MAURITIUS RESEARCH COUNCIL

# GENETIC VARIANTS OF BANANA STREAK VIRUS IN MAURITIUS

**Final Report**

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# Genetic variants of *Banana Streak Virus* in Mauritius

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Start of Project April 2001

Appointment of first research assistant Ms Nisha Dabysing who left two months after her appointment (April-May 2001)

Appointment of second research assistant Mr Ahswin Khorugdhary for a period of two years (June 2001 –June 2003)

# GENERAL INTRODUCTION

Banana Streak Virus (BSV) is a very common viral pathogen of bananas and plantains *(Musa* spp) and has been reported in most countries where this crop is grown. It was first reported in Ivory Coast in 1974 (Lassoudiere, 1974). Since then it has been identified in plantations in other African, South American and Carribean countries as well as China and India. The virus was first isolated in Morocco in 1986 (Lockart, 1986) and characterized. Detection methods have been set up to establish the extent of the problem and to follow the spread of the pathogen. It is primarily spread through vegetative propagation of the mother plant.

The observation that in vitro propagated Musa genotypes showed evidence of virus infection even when starting with clean materials, has prompted research to explain this phenomenon (Ndowora et al 1999). Virion particles were present in the plantlets even if parents from which hybrids were produced were free of the virus. The appearance of virus from such cultures was explained by the fact sequences of BSV had been found integrated in the Musa genome (Ndowora et al.,1999;Geering et al., 2001). It is now well established that many Musa genotypes screened so far, have integrated BSV sequences. This has meant that current Musa breeding programmes are faced with a difficult issue to resolve.

**Symptoms and Detection**

The disease symptoms vary and appear as clear yellow streaks, or as black or brown necrotic ones. Necrosis of the pseudostem and deformed bunches are also typical symptoms. A particular feature of this disease is the periodic variations in symptoms. They can be conspicuous at times and may disappear altogether at a later stage. This is most likely an indication of the viral titre at the different times and can be ambiguous for detection tests. Dahal et al. (1998) have emphasized the need to collect material during cool and rainy periods when the viral titre is higher than during hot and dry conditions. The guidelines for safe international movement of Musa germplasm (Diekmann and Putter, 1996) recommends that the indexing by serological methods of *in vitro* plantlets,

be carried out after growing them over a period of nine months in a cool environment (22 ° C). Younger leaves do not seem to have higher concentration of virus.
Detection methods were first based on serology (ELISA) even if the sensitivity is sometimes lower than what is required. But for large-scale detection, this method is adequate. Antibodies are available commercially from AGDIA while many laboratories have their own. In immunosorbent electron microscopy, antibodies are used to trap the virus for electron microscopy. Although the sensitivity is thus greatly enhanced this approach requires sophisticated equipment and appropriate expertise.
More practical methods of detection have been designed mainly using the polymerase chain reaction (PCR). This has followed the sequencing of various segments of the genome and the design of oligonucleotide primers. However, in order to detect episomal DNA it is important that the virion particles be first trapped using antibodies. This will ensure that what is being amplified are not from the integrated sequences.

The consequences of the disease vary among different varieties. It has been reported to have detrimental effects on the yield and quality of fruits. In many cases death of the plants do occur.

**Taxonomy and Characteristics of the Virus**

Family: Caulimoviridae

Genus : Badnavirus

BSV is a member of the genus badnavirus. The virion particles are non-enveloped and bacilliform. It is reported that the virion-associated-protein assemble into tetramers. The genome consists of a circular, double stranded DNA molecule of 7.4 kb. Replication occurs through reverse transcription. Serological data have pointed to the very heterogeneous nature of the virus. This has been further confirmed by molecular studies (Geering et al. 2000).
Plant pararetroviruses belong to the Caulimoviridae family which include

six genera of viruses each having different biological, serological, and molecular characteristics. They have several conserved motifs found in retroelements and these include the polymerase, the primer binding site, aspartic protease, RNase H, and Zn finger. Similarly there are also some domains which are specific to plant pararetroviruses such as the movement, proline-rich, nucleic acid-binding, and transactivator domains. Genomic sequences for four members of the badnavirus genus are available. They include those for *Commelina* Yellow Mottle Badnavirus (CoYMV), which is the type member, rice tungro bacilliform virus (RTBV), sugarcane bacilliform virus (SCBV), banana streak virus (BSV) and cacao swollen shoot virus (CSSV). They all show the same genomic organization with three open reading frames (ORFs), except for RTBV which has four ORFs. ORFs I and II encode small proteins whose function are not known. ORF III encodes a polyprotein which is cleaved by an aspartic protease. The products of this proteolytic activity are the coat protein, an aspartic protease, reverse transcriptase and RNase H. A putative cell-to-cell movement protein is also produced in *Commelina Yellow Mottle Badnavirus*. Using deletion mutants of the putative cell-to-cell movement protein of CoYMV, Tzafrir et al (1997) have shown that the N-terminal portion of the polyprotein of ORF III is responsible for this function. Deletion mutants in this terminus that maintain the reading frame were used. Aspartate or histidine of this region were deleted as these are known to be highly conserved in cell-to-cell movement proteins of other viruses. Commelina diffusa were agro-inoculated with these mutant constructs in vitro and viral replication was observed. Virion particles were isolated from such infected tissues. In contrast replication-deficient mutants did not produce virion particles.

RTBV has an additional ORF IV.

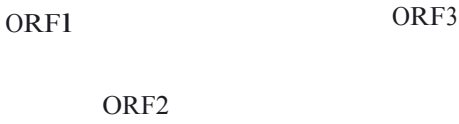



Figure 1 Genome Organisation of Banana Streak Virus, 7389 nts. The sequences for Aspartate Protease, Reverse transcriptase and Rnase H are found in ORF3.

Replication happens through a longer than genome length +ve sense RNA . An initiator methionine tRNA primes the negative strand synthesis. Translated viral products then appear in the cytoplasm. The viral reverse transcriptase converts viral RNA into DNA forming a RNA/DNA complex. The latter is then converted into gapped double stranded DNA by virion RT. Assembly of viral proteins and ds DNA produce mature viral particles. The normal infection cycle does not include an integration stage. As a result of the replication mode, there are discontinuities in the genome which is a feature of pararetroviruses. They are site-specific discontinuities which contain overlaps between the 5' end and the 3' end of the two strands.

The molecular characterization of BSV has been possible following the sequencing of a Nigerian isolate of the virus. Harper and Hull (1998) have cloned and sequenced the whole genome. It is made up of 7389 bp (accession number AJ002234 in EMBL database) and show some similarities to other badnaviruses. Its similarity is highest with ScBV but it is different enough to be a virus of its own. It is phylogenetically furthest away to RTBV. ORF I and ORF II overlap at ATGA sequence which has the start codon for ORF II and stop codon for ORF I, while ORFs II and III overlap at a TAATG sequence.

## Detection of BSV DNA

From these sequence data, primers were designed to amplify the reverse transcriptase and aspartate protease region (Harper and Hull 1998). This set of primers has proven effective in amplifying the viral DNA from many different *Musa* varieties.

Similarly, Geering et al. (2000) have sequenced DNA fragments (the Rnase H and tRNA binding site) from four different isolates of BSV originating from different banana cultivars, Red Dacca, Williams( Cavendish), Mysore and Goldfinger. This allowed the design of primers specific for the four isolates and comparison of the sequences obtained. It was found that the sequence of BSV-RD was identical to that of BSV-Onne Nigerian isolate) BSV-Cav had 95.1 % identity with BSV-Onne, which is more than what BSV• Mys and BSV-GF had.

## Integration in the *Musa* genome

It has been found that the virus, from tissue cultured plantlets, all had the same restriction digestion pattern and therefore very likely to be of the same origin. Two classes of integrants have been been identified. One type consists of only parts of the BSV genome that do not seem to be able to produce infectious particles. The other type however, is found mostly in the *Musa balbisiana* genome and is made up of multiple copies of the complete BSV genome. It is likely that the tissue-cultured induced stress results in the a homologous recombination process thereby forming infectious virus.

Geering et al (2001) have also studied the restriction hybridisation patterns of Musa genomic DNA with labeled BSV probes and have found that BSV-OL integrants are associated with the B-genome of cultivated Musa (eg Obino l'Ewai) and while the badnavirus DNA from cultivar Williams (AAA) could be found in the A-genome cultivars only.

## Aim of Project

This work was carried out to identify genetic variants of BSV in different cultivars of banana in Mauritius and to investigate the varietal correlation. It also aimed at determining the extent to which the BSV sequences are related to those of Sugarcane Bacilliform Virus (ScBV) which is also widely present, even in noble canes in Mauritius. As has been shown elsewhere, the virus is heterogeneous (Lockhart and Olszewski, 1993). No information was available on the exact nature of BSV in Mauritius and since

many new cultivars have been introduced from other countries, there was a possibility that different strains of the virus are present

**Objectives of the project**

The main objectives of the work are:

1. To test primers which were already available in the literature on local samples
2. To use those primers to analyse the genetic variability among the samples
3. Optimisation of the detection methods on virus extracts
4. Virus Purification to confirm the nature of the virus through electron microscopy
5. Field assessment of the presence of possible vectors
6. Isolation and cloning of PCR products
7. Nucleotide sequence analysis

# RESULTS

## *1.* Primer selection for the detection of local isolates of *banana streak virus*

A first screening procedure was adopted by amplification from leaf DNA extracts. All samples were used for DNA extraction and the primers tested. The preliminary results were then analysed and samples were selected for virus purification and immunocapture•

r BSV was to detect the virus either in virus

PCR. The approach that was appropriate fo

preparations or through the technique of immunocapture-PCR (IC-PCR). Both were tried and found to work with this virus. However, virus purification is a lengthy process and cannot be performed on a large number of samples. Therefore IC-PCR was used to confirm results obtained with the DNA extracts.

### 1.1 Virus Purification

This was done according to Harper et al (1998).

- Leaf material was ground in liquid nitrogen and a buffer A (50 mM sodium phosphate pH 6.1, 5mM DTT( dithiothreitol), 5 mM diethyldithiocarbonate and 0.5 % polyethylene glycol (PEG)
- Celluclast was added to 2 % and the mixture incubated overnight at room temperature
- Triton X-100 was then added to 1 % and incubation for 30 minutes
- After a centrifugation at 10,000 g for 10 min the supernatant was subjected to a high-speed centrifugation at 120,000 for 90 minutes
- The pellet thus obtained was then resuspended in 100 ml of buffer A and centrifuged for another 2.5 hr at 120,000 g
- The final pellet was resuspended in 5ml buffer A.

This preparation was used for electron microscopy and bacilliform particles were visualized on most of the samples tested.

The virus preps were also used for IC-PCR and for direct PCR.

## 1.2 Immunocapture PCR

This requires that the virus particles are first immobilized onto the surface of the PCR tubes before the reaction is done.

- Tubes were coated with anti-BSV antibodies (AGDIA) at a concentration of 1µg/ml in 0.05M sodium carbonate buffer, pH 9.6 and incubated for 2 hours.
- Washing was done twice with phosphate buffered saline+ Tween 20 0.05 %( PBS-T)
- Leaf extracts were made by grinding the leaf tissues in 2 ml of 0.05 M Tris-HCl, pH 7.4 containing 5 % skim milk and 0.5 % sodium sulphite
- After centrifugation, the supernatant, 25 µl, was added to the coated tubes followed by an overnight incubation at 4° C
- After a thorough washing with PBS-T, the PCR mix (see below) was added and tubes were placed in a thermal cycler with the appropriate cycling programme

PCR MIX

| | |
|---|---|
| Buffer (10X) | 3 µl |
| dNTPs (2.5 mM) | 2.4 µl |
| primer 1(10 µM) | 1.5 µl |
| primer 2 (10µM) | 1.5 µl |
| Taq polymerase (5U/µl) | 0.2 µl |
| DNA template | 2µl |
| Water | up to 30 µl |

The $MgCl_2$ was adjusted to give a concentration of 1.5 mM

### 1.3 Primers tested

The sequences of primers used were from two different sources.

Harper et al 1999. had designed several sets of primers.

The ones used here are referred as: 3012 (BSV4673) and 1573( BSVr5317)

and 3068 which was used with 3012 to give a longer fragment of the same region. This part of the genome codes for the aspartate protease and reverse transcriptase.

The sequence of the primers are :

3012 (BSV4673) 5' GGAATGAAAGAGCAGGCC3'

1573( BSVr5317) 5' AGTCATTGGGTCAACCTCTGTCCC3'

Geering et al 2000. Four sets of primers were tested.

| Virus isolate | Primer name | Primer sequence | Size of product (bp) |
|---|---|---|---|
| BSV-Red | RD-F1 | 5' ATC TGA AGG TGT GTT GAT CAA TGC 3' | 522 |
| Dacca | RD-R1 | 5' GCT CAC TCC GCA TCT TAT CAG TC 3' | |
| BSV- | Cav-F1 | 5' AGG ATT GGA TGT GAA GTT TGA GC 3' | 783 |
| Cavendish | Cav-R1 | 5' ACC AAT AAT GCA AGG GAC GC 3' | |
| BSV- | Mys-F1 | 5' TAA AAG CAC AGC TCA GAA CAA ACC 3' | 589 |
| Mysore | Mys-R1 | 5' CTC CGT GAT TTC TTC GTG GTC 3' | |
| BSV- | GF-F1 | 5' ACG AAC TAT CAC GAC TTG TTC AAG C 3' | 476 |
| Goldfinger | GF-R1 | 5' TCG GTG GAA TAG TCC TGA GTC TTC 3' | |

The above primers were tested on the virus isolates and the sets that produced amplification products of the given sizes were: 3012/1573 and F1/R1 cav.

These two sets were therefore used for the screening of all samples.

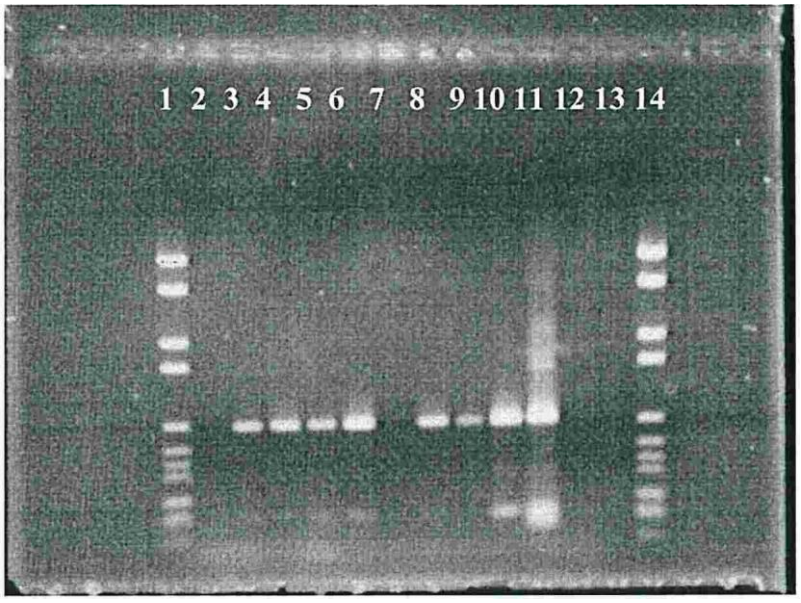



**Figure 1. PCR products using primers 3012/1573. Lanes 1 & 14: Molecular weight marker; lanes 3–6, 8–10: Mauritius isolates (Variety Mamzelle); lane 11: Obino isolate**

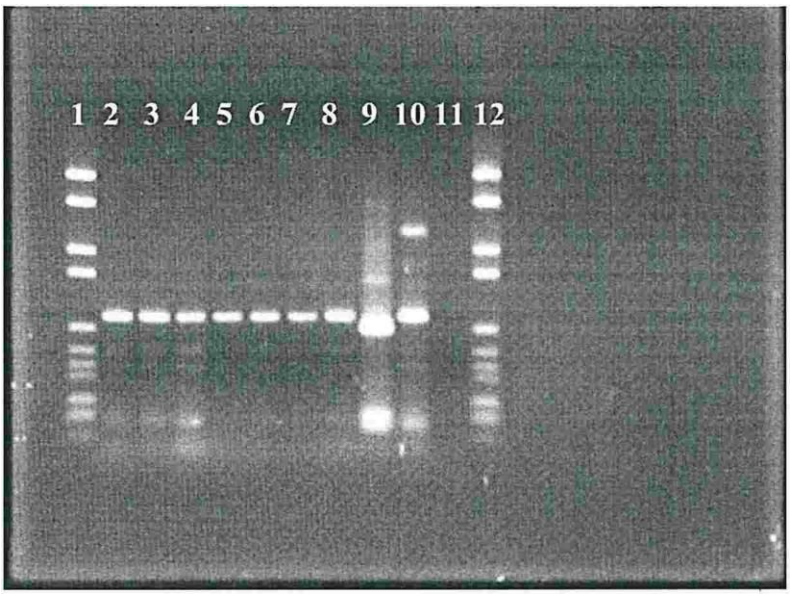



**Figure 2. PCR products using primers 3012/1573. Lanes 1 & 12: Molecular weight marker; lanes 2 -8, 10: Maur i• tius isolates (Variety Williams); lane 9: Obino isolate.**

Figure 3 shows amplification polymorphism using the primers F1/R1cav. The bands from the Mauritius isolates produced bands of two different sizes both larger (approx. 700 and 750 bp) than the Obino control isolate.

One sample, isolated from variety Ollier (lane 7), gave a clear band of higher molecular weight.

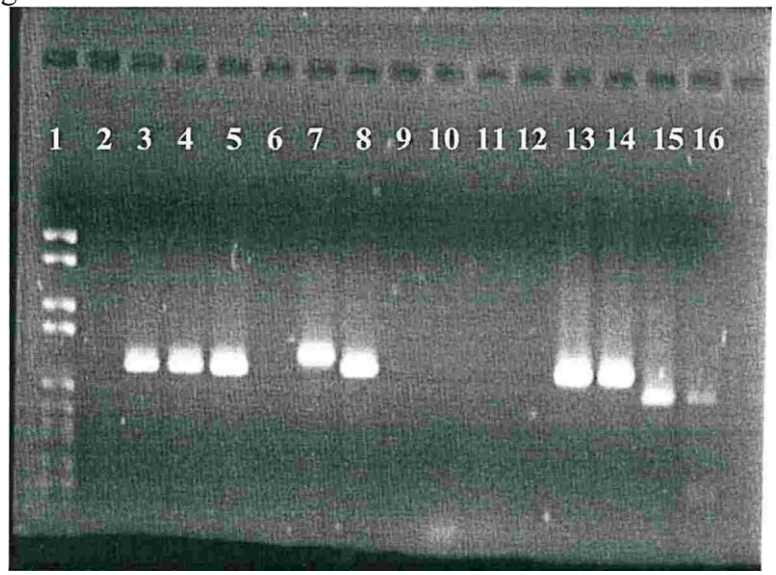



Fig.3 PCR products of F1/R1cav.

Lanes 3, 5,7,813,14: Mauritius isolates
Lanes 15, 16: Obino isolate

**Hybridisation**

In order to ensure that the amplified DNA is of BSV origin, they were hybridized with the Obino (Nigerian) product which had been labeled with DIG (Boehringer Mannheim).

*Blots with FJRJ cav products*

Blot 1 showed the signals obtained with the samples collected in Mauritius and hybridizing with the Nigerian virus probe.

Blot 2 gave bands hybridizing with the Obino probe but differentially as the intensity of the signal differs. A product originating from an isolate of variety Gingeli hybridizes

strongly with the OL probe while the other three from Dwarf Cavendish isolates give fainter signals. It was thus confirmed that the amplified products were of BSV origin. The differential intensity might point to the divergent sequences which are not highly homologous to the Obino isolate.

Another blot revealed a very strong signal with the band from isolate 8M( Mamzelle) thus indicating a high homology with the Obino isolate. The signals from other virus isolates were present but much fainter than with sample 8M.

## 2. Detecting variability among the local isolates of *Banana Streak Virus*

The two sets of primers 3012/1573 and F1/R1cav were used to amplify the corresponding region in the isolates of BSV collected from several different Musa accessions of Mauritius as well as from sugar cane bacilliform virus samples.

1. Amplification polymorphism using 3012/1573

Among the samples tested, most of them produced a band of size slightly larger than the Obino isolate, that is about 750 bp. This was seen with the following varieties: Williams, Banane La Grain, Grande Naine, Ollier, Mamoule and the imported variety Pisang Mas.

Some local samples produced a band of similar size to the Obino isolate and they are: the isolates from Mamzelle, Gingeli and Banane Rouge. Sugar cane bacilliform virus also produced a band of same size.

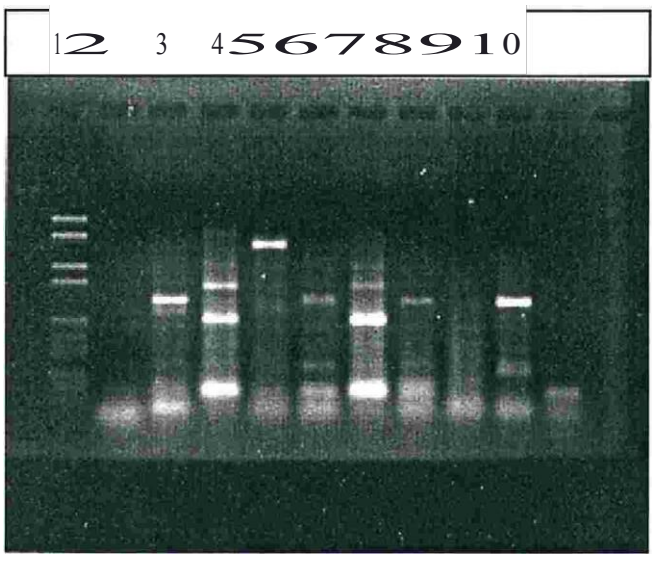



Figure 4. Amplification with primers 3012/1573 . Lane 1: molecular weight marker; lane 2: negative control; lane 3: BLGR; lane 4: Obino; lane 5: BRW; lane 6: PMR; lane 7: BSV pure DNA: lane 8: MR; lane 9: SCBV; lane 10: GNR

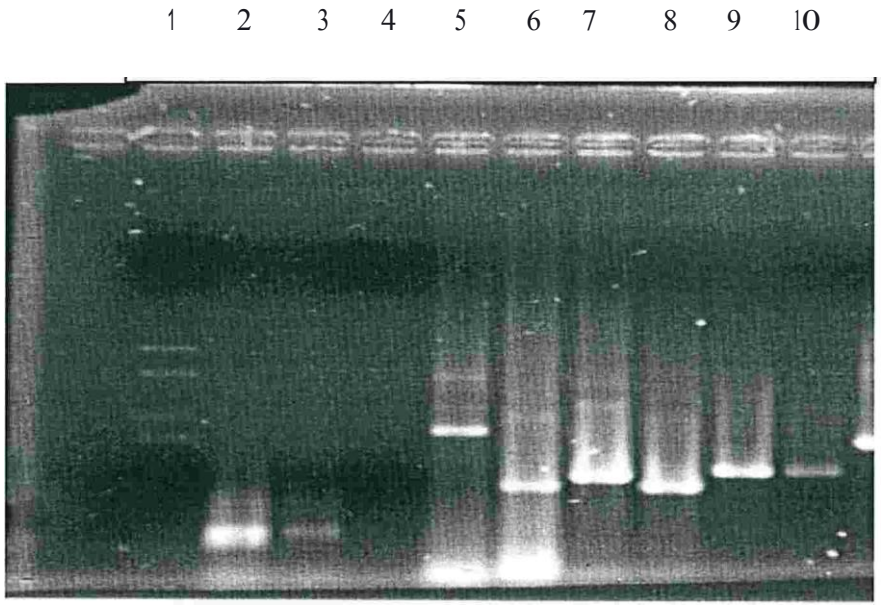
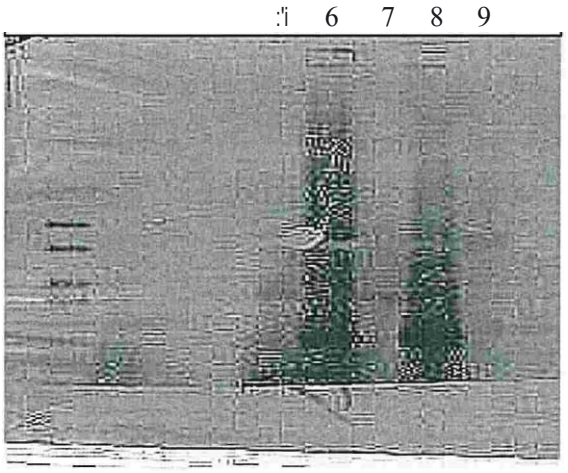



Figure 5 Amplification with primers 3012/1573

Lane 1: Molecular weight marker; lane 5: W5; lane 6: BSV DNA control; lane 7: F10; lane; lane 8: 8M; lane 9: CP5; lane 10: Obino.

Figure 5A Hybridisation with probe from Obino (amplified with 3012/1573)




Lanes have same loading as for figure 4 above. The strong signals in lanes 6 and 8 are from samples Obino and 8M which is of the variety Mamzelle. In original blot signals were visible for the samples in lanes 5,6,7,8,9 and 10 correponding to band sizes as for the gel above.

2. Amplification with primers F1/R1cav

The same approach of immunocapture and viral preps were done to confirm that the fragments obtained in the amplification were of viral origin.

With this set of primers, none of the local samples produced a band of the same size as the Obino isolate. The Obino isolate produced a band of 650 bp while all of the samples (except for S1) amplified a band of 750 bp. Sugarcane bacilliform also produced the same sized band while one sample, S1, amplified a band of 800 bp that is larger than the other samples.

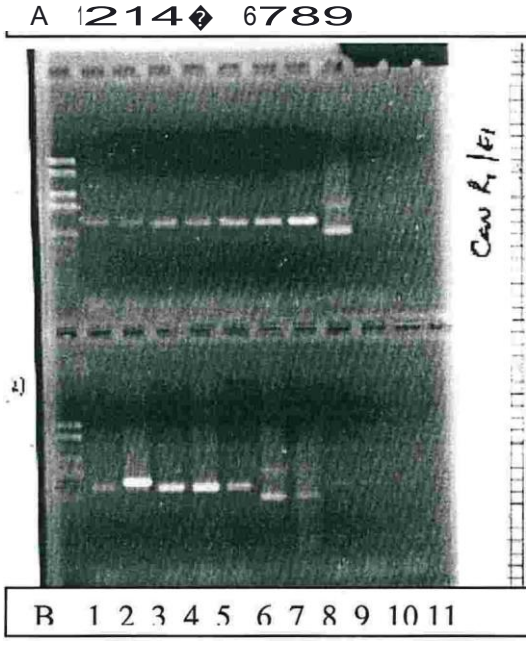



Figure 7. Amplification with primers F1/R1cav. PCR with virus preps.
A. Lane 1: Molecular weight marker; lane 2-7: samples from Cavendish types; lane 8: SCBV; lane 9: Obino.
B. Lane 1: Molecular weight marker; lane 2 LV1 (Ollier); lane 3: S1 (Cav);lane 4: P1(cav); lane 5: SCBV; lane 6: LC1(cav); lane 7 & 8: Obino, BSV; lane 9-11: Cavendish isolates.

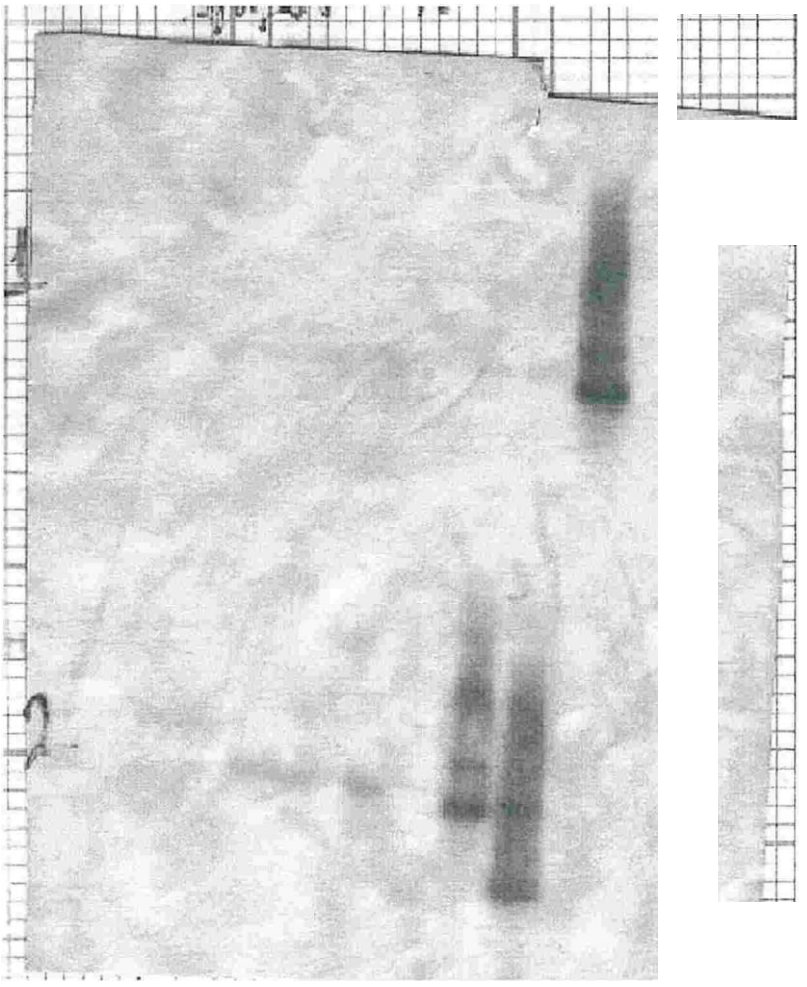


Figure 7A. Hybridisaton with a F1/R1 cav probe from Obino. Gel loading same as for figure 7. The strong signal is from the Obino isolate while the other samples did hybridise to the probe although the signal intensity is significantly lower.

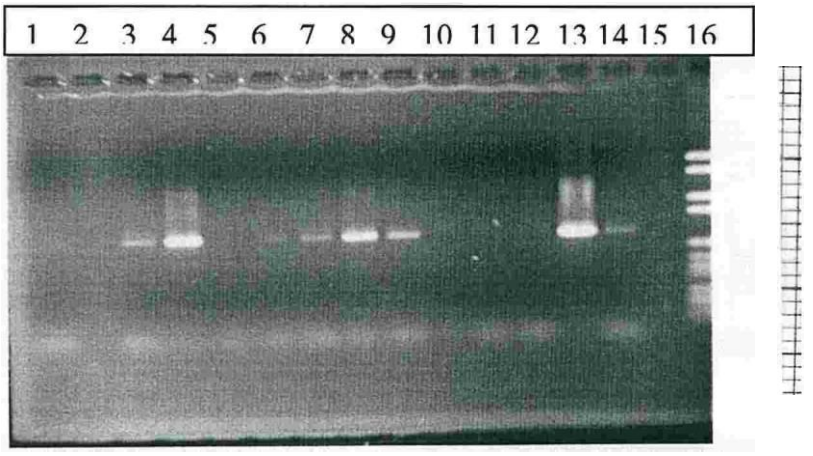



Figure 9. Amplification with Primers F1/R1cav. hnmunocapture-PCR

Lane 1: negative control; lane3: PM1 (Mamzelle); lane 4: SCBV; lane 5: L1( Gingeli); lane 6 & 7: F 7 and F5(cav); lane 8: LV1 (Ollier); lanes 9-12: Cavendish isolates! lane 13: SCBV; lane 14 F3 (cav); lane 16: molecular weight marker.

## 3. Isolation and cloning of PCR products

Following the amplification of viral genome segments using the two sets of primers, the bands of differing sizes were purified from the gel and cloned. They were sequenced so as to compare the nucleotide sequences with the reference isolate, Obino.
It can be concluded that there are several strains of BSV among the local accessions of Musa in Mauritius. With the first set of primers two variants were identified: one having the same size band as the Obino, 650 bp, and one that gave a larger band of 750 bp.
With the second set of primers, again two types of variants were obtained differing in band sizes. However, neither had the same size as the Obino isolate.

Hence the main conclusion is likely to be that in Mauritius, the BSV isolates of Mauritius are significantly different from the Nigerian isolate and that among those there exist at least three variants.

### 3.1 Purification of band from the gel

This was achieved by running the PCR product on a low-melting point agarose and cutting the band out of the gel under UV. Once the band was in a tube the DNA fragment was isolated by using a column purification kit (Promega).

### 3.2 Ligation into a vector

The cloning procedure started with the ligation reaction into pGEM (Promega). This vector is a plasmid vector with a poly-T site for ligation of PCR products. It is supplied with the buffer and ligase. The reaction was set up as follows:

| | |
|---|---|
| Buffer 2X | 5 µl |
| pGEM | 1 µl |
| insert | 3 µl |
| Ligase(3U/µl) | 1 µl |

The reaction was incubated at 4° C overnight.

The ligation mix was used directly for transformation of *Escherichia coli* DH5a. Made competent using a CaCl2 treatment. Transformation was done by a conventional heat shock at 45 ° C for 45 seconds and then placed on ice.

The cells were spread onto LB+ampicillin at 100 µg/ml.

White colonies were selected for testing the presence of the inserts using M13 and T7 primers which correspond to the sequences on either sides of the pGEM cloning site.

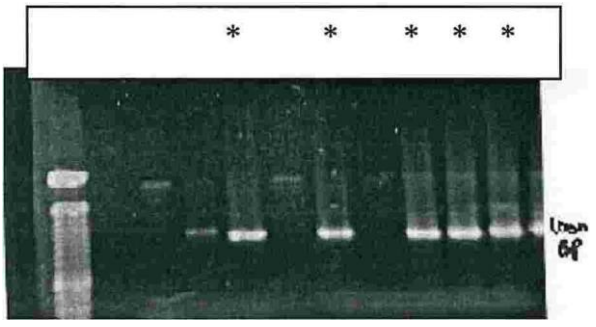



Figure 10. Amplification using pUC primers M13 and T7 for selection of colonies with inserts. The colonies with the band of appropriate size (*) were cultured.

The cloned fragments were:

- From 3012/1573: 8M (Mamzelle); BLGR (Banane La Grain) and GNR (Grande Naine)
- From F1/R1cav: S1 (cav); SCBV; W5 and CPS (cav.)

3.3 The inserts were sequenced using the dideoxy termination method.

The sequences for CPS, W5 and SCBV fragments amplified with R1/F1 are given below.

>cp5R1 F1

TACCAATAATGCAAGGGACGCGTCACAGCATACCTCTGGACCTACCGCTT
TGCGTACATCTCACGCAAAGTAATCTAAAGTCTTTGTGCGTGACACAGCA
TCGCACCTTA GACAGACTAT AGTGGAGTTG GTACGCAGAC CACTCTTTAT
ATAAAGTGAG TGCTATCCAG CTCACTTGGC ATCTCGTATC CCTGTCACAC
TGGTTAGTGC CATCCAGTTG TATGGGGTCC ATCACTTCCG CTACGTCATT

GCTTACGACC TTGCCCATTG ATCGTCATTG CTGACGTCTA GCAGATCGAA
TGCGTCTTCA ATTGTGCATC CTTCGTTGTG GCCTTCTGGT TCTTCTGGCA
ACTGGCTTAC TTGGTCGTTC CACGTCATCA CCTCTTGAAT GTACTCATCA
ATTTGATCCT TCCATACCCA AGTGAAACAT GCTTTGTTTT CACATGAGTA
GAATTCTCTT CCTGGATTTC TTGATGTTTT GACGTATGA AGCCTAGCCG
GTTTCCTGCA ACCACATTTA AAAATTGGTT TTCGATAAG TGTTAGTGTC
TTGATTTTGT GTTCTTGGAT TCTTGTCATC CATGCTTCAT AACCACTTAT
CATTTCTCCC AATTTTCTTT TTGCTCCAAT ACTTCCTTTC TGAAGTATTT
CTTCTGCGAC GTTGATCAAT ATTGTCTCTG ATGGATGTTT CTCCTTATGA
CATACGATTC TTACTAGCCT TGACAGAGG TCTGCTAGCA CATTATCTTT
GCGTCAATATGCTCAAACTTCACATCCAATCCT

>ScbvR1 F1 TACCAATAATGCAAGGACGCGTCACAGCA CACCTCTGGA CCTACTGCTT
TGCGTACATCTCACGCAAAGGAATCTAAAGTCTCTGTGCGTGACACAGCA TCGCACCTCA
GACAGACTAT AGTGGAGTTG GTACGCAGAC CACTCTTTAC
ATAAAGTGAG TGCTATCCAG CTCACTTGGC ATCTCGTATC CCTGTCACAC
TGGTTAGTGC CATCCAGTGG TATGGGTCCA TCACTTCCGC TACGTCATTG
CTTACGACCT TGCCCATTGA TCGTCATTGC TGACGTCTAG CAGATCGAGT
GCGTCTTCAA TCGTGCAACC TTCATTGTGG CCTTCCAGTT CTTCTGGCAA
CTGGCTCACT TGGTCGTTCC ACGTCATTAC TTCTTGAACG TATTCATCAA
TCTGATCCTT CCATACCCAA GTGAAACATG TTTTACTTTC ACATGAGTAG
AATTCTCTTC CTGGATTTCT TGATGTCCTG GACGTGTGAA GCTTAGCCGG
TTTCCTGCAA CCACATTTAA AAATTGGTTT TTCGATAAGT GTTAGTGTCT
TGATTTTGTG TTCTTGGATT CTTGTCATCC AAGCCTCATA TCCATTTATC
ATCTCTCCCA ACTTTCTTTT TGCTCCAATA CTTCCTTTCT GAAGTATTTC
TTCTGCAACG .TGATCAATA TTGTCTCCGA TGG.ATGTTT CCCCTTGGG
GCATATGATT TTCACTAGCC TCGACAGAGT GTCTGCTAG A.ATTATCCT
TGCCGGCAATATGCTC.AAACTTACATCC AATCCTA

>W5R1 F1AGGATTGGATGTGAAGTTTG AGCATATTGA CGGCAAAGAT AATGTGCTAG
CAGACACTCT GTCAAGGCTA GTAAAAATCA TATGCCACAA GGAGAAACAT
CCATCAGAGA CAATATTAAT CAACGTTGCA GAAGAAATAC TTCAGAAAGG
AAGTATTGGA GCAAAAAGAA AGTTGGGAGA GATGATAAGT GGATATGAAG

CTTGGATGAC MGMTCCAA GAACACAAAA TTAAGACACT AACACTTATC
GAAAAACCAA nmAAATG TGGITGCAGG AAACCGGCTA GGCTTCACAC
GTCTAGAACATCAAGAAATCCAGGAAGAGAAITCTACTCATGTGAAAACA
AAGCATGTIT CACTTGGGTA TGGAAGGATC AAATTGATGA GTACGTTCAA
GAGGTGATGA CGTGGAACGA CCAAGAAAGC CAGTTGCCAG AAGAACCAGA
AGGCCACAAT GAAGGATGCA CAAITGAAGA CGCATTCGAT CTACTAGACG
TCAGCAATGA CGATCAATGG GCAAGGTCGT AAGCCATGAC GTAGCGGAAG
TGATGGACCC CATACCACTG GATGGCACTA ACCAGTGTGA CAGGGATACG
AGATGCCAAG TGAGCTGGAT AGCACTCACIT

All three above sequences aligned with the corresponding sequence of ORF iii from an Australian isolate.

Sequences of fragments amplified with primers 3012/1573 are given below

8M

AGTCATTGGGTCAACCTCTGTCCCAGATTCTACTATCATTGCCGTTGTTCTATGTTTTGA
GCTTGAAGGCCTGATCACTTTAAGTTCTAAAAGCTTCTGGACATGCTTAGCCATGGTTTC
TTTCATTGTAGGGGTAACATGTTTTAATGGCCTATCTTCAATAATCATATCAGGGTTTTT
TACTTCAATCCTACATTTCACCTGATTTTTCTCCCAATGTTTGAGAGGTTCTTCTCCAAT
ATATCCCAATTTTTTCAI [illegible] CCAATAATGTCAGGAGATATAAATTCTTCAGAAATTTC
CCCTCTAGAACTCTCAGCTGCACATATGTTATAGTATTCATGAAGTTCCAGTTCTAGTTC
ATCAATATAATTCAGCTCATGAACTATTGGTGGGGCTTGTACTGTTGTCATGATCTTATA
AATTGTGACCTCACCATTTTCTATCCTTAGGCCAACAGTTCTAATAAAATTCATGCCTAT
GATCATATGAACTCCATCAGCTAATGAAGGCATAATAAAAGTTTGAGGGAGGTAGAACCA
TTGCTTACCAACCCATAGCTTTCCCGCTGATGTCACCTCGTTTACTTCAGTGACTCCGTT
GACTCCTCGAATAATGATTCTGTTTTTGGCCTGCTCTTTCATTCC

F3

GGAATGAAAGAGCAGGCCAAAAACAGAATCATTATTCGAGGAGTCAACGGAGTCACTGAA
GTAAACGAGGTGACATCAGCGGGAAAGCTATGGGTTGGTAAGCAATGGTTCTACCTCCCT
CAAACTTTTATTATGCCTTCATTAGCTGATGGAGTTCATATGATCATAGGCATGAATTTT
ATTAGAACTGTTGGCCTAAGGATAGAAAATGGTGAGGTCACAATTTATAAGATCATGACA
ACAGTACAAGCCCCACCAATAGTTCATGAGCTGAATTATATTGATGAACTAGAACTGGAA
CTTCATGAATACTATAACATATGTGCAGCTGAGAGTTCTAGAGGGGAAATTTCTGAAGAA
TTTATATCTCCTGACATTATTGGAAAAATGAAAAAATTGGGATATATTGGAGAAGAACCT
CTCAAACATTGGGAGAAAAATCAGGTGAAATGTAGGATTGAAGTAAAAAACCCTGATATG
ATTATTGAAGATAGGCCATTAAAACATGTTACCCCTACAATGAAAGAAACCATGGCTAAG
CATGTCCAGAAGCTTTTAGAACTTAAAGTGATCAGGCCTTCAAGCTCAAAACATAGAACA
ACGGCAATGATAGTAGAATCTGGGACAGAGGTTGACCCAATGACT

Both sequences aligned with BSV Obino (Nigerian strain)

The second set of primers used (3012/1573) indicated two variants on the basis of the sizes of PCR products. They were either 650 bp, which is similar to the Obino isolate or 750 bp. A 650 bp band from the Mamzelle isolate, 8M, was cloned and sequenced and found to be nearly identical to the corresponding Obino isolate.

The samples from Chamarel were amplified with both sets of primers. With F1/R1cav samples cha1 to cha 9 produced a band of 650 bp. While only three of those i.e cha 3, cha 4 and cha 5 produced a band with primer set 3012/1573

With the samples from FUEL, a band of 750 bp was obtained from F3, F5 and F6 with primer set 3012/1573 and only F3 and F5 gave the same band with primers F1/R1cav. The primer set F1/R1 cav had previously been shown to work exclusively on Dwarf Cavendish samples and they did not amplify the one from Goldfinger variety and other DC samples.

**Virus Purifications were made on the following samples:**

- BRW
- LV1
- PM1
- S1
- BLGR
- CPS
- PMR
- L1
- R1
- MR
- NIRA
- F5
- GNR
- F3

- SCBV

All amplicons for cloning and sequencing were obtained either from the virus preps directly or after immunocapture.

Table 1 Samples of BSV infected plants collected for analysis

| Code | Variety | Location | Date | PCR test |
|---|---|---|---|---|
| UOM1 | Olli er | Q. Militaire | May2001 | - |
| UOM2 | Olli er | Q. Militaire | May2001 | + |
| UOM3 | Olli er | Q. Militaire | May2001 | - |
| UOM4 | Olli er | Q. Militaire | May2001 | + |
| UOM5 | Olli er | Q. Militaire | May2001 | + |
| UOM6 | DC | Providence | May2001 | + |
| UOM7 | DC | Providence | May2001 | - |
| UOM8 | B.Carree | C. de Masque | May2001 | + |
| UOM9 | B.Carree | C. de Masque | May2001 | + |
| Cha 1 | DC | Chamarel | June 2001 | + |
| Cha2 | DC | Chamarel | June 2001 | + |
| Cha3 | DC | Chamarel | June 2001 | + |
| Cha4 | DC | Chamarel | June 2001 | + |
| Cha5 | DC | Chamarel | June 2001 | + |
| Cha6 | DC | Chamarel | June 2001 | + |
| Cha 7 | DC | Chamarel | June 2001 | + |
| Cha8 | DC | Chamarel | June 2001 | + |
| Cha9 | DC | Chamarel | June 2001 | + |
| Cha 10 | DC | Chamarel | June 2001 | + |
| Cha 11 | DC | Cham arel | June 2001 | + |
| Cha 12 | DC | Chamarel | June 2001 | + |
| F1 | FUEL | GF(PHIA 01) | August 2001 | - |
| F2 | FUEL | DC | August 2001 | - |
| F3 | FUEL | DC | August 2001 | + |
| F4 | FUEL | DC | August 2001 | - |
| F5 | FUEL | DC | August 2001 | + |
| F6 | FUEL | DC | August 2001 | + |
| F7 | FUEL | DC | August 2001 | + |
| F8 | FUEL | DC | August 2001 | - |
| F9 | FUEL | DC | August 2001 | - |
| F10 | FUEL | DC | August 2001 | - |
| F11 | FUEL | DC | August 2001 | - |
| F12 | FUEL | DC | August 2001 | - |
| LC1 | Union Lachan | DC | August 2001 | + |
| LC2 | Union Lachan | DC | August 2001 | + |
| P1 | Chamouny | DC | August 2001 | + |

| | | | | |
|---|---|---|---|---|
| P2 | Chamouny | DC | August 2001 | + |
| FM1 | Reduit | Williams | September 2001 | + |
| W1 | Wooton | DC | September 2001 | + |
| W2 | Wooton | DC | September 2001 | + |
| W3 | Wooton | DC | September 2001 | + |
| W4 | Wooton | DC | April 2002 | + |
| W5 | Wooton | DC | April2002 | + |
| W6 | Wooton | DC | April2002 | - |
| W7 | Wooton | DC | April2002 | + |
| CP1 | Chamarel Paroisse | DC | February 2002 | - |
| CP2 | Chamarel Paroisse | DC | February 2002 | + |
| CP3 | Chamarel Paroisse | DC | February 2002 | + |
| CP4 | Chamarel Paroisse | DC | February 2002 | + |
| CPS | Chamarel Paroisse | DC | February 2002 | + |
| CY2 | Chamouny | DC | Septem.2001 | + |
| L1 | Labourdonais | Gingeli | Septem.2001 | - |
| LV | Le Val | Ollier | Septem.2001 | - |
| PM1 | Pellegrin | Mamzelle | Septem.2001 | + |
| S1 | Sebastopol | DC | Septem.2001 | + |
| R1 | Reduit | DC | Septem.2001 | + |
| R2 | Reduit | DC | Septem.2001 | + |
| GNR | Richelieu | Grande Naine | July 2002 | + |
| BLGR | Richelieu | Banane La Grain | July 2002 | + |
| PMR | Richelieu | Pisang Mas | July 2002 | + |
| HDR | Richelieu | Hybrid Ducasse | July 2002 | - |
| MR | Richelieu | Mamoule | July 2002 | + |
| GiR | Richelieu | Gingeli | July 2002 | + |
| BRW | Wooton | B. Rouge | July 2002 | + |

## DISCUSSION

Banana streak virus has been reported in many countries including Morocco, Jordan, Rwanda, Brazil, China and India. It is a major hindrance to Musa breeding programmes and affects banana and plantains. Current control measures rely on the use of relatively clean material. Serological detection methods were only possible with the availability of antibody from Lockart and eventually from the commercial supplier AGDIA. The virus spread mainly thorough vegetative propagation. Symptoms can be very variable and thus are difficult to rely on for visual assessment. It has been reported in Mauritius since 1997 (L.Chung (MoA), personal communication). A surveillance programme was already on the way at AREU using serological based method of ELISA.

It was known then that the virus has been spreading among the cultivated varieties and the mode of control was to destroy the infected plants. This study was started to investigate into the extent to which the virus is present and if it is found in all of the varieties.

The virus cannot be mechanically transmitted and it has been reported to be transmitted by the mealybug Planococcus citri. A survey of most banana growing areas in Mauritius was done and this mealybug was not found. Instead aphids, which do transmit BSV, were found to be present on a large number of the plants.

The use of BSV antibodies reveal a significant heterogeneity among the isolates and pointed to the variability of the virus from different origins. In order to have a reliable detection method, the use of a mixture of antibodies made using different BSV isolates was recommended initially. ELISA together with immunosorbent electron microscopy and immunocapture-PCR are appropriate detection tools. BSV is serologically and genetically very closely related to the sugacane badnavirus (SCBV).

In addition, molecular tools of detection by PCR were likely to increase the sensitivity of the assays. Hence, various groups have developed primers for the identification of BSV. Degenerate primers based on badnavirus sequences were first designed by Lockart , B.E.L. This set works very well with BSV and other badnaviruses.

Following the cloning and sequencing of a whole viral genome of the BSV from Obino l'Ewai, (Harper and Hull,1998) primers targeting the conserved aspartate protease and reverse transcriptase region, were available. Those primers were used in this study. Geering et al (2000) have developed primers for four different isolates of BSV: BSV-Red Dacca, BSV-Cavendish, BSV-Mysore and BSV-Goldfinger. Out the four only the primers for BSV-cavendish (R1/F1cav) were found to amplify the virus isolates from Mauritius.

This study was also carried to compare the isolates from Mauritius with the reference strain from Nigeria, for which the whole genome sequence is available. The results from the primer set R1/F1cav. gave a band which was larger (750 bp) than the Obino control (650 bp), from all the Mauritius samples except for one. Sample S1 produced a band which was larger than the rest of the isolates and was about 850 bp. S1 was therefore different from all the rest. Is was an isolate from a dwarf Cavendish plant in Sebastopol.

With the 3012/1573 primers (Harper and Hull, 1998), some isolates produced a band of the same size as the Obino control while most of them gave a larger band of about 750 bp. Two of the 650 bp fragments were sequenced and found to be nearly identical to the Obino isolate.

The use of the degenerate primers on whole genomic DNA, i.e containing the plant DNA in addition to the viral DNA, produced band of the same size in several samples and upon sequencing revealed BSV sequences integrated into the genome.

The main conclusions which can be made from this work are:

- that BSV is widely spread in Mauritius and occurs amongst all the varieties tested. The virus was visualized under electron microscopy from virus preparations.
- It is possible to relate the genetic variant to the Musa variety in some cases. For example: All Mamzelle isolates gave the 650 bp band with primer set 3012/1573. Gingeli and Banane Rouge as well as SCBV also produced that same band. On

the other hand all the Dwarf Cavendish isolates gave the larger band of 750 bp. Isolates from Ollier, Banane La Grain, Grande Naine, Pisang Mas, and Mamoule also gave the same band.

- Amplification with primer set R1/F1 did not produce much polymorphism among the Mauritius isolates but pointed to a significant difference from the Obino isolate, in that none of the local virus isolates produced the same size of band as the Obino. All local isolates gave a band of 750, except for S1 which was larger.
- Sequencing of the R1/F1 amplicons revealed even finer differences between two bands of the same size (CPS and W5). It is therefore likely that the many band of the same size are not of the same DNA group and this can only be confirmed by sequencmg.

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