain even better resistant breeding lines and onto polyploids to produce hybrids which will be evaluated for commercial characteristics.

The Pisang Jari Buaya (PJB) nematode-resistant clones have relatively good bunch features, but their lack of pollen, very sparse seed production, and low percentage of seed germination were hindrances to using them in cross-pollinations. Some 10 000 bunches of PJB were pollinated with several different diploids and a few hybrid seedlings were

obtained by using embryo culture to germinate the very limited numbers of seeds produced. Only one selected hybrid, SH-3142, resulted from this very exhaustive endeavour, but it proved to have outstanding qualities. It is the only known male and female fertile source of resistance to the burrowing nematode, and subsequent tests showed that it is also resistant to the new race 4 of the Panama disease pathogen which is now attacking the Cavendish commercial clones in some countries. In addition to these disease-resistance properties, SH-3142 has a nice bunch and provides a much needed source of genetic diversity in breeding for agronomic qualities.

These accounts of obstacles and accomplishments show that banana breeding was for many years handicapped by the time required to upgrade the diploids which could be used to breed commercial hybrids. Now, some recently selected tetraploids have illustrated that these breakthroughs described in diploid breeding have placed banana breeding in the realm of reality. Bunch features of the SH-3446 tetraploid from the cross, Highgate  $\times$  SH-3362, are shown (Fig. 1). This 15-hand bunch weighed 71 kg and the fruit flavour was very good. To place this achievement in proper perspective, it is noteworthy that the parental lines of SH-3362 are the diploids SH-3217 and SH-3142 which were results of the very extensive efforts explained earlier.

Another recently selected tetraploid, SH-3445, had a 61-kg bunch. The diploid parent of this tetraploid was SH-3413 which was derived from a cross between SH-2095 and SH-3142. The bunches of SH-3445 and SH-3446 are the largest which have been observed of tetraploids. These are just the kind of bunches needed in parental lines for  $4N \times 2N$  crosses for producing triploids for commercial possibilities. The open-pollinated bunch of SH-3445 had one seed, so it is already known that seeds will be produced in this tetraploid under controlled pollinations. The leaves of SH-3445 were completely free of Black Sigatoka spotting, but a more reliable reading will be made when additional plants are available for evaluation.

While the above references have been to bananas, it should be emphasised that preliminary results have shown that the bred diploids are equally useful in breeding plantains. Indeed, some progenies from crosses of diploids onto the AVP-67 seed-fertile plantain were quite good in regard to plantain-like appearance and flavour. The number of bunches pollinated in this plantain breeding effort have been increased greatly with the multiplication of AVP-67 to several hundred plants, and the results from crosses like AVP-67  $\times$  SH-3437 are being awaited with a great deal of optimism.

Approaches to plantain breeding have now been expanded to also include the seed-fertile Maqueño,

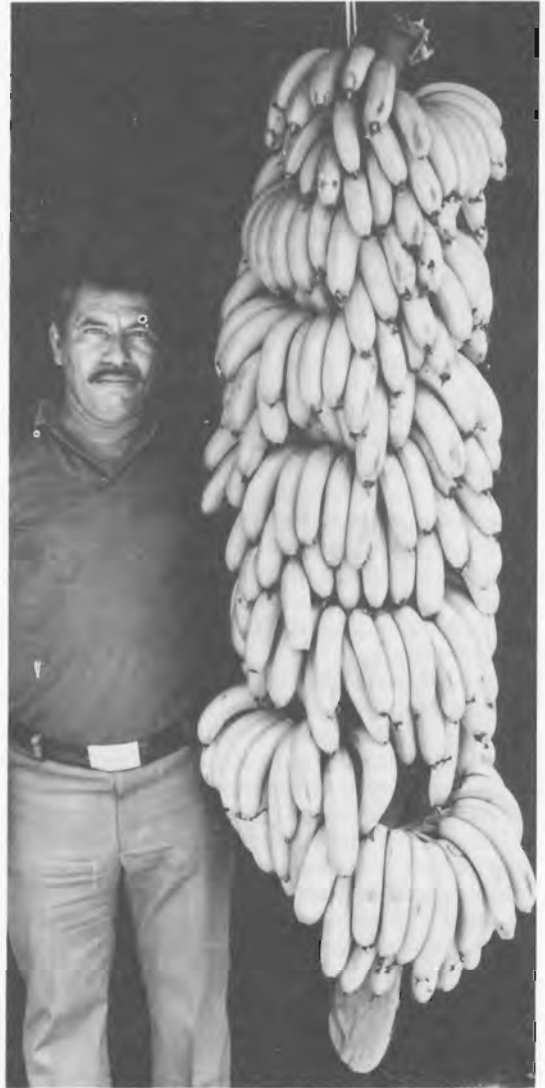


Fig. 1. Bunch features of the SH-3446 tetraploid which was derived from the cross, Highgate  $\times$  SH-3362.

an AAB clone from Ecuador, as a fixed female parent. Maqueño has the largest bunch size of any known cooking-type clone and has an excellent flavour. Like the other AAB plantains, it is susceptible to Black Sigatoka, but offers an excellent parent for more diversity in breeding for disease-resistant plantains. A pollination block of 250 Maqueño plants will begin to flower in a few months, and additional increases of this parental line are planned.

### Recommendations

Conventional breeding of *Musa* has long been

handicapped by the inferiority of the available disease-resistant diploids in regard to agronomic qualities. The progress described and results shown illustrate that the current bred diploids provide a basis for concluding that this approach to genetic improvement is at a very advanced stage of development. It is now apparent that diploids once considered as parental lines for only banana breeding are equally useful in plantain breeding. The only obvious obstacle to further progress is the large number of bunches, of both diploids and polyploids, which must be pollinated to obtain optimum quantities of seeds. Adequate funding would provide for developing new disease-resistant banana and plantain hybrids at a much faster pace. The urgent need for new varieties, especially for Black Sigatoka-resistant plantains, is indicative that this conventional approach to genetic improvement

should receive high priority.

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# Banana Breeding in Brazil

K. Shepherd, J.L.L. Dantas, and E.J. Alves \*

THE Brazilian banana breeding program is located in the Centro Nacional de Pesquisa de Mandioca e Fruticultura (CNPMPF), one of the national centres of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), situated at Cruz das Almas in Bahia State. The program effectively started with the first controlled pollinations of inflorescences at the end of 1982, although at that time the useful available germplasm was severely restricted. The initial collection included the country's cultivars, with some duplications and a very few omissions, and with fertile diploid material comprising five accessions of wild *Musa acuminata*, received as seeds from Jamaica, three of *M. balbisiana* and one of *M. ornata*.

## Breeding Aims

The AAA group is not the major component of Brazil's vast banana production, of which fruit for export is not an important proportion. No high priority has been assigned to the genetic improvement of the group. Nevertheless, it has been confirmed that clones of the Cavendish subgroup are as seed-sterile in Bahia as has been reported elsewhere. Furthermore, a small stock of 'Highgate' is maintained for possible future exploitation.

The usual Brazilian banana preference is for dessert varieties of the AAB group which, despite their smaller fruit size and low productivity, command a higher unit price on domestic markets. 'Maçã' ('Silk') was formerly very important, until widely destroyed by Panama disease, and now 'Prata' ('Pome') occupies much the largest area under cultivation, sometimes substituted by its larger-fruited mutant 'Pacovan.' 'Prata Anã' is a semi-dwarf cultivar not derived from 'Prata' but of closely similar fruit flavour. It has been cultivated chiefly in the southern, subtropical state of Santa

Catarina but lately has been recommended as a more productive (higher planting density) replacement for 'Prata' in the tropical states. 'Mysore' is a fairly recent introduction which is only starting to establish itself with growers and consumers.

These are the cultivars of highest priority in the Brazilian program, for improvement or substitution, and their principal individual defects, to be remedied as far as possible, are shown in Table 1. Black Sigatoka has not been included since it has not yet reached Brazil. For want of more precise information it is assumed that all of these AAB dessert types will prove to be susceptible and resistance to the disease is therefore a major objective. Universal susceptibility to Moko disease is also considered as highly likely although published information on this is scanty, as is information on resistance sources accessible for hybridisation. Resistance has not been treated as a formal objective. Nematodes have not been shown to be a serious pest of these cultivars, which also may not be among the most prone to attack by *Cosmopolites sordidus*.

Apart from disease problems none of them produces a conspicuously heavy bunch and none of them crops at rapid intervals. Breeding for enhanced productivity is an additional and realistic aim, whether arising from shorter stature and higher planting densities, from shorter cropping intervals, from higher fruit numbers per bunch or from larger fruits.

Attention is also given to plantains, which make a significant contribution to the basic food supply in certain regions of Brazil. One of these is the Amazon basin where Moko disease is prevalent. Current efforts are concentrated on eradication of the disease but acceptable new varieties of cooking banana would clearly be of value if resistant to Moko and also resistant to Black Sigatoka.

## Germplasm Bank and its Utilisation

Even before the first pollinations, the labour had started of rapidly constructing a large and diverse

\* Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Centro Nacional de Pesquisa de Mandioca e Fruticultura, Caixa Postal 007, CEP 44.380, Cruz das Almas, Bahia, Brazil.

**Table 1.** Principal defects of AAB group dessert cultivars in Brazil.

Cultivar	Panama disease (race 1?)	Yellow Sigatoka	Plant stature	Cycle of production	Fruits per bunch	Fruit size
'Maçã' (Silk)	extremely susceptible		tall		few	small
'Mysore'			tall			very small
'Prata' ('Pome')	susceptible	susceptible	very tall	very slow	few	small
'Pacovan' (mutant)	susceptible	susceptible	very tall	very slow	few	small
'Prata Anã'	susceptible	susceptible			few	small

**Table 2.** Origins and numbers of imported accessions in Brazil's breeding program.

Type of material	Jamaica	Honduras	Ecuador	Costa Rica	French Antilles	Papua New Guinea	Thailand	Java	Hawaii
<i>Species:</i>									
<i>M. acuminata</i>	5	3	—	—	4	1	5	4	1
<i>M. balbisiana</i>	—	—	—	—	1	—	—	1	1
Other	—	—	—	—	—	—	1	—	—
<i>Cultivars:</i>									
AA	—	7	—	—	—	39	7	5	1
AS	—	—	—	—	—	2	—	—	—
AAA	—	1	1	3	3	27	9	4	1
AAB	—	1	2	1	14	10	6	5	4
ABB	—	2	1	3	6	—	5	1	1
Tetraploids	—	—	—	—	—	2	1	—	—
AA hybrids	—	—	5	—	—	—	—	—	—

germplasm bank, with emphasis on the collection and maintenance of wild and cultivated fertile diploids. To add to the wild material previously secured from Jamaica, more accessions of both types were donated by the Honduras collection in 1982 and two major collecting missions have been mounted, in 1982 and 1985. Existing national collections in Asia have been visited and some have contributed material to the Bahia bank, augmented also by visits to Hawaii, Ecuador and the French Antilles, as well as by further donations. The present content of imported stocks in the field is summarised in Table 2. Duplications of genotypes undoubtedly exist but these are not believed to represent a substantial number. It may be noted that wild species other than *M. acuminata* and *M. balbisiana* are poorly represented.

Establishment of new accessions has been accompanied, where advisable, by chromosome counts. Morphological characterisation of cultivars is in progress, based on a system of descriptors which has been developed in Brazil. Finally, by repeated pollinations, assessments are being made of seed productivity, both of diploid and polyploid cultivars. At least one starchy fruited AAB accession from New Guinea may prove to be a source of

plantain-like tetraploids. Others of both AAA and AAB Groups could merit tests of productivity and acceptance as cooking bananas.

Three hybridisation projects were launched initially, within the limitations of the germplasm available, and a fourth is contemplated, as follows: (1) genetic improvement by recombination in diploid AA germplasm; (2) feasibility of modifying diverse existing triploid genotypes by tetraploid hybrid production; (3) feasibility of synthesising new triploid AAB genotypes directly from crosses of diploids (AB × AA); (4) production of new triploid AAB recombinants from tetraploid-diploid crosses.

#### Breeding Improved AA Diploids

As is generally agreed, such improved diploids are fundamental to the success of other hybridisation methods for the production of new polyploid cultivars. Consequently, Brazil cannot expect optimal results until diploid breeding is reasonably advanced in Bahia or, alternatively, until the program has ready access to advanced diploids selected elsewhere.

In an early phase of the relevant project in Bahia, a neglected aspect is being explored of possible gains by hybridisation between subspecies of *M.*



*acuminata*. As in the Jamaican program but with a different accession, ssp. *banksii* has shown promise in the fruit size of its hybrids, which might offer a useful bridge between the other, smaller-fruited subspecies and later generations of parthenocarpic hybrids. Presently in progress also is a broad and empirical survey of hybrids between wild forms and diploid AA cultivars, hoping to establish which of these latter have most potential in transmitting good bunch and fruit characters.

Altogether, 3150 AA hybrids have gone to the field between October 1983 and August 1986, of which number about 2500 remain in the stage of primary evaluation as single plants of each genotype.

### **Breeding Tetraploid Hybrids of Triploid Cultivars**

The principal results have been obtained with female parents of the AAB group and the preliminary ones solely with wild type pollens of *M. acuminata*. The most intensively used pollen was of ssp. *burmanica*, because of its high potency in seed setting and of the possible inheritance of resistance to *Mycosphaerella*. The analysis had to consider rates of seed set, germination capacity of the seeds and the proportion of tetraploid plants generated, leading to the following conclusions (Shepherd et al. 1985, 1986):

'*Maçã*' ('Silk'): seed production is fair, germination is very poor and no tetraploid hybrid has yet been identified;

'*Mysore*': seed production in Bahia is satisfactory to good only from pollinations in the hot, dry season, germination is very poor but the tetraploid frequency is high;

*Prata subgroup*: seed production, germination and tetraploid frequency are all satisfactory;

'*Prata Anã*': seed production is quite low but germination and tetraploid frequency are both satisfactory;

'*Terra*' (tall French plantain): occasional seeds have been found but none has yet germinated;

'*Terrinha*' (small French plantain): seed production is fair if sporadic, germination is very poor with some tetraploid hybrids;

*various false horn plantains*: no seeds have been obtained.

On the aspect of germination, the in vitro culture of excised mature embryos has been tried but has so far given no better success than natural sowings. One reason for this is that a significant proportion of apparently sound seeds from AAB fruits is either lacking an embryo or has the embryo variously defective.

A serious fall in seed production has resulted from a switch in pollens, from wild type to those of cultivars or parthenocarpic hybrids. It seems that

AAB female parents are very sensitive to differences in the potency of the pollen used, perhaps in terms of differential pollen tube growth rates. In an extreme case not a single seed has yet resulted from crosses of 'Terra' or 'Terrinha' with these later pollens. Such behaviour must impose an additional criterion on male parent selection and perhaps will necessitate the breeding of such parents retaining a high proportion of wild type parentage.

More than 500 tetraploid AAB hybrids reached the field between October 1983 and July 1986, of which about 200 remain in the initial round of evaluation. More than 90% of those planted have been hybrids of the Prata subgroup. Many selections are in the course of further evaluation as clonal material, these seeming to offer resistance to yellow Sigatoka, at least, along with bunch characters not inferior to those of their respective female parents. Some urgency attaches to the identification of even such simple hybrids for commercial production, where there is a possibility of their inheriting useful resistance to Black Sigatoka from ssp. *burmanica* (Rowe 1981).

A small number of tetraploid AABB hybrids has also been raised and these will be discussed in a later section.

### **AAB Triploids from Backcrosses of AB Diploids**

The studies undertaken, regrettably, have not been able to cover a broad range of sources of AB hybrids and have almost excluded parthenocarpic ones. Now, because of staff and space limitations, as well as of discouraging results, they are being abandoned for the present time.

A provisional conclusion is that AB hybrids, despite their postulated role in the evolution of hybrid triploid cultivars, are only rarely capable of generating triploids in acceptable numbers in experimental backcrosses, so that only a small part of the total germplasm could be initially involved. An interesting fact revealed is that many AB hybrids set seeds rather freely, in backcrosses with AA parents, and that a high proportion of the plants resulting are diploid or nearly so ( $2n = 22$  to  $25$ ). The transference of genes from *M. balbisiana* to *M. acuminata* may be possible and is to be further investigated.

### **AAB Triploids from Tetraploid-Diploid Crosses**

This was not initially defined as a project in Brazil for the obvious reason that there were neither suitable tetraploids nor suitable diploids on hand. The process was foreseen, nevertheless, as a way to generate triploids of AAB constitution in considerable numbers, expectedly enough to offset the enormous variability that might be released.

An early but limited study was made of triploid offspring from spontaneous tetraploid AAAB cultivar indigenous to Brazil ('Ouro da Mata', an evident 'Prata' hybrid). Not surprisingly, there was a segregation in morphological aspects of the B component. The starting point for AAB triploids would clearly have to be AABB tetraploids crossed with AA. Unpublished cytological data indicate that an AABB plant would not necessarily produce exactly AB spores but they would be the best possible approximation.

Some hybrids raised from crosses of 'Prata' (AAB) with *M. balbisiana* have been seen and rejected as possible AABB female parents. The better approach will assuredly be  $ABB \times AA$ , which will permit a two-stage use of developed AA germplasm, in producing tetraploids and subsequently in producing triploids.

Again, some few AABB hybrids have been studied from crosses to 'Figo' ('Bluggoe') clones with *M. acuminata*. They constitute not more than 2% of the plants germinated from such crosses and can thus be produced and identified only by considerable effort. The best of the hybrids with ssp. *burmanica* inherited much of the fruit size of the ABB parent, have been selected and should afford more concrete data, in the near future, on the secondary triploid AAB plants attainable. There is also contemplated the production of tetraploid hybrids from 'Pisang Awak' and other ABB female parents, including 'Pelipita' should this be possible.

### Screening Approaches and Facilities

As the Brazilian program is a new one, some aspects of the screening methodology have yet to be defined and some problems have yet to be overcome. One of these is that of how to achieve uniformity of field conditions for the evaluation of plants, in the face of a prolonged dry season, uncertain rains and insufficient water for irrigation. Mulch is used to the greatest extent possible, but only with banana plant remains which are not regularly in adequate supply.

It is certain that new hybrids will have to be evaluated in the field in at least two stages, the first of these based predominantly on the first seedling fruiting cycle, since space imposes a maximum span of about 18 months on these first plantings. A further clonal evaluation of selected genotypes may then be based on a variable number of plants of each, usually few in the case of diploids. Assessment of resistance to yellow Sigatoka is concurrent and without serious difficulty, in spite of variation in disease intensity from mild in the dry season to perhaps exceptionally virulent in the wet.

Efforts have been made at rapid clonal

propagation of initial tetraploid selections, using a borrowed technique of wounding meristems on whole rhizomes (Dantas et al. 1985, 1986). However, their response has been erratic and there has sometimes been an alarming incidence of somaclonal variations among adventitious plants obtained.

There is yet no screening facility for resistance to Panama disease, nor even a clarification of the pathogen races present in Brazil. Attempts are being made to demarcate differential hosts among inoculated young seedlings of *M. acuminata* strains, test plants easy to produce and with rapid symptom expression (Vakili 1965).

Meanwhile it is assumed that race 1 is prevalent in the country and in the CNPMF experimental area. Unfortunately, in the latter case the soils appear to be 'resistant.' 'Maçã' is all too readily attacked but 'Gros Michel' and 'Highgate' are little affected and disease incidence in the Prata subgroup is rare indeed. Field screening may depend on the use of another area, not too distant, where 'Prata' is evidently susceptible. Alternatively, pot tests of suckers or inoculation of tissue culture plantlets may be considered, despite the great labour of testing a very large number of genotypes.

After basic assessment at the CNPMF, it is probable that any hybrid of commercial promise will be multiplied for trials in state research centres in the diverse regions of Brazil.

### The Breeding Program

The first problem of land area is that of available water for irrigation. At present the breeding program uses 2.7 ha with a drip system and 1.3 ha with micro-sprinklers. In the dry season, however, even with the use of mulch, the quantity of water applied is insufficient for optimal plant growth. The irrigated area is currently occupied in the following manner: germplasm bank 1.0 ha, additional plants for pollination 1.2 ha, new diploid hybrids and a few triploids 1.2 ha, new tetraploid hybrids 0.2 ha and clonal evaluation of selections 0.4 ha.

With an additional water source, and additional equipment, perhaps 5 ha more could be irrigated. Thereafter, land might be a constraint.

### Cooperation in International Activities

The present policy of the Brazilian program is in favour of international cooperation in breeding and, so far as it is possible to foresee, that policy will continue. On the particular aspect of availability of germplasm, no restriction is envisaged in the liberation of material to other cooperating countries.

## Recommendations for Future Action

### Genetic Resources

We *recommend* that immediate steps be taken to designate a new world centre, perhaps at Turrialba, as an international collection and clearing house for banana germplasm, with appropriate funding.

Such a new world centre was foreseen by the first IBPGR working group on the genetic resources of bananas and plantains (IBPGR 1978).

Four collections of appreciable size now exist in the Americas (Jamaica, Honduras, Brazil and French Antilles) and each has basic material that the others lack. One useful function of the designated centre would be to amalgamate these diverse resources.

The collection would be further fortified with additional old world stocks, either from the existing regional collection in the Philippines or directly from national collections.

We *recommend* that INIBAP stimulate and support further banana collecting initiatives of two kinds: (a) those areas as yet relatively unexplored, including Burma, Indochina, Borneo, Sulawesi and Irian Jaya; initial collecting would foreseeably be on a national scale, but with international aid to cover collection, good standards of maintenance and documentation; and (b) those with special interest in the wild species of *Musa*. Some of the above-mentioned areas were specified by the IBPGR working group in 1977 and remain under- or unexplored.

Collections now maintained in Malaysia and Indonesia are almost exclusively of material from mainland Malaysia and Java, respectively.

The level of maintenance of national collections in Asia could be improved with additional resources. The essential phase of elimination of duplicates and orderly replanting is to a varying degree neglected. Only one or two plants may be maintained of each genotype and cases exist or have existed of serious genetic erosion within collections.

Asian collections tend to have inadequate representation of wild species or, more specifically, of variation within *M. acuminata*. Alternatively, as in Papua New Guinea for instance, these have been among the first accessions to be lost.

We *recommend* that urgent attention be given to the development and dissemination of reliable methods for the detection of bunchy-top virus and for its elimination from the meristems of infected plants.

In the present state of knowledge of this virus, it remains as a serious deterrent to the international movement of germplasm of *Musa*.

By 1982 the virus was a major problem in South India, very evident in the vicinity of the Coimbatore collection (Shepherd and Ferreira 1982). In the same

year these authors also saw symptoms in two separate plantings at Los Baños, in an old national collection and in material newly introduced from Malaysia. In 1985 they were warned of possible bunchy-top contamination in the collection at Cibinong, near Bogor, and refrained from collecting there. Until such germplasm can be safely moved, these collections will continue to be dubious sources.

### Approaches to Breeding and Selection

We *recommend* that, for the genetic improvement of the AAB dessert cultivars, such as Prata or Pome and Mysore, the principal approach shall continue to be by the generation of AAAB tetraploid hybrids.

This approach is likely to yield particularly rapid results with 'Pome' itself or, preferably, with the larger-fruited 'Pacovan.' However, the rate of progress is obviously dependent on the availability of resistant male parents with good bunch characters and with potent pollen.

We *recommend* that the production of large numbers of triploid hybrids be more thoroughly explored, as a longer term approach to banana breeding.

Despite the commonly accepted mode of evolution of existing triploid cultivars, their production in large numbers now is more likely to be achieved through tetraploid-diploid crosses, of which AAAA × AA and AABB × AA should have the highest priority.

Looking pessimistically to the more distant future, traditional cultivars with their acquired disease problems may decline in importance, leaving space for the development of new consumer tastes. In such circumstances, the similarity of newly synthesised triploids to traditional ones may be less critical than their resistance and productivity.

We *recommend* that, in the improvement of diploid AA germplasm, selection criteria should include potency of pollen.

As a corollary to this, it would be valuable to confirm a valid relationship between pollen tube growth rates and seed sets obtained in standard crosses using the pollen. Such a correlation would permit a quite rapid evaluation of pollen tubes in styles of Cavendish clones, paradoxically, or perhaps of 'Bluggoe' or 'Figo.'

The pollen potency of male parents appears likely to be of the most critical importance in the generation of tetraploid hybrids from French plantains, or from other cultivars whose output of viable seeds is extremely low.

We *recommend* that immediate and intensive attention be given to the development of techniques for the fertilisation of banana ovules and the recovery of viable embryos in vitro.

The greatest obstacle to genetic improvement of bananas and plantains by hybridisation continues

to be the very low production of viable seeds by most parthenocarpic forms, even diploid ones, after natural pollination.

In the majority of cases, the true potential female fertility has yet to be determined, but there are sundry lines of evidence to suggest that seeds encountered often represent only a fraction of this potential, because of the inefficiency of the process of fertilisation:

- The growth of pollen tubes in styles of female flowers of cultivars can be irregular or retarded (Shepherd 1960);
- Great variations in seed set capacity ('potency') exist between pollens of different AA sources, and these may be related to growth rates of pollen tubes (Shepherd et al. 1985);
- Many unfertilised ovules, without pollen tubes, have been directly observed in ovary sections of the AA diploid cultivars Lili or Lidi and Tongat and in the ABB cultivar Ney Mannan, sufficiently long after tubes should have reached these ovules (Dodds 1945; Shepherd, unpublished data);
- In samples of 'Gros Michel' ovaries from two localities in Jamaica, the frequency of ovules with mature embryo sacs was of the order of 10% or, say, 15 or more per ovary, far in excess of numbers of seeds recovered after pollination (Shepherd 1954);
- These frequencies were found both in early and late hands of the bunch, but seeds in 'Gros Michel' and 'Highgate' are found predominantly in the early hands (Shepherd 1954 and unpublished data);
- It is a universal factor in seed set of cultivars that seeds occur predominantly or almost solely in the apical half of the fruit (Shepherd 1954, 1960);
- At least in some cultivars there is a tendency for the nectary of the female flower, in the ovary apex and close to the path of pollen tubes, to be already necrotic at the time of flower opening, and pollen tube failure has been observed in this region (Shepherd, unpublished data).

It follows that, should it be possible to bring pollen tubes directly to the ovules of cultivars,

without the evident hazards of a long passage through style and ovary tissues, there would be a chance of recovering greatly increased numbers of viable zygotes. Certain crosses might be accomplished that are not possible by natural pollination.

First efforts in Brazil are still seeking culture media favourable for pollen germination and for continued tube growth.

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# Banana Breeding in France and Guadeloupe

H. Tezenas du Montcel \*

FRANCE has long been concerned about the banana industry for two important reasons:

(1) Two French départements, Guadeloupe and Martinique, situated in the West Indies, produce and export bananas to Europe; and

(2) France has a long tradition of close cooperation with the West African countries which are also producing and exporting dessert bananas as well as cooking bananas — mainly plantain types.

The French market (more or less 500 000 t) for the dessert banana is supplied by Guadeloupe and Martinique as well as by our traditional African partners (Ivory Coast, Cameroon, etc.).

African countries produce for themselves around 1 million t of plantains which corresponds to a consumption of more than 300 g per day per inhabitant.

In France the central organisation which covers all the research on tropical crops is the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD). This organisation has various departments corresponding to the major tropical crops.

One of these departments is the Institut de recherches sur les fruits et agrumes (IRFA), and as its name indicates, it is the one in charge of fruit research (banana, pineapple, citrus, avocado, mango, etc.).

IRFA carries out its research in various stations and in cooperation with various countries.

For banana research the most important stations are at Guadeloupe and Martinique and we cooperate with Cameroon, Ivory Coast and Gabon. The main office and laboratories are in Montpellier, France.

## French Program on Banana Improvement

The general aims of our program are the improvement of dessert and cooking bananas. The scope of the research operations, using traditional

approaches and more recent technologies, is summarised in Tables 1 and 2 respectively. The desired characteristics for dessert bananas of the Cavendish type are resistance to Black Sigatoka and Panama disease (race 4), plus others. For cooking bananas the desired characteristics for the plantain type are borer resistance, Black Sigatoka resistance, and others (e.g. rusticity or hardiness).

For the ABB type (Bluggoe, Saba, Pelipita), resistance to Panama disease (race 2, race 4), high quality of fruits, and other characters (e.g. Moko disease resistance).

Cavendish, plantain, and ABB types refer to banana trees that look like a Grande Naine, a plantain or a Pelipita. We are not looking for an ideal type, but for various plants which could be proposed to the farmers.

Table 1. Traditional research operations.

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### *Germplasm*

Germplasm constitution — Prospects — Exchange  
Taxonomy (morphotaxonomy)  
Characterisation — Determination  
Evaluations (fertility, disease resistance, agronomic characters, etc.)

### *Hybridisation*

Improvement of diploids  
Self-pollination of diploids  
AB formation  
AAAA and AABB formation  
Study of the residual female fertility of triploid cultivars

### *Cytology*

Study of banana sterility (meiosis)  
Colchicine treatments  
Chromosome counting

### *In Vitro Methods*

In vitro proliferation (meristem cultures)  
Conformity or non-conformity field tests  
Search for variants in in vitro plant populations  
Embryo cultures

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\* CIRAD-IRFA, Station de Neufchateau, 97130 Capesterre Belle Eau, Guadeloupe (F.W.I.).

**Table 2.** Operations using new breeding methods or new technology.

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*Germplasm*  
 Taxonomy (chemotaxonomy)  
 Determination (XPER system)  
 Conservation (in vitro collection)  
 Evaluation (in vitro tests; rhyzotron)  
 Characterisation (constitution of a data bank)

*Hybridisation*  
 In vitro fertilisation

*Cytology*  
 New observation techniques  
 Colchicine treatments on in vitro plants or embryos

*In Vitro Methods*  
 Proliferation (from embryo culture)  
 Neo-formation ('neo-corm,' 'neo-pseudostem,' floral reversion)  
 Callogenesis  
 Somatic embryogenesis  
 Protoplasts  
 Immature embryo culture

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**Results**

The evaluation of our germplasm cultivars is mainly based on agronomic characters. We are also looking at male and female fertility.

Black Sigatoka, nematodes and borer resistance are studied in cooperation with Cameroon researchers.

In our hybridisation studies, the improvement of diploids has just started, with the new diploids coming from Papua New Guinea and Southeast Asia.

Our first results for self-pollination of the diploids show that we have a low percentage of germination. The seedlings show low vigour and a high mortality compared to intersubspecific hybridisation seedlings.

Various crosses for AB formation (AABB formation linked with the residual female fertility of triploid cultivars) have been tried. All gave a lot of seeds (100 to 5000 per bunch) but the percentage of good seeds varies between 0 to 90% depending on the parents used and the female parent.

At the present time only four crosses give us seedlings (one cross *M. balbisiana* × *P. liliin* and three crosses *M. acuminata* ssp. *malaccensis* × *M. balbisiana*).

The cytology work has included colchicine treatments, studies of banana sterility (meiosis), and chromosome counting.

The most important mutations (somaclonal mutations) obtained with the plantlets issued from in vitro plants are similar to the ones obtained by Dr. Hwang from Taiwan (see these Proceedings) (i.e. they concern the stature, dwarfism and giantism). We obtained variations of the foliage (drooping leaves), fruit characteristics, plant colour, suckering and pseudostem characteristics, but we

**Table 3.** Results achieved on in vitro methods.

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*Somatic embryos*  
 The De Langhe method was tried on ABB. The various steps of this method are:  
 Vegetative apex culture  
 Proliferation of this culture  
 Formation of pro-embryo on a shaken liquid medium  
 Evolution from pro-embryo to somatic embryo (not yet achieved)  
 Regeneration of the somatic embryo (achieved by De Langhe on AAA)

*Callogenesis*  
 Very young banana fruits take on female inflorescence (AAA, AAB, ABB)  
 Embryo  
 Young pseudostem from in vitro plants  
 Apex of a male bud (AAA)  
 (not yet achieved with plantain and ABB)  
 Ovary slices

} callus

*Neoformations*  
 From the base of young ovaries  
 From the youngest tissues of a male inflorescence (AAA)  
 From pseudocorm (neo-bulb) (AA, AAA, AAB)  
 From embryo — pseudocorm  
 From callus (embryo and male bud apex)

*Protoplasts*  
 From young in vitro plant leaves base  
 From male inflorescence bud callus

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believe that these variations are not stable and that they are going to disappear during the second ratoon. These variations reached 10% in a non-conformity test (floral reversion). The results achieved in the in-vitro research are summarised in Table 3.

### **Recommendations for Future Research**

#### **Germplasm**

In our opinion it is very important to have in a single place as wide a collection as possible. This collection would be useful as a reference collection for the international community, as a gene pool for all researchers, for commercial/subsistence farming, to train interested people on the classification of the bananas and on morphotaxonomy, to put at the disposal of the

international community a data bank on the most important characteristics of the bananas, and for many other purposes.

The choice of location should take into consideration the ecological environment, the presence of diseases, the facilities offered by the country and the political stability of the country.

#### **Breeding and Selection**

In our opinion all efforts should be concentrated on the improvement of the diploids. This means that we have to look for further collections of more wild or cultivated diploids; to study the characteristics of these diploids (cytology, disease resistance, agronomic characters); to increase the variability of these primary diploids and/or of the bred diploids; and to improve these diploids keeping in mind the final results we are looking for (Cavendish, Plantain, Silk type).

# Producing Disease-Resistant *Musa* Cultivars by Genetic Engineering

Jane Murfett and Adrienne Clarke \*

DESPITE many years of effort, classical breeding programs have achieved limited success in producing disease-resistant cultivars of *Musa* species. This raises the question of whether useful genes can be transferred to *Musa* species by genetic engineering techniques.

A number of different methods have been used to introduce foreign DNA into plant cells. At present the most widely used vector for gene transfer is the *Agrobacterium* Ti plasmid; however other vectors, such as modified plant viruses, are being investigated. In addition, a number of direct transfer techniques, such as microinjection and electroporation are available.

In this paper we will outline the methods for gene transfer based on the Ti plasmid, and briefly describe the alternative methods mentioned. We will then consider whether these methods could be used for banana improvement. Finally we will discuss possible approaches to defining genes which might be useful in improving the resistance of *Musa* species to fungal and viral diseases.

## The *Agrobacterium* Ti Plasmid

*Agrobacterium tumefaciens* is a Gram negative soil bacterium that causes Crown Gall disease in many dicotyledonous plants (for reviews see Barton and Chilton 1983; Drummond 1979; Nester et al. 1984). Virulent strains carry a large tumour-inducing, or Ti, plasmid which has two distinct regions necessary for tumour formation: the T-DNA region, and the virulence or *vir* region (see Fig. 1).

After infection of the host plant at a wound site, the T-DNA is transferred to the plant genome. It carries bacterial genes which are expressed in the plant cells. Some of these genes encode enzymes involved in the synthesis of the plant hormones

auxin and cytokinin (Akiyoshi et al. 1984; Schröder et al. 1984), so that infected plant cells multiply rapidly. This leads to formation of a gall. Other T-DNA genes direct the synthesis of compounds called opines, which are used as metabolites by the *Agrobacterium* cells (De Greve et al. 1982a; Depicker et al. 1982). Ti plasmids can be classified according to the opine synthase gene they carry. These include the octopine, nopaline and agropine synthase genes (Guyon et al. 1980).

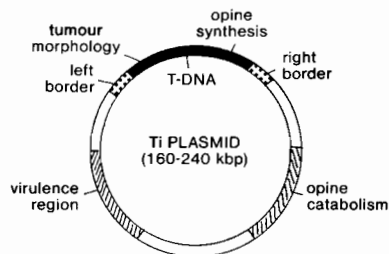


Fig. 1. The essential features of an *Agrobacterium tumefaciens* Ti plasmid. The virulence region contains genes required for insertion of the T-DNA into plant cells. The T-DNA is flanked by 25 base pair border sequences. Between these are genes controlling tumour morphology, as well as opine synthesis. The plasmid also carries genes controlling degradation of opines, which are used as a nutrient source by the *Agrobacterium*.

The T-DNA is flanked by nearly identical 25 base pair (bp) border sequences, and only the DNA between these border repeats is transferred to the plant genome. While deletion of the left border repeat has no significant effect on T-DNA transfer (Joos et al. 1983), deletion of the right border totally abolishes it (Shaw et al. 1984). An additional 24 bp sequence, located within 40 bp to the right of the right border, is also necessary for efficient T-DNA transfer (Peralta et al. 1986).

The virulence region of the Ti plasmid carries genes necessary for the insertion of the T-DNA into the plant cells. These genes are activated by specific

\* Plant Cell Biology Research Centre, University of Melbourne, Parkville, Vic., Australia.



phenolic compounds present in plant exudates, which are released when plant cells are wounded (Bolton et al. 1986; Stachel et al. 1985).

The mechanism by which T-DNA is transferred to plant cells and integrated into the plant genome are not yet understood. However a recent study has shown that the T-DNA is transferred as a linear, single-stranded molecule. Formation of this molecule is initiated at the right border, after activation of the virulence genes (Stachel et al. 1986).

The site of insertion of the T-DNA in the plant genome appears to be random (Chyi et al. 1986). Most often, insertion occurs at a single locus, and the foreign genes are passed on to the progeny in a Mendelian fashion (Chyi et al. 1986; De Block et al. 1984). A variable number of T-DNA molecules may insert at the locus, and these can be orientated either head-to-tail, or as direct repeats (Barton et al. 1983; Czernilofsky et al. 1986; Zambryski et al. 1980).

### Genetic Engineering Using the Ti Plasmid

It is now established that any piece of DNA, up to 35 kilobase pairs (kbp) in length (An et al. 1985), can be stably integrated into a plant genome simply by inserting it between the T-DNA border regions of a Ti plasmid, and then infecting plant material with *Agrobacterium* cells carrying this modified Ti plasmid. Plant cells transformed in this way with tumour-inducing plasmids can then be selected by their ability to multiply and form callus on hormone-free medium, as the plasmid carries genes for synthesis of plant hormones. However plants regenerated from this callus grow abnormally.

In order to use this transformation method to regenerate normal plants, Ti plasmids have been 'disarmed' by removing all the genes involved in tumour formation from the T-DNA (De Block et al. 1984; De Greve et al. 1982b; Fraley et al. 1985; Zambryski et al. 1983). The opine synthesis genes are generally retained, since they can be useful as genetic markers to identify transformed plant material (Aerts et al. 1979; Otten and Schilperoort 1978).

As the ability to grow on hormone-free medium is not acquired by cells transformed with the 'disarmed' Ti plasmid, an alternative method of selection is necessary. For this purpose, chimeric genes have been constructed which contain bacterial genes for antibiotic resistance, modified so that they can be expressed in plant cells (De Block et al. 1984; Fraley et al. 1983). One commonly used construct contains the coding sequence of a bacterial kanamycin resistance gene, inserted between the regulatory regions of the nopaline synthase gene. Plant cells generally do not grow well in the presence

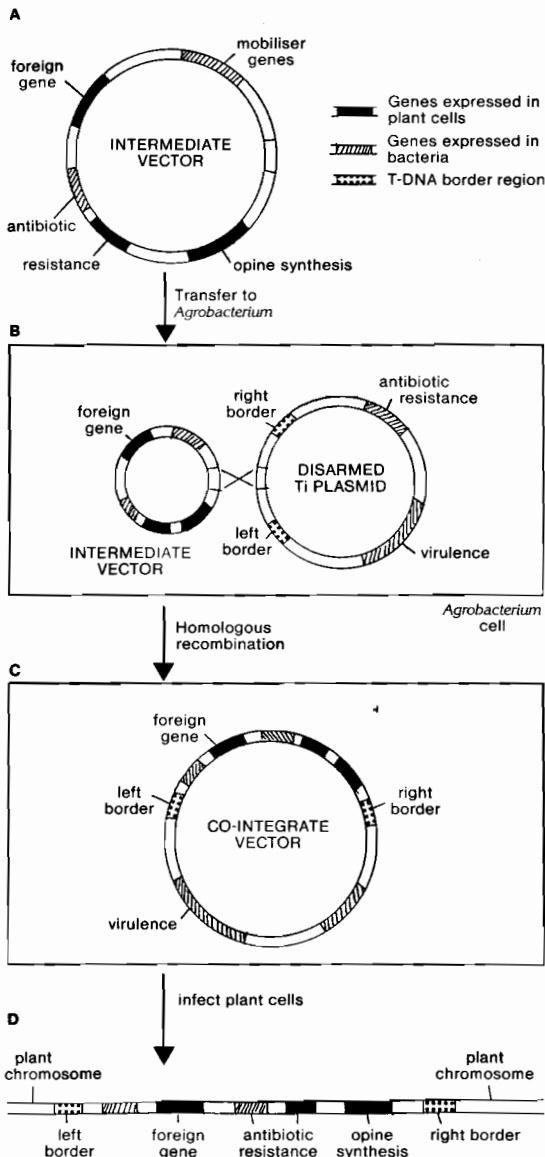
of kanamycin, but plant material transformed with this construct will grow; transformants can therefore be selected on medium containing kanamycin.

Manipulation of DNA can be performed most easily in the bacterium *Escherichia coli*, for which a wide range of cloning vectors exist. Cloning systems have therefore been developed in which vector constructs are made in *E. coli*, and then transferred to *Agrobacterium* by conjugation. Currently there are two different vector systems: one is referred to as the cointegrate system (Fig. 2) where the T-DNA and the virulence genes are present on the same plasmid (Fraley et al. 1985; Zambryski et al. 1983); the other is the binary vector system (Fig. 3), where the T-DNA and the virulence genes are present on two separate plasmids (An et al. 1985; Bevan 1984; van den Elzen et al. 1985; Hoekema et al. 1983).

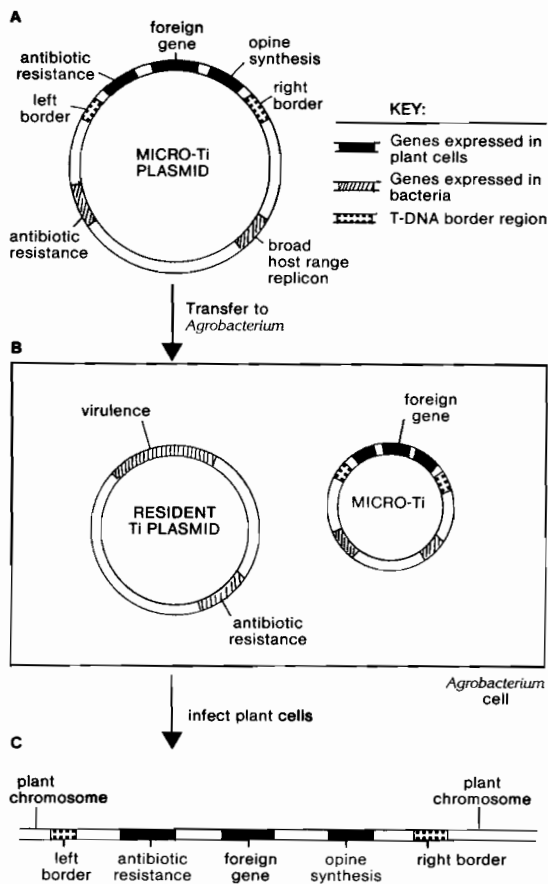
A typical co-integrate system utilises an intermediate vector with a number of important features. It carries antibiotic resistance genes for selection in both bacteria and plants, and often it carries an opine synthase gene which is used as an additional genetic marker. It has a cloning site for insertion of foreign DNA, and mobiliser genes which allow it to be transferred between bacterial cells.

After the gene of interest has been cloned into the insertion site of the intermediate *E. coli* vector, it is transferred to *Agrobacterium* via a triparental conjugation. The donor *E. coli* strain, carrying the intermediate vector, is incubated with the recipient *Agrobacterium* in the presence of a second *E. coli* strain. This strain carries a mobiliser plasmid which provides genes necessary for transfer of the intermediate vector. First the mobiliser plasmid moves into the cell carrying the intermediate vector, then both plasmids are able to move into the *Agrobacterium* cell. The recipient *Agrobacterium* strain carries a disarmed Ti plasmid, which also carries a bacterial antibiotic resistance gene, different from the one on the intermediate vector. Between the T-DNA borders is a region which is homologous to part of the intermediate vector, so that the intermediate vector becomes integrated into the modified Ti plasmid by homologous recombination. The resulting co-integrate vector carries two bacterial antibiotic resistance genes, and *Agrobacterium* cells carrying the co-integrate can be selected on medium containing both the antibiotics. In this way the foreign DNA becomes inserted between the two T-DNA borders, so that it can be transferred into plant cells along with the rest of the T-DNA.

The binary vector system utilises a 'micro-Ti' plasmid, which is much smaller and therefore easier to manipulate than the large, wild-type Ti plasmid. It carries a broad host range replicon (Simon et al.



**Fig. 2.** Plant transformation using the cointegrate vector system: **A.** The intermediate vector, with a foreign gene inserted into its cloning site. This manipulation is done with the vector in *E. coli*. **B.** After insertion of the foreign gene, the intermediate vector is transferred to *Agrobacterium* by triparental conjugation. The *Agrobacterium* cell contains a disabled Ti plasmid, with a region homologous to part of the intermediate vector, carried between its left and right border sequences. **C.** The intermediate vector becomes integrated into the disabled Ti plasmid by homologous recombination, producing a 'cointegrate' vector. **D.** Leaf discs or protoplasts are infected with *Agrobacterium* cells carrying the cointegrate vector. The T-DNA region, which includes all the genes carried between the left and right borders, is transferred to the plant cells and becomes integrated into the plant genome.



**Fig. 3.** Plant transformation using the binary vector system. **A.** The micro-Ti plasmid with a foreign gene inserted into a cloning site between the left and right borders. **B.** The micro-Ti plasmid bearing the foreign gene is transferred to *Agrobacterium* by triparental conjugation. The *Agrobacterium* cell contains a resident Ti plasmid with an intact virulence region, but no T-DNA. **C.** Leaf discs or protoplasts are infected with *Agrobacterium* cells carrying the resident Ti plasmid and the micro-Ti plasmid. The virulence genes act in trans to cause transfer of T-DNA from the micro-Ti plasmid into the plant genome.

1983) so that it can replicate in both *E. coli* and *Agrobacterium* cells. It carries the T-DNA left and right border regions, and between these are antibiotic resistance genes, opine synthesis genes, and a cloning site for insertion of foreign DNA. The gene of interest is cloned into this site, then the construct is transferred to *Agrobacterium* by triparental conjugation. The *Agrobacterium* cell carries a resident Ti plasmid which has had all its T-DNA removed. The virulence genes on this resident plasmid act in trans to cause the transfer of T-DNA from the micro Ti plasmid, into plant cells.

Two commonly used in vitro methods have been developed for infecting plant cells, and regenerating transgenic plants. The first, termed 'co-cultivation' (Willems et al. 1981), involves the use of plant protoplasts, which are prepared by enzymically removing the cell wall. These are mixed with *Agrobacterium* cells and incubated in liquid medium at room temperature for several days. The *Agrobacterium* cells are then removed by centrifugation, and the protoplasts are grown on a solid medium containing antibiotics to select for those transformed with T-DNA. Appropriate hormones are added to the medium to allow callus, then whole plants to regenerate.

A 'leaf disc transformation' method was developed by workers at the Monsanto laboratories (Horsch et al. 1985). This involves cutting small pieces of sterile leaf tissue and incubating them with *Agrobacterium* cells on solid medium for 2 or 3 days. The discs are then transferred to a shoot regeneration medium containing kanamycin, which selects for transformed plant cells, and another antibiotic such as carbenicillin, which kills the *Agrobacterium* cells. Regenerated shoots are transferred to root-inducing medium after several weeks.

#### Limitations of the Ti Plasmid System

Until recently it was believed that *Agrobacterium tumefaciens* would only infect dicotyledonous plants, since inoculation of monocots did not result in the formation of galls. It was assumed that T-DNA could not be transferred to monocot cells, either because the *Agrobacterium* could not attach to monocot cell walls, or because the phytohormone balance in monocot cells was incompatible with T-DNA insertion (Hooykaas-Van Slogteren et al. 1984).

Recent experiments, however, have shown that some monocotyledonous plants can be infected with *Agrobacterium*, and the T-DNA can be stably integrated in the infected cells. Wounded stem tissue was inoculated with *Agrobacterium*, and 2 weeks later, swelling was observed in the inoculated area. The infected cells were found to contain opine synthase enzymes, indicating that T-DNA, carrying the genes for these enzymes, had been transferred to the plant cells. This was demonstrated for members of the families Liliaceae (Hernalsteens et al. 1984), Amaryllidaceae (Hooykaas-Van Slogteren et al. 1984), and Gramineae (Graves and Goldman 1986).

A further problem, which applies to other gene transfer techniques as well as the Ti plasmid methods, is that of plant regeneration. It is still difficult to regenerate whole, monocotyledonous plants from single transformed cells. In fact only a

few genera of dicots, including *Nicotiana*, *Petunia*, *Lycopersicon* and *Solanum* have been reliably regenerated from single transformed cells. Since many of the agriculturally important crops, particularly the cereals, are monocots, there is currently intensive effort directed to establishing ways of regenerating graminaceous plants. To date, maize protoplasts have been induced to re-synthesise cell walls and initiate cell divisions (Imbrie-Milligan and Hodges 1986), however the resulting microcalli have not been regenerated to whole plants. Greater success was recently reported for rice (Yamada et al. 1986) where whole plants have been regenerated from protoplasts.

#### Viral Vectors

The problems of host range encountered with using the Ti plasmid as a gene transfer vector could theoretically be bypassed by using modified plant viruses as vectors. Since viruses infect plants systemically, regeneration of single transformed cells would not be necessary. Viruses replicate to high copy numbers within plant cells, so that high levels of expression of foreign genes might be attained. It should be possible to remove the genes responsible for disease symptoms from the viral genome, just as the tumour-inducing genes were removed from the Ti plasmid.

Most work in this area has centred on the cauliflower mosaic virus (CaMV) which has a double stranded DNA genome that is easily manipulated in vitro. In contrast, the genomes of many other plant viruses consist of RNA, which is more difficult to manipulate. In 1984, CaMV was used to transfer a bacterial antibiotic resistance gene into turnip plants (Brisson et al. 1984). The virus carrying the bacterial gene infected the plants systemically, and expression of the foreign gene occurred throughout the plant. The major limitation to the use of CaMV as a vector is the relatively small piece of DNA which it can carry (250 base pairs; Gronenborn et al. 1981).

A general problem with the use of viral vectors is that viral genes do not become integrated into the plant genome, but replicate autonomously in the plant cell, and are not passed on to the plant progeny. Furthermore, many plant viruses are transmitted solely by insect vectors. Thus, alternative delivery systems are required if plant viruses are to be used to transform plants routinely. Recently Grimsley et al. (1986) inserted tandem arrays of the CaMV genome into the T-DNA of a Ti plasmid. After infecting turnip plants at wound sites with *Agrobacterium* containing the engineered Ti plasmid, systemic viral infection occurred. Hence the virus was able to escape the plant genome after integration with the T-DNA and move throughout

the plant. This use of the *Agrobacterium*-Ti system to carry viral vectors into plants may prove to be more generally applicable.

### Direct Transfer Techniques

When plant protoplasts are incubated in liquid culture containing DNA and polyethylene glycol, which permeabilises the protoplast membrane, the DNA will enter the cells and become integrated into the plant genome. The method was first developed by Krens et al. (1982), and has been used to transfer bacterial genes into tobacco plants (Paskowski et al. 1984), ryegrass cells (Potrykus et al. 1985) and rice cells (Uchimiya et al. 1986).

Fromm et al. (1986) found that by subjecting maize protoplasts to an electric pulse of high field strength, the membranes were reversibly permeabilised, and foreign DNA could enter and become stably integrated. This procedure is called electroporation.

Another technique under investigation is the microinjection of DNA directly into the nuclei of isolated cells. This method requires expensive equipment and a great deal of skill. At the CSIRO Division of Plant Industry in Canberra, microinjection has been successfully used to transform tobacco protoplasts, with a 50% survival rate of injected cells (E. Dennis, pers. comm. 1986).

Although direct transfer techniques have some advantages over the Ti plasmid — for example they are not limited by host range — they also have some problems. These techniques require regeneration of whole plants from single cells; and there is evidence that DNA transferred by direct techniques often undergoes structural changes and concatemerisation (Czernilofsky et al. 1986).

### Candidate Genes for Transfer to *Musa* to Confer Disease Resistance

It seems likely on the basis of recent experience with other crop plants, that with appropriate resources it would be possible to develop a transformation system for *Musa*. The next question is, what genes would be transferred. This is a problem not only for *Musa*, but for all crop species as our technology for transferring genes is ahead of our ability to identify agronomically useful genes.

### Fungal Diseases

A major problem for bananas and plantains is susceptibility to the fungal disease caused by *Fusarium oxysporum* f. sp. *cubense*. Ideally, genes conferring 'resistance' to this disease could be transferred to susceptible cultivars to create new

resistant cultivars. However, genes conferring resistance are not available as defined DNA sequences, and the phenomenon of resistance itself is poorly understood at the molecular level.

Resistance of plants to invasion by pathogens is described at two levels, non-host and host resistance. Non-host resistance is that expressed by a potential host species to all biotypes of a potential pathogen. Host resistance refers to that shown by certain cultivars of a plant species, normally considered host to a particular pathogen, to particular biotype(s) of the pathogen (Heath 1981; Ralton et al. 1986). Host resistance may be determined by major dominant resistance genes, as in gene-for-gene relationships (Flor 1955; Ellingboe 1981) or the combined action of many genes, as in 'horizontal' (or field) resistance (Vanderplank 1982). Non-host and 'horizontal' resistance are frequently more durable in the field than major gene host resistance. There is virtually no understanding of these forms of resistance at the molecular level.

Generally, the systems in which host resistance can be described by the gene-for-gene theory have been developed by classical breeding strategies, aimed at producing disease-resistant cultivars of agriculturally important plants by including genes conferring resistance originating from different, but related, species (Day et al. 1983). Transfer of the genes is usually assessed by examining symptom expression in the host plant, and to date none of these genes can be defined molecularly in terms of a particular DNA sequence.

We do, however, have molecular information on some of the plant responses associated with expression of resistance. For example:

(1) Hydroxyproline-rich cell wall glycoprotein: This glycoprotein is expressed at increased levels in several host plants after fungal infection. Genomic clones and partial cDNA clones for several species are available (for review see Showalter and Varner 1986).

(2) Phytoalexins: These are low molecular weight antimicrobial compounds which accumulate in localised areas of infected plant tissue. The structure and the pathways of biosynthesis of a number of phytoalexins are known and a number of enzymes such as phenylalanine ammonia lyase and chalcone synthase have been cloned for different species (Kreuzaler et al. 1983; Kuhn et al. 1984).

(3) Formation of callosic papillae: Papillae are deposits of material, commonly containing a (1→3)- $\beta$ -glucan which are found between the host plasma membrane and the existing cell wall in the region adjacent to the area of pathogen penetration (Aist 1983). They may function as mechanical barriers to penetration. The formation of papillae correlates with non-host resistance and is also associated with gene-for-gene interactions. Efforts are currently

being made to isolate the (1→3)- $\beta$ -glucan synthase, as the basis for strategies to clone the gene encoding the enzyme.

In some cases, it seems that these responses are associated with fungal penetration of a resistant as well as a susceptible host, the major difference being the level of expression and the timing of the expression which is delayed in the susceptible compared with the resistant host.

Our lack of knowledge of the biochemistry of fungal infection in resistant and susceptible hosts does not allow us to pinpoint critical genes, and transfer them to susceptible hosts at present.

Nonetheless, while this information is accumulating, there are several approaches which might be worth trying.

1. *Transfer genes associated with the resistance response to susceptible plants.* It may be that if genes for particular responses, for example phytoalexin and hydroxyproline-rich glycoprotein production, were transferred to susceptible species and expressed at high levels in tissues at the site of infection, a greater generalised resistance to infection would be induced. This would involve using tissue specific promoters which would express high levels of the gene product in particular tissue.

2. *Transfer genes encoding enzymes which would destroy the fungus but not the plant.* This strategy has been used by Jones et al. (1986) who transformed tobacco plants with a gene encoding the enzyme chitinase. This enzyme hydrolyses chitin which is a fungal cell wall component but is not a component of higher plant cell walls. Expression of the enzyme in higher plant tissue would not therefore cause breakdown of the host tissues but does cause degradation of the fungal walls. Their preliminary data indicated that the transformed plants were more resistant to infection by certain fungi than control plants.

There are many problems associated with this sort of approach; for example if pathogenic fungi are indeed lysed by the enzyme, it is likely that non-pathogenic fungi which have chitinous cell walls, for example mycorrhizae, may also be vulnerable.

3. *Transfer genes encoding antibodies directed to specific pathogens.* It may be possible to transform plants with cDNAs encoding the heavy and light chains for antibodies raised to essential components of the pathogen. To be effective, the individual chains would need to assemble to give a molecule with functional antigen binding sites, and the interaction would need to cause arrest for fungal growth. Although this sounds rather far-fetched, we should not dismiss such conceptually possible ideas out of hand, because of the remarkably rapid progress currently being made in plant molecular biology.

## Viral Diseases

In 1986, Abel et al. reported that it is possible to produce plants resistant to development of viral infection by transformation with a gene encoding the viral coat protein. In this study a cDNA encoding the coat protein of tobacco mosaic virus (TMV) was transformed into tobacco plants using a Ti plasmid of *Agrobacterium tumefaciens*. The transformed plants expressed the coat protein and when these plants were inoculated with TMV, there was a delay or failure to express symptoms of disease. The mechanism by which resistance is induced in this way is not yet understood, but preliminary data indicate that it might be a generally applicable practical way of producing plants resistant to particular viruses.

In all these approaches, there is the overriding question of whether, when and under what circumstances transgenic plants can legally be released into the environment. The first permits to field test engineered plants have been issued in the USA and UK, but there is still considerable uncertainty regarding the regulations (Beardsley 1986).

## Conclusions

1. Transformation of *Musa* species by genetic engineering techniques is worth considering as a potential route to producing disease-resistant varieties.

2. There is no system for transformation of *Musa* species yet established. It is possible that a system based on the use of the Ti plasmid of *Agrobacterium tumefaciens* could be developed.

3. At present there are no cloned genes available which represent proteins acting directly to confer host resistance to fungal disease for any host-pathogen system.

4. There are, however, a number of genes associated with expression of resistance which might be used experimentally in attempts to confer resistance. Genes for enzymes destroying fungal components which are not common to the host tissue are also potentially useful.

5. One effective approach to control of viral disease is to transform plants with the coat protein gene. The transformed plants show delayed symptom development. Such an approach might be applicable to certain viral diseases of *Musa* species.

6. Attempts to develop a transformation system for *Musa* species should be encouraged now, so that when useful genes become available in the future, a route for transfer will be available.

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# Disease Susceptibility and Genetics in Relation to Breeding of Bananas and Plantains

Ivan W. Buddenhagen \*

It is realistic to state that the real reason for both this conference and the interest today in breeding bananas and plantains lies in only one crop attribute — disease susceptibility. Hence the title of this paper, and its emphasis: *susceptibility*. Although resistance may be the goal of research investment, the real driving force for investment is susceptibility, which has been, or is perceived to be, changing in the last decade or two. The most important change has been the appearance and spread of more virulent forms of Sigatoka leaf spot which are now present in the Pacific Islands, Latin America, and Africa. The 'new' forms are not only more virulent on their traditional banana hosts but they now endanger the plantains, unlike the old Sigatoka. Thus they generate much more interest among organisations concerned with peasant agriculture and local food supplies in the lowland wet tropics.

The second change is the appearance of a race or races of the *Fusarium* wilt pathogen on Cavendish bananas formerly considered resistant. This has occurred not only in widely separated locations in the subtropics (Taiwan, Australia and South Africa) but also in the equatorial climate of Mindanao in the Philippines. Although it has long been considered that the great banana export industries in the tropics were not endangered by wilt after successfully converting to resistant Cavendish clones, the present experience in Mindanao belies that assumption. However, the fact that wilt has not appeared in Cavendish clones in Latin America or Africa, and that the AAB plantains remain unaffected everywhere, tends to reduce the urgency of concern. There is localised concern in a few places, but largely, the problem is seen as a potential threat rather than an actual one. Brazil is seen as a special case, where there is a need for wilt resistance for the widely planted and susceptible AAB cultivars.

Once one considers embarking on a resistance

breeding program, driven by the actual or perceived threat by only these two pathogens, other useful objectives become apparent, especially, reduced susceptibility to various nematodes, and in some areas, reduced susceptibility to bacterial wilt (Moko disease) and to fruit spotting fungi. Then one may question if resistance to bunchy-top or other viruses should be considered. That leads to the question of resistance to the virus vectors and even to other insects. Does one include such objectives in a breeding program? Such reasoning may lead one to question to what degree susceptibility to minor pathogens may be *increased* by any breeding effort targeted only at the two major pathogens, and how much one should be concerned with 'preventive' breeding.

And last, but not least, is the need to be concerned with all of the diverse agronomic and quality aspects of a highly sophisticated industry for dessert bananas and of different consumer groups. It becomes apparent that beyond the worldwide needs there are localised and specific needs in different regions. Given the diversity of needs and the difficulty of obtaining recombination in this parthenocarpic triploid crop and the very limited number of sustained breeding programs, it is not too surprising that success has not yet been achieved: All the bananas and plantains that we grow and eat were selected in prehistory by primitive peoples!

## Breeding for Resistance: General Considerations

It is important to know as much as possible about the existing variability of the pathogens against which one is trying to breed, and also about their potential variability and the ease with which a new virulent form, once selected, can replace the old pathogen population and thereby render ineffective the results of a resistance breeding program.

For *Musa* the world may be divided into two parts: 1) a coevolved pathosystem region of Southeast Asia; and, 2) the rest of the world where *Musa* is of fairly recent introduction.

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\* Agronomy Department, University of California, Davis, CA 95616.



The areas of recent introduction are Latin America and Brazil, the Caribbean, and Australia. These represent the locations of most research on bananas. Pathology research has been most intensive in Central America but with some research also connected with the export trades in the other new areas and in the Philippines and Taiwan. The new areas are all radically different from the coevolved pathosystem areas in that most research has been confined to only two basic AAA clones: Gros Michel and Cavendish (all Cavendish clones are grouped in this paper as one for pathosystem analysis). A small amount of research has been conducted on Silk/Pome (AAB) types in Brazil and on Bluggoe (ABB) and plantains in Central America but basically only a few clones have sampled the environment for local non-coevolved but potential pathogens over wide areas. Moreover, it means that any pathogens occurring in the new areas following their limited introduction from the centre of host origin have had very little host diversity on which to propagate and diversify.

This means that any sampling of Cavendish, for example, for the Sigatoka pathogen, in any new region should result in cultures with identical pathogenicity genes. At least there would have been no reason for selection and/or preservation of pathogen diversity. Now that Sigatoka forms exist that attack plantains, one could consider that the plantains might select a different pathotype; at least the possibility exists.

The presence of a sexual stage and the rapid aerial dispersion of the Sigatoka pathogen, which has been amply demonstrated with Black Sigatoka in Africa and Latin America recently, show that deployment of a new cultivar, with new and different resistance genes, would exert great selection pressure on the existing Sigatoka population. A 'breakdown' of resistance would be limited only by the flexibility of that pathogen population in mutating to new virulence. Once mutated, a new pathogenic form would be dispersed rapidly, especially since, on the new 'resistant' clone, spraying would have been stopped or be at a low level.

In thinking about breakdown potential, one must consider pathogen population size and location. In the case of Sigatoka, if a banana industry converted to a resistant clone, there would be only a short period of time (a few years at most) for complete replacement, during which abundant spores (from the old variety) would be challenging the new one, mostly at the transition zone. After that, challenge inoculum would no longer exist within the plantation and the risk of breakdown would be extremely low. Presumably the region would preserve a limited inoculum on household plantains scattered about. If a resistant plantain were also introduced, the Sigatoka population would become

so low that it could become locally or regionally extinct.

The point of importance right now in assessing Sigatoka variability in the laboratory is that cultures collected from the *same host* over a regionally intact area outside the area of coevolution should have very low variability. Thus, their analysis will tell us little about potential variability. What is needed is to collect anew, from as many *different* genotypes as possible, in each region. The best place is a banana collection. Lacking that, a diverse set of genotypes could be planted in each region purposely to act as a trap for pathogen variability. Such a genotype set should contain the major parents being used in breeding programs around the world, and be updated periodically.

For the *Fusarium* wilt fungus, the story is somewhat different. The presumption is strong in Central America, the Caribbean, West Africa, Australia, etc. that the wilt fungus was moved about extensively in seed pieces of Gros Michel. Whether or not the fungus was originally introduced from Asia or had a separate origin in Latin America, etc. is not known. New research could provide a probability answer. If it is an introduction, then one would expect little variability; again, because it had only one static host. Cultures from banana from one introduced region should be very limited in pathogenic variation. If, on the other hand, indigenous wilt fungi in the new areas evolved to attack the introduced host, there should be much more variation.

Certainly, the very discovery and existence of the races of *Fusarium oxysporum* f.sp.  *cubense* were due to appearance of wilt symptoms on banana/plantain cultivars earlier considered resistant to the fungus attacking Gros Michel. The exception is race 3 which was discovered on *Heliconia*, where a *Fusarium* was purposely sought after it was discovered that *Heliconia* was the original host of the bacterium that caused the new-encounter Moko disease of banana in Latin America (Buddenhagen 1964). There is no question that the *Heliconia* wilt *Fusaria* are locally evolved in Latin America on this genus. Whether their diversity includes forms that created the American Panama wilt of banana was never proven, but it still remains a good possibility. New research should be able to answer this question.

The question of separate origin in Latin America for the banana wilt *Fusarium* is not all irrelevant to a consideration of breeding for resistance because it should influence greatly the possibility of resistance breakdown of new cultivars bred from Gros Michel.

Unfortunately, a further unknown is the level of challenge that will confront a new cultivar. After 25 years of cultivation of resistant Cavendish on soils where wilt devastated Gros Michel, what has happened to the inoculum? Is it still there at high or

moderate levels, even now, and more genetically diverse due to its life as a saprophyte or rhizosphere organism? I am not aware of information that answers this question, important to a banana breeding program that is considering the deployment of a new cultivar.

If a new cultivar became widely planted and a single rare mutation (or even a few) to new virulence then occurred, spread to become important should be slow if what we think we know about spread is correct.

In the region of the world where *Musa* and its two great pathogens are coevolved the situation should be very different in terms of diversity of genes both for pathogenicity and for resistance. But exactly what part of the *Musa* homeland also contains these pathogens as indigenous in any active coevolving relationship? Does it extend to India, to Fiji, to Papua New Guinea? Much pathosystem analysis is needed in the presumed centres of origin. Since *Fusarium* wilt is endemic in Southeast Asia in AAA, AAB and ABB karyotypes (Vakil 1965), it is highly probable that its variability there is much greater than is represented by our concept of the four races.

**Clonal replacement** Although those in the banana business have some idea of the cost and magnitude of effort required to restock lands with a new clone, newcomers may underestimate how these considerations may influence strategy and feasibility of banana breeding schemes.

For the established banana trades where Cavendish is not apparently threatened by *Fusarium*, a *Cercospora*-resistant clone based on *Fusarium* wilt susceptible Highgate would find considerable opposition in company management. They would be concerned with the risk of breakdown of the *Fusarium* wilt resistance, and have to balance that against the financial advantages of Sigatoka resistance.

Plantain culture, now threatened in many places by Black Sigatoka, is in a completely different position. If a new plantain can be constructed from two *Fusarium* wilt resistant (both races 1 and 2) parents, it would be accepted immediately and would be pushed by agencies concerned with local farmer welfare.

For Pacific Islands where black leaf streak or Black Sigatoka devastates formerly successful banana export production and where *Fusarium* wilt is not a threat, a *Cercospora*-resistant clone somewhat less perfect than ideal might still work and be a great boon.

In Brazil, where special clones of AAB are desired, a *Fusarium*-resistant clone with good Pome/Silk type taste would be readily adopted. The question of Black Sigatoka resistance has to be handled by the breeding program outside Brazil since it does not yet occur in the country.

Is it really realistic to think of a continuing breeding program that would result in one improved variety after another — or the need for replacement of 'collapsed resistance'? Or are we indeed confined to a 'one-shot' deal for either or both *Fusarium* and *Cercospora* resistance? Would sufficient financial support continue for maintaining a good breeding program? The answers are not clear but they will differ from place to place and they require local analysis.

The economics and the feasibility of variety replacement should be studied in each region as a prerequisite to making decisions on breeding and on breeding strategy.

In judging breeding for resistance and the benefits of resistance to the one or two major pathogens, one should also estimate the cost of nematodes to the local industry in terms of control, yield reduction, and either field shifting or replanting frequency. Certainly for plantains, resistance to several nematodes, and probably to their accompanying fungi, needs to be considered a primary target, along with resistance to *Cercospora*.

The potential for increases or decreases in yield and quality must also be considered in breeding and selection for resistance. Each region has different standards and different possibilities and dangers. If yield and quality are high, as for Grande Naine in Latin American export trades, the difficulties of matching these standards are great. For less sophisticated industries, the situation is different. For plantains, inherently low yielding, the possibilities and needs for combining both higher yielding ability with higher nematode and *Cercospora* resistance offer added incentives for breeding.

### The *Cercospora* Pathogens

The banana breeding programs in the Caribbean and Honduras were, prior to about 1970, confronted only with Yellow Sigatoka (YS). Presently, only the breeding program in Honduras has Black Sigatoka (BS) challenging its parents and progenies. The programs in Jamaica, Brazil and Guadeloupe are located in areas where neither Black Sigatoka nor Black Leaf Streak (BLS) exist. The nascent program in Guadeloupe plans to use Cameroon as a screening site for Black Sigatoka. Thus, the only breeding program with considerable experience and information on resistance to Black Sigatoka is that in Honduras. However, collections exist in several areas where BLS/BS occurs in favourable environments and these either have been scored for relative susceptibility, or they could be (Pearson et al. 1983; Foure 1985). Local reports exist for scoring of collections in the Philippines, Nigeria, Cameroon and Honduras. Relative susceptibility of

the same material in these diverse sites should be assessed. Apparently genotypes rank in the same order of susceptibility for Black Leaf Streak as for Black Sigatoka. On limited observations to date there is no evidence that these two names represent different virulence or pathogenicity genes. Contrarily, both, when compared with Yellow Sigatoka, have different genes which increase their host range and their virulence. General observations indicate similar ranking but less susceptibility with YS, but careful comparisons have not been made.

Almost all our knowledge of the Sigatoka pathogens and their biology comes from their existence in banana plantations, on Gros Michel or Cavendish. An exception is the work of Brun (1963) in Guinea. Another exception is Meredith's work (1970) in Hawaii where his studies were based on an isolated varietal collection. In this case, however, he was working only with *Mycosphaerella fijiensis*, the Black Leaf Streak pathogen. A flurry of work followed the appearance of Black Sigatoka in Honduras wherein the essentially identical disease symptoms were considered to be caused by a form (var. *difformis*) of *M. fijiensis* (Stover 1976).

Of considerable scientific interest would be to determine if the occurrence in Honduras was an introduction of a new and separate evolutionary event. I have hypothesised that since it occurred in the only location in Latin America where great host genetic variability existed, it would have been possible for new and different mutants to have been conserved on different hosts and for them to have recombined virulence genes to create a 'new' pathogen. It should still be possible to investigate this hypothesis by assaying the fungal variability in this site of possible evolution and comparing it with that in both the distant areas into which the 'new' form subsequently spread and the areas from which it might have been introduced (such as the Pacific Islands). A key question also is to determine how genetically different the BS pathogen is from the YS one. Alternative to the idea of a new evolutionary event would be that of introduction from the original coevolved centre. This clearly happened for BLS in West Africa (from Taiwan) and it could well have been so for the Honduran event since the germplasm collection and breeding program are the site for international visitors and traffic as well as for planting stock introductions. At first, the difference from BLS in sporodochial production indicated a new evolutionary event, but the findings by Stover (1976) that pathogens indistinguishable from the Honduran BS were widespread in the Pacific Islands strengthens the introduction hypothesis.

The most interesting and perplexing phenomenon has been the replacement (and apparent extinction) of the Yellow Sigatoka pathogen by the BLS/BS

pathogens as they have spread in Fiji, Central and South America and Africa. Stover (1976) travelled through the Pacific area and Asia in 1975 and concluded that the BLS pathogen was present (and not BS) in Hawaii and the Philippines; that the BS pathogen was present (and not BLS) on all other Pacific Islands (including the Solomons, Papua New Guinea) and Taiwan. His collections from Fiji revealed only BS, but he cites Meredith and Firman (1970) wherein they found in the Nadi area of Fiji the Yellow Sigatoka pathogen as well as that of BLS. At that time the BS pathogen (distinguished from that of BLS only by sporodochial production) was not known. Stover found that the BLS and BS pathogens could not be distinguished in culture and that they were clearly separate from the Yellow Sigatoka pathogen. Collections received by Stover in 1975 from Bogor (Java), Indonesia and Kuala Lumpur in Malaysia revealed only *M. musicola*. My observations in Sri Lanka in 1984 were that only *M. musicola* was present. Of considerable interest is Stover's re-examination of herbarium material dated 1927 from Taiwan, material which proved to have the pathogen of BS. Pearson et al. (1983) confirmed the presence of only BS in the Port Moresby area of Papua New Guinea.

No one has gone back to the pathogens' homeland in Southeast Asia and either collected extensively for mycological and genetic work or has studied the diseases and pathogens in situ in scattered domestic plantings or in the wild. Thus, we do not know if the three Sigatoka forms, or many more, are living together there in a compatible fashion. We also do not know the most important point of all — if they can interbreed. My prediction is that the BLS and BS pathogens will intercross freely and that they will segregate the sporodochial trait. If this is so, then collections from BLS/BS symptoms in the original area of evolution will reveal both sporodochial and non-sporodochial isolates. If indeed there are islands or continents where only one type occurs it would mean limited introduction and then spread of only one or the other. Although the probability of crossing of BLS/BS with YS is less, on observations which indicate a lack of intermediate symptom forms, crossing should certainly be considered and attempted. It could be that simple 1:1 inheritance occurs (in symptom expression) and that the extinction of YS is purely epidemiologically based.

The Yellow Sigatoka pathogen (*Mycosphaerella musicola*) was proven to be hermaphroditic but heterothallic, with a single pair of alleles controlling ascospore production (Stover 1963). These findings were from pairings conducted on banana leaves, since ascospore production in culture has not been obtained. The BLS/BS pathogen also produces abundant ascospores in nature. The type of its

sexuality has not been reported.

There is little work on genetics of resistance/pathogenicity with other *Cercospora/Myco-sphaerella* species. Thus, we have no guidelines to help us as we approach the subject for *Musa*.

### Susceptibility to the *Cercosporas*

Apparently all *Musa* can be infected even by YS but subsequent fungal growth is more rapid and extensive in some than in others. The more rapid and extensive the fungal growth the greater the symptoms. Resistance is relative and is seen as lesions appearing on lower (older) rather than upper leaves, and fewer rather than more lesions in a certain group of numbered leaves. Various refinements based on timing of the key stages of the infection cycle and on sporulation have been used, but basically, *relative* susceptibility is easy to estimate. FAO (1971) has produced a rating system. The key need is to remove environmental variance across sites and dates, by the use of a set of standards with known levels of resistance/susceptibility. No one has used such clones as standards in evaluating progeny in breeding programs. A new set of differentials is now needed for BLS/BS. This is especially important since environmental conditions markedly influence lesion numbers and development time. Much less disease occurs in the dry season. Even temperature alone changes the host-parasite interaction to the extent that AAB plantains, disease-free in the lowlands, show considerable Sigatoka (YS) at 1000 m in the coffee-growing areas of Colombia.

It is generally considered that ABB cultivars are more resistant, but too much reliance on genomic origin obscures the point that plenty of resistance occurs within the species *M. acuminata* and this frequency varies by subspecies and original location. The most extensive and interesting work on comparative susceptibility was done 20 years ago in connection with the early breeding work in Honduras (Vakili 1968). Seventy per cent of seeded AA diploids were classed as resistant. This decreased to 26% for edible AAs and 19% for AAAs (Table 1). The frequency of resistance (to YS) based on geographical origin differed markedly: 94% of entries originating from Southeast Asia/Philippines were resistant versus only 19% of those from Papua New Guinea/Solomons (Table 2). This must tell us something about the original area of evolution of YS and/or of contrasting environments. The frequency of resistance in edible diploids decreased compared with seeded diploids and the frequency of resistance in triploids decreased even more. Although dosage effects for increased susceptibility might be implicated in the triploids, the lower frequency in edible diploids must indicate that either the resistant wild progenitors gave people fewer

**Table 1.** Yellow Sigatoka resistance/susceptibility of *Musa acuminata* in relation to parthenocarpy and ploidy.\*

Type	Resistant (%)	Susceptible (%)
Seeded AA	70	19
Edible AA	26	60
Edible AAA	19	76

\* After Vakili 1968. Based on 341 accessions.

**Table 2.** Yellow Sigatoka resistance/susceptibility of *Musa acuminata* in relation to geographic origin of accessions and their ploidy and parthenocarpy.\*

Region	Resistant (%)	Susceptible (%)
<i>Southeast Asia/Philippines</i>		
Seeded AA	94	6
Edible AA	34	66
Edible AAA	27	73
<i>New Guinea/Solomons</i>		
Seeded AA	19	81
Edible AA	14	86
Edible AAA	10	90

\* After Vakili 1968. Based on 433 accessions.

'good' edible types and/or that Yellow Sigatoka was not important enough in scattered village plantings to be selected against. All of this should be reexamined for BLS/BS with more diploid collections. The areas of coevolution of BLS/BS forms on *Musa* are possibly different from areas of YS evolution.

Regarding genetics, little is known publicly beyond the work of Vakili (1968) on YS with crosses of susceptible *M. acuminata banksii* with a resistant clone of *M. acuminata microcarpa* and with resistant *M. acuminata errans*. He concluded that multiple factors were involved and that resistance was partially dominant. Both resistant parents were partially heterozygous for resistance and the Zebrina clone of *microcarpa* had more resistance genes than the *errans* parent. It is of considerable interest that some resistant clones are carrying genes for susceptibility.

A cross between the homozygous susceptible *banksii* and a resistant edible diploid, 'Lidi,' gave all resistant F<sub>1</sub> progeny, indicating that at least one dominant gene conditioning resistance is present in the homozygous condition in 'Lidi.' Yet, when 'Lidi' was crossed with a short Gros Michel (Cocos, AAA) most progenies were susceptible. Although Vakili speculated that this was due to a dosage effect, additional reasons could be advanced. Since some F<sub>1</sub> progenies were resistant it could be that resistance in 'Lidi' is conditioned by multiple factors, some of which are in the heterozygous state.

Unfortunately, no further information on genetics is available from this work. Twenty years earlier a report from Jamaica suggested that resistance to Sigatoka (YS) was a dominant character (Larter 1947 see in Rowe and Richardson 1975).

Without further genetic work on resistance and susceptibility, using diploids and carrying through to F<sub>2</sub> and backcross generations, it is impossible to be more precise about genes conditioning resistance. Moreover, such work needs to be redone now for BLS/BS as well as YS.

With the ready sexuality of the pathogens, the genetics of pathogenicity and virulence and the genetics of the various diploid hosts for resistance/susceptibility should be readily determinable. This is crucial to any banana/plantain breeding program and of considerable scientific value as well. If unpublished data are available on this subject, every effort should be made to review the work and publish it.

It is probable that the degree of susceptibility is conditioned by several or many genes since 'susceptibility' across genotypes appears to be a continuous spectrum of degree. It must reflect many host processes which interact, over considerable time, to limit or not limit fungal development. On the other hand, it is quite probable that resistance can be conditioned by a single gene, blocking fungal development at one of many potential interacting sites. Thus, 'resistance' will be found to be due to different single genes or combinations thereof, in different materials.

*Toxins* Pertinent to discussions of susceptibility/virulence is work on a toxin produced by many cercosporas (Daub and Briggs 1983; Assante et al. 1977). It is especially interesting in relation to possibilities of tissue culture selection for resistance (Daub 1986). The toxin, cercosporin, is unique among fungal toxins in being a photosensitising agent. In the presence of light, cercosporin causes the peroxidation of polyunsaturated fatty acids in membrane lipids, leading to rapid membrane leakiness. The symptoms of the Sigatokas, with tissue watersoaking and the suppression of the disease under shade certainly fit a hypothesis of cercosporin-mediated pathogenesis. Relative susceptibility and relative virulence (of BS vs YS) could reflect relative production of cercosporin in host tissue. Thus, resistance could be seen as a suppression of cercosporin production. Thus, one could believe that selection for cercosporin resistance in tissue culture would be a potential avenue to pursue. But this may be a trap for the unwary. First, studies on testing *C. musae* for cercosporin production (amongst 60 other cercosporas) listed it as negative (Assante et al. 1977). This cursory screen may need rechecking. Either cercosporin or some other secondary

metabolite may be produced that governs relative host susceptibility. It would be of interest to see if the cercosporas which produce or do not produce cercosporin fit into different perfect genera of fungi.

If cercosporin is the important compound in pathogenesis, can one expect success using it as a screening agent? Daub (1986), in a review of tissue culture for selection of resistance to pathogens argues convincingly that the answer is No. This follows her own failure to select resistant cells of sugarbeet and protoplasts of tobacco. An appraisal of the original thinking of why cercosporin seemed an ideal candidate for in vitro selection reveals why this thinking was illogical (Daub 1986). Not only is cercosporin toxic to plants carrying resistance to the pathogen itself but it is toxic to all plants tested. Moreover, it is the basic structure of the polyunsaturated fatty acids that makes them susceptible to peroxidation and their essential function is tied to this basic structure. The idea that cells might be able to detoxify cercosporin is not favored by the nature and site of action (plasma membrane). If, indeed, cercosporin is effectively the mediator of pathogenesis, then increased resistance has to lie in host suppression of cercosporin production. Thus, resistance/susceptibility would have to be judged directly and we would not be able to direct the selection of higher resistance levels except through choice of parents and use of a standardised realistic challenge wherein the suppressive host effect can be judged accurately.

That somatic mutagenesis might result in resistance is not an impossibility and this could be pursued through tissue culture and somaclonal variation. No positive results are known to have been obtained so far, but would they be noticed in commercial operations where the plantings are sprayed anyway? An old report (Drummond 1964 — mentioned in Meredith 1970) mentioned suckers of a susceptible mother plant of an AAB clone being resistant to Sigatoka.

### The *Fusarium* Pathogens

There is abundant literature on the fusaria in general (Nelson et al. 1981) and on *Fusarium oxysporum* f.sp. *cubense*, the banana wilt pathogen (Stover 1962, 1972), and on other formae specialis of *F. oxysporum*, the xylem-invading *Fusarium* causing wilt of many crops.

Much of the literature on pathogen variability both in culture and in nature is confusing and conflicting and for *oxysporum*, a species without a known perfect stage, genetic information is entirely lacking. Identification of fusaria is an art usually left to a few specialists, and *Fusarium* taxonomy is a continuing bone of contention among specialists.

It is conventional to throw all fusaria isolated

from plants suffering a wilt disease into the species *F. oxysporum*, but not all oxysporums induce wilt symptoms.

It is also conventional to consider that fusaria which wilt different host species are different themselves and they are usually given different names at the level of 'formae speciales.' If the hosts, however, are considered sufficiently related, their wilt fusaria may be left with the same name but given a 'race' designation. Thus, we have race 3 of the banana wilt fungus, which is really a pathogen of the different genus *Heliconia*. Most 'races' however are described when a variety or cultivar, earlier considered resistant to a known wilt disease, is seen as susceptible. If, on inoculation, the first host is not attacked by the isolates from the newly susceptible cultivar then a new race is established and one has two races and two differential cultivars. This is what happened when Bluggoe was first found diseased in Honduras and race 2 was established. However, if a cultivar is newly found to be susceptible and the isolates still attack the old cultivar, then a new race is still established. The two hosts do not differentiate the races; rather, only the new cultivar detects that there is a different pathogenicity. This is the case for race 4 in Taiwan (Table 3). The situation of *Fusarium* wilt of Cavendish bananas in Taiwan is reviewed by Su et al. (1986).

**Table 3.** Races of *F. oxysporum* f.sp. *cabense* as defined by the reaction of three cultivars. The differentiating characteristics are shown in dotted boxes.\*

Cultivar	Cocos AAA	Bluggoe ABB	Cavendish AAA
Race 1	+	-	-
2	-	+	-
4	+	+	+

\* Race 4 data are for the Taiwan strain.

The implications in the first case, if one considers the new race to be derived from the old, is that a single virulence gene mutated to avirulence for the first host while simultaneously acquiring virulence to the second. The implication in the second case is that the fungus has retained the old gene for virulence but has acquired another virulence gene at a separate locus, giving it new virulence. Of course this is pure speculation since no genetic studies have been conducted. Since race 4 is also virulent on the race 2 host, one could just as well suggest an alteration of the race 1 virulence gene to a third form, enabling it to expand its hosts without losing its race 1 virulence. Thus, without a set of differential hosts, the situation we have today is that any strain capable of readily attacking Cavendish

clones would be called race 4. That essentially is the current definition of race 4.

The key questions needing answers in relation to a rational resistance breeding program for *Fusarium* wilt are:

- (1) Are the strains from Cavendish from Taiwan, Philippines, Australia, South Africa, etc. the same, with the same virulence genes, or not?
- (2) Do they represent different evolutionary events?
- (3) Has Cavendish successfully selected its pathogen in each case due to environmental factors acting on the host/parasite system, or is it due to the greater genetic potential of the *cabense* already in those particular soils?
- (4) If it is due to the greater genetic potential of the local *cabense*, was this potential really a result of the presence of prior different *Musa* hosts such as Abaca in the Philippines, Silk in Australia and Latundan and others in Taiwan?
- (5) If it is due to the latter, were these host/cultivar systems indigenous or were they themselves brought from other centres of origin?
- (6) What is the genetic variability of the wilt fusaria in the centre of origin of *Musa*? (a) In what areas is it indigenous? (b) Is selection pressure exerted on the wild species? (c) Do all the known races, and many more, representing different virulence and pathogenicity genes, occur? (d) What wilt fusaria occur on other Scitamineae in Asia?
- (7) Are the *cabense* in Latin America indigenous and if so, are they coevolved with certain *Heliconia* species in certain places and have they spread from there?

Stover and Buddenhagen (1986) proposed a greater pathogen variation in the centre of origin and a need for testing identities of race 4 strains from different locations. They also suggested that the Philippines race 4 might have been introduced from Taiwan, but this is probably incorrect, based on the mild pseudostem symptoms and on colony cultural differences detailed by Dr Shirley Nash-Smith (pers. comm.).

Dr Nash-Smith believes that the Taiwan and Philippines isolates differ markedly from each other (in culture) but they are similar in each region. She believes that they are not related and that the Philippines Cavendish-attacking *cabense* derives from Abaca. She also believes that the Latin American *cabenses* have great variability (the old Gros Michel ones) unlike the Philippines and Taiwan Cavendish isolates. She has not seen the Australian isolates of race 4.

Thus, we need many more definitive studies, using modern methods, to explore genetic relatedness as well as virulence/pathogenicity genes of *F. oxysporum* f.sp. *cabense*.



One point remains clear. The reason Cavendish remains resistant in the vast areas of Latin America where it was introduced into soils containing untold billions of *cubense* propagules is that the gene or genes necessary for virulence to Cavendish are not easy to acquire. If the local fusaria had those genes, Cavendish would be attacked there as well. It speaks for tremendous stability of this resistance and it leads one to believe that the capacity for appropriate virulence genes must come from prior coevolved systems.

*Information from other wilt/host systems* Is there any information on other wilt/host systems which might influence our thinking? *Fusarium* wilt of peas in Washington State became important shortly after the pea industry started in 1924 and within 6 years resistance to race 1 was needed and shortly incorporated (Kraft et al. 1981). Race 1 resistance due to a single dominant gene was adequate for 30 years but then two new races became important. Subsequently, up to 1980 three more races or strains have appeared. Thus, under monoculture, the deployment of varieties with different genes for resistance resulted, with varying lag times, in the appearance of 'races' overcoming the resistance. Even in this easily bred crop no satisfactory variety exists carrying resistance to all the races, so soils are assayed to determine the race present to enable the right variety to be grown.

The tomato story is somewhat different. Wilt was once the most common and destructive disease of tomato (Jones and Woltz 1981). A wild species was found to carry a single dominant gene conferring resistance and by 1941 resistant commercial tomatoes were in use. Race 2 was first reported in 1945 but it did not become of widespread concern until 1961. It was then reported from Florida, USA and rapidly from other states in the USA and countries as distant as Australia, Brazil, Morocco, and England. This nonimportance for years and then rapid importance in widely separated areas may mean either (1) seed transmission, or (2) wide deployment of cultivars which act as a specific screen for the new race. If earlier cultivars were not selective and since all had the same dominant resistance gene, then this selectivity trait has to be due to background genes. Resistance to both race 1 and race 2 was found in an interspecies hybrid and it was quickly incorporated into tomatoes everywhere. Two dominant single genes are said to be responsible but all known lines resistant to race 2 also carry resistance to race 1. Jones and Woltz (1981) argue that these major genes are adequate and better than the use of polygenes conferring tolerance. They also argue that in spite of reports on nematodes reducing the resistance conferred by the major genes, there is no field evidence to support this contention. Since their 1981 report, a race 3 has

been reported in Australia and elsewhere, so far with limited distribution.

The cotton wilt pathogens are designated as five races which are geographically separated (Smith et al. 1981). Armstrong and Armstrong (1978) reported six races on cotton. The pathogens are not confined to cotton and races 1 and 2 are differentiated artificially on soybean and tobacco. Seed transmission could explain the widespread presence of race 1 in USA and also in East Africa. Although breeding for resistance has been conducted since 1900 greater resistance is still needed in combination with high quality cotton. Studies on the inheritance of resistance have indicated everything from one dominant gene to additive polygenes, depending on the cross and the methods of challenge. It appears that adequate screening facilities and techniques are not available or used in many breeding programs and this lack limits progress.

The only paper wherein I have found a concern for and experimental work on what formae specialis and races really mean and where they come from is that of Bouhot (1981) in France, working with the melon wilt fungus. Salient points are that prior to 1963 only melons not carrying resistance genes were cultivated in France. Yet when varieties carrying either one or a second gene for resistance were introduced they were widely attacked in the second year. Moreover, some cultures made before introduction of the resistance genes were shown to carry virulence for the genes. Bouhot easily induced mutations in microconidia with nitro-soguanidine, and could create one race from another. He even made new races, not yet known in the field. He then attempted to change formae specialis. He was unable to mutate a gladiolus or leek *Fusarium* to virulence on melon. But when he used fusaria from Cucurbitaceae he was able to switch a f.sp. *niveum* (watermelon) to a race 0 of f.sp. *melonis*. Moreover he found that some isolates from either cucumber or melon contained spores that attacked watermelon. Thus, these cucurbitaceous pathogens are probably all one group, having minor differences in virulence genes only some of the time.

Although interesting, what do these examples really tell us? First, the durability of resistance seems to vary from short to long. There is no rule. It must depend on both the kind of gene added to the host as well as (or possibly even more so) on the variability of the pathogen. Obviously the ability to replace the old race depends upon dissemination as well as frequency of mutation of the new race, and both differ by crop and pathogen. There has been no concern for origin or coevolution and no attempt to determine the situation in the centre of origin of the crop species. Even where the cotton wilt races have such specific geographic locations I see no

speculation as to why or what it might mean to breeding. An enigma remains, the fairly long durability of resistance and then a report of a new race in one place followed by its appearance everywhere. Why is this? What does it mean? We do not know.

It seems to me that we must be more analytical for *cubense* and bananas. We must apply both good logic and modern techniques to understanding fungal variability in order to obtain durable resistance.

#### **Susceptibility to *Fusarium oxysporum* f. sp. *cubense***

The classical breeding scheme employed since the beginning has had the underlying assumption that resistance will be dominant and of high penetration. Also it assumes that the resistance will be simply inherited and that the male parent will be homozygous for resistance. Also it assumed that the fungal pathogen is uniform everywhere for virulence genes and that it will remain that way. Larter (1947) reported that wilt resistance was dominant and that virtual immunity was imparted to Gros Michel in the cross with a resistant diploid (see in Rowe and Richardson 1975). Vakili (1965) gives considerable data on susceptibility/resistance based on many crosses; little else is available publicly. Vakili concluded that resistance to race 1 in the edible diploid 'Lidi' is due to a single dominant gene in the heterozygous state. Also, that different accessions of *M. acuminata* subspecies *banksii* differed in susceptibility, to both race 1 and race 2. Subspecies *errans* was susceptible to race 1 but resistant to race 2. *Mycosphaerella balbisiana* was susceptible as a seedling but resistant as an adult plant. Vakili suggested that the subspecies *banksii*, from New Guinea, is not attacked in its homeland and that probably the wilt fungus is not present nor has it influenced *banksii* evolution. The subspecies *burmanica*, *malaccensis*, *microcarpa*, and *siamea*, on the other hand, are highly wilt resistant and they originate from Burma to Vietnam, Malaysia and the Soenda Islands where *Fusarium oxysporum* f. sp. *cubense* is endemic. Seedlings from the majority of the accessions from these subspecies segregated for resistance to both races.

In Jamaica, various diploids were considered to have polygenic resistance and a seedling screen of the potential parents was necessary to ensure sufficient resistance in the tetraploids (Shepherd and Lacy 1968).

Waite (1977) reports on reaction of container-grown plants of Gros Michel and Bluggoe in Honduras to inoculations of fusaria from Australia, Philippines, Malaysia, Thailand and various Latin American locations. The results were consistent with expectations based on source of the isolates, but

with only two differentials, unique pathogenicity genes would not have been detected. Waite concluded that further studies to determine the pathogenic characteristics of *Fusarium* clones attacking the Cavendish group and the AAB group in various countries is obviously needed. This statement is still true now, 10 years later. In fact, the cumbersome nature of tank-grown tests, and the reluctance to introduce foreign isolates for field tests and the uncertain correlation between pot-grown tests and field results and the lack heretofore of tissue culture 'plantlets' have all combined to limit knowledge of comparative virulence and pathogenicity of the banana wilt fusaria. Improved methods with small containers and tissue-grown plantlets such as that used by Sun and Su (1984) should be utilised not just for screening for resistance but for comparative virulence and pathogenicity studies to answer the questions posed above. Probably important and mostly neglected is to standardise temperatures for such tests, an emphasis for many years in *Fusarium* work in Wisconsin (Bosland and Williams 1984).

#### **Modern Methods for Understanding *Fusarium***

The lack of definitive genetic information on virulence and pathogenicity of the wilt fusaria, and the lack of genetic meaning to the terms race and formae specialis leave the person working with the system in a quandary as to what is worth doing.

Obviously new techniques must be applied. Some information on electrophoresis (Glynn and Reid 1969; Reddy and Stahmann 1972) and restriction fragment work applied to the wilt fusaria exists. Both should be tried anew, but they must be tried with the right isolates which will give evolutionary and practical scientific information.

An interesting new technique has been applied to obtain information on possible evolutionary relationships. The basic idea is that only closely related isolates will form heterocaryons and isolates can be grouped into 'vegetative compatibility groups' (VCGs) that represent relationships (Puhalla 1985; Correll et al. 1986). Preliminary studies indicate that different formae specialis belong to different compatibility groups and that even some races may be distinguished. This latter means that some formae specialis must contain different compatibility groups. The new technique which makes heterokaryon detection easy is the formation at high frequencies of nitrogen reductase deficient (*nit*) mutants by growing the fungi on a potassium chlorate medium. When subsequently grown on a minimal medium, thick growth occurs where heterokaryons are formed. Apparently the heterokaryons are unstable, however, so that they are not automatically useful for subsequent



pathogenicity tests to determine different virulence or pathogenicity gene effects.

Although data are still limited, it would seem this compatibility grouping should be tried for *cubense* isolates from different continents and those considered to be different races. The inference is that relatedness and descent are revealed because compatibility relies on having all of the many (6 to 10?) genes governing compatibility identical in each strain, whereas the races could differ by only one pathogenicity gene. If the pathogenic mutation has been from different parental stocks it could be revealed by the compatibility grouping.

A recent paper describes linear mitochondrial plasmid-like DNA from *Fusarium oxysporum* f.sp. *conglutinans* (Kistler and Leong 1986). All isolates of races 2 and 5 contained the same plasmid, even though they were from locations as separate as Japan, California and Germany. All race 1 isolates (a pathogen of radish, not cabbage) had a different plasmid, similar in size but nonhomologous. The suggestion was made that these elements may be carrying genes for host specificity. Even if not, it was suggested their presence could be diagnostic for determining the fungal race. (But in reality the difference here reflects a formae specialis difference, not a race difference.) The two races attacking cabbage varieties which differ by a single resistance gene were not separated.

In fact, for the *conglutinans* group all three modern methods (VCG, electrophoresis and RFLPs) gave the same differentiation of the races (Bosland, pers. comm.). Races 1 and 5 (cabbage pathogens) were separate from races 3 and 4 (stock pathogens) and race 2 was separate from the other two groups (see Armstrong and Armstrong (1966) for a discussion of *conglutinans*). Although this would seem to be a beautiful confirmation of pathotype with modern molecular characterisation techniques, a careful review of the key paper (Ramirez-Villupadua et al. 1985) gives me pause. The label race 5 (the first race to break Walker's famous type A resistance in cabbage) was given to a fungus isolated from cabbage in California from a field where no crucifer yellows was known previously. Moreover, race 1 is not known in California and thus cabbage varieties susceptible to race 1 can and are being grown. The variety which became diseased in this one field is susceptible to race 1. Thus, the 'new' race was not selected by a 'resistant' cultivar containing the A or race 1 resistance nor did it appear from a population of race 1 in wilt-affected soils. Moreover, it is not just a converted race 1 because it also was highly virulent on broccoli, cauliflower and some stock varieties not attacked by race 1, as well as being virulent to both old and new cabbage varieties. Thus, it is not just a simple race 1 change and it is a very different

pathotype in pathogenic capability. To me, the presumption is strong that it is a 'new' evolutionary event and that it arose out of saprophytic or other pathogenic (than race 1) fusaria. All the molecular methods, including the mitochondrial plasmid DNA homology work, could not pick up that it is a different pathotype with a different origin.

The work on compatibility groups and on plasmids is of interest but we do not know if it will prove to be general and useful for other formae specialis. It is certainly promising enough to examine for *cubense*.

Several laboratories are just beginning to work with ribosomal RNA homologies and with DNA restriction fragment length polymorphisms for *Fusarium* (Sally Leong and Tom Gordon, pers. comm.).

Much new work is underway in many laboratories, as is revealed by the recent book 'Molecular genetics of filamentous fungi,' (Timberlake 1985), and by papers such as that of Gilchrist and Yoder (1984). It would be well for those of us interested in practical but very scientific questions to interest molecular people in our questions so the elegant new techniques can be applied to our very important wilt disease pathogen.

Molecular biologists, however, want a clean system to work with, and one with a perfect stage would be chosen as a model unless special reasons existed for not so choosing. *Fusarium oxysporum* would not be an organism of choice. To clean up terminology in relation to communication and genetic research in this area, a very recent paper should be consulted, entitled 'Genetic terminology and practice for plant pathogenic fungi' (Yoder et al. 1986).

However, there are really two basic questions to explore. One concerns the genes for pathogenic specificity, the other concerns the genetic relatedness of the pathogens. This latter point could be addressed with existing modern methods, even for the wilt pathogens. Thus, the questions posed earlier on pathogen evolution and pathogen/host coevolution could be addressed for the banana wilt system.

### Nematodes

Nematodes have received much less research than the other major pathogens, in spite of their importance. Million of dollars are spent on nematode control in bananas and undoubtedly many more millions are lost through their effect in lowering yields. The reasons for this neglect are the insidious and hidden nature of the injury and the ability of *Musa* in the right moist environments to bear and continue to grow with considerable nematode damage.

Although some research on nematodes has been

carried out in connection with the Honduran breeding program, there has been little in-depth research. Most research worldwide on nematodes in relation to bananas has been on pragmatic aspects of control. Chemical control is commonly practiced by the export industries but for the low resource banana farmer and the plantain farmer, such control is too costly. Moreover, control chemicals are environmentally damaging.

Although it has long been known that nematodes were moved about in planting stock, that knowledge has not prevented their continuing dissemination. How much the indigenous nematode fauna is really responsible for the depredations and how much is due to the introduced nematode biotypes has never been addressed in spite of the great opportunity to explore this question as new isolated jungle lands have been developed into plantations over the years.

Thus, we know even less of nematode 'races' on bananas in different parts of the world than we know of the other major pathogens. Some interesting work in Central America on variability of *Radopholus similis* indicates that two banana biotypes exist there, differing in virulence (Pinochet 1979; Tarte et al. 1981). Earlier work on 'races' was published in 1971 (Edwards and Wehunt). A more general account of nematode problems of bananas by Tarte and Pinochet (1981) is very useful. This bulletin includes 43 references to nematode papers, most of which involve nematodes on *Musa*.

The most extensive published information on resistance is that of Wehunt et al. (1978), reporting on some of the work carried out in the 1960s in Honduras. In the Honduran work, 64 clones of interest to the breeding program were screened and it is clear that the clones range in resistance to *Radopholus similis* from high susceptibility to virtual immunity. The cultivar group 'Pisang Jari Buaya' (PJB) was identified as having considerable resistance in some accessions, but not in all. Some *banksii* derivatives, and others, were more susceptible than the susceptible check, 'Valery.' The results confirmed earlier observations that the Cavendish group is more susceptible than the Gros Michel group.

In more recent work the *Radopholus* resistant diploid parent 'Pisang Jari Buaya' (PJB) was found to be highly susceptible to *Pratylenchus coffeae* (Pinochet and Rowe 1978). Valery had similar root lesion indices with *P. coffeae* and *R. similis* and both Valery and PJB were good hosts for *Meloidogyne incognita*. Plantains are a preferred host for *P. coffeae* so plantains also would need resistance to this nematode.

Very little information has been published on genetics of resistance but PJB apparently carries one or more dominant genes for resistance to *Radopholus* (Pinochet and Rowe 1979). This

parent, crossed with SH1734 has produced an outstanding diploid (SH3143) with resistance even higher than that of PJB (see Rowe, these Proceedings).

Most of the comments made for the fungal pathogens apply also to the nematodes. It is not known how potentially variable they will be if resistant clones are deployed in one area, or if other areas will have different 'races,' differing in virulence genes. No one has studied their variability in the centre of origin of *Musa* and no one has assayed the genetics of the many wild subspecies in terms of nematode reaction. Much needs to be learned.

### Moko Disease

Moko disease (bacterial wilt of *Musa*) caused by a special race (race 2) of *Pseudomonas solanacearum*, has received little attention from the banana scientific community because of its limited distribution and its ease of control by prophylactic measures. However, it is potentially a very destructive disease. One strain is readily transmitted among inflorescences by insects and has caused major epidemics (Buddenhagen and Elsasser 1962). If this strain were introduced into the homeland of *Musa* in Asia it would probably spread quickly throughout Asia and devastate much local production. The Moko pathogen evolved in Latin America on *Heliconia* and is absent from Africa and Asia, except for Mindanao in the southern Philippines where the Moko pathogen (a non insect-transmitted strain) was inadvertently introduced in seed pieces in the 1960s, from Honduras. The bacterial wilt pathogens of other crops are ubiquitous in Africa and Asia (Buddenhagen 1964; Buddenhagen and Kelman 1964). Some seeded diploids are attacked, however, by the ubiquitous and omnivorous *Pseudomonas solanacearum* race 1, in Honduras (Buddenhagen 1962), and thus they should be attacked in the Asian homeland as well, but there are no such reports.

Little is known regarding inheritance of resistance except for one seedling resistance study by Vakili. Resistance is mediated by plant response mechanisms which limit or slow down systemicity of the pathogen within the xylem vessels (Buddenhagen, unpublished data). Such limiting of systemicity is rare in *Musa* and it is not known if the resistance levels that exist would be useful in limiting the disease under natural field conditions. No one has bred for or selected progeny for resistance to Moko disease.

A second form of resistance occurs which operates as an escape mechanism for the insect-spread strain. This consists of persistence of small hemaphroditic fruits on the peduncle rachis, instead

of the conversion to male flowers which abscise. It is the site of male flower abscission, which has open xylem vessels and which exudes a nectar attractive to many bees and other insects, where infection occurs during insect-transmitted epidemics. These sites and others on the peduncle, bud and fruits also exude the bacterial pathogen in great quantities.

These Bluggoe type cultivars, such as Pelipita, which hold their hemaphroditic fruits/flowers, escape the disease. In any breeding program for AAB silk types or ABB Bluggoe types, selection for this escape mechanism should be practised. This is especially important in Brazil and elsewhere in Latin America, where insect-transmitted strains are prevalent and expanding in area.

A recent collection of papers on bacterial wilt in Asia has appeared (Persley 1986) which covers bacterial wilt of non-*Musa* species. One paper in this collection (Buddenhagen 1986) reviews races, genetics, and resistance and includes a discussion of Moko disease over all crops.

### Breeding

Breeding has been reviewed in two quite different treatments (Rowe 1984; Stover and Buddenhagen 1986). There are three basic breeding targets in terms of the final quality product: (1) Gros Michel/Cavendish (AAA), (2) Silk (AAB), and (3) Plantain (AAB). At present there are three breeding programs for the first target and one in Brazil for the second. For the third target a small effort has been made in Honduras but no major program exists anywhere. A fourth breeding target that should merit consideration would be for a Bluggoe (ABB) type fruit from plants resistant to both *Fusarium* wilt and Moko disease.

Everyone essentially would be starting from scratch for objectives 3 and 4. For objective 1, those who have invested years and millions in background diploid breeding (in Jamaica and Honduras) are leagues ahead of anyone beginning, and thus whether or not the advanced diploids are to be shared is a critical question for consideration by anyone thinking of beginning breeding for target 1. In fact, I think the presence of excellent bred diploids inhibits the advanced breeding programs from considering going back to any wild species or any cultivated diploids for use in breeding. This effectively cuts off a broader germplasm base. (In fact, for most crop species, this is also the case.)

For objective 2 (Silk types) Brazil should be ahead of everyone, but their lack of Black Sigatoka is a major difficulty. No one else has been very interested in investment into 'Silk' types.

The problem with too few localised breeding programs is that they easily become stultified if not connected with current good university research

advances and at the same time their target horizons may be much narrower than real needs in different parts of the world. To back these points, one may ask how many doctoral theses have been produced at a good university since the United Fruit Company cut back its research in the late 1960s? Also one could cite lack of any concerted effort to utilise the good dessert tetraploids resistant to BS and *Fusarium* for local use in low resource farming anywhere. Jamaica is just starting. Also there has been a lack of any real concerted effort to produce a BS-resistant plantain, now some 16 years after BS invaded Latin America.

Above and beyond resistance considerations is a consideration of the agronomic status of the existing cultivars. If Grande Naine is the excellent and ideal ideotype already existing for the AAA target, then matching it is, indeed, difficult (Stover and Buddenhagen 1986). However, for breeding targets 2, 3, and 4, existing desired clones are agronomically quite deficient from the ideal in terms of yielding ability and stature, so matching or improving them should be relatively easy.

We have argued that the classical breeding approach of creating a tetraploid from an intact 'Highgate' genome plus a gamete from a resistant diploid should be changed (Stover and Buddenhagen 1986). Very good tetraploids have been produced which would be very useful in low resource agriculture in many parts of the world. However, the approach is a dead-end since any further attempt to use the tetraploid in breeding results in break-up of the 'Highgate' genome. After reviewing breeding for *Fusarium* resistance in other crops I am convinced that the *Fusarium* susceptibility of the 'Highgate' genome is a major danger and a more important concern than the slightly poorer crop physiological characters of known tetraploids. The same may be true for making tetraploids with the AAB Brazilian bananas.

Certainly for breeding targets 2, 3, and 4 there is no need to even start with the tetraploid approach. Rather, one should start by exploring where the centres were for natural synthesis of those triploids and utilise diploids in great numbers from those regions. They could be screened for resistance, improved themselves, and utilised for resynthesising the desired triploids.

In such a program, various advantages accrue which are common in other crop-breeding programs. Progenies can be large enough for heritability studies, tester lines can be used and breeding values estimated, inbreds can be made, heterotic values calculated, and most important, recurrent selection can be carried out. Moreover, the strong need to have absolute parthenocarpy can be backed away from, making for much greater flexibility in breeding at the diploid level.

No one has mentioned inbreeding depression in *Musa*. Vakili (1968) showed that wild accessions were heterozygous even for resistance genes but there is no mention of general effects of inbreeding. Could inbreeding be practiced to 'clean up' materials so that superior breeding value can come from the surviving inbreds, then homozygous for better alleles? Although high heterozygosity is essential in the final product (Bingham 1980), having many inbreds as parents may enhance identification of, and obtaining, superior heterotic products.

In the past, some diploids were known to undergo natural restitution at a moderate level and the idea of utilising restitution to produce triploids was thus limited to these. Secondary triploids resulting from a breakup of the 'Highgate' genome in tetraploids also was very limiting. But now with tissue culture and the production of meristems of small size the possibility of inducing tetraploids with colchicine, as was done for true seedlings (Vakili 1967) is a major opportunity. It opens great opportunities for resynthesis of new superior triploids, starting from scratch. This could be done for all the breeding targets.

The other great advantage of using only diploids in breeding is that their resistance genetics can be studied and different resistance genes can be pyramided before triploid synthesis. This will be enhanced if diploids are bred which are more fertile, a definite possibility if the final product is a triploid. Earlier, with a tetraploid target, parthenocarpy had to be intense to preclude an occasional seed in pollen fertile and potentially female fertile tetraploids. This strong parthenocarpy at the diploid level limited (and still limits) flexibility in diploid improvement.

### Tissue Culture Techniques

Somaclonal variation so far obtained in banana is significant and, thus, this new methodology should be examined for breeding. No one has attempted gametoclonal work. A good overview of somaclonal and gametoclonal variation up to 1984 is provided by Evans et al. (1984).

The more recent review by Daub (1986) is directly pertinent to tissue culture in relation to resistance to pathogens and it includes references to three successes with *Fusarium oxysporum*, two of which were proven to be transmitted to progeny. It also gives a good discussion of possibilities and problems in using selective agents versus no selective pressure.

Tissue culture techniques should be applied to obtain dwarfness, possibly with the addition of mutagens. Dwarfness is an important need in breeding and it would be most useful to have dwarfness more general in diploids, to be not confined to only a few dwarf donors. The stature series of the Cavendish group should also exist with

the 'Silk' types, the plantains and with 'Bluggoe.' Although a few dwarfs are known, they are not abundant and these groups need short clones as breeding goals, just as is needed for the dessert bananas. It would be most interesting to do abundant somaclonal work with the diploid parents, not only to obtain dwarfs but other variations as well. As one approaches biotechnology in relation to plant breeding one should be aware of some of the implications in relation to public versus private research support, propriety rights, patents, and the social and political impact (Hansen et al. 1986).

### Cytology

The cytological work with *Musa* has been very limited and it is now 40-60 years old. Could new cytological work on parents of interest and on Cavendish be helpful in breeding? If one wishes to continue with triploid restitution breeding the logical first step would be to apply cytology and physiology to understand sporogenesis and pollen tube growth in Grande Naine in order to enable it to enter into breeding. It may well be possible to manipulate the system to get either 'n' or '3n' products in Grande Naine. No one has really tried, using modern methods. Even without a restitution approach it would certainly be of interest to have Grande Naine enter into the breeding parentage in some way. Indeed, Cavendish is in the parentage through the improbable single case of trapping a single haploid gamete (Rowe 1984). If this could be done with Grande Naine (with many haploid gametes), there could be many recombinants of interest.

### Conclusion

The practical and scientific worth of a much more in-depth approach to banana and plantain breeding and genetics is very great. With the abundant talent available today wishing to work on interesting problems in breeding, genetics, molecular genetics, and pathology, great progress could be made if there were funds, a focus, good leadership and sufficient communication between the past knowledge and the present and future technology. It is amazing how neglected *Musa* has been for basic studies. It provides very important crops for people, yet, like so many tropical crops, it has been badly neglected. I think the failure to support and carry out basic work has to be laid at the lack of a strong connection of good universities in the tropics with the botany and agriculture of their locales. In addition, the corporate management of profitable production has not been wise enough to see the needs and advantage of long-term basic research and thus their support has been sporadic. Moreover, they have not linked up with universities for a better addressing of their

problems and their opportunities. And finally, the international agricultural research system has largely ignored *Musa* due to the agronomic bias of original leadership and the bias against any 'commercial' crop.

The opportunities are there. I hope this wonderful crop can now receive its just due from science.

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# Varietal Reactions of Bananas and Plantains to Black Leaf Streak Disease \*

E. Fouré \*\*

SIGATOKA diseases in bananas and plantains are caused by *Mycosphaerella musicola*, the agent of Yellow Sigatoka, and by *Mycosphaerella fijiensis*, the agent of Black Sigatoka or black leaf streak disease.

These two diseases are the most important diseases of banana, and may cause the total collapse of the plant, in highly susceptible varieties.

The initial symptoms are foliar leaf streaks, with consequent reduction in photosynthetic leaf surface. This results in a loss in gross yield at harvest time and also in the premature ripening of the fruit on the trunk, which prevents the marketing of the crop with a consequent net yield loss in exportable tonnage.

*Mycosphaerella musicola* (Yellow Sigatoka) attacks bananas but not plantains. However, *Mycosphaerella fijiensis* attacks both bananas and plantains. Plantains are a staple food in many West African countries. Black Sigatoka appeared only recently in Africa. It was identified in Gabon in 1980; the neighbouring countries (the Congo, Equatorial Guinea and Cameroon) are also now affected by the disease, which is rapidly replacing Yellow Sigatoka. Black Sigatoka has also been reported recently in the Ivory Coast and in Nigeria in the Port Harcourt area.

A research program to study the pathogenic activity of the fungus was established in Gabon in 1981. Its aim has been to develop improved methods to control the disease and to become better acquainted with the biology and epidemiology of the fungus. Such a program has also been undertaken in Cameroon since November 1985. The research conducted in Africa into *Mycosphaerella fijiensis* has benefited from the work undertaken for many years by IRFA on *Mycosphaerella musicola*.

In our investigation into the biology of the Black Sigatoka pathogen and into the evolution of the

disease, we have made use of previous research and are now able to launch a systemic control program for the disease as soon as notification reaches us of new outbreaks.

Although encouraging results have been obtained in our research into chemical control, it seems that the genetic approach is the only control strategy that allows the maintenance of production levels in the long term. Genetic improvement is now given priority in research, with the aim of finding varieties which are resistant or tolerant to Black Sigatoka.

We have studied under conditions of high incidence of Black Sigatoka the behaviour of cultivars belonging to the diploid (AA) and triploid (AAA) *acuminata* groups, and the hybrid triploids AAB and ABB.

We have also completed our studies on phases of incubation and development of the disease along the following parameters: morphological traits of asexual and sexual phases; intensity of sporulation; study of the lateral transportation of ascospores; and distribution of symptoms on the plant.

The evolutionary cycle of *Mycosphaerella fijiensis* comprises, as in the case of all other parasites, two distinct phases: 1) dispersion and establishment, followed by 2) development of the interaction with the host.

## Dispersion

Dispersion takes place in two successive stages: release of the inoculum and its dispersal.

## Production and Release of Ascospores

The principal factor in the release of ascospores is the rain or, at the minimum, the presence on the leaf of the liquid pellicule, which can also form in the wake of dew or mist. The tissue of the perithecium must be permeated by water. This phenomenon, however, results in one liberation only; others will be achieved if a period of time elapses between two consecutive rains, during which the tissues can dry.

\* Translation of paper written in French.

\*\* Institut de recherche agronomique (IRA), B.P. 832, Douala, Cameroon.



The tornado (hurricane) season with its short and violent rains, separated by sunny periods, is the most favourable period for the release of the ascospores. The bicellular ascospores can be produced quickly; the precocity of the production depends on a number of factors among which climate is the most important.

If a significant quantity of inoculum is present, and if climatic factors are favourable to the development of the disease, the perithecium will form very quickly; if stages two or three coalesce, necrotic areas will appear on the foliage. The speed with which sexual fructification of the fungus takes place, being proportional to the speed with which lesions evolve, also depends on the cultivar.

Results from Gabon indicate that in humid or equatorial conditions the production of ascospores can continue throughout the year. The Cavendish subgroup, in particular the cultivar Poyo, has always shown significantly higher values for ascospore production compared with other cultivar studies.

### Production and Liberation of Conidia

For the majority of the observed cultivars, the study of asexual sporulation in Gabon has shown that the production of conidia by *Mycosphaerella fijiensis* is very low compared to that of *Mycosphaerella musicola* and is strongly affected by the climate.

The first conidiophores become visible with the brown coloration of stage 1, but the production of conidia, even though more abundant during the first stages of the disease, can continue up to the stage preceding the appearance of necrotic areas. It is also subject to significant variations depending on the climate.

There is a correlation between the number of conidia produced and the sensitivity of the cultivar. The most significant values have been obtained with the cultivars Poyo (AAA) and Ebang (plantain AAB) which presented shorter evolutionary periods of the disease, when compared to other cultivars.

In contrast no sporulation could be observed on the cultivar Yangambi, which has shown high resistance to the Black Sigatoka.

### Dispersal of the Inoculum

The inoculum (conidia or ascospores) is located at the level of young leaves (production of conidia in the first stages of the disease on the leaves of rows 2 or 3) or at the level of the older leaves.

Dispersal can take place in two directions: 1) conidia are carried downward by water towards the leaves of young sprouts or towards the older leaves of the mother plant (reinfestations); they can also be transported by wind due to the way the spores are attached to the conidiophores. 2) Ascospores are

carried towards the young leaves by rising air currents, but are mainly transported laterally by the wind. This mode of dispersal explains why the disease is disseminated over long distances, and why this type of spore acts as the main inoculum.

### Host and Parasite Interaction

After the spores have germinated and the germinative tubes have penetrated, if internal conditions are favourable to the development of the fungus, the first symptoms will appear after varying incubation times, and will develop until the host is totally or partially destroyed.

### Incubation

The incubation period is defined as the period in the biological cycle of the fungus which follows penetration and ends with the appearance of the first symptom of the disease.

The duration of incubation is a highly variable phenomenon, depending on several factors such as climate, cultivars, and quantity of the inoculum.

For a given temperature, the duration of the incubation period is inversely proportional to the quantity of the inoculum deposited on the leaf. In regions with a regular climate, which is favourable to the disease (regardless of temperature), the quantity of the inoculum is the most important factor.

In Gabon, however, significant variations were recorded as a function of climatic conditions and of the cultivars of the bananas and plantains under study.

The results show that the length of the incubation period is not a sufficiently reliable criterion to determine the susceptibility of a cultivar. There is no correlation between incubation time and susceptibility.

Cultivars such as Yangambi (AAA) and Sweet Plantain (AA) have short incubation times compared to other cultivars, particularly during the period favourable to the disease; the later evolution of the lesions is either blocked at stage 2 (cv. Yangambi, no canker) or is very slow (cv Sweet Plantain).

### Symptoms of Black Sigatoka

*Stage 1* is the first external symptom of the disease. It appears as a small depigmentation spot whose whitish or yellow colour resembles stage 1 of Yellow Sigatoka disease. These symptoms are not visible in transmitted light and can be observed only on the underside of the leaf.

This stage can be regarded as preceding stage 1 of Meredith and Lawrence (1970), which is manifested by the rusty-brown coloration of the lesion. This symptom becomes distinctly visible due to its transparency.



## Methods of Disease Control

*Stage 2* appears as a stripe, generally brown in colour 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 colour of which resembles the stripe at stage 1 of Yellow Sigatoka. The colour of this stripe will change progressively to brown and later to black on the upper side of the limb, but will retain the brown colour 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 unfavourable 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 limb. It is surrounded by a yellow halo with the centre beginning to flatten out.

*Stage 6* is when the centre 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.

### Evolution of the Lesions

Following the appearance of the first symptom, the disease continues its evolution until the final stage of the lesion's development, when the cankers (which appeared at stage 2 or stage 6 above) coalesce.

The number of leaves still functioning at harvest time depends on the time taken for the lesions to develop. The number of functioning leaves is one of the criteria in determining the sensitivity of a cultivar.

Climatic conditions have a strong influence on the time taken for the lesions to develop; it may be 10 to 36 days with the Ebang (AAB) cultivar; 30–78 days with the Fougamou (ABB) cultivar. In general, apart from the strongly pronounced resistance of the triploid *acuminata* Yangambi (AAA), it is the groups ABB, represented by the cultivars Fougamou and AA, represented by Figue Sucrée, that offer the highest resistance to the Black Sigatoka.

However, significant differences have been observed between cultivars belonging to the same genetic group. This is the case, for instance, with the cultivars Poyo and Yangambi (AAA).

It is, in general, inadvisable to establish a classification of cultivar sensitivity, taking into account only one series of measures carried out during a well-defined climatic period. Research undertaken in Africa has shown that, depending on the effect of the climatic conditions and of the quality of the inoculum, the symptoms continue to develop throughout the year.

By making use of appropriate notification within the framework of an integrated strategy against Black Sigatoka, the number of chemical treatments employed can be reduced to the minimum while maintaining sanitary conditions still satisfactory for the culture of bananas and plantains. The zone by zone evolution of the disease may be followed by continual analysis of two types of parameters:

1) *Biological parameters* which consist of systematic control on the development of the disease in diverse ecological zones by observations on the foliage of the various stages of the disease.

2) *Climatic parameters*, such as evaporation and temperature, which allow the determination of an effective period of treatment in accordance with the climatic or geographic zone concerned.

In Guadeloupe climatic notification is dominant in the control of Yellow Sigatoka (*Mycosphaerella musicola*). The presence of centres of infestation in certain zones make the maintenance of an observation network obligatory in order to follow the evolution of the pathogen and to report on the effectiveness of the climatic notification system.

The climatic situation is characterised by the evaporation 'Piche' (measured under AMPS); it allows the distinction between conditions more or less favourable to the evolution of the fungus. (Temperature data are no longer taken into account since temperature rarely has a restrictive effect on the fungus.)

The principle of climatic notification is as follows:

The theoretical duration of effectiveness 'd' is estimated on the basis of the last spray treatment beginning with Ep. The weekly evaporation is balanced against the weekly evaporations since the last treatment:

$$dn = 0.5 EPn \quad Epn = \frac{En + Ep(n-1)}{2}$$

In Cameroon the control of Black Sigatoka is still based solely on biological notification. Meteorological stations have been installed on 3500 ha of industrial banana plantations, but since the climatic requirements of *Mycosphaerella fijiensis* are not fully understood as yet, reliable climatic notification has not been established.

From fungicidal tests we have found that the principles currently applied for the control of *Mycosphaerella musicola* cannot be systematically transposed to *Mycosphaerella fijiensis*, a pathogenic agent different from the former not only in certain characteristics but also in its pathogenic activity.

Since *Mycosphaerella musicola* strains resistant to Benzimidazoles have appeared in the Antilles, Cameroon, and the Ivory Coast, systemic fungicides belonging to another chemical family had to be employed. The chemical industry has proposed the

production of a series of fungicidal molecules whose common function is to act as inhibitors of the synthesis of sterols. Tests so far conducted have allowed us to document and to confirm the excellent action of systemic fungicides of the triazole group on *Mycosphaerella fijiensis*, both in foliar application (oily atomisation) and in distribution for contact with the pseudo trunk.

Other methods at our disposal made possible the treatment of small industrial plantations, difficult to treat from the air. Interesting results have been obtained with fungicides deposited in foliar sheaths.

Notification strategies, making use of systemic fungicides, have significantly reduced the number of treatments in comparison with other systemic processes.

In Guadeloupe, 25 annual treatments have been reduced to 6. In Cameroon, using a system of biological notification in connection with *Mycosphaerella fijiensis* 10-12 treatments are carried out on average each year.

### Conclusion

Work on hybridisation for the purpose of creating new varieties has proved difficult with *Musa*, chiefly

because of the almost total gametic sterility of all the clones presently cultivated, clones whose fruit development is parthenocarpic.

Methods of improvement at the present time rest essentially on in vitro culture (in vitro fertilisation, mutagenesis, fusion of protoplasts). Nevertheless, taxonomic studies and collections remain very important. Research programs in progress in Cameroon on varietal sensitivity integrate into broader plant improvement programs for banana and plantains.

The different clones tested have enabled us to provide evidence of a continuous gradation in behaviour from extreme sensitivity to resistance. The host-parasite interactions between the host genus *Musa* and the pathogen genus *Mycosphaerella* must be governed by a large number of genes which, as a function of their recombination, confer on *Musa* a gradient of reaction from susceptibility to resistance.

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# Measuring Response of *Musa* Cultivars to Sigatoka Pathogens and Proposed Screening Procedures

R.H. Stover \*

SINCE the first detailed study of resistance of edible banana varieties and diploids to *Mycosphaerella musicola* by Vakili (1968), there have been several reports of the response of edible clones to *Mycosphaerella fijiensis* (Meredith and Lawrence 1970; Meredith 1970; Firman 1972; Fouré 1982, 1985; Laville 1983). A survey of the Papua New Guinea banana collection classified most diploids with respect to response to *M. fijiensis* var. *difformis* (Pearson et al. 1983). All released bred tetraploids have had some resistance to Yellow and Black Sigatoka (Rowe 1984; Stover and Buddenhagen 1986). All of the disease ratings and resistance levels were based on natural field inoculum and mature plants.

With renewed international interest in banana breeding and the widespread occurrence of somaclonal variation in banana plants produced in vitro (Stover and Buddenhagen 1986), improved methods for rapidly screening large numbers of banana plants for response to *Mycosphaerella fijiensis* are needed.

This paper reviews the response of banana foliage to infection by the Sigatoka pathogens, and suggests some inoculation procedures for screening for resistance to *M. fijiensis* large numbers of young plants produced by conventional breeding procedures and in vitro.

## Methods

*Mycosphaerella fijiensis* does not produce ascospores in vitro and produces only a few conidia in freshly isolated cultures. Therefore, it is necessary to collect natural ascospore inoculum or place plants in areas where inoculum is abundant from nearby susceptible plants. Ascospores are present in large numbers in heavily infected leaves soon after mass spotting first appears in susceptible cultivars. Methods for collecting and detecting this inoculum

have been described (Anon. 1983) and were utilised for artificial inoculations.

Ascospore-bearing leaf tissue is utilised in two ways: (1) placing the leaves below young plants where natural or artificial rain leads to discharge and leaf deposition, or (2) stapling small pieces of ascospore-bearing leaf tissue directly onto the unfurled heart leaf or lower surface of the first open leaf after thoroughly wetting the dry pieces. In dry weather, a fine mist is applied over the plants from dusk to daylight. At the time of exposure to inocula the furled heart leaf and leaf number one are tagged and dated. Time to appearance of streaks, spots and ascospores can then be recorded as well as the area affected. The leaf number counting down from the first unfurled leaf as number one is also noted with respect to streak and spot formation.

Vakili (1968) proposed four diploid clones as standard varieties (Table 1). The response of these clones to Yellow and subsequently Black Sigatoka in Honduras was recorded.

**Table 1.** Edible diploid clones and origin proposed by Vakili (1968) for Sigatoka standard varieties.

Honduras accession No.	Group	Origin	Classification by Vakili
II-249	Siamea	Singapore	Resistant
III-154	Kokodja	Solomon Is.	Partially resistant
III-193	Inarnibal	North Borneo	Susceptible
III-100	Pisang mas	Philippines	Highly susceptible

## Results

Stover and Fulton (1966) placed heavily diseased ascospore-bearing leaves on the ground beneath disease-free Valery. Discharge of the *Mycosphaerella musicola* ascospores was triggered by natural rainfall and resulted in a distinct spotting pattern along the left lamina as a result of infection

\* C/o United Fruit Company, Tela Railroad Co., Apartado Postal 30, San Pedro Sula, Honduras.

of the furled heart leaf. When referring to the left and right laminae, reference is made to the view of the upper (ventral) surface from the base towards the apex. Spotting patterns were distinctly characteristic of natural infection with *Mycosphaerella musicola* ascospores. Thus far this technique has not been utilised with *M. fijiensis* but field observations indicate streaks resulting from ascospore infection appear first along the edge of the left lamina as with *M. musicola* indicating that most early infection occurs on the furled heart leaf.

Young plants in small and large containers in the greenhouse and in the field were inoculated by attaching pieces of ascospore-bearing tissue to the undersurface of leaf No. 1 (Fig. 1). Streaks appeared in 12–18 days (Table 2). In the field in sprayed areas streaks from natural infection did not appear until 60 days (Table 2). Streaks on unsprayed young plants developed into mass spotting in less than 40 days (Fig. 2).

The response of the four standard varieties to *M. musicola* as indicated by the youngest leaf spotted was followed through the dry and wet seasons (Fig. 3). Following the appearance of Black Sigatoka in Honduras in 1972, the response of the same varieties to *M. fijiensis* var. *difformis* was recorded (Table 3). There was no pronounced separation of the



Fig. 1. Inoculating the lower surface of leaf No. 1 with ascospore-bearing tissue. The dried tissue is soaked in water for 5 min and then stapled to the green leaf for 1 hour.

Table 2. Days to streaks and spots in field grown plantation bananas sprayed for Black Sigatoka control and naturally or artificially inoculated.

Date leaf No. 1 marked or inoculated	Inoculation method	Days to streaks	Leaf No. where streaks appeared	Days to spots	% plants shot when spots appeared
December 31	natural	61	6.3	104	80
April 28	natural	77	8.3	121	100
June 11	natural	77	8.2	119	100
March 9	artificial	15	3.0	61	—
July 26	artificial	18	3.2	75	—

Notes: Spots appeared on leaf 8.6 for March 9 and leaf 9.6 for July 26 inoculations; artificial inoculations made by stapling ascospore-laden leaf pieces to the lower surface of leaf No. 1. Fungicide applications made weekly to the upper leaf surface by aircraft did not prevent infection of the lower leaf surface but did delay spot development. Variety Grande Naine.

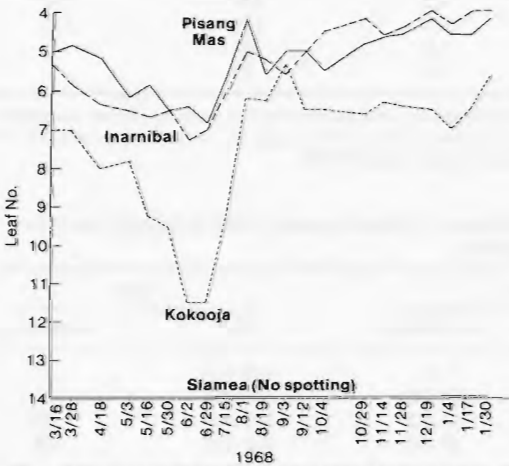
Table 3. Response of Vakili's Sigatoka standard diploid varieties to Yellow Sigatoka (1968) and Black Sigatoka (1976) in Honduras.

	1968		1976	
	YLS	% spotting	YLS	% spotting
Resistant (Siamea)	11.0–13.0	0	4.0–4.6	12
Partially resistant (Kokodja)	5.3– 9.5	18–20	3.7–4.2	19
Susceptible (Inarnibal)	4.2– 6.8	23–32	3.1–4.0	36
Highly susceptible (Pisang mas)	4.0– 7.3	27–36	3.0–3.8	38
Valery AAA	3.7	43	—	—

Notes: YLS = youngest leaf spotted indicating range of variability between wet and dry season for 1968 and wet season only for 1976; % = per cent of leaves in disease categories 3 (16–33% destroyed) and 4 (> 33% destroyed).



**Fig. 2.** Number one leaf from a young potted banana plant inoculated by attaching ascospore-laden leaf pieces to the undersurface after wetting. Upper right and lower left inoculum from Panama; upper left and lower right inoculum from Honduras. Photographed 54 days after inoculation.



**Fig. 3.** Response of four standard diploid varieties to *Mycosphaerella musicola* as indicated by the youngest leaf spotted (YLS). Mid-March through May is the dry season in Honduras and July to December the rainy season.

varieties Pisang mas and Inarnibal classified by Vakili as highly susceptible and susceptible, respectively. In contrast, the resistant and partially resistant Siamea and Kokodja varieties were widely different in disease response (Fig. 2). Siamea, Kokodja and Pisang mas could serve as standard varieties for resistant, partially resistant and susceptible categories, respectively.

## Discussion

The criteria utilised (Table 4) for evaluating foliage response to infection by the Sigatoka pathogens have been used for rating disease resistance and the effectiveness of fungicide control measures. To these could be added the amount of conidia and ascospore production since there is evidence that conidia production is reduced in resistant varieties. However, this involves tedious microscope work and the rate and amount of lesion development is much more easily evaluated, and are good indicators of resistance and susceptibility.

**Table 4.** Criteria for evaluating different levels of resistance and susceptibility in Sigatoka screening (modified after Vakili 1968, with additions).

1. Number of days from heart leaf and leaf No. 1 exposure to streak formation (first symptoms) Stage 2, (Fouré 1982); Fig. 5.
2. Number of days from streak appearance to mature lesions (dark brown to black spots) Stage 4, (Fouré 1982); Fig. 5.
3. Number of youngest leaf with mature lesions (youngest leaf spotted or YLS).
4. Percentage of leaves younger than No. 8 spotted.
5. Percentage of leaves in disease grades 1 and 2.
6. Percentage of leaves in disease grades 3 and 4.

**Notes:** The furled but fully exposed heart leaf and leaf No. 1 should be tagged and dated when plants first exposed to inoculum. Disease grades from Stover (1971).

Standard varieties are needed in order to compare response to the pathogen in different areas and seasons. The four diploid varieties proposed by Vakili (1968) for four disease levels actually measure only three categories clearly: resistant, partially resistant and susceptible. Another variety is needed to clearly separate susceptible from highly susceptible.

A small area of susceptible, unsprayed bananas can supply abundant ascospore inoculum for artificial inoculations. The area could be supplied with simple above-canopy misting equipment to ensure abundant infection and ascospore production during dry seasons. More research is needed to determine how sensitive the method is of attaching inoculum directly onto the undersurface

of the leaf in separating various levels of resistance or susceptibility. The amount of ascospore dose will vary and is not easily controlled. What influence this could have on plant response needs to be determined. The possibility of discharging ascospores into water and regulating the dose needs investigation.

Another method of producing inoculum is to grow single ascospore cultures on cheesecloth squares in bottles of liquid medium (Stover 1963). After 9-10 days growth the cloth squares containing fungus colonies are rubbed over the undersurface of the leaves. Inoculum consists mostly of hyphae and a few conidia. Cultures must be frequently renewed from leaf tissue because virulence declines with time in vitro.

A proposed system for screening large numbers of plants for resistance is outlined in Fig. 4. The first screen would be in a nursery with natural inoculum from infected leaves placed on the ground. Heavily infected leaves placed on the ground will continue to discharge ascospores in quantity for several weeks. This would be followed by a second screen using artificial inoculation and a comparison of the response with differential varieties.

There is no evidence that races of the Sigatoka pathogens exist. Ranking of varieties for resistance to *M. musicola* and *M. fijiensis* are similar although the latter is more virulent and causes much more

defoliation. However, *Mycosphaerella musicola* is heterothallic (Stover 1963) and recently there is an indication that *M. musicola* and *M. fijiensis* can form heterokaryons (Monier 1986). This suggests that the separation into two species (Mulder and Stover 1976) is not justified and we are actually dealing with two or three varieties. Further studies are needed to verify this.

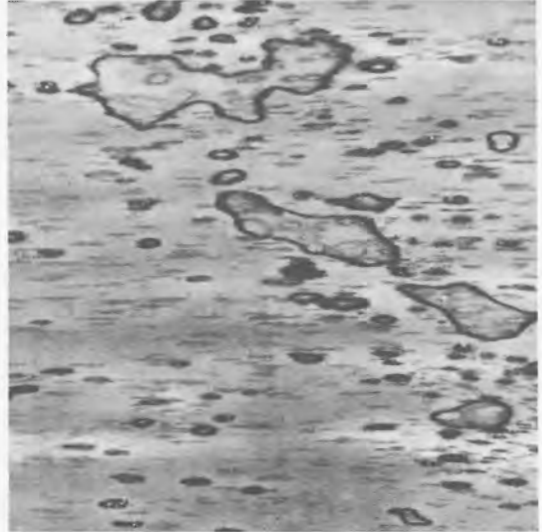


Fig. 5. Young streaks and mature spots with a dark border on Valery infected with *Mycosphaerella fijiensis* var. *difformis*. The smallest streaks correspond to stage 2 and the elliptical black spots to stage 4 of Fouré (1982). The large areas surrounded by a black border are coalescing areas of mass-infection. These turn grey in colour and are excellent sources of ascospores.

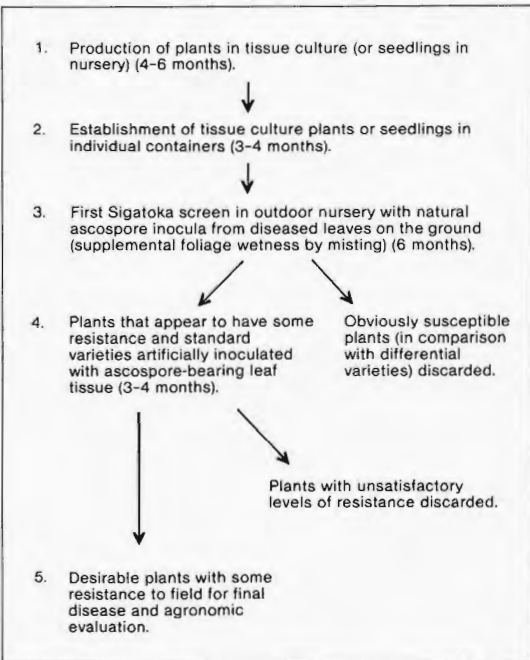


Fig. 4. Proposed outdoor screening procedures for resistance to *Mycosphaerella fijiensis*. Stages 3 and 4 could also be used for a combined *Fusarium* wilt screen.

### Recommendations for Research

Studies should be continued on: the inoculation methods outlined using the standard varieties Seamea, Kokodja, and Pisang mas and one Cavendish subgroup clone; measuring the variation in disease development throughout the year; developing a method of using a standardised dose of inoculum; measuring disease response in controlled environment chambers with a standardised inoculum dose; comparing the response of the standard varieties to geographically different pathogen populations to see if differences in virulence can be detected.

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# ***Fusarium* Wilt (Panama Disease): A Review**

**K.G. Pegg and P.W. Langdon \***

VARIOUS names have been applied to this disease. The name Panama disease was originally used in the literature by Rorer (1911) and Drost (1912), and was derived from that Central American country where wilt caused by *Fusarium oxysporum* f.sp. *cubense* first became epidemic. The more descriptive term banana wilt, first used in Jamaica in 1915, is preferable to Panama disease but there is a need to distinguish the fusarial from the bacterial wilt (or Moko disease). With races of *F. oxysporum* f.sp. *cubense* now attacking an increasing range of banana cultivars around the world, the name *Fusarium* wilt seems more appropriate as it is descriptive and in accordance with the naming of *Fusarium* wilts of other host species.

*Fusarium* wilt of banana is one of the most destructive diseases of tropical plants. The large volume of literature published on the various aspects of the disease has been thoroughly reviewed (Wardlaw 1972; Stover 1962, 1972; Simmonds 1966; Meredith 1970). The disease has been most destructive in the western tropics, particularly in Central America and the Caribbean, where it almost destroyed the banana export trade in the 1940s and 1950s, which was based on the clone Gros Michel. The industry was saved by replacing Gros Michel with resistant Cavendish clones and these have remained resistant to this day in the tropical regions. This has been heralded as one of the great successes of plant selection. However, in the subtropical areas of Taiwan (Hwang 1985), South Africa (Deacon 1984) and Queensland these Cavendish clones are being seriously damaged by *Fusarium* wilt. A Cavendish resistance-breaking strain (race 4) of the pathogen is present in these areas. There have also been periodic reports of *Fusarium* wilt in Cavendish clones in the Canary Islands since 1926 but the disease occurs only in some soils and it is not known if a new race is involved (Stover 1986). Race 4 was apparently introduced into the Philippines

(Mindanao) with planting material from Taiwan where it occasionally attacks Cavendish clones but apparently does not cause serious losses (Stover 1986). However, there are conflicting reports on the economic importance of this race in the Philippines. It is difficult to understand why *Fusarium* wilt in Cavendish clones became recognised as a serious problem almost simultaneously in much widely separated subtropical areas such as Australia, South Africa and Taiwan.

## **Present Status**

### **Queensland, Australia**

It is interesting to note that the first world recording of *Fusarium* wilt of banana was made in southeast Queensland in 1874 (Bancroft 1876) when Sugar [(Silk) AAB, dessert cultivar] was found to be highly susceptible. This was also the first plant disease recorded in that State. There are appreciable areas of Lady Finger (Pome, AAB, dessert cultivar), which represents 5% of Australian banana production, grown in southern Queensland and *Fusarium* wilt has been a major threat to this industry since early this century. Since the 1940s it has been common practice to replant devastated Lady Finger plantations with the wilt-resistant Cavendish clone Williams (Mons Mari, Giant Cavendish type, AAA, dessert cultivar).

In the early 1950s a wilt disease occurred in three Williams plants at Woongoolba (latitude 27°50') in southern Queensland (Purss 1953). An unidentified *Fusarium* sp. was isolated which proved to be pathogenic to Lady Finger and Williams, but in the same experiment Purss found that an isolate of *F. oxysporum* f.sp. *cubense* from Lady Finger did not infect Williams but produced typical wilt symptoms in Lady Finger plants. In 1976 R.A. Peterson (pers. comm.) observed *Fusarium* wilt in Williams at Wamuran in southern Queensland. Wilt had apparently been observed periodically by growers in Cavendish clones in this district for at least the preceding decade, but since only occasional plants were affected it was considered unimportant. The

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\* Plant Pathology Branch, Queensland Department of Primary Industries, Indooroopilly, Brisbane, Qld., Australia.



plants affected in 1976 were growing in heavy soils subject to waterlogging and since the rate of disease spread was much slower than in wilt-affected Lady Finger plantations it was at first thought that the problem was associated with edaphic conditions rather than a change in the organism.

In 1981 P.E. Mayers, alarmed at the increasing incidence of *Fusarium* wilt in Cavendish clones, considered that Cavendish was being attacked more severely than the physical environment (soil structure, fertility and water relations) could possibly warrant. He initiated pathogenicity tests which confirmed that a Cavendish resistance-breaking strain of *F. oxysporum* f.sp. *cubense* was present. *Fusarium* wilt now occurs, sometimes severely, in 42 Cavendish plantations located between Caboolture (latitude 27°) and Yandina (latitude 26°40') in southern Queensland. The disease was also detected in a small Cavendish planting at Byron Bay (latitude 28°30') in northern New South Wales in 1983. Nearly all outbreaks have occurred in plantations where wilt-susceptible Lady Finger was replaced with Cavendish clones. The new race (race 4) can probably be accounted for by chance mutation, dispersal and subsequent selection by the host. The *Fusarium* wilt pathogen was well established in Queensland in 1876 and what was possibly the first record of a new race with proven pathogenicity was in 1953 (Purss 1953). More than 30 years later we are now seeing the early stages of the development of a major epidemic.

#### South Africa

In the past 10–15 years *Fusarium* wilt has caused serious crop losses in South Africa. The disease is present on at least 24 plantations in the Transvaal (Kiepersol/Burgershall, latitude 25°), and on six of these properties it has reached serious proportions (Deacon 1984). It is also widespread in Natal but is not present in the Levubu/Soutpansberg, Tzaneen/Lataba and Malelane production areas, which supply about half the South African production.

The disease has been known in Natal since the turn of the century, but until 1974 Dwarf Cavendish was the only cultivar grown commercially in South Africa. A very limited occurrence of wilt was recorded in Dwarf Cavendish plantations. There has apparently never been an industry based on a race 1 susceptible cultivar in South Africa. The rapid and damaging spread of the disease has apparently only occurred since the Williams cultivar was released to the industry in 1974. However, the occurrence and development of the disease in South Africa does not seem to have followed any traceable evolutionary pattern.

#### Taiwan

Race 4, formerly referred to as T race, was first

recorded in Cavendish clones in the southern part of Taiwan in 1965. In 1984 *Fusarium* wilt destroyed about 500 out of 5000 ha of banana plantations in southern Taiwan (latitude 22° to 23°). Currently, an average of 15% of banana plants are affected by the disease in that area (Hwang 1985). There is the possibility that race 4 originated from race 1 in Taiwan, and since all race 4 isolates were identical in colony morphology it has been suggested that they may have originated from a single mutation (Sun et al. 1978).

#### Races of *Fusarium oxysporum* f.sp. *cubense*

Resistance breeding requires an understanding of the pathogen and its range of variability.

At present four races (pathotypes) of *F. oxysporum* f. sp. *cubense* have been differentiated and designated (race 1 attacks: Gros Michel (AAA), Apple (AAB), Silk (AAB), Taiwan Latundan (AAB), IC<sub>2</sub> (AAAA); race 2: Bluggoe (ABB) and close relatives, some Jamaica tetraploids (AAAA); race 3: *Heliconia* spp. in Honduras, Costa Rica; race 4: Cavendish (AAA), Taiwan Latundan (AAB), Gros Michel (AAA), Bluggoe (ABB), bred tetraploids (AAAA). One race (race 3) attacks *Heliconia* spp. in Honduras and is only mildly pathogenic to bananas (Waite 1963). The three races attacking *Musa* spp. can be separated on the varieties of Gros Michel (AAA) which is susceptible to races 1 and 4 but resistant to race 2; and Bluggoe (ABB) which is susceptible to races 2 and 4 but not race 1; and Cavendish (AAA) which is attacked by race 4 only. Originally there were only two races (1 and 2) causing wilt of *Musa* spp. in most banana-growing countries (Stover 1972). In 1967 a new race (4) capable of attacking Cavendish appeared in Taiwan (Sun et al. 1978). This was initially identified as a new race by its cultural morphology (lacinated colonies) on a refined agar medium (K-2). Race determination has subsequently been made using banana plantlets derived from tissue culture (Sun and Su 1984). In Queensland a comparison has been made of the esterase patterns of race 1 (Queensland) and race 4 isolates (Taiwan, South Africa and Queensland) using isozyme analysis (unpublished data). Race 1 isolates had two loci for esterase whereas all Cavendish isolates had only one locus. Culture morphology and isozyme patterns are associated characters and not necessarily a pleiotropic effect of the gene for virulence. Race determination must be based on virulence studies using differential varieties (host resistance genes).

The designation of race 4 is given to any Cavendish resistance-breaking strain of *Fusarium oxysporum* f.sp. *cubense*. However, these strains

(biotypes) may be of separate origin and thus may differ in virulence and even host range.

Virulence and aggressiveness are important and separate attributes of a pathogen. Virulence is defined as the relative capacity of a given isolate to produce severe disease, i.e. the capacity of the isolate to overcome resistance mechanisms and build up within the root-rhizome region. Aggressiveness is a measure of the rate of growth or reproduction of an isolate, or other attributes which confer ability to produce a given size lesion in less time, or produce more inoculum for infection of other tissues.

Cavendish was assumed to possess general resistance (horizontal, multigenic, polygenic or field resistance) to the wilt pathogen. However, the occurrence of race 4 and the demonstration of cultivar by isolate interaction suggests that the resistance must have a large vertical component. General resistance, which provides a variation in degree of attack from susceptibility to resistance, is only present if there is no differential interaction among genotypes of the host and genotypes of the pathogen. The vertical component in Cavendish clones is apparently greatly influenced by the environment and pathogen inoculum levels. This could explain the occasional breakdown in resistance to races 1 and 2.

### Histopathology

No cultivars are available that are immune to the wilt pathogen. Regardless of the pathotype or the cultivar used, the fungus is able to penetrate and establish in the vascular system of the root. Infection then induces a resistance reaction in cultivars possessing resistance genes, and this prevents proliferation of the pathogen by formation of a mechanical barrier (i.e. gels, gums, tyloses and vascular collapse). Gums and gels localise the pathogen by trapping conidia in the vessel elements. If the gels persist long enough to allow tyloses to form the pathogen is successfully contained (Beckman et al. 1962). There are apparently no structural differences between the xylem elements of resistant and susceptible cultivars. The crucial difference is how quickly the host plant responds to invasion.

The resistance reaction is apparently temperature sensitive. Beckman et al. (1962) found that the mechanical barrier was effective in resistant cultivars at 21, 27 and 34°C. It was effective at 34°C in a susceptible cultivar but not at 27°C when the gel disappeared rapidly and tylose formation was delayed. There is an urgent need to study patterns of colonisation associated with resistant and susceptible reactions when plants are challenged with race 4 at different temperatures. This will assist in developing realistic selection methods for resistance.

### Cultural Morphology and Biochemical Properties

In an international breeding or selection program the precise classification of the pathogen used for screening for resistance must be known in relation to isolates of the pathogen from other areas. Races which are differentiated on the basis of pathogenicity may not have a common origin and therefore may differ in virulence and host range.

It is important to determine whether other characters may be used which can be related to virulence or host range. 'Characters' which may be useful include those derived from studies of cultural morphology, protein analyses, enzyme analyses and serological techniques including polyclonal or monoclonal antisera.

Race 4 isolates, for example, were originally identified by the production of lacinated colonies on a refined agar medium (K-2) (Sun et al. 1978). W.C. Wong (pers. comm.) has also been able to identify race 4 isolates by their colony appearance on a synthetic medium. However, these morphological characters do not reflect the extent of genetic variations between isolates.

Protein patterns from one dimensional gel electrophoresis are generally only suitable for identification at the species level, and may not differentiate further divisions within the species. Two dimensional gels are more likely to differentiate these divisions as even minor changes in molecular weight or change can be detected. Closely related organisms can at times also be differentiated on the basis of single amino acid changes in one or more enzymes. Preliminary results with *F. oxysporum* f.sp. *cabense* staining for the enzyme esterase have revealed isolate relationships. Isozyme patterns for a number of enzyme systems may allow us to assess the origin of race 4 isolates in Australia, South Africa and Taiwan. Do these isolates have a common origin or has independent evolution occurred in each country? It seems most unlikely that race 4 could have been accidentally introduced from Taiwan into Australia. Two dimensional electrophoresis may allow us to determine whether race 4 has descended from race 1 by mutation at loci governing pathogenicity, or whether it has evolved from an unknown progenitor. Even though cultural morphology and biochemical properties may not be related to pathogenicity or virulence, they may prove useful for placing isolates in different evolutionary groups.

### Resistance Screening

Plantlets from meristem culture are considered promising for screening for wilt resistance. Sun and Su (1984) found that root dipping plantlets in a spore suspension ( $3 \times 10^4$  spores ml<sup>-1</sup>) before

replanting provided a rapid method for race determination and also suggested that it was useful for screening for wilt resistance. They found that the results of such pathogenicity tests after 1 month agreed with those with mature banana plants which took an average of 9 months for expression of external symptoms. We have found in Queensland that root dipping plantlets in spore suspensions of some isolates before replanting is a very severe form of inoculation and resistance genes cannot always cope with this type of challenge. For example, an isolate from wilt-affected Bluggoe plants in North Queensland growing in a field where Lady Finger and Cavendish clones remain unaffected, and which therefore by definition is race 2, is so virulent in glasshouse root dipping experiments that it produces significant vascular discoloration in Cavendish, Lady Finger and Bluggoe plants often resulting in their death. In this case, wilt expression in the glasshouse cannot be correlated with wilt expression under field conditions. Inoculum concentration is an important variable in typing races and in resistance selection and it may have to be varied depending on the level of virulence in a particular isolate.

The glasshouse screening technique used by Hwang (1985) may be a more reliable plantlet inoculation procedure. Plantlets for testing are planted in soil containing 600 cfu/g soil (range 400–1200 cfu/g soil) and are examined for disease 2 months later. Resistance genes are more likely to be able to cope with this type of inoculation.

Once resistant plants have been selected by glasshouse tests they must then be grown for several years in plantations where the disease occurs to determine if they remain resistant.

Hwang (1985) also conducts resistance screening in the field. Plantlets from meristem culture are grown in the glasshouse for 2 months (i.e. until 30 cm tall with well-developed corms) before transplanting into a field soil containing 1200 cfu/g soil (range 600–1500 cfu/g). Plants are examined for vascular discoloration 4 months later. Using his glasshouse and field screening techniques, Hwang has now screened 13 878 plants of Taiwan Cavendish and 7 have remained healthy. Plantlets derived from these seven survivors are currently being field-evaluated in race 4-infested soil in southern Queensland. A tremendous effort and cost is involved in developing field screening facilities and the amount of material which can be processed is limited. A glasshouse technique is urgently required which gives consistent and reproducible results which can be correlated with field performance.

We have tried to select for resistance by repeatedly flooding proliferating meristem cultures with fusaric acid or culture filtrates of *F. oxysporum* f.sp.

*cubense*. Surviving plantlets were grown in the glasshouse and then established in a race 4-infested plantation. However, these plants have succumbed to wilt thus providing further evidence that *F. oxysporum* f.sp. *cubense* does not overcome host resistance through the action of toxins on host protoplasts. Resistance is due to physical occlusion of the pathogen by the host. Results indicate that fusaric acid or culture filtrate probably cannot be used as a selection tool in vitro.

Resistance screening needs to be organised on an international basis. There are numerous reports of varieties being resistant in one area, but succumbing when introduced into another area. For example, tetraploids which were resistant in the area where they were selected have proved to be susceptible in other areas. This may be due to differences in pathogen virulence or in the host/pathogen/environment interactions. It is interesting to note that the resistance to race 4 in Cavendish clones is apparently adequate in the Philippines to prevent crop losses provided it is combined with appropriate crop management practices; practices which were developed after a thorough study of field parameters which affect susceptibility.

A set consisting of standard differentials, 'resistant' varieties and parents likely to be used in breeding programs needs to be assembled and established in all regions where the crop and disease are important. The ultimate objective should be to breed or select a genotype which will retain its resistance under conditions different from those under which it was originally selected.

### Suggested Areas of Research

A high rate of genetic variability can be generated from banana meristem culture. However, obtaining individuals with resistance to race 4 will be controlled by chance. They will occur at a very low frequency and this will necessitate screening a large number of plants. A reliable glasshouse screening technique is urgently needed. Plants resistant to *Fusarium* will confine the pathogen to the roots or relatively few vessels of the rhizome by formation of mechanical barriers. This type of resistance may not be expressed in small plantlets and material may have to be assessed in the field. Root dipping plantlets in a suspension of spores prior to replanting is a severe form of inoculation and it seems that the resistance genes cannot cope with this type of challenge. Planting in soil infested with a determined number of resting propagules, a technique developed by Hwang (1985), may be a much more reliable glasshouse screening procedure. It warrants further evaluation to determine the

correlation between resistance in young and field-grown plants.

A study is required on the anatomical and physiological responses to invasion in clones resistant and susceptible to race 4. This will assist in the development of more reliable screening methods.

Resistance breeding and selection to be successful on an international scale requires more knowledge of the pathogen and its range of variability (host range, physiologic race, and different degrees of virulence and aggressiveness). Cultural morphology and biochemical studies such as electrophoretic analysis may provide information on the origin of race 4. It may be possible to develop a relationship between these biochemical and cultural characters and pathogenicity.

A standard set of differentials plus cultivars with resistance to race 4, e.g. AAB plantains (Stover and Buddenhagen 1986), SH3142 and SH3362, plus breeding material should be assembled and distributed worldwide to give more information on the variability of the fungus and on the stability of resistance in the different banana-producing countries where *Fusarium* wilt is present. Areas such as southern Queensland provide a unique opportunity to study the susceptible/resistant reaction of a large number of clones as both race 1 and race 4 infested fields are available.

The inheritance of resistance in the banana is still not defined. The Cavendish  $\times$  race 4 interaction is apparently highly dependent on environmental factors, since race 4 is apparently under control in well-managed Cavendish plantations in the Philippines. Factors which may influence disease development with race 1 have been studied and thoroughly reviewed by Stover (1962) in his monograph. Expression of resistance to race 1 is apparently influenced by soil type, soil moisture, soil fertility, soil temperature, the condition of the root system, the inoculum threshold and virulence of the pathogen. The same factors probably apply to resistance to race 4, and any resistance to this race may need to be complemented by crop management practices based on sound epidemiological principles.

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# Banana Bunchy-Top Virus: a Continuing Threat

J.L. Dale \*

BANANA bunchy-top virus (BBTV) is the most important virus infecting bananas. It causes a serious debilitating disease. The potential threat of this virus may not be fully realised as its geographical distribution is limited. The first record of BBTV is from Fiji in 1889 and 1890 but the virus was probably present there as early as 1879. By 1901, BBTV had been recorded in Taiwan and Egypt, and from Sri Lanka and Australia in 1913. Up to 1986, the disease has been confirmed in 19 countries with unconfirmed reports from a further 11 countries. As recently as 1982, reports of the presence of the virus in a new area have been recorded (Manser 1982). Despite the large number of 'infected' countries, BBTV is essentially confined to the Asian-Pacific basin as well as a few countries in Africa. Fortunately, the virus has seemingly as yet not travelled to Central and South America nor to the Caribbean. Thus the major banana-exporting countries are at the present time free of banana bunchy-top virus with the exception of the Philippines.

## Current Status of BBTV Research

### Viral Etiology

By the end of the 1930s BBTV research was very much 'state of the art' due mainly to the work of C.J.P. Magee in Australia (Magee 1927; 1936). By 1927, Magee had discovered that bunchy-top disease was caused by a virus which was not sap transmissible but was transmitted by the aphid *Pentolonia nigronervosa* in a persistent manner. This was only 7 years after the first persistently aphid-transmitted virus had been recorded. Unfortunately, in the ensuing years, research on and knowledge of BBTV has not kept pace with plant virology in general. There are a number of possible reasons for this (Dale 1987).

Banana bunchy-top virus is now classified as a

possible luteovirus (Matthews 1982) based on: (a) persistent aphid transmission; (b) yellows-type disease; (c) phloem damage after infection; and (d) non-sap transmissibility. However, there have been no published reports on virus morphology. Spherical particles have been observed in 'purified' preparations (Dale and Behncken, unpublished data) but in too low a concentration for either further characterisation or confirmation that they are virus particles.

Further evidence that BBTV is a luteovirus was provided by double-stranded ribonucleic acid (ds RNA) analysis (Dale et al. 1986). Ds RNA usually only occurs in plants infected with RNA viruses (Dodds et al. 1984) even though some instances of apparently non-viral ds RNA in plants has been recorded (Wakarchuk and Hamilton 1985). We extracted ds RNA from bananas infected with BBTV but were unable to extract ds RNA from corresponding healthy plants. The number and molecular weights of the extracted ds RNAs from BBTV-infected plants closely resemble those of barley yellow dwarf virus (Gildow et al. 1983), the type member of the luteovirus group, thus adding credence to the classification of BBTV as a possible luteovirus.

Ultimate proof that BBTV is a luteovirus or, in fact, a virus awaits purification and full characterisation of the virus particles.

### Transmission

The aphid *Pentolonia nigronervosa* colonises *Musa* spp. but has a limited host range outside this genus. It occurs in most banana-producing countries. Most common transmission by aphids is short distance. Allen (1978) showed that 99% of new infections were within 86 m of their source of inoculum. However, it is possible for wind-borne aphids to spread BBTV over long distances.

BBTV is also efficiently transmitted in all banana-propagating material. Infected plants give rise to infected planting material allowing for very long-distant spread of the virus, including internationally. There is also evidence that BBTV is

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\* Plant Pathology Branch, Queensland Department of Primary Industries, Indooroopilly, Brisbane, Qld, Australia.

not eliminated by meristem tip culturing infected bananas.

### Host Range and Varietal Reactions

There has been relatively little effort expended on host range studies of BBTV. There are, of course, a number of problems associated with experimental host range studies of this virus; firstly, it is not sap transmissible and, secondly, its aphid vector, *P. nigronervosa*, has very strong host preferences itself. Magee (1927) recorded four species of *Musa* as being susceptible as well as *Ensete ventricosum* (family Musaceae). The only reported non-musaceous host is *Colocasia esculenta* which became symptomless infected after experimental inoculation (Ram and Summanwar 1984). This plant occurs in many countries commercially growing bananas and, therefore, it is very necessary to confirm this report as well as to determine whether this species is a natural host of BBTV.

Unfortunately, all species, cultivars and types of the genus *Musa* which have been experimentally challenged with BBTV have become infected. However, differences in susceptibility have been noted. Magee (1948) reported that the cultivar Gros Michel was more resistant to infection than Dwarf Cavendish and Jose (1981) inoculated 19 cultivars of banana of which Kanchikela and Venattukunnan were the most resistant.

### Strains of BBTV

Again there has been little work reported on strain variation within BBTV. Abaca bunchy-top virus (ABTV), recorded in the Philippines (Ocfemia 1926), is most probably a strain of BBTV. Both viruses are transmitted persistently by *P. nigronervosa*, and cause a yellows-type disease. ABTV infects *M. textilis* but not banana whereas BBTV infects both (Magee 1927). The possibility of a mild strain of BBTV was reported by Magee (1948) in very mildly infected plants of the banana cultivar Veimama. And Vakili (1969) reported a 'banana' strain and an 'abaca' strain of BBTV on the cultivar Giant Cavendish in Vietnam, based on symptoms.

### Control of BBTV — The Ultimate Aim

Before possible future research requirements can be identified for BBTV, it is necessary firstly to define the aim of the research, which in this case is control of the disease. Secondly, it is necessary to explore the possible research directions to attain control. There are a number of avenues open for controlling plant viruses that are applicable to BBTV.

### Quarantine

Fortunately, a significant proportion of the world's bananas are grown in countries where BBTV

has never been recorded, and, obviously, it is imperative to continue to exclude the disease from these countries. Countries, at present free of BBTV but most at risk are in the Asian-Pacific Basin and Africa because this virus is well established in these regions and often 'infected' countries share borders with 'virus-free' countries. At lesser risk are the countries of Central and South America and the Caribbean because of their distance from sources of infection.

To maintain virus freedom, a total ban on importation of banana material can be imposed or strict quarantine measures adhered to. Unfortunately, there is no sensitive and reliable diagnostic technique at present for BBTV. Currently, diagnosis is based on visual inspection for symptoms which is notoriously unreliable for most plant virus infection. Currently, therefore, there is a real and constant risk involved with international movement of banana material.

### Avoidance

Avoiding plant virus diseases can be achieved in a number of ways but in relation to BBTV this depends primarily on reducing or eliminating sources of infection. The highly successful campaign to control BBTV in southern Queensland and northern NSW beginning in the late 1920s was based on avoiding the disease. The strategy was to reduce sources of infection by providing virus-free planting material, eliminating (by roguing) infected material in plantations and limiting the spread of infected planting material by quarantine. While eradication is strived for by these measures, it has not been achieved, and BBTV continues to infect banana plantations in these areas, be it at a low level. BBTV was eradicated from north Queensland, an area which remained free of the disease during the devastating epidemic of the 1920s. Infected planting material was accidentally introduced into this area but subsequent infections were destroyed before the disease became established.

Whether the control measures used in Australia are applicable to other 'infected' countries is open to conjecture.

### Selection and Breeding for Resistance

Selection or breeding programs for resistance in this case to BBTV can take one of three approaches. Firstly, there are the conventional techniques which are based on the selection of immune, resistant or tolerant cultivars that can be used as is or in a breeding program. To date, no cultivars or even *Musa* spp. have been identified as immune, while cultivars with enhanced resistance to infection have been selected (Jose 1981). There have, however, been no reported attempts to put those resistances into the recognised commercial cultivars.

Conventional resistance breeding for BBTV suffers three major obstacles: bananas are difficult to breed, there are no known BBTV-immune lines to work with, and there is no reliable and adaptable screening technique for BBTV infection currently available. Where conventional techniques fail, newer techniques might succeed, such as somaclonal variation. This technique, already being used to generate new variability within banana cultivars, is potentially useful where desirable traits, such as BBTV-immunity, cannot be found in existing material. The major obstacle to this approach at the present time is again the lack of screening technique for BBTV infection. The final and least conventional approach to developing BBTV-immune cultivars is genetic engineering. There is considerable interest in and some evidence to suggest that virus-resistant plants can be generated by integrating into their genomes part of the viral genome (either as the viral coat protein gene or as 'anti-sense' RNA). This approach is exciting but depends firstly on an intimate molecular knowledge of the causal virus and also an efficient plant transformation technique both of which are lacking for BBTV and bananas.

#### **Mild Strain Protection**

This technique of deliberately inoculating a plant with a mild or symptomless strain of a virus so as to protect that plant against infection by severe strains is not widely used as a control measure for plant viruses, but as a 'last resort' in the absence of more effective measures may be useful. As yet, however, no mild strains of BBTV have been conclusively identified or isolated.

#### **Prospects for Control of BBTV**

As outlined above, BBTV has been controlled by avoidance in Australia, i.e. reducing the number of infection sources. Its application to other environments is unknown. In the short term, there seems no easy answer to controlling BBTV. In the long term there are a number of options but all require considerably more research to understand this virus.

#### **Research — Possible Directions**

In this section, I have attempted to identify areas of priority in research at this time.

#### **Diagnosis and Detection**

Diagnosis of the disease today is invariably based on visual inspection for symptoms. This technique is fraught with danger due to the vagaries of symptom variation due to environment, age of infection, cultivar, virus strain, etc. It is essential that a rapid, sensitive and reliable diagnosis and

detection technique be developed. This would have immediate application to quarantine, eradication or avoidance programs as well as being invaluable for screening in any breeding or selection program. Two such techniques are now in common usage in plant virology but have not been developed for BBTV because of lack of knowledge about the virus. The techniques are nucleic acid hybridisation (using DNA probes) and the serological technique Elisa (enzyme linked immunosorbent assay). Both these methods would adequately serve as a reliable, sensitive and rapid diagnostic technique and both are adaptable to large-scale screening. We are currently attempting to develop a nucleic acid hybridisation technique for BBTV.

#### **Strain Variation**

If breeding programs are to succeed, the range of strains of BBTV must be determined so that resistance is not to only one or a few strains. Also, it is necessary to confirm that the diseases assumed to be caused by BBTV are, in fact, caused by the one virus. There are well-documented instances of two different viruses causing similar, if not identical, diseases and also being transmitted by the same aphid species (i.e. leafroll in potatoes can be caused by either potato leafroll virus or beet western yellows virus). Both are luteoviruses transmitted persistently by the aphid *Myzus persicae*. An important component of research into strain variation would be an adequate diagnosis technique outlined above.

#### **Cultivar and Species Reaction**

If a breeding or selection program is undertaken or contemplated to incorporate BBTV resistance into commercial cultivars, then sources of resistance must be identified either within *Musa* × *paradisiaca* or within the genus *Musa*. Also the reaction of identified lines to a number of strains of BBTV must be ascertained so as to avoid fragile or transient sources of resistance.

#### **Biochemical Characterisation of the Virus**

Biochemical characterisation of BBTV will be necessary if genetic engineering techniques are employed in an attempt to generate resistance. An understanding of the viral genome is required before part of that genome can be incorporated into the banana genome assuming a transformation system can be developed. There are a number of other benefits which would accrue from such biochemical characterisation including determination of strain variation and facilitating the development of diagnostic techniques (even though these can be developed without complete characterisation).

#### **Geographical Distribution**

It is important to confirm the international



distribution of this disease as diagnosis has been based primarily on symptoms and transmission. The movement of banana propagation material from one country to another always carries a certain amount of risk but this risk can be minimised if the disease status of the country of origin is known.

### Host Range

Knowledge of the host range of BBTV is inadequate. Reports of the susceptibility of *Colocasia esculenta* need to be investigated as well as the possibility of other alternate hosts. Any eradication or avoidance program depends on the exclusion or reduction of sources of infection. Unknown alternate hosts of the virus could render such programs useless.

### Conclusion

Knowledge of banana bunchy-top virus has progressed very little in the past 50 years particularly in comparison to other serious virus diseases of economically important crops. If this situation is allowed to persist, then further losses due to the disease and further spread of the virus are inevitable. This is especially so with the increasing emphasis on rapid international movement of banana-propagating material.

Finally, we are moving into an exciting area of possible new control techniques for plant viruses based on genetic engineering. For the world banana industry to take advantage of this era, more effort must be made to understand BBTV.

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# Callus and Cell Culture, Somatic Embryogenesis, Androgenesis and Related Techniques for *Musa* Improvement

Abraham D. Krikorian \*

PLANT cell, tissue and organ culture is a frontier area of biotechnology which is gaining in importance. Research carried out in most countries has primarily been academic, but in many places it has been recognised for a number of years that a great potential exists for utilising aseptic culture and related techniques in the improvement and management of horticultural and agricultural crops. The techniques of tissue and cell culture that can be utilised most profitably fall into the following broad areas: (1) rapid and massive clonal multiplications; (2) production of haploids or homozygous diploids and triploids through ovule, anther, pollen and endosperm culture respectively; (3) embryo culture for rescuing progeny of difficult crosses; and (4) generation of 'somaclones' or tissue culture-associated variants.

A major constraint to the effective formulation of research and development initiatives in less economically developed and developed places alike, has been, in part, failure to appreciate fully the *limitations* as well as the capabilities of the available methodologies. In defence of all concerned, it should be stated that the new biotechnologies have until only recently been naively heralded by many, virtually everywhere, as offering the ultimate guarantee of realising broad and sweeping solutions to the world's agricultural and plant biological problems. Like other evolving methodologies and trends, it is often difficult to separate possibility from accomplishment and reasonable expectation from fantasy. There is no doubt that aseptic culture and molecular biology methods offer powerful tools for investigation, and in some cases for development, but before we can expect routine implementation at the practical level, we need to learn a great deal more about fundamental

physiological and cellular processes of in vitro systems. This conservative view of *Musa* improvement and biotechnology is justified and continues the stance taken in earlier statements on the potential role of tissue culture and the genetic engineering of bananas and plantains and indeed, virtually all plants (Krikorian 1982, 1986; Simmonds 1983; Krikorian and Cronauer 1984 a,b).

## Morphogenetic Competence and Totipotency

Most experiments in vitro on the apical growing regions of *Musa* have been aimed at showing potentialities of the shoot tip, apical meristems with or without leaf primordia or subjacent tissues, and their growth and development under particular conditions. As a result of this work, the means whereby select germplasm can be rapidly multiplied in a specific-pathogen-free state is now more or less worked out. Shoot tips comprising a few or several leaf primordia or, with greater difficulty even strict apical meristems, can be excised under aseptic conditions, induced to proliferate and form multiple shoots which can, in turn, be separated mechanically and rooted at a high level of efficiency (e.g. Krikorian and Cronauer 1984a; Vuylsteke and De Langhe 1985; Cronauer and Krikorian 1985a, 1986b and references there cited). These plants can be reared to maturity ex vitro. The apex of that part of the inflorescence bearing the male flowers of what we call *indeterminate* clones — that is clones which have, in theory at least, the morphological capacity for continued elaboration of primordia on the flanks of their floral apical meristems — can similarly be excised and stimulated in vitro to reinitiate growth and form vegetative leaf primordia and shoots that behave as if they were derived from apices of non-flowering corms or suckers (Cronauer and Krikorian 1985b). While the morphological origin of the response differs, terminal growing point axes of male flower buds from *Musa* clones

\* Department of Biochemistry, University of New York at Stony Brook, Stony Brook, New York 11794-5215, USA.

which are *determinate* as to their growth mode, can also give rise to vegetative shoots. If intervention by *in vitro* techniques is not implemented in this kind of male bud axis, it is a morphological impossibility, as it were, to form additional bract or leaf primordia and buds on the 'terminal' structure (Cronauer and Krikorian 1986a and unpublished data). The dessert bananas and the 'French type' plantains and cooking bananas are, on the criteria given above, of the indeterminate male bud types; the 'False or True Horn' plantains would be categorised by us as determinate (Tezenas du Montcel et al. 1983). All this work underscores the full developmental potential of shoot apical tissues of *Musa* and their competence to yield complete plants.

Despite this technical capacity, we still do not know the extent to which one can infallibly rely on the *in vitro* methodology that flows from the above in terms of clonal fidelity of *Musa* plants produced from meristem or shoot tip culture. There is confusion as to whether plants multiplied by these means are clonally stable, or whether they show varying amounts of variability. The finding of tissue culture-associated variation among bananas and plantains reared via shoot tip culture (Pool and Irizarry 1985; Ramcharan et al. 1985; Vuylsteke et al. 1986), early recognised by me as a possibility, has made it all the more urgent to determine whether one can delineate in the first instance, and ultimately to understand the basis of, methods whereby aseptic shoot and tissue culture systems can be used as a vehicle to achieve production with strictly clonal or true-to-type fidelity on the one hand, or to generate useful change on the other.

The question arises whether or not additional shoots can develop in *Musa* shoots from one or only a few cells under certain circumstances — including *in vitro* culture. Adventitious meristems can arise from a single cell in some plant species. While some shoots arise from pre-existing buds in the *in vitro* multiplying *Musa* shoot systems, adventitious buds can, and do, form in large numbers on multiplying shoot complexes. It is not yet known whether any originate from single cells (Banerjee et al. 1986; Cronauer and Krikorian 1987).

This means that at present, and until shown otherwise, one seems to be dependent on shoots of multi-cell origin (Stewart and Dermen 1979). The implications of this for generation of variants or mutants from aseptically cultured shoots of *Musa* will become apparent (De Guzman et al. 1978; Menendez and Loor 1979; Novak et al. 1985). In tissue culture, it is generally supposed that the more organised the starting explant, the less the variation in a culture-generated propagule. The less organised the cultured starting material, the more the variation. On this view, meristems and shoot tips would generate less variation, and callus, cell

suspension or protoplast procedures would generate the most varied plantlets.

In the majority of cases that I can think of at present, genetic engineering procedures such as gene insertion will, perforce, depend on successful regeneration of a plant from cells and protoplasts. Even procedures involving pollen genome modification will benefit from such capability and screening embryoids from pollen will require androgenesis to be achievable at a reasonably high efficiency (Knox et al. 1986). In short, in my opinion, all prospective advantages that might accrue to banana-plantain breeding or improvement program, other than the obvious ones such as embryo rescue and multiplication via meristems and shoot tips, demand availability of dependable cell, protoplast, anther-pollen-ovule culture procedures (Krikorian and Cronauer 1984b).

### Studies on Callus and Cell Culture

This laboratory embarked upon a broadly based tissue culture of *Musa* effort because of our interest in the problems of growth and development, and our expertise in aseptic culture methodologies. A long-standing interest in monocotyledons, especially perennial ones, and early exposure to tissue and cell culture of banana, first of fruit, at Cornell University while I was a student in F.C. Steward's laboratory, and then through close friendship with the late Emerita de Guzman of the University of the Philippines at Los Baños, with shoot tip methods, provided additional bases of incentive. We knew at the outset of our studies that it would not be an easy undertaking. Our successes with daylily (Krikorian et al. 1986) and oil palm (Krikorian and Kann 1986), however, served to encourage us. Most of the available literature on *Musa*, such as was then available, was not detailed enough to be very helpful. The work of Mohan Ram and Steward, essentially terminated in 1961, still provided us with the best leads for callus and cell culture work (Mohan Ram and Steward 1964). We were able essentially to repeat the fruit callus and cell culture work but healthy cultures from virtually all morphological origins which were readily sustainable as to vigour were very difficult to obtain. Cells in suspension were almost inevitably of the 'wrong kind' and lacked those qualities that experienced investigators identify as of high likelihood to be morphogenetically competent. Darkening and oxidation was frequently a problem. A frank assessment of our ability to initiate and reliably sustain cultures of either bananas or plantains using 'updated know-how' led to the conclusion, and still leads us to feel, that *Musa* truly seemed to be an extremely recalcitrant system (Bakry et al. 1985).

In the course of our work Sandra S. Cronauer was able to initiate fine, healthy cell suspensions from which globular or proembryonic structures were derived. The structures could be obtained in relatively large numbers from the cooking bananas (ABB) 'Saba' (actually later found out to be more accurately called 'Cardaba') and 'Pelipita.' So-called 'Horn Plantain' (actually a 'False Horn,' the 'Platano Commun' or 'Harton' or 'Platano Cuerno,' AAB) also responded. Although it was not possible to report the development of a well-formed shoot apex from the structures, we interpreted them as somatic embryos based on their gross morphology and great similarity to embryos of seeded members of the Scitaminae (Cronauer and Krikorian 1983). We did histology on this material as well. Since a number of embryo mutants exist which are unable to form leaf primordia (Caruso 1967; Sheridan and Neuffer 1982), we were not then preoccupied with the failure of our somatic embryos to develop shoots. Since *Musa* has generally proven itself as difficult to work with from the perspective of callus and suspensions, we saw and still see, this work as a step forward. Also, 'neomorphs,' or embryonic structures which reflect aberrations and deviations or alternative pathways in development, had been encountered in daylilies and oil palm and could be controllably reprogrammed to yield plantlets (Krikorian and Kann 1981). Rowe and Richardson (1975) show several zygotic embryos of *Musa* grown in vitro that are very suggestive of some of our 'germinated' somatic embryos, or perhaps more precisely, neomorphs.

Since then, additional efforts have been placed on reassessing our strategies and further probing the responsiveness of some clones to various modifications to our existing procedures for initiating competent cultures and inducing organised growths. Our outlook continues to be that *Musa* will, eventually, fully respond in a controlled way.

The general plan of work has been: (1) to concentrate on meristem work with plantains and cooking bananas since this has been the focal point of our financial support. But, as time and resources have permitted we have tried; (2) to induce active growth and cell proliferation from cells as they exist in situ from starting materials which derive from multiplying shoots and a wide range of growing areas of plantlets which, preferably, are aseptically derived by vegetative and floral meristem culture. Also, we try to use explants from mature and young plants maintained by us either in vitro or ex vitro or such as may be made available to us from time to time through the cooperation of others; (3) to obtain, maintain and multiply in liquid and semi-solid culture relatively large units and masses, cell clusters and even free cells, the behaviour of which can then be compared and contrasted as they

develop when transferred or subcultured into fresh medium (or distributed into or onto agar media) into unorganised masses or callus, on the one hand, and into organised structures on the other; (4) to investigate the best physical and exogenous chemical environments which promote organised growth or even somatic embryogenesis to proceed from large or small units, clumps or even single units; (5) to examine the relationship between morphological origin of starting material, physiological status of primary explant, genotype and capability for organised response.

The investigative procedures being used, by the nature of the problem and by the emphasis upon interactions between factors, are necessarily different from those in which experimental variables at any one time can be reduced to one. The number of cultures in a given experiment needs to be large to comprehend the range of interactions upon which evidence is sought. Where possible, trials are laid out in Latin squares with blocks of treatments. In tissue culture work, research represents a sequence or a series of individual experiments, each one of which is designed to study one or more specific factors — such factors as medium composition, growth regulator types and concentration often interact with the environment and it is very helpful to visualise subtle sensitivities. This permits one to base better the design of a new experiment on the results obtained from the previous one. In this laboratory, photography is used to a substantial degree as a supplement to the more conventional recording of data.

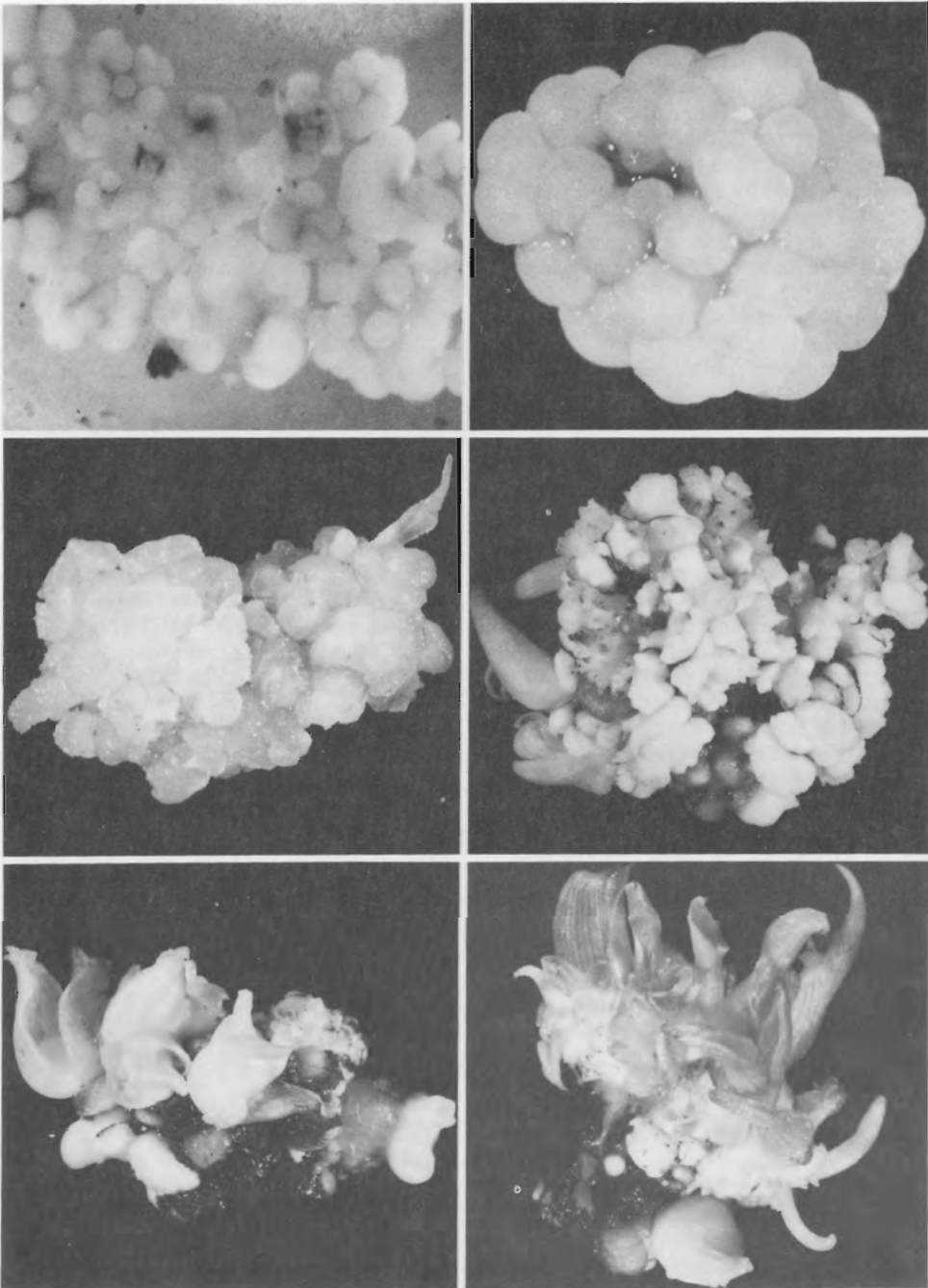
At this point, the systems are so labour-intensive to maintain that it is difficult to work with very many clones at a given time. Moreover, it has not seemed worthwhile to attempt quantification of growth responses. Indeed, we predict that if meaningful quantitative data are readily obtainable, many of the problems which we seek now to solve, will have been solved.

The more recent significant findings from our efforts are:

(1) Nodular masses obtained from some ABB cooking bananas ('Cardaba' [of the 'Saba' affinity (?)], 'Chato' ['Bluggoe' group], 'Pelipita' [Bluggoe group]), can be serially subcultured at 3–4-week intervals and maintained on semi-solid medium and in liquid culture in the presence of cytokinins like N<sup>6</sup>-benzylaminopurine (BAP).

(2) These largish (order of 5–10 mm diam) nodular masses are competent, replete with peripheral growing zones, and readily give rise to shoots either in liquid or on semi-solid media, and from these, rooted plantlets can be produced.

(3) With added synthetic auxins such as 2, 4–5 trichlorophenoxyacetic acid (2,4,5-T), or 2,4–dichlorophenoxyacetic acid (2,4-D) these nodular



**Fig. 1.** Development of shoots of a cooking banana 'Cardaba' (ABB) derived from small, compact globular masses (nubbins) cultured first in liquid media through several stages and then transferred to a semi-solid medium for further shoot growth. A, Close-up of a flask of proliferating nubbins in liquid media,  $\times 2.4$ ; B, close-up of some nubbins clusters generated from a discrete unit and removed from culture vessel for photography,  $\times 9.9$ ; C, nubbins cluster that has further developed and shows organisation,  $\times 2.4$ ; D, further organisation from a culture similar to that at C,  $\times 2.1$ ; E, emergence of well-formed shoots on a semi-solid media,  $\times 2.2$ ; F, proliferation shoot culture,  $\times 2.1$ . From work of Krikorian and Scott.

masses can be serially subcultured and maintained in liquid culture in a smaller and more compact growth mode which we call calloid.

(4) These compact, calloid masses in the smaller growth mode are also competent.

(5) With selection and further exposure to auxins such as 2,4-5 T, small, compact, globular masses (order of a few mm  $\pm$ ) form on these larger calloid masses. Following terminology peculiar to my laboratory at Stony Brook, these compact globular masses are referred to, for convenience, as 'nubbins' (Krikorian et al. 1986).

(6) These nubbins can be produced and serially maintained in liquid media.

(7) The morphogenetic competence of the nubbins is beyond all doubt.

(8) Shoots can be generated from these nubbins.

(9) Plantlets can be increased from these.

It remains to be seen whether (a) we can speed things up, and (b) whether further breakdown in unit size of these nubbins along the lines that we have successfully adopted for daylily (*Hemerocallis*) (Krikorian et al. 1986 and references there cited) can be achieved. Because we are making progress towards achieving competent cell suspensions, it will perhaps now become superfluous to worry about production of germinable somatic embryos in large numbers as long as we can generate propagules from cells multiplied in liquid. Even so, we continue to work towards this goal for it provides basic research opportunities and would offer many advantages.

Figure 1 shows a sequence of development from nubbins of a septically cultured cooking banana, 'Carbaba.' It would serve no useful purpose here to recapitulate the precise procedures necessary to induce these. A manuscript providing full details is in preparation by Krikorian and Scott. What is significant to this report is that we know that the system sought is achievable but it must be reduced, or elevated, if you will, to a level so that efficient cell culture techniques which can have practical potential can emerge. The finding that liquid-cultured globular masses of *Musa* can yield propagules is an important first step. But the full exploitation of the methods, despite our hopes for their routine application, must await a more complete and basic scientific understanding of not only the system in question, but those involving cells from germplasm of high priority to breeders or improvers. Studies on the nature of the developmental controls which are brought into play during the culture process and release of morphogenetic competence in banana-plantain cells, and their protoplasts could not only lead to the production of large numbers of plants but could help us achieve the variability and develop the selection procedures that are so necessary in the kind of improvement work envisioned (Krikorian and

Cronauer 1984b; Stover and Buddenhagen 1986).

The flow of work from the above should be to: (1) examine the growth characteristics of the nubbin technique-derived progeny and to establish their behaviour at the plantlet/plant level; (2) extend methods that suffice for generation of competence from nubbins to cultures of free cells to free protoplasts with the emphasis that, in order to be useful, the protoplasts should be capable of organised development; and (3) determine some of the physiological and biochemical mechanisms involved in the release of the competence or totipotency.

A scheme for possible use of cell, protoplast, and shoot apical meristem techniques for producing novel bananas or plantains is shown in Fig. 2. While the part of the scheme dealing with the protoplasts is the most speculative, in the longer run it is likely to be most productive for generation and selection of variants. This is not a criticism, simply a statement of fact, and it is recognised that this part of the work is likely to take a fair amount of effort, and hence time. We learned some time ago to make protoplasts from certain *Musa* preparations (Cronauer and Krikorian 1987). Our procedures do not differ drastically from those already published (Bakry 1984; Chen and Zu 1985).

I reemphasise, however, that *speculations such as those in our scheme are useless unless a capacity to produce or isolate competent cells and protoplasts and to regenerate protoplasts in large numbers has been developed*. Success is affected by many factors. The entire process can be subdivided into three steps: protoplast isolation, protoplast culture, and plant regeneration. The hope at Stony Brook is that

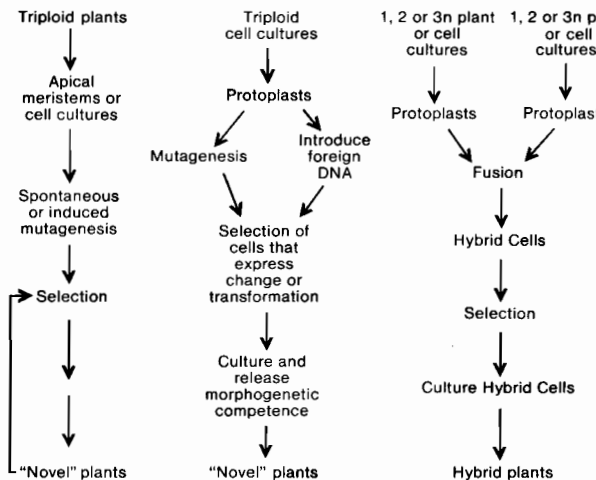


Fig. 2. Schemes for possible use of cell, protoplast and apical meristem culture techniques for producing novel plantains or bananas from Krikorian and Cronauer (1984b).

we will have the opportunity to examine each of these steps in depth and to ascertain the conditions necessary for the successful isolation and regeneration of plantain and banana protoplasts.

### Conclusions

(1) *Musa* continues to present challenges and opportunities to the tissue culture worker.

(2) Progress has been substantial but we have a long way to go.

(3) Generation of shoots and plantlets from competent liquid culture-grown small, compact, globules ('nubbins') from some triploid (ABB) cooking bananas has been achieved.

(4) Preliminary protocols for protoplast preparation and collection have been developed.

(5) No reports on successful androgenesis or microspore/pollen culture have appeared so far.

(6) Ovule culture studies are just starting in Panama.

(7) We are at the 'definition of the problems' stage of research.

### Recommendations

#### Research Areas

(1) Production of morphogenetically competent globules, 'nubbins,' in liquid media must be assessed in a broader range of germplasm.

(2) Various parameters to enhance the level and speed of response from each competent unit must be investigated and evaluated.

(3) Special attention must be given to evaluating potential for competent globule production from material of special interest for improvement and to breeders.

(4) Efforts must be made to reduce further the unit size of the competent globules used for regeneration, preferably down to the single cell level.

(5) Studies on protoplasts should be intensified to define better how increased numbers can be produced and collected and, eventually regenerated through reliable protocols, into morphogenetically competent units and plantlets.

#### Research Support

(1) The directions for future work fall completely within the resolutions passed at the 3rd International Conference of IARPCB in Abidjan, Côte d'Ivoire 28-31 May 1985, aimed at fostering *in vitro* work on bananas and plantains (see Krikorian 1985, in press).

(2) More frequent and in depth communication between breeders, pathologists and tissue culturists is needed to facilitate full understanding and coordination of needs, strategies and objectives.

(3) A 'wish' list should be produced by breeders and pathologists in conjunction with tissue culture experts in light of the very latest views on breeding, pathology and improvement. This should be circulated to all interested.

(4) Full access to important germplasm should be given to all *bona fide* tissue culture investigators. Limitations should be strictly on the basis of availability and legal considerations such as quarantine, etc.

(5) A tissue culture expert group should meet every 2 years or some other regular basis in a workshop and prescription clinic format so that a detailed 'state of the art' assessment can emerge.

(6) This working group should be so structured and constituted that membership comprises representation from all those laboratories who are actively engaged in *in vitro* work on *Musa* from all perspectives *except* nominally clonal multiplication via shoot tips and meristems.

(7) Regular and updated reports of progress should emerge from the group and be circulated through INIBAP.

(8) An active program of education of potential granting agencies should be undertaken by INIBAP, and all others able to bring their influence to bear, towards securing a significant and legitimate place for research support on export, dessert bananas (AAA clones). The policy on the part of some to limit research support to plantains and cooking bananas (AAB and ABB clones), and equating support for research on export dessert bananas as contrary to the spirit of helping the peoples of developing countries must be shown to be unjustified.

(9) Finally, the culture methods in the title of this report will undoubtedly demand considerable effort for full practical development.

(10) A case can readily be made to justify sustained financial support until that end is achieved.

### Acknowledgments

This appraisal derives from research efforts supported by the U.S. Agency for International Development. Earlier phases were supported by the U.S. National Science Foundation, Division of International Programs. The U.S. National Aeronautics and Space Administration is also to be thanked since it has supported the tissue culture work of the laboratory for many years. The opinions voiced, however, are my own. Special recognition is made here of the work of Dr Sandra S. Cronauer (now Mitra), Mrs Mary E. Scott and Mr David L. Smith. Mr Robert P. Kann and Mr G. David Whitmore have also played a significant role. The encouragement of Dr Ben Waite and others at



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# Somaclonal Variation in Grande Naine and Saba Bananas in the Nursery and Field

R.H. Stover \*

THE classical breeding techniques consisting of recombination and selection procedures are the most efficient for obtaining new clones of most crops. Nevertheless, 60 years of banana breeding using these techniques has failed to yield a single variety that replaced or substituted for established varieties. Some of the reasons for this have recently been reviewed (Stover and Buddenhagen 1986).

With the increased use of *in vitro* plant multiplication, the widespread presence of somaclonal variation was detected. This variation, unique to the *in vitro* environment, has been studied since the early 1970s but the causes are still obscure (Scowcroft and Larkin 1982; Scowcroft 1985). When *in vitro* multiplication of bananas and plantains began, somaclonal variation was found to be widespread in the Cavendish subgroup. This report describes the preliminary observations on somaclonal variation in Grande Naine and Saba plants produced *in vitro* on a large scale for commercial use.

## Methods

Shoot tips (the vegetative meristem) plus several leaf primordia were removed from the field-grown rhizomes of Grande Naine (Cavendish Subgroup AAA) and Saba. Saba is a cooking banana popular in the Philippines with an ABB or BBB karyotype (Valmayor et al. 1981). Standardised techniques were used to assure sterility (Krikorian and Cronauer 1984a, b). The shoot tips of Grande Naine were maintained *in vitro* for about 1 year before mass production of plantlets began.

Explants were cultured on a medium similar to that described by Hwang et al. (1984). Both solid and liquid media were used. The tops of explants when 5–8 cm tall were removed to form new bases for growth. From 9–12 shoots were produced per explant every 5 weeks. Hwang et al. (1984) obtained

5–10 adventitious buds from each explant every 6–8 weeks.

Rooted plantlets 5–8 cm in height were shipped in aluminium or plastic containers (50 plants per container) to the final destination in the tropics. The agar was washed from the roots and the roots were trimmed. Plants were set in 'Speedling' trays containing an artificial soil mixture ('Metro-mix 300') and fertilised with a soluble 20–20–20 with micronutrients. At first plants were hardened in reduced light (70%) and high humidity for 1–2 weeks and then moved to 30–40% shade for 2 weeks and then to full sunlight.

After 4–5 months in the nursery, plants have a large, dense ball of root and are moved directly to the field. With good irrigation and weed control about 90% of the plants survived in the field. More than 1 million *in vitro* Grande Naine plants were produced from original shoot tips and grown to the fruiting stage. About 5000 Saba plants were produced *in vitro* and grown to fruiting.

## Results

### Somaclonal Variation Observed

Off-type plants could not be detected with certainty in the nursery because plants become somewhat root-bound in the small container (Fig. 1–2) thus restricting plant height prior to transfer to the field. There was considerable variation in the amount of brown to purple pigmentation in the foliage but it was not possible to correlate plants with little or no pigment in the foliage (considered abnormal) with subsequent off-types in the field.

About 5–6 months after planting and 2 months before shooting, from 6–10% of the plants showed abnormal foliage characteristics. This consisted of darker green, leaves upright, thickened leaves, closer internode spacing, shorter-wider leaves, narrow leaves and leaf variegation (yellow-green mottling). Occasionally, plants had abnormal foliage characteristics suggesting aneuploids and tetraploids.

\* C/o United Fruit Company, Pela Railroad Co., Apartado Postal 30, San Pedro Sula, Honduras.

The percentage of identifiable off-types increased greatly following flowering and fruit maturation and totalled about 25% of the population. It was then apparent that changes in plant stature and bunch characteristics were the most common off-types. Dwarfism as indicated by mutation to the Dwarf Cavendish height class accounted for 75% of the off-type mutants (50% Dwarf Cavendish, normal fingers; 25% Dwarf Cavendish, abnormal fingers; 10% Valery; 10% Extra Dwarf Cavendish; 2% Grande Naine, abnormal fingers; 3% miscellaneous: aneuploids, tetraploids, leaf abnormalities, flower malformation, short fingers, all male flowers, variegation, etc.).

The off-type mutants were classified into six groups as above. Mutation to short-fingered fruit with abnormally constricted finger tips (Fig 9-10) occurred independently of changes in plant stature. This fruit tended to ripen sooner and had a bland flavour.

Off-type plants were distributed at random throughout 250 ha observed in detail from the beginning to the latest planting (a period of about 2 years) but incidence varied from 10% in localised sections up to 35%. Off-type mutants were stable and there was no reversion or change in plant characteristics through the second and third ratoons.

#### Height Measurements and Leaf Index

The height of 20 typical Grande Naine plants with excellent growth and 12-14-hand bunches from the first plants set out was measured. The average was  $302.7 \pm 2.5$  cm and the range 287-325 cm. Additional measurements, including the leaf index, taken at random throughout 250 ha are shown in Table 1. These measurements served as a guide in establishing Grande Naine type plants for selection for vegetative production to replace off-type dwarfs. Almost all bunches from off-type dwarfs had finger lengths that were too short to meet export qualification and had to be rogued. A few Dwarf Cavendish-height plants with superior bunches were

**Table 1.** Average plant height and range, and leaf index of five clonal classes with second ratoon fruit all derived from Grand Nain produced in tissue culture.

Clonal class	Height (cm)	Leaf index:
		length width
Valery	373.1 (359-384)	2.76
Grande Naine	299.1 (287-320)	2.46
Dwarf Cavendish normal fingers	208.0 (184-229)	2.08
Dwarf Cavendish, abnormal fingers	200.1 (180-221)	2.41
Extra Dwarf Cavendish	152.4 (132-168)	-

*Notes:* Measurements made on leaves 6-8 in areas with excellent growth. Ten plants measured for each type. Height measurements are from ground along pseudostem to top peduncle where it emerged from pseudostem. Abnormal fingers are small fingers with a constricted tip ('nipple-like'). In Honduras, depending on soil type, Valery is from 322-391 cm tall and Grande Naine 272-320.

selected for further observation and comparison with Grande Naine. The minimum finger length (outer curve) for export is 19 cm in first-class packs.

#### Discussion

Mutation in plants produced in tissue culture was noted in Honduras in 1978 when some Grande Naine plants produced in vitro had reverted to the Valery height (see earlier for heights). In 1984 and 1985 numerous reports of somaclonal variation were recorded (Table 2). All of these reports except one referred to clones in the Cavendish subgroup and the large majority of mutants involved dwarfism. Along with reduced plant stature there was a reduction in finger length to the extent that fruit from most dwarfs was of no commercial value. In contrast, not a single off-type was found in 5000 Saba plants produced in tissue culture using the same technique as for Grande Naine. Saba has a ABB or more likely a BBB genome.

The factors involved in somaclonal variation have

**Table 2.** Reported mutations in banana and plantains produced in tissue culture.

Country	Year	Source	Mutants and source	
Honduras	1978	Unpublished data	Grande Naine	Valery height
Jamaica	1984	Unpublished data	Grande Naine	Dwarf and other off-types
Israel	1984	Reuveni et al. (1984)	Williams	Dwarfs, variegated foliage
Virgin Islands	1984	Ramcharan and Knausenberger (1984)	Horn plantain	French plantain
Taiwan	1985	Stover (1985); Hwang (1986)	Poyo	Dwarfs and many other off-types
Puerto Rico	1985	Pool and Irizarry (1986)	Grande Naine	Dwarfs and other off-types
Australia	1985	Johns (1986) and Banana Bulletin	Williams	Dwarf Cavendish and other off-types

been discussed by Scowcroft (1985). In asexually produced plants there is a great increase in the frequency of chromosomal abnormalities during tissue culture. These seem to increase with prolonged periods in vitro. There is little evidence that tissue culture media per se is mutagenic. The fact that somaclonal off-types are stable and are not chimeras indicates that most mutations occurred in single or at least clumps of very few cells that multiplied into adventitious shoots with a uniform genotype. With Grande Naine the frequency of mutants produced over a 30-month period from the same source of material did not significantly change. This suggests that the mechanical manipulations including wounding and method of rapid multiplication in series was more important than the length of time in culture. Wounding tissue by incision is often used to stimulate adventitious bud formation (Hwang et al. 1984; Krikorian and Cronauer 1984a, b). This, along with cutting tissue for transfer, could favour chromosomal aberrations. By rapidly turning over thousands of plants each week some in vitro mutants were undoubtedly multiplied. In Israel by limiting in vitro produced plants to a maximum of 1000 per original meristem, 95% of the plants produced were true-to-type (Reuveni, pers. comm.).

There is only one published report on the effect of radiation on banana tissue culture (Kao, Dein-Lin 1979). The use of mutagens on banana rhizome tissue did not yield results of significance (Stover and Simmonds 1987). With the high level of somaclonal variation in the absence of mutagens, at least with varieties in the Cavendish subgroup, mutagens could be counterproductive.

Preliminary surveys of the somaclonal variants in Jamaica did not indicate any increase in resistance to Sigatoka leaf spot (*Mycosphaerella musicola*) with the possible exception of some useless thick-leaf dwarfs. However, the possibility of finding some agronomically superior Dwarf Cavendish clones is under study. It will take from 3–5 years to select and evaluate possible useful variants with respect to agronomic and disease resistance characteristics. In Taiwan some mutants of Poyo with resistance or at least greater tolerance to race 4 of *Fusarium oxysporum* f. sp. *cubense* have been screened for (Hwang 1986). At present it appears that all the somaclonal variants are agronomically inferior to the Grande Naine variety from which they are derived. However, much more study is needed to determine if rare but useful mutants can be identified among the large populations of somaclonal variants that can be readily obtained from the Cavendish subgroup varieties. A most useful mutant for breeding purposes would be one that has some female fertility as the Cavendish subgroup is completely female sterile.

## Recommendations

High levels of somaclonal variation in the Cavendish subgroup in plants produced in vitro precludes the use of the somaclonal technique, for the present, for large-scale commercial production of plants. Research is needed to determine how to multiply the Cavendish subgroup clones in vitro with less than 5% off-types, which would be commercially acceptable.

Field observations need to be carried out over a period of 2–3 crops on selected somaclonal mutants to determine if they have any useful agronomic, disease resistant or breeding characteristics. Dwarfism with no sacrifice in fruit quality and yield and female fertility should be looked for.

Mass screening should be undertaken to see if somaclonal mutants can be selected with increased tolerance to *Mycosphaerella fijiensis*. Experience already obtained with *Fusarium oxysporum* f. sp. *cubense* race 4 will be valuable.

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# Somaclonal Variation in Bananas: A Case Study With *Fusarium* Wilt

Melvin D.Epp \*

PANAMA Disease (PD) has been a component of the culture of bananas in the Philippines for many years. In 1920, Lee and Serrano reported the infection of Latundan and Calamba. Subsequently, they studied the impact of PD on the abaca cordage industry which flourished during the pre-World War II era in the Philippines (Lee 1923; Lee and Serrano 1923; Teodoro 1925; Leoncio 1930; and Castillo and Celino 1940).

In the late 1960s there was a major expansion of the dessert banana industry in the Philippines. Cavendish cultivars were used. But by 1974, Philippine Packing Corporation (PPC) had confirmed the first case of PD in their Cavendish plantings. What was more disconcerting was the report from Taiwan of a new fungal race of *Fusarium* (Sun et al. 1978). Were all the Cavendish bananas grown in Asia now going to succumb like the Gros Michel of Central America? I was asked in late 1977, to answer that question and to develop a clone of Cavendish that was resistant to the *Fusarium* of the Philippines.

Assisting me with this project were Dr. A.M. Pedrosa, Plant Pathologist; Dra. Aurea Almazan, Plant Biochemist; Mrs. Chesed Sison, Plant Physiologist; and Dra. Lulu Bongcayao, Plant Geneticist. Collectively, we studied the banana plant, the fungal pathogen, the cultural practices which might influence the epidemiology of PD within the plantations, and the interaction of the fungus and the banana plant.

## Studies of the Banana Plant

I view plants in terms of seeds and plants. If you have a plant system where tissue culture techniques are well developed, you can go from meristems to callus to suspension cultures to protoplast and then back to an intact plant. If the economical return is a plant part, you always evaluate the end point of

an experiment after you have returned experimentally to an intact plant.

When I began working with bananas, a major experimental choice was made to design a protocol using meristems for mutation breeding and plant propagation rather than spend time initially developing callus and suspension culture techniques. Meristem culture techniques had been developed by Berg and Bustamante (1974). Of particular value also was the research of the late Dra. Emerita de Guzman (de Guzman et al. 1976; de Guzman and Tolentino 1978; de Guzman et al. 1980) at the University of the Philippines and of Professor Ma (Ma et al. 1978) at the National Taiwan University, Taipei. They briefly describe how to propagate banana meristem in tissue culture. These papers were sufficient to give us a start and to realise that for the banana system, going from plants to meristems to plants was a system that we could use immediately.

We initiated our project with adapted plantation cultivars and then selected for disease resistance, trying to retain all the characteristics of the plantation cultivar with the exception of altering disease susceptibility to disease resistance. We first created variability and then we selected for increased resistance among the clones of plants. A similar protocol has been successfully used to select *Fusarium* resistance in celery (Heath-Pagliuso and Rappaport 1986).

To explant meristems, 30–45 cm sword suckers were trimmed to yield a block of tissue (6 cm × 6 cm at the base and 10 cm long). These blocks were taken into the laboratory and surface-sterilised in 10% commercial bleach (0.0525% NaOCl) plus several drops of liquid soap solution for 15 min. The blocks were trimmed further to about 4 cm × 4 cm and 6 cm long and re-sterilised as above. The blocks were trimmed aseptically the third time until the leaf cone was about 1 cm in diameter. The meristem and the surrounding tissues (1 × 1 × 1.5 cm) were excised and placed individually into test tubes or flasks with MS (Smith) (Smith and Murashige 1970)

\* ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568.

supplemented with 11.4 $\mu$ M indole-3-acetic acid (IAA) and 9.3 $\mu$ M kinetin (K). The cultures were maintained for 10–15 days under constant light to verify that the explants were free of systemic contamination.

These meristems were the starting materials for all experiments of rapid propagation and mutation breeding. The axillary buds present in such large explants increased the proliferation of shoots for micropropagation and increased the number of targets during irradiation for mutation breeding. Our purpose was not to rid the plants of viruses. Vuylsteke and De Langhe (1985) have recently evaluated *in vitro* banana propagation and emphasise its utility also for germplasm exchange. Wong (1986) has studied meristem cultures of 22 banana cultivars and together with Hwang (1984) utilise the fungal-free condition of aseptic meristem cultures.

### Micropropagation Using Meristem Culture

From the literature, we knew that MS medium (Murashige and Skoog 1962) supported growth of meristems and axillary buds (de Guzman et al. 1976). We found that MS medium with altered organics (Smith and Murashige 1970) stimulated growth of banana cultures: 340 mg sodium phosphate monobasic, monohydrate, 160 mg adenine sulfate, 100 mg L-tryosine and 0.4 mg thiamine HCl of each per litre. We labelled this medium MS (Smith).

Additionally, we were interested in optimising the components of the medium used by de Guzman et al. (1976) to obtain maximum growth of the cultivar, Umalag, the Grande Naine-type Cavendish used extensively by PPC. Bananas respond negatively to the addition of agar to the medium (Ma et al. 1978). This was true with two different brands. Consequently, we eliminated agar from the medium and used static liquid cultures. The amount of medium was adjusted to cover the tissue to a depth of one half. The sucrose concentration was optimal at 20 g/l. Coconut water gave an enhanced response at the low concentrations of sucrose probably because it supplied complex sugars useful with limiting amounts of sucrose. Normally, we added 30 g/l of sucrose to provide more flexibility in timing subcultures. We subcultured every 3–4 weeks until the workload became excessive, then subcultures could stretch to 5–6 weeks. We did not want the sucrose to become limiting. Additionally, we tested hormone concentrations. MS (Smith) supplemented with 11.4  $\mu$ MIAA, 9.3  $\mu$ M K and 2.2 to 22.2  $\mu$ M N-6-benzylaminopurine (BAP) gave the most shoots with roots with both whole meristems and halved meristems. Halving the meristems eliminated much of the apical dominance and allowed more axillary buds to grow. Coconut water

appeared to suppress shoots formation and so was not used routinely.

In summary, the medium used for meristem culture and subsequent growth of axillary buds was liquid MS (Smith) supplemented with 11.4  $\mu$ MIAA, 9.3  $\mu$ M K and 22.2  $\mu$ M BAP, 30 g/l sucrose, pH 5.7. The medium supported the continual proliferation of shoots from axillary buds and roots on the shoots. About 20 ml of medium was used per 12-oz glass canning jar and covered with a double layer of aluminium foil secured with a rubber band. The jars were maintained statically on culture shelves continuously illuminated with cool white and warm white fluorescent lights about 40 cm above the jars.

With this medium 130 000 banana plants were grown in the laboratory and transferred to the greenhouse for propagation and mutation breeding purposes. The Umalag cultivar, as well as the other commercial cultivar, Hijo Giant Cavendish, grew well. Cavendish clones in general responded well to this medium. However, there are clonal differences in response; the cultivars Roy Kerr, Saba and Latundan grew only if placed on a gentle reciprocal shaker. Some clones are sensitive to the submersion in the liquid medium; had we used Gelrite or some other gelling agent, the growth of sensitive cultivars may have been enhanced.

The numerical data of two propagation experiments are shown in Table 1. In the first, 21 Umalag meristems were cultured individually, and the total number of plantlets produced was recorded. The impact of the large explant was evident by more shoots being produced per month in the first 3.5 months than in the second 3.5 months; new axillary buds would be more important than pre-existing buds as time moves the cultures away from explant. After nearly 4 years the number of plants produced per meristem ranged from 152 to 2738. In the second experiment, the cultivars Morado and Gloria were propagated. Within 14 months, 5000 Gloria plantlets had been produced and within 17 months 20 000 Morado were in the greenhouse (Table 1). Had these 30 meristems been propagated conventionally assuming an 8-fold annual increase, 3–4 years would have been required. Approximately 10–20% of all Morado plantlets were green rather than pigmented red. The green plantlets were discarded at every transfer but the frequency persisted. This phenomenon occurs regularly in nature; thus the many synonyms for Gloria which is a green Morado.

### Propagation by Culturing Inflorescence Tips

Following the procedure published by Ma et al. (1978), we collected male cones on a collecting trip to the island of Mindoro in March 1979. Also cones are free of soil with possible pathogens; they weigh

**Table 1.** Rapid propagation of banana through tissue culture. Twenty Morado suckers and 10 Gloria suckers produced 26 000 plants in 17 months. Conventional propagation would have hypothetically yielded 2000 plants during the equivalent time period.

Month	Plants in culture in the laboratory		Plants moved to the greenhouse from the laboratory		Hypothetical conventional propagation
	Morado	Gloria	Morado	Gloria	No. of Plants
April 1979	20	10	0	0	30
May	-	-	0	0	
June	47	23	0	0	240
July	-	-	0	0	
August	140	50	0	0	
September	-	-	0	0	
October	655	305	0	0	
November	-	-	0	0	
December	1 718	959	0	0	
January 1980	-	-	1 000	0	
February	-	-	1 000	500	
March	-	-	1 300	100	
April	-	-	2 700	2 500	
May	-	-	2 500	1 777	
June	-	0	3 500	0	
July	-	-	3 150	-	
August	-	-	5 674	-	
September	0	-	0	-	
October	-	-	-	-	
November	-	-	-	-	
December	-	-	-	-	
January 1981	-	-	-	-	
February	-	-	-	-	
March	-	-	-	-	
April	-	-	-	-	1 920

less than corms and are more convenient to ship. Many of the wild bananas which we collected were on steep slopes and so digging would have been cumbersome.

Of the 10 cones which we collected, six produced plantlets, three were either contaminated or died and one cone produced florets perpetually. It required 5-11 months of laboratory time for us to produce plantlets for shifting to the greenhouse.

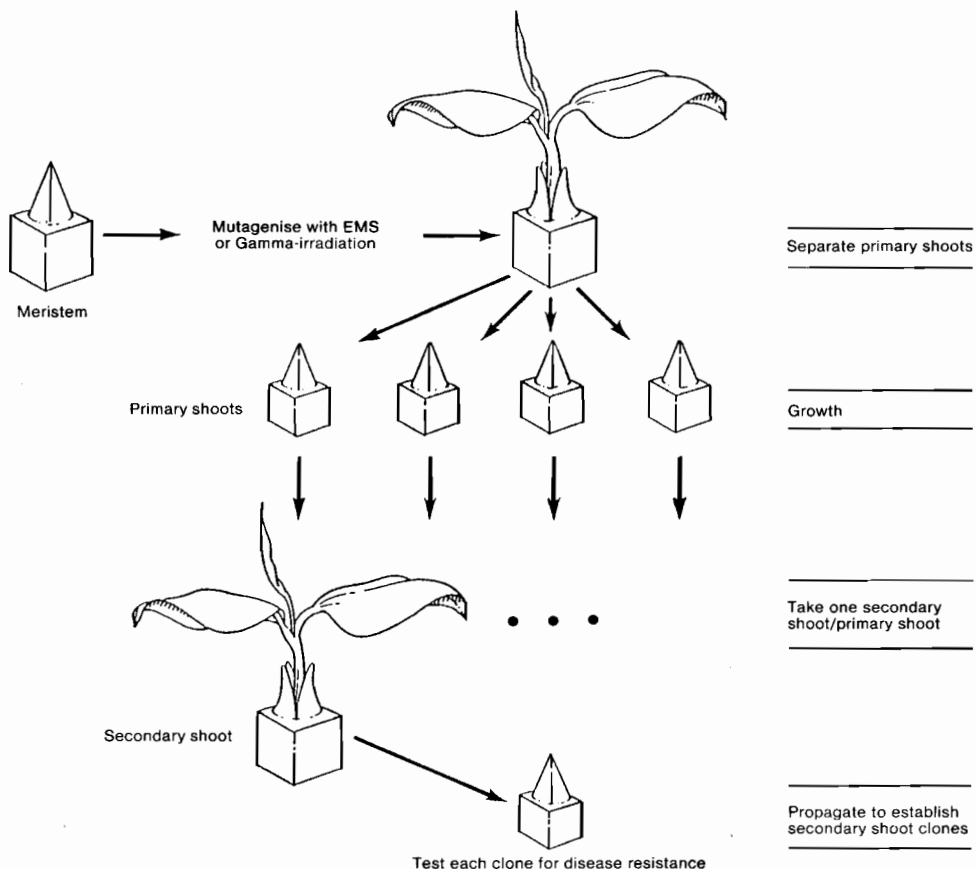
The survival rate of collected suckers was near 100%, so convenience has a price. But this technique will surely continue to find utility for collecting and as a source of meristematic tissue (Cronauer and Krikorian 1985).

### Mutation Breeding

The induction of somatic mutations as a breeding tool for the improvement of bananas has been proposed in the past by numerous researchers. This literature has been reviewed by Broertjes and van Hartman (1978), Krikorian and Cronauer (1984) and Novak et al. (1985). Of particular value was the experimental outline of Menendez (1973) and the dose response curve developed by de Guzman et al. (1976).

For the gamma-irradiation, meristems in individual test tubes were taken to the Philippine Atomic Energy Commission. We used the same dose range as de Guzman et al. (1976). Following treatment, the meristems were cultured in liquid medium and shoot-cloned (Fig. 1). Since the explants were large, each explant had many axillary buds. One axillary bud per quarter explant was allowed to form a shoot. Likewise, one axillary shoot was allowed to grow per primary shoot. The secondary shoots were then multiplied to provide clonal material for disease testing. Data from one irradiation experiment is shown in Table 2.

For the ethyl methanesulfonate (EMS) treatments, 50 aseptic freshly trimmed shootlets from advanced cultures were placed into culture jars containing 50 ml of liquid medium. The culture jars were covered with two layers of aluminium foil and the jar lid. The lid had been modified to include a serum bottle stopper in the middle. EMS was measured with a syringe and injected through the serum stopper. The cultures were placed on a gyratory shaker. To terminate the mutagenic treatment of EMS, a filter sterilised solution of thioglycolic acid (mercaptoacetic acid) was injected



**Fig. 1.** Protocol for mutagenesis of banana meristem followed by shoot cloning.

**Table 2.** Experiment B13. Meristems irradiated in individual test tubes at Philippine Atomic Energy Commission with gamma irradiation (Co-60) at 10 Kr/hr, 3-5 October, 1978.

Label	Treatments	Number meristem treated	Discarded due to contamination within first month post-treatment 11/2/78	After 6 months 19/3/79			As of 31/12/80			1/1/82
				Actively growing	Alive but no growth	Dead	Number of meristem yielding plants	No. of primary clones for testing	No. of clones passing primary PD test	No. of clones passing secondary PD test
B13-1	1 Kr	249	19 8%	124 50%	23 9%	83 33%	133	368	27	1
B13-2	2 Kr	250	15 6%	95 38%	44 18%	96 38%	104	255	14	0
B13-4	4 Kr	249	50 20%	2 1%	53 21%	144 58%	4	4	0	0
B13-6	6 Kr	252	34 13%	0	53 21%	165 65%	0	0	0	0
B13-10	10 Kr	147	25 17%	0	35 24%	87 59%	0	0	0	0



into the culture jar. Thioglycolic acid disrupts the ester bond of EMS and renders the solution nonmutagenic. The final molar concentration of thioglycolic acid always exceeded the molar concentration of EMS. After one hour the meristems were removed and washed three times with sterile water in a buchner funnel and placed into liquid medium for growth and shoot cloning (Fig. 1).

By using 0.1–0.2% EMS and treating the meristems for 4–5 days 50–60% of the meristems continued to grow and proliferate shoots (Table 3). With both the gamma-irradiated and the EMS-treated material, signs of mutagenic damage were evident in the first leaves after treatment.

The shoot cloning steps were included in the protocol because meristems and axillary buds are multicellular structures. But mutagens function on individual cells and so would create mosaic shoots containing both wild-type and mutant tissue. The shoot cloning steps were intended to produce clones of plants wholly wild-type or wholly mutant. I believe the cloning was effective because in one clone of four plants all plants had rolled leaves. This encouraged us to consider the plants within a clone as genetically uniform and individual plants could function as replicates in the disease-testing process.

Since Umalag is a triploid, by using this experimental protocol the only mutations which we would be able to detect would be dominant phenotypes. But this was fine, because most genes reported to confer *Fusarium* wilt resistance in other crops are dominant in phenotype anyway. The same is true for banana; Vakili (1965) reported the genetic segregation of hybrid seedlings of three *Musa* species crossed with Lidi indicating a single dominant factor for resistance to race 1 in Lidi. For

additional discussion on this topic see Buddenhagen (these Proceedings).

The clones produced in the laboratory were moved to the greenhouse. A soil bed test was developed to ascertain levels of PD sensitivity. Large concrete tanks 1 × 3 × 1 m deep divided into three chambers to contain 1m<sup>3</sup> of soil each were found ideal. The inoculum of separate isolates or mixtures were mixed with the top 5–7-cm layer of soil. The fungus was allowed to colonise the soil for 5 days. It was experimentally determined that 2-month-old seedlings of *M. balbisiana* and meriplants with 6–8 leaves gave results that were reproducible and were very similar to field responses. More than 2500 experimental clones and most cultivars in our Accession Nursery were tested using this procedure.

The above-ground symptoms were scored after 60 days using a disease severity index (DSI) with a scale of 1 to 5 (1 = no yellowing, plants appear healthy; 2 = slight yellowing of the lower leaves; 3 = extensive yellowing of leaves with pronounced wilting; 4 = severe yellowing with indications of recovery in the unopened or newly-opened leaves; and 5 = plants were dead). The below-ground symptoms were also scored at 60 days using a rhizome discoloration index (RDI) with a scale of 0 to 5 evaluating rhizomes sliced open (0 = no vascular discoloration; 1 = slight discoloration involving less than 1/3 of the rhizome; 2 = discoloration of several bundles involving 1/3 to 1/2 of the rhizome; 3 = the whole stellar region of the rhizome had discolored vascular bundles; 5 = dead plant). The rhizome with an RDI of 1–3 were plated on Smith's PCNB (Nash and Snyder 1962) to verify the growth of *Fusarium*. The percentage of rhizome infection was used in combination with DSI and RDI to evaluate the resistance of the test materials.

**Table 3.** Summary of meristem treated with ethyl methanesulfonate. These experiments were conducted between 27/9/80 and 9/1/81 and evaluated for survival between 30/3/81 and 4/8/81.

Experiment number	Treatments		Total treated	Number of Meristem		
	Dose	Time (days)		Response after 5–9 months		
				Contaminated	Dead	Grew
E27-6	0.3% 0.024 M	3.5	800	109	567	124
E27-7	0.2% 0.016 M	4.0	701	32	272	397
E27-8	0.1% 0.008 M	5.0	500	184	59	257
E27-9	0.2% 0.016 M	5.0	300	42	70	188
E27-11	0.1% 0.008 M	5.0	400	101	39	260

When the DSI and RDI of standard cultivars and selected clones are plotted together, their sensitivity to PD can be illustrated (Fig. 2). Gros Michel and *M. balbisiana* were the most sensitive. The Cavendish cultivars were less sensitive. Umalag and Hijo Giant were the two commercial cultivars used by PPC. Umalag is entered twice in Fig. 2, to show the reproducibility of two independent experimental runs. Numbers 1-4 represented four Umalag clones from the mutation breeding program which showed the lowest level of infection.

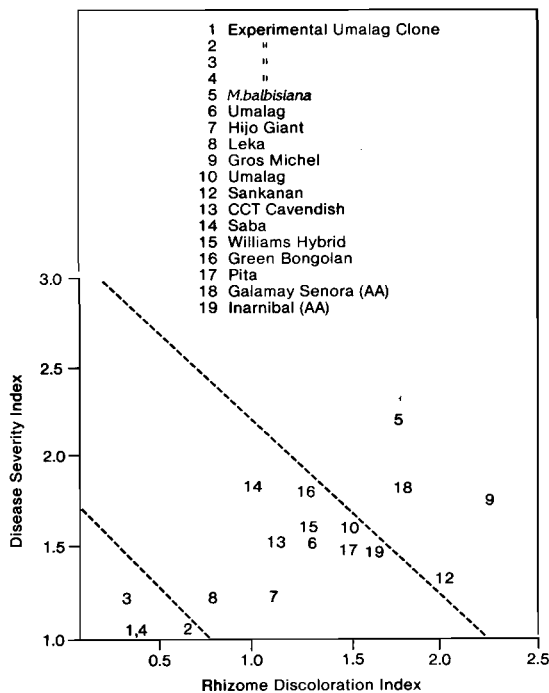


Fig. 2. The sensitivity of banana cultivars and experimental clones to infection by FOC in soil bed tests. See text for details.

The four selected clones were evaluated twice in the soil bed and in culverts used as microplots. Their resistance was excellent in the soil bed tests. In the culverts, by varying the inoculum titre, the clones were shown not to be immune to PD, however, their response was superior to the control Umalag. Three of these clones were from 1 Kr treatments; one was from a control treatment. But each represented a single shoot from an originating meristem. The other primary shoots from these same meristems were rejected by the disease screen indicating differences between shoots having a common explant. The four clones were subsequently planted into a plantation on heavily infected soil for field studies of both agronomic quality and disease resistance. Again, like in the culverts, the plants

within the selected clones were not immune, but really the critical question is whether or not it took them longer to become infected. I am not sure that the plot sizes are large enough to show small differences. These field plantings were made after I left the project and I have never observed these plants in the field. The scant information I have received indicates that the selections are now considered to represent no major increase in levels of field resistance, but testing continues. The agronomic and fruit characteristics appear unchanged and similar to Umalag.

If a new race of *Fusarium* becomes established in Davao at some future date, these selections should be the initial candidates for testing. Also, these selected clones should be tested as replants in areas of low PD infection. There they may be sufficiently superior to Umalag to make fruit production possible in fallowed, disease-eradicated sites. The selections would blend in with the existing Umalag plantings.

The clones treated with EMS had not advanced through the screening process sufficiently to identify prospective candidates at the time the project was terminated.

Callus and protoplast cultures of bananas were investigated in the final year of this project. The callusing capacity of 89 AAA and AA bananas in our accession nursery were tested on MS with 10  $\mu$ M 2,4-dichlorophenoxyacetic acid and 0.1  $\mu$ M K. After 30 days, the cultivars with the most growth were Cuba Congo, Tumuk, Sankanana Variety, Kluai Nam Wah Kon and Bongoran Tsina. These five are all AAA types and show the general rapid growth patterns of the Cavendish bananas.

Protoplasts of banana are difficult to isolate. The wall digesting enzymes used with solanaceous plants do not readily digest the walls of banana cells, particularly cell walls with secondary thickenings. Meristematic regions do yield protoplasts but the block of usable tissue per meristem is small and yields few protoplasts. Banana cells from suspension cultures also do not readily become protoplasts. However, there is a recent report of research using banana protoplasts isolated from meristematic regions and fruit placental tissue for protoplast fusion experiments (Bhagyalakshmi 1986). Also noteworthy is the report of somatic embryos from cultured tissues of plantains (Cronauer and Krikorian 1983). Initiating cultures from immature embryos from seedy varieties may be a useful source of embryonic cultures.

### The Philippine Fungal Pathogen

While we were creating banana clones for testing, we simultaneously tried to develop an understanding of what constituted an adequate inoculum for PD

screening. An extensive collection was made of *Fusarium oxysporum* Schlecht. ex Fr. f.sp. *cubense* (E.F. Smith) Snyder & Hans. (FOC) isolates from infected plants within the plantation and infected plants adjacent to the plantation.

The Davao isolates were morphologically and culturally very similar to FOC races 1 and 2 of Central America. It was recognised early that all the isolates from Latundan produced purple to violet pigmentation in PDA medium. All isolates from field-infected Cavendish (Umalag and Hijo Giant) invariably produced flesh or salmon colour. There were no observable changes in the cultural characters and pathogenicity of the isolates after five passages of the isolates on *M. balbisiana*. The colour and type of growth of aerial and submerged mycelia, and pigment produced in the culture medium of the reisolated cultures were the same as the original inoculum. When manual mixtures of 50% purple isolates and 50% flesh-coloured isolates were used for inoculation of *M. balbisiana*, among the cultures recovered by reisolating from infected rhizomes only 8% were purple, 6% intermediate pink and 82% white to flesh-coloured. Additionally, it was observed that the Latundan isolates were more virulent on *M. balbisiana* than the isolates from Cavendish. This may indicate that the ability to compete and survive is not necessarily associated with pathogenicity. On Cavendish, the Cavendish isolates were more virulent than the Latundan isolates.

Seventy-two isolates were grown on steamed rice to determine if they belonged to the 'odoratum' group (Stover 1959; Waite and Stover 1960). All isolates developed the formaldehyde-benzoic-acid-like odour as described by Leoncio (1930), except one isolate from a Latundan and one from an abaca. This same collection of isolates was analysed for biochemical differences. The electrophoretic patterns of the peroxidase isozymes of FOC did not delineate the Latundan fungal isolates from the Cavendish fungal isolates indicating that all FOC in Davao are probably from one fungal population. The common bands among the FOC isolates were uniquely different from the peroxidase isozyme patterns of the saprophytic *Fusarium* and other *Fusarium* used in this set.

For the determination of the race(s) extant in the Davao area, host range studies were conducted using the soil bed test, 30 cm clay pots and field trials. For the soil bed test, meriplants of cultivars were tested as described above. Meriplants grown in clay pots were tested by adding 20–50 g of a 14-day-old corn meal-sand culture of a fungal isolate in a shallow trench around each plant growing in sterilised 1:1 sand-soil mixture. The rhizomes were scored for RDI after 60 days. Except for the occasional wilting of Gros Michel and *M. balbisiana* seedlings, very

few above-ground symptoms were observed within 60 days. The cultivars were also planted to field sites where infected plants had been recently eradicated.

The results showed that both a Cavendish isolate and a Latundan isolate were highly pathogenic to most banana cultivars tested. There were slight differences in levels of infection but no apparent differential reactions. Several abaca cultivars are susceptible to both sources of isolates and several were immune to both. When Saba (ABB) was tested using meriplants, it was equally susceptible to both isolates; however, in the field trials, Saba continues to show resistance. Saba is widely planted in Davao but it is very rarely infected. Healthy Saba are commonly seen growing in Latundan orchards which are heavily damaged with PD.

Bluggoe was used by Vakili (1965) and Waite (1977) to differentiate between races 1 and 2. Our extensive host range studies do not readily delineate two different races. The isozyme data described above are supportive. We feel the field data would indicate that the FOC which we studied has many race 1 characteristics.

Additional tests showed that *Heliconia* species were resistant to all isolates of FOC tested and so the Philippine FOC was not race 3. Also when single spore cultures of 73 FOC isolates, selected from the collection to represent different geographical locations around Davao and various banana cultivars, were grown on modified Komada's medium (Sun et al. 1978) no lacinated colonies developed. All the isolates grew slowly with appressed mycelia with very sparse sporulation. This would indicate that the Philippine FOC also was not race 4. However, by the definition of race 4 as suggested by Stover (these Proceedings), the Philippine FOC would need to be considered as race 4 because it infects Cavendish. For additional information see Buddenhagen (these Proceedings).

### Epidemiology Within Plantations

As I have mentioned above, PD was a disease in the extensive abaca industry which flourished prior to World War II. The land was subsequently converted to citrus, to Latundan, or simply fallowed. Much of the area now occupied by the dessert banana plantation was at some time in the past planted to abaca or Latundan.

The Cavendish plantations were planted in 1969 using Hijo Giant Cavendish. Plantings of Umalag and the gradual conversion to Umalag of some areas began in 1970. In 1974, the year of the first confirmed PD diseased plant, 500 diseased mats were reported on about 5000 ha of Cavendish. By 1979, the number reported had tripled. The number decreased to near 1000 by 1981.

Contributing to this annual decrease after 1979

was the improved accuracy of disease identification. A monitoring system was established to obtain accurate field data. Tissue samples of most diseased plants were sent to the laboratory to verify PD infections. Improvements in the eradication procedures including an enlarged buffer zone and the cessation of the immediate replanting of eradicated sites were helpful. Some marginal lands of Class III and IV soils were taken out of production because they were uneconomical for production; soils of Class I and II tended to have fewer PD cases than marginal lands.

Using the information available from Central America (Stover 1962; Simmonds 1966) many parameters were investigated to develop a better understanding of the epidemiology of the Davao PD problem. The cyclic patterns of the average cases per month followed the precipitation cycle, only the PD cycle was offset by 3 months (Fig. 3); 95% of the infected plants in the field were about to shoot or had already shot. Few infected plants were less than 6 months old. This pattern for infection differs somewhat from those published for Gros Michel plantations (Stover 1962) and may reflect the increased resistance of Umalag. The events of root infections appeared to be similar to those of Gros Michel but with the Cavendish, the sequence of events leading to infection takes more time. In our plantings, even young plants of Gros Michel showed PD symptoms.

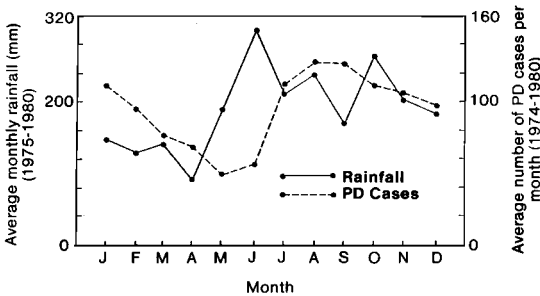


Fig. 3. The average monthly rainfall and average number of PD cases per month are superimposed to show the coordinated cycling of both events. The cases of PD follow the rainfall pattern 3 months earlier.

In culvert experiments, when plants were fertilised with the  $\text{NO}_3\text{-N}$  forms rather than  $\text{NH}_4\text{-N}$  fertiliser, the time duration between onset of PD symptoms and death of the plants was significantly lengthened, possibly indicating that the tolerance of the plant had been improved. However, all plants regardless of fertiliser application died due to PD. In another experiment, both the RDI and DSI increased with increasing rates of  $\text{NH}_4\text{Cl}$  fertilisation while the reverse was true with  $\text{KNO}_3$ . FOC grown in the

laboratory did not show any distinct preference for either form of nitrogen.

Again in culverts, when the equivalent of 0, 300, 600 and 900 g  $\text{K}_2\text{O}/\text{ha}/\text{yr}$  was used, the systemic spread of the pathogen within the plant body seemed to be enhanced because the number of days between first symptoms and death was shortened. The fertiliser treatments resulted in a build-up of potassium in the soil and in the leaves. Potassium is needed by both host and pathogen and whatever exceeds the plant requirement may just nurture the fungus. In the laboratory, when FOC was grown in different concentrations of potassium, the mycelial weight of the fungus increased with potassium concentration over the range tested from  $3.7 \times 10^{-5}$  to  $10^{-2}$  M.

As the pH of test plots soils increased from 5 to 9, there was a decrease in the severity of symptoms but no curtailment of infection. We could never show an association of nematode infection (*Radopholus* and *Meloidogyne*) and PD infection.

*Fusarium oxysporum* could be isolated from the roots of 40 out of 41 species of weeds growing in the plantations and tested. Pathogenicity tests verified that some of the *Fusarium* were FOC (Table 4). FOC could be isolated from the roots of weeds even from a plantation that had not reported any PD infected mats. By growing selected weed species in the soil beds inoculated with OC, we showed that FOC can survive via the roots of weeds externally in most and systemically in others.

Table 4. Test of pathogenicity of *Fusarium* spp. isolated from the roots of weeds and inoculated on 2-month-old *M. balbisiana* seedlings.

Weed source	Species identified by morphocultural characters	Number of isolates tested	Number of isolates that caused PD infections
Plantation No. 1 with PD cases			
	<i>F. oxysporum</i>	4	2
	<i>F. solani</i>	1	0
	<i>F. dimerum</i>	1	0
	Other <i>Fusaria</i>	2	0
Plantation No. 2 with PD cases			
	<i>F. oxysporum</i>	5	1
	<i>F. dimerum</i>	1	0
	<i>F. equiseti</i>	1	0
Plantation No. 3 with no PD cases reported			
	<i>F. oxysporum</i>	4	2
	<i>F. solani</i>	1	0

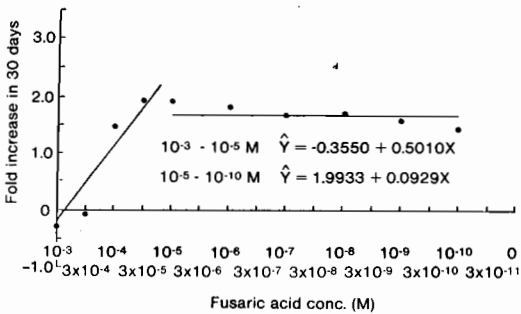
#### Interaction of the Fungus and Plant

One of the earliest symptoms of PD infection is the yellowing of the lamina at the base of the oldest

leaf. Since PD is initiated by root infection and the pathogen is normally not extractable from tissues 1 m above the soil line, it would appear that a translocatable factor moves from the lower portions of the plant to the leaves.

When FOC is grown in liquid medium, it contributes compounds to the medium. These compounds inhibit the growth of banana meriplants. Culture filtrates of *Fusarium* have been used to select for disease resistance in potato and alfalfa (Behnke 1980; Hartman et al. 1984). Fusaric acid is a major component of *Fusarium* culture filtrates.

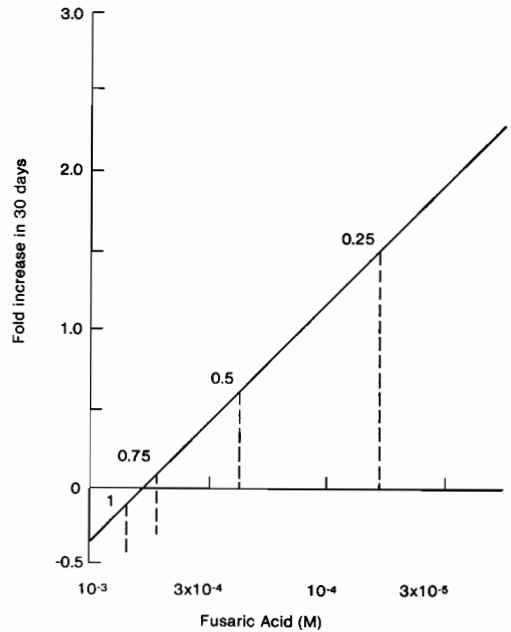
When Umalag meriplants are grown in increasing concentrations of fusaric acid (Sigma Chemical Company, St. Louis, Missouri) a biphasic curve develops (Fig. 4). The curve at concentrations higher than  $10^{-5}$  M is the interesting portion of the curve. At concentrations below  $10^{-5}$  M, the growth was not inhibited. Using the inhibition curves of fusaric acid, the concentrations of fusaric acid equivalence in fungal filtrates could be estimated (Fig. 5).



**Fig. 4.** Growth response of Umalag meristems with fusaric acid added to the medium. Fold increase of fresh weight is calculated as day 30 — day 0/day 0.

In a preliminary comparison of the responses of different cultivars to fusaric acid, there appears to be a tendency for resistant cultivars to have a steeper slope indicating less sensitivity at  $10^{-4}$  and  $10^{-5}$  M (Table 5). It may be possible to use growth parameters of meriplants in culture to determine disease sensitivity. This concept will require additional verification.

Using fungal filtrates and fusaric acid we were unable to develop a plantlet leaf wilt test or leaf disc assay which delineated the field PD resistance of cultivars. The leaf disc assay did show, however, that fusaric acid induced concentric black rings on the discs floating in solutions while fungal filtrates caused the discs to become yellow green or olive green. This indicates that there are other compounds



**Fig. 5.** Estimation of the fusaric acid equivalence in the filtrates of a Cavendish fungal isolate on Umalag. The fold increase in growth in medium with varying concentrations of filtrate was plotted on the curve developed in Fig. 4 and the fusaric acid equivalence extrapolated.

**Table 5.** Comparison of inoculation response to FOC and growth inhibition after 30 days by fusaric acid in different banana clones.

Clone	Inoculation response to FOC	Slope of $10^{-3}$ to $10^{-5}$ M fusaric acid growth curve
Ipod	Resistant	1.025
PD-resistant Umalag clone	Resistant	0.697
Roy Kerr	Resistant	0.624
Hijo Cavendish	Susceptible	0.550
Sankanana	Susceptible	0.545
Umalag	Susceptible	0.501

in fungal filtrates that may be more important to pathogenicity than fusaric acid.

### Summary

In retrospect, it does not appear that we were working with a new race of PD in 1977. By 1982, Panama disease was no longer considered an impending epidemic and the impact of the disease was considered minor. Through the use of good

cultural practices, i.e. accurate identification and eradication of PD-infected plants, adequate buffer zones around infected mats, proper fertilisation practices and continual weed control, the current level of PD infection could be managed and tolerated. Diseases like Sigatoka, Moko and bunchy-top are now considered diseases of economic importance.

The techniques of tissue culture are adequate in banana to design experiments in micropropagation and mutation breeding. Although the EMS-treated plants were not evaluated in this project, the early indications were that EMS was far more effective in producing variation than gamma irradiation. This needs to be pursued.

There is always a need to correlate laboratory and greenhouse tests and field responses. It would appear that greenhouse evaluations identified plants that while showing improvements in the greenhouse, were insufficiently better than the nearly field-resistant Umalag.

There is a need to improve the assays for PD resistance. Using meriplants for disease screening may be convenient, but Saba meriplants were more sensitive in the soil bed tests than Saba tends to be in the field. Conversely, the Umalag meriplant selections showed increased resistance in the soil bed tests, but apparently little improvement under field conditions. Additional studies are needed to understand the effects of plant age and maturity relative to disease sensitivities. Improved field techniques to evaluate the differences in disease sensitivities among nearly resistant cultivars is also needed.

By the design of the mutation experiment, it was impossible to determine if the increased resistance observed in the soil bed tests of the four experimental clones was present in the original meristems, induced by the mutagenic treatments or the product of somaclonal variation (genetic-variation induced because the plants were put through tissue culture). In the final analysis, the shoot cloning procedure was able to create and identify clones which consistently differed in their disease reactions. The fact that sibling shoots gave rise to clones which differed would argue against variation between original meristems, but one selection did not have any mutagenic treatment in its cultural history. The protocol of the mutation breeding experiment would have eliminated any clone which was slow-growing or with growth defects because the meriplants were tested at the 6–8-leaf stage. The rapidly growing clones would be tested first.

There is increasing evidence that somaclonal variation is cultivar-dependent. Selecting the right cultivar is then an important consideration. If the variation level is low, one can discard the odd plant

and continue. But if one wishes to select for useful variation, it is probably best to establish screening procedures adequate for a mutation run, so that very rare events (one in  $10^4$  to  $10^6$ ) will be identified. Otherwise, there is a tendency to become overly enamoured with useless variation.

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# Somaclonal Variation of Bananas and Screening for Resistance to *Fusarium* Wilt

S.C. Hwang \* and W.H. Ko \*\*

FUSARIAL wilt of banana, also popularly known as Panama disease, is the most destructive disease of bananas. It has destroyed more than 40 000 ha of bananas in Central and South America over a period of 50 years (Stover 1972). Since first noticed in 1967, the disease has become the major threat to banana production in Taiwan. At present about one-third of the 5000 ha of bananas are affected, and within infested areas about 10–15% of the bananas are diseased. Attempted control measures include injection of chemicals, fumigation, and soil treatments. Although some of the treatments suppressed the disease, none were commercially applicable.

The pathogen *Fusarium oxysporum* f. sp. *cubense*, attacking the Taiwan Cavendish, formerly considered to be resistant, is race 4 (Su et al. 1977), which has a broader host range than race 1 (Hwang et al. 1984). Varieties attacked by race 4 must be replaced by a resistant variety. However, no commercial varieties tested so far had a desirable level of resistance (Hwang et al. 1984).

Due to the heavy losses of banana production to fusarial wilt, a new planting program has been initiated in Taiwan since 1983, using pathogen-free in vitro-produced meristem plants for planting (Hwang et al. 1984b). These meristem-derived plants showed up to 3% somaclonal variation (Hwang 1986), which would be considered more desirable as material for screening for wilt resistance because of their genetic variability potential much greater than is occurring in nature. We therefore set up a mass-screening program that began in 1984 with the objective of producing large numbers of plants in vitro and to screening these for wilt-resistant mutants. We examined the types and frequency of variation occurring in in vitro-produced plants, and

report here the progress of the screening program made in the past 2 years.

## Mass Propagation of Planting Material In Vitro

Because of heavy losses of bananas caused by fusarial wilt, a new planting program was initiated in Taiwan in 1983 to ensure an abundant banana production for the export trade and for local consumption. Seed corms obtained from wilt-infested areas are important sources of inoculum (Stover 1972; Su et al. 1977). To prevent spreading of the disease to a disease-free orchard, it is necessary to obtain seed corms for planting from wilt-free areas. This is becoming increasingly difficult because of the widespread nature of the disease. A meristem culture technique was, therefore, developed for mass propagation of the pathogen-free banana plantlets for commercial planting (Hwang et al. 1984b).

Plantlets derived from meristem culture became well established under field conditions and resulted in mature plants with uniform growth and normal fruit yield. They had a bigger pseudostem and retained more healthy leaves than those originating from suckers. The harvest period was shortened from the usual 3 months to 1.5 months because of the uniform growth. Plantlets are cheaper and easier to propagate and transport than suckers. More and more banana growers have begun using meristem culture plantlets to establish their fields. By July 1986, plantlets have been planted in over 1200 ha of banana orchards. A total of 3 million plantlets are to be produced for planting in 1987, which will be established in 1500 ha, or about one-third of the banana plantations in the major production areas. With so many small producers under an annual replanting system in Taiwan, the clean-seed program is especially important in not only providing disease-free planting material for the growers, but also paving the way for solution of the seasonal overproduction problem that occurs at some time each year.

\* Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan, Republic of China.

\*\* Department of Plant Physiology, University of Hawaii, Hilo, Hawaii 96720, USA.



### Somaclonal Variation In Vitro

About 3% of Cavendish plantlets derived from meristem culture were variants (Hwang 1986). Some are detectable when plants are young and others after flowering. Two types of variants with variegated leaves and abnormal leaf shape were recognised at the young stage. Of 30 000 2-month-old meristem-derived plants examined, about 0.25% of them were found with variegated leaves, and 0.12% with abnormal leaf shapes distinctively different from those of ordinary plants. In mature plants, four types of variants were recognised differing from the ordinary plants in stature, leaf shape, pseudostem colour, and bunch characters, and occurring at a rate of 1.4, 0.5, 0.1 and 0.4%, respectively, in 46 260 plants surveyed (Table 1). Dwarf variant appeared to be the most common, occurring at a rate of 1.4%. With the exception of variegated variant which is unstable, all other types of variant were preserved consistently in three successive clonal generations. No variations were detected among 40 000 mature plants propagated from suckers, the conventional planting material.

The rate of variability in Giant Cavendish produced in vitro, observed over the past 4 years,

was rather constant. Recently, the same method also was used for propagating Williams Hybrid, Valery (a dwarf-type) and Umalag. However, the rate of variability varied from 12% in Valery to 3–5% in Williams Hybrid and Umalag. It would appear that members of Cavendish group have different potential for producing variability in vitro. Variability rates of up to 20% for a Cavendish variety, and of 19% for Grande Naine were recorded in Jamaica (Stover and Buddenhagen 1986) and Puerto Rico (Pool and Irizarry 1985), respectively. Whether the kinds of medium used, the incubation conditions, or the number of subculturing also affect the range of variability in each variety remains to be studied.

### Screening Mass-Produced In Vitro Cavendish Plants

#### Screening Procedures

A large number of 2-month-old meristem-derived plants, propagated by the method of Ma and Shii (1972) and of Hwang et al. (1984b), were planted in nursery soil heavily infested with diseased tissue of Cavendish plants. The diseased tissue was ploughed

**Table 1.** Feature and rate of variation in shooted banana plants propagated through tissue culture.

Variant type	Variant characteristics *	Variation Rate (%)
Stature	1. The plant is 180 cm in height, with thicker pseudostem, shorter petiole, and a leaf ratio 1.79–1.95. Choking usually occurs when shooting. The peduncle is much shorter and hands packed much closer to each other than the normal	1.30
	2. The plant is 150 cm in height, with thinner pseudostem, fewer hands and long tapering bunches	0.05
	3. The plant is 180 cm in height, but otherwise similar to ordinary plants	0.04
	4. The plant is 310 cm in height, about 50 cm higher than the normal	0.05
Leaf shape	1. The leaf is narrow with a leaf ratio 3.41, and drooped upon unfurling. The pseudostem is thinner than the ordinary, and fruit is abnormal	0.04
	2. The leaf is narrower and thicker than the ordinary, with numerous clear spots scattered on it. The fruit may be normal or smaller than usual	0.42
Colour of Pseudostem	1. The pseudostem, petiole and midrib, turns black upon shooting stage. The plant produces fruit normally or fruit with fewer and smaller hands	0.04
	2. The plant has purple-black pseudostem, but otherwise similar to the ordinary	0.02
	3. The plant has greenish pseudostem, petioles and midribs	0.5
Bunch characters	1. The inflorescence has only 4–6 female flowers developing into fruit hands. The leaves are dark pigmented and droop over	0.04
	2. Hairy fruit	0.01
	3. More hands but smaller fruit	0.01

\* The ordinary plant is about 260 cm in height, with a leaf ratio 2.29.

into soil in which infectivity was made very high and uniform. The soil had a pathogen population about 500–2000 propagules/g soil throughout the testing period. After 3–4 months, depending on seasonal temperature, the surviving plants were dug up and the rhizomes examined for infection. Those free of infection were again multiplied in vitro for additional tests. Whenever suckers became available, they were also used as planting material for testing.

### Resistant Clones Identified

Out of 17 979 meristem-derived plants screened thus far, 45 were free from infection initially. When suckers of these plants were planted for additional tests, 10 clones remained in the selection (Table 2). Further tests involving 2–50 suckers of second generation of each clone showed that six clones had a high level of resistance with disease incidence less than 5%, while another four clones were more tolerant to disease, with disease incidences of 9–29%, which were much lower when compared with standard reference clones, GCTCV-71 and GCTCN-25. Similar results were obtained when 2-month-old

plantlets derived from meristem culture of these clones were tested in a heavily diseased field (Table 2). Percentage of the disease of GCTCV-40, -44, -46, -53, -62, and -119 again was less than 5% compared to 63 and 75% of the check clones GCTCV-71 and GCTCN-25, respectively. Plantlets of GCTCV-41 and GCTCV-63 also displayed a higher level of disease tolerance than the check clones. Plantlets of GCTCV-73 and GCTCN-100 were not included in the test because their meristem cultures were contaminated.

### Major Agronomic Characteristics

All six resistant clones obtained thus far are variants, differing from the normal plants in stature, plant colour, or leaf shape. They all produced inferior bunches smaller than usual. Their major agronomic characteristics are described in Table 3.

### Variability of Resistant Clones

Although most plants of these resistant clones had inferior agronomic traits and yielded poor bunches, a few plants were found in each clone with better agronomic characters and better fruits. The variant

**Table 2.** Response to fusarial wilt of banana (*Fusarium oxysporum* f. sp. *cubense* race 4) of some clones of Giant Cavendish derived from meristem culture in a field test.

Clone	1st generation		2nd generation	
	No. of plants tested	Disease (%)	No. of plants tested	Disease (%)
*GCTCV-40	12	0	38	0
GCTCV-41	6	0	21	29
GCTCV-44	5	0	20	5
GCTCV-46	9	0	43	5
GCTCV-53	2	0	9	0
GCTCV-62	3	0	4	0
GCTCV-63	7	0	34	9
GCTCV-73	2	0	10	10
GCTCV-100	2	0	6	17
GCTCV-119	1	0	2	0
GCTCV-71 (CK)	7	19	10	40
GCTCV-25 (CK)	50	60	50	74

In vitro-produced plantlets used as planting material		
Variant	No. of plants tested	Disease (%)
GCTCV-40	44	5
GCTCV-41	35	40
GCTCV-44	40	0
GCTCV-46	68	3
GCTCV-53	49	4
GCTCV-62	16	0
GCTCV-63	51	12
GCTCV-119	28	0
GCTCV-71 (CK)	19	63
GCTCV-25 (CK)	50	75

\* GC = Giant Cavendish; TC = Tissue culture; V = Variant; N = Normal.

**Table 3.** Major agronomic characteristics of in vitro-derived Cavendish resistant clones.

Clone	Salient features
GCTCV-40	Tall, thinner pseudostem with wide spacing of internodes, weak petioles, narrow drooping leaves (resembles tetraploid)
GCTCV-46	Black spotting in pseudostem, petiole and leaf sheaths, more upright leaves, smaller bunches with short fingers
GCTCV-44 GCTCV-53	Darker green pseudostem and leaves, drooping leaves, narrow and elongate male bud, smaller bunches with short fingers
GCTCV-62	Pale green pseudostem and leaves, slow in producing suckers, smaller bunch and smaller fingers
GCTCV-119	Wavy lamina with pronounced purple spotting, rose shades in pseudostem, petioles and leaf sheath at young stage, loosely-packed pseudostem in mature plant.

was detectable among plants grown either from suckers or from meristem plantlets. The variant of GCTCV-40, originated from suckers, designated as GCTCV-40-1, yielded a bunch weighing 24 kg, or 41% more than GCTCV-40. Weight per bunch of the variants found in GCTCV-46, and -53 was double those of their originals. However, the fruits produced by these variants in each clone, even the best, did not reach the same level as those produced by the normal plants which produced an average of 26 kg/plant in the same field. With the exception of productivity, these variants otherwise displayed agronomic characteristics very close to the normal plants. For instance, GCTCV-40-1 was 260 cm tall, with circumference of pseudostem measuring 68 cm, and a leaf ratio of 2.33; the corresponding figures for the normal plant were 260, 70 and 2.27 cm, respectively (Table 4). Similar to their original parents, the variants of GCTCV-40, -46, -53 and -119 were free from infection in the first crop. Observation of response to fusarial wilt of variants derived from GCTCV-40 (of plantlet origin) and GCTCV-44 is continuing.

### Conclusion and Future Prospects

At present, the Cavendish varieties of banana grown in Taiwan are affected markedly by the fusarial wilt problem where race 4 of wilt pathogen is involved. Numerous control measures have been attempted with no success. It is a major challenge of research to find a resistant variety to replace the present susceptible varieties. In the past 60 years, breeding bananas for resistance to fusarial wilt,

mainly conducted in Jamaica and Honduras, has been an expensive undertaking but with limited success. Breeding for resistance to race 4, a strain with additional virulence to race 1, would be even more difficult. For several reasons, the approach to breeding for resistance through conventional hybridisation has not been attempted in Taiwan. First, the only triploid used for the female parent, Gros Michel or its shorter versions, was susceptible to both race 1 and race 4. Second, in the traditional breeding strategy, disease resistance genes were to be added in a single dose ( $n$ ) from the diploid male parent to a  $3n$  susceptible triploid. Some important diploids widely used as resistant parents previously such as Pisang Lilin, SH2095, and SH2982 were later found to be susceptible to race 4 (Hwang et al. 1984a; Stover and Buddenhagen 1986). So far, SH3142 was the only diploid tested which demonstrated race 4 resistance reaction in a container test (Stover and Buddenhagen 1986). Since race 4 has a broadened host range, it would appear that sources of resistance to race 4 in diploids probably will be very limited in numbers. Third, the bred tetraploids generated to date appear to have agronomic or quality defects; none has been yet planted commercially as a replacement for the present commercial Cavendish clones. Thus, even without considering race 4 resistance, it is highly unlikely to produce an ideal bred variety that has superior agronomic characters and high productivity.

Two tetraploids, Hybrid 972 and SH2742, resulting from a cross between Cocos and Pisang Lilin, resistant to fusarial wilt in Honduras, had been tested in Taiwan. Both clones were susceptible (Hwang et al. 1984a). Lack of resistance to race 4 in both clones is not unexpected, since Pisang Lilin used as the diploid side, although resistant to race 1, was highly susceptible to race 4 (Hwang et al. 1984a). Recently, the Honduras breeding program has produced a promising tetraploid SH3436 derived from the cross between Highgate and SH3142, the latter being resistant to race 4 based on an inoculation test in the container (Stover and Buddenhagen 1986). Both SH3436 and SH3142 (kindly supplied by Dr R.H. Stover) are now being tested under field conditions in Taiwan.

For practical reasons, our first approach was to seek resistant mutants in nature. Bananas have been cultivated for centuries and the probability exists that mutants carrying different levels of disease resistance exist in nature. Screening for race 4 resistance began in the early 1970s. First we collected as many as 150 varieties and tested them in an area where Cavendish varieties were devastated by race 4, but with negative results. None of the introduced commercial varieties tested had a desirable level of resistance (Hwang et al. 1984a). So far, our

collections have been largely limited to the countries in three regions, with only one plant for each clone introduced from each country. Perhaps many more collections should be made in Southeast Asia where bananas originated and much greater variability of bananas exists in this region.

Beginning in 1974, selections were made from more than 3000 suckers obtained from healthy Cavendish bananas grown in severely diseased banana fields. At the end of the fifth year, eight clones appeared to possess some resistance because none of their offspring showed disease symptoms. However, at the end of the sixth year disease also occurred among members of these eight clones (Hwang 1985).

The program was subsequently discontinued, but was reconsidered recently. Since bananas in Taiwan are replanted each year, local commercial varieties propagated by suckers are being tested again to determine if it is possible to obtain a segregant which will tolerate disease for just 1 year rather than 6 years as previously attempted.

With the development of a meristem culture technique, banana plantlets have been mass-produced for commercial planting in Taiwan since 1983 (Hwang et al., 1984b). These plants produced *in vitro* showed up to 3% variations affecting stature, colour of pseudostem and leaves, and shape of leaves and fruit (Hwang 1986). Because of the high frequency of variation among Cavendish plantlets, they were tested recently for resistance to race 4. Out of 17 979 plantlets tested in nursery soil heavily infested with diseased tissue, 45 were free from infection after 4 months. Additional tests of those healthy clones using suckers and plantlets for planting showed that six clones remained to have a high level of resistance to race 4, while another four clones had a higher level of disease tolerance than standard reference clones in the check (Table 2). All six clones, designated as GCTCV-40, -44, -46, -53, -62, and -119, were morphological variants with inferior quality (Table 3). So far, the screening tests have been limited in three small experimental farms, each clone with no more than 70 plants tested. It is still uncertain if resistance of these clones will be sufficiently stable to be maintained if they are planted over a wide area with environmental conditions covering the normal field range. A much more widespread testing of the six clones in diseased areas is still in progress.

It is of interest that, although plants of these resistant clones were mostly inferior variants producing smaller fruits than normal, a few plants with improved agronomic characters and higher productivities were recognised in this study (Table 4). Most important of all, these normal-like plants also were free from infection in the first crop. Research needs to be carried out to determine the

**Table 4** Variation in agronomic characteristics of the clone GCTCV-40 grown from suckers.

Plant No.	Height (cm)	Circumference* of pseudostem (cm)	Leaf ratio
GCTCV-40- 1	260	68	2.33
GCTCV-40- 2	285	57	3.61
GCTCV-40- 3	255	47	3.70
GCTCV-40- 4	260	50	3.71
GCTCV-40- 5	270	54	3.71
GCTCV-40- 6	310	56	3.75
GCTCV-40- 7	265	51	3.48
GCTCV-40- 8	300	58	4.15
GCTCV-40- 9	270	52	3.95
GCTCV-40-10	290	52	3.82
GCTCV-40-11	290	57	3.76
GCTCV-40-12	295	57	3.83
GCTCV-40-13	295	54	3.81
GCTCV-40-14	280	52	3.73
GCTCV-40-15	310	57	3.98
GCTCV-40-16	300	48	4.13
GCTCV-40-17	275	55	3.50
GCTCV-40-18	305	57	3.79
GCTCV-25	260	70	2.27

\* Measured at 30 cm above ground.

resistance and genetic stability of these plants. It is possible to obtain a commercially acceptable resistant clone through this approach.

Even if the above approach cannot produce commercially acceptable resistant clones, desirable resistant traits will be identified in the process. With rapidly advancing biotechnology in the areas of somatic hybridisation through protoplast fusion and genetic transformation, such traits will be useful in the future for the development of new somatic hybrids and genetically engineered bananas that are commercially acceptable and disease-resistant.

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# Cytotaxonomic and Morphological Studies of Thai Banana Cultivars

Benchamas Silayoi \* and Narong Chomchalow \*\*

BANANA is the most common fruit crop in Thailand. As the centre of diversity is believed to be in Southeast Asia, large numbers of cultivars are present in Thailand and its neighbouring countries as the result of a long history of cultivation. Due to advances in agricultural technology and the pressure of increasing populations, genetic erosion is taking place on banana at a very rapid rate. Thanks to the International Board for Plant Genetic Resources which provided a grant to the authors, a total of 323 accessions have been collected from all over the country (Silayoi and Babprasert 1983). They are now being conserved at a field genebank at Kasetsart University Student Training Farm, Pak Chong, Nakhon Ratchasima. A number have been transferred to the Regional Banana Germplasm Center in Davao, the Philippines, Brazil, France and Guadeloupe, as well as the collection of the Thai Department of Agriculture Tha Chai Horticultural Experiment Station.

The present paper deals with cytotaxonomic and morphological studies of some of the collection.

## Cytotaxonomic Study

Based on Simmonds and Shepherd's (1955) scoring method of 15 characters, morphological grouping of 137 accessions has been made. Supplemented by cytological determination of the chromosome number, genomic grouping has been attempted, with the following results:

Score*	Genomic constitution	2n	No. accessions
15-25 <sup>1</sup>	AA	22	37
15-25 <sup>1</sup>	AAA	33	25
26-46	AAB	33	29
59-63	ABB	33	33
67-69 <sup>2</sup>	ABBB	44	3

\* Department of Horticulture, Kasetsart University, Bangkok, Bangkok 10903, Thailand.

\*\* IBPGR Regional Field Officer, Southeast Asia, Maliwan Mansion, Phra Atit Rd., Bangkok, Thailand.

70-75 <sup>3</sup>	BB	22	9
70-75 <sup>3</sup>	BBB	33	1

\* Scores have been modified from the original scheme as follows:

<sup>1</sup>original: 15-23, <sup>2</sup>original: 67, <sup>3</sup>original: not given.

## Preliminary Morphological Study

In order to reduce the synonyms of the cultivar names, a preliminary study has been made on 96 accessions with distinct cultivar names collected from various places. Morphological characters which have been selected for the present study were fruit characters (including shape, colour, texture and taste), petiolar canal, stem colour, and suckering habit. From this study, assignment of 41 'type' cultivars has been made, based on the popularity of cultivar names. The 54 remaining cultivars have been assigned as synonyms based on their morphological similarity to the 'type' cultivars (Silayoi et al. 1981).

The genomic constitution and the 'type' cultivars and their different synonyms are enumerated (Table 1).

## Characterisation

In order to further reduce the duplicates of the collected material and to obtain information related to the accessions, preliminary work on characterisation has been made on 64 selected accessions using 23 morphological characters based on the IBPGR Minimum List of Descriptors (1978). The results are contained in a report to IBPGR (Silayoi and Babprasert 1983).

## Discussion

From the results of the studies reported herein, Thai banana cultivars have been grouped into seven distinct genomic groups (Chomchalow and Silayoi 1984). In each group 'type' cultivars are given together with their synonyms. The complete list of Thai banana cultivars is contained in IBPGR (1978).

**Table 1.** Thai banana names and synonyms.

Common name	Synonym	Remarks
<b>Wild Acuminata (<i>Musa acuminata</i>)</b>		
K. Pa	K. Thong, K. Thong Khi Mao (Songkhla-S) K. Khae (Phrae, Lampang and Uttaradit-N)	Distribution: pan Thailand (Subspecies have not been evaluated.)
<b>Wild Balbisia (<i>Musa balbisia</i>)</b>		
K. Tani	K. Pa (Phrae, Lampang-N) K. Phongla (Nakhon Si Thammarat-S) K. Tani Nai (Udon Thani-NE)	Distribution: pan Thailand.
<b>Wild Ornata (<i>Musa ornata</i>)</b>		
K. Bua	K. Pa (Lampang-N)	Common in the Northern Region.
<b>Acuminata Cultivars</b>		
<b>(A) AA GENOME</b>		
K. Khai	Jek Bong (Surin-NE)	'Khai' means egg. Widely grown as backyard fruit and in commercial farm, est. at Kamphaeng Phet-N.
K. Lep Mu Nang	K. Thong Dok Mak (Phatthalung-S) K. Mak (Nakhon Si Thammarat-S) K. Lep Mu (Nakhon Sawan-N)	Very popular in the Southern Peninsula.
K. Thong Ruang	K. Khai Thong Ruang (Nakhon Si Thammarat-S) K. Khom Bao (Songkhla-S)	Fruits fall off easily when ripened.
K. Nom Sao	—	Common in the Southern Region.
K. Lai	—	Common in the Southern Region.
K. Sa	—	Common in the Southern Region.
K. Hom	—	'Hom' means fragrant. Fruits are as short as K. Khai, but bigger in size. Grown in the Northern and North-eastern Regions as a backyard garden.
K. Thong Kap Dam	—	Common in the Southern Region.
K. Hom Thong San	—	Common in the Northeastern Region.
<b>(b) AAA GENOME</b>		
K. Nak	K. Nam Khrang (Phayao, Phrae-N) K. Khrang (Surin-NE) K. Kung (Nakhon Si Thammarat-S)	'Nak' means purplish red.
K. Kung Khieo	K. Hom Thong (Phrae-N)	A mutant of K. Nak.
K. Hom Khieo	K. Khrao (Phrae-N) K. Hom Khrao (Phayao-N) K. Khieo (Nakhon Si Thammarat-S) KK. Khieo Kho Hak (Nakhon Si Thammarat-S)	'Khieo' means green. When the fruit ripens, the peel is still green.
K. Hom Thong	K. Hom (Chanthaburi-E)	'Thong' means gold. Fruit turns yellow when ripened. Very popular dessert cultivar. Belongs to Giant Cavendish group.
K. Dok Mai	—	Belongs to Gros Michel group.
K. Hom Khom	K. Pha (Chiang Rai-N) K. Hom Tia (Khon Kaen-NE, Kamphaeng Phet-N) K. Khieo (Ubon Ratchathani-NE) K. Tia (Ubon Ratchathani-NE) K. Mu Si (Ubon Ratchathani-NE) K. Hom Khieo Tia (Buriram-NE) K. Tia Hom (Nakhon Si Thammarat-S) K. Tia (Phatthalung-S)	'Khom' and 'tia' mean dwarf. Belongs to Cavendish group.

K. Khlong Chang	—	Like Pisang Umbol.
K. Khai Bonng	K. Khai Pra Ta Bong (Nakhon Ratchasima-NE)	Fruits are bigger than K. Khai.
<b>Acuminata × Balbisiana Hybrids</b>		
<b>(A) AAB GENOME</b>		
K. Nam Phat	K. Nam Kap Dam (Chanthaburi-E)	—
K. Lanka	K. Chin (Phatthalung-S)	Belongs to Mysore group.
K. Roi Wi	—	Pisang Serebu.
K. Ngoen	—	Very rare cultivar, found only in Songkhla-S.
K. Khai Boran	—	Very rare cultivar, found only in Trat-E.
K. Thong Det	—	Common in Songkhla-S.
K. Nang Nuan	—	Common in Nakhon Sawan-C.
K. Nam	K. Hom Chan (Kalasin-NE) K. Hom Lek (Yasothon-NE) K. Hom (Sakon Nakhon and Chaiyaphum-NE) Kracho Nok (Surin-NE) K. Kaeo (Nakhon Si Thammarat-S) K. Hom Nang Nuan (Nakhon Nayok-C)	'Nam' means water.
K. Khom	—	'Khom' means bitter. Similar to K. Nam, except for bitter taste of fruit. Common in the Southern peninsula.
K. Khom Nak	—	Common in the Southern Region, like K. Khom but the fruiting period is later than K. Khom.
<b>(B) ABB GENOME</b>		
K. Pluak Na	K. Hom Thong (Buriram-NE) K. Som (Yasothon-NE) K. Tip Yai (Ubon Ratchathani-NE) K. Nam Thai (Nakhon Nayok-C) Phama Haek Kuk (Ang Thong-C)	Common in the Northeastern Region.
K. Nom Mi	—	—
K. Phaya	—	Common in Songkhla-S.
K. Hakmuk	—	Belongs to Silver Bluggoe group. Cooking variety.
K. Som	K. Hakmuk (Chanthaburi-E)	Very similar to K. Hakmuk but no bloom on fruit. Cooking variety.
K. Tip	K. Tip Kum (Chiang Rai-N)	Similar to K. Hakmuk and K. Som, but the finger tip is blunt.
K. Namwa	K. Tai (Chiang Rai-N) K. Namwa Luang (Phrae-N)	Most common as cooking and dessert banana. Distribution: pan Thailand. The flesh is yellow. 'Luang' means yellow.
K. Namwa Khao	—	A mutant of K. Namwa. The flesh is white. 'Khao' means white.
K. Namwa Daeng	K. Namwa Nai On (Phrae-N) K. Ong (Chaiyaphum-NE) K. Mali Ong (Chanthaburi-E) K. Luk Sai Daeng (Nakhon Sawan-C)	A mutant of K. Namwa. The flesh is reddish. 'Daeng' means red.
K. Namwa Khom	—	A mutant of K. Namwa. The plant is dwarf.
<b>(C) ABBB GENOME</b>		
K. Teparot	K. Pli Hai (Chiang Rai-N) K. Tin Tao (Yasothon-NE) K. Sin Pli (Nakhon Si Thammarat-S) K. Palo (Ubon Ratchathani-NE) K. Tiparot (Bangkok-C)	Normally the fruit bunch does not have male bud, but sometimes the bunch may appear.



### Recommendations

The scoring method of 15 characters by Simmonds and Shepherd (1955) should be modified because: (1) there are some variations in the expression of soma characters when planted in different environmental conditions; (2) some plantains have no male bud. A scoring method, supplemented with cytological determinations of the chromosome number, would be superior.

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# Taxonomic Classification of Philippine Bananas

O.C. Pascua \* and R.R.C. Espino \*\*

THE Philippines is one of the countries in Southeast Asia where bananas abound in great diversity. Today due to pressures of agricultural and technological development, this rich diversity of wild species and primitive banana cultivars has rapidly diminished. Realising the urgency to collect and conserve this important but vanishing germplasm, a field genebank for bananas and plantain was established. The collection, evaluation and conservation undertaken expanded considerably when the International Board for Plant Genetic Resources (IBPGR) designated the Philippines to be the seat of the Southeast Asian Banana Germplasm Resource Center. The establishment of the Center based at the Davao Experiment Station, Davao City, allowed the inclusion of banana germplasm not only from this country but also from the neighboring countries of Malaysia, Thailand and Indonesia. Since the Philippine collection was the first to be established, the characterisation and evaluation work done from the Philippines was more extensive compared with the rest. Presently, there are 80 distinct Philippine cultivars already characterised (Pascua et al. 1984). However, this is expected to increase as more undescribed banana cultivars are likely to be uncovered in the near future.

## Characterisation and Parameters Used as Indicators

The IBPGR Revised Descriptors for Bananas (1984) has been adopted in the characterisation of the different Philippine banana cultivars. Also the use of the taxonomic scoring developed by Simmonds and Shepherd (1955) was likewise utilised.

In the current work on Philippine cultivars and those coming from Malaysia and Thailand, several morphological characters were used as visual

indicators of the ploidy level of the various cultivars. Although the characters adopted were found useful in the visual judgment, this required supplemental experience during the process of observation to make the data very reliable.

There are several vegetative characters that can be used as an indicator to distinguish the ploidy level. One of the most important is the leaf morphology. As observed and manifested by the different cultivars in the field genebank, leaves of different banana cultivars somehow differ in length and width. Measurement of lamina width of known diploid ('Amas,' 'Lakatan,' and 'Kinamay Dalaga') and triploid ('Morado,' 'Bungulan,' and 'Umalag') cultivars (PCARRD 1985) had shown that the former group had leaf width ranging from 27 to 65 cm while the latter had higher figures ranging from 47 to 86 cm. This result apparently pointed to the fact that diploid cultivars have narrower leaves than their triploid counterparts.

Another discernible character that may indicate the ploidy level of a cultivar is how the leaves are held or 'carried' by the plant itself. Owing probably to their narrower width, the leaves of the diploid cultivars are more upright when compared to the triploid which are characterised as drooping. In the descriptor for bananas, the drooping of the leaves serves as one of the vegetative characters used in the visual judgment for a triploid (AAA) cultivar (IBPGR 1984).

On the other hand, leaf size and the way it is held or whether they are upright or drooping could not serve as an indicator of the ploidy in *balbisiana* cultivars. 'Turangkog' which is a triploid (BBB) has broad leaves and are held upright while 'Abuhon' (BB), being a diploid, has drooping leaves. In another case, 'Saba' (BBB) has drooping leaves. However the structure of the leaf margin can be used as a means of differentiating this group with that of the *acuminata*. The petiole margin of the *balbisiana* cultivar is enrolled whereas it is generally spreading with that of the *acuminata* cultivars.

Another probable vegetative character that may be considered in the visual judgment of ploidy for

\* Bureau of Plant Industry Research Station, Davao, Philippines.

\*\* University of the Philippines, Los Baños, Philippines.

*acuminata* cultivars is the pseudostem size. Specifically it is the girth that is measured 50 cm from ground level. In general, diploid (AA) cultivars have a slimmer pseudostem than the triploid (AAA) cultivars. This can be exemplified by the observation in 'Amas' (AA) wherein its girth ranges from 11 to 13 cm while 'Bungulan' (AAA) has a girth of 15 to 20 cm. This observation also conforms with what is stated in the banana descriptor that diploid cultivars are slim while triploid cultivars are more robust.

For *balbisiana* cultivars, they are strikingly big and robust, with girth measurement ranging from 28 to 31 cm. Even when viewed from a distance, the robustness of the *balbisiana* cultivars is unmistakable.

There are other vegetative characters that may be used to indicate ploidy or cultivar grouping, but so far those just mentioned in this paper are the more pronounced ones that have been utilised in the characterisation work prior to the stage when the plants produce inflorescence and fruit. Visual judgment of vegetative characters as a means to determine ploidy could be considered as a very reliable parameter complementary to and in the absence of the more conclusive chromosome counts that are required.

### Cluster Analysis

Cluster analysis was conducted on several measurable characters namely, pseudostem height, pseudostem diameter, leaf length, leaf width, leaf length/leaf width ratio, bunch weight, number of fingers/hand, finger length, finger diameter, number of days from planting to shooting and number of days from shooting to harvesting. Its primary purpose is to develop some groupings of the various cultivars and relate this to its genomic constitution. Moreover, these will also be useful in the designation of the range and limits of each group which will be very helpful in assigning numerical designation based on the IBPGR descriptor.

Three predetermined numbers of clusters were made to determine how well the data were separated through minimisation within cluster variation and maximisation between cluster variation. This is measured through the cubic clustering criterion (CCC). A value of greater than three is indicative of acceptable clustering (SAS 1982). Table 1 shows the CCC value obtained using 3, 4 and 5 cluster groupings. Leaf length, bunch weight, number of fingers/hand and finger diameter had the highest CCC when grouped into three. While pseudostem diameter, leaf width, finger length and number of days from shooting to harvesting had the highest CCC when grouped into four. Pseudostem height, leaf length, leaf width ratio and number of days

**Table 1.** Cubic clustering criterion (CCC) obtained using predetermined number of clusters on various characters of banana.

Characters	Number of clusters		
	Three	Four	Five
Pseudostem height (cm)	3.2	2.8	3.9
Pseudostem diameter (cm)	4.0	4.5	2.9
Leaf length (cm)	6.1*	4.1	2.6
Leaf width (cm)	4.8	6.2	5.2
Leaf length: leaf width ratio	2.6	4.6	5.8*
Bunch weight (kg)	5.5*	3.1	2.8
Number of fingers/hand	6.6*	4.6	4.9
Finger length (cm)	4.3	5.2	4.9
Finger diameter (cm)	6.9	5.1	5.5
Number of days from planting to shooting	4.6	5.0	5.4*
Number of days from shooting to harvesting	5.8	6.7	4.3

\* Highest cubic clustering criterion.

from planting to shooting had the highest CCC when grouped into five.

Table 2 shows the distribution of the various cultivars on the characters which are grouped into three classes. For bunch weight, diploid (AA) cultivar has generally a small-medium sized bunch while triploid (AAA, BBB) cultivars have a bigger and heavier bunch. Most of the cultivars exhibited medium-sized bunch with a weight ranging from 10.4–21.8 kg.

This is also true for leaf length and finger diameter wherein diploid (AA) cultivars are grouped in classes with lower values. For the number of fingers/hand, cultivars belonging to the various genome groupings are almost represented in each class level. Diploid (AA) and triploid (AAA, AAB, ABB and BBB) cultivars have fingers ranging from 8 to 25 per hand.

The distribution of the various cultivars on the characters which are grouped into four classes is presented in Table 3. The number of diploid (AA) cultivars is concentrated on the values ranging from 10.9 to 19.6, 47.8 to 67.7 and 7.1 to 16.1 cm for pseudostem diameter, leaf width, and finger length, respectively. This represents the first and second group. This reinforces the earlier statement that pseudostem diameter and leaf width can be used as a possible index for visual assessment of the ploidy level. However, one should be careful not to use these characters solely since there are triploid cultivars that are also included in its grouping. Moreover, diploid cultivars tend to mature earlier as indicated by shorter number of days from shooting to harvesting.

Table 4 shows the distribution of the various cultivars according to their genomic constitution for

**Table 2.** Distribution of banana cultivars according to its genomic designation in characters grouped into three (3) classes.

Character	Grouping	Number of cultivars/genome					
		AA	AAA	AAB	ABB	BB/BBB	ABBB
Bunch weight (kg)	I (3-10)	17	3	2	-	2	-
	II (10-22)	6	16	17	4	5	1
	III (22-31)	-	-	1	1	3	-
Leaf length (cm)	I (135-185)	12	8	3	1	-	-
	II (186-250)	12	8	15	6	4	1
	III (252-304)	-	3	-	-	6	-
Finger diam (cm)	I (2-3)	21	10	1	-	5	-
	II (3-5)	2	8	16	6	4	-
	III (5-6)	-	-	2	-	1	1
No. of fingers/hand (2nd)	I (8-13)	6	3	6	1	-	1
	II (14-20)	16	14	13	4	9	-
	III (21-25)	2	1	1	-	1	-

**Table 3.** Distribution of banana cultivars according to its genomic designation in characters grouped into four classes.

Character	Grouping	Number of cultivars/genome					
		AA	AAA	AAB	ABB	BB/BBB	ABBB
Pseudostem diam (cm)	I (1-14)	12	2	-	-	-	-
	II (15-19)	11	9	8	2	-	-
	III (20-25)	1	8	10	3	7	1
	IV (26-32)	-	-	1	-	4	-
Leaf width (cm)	I (48-55)	5	1	1	-	-	-
	II (56-68)	18	8	6	2	4	1
	III (68-79)	1	7	9	6	4	-
	IV (81-91)	-	3	1	-	2	-
Finger length (cm)	I (7-11)	10	2	2	-	4	-
	II (11-16)	11	12	11	3	6	1
	III (17-21)	1	3	5	2	-	-
	IV (26-28)	-	-	3	-	-	-
Number of days from shooting to Harvesting	I (40-75)	12	-	2	1	-	-
	II (76-128)	11	16	16	2	4	-
	III (129-177)	-	1	2	2	5	1
	IV (277)	-	-	-	-	1	-

**Table 4.** Distribution of banana cultivars according to its genomic designation in characters grouped into five classes.

Character	Grouping	Number of cultivars/genome					
		AA	AAA	AAB	ABB	BB/BBB	ABBB
Plant height (cm)	I (138-170)	3	3	-	-	-	-
	II (184-240)	10	4	1	-	-	-
	III (246-315)	10	6	9	-	2	-
	IV (318-385)	1	5	11	1	3	-
	V (396-449)	-	-	-	4	6	1
Leaf length/leaf width ratio	I (2-2.3)	-	3	1	-	-	-
	II (2.4-2.8)	6	5	4	2	-	-
	III (2.9-3.3)	13	4	10	2	5	-
IV (3.3-3.7)		2	6	2	3	-	1
	V (3.7-4)	3	1	1	-	2	-
Number of days from planting to shooting	I (153-180)	2	1	-	-	-	-
	II (210-238)	3	6	1	1	-	1
	III (274-336)	7	10	9	5	5	-
	IV (357-406)	5	-	4	-	3	-
	V (447-476)	-	2	2	-	-	-

the various characters that can be grouped into five classes. One can easily discern that the distribution pattern of cultivars of each class for each character follows a bell-shape wherein the middle class has the most number of entries. Moreover, the diploid (AA) cultivars were distributed in the various classes such that it becomes difficult really to distinguish it from the triploid. This holds true for plant height, leaf length/leaf width ratio and number of days from planting to shooting. Thus, these characters can not be used to distinguish various genome groupings in bananas.

### Conclusion

So far, there are several characters both quantitative and qualitative that can be used for distinguishing the ploidy level of the various cultivars of banana and plantain. These are leaf width, pseudostem girth, bunch weight, petiole margin, etc. However, this should not be used singly but rather in combination. Moreover, actual field experience is necessary to ensure its reliability.

The ultimate goal in taxonomic classification in banana and plantain is to develop a reliable index in distinguishing the various cultivars. At present, the best index used is through the morphological

characteristics of the fruits. This would mean that one had to wait for 12-18 months in order to distinguish a particular cultivar. Moreover, this is confounded with the use of different names for each cultivar in various localities.

Activities in taxonomic classification of the various cultivars of banana and plantain need to be looked at further to develop an index to distinguish one from the other, both at the vegetative and reproductive stage of growth.

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# Morphological Taxonomy of Plantain (*Musa* Cultivars AAB) in West Africa

R. Swennen and D. Vuylsteke \*

PLANTAIN bananas, hereafter called 'plantains', form a special subgroup among the AAB bananas (Simmonds 1966). These perennial giant herbs are interspecific hybrids produced as a natural crossing between *Musa acuminata*, which provided genome A, and *Musa balbisiana*, which provided genome B (Simmonds and Shepherd 1955). They are characterised by the orange-yellow colour of the compound petal in the flowers and the rather orange-yellow starchy flesh when ripe (Simmonds 1966). Fruits are slender (ratio fruit length-fruit girth is high) and are usually cooked, roasted or even boiled before consumption because they are unpalatable when raw. Although the centre of origin is supposed to be in South India (Simmonds 1966), a remarkable diversity exists in Central Africa (De Langhe 1961a, 1964a). Plantain was suggested to be among the oldest cultivated bananas in Africa (De Langhe 1964b). Four bunch types are distinguished (Tezenas du Montcel and Devos 1978): (1) *French plantain*: inflorescence is complete at maturity (many hands consisting of many rather small fruits followed by inflorescence axis covered with persisting hermaphrodite flowers and male flowers; the male bud is large and persistent); (2) *French Horn plantain*: inflorescence is incomplete at maturity (hands consisting of large fingers followed by few hermaphrodite flowers); (3) *False Horn plantain*: inflorescence is incomplete at maturity (hands consisting of large fingers followed by few hermaphrodite flowers); (4) *Horn plantain*: inflorescence is incomplete (few hands consisting of few but very large fingers; no hermaphrodite flowers and no male inflorescence; inflorescence axis is terminated by a tail or a deformed glomerule).

Hence within the plantain subgroup there exists a continuous variation from inflorescences with many hands and small fingers to inflorescences with few

hands and large fingers. One extreme is represented by the Congolese cultivar 'Nazika' (with 22 fruit-bearing hands, personal observation) and the other by the Cameroonian cultivar 'nothing but green' (no fruits, Tezenas du Montcel et al. 1983). That this variation is continuous is clearly indicated by the fact that some cultivars vary between two inflorescence types. The Horn plantain cultivar 'nothing but green' which normally does not produce fruits, occasionally produces one or two hands (Tezenas du Montcel et al. 1983). Similarly the False Horn plantain cultivar 'Agbagba' occasionally becomes a French plantain (Tezenas du Montcel et al. 1983; Vuylsteke et al. 1986). The prototype plantain is believed to be a French plantain (Simmonds 1966), the other bunch types being more degenerated and more parthenocarpic (De Langhe 1964b, c).

De Langhe was the first to classify the plantain cultivars according to the size of the pseudostem (De Langhe 1964a). In Zaire at the Yangambi field genebank he distinguished 'giant,' 'medium' and 'small' plant sizes which were supposed to be of parental origin.

The plant size was positively correlated with the number of hands per bunch produced, time till harvest and number of leaves produced till flowering. Mutants of the plant size types were considered either as 'semi-dwarf' or 'dwarf.'

The previous consideration leads to the conclusion that the plantain subgroup manifests an extremely wide morphological variation despite botanical homogeneity. This is the main reason why identification of plantain cultivars is so difficult and why in many reports the cultivars are not precisely identified. This makes the meaningful exchange and interpretation of research findings and results difficult and sometimes impossible.

This paper is based on field data related to cultivars from the field genebank at the High Rainfall Substation of the International Institute of Tropical Agriculture (IITA) at Onne in southeastern Nigeria. It is hoped that the information may

\* International Institute of Tropical Agriculture, High Rainfall Substation One — Port Harcourt, P.M.B. 5320, Oyo Road, Ibadan, Nigeria.

provide a better understanding of plantain morphology.

### Materials and Methods

The plantain field genebank was established in 1983 in the beginning of the rainy season (April). Sword suckers were planted in plant holes 30–40 cm deep at a spacing of 3 × 2 m. Row spacing between cultivars was 3 m, and plants of the same cultivars were separated by a 2 m distance.

Annual rainfall at Onne averages 2400 mm. The rainfall is distributed over a 10-month period extending from February through December. Temperatures are moderate, averaging 27°C in the warmest months (February, March and April) and 25°C in July, the coolest month. Relative humidity remains high throughout the year, with average values ranging from 78% in February to 89% in July and September. On the average, there are only 4 hours of sunshine each day. The range is from 2 hours/day in September to 6 hours/day in February. The soil is an Oxyc Paleudult derived from the coastal plain sediments of the Niger Delta region. It is deep and well drained. The soil is acid with pH of 4.2.

The field was mulched with *Pennisetum purpureum* at a rate of 80 t/ha/year (fresh weight). Total fertiliser rate per ha was 300 kg N and 456 kg K applied in six equal applications during the annual rainy season. The Onne field genebank contains at least 46 plantain cultivars (more accessions await a final conclusion); these are part of 85 cultivars we have seen in West Africa and which are now in the process of being introduced to Onne from Cameroon, Gabon and Congo. Interpretation of the determination key of the 56 Zairean plantain cultivars (De Langhe 1961a) suggests that among these, 32 cultivars are common to our genebank. Hence the total number of plantain cultivars identified is at least 109.

Twenty-five plantain cultivars were selected for discussion as they are believed to represent the major variability of the plantain subgroup in West Africa. They were introduced from Ghana, Ivory Coast, Nigeria and Cameroon but are also present in other countries under different names (Table 1).

The following characters were observed or measured: (1) Rhizome size, sheaths, petiole margins and bases, lamina bases, peduncle, basal flowers, bunch density, pedicels, fruit skin, fruit dehiscence, male bud and male bracts. They were classified according to Simmonds' criteria (Simmonds 1962); (2) Bunch weight: fresh fruit weight at harvest including the inflorescence axis bearing the fruits (fingers); (3) Number of fruit bearing hands; (4) Total number of fruits; (5) Average fruit weight calculated as the ratio of fresh

fruit bunch weight to number of fruits; (6) Total number of flowers of each hand; (7) Height at flowering: the main pseudostem was measured from soil level to the point where the two highest petioles meet at the time of bunch emergence (shooting); (8) Circumference at flowering: measured on the main pseudostem 50 cm above soil level at the time of bunch emergence; (9) Time to flowering: number of days between date of planting and date of bunch emergence; (10) Leaf number: number of asymmetric leaves (i.e. foliage leaves) produced till flowering; (11) Leaf ratio: ratio leaf length — leaf width, the width being measured at the middle of the leaf. This was measured on the 7th youngest leaf at shooting; (12) Height of ratoon at harvest: height of the tallest sucker at the time of harvest of its mother plant. Height is measured in the same way the main pseudostem is measured; (13) Bunch orientation: either pendant (positively geotropic) or subhorizontal (ageotropic) (De Langhe 1964a; Tezenas du Montcel et al. 1983); (14) Fruit orientation: either erect (negatively geotropic), subhorizontal (ageotropic) or pendant (positively geotropic) (De Langhe 1961a; Tezenas du Montcel et al. 1983); (15) Fruit apex: either blunt, straight or salient (bottlenecked) (Tezenas du Montcel and Devos 1978; Tezenas du Montcel et al. 1983); (16) Fruit shape: either curved or S-shaped (Tezenas du Montcel et al. 1983).

The aforementioned data were recorded on just five plants of each cultivar from the plant crop only. Because of this and since the cultivars were not randomly planted, comparisons among cultivars can be made only with caution.

### Results and Discussion

The corms of the different plantain cultivars are about the same size. Sheaths are slightly waxy, petiole margins are incurved and petiole bases are clasping. The lamina bases are rounded. The peduncles of the bunches of the different plantain cultivars are slightly hairy, all basal flowers are biserial and parthenocarpic (except in 'nothing but green' which has no flowers and in 'Madre del Platanar' which has only hermaphrodite flowers).

Hermaphrodite flowers of French plantain cultivars are biserial but are uniserial in the French Horn and False Horn plantains. The bunch density of French plantain cultivars are either dense or medium, while the other bunch types are rather lax. Pedicels of all plantain cultivars are of the same size. The fruit skin is glabrous. Fruits are indehiscent. The male bud of French plantain cultivars is imbricate. Their male bracts are deciduous but this happens rather slowly. In several cultivars bracts start to wither before dropping off. The male bracts have a dull surface and are not revolute.

**Table 1.** Local names and synonyms of 25 cultivars representing the major variability in the plantain subgroup in West Africa.

cv. No.	Ivory Coast	cv. No.	Ghana	cv. No.	Nigeria	cv. No.	Cameroon	cv. No.	Gabon	cv. No.	Congo	
				P 1	<i>Ntanga 2*</i>	P 1	Mbouroukou No. 2*					
				P 2	<i>Ntanga 5*</i>	P 2	Nyombe No. 1*	P 2	Digondi Dibala			
						P 3	<i>Ovang</i>	P 3	Baka			
						P 4	<i>Njock Kon</i>					
P 5	3	<i>Vert</i>				P 5	Batard					
						P 6	<i>Big Ebanga</i>					
				P 7	<i>Mimi Abue</i>					P 7	***	
				P 8	<i>Ukom</i>							
				P 9	<i>Obino L'Ewai</i>	P 9	French Clair	P 9	Biri	P 9	***	
					Ukom Ntanga				Digondi Di-dine			
				P 10	<i>74.79<sup>3</sup></i>			P 10	Kokou	P 10	??	
			P 11	Nyiretia apem	P 11	<i>Egjoga</i>						
					P 12	<i>Obubit Ntanga 1*</i>				P 12	***	
					P 13	<i>Akpakpak</i>						
							P 14	<i>Bobby Tannap</i>				
				P 15	<i>Mbang Okon</i> Ntanga							
									Ebang			
			P 16	Borodehene	P 16	<i>Agbagba</i>	P 16	Corne type	P 16	Guibangi	P 16	Libanga
									Didoungou			
					P 17	<i>Ngok Egame</i>						
			P 18	Borodewuio	P 18	<i>Obubit Ukom</i>					P 18	Tjei
					P 19	<i>Orishele</i>	P 19	Mbeta 2*				
						Okinima Obara						
					P 20	<i>Kiogo</i>						
					P 21	<i>Ihitisim</i>						
			P 22	<i>Asamiensa</i>			P 22	2 Hands planty				
					P 23	<i>Ubok Iba</i>						
			P 24	<i>Osoaboaso</i>								
			P 25	<i>Kaamenko</i>								

Notes: Names in italics indicate the country from which introduction was made.

\* In the field genebanks a number was given whenever different cultivars were introduced with the same name.

\*\* Present but no name was obtained.

### Inflorescence and Related Characters

Bunch type is the most striking difference among plantain cultivars. The bunch types have already been described adequately by several authors (De Langhe 1961a, 1964a; Simmonds 1966; Tezenas du Montcel and Devos 1978; Tezenas du Montcel et al. 1983).

Here it is convenient to anticipate what we have to do with three plant size categories (see further). For the discussion of the inflorescence and related characters, comparisons should therefore only be made among the giant plantains (P<sub>1</sub>-P<sub>3</sub>, P<sub>5</sub>-P<sub>8</sub>), the medium plantains (P<sub>9</sub>-P<sub>23</sub>) and the small plantains (P<sub>24</sub>-P<sub>25</sub>).

French plantain cultivars (P<sub>1</sub>-P<sub>4</sub>, P<sub>9</sub>-P<sub>14</sub>) produced heavier bunches than French Horn plantain cultivars (P<sub>5</sub>, P<sub>15</sub>) and False Horn plantain cultivars (P<sub>6</sub>-P<sub>8</sub>, P<sub>16</sub>-P<sub>20</sub>) (Table 2).

This is due to the greater numbers of fruit produced by French plantain cultivars than by French Horn and by False Horn plantain cultivars. Similarly French Horn plantain cultivars produce heavier bunches than False Horn plantain cultivars because of the greater number of fruits (compare P<sub>5</sub> with P<sub>6</sub>-P<sub>8</sub>, P<sub>15</sub> with P<sub>16</sub>-P<sub>20</sub> and P<sub>24</sub> with P<sub>25</sub>) (Table 2). The number of fruit-bearing hands and average fruit weight is smaller in French plantain cultivars than in French Horn and False Horn cultivars (except for the False Horn plantain cultivars P<sub>8</sub> and P<sub>20</sub>).

French Horn plantain cultivars produce about the same number of hands as the False Horn plantain cultivars but the average fruit weight is less for the former than for the latter (Table 2). With the exception of P<sub>21</sub>, bunch weight of Horn plantain cultivars does not seem to differ in weight from the False Horn plantain cultivars although the former



**Table 2.** Some bunch characters of 25 cultivars representative of the major variability in the plantain subgroup in West Africa.

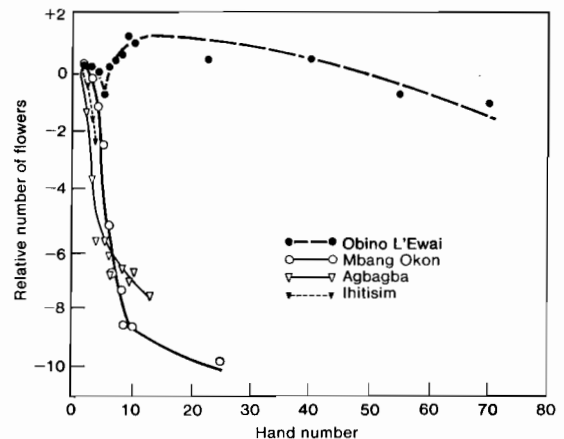
Cultivars	Bunch characters				
	Bunch weight (kg)	Number of hands*	Number of fruits	Fruit weight (kg)	
P 1	Ntanga 2	32.2 ± 3.7	8.8 ± 1.5	132.8 ± 26.3	0.25 ± 0.03
P 2	Ntanga 5	25.9 ± 1.6	9.3 ± 0.5	133.3 ± 13.5	0.20 ± 0.03
P 3	Ovang	28.6 ± 10.1	8.8 ± 2.2	132.6 ± 45.9	0.22 ± 0.04
P 4	Njock Kon	35.9 ± 2.7	9.2 ± 1.9	134.0 ± 33.4	0.23 ± 0.01
P 5	3 Vert	21.5 ± 2.6	8.8 ± 0.8	71.0 ± 12.9	0.28 ± 0.02
P 6	Big Ebanga	14.4 ± 2.0	9.4 ± 0.9	36.2 ± 3.7	0.40 ± 0.03
P 7	Mimi Abue	22.5 ± 2.3	10.6 ± 1.1	67.8 ± 13.1	0.33 ± 0.05
P 8	Ukom	8.6 ± 3.1	7.6 ± 1.3	23.2 ± 8.4	0.37 ± 0.02
P 9	Obino L'Ewai	13.6 ± 1.6	6.4 ± 0.9	80.8 ± 18.8	0.16 ± 0.02
P 10	74.79	13.5 ± 2.9	6.4 ± 0.5	75.0 ± 14.8	0.18 ± 0.02
P 11	Egjoga	16.8 ± 4.1	6.8 ± 0.8	82.4 ± 16.8	0.20 ± 0.03
P 12	Obubit Ntanga 1	10.1 ± 2.9	6.2 ± 0.4	70.4 ± 15.6	0.15 ± 0.04
P 13	Akpakpak	11.4 ± 1.1	6.0 ± 0.7	69.2 ± 12.9	0.15 ± 0.01
P 14	Bobby Tannap	15.9 ± 3.0	7.0 ± 0.0	93.4 ± 9.3	0.17 ± 0.04
P 15	Mbang Okon	14.6 ± 1.2	7.8 ± 0.8	69.2 ± 5.0	0.21 ± 0.02
P 16	Agbagba	11.2 ± 2.3	7.4 ± 0.5	31.8 ± 4.3	0.35 ± 0.04
P 17	Ngok Egome	8.2 ± 1.5	8.0 ± 0.0	31.2 ± 2.9	0.26 ± 0.03
P 18	Obubit Ukom	9.5 ± 1.6	7.6 ± 0.5	31.4 ± 3.6	0.30 ± 0.05
P 19	Orishele	12.8 ± 2.7	8.8 ± 0.8	48.0 ± 11.5	0.27 ± 0.04
P 20	Kiogo	7.9 ± 1.5	5.4 ± 1.3	15.2 ± 4.1	0.46 ± 0.07
P 21	Ihitisim	13.2 ± 2.8	4.2 ± 0.4	45.2 ± 4.7	0.29 ± 0.04
P 22	Asamiensa	9.0 ± 3.1	1.8 ± 0.4	18.2 ± 4.9	0.52 ± 0.07
P 23	Ubok Iba	10.0 ± 2.2	1.6 ± 0.5	23.0 ± 8.3	0.45 ± 0.08
P 24	Osoaboaso	10.8 ± 1.7	7.3 ± 0.5	41.5 ± 8.1	0.23 ± 0.02
P 25	Kaamenko	9.4 ± 1.8	6.6 ± 0.5	30.0 ± 3.2	0.31 ± 0.03

\* Fruit bearing hands only.

have fewer hands and fingers (compare P<sub>16</sub>-P<sub>20</sub> with P<sub>21</sub>-P<sub>23</sub>) (Table 2). However fruit weight of Horn plantain cultivars is much greater than that of False Horn plantain cultivars (except P<sub>20</sub>).

If the different Horn plantain cultivars with one hand (lacking in our field genebank) had been included in our sample, it would have become clear that the average bunch weights of Horn plantain cultivars in general are less than of the False Horn plantain cultivars.

The relative decrease or increase in number of flowers per hand shows some distinct difference between French plantain, French Horn, False Horn and Horn plantain cultivars (Fig. 1). In the French plantain cultivar 'Obino L' Ewai,' the relative number of flowers increases in the second and third hand as compared with the first hand. Then it decreases sharply till the fifth hand followed by a new increase till the tenth hand. Thereafter there is a steady decrease in the number of flowers per hand. In the French Horn, False Horn and Horn plantain cultivars 'Mbang Okon,' 'Agbagba' and 'Ihitisim,' respectively, the relative number of flowers per hand decreases sharply from the first and second hand onwards. Similar figures can be drawn for other cultivars with the same bunch-type.



**Fig. 1.** Relative number of flowers/hand for four cultivars.

### Plant Size and Related Characters

A second striking difference among plantain cultivars is the size of the main pseudostem at flowering. Although absolute figures have little meaning generally, because the plant size is influenced by soil fertility and plant density, the relative differences in field genebanks are invaluable.

Tall plants have a larger girth at flowering than shorter plants (Table 3) (except P<sub>4</sub>) and need more time to flower (compare P<sub>1</sub>-P<sub>8</sub> with P<sub>9</sub>-P<sub>23</sub> and with P<sub>24</sub>-P<sub>25</sub>). These tall plantain cultivars (P<sub>1</sub>-P<sub>3</sub> and P<sub>5</sub>-P<sub>8</sub>) are the 'giant' plantains. Because the size and circumference are less, the plantain cultivars P<sub>9</sub>-P<sub>23</sub> and P<sub>24</sub>-P<sub>25</sub> are considered 'medium' and 'small' plantains respectively.

Giant plantains flower later than medium plantains and medium plantains flower later than small plantains (Table 3). This becomes apparent if one looks especially at those cultivars which are morphologically very similar (compare P<sub>6</sub> with P<sub>16</sub> and P<sub>25</sub>; compare P<sub>5</sub> with P<sub>15</sub> and P<sub>24</sub>).

The plantain cultivars belonging to the giant and medium size categories are taller but flower earlier than the ones observed in Yangambi (De Langhe 1964a). This is almost certainly due to the different ecological conditions, despite the fact that some of the cultivars under observation were different from ours.

Giant plantain cultivars produce more foliage leaves than do medium plantain cultivars but any differences in the number of foliage leaves produced by medium plantain cultivars and small plantain

cultivars is less clear, probably due to the low number of plants under observation. The medium Horn plantain cultivars tend to produce more foliage leaves than do other medium plantain cultivars with another bunch-type (Table 3). The number of foliage leaves recorded for each plant size category agrees well with the ones from Yangambi (De Langhe 1964a). This suggests that despite different ecological conditions the number of foliage leaves produced till flowering remains an important character for the identification of a plant category.

Giant plantain cultivars produced heavier bunches with more fruit-bearing hands and more fruits than medium and small plantain cultivars (Table 2) (compare P<sub>1</sub>-P<sub>3</sub> with P<sub>6</sub>-P<sub>14</sub>; compare P<sub>6</sub>-P<sub>7</sub> with P<sub>16</sub>-P<sub>20</sub>; compare P<sub>5</sub> with P<sub>15</sub> and P<sub>24</sub>). The fruit weight, however, seems to depend not on the plant size category but on the bunch type (Table 2).

The leaf ratio of the medium plantain cultivars does not differ among cultivars with different bunch types. The picture is not clear among the giant plantain cultivars.

The French plantain cultivar 'Njock Kon' (P<sub>4</sub>) needs comment. It is a cultivar with characters such as circumference, time to flowering, leaf number,

**Table 3.** Some vegetative characters of 25 cultivars representative of the major variability in the plantain subgroup in West Africa.

		Vegetative characters				
Cultivars		Height at flowering (cm)	Circumference at 50 cm at flowering (cm)	Time to flowering (days)	Leaf number	Leaf ratio
P 1	Ntanga 2	424.0 ± 21.6	90.6 ± 2.7	397.2 ± 33.5	45.8 ± 1.3	—
P 2	Ntanga 5	467.5 ± 26.6	94.3 ± 10.0	429.0 ± 73.2	42.5 ± 6.5	—
P 3	Ovang	430.8 ± 33.5	87.5 ± 4.7	340.0 ± 41.3	40.8 ± 2.8	3.24 ± 0.01
P 4	Njock Kon	306.0 ± 28.2	87.8 ± 6.3	361.0 ± 46.7	41.8 ± 4.1	2.08 ± 0.12
P 5	3 Vert	371.0 ± 24.8	78.8 ± 6.5	338.2 ± 74.3	40.8 ± 3.5	2.81 ± 0.11
P 6	Big Ebanga	398.0 ± 11.0	76.4 ± 0.9	324.2 ± 29.3	39.4 ± 3.5	2.82 ± 0.14
P 7	Mimi Abue	417.0 ± 24.9	83.4 ± 3.1	350.2 ± 87.2	37.6 ± 2.3	2.74 ± 0.05
P 8	Ukom	390.0 ± 22.4	77.4 ± 4.5	—	40.5 ± 1.3	3.16 ± 0.22
P 9	Obino L'Ewai	334.0 ± 18.5	63.2 ± 5.8	253.6 ± 31.0	32.6 ± 0.5	2.77 ± 0.17
P 10	74.79	326.0 ± 20.4	61.6 ± 6.2	237.4 ± 15.8	31.5 ± 0.6	2.68 ± 0.21
P 11	Egjoga	322.6 ± 13.2	64.6 ± 4.9	252.4 ± 54.2	31.6 ± 1.5	2.55 ± 0.15
P 12	Obubit Ntanga 1	329.0 ± 29.0	60.4 ± 4.0	256.0 ± 29.7	30.6 ± 3.7	2.87 ± 0.18
P 13	Akpakpak	318.4 ± 19.9	60.6 ± 5.9	235.4 ± 10.6	30.4 ± 1.6	2.69 ± 0.21
P 14	Bobby Tannap	302.8 ± 11.6	63.2 ± 1.5	226.8 ± 14.0	31.6 ± 0.9	2.54 ± 0.22
P 15	Mbang Okon	351.0 ± 9.6	65.2 ± 1.9	235.6 ± 17.7	32.6 ± 1.3	2.73 ± 0.11
P 16	Agbagba	330.0 ± 16.2	62.0 ± 3.5	251.6 ± 25.8	32.8 ± 2.0	2.70 ± 0.16
P 17	Ngok Egome	332.0 ± 20.2	61.4 ± 3.0	258.4 ± 22.7	31.4 ± 1.1	2.79 ± 0.19
P 18	Obubit Ukom	342.0 ± 9.7	64.0 ± 1.2	238.4 ± 9.3	31.5 ± 1.3	2.71 ± 1.58
P 19	Orishele	349.0 ± 14.7	63.4 ± 5.1	274.4 ± 51.7	32.0 ± 3.2	2.66 ± 0.19
P 20	Kiogo	343.0 ± 8.4	60.4 ± 1.7	269.4 ± 13.0	33.2 ± 2.2	2.77 ± 0.12
P 21	Ihitisim	349.2 ± 10.7	67.2 ± 2.9	283.4 ± 17.4	34.2 ± 2.0	2.76 ± 0.07
P 22	Asamiensa	328.6 ± 12.3	69.2 ± 5.0	291.8 ± 36.2	36.4 ± 3.0	2.66 ± 0.11
P 23	Ubok Iba	378.0 ± 7.6	83.2 ± 3.0	270.8 ± 18.3	34.3 ± 1.0	2.81 ± 0.10
P 24	Osoaboaso	301.3 ± 8.5	59.5 ± 2.5	238.3 ± 22.1	32.8 ± 1.5	2.76 ± 0.08
P 25	Kaamenko	305.4 ± 22.7	58.4 ± 4.2	228.2 ± 6.8	29.2 ± 2.5	2.49 ± 0.18

bunch weight, number of fruit-bearing hands and fingers which should place it among the giant plantain cultivars (Table 2 and 3). Its pseudostem size, however, is small. Its false internodes are very reduced since the same number of foliage leaves are produced. Hence it is a dwarf mutant of a giant French plantain. This dwarf mutant has a low leaf ratio, i.e. the leaves are relatively wide. De Langhe has already pointed out that dwarfism is correlated with a lower leaf ratio (De Langhe 1964a).

Within-variety variation of the length of the ratoon was so high that no picture emerged in relation to plant size category or bunch type.

### **Bunch Orientation**

Generally speaking the French plantain cultivars have pendant bunches (P<sub>1</sub>-P<sub>4</sub>, P<sub>9</sub>-P<sub>13</sub>) and the French Horn, False Horn and Horn plantain cultivars have subhorizontal bunches (P<sub>5</sub>-P<sub>8</sub>, P<sub>15</sub>-P<sub>25</sub>). A French plantain cultivar with subhorizontal bunch is Bobby Tannap (P<sub>14</sub>).

### **Fruit Orientation**

Among the 25 cultivars considered P<sub>1</sub>-P<sub>4</sub> manifest erect fruits, P<sub>5</sub>-P<sub>19</sub> and P<sub>21</sub>-P<sub>25</sub> manifest subhorizontal fruits and P<sub>20</sub> manifest pendant fruits.

Subhorizontal fingers more or less point either randomly away from the inflorescence axis (P<sub>5</sub>-P<sub>18</sub>, P<sub>20</sub>, P<sub>21</sub>, P<sub>24</sub>, P<sub>25</sub>) or they all point towards the illuminated side (P<sub>19</sub>, P<sub>22</sub>, P<sub>23</sub>).

### **Fruit Apex**

Among the 25 cultivars considered P<sub>11</sub>, P<sub>13</sub> and P<sub>17</sub> have a blunt apex; P<sub>1</sub>, P<sub>2</sub>, P<sub>4</sub>, P<sub>6</sub>-P<sub>10</sub>, P<sub>12</sub>, P<sub>14</sub>-P<sub>16</sub>, P<sub>18</sub>, P<sub>19</sub>, P<sub>21</sub>, P<sub>24</sub>, P<sub>25</sub> have a straight apex, and P<sub>3</sub>, P<sub>5</sub>, P<sub>20</sub>, P<sub>22</sub> and P<sub>23</sub> have a salient apex.

### **Fruit Shape**

All cultivars but two have curved fingers. P<sub>2</sub> and P<sub>10</sub> have S-shaped fingers.

## **Recommendations**

### **Determination of the Potential for Perennial Production**

Giant plantain cultivars produce heavier bunches than medium plantain cultivars (Table 2). However, the maturation time for the planted crop and following ratoon crops is very important. It is the yield per unit time which determines the perennial production potential. Therefore the perennial production potential for giant plantain cultivars which need more time to flower (Table 3), and hence to be harvested, may be the same as for medium plantain cultivars. The same could apply to small plantain cultivars.

Yield and dry-matter distribution (mother pseudostem vs. bunch vs. suckers) seems to depend on the bunch type (Table 2; Stover, in press). This could influence the type of ratooning, hence the capacity for perennial cultivation. Therefore there is a need to determine the perennial production potential of plantain cultivars belonging to the different bunch-type categories.

The aforementioned considerations can be combined in one experiment which should last at least 5 years. This would give allowance for the harvest of three crops of the giant plantain cultivars. Results would make it clear which plant size and bunch-type category is associated with high yield potential. The plantain cultivars needed for this experiment are all available in Onne and can be multiplied rapidly there by the *in vitro* technique. Hence the experiment should be conducted in Onne.

### **Screening for Female Fertility**

There are reports which indicate that some plantain cultivars are female-fertile. At Yangambi, eight female fertile plantain cultivars were identified. They produced about one seed per bunch after cross pollination (INEAC 1959). These cultivars were all of the French bunch type (INEAC 1985, De Langhe 1961b). The female fertile cultivar 'AVP-67,' also called 'Elat' or 'Terrinha' is also of the French-bunch type (Rowe 1986; Tezenas du Montcel 1985).

Hence some plantain cultivars could possibly be used as a triploid parent instead of the AAB Laknau with its inferior cooking qualities.

In Onne we have 18 French plantain cultivars. The *in vitro* facilities enable us to introduce more cultivars which can be screened for female fertility. Alternatively the French plantain cultivars from the Onne field genebank can be forwarded to other institutions screening plantain for female fertility.

### **Evaluation of Existing Plantain Germplasm**

Four plantain field genebanks exist in Africa, namely in Onne (Nigeria), Ekona and Nyombe (Cameroon) and Azaguie (Ivory Coast). The N'Toum field genebank (Gabon) was abandoned this year due to lack of funds. However, it was transferred to Cameroon. The Onne and Nyombe field genebanks are the largest plantain field genebanks in Africa but none can be considered as a reference field genebank since both lack many cultivars. The exchange of germplasm has just started. All these cultivars should be clearly identified and receive a common name. They should be evaluated systematically over three cycles and their characters (vegetative and inflorescence traits, disease resistance, etc.) should be measured and recorded. The data collected should serve as a base for exchange and for training.

## Germplasm Collection in Zaire and Congo

Among the 26 plantain cultivars with a short habit (either small, semi-dwarf or dwarf), 14 are French plantain cultivars, five are False Horn, and two are Horn plantain cultivars. Eighteen cultivars come from Zaire (De Langhe 1964a), four from Cameroon (Tezenas du Montcel 1983), two from Nigeria and two from Ghana (personal observation). The Zairean cultivars, of which 13 are French and five are False Horn-type, are not readily available because the Yangambi field genebank has been discontinued. They should be collected again so that the French plantain cultivars can be screened for their female fertility.

As the Congo has never been systematically explored, a plantain collection expedition should be conducted to conserve valuable but unknown germplasm.

## Root Ramification of Different Plantain Cultivars

Root branching of plantain cultivars is very much inferior to root branching of AA and AAA bananas (Swennen 1984; Swennen et al. 1986). The poor root branching is believed to be one of the causes of the rapid yield decline in plantain (Swennen 1984). Therefore a study should be undertaken to find out if genotypic differences in root branching exist. Plantain cultivars with superior branching should be tested for perennial cultivation.

Small in vitro plants grown in hydroponics will greatly facilitate this research which should not take more than 2 years. It could be carried out in Onne after installing some equipment needed for hydroponic culture or at the Catholic University of Louvain (Belgium) where these facilities exist.

## Conclusion

Approximately 30 characters were studied on entries in the IITA plantain genebank located at Onne Substation. Most characters showed no variation between entries; however, dissimilarities were found in bunch-type (French, French Horn, False Horn and Horn) and plant size (giant, medium and small), bunch and fruit orientation, fruit apex shape and fruit curvature.

The bunch weight decreases and average fruit weight increases progressing from the French to the French Horn to the False Horn bunch-type. Horn plantain cultivars produce the heaviest fingers. Number of flowers per hand decreases slowly with increasing hand number in the French plantain cultivars. This decrease however is very sharp in the other three bunch-types.

Giant cultivars are taller and thicker, and flower much later than medium ones. They produce more foliage which results in heavier bunches consisting

of more hands and fingers than is the case with medium cultivars. Medium cultivars are superior to small cultivars for analogous reasons. Data for size at flowering and time to flowering for the giant and medium cultivars from the Onne field genebank differ from the data recorded in the Yangambi field genebank but the total number of leaves does correspond. This suggests that the latter character is independent of ecological conditions. A mutation towards a dwarf habit seems to lower the leaf length: width ratio.

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# Morphological Taxonomy of *Musa* in Eastern Africa

Kabonyi Sebasigari \*

In the late 1940s, Baker and Simmonds (1951, 1952) toured the anglophone countries of East Africa (Kenya, Uganda, Tanzania mainland and Zanzibar) to investigate the cultivated and wild bananas in the region.

Subsequent to that survey, 47 East African clones, together with four previous introductions (Shepherd 1957) were established at the Imperial College of Tropical Agriculture in Trinidad for study. Shepherd (1957) classified these 51 accessions according to their genomic constitution and divided the clones known from East Africa into two groups, namely Mukita and Lujugira. Baker and Simmonds (1951) and Shepherd (1957) have given estimates of 45 and 70, respectively, for the total number of banana clones grown in East Africa.

Champion (1965) studied banana cultivation in Rwanda and described some varieties grown in that country. De Langhe (1960) established a banana collection at Mulungu Research Station in eastern Zaïre. Later, in 1983, De Langhe and Sebasigari (De Langhe 1983b) visited Burundi, Rwanda and eastern Zaïre to study banana cultivation and develop a research program for these countries.

This paper gives a preliminary understanding of the variation and classification of banana cultivars grown in eastern Zaïre, in Rwanda and Burundi. Emphasis is put on the genomic constitution and general characteristics of clones within the four types of bananas: beer, boiling, flour (plantain) and sweet bananas as they are distinguished in these countries. This study is mainly based on the IRAZ germplasm collection but also includes information collected during two short visits to the Tanzania mainland and Zanzibar (Ngendahayo and Sebasigari 1984; De Langhe et al. 1985).

## Overview of Banana Cultivation

In East Africa bananas are produced in smallholdings of a few hectares which are characterised by a mixture of banana varieties, mixed intercropping with food crops, no mechanisation and no renewal of the banana plot (plantation) for at least 30 years, and even a century!

In this system the banana crop is the basis of the food production and is, at the same time, a food and a cash crop.

Bananas are a dominant feature of the landscape in Burundi, Rwanda and eastern Zaïre. The plantations of individual farmers join and form broad fields that keep their green colour throughout the year.

In this system practiced by the smallholders, all banana products (cider, bunches, fibers, etc.) are consumed and marketed locally and, as a consequence, the mass of money generated by this important crop only circulates at the village level without contributing to foreign exchange earnings.

Based on the use of the fruits, four groups of bananas are distinguished in East Africa: beer, boiling (or steaming in some areas), flour or roasting (i.e. plantain that can also be boiled) and sweet (eaten raw and ripe). These uses are flexible in some areas (Baker and Simmonds 1951) but in Eastern Zaïre, Rwanda and Burundi this is especially for beer-making where boiling and sweet bananas are often mixed with the true beer-types to make a traditional cider commonly called 'banana beer.'

Beer and boiling types are adapted to highland regions with high rainfall between 1200 and 1900 m above sea level. Some clones, however, perform well over 2000 m on the slopes of Mount Kilimanjaro in Tanzania. These high-altitude bananas are grown in eastern Zaïre, Rwanda, Burundi, Uganda, Kenya and the Tanzania mainland. They are a staple food in Burundi, Rwanda, Uganda and many areas of Tanzania, and for some ethnic groups of eastern Zaïre and Kenya.

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\* Head, Banana Research Program, Institut de Recherche Agronomique et Zootechnique (IRAZ) de la CEPGL, Box 91, Gitega, Burundi.

## Classification

The IRAZ collection on which this study is based was established in October 1984 on a site at 1520–1620 m elevation near Gitega, Burundi. It contains 155 entries which are being characterised in order to identify duplicates and classify the true clones. This germplasm collection is almost complete for eastern Zaïre, Rwanda and Burundi, and continues to be enriched.

Where possible, cultivars were collected from areas where they occurred most frequently and the names listed for the collection are those from the collecting areas. However, other synonyms given by the farmers have been recorded and some names match those recorded by Baker and Simmonds (1952) and Shepherd (1957).

## Alien Types

### Sweet Bananas

All farmers agree on the foreign origin of all the cultivars listed in Table 1 and they can even trace back the introduction dates of some of these cultivars.

**Table 1.** Cultivars in IRAZ banana collection whose fruits are eaten raw and ripe (sweet bananas) in Rwanda, Burundi and Zaïre.

Collection name	Name in literature	Genomic constitution
Kigurube	Kinguruwe Naine de Chine Dwarf Cavendish Dwarf Chinese	AAA*
Grande Naine	Grande Naine	AAA
Poyo	Poyo, Robusta	AAA
Americani	?	
Kigurube — Malaya	Lacatan	AAA
Gros Michel (Bogoya)	Gros Michel	AAA
Igisukari	Banane rouge Red banana Red Green Banana (a mutant of the red)	AAA
Ibotabota or km 5	—	AAA
Kamaramasenge	Ney poovan	AB*
Prata	Prata, Pomme Kijakazi	AAB
Unnamed from Burhale (Zaïre)	Paka ?	AA?

\* A stands for a haploid chromosome set of *Musa acuminata*, B for *Musa balbisiana*.

As shown in Table 1, these cultivars have different genomic constitutions (one AB, one AAB, seven AAAs, one unknown). Furthermore, they tend to be adapted to different environments.

There are apparently no more uncollected clones belonging to this category of bananas in East Africa.

The first four clones in Table 1 belong to the Cavendish group. Poyo and Americani seem to be the same clone since there is no apparent characteristic which can distinguish them. Both entries were taken from the national banana collection of Rwanda at the Institut des Sciences Agronomiques du Rwanda (ISAR) research station in Rubona.

Entry 10 has a pale-green slender pseudostem about 3 m tall pigmented with a few black blotches; leaf ratio 0.30; margins of leaf petiole slightly open like those of the Cavendish group. Bunches subpendulous; fruit apex obtuse; neuter flowers persistent. Ripe fruit remains green and has a sweet, pleasant, aromatic flavour. Bract scars as prominent as those in the Cavendish group. Male bud is yellow, male flowers have six white staminodes, compound and free tepals are white but the lobes of the compound tepal are yellow; stigma is orange. This clone may be the 'Paka' of Zanzibar described by Shepherd (1957).

### Alien Cultivars Used for Beer-Making

The other cultivars known by farmers as being of recent introduction are one AB and three ABBs (Table 2). They are mixed with local varieties to make banana beer.

**Table 2.** Banana cultivars in the IRAZ collection of alien origin which are used to make beer in Rwanda, Burundi and eastern Zaïre.

IRAZ collection name	Name in literature	Genomic constitution
Kisubi	Kisubi	AB
Kayinja	Pisang awak legor	ABB
Kisandugu	Pisang awak	ABB
Gipungusi or Sembe	Bluggoe	} ABB
Kivuvu	Silver Bluggoe	

The ABBs are mainly grown in dry areas where highland bananas do not perform well or do not grow at all. One of them, Kayinja (so-called in Uganda but has many other names in Zaïre, Rwanda and Burundi) is very popular. Kisubi has a pale-green slender pseudostem about 2.80 m tall covered with a few brown blotches and about 0.39 m of circumference at 1 m from ground; leaf ratio 0.32; petiolar canal is closed; bunch subhorizontal; fruits strongly constricted (bottle-

necked); bract scars are weakly prominent. Fruit does not ripen completely and is unpleasant to eat. Outer face of bracts are dark violet and waxy, inner face is uniformly crimson. Male flowers have five creamy white staminodes; Compound tepal is deep pink inside but its lobes are yellow. Free tepals are white and slightly tinted with pink inside. Stigma is orange.

Kayinja and Kisubi were introduced to Rwanda, Burundi and eastern Zaïre from Uganda between 1940 and 1960. Kayinja is called Kisubi in some parts of the region and Bukoba in Tanzania although it is unnamed along the Dar Es Salaam-Arusha road from Kibaha to Arusha. In the Rwandan national collection, it is called 'Ducasse hybride' and Champion (1965) refers to it as 'a banana of Indian origin but not a Bluggoe.' Kisubi is a very infrequent cultivar and Shepherd (1957) noted that it was a recent introduction to Uganda.

### Flour Bananas or Plantains

This category of bananas is a subgroup of the AAB group (De Langhe 1961).

During the collecting missions in Burundi, Rwanda and eastern Zaïre, clones of plantain grown in highland areas were collected together with the other banana groups.

The IRAZ collection contains 28 entries which include some French and Horn types and one False Horn. Although many of them are duplicates it seems that, at the present stage of the germplasm study, there are nine different clones of which six come from Walungu, an administrative zone close to the eastern edge of the Zaïre River basin in Zaïre, and the other three clones have been grown together with high-altitude bananas in the three countries (Zaïre, Rwanda and Burundi) for a long time.

The Zaïre River basin, in the heart of Central Africa, has an altitude of about 500 m and contains most of the clones of plantain grown in this part of Africa. These bananas share the same ecology with *Coffea robusta*, but farmers do not intercrop bananas and coffee. Plantain production is mainly a backyard activity. De Langhe (1961) described in detail 56 clones grown in the Zaïre River basin. IRAZ is planning to establish a working collection of plantains in that area.

### Beer and Boiling Bananas

The two types can be distinguished as follows: when the green banana is peeled, the pulp of the beer banana produces sticky, brownish excretions all along its surface. None are seen on the pulp of the boiling banana; when the green fruits are peeled and the pulp eaten raw, the boiling bananas are rather insipid, whereas the beer bananas taste bitter. Chewing styles of male flowers gives the same information; when boiled the pulp of the boiling

banana has a salty taste, whereas the beer bananas stay bitter.

Nevertheless, it is worth noting that the pulp of the Cavendish fruit produces very few and very scattered excretions. Also when chewed, the pulp or the style of the male flower tends to have a less sour taste than that of the beer banana. However, Gros Michel and Poyo (Cavendish) seem to be as sour as beer bananas.

In Rwanda and Burundi, boiling bananas are called Ibinyamunyu and Ibinyamushanga, respectively, which means with salt or salty. The beer bananas are called Ibikashi, in Rwanda, meaning bitter of beer, or Mazizi in Burundi, which refers to the bitter and sticky sap in the pulp.

Some of the major characteristics that beer and boiling bananas share are listed below: (1) Pseudostems are more or less upright but one cultivar, Mushayija Naranda (name coming from Bukoba in Tanzania), is somewhat stoloniferous; (2) Pseudostems, bunches and male buds are variable in sizes, pseudostem height 2.3–3.6 m, ratios of male bracts 0.24–0.34, leaf ratio 0.3–0.5; (3) Pseudostems and the base of the petioles are slightly or heavily pigmented with brown or black blotches. According to Champion (1965) this feature is sufficient to assign them to the *acuminata* group; (4) Petiole canals are wide and the margins of the petiole bases are spreading and turned outwards; (5) Leaf laminae are green but in one cultivar, Nailoni (Rwanda) and Ntukula (in Maruku collection in Bukoba, Tanzania), they are uniformly green-red; (6) The axis is hairy; (7) Fingers are green before ripening, but in one cultivar Nshule (in Maruku Collection in Bukoba, Tanzania) about one-quarter of the fingers have a light yellow colour (these are chimera) but are otherwise normal; (8) Fingers are biserially arranged on the cushion, but in one cultivar Mujuba (Burundi, Rwanda and Zaïre) and Muvuba (Uganda), cushions bear uniseriate flower clusters (or hands). In another cultivar, Inzinga (Rwanda) or Inzingwa (Burundi), all flower clusters are grouped into a single spiraloid hand; (9) Fruits are variable in size, generally fat with a short and strong pedicel; (10) By maturity, the cross-section of the fruit is somewhat angular or smoothly rounded; (11) Fruit apices are blunt, somewhat constricted (bottle-necked) or strongly constricted; (12) When naturally ripe, the fruit apices of bottle-necked fruits remain green; (13) By physiological maturity almost all male bracts have fallen in some varieties whereas the buds remain quite big in other varieties; (14) In most varieties, the tips of the male bud bracts are completely overlapping and the bract apices are acute. There are varieties with weak to strongly imbricate male bud bracts with obtuse apices in which the male bracts and neuter flowers are always persistent;

(15) Bracts of the male bud are curled (involute) after opening and then lift free of the bud; (16) Bract scars are strongly prominent compared with those of ABBs; (17) Male flowers are white, petals transparent and the anthers are pigmented with a dark pink colour (Table 3); (18) The ovary has three loculi and in each loculus, ovules are two-rowed.

Some of the foregoing characteristics (e.g. 3, 4, 6, 15, 16) readily assign these two types of bananas to the *acuminata* group whereas other characteristics discriminate between clones.

Champion (1970) suggests that a very ancient introduction is probably the parent of these cultivars which have developed into different varieties through successive mutations which have occurred on the high plateaus. Shepherd (1957) and De Langhe (1983a) indicated that these bananas (i.e. beer and boiling types) are only known to be from the East African region.

At the present stage of the germplasm study at IRAZ and having visited some banana-growing areas in Tanzania, I agree with Shepherd (1957) that the number of true banana varieties known to be

from East Africa may reach 70 including those endemic to the Island of Zanzibar.

However, in order to continue this study and devise a determination key for East African banana clones, all presumed different clones should be collected from Uganda, Kenya and Tanzania (mainland and Zanzibar).

Furthermore, even though some of the same indigenous varieties are found in all these East African countries they often bear different names. As far as names are concerned, one can probably trace back the history of banana cultivation or varietal establishment in East Africa if one studies the different local names in different languages from the coast to inland (e.g. Kinyarwanda, Kirundi, Luganda, Kihaya, Kichaga).

### Prospects for the Future

To study and completely classify banana clones in East Africa the following perspectives for the future are offered:

(1) Collections will continue in Zaïre, Rwanda and

**Table 3.** Comparison of male flower colour of different types of bananas.

Clones	Flower parts						
	Tepals			Staminodes		Pistil	
	Compound tepal	Lobes of compound tepal	Free tepal	Filament	Anthers	Style	Stigma
Kayinja (ABB)	deep pink	orange	white with pink tint	creamy white	yellow	white	yellow
Bluggoe (ABB)	deep pink	orange	pink	pink	yellow	white	creamy white
Prata (AAB)	slightly pink	yellow	white	creamy white	white with pink tint	white	yellow
Kisubi (AB)	deep pink	yellow	white with pink tint	white	creamy white	white with pink tint	orange
Kamaramasenge (AB)	slightly pink	yellow	white	white	white	white	orange
Unnamed from Burhale (Paka?) (AA?)	white	yellow	white	white	creamy white	white	orange
Gros Michel (AAA)	white	yellow	white	white	creamy white	white	yellow
<b>Groups</b>							
Cavendish series (AAA)	white	yellow	creamy white	creamy white	orange	white	orange
Plantains (AAB)	slightly pink or yellow	orange	creamy white or yellow	creamy white	creamy white or yellow	white	yellow
Beer and boiling (AAA — East Africa)	white	yellow	white	white	pink	white	orange



Burundi for high-altitude bananas. The INIBAP mission in East Africa (De Langhe et al. 1985) recommended the extension of the IRAZ germplasm collection to include material from other East African countries. IRAZ is also planning to establish a working collection of plantain at the Kondo or Yangambi stations of the Institut National pour l'Etude et la Recherche Agronomique (Zaire) (INERA) in Zaire and would like to seek the help of the West African Regional Corporation for Research on Plantains (WARCORP) and the International Network for the Improvement of Bananas and Plantains (INIBAP) for this.

- (2) IRAZ would like to request also the assistance of INIBAP to train a scientist and equip a laboratory for the chemical characterisation (e.g. electrophoresis) and chromosome counting of banana clones.
- (3) IRAZ will carry out varietal trials for environmental adaptation and field screening for resistance to banana weevil, Panama disease, bunchy-top and cigar disease. These pests and diseases are responsible for high yield losses in Rwanda, Burundi, Zaire and other East African countries.
- (4) IRAZ also intends to test different clones for male fertility and female receptivity in order to advise banana breeders on this issue. The help of INIBAP is also needed in this area of research.

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# Banana and Plantain Germplasm Conservation and Movement and Needs for Research

J.T. Williams \*

DUE to numerous recent developments the scenario for genetic conservation for *Musa* has changed greatly in the past 5 years. This change results, in the main, from action taken by the International Board for Plant Genetic Resources through its support both for research and also for practical field work. This work is summarised in this paper and also priority research required is stressed so that a scientifically sound, truly international network will emerge to ensure genetic conservation, ready availability and movement of *Musa* germplasm.

## Historical Perspective

In the past, improvement of *Musa* has been based on a limited number of germplasm collections. None of these collections were comprehensive nor encompassed representative variability. This was because the collections were built up in an *ad hoc* manner to fit the then-current interests of the scientists using them. At the same time it should be recalled that our knowledge of the genomic relationships of *Musa* only goes back a few decades, and long before that colonial powers were attempting to develop banana export trades, but particularly only over the past 100 years. Such trades were based on few clones adapted to the newer cultivation techniques being introduced; breeding only began in the West Indies in the 1920s but attempts to produce acceptable new varieties are still underway. Prior to the colonial era, banana had spread throughout the tropics in relation to the movements of early human cultures.

The extant collections thus contain materials which have been used in these improvement efforts of the past 60-odd years. They have provided backup to plant introduction efforts rather than forming the basis for gene transfers so typical of breeding in the major staple crops. Some

collections, of course, include synthetic diploids, stemming from the early breeding efforts in Trinidad, but all breeding efforts have relied on a very narrow genetic base (Simmonds 1979).

When the IBPGR was created in 1974, it was in response to the widespread concern that genetic variability was being lost as new high-yielding varieties of crops were being bred and diffused rapidly over large areas. Hence its early efforts related to crops such as cereals and food legumes. Far less concern was expressed about fruits, vegetables and forages, but as agricultural practices were rapidly modernised in the developing world these also came within IBPGR's program.

In parallel IBPGR inherited, by default, the need to do something about the older historical collections of cash crops which no longer attracted funding from the donor community.

Since IBPGR was charged with developing a global network of genetic resources activities — from collecting to conservation, documentation and use — its strategy was to cull scientific opinion on a crop-by-crop basis and then to develop action programs in collaboration with existing international or national programs and to initiate such programs where needed (Williams 1984).

In the case of *Musa* the IBPGR convened an international Working Group in July 1977, which met under the chairmanship of Professor N.W. Simmonds (IBPGR 1978). A second Working Group met in October 1982 to review progress (IBPGR 1982). In parallel, conservation methodology had received enhanced attention by IBPGR following a review of recent developments in tissue culture (Withers 1980) and an international workshop in 1979 on the conservation of difficult material organised by the International Union of Biological Sciences, the International Genetics Federation and IBPGR (Withers and Williams 1982).

At the regional level, a number of countries of Southeast Asia expressed concern over the genetic erosion of local *Musa* cultivars and, through an

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\* Director, International Board for Plant Genetic Resources, FAO, Via Delle Terme di Carcalla, Rome, Italy.

IBPGR regional cooperative program, started to salvage and conserve such materials.

I do not propose to summarise these activities here because they are all fully documented, but I will draw upon the experience gained in the discussion which follows.

### Complementary Methods for Genetic Conservation

The collections of material used by breeders are called 'working collections.' These are outside the scope of genetic conservation collections which comprise one or more of the following: *Base collections*: for long-term preservation and security. These are not used for distribution purposes; *Active collections*: for medium-term preservation for duplication of samples stored in base collections and also for supplying material to users. Work in the active collections includes regeneration and documentation.

There are various types of base and active collections depending on whether the plant materials are in the form of seed, which can be dried and stored at low temperatures, or in the form of clonal material. They form genebanks as follows: *Base seed genebank*: seeds dried and stored at sub-zero temperatures; *In vitro base genebank*: tissues cryopreserved at the temperature of liquid nitrogen; *Active seed genebank*: seeds dried and stored at or a little above 0°C; *In vitro active genebank*: tissues in conditions of slow growth; *(Active) field genebank*: collections of plants in plantations, orchards, 'clonal repositories,' etc.; *In situ genebank*: materials maintained in ecosystems as genetic reserves.

The principles and technologies for both base and active seed genebanks have been worked out and are now widely applied for most major annual seed crops. The conceptual framework and specifications for in vitro genebanks have only recently been determined for the following reasons: (i) most research on tissue culture has related to model systems rather than to being specifically directed to economic species which present problems for conventional genetic conservation; (ii) most of the research was directed to rapid propagation and culture associated with thermotherapy to free stocks from pathogens; (iii) research on slow growth and cryopreservation has been inappropriately directed; (iv) culture systems for tissues of particular species were often genotype-specific and unsuitable for use on material which was by definition genetically diverse; (v) basic scientific data on the genetic stability of tissue cultures was lacking; the recognition of somaclonal variation led to lack of confidence by many scientists in attempting to develop genetic conservation in vitro.

All these topics have been addressed by an IBPGR Advisory Committee on In Vitro Storage, chaired by Prof. E.A.L. De Langhe (IBPGR 1983, 1986).

The principles for use of field genebanks, often as backup to either seed or in vitro collections are not scientifically specialised; they involve careful maintenance and labelling of plants. The principles for the designation of special genetic reserves, in situ, however, have received less attention but have been discussed in detail (Frankel 1983; Frankel and Soule 1981; Ingram and Williams 1984). Better coordination between national planners, nature conservationists and germplasm users is now required.

In the case of *Musa*, genetic conservation may be achieved using all types of genebanks listed above; each should be complementary, but results of research point clearly to the need to move away from clonal collections (field genebanks) to seed storage and in vitro preservation.

### Seed Storage

Until recently it was thought that *Musa* species which produce seed could not be stored by the usual procedures of seed drying and conservation at low temperatures. IBPGR-funded research at the Universiti Pertanian Malaysia has shown that this is not the case and with the correct techniques seeds can be stored for long periods. This opens up a new perspective on the conservation of the wild *Musa* gene pool, and offers the possibility of cheap and effective conservation of diploid diversity as an alternative to the hazardous clonal method of field genebanks.

Since the wild gene pool covers vast areas of South, East and Southeast Asia, careful plans now need to be developed to sample this untapped gene pool and in some cases to combine the ex situ seed storage with in situ conservation. Primary attention should be paid to the *Eumusa* species in the region of domestication comprising the Malaysia Peninsula and adjacent areas as well as to Papua New Guinea and other areas of Southeast Asia where species of *Rhodochlamys*, *Australimusa* and *Callimusa* are to be found. Edible diploids which are seedless or seed sterile, of course, will still need to be conserved by methods other than seed storage.

### Tissue Culture

In the conceptual framework for in vitro conservation, adequate in vitro methods for propagation are necessary in order to multiply stocks through subculturing. In the past few years a number of studies have indicated that techniques for shoot-tip culture from dormant buds, corms, eyes etc. (the preferred method in relation to conservation) are now worked out in relation to explant preparation, culture medium and initiation

of proliferation when multiplication is required (Banerjee and De Langhe 1985; Berg and Bustamente 1974; Cronauer and Krikorian 1984; De Guzman et al. 1980; Dore Swamy et al. 1983; Jarret et al. 1985; Vuylsteke and De Langhe 1985). The techniques are successful with *Musa* cultivars and species and have been summarised for the IBPGR in a status report by Jarret (1986). Data are also maintained in an IBPGR data base on in vitro research freely available to all enquirers (Wheeler and Withers 1984).

There is reason to be optimistic that medium-term storage of germplasm in vitro is possible and further refinement of chemical and environmental variables will extend the storage interval between subculturings. Hence in vitro active genebanks can be established.

Work on cryopreservation of tissues has not progressed very far and much more strategic research is required to develop suitable protocols. Hence, in vitro base genebanks are not yet feasible.

The IBPGR has also developed a scheme for the initiation of in vitro genebanks in association with quarantine, disease indexing and with disease eradication procedures before the sample enters the in vitro culture system. It is essential for an institute setting up the in vitro genebank to pay particular attention to these matters to avoid threats to plant health in the introducing country. False assumptions of disease freedom have been made simply because material was in in vitro culture (IBPGR 1986).

No true in-vitro active genebanks exist and to rectify this, and to test the standards proposed, IBPGR has jointly with CIAT, Colombia, agreed to establish a model in vitro genebank using cassava. Major aspects which require attention are the optimum size of the genebanks, the ability to test for stability and to monitor it through subculturing cycles. The evidence points to the fact that large numbers of cultures and routine biochemical testing will be the norm. Up to nine staff may be required to maintain in vitro about 500 clones and planning for in vitro genebanks must take into account the high investment in qualified manpower but relatively low costs in terms of equipment and consumable items.

Concern for stability of in vitro cultures led IBPGR to commission a status report (Scowcroft 1984) and to support research on variability in tissue culture-derived plants of *Musa* (O. Reuveni, unpublished data). Whereas on current knowledge, cryopreservation of embryos/meristems/shoot tips will provide security against genetic instability, it is certain that there will always be a degree of instability in tissue cultures under slow growth. IBPGR has commissioned special research to further understand the causes and types of instability so that they can be monitored, checked

as necessary and practicable conservation systems devised which take it into account.

### Field Genebanks

The IBPGR Working Groups had advised of the need for the existing collections to be viewed as complementing each other and that IBPGR should request the authorities holding the collections in the Philippines, Honduras, Jamaica and Ekona, Cameroon, to accept responsibility to maintain the materials as conservation collections. To date only the governments of the Philippines and Cameroon have agreed. At the same time IBPGR was asked to approach the Indian authorities to build up a well-described and classified collection in India but to date national policy is not clear.

Despite this situation IBPGR has provided ad hoc assistance to the Philippines and Jamaica to put their collections in order. In addition collections of cultivars have been made by IBPGR in Thailand, Malaysia and Indonesia and support given for their transfer to the regional collection in the Philippines. Also materials from the Papua New Guinea collection are being transferred to the Philippines while limited help has been given by IBPGR to collect in Burundi and Rwanda.

In total there are 42 collections of *Musa* in 33 countries but many of them must be viewed as working collections rather than field genebanks (IBPGR 1984).

Exchange of material between collections from widely separated geographical regions poses pathogen transfer hazards, although care has been taken where movement has taken place (e.g. in vitro movement is practised in accordance with the principles outlined above).

The size of the field genebanks will be necessarily finite. For instance it is estimated that the regional collection in Davao, Philippines, need not exceed about 250 cultivars and that at Ekona, Cameroon, about 100 (IBPGR 1982).

IBPGR has also issued standardised descriptors (IBPGR 1984a being a revision of a list produced in 1977) to be used in describing materials (IBPGR 1984); these are now in use in most field genebanks.

### Expanding the Genepool Available for Use

The IBPGR Working Groups stressed the need to make available and conserve *Musa acuminata* and to study the variability of *M. balbisiana*; further it foresaw that other wild species might prove to be useful in breeding and small collections of these would be desirable.

These recommendations were not acted on by the scientists involved with breeding and IBPGR had therefore agreed to take direct action, starting in Papua New Guinea in 1986. It has been necessary to postpone the start of this work for one year.

Two approaches are possible: (i) to engage in limited collecting based on known species and on intraspecific variation and assume that wild species will eventually be conserved in part, *in situ*; or (ii) to carry out research on the materials readily available to evaluate genetic diversity and then to return to the field to sample diverse populations with different ecogeographic origins.

Whereas isozyme analysis was first applied to *Musa* for cultivar identification (Bonner et al. 1974), Jarret and Litz (1986), have studied enzyme polymorphisms in numerous taxa of the genus using young leaf material derived from the La Lima collection. Differentiation between fertile *M. acuminata* subspecies can be shown by as few as six enzyme systems; differentiation within the AAA cultivars is more difficult and may reflect a narrower genetic base; and that within AAB and ABB groups is readily seen by isozyme analysis. These results were based on samples in an *ex situ* collection. Further study combined with field work is proposed by the IBPGR in order to follow the second alternative above. Newer molecular techniques such as RFLP or cpDNA analysis are potential tools for understanding more about the patterns of variation in the genus. Such understanding is an important requisite for ensuring that genebanks contain adequate representation of the wide range of variation.

### Task To Be Resolved

I have mentioned above the need to initiate *in vitro* active genebanks for *Musa* both for conservation and also for safer exchange of germplasm; IBPGR hopes that INIBAP also views this as a priority task. Whereas INIBAP is likely to lay more emphasis on working collections these are outside the scope of IBPGR's work, but cooperative action on the former could only be beneficial. We should also recall that enhanced *in vitro* research interfaces closely with newer biotechnological work; such work would be of great interest in breeding materials such as banana.

A major constraint in the movement of materials which are normally clonally propagated related to the slow development of indexing procedures for disease so that improved strategies may be developed to obtain and move vegetative germplasm free of known pests especially viruses. As a result, IBPGR has convened a specialist working group which will discuss this topic in 1987. In particular, attention will be paid to guidelines or strategies for facilitating the movement of vegetative germplasm through quarantine without compromising the quarantine process and to identify areas which need research support.

IBPGR is likely to consider modest support to

disease indexing of banana to help the establishment of *in vitro* genebanks and it has already made preliminary contacts with the Queensland Department of Primary Industries to this end.

IBPGR functions largely as a catalyst, in initiating and promoting action. It hopes to see the results of its enterprise recognised and adopted by other organisations. It expects to continue in its active rôle of identifying gaps in our scientific knowledge and of stimulating research to fill more gaps.

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# Biochemical/Genetic Markers and Their Uses in the Genus *Musa*

Robert L. Jarret \*

GENETIC studies in the genus *Musa* have lagged behind those conducted with far less important crops. The paucity of available data can be attributed to a number of factors which have hindered such studies. These include: the sterile nature of the triploid clones, the inaccessibility and lack of interest in the fertile diploids, the size of the land area required for the collection of segregation data, and perhaps due to a general belief that the collection of genetic data on such a conspicuously sterile crop would be of little value. The study of Vakili (1965) demonstrates otherwise. Information on genetic mechanisms and on the organisation of the *Musa* genome will become increasingly important as crop improvement efforts, both conventional and biotechnological, accelerate as a result of the formation of INIBAP.

In view of the above, some effort has been made to develop a series of genetic (isozyme) markers for use with bananas and plantains. The many uses for genetic markers have been described elsewhere (Tanksley and Orton 1983) and will not be repeated here. The major emphasis of my research has been, and continues to be, the use of genetic markers to study the evolution, taxonomy and the extent of genetic diversity in the genus *Musa*. In order to accomplish these objectives a number of enzyme-coding loci have been identified and characterised using starch and polyacrylamide gel electrophoresis. Fertile diploid *acuminata* subspecies and *M. balbisiana* (Jarret and Litz 1986a), commercial cultivars of various ploidy levels (Jarret and Litz 1986b) and several species (Jarret, in preparation) have been examined (Table 1).

This paper reviews the present uses of isozymes as genetic markers in the genus *Musa* and suggests how they may be utilised in future investigations.

**Table 1.** *Musa* species and subspecies examined for enzyme polymorphisms.

Genus	Species	Subspecies
<i>Musa</i>	<i>acuminata</i>	<i>banksii</i>
"	"	<i>burmannica</i>
"	"	<i>errans</i>
"	"	<i>malaccensis</i>
"	"	<i>microcarpa</i>
"	"	<i>siamea</i>
"	"	<i>truncata</i>
"	"	<i>zebrina</i>
<i>Musa</i>	<i>balbisiana</i>	
"	<i>coccinea</i>	
"	<i>fehi</i>	
"	<i>luikiuensis</i>	
"	<i>ornata</i>	
"	<i>velutina</i>	
<i>Ensete</i>	<i>ventricosum</i>	

## Methods

The electrophoretic conditions for separation of isozymes from leaf extracts are detailed in Table 2. In reality, a wide array of buffers, running times and gel matrices have been evaluated and those listed in Table 2 represent the most useful. Resolution of the gene products at additional loci will probably require modification of these systems.

Leaf continues to be the tissue of choice in these studies. This is chiefly due to its ease of acquisition and preparation. Root tissue has also been used successfully for assays of alcohol dehydrogenase (ADH) and peroxidase (PRX) activity. An extraction buffer consisting of 0.05M Tris/HCl (pH 7.5), 5% (w/v) polyvinylpyrrolidone (PVPP) or polyvinylpyrrolidone (PVP-40), 3% (v/v) Triton X-100, 5% (v/v) glycerol and 10 mM dithiothreitol (DTT) is suitable for the extraction of a number of active enzymes. PVPP is generally removed from the extracts by centrifugation. The pH and composition of the extraction buffer has considerable effect on the subsequent resolution of

\* U.S. Department of Agriculture /ARS/SAA, Regional Plant Introduction Station, Experiment, Georgia, 30212, USA.

isozymes and modifications of it may be required in certain instances. Details of the extraction procedure have been described by Jarret and Litz (1986a).

A list of enzyme systems and characterised loci are presented in Table 3.

**Table 2.** Principal buffer systems used for separation of *Musa* isozymes.

Buffer	Gel matrix	Reference
Histidine/citrate pH 6.5	12% starch	Cardy et al. 1981
Tris/malic acid/ EDTA/MgCl <sub>2</sub> pH 7.4	12% starch	Selander et al. 1971
Tris/glycine pH 8.9 (electrode)	7.5% acrylamide	Jarret and Litz 1986
Tris/HCl pH 8.3 (gel)		

**Table 3.** Polymorphic enzyme-coding loci identified in *Musa*.

Enzyme	No. loci (est.)
Malate dehydrogenase (MHD)	4
Glutamate oxaloacetate transaminase (GOT)	4
Shikimate dehydrogenase (SKDH)	2
Phosphoglucosmutase (PGM)	3
Esterase (EST)	5
Peroxidase (PRX)	4
Catalase (CAT)	2
Tetrazolium oxidase (TO)	3
Phosphoglucose isomerase (PGI)	3
Malic enzyme (ME)	1
Acid phosphatase (ACP)	1
Leucine aminopeptidase (LAP)	1
6-Phosphogluconate dehydrogenase (6PGD)	3
Alcohol dehydrogenase (ADH)	2

## Results and Discussion

### Diploid *Musa acuminata* and *M. balbisiana*

The species *acuminata* apparently contains five or more subspecies. Few of these have been adequately characterised. Efforts to delineate individual subspecies have been thwarted as a result of inadequate plant collection over the species range and due to the extensive overlap in their geographical distributions (Simmonds 1962). Considerable interest has recently been shown on the possible involvement of individual subspecies in the evolution of the edible clones. Morphological markers have been utilised for this purpose

(Simmonds 1966). Apparent subspecies-specific alleles have been identified at several enzyme-coding loci (Jarret and Litz 1986a). However, a more extensive examination of the diploid germplasm is essential before these subspecies markers are utilised in taxonomic or evolutionary studies.

At the present time, little is known about the extent of genetic diversity within and between *acuminata* subspecies although Jarret (1986) has suggested that allelic diversity within the fertile *acuminata* diploids is considerably greater than that observed in the cultivated triploids. The same cannot be said for *M. balbisiana*. In this species, all isozyme alleles identified in the triploid AAB and ABB clones yet examined have been accounted for in a single *M. balbisiana* (BB) accession. More information is needed on the extent of genetic diversity within *balbisiana*.

Fertile diploid *acuminata*, *balbisiana*, and interspecific *acuminata* × *balbisiana* hybrids are ideal plant material for studying the hereditability of isozyme and other genetic/molecular markers. The hereditability of numerous isozyme markers in *Musa* has been determined in this manner and these data have been used to construct a rudimentary linkage map of the *M. acuminata* genome. At present, five linkage groups have been identified. Genetic linkage maps provide valuable information on the organisation and evolution of the nuclear genome and on the physical location of agriculturally important genes. With the appropriate crosses it is possible to map numerous biochemical, molecular, morphological and disease or insect-resistant genes simultaneously (Bernatzky and Tanksley 1986). Knowledge of the physical location of a disease-resistance gene can provide the basis for its subsequent isolation without the need to identify and isolate a specific gene product. The construction of a detailed linkage map of the *acuminata* and *balbisiana* genomes would do much towards improving the chances for the isolation and transfer of genes within this genus.

### The Cultivated Clones

An understanding of the evolution of today's cultivars could have a profound effect upon present and planned banana and plantain improvement and collection activities. Such knowledge, were it available, may indicate specific subspecies likely to contribute to these improvement efforts and may help to identify geographic areas deserving of more intensive collection.

A means of unambiguously identifying banana and plantain cultivars would be of great interest to germplasm curators, plant collectors and others who find themselves confronted with the need to differentiate between apparently identical clones possessing different vernacular names. Isozymes



have been used successfully for clonal identification in numerous crops. Jarret and Litz (1986b) screened an array of *Musa* germplasm for enzyme polymorphisms and were able to confirm the occurrence of duplicates in the University of Florida's *Musa* collection in Homestead. Differentiation between AAA cultivars was generally more difficult than differentiation between members within the AAB and ABB groups. In addition, it was not possible to differentiate between a parental clone and its sport(s) (i.e. 'Highgate' vs 'Gros Michel' or between the Cavendish cultivars). However, additional enzyme markers have been identified since this initial report (Jarret and Litz 1986b) and these should provide greater resolving power to these techniques.

The available evidence suggests that only a small portion of *acuminata* diversity evident in the diploids is present in the cultivated clones. This may reflect either a very localised origin for these clones or the breeding behaviour of individual subspecies. A rational basis for the improvement of today's cultivars depends upon an understanding of their evolution. This information may have a profound effect upon present and planned plant improvement and collection activities in that it may indicate specific subspecies or species likely to be important in future plant improvement activities or geographic areas deserving of more intensive collection.

Simmonds (1966) divided the ABB genome group into seven subgroups one of which includes the plantains. Isozyme markers suggest considerable diversity between these groups (Silk, Plantains, Pome, Pisang Rajah etc.). However the contribution that somatic mutations have made to intrasubgroup diversification remains unknown. An extensive investigation of all the clones within individual subgroups should enable an estimate to be made since somatic mutants would likely be indistinguishable from the parental clone based on isozyme analysis. Such a study may also indicate whether individual subgroups resulted from one, or more than one, ancestral hybridisation.

### *Musa* Species

The genus *Musa* contains an as yet undetermined number of species (approximately 30–40) and little information is available on most of these in the scientific literature. Of greatest interest to the plant breeders and crop improvement enthusiasts are the diploid *acuminata* subspecies and *M. balbisiana*. However, there is reason to believe that with the appropriate technology desirable genes from other species may be of use in future banana plantain improvement efforts. The establishment of a seed bank or clonal repository for these plant materials is to be strongly encouraged.

An examination of various *Musa* species (Table

1) revealed that all species examined, except *M. liukuensis*, are readily distinguishable from one another based on enzyme polymorphisms for any one of several enzyme systems. *Musa liukuensis* was included in this study since no reference to it could be found in the available literature. At the time of sampling, only leaf material from a small sword sucker was available. A brief description of its morphological characteristics, taken at the time of its collection in Okinawa, suggested it to be related to or identical to *M. balbisiana*. An examination of enzyme polymorphisms at eight enzyme-coding loci failed to reveal any differences between *M. balbisiana* and *M. liukuensis* further supporting the hypothesis that these two species are identical.

An examination of the banding patterns of several *M. banksii* introductions supports Simmonds' (1956) reclassification of this species to *M. acuminata*.

Additional areas for future investigation which might be explored using species-specific isozyme markers include: an examination of putative *balbisiana*-derived triploids (BBB), confirmation of the interspecific origins of the AAS, AAAT and the ABBT groups described by Shepherd and Ferreira (1984), early detection of successful *acuminata* × *balbisiana* (or other) species hybridisations at the seedling stage of development and detection of the progenitor species in the evolution of the Feh'i bananas.

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## Participants

- G. Behncken**, Department of Primary Industries, Horticulture Branch, Brisbane, Qld. Australia
- I.W. Buddenhagen**, Department of Agronomy, University of California, Davis, Calif., USA
- A. Clarke**, School of Botany, University of Melbourne, Parkville, Vic. Australia
- B. Cull**, Maroochy Horticultural Research Centre, Nambour, Qld. Australia
- J. Dale**, Department of Primary Industries, Plant Pathology Branch, Indooroopilly, Qld. Australia
- G. Daniels**, Department of Primary Industries, South Johnston, Qld. Australia
- J. Ddungu**, Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda
- E.F. De Langhe**, Director, International Network for Improvement of Banana and Plantain (INIBAP), Avenue du val Montferrand, B.P. 5035, 34032 Montpellier Cédex, France.
- M. Epp**, ARCO Plant Cell Research, 6550 Trinity Court, Dublin, California, USA\*
- R. Espino**, University of the Philippines, Los Baños, Philippines
- M. Fouré**, Institut de recherche agronomique, B. P. 832, Douala, Cameroon
- R.A. Fullerton**, Department of Scientific and Industrial Research, Plant Pathology Branch, Mount Albert, Auckland, N.Z.
- S.C. Hwang**, Director, Taiwan Banana Research, P.O. Box 18, Chiuju, Pingtung, Taiwan 901, Republic of China
- R. Jaramillo**, Apartado 4824-1000, San Jose, Costa Rica
- G.G. Johns**, New South Wales Department of Agriculture, Tropical Fruit Research Centre, P.O. Box 72, Alstonville, N.S.W., Australia
- D. Jones**, Department of Primary Industries, Plant Pathology Branch, Indooroopilly, Qld. Australia
- A. Krikorian**, Department of Biochemistry, State University of New York at Stony Brook, N.Y. USA
- R. MacIntyre**, Australian Centre for International Agricultural Research, G.P.O. Box 1571, Canberra ACT 2601, Australia
- H. Tezenas du Montcel**, Station de Neufchateau, Sainte Marie, 97130 Capesterre Belle Eau, Guadeloupe, F.W.I.
- P. O'Farrell**, Department of Primary Industries, South Johnston, Qld. Australia
- O. Pascua**, Bureau of Plant Industry Research Station, Davao, Philippines
- K. Pegg**, Department of Primary Industries, Plant Pathology Branch, Indooroopilly, Qld. Australia
- G.J. Persley**, Australian Centre for International Agricultural Research, G.P.O. Box 1571, Canberra ACT 2601, Australia
- A. Putter**, FAO, Plant Protection Service, via Delle Terme di Caracalla, Rome, Italy
- M. Ramsay**, Department of Primary Industries, Mareeba, Qld. Australia
- P.R. Rowe**, Fundacion Hondurena de Investigacion Agricola, Apartado Postal 2967, San Pedro Sula, Honduras
- K. Sebasigari**, IRAZ, P.O. Box 91, Gitega, Burundi
- B. Silayoi**, Horticulture Department, Kasetsart University, Bangkok, Thailand
- M. Smith**, Maroochy Horticultural Research Centre, Nambour, Qld., Australia.
- H. Stover**, C/o United Fruit Company, Tela Railroad Co., Apt. Postal 30, San Pedro Sula, Honduras

- R. Swennen**, International Institute for Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria
- D. Turner**, School of Agriculture, University of Western Australia, Nedlands, W.A., Australia
- N. Vakili**, Department of Plant Pathology, Iowa State University, Ames, Iowa, USA
- R.V. Valmayor**, Executive Director, Philippines Council for Agriculture Research and Resource Development, Los Baños, Philippines
- G. Watson**, Department of Primary Industries, Cairns, Qld. Australia
- J.T. Williams**, Director, International Board for Plant Genetic Resources, FAO, via Delle Terme di Caracalla, Rome, Italy
- G. Wilson**, International Institute for Tropical Agriculture, P.M.B., 5320, Ibadan, Nigeria
- A. Zamorra**, University of the Philippines, Los Baños, Philippines

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