Banana streak virus: a unique virus-*Musa* interaction?

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A Global Programme for *Musa* Improvement

International Plant Genetic Resources Institute
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- to strengthen the ability of NARS to conduct research on bananas and plantains;
- to coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

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Cover illustration:
Background photograph: Symptoms of BSV (photo: J. d'A. Hughes, IITA, Nigeria);
Insert photograph: Electron micrograph of bacilliform virus particles
(photo: courtesy of R. Pérez, Universidad de Javeriana, Colombia).

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Foreword

In recent years, banana streak virus has proven to be a major challenge to both the virologists working with the virus and to banana researchers in general. The unusual characteristics of this virus have numerous consequences for banana research and development, far beyond the usual phytosanitary aspects linked with the movement of germplasm and the control of the disease in the field.

It is therefore not surprising that BSV was identified among the highest priorities by the Virology working group of PRO*MUSA*, the Global Programme for *Musa* Improvement (Frison *et al*. 1997).

In order to develop a common understanding about the complex features of the virus and to take stock of existing information and knowledge on BSV, it was decided to organize a workshop, in the framework of the Virology working group of PRO*MUSA*, bringing together the virologists working on BSV worldwide.

This workshop, held in Montpellier from 19 to 21 January 1998, was also an opportunity to identify and prioritize research needs, to discuss informally possible collaborative projects and to identify comparative advantages and complementarities among the various research teams.

The workshop indicated that significant progress has recently been made, leading to the current thinking that three forms of BSV exist (an encapsidated episomal form, an unencapsidated episomal form and an integrated form) and it allowed some interim recommendations to be made. However, it also showed that many questions remain to be answered and that a significant research effort has to be undertaken to overcome the problems caused by this unique virus-*Musa* interaction.

Recommendations from the meeting

In January 1998, INIBAP organized a meeting of *Musa* virologists, in the framework of the Virology working group of PRO*MUSA*, in order to discuss the latest developments in relation to banana streak virus (BSV). Until very recently, little research had been carried out on this virus which seems to have a number of unique features not found in most other plant viruses. While it is clear that many questions remain to be answered, certain interim recommendations emerged from the meeting. These are summarised below.

- BSV is a badnavirus, which appears to have a worldwide distribution and is clearly not a new virus. It has been present for very many years and during its long history, has not caused widespread epidemics. However, BSV infection has caused very significant yield loss in some locations.
- The BSV indexing procedure described in the *Technical Guidelines for the Safe Movement of* Musa *Germplasm* provides the most reliable current method for detecting the virus. It is therefore recommended that these guidelines continue to be used by indexing centres. New methods are being developed for detection of the presence, and potential for, BSV infection.
- The following points should be taken into consideration when analysing the potential risks and benefits in relation to germplasm movement:
  1. It is recommended that only virus-tested *Musa* germplasm be distributed. Virus infected material should not be distributed (except in specific instances to contained and monitored environments in the receiving country and for specific research purposes).
  2. In some cases, and under certain conditions, virus-tested (negative) material may develop BSV symptoms, and these symptoms may result in yield loss. This phenomenon of apparently "*de novo* generation of BSV infection" has been observed particularly with new hybrids produced by breeding programmes which have been multiplied by tissue culture. It is therefore recommended that if such material is introduced, it should be closely observed for BSV symptoms and plants showing symptoms should be destroyed.
  3. Tissue culture remains a useful technique for the production of clean planting material (nematode, weevil and fungal-free) despite the apparent potential for tissue culture to initiate BSV infection in some varieties. It should be noted that there has been no evidence of "*de novo* BSV-infections" in Cavendish varieties multiplied by tissue culture.
  4. BSV can be spread by natural vectors (e.g. mealybugs) but few studies have so far been carried out. Transmission by mealybugs is considered to result in slow disease spread. Observations indicate that BSV spread is limited under field conditions. Because of this, BSV infections appearing in the field can be relatively easily eradicated.
  5. Improved hybrids may perform significantly better than local varieties under conditions of high disease pressure, despite BSV infection. The potential risks and benefits should be evaluated on a case-by-case basis.

Report of the meeting

Background

Banana streak disease was first described from bananas in the Ivory Coast (Lassoudière 1974), following previous reports of a mosaic disease in Africa thought to be caused by a strain of cucumber mosaic virus (CMV). Despite the fact that these first recordings of BSV were from Africa, in a relatively short period of time, the disease had been observed or identified in most banana and plantain producing areas of the world. This suggests that the causal agent, banana streak virus (BSV), has been widely distributed for many years, and that its geographical origin is still uncertain. It is clear that in the past the symptoms caused by BSV were confused with those caused by CMV. Indeed photographs of what is almost certainly BSV infection have even appeared labelled as CMV infection in reference texts.

The virions of BSV were first isolated in 1985 (Lockhart 1986) and are bacilliform in shape (*ca* 30 x 120 nm), containing a dsDNA genome of *ca* 7.4 kbp. A high degree of genomic and serological heterogeneity between BSV isolates has meant that indexing can be problematic. The virus is transmitted in a semipersistent manner by mealybugs and also through vegetative planting material. Long distance spread is primarily due to the use of infected planting material, including micropropagated plants. The mealybug vector can spread the disease locally, though this is thought to occur very slowly and over short distances. The use of infected planting material can also be responsible for local spread. It has been reported that the disease may be seed transmitted and this possibility is being further investigated.

A wide range of cultivars of all genotypes, from Cavendish types to plantain landraces, has been observed with the disease. *Ensete* is also susceptible. The symptoms can be very variable, but generally consist of chlorotic and necrotic streaks on the lamina tissue. In some severe or extreme situations, additional symptoms including distorted bunches, splitting of the pseudostem and even heart rot and plant death have been noted. In some cases, the effect of the disease has been sufficiently severe for smallholders in countries such as Rwanda and Uganda to be aware of, and concerned about, the disease. On the other hand, symptoms may sometimes be mild and/or irregular, occurring on only some leaves, or sporadically during the year. In such cases infection may go unnoticed or farmers may be reluctant to remove such plants. Symptom expression in at least some cultivars and with some strains of the virus depends on climatic conditions. In experiments at the International Institute of Tropical Agriculture (IITA), symptoms were more prevalent and severe at lower temperatures (22-24°C) than at higher temperatures (28-32°C).

There is little published information on the agronomic and economic impact of the disease and little is known of the interaction between virus strains and cultivars. Studies on the cultivar Poyo (AAA Cavendish subgroup) in the Ivory Coast demonstrated yield losses over two cropping cycles of between 7% on plants with mild symptoms and 90% on plants

with severe symptoms. It is probable that the effect on yield is more pronounced when plants are growing under sub-optimal conditions, such as low nutrition or water stress.

Although BSV has been widely distributed for many years, the disease has only recently become a cause for concern. The increased incidence seems at times, but not always, to be linked to the use of micropropagated tetraploid hybrids. The ease of worldwide germplasm distribution as micropropagated plantlets may also be a factor, as the virus is readily transmitted by this means.

Recent developments

During the past five years, reports of BSV incidence have increased greatly. Because many of these reports have involved local consumption banana and plantain in a number of countries, this has led to concern. The reasons for the increase in the number of reported cases of BSV are threefold:

- An increased awareness of BSV symptomatology and its distinction from that of cucumber mosaic virus (CMV). This has been due in considerable measure to the wide distribution of INIBAP fact sheets and related publications.
- Availability of more effective diagnostic protocols utilising polyclonal antisera capable of recognising a wide range of isolates of this serologically highly heterogenous virus.
- The recognition of the propensity of certain *Musa* cultivars to develop BSV infections when plants with apparently no detectable virus are subjected to *in vitro* propagation. This situation is of particular concern because a majority of improved disease and pest resistant tetraploid hybrids have exhibited this characteristic. In contrast, no incidence of this phenomenon has been reported for AAA Cavendish bananas, which are also increasingly propagated by *in vitro* multiplication.

Using oligonucleotide sequences which specifically prime the amplification by PCR of badnavirus genomic sequences, it has been shown that the *Musa* genome contains integrated badnavirus sequences, which can be detected in plants which contain no BSV particles or antigen detectable by any available diagnostic protocol. Recent results indicate that, while some of these integrated viral sequences are unlikely to act as templates for replication of the viral genome, one or more other integrated sequences may be able to do so. Current research is therefore focused on identifying and characterising these integrated viral sequences, and accumulating both correlative and direct evidence that they may be the source of *de novo* BSV infection in certain *Musa* genotypes. The possibility that *in vitro* propagation may be one of several factors, including perhaps the breeding process itself as well as environmental stresses, which cause the activation of integrated viral sequences which can then function in viral replication, is also being investigated.

Attempts are being made to develop methods to identify the presence of "activateable" BSV integrated sequences in different *Musa* genotypes. This would be of particular interest in *Musa* breeding, so that parental genotypes containing such integrated viral sequences could be identified. Initial results suggest that these

sequences may occur in a range of *Musa* genotypes, including AA, AB, AAB and ABB, which are used in *Musa* breeding programmes.

Current molecular understanding of banana streak virus

Recent molecular studies on banana streak virus (BSV) have indicated that this virus has several unique features not found in most other plant viruses. The current thinking is that there are three forms of BSV (an encapsidated episomal form, an unencapsidated episomal form and an integrated form).

Encapsidated episomal form

This is the conventional form of the virus with the DNA viral genome encapsidated in the viral coat protein. This form is found in all cases of BSV symptom expression and sometimes in asymptomatic plants. The genome of an isolate of BSV has recently been sequenced confirming that it is a badnavirus, with the genome organization characteristic for the mealybug-transmitted genus of this subfamily.

Unencapsidated episomal form(s)

One of the features of BSV infections is the periodic appearance and disappearance of symptoms. In this symptom suppression phenomenon, BSV resembles the behaviour of another pararetrovirus, cauliflower mosaic virus (CaMV), in certain hosts. Studies on CaMV symptom suppression have shown that it is associated with the perturbation of viral replication. Pararetrovirus replication has two stages, transcription from a supercoiled form of the viral genome in the nucleus and reverse transcription of the resulting RNA in the cytoplasm. The perturbation in CaMV is in the transcription stage and leads to an accumulation of the supercoiled viral genome relative to other unencapsidated replication intermediates. The supercoiled form can be distinguished from the other unencapsidated viral replication intermediates by its migration on gel electrophoresis. Currently, experiments are being planned to determine if the molecular biology of the symptom suppression phenomenon of BSV resembles that of CaMV.

Integrated form(s)

Some of the recent developments of the BSV problem (see section on Recent developments above) led to the conclusion that there are integrated forms of BSV which, in some cultivars, could be activated by certain stresses to give episomal infections.

There are several lines of evidence confirming that there are in fact BSV-like sequences integrated into the *Musa* genome. These include:

- Southern blots of *Musa* chromosomal DNA probed with BSV sequences show hybridization with molecules of much higher molecular weight than that of the BSV genome.

- Badnaviral sequences have been found in genomic libraries of *Musa* chromosomal DNA.
- The presence of BSV sequences in the banana genome has been confirmed by fluorescent *in situ* hybridization (FISH) using BSV probes on several *Musa* cultivars.

The question remains, do any of these BSV integrants have the potential for activation?

The sequence of one of the badnaviral inserts from the *Musa* chromosomal DNA library showed that it differed significantly from that of the sequenced episomal isolate and that this insert would not be capable of giving an episomal infection. By analogy with retrotransposons, it is suggested that there might be two forms of BSV integrants, inactive inserts, which can not lead to episomal infections because of mutations or other perturbations of their sequence, and potentially active forms.

In devising approaches to identifying potentially active inserts, account has to be taken of the fact that the most likely way that such inserts become episomal is that the more-than-genome length RNA transcript, which is the template for reverse transcription, is produced. Sequencing of another badnaviral insert detected in the *Musa* genomic library and sequence-specific amplification of *Musa* total chromosomal DNA using a primer from the BSV sequence near the transcript initiation point and random *Taq*1 primers, have revealed a BSV sequence integrated into the *Musa* sequence, with the interface being at the transcription initiation point. This BSV integrant has very close sequence similarity to that of the episomal form of the previously sequenced isolate but, although it contains the complete BSV genome, the sequence is perturbed in such a way that expression of an active form would require recombination.

FISH has been conducted on chromosomal spreads of various *Musa* cultivars using probes based on the adjacent *Musa* and BSV sequences of the above insert. These show several chromosomal sites where the fluorescence from the two probes co-localizes. Probing of fibre stretches of *Musa* chromosomal DNA reveals a series of tandem repeats of *Musa* and BSV sequences. Thus, there appear to be several copies of this BSV integrant.

Thus, the present picture of active BSV integrants is that various candidates are being isolated and being tested against various models. The simplest is that there is a direct integrant which can yield the essential intermediate of activation, the more-than-genome length transcript. It may be that this transcript arises by recombination from two or more pieces of BSV sequence. Another point being addressed is whether the promoter for activation of transcription is that of BSV or is in the *Musa* sequence adjacent to the insertion.

There are still numerous questions about integrated BSV DNA. These include:

- While there is no doubt that BSV sequences integrate into the *Musa* genome it still has to be formally proved that, at least some of these are activateable. This raises the question of how to identify the activateable integrated form(s)? One circumstantial approach is to demonstrate that it/they are present in cultivars which have a history of activation and absent from those which do not have such a history. However, there will also be a need for formal direct evidence for these integrated forms and this will require extremely complex and difficult experimental approaches.

- Is the sequenced isolate integrant the only activateable one, or do others exist, possibly activated by different stresses?
- Are there different forms of the sequenced isolate integrant which are activateable to different levels or extents?
- How does the activation event operate and will it be possible to eliminate or control it?

As noted above, the molecular situation with BSV is unique and these, and other questions are currently being addressed to provide a better understanding of the BSV situation.

Research needs

Studies on the activation of integrated sequences of BSV

Variation in the episomal form of the virus

The working hypothesis is that stress, such as that caused by tissue culture, can activate BSV activateable integrated sequences where they are present in *Musa* varieties/species. Studies so far conducted indicate that the episomal form of the virus isolate produced by tissue culture-induced activation is the same in all cases. However, only 10 -14 genotypes have been tested and this does not represent the full range of variation. Testing of a wider range of cultivars is required.

Mechanisms of activation and the effects of tissue culture

Further research is necessary to understand the mechanisms involved in activation. What turns the process on? Such research requires an investigation into the molecular aspects of integration and activation and would require collaboration between a molecular and a tissue culture laboratory.

It is not known if all tissue culture techniques cause activation and if there may be ways of reducing the risks. Existing evidence in relation to this should be collated and analysed. Experiments are also required using a range of cultivars of which the mother plants are negative for BSV and which have never been in tissue culture. Using these cultivars, various tissue culture techniques could be tested in order to identify any variation in virus development. In addition, other methods of mass propagation could be investigated.

Non-tissue culture activation

Research is required to determine if other stresses (water stress, mixed viral infections, bacterial infections, wounding, environmental stress etc.) cause activation of integrated sequences, and if so, to determine if these stresses produce the same episomal form of the virus as tissue culture stress.

Genetic hybridization

There is a need to determine if the process of genetic hybridization causes BSV activation. Hybrids grown from seed and which have not been through tissue culture

must be tested for the presence of BSV. Studies are also required on the possibility of seed transmission of BSV.

Transmission and effect of the virus

Two levels of research in this area are required:

- Determination of the situation in the field under natural conditions.
- Basic studies on virus/vector interactions.

Studies on the spread of the disease under natural conditions can be carried out by National Agricultural Research Systems (NARS) under their own conditions. A simple experiment would involve planting a plot of a locally occurring infected variety and surrounding this by a known susceptible but uninfected variety (Williams) and monitoring the spread of the disease from the trial plot over a significant period (3-5 years).

Basic studies on virus/vector interactions could be conducted, for example, as a graduate student research project in a third country, using a range of introduced species and biotypes of mealybugs. This research should be carried out in a non-banana producing country.

Countries wishing to introduce an improved variety which is known to be infected by BSV, should be encouraged to initially establish small trial plots of the improved variety and surround these with a known susceptible but uninfected variety (Williams/local Cavendish variety). The spread of the disease, and its effect on both the introduced variety and the surrounding variety could then be monitored over a significant period (3 - 5 years).

Diagnostics

Active integrated sequences

Based on the hypothesis that some of the integrated badnavirus-like sequences are activateable to produce episomal forms of BSV, there is a need for the development of diagnostics for activateable integrated sequences. This is particularly in relation to being able to provide information to breeding programmes on the suitability of germplasm for use as parental material in breeding programmes and the detection of these sequences in their progenies.

Related to this is the need for further mapping of the *Musa* genome and the development of molecular libraries for a number of varieties. This is on going for cultivar Obino l'Ewai at the University of Minnesota and may be available for other cultivars from work being carried out by CIRAD and other laboratories. The aim should be to concentrate on potential parental material for breeding programmes so that such material can be screened for the presence of the activateable integrated sequences.

Encapsidated episomal forms of the virus

The IC-PCR technique, which has been developed to detect encapsidated episomal forms of the virus, should be further refined in order to increase its reliability and usefulness

for the routine diagnosis of the entire range of BSV isolates. There is a need for molecular characterization of a range of strains of the virus so that the major strains are recognised and the information made available for IC-PCR. The coat protein genes of the major strains should also be cloned into bacterial expression vectors to provide a reliable source of antigens for future diagnostic work.

Symptom suppression

Research is required to investigate the "symptom suppression" hypothesis. This would consist of checking for the presence of an episomal form of the virus e.g. in super-coiled form, in plant cells even when symptoms in the plant are not apparent. A southern blotting technique could be developed for the detection of this form of the virus.

Encapsidated episomal forms of the virus

Further research is required in order to determine the impact and effects on yield of the various episomal forms of the virus. A number of institutes are already conducting some trials in relation to this.

It is suggested that NARS be supported in this activity, through advice on trial design and assistance with indexing. It is recommended that all trials be conducted using symptomatic vs. indexed, asymptomatic material.

Musa genetics and cytogenetics

There is a recognised need for an increased understanding of the genetics and cytogenetics of *Musa*. This would allow a better understanding of the mechanisms of inheritance and any possible role of non-nuclear DNA in the BSV situation. This could be facilitated for example, by joint meetings between virologists and geneticists.

Information needs

There is a need for increased information exchange in relation to BSV. Within the framework of the Virology working group of PRO*MUSA* (Frison *et al.* 1997) information exchange in relation to BSV can be facilitated, for example by email. Information exchange can also be enhanced through meetings between members of the working group.

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Banana streak virus in Australia

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Detection and distribution of BSV in Australia

BSV was first detected in Australia in 1992 in the cultivar Mysore (Thomas *et al.* 1994) and confirmed by ISEM. The virus has since been detected in at least 15 cultivars from both New South Wales and Queensland (Table 1). Infections have been detected in both commercial plantings of Williams (AAA, Cavendish Subgroup) and Lady finger (AAB Group, Pome subgroup) cultivars and in germplasm collections. In all cases, symptoms consisted of chlorotic and/or necrotic streaks and infections were confirmed by electron microscopy, and in many cases also by ELISA or PCR.

Table 1. Records of BSV from Australia

Cultivar	Isolate number	Location	Diagnosis
Mysore	417	Thursday Island, Qld	ISEM
Mysore	418	South Johnstone, Qld	ISEM
Mysore	419	Wamuran, Qld	Miniprep/ISEM, ELISA
Da Jaio	420	Wamuran, Qld	Miniprep/ISEM, ELISA
Cavendish	517	Babinda, Qld	Miniprep
Cavendish	540	Kiamba, Qld	Miniprep
Cavendish	565	Innisfail, Qld	Miniprep/ISEM
Lady Finger	573	Flaxton, Qld	Miniprep/ISEM, ELISA
Dwarf Cavendish	576	Alstonville, NSW	Miniprep/ISEM, ELISA
Goldfinger	580	Alstonville, NSW	Miniprep/ISEM, ELISA
Cavendish	581	Alstonville, NSW	Miniprep/ISEM, ELISA
Ho Chu Chu	587	Alstonville, NSW	Miniprep/ISEM/ELISA
Pisang Awak	593	Alstonville, NSW	Miniprep/ISEM, ELISA
Dai Jiao	594 - 598	Alstonville, NSW	Miniprep/ISEM, ELISA
FHIA 17	621	Cudgen, NSW	Miniprep/ISEM, ELISA
French Reversion	643	South Johnstone, Qld	Miniprep/ISEM, ELISA
Cavendish	668	South Johnstone, Qld	Miniprep/ISEM, ELISA, PCR
Mysore	669	South Johnstone, Qld	Miniprep/ISEM, ELISA

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Table 1. (cont'd.)

Cultivar	Isolate number	Location	Diagnosis
Musa acuminata	670	South Johnstone, Qld	Miniprep/ISEM, ELISA
Tempua	671	South Johnstone, Qld	Miniprep/ISEM, ELISA
Cavendish	674	New Weipa, Qld	ISEM
Lady Finger		Wamuran, Qld	ISEM
Pisang Rajah	778	Red Hill, Mareeba, Qld	Miniprep/ISEM

Strain variation

PCR amplicons covering the 3'untranslated region and RNaseH region of ORF 3 have been cloned from BSV-Mysore, BSV-Cav and BSV-Agbagba. Amino acid sequence alignments of the RNaseH region indicate that BSV-Cav and BSV-Mysore are distinct badnaviruses (Table 2), and preliminary analysis suggests that BSV-Agbagba is also distinct.

Table 2. Percentage amino acid identities in the RNaseH region of ORF 3 of several badnaviruses

	BSV-Cav	CSSV	RTBV	SCBV	CYMV	BSV-Mys
BSV-Cav	100	69.5	42.9	59.4	72.7	72.9
CSSV		100	44.4	62.5	69.5	57.3
RTBV			100	40.5	45.2	41.5
SCBV				100	59.4	46.9
CYMV					100	63.5
BSV-Mys						100

CSSV = cacao swollen shoot virus, Genebank accession number L14546; RTBV = rice tungro bacilliform virus, X57924;CYMV = commelina yellow mottle virus, X52938; SCBV = sugarcane bacilliform virus, M89923.

Detection of BSV

Detection of BSV is problematic due to the serological and genomic heterogeneity of virus isolates (Lockhart and Olszewski 1993), the erratic appearance of symptoms (Dahal *et al.*1998) and the uneven distribution of the virus in plants. In the authors' laboratory, ISEM of viral minipreps, using a multi-strain antiserum to BSV (Diekmann and Putter 1996), appears to be the most reliable method for the detection of a variety of strains of BSV. This combined assay is more sensitive than either assay alone.

The commercial AGDIA ELISA kits for BSV and SCBV were only able to detect some isolates of BSV (Table 3). The SCBV kit did however, detect BSV-Cav and the sensitivity could be increased with a modified extraction buffer (0.05M Tris-HCl, pH 7.4, 0.5% sodium sulphite, 5% skim milk). This test was suitable for screening the South Johnstone field trial (see below).

Immunocapture-PCR (Wetzel 1992) was also developed for the more sensitive detection of BSV isolates. For the reliable detection of BSV-Cav, microcentrifuge tubes were coated with AGDIA SCBV antibodies for trapping of virions from ELISA sap extracts. Specific primers located in the 3' untranslated region and RNaseH region were used which gave a product of 740bp which was not detected in amplifications from healthy Williams plants. For more general detection of isolates, the BSV multi-strain or the AGDIA BSV antiserum was used for trapping of virions from partially purified viral minipreps, and the degenerate primers badnaT and badna3 used.

Table 3. Detection of BSV isolates by ELISA and virus minipreps +/- ISEM

Cultivar	BSV isolate	Source	BSV ELISA*	SCBV ELISA	Miniprep	ISEM
M. acuminata	643	*Qld*	+/-	+	3†	178
FHIA-17	621	NSW	-	-	ND#	52
Cavendish	568-2	Indonesia	+	-	ND	21
Cavendish	581	NSW	+/-	+	1	50
Agbagba	-	Nigeria	-	+	ND	16
Lady Finger	-	Qld	-	-	1	19
Williams	BSV-Cav	Qld	+/-	+	-	110

*ELISA results using AGDIA BSV or SCBV kits: - = negative; + = positive; +/- = borderline result; # ND = not detected; † = average particle count from five fields of view.

South Johnstone field trial

A field trial was established on the South Johnstone Research Station, north Queensland, to investigate:

- the effect of BSV-infection on yield and fruit quality;
- symptomatology throughout the plant and ratoon crop cycles;
- virus levels in plants throughout the cropping cycles.

A replicated planting of 120 plants each of BSV-infected and healthy cv. Williams was established. The percentage of infected plants with levels of virus detectable by ELISA increased during the cropping cycle (Figure 1). The percentage of plants with high virus titres also increased during both cropping cycles, up to the time of bunch emergence, after which the percentage dropped again (Figure 2). The detectability and symptom expression of the virus in individual plants throughout the cropping cycle was erratic. No spread of BSV to healthy plants was detected by ELISA, IC-PCR or symptoms.

When expressed in infected plants, symptoms included chlorotic and necrotic streaks on the leaf lamina, and sometimes splitting of the pseudostem, and a "traveller's palm" appearance of emerging leaves (*i.e.* leaves unfurling in a single plane). Yield trials are still in progress. Initial analysis of the plant crop yield data indicates a delay in time to harvest in infected plants (355 days vs 376 days), but little difference in average yield per plant (22.7 kg healthy vs 22.3 kg infected).

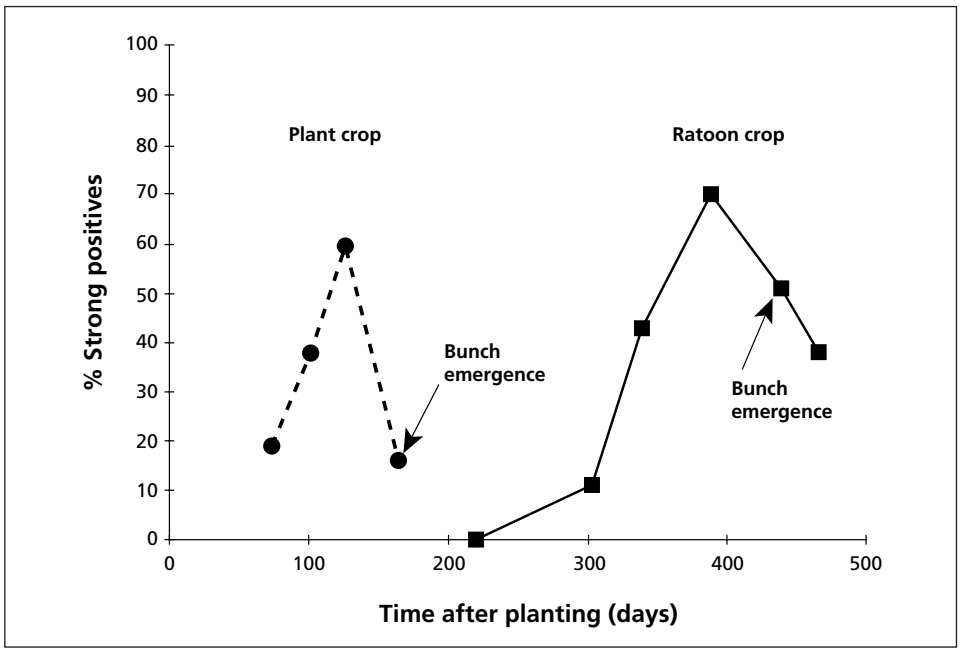

Figure 1. Ratio of strong to weak positive ELISA values at various sampling dates in plant and ratoon crops

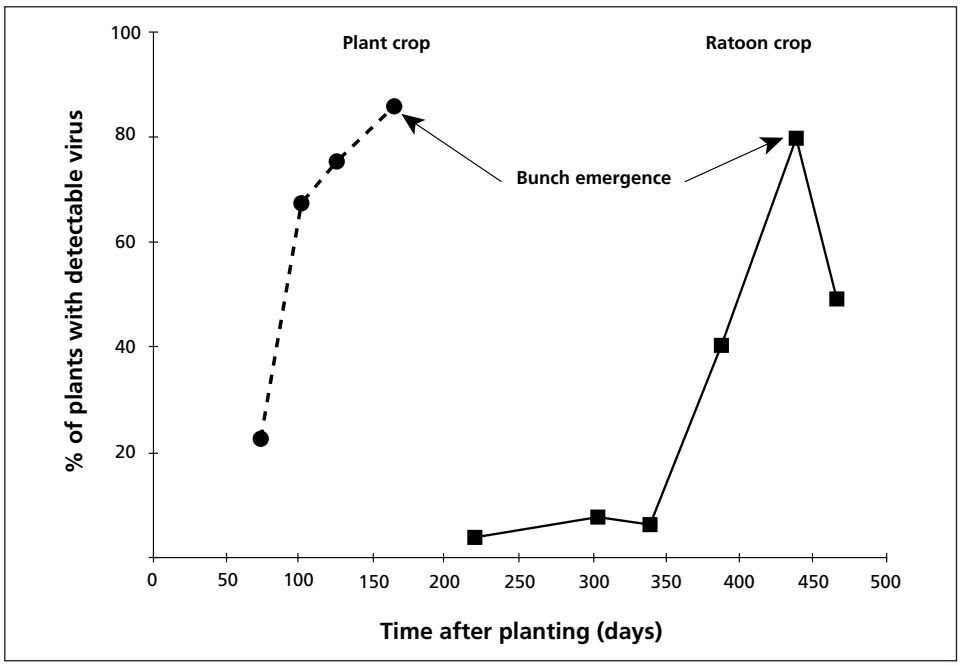

Figure 2. Percentage of infected plants with levels of virus detectable by ELISA

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First occurrence of banana streak badnavirus and studies on its vectorship in Taiwan

Hong-Ji Su[1]

Banana is Taiwan's most important fruit crop, both for local consumption and for export. Virus diseases have been causing considerable damage to banana production in tropical Taiwan. They are commonly spread through vegetatively propagated suckers and tissue-culture (TC) seedlings and can be rapidly transmitted by insect vectors. Banana bunchy-top disease caused by banana bunchy-top virus (BBTV) has been the most destructive virus disease of banana in Taiwan since 1892. Several epidemics of BBTV resulted in it becoming the limiting factor for the banana industry on this island. This decade, banana mosaic disease, caused by cucumber mosaic cucumovirus (CMV), has also become seriously epidemic in Taiwan since tissue culture-plantlets have been widely planted. Recently, leaf symptoms characteristic of banana streak badnavirus (BSV) (Lockhart 1986) were found on banana cv. Mysore (AAB) introduced from Australia into the germplasm collection of the Taiwan Banana Research Institute in 1994.

The first occurrence of banana streak badnavirus infecting banana cultivars in Taiwan was reported through transmission trials and PCR detection in 1997 (Su *et al*). The present report presented during the INIBAP BSV meeting held in January 1998, concerns the experimental results so far obtained in vectorship and ecological studies made in Taiwan.

Materials and methods

Collection of BSV-infected samples and indexing

Diseased leaf samples and suckers were collected from plants of cv. Mysore (AAB) showing streak and mosaic symptoms, growing in the germplasm collection (banana cultivar plot) of Taiwan Banana Research Institute, Pingtung, Taiwan. Some other leaf samples of AAB cultivars and Cavendish (AAA) plants showing mosaic and/or streak symptoms were also collected from the cultivar plot and nearby commercial plantations. The diseased and healthy samples were subjected to CMV detection by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies against banana isolates of CMV (Wu *et al*. 1997), and BSV detection by polymerase chain reaction (PCR) amplification with primer pairs followed by electrophoresis analysis of PCR products.

BSV-primer pairs:

- Primer F1: CAA CTC AAG AGC CTA GTA TGC;
- Primer R2: TAC CTC CGA CCG TAT TTC CAG.

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PCR cycles: (using Taq polymerase, BRL)

- 94° 4 min; 50° 1 min; 72° 2 min for 1 cycle. 94° 1 min; 50° 1 min; 72° 2 min for 30 cycles. 72° 10 min. 4° soaking.

The BSV-infected Mysore plants showing streak symptoms, produced dark colored stripes on the pseudostem such as those caused by banana bract mosaic virus (BBrMV) (Magnaye 1990). The following reverse transcription (RT) PCR method was formulated for detecting BBrMV. The primer pairs were derived from the nucleotide sequence of BBrMV gene - documented by Bateson and Dale (1995).

BBrMV primer pairs:

- F: GAC ATC ACC AAA TTT
- R: ACT ACG AAC AGG GCTAGA GAA

PCR cycles: (using Transcriptase II and Taq polymerase, BRL)

- 50° 35 min; 94° 2min; 94° 30 sec; 56° 30 sec; 68° 45 sec for 10 cycles. 94° 30 sec; 56° 30 sec; 68° 45 sec for 25 cycles. 68° 7min.

Receptor plants and vector insects

Healthy tissue-culture plantlets of Cavendish bananas with different heights (5-20 cm) were used as receptor plants for transmission trials with mealybugs. Citrus mealybug (*Planococus citri* Risso), which were used as the BSV vector, were collected mainly from citrus plants, and occasionally from banana plants. Two biotypes (short and long tail) of *P. citri* were collected from citrus plants. Mealybug clones were propagated and maintained on healthy citrus seedlings. Gray mealybugs (*Ferrisia virgata* Cockerell) were collected from citrus plants, and propagated and maintained on healthy citrus.

Donor leaf-tissue with distinct streak symptoms collected from Cavendish and Mysore plants, was kept moist within petridishes. The nonviruliferous mealybugs of different instars and adults were fed on the donor tissues in a petridish for varying acquistion feeding periods (10, 30, 60 minutes, and one day). The viruliferous mealybugs were then transferred with a moist brush to healthy Cavendish TC-plantlets for inoculation feeding for more than one day. All the test plants were kept in a ventilated green house.

Results and discussion

First occurrence of BSV infection in Taiwan

In the summer of 1994, Mysore banana plants (AAB) grown in the cultivar collection of the Taiwan Banana Research Institute were found to show mild mottle symptoms including diamond-shaped chlorotic spots and fine striate streaks. The suspected leaf samples with mosaic symptoms were collected for CMV detection by ELISA using common monoclonal antibodies prepared against CMV strain isolates from mosaic-diseased banana plants. Negative results were obtained with the all test samples. In addition, BBTV detection by ELISA with monoclonal antibodies was carried out, but no BBTV infection was detected. Accordingly, the mottling symptoms of Mysore were attributed to BSV infection.

An attempt was made to confirm the causal virus by PCR amplification of the virus with primer pairs derived from the nucleotide sequences of sugarcane badnavirus (Braithwaite *et al*. 1985) and primer pairs derived from banana streak badnavirus from Australia (J. Thomas, personal communication). Specific PCR products of 221 bp in the PCR amplification with the former primer pairs, and DNA products of 220 bp with the BSV primer pairs were obtained. The resulting data was the first evidence of BSV infection in Taiwanese banana plants. In 1995, badnavirus was first found in sugarcane cultivars in Taiwan (Liang *et al*. 1995). No evidence suggested that the BSV in Mysore was associated with the sugarcane badnavirus. It seems that the isolate of BSV from Mysore might be banana BSV from Australia.

The BSV detection by PCR amplification with the primer pairs followed by electrophoretic analysis showed a constant positive reaction with BS-diseased Mysore plants. Using the PCR detection, a survey of BSV infection in the cultivar collection plot and the nearby commercial plantations of Cavendish (AAA) bananas was made. BSV was detected in some Latundan (AAB) plants, in two other AAB cultivars with mild mottle symptoms and in a few Cavendish plants with distinct mosaic symptoms consisting of yellow stripes. Most of the plants of Cavendish banana in the plantation, which showed mosaic symptoms with or without malformation, were found to be infected by CMV in ELISA tests. So far, the spread of BSV through vector transmission in commercial plantations of Cavendish bananas in Taiwan is very slow, however the survey of BSV infection must be continued.

Transmission trials

The citrus mealybug (*P. citri*) has been shown to transmit BSV but not BBTV or CMV (Lockhart 1995). Citrus mealybugs were fed on leaf tissue of diseased Mysore bananas, and transferred to healthy tissue-cultured plantlets (5 to 10 cm high) of Cavendish banana under green house conditions. The TC seedlings developed distinct chlorotic streaks characteristic of symptoms caused by BSV within three to four weeks during spring. The Taiwan cultivar of Cavendish banana was therefore assumed to be considerably susceptible to the BSV isolate from Mysore. The artificial inoculation onto Cavendish TC-plantlets induced severe streak symptoms, including uniform vein yellowing, and chlorotic banding and stripes.

The two mealybug biotypes used in the trial - wild biotype with short tail (*P. citri*-ST) and a new biotype with long tail (*P. citri*-LT) - have both been found on citrus. The *P. citri*-ST biotype has commonly been used for transmission trials and shows a high ability of transmission. The *P. citri*-LT biotype showed a similar ability to transmit BSV among banana plants as *P. citri*-ST mealybug. Both biotypes of citrus mealybug grew and propagated well on citrus and banana, but they both preferred citrus to banana plants.

Gray mealybug (*Ferrisia virgata*) was found on citrus plants. This mealybug also grew and propagated on banana plants. Several trials of BSV transmission were carried out with the gray mealybugs, but no transmission was demonstrated.

Acquisition feeding period for the citrus mealybug with BSV, seemed to be 1 day, since no successful transmission of BSV was demonstrated on the receptor banana plantlets inoculated with *P. citri* mealybugs fed on donor tissues for 10, 30, and 60 minutes. In order to clarify the stage of mealybug life cycle affecting transmission of BSV, banana TC-plantlets were inoculated with young instars (1st and 2nd instars), old instars (3rd instar and adult), and alate adults which had fed on donor tissue. Over three quarters (> 75%) of plantlets fed with young instars became infected and showed BS symptoms, while a low percentage (one quarter) of plantlets fed with old instars and adults developed symptoms. It may thus be assumed that young instars play a more important role in BSV transmission than adults and old instars. The alate adults with degenerated mouth parts cannot transmit BSV. The young instars are tiny enough to be spread by wind and accordingly, transmission over distance may occur during strong winds. Higher populations of citrus mealybugs were commonly noticed in banana orchards mix-planted with citrus trees or near to citrus orchards.

Host factor affecting transmission and symptom expression

Different cultivars of banana showed different degrees of disease resistance. The Peichiao of Taiwan Cavendish (AAA) produced severe typical streak symptoms, while only mild streak symptoms developed on Mysore (AAB) banana plants. No typical streak symptoms developed on Taiwanese and exotic cultivars of Latundan banana (AAB). The infected Latundan plants were symptomless or produced mild chorotic mottling. The concentration of BSV replicating in the three cultivars was detected by PCR followed by electrophoresis analysis. The highest content of BSV was detected in the Cavendish plants, with a lower content in Mysore plants and the lowest content in plants of Latundan.

The maturity of banana plants also affected symptom expression. The smaller (less than 20 cm) TC-plantlets inoculated produced severe streak symptoms. Some of these diseased plants developed severe leaf necrosis causing heart rot of spindle leaves characteristic of symptoms caused by CMV. Milder symptoms developed in larger or older plants. The symptoms on young and spindle leaves were distinct and severe and became milder or indistinct towards maturity of the leaf.

Temperature can affect symptom development in Cavendish plants (Dahal *et al*, 1998). Symptoms of varying severity developed on leaves emerging in different seasons on the same plant. Leaves which emerged during the cold winter (December to February) did not produce symptoms or produced very mild symptoms, while leaves emerging during spring (March to May) produced distinct symptoms. Milder symptoms developed or were masked on leaves growing during the hot summer (June to August).

Dark colored stripes on the pseudostem, as caused by bract mosaic virus (BBrMV) (Magnaye and Espino 1990), were found in Mysore plants showing streak symptoms. However the plant was confirmed to be infected with BSV as mentioned above. Samples of leaf and pseudostem tissue were subjected to BBrMV detection of RT-PCR with primer pairs derived form nucleotide sequences of BBrMV (Bateson and Dale 1995). Negative

results were obtained in the RT-PCR analysis with Mysore samples, while positive detection of BBrMV was demonstrated by RT-PCR with banana samples infected by BBrMV from Davao, Philippines. It may be assumed that the Mysore plants showing dark stripes on the pseudostem were free of BBrMV. The same pseudostem symptoms were also commonly observed on pseudostems of Cavendish plants inoculated with BSV. No BBrMV was detected by RT-PCR in BSV-infected Cavendish plants showing streak symptoms on the leaves and dark colored stripes on the pseudostem.

Conclusion

The first occurrence of banana streak badnavirus (BSV) in Taiwan was confirmed by PCR detection of BSV in Mysore (AAB) plants showing streak symptoms growing in the germplasm collection of the Taiwan Banana Research Institute. The BSV isolate was transmitted by the citrus mealybug (*Planococus citri*) to Cavendish (AAA) tissue-culture plantlets which produced severe streak symptoms within three to four weeks after inoculation feeding. TC-plantlets of Cavendish banana were very susceptible to BSV. Severe streak symptoms on young and spindle leaves became mild toward the maturity of leaf. Temperature affected symptom expression of BSV, as severe symptoms developed on leaves emerging in the cooler spring season and no symptoms or mild symptoms developed on leaves grown during the cold winter or hot summer. AAB cultivars such as Mysore and Latundan were more resistant to the virus than the Taiwan Cavendish cultivar. Natural infection of BSV was detected in the above-mentioned AAB cultivars and in Cavendish cultivars grown in the cultivar plot and in nearby Cavendish orchards. The field spread of BSV in the commercial plantation was still very slow. Two biotypes (short tail and long tail) of citrus mealybug were demonstrated to transmit the virus equally efficiently. The preliminary transmission trial revealed that the acquisition-feeding period of citrus mealybug was one day. Young instars of the mealybug showed a higher ability to transmit virus than old instars and adults. No transmission by alate adults with degenerated mouth parts took place. Gray mealybugs (*Ferrisia virgata*) did not transmit the virus.

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Activities of CIRAD virus indexing centre

M.L. Caruana[1]

The CIRAD Virus Indexing Centre, located in Montpellier, France, is concerned with banana virus detection. In recent years there has been a special focus on the detection of banana streak virus (BSV) which has become the most commonly detected virus, followed by filamentous particles. Plants undergoing virus indexing are planted in the greenhouse and classical symptoms of BSV infection can be observed. These include yellow streaks, which later turn necrotic as well as crinkling, suberisation and thickening of foliage. Symptom expression and intensity is however correlated with ambient temperature and season.

Virus detection methods

The virus detection methods used at the Virus Indexing Center (VIC) are described in the IPGRI/FAO Technical Guidelines for the Safe Movement of *Musa* Germplasm. In relation to BSV, this involves obtaining a concentration of potential viral particles from banana leaves. This is analysed by ISEM techniques using the BSV polyclonal antisera according to the procedure of Dr Lockhart (1986). The observation of bacilliform particles confirms the presence of BSV. Each accession is tested twice during the indexing period. Attempts have been made to reduce the workload by prescreening using PTA-ELISA and DTBIA. However the background readings obtained were always too high and this has therefore been discontinued.

BSV detection

Two different couples of primers have been published for the detection of sugar cane bacilliform virus (ScBV) and badnavirus. These are VF and VR primers described by Braithwaite *et al.*, 1995, and Badna 2 and Mys 3 degenerate ologonucleotide primers described by Ahlawat *et al.*, 1996.

The VF and VR primers are defined by aligning the conserved amino acid sequences within the reverse transcriptase coding region:

- SCBV VF5 : 5' - TCA AAG TTT GAT TTG AAG AGC GGG
- SCBV VR5 : 5' - CTC CGA GAA AAC CAA TAT GTC ATC

[1] URPV – Laboratoire de Virologie, Station INRA, Petit Bourg, Guadeloupe, F.W.I.

The Badna 2 and Mys 3 degenerate primers are based on consensus sequences located in the reverse transcriptase and Rnase H domains of ORF 111 of the badnavirus genome:

- Badna 2 : 5' - TAY ATH GAY GAY ATH YT
- Mys 3' : 5' - CCC CAT RCA NCC RTC NGT YTC

In an attempt to reduce the indexing procedure, and make it applicable to direct indexing of vitroplants, it was decide to use the different primers in sequence. All manipulations were carried out on banana plants infected with the Guadeloupe strain of BSV. Mother plants were grown in the greenhouse in Montpellier and vitroplants were obtained from suckers of the mother plant.

Amplification was carried out using the primers following the recommended procedures. Infected sugar cane was used as a positive control for the VR-VF primers. The only amplification obtained from infected banana plants was using the miniprepared samples according to the procedure of Dr Lockhart. No amplification of the sugar cane positive control was obtained with the badnavirus primers.

The test was also conducted on vitroplants at two different growth stages, an early stage and the final stage, just before planting out. No amplification was obtained with the infected vitroplants at the early stage. Amplification was only obtained with the positive controls.

For the final stage vitroplants, amplification was observed with the badna primers and for all infected vitroplants. No amplification was observed from healthy vitroplants. The same profile was obtained for all infected vitroplants: one band of the expected size and three additional other bands at 900, 800 and 200 pb. Efforts have been made to explain or eliminate the additional bands by changing the extraction protocol and the conditions of amplification, but to date this has not been successful.

The presence of BSV in infected plants was confirmed by electron microscopy three months after planting out.

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Banana streak badnavirus research at the International Institute of Tropical Agriculture

J. d'A Hughes[1]

The International Institute of Tropical Agriculture (IITA) is working on the following aspects of banana streak badnavirus (BSV) as part of IITA's core research agenda, with supplementary funding from the World Bank (CFC/BIP), Gatsby Charitable Foundation, UK and BADC, Belgium.

- Natural and experimental transmission of BSV
- Epidemiology of BSV
- Yield loss assessment
- Detection of BSV
- Virus-host interactions
- Control of BSV
- BSV elimination from infected plant material
- Safe movement of *Musa* germplasm in Africa

Other IITA scientists involved in these studies at IITA Ibadan (Nigeria), IITA High Rainfall Station, Onne (Nigeria) and IITA's East and Southern Africa Regional Centre (ESARC, Uganda) are J. Ikea, S.Y.C. Ng, M. Pillay, A. Tenkouano, G. Thottappilly and D. Vuylsteke. J. Crouch, H. Crouch, G. Dahal, F. Gauhl, R. Ortiz and C. Pasberg-Gauhl are no longer at IITA but were involved with the initiation of the research at IITA on BSV and the subsequent development and implementation of the research programme.

Natural and experimental transmission of BSV

BSV can be transmitted experimentally by the mealybugs *Planococcus citri* and *Saccharicoccus sacchari* (Lockhart & Autrey 1988).

Mealybugs are found on banana and plantain growing in the field in West Africa. In Nigeria the main species are predominantly *Ferrisia virgata*, *Dysmicoccus brevipes* and *D. grassii*. The numbers and proportion of nymphs of these species varies according to the time of year. In spite of large numbers of mealybugs (including a new species *Planococcus musae*, described by Matile-Ferrero & Williams, 1995) on the bracts, pseudostems and roots, there is no evidence from IITA of field spread of BSV by these possible vectors.

There is some evidence of seed-transmission of BSV in *Musa* (Daniells *et al*. 1995). However, at IITA it has not been possible to confirm conclusively that seed transmission

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does occur. Preliminary studies, albeit with seedlings which were not grown in an insect-proof environment, suggest that seed transmission of BSV in hybrids does occur. Further studies are in progress using seed derived from BSV-infected and virus-tested mother plants to determine if seed transmission occurs and, if so, whether this occurs through the male or female parent.

Epidemiology of BSV

Trials at IITA with foci of BSV-infected material showing severe symptoms planted in the middle of blocks of 'healthy', symptomless plants of two IITA hybrids do not show any evidence of radial spread of the disease or new outbreaks which would be expected through mealybug transmission. The main means of transmission of BSV in Nigeria appears to be through dissemination, by farmers, of BSV-infected suckers.

Water stress and sustained cooler temperatures (Dahal *et al*. 1997b) seem to be the cause of localized 'outbreaks' of symptoms. Where there remains an adequate supply of water and other stresses are minimised, the plants generally appear to be healthy with few if any symptoms, except during the cooler season in southern Nigeria. Some varieties/hybrids appear to be more susceptible to the stress and cooler temperature induction of symptom expression than others.

Yield loss assessment

Studies are in progress at IITA-Ibadan to determine the relative yield losses incurred by banana and plantain landraces and hybrids which have been recorded as exhibiting symptoms of BSV compared to plants which have not been recorded as exhibiting symptoms at any time during the season.

Detection of BSV

Several detection methods are used at IITA to confirm the presence of BSV in germplasm. Usually the tests are not done singly; several tests are applied to each plant. The following tests are used:

Visual

The symptoms of BSV are fairly characteristic although they may occasionally be confused with those caused by CMV. Where distinct symptoms are seen, BSV can usually be implicated although it may be necessary to confirm the diagnosis through other techniques. The use of a controlled temperature room at approximately 22°C (compared with ambient temperatures of about 28°C - 32°C) promotes symptom expression in 2-5 months and concurrently promotes a higher virus titre in the plant tissue enabling more reliable detection by serological tests and electron microscopy.

Triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA)

Several different TAS-ELISAs are used at IITA depending on the purpose of the tests and the antibodies used. Both rabbit (B.E.L. Lockhart, University of Minnesota) and mouse (G. Thottappilly, IITA) polyclonal antibodies are used in one TAS-ELISA. In a second, the rabbit polyclonal antiserum is combined with one raised in chickens (two antisera available: B.E.L. Lockhart, University of Minnesota and G. Thottappilly, IITA). In addition, monoclonal antibodies against a Nigerian isolate of BSV are being produced at IITA.

Electron microscopy

Electron microscopy, using sap and/or partially-purified preparations, can often be useful for making a preliminary diagnosis of BSV infection in symptomatic, and some asymptomatic, plants. CMV will not be readily seen by electron microscopy without the use of immunosorbent electron microscopy (ISEM).

Immunosorbent electron microscopy (ISEM)

ISEM is routinely used for BSV diagnosis either from sap extracts or partially-purified preparations. Generally, sap extracts are first tested and, if negative, re-tested using partially purified preparations. To detect BSV in samples from Nigeria, mouse polyclonal antiserum (G. Thottappilly, IITA) is used, with random back-up tests using rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota). For leaf samples from outside Nigeria, BSV test results are always confirmed using the rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota) which is known to be effective in detecting a wide range of BSV isolates.

Immunocapture polymerase chain reaction (IC-PCR)

IC-PCR is carried out on sap extracts. The efficiency of IC-PCR using sap rather than partially purified preparations as well as the specificity of the test for episomal BSV at IITA has been confirmed. Rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota) is usually used to trap the virus (although the other antisera can also be used) and the primer obtained from R. Hull (John Innes Centre) is subsequently used for the PCR. The IC-PCR is reliable, detecting BSV in those plants exhibiting symptoms as well as in some symptomless plants. The presence of BSV in the symptomless plants can later be confirmed using other techniques such as ISEM after transfer of the plants to 22°C to promote an increased virus titre.

Polymerase chain reaction (PCR)

PCR is also carried out on extracted DNA. The results from these tests are however not conclusive after confirmatory testing with other diagnostic methods. Because the

significance of detection of integrated BSV sequences in *Musa* is not fully understood, PCR for diagnosis of BSV at IITA remains a research technique and is not, as yet, a diagnostic tool.

Virus-host interactions

It has been noted in the past that tetraploid hybrids seem to be more 'susceptible' to BSV. Studies at IITA on micropropagated banana and plantain hybrids confirmed that plantain hybrids had a high incidence of symptomatic plants which were negligible or absent in their parental lines and other landraces (Dahal *et al*. 1997a). In addition it has been noted that while plantain hybrids express symptoms under cooler (22°C) as well as ambient temperatures, most cooking bananas did not express symptoms under either regime although virus could be detected by ISEM in some of the plants (Dahal *et al*. 1997b, Dahal *et al*. 1998).

Control of BSV

Studies on controlling BSV are centred around aspects of phytosanitation, good management of the plants and their environment, the possible use of resistant or tolerant germplasm and use and distribution of healthy planting material.

Phytosanitation

Roguing is traditionally used to remove plants which are apparently unhealthy so that healthy plants can be substituted. In the case of BSV-infected plants, severely affected plants may not produce acceptable yield. It can therefore be useful to remove plants exhibiting severe symptoms and replant with 'healthy' suckers. It must be noted however that symptomless suckers can be obtained from mother plants showing severe symptoms, and that suckers with symptoms can grow from apparently symptomless mats. In the former case, the symptomless suckers are virus-infected and may later develop symptoms.

Good management

Good farm management appears to allow plants to attain a high percentage of their yield potential, despite infection with BSV. Field trials using severely BSV-infected suckers of many cultivars and hybrids, when well-managed result in plants with reduced symptom expression and apparently much improved yields, as compared to plants of the same cultivars and hybrids exhibiting severe symptoms under poor management conditions.

The major factors which seem to reduce symptom expression and thus have a subsequent effect on yield are: reduced water stress, mulching, reduced weed competition and minimal stress from other pests and diseases. Mulching is likely to be effective in reducing the effect of BSV on the plants by helping to reduce water loss and therefore also reducing water stress. Removing or reducing other stresses (such as

weeds competing for available nutrients, and other pests and diseases) also appear to be beneficial in reducing the effects of BSV.

Resistant/tolerant germplasm

IITA has identified several hybrids which are apparently resistant to BSV based on symptom expression. These hybrids are described by Ortiz and Vuylsteke, 1998a; Ortiz and Vuylsteke, 1998b and Ortiz *et al*., 1998. Some recent results show that the incidence of BSV symptom expression in tetraploid *Musa* hybrids in South-eastern Nigeria varies from over 43% in one hybrid to less than 4% in another. This may indicate a degree of resistance or tolerance of the germplasm to BSV.

Healthy planting material

To produce healthy planting material, national tissue culture laboratories are being encouraged to micropropagate virus-tested germplasm for distribution. Additionally, training in the aetiology of BSV has enabled national programme scientists to be able to make decisions on the health status of banana and plantain and make appropriate recommendations for extension workers and farmers.

BSV elimination from infected plant material

The elimination of BSV from infected plants has been attempted by both meristem-tip culture and the use of embryonic cell suspension culture.

In vitro meristem-tip culture

Meristem-tip culture alone, with chemotherapy and/or thermotherapy has been unsuccessful in eliminating BSV from infected plants.

Embryonic cell suspension (ECS) derived plants

ECS derived plants of three lines (Three Hand Planty, Bise Egome and Nakitengwa) have been produced by the Katholieke Universiteit Leuven (KUL). However, it appears unlikely that any of the plants will be virus-free as all plants have exhibited symptoms at some time. In addition, many have tested positive by ELISA, ISEM and/or IC-PCR. Some plants, sent to the John Innes Centre for fluorescent *in situ* hybridization studies, showed positive signals of integrated BSV genome. Final testing of the ECS plants is currently in progress.

Safe movement of *Musa* germplasm in Africa

IITA has considered the following points in taking the decision to distribute improved hybrids, upon request, within Africa:

- There is no evidence of natural transmission of BSV under field conditions,

- There is no direct evidence of field spread of BSV,
- There is uncertainty over the significance of the results of direct PCR (*c.f.* IC-PCR) and the consequences/detection of BSV genome integrated within the *Musa* genome,
- The effects of BSV in Nigeria appear to be relatively mild and can be managed by good farming practices,
- The risks associated with moving virus-tested germplasm from Nigeria are relatively low,
- The need for Sigatoka-resistant material and improved hybrids of banana and plantain with other characteristics to alleviate poverty in sub-Saharan Africa is very high,
- National programme scientists have been trained in BSV symptom recognition and BSV diagnostics and there are national laboratories with diagnostic capability in Ghana, Nigeria and Uganda,
- There is a strong commitment to good field sanitation of imported IITA germplasm in quarantine plots in receiving countries,
- There will be a follow-up programme of visits by IITA scientists to monitor the germplasm in the quarantine plots in the receiving countries,
- The consignments are always sent with safety notes and in future will be accompanied by further explanatory documentation, similar to that in Appendix 3.

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Development of techniques for the elimination of virus diseases from *Musa*: Progress report

H. Muylle[1]

In the framework of the INIBAP-funded project 'Development of techniques for the elimination of virus diseases from *Musa*', research concerning BSV detection and BSV eradication is on going at the Faculty of Agricultural Sciences of Gembloux, Belgium, and the Katholieke Universiteit Leuven, Belgium.

BSV detection

Immunological detection

Comparison between the AGDIA commercial BSV ELISA kit and the TAS protocol developed by Dr Lockhart

Detection was first carried out using the commercial ELISA kit from AGDIA and further compared to the TAS protocol developed by Dr Lockhart (University of Minnesota, USA). According to the TAS protocol, antisera raised in chicken and rabbit against BSV were used. The rabbit IgG was used as primary antibody, the chicken antisera as second antibody and an anti-chicken conjugate as third antibody.

The AGDIA serological test did not give reliable results for BSV detection as it did not reliably recognise some BSV strains (e.g. infected FHIA-20 plants obtained from ITC Leuven and infected FHIA-21 plants obtained from Costa Rica). Moreover the test frequently gave intermediate values which were difficult to interpret.

The TAS-protocol using the antisera provided by Dr Lockhart seemed to be more reliable than the AGDIA test as it recognised a wider range of BSV isolates. However, some contradictory results were obtained in three cultivars using the TAS ELISA protocol as compared with the AGDIA ELISA protocol.

Results obtained with the two ELISA protocols, ISEM and EM are compared in Table 1.

The positive control used in the AGDIA serological test (strain originating from Burundi, cv. Gros Michel) appeared to be BSV-free when analysed with the TAS-protocol. However an ISEM carried out on frozen plant extract infected with this strain showed bacilliform particles (laboratory of Gembloux). Observation by electron microscopy (Dr Lockhart) carried out on the same frozen plant extract containing this strain showed bacilliform particles exhibiting the same size as BSV particles but also particles with a

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Table 1. EM and ELISA results (obtained with the AGDIA test and the TAS protocol).

Plant	AGDIA test	TAS protocol	ISEM[1]	EM
Negative control (Petite Naine)	0.110[5]	0.170[5]	Not done	Not done
Positive control (FHIA20)	0.554[5]	1.806[5]	+[2]	Not done
Gros Michel (originating from Burundi)	2.519[5]	0.012[5]	-[3]	+[2]
cv. Bluggoe (originating from Nigeria)				+[4]
plant 1	0.169[5]	0.194[5]	-[2]	
plant 2	1.901[5]	0.163[5]	-[2]	
cv. Monthan (originating from Ivory Coast)				+[4]
plant 1	0.866[5]	0.205[5]	-[2]	
plant 2	0.524[5]	0.191[5]	-[2]	

[1] Using Dr Lockhart's Rabbit IgG.
[2] Done by Dr Lockhart at the University of Minnesota.
[3] Done at the laboratory of Gembloux.
[4] Accessions Monthan and Bluggoe were tested by the Virus Indexing Centres and indexed as positive for bacilliform particles using EM.
[5] Values are means of two absorbance readings at 405 nm, recorded 20 hours after adding the substrate and incubation at 4 °C.

smaller dimension than BSV particles. This might be due to proteolytic degradation of the sample. A sucker of this plant is being kept at the University of Minnesota and will be examined later for the presence of BSV.

Some plants originating from ITC accessions (cv. Bluggoe and Monthan) which tested positive for bacilliform particles (EM test) at the INIBAP Virus Indexing Centres, appeared BSV-positive with the AGDIA test. However the TAS protocol (see Table 1) and an ISEM test (carried out during a visit to the laboratory of Dr Lockhart) gave rise to negative results.

The contradictory results were obtained with plants derived from East and Central Africa. To evaluate the specificity of the TAS ELISA protocol towards African BSV isolates, more African isolates will be examined. Similarly, plants of cv. Monthan and cv. Bluggoe will be examined again using the IC-PCR protocol to determine if the results obtained with the AGDIA ELISA test were due to a non-specific immunological reaction.

Molecular based detection

PCR based detection

DNA was extracted from leaves showing BSV symptoms (strain originating from Burundi, positive in AGDIA ELISA test and negative in TAS ELISA test) using the DNA extraction method of Braithwaite *et al.* (1995). Degenerate primers, which were designed from

conserved regions of badna genome (targeting the region coding for the reverse transcriptase), were tested:

- Upstream primer: BSV1 : 5' TAC GAR TGG YTN GTN ATG CC 3'
- Downstream primer: BSV3 : 5' CCA TTT RCA NAC NGC NCC 3'

An amplified product of approximately 600 bp fragment was detected but the origin of this fragment remains unknown (*Musa* genome or BSV genome). The amplified fragment was cloned and a badna-like sequence (60% of homology with other badnaviruses) was found after sequencing it.

Using total genomic DNA (plant and viral DNA) as a template, PCR will give rise to amplified products if viral sequences are included in the genome of the plant whatever the result of the ELISA test. Another constraint of this PCR approach is that revelation carried out after separation by electrophoresis and visualisation by ethidium bromide is not very sensitive and can thus give rise to false negative results.

IC-PCR

In order to develop a more sensitive detection technique, specifically targeting viral particles, an immuno-capture PCR (IC-PCR) protocol is currently being investigated. Preliminary results have shown that BSV detection by IC-PCR is possible, but only if high dilutions of plant sap are used, probably due to the presence of PCR inhibitors.

It was recently observed that the addition of Triton X 100 in the PCR mix, allowed the amplification of a BSV-DNA specific fragment from undiluted plant sap.

Method

Tubes were coated with the IgG PMxR-2-C provided by Dr Lockhart. After four hours incubation at room temperature, tubes were rinsed with PBST, three times for three minutes. 100 mg of leaves were ground in liquid nitrogen and 400 µl of extraction buffer (PBST + 1% Na_2SO_3) added. 100 µl of the homogenate was added per antibody coated tube. After incubation overnight at 4°C, tubes were again rinsed with PBST three times for three minutes. 50 µl of PCR-mix (BSV1 and BSV3 primers dNTP, buffer, Taq polymerase, Triton) was added to the tubes and the following PCR programme was run : 5 min 94°C, 40 cycles of 30 sec 94 °C, 30 sec 50 °C, 1 min 72°C and finished with 10 min at 72°C. Amplification products were visualised by electrophoresis on a 1% agarose gel after staining with ethidium bromide.

Some problems associated with oxidation reactions occurring during extract preparation (blackening of plant extract), seemed to inhibit the amplification step. Different buffers containing antioxidants are currently being evaluated in an attempt to overcome this problem. A buffer containing PVP and Na_2SO_3 gave promising preliminary results but needs further evaluation.

Five different BSV isolates were used during this study: 2 isolates infecting two different clones of FHIA-21, one isolate present in FHIA-20, one isolate present in cv. Agbagba and an isolate present in TMPx hybrids. All isolates were detected at least once by IC-PCR.

After optimization of the extraction buffer, the IC-PCR protocol will be evaluated for its sensitivity and frequency of false results. The frequency of false positives, due to the presence of traces of plant DNA after immuno-capture and rinsing, as well as the frequency of false negatives, will be examined by performing a significant number of tests.

A disadvantage of this IC-PCR protocol is that detection of amplified products was carried out after separation on an agarose gel and staining with ethidium bromide. This is not a very sensitive technique and can give rise to possible false negatives. Developing an IC-PCR-ELISA protocol, to detect the amplified products, could solve this problem. Development of an IC-PCR-ELISA protocol will be the next step towards a more sensitive technique.

BSV eradication

Results obtained with cv. Three Hand Planty and cv. Agbagba

Plants of the accessions Three Hand Planty and Agbagba (both formerly indexed as positive for bacilliform particles by the virus indexing centre) were regenerated after exposure to different tissue culture techniques. They were then tested for the presence of BSV using the TAS protocol of Dr Lockhart (positive control = FHIA-20, negative control = Petite Naine).

Different techniques were evaluated:

- Storage at 15 °C and proliferation on 10^{-5} µM BAP (ITC collection)
- Multiplication at 25°C on proliferation medium containing 10^{-4} µM BAP
- Cell suspension
- Cryopreservation of cell suspensions
- Cryopreservation of meristems

Table 2. Summary of results of the eradication test obtained with cv. Three Hand Planty using different tissue culture techniques.

Tissue culture technique plants tested	BSV positive	Doubtful result	BSV negative	Total
Proliferation on 10^{-5} BAP	8	1	7	16
Proliferation on 10^{-4} BAP	9	4	5	18
Cell suspension	7	2	7	16
Cryopreserved suspension	11	2	4	17
Cryopreserved meristems				
Control	3	3	0	6
Cryopreserved	4	2	6	12

Table 3. Summary of results of the eradication test obtained with cv. Agbagba using different tissue culture techniques.

Tissue culture technique	BSV positive	Doubtful result	BSV negative	Total plants tested
Proliferation on 10^{-5} BAP	10	1	6	17
Proliferation on 10^{-4} BAP	15	1	1	17
Cell suspension	14	0	0	14

Several treatments gave a percentage of plants which appeared BSV-negative on the basis of the TAS ELISA protocol. However, these results should be treated with great caution, as it is known that BSV can be present but not detectable for a long period of time. Plants are being maintained in the greenhouse and will be examined again later.

It was also found that, within the same accession from the ITC collection, some plants appeared to be BSV-negative while others were BSV-positive. This finding has implications for virus indexing procedures. Conclusions made about the sanitary status of an accession, based on the indexing results of a few plants of the accession, cannot be generalized to the other plants of the same accession. In this respect, it is recommended that an accession should start from one single plant which tested negative for the viruses under consideration.

Indexing activities

FHIA-18

In vitro plants received from the Katholieke Universiteit Leuven and acclimatized at Gembloux on 31 October 96.

Table 4. BSV-indexing results for FHIA-18.

plant	test 18/3/97	test 7/4/97	test 12/5/97	test 10/06/97	test 22/07/97	test 25/08/97	test 1/10/97	test 5/11/97
Neg. Control FHIA-18.8	-0,111	0,002	-0,054	-0,152	0,024	-0,028	0,102	-0,029
Pos. Control FHIA -20.20	**1,677**	**1,205**	**0,645**	**1,724**	**1,528**	**1,900**	**1,286**	**1,852**
FHIA -18.1	-0,136	went *in vitro*						
FHIA -18.2	-0,146	-0,020	-0,049	-0,107	0,032	-0,028	0,072	0,131
FHIA -18.3	-0,111	-0,032	-0,027	-0,151	0,033	0,110	0,045	-0,024
FHIA -18.4	-0,065	-0,050	-0,024	-0,129	0,027	0,009	0,093	0,087
FHIA -18.5	-0,103	-0,042	-0,038	-0,145	0,102	-0,047	0,055	0,105

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in roman: BSV negative.

Table 4. (cont'd.).

plant	test 18/3/97	test 7/4/97	test 12/5/97	test 10/06/97	test 22/07/97	test 25/08/97	test 1/10/97	test 5/11/97
FHIA -18.6	-0,069	-0,049	-0,067	-0,074	0,077	-0,069	0,093	0,167
FHIA -18.7	-0,095	-0,016	-0,066	-0,251	0,018	0,121	0,115	0,005
FHIA -18.8	-0,111	0,002	-0,054	-0,152	0,024	-0,028	0,102	-0,029
FHIA -18.9	-0,105	-0,018	-0,053	-0,150	-0,039	-0,018	0,093	-0,021
FHIA -18.10	-0,143	-0,018	-0,056	-0,127	0,030	-0,003	0,103	0,051
FHIA -18.11	-0,131	-0,035	-0,038	-0,188	0,087	0,090	0,055	0,036
FHIA -18.12	-0,112	-0,037	-0,059	-0,099	0,036	-0,093	0,096	0,023
FHIA -18.13	-0,095	-0,042	-0,060	-0,079	0,080	-0,063	0,068	0,079
FHIA -18.14	-0,059	-0,024	-0,068	-0,181	-0,054	-0,053	0,060	-0,046
FHIA -18.15	-0,082	-0,019	-0,093	-0,215	0,007	0,080	0,078	0,013
FHIA -18.16	-0,122	-0,041	-0,010	-0,142	0,138	0,024	0,097	0,012

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in roman: BSV negative.

According to the results gathered over a period of at least eight months, we can conclude that the accession FHIA-18 is free of BSV. One plant (FHIA-18.1) was put *in vitro* for multiplication and indexing at the INIBAP Virus Indexing Centre.

FHIA-20

In vitro plants received from KUL and acclimatized at Gembloux on 31 October 96.

Table 5. BSV-indexing results for FHIA-20.

plant	test 18/3/97	test 7/4/97	test 12/5/97	test 10/06/97	test 22/07/97	test 25/8/97	test 1/10/97	test 5/11/97
Neg. control FHIA-20.11	-0,055	-0,025	-0,054	-0,297	0,138	0,070	0,071	0,044
Pos. Control FHIA-20.20	**1,677**	**1,205**	**0,645**	**1,724**	**1,528**	**0,830**	**1,286**	**1,852**
FHIA-20.1	**1,928**	0,110	0,076	*0,497*	0,075	**2,204**	**1,493**	**1,781**
FHIA-20.2	-0,094	-0,015	-0,066	**1,701**	**1,841**	**1,668**	plant died	
FHIA-20.3	-0,073	-0,012	-0,060	**1,076**	0,091	0,104	0,164	0,106
FHIA-20.4	-0,056	-0,026	-0,069	-0,277	0,069	-0,117	0,062	0,015
FHIA-20.5	-0,021	**1,245**	**0,986**	**1,358**	**1,600**	**1,180**	**1,728**	**1,858**
20.6	-0,046	-0,045	-0,092	-0,156	-0,076	-0,104	0,061	0,078

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in italics: doubtful result ; Figures in roman: BSV negative.

Table 5. (cont'd.).

plant	test 18/3/97	test 7/4/97	test 12/5/97	test 10/06/97	test 22/07/97	test 25/8/97	test 1/10/97	test 5/11/97
FHIA-20.7	-0,026	-0,013	-0,075	-0,240	-0,040	-0,079	0,056	-0,020
FHIA-20.8	**0,537**	**1,206**	**1,017**	**1,354**	**0,609**	**0,867**	**1,861**	**1,605**
FHIA-20.9	**1,480**	**1,038**	**0,715**	**1,663**	**1,265**	**0,835**	**1,594**	**2,120**
FHIA-20.10	-0,093	-0,021	-0,053	-0,111	0,057	0,064	0,063	0,053
FHIA-20.11	-0,055	-0,025	-0,054	-0,297	0,138	0,070	0,071	0,044
FHIA-20.12	**1,717**	0,156	**0,917**	**1,591**	**1,446**	*0,321*	**1,514**	**2,203**
FHIA-20.13	-0,050	-0,045	-0,070	-0,241	**1,648**	**0,816**	**1,642**	**1,944**
FHIA-20.14	-0,053	-0,037	-0,072	-0,214	-0,092	-0,021	*0,569*	*0,272*
FHIA-20.15	-0,061	-0,029	-0,073	**0,906**	**2,162**	**0,507**	*0,218*	**1,425**
FHIA-20.16	-0,068	went *in vitro*						

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in italics: doubtful result ; Figures in roman: BSV negative.

According to the results obtained over a period of at least eight months, two plants appeared BSV positive throughout the whole screening period, others showed positive sometimes, and negative at other times. Five plants have not yet given a suspect result. It is recommended that these plants be established *in vitro* for multiplication and re-indexing.

FHIA-21

In vitro plants received from KUL and acclimatized at Gembloux on 31 October 96.

Table 6. BSV-indexing results for FHIA-21.

plant	test 18/3/97	test 7/4/97	test 12/05/97	test 10/06/97	test 22/07/97	test 25/8/97	test 1/10/97	test 5/11/97
Neg. Control FHIA-21.4	-0,080	-0,025	-0,081	-0,197	-0,006	0,083	0,054	0,097
Pos. Control FHIA-20.20	**1,677**	**1,205**	**0,645**	**1,724**	**1,528**	**0,830**	**1,286**	**1,852**
FHIA-21.1	**1,036**	**0,742**	*0,264*	**1,191**	**1,618**	**0,696**	**1,538**	**1,849**
FHIA-21.2	-0,088	went *in vitro*				0,146 sucker of plant which was put *in vitro*	0,092	plant died

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in italics: doubtful result ; Figures in roman: BSV negative.

Table 6. (cont'd.).

plant	test 18/3/97	test 7/4/97	test 12/05/97	test 10/06/97	test 22/07/97	test 25/8/97	test 1/10/97	test 5/11/97
FHIA-21.3	-0,086	**0,974**	**0,999**	**1,382**	**0,866**	**0,699**	**2,053**	**1,909**
FHIA-21.4	-0,080	-0,025	-0,081	-0,197	-0,006	0,083	0,054	0,097
FHIA-21.5	-0,056	-0,015	-0,070	-0,208	-0,072	0,108	**0,651**	0,182
FHIA-21.6	-0,106	-0,017	-0,039	-0,225	**0,840**	*0,379*	**1,843**	**1,354**
FHIA-21.7	-0,099	-0,034	-0,080	0,191	0,067	0,117	**0,512**	0,084
FHIA-21.8	-0,077	-0,024	-0,072	-0,271	0,050	0,013	**1,161**	**0,869**
FHIA-21.9	-0,078	-0,038	-0,092	-0,243	-0,029	0,086	0,116	**1,889**
FHIA-21.10	-0,193	-0,031	-0,012	-0,232	-0,060	0,078	0,101	-0,031
FHIA-21.11	-0,115	-0,047	-0,003	-0,304	-0,056	0,096	0,190	0,277
FHIA-21.12	-0,063	-0,032	-0,001	-0,178	0,062	plant died		
FHIA-21.13	0,009	-0,038	0,019	-0,062	plant died			
FHIA-21.14	-0,080	-0,032	-0,021	-0,127	0,139	0,064	0,070	0,089
FHIA-21.15	**1,536**	**0,473**	**0,582**	0,219	**1,128**	**0,850**	**2,006**	**1,813**
FHIA-21.16	-0,062	-0,012	-0,038	-0,259	0,001	0,014	0,159	0,102
FHIA-21.17	-0,002	*0,297*	**0,574**	**1,236**	**1,696**	**0,794**	**1,422**	**2,191**
FHIA-21.18	-0,148	-0,019	-0,013	-0,142	0,009	0,095	0,113	0,102

*TAS-protocol: First antibody: IgG PMxR-2-C, second antibody: As PMxC-2-F and third antibody: IgG antichicken antibody (Sigma).
Figures in bold: BSV positive; Figures in italics: doubtful result ; Figures in roman: BSV negative.

Similar results were obtained with FHIA-21 as those obtained for FHIA-20. Six plants were BSV negative for all tests. It is recommended that these be established *in vitro* for multiplying and re-indexing.

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Studies on integration of banana streak badnavirus sequences in *Musa*: Identification of episomally-expressible badnaviral integrants in *Musa* genotypes

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Banana streak badnavirus (BSV) the causal agent of viral leaf streak of *Musa* spp. (banana and plantain) is of worldwide occurrence and is now considered to be the most frequently occurring virus of *Musa*. One disturbing factor in the global epidemiology of banana streak disease is the occurrence of BSV in a significant proportion of improved tetraploid hybrids which have been bred for resistance to diseases and pests and for increased yields. These improved hybrids have developed BSV infection under conditions which seem to preclude field infection by mealybug vectors. The discovery that many *Musa* genotypes contain integrated badnaviral sequences (LaFleur *et al*. 1996) led naturally to the speculation that *de novo* virus infection might arise from integrated viral sequences even though no parallel phenomenon had previously been documented for either plant or animal pararetroviruses. If the hypothesis proved to be correct, and if it were possible to identify potentially functional integrated viral sequences, then it would be possible, over the short term to identify *Musa* genotypes which plant breeders could use without danger of BSV infection arising in the progeny. Over the longer term, it would be necessary to understand the cellular and molecular mechanisms through which integrated badnaviral sequences are expressed episomally, with a view to developing strategies which might interfere with this process.

Background to the current situation

In an attempt to develop a reliable method for BSV indexing of *Musa* that would overcome the problem posed by wide serological and genomic diversity among BSV isolates, a PCR-based method was developed (Lockhart and Olszewski 1993). This method was based on the use of degenerate badnavirus-specific oligonucleotide primers which were shown to be able to prime the amplification of all definitive badnaviruses with the exception of rice tungro bacilliform virus (RTBV). It was discovered however, that these badnavirus-specific primers generated a similar-sized badnavirus-related product from plants that were either BSV-positive or BSV-negative (LaFleur *et al*, 1996). When a wide range of virus-free *Musa* genotypes were tested by PCR, a similar PCR product was obtained in all cases. This

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observation led to the hypothesis that badnavirus genomic sequences, not associated with virions, were integrated into the genomic DNA of a wide range of *Musa* genotypes. A similar badnavirus-specific PCR product was also obtained from *Ensete*, suggesting that the hypothetical integration event(s) was not of recent origin.

To confirm that the badnavirus-specific sequences being amplified were indeed contained within the *Musa* genome and not simply associated with it, hybridization analysis was carried out, using the labelled PCR product as a probe. This showed that in virus-free plants, the label was associated with DNA of a molecular weight (>20 kb) much greater than that of virion DNA (7.4 kb). Similar hybridization following restriction enzyme digestion of cellular DNA showed that label was associated only with some fragments, confirming the specificity of the hybridization signal (Figure 1).

At this point, there was substantial evidence for the presence of integrated BSV sequences in a wide range *Musa* genotypes. It remained to be determined whether this had any biological significance. The first indication of this was the observation that with certain *Musa* genotypes, BSV-free source plants appeared to yield BSV-infected progeny when propagated by tissue culture under conditions where there appeared to be little likelihood of infection coming from an external source.

It was therefore hypothesized that:

- All *Musa* genotypes contain integrated badnavirus sequences (integrated form of BSV).
- Under certain conditions (e.g. tissue culture), virions (episomal form of BSV) are produced from the integrated form and disease symptoms develop.

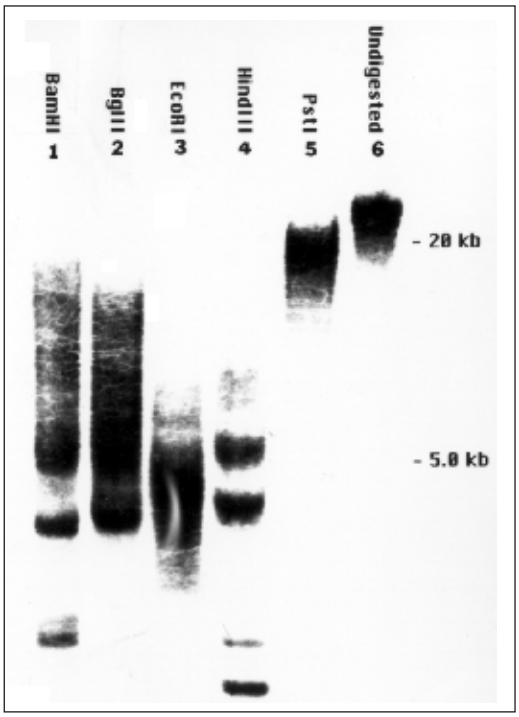

Figure 1.
Detection of badnavirus-related sequences in genomic DNA of *Musa* cv. Calcutta 4. Genomic DNA, undigested (lane 6) or cut with one of five restriction endonucleases (lanes 1 – 5), was hybridized to the ^{32}P-labelled product obtained by PCR amplification of BSV using badnavirus specific oligonucleotide primers. The pattern of hybridization indicates that target sequences were not virion genomic DNA (7.4 kb).

If the above hypothesis is correct, then the following questions arise:

- What is the nature of the integrated viral sequences?
- What is the relationship between the integrated and episomal forms of BSV occurring in different *Musa* genotypes?
- Does any correlation exist between genotype and/or pedigree and presence of episomally-expressible BSV integrants?

To characterize integrated BSV sequences in *Musa*, genomic libraries were constructed of Calcutta 4 (AA) and Obino L'Ewai (AAB), two parental genotypes used in breeding programmes to obtain improved tetraploid plantain (AAAB) hybrids. Calcutta 4 genomic clones were screened by hybridization with a probe derived by PCR amplification using badnavirus specific degenerate oligonucleotide primers (LaFleur *et al*. 1996). The integrated BSV sequence in one genomic clone of Calcutta 4 was characterized and found to be an incomplete, non-functional viral sequence (LaFleur *et al*. 1996). When the Obino L'Ewai genomic library was screened using a probe consisting of a Pst I fragment of genomic DNA from episomal virus isolated from a tetraploid Obino L'Ewai x Calcutta 4 hybrid, integrated BSV sequences were identified in 11 genomic clones (Ndowora 1998). Sequence analysis revealed a high degree (> 99%) of homology between the viral sequences integrated in Obino L'Ewai and that of the episomal virus occurring in the tetraploid hybrid progeny derived from it. Interestingly, genomic DNA of Calcutta 4 did not hybridize with the probe derived from genomic DNA of the episomal virus occurring in the tetraploid hybrid of which it was the male parent.

The preceding observations indicated that in the case of tetraploid *Musa* hybrids resulting from a Calcutta 4 x Obino L'Ewai cross, episomal virus occurring in tetraploid progeny contained genomic DNA very similar to that of integrated BSV sequences identified in the female parent, Obino L'Ewai. To determine whether integrated and episomal BSV sequences in other *Musa* genotypes might be related to hybrid progeny, virion DNA identified in Obino L'Ewai and from its hybrid progeny from a variety of sources, was digested with the restriction endonucleases Pst I, Sac I and Xba I. The electrophoretic profiles of the digestion products were then compared. The results showed that whereas genomic DNA of naturally-occurring isolates of BSV had diverse restriction endonuclease digestion profiles, genomic DNA of virions, assumed to have originated from integrated viral sequences, had a common restriction enzyme digestion pattern. These results suggested that the episomal virus might be derived from one or more integrated viral sequences, which were largely similar across a range of genetically diverse *Musa* genotypes.

Additional evidence to support the hypothesis that episomal virus appearing in tissue culture–derived plants originated from integrated viral sequences was obtained from nucleotide sequence comparisons between the integrated and episomal forms. It was found that a BSV sequence integrated in Obino L'Ewai shared > 99% sequence homology with genomic DNA of episomal virus isolated from one of its tetraploid progeny which had been propagated *in vitro*. Once again, this is in contrast to naturally occurring BSV isolates, which are characterized by a high degree of genomic heterogeneity (Lockhart and Olszewski 1993).

BSV infection arising from integrated viral sequences: developing predictive models

In order to assist plant breeders in avoiding the use of parental *Musa* genotypes, which might predispose hybrid progeny to BSV infection following *in vitro* propagation, it would be useful to develop predictive criteria for the selection of such parental genotypes. To do this, two possibilities were considered, namely that:

- a correlation be found between genotype and/or pedigree and the presence of "activatable" integrated viral sequences;
- a method might be found to detect such integrated viral sequences.

The phenomenon of *de novo* BSV infection arising during *in vitro* propagation was first observed in tetraploid AAAB plantain hybrids. It was initially thought that it might be related either to the process of hybridization per se, or that it was associated with the *balbisiana* component of the genome. This was because AAA dessert bananas such as Grand Naine and Williams do not appear to exhibit this phenomenon, even though these cultivars are produced in very large numbers by *in vitro* propagation. Both of these hypotheses were subsequently found to be inconsistent with the observation that an apparently similar tissue culture–induced activation of integrated viral sequences occurred in *Musa* genotypes which either had not undergone hybridization or contained no *balbisiana* component. These *Musa* cultivars included Obino L'Ewai (AAB), French plantain (AAB), Red (AAA) and Pisang lilin (AA). It is interesting to note that episomal BSV occurring in these diverse genotypes, as well as in tetraploid plantain hybrids of totally different pedigree (e.g. PITA, FHIA-21), had similar restriction enzyme profiles. This suggests that one or more similar integrated viral sequences were present in a diverse range of *Musa* genotypes, and that it might be impossible to establish a correlation between either genotype or pedigree and presence of "activateable" integrated viral sequences.

Since it appears doubtful that the presence of episomally expressible badnaviral integrants can be deduced from information on plant genotype and pedigree, a logical alternative would be to develop a diagnostic process for identifying such integrated sequences. The particular approach chosen was to design oligonucleotide primers which would generate PCR amplification product spanning the *Musa*–BSV integrant junction, as this would obviate spurious amplification of any episomal viral sequences present. Primers for PCR amplification were derived from sequences in an Obino L'Ewai genomic library clone that included the *Musa*–BSV integrant junction. Initial results of these experiments, listed in Table 1, show a strong positive correlation between the presence of the *Musa*–BSV integrant junction, as detected by PCR amplification, and the propensity for BSV infection in tissue culture–derived progeny. This suggests that this or a similar PCR amplification procedure may be useful in detecting the presence of "activateable" integrated sequences. However the model requires more rigorous testing, and a wider range of *Musa* genotypes will have to be tested in order to determine its validity and reliability. If this procedure, or some modification of it, proves to be a dependable method of identifying integrated viral sequences that are potentially

activateable by *in vitro* propagation or other stress factors, then *Musa* improvement programmes would have the means to avoid the problem of inadvertent BSV infection that has arisen in all current banana and plantain breeding programmes.

The data, observations and discussion presented above describe a novel virus-host interaction of both practical and scientific importance. The most important conclusion arising from this study is that it is imperative that research be continued to fully characterize integrated BSV sequences occurring in different *Musa* genotypes. It is equally important that an integrated approach involving plant breeders, cytogeneticists and molecular biologists be adopted in order to develop effective practical solutions to the problem posed by the occurrence of integrated viral sequences in *Musa*.

Table 1. Results of PCR amplification using a pair of oligonucleotide primers that sit on either side of the *Musa*-BSV integrant junction in Obino L'Ewai.

Genotype	Genome	PCR amplification	Genotype	Genome	PCR amplification
Pisang Lilin	AA	+	PV 03-22	AAAB	+*
Inarnibal	AA	+	PV 03-44	AAAB	+*
Pamotion	AA	-	JV 03-15	AAAB	-*
Pisang Jari Buaya	AA	-	SH-3648	AABB(?)	-
Pisang Mas	AA	-	Bobby Tannap	AAB	+*
Veinte Cohol	AA	-	Pisang Ceylan	AAB	-
Rose	AA	-	Popoulu	AAB	+
Gros Michel	AAA	-	Silk	AAB	-
Lacatan	AAA	-	Sugar	AAB	-
Dwarf Cavendish	AAA	-*	Kofi	AAB	-
Williams	AAA	-*	AVP 67	AAB	+
Yangambi Km5	AAA	-	Maqueño	AAB	+
FHIA-02	AAAA	-	VI	AAB	+
FHIA-23	AAAA	+	Prata Aña	AAB	-
FHIA-17	AAAA	+	Kunnan	AB	-
FHIA-01	AAAB	+	Ney Poovan	AB	-
FHIA-07	AAAB	+*	Pelipita	ABB	-
FHIA-09	AAAB	+*	Ducasse	ABB	+
FHIA-21	AAAB	+*	Bluggoe	ABB	-
CRBP-39	AAAB	+*	Saba	ABB	+
CRBP-01	AAAB	+*	Obino L'Ewai	AAB	+*
CRBP-15	AAAB	+*	Calcutta 4	AA	-*
TMPx 4698-1	AAAB	+*	Virion DNA		-*
TMPx 5511-2	AAAB	+*	No template		-*
TMPx 5860-1	AAAB	+*			

* Confirmed by Southern hybridization

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Banana streak virus studies at the John Innes Centre

R. Hull and G. Harper[1]

The study of banana streak virus (BSV) at the John Innes Centre is part of a collaborative project with the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria to develop diagnostics for the virus. The approach has been to gain an understanding of the virus so that targets for diagnostics can be identified. Current thinking is that there are three forms of BSV (encapsidated episomal form, unencapsidated episomal form, integrated form) which would require different diagnostic approaches. Concurrent with developing diagnostics, information is being accrued which could be used in approaches to controlling the virus.

Encapsidated episomal form

This is the conventional form of the virus with the DNA viral genome encapsidated in the viral coat protein. All the current diagnostic approaches target this form. The genome of a Nigerian isolate of BSV has been sequenced confirming that it is a badnavirus (Harper and Hull, submitted). Using the sequence data the authors, together with Drs Thottappilly and Dahal from IITA, have developed an immune capture - polymerase chain reaction (IC-PCR) technique for detecting this form of the virus. The virus particles are captured in plastic tubes or microtitre plates using broad spectrum antiserum (from Dr Lockhart) and a portion of the DNA genome amplified by the PCR using primers based on the sequence and known to be specific for BSV. One possible problem was that the immune capture stage could be contaminated with banana chromosomal DNA containing integrated BSV sequences (see below) which would be amplified by PCR. Using internal controls of primers to *Musa* nuclear intermediate repetitive DNA and to chloroplast intergenic region DNA it was shown that there was no chromosomal contamination.

The IC-PCR technique has proved to be efficient and easy for the detection of encapsidated episomal BSV in Nigerian material. However, it is recognised that the variation of BSV may cause problems for its world-wide use. The antiserum must be able to capture the particles and it is possible that previously unrecognised serotypes may be missed. The PCR stage depends on the primer sequence and the current primers may not anneal to sequence variants. As further information is gained on the variation of BSV this technique could be refined to make it more reliable.

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Unencapsidated episomal form(s)

One of the features of BSV infection is the periodic appearance and disappearance of symptoms. In this symptom suppression phenomenon BSV resembles the behaviour of another pararetrovirus, cauliflower mosaic virus (CaMV), in certain hosts. Studies on CaMV symptom suppression have shown that it is associated with the perturbation of the viral replication cycle (Covey *et al*. 1997). Pararetrovirus replication has two stages, transcription from a supercoiled form of the viral genome in the nucleus and reverse transcription of the resulting RNA in the cytoplasm. The perturbation in CaMV is in the transcription stage and leads to an accumulation of the supercoiled viral genome relative to other unencapsidated replication intermediates. The supercoiled form can be distinguished from the other unencapsidated viral replication intermediates by its migration on gel electrophoresis. Thus, the diagnostic test that is being considered would involve electrophoresis of total DNA from the plant followed by blotting onto a membrane and probing for BSV-specific sequences.

Integrated form(s)

One of the outcomes from the initial attempts to develop a PCR-based diagnostic technique was the recognition that BSV sequences are integrated into *Musa* genomes. This meant that PCR could not be used directly on DNA extracted from *Musa* plants as all gave positive results irrespective of whether they contained episomal virus or not. The presence of BSV sequences in the banana genome has been confirmed by fluorescent *in situ* hybridization (FISH) using BSV probes on several *Musa* cultivars (Osuji, Heslop-Harrison, Harper and Hull, unpublished observation).

Analyses of the occurrence of BSV infections in progeny from tissue culture and/or crossing of certain banana cultivar parents which do not show infection has led to the suggestion that there might be integrated BSV sequences which are activated by certain stresses to give episomal infection. By analogy with retrotransposons, it is suggested that there might be two forms of BSV integrants, inactive inserts which can not lead to episomal infections because of mutations or other perturbations of their sequence and potentially active forms.

The most likely way that potentially active inserts become episomal is that the more-than-genome length RNA transcript, which is the template for reverse transcription, is produced. The 5' end of this transcript in episomal infections was mapped onto the viral genome (Harper and Hull, submitted). Using a primer from the BSV sequence near the transcript initiation point and random *Taq*1 primers, sequence-specific amplification was undertaken on several cultivars which had a history of potential activation. Various products were obtained and are being characterized. The sequence of one of these products shows the BSV sequence integrated into the *Musa* sequence with the interface being at the transcription initiation point. Using a primer based on the *Musa* sequence (*MUSA*1) and various primers based on the BSV sequence, it was shown that not all the viral sequence was present in a linear form; there appeared to be some perturbation of

the BSV sequence. A wide range of amplifications has been undertaken using *MUSA*1 primer, its complement (ASUM1), another *Musa* sequence (*MUSA*2) and ASUM2, together with BSV primers in various orientations. The products of these amplifications are currently being analysed.

FISH has been conducted on chromosomal spreads of various *Musa* cultivars using probes based on the *Musa* and BSV sequences. These show several chromosomal sites where the fluorescence from the two probes co-localizes. Probing of fibre stretches of *Musa* chromosomal DNA reveals a series of tandem repeats of *Musa* and BSV sequences. Thus, there appear to be several copies of the BSV integrant which is being characterized by the sequence-specific amplification described above.

Thus, the present picture of active BSV integrants is that various candidates are being isolated and being tested against various models. The simplest is that there is a direct integrant which can yield the essential intermediate of activation, the more-than-genome length transcript. It may be that this transcript arises by recombination from two or more pieces of BSV sequence. Another question being addressed is whether the promoter for activation of transcription is that of BSV or is in the *Musa* sequence adjacent to the insertion.

Once the active integration form is identified a diagnostic can be developed using PCR across the *Musa*/BSV interface with primers from the *Musa* and BSV sequences.

Some questions posed for BSV

These and other studies on BSV raise various questions in relation to the direction of future work on this virus. As well as gaining a basic understanding of this unique plant viral system these questions can be directed at diagnostics and control.

Diagnostics

Firstly, do we need diagnostics for BSV? It is generally assumed that diagnostics will lead to control both in the field and in the movement of planting material. Thus, if we need diagnostics, for what stages are they required? It can be argued that, as BSV is integrated into the host genome, there would be no need for detection of the virus or its sequence. However, it is uncertain as to how many cultivars carry activateable integrated BSV. So, assuming that diagnostics are needed, the following points arise in relation to the three viral forms.

As noted above, the current and developing techniques for detecting the encapsidated episomal form depend on understanding the variation of the virus. Thus, we need to have a full picture of the serological and sequence variation of BSV. We already know of a wide range of "strains" and further ones will no doubt be revealed by failures of the current techniques to recognise other viral isolates. This raises the further question that, if the current approaches have potential limitations, are there any non-variation specific approaches? Can diagnostic tests be based on any gene function conserved across all variants?

The understanding of the symptom suppression form of BSV infection is currently in the hypothesis stage and experiments are being undertaken to determine if this phenomenon resembles that of CaMV described above. Even if it does not, the probing of unencapsidated DNA replication intermediates might be another approach to diagnostics which could be non-strain specific. However, it requires more sophisticated technology than that for detecting the encapsidated episomal form and should be used as a secondary backup to test plants in which encapsidated virus was not detected.

There are numerous questions about integrated BSV DNA. While there is no doubt that BSV sequences integrate into the *Musa* genome, it still has to be formally proved that at least some of them are activateable. This raises the question of how do we identify the activateable integrated form(s)? One circumstantial approach is to show that it/they are present in cultivars which have a history of activation and absent from those which do not have such a history. This means generating information, which can be used to identify such cultivars. A recently initiative involves collating the data concerning the propensity of cultivars to show BSV infection after tissue culture. From this information and analyses of integrated forms we should also be able to determine if there is more than one form of activateable integrated BSV sequence. This raises the questions of how this information should be used and whether there should be "parental health testing" to identify cultivars which could potentially have BSV problems in tissue culture or breeding progeny.

Control

There are currently no really effective ways of controlling episomal BSV apart from ensuring that planting material is disease free and by eradication. There is conflicting evidence on the extent and significance of horizontal vector-borne spread of BSV by mealybugs. Thus, there is a need to gain firm data on this and on whether BSV can spread from sugarcane to banana. As there appear to be few or no natural forms of resistance to BSV which could be bred in by conventional crossing, possible transgenic approaches should be considered. Although such approaches are being used widely and successfully with other virus-host combinations, the potential complications of integrated viral sequences, whether active or inactive, have to be considered in the case of BSV. Similarly, there is no case history which can show the potential of the transgenic approach in overcoming the active integrated form.

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Current status of banana streak virus in Costa Rica

P. Ramírez[1] and C. Rivera[2]

Banana and plantain production in Latin America and the Caribbean generates very important economic and social benefits. In Costa Rica there are around 49,000 ha planted with banana and 7,700 ha cultivated with plantain. Both fruits are important elements in the Costa Rican's diet and they also constitute subsistence crops for many families. In recent times, plantain production has become oriented more towards the export market. This has allowed an increase on the activity's aggregated value from US$195,600 to US$335,600; a 16% average annual growth rate and a 71.6% increment for the period 1992-1996. The plantain production growth rate has placed this activity in fifth place nationally in terms of relative importance, close to rice production (Work Document of the National Commission of Agriproductive Systems, 1998).

Virus diseases are limiting factors for banana and plantain production world-wide. Among the main virus diseases which affect these crops are: banana streak virus (BSV) (Brunt *et al*. 1987), cucumber mosaic virus (CMV) (Francki *et al*. 1979), banana bunchy top virus (BBTV) (Dale 1986) and banana bract mosaic virus (BBrMV) (Brunt 1991) (Ramirez and Rivera 1994). The only virus disease reported previously in the literature affecting banana and plantain in Costa Rica was mosaic disease caused by cucumber mosaic virus (Rivera *et al.* 1993). Chlorotic streak symptoms with subsequent necrosis similar to those reported for BSV (Jones and Lockhart 1993) have been observed in Costa Rica for many years. Nevertheless, it was not until 1994 that BSV was detected using a specific diagnostic method (Ben Lockhart, personal communication).

This paper reports on the presence of BSV in Costa Rica, its current status, the existing capacity for research and diagnosis of this disease, as well as future plans and research perspectives regarding the virus.

Today, the Cellular and Molecular Biology Research Center (CIBCM) of the University of Costa Rica has the necessary technology to diagnose and to detect BSV (electronic immunomicroscopy and TAS-ELISA). This technology was transferred by Dr Lockhart from the University of Minnesota. During 1997, 144 banana and plantain samples from the field and from *in vitro* cultures, were analyzed using ISEEM and TAS-ELISA techniques; 38 of them tested BSV positive (Table 1).

In October 1997, the Ministry of Agriculture and Husbandry (MAG) organized a meeting to define the status of BSV in Costa Rica. The main conclusions of this meeting were the following:

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Table 1. BSV diagnosis results according to the origin of samples and techniques employed.

BSV positive samples/total samples analyzed				
Techniques	**Field samples**		***In vitro* culture samples**	
ISEM	1/1	3/14	0/11	5/13
TAS-ELISA	6/10	6/11	13/75	4/9

- BSV was already in Costa Rica affecting banana and plantain before the introduction to the country of some new materials from banana improvement programmes;
- There is a lack of knowledge regarding BSV epidemiology and its economic impact in the country;
- The University of Costa Rica's CIBCM has the technology and installed capacity to conduct research as well as routine diagnosis of BSV;
- There are no regulations covering banana and plantain micropropagation.

Several activities were proposed during the same meeting to address the problem of BSV:

- To eliminate any BSV suspicious *Musa* material,
- To establish quarantine measures for the introduction of material from other countries, either from public or private entities and improvement programmes,
- To regulate the *in vitro* propagation and distribution of suspicious materials to present possible viral infections after multiplication,
- To establish routine evaluations of introduced materials and *in vitro* culture mother plants through specific detection methods.

These recommendations are still under discussion.

The CIBCM, within its Virus, Viroid and Phytoplasm Diagnostic and Characterization of Food and Economic Importance Crops Programme (PCDV-CIBCM), offers banana and plantain virus diagnosis services. The CIBCM, in collaboration with different private and public, national and international organizations, is also developing BSV epidemiology research with the purpose of determining the geographical distribution, incidence, and economic impact of the disease, and identifying alternate host plants and virus vector insects. A collaborative project is also being developed which will study the relationship between *in vitro* culture and BSV infection.

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Banana streak badnavirus infection in *Musa* plantations in Colombia

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Plantains and bananas (*Musa* sp.) are a basic food source in many regions of the world. In Colombia, approximately 1 000,000 acres are planted with plantains (mostly for local consumption) and 120,000 acres are planted with Cavendish bananas for export. Colombia is the second largest exporter of bananas in South America. Both crops are important for social and economic reasons since they play a significant role in income generation, as well as providing a basic food and employment opportunities. Of the five viral diseases affecting *Musa* spp. (abaca mosaic, banana bract mosaic, banana bunchy top, banana mosaic and banana streak), only banana mosaic and banana streak disease have been reported in Colombia.

The causal agent of banana mosaic disease is cucumber mosaic virus (CMV). Banana mosaic disease has been found in most banana-growing areas of the world and in Colombia it was first observed in 1940 affecting plants of plantain (Belalcázar 1992). This virus was recently isolated and partially characterized in Colombia (Casta. *et al*. 1995, Reichel *et al*. 1996), and has been reported to have a major impact on banana production in the country (Belalcázar *et al*. 1996). The other viral disease affecting *Musa* sp. in Colombia is viral leaf streak of banana. This disease was first described from Côte d'Ivoire, Africa in 1968 (Lassoudière 1974) and the causal agent banana streak virus (BSV) was identified in 1985 (Lockhart 1986). In Colombia banana streak disease was discovered in late 1995 in the locality of Andes (Antioquia) affecting plants of plantain 'Dominico-Hartón (*Musa* AAB Simmonds, Reichel *et al*. 1996).

The objectives of this study were:

- to isolate the causal agent of banana streak disease (BSV) affecting plantains 'Dominico-Hart on' from the locality of Andes, Antioquia (Colombia);
- to determine if other plants (sugarcane, arrowroot, coffee, etc.) from the vicinity of the BSV-affected plantain plantation were also affected by a badnavirus in order to later determine if these plants might be part of the host range of BSV.

Results

A badnavirus was isolated from a BSV-affected plant of plantain 'Dominico-Hartón' with typical banana streak symptoms from Antioquia, Colombia. The badnavirus is bacilliform and measures ca. 150 x 30 nm (Figure 1) with an A 260/280 ratio of 1.2. The virus was

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detected by serological tests (DAS ELISA) and by electron microscopy. This is the first report of the isolation of BSV from *Musa* sp. in Colombia.

An association of BSV with flexuous viral particles (possibly potexvirus) was detected in a viral purification from a BSV-infected plant of plantain 'Dominico-Hartón' with symptoms of chlorotic streak characteristic of banana streak disease. (Figure 2) This is the first report of an association of BSV with flexuous viral particles affecting *Musa* spp. in Colombia.

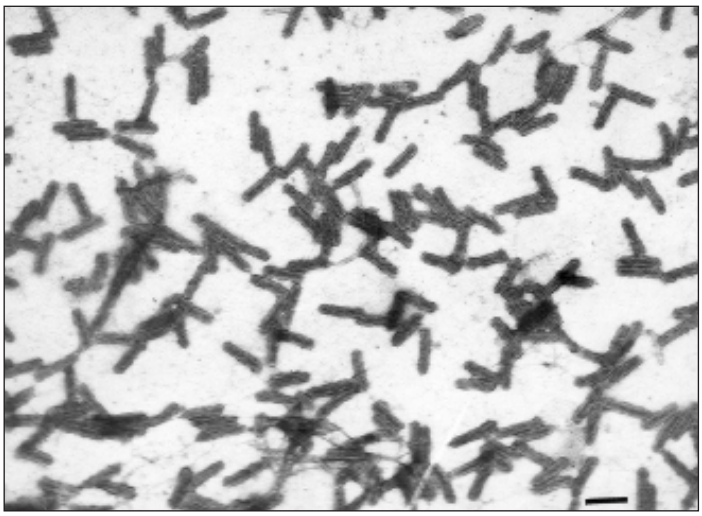

Figure 1.
Electron micrograph of bacilliform virus particles (ca. 150 x 30 nm) isolated from a BSV-infected plant of plantain that showed foliar chlorotic streak symptoms, typical of banana streak disease from the locality of Andes, Antioquia (Colombia). Scale bar represents 150 nm. (Courtesy of R. Pérez).

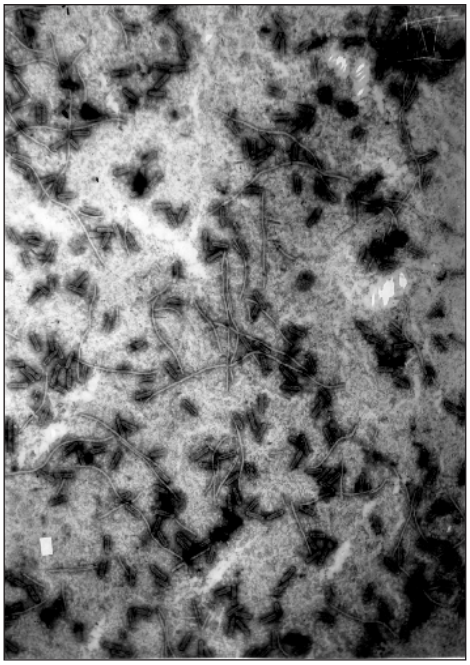

Figure 2.
Electron micrograph of flexuous, viral particles associated with BSV in a viral purification from a plantain leaf with chlorotic streak symptoms (which was confirmed to be BSV-infected by DAS-ELISA tests with polyclonal antiserum to BSV). Scale bar represents 150 nm. (Courtesy of R. Pérez).

Positive serological reactions to badnavirus with polyclonal antisera to BSV were obtained when serological tests (DAS-ELISA) were carried out on sugarcane, arrowroot and coffee leaves with virus-like symptoms (mosaic, chlorosis). Electron microscopy studies confirmed the presence of bacilliform particles in sugarcane and arrowroot leaves by ISEM. Electron microscopy studies are being carried out on coffee leaves, which gave positive serological reactions for badnavirus.

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Appendix 1. Indexing for banana streak badnavirus

Conventional indexing for BSV has been described in the FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* Germplasm. This involves symptom observation and indexing plants from tissue culture at three and six months by immunosorbent electron microscopy using minipreps. This procedure is currently considered to be the most reliable to detect a wide range of BSV isolates.

It was agreed that testing for BSV should continue to based upon the detection of virus particles (episomal form).

It has been observed that temperature changes promote symptom expression. In some cases temperature changes can occur due to natural changes in ambient environmental conditions. In other situations, this can be simulated by moving the plants to 20-25°C for at least six weeks. Virus Indexing Centres are already implementing the use of changes in temperature to promote symptom expression.

It is recommended that when a large proportion of samples is expected to be positive, triple antibody sandwich (TAS) – ELISA, using broad-spectrum antisera able to detect a wide range of different isolates of BSV is carried out before detailed ISEM investigation.

Appendix 2. Recommendations for tissue culture propagation of *Musa*

In view of the problems encountered in relation to the appearance of BSV symptoms in banana plants of certain genotypes following multiplication by tissue culture, the application of tissue culture for *Musa* mass propagation needs to be reassessed.

However, it should be noted that mass propagation is frequently of Cavendish-types of banana and currently there is no evidence of BSV expression in these banana cultivars following passage through tissue culture. In such genotypes therefore, BSV is considered unlikely to be a cause for concern.

If micropropagation of other *Musa* genotypes is proposed, the risk of BSV expression exists. The recommendations for the mass propagation of non-Cavendish genotypes are as follows:

- test mother plants for the presence of the virus before initiating tissue cultures;
- test representative samples of the progeny;
- consider utilizing other mass propagation techniques if the genotypes are being proposed for commercial multiplication.

Appendix 3. Modified INIBAP germplasm health statement

(to accompany tissue culture material when distributed)

The germplasm designated below was obtained from a shoot-tip cultured *in vitro*. Shoot-tip culturing is believed to eliminate the risk of the germplasm carrying fungal bacterial and nematode pathogens and insect pests of *Musa*. However, shoot-tip cultures could still carry virus pathogens.

Screening for virus pathogens

A representative sample of four plants, derived from the same shoot-tip as the germplasm designated below, has been grown under quarantine conditions for at least six months, regularly observed for disease symptoms and tested for virus pathogens as indicated below following methods recommended in the FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* Germplasm for the diagnosis of virus diseases.

ITC-code	accession name	origin	serology - ELISA				Electron microscopy		
			CMV	BBTV	BSV	BBrMV	Bacill	Iso	Fil

CMV = cucumber mosaic virus
BBTV = banana bunchy top virus
BSV = banana streak virus
BBrMV = banana bract mosaic virus
Bacill = bacilliform virus particles - includes BSV
Iso = isometric virus particles - includes CMV
Fil = filamentous virus particles - includes BBrMV
[+] = test positive
[-] = test negative
[o] = test inconclusive
[ ] = test not undertaken

The information provided in this germplasm health statement is based on the results of tests undertaken at INIBAP's Virus Indexing Centres by competant virologists following protocols current at the time of the test and on present knowledge of virus disease distribution. It should be noted that some virus-tested (negative) hybrids, after *in vitro* multiplication, have developed BSV infection and may express symptoms.

Neither INIBAP, nor its Virus Indexing Centre staff assume any legal responsibility in relation to this statement.

Signature: Date:

This statement provides additional information on the phytosanitary certificate of the plant germplasm described herein. It should not be considered as a substitute for the official "Phytosanitary Certificate" issued by the plant quarantine authorities of Belgium.

Appendix 4. Production of virus-tested *Musa* germplasm for international distribution by IITA

IITA has produced a number of plantain and banana hybrids that are resistant to black Sigatoka disease; they are high-yielding and have a fast rate of cycling or ratooning through regulated suckering (Vuylsteke *et al*. 1993, Vuylsteke and Ortiz 1995, Vuylsteke *et al*. 1995). In addition, some hybrids have other attributes such as earliness, shorter stature, large fruit and good fruit quality. Some hybrids have also shown a lower incidence of virus symptoms, suggesting field tolerance to virus (Ortiz and Vuylsteke 1998a, 1998b, Ortiz *et al*. 1998). IITA also has FHIA hybrids of banana and plantain, which are available for distribution. IITA works closely with the Nigerian Plant Quarantine Service and Plant Quarantine Services from other countries to ensure safe movement of germplasm. With assistance from IITA's Germplasm Health Unit, the FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* Germplasm (Diekmann and Putter 1996) are followed. As the *Musa* germplasm is multiplied *in vitro* on medium free from charcoal, antibiotics and fungicides, freedom from insects, fungi and bacteria can be assured. Virus indexing for a range of viruses known to infect *Musa* is then carried out. This virus-tested improved germplasm is available to NARS and other agricultural organizations with interest in *Musa* improvement. IITA hybrids and other germplasm are available from IITA upon request from countries in Africa. Countries outside Africa should continue to obtain their IITA germplasm through INIBAP Transit Centre at the Katholieke Universiteit Leuven, Belgium.

Virus diseases of *Musa*

Several viruses have been reported to infect *Musa* and to cause disease. These diseases, their causal agents and their international distribution are shown in Table 1. Banana bunchy top, banana mosaic and banana streak are found in Africa. Banana bunchy top is of limited distribution while banana mosaic and banana streak appear to be widespread. Abaca mosaic and banana bract mosaic have yet to be found in Africa. Another disease, banana die-back, has recently been described only from Nigeria (Hughes *et al*. 1998). The distribution of banana bunchy top and banana streak in Africa are shown in Figure 1.

Table 1. Virus diseases of *Musa,* their causal agents and geographical distribution.

Virus disease	Causal agent	Geographical distribution
Abaca mosaic	A potyvirus, possibly a strain of sugarcane mosaic potyvirus	Asia (Philippines)
Banana bract mosaic	Banana bract mosaic potyvirus	Asia (Philippines, India and Sri Lanka)
Banana bunchy top	Banana bunchy top virus	Africa, Asia, Australia and Pacific Islands
Banana mosaic	Cucumber mosaic cucumovirus	Occurring in all continents and many countries of each continent
Banana streak	Banana streak badnavirus	Europe, Africa, Asia and Oceania
Banana die-back	Banana die-back virus	Africa (Nigeria)

Figure 1. Geographical distribution of banana bunchy top virus (BBTV) and banana streak virus (BSV) infecting *Musa* spp. in Africa (Diekmann and Putter 1996).

Virus indexing

The following virus-indexing procedures are followed at IITA. The procedure is shown in Figure 2.

Seven clonal plantlets are produced *in vitro* from a single banana or plantain meristem. Two of the plantlets remain in tissue culture (*in vitro*) to serve as the source material for the clonal lines if the plantlets test negative for all characterised viruses known to infect *Musa*, and no virus-like symptoms are seen or virus-like particles observed by electron microscopy.

The five plantlets, which are to be virus-indexed, are potted in sterile soil and 'hardened' in an insect-proofed screenhouse. After about three weeks, when they are well established in the pots, the plantlets are moved to a controlled-temperature room (23°C (2°C) under fluorescent lights on a 12 hour day/12 hour night regime. This environment promotes virus symptom expression in *Musa*, particularly symptoms expressed by plants infected with banana streak badnavirus (BSV), and also promotes a concurrent increase in virus concentration in the leaf tissue. This enables virus detection to take place more readily using conventional serological diagnostics.

The plants are grown in the controlled environment for a period of six months. They are examined weekly for symptom expression, tested after three months by enzyme-linked immunosorbent assay (ELISA) using sap samples for cucumber mosaic cucumovirus (CMV) and BSV (both endemic in Nigeria). Plants of a clonal group from which all plants test negative for these two viruses are additionally checked for banana die-back virus (BDBV). The serological tests performed on the plantlets are detailed in Table 2. After a

Table 2. Diagnostic tests performed on *Musa* plantlets for virus-indexing after transfer to controlled-environment conditions.

Viruses	Diagnostic test	Antibody source	Time
Sugarcane mosaic potyvirus	ELISA[1], EM[2]	ATCC[3] (using PTY general potyvirus group antibodies)	6 months
Banana bract mosaic potyvirus	ELISA, EM	QDPI[4]	6 months
Banana bunchy top virus	ELISA	QDPI	6 months
Cucumber mosaic cucumovirus	ELISA, HIP[5]	IITA[6]	3 and 6 months
Banana streak badnavirus	ELISA, ISEM[7], IC- PCR[9]	IITA, UM[8]	3 and 6 months
Banana die-back virus	ELISA, HIP	IITA	3 and 6 months

[1] Enzyme-linked immunosorbent assay
[2] Electron microscopy
[3] American Type Culture Collection, USA
[4] Queensland Department of Primary Industries, Australia
[5] Herbaceous indicator plants
[6] International Institute of Tropical Agriculture, Nigeria
[7] Immunosorbent electron microscopy
[8] University of Minnesota, USA
[9] Immuno-capture polymerase chain reaction

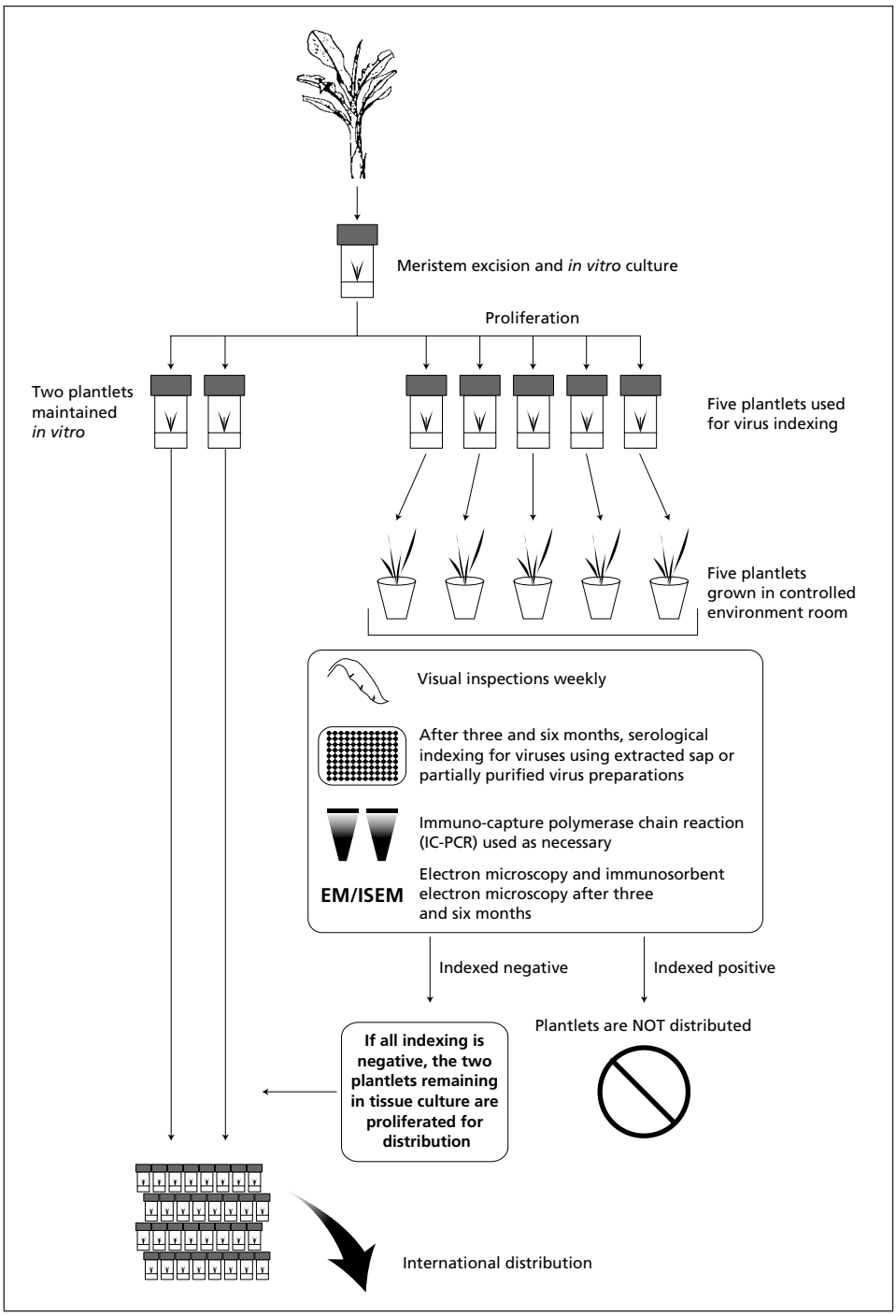

Figure 2. Virus testing of IITA *Musa* germplasm.

further three months (total of six months) of weekly visual inspection, the plants are again tested for CMV and BSV by ELISA using both sap extracts and partially purified virus preparations. For detection of CMV, herbaceous indicator plants may be inoculated. If the banana and plantain plantlets test negative, they are again screened for BDBV (possibly including inoculation of partially-purified virus preparations to herbaceous indicator plants) and also for banana bunchy top virus (BBTV). At this stage they may also be tested for abaca mosaic and banana bract mosaic if warranted. Both sap extracts and partially purified preparations are then tested by immunosorbent electron microscopy (ISEM) for BSV, and both the sap extracts and partially purified preparations examined directly by electron microscopy for the presence of virus-like particles. For those plants which test negative, immuno-capture polymerase chain reaction (IC-PCR) is also carried out as a final check for the presence of BSV.

If no symptoms are seen, no viruses are detected in the plants and no virus-like particles observed by electron microscopy, the two plantlets of the clone remaining in tissue culture are proliferated. For distribution, the plantlets will be accompanied by a Plant Health Statement from IITA's Germplasm Health Unit listing the tests performed on the plantlets and the results obtained. Prior to distribution, the plantlets will receive a phytosanitary certificate from the Nigerian Plant Quarantine Service who will inspect the plantlets and laboratory test results prior to certification. The micropropagated plantlets are then available for distribution.

Plant health follow-up

IITA has held several training courses including diagnosis of virus diseases of *Musa* spp. based on symptom expression as well as diagnosis of the virus diseases using serological diagnostics, electron microscopy and polymerase chain reaction. This has resulted in the training of 42 national staff from eight countries (Ethiopia, Ghana, Kenya, Madagascar, Malawi, Nigeria, Tanzania and Uganda) in addition to postgraduate training of Nigerian and Cameroonian students. The diagnostic reagents have been distributed to trained collaborators in several countries. Where specific diagnostics can not be carried out, leaf samples can be sent (with prior arrangement) to IITA (Ibadan) for serological diagnostics and electron microscopy.

This training and availability of diagnostics either *in situ* or at IITA, in addition to visits by IITA scientists, help to ensure the continued health of banana and plantain germplasm distributed by IITA within Africa.

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Acronyms and abbreviations

BADC	Belgian Administration for Development Cooperation
BBrMV	banana bract mosaic virus
BBTV	banana bunchy top virus
BDBV	banana die-back virus
BSV	banana streak virus
CaMV	cauliflower mosaic virus
CFC	Common Fund for Commodities, the Netherlands
CIBCM	Cellular and Molecular Biology Research Center
CIRAD	Centre de coopération internationale en recherche agronomique pour le développement, France
CMV	cucumber mosaic virus
CORPOICA	Corporación Colombiana de Investigación Agropecuaria
CSSV	cacao swollen shoot virus DNA
CYMV	commelina yellow mottle virus
DNA	deoxyribonucleic acid
ECS	embryonic cell suspension
ELISA	enzyme-linked immunosorbent assay
ESARC	East and Southern Africa Regional Centre, IITA
FISH	fluorescent *in situ* hybridization
IC-PCR	immunocapture polymerase chain reaction
IITA	International Institute of Tropical Agriculture, Nigeria
INIBAP	International Network for the Improvement of Banana and Plantain, France
ISEM	Immunosorbent electron microscopy
ITC	INIBAP Transit Center, Belgium
KUL	Katholieke Universiteit Leuven, Belgium
NARS	National Agriculture Research System
ORF	open reading frame
PCR	polymerase chain reaction
RNA	ribonucleic acid
RnaseH	ribonucleaseH
RTBV	rice tungro bacilliform virus
SCBV	sugarcane bacilliform virus
TAS-ELISA	triple antibody sandwich enzyme-linked immunosorbent assay
TC	tissue culture
VIC	Virus Indexing Center

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