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ORIGINAL ARTICLE

Prevalence and diversity of Banana streak viruses in the Dominican Republic

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Abstract

An extensive survey was carried out in the Dominican Republic in order to monitor the prevalence of the three most widespread BSV species: *Banana streak OL virus* (BSOLV), *Banana streak GF virus* (BGFV) and *Banana streak IM virus* (BSIMV), in Macho x Hembra (MxH) and FHIA-21 plantains and in Cavendish dessert banana throughout all banana-producing areas. This survey led to the first detection of BSVs in the Dominican Republic. BSGFV was the most prevalent of the three targeted BSV species in both FHIA-21 and MxH varieties. Prevalence of BSGFV and BSOLV were substantially higher in FHIA-21 than in MxH. Molecular analysis of the eBSV pattern of sampled FHIA-21 and MxH plants showed that both varieties harbor infectious alleles OL1 (eBSOLV) and GF7 (eBSGFV) and are devoid of eBSIMV. Very low prevalence was recorded for both BSOLV and BSGFV in Cavendish banana, which is devoid of infectious eBSVs. BSIMV was not detected at all. Our results point to a marginal role of insect-borne transmission in the epidemiology of BSVs in the Dominican Republic.

Keywords *Musa* spp. · Interspecific hybrid · Endogenous BSV sequence · Plant virus survey

Introduction

Dessert banana and plantain are major subsistence and cash crops throughout the tropics and subtropics. Cultivation and trade of banana are sources of jobs and income for rural communities and also play a central role in food security and in reducing rural poverty. In the Dominican Republic, three main varieties are cultivated by small and medium-scale farmers: synthetic tetraploid FHIA-21 (AAAB) and natural triploid Macho x Hembra (MxH) (AAB) plantains and Cavendish dessert banana (AAA).

Both FHIA-21 and MxH are interspecific hybrids harboring the *M. balbisiana* genome, which contains infectious Banana streak virus endogenous sequences (eBSVs), whereas Cavendish banana harbours only the *Musa acuminata* genome, which is devoid of eBSVs. Banana streak viruses (BSVs) are members of the genus *Badnavirus* in the family *Caulimoviridae* (Fauquet et al. 2005). They have bacilliform-shaped virions with a circular dsDNA genome of 7.0 to 7.8 Kbp (Harper and Hull 1998; Lockhart and Jones 1999; Hull et al. 2005). The viral genome replicates *via* a ssRNA intermediate, which is converted back to dsDNA through the action of a virus-encoded reverse transcriptase (RT). The genome of BSVs has three open reading frames (ORFs). ORF1 encodes a small protein, whose function has not been elucidated; ORF2 encodes a virion-associated protein; ORF3 encodes a large polyprotein containing the viral capsid (CP) and domains associated with movement proteins (MP), aspartic protease (AP), reverse transcriptase (RT) and

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ribonuclease H (RNaseH) functions (Harper and Hull 1998). BSV infections cause typical leaf streak symptoms, which tend to be intermittent in expression. Diseased plants may be stunted, showing pseudostem splitting and necrosis of the leaf cigar, and fruit may be distorted (Gauhl and Pasberg-Gauhl 1995; Lockhart and Jones 1999; Fargette et al. 2006; Carnelossi et al. 2014). BSV is transmitted by several mealybug species in a non-persistent manner (Kubiriba et al. 2001; Meyer et al. 2008). Horizontal transmission has been reported for viral species *Banana streak OL virus* (BSOLV), *Banana streak GF virus* (BSGFV) and *Banana IM virus* (BSIMV) through activation of infectious eBSVs by biotic or abiotic stresses, such as temperature differences (Dallot et al. 2001; Côte et al. 2010; Karanja et al. 2013).

Yield losses ranging from 6% -15% were reported in banana fields infected by BSVs (Dahal et al. 2000; Daniells et al. 2001). Disease severity and losses depend upon several factors, including banana varieties, virus species and climatic conditions (Muturi et al. 2013, 2016). BSV-like symptoms were first observed in the Dominican Republic upon the introduction of interspecific hybrid FHIA-21, which was introduced in the 1990's to control a destructive disease, Black Sigatoka, caused by the fungus *Mycosphaerella fijiensis* Morelet (Churchill 2011). No further work was performed to investigate the epidemiology and prevalence of BSVs in the country. The work reported here was designed to assess the diversity of BSV species and their prevalence in the Dominican Republic.

Materials and methods

Field surveys and sample collection

A nationwide survey was carried out in order to assess the prevalence and diversity of BSV species BSOLV, BSGFV and BSIMV in interspecific hybrids and dessert banana in the Dominican Republic. Leaf samples were collected from January 2012 to April 2014 from 36 plantations across 11 provinces, representing the diversity of environments and agronomic practices utilized by farmers in banana growing areas. Nine to twenty leaves were collected in each sampled plots: 299 samples from MxH, 291 samples from FHIA-21 and 248 leaf samples from Cavendish, leading to a total of 848 samples. Samples were labelled, wrapped in wet paper towels, placed in plastic bags, transported in a cooler and stored at 4 °C in the laboratory until processing. A comprehensive range of criteria were recorded for each sample and/or sampling site, including GPS coordinates, altitude, surface and age of sampled plots, nature of planting material (vitroplant, sucker), plant density, crop growth cycle, presence and nature of an irrigation system, association with other crops, weed control, presence of banana in nearby plots, presence of mealybugs and extent of Black Sigatoka and/or BSV symptoms in sampled plots. Data on weather and environmental conditions in sampled plots were also collected during surveys.

Based on the results of the nationwide survey, two locations, Montecristi and Puerto Plata, were selected for a regional survey that was carried out in October 2013. In both locations, 100 leaf samples were collected randomly from FHIA-21 and MxH plants, leading to a total of 400 collected leaf samples. The presence of BSV symptoms and mealybugs was recorded for each sampled plant. Samples were preferentially collected from plots of FHIA-21 and MxH adjacent to one another.

Virus indexing by multiplex immunocapture PCR (M-IC-PCR)

Collected leaf samples were indexed for the presence of BSV species BSOLV, BSGFV, and BSIMV by multiplex immunocapture PCR (M-IC-PCR), according to Le Provost et al. (2006) with modifications. A rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota, USA) was used for the immunocapture step. This antiserum has the ability to detect a wide range of badnaviruses, including most BSV species (Ndowora et al. 1999; Ndowora and Lockhart 2000). Indexings were performed in 96-well microtiter plates (Agdia). Plate wells were coated overnight at 4 °C with 25 μl of IgG purified from the antiserum and diluted at 2 μg/ml in coating carbonate buffer (15 mM sodium carbonate, 34 mM sodium bicarbonate, pH 9.6). Wells were then washed three times with 100 μl of PBT washing buffer (136 mM NaCl, 1.4 mM KH2PO4, 2.6 mM KCl, 8 mM NA2HPO4, 0.05% Tween-20, pH 7.4) and dried.

Crude extracts were prepared from leaf samples in meshed plastic bags (Agdia) by grinding 0.5 g of leaf material in 5 ml of grinding buffer (2% polyvinylpyrrolidone 40, 0.2% sodium sulfite and 0.2% bovine serum albumin, prepared in PBT), using a bead grinder, then transferred into microfuge tubes. Extracts were clarified by centrifugation at 8000 rpm for 2 min at room temperature, and 25 μL of the supernatant was deposited in antibody pre-coated microtiter plates for the immunocapture step. Plates were incubated for 30 min at room temperature, then rinsed four times with 100 μl of PBS-Tween and twice with ultrapure sterile water (Corning). A DNase I treatment was performed after the immunocapture step and prior to the PCR step with 2 U of RQ1 DNAse (Promega) as described by Gambley (2008), in order to eliminate plant genomic DNA contaminants and prevent false positives resulting from the amplification of eBSV sequences. Following this step, RQ1 DNAse was heat denatured (10 min at 95 °C) and PCR amplifications were performed separately for each BSV species. For this, 25 μL of PCR mix was added in in each well. PCR mix contained 2.5 μl of 10x reaction buffer (400 mM Tris-HCl pH 8, 100 mM MgSO4, 10 mM CaCl2), 0.75 μl of 50 mM MgCl2, 2 μl of

2.5 mM dNTPs, 1 U of Taq DNA polymerase (New England Biolabs), 1 µl of 2 µM forward and reverse BSIMV-specific primers, 0.75 µL of 2 µM forward and reverse BSOLV-specific primers or 0.5 µL of 2 µM forward and reverse BSGFV-specific primers and 0.75 µl of 2 µM forward and reverse Monkey transposon-specific primer pair MonR-MonF, for the detection of contaminations by *Musa* genomic DNA (Gayral et al. 2008) (Table 1).

PCR conditions were an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30s at 94 °C, 30 s at 58 °C, 30s at 72 °C and one cycle of 10 min at 72 °C. Ten µl of the reaction mixes were analyzed by electrophoresis on a 1% agarose gel prepared in 0.5xTBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) containing 0.005% of GelRed (Biotium). Amplification products were visualized under UV light. Each indexing experiment included healthy and infected samples used as negative and positive controls, respectively. Every sample found infected was re-tested for confirmation.

eBSVs genotyping of FHIA-21 and MxH samples by PCR using dCAPS markers

eBSOLV, eBSGFV and eBSIMV patterns were assessed by PCR-based screening on a selection of 49 non-infected FHIA21 and MxH hybrids, using the 23 PCR and dCAPS (derived cleaved amplified polymorphic sequences) primer pairs and conditions described by Gayral et al. (2008) and Chabannes et al. (2013).

PCR mixes contained 2.5 µL of genomic DNA, 2.5 µl of 10x reaction buffer (400 mM Tris-HCl pH 8, 100 mM MgSO4, 10 mM CaCl2), 1.5 µl of 50 mM MgCl2, 1 µl of 2.5 mM dNTPs, 1 U of Taq DNA polymerase (New England Biolabs), 2.5 µL of forward and reverse primers at 2.5 mM each and ultrapure sterile water to 25 µL. Different PCR conditions were used depending on the annealing temperatures of the primers used. For eBSGFV- and eBSIMV- specific primers, these conditions were an initial denaturation step at 94 °C for 5 min, followed by 25 cycles of 30s at 94 °C, 30 s at 58 °C, 1 min at 72 °C and one cycle of 10 min at 72 °C. For eBSOLV-specific primers, these were an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 30s at 94 °C, 30 s at 65 °C, 1 min at 72 °C and one cycle of 10 min at 72 °C. For primer couple DifGF/DifGR, PCR conditions were an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30s at 94 °C, 30 s at 55 °C, 30s at 72 °C and one cycle of 10 min at 72 °C. An aliquot of 7 µL of the amplification products was digested for 3 h by 1 U of restriction enzyme HpyCH4 III (New England Biolabs), and isomers of restriction enzyme TaaI. The expected size of digestion products were 442 bp, 227 bp and 76 bp for allele GF9 and 366 bp and 227 bp for allele GF7, respectively.

Analysis of electrophoresis products was performed as described above. eBSV patterns were compared to reference patterns of model species Pisang Klutuk Wulung (Gayral et al. 2008; Chabannes et al. 2013).

Mealybug collection and identification

A total of 400 mealybugs at various developmental stages were collected from the pseudostem of plants sampled in Montecristi and Puerto Plata during the regional BSV survey. Plants displayed various infestation levels; some were heavily infested, whereas others were not infested at all. Therefore, several insects were collected on heavily infested plants whereas none were collected from mealybug-free plants. Upon collection, insects were immediately placed in microfuge tubes containing 70% alcohol and kept at room temperature or at −20 °C until further use.

Each collected insect was dried, placed at −80 °C for 30 min in a 2 ml Safe Lock microfuge tube (Eppendorf) with a 5 mm steel bead (Loudet Industries) and ground in 150 µl of PBS (137 mM NaCl, 2, 7 mM KCl, 10 mM Na2HPO4, 1,76 mM KH2PO4) using a Tissue Lyzer (Qiagen). Total genomic DNA was extracted from each insect using the NucleoSpin® 96 Virus Core Kit (Macherey Nagel) and the automated extraction station Biomek 4000 (Beckman Coulters). Purified DNA was eluted in 200 µl of ultra-pure water. Molecular characterization of mealybugs was carried out by PCR, using the primers listed in Table II-3. Universal primers C1-J-2183 and TL2-N- 3014 (Simon et al. 1994) were

Table 1 Nucleotide sequences of the primers used for M-IC-PCR indexings

Target	Primer name	Primer sequence (5′-3′)	Expected size of PCR product	Reference
BSOLV	RD-F1 RD-R1	ATCTGAAGGTGTGTTGATCAATGC GCTCACTCCGCATCTTATCAGTC	522	Geering et al. 2000 Geering et al. 2011
BSGFV	GF-F1 GF-R1	ACGAACTATCACGACTTGTT CAAGC TCGGTGGAATAGTCCTGAGTCTTC	476	
BSIMV	IM-F1 IM-R1	CACCCAGACTTTTCTTTCTAG C TGCCAACGAATACTACATCAAC	384	
Monkey	MonF2 MonR2	GTC GAC ACA TGG GAG GAC TT CTT GTT GGG TCT TCA GAG GAA	300	Gambley 2008

used to amplify a 831 bp fragment in a highly conserved region of the gene encoding mitochondrial cytochrome c oxidase subunit 1 (COI).

Molecular identification of species *Planococcus citri*, *Planoccocus ficus*, *Pseudococcus longispinus*, *Planococcus minor* and *Dysmicoccus brevipes* was also attempted by PCR, using species-specific primers. For *P. citri*, *P. ficus* and *P. longispinus*, primer TL2N-3014 was used in a multiplex assay with primers C1-J-2260, C1-J-2427 and C1-J-2608 (Simon et al. 1994; Saccaggi et al. 2008). PCR conditions were an initial denaturation step at 94 °C for 4 min, then 35 cycles of 1 min at 94 °C, 1 min at 51 °C, 1.5 min at 72 °C and one cycle of 72 °C for 4 min. For *P. minor*, primer couple C1-J-2183 / 3014-R2 (Rung et al. 2009) was used. PCR conditions were an initial denaturation step at 94 °C for 5 min, then 35 cycles of 45 s at 94 °C, 45 s at 48 °C, 1 min at 72 °C and one cycle of 72 °C for 10 min. For *Dysmicoccus brevipes,* primer couple Dybr and Dybf was used (Simon et al. 1994). PCR conditions were an initial denaturation step at 95 °C for 30s, then 35 cycles of 45 s at 95 °C, 45 s at 61 °C, 1 min at 72 °C and one cycle of 72 °C for 5 min. PCR mixes contained 1 μl of extracted DNA diluted 1:10, 2.5 μl of each forward and reverse primer at 2.5 mM, 2.5 μl of 10x reaction buffer (400 mM Tris-HCl pH 8, 100 mM MgSO4, 10 mM CaCl2), 2 μl of 50 mM MgCl2, 0.5 μl of 2.5 mM dNTPs, 1 U Taq polymerase (New England Biolabs), and nuclease-free water up to a final volume of 25 μl. min. Analysis by electrophoresis was performed as described above. Amplification products were purified using the Qiaquick PCR purification kit (Qiagen) and sequenced by Beckman Coulter Genomics (Takeley, UK).

Results

National survey

A total of 838 samples was collected from 36 plantations throughout 11 provinces (Fig. 1; Supplementary Tables 1–4), corresponding to MxH (299 samples), FHIA-21 (291 samples) and Cavendish desert banana (248 samples).

BSIMV was not detected in any of the samples whereas both BSGFV and BSOLV were detected in sampled plants. BSGFV was the most prevalent species, infecting 19.59% and 4.01% of FHIA-21 and MxH samples, respectively. BSOLV was detected in 12.37% of FHIA-21 samples and 0.67% of the MxH samples, respectively (Table 2). Overall prevalence of BSGFV and BSOLV was significantly higher in FHIA-21 than in MxH. Co-infections by BSGFV and BSOLV were detected in 3% of the FHIA-21 samples but not in any of the MxH samples.

A total of 57 plots were sampled: 28 FHIA-21 plots and 29 MxH plots (Supp. Tables 1 and 2). BSGFV was present in 23/28 (82.1%) of the sampled FHIA-21 plots, in 7/29 (24.1%) of the sampled MxH plots, and in 5 of the 11 provinces where samples were collected. BSOLV was present in 18/28 (63.4%) of the sampled FHIA-21 plots, in 2/29 (6.9%) of the MxH sampled plots, and in 10 of the 11 provinces where samples were collected. BSOLV and BSGFV were more prevalent in FHIA-21 than in MxH. Overall, these results showed that BSV species are well distributed across the Dominican Republic's banana producing areas. The fact that 20 of the sampled FHIA-21 and MxH plots were adjacent did not influence the general results.

Very low prevalence was registered in Cavendish banana (Table 2): 1.61%, for BSOLV and 0% for BSGFV and BSIMV. Considering that the genome of Cavendish banana is devoid of eBSVs, infections occurred from the use of infected planting materials and/or from mealybug-mediated transmission. BSOLV-infected samples originated from three separate plots aged 10, 12 and 15 years, respectively. The absence of spread of BSOLV within these plots, even after such a long period of time, suggests that vector-borne transmission occurs at very low rates, perhaps because mealybug species associated with BSV transmission are not present, because they do not move around in these plots or because farmers carry out particularly thorough insect control, as would be expected for Cavendish bananas, which is grown for export in the Dominican Republic.

Most infected plants did not exhibit symptoms. Characteristic symptoms of BSV such as leaf streak, pseudostem splitting, cigar or petiole necrosis were observed only in a few infected FHIA-21 plants (Fig. 2). Therefore, no significant correlation could be established between the presence of symptoms and the presence of BSVs, confirming previous observations that diagnostic of BSV based on the presence of symptoms is not reliable.

Regional survey

A regional survey was performed in October 2013. It was focused on two provinces where the highest numbers of infected samples were registered during the nationwide survey, Monte Christi and Puerto Plata. Four sites were selected for sampling. FHIA-21 samples were collected from two sampling sites previously used for the nationwide survey in Montecristi: Jaramillo for plants originating from vitroplants and in Puerto Plata (Belloso) for plants originating from suckers. MxH samples were collected in Montecristi (Palo Verde) and in Puerto Plata (Belloso).

In Puerto Plata (Belloso), BSOLV and BSGFV were detected in 0% and 31% of the FHIA-21 samples, respectively (Supp. Table 4). Similar rates of infection of 30% and 30% were obtained from the same plot during the nationwide survey for BSOLV and BSGFV, respectively. In Montecristi (Jaramillo), BSOLV prevalence levels were 5% and 0% for samples originating from vitroplants and suckers, respectively

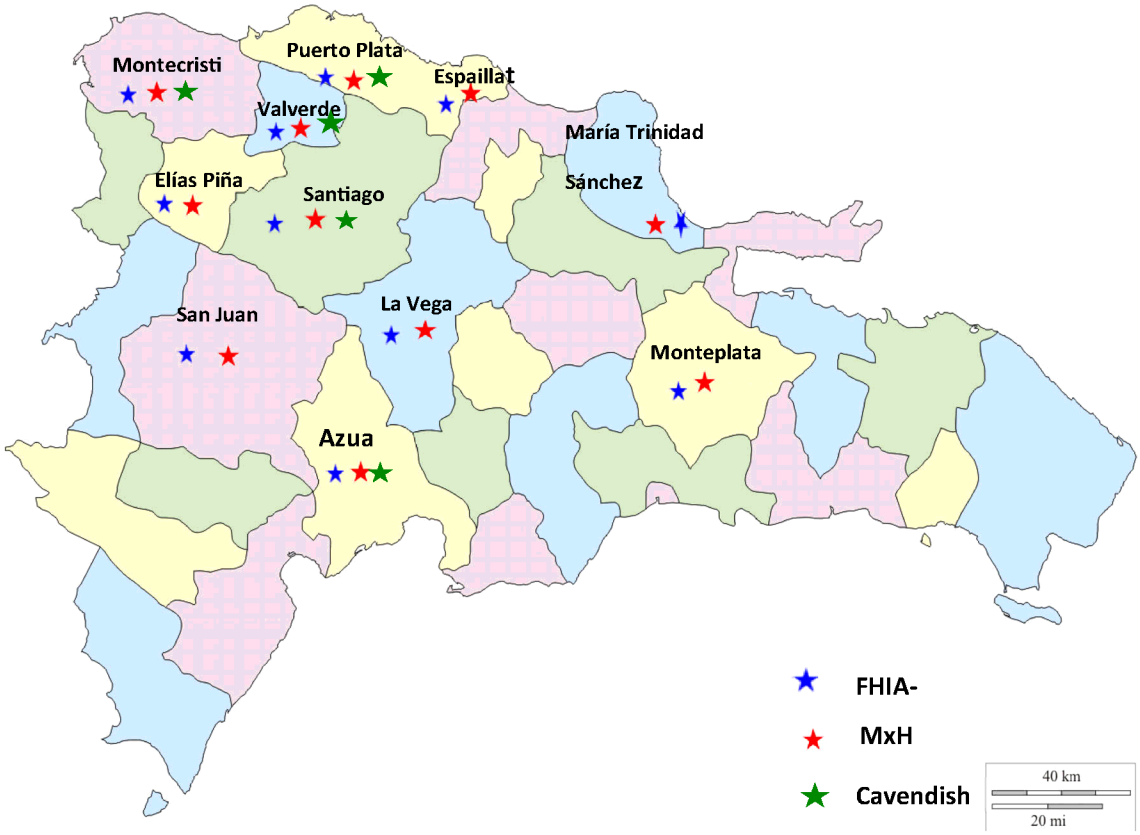

Fig. 1 Map of the collection sites used for the national and regional surveys

(Supp. Table 4). The prevalence rates for BSGFV were 29% and 31% (Supp. Table 4). BSIMV was not detected in any of the analyzed samples from the four sites (data not shown). These results confirmed those of the nationwide survey.

In Montecristi (Palo Verde), BSOLV and BSGFV were detected in 0% and 6% of the MxH samples, respectively (Supp. Table 4). Prevalence levels of 0% were obtained in the same plot during the nationwide survey for both BSOLV and/or BSGFV. The difference between average BSGFV and BSOLV prevalence in FHIA-21 (19.59%) and in MxH (12.37%) registered during the nationwide survey was confirmed by the regional survey, with values of 30% and 2.5%, respectively. Likewise, the average BSOLV and BSGFV prevalence in MxH was very similar for the nationwide survey (0.67% and 4.01%, respectively) and for the regional survey (0% and 3.5%, respectively).

Results of the regional survey confirmed those obtained for the nationwide survey, with BSOLV prevalence levels of 5% and 0% registered for samples originating from vitroplants and suckers, respectively, whereas BSGFV prevalence levels were 29% and 31%, respectively (Supp. Table 4). The regional survey also confirmed the absence of BSIMV.

BSGFV prevalence was significantly higher than that of BSOLV in FHIA-21 in the analyzed samples and was not influenced by the nature of the planting material (vitroplants or suckers). Among the 72 plants that were indexed positive

Table 2 Total plants indexed and prevalence of BSOLV, BSGFV and BSIMV in samples collected during the national survey

Cultivar	Total Indexed	Indexing results						Total positive samples	Percentage of positive samples (%)
		BSOLV		BSGFV		BSIMV			
		+	%	+	%	+	%		
FHIA-21	291	36	12.37	57	19.59	0	0	93	31.9
M x H	299	2	0.67	12	4.01	0	0	14	4.7
Cavendish	248	4	1.61	0	0	0	0	4	1.6
Total	838	42	5.0	69	8.2	0	0	111	13.2

+: number of infected plants

%: percentage of infected plants

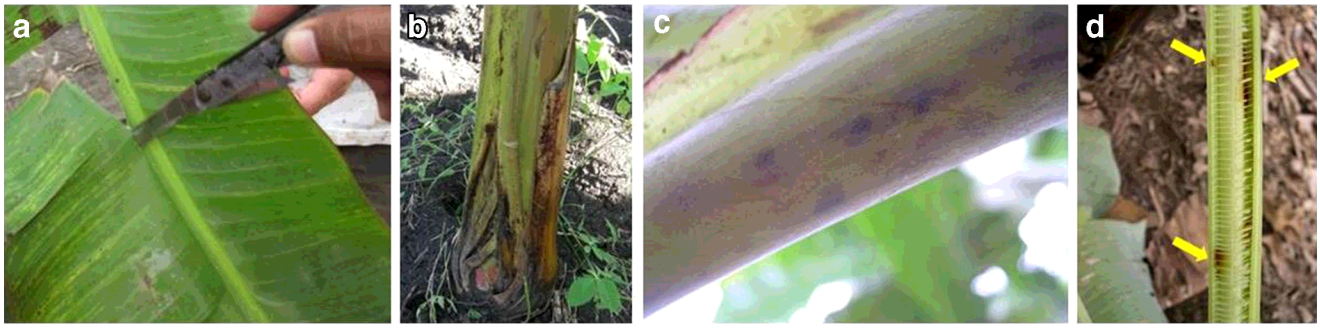

Fig. 2 BSV symptoms on leaf (**a**), pseudostem (**b**) and petiole (**c**, **d**) observed on infected FHIA-21 plants during the nationwide survey. Yellow arrows point to internal necrosis symptoms observed in the section of a petiole (**d**). Source: R.T. Martinez, ©IDIAF

(Supp. Table 4), only two FHIA-21 plants infected by BSGFV were infested by mealybugs, suggesting that BSV infections occurred either from the use of infected planting material or the activation of infectious eBSVs rather than from mealybug-mediated transmission.

Analysis of the temperature data collected during six months prior to the survey did not show any correlation with infections rates (data not shown).

eBSV characterization in FHIA-21 and M x H cultivars

During the nationwide survey, 49 leaf samples were collected randomly from 24 of the FHIA-21 plots and 25 of the MxH plots that were sampled. Multiple plants were purposely analyzed in order to assess that all samples from a given variety shared the same eBSV pattern, considering that mislabeling of varieties is frequent. All analyzed samples displayed identical eBSV patterns: they harbored infectious alleles OL1 and GF7 (Fig. 3) and were devoid of eBSIMV. Therefore in both FHIA-21 and MxH hybrids, BSOLV and BSGFV infections result from the activation of OL1 or GF7 infectious alleles and/or from mealybug-mediated transmission and/or the use of infected planting materiel, whereas BSIMV infections could result only from mealybug-mediated transmission and/or the use of infected planting material. The fact that no BSIMV infection was registered sustains the hypothesis that mealybug-mediated transmission plays a marginal role in the epidemiology of BSVs in the Dominican Republic.

Diversity of mealybug species

The diversity of mealybug species in the FHIA-21 and MxH plots that were sampled during the regional prevalence survey was assessed by PCR. Total genomic DNA was extracted from the 400 individual insects collected in these plots and used for PCRs using either generic primers or specific primers for species *Planococcus citri*, *Planoccocus ficus*, *Pseudococcus longispinus*, *Planococcus minor* or *Dysmicoccus brevipes*, which are vectors of BSVs. All primers targeted the mitochondrial cytochrome c oxidase subunit I (COI).

A total of 297 samples gave rise to an amplification product of the expected size (536 bp) using generic primer pair TL2N-3014 / CJ-J-2183. It is likely that the remaining 103 samples contained insufficient DNA quantities to raise PCR products. PCR products were also raised using specific primer pairs specific of *P. citri* (CitriFor1 / CitriRev1), *P. longispinus* (C1-J-2608 / TL2-N-3014) and *D. brevipes* (Dyb-1F / Dyb-1R). Amplification products obtained using the generic primer pair TL2N-3014 / CJ-J-2183 were sequenced and sequence comparisons were performed using all mealybug COI sequences available from GenBank, except the one from *Saccharicoccus sacchari* because it overlaps only on a short

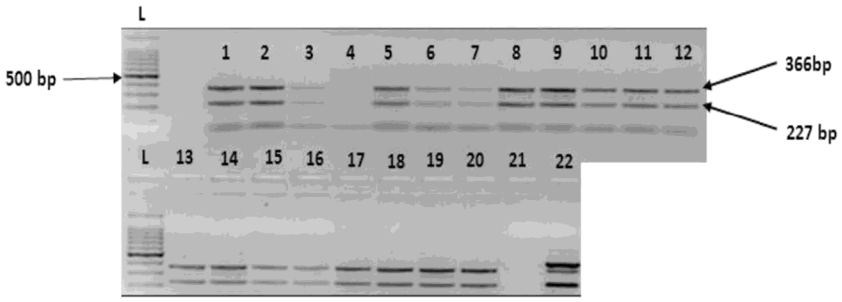

Fig. 3 eBSGFV genotyping of FHIA-21 and MxH samples by PCR using dCAPS markers. Amplification products obtained using the DifGFF / DifGFR primer pair were digested by restriction enzyme HpyCH4III prior to electrophoresis. Size of digestion products is shown on the right side of figure; 500 bp band of DNA size marker is shown on the left side of figure. L: 100 bp DNA ladder; 1–10: FHIA-21 samples; 11–20: MxH samples; 21: reaction mix only; 22: Pisang Klutuk Wulung

stretch (137 bp) with the other 536 bp sequences used in our analyses.

Amplified sequences from the mealybugs collected on sampled FHIA-21 and MxH grouped in two clades on phylogenetic branches that are well supported by high bootstrap values (Fig. 4). One of the clades does not group with any of the known mealybug species for which COI sequences are available; the other clade groups with COI sequences from *D. neobrevipes*, which is closely related to *D. brevipes.* None of these two clades groups with any of the mealybug species known to transmit BSVs, which were not represented in the mealybugs collected on FHIA-21 and MxH plants. Overall, our analyses further support the hypothesis that mealybug transmission plays a marginal role in the dynamics of BSVs in FHIA-21 and MxH in the Dominican Republic.

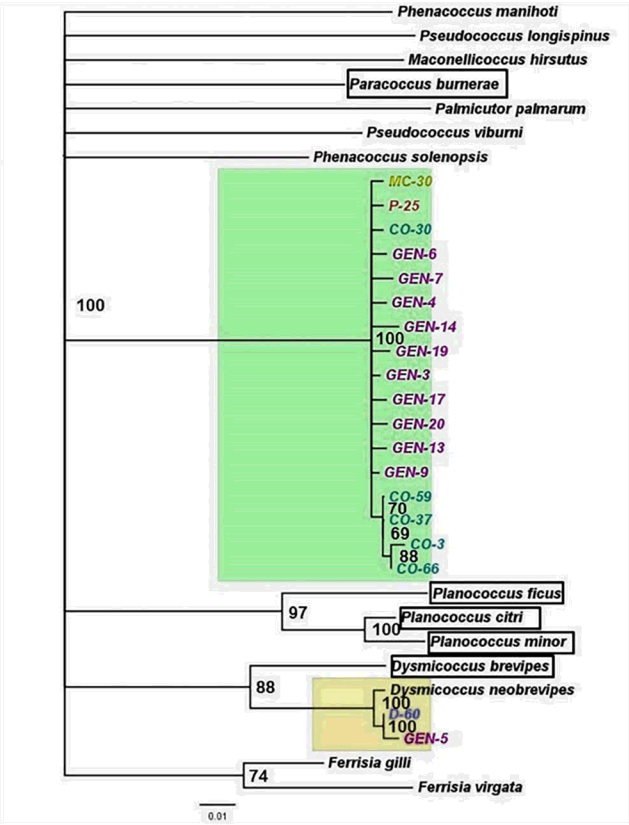

Fig. 4 Phylogenetic neighbor-joining tree built from partial 536 bp cytochrome C oxydase nucleotide sequences amplified from mealybug samples and from homologous sequences from known mealybug species. Bootstrap values of 1000 replicates are given above nodes when above 50% (Tamura et al. 2004). Alignments were performed using CLUSTALW (Larkin et al. 2007) and phylogenetic tree was built with MEGA6 using the Neighbor-Joining method based on the HKY model (Hasegawa et al. 1985; Tamura et al. 2011). Only sequences displaying more than one substitution per base to each other were retained for the construction of the phylogenetic tree in order to avoid redundancy. The scale bar shows the number of substitutions per base. Partial cytochrome C oxidase sequences from mealybug species *Phenacoccus manihoti* (EU267196), *Palmicutor palmarum* (EU267218), *Pseudococcus longispinus* (EU267194), *Paracoccus burnerae* (FJ786962), *Maconellicoccus hirsutus* (EU267200), *Phenacoccus solenopsis* (EU267212), *Pseudococcus viburni* (EU267207), *Planococcus citri* (DQ238221), *Planococcus ficus* (DQ238220), *Dysmicoccus brevipes* (EU267214), *Dysmicoccus neobrevipes* (AF483206), *Ferrisia gilli* (EU267203) and *Ferrisia virgata* (EU267205) were used. Selected sequences amplified from collected mealybugs are shown in the green and yellow boxes. They were amplified using generic primer pairs C1-J-2183/TL2-N- 3014 (GEN and CO; Simon et al. 1994) and species-specific primer pairs CitriForl / CitriRevl (MC; Rung et al. 2009) and Dybr/Dybf (D; Simon et al. 1994). COI sequences of mealybug species known to transmit BSVs are boxed

Discussion

This paper reports on the first study of the prevalence and diversity of BSVs in the Dominican Republic. Leaf samples were collected from 590 interspecific hybrids with AAB or AAAB genotypes and from 248 dessert bananas with AAA genotype throughout all banana growing areas of the country. An additional 200 samples were collected for each interspecific hybrid in two locations, bringing the total sample count to 1238. BSV species BSOLV and BSGFV were found to be present in the Dominican Republic, but not BSIMV.

Surveys' results highlighted the higher prevalence of species BSOLV and BSGFV in interspecific hybrid FHIA-21 (AAAB) than in interspecific hybrid MxH (AAB) or Cavendish banana (AAA) in which only BSGFV was detected. Similar findings were reported previously in another Caribbean island, Guadeloupe, where prevalence of BSGFV and BSOLV was also found to be significantly higher in interspecific AAB hybrids than in Cavendish cultivar (Péréfarres et al. 2009). We established the molecular eBSV patterns of FHIA-21 and MxH and showed that both hybrids harbour infectious alleles OL1 and GF7. This makes FHIA-21 and MxH prone to spontaneous BSOLV and BSGFV infections following the activation of infectious alleles OL1 and GF7 by abiotic stresses (Dallot et al. 2001; Meyer et al. 2008; Côte et al. 2010), as opposed to Cavendish which does not harbor eBSVs (Gayral et al. 2008). Infestation by mealybugs was low in all sampled plots. Similar low infestation was also reported in Guadeloupe (Péréfarres et al. 2009) and in Mauritius (Jaufeerally-Fakim et al. 2006). Furthermore, the molecular analyses reported in this work showed that none of the mealybug species that can transmit BSVs were present in the sampled plots. Overall, our results suggest that mealybug transmission plays a marginal role in the dynamics of BSVs in the Dominican Republic. This is exemplified by the very low prevalence of BSOLV (1.61%) and absence of BSGFV registered in Cavendish during the national survey. In consequence, the spread of BSOLV and BSGFV in the Dominican Republic is likely to result from the activation of infectious eBSVs in interspecific hybrids by abiotic stresses such as temperature differences (Dahal et al. 1998; Gauhl and Pasberg-Gauhl 1995; Ortiz 1996) and/or the use of infected plant material. However, no statistically supported correlation

could be established between temperature differences and prevalence levels in the sampled plots (data not shown).

Prevalence differed for BSOLV and BSGFV in FHIA-21 and MxH, although both hybrids share similar eBSV patterns. This suggests that OL1 and GF7 infectious alleles display differential activation potentials in FHIA-21 and MxH, maybe because of their different ploidy or their respective genetic background, as previously hypothesized for other interspecific hybrids (Côte et al. 2010).

The majority of infected plants showed no BSV symptoms. A similar situation was reported in Cuba and Guadeloupe during nationwide BSV prevalence surveys (Javer-Higinson et al. 2014; Péréfarres et al. 2009). The fact that most BSV-infected plants are asymptomatic underlines the outermost importance of accurate molecular diagnostic methods to prevent the spread of BSVs and suggest that BSV infections have a low impact on FHIA-21, MxH and Cavendish grown in the Dominican Republic.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest

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