WORKSHOP ON BANANA DISEASE IDENTIFICATION, MANAGEMENT AND PRODUCTION OF HEALTHY PLANTING MATERIAL

26th - 29th October 2004

conducted by the

Horticultural Crops Research and Development Institute,
Department of Agriculture and Banana Asia Pacific Network.

under the aegis of the

Sri Lanka Council for Agricultural Research Policy

Programme for
Workshop on Banana Disease Identification, Management and production of healthy planting materials.

26.10.2004 - Morning session

8.30 - 9.30	**Registration**
9.30 - 10. 30	**Session 1: Opening ceremony** Welcome address: by Dr. C. Kudagamage, Director/HORDI Opening remarks: by Prof. Gunasena, Director/CARP Address by -Dr. S. Weerasena, Director General of Agriculture Address by -Dr. Agustin A. Molina, Regional coordinator, INIBAP
10.30 – 11.00	**Tea**
11.00 – 12.00	Concerns & Problems of banana planting material production through tissue culture Dr. C. Kudagamage, Director / HORDI
12.00 – 13.00	**Lunch**

26.10.2004 - Afternoon session

13.00 – 14.00	Banana industry, global prospects, challenges and INIBAP programmes. Dr. A.A.Molina
14.00 – 15.00	IDM with virus indexing and the use of clean planting materials. Prof. Hong Ji Su. Virologist, Taiwan
15.00 – 15.30	**Tea**
15.30 – 16.30	Banana diseases recorded in Sri Lanka and Identification and management of banana Anthracnose and leaf diseases. Dr. I.J. De Zoysa, Deputy Director Research II /HORDI

27.10.2004 - Morning session

9.00 – 10.00	Enzyme-linked Immunosorbent assay as a diagnostic tool for identification of banana virus diseases and establishment & maintenance of national repository of virus free planting material Dr. Indra Ariyaratne
10.00 – 10.15	**Tea**
10.15 – 11.15	Diagnosis of banana virus diseases by polymerase chain reaction (PCR). Dr. Manel Dassanayake
11.15 – 12.15	Production systems using tissue culture planting materials. Dr. S.C. Hwang from Taiwan
12.15 – 13.00	**Lunch**

27.10.2004 - Afternoon session

13.00 – 14.00	Management of panama disease of banana Dr. R.G.A.S. Rajapaksa
14.00 – 16.30	**Practical demonstration** ELIZA with banana bunchy top *Banovirus* (BBTV) and cucumber mosaic *Cucumovirus* (CMV) Prof. Hong. Ji.Su. assisted by Dr. I. Ariyaratne

28.10.2004
Field tour
Mahaweli system H-Tissue-cultured banana cultivation.

29.10.2004 - Morning session

8.30 -9.30	Multiplication of disease-free banana plants through tissue culture and other methods. Mrs Darshani Premathilake
9.30 -10.30	Management of banana virus diseases. Prof. Hong Ji. Su
10.30 – 10.45	**Tea**
10.45 – 11.45	Cultural management of banana diseases Dr. S. Weerasinghe
11.45 – 12.45	**Lunch**

29.10.2004 - Afternoon session

12.45 - 16.00	**Practical demonstration** Identification of banana viruses by PCR Prof. Hong Ji. Su. Assisted by Dr. M. Dassanayake and Dr. I.Ariyaratne

Sustainable Banana Production Through the Use of Tissue-Cultured Seedlings in Taiwan

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ABSTRACT:

Banana production in Taiwan is seriously threatened by fusarial wilt. This paper describes such devastating disease has been controlled on thousands of small banana farms in Taiwan. A key technology is the production of disease-free seedlings by tissue culture (TC). The TC program, begun in 1983, originally started to produce plantlets free of fusarial wilt, it is now also used to ensure that TC seedlings are free of viruses. A total of 44 million plantlets have been produced for use by farmers for planting on over 22,000 ha so far, thus maintaining banana production for export. The advantages of TC seedlings are discussed, and also their disadvantages, such as the increased degree of susceptibility to CMV infection, herbicide damage, and occasional uprooting problem. This paper describes a series of cultural methods taken to overcome these problems, especially to protect disease-free seedlings from CMV reinfection in the field.

INTRODUCTION:

The oldest international banana trade in Asia began in the early 1900s in Taiwan, where bananas were produced for exporting to neighboring Japan. The banana industry, involving mostly small producers, expanded rapidly and reached peak production on over 50,000 ha in the mid-1960s, ranking Taiwan as one of the largest banana exporting countries in the world, second only to Ecuador. The industry began to decline in the early 1970s due to high production cost and foreign competition. During the last decade, only around 6,000 ha have remained in production. However, because bananas produced in Taiwan's subtropical climate are excellent in eating quality, they are still highly prized in the Japanese market.

Key words: Banana, fusarial wilt, tissue culture

Cavendish is the most common banana cultivar cultivated in Taiwan. While the ratoon cropping system is practiced in most banana producing countries, most bananas in Taiwan are replanted each year so that fruit production coincides with spring export season and to reduce the risk of typhoon damage during the summer and fall. In addition to the risk of wind damage, the major constrains for banana cultivation in Taiwan are high labor cost, chilling damage during the coldest winter months, and most important of all the outbreaks of fusarial wilt.

Fusarial wilt of banana, commonly known as Panama disease, is one of the most catastrophic plant diseases, destroying more than 40,000 ha of banana in Central and South America over a period of 50 years (Stover 1972). Fusarial wilt on the Cavendish cultivar in Taiwan, first noticed in 1967 in the main banana producing area of southern Taiwan, spread rapidly and reached to the epidemic proportions only in 10 years (Su *et al.* 1986). Since seed corms obtained from wilt-infested areas are the most important source of inoculum (Stover 1972, Su and Chuang 1977), it is necessary to obtain pathogen-free seedlings for planting to prevent spreading of the disease to wilt-free areas. A tissue culture (TC) program was, therefore, developed for mass propagation of pathogen-free banana plantlets for use by farmers (Hwang *et al.* 1984). The program, begun in 1983, originally started to produce pathogen-free seedlings of traditional Cavendish cultivars, it is now also used to produce seedlings of wilt-resistant varieties for planting on diseased farms. The resistant varieties were developed also based on TC technology (Hwang et al. 1994, Hwang and Ko 2004). The TC technology has proved to be very useful for improving banana production in Taiwan. This paper discusses the advantages and disadvantages of TC seedlings, TC seedling production and extension system, and cultivation of TC seedlings on farm.

ADVANTAGES AND DISADVANTAGES OF TC SEEDLINGS:

As of 2003, the TC program set up at the Taiwan Banana Research Institute (TBRI) has produced a total of 44 million plantlets for distribution to farmers. Compared to suckers, the conventional planting material, TC plantlets are not only disease-free, they also have other benefits. They are cheaper and easier to propagate and transport. They have a higher rate of survival in the field. They have uniform, vigorous growth, and give higher yields of better fruit quality (Hwang *et al.* 1984). The uniformity of growth makes it easier to control the time of flowering and harvesting to meet the market demand. Because TC plantlets are superior to suckers, in recent years, more and more farmers have begun using planting material of this kind to establish their fields.

However, TC plantlets also have disadvantages. Compared to suckers, they are more susceptible to CMV, and are more vulnerable to herbicide damage at early sage of growth. Occasionally, they have uprooting and heart rot problems (Hwang and Su 1998).

TC SEEDLING PRODUCTION AND EXTENSION SYSTEM:

There are 3 major Cavendish cultivars including *Giant Cavendish, Tai-Chiao No. 2,* and *Formosana* (wilt-resistant) cultivated in Taiwan at the present time. Every farmer has his preference for the kind of cultivar chosen for planting. As an annual crop, most bananas are planted during the period from February to May each year. To determine the kind of variety, and the quantity of seedlings of each variety to be propagated in each month, TBRI has set up a TC seedling production and extension system through the cooperation of the Taiwan Provincial Fruit Marketing Cooperative (TPFMC). With this system, the TC program is able to produce sufficient amounts of seedlings of top quality to meet the demand of farmers each year.

Under the system, TBRI is in charge of the operation of TC laboratory. TPFMC is responsible for taking the order of seedlings from farmers, nursery management, and distribution of seedlings to farmers. Farmer who needs seedlings has to order with a small amount of deposit at least 6 months before the planting time. For facilitating distribution of seedlings to farmers, a total of 15 nurseries are established in the major banana producing areas. These nurseries are insect-proof and vector-free. In recent years, around 3 million seedlings have been produced and sold out to farmers each year through this system. After planting, TBRI conducts field inspection regularly for the quality of seedlings, such as mutation rate, and incidence of BBTV, CMV and heart rot disease, etc.

CULTIVATION OF TC SEEDLINGS:

As mentioned above, the use of TC plantlets also bring some new problems. There is strong evidence that TC plantlets are more susceptible to CMV than suckers. In some orchards where TC plantlets were grown, outbreaks of CMV with infection rates up to 65% were observed (Hwang and Su 1998). Outbreaks of CMV are usually associated with poor weed control, or occur in the neighborhood of vegetable crops such as bean, cucumber, and pepper which are alternate hosts of CMV. Measures for integrated control of CMV are avoid alternate hosts of CMV, use large plantlets, selection of

proper planting time, shiny plastic mulching, and early detection and removal of diseased plants.

At early stage of growth, TC plantlets are more sensitive to herbicide than suckers. For weed control, soil mulching with 3 or 4-feet wide shiny plastic on planting rows is recommended. Plastic mulch also promotes the growth of banana plants, probably because of the higher moisture underneath the plastic. Another problem is that mature plants grown from TC plantlets tend to develop " floating mat" which makes them likely to topple over after shooting. This problem can be avoided by planting the TC plantlets deeper, about 10-15 cm below soil surface.

The fertilization program for plantlets can follow the standard recommendations for commercial banana plantations, except that the first application of fertilizer should be made earlier for plantlets than for suckers. While suckers receive fertilizer about one month after planting, plantlets should be fertilized about 10 days after planting. Heart rot of TC plantlets caused by boron and calcium deficiency is occasionally observed in Taiwan (Ko et al. 1997). In plantations where the soil is sandy and acidic, the application of borax (2 kg/ha) and lime is recommended to control heart rot disease.

CONCLUSION:

This paper describes how TC technology has been applied successfully for improving banana production in Taiwan, which otherwise would haven been destroyed by the epidemic of fusaial wilt. The TC program undertaken in Taiwan could also be effective and feasible for other banana producing countries in the Asia and Pacific region where small banana producers are suffering considerable losses to diseases, provided they are given the necessary technical support.

Banana production in many countries in the region, similar to that of Taiwan, is also based mostly on small producers. Recent surveys and field visits indicate that many small banana farms are seriously threathened by epidemic of diseases such as bunchy top virus, fusarial wilt, bacterial wilt, as well as black Sigatoka. The impact of these diseases is severe for most small producers do not have the economic and technical capabilities to manage these problems. The key to solution is that disease-free seedlings of some banana varieties of local importance should be made available to farmers. In many countries, although advanced TC technology is practiced on large plantations, it is still completely unknown to the majority of small producers.

In response to this need, the Food and Fertilizer Technology Center (FFTC), co-sponsored by the International Network for the Improvement of Banana and Plantain (INIBAP), therefore, launches a 3-year project entitled " Establishment of National Germplasm Repository, Multiplication and Distribution Center for Improved Varieties of Banana and Plantain". This project, begun in 2002, with technical support of TBRI and the National Taiwan University (NTU), aims to ensure the availability and enhance the distribution of high-yielding and disease-resistant varieties for use by farmers of a number of selected countries in the region.

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CONCERNS AND PROBLEMS OF BANANA PLANTING MATERIAL PRODUCTION THROUGH TISSUE CULTURE

Dr. C. Kudagamage, Director, Horticultural Crops Research and Development Institute

Banana is the most important fruit crop in Sri Lanka, cultivated to around 53,000 ha. It is cultivated in parts of Sri Lanka except in most higher elevations in Nuwara Eliya district. However, highest acreage is found in North Western Province and in Southern Province.

The productivity of Banana in Sri Lanka compared to other countries in the region is low. The contributory factors for low productivity are use of low quality planting material, non - adoption of improved cultural practices and pest and disease epidemics.

There are over 30 varieties of Banana cultivated in Sri Lanka among them Embul, Anamalu and Ambun are the most popular varieties. Farmers mostly use either their own planting material or obtain them from their neighbours. As there is only limited supply of planting material from government farms or registered private nurseries. Sword suckers are the most popular type of planting material used by the farmers. Additionally, maiden suckers, bits and plants obtained by rhizomes pieces and to an lesser extent tissue cultured plants are also used as planting materials. There are several disadvantages of using conventional planting material. Such as,

1. Higher degree of susceptibility to disease and transmission of diseases caused by fungi, nematodes, and weevil.
2. Poor establishment due to damaged roots in transplanting.
3. Difficult to maintain uniform cultivation due to growth rate differences after transplanting.
4. Owing to large bulk involved, transport of planting material is difficult.
5. Non availability of sufficient planting material in a given time.

Planting material produced by tissue culture also, has several disadvantages compared to conventional planting material. Non - adoption of standard protocol and better management may cause some problem when tissue cultured material are used for propagation. If standard protocol are not followed during tissue culturing process, foliage and bunch variation may be observed in the field.

The greatest disadvantage of using tissue cultured plants is the possibility of viral infection if proper, indexing and multiplication facilities are not followed. Among the viruses the probability of transmission of banana streak virus is more common due to integration of viral genome into banana genome.

Taking the above problems and concerns of tissue culture multiplication, following scheme is proposed for virus indexing and tissue culture propagation.

1. Index apparently healthy plants (1M after establishment)
2. ELISA Negative samples once again indexed by ELISA 3-6 M after collection
3. PCR tests conducted for final ELISA Negative samples
4. Multiplication of healthy banana through conventional methods under aphid proof green house condition
5. Issue of healthy banana material for rapid multiplication in tissue culture laboratories
6. Random indexing after tissue culture propagation

However, practices followed in the above scheme is expensive to practice and hence more simplified scheme is proposed for production of planting material which is given below.

Proposed Planting Material Production System

Mother Plant Selection and virus indexing

↓

Multiplication of selected plants in Aphid – free plant house

↓

Rapid multiplication through tissue culture

↓

Multiplication of suckers in high density planting in the field

↓

Issue to growers

The ultimate output of this training workshop is to acquire knowledge and skills in the production of healthy planting material of banana in an economical manner and to manage banana plantations in order to increase productivity without affecting the environment.

The most appropriate scheme for production of planting material will be developed with the experience of the resource personnel from INIBAP, HORDI with the participation of trainees from various divisions of DOA, Universities and Private Sector.

Management of Panama disease of banana

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Panama disease or Fusarium wilt, of banana is recognized as one of the most widespread and destructive plant diseases in the recorded history of agriculture. The first description of Fusarium wilt of banana was published in 1876 by Joseph Bancroft who described disease plant of variety 'sugar' observed in Brisbana, Australia. This disease has reached epidemic in most of the banana cultivations in Asia, Africa, Central America and Caribbean Islands.

Of the numerous fruits grown in Sri Lanka banana are cultivated 52000 ha of area and it contributes to 46% of the total fruit production (Censes & Statistics Department, 2002). *Fusarium* wilt (Panama disease) caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) is the most destructive banana disease in Sri Lanka, which seriously threaten banana cultivation in the country. No bunches or poorly developed bunches could be obtained from infected plants. At present, this disease has reached epidemic level in most of the banana growing areas (Gamini, 1999). All banana and plantain varieties except Embul (Pisang Awak), cultivated in Sri Lanka are susceptible to panama disease (Gamini, 1999). Among the popular varieties grown in Sri Lanka, Kolikuttu (silk) and Ash plantain (Pisang Awak) are highly susceptible and Seeni Kesel (Pisang Awak), Ambon and Anamalu (Gros Michel) are moderately susceptible to the disease. It has reported that cultivation of some banana cultivars especially, Kolikuttu in Ambilipitiya, Udawalawa, and Rajanganaya areas has eliminated from farmers field due to this disease (*Personal communication of extension officers of department of Agriculture*). Variety Kolikuttu cannot be cultivated in the wet zone due to high incidence of panama. Survey conducted on banana cultivation in the Hambanthota District by Economic and Data Management Division of the Horticultural Crops Research and Development Institute, Sri Lanka (HORDI) revealed that cultivating percentage of Kolikuttu was 45% compared to other varieties in the district and

among the Kolikuttu cultivation, 74% fields have infected with *Fusarium* wilt. Among the *Fusarium* wilt infected Kolikuttu fields, 25% fields showed epidemic level of disease and 42% and 29% fields showed severe and moderately severe infestation respectively. These results clearly indicate the threat of *Fusarium* wilt disease for cultivation of popular banana cultivars in Sri Lanka

Different methods have been proposed to control *Fusarium* wilt including cultivation of resistant varieties, chemical control methods by fungicides, changes of soil pH by liming, application of biological control agents and *in situ* burning. However, Meng *et al.*, 1999 reported that cultural and chemical control measures have failed to control the spread of *Fusarium* wilt in Malaysia and varietal resistance could be used to control this disease. Most of the countries have their own cultivars resistant to *Fusarium* wilt to substitute panama susceptible varieties in FOC infected fields (Beer *et al.*, 1999, Thangavelu *et al.*, 1999). In Sri Lanka, tentative recommendations have given by Department of Agriculture, Sri Lanka to manage the disease. These are uprooting and removing of panama infected plants from soil and alternate planting of kolikuttu with Embul under improved drainage condition. At present, these practices are not being success in controlling panama, as most of the banana cultivation has infected with FOC. Embul is also not a suitable substitute to fulfil the high consumer demand of kolikuttu in local banana market. Therefore, an appropriate method, which is effective in disease control and easily adopted under local condition is an important and timely issue.

Detailed studies of pathogen have been conducted in many countries. It has been identified that four races of FOC are involved in development of *Fusarium* wilt in different banana varieties in the world. Race 1 has been reported to be pathogenic to Gross michel (cultivar- Emban & Anamalu), Silk (Cultivar- Kolikuttu,), Pome and Pisang awak (Cultivar- Seeni Kesel & Ash plantain). Race 2 affects closely related ABB cooking banana cultivars (Cultivar- Monthan) whereas race 4 is pathogenic on Cavendish and all cultivar are attacked by other races. Race 3 is slightly pathogenic to Gross michel (Ploetz and Pegg, 2000). In 1996, Simmnds identified that

Embul is resistant to *Fusarium* wilt (panama disease). In 1997, Dr. Natalie More identified FOC race one from two different locations (Peradeniya and Kirinda) from two different cultivars, *i.e.* Kolikuttu and Emban (Source: S. Udugama, HORDI). Initial control measure of panama disease is removing the whole diseased plant from soil to eliminate the inoculum source and replant with resistant/tolerant cultivars. However, these practices are not success in controlling *Fusarium* wilt when cultivation has done with susceptible varieties and soil has highly infected with FOC. It has been examined the potential of developing chemical and biological controls for susceptible cultivars in India under experimental level. Thangavelu *et al.*, (1999) reported that field inoculation with different bio-control agents such as *Trichoderma viride* and *Trichoderma harzianum* caused significant reduction of incidence of *Fusarium* wilt. Lakshman *et. al.*, (1987) showed that corm injections with fungicide (2% carbendazim) reduce *Fusarium* wilt development in susceptible cultivars. Germplasm screening technology of banana against different races of FOC is available under field condition (Jean carlier *et al.*, 2002). Preliminary studies conducted at the HORDI has shown that isolates of mycoparasitic bio control agent: *Trichoderma harzianum* and *Trichoderma koningi* control isolates of FOC, collected from variety Kolikuttu in two different locations *i.e.* Angunakolapallassa and Kegalle *in vitro* (Rajapakse, 2003). Inoculum production technology for *Trichoderma* using locally available materials has developed by HORDI. Field studies were established in Udawalawe area to test the efficacy of *Trichoderma* as a bio control agents under field condition. Studies have been started at the HORDI to identify FOC race specificity with different banana cultivars and selection of natural resistant/tolerant germplasm from panama susceptible varieties such as Emban, Kolikuttu, Seeni Kesel and Ash plantain (Rajapakse, 2003, CARP project no. 12/573/474).

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ELISA as a Diagnostic tool for Identification of Banana Virus Diseases & Maintenance of National Depository of Virus-free Planting Material

I. Ariyaratne, HORDI, Gannoruwa
2004-10-27

Methods of Virus disease identification:

- Symptomatology – Identification by visual symptoms of the infected plant induced by the pathogen
- Host range studies – Study on symptoms induce on an experimental host plants.
- Mode of transmission – Insect, sap, dodder, seed
- Electron microscopy – Can see size and shape of the virus
- Inclusion bodies – Protein crystals in cytoplasm of infected plant cells
- Enzyme-linked Immunosorbent Assay -ELISA
- Polymerase Chain Reaction -PCR

ELISA as a diagnostic tool

- Sensitive method
- Practical and not laborious
- Quick method. Can complete within 2 days
- Inexpensive compared with PCR
- Can test more samples in single test
- Need small quantity of antiserum
- Can detect mix infections by two or more viruses

But less sensitive than PCR

Banana Virus diseases

Banana streak virus (BSV)
Banana mosaic virus (BMV)
Banana bunchy top virus (BBTV)
Banana bract mosaic virus (BBrMV)

Symptoms of banana virus diseases

Banana streak virus (BSV)

Yellow-streaks parallel to veins of the leaf
Yellow-streaks turn to Black streaks with time
Vein thickening, leaf curling & rolling
Reduction of fruit size and fruit distortion

Banana mosaic virus (BMV)

Severe strain cause Severe Mosaic and leaf distortion Heart rot and death of plant. Mild chlorosis and leaf mottling cause by mild strain.

Spread by aphids, *Aphis gossypii, Myzus persicae, Rhopalosiphum maidis and R. prunifoliae*

Banana bunchy top virus (BBTV)

Gathering of leaves around apical region of the plant and stunting.
Narrowing and reduction of leaf size, marginal chlorosis and necrosis
Dark green lines on petioles, midribs and veins.
Early infection prevent fruit formation, late infection cause small distorted bunch
Spread by banana aphid *Pentalonia nigronervosa*

Banana bract mosaic virus (BBrMV)

Diamond shaped red patches on pseudo stem, leaf mid rib and bracts
Reduction of fruit size and fruit distortion
Spread by banana aphid *Pentalonia nigronervosa, Aphis gossypii, and Rhopalosiphum maidis*

Economical importance of virus diseases

Ivory Coast - BSV in cultivar Poyo caused,
7% yield reduction with mild BSV symptoms
90% yield reduction with severe BSV symptoms *(Frison and Sharrock, 1998)*

Once the mother plant is infected, all the suckers produced are infected. No treatments for virus diseases.

Certification Scheme for Banana Health Indexing

Establishment of Banana virus-free foundation stocks

Banana Foundation Depository – Place where virus-free plants maintain under insect-proof conditions. Periodical indexing should be practiced in four seasons of the year.
All the plants inside a depository must subject to 100% virus indexing.

Indexing Techniques for Banana Foundation Depository

Target pathogen	Indexing method
Banana bunchy-top (BBTV) *Banovirus*	ELISA or PCR
Banana mosaic (CMV) *Cucumovirus*	ELISA, Bioassay with *Chenopodium amoranticolor*
Banana streak virus (BSV)- *Badnavirus*	ELISA or PCR
Banana bract mosaic virus (BBrMV)- *Potyvirus*	ELISA or RT-PCR

Production of Virus-free plants by Corm-propagation

Place: At experimental stations or private nurserymen.
Virus-free plants derived from foundation stock grow in Corm-propagation screen-house and propagation of virus free plants can be done inside the Corm-propagation screen-house.

Indexing Techniques for by Corm-propagation

Target pathogen	Indexing method
Banana bunchy-top (BBTV)	Symptom inspection & ELISA
Banana mosaic (CMV)	Symptom inspection & ELISA
Banana streak virus (BSV)	Symptom inspection & ELISA or PCR
Banana bract mosaic virus (BBrMV)	Symptom inspection & ELISA or RT-PCR

Virus-free plants through Tissue Culture propagation

Indexing of mother plants should be done just before collecting corms for Tissue Culture. Indexing of each TC clones is necessary prior to mass propagation of virus-free Tissue Culture plantlets

Certification Indexing of Banana TC plantlets

Target pathogen	Indexing method
Banana bunchy-top (BBTV)	Symptom inspection & sample indexing with ELISA
Banana mosaic (CMV)	Symptom inspection & sample indexing with ELISA
Banana streak virus (BSV)	Symptom inspection
Banana bract mosaic virus (BBrMV)	Symptom inspection

Certification Indexing of Banana TC plantlets

Production: In Tissue culture laboratory
Hardening: In insect-free screen-house
Indexing 1% randomly selected samples of seedlings before releasing
Tags of health certificate must issue after quarantine inspection

Maintenance of Banana virus-free cultivations

1. Pathogen-free seedlings – produced by corm-propagation or TC-propagation from virus indexed mother plant.
2. Elimination of pathogen
3. Elimination of inoculum source
4. Prevention of 2ry spread by vectors

Elimination of pathogen-Heat Therapy

Banana plants with 3-5 cm pseudo stem diameter well established in potted soil are suitable for virus elimination. Keep inside a heat chamber 40^0C for 3 months. Plant dies but growing point can use for TC and keep at 28^0C for 2 weeks. When primordial tissues appear, move to 40^0C for 10 days. If the plant starts to wilt, move to 28^0C. Total accumulation time at 40^0C is 3 months. Index again and if it is virus-free, keep inside virus-free banana depository.

Elimination of inoculum source

Kill weeds by weedicides
Kill alternate host
Physalis spp., Solanaceous,legumes, Cucurbits –CMV
Canna, Alpine, Garland flower(Hedychium coronarium) – BBTV
Arrow root, Sugarcane, coffee –BSV

Prevention of 2^{ry} spread by vectors

Shining mulches to repel aphids - for CMV/BBT
Use one year crop
Use Yellow sticky traps
Regular inspection for insects-if the population is high use systemic insecticide
Stylet oil / Banana oil / Mineral oil- good for CMV, BBrMV vector control

Requirements for
Enzyme-linked Immunosorbent Assay (ELISA)

1. Leaf grinder or mortar and pestles to grind samples.
2. Sample extraction buffer-to extract sap from the sample *
3. Micropipettes and tips
4. ELISA Plates: Polyvinyl plates or Polystyrene strips/plates
5. Specific antiserum**
6. Enzyme-conjugated antiserum***

Enzymes using for conjugation:

   a. **Horse radish peroxides (HRP)**

   b. **Alkaline phosphatase (AIP)**

6. Substrates****

Substrate: p-nitrophenol (PNP) - use for enzyme Alkaline phosphatase (AIP)

Following two substrates can use with enzyme Horse radish peroxidase (HRP)

1. **Substrate: 3,3',5, 5' – tetra methyl benzidine dihydrochloride (TMB)**

   This substrate is non-carcinogenic and nonmutagenic.

2. **Substrate: O- phenylenediamine (OPD)** - This substrate is mutagenic.

   Both substrates require the addition of freshly diluted H_2O_2.

7. PBS-T buffer (phosphate- buffered saline with Tween 20) /wash buffer – to wash ELISA plates*****
8. 3M Sodium hydroxide-To stop reaction between enzyme and substrate.
10. ELISA Microplate reader, 405 nm filter and printer – To get ELISA absorbance.

Buffers need for ELISA procedure:

1. PBS-T buffer (phosphate- buffered saline with Tween 20) /wash buffer

Dissolve in distilled water to 1000 ml.:

Sodium chloride (NaCl)	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15g
Potassium phosphate, monobasic (anhydrous)	0.2g
Potassium chloride	0.2g
Tween 20	0.5g

Adjust pH to 7.4

2. General extraction buffer: -To extract plant sap of BSV and BBrMV

Dissolve in 1000ml 1 x PBS-T	
Sodium sulfite (anhydrous)	1.3g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0g
Sodium azide	0.2g
Powdered egg (chicken) albumin, Grade 11	2.0g
Tween 20	20.og

Adjust pH to 7.4 and store at $4C^0$

Using extraction buffer powder:

Buffer powder	16.5g
Distilled water	500ml.
Tween 20	10.0g

3. BBTV extraction buffer (Tris DIECA- Sucrose buffer):

Tris base	1.18g
Tris HCl	6.35g
Na-DIECA (Sodium n, n-Diethiocarbomate)	0.1g
Sucrose	5%
Distilled water	1L

Adjust pH to 7.5 and store at $4C^0$ + skim milk 0.5%

1. CMV extraction buffer (0.2 M Potassium phosphate buffer)

K2HPO4	27.86g
KH2PO4	5.44g
Distilled water	1L

Adjust pH to 7.4 and store at $4C^0$ + 0.5% Na_2So_3

2. **CTV extraction buffer (MEB extraction buffer)**

Dissolve in PBST buffer to 250ml.

Tween 20	1.25g
Nonfat dried milk	1.0g

Stir for 30 min. Adjust pH to 7.4 and store at $4C^0$

3. **Grape sample extraction buffer:**

Tris (hydroxymethil) aminomethane (Tris)	60.5g
Sodium chloride	8.0g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0g
Polyethelene glycol	10.0g
Sodium azide	**0.2g**
Tween 20	**0.5g**

Adjust pH to 8.2 and store at $4C^0$

or

Buffer powder	98.8g
Distilled water	1000ml.
Tween 20	0.5g

Adjust pH to 8.2 and store at $4C^0$

4. **ECM buffer (to dissolve enzyme-conjugated antibody of CTV)**

Dissolve in PBST buffer to 100ml.

Non-fat dried milk	0.4g

Stir for 30 min. Adjust pH to 7.4 and store at $4C^0$

3. ** **Coating buffer:** To coat uncoated ELISA wells with specific antiserum (50Mm-bicarbonate buffer)

Na_2Co_3	1.59g
$NaHCo_3$	2.93g
Distilled water	1L

Adjust pH to 9.6 and store at $4C^0$

4. ***ECI buffer: To dilute enzyme-conjugated antiserum**

Bovine serum albumin (BSA)	2.0g
Polyvinylpyrrolidone (PVP) MW 24-40,000	20.0g

Sodium azide	0.2g

Adjust pH to 7.4 and store at $4C^0$

5. ****PNP Substrate buffer: To dissolve PNP substrate tablets

Dissolve in 800 ml distilled water	
Magnesium chloride	0.1g
Sodium azide	0.2g
Diethanolamine	97.0ml

Adjust Ph to 9.8 with HCl acid adjust volume to 1000ml using distilled water and store at $4C^0$.

Double Antibody Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA) or Direct ELISA Method

1. Dissolve special antibody in coating buffer and dispense 100ul antibody into each un-coated ELISA well. Keep ELISA plate overnight at $4C^0$ for coating.

2. Wash the ELISA wells with wash buffer (1 X PBS-T). Wash 4 times. Dry the ELISA plate well.

3. Grind 0.5g-sample using 1ml sample extraction buffer. Use Leaf grinder or mortar and pestles for sample grinding. Dispense 100ul-plant sap into each ELISA well and incubate overnight at $4C^0$.

4. Wash ELISA wells with wash buffer (1 X PBS-T). Wash 4 times. Dry the ELISA plate well.

5. Dilute enzyme-conjugated antibodies in ECI buffer (make 10min. before use)and Dispense 100ul solution per ELISA well and incubate at 37 C^0 for 2hrs.

6. Wash ELISA wells with wash buffer (1 X PBS-T). Wash 4 times. Dry the ELISA plate well.

5. Add one PNP tablet in 5ml PNP buffer and dissolve well to prepare the substrate Solution (1mg/ml.). Dispense 100ul-substrate solution into each ELISA well and incubate at 37 C^0 for 30-60min. inside a humid box (Do not touch PNP tablets or expose PNP solution to sunlight).

6. Add 50ul of 3M Sodium hydroxide to each well.

7. Examine the wells by eye or measure on an ELISA micro-plate reader at 405nm.

Wells in which color develops indicate positive results. Wells in which there is no significant color develops indicate negative results. Test results are valid only if positive sample should give color development and buffer wells remain colorless.

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Workshop on Banana disease identification, Management and production of healthy planting materials

26th – 29th October 2004

MULTIPLICATION OF DISEASE-FREE BANANA PLANTS THROUGH TISSUE CULTURE AND OTHER METHODS

Darshanie Prematilake

Research Officer, HORDI

Banana is the number one fruit crop in Sri Lanka. The land extent under banana cultivation is around 45000 Ha (Department of Census & Statistics, 2001). The recommended banana planting intensity is around 1000 plants/Ha under normal planting and 3000 plants/Ha under intensive planting. Due to rapid spread of banana viral diseases in Sri Lanka, (BBrMV, BSV, BBTV and CMV) annual planting of banana without permitting a second-generation crop is recommended. Therefore, a large number of banana planting materials are needed to fulfill the annual requirement.

Conventionally, banana is propagated through suckers (i.e. sward suckers) and bits (portions of the corm). A single mother plant will produce only a limited number of such materials in its life span. Since banana is a vegetatively propagated crop, any disease that may be present in the mother plant will be transmitted to its progeny through these materials, thereby posing the risk of multiplying contaminated plants. The only practical way to overcome these problems is to use clean planting material. These factors necessitate a high demand for a rapid propagation system that produces pathogen-free plantlets in a large-scale and in a shorter time.

Micro propagation of *Musa* through shoot tip culture is a well-accepted technique worldwide. The principal of *in vitro* banana multiplication is the 'direct organogenesis from axillary buds by *in vitro* culture'. This is the basis of mass propagation of banana with the aim of large-scale commercial distribution of disease-free plants.

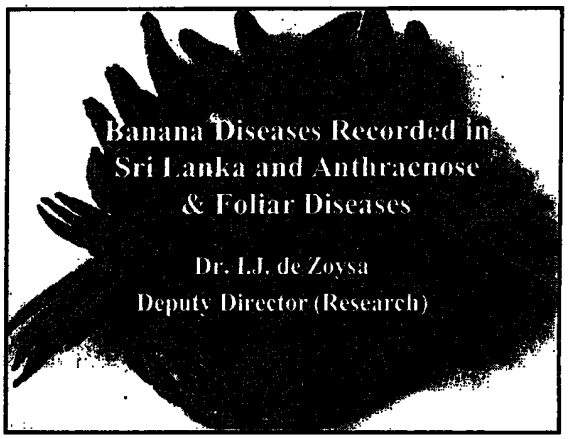
The procedure of *in vitro* banana micro propagation consists of several steps, i.e. selection of mother plants, disease indexing, culturing shoot tips under aseptic conditions, shoot

multiplication, plantlet formation and soil transfer. Each of these steps is equally important to ensure the production of quality planting material of banana.

Micro propagation efficiency in tissue culture is affected by culture medium composition, contact area of the explant and oxygenation. Modern techniques of banana micro propagation such as embryogenic cell suspension culture and temporary immersion system used in automated bioreactors are based on these factors.

There are several factors that affect *in vitro* micro propagation efficiency of banana. *In vitro* contamination with microbial agents such as fungi and bacteria is the most common problem encountered in *Musa* tissue culture. Presence of internal (systemic) bacteria in the initial tissue further complicates this problem. Appearance of somaclonal variations or off-types in micro propagated progeny is another disadvantage in tissue culture. Dwarfing, color difference in leaves and pseudostems, abnormality in fingers are some such differences observed by banana workers. Genotype, growth hormones and multiplication protocol may be the reasons for these variations.

Within this context, attention should be paid to certain factors in developing a program for mass propagation of banana using tissue culture technology. Selection and maintaining a healthy mother plant stock, selecting varieties with good in vitro response that are to be mass propagated and target-oriented production programme is envisaged.



Banana Diseases Recorded in Sri Lanka and Anthracnose & Foliar Diseases

Dr. L.J. de Zoysa
Deputy Director (Research)

Introduction

- Banana crop was originated in Southeast Asia and South Asia.
- Therefore diversity of banana in this area is wide.
- Diseases are major constraints for banana production.
- Diseases cause losses sometimes up to 100%

Banana Diseases recorded in Sri Lanka

1. Fungal Diseases

A. Leaf Diseases

1. Sigatoka - * Yellow Sigatoka (Common) *Mycosphaerella musicola*
   * Black Sigatoka *M. Figiensis*

B. Other leaf diseases

- Septoria leaf spot *Mycospherella eumusae*
- Phaeoseptoria leaf spot *Phaeoseptoria musae*

} Sigatoka like leaf spots

- Cordana leaf spot - *Cordana musae* (Associated with *Khuksia musae*)
- Cladosporium speckle- *Cladosporium musae*

- Pyricularia leaf spot
  Magnaporthe (Pyricularia) grisea
- Pestalotiopsis leafspot
  Pestalotiopsis palmarum (P. leprogena)
- Anthracnose
  Colletotrichum musae(Glomerella musarum)
- Mycospherella speckle
  Mycospherella musae
- Freckle
  Guignardia musae (Phylosticta musae)

B. Diseases of root, corm & pseudostem

- **Panama**
  Fusarium oxysporum f. sp. *cubense*
- **Pseudostem, corm & root rot**
  Ceratosystis paradoxa
  Macrophomina phaseolina
  Marasmiellus inoderma
  M.stenophyllus
  Rhizoctonia sp.
  Fusarium solani

C. Fruit diseases

- Anthracnose - *Colletotriclum musae*
  Glomerella cingulata, G. musarum
- Crown rot - *Fusarium* spp., *F. pallidoroseum*, *C. musae*, *Nigrospora* spp., *Lasiodiplodia theobromae*
- Fruit rot - *Diplodia radula*
- Freckle - *Guignardia musae (Phyllosticta musarum)*
- Fruit speckle - *Deightoniolla torulosa*

D. Diseases of Pseudostem

Pseudostem heart rot- *Fusarium moniliformae* & *F. solani*

Followed by bacterial infection

Mainly *Erwinia* spp

After stress ie. drought period or high winds.

Diseases recorded in Sri Lanka (Contd.....)

2. Virus diseases

1. Banana Bunchy Top
2. Banana Bract Mosaic
3. Cucumber mosaic (Banana mosaic)
4. Banana streak
5. Banana mild mosaic

Anthracnose
Colletotrichum musae
Glomerella musarum

- Normally a post-harvest disease
- But some varieties are susceptible at pre-harvest stages
- In Sri Lanka Alukesel is commonly affected before harvest if the whether conditions are conducive.

Anthracnose

C. Musae - Longer and wider conidia

Faster growth rate in artificial media at 24°C

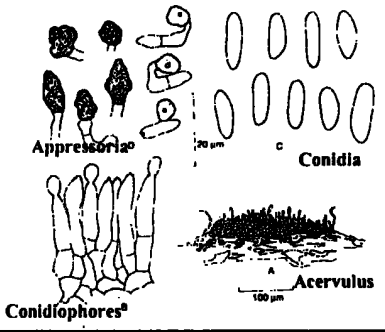
than *C. gleosporioides*

C. gleosporioides and *G. cingulata* are present in decaying bananas but C. *musae* is the most predominant pathogen of banana anthracnose

Disease symptoms

- Small, black circular specks appear on the flowers and peels of the fruits.
- They enlarge and coalesce to form large, sunken, black areas.
- Finally flowers wither and fingers get covered with the lessons.
- The fruits rot and eventually shrivels.
- In ripe fruits large black lesions with pink conidia.

Colletotrichum musae



Favourable climatic factors

1. High humidity
2. High temperature
3. High rains or mist

At flowering and premature fruit stages.

Mature fruits are tolerant.

Ripening fruits are susceptible.

Other factors – Damage during transport & storage.

Epidemiology

- *C. musae* is a common component of the micro flora of banana canopy.
- Floral & bract parts are most important sources.
- Removal of bract after bunch formation reduced anthracnose
- Conidia form in wet weather
- Disperse in rain
- Rain & mist enhance infection
- Disease severity significantly correlated with cumulative rain fall at flowering
- In mature fruits conidia germinate and form appressoria but the infection remain latent until ripening.

Management

- Protection of bunches from rain during the first month after flowering (for susceptible cultivars)
- Removal of flower and leaf debris
- Dipping fruits in hot ethylene – 52°C.
- Some countries use fungicides for dipping.

Foliar Diseases

Introduction

1. Yellow sigatoka
2. Black sigatoka (Black leaf streak)
3. Phaeoseptoria leaf spot
4. Eumusae leaf spot

- Do not kill the plants immediately
- Result in leaf necrosis
- Cause severe decrease in functional leaf area

These 4 disease have similar symptoms

- Reduce quantity & quality of fruits
- Premature fruit ripening

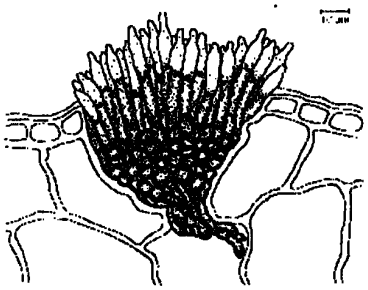
Symptoms

Yellow sigatoka	Black sigatoka
1. Light green narrow specks appear upper leaf surface elongate parallel to the veins.	1. Reddish brown specks appear lower leaf surface elongate parallel to the veins.
2. Expand laterally and become elliptical	2. Expand laterally and become elliptical
3. Turns rusty red with water soaked halo	3. Turns dark brown or black.
4. Centre of the spots become dark brown and turn grey with a brown halo.Tissues surrounding the spots turns yellow	4. Centre become grey with dark brown/black borders.
5. Spots coalease and leaf become necrotic	5. The spots coalese and the entire leaf get blacken and necrotic.

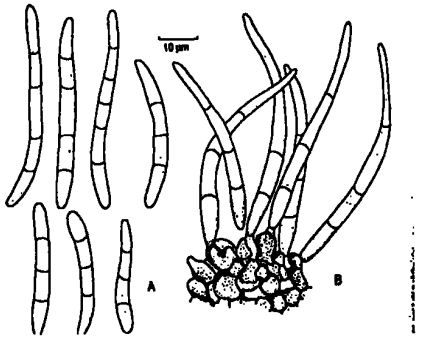
These two species can be distinguished by PCR

Yellow Sigatoka *Mycospherella musicola*	Black sigatoka *M. fijiensis*
1. Greater geographic distribution 2. First reported in Java in 1902. Later in Fiji Sigatoka valley (Viti Levu) 3. Spread to most banana growing countries by 1930	1. Less geographic distribution 2. First reported in Fiji Sigatoka valley (Viti leuv) in 1963 3. Spread to Pacidic region Southeast Asia, Latin & Central America, Africa, Australia by 1985

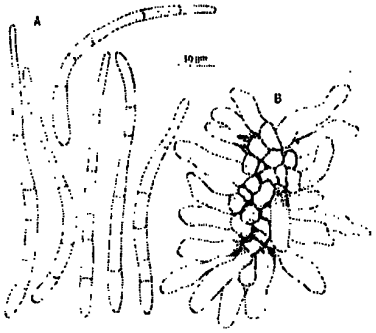
Sporodochium of *M. musicola*

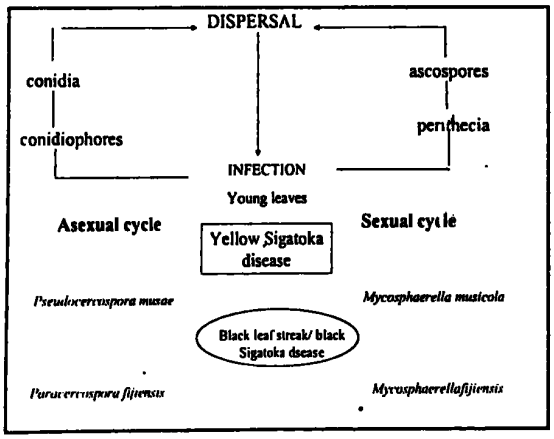


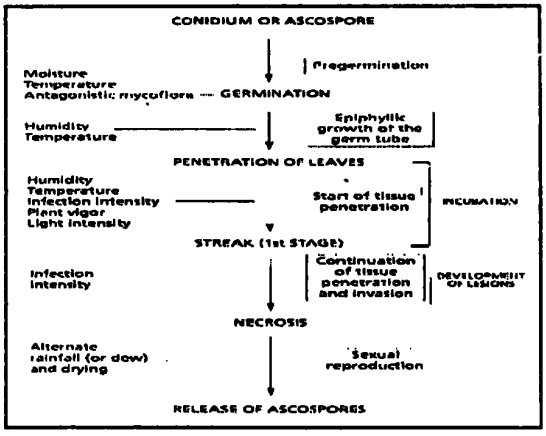
Conidia and conidiophores of *M. musicola*



Conidia and conidiophores of *M. fijiensis*







Yellow Sigatoka *Mycospherella musicola*	Black sigatoka *M. fijiensis*
4. Not reported in Egypt, Canary Islands and Israel.	4. Not reported in India, Burma & Bangladesh
5. Cause moderate losses	5. Cause heavy losses in Pacific region, Latin & Central America and Africa. But not in Asia.
6. Conidiophores form in sporodochia. No scars in conidiophores. No scars in conidia Septate 2-5 (CMI discription 414)	6. Conidiophores form in sporodochia Scar present at the base Septate 2-7 (CMI discription 413)

Phaeoseptoria musae	*Mycospherella cumusae* (*Septoria* anamorph)
1. Mainly distributed in Asia	1. Mainly distributed in Asia
2. First reported in Trivendrum, Kerala, India in 1963	2. First reported in 1995 from samples collected from India Sri Lanka, Malaysia, Thailand and Vietnam
3. Reported from many countries by 2000.	3. Reported from many countries by 2000
4. Cause moderate losses	4. Cause moderate losses
5. Conidia form in flask shaped pycnidia. Pycnidia large. Conidia small (CMI discription 772)	5. Conidias form in flask shaped pycnidia Pycnidia small. Conidia large

Cordana leaf spot

Cordana musae – 1902 from Java

C. Johnstonii – 1971 from Malaysia and Tonga (tole rate cooler climates)

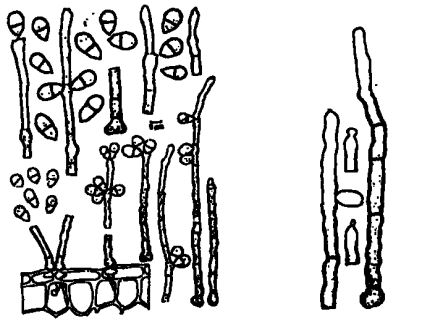
- Associated with *Khuksia musae* in Sri Lanka

Symptoms

- Pale brown oval patches
- Lesions surrounded by yellow halos
- Light grey, necrotic centres with concentric zonations
- Later lesions coalese large portions of the leaves may be necrotic.

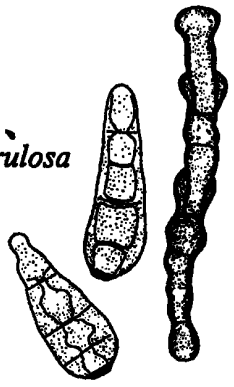
Conidia and Conidiophores of

Cordana musae *Cordana johnstonii*



Conidia and conidiophore of *Pyricularia grisea*

Conidia and conidiophore of *Deightoniella torulosa*



Management

1. Good sanitation – Remove infected leaves and other plant debris
2. Drainage – This will help to reduce RH inside the cultivation
3. Plant density – should be ideal
4. Nutrient condition – blanced nutrition

   High K
5. Fungicide – Not necessary if the disease is not severe.

Mancozeb, Chlorothalonil } Protectants

Propiconazole, Bencimidazole, Tebuconazole } Systemic

Protectants / Systemic } With Petrolium oil; Unsufonated residues – 90% or more; Aromatic content – less than 12 %

Diagnosis of Banana Viruses by Polymerase Chain Reaction (PCR)

Dr. (Mrs) E. M. Dassanayake
Plant Virus Indexing Centre, Homagama

Banana is a widely adaptable crop and consumed nationwide in Sri Lanka.. This crop could be grown in the wet, dry and intermediate zones. Some systemic viruses have been one of the serious constrains to banana production. They have caused considerable damage to fruit yield and its quality. For viruses that infect banana have been identified as banana bunchy top virus, banana bract mosaic virus in Sri Lanka was recently confirmed by Dr. John Thomas (personal communication)

Banana Virus Detection Methods

Prior to 1990, identification and detection of virus in banana was based largely on symptomatology. However, results from these methods were both inaccurate and unreliable. In the last decade advances in bio-technological studies on banana viruses at the Plant Virus Indexing Centre, Homagama has led to the development of reliable and sensitive methods for detection of viruses.

Molecular based virus detection

In April 1983, Karry Mullis took a drive on moonlight California Mountain road and changed the course of Molecular Biology. During that drive he conceived the Polymerase Chain Reaction (PCR). The PCR technique is now so pervasive in molecular diagnosis of plant virus in Agricultural Crops.

2nd page

(2)

What is PCR?

PCR is an in-vitro method for enzymatically synthesizing defind sequence of DNA. The reaction uses two olegonucleotide primer that hybridise to oppose strands and flank the target DNA sequence that is to be amplified. The elongation of the primers is catalysed by Taq polymerase a heat stable DNA polymerase that is isolated from thermophilic eubacterium. Thermus aquaticus. A repetitive series of cycles involving template denaturation ,primer annealing and extention of the annealed primers by Taq DNA polymerase results in exponentiol accumulation of specific DNA fragment.
Several primers pairs, and protocols for PCR derived from CARP project 12/371/288 were used for the detection of banana viruses present in Sri Lanka .

Different buffers and protocols used for the extraction of DNA/RNA in Banana viruses

Virus	Buffers used for the DNA/RNA extraction	Method
Banana Bunchy Top Virus	♦ 100 mM Tris HCl , pH 7.4 1m KCl, 10mM EDTA	Leaf soak extraction
	♦ 1% Sarcosine 0.8M NaCl 0.022M EDTA pH 8 0.22M Tris HCl pH 7.8 0.8% CTAB 0.14 Mannitol	CTAB method
Banana Bract Mosaic Virus	100mM Tris HCl 100mM EDTA 250 mM NaCl 1% Sarkosyl	Hang - Ji su's protocol

(3)

Banana Streak Virus	100 mM Tris -HCl, 100mM EDTA 250 mM Nacl 1% Sarkosyl	Hang - Ji su's protocol
Cucumber Mosaic Virus	0.05M Citrate 0.5 mM EDTA 1% Skimmed milk powder 0.5% Tween 20 0.5% monothioglycerol	IC -PCR method

Suitable conditions for PCR methodology

Virus	Primers Tested	Selected primer pair	Amplified product size (bp)
BBTV	BBT_1 & BBT_2	BBT_1 & BBT_2	350
	$FPCR_4$ & 30mer F_3	--	--
BSV	F_1/R_2	F_1/R_2	220
	BSV - Mysore	BSV-Mys	589
BBrMV	Bract $_1$& Bract $_2$	F_1 /R	324
CMV	93.309 & 93.35g	--	--
	CMV 3' & CMV 5'	CMV 3', CMV 5'	500

In PCR methodology nucleic and extraction was the time consuming part. This problem was somewhat overcome with simple methods such as leaf soak method & immuno-

capture PCR method. However leaf soak extraction method could be worked only with banana bunchy top virus. Similarly immuno capture method was easily applied for banana bract mosaic virus. For banana bunchy top virus amplification can be completed within one day; while for other 3 viruses it take at least 2 days.

Molecular methods of virus detection are more sensitive then the serological methods. However due to the costs of chemicals and tedious sample preparation procedure, it is applied only at the final step of indexing programme. Other than the serological methods molecular based detection methods are more helpful to identify virus infected banana samples using perfected protocols.

The National Repository, Multiplication and Dissemination Centers: An instrument to enhance the distribution and adoption of improved varieties within Asia and the Pacific

Agustin B. Molina*

The members of BAPNET have identified pests and diseases as the main constraint to *Musa* production in Asia. The impact of these pests and diseases is most severe to small-scale farmers who do not have the economic and technical capabilities to manage such problems. Large plantation owners can control pests and diseases through the intensive use of chemicals and pesticides, but aside from having an adverse effect on the environment and human health, these chemicals are beyond the means of majority of the growers, and other options are needed.

The need for National Repository, Multiplication and Dissemination Centers (NRMDCs)

In the last 15 years, *Musa* researchers worldwide have made several important breakthroughs in developing a number of high-yielding, pest- and disease-resistant varieties. Although the major *Musa* breeding programmes are located outside Asia, many of the new hybrids being produced by these programmes may be of interest for production in Asia. However, the availability of these improved materials for wide distribution is limited by the capability of the INIBAP Transit Center (ITC) to respond to the many requests for materials worldwide. Hence, in the BAPNET (formerly ASPNET) meeting in Bangkok, Thailand in 2000, the Steering Committee adopted the recommendation that NRMDCs be established in each BAPNET member country to facilitate and enhance expanded evaluation and adoption trials. These centers maintain not only the introduced cultivars but also popular local cultivars. The NRMDCs, thus serve as repository of virus-free foundation stocks of improved varieties as well as popular and superior land races.

*Regional Coordinator, INIBAP-AP, Los Banos, Laguna, Philippines

The NRMDCs were established in almost all BAPNET member countries (Table 1) in 2002-2003. Twenty-one accessions (Table 2) were already turned over to Bangladesh, China, India, the Philippines (Figure 1), Indonesia (Figure 2), Sri Lanka, the South Pacific Commission and Vietnam, while Letter of Agreements (LOA) were signed with Cambodia, Malaysia, Papua New Guinea, Thailand and the Taiwan Banana Research Institute. The improved varieties and the local cultivars are maintained *in vitro* as well as in insect-proof screenhouses to keep them from infection from virus diseases, particularly the Banana Bunchy Top Virus (BBTV), the most destructive virus disease in Asia Pacific.

Table 1. National Repository, Multiplication and Dissemination Centers (NRMDCs).

Country/Institution	Participating Institution
Bangladesh	Bangladesh Agricultural Research Institute (BARI)
Cambodia	Cambodian Agricultural R&D Institute (Cambodia)
China	Guangdong Academy of Agricultural Sciences (China)
	South China Agricultural University (China)
India	National Research Center for Banana
Indonesia	Indonesian Center for Horticultural R&D
Malaysia	Malaysian Agricultural R&D Institute
Papua New Guinea	National Agricultural Research Institute
Philippines	Bureau of Plant Industry
	Institute of Plant Breeding
Sri Lanka	Horticultural R&D Institute
Vietnam	Vietnam Agricultural Science Institute
Pacific Island countries	Secretariat of the Pacific Community
Taiwan	Taiwan Banana Research Institute

Figure 1. Ceremonial turnover of banana germplasm in the Philippines by Dr. Geoffrey Hawtin, IPGRI DG, to Philippine Department of Agriculture Secretary Leonardo Montemayor.

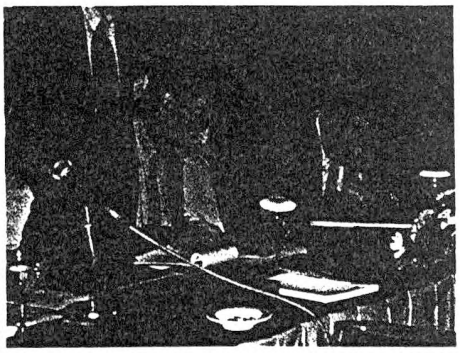


Figure 2. Signing of the MTA in Indonesia by Dr. Richard Markham, INIBAP Director, and Dr Sumarno, Director General of Horticulture Production of Indonesia.

Table 2. List of accessions maintained in the NRMDCs.

Name of accession/ synonym	ITC Code	Type	Stature	Crop cycle	Bunch weight	Fruit weight	Resistance/tolerance (R/T)
Pisang Jari Buaya	0312	dessert	tall	Late	low	Low	Reference clone for *Radopholus similis*
FHIA-01, SH-3481, Goldfinger	0504	dessert/ cooking	medium	medium	low	medium	R to black sigatoka and fusarium
FHIA-02, SH-3486, Mona Lisa	0505	dessert/ cooking	medium	medium	low		R to black sigatoka
FHIA-03, SH-3565	0506	dessert/ cooking	medium	medium	low	medium-high	R to black sigatoka and fusarium
Williams (Bell, S Johnstone)	0570	dessert	dwarf	late	low	low	Reference clone for fusarium
Cachaco	0643						Reference clone for fusarium
AA Cv. Rose	0712	dessert	medium	medium	very low	very low	Reference clone for fusarium
Gros Michel	1122	dessert	tall	very late	very low	medium	Reference clone for fusarium
Yangambi Km 5	1123	dessert/ cooking	medium	medium -late	low	low	Reference clone for sigatoka
FHIA-17, SH-3649	1264	dessert/ cooking	tall	late	medium	medium	T to black sigatoka, R to fusarium R1
FHIA-23, SH-3444	1265	dessert/ cooking	tall	late	medium	medium	T to black sigatoka and fusarium
GCTCV-119	1282	dessert	medium	late	very low	low	R to fusarium R1
SH-3436-9	1283	dessert	medium	medium	medium	medium	T to black sigatoka
BITA-2, TMBx1378	1296	cooking	tall	early-medium	medium		R to black sigatoka
BITA3, TMBx5295-1	1297	cooking	medium -tall	medium	medium		R to black sigatoka
SH-3640	1307	dessert/ cooking	medium	medium -late	high		R to black sigatoka
FHIA-18, SH-3480	1319	dessert	medium		medium		R to black sigatoka
FHIA-21 (#68)	1332	plantain	tall-very tall	early	low-medium	high-very high	R to black sigatoka
CRBP-39	1344	plantain	tall				R to black sigatoka
FHIA-25	1418	cooking	medium -tall	early	high-very high		R to black sigatoka
Pisang Ceylan	1441	dessert	tall	medium	low	low	Reference clone for sigatoka
GCTCV-106	1442	dessert					R to fusarium R1
GCTCV-247	1443	dessert					R to fusarium R1

Height: dwarf(<2.0m), medium(2.1-3.0 m),tall(3.1-4.0m),very tall(>4.0m); Crop cycle: very early(<10 months),early(11-13 months),medium(14-16months),late(17-19 months),very late(>19 months); Bunch weight: very low(<10 kg),low(11-20kg),medium(21-30kg),high(31-40kg),very high(>40kg); Fruit weight: very low(<50g), low(51-1oog),medium(101-150g),high(151-200g), very high(>200 g)

Support has been provided to access the new, improved hybrids and superior varieties from INIBAP and multiply them locally, in order to provide material to national programmes for more expanded evaluation activities and eventual adoption by farmers. The NRMDCs play a central point in the local multiplication and distribution of healthy planting materials. Local tissue-culture facilities in the region are being used for multiplying initial planting materials supplied by the NRMDCs. All germplasm movement, both into the region from

outside and between countries in the region, is carried out according to the FAO/IPGRI Guidelines for the Safe Movement of *Musa* Germplasm.

Production and use of healthy planting materials

The NRMDCs serve as a launching pad for the rehabilitation of the local banana industry through the use of clean planting materials to combat virus diseases ravaging the small scale banana farms. The type of planting material is an important aspect in the battle against pests and diseases. It has been shown that yield losses due to pests and diseases, especially virus diseases, can be reduced substantially by starting crop cycles with clean plants. One of the most efficient ways of producing clean planting material of banana is through tissue culture. Nematodes, fungal and bacterial pathogens are not transmitted through tissue culture. Virus diseases may be transmitted through tissue culture, but a screening procedure can be used to exclude infected mother plants. Research has shown that tissue-cultured plants have many advantages over conventional sucker material, such as shorter harvest-to-harvest periods, higher bunch weight and increased annual yield - all these remain measurable up to the third year after planting. Another advantage of tissue culture is that new, improved banana varieties can be rapidly multiplied and quickly introduced in response to outbreaks of diseases to which traditional varieties are susceptible.

The NMRDC as a national programme

It is the implementing strategy of this programme to tap national supports to achieve sustainable activities and accomplishments. Evaluation and promotion of the improved varieties is a national activity and thus supported by national resources. However, to ensure the immediate establishment of the improved varieties received from ITC, INIBAP provided a small-fund support to participating BAPNET countries. In the Philippines and Sri Lanka, national funding support to their NRMDCs allowed them to carry out more expanded field trials. The Department of Agriculture through the Bureau of Agricultural Research (DA-BAR) in the Philippines, provided a grant of $150 000.00 to support more activities to field evaluate the improved varieties together with the local cultivars using clean planting materials as a means to rehabilitate the BBTV-ravaged small-scale banana industry. The Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) also plans to embark to fund this initiative.

Strategic partners

A very critical and important component of the programme is the maintenance of virus-free foundation stocks and the mass production of healthy planting materials. In the BAPNET region, the Taiwanese certainly have the expertise in the field of mass production of virus-free tissue-cultured planting materials. INIBAP forged partnerships with Taiwanese institutions and scientists to provide capacity building to the BAPNET NRMDC banana development programme. A 3-year collaborative project between INIBAP and the Food and Fertilizer Technology Center (FFTC) in Taiwan, the National Taiwan University (NTU), and the Taiwan Banana Research Institute (TBRI), through funding from the Council of Agriculture of Taiwan is currently in place to strengthen the capacity of some BAPNET-member countries in the area of mass production of healthy planting materials through tissue culture and virus-indexing. Two training programmes were carried out in 2002 in TBRI, Pingtung and NTU, Taipei. These were co-organized by INIBAP and FFTC. Participants from Indonesia, Malaysia, Sri Lanka, Bangladesh, Thailand, Philippines, Vietnam, and Cambodia benefited from the training programmes. Follow-up in-country trainings ~~will be~~ are done in 2003.

The NRMDC as a platform for an improved production system

The NRMDC may serve as the launching pad for a program to develop and adapt an improved production system using healthy seedlings. With tissue-cultured seedlings, an improved cropping system may be developed. Production practices such as fertilization, irrigation and drainage, population management, other IPM tactics, and even annual cropping may be evaluated and adapted for small scale growers in some Southeast Asian countries. Such system is already in place in Taiwan, China and India. Moreover, it is hoped that the introduced varieties will provide genetic variability and option in the management of banana diseases in the Asia Pacific region

INIBAP will continue to assist and facilitate activities and collaboration to ensure the advancement of the programme. However, the success of this program may well depend on national initiatives and support, as well as the benefits and impacts to the small-scale banana farmers.

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