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# Occurrence, prevalence and molecular diversity of banana streak viruses in Cuba

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**Abstract** Banana streak viruses (BSV) belong to the genus *Badnavirus* of the family *Caulimoviridae*. They cause banana streak disease on banana and plantains worldwide. The recent detection of BSV in Cuba has prompted a nationwide research effort focused on the occurrence, prevalence and diversity of BSV species on dessert type banana on the island. Indexing by multiplex immunocapture-PCR (M-IC-PCR) performed on samples collected throughout the country showed that the overall prevalence of Banana streak OL virus, Banana streak GF virus and Banana streak IM virus is low in *Musa acuminata* genotypes in Cuba. However, the prevalence of BSV species Mysore (BSMYV) was surprisingly high in samples of cv Yangambi km5 collected from distinct and distant locations. The presence in Cuba of an as yet unreported BSV species was also investigated, showing that Banana steak VN virus is also present in *Musa acuminata* genotypes.

**Keywords** Banana streak viruses · Molecular diversity · Diagnostic

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## Introduction

Banana streak viruses (BSVs) cause banana streak disease (BSD) on banana and plantains worldwide. Disease symptoms include yellow leaf streaks that can turn necrotic, pseudostem splitting and internal necrosis, aberrant bunch emergence, fruit peel splitting and necrotic fruit spots (Jones 2000). BSVs belong to the genus *Badnavirus* of the family *Caulimoviridae*. They have a noncovalently closed, double-stranded circular DNA genome of approximately 7.2 to 7.8 kbp (Hull 2002). The genomes of several distinct BSV species have been fully sequenced, and partial genomic sequences are also available for numerous strains of these and other species. Phylogenetic analyses showed that BSVs display a high level of molecular diversity (Geering et al. 2000; Harper et al. 2004, 2005; Jaufeerally-Fakim et al. 2006). They are also serologically very diverse (Lockhart and Olszewski 1993). BSD was traditionally not considered a serious problem until the discovery of endogenous BSV (eBSV) sequences in the nuclear genome of *Musa balbisiana* (Harper et al. 1999; Ndowora et al. 1999; Gayral et al. 2008; Gayral and Iskra-Caruana 2009) and, more recently in that of *M.*

*acuminata* (d'Hont et al. 2012). It is likely that endogenization of BSV sequences occurred through illegitimate recombination during DNA repair mechanisms (Teycheney and Geering 2011). Most eBSV sequences are replication defective because they are fragmented and decayed. However, several infectious eBSV sequences have now been characterized in *M. balbisiana* genomes (Iskra-Caruana et al. 2010; Chabannes et al. 2013). Although some are highly rearranged, they are replication-competent and can be activated to cause infection under abiotic stresses in particular host genotypes, such as interspecific hybrids harbouring both the *M. acuminata* and *M. balbisiana* genomes (Iskra-Caruana et al. 2010). It is suspected that infectious eBSV could cause outbreaks following activation by tissue culture, hybridization or temperature differences in newly-created banana interspecific hybrids (Ndowora et al. 1999; Dallot et al. 2001; Côte et al. 2010), then transmission of viral particles by mealybugs (Meyer et al. 2008).

*Musa* spp are vegetatively propagated either by tissue culture or the production of suckers. Viruses are readily transmitted by vegetative propagation. In the case of BSV, the risk of transmission through vegetative propagation is increased due to potential activation of infectious eBSVs by tissue culture. Therefore accurate and sensitive diagnostic methods are critical for assessing and managing the risk of spreading BSV through the distribution of vegetatively propagated planting material. However, the important level of serological and molecular diversity between BSV species and the presence of endogenous BSV sequences in the genome of *Musa* spp make diagnosis difficult. Hence polyvalent diagnostic techniques such as multiplex immunocapture PCR (M-IC-PCR) and rolling circle amplification were optimized for the detection of BSV (Le Provost et al. 2006; James et al. 2011).

Banana is the third most important fruit crop in Cuba in terms of production and consumption (Pérez-Vicente 2011). Cuba grows more interspecific disease-resistant hybrids than any other country in the world, totaling 11,000 ha (Pérez- Vicente et al. 2009). Following the discovery of infectious eBSVs in these hybrids and the first report of BSV species in Cuba (Javer-Higginson et al. 2009), a nationwide research effort was focused on determining the occurrence, prevalence and diversity of BSV species in dessert banana, in order to assess the schemes of production of vegetatively propagated planting material and the risk of spreading BSV from interspecific hybrids –following activation of infectious eBSVs- to dessert banana. Dessert banana samples were collected throughout the country. Their analysis showed that the overall prevalence of *Banana streak OL virus* (BSOLV), *Banana streak GF virus* (BSGFV) and *Banana streak IM virus* (BSIMV) is low in *Musa acuminata* genotypes in Cuba. In contrast, prevalence level of *Banana streak MY virus* (BSMYV) was surprisingly high in samples of introduced cultivar Yangambi km5 that were collected in distinct locations from plants originating from vitroplants, suggesting a contamination of the original plant introduced in Cuba. The presence in Cuba of a previously unreported BSV species was investigated, showing for the first time that *Banana streak VN virus* (BSVNV) is also present in Cuba.

## Materials and methods

### Sample collection and multiplex IC-PCR

Leaf samples were collected from distinct *Musa* cultivars and accessions with *Musa acuminata* AAA genotypes between 2008 and 2010 (Table 1). The presence or absence of mealybugs was monitored for each plot where samples were collected. Crude leaf extracts were prepared from symptomatic and symptomless plants and used to perform M-IC-PCR according to Le Provost et al. (2006) with the following modifications. Immunocapture times were reduced to 30 min; PCR amplification was performed using either BSV species-specific primers (Geering et al. 2000) or *Badnavirus* degenerate primers (Geering et al. 2005; Yang et al. 2003) for the detection of BSV and Monkey retrotransposon primer pair MonR/MonF (Gambley 2008) for the detection of contaminations by *Musa* genomic DNA, respectively (Table 2). PCR reaction mix of 25 µl contained 1× PCR buffer, 100 µM each dNTP, 0.2 µM of each primer and 1 U Taq DNA polymerase (Eurogentech, Seraing, Belgium). PCR cycle conditions used with BSV species-specific primers were an initial denaturation step at 94 °C for 5 min, then 35 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s followed by a final elongation step of 5 min at 72 °C. Similar conditions were used with degenerate primers except that the primer annealing temperature was decreased to 52 °C. PCR products were electrophoresed through a 1 % agarose gel in 0.5 X Tris-

borate-EDTA and visualized under UV light following ethidium bromide staining.

### Cloning, sequencing and phylogenetic analyses

Amplification products were gel purified using the Qiaquick purification kit (Qiagen, Courtaboeuf, France) and cloned into pGEMT-Easy vectors (Promega, Madison, USA). Recombinant clones were fingerprinted according to Geering et al. (2005) and representatives from each restriction pattern group were selected for sequencing and sequenced by Beckmann Coulter Genomics (Takesley, UK). Nucleotide sequence analyses were performed using CLUSTALX (Thompson et al. 1994) and BLASTN (Altschul et al. 1990). Phylogenetic trees were generated using the Mega 5 programme (Tamura et al. 2007).

### Rolling circle amplification (RCA) of BSV genomic DNA

Total genomic DNA was extracted from banana leaves according to Gawel and Jarret (1991). One µl of purified DNA was used as template in RCA experiments according to James et al. (2011), using Illustra TempliPhi 100 Amplification Kit (GE Healthcare, Buckinghamshire, United Kingdom). One µl of the amplification products was digested overnight using 20 U of restriction endonucleases (Promega, Madison, USA), then electrophoresed through a 0.8 % agarose gel in 0.5 X Tris-acetate-EDTA buffer. Selected digestion products were gel purified as described above and cloned into predigested and dephosphorylated pUC19 vector (New England Biolabs, Evry, France). Recombinant clones were sequenced and sequences were analyzed as described above.

## Results

### Prevalence of BSOLV, BSGFV, BSMYV and BSIMV in Cuba

A total of 512 leaf samples from seven *M. acuminata* cultivars were collected from 11 farms scattered among eight Cuban provinces (Table 1). Sampling was representative of the local cultivars grown in each province. Only 7.6 % (39/512) of the collected samples showed symptoms (Table 3), mostly chlorotic streaks. However, two Gran Enano plants from Ciego de Avila showed symptoms on bunches.

Overall, 64 % (25/39) of the samples collected from symptomatic plants were indexed positive for BSV species when using BSV species-specific primers, whereas 16 % (78/473) of the samples collected from asymptomatic plants were indexed positive (Table 3). In contrast, 14 of the 39 samples (36 %) collected from symptomatic plants were indexed negative for BSV. Symptoms on these samples are likely to result from infections by *Cucumber mosaic virus* whose symptoms can be mistaken for those caused by BSV, or from abiotic stresses such as nutrient deficiencies. n all multiplex PCRs, the presence of banana genomic DNA was assessed and faint residual bands could occasionally be observed, showing that traces of plant genomic DNA can remain in samples. However, this residual banana genomic DNA does not interfere with IC-PCR diagnostic in *M. acuminata* genotypes, which are devoid of eBSVs for the viral species targeted by the primers used in this part of our work.

The level of prevalence for BSOLV, BSGFV and BSIMV in single infections did not differ significantly (Table 4) and was low, with values ranging from 1.1 % (6/512) for BSGFV and BSIMV to 2.1 % (11/512) for BSOLV. Taking mixed infections into account did not significantly change the situation, with total levels of prevalence reaching values of 1.8 % (9/512) for BSGFV, 2.3 % (12/512) for BSOLV and 2.5 % (13/512) for BSIMV. Single and mixed infections were detected in samples originating from several cultivars (Gran Enano, Cavendish and Johnson) collected from distinct locations, suggesting the absence of an outbreak in any specific location for these three species.

In contrast, a high prevalence was registered for BSMYV specifically on cultivar Yangambi km5. A total of 81.4 % (22/27) of symptomatic Yangambi km5 samples and 50 % (54/108) of asymptomatic samples, including nine samples with mixed infection, were indexed positive for BSMYV (Table 3). Yangambi km5 samples infected by BSMYV originated from several provinces: with 10/10 samples from Mayabeque infected, 9/9 from Villa Clara, 13/54 from Cienfuegos, 17/20 from Granma, and 27/42 from Santiago de Cuba. Overall, 56.3 % (76/135) of Yangambi km5 samples were infected by BSMYV, whereas only 0.4 % (1/263) and 2.6 % (1/38) of the Gran Enano and Johnson

**Table 1** Origin and genotype of plant samples used in this study

| Cultivar | Genotype | Mayabeque | Cienfuegos | Villa Clara | Sancti Spiritus | Ciego de Ávila | Santiago de Cuba | Granma | Guantánamo | Grand total |
|---|---|---|---|---|---|---|---|---|---|---|
| Americani | AAA | | | 11 (3) | | | | | | 14 |
| Cavendish | AAA | 20 | 15 | 4 (3) | | | | | | 42 |
| Gran enano | AAA | | 18 | | | 215 (2) | 25 (3) | | | 263 |
| Johnson | AAA | | 25 | | 10 | | | | 3 | 38 |
| Robusta | AAA | | | | | | | | 13 | 13 |
| Vietnam | AAA | | | 6 (1) | | | | | | 7 |
| Yangambi km5 | AAA | 10 | 36 (18) | 0 (9) | | | 42 | 20 | | 135 |
| Total | | 30 | 94 (18) | 21 (16) | 10 | 215 (2) | 67 (3) | 20 | 16 | 512 |

Number of asymptomatic plants is indicated, with that of symptomatic plants shown in brackets

cultivar samples were infected by BSMYV respectively (Table 3).

Search for other BSV species

Two distinct approaches were used to search for BSV other than BSOLV, BSGFV, BSMYV and BSIMV in the samples used in this study. Firstly, all samples found uninfected by BSOLV, BSGFV, BSMYV or BSIMV using BSV species-specific primers were indexed using degenerate primer pairs Badna 1A/4′ (Geering et al. 2005) and Badna FP/RP (Yang et al. 2003). These primers target the same conserved sequences in the reverse transcriptase/RNase H region of badnaviruses open reading frame III as BSV species-specific primers. While only one amplification product was produced using the former primer pair, a large number of amplification products was produced using the latter (Table 4). A selection of amplification products was cloned and sequenced, resulting in 33 distinct nucleotide sequences. These sequences were compared to those of other badnaviruses, including all BSV species whose genomes have been totally or partially sequenced. The sequences were deposited in GenBank under accession

**Table 2** Primers used for multiplex immunocapture PCR

| Target | Primer | Sequence (5′-3′) | Size of PCR product (bp) |
|---|---|---|---|
| BSOLV | RD-F1[a]<br>RD-R1[a] | ATCTGAAGGTGTGTTGATCAATGC<br>GCTCACTCCGCATCTTATCAGTC | 522 |
| BSGFV | GF-F1[a]<br>GF-R1[a] | ACGAACTATCACGACTTGTTCAAGC<br>TCGGTGGAATAGTCCTGAGTCTTC | 476 |
| BSMYV | Mys-F[a]<br>Mys-R1[a] | TAA AAGCACAGCTCAGAACAAACC<br>CTCCGTGATTTCTTCGTGGTC | 589 |
| BSIMV | IM- F1[a]<br>IM- R1[a] | CACCCAGACTTTTCTTTCTAG C<br>TGCCAACGAATACTACATCAAC | 384 |
| *Badnavirus* | Badna FP[b]<br>Badna RP[b] | ATGCCITTYGGIITIAARAAYGCICC<br>CCAYTTRCAIACISCICCCCAICC | 579 |
| *Badnavirus* | Badna 1A[c]<br>Badna 4′[c] | CTNTAYGARTGGYTNATGCCNTTYGGTCC<br>TCCAYTTRCANAYNSCNCCCCANCC | 591–597 |
| Monkey | MonR[d]<br>MonF[d] | GCTGACACATGGGAGGACTT<br>CTTGTTGGGTCTTCAGAGGAA | 300 |

[a] Geering et al. 2000

[b] Yang et al. 2003

[c] Geering et al. 2005

[d] Gambley 2008

**Table 3** Prevalence of BSOLV, BSGFV, BSMYV and BSIMV

| Cultivar | Symptomatic plants | | | | | Asymptomatic plants | | | | | | | | | Total indexed samples | Total positive samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total indexed | Indexed positive | | | | Total indexed | Indexed positive | | | | | | | | | |
| | | BSOLV | BSGFV | BSMYSV | BSIMV | | BSOLV | BSGFV | BSMYSV | BSIMV | BSMYV + BSIMV | BSMYV + BSGFV | BSMYV + BSOLV | BSGFV + BSIMV | | |
| Americani | 3 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 |
| Cavendish | 3 | 0 | 0 | 0 | 0 | 39 | 0 | 3 | 0 | 5 | 0 | 0 | 1 | 1 | 42 | 10 |
| Gran enano | 5 | 3 | 0 | 0 | 0 | 258 | 8 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 263 | 16 |
| Johnson | 0 | 0 | 0 | 0 | 0 | 38 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 38 | 1 |
| Robusta | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 |
| Vietnam | 1 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| Yangambi km5 | 27 | 0 | 0 | 22 | 0 | 108 | 0 | 0 | 46 | 0 | 6 | 2 | 0 | 0 | 135 | 76 |
| Total | 39 | 3 | 0 | 22 | 0 | 473 | 8 | 6 | 48 | 6 | 6 | 2 | 1 | 1 | 512 | 103 |

numbers KF318340-KF318357, KF386728, KF386731-KF386741, KF386745, and KF386747-KF386748.

The results of phylogenetic analyses (Fig. 1) showed that all the sequences amplified in this study by degenerate primers Badna FP/Badna RP grouped within the clades I and II defined by Harper et al. (2005). Clade I contains sequences from BSV species that are known to have both endogenous and episomal forms and from other BSV species for which no endogenous form has been reported yet. This group includes clones EJ-5, E391-94 and E372-111, and EJ-34 amplified from cultivars Americani, Gran Enano and Yangambi km5, respectively. Nucleotide sequences of these clones displayed 99–100 % identities with *Musa acuminata* eBSV sequences FP4, FP6 and FP28, respectively, for which no episomal form is known. A second group clustered also within clade I with *Banana streak acuminata Yunnan virus* (BSAcYNV) and BSVNV for which no endogenous sequence has been reported. This group includes clones E63, E1063-25 and E1062-34 amplified from cultivars Vietnam, Americani and Dwarf Cavendish respectively. Nucleotide sequences of these clones displayed 89 to 91 % sequence identities with sequences of BSAcYNV and BSVNV.

Another group of sequences grouped within clade II, which contains endogenous BSV sequences for which no episomal form has been identified. In this group, sequences E408-125, E375-114, E324-107 and E308-76 amplified from cultivar Gran Enano grouped together with *Musa acuminata* eBSV sequences (Gayral and Iskra-Caruana 2009) and endogenous *Badnavirus* sequences of *Musa banksii* (Geering et al. 2005).

A second approach using rolling circle amplification (RCA) was used to search for episomal forms of BSV species in samples from cultivars Vietnam, Americani and Dwarf Cavendish, from which clones E63, E1063-25 and E1062-34 were obtained (see above). RCA products were only obtained from cultivars Americani and Vietnam. Very similar patterns were obtained following the digestion of amplification products by restriction enzymes KpnI, BamHI, HindIII and PstI (Fig. 2). These restriction patterns were compared to those of BSV species whose genomes have been fully sequenced, and shown to match those of BSVNV (Lheureux et al. 2007) and not those of any other fully sequenced BSV genome. Digestion of RCA products by KpnI resulted in a single fragment of 7.8 kbp (Fig. 2a), which was cloned and whose 5′ and 3′ ca 800 bp

**Table 4** Results of indexing using *Badnavirus* degenerate primers

| Cultivar | Total indexed | Positive samples using species-specific primers | Positive samples using Badna1A/Badna4 primer pair[a] | Positive samples using BadnaFP/BadnaRP primer pair[a] |
|---|---|---|---|---|
| Americani | 13 | 0 | 0 | 8 |
| Cavendish | 42 | 10 | 0 | 15 |
| Gran enano | 263 | 16 | 0 | 14 |
| Johnson | 38 | 1 | 0 | 16 |
| Robusta | 13 | 0 | 0 | 0 |
| Vietnam | 8 | 0 | 0 | 6 |
| Yangambi km5 | 135 | 76 | 1 | 10 |
| Total | 512 | 103 | 1 | 69 |

[a]Only those samples indexed negative using BSV species-specific primers were used

extremities were sequenced. These 5′ and 3′ sequences displayed 89 and 91 % identity to BSVNV.

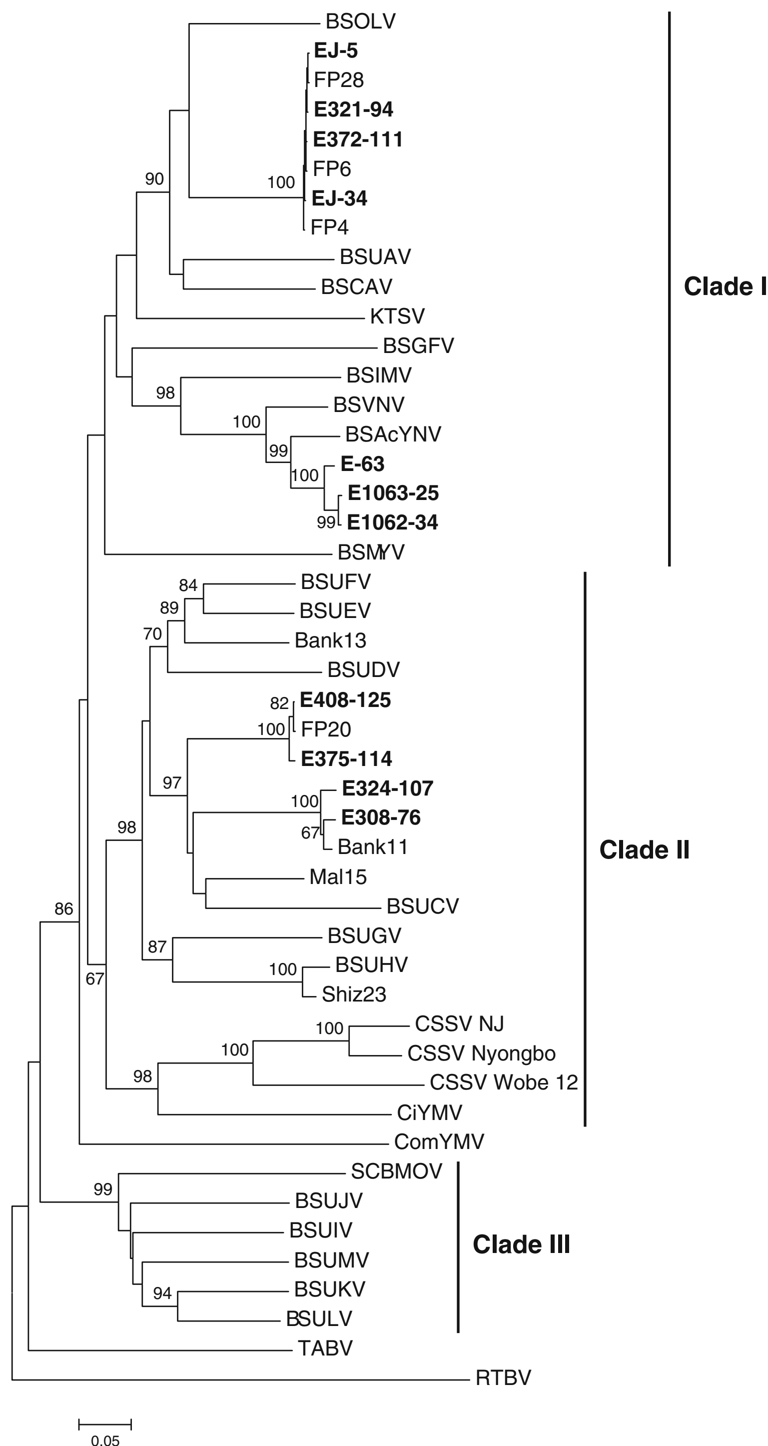
## Discussion

This work reports on a nationwide survey carried out to assess the prevalence and diversity of BSV species in dessert banana in Cuba. Leaf samples from 512 plants of seven *Musa* cultivars of dessert bananas with AAA genotypes were collected throughout Cuba in the main dessert banana production areas and indexed by M-IC-PCR for BSV infection.

Our results showed that a substantial proportion of samples showing symptoms (36 %) tested negative for BSV when indexed by M-IC-PCR and conversely a significant proportion of samples showing no symptoms (16 %) did test positive. They also show that more than 75 % (78/103) of infected samples were collected on asymptomatic plants (Table 3). These data confirm the importance of asymptomatic infections in the spread of BSV and that symptomatology-based diagnosis is ineffective for accurately monitoring BSV infections.

Indexing with BSV species-specific primers confirmed the presence in Cuba of the four BSV species BSOLV, BSGFV, BSIMV and BSMYV previously reported by Javer-Higginson et al. (2009). Overall levels of prevalence of BSOLV, BSGFV and BSIMV did not exceed 3.1 %, indicating that the dessert banana certified planting scheme results in efficient control of these BSV species in Cuba. A similar situation was reported previously in Guadeloupe, where the use of certified virus-free vitroplants is enforced and results in very low levels of prevalence of BSV species (Péréfarres et al. 2009). These low levels of prevalence may also reflect low levels of transmission by mealybugs, although several mealybug species are widespread in Cuba, including the ones known to transmit BSV (González et al. 2002; Blanco 2007). Moreover, samples used in this study were purposedly collected in dessert banana plantations adjacent to plantations of interspecific

**Fig. 1** Phylogenetic neighbour-joining tree generated from the nucleotide sequences of RT/RNaseH domain of ORFIII of different badnaviruses. Bootstrap values of 1,000 replicates are given above nodes when above 60 %. The evolutionary distances were computed using the Kimura 2-parameter method. Selected sequences representative of all the sequences amplified from leaf samples collected in Cuba are in bold. Abbreviations: *Banana streak CA virus* (BSCAV); *Banana streak MY virus* (BSMYV); *Banana streak GF virus* (BSGFV); *Banana streak IM virus* (BSIMV); *Banana streak OL virus* (BSOLV); *Banana streak VN virus* (BSVNV); *Banana streak Acuminata Yunnan virus* (BSAcYNV) *Banana streak Uganda A virus* (BSUAV), *Banana streak Uganda C virus* (BSUCV), *Banana streak Uganda D virus* (BSUDV); *Banana streak Uganda E virus* (BSUEV); *Banana streak Uganda F virus* (BSUFV); *Banana streak Uganda G virus* (BSUGV); *Banana streak Uganda H virus* (BSUHV); *Banana streak Uganda I virus* (BSUIV); *Banana streak Uganda J virus* (BSUJV), *Banana streak Uganda K virus* (BSUKV); *Banana streak Uganda L virus* (BSULV); *Banana streak Uganda M virus* (BSUMV); *Cacao swollen shoot virus isolate New Juaben* (NJ-CSSV); *Cacao swollen shoot virus isolate Nyongbo2* (Nyongbo2-CSSV); *Cacao swollen shoot virus isolate Wobe 12* (Wobe 12 -CSSV); *Citrus yellow mosaic virus* (CiYMV); *Commelina yellow mottle virus* (ComYMV); *Kalanchoe top-spotting virus* (KTSV); *Sugarcane bacilliform MO virus* (SCBMOV); *Taro bacilliform virus* (TaBV); *Musa schizocarpa* EPRV isolate 23 (Shiz23); *Musa acuminata* cv. 'grande Naine' (AAA) EPRVs isolates FP4, FP6, FP20, FP28; *Musa acuminata* subsp. malaccensis isolate Mal15; *Musa acuminata* subspecies banksii isolates Bank 11 and Bank 13; *Rice tungro bacilliform virus* (RTBV)




banana hybrid FHIA21, which can be a source of BSV viral particles for transmission by mealybugs, following activation of infectious eBSV under field cultivation conditions (Meyer et al. 2008). Yet the overall prevalence of BSV was low, although all samples were collected from plots infested by mealybugs. Our observations support those made previously in Australia and South Africa (Meyer 2005; Daniells et al. 2001) and confirm

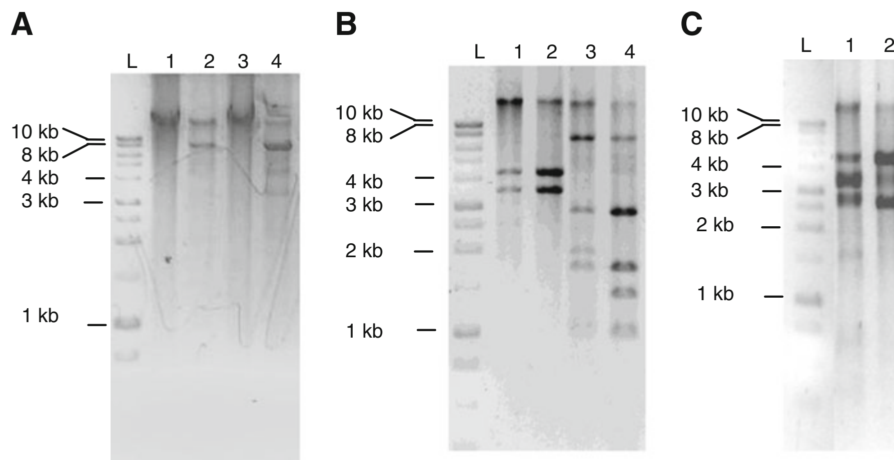



**Fig. 2** Restriction patterns of rolling-circle amplification (RCA)-amplified DNA. **a** Undigested (lanes 1 and 3) and KpnI-digested (lanes 2 and 4) RCA products derived from samples 2015 (Americani, lanes 1 and 2) and 2024 (Vietnam, lanes 3 and 4). **b** BamHI-digested RCA products derived from samples 2015 (lane 1) and 2024 (lane 2); **c** PstI-digested RCA products derived from samples 2015 (lane 1) and 2024 (lane 2). L: 1 kb ladder

that the spread of BSV by mealybugs may be limited. The risk of BSV outbreaks on dessert banana resulting from mealybug transmission of BSV following activation of infectious eBSV in interspecific hybrids such as FHIA 18 and FHIA 21, which have been massively distributed in Cuba since the 1990s, appears to be low.

The situation encountered for BSMYV was very different. A high prevalence of 56.3 % (76/135) was observed for BSMYV in cv Yangambi km5. In contrast, prevalence of BSMYV in other cultivars was similar to that monitored for BSOLV, BSGFV and BSIMV: 2.3 % (1/42) in cv Cavendish, 0.4 % (1/263) in cv Gran Enano and 2.6 % (1/38) in cv Johnson. Considering that infected Yangambi km5 samples originated from distinct and distant locations and that partial BSMYV sequences amplified from these samples displayed 97–99 % homology at the nucleotide levels (data not shown), we hypothesize that infected Yangambi km5 plants may originate from a single source of infected mother plants introduced in Cuba in the early 1990s for the multiplication of this cultivar by cell culture. At the time of introduction, no specific and sensitive diagnostic method was available, and it is likely that the presence of the virus went unnoticed because plants were symptomless. Virus-free sources of planting material are now available for this cultivar, and should result in a better control of the BSMYV.

In order to tackle the issue of BSV diversity in Cuba, we used a similar approach to that used by Harper et al. (2005). However, even when using primer pair Badna FP/RP (Yang et al. 2003), which is more degenerate than primer pair Badna 1A/4′ used by Geering et al. (2005), the diversity of BSV species detected in the samples used in our study was very low: besides BSOLV, BSGFV, BSIMV and BSMYV, only one additional species, BSVNV, was identified for the first time in Cuba. Since no potentially infectious endogenous counterpart of BSVNV has been reported in *Musa* genomes, episomal forms of this species in infected samples may also originate from the introduction of infected germplasm.

Our work shows that in a limited but significant number of uninfected samples, eBSV sequences can be amplified following M-IC-PCR. This result confirms the presence of eBSVs in *M. acuminata* genotypes. These eBSVs do not have episomal counterparts and do not interfere with the diagnosis of species BSOLV, BSGFV, BSIMV and BSMYV. However, this finding also highlights the risk of false positives when using M-IC-PCR for the detection of one of these species in genoytypes containing the *M. balbisiana* genome, which harbours eBSOLV, eBSGFV, eBSMYV and eBSIMV. In which case, multiplex PCR using sensitive primers targeting Musa genomic DNA and DNase I treatment of samples following immunocapture and prior to the PCR step should be enforced, as recommended by Gambley (2008).

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