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ORIGINAL ARTICLE

Sequencing and assembly of small RNAs reveal the presence of several begomoviruses, potyviruses, badnaviruses and mastreviruses in the sweet potato leaf virome in Barbados

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Abstract

Sweet potato [*Ipomoea batatas* (L.) Lam] is increasingly becoming an important food and industrial crop worldwide that is affected by over thirty viruses belonging to different virus families. Little is known about these viruses on the many local and commercial sweet potato cultivars in the Caribbean, including Barbados. Therefore, polymerase chain reaction (PCR) and small RNA sequencing and assembly (sRSA) were used to detect viruses in the sweet potato leaf virome in Barbados. The viruses detected by sRSA were mainly from two virus families in sweet potato: *Geminiviridae* (genus *Begomovirus*) and *Caulimoviridae* (genus *Badnavirus*). Together, these viruses accounted for more than 75% of the virus contigs found in the sweet potato leaf virome in Barbados. Although sweet potato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) was frequently detected by PCR in infected leaf samples, only 10% of the sequenced contigs matched to these viruses in the leaf virome. The results suggest the absence of the sweet potato virus disease complex (SPVD) in Barbados but reveal possibly new, severe single virus infections or emerging virus associations in the virome largely composed of begomoviruses, badnaviruses, potyviruses and mastreviruses previously unreported in sweet potato in the Caribbean.

Keywords Sweet potato · Sweepovirus · sRSA · RNAseq

Introduction

Sweet potato infecting viruses belong to several different families: *Caulimoviridae*, *Geminiviridae*, *Bromoviridae*, *Closteroviridae*, *Flexiviridae*, *Comoviridae*, *Luteoviridae* and *Potyviridae* (Kreuze et al. 2009; Clark et al. 2012). Viruses within these families may either affect crops singly or participate in synergistic co-infections along with other viruses. These latter virus partnerships may promote the development of virus disease complexes that severely affect crop quality and yield (Gutiérrez et al. 2003; Gibson and Kreuze 2015). Yield losses of 100% and even plant death have sometimes been reported for sweet potato virus disease complex (SPVD) (Untiveros et al. 2008). Similar occurrences of such yield limiting patterns have been observed in the sweet potato crop in some parts of the island of Barbados since 2001 (James et al. 2003) and continue to threaten the industry today.

SPVD is the most frequently described disease complex (Gibson et al. 1998; Karyeija et al. 2000; Tairo et al. 2005; Kokkinos and Clark 2006) and is composed of two major virus families: *Potyviridae* and *Closteroviridae*. General symptoms of SPVD include chlorosis, stunting, leaf strapping, crop yield reduction, vein clearing and leaf distortion (Kokkinos and Clark 2006; Untiveros et al. 2008). *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*, family *Potyviridae*), an aphid-transmitted virus, is the main virus found in SPVD, and is singly the most common pathogen affecting sweet potato crops worldwide (Tugume et al. 2010; Kashif et al. 2012). *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*, family *Closteroviridae*) is equally important

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in SPVD and is transmitted by whiteflies (Untiveros et al. 2008). In some African countries, SPCSV is the main synergistic virus found in partnership with SPFMV (Gibson and Kreuze 2015), but it may be difficult to detect because it decreases its own viral RNA titre, when synergized in mixed infections (Gibson and Kreuze 2015).

Enzyme linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) was previously used in the detection and diagnosis of sweet potato infecting viruses in Barbados (James et al. 2003) and showed a higher incidence of virus infection mixtures involving SPFMV, sweet potato virus G (SPVG) and sweet potato virus 2 (SPV2 [69%]), than mixtures including SPCSV (7%) (James et al. 2003). Since then, new techniques such as species-specific PCR and small RNA sequencing and assembly (sRSA) in the sweet potato virome have revealed a diverse collection of virus families and new virus associations affecting commercial sweet potato globally (Kreuze et al. 2009; Cuellar et al. 2015). Currently, more viruses are being detected through deep sequencing of small RNA (sRNA) molecules which are produced against viral infection (Kreuze et al. 2009; Mbanzibwa et al. 2011; Kashif et al. 2012; Gibson and Kreuze 2015).

Accordingly, to improve the previous NCM-ELISA studies on the island, the main objective of the current study was to identify the specific viruses found infecting sweet potato plants showing SPVD-like symptoms in Barbados by using specific molecular markers and small RNA sequencing and assembly.

Materials and methods

Sample collection A total of 150 symptomatic leaf samples from available sweet potato cultivars 'CBS 49', 'C-104', 'CBS 32' and 'Carolina Lee' were collected (five leaves per cultivar) from a total of 10 fields located in three rainfall zones within Barbados with dry, southern parishes (1100–1500 mm of precipitation), intermediate, south-western parishes (1500–1600 mm of precipitation) and wet, central parishes (1600–1900 mm of precipitation) in these fields. Sweet potato plants from the cultivars 'CBS 49' (cream skin, with orange flesh and elongated tuberous roots), 'C-104' (red skin, with white flesh and round tuberous roots), 'CBS 32' (red or brown skin, with deep orange flesh and oval tuberous roots) and 'Carolina Lee' (white or cream skin, with white or cream flesh and round tuberous roots) ranged in age from three weeks to three months. Leaf samples collected that displayed typical virus symptoms (from mild to severe leaf curling, distortion and chlorosis) were dissected and then immediately placed in 5 mL of RNA*later*® (Life Technologies, San Juan, Puerto Rico) for storage. Each sample was then refrigerated at −20 °C before RNA extraction. A disease-free leaf of *Bougainvillea glabra* L. (Bougainvillea) representing a distinctly different genus was used as the negative control due to a lack of *Ipomoea* spp. disease-free tissue culture material. For PCR, only leaves from 'C-104', 'CBS 49' and 'Carolina Lee' were used, and for sRSA, leaf samples from 'CBS 32' were used instead of those from 'Carolina Lee'. 'CBS 32' is a recently introduced cultivar and is now grown more widely than 'Carolina Lee', a traditional local cultivar that was not always available during the study.

RNA extraction A total of thirty RNA extractions were performed for PCR reactions from the collected leaf samples representing cultivars 'CBS 49', 'C-104' and 'Carolina Lee', using a QIAGEN RNeasy Mini kit (QIAGEN®, CA, USA) and following the manufacturer's instructions. A single RNA extraction was conducted in duplicate from leaf tissue (100 mg, three leaves combined) for each sweet potato variety. RNA was eluted in a final volume of 50 μL of RNase-free water (RFW). Each extraction was performed in duplicate for each sweet potato variety, and its quality and quantity were measured using a Beckman Coulter DU730 UV spectrophotometer and by agarose gel electrophoresis (1%). Five RNA extracts were discarded due to poor quality RNA assessed by gel electrophoresis and $A260/280_{nm}$ readings. The aliquots of RNA were stored at -20 °C until further processing. RNA was also similarly extracted from a disease-free leaf as described above as a negative control.

Total RNA, purified with the Trizol RNA extraction method was used in the preparation of three sRNA libraries with each one representing a single cultivar namely, 'C-104', 'CBS 49' and 'CBS 32'. Sweet potato leaf tissue (100 mg) was homogenized with 1 mL of Trizol reagent (Invitrogen®, Life Technologies, San Juan, Puerto Rico) according to the manufacturer's instructions. RNA was suspended in 200 μL of TE buffer and measured in a UV spectrophotometer, as previously described.

Polymerase chain reaction of the *Crinivirus* and *Potyvirus* infecting sweet potato A PCR protocol based on methods used by Winter et al. (1997) was performed to detect the conserved HSP70h gene fragment of SPCSV. Complementary DNA (cDNA) synthesis was conducted in a 10 μL final volume containing RNA (0.5 μg), 200 U of SuperScript III RT polymerase (Life Technologies®, Invitrogen, San Juan, Puerto Rico), 0.2 mM dNTPs, 0.5 μM primer Hsp70-R (Table 1) and 1X RT buffer. Each sample was then incubated at 37 °C for 45 min in a heating block and then stored at -20 °C. PCR was performed in a 20 μL total volume containing 1X buffer, 4 μL of cDNA, 1 mM dNTPs, 4.5 mM $MgCl_2$, 0.4 μM primers Hsp70-F and Hsp70-R (Table 1), and 1 U of Go*Taq* DNA polymerase (Promega Corp., Madison, WI, USA). The PCR conditions were 30 cycles of 92 °C for 60 s, 50 °C for 30 s and 72 °C for 60 s, and then a final extension at 72 °C for 10 min. PCR products were separated

Table 1 Primer sequences and expected amplicon size for selected sweet potato viruses

Virus	Primers	Sequence 5' ➡ 3'	Amplicon size (bp)	Reference
FMV	POT1 POT2	GACTGGATCCATTBTCDATRCACCA GACGAATTCTGYGAYGCBGATGGYTC	1300	Colinet *et al.* (1997)
SPCSV	Hsp70-F Hsp70-R	ATCGGCGTATGTTGGTGGTA GCAGCAGAAGGCTCGTTTAT	486	Winter *et al.* (1997)
SPVG	SPVG -F SPVG-R	TATACCGCGGAAAAGCACCCTACATAGCT TATATGAGCTCCACTGAAGGCGAAACTGAAA	1287	Rännäli et al. (2008)
SPV2	SPV2-F SPV2-R	CGAACTTGCTCGAGTAGGCAG TCCGTCCATCATCACCCA	753	Ateka et al. (2004)
SPLCV	SPG1 SPG 2	CCCCKGTGCGWRAATCCAT ATCCVAAYWTYCAGGGAGCTAA	912	Li et al (2004)
BadnaV	BaVC1-F BaVC1-R	ATTGCCCTGCATGTTTGCTCAC AAATCCTCAACTGTCATTGTTACG	302	DNASTAR V. 1.5
MasTV	MaVC18-F MaVC18-R	GACGTCGTATTCTTATCTTATCAG ATCGTCTTCCATTTTCTTTTTACT	840	DNASTAR V. 1.5

on 1.5% agarose gels stained with ethidium bromide (0.5 mg mL^{-1}) for visual detection of presence or absence of the expected 486 bp amplicon. For potyviruses first strand cDNA synthesis was performed with the POT1 primer for SPFMV, SPVG and SPV2 (Colinet et al. 1997) and superscript III as described above.

PCR conditions for SPMFV were as follow: 40 cycles of 94 °C for 30 s, 60 °C for 60 s and 72 °C for 90 s and a final extension at 72 °C for 5 min. PCR reactions for SPV2 and SPVG were based on these published protocols (Ateka et al. 2004; Rännäli et al. 2008).

All PCR reactions were conducted on a Veriti thermocycler (Life Technologies®, Applied Biosystems, San Juan, Puerto Rico). RFW and RNA extracted from disease-free Bougainvillea leaf were used as no template and negative controls, respectively. A positive identification of the suspected virus was done by the presence of expected size of the amplified PCR product (Table 1) followed by verification of its sequence in GenBank. Selected positive amplicons for SPFMV were sequenced after PCR clean-up with a ChargeSwitch® Pro PCR Cleanup Kit (Life Technologies™, San Juan, Puerto Rico) as described by the manufacturer. Sequencing was conducted at GENEWIZ® (Washington D.C., USA) using the POT2 primer.

Small RNA (sRNA) sequence analysis TRIZOL-extracted total RNA (previously described) from leaf samples of each sweet potato cultivar 'C-104', 'CBS 49' and 'CBS 32' was subjected to next-generation sequencing (NGS). 'CBS 32' replaced 'Carolina Lee' as no positive PCR results were obtained from 'Carolina Lee' for the viruses that were tested as described above. RNA processing consisted of separation of RNA by 3.5% agarose gel electrophoresis and isolation of RNA bands <30 nucleotides (nts), followed by ligation with single-stranded 3′ and 5′ adapters. These ligated fragments were then reverse transcribed and amplified by PCR to generate a library of short inserts representing a bulk sample of each cultivar (Chen et al. 2012). Three libraries, GAF 318-41, GAF 318-42 and GAF 318-43 composed of suspected virus-infected leaves of cultivars 'C-104', 'CBS 49' and 'CBS 32', respectively, were constructed in vitro from gel-purified sRNA fragments. Each library was then sequenced on an Illumina Hi-Seq (Fasteris Inc.) (http://www.fasteris.com) (Kreuze et al. 2009; Kashif et al. 2012).

sRSA and genomic analysis Data were reported using Hi-Seq control software 2.2.38. Adapter sequences at both ends were removed, and sRNA reads were analysed using VirusDetect software v 1.5 (Li et al. 2012; Zheng et al. 2017). Additionally, sRNA reads for each library were also imported into Geneious 11.02 http://www.geneious.com, (Kearse et al. 2012), and the assembled contigs were mapped to reference sequences and further analysed by Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1990). Each library was then mapped in Geneious (11.02) to four known reference sequences represented by the most frequently occurring contigs for each virus species, These were as follows: NC_001841, sweet potato feathery mottle virus complete genome; FJ560945, sweet potato symptomless mastrevirus 1 movement protein (V1) gene, complete cds; and coat protein (V2) gene, partial cds; JQ902104, sweet potato badnavirus C isolate SCCD polyprotein gene, partial cds and HQ333144, sweet potato leaf curl South Carolina virus isolate US:SC:648B-9, complete genome.

PCR amplification of identified contigs Positive contigs of badnavirus and mastrevirus found in the genomic libraries representing infected sweet potato in Barbados were confirmed by DNA extraction from fresh leaf material of 'CBS 32' and 'CBS 49' using a Qiagen DNeasy Plant Mini Kit

(Qiagen®, USA) followed by PCR and sequencing of the PCR products. The primers for badnaviruses A and B, (BaVC1-F and BaVC1-R) and mastreviruses (MaVC18-F and MaVC18-R) were designed (Table 1) using PrimerSelect from the Lasergene software package v.5.00 (DNASTAR). Consensus of the sRNA sequences from GAF 318–41, −42, and − 43 aligned with the reference sequences of those viruses available in the GenBank database was used as template for designing primers. In addition, DNA extracted from virus-infected samples of 'CBS 49' was used to confirm the presence of begomovirus using the standard primers SPG1 and SPG2 reported by Li et al. (2004) (Table 1).

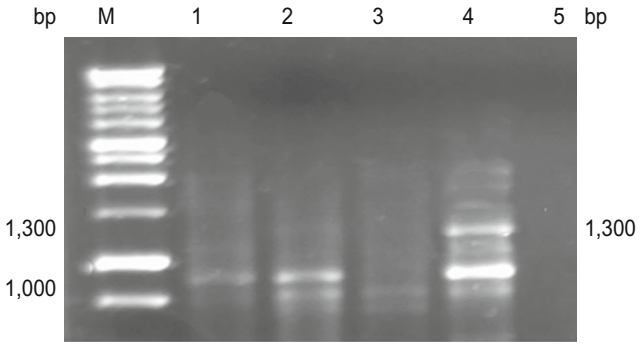

Fig. 2 Agarose gel of PCR amplicons produced with the POT1/POT2 PCR primer pair specific for potyviruses in CBS-104 and CBS 49 leaf tissues. The 1.5% agarose gel contains DNA from: M, 1Kb DNA ladder (Promega); Lane 1, amplified fragments from asymptomatic Bougainvillea leaf; lane 2, amplified fragments of C-104 (from Armag field); lane 3, CBS 49 (from Colleton field); lane 4, C-104 (from Fisherpond field); and lane 5, no template control (nuclease-free water)

Results

Virus disease symptoms and viruses detected by the polymerase chain reaction Generally, contrasting virus symptoms were observed in dry and intermediate rainfall zones (Fig. 1), with more severe symptoms in dry areas. Leaf samples taken from plants in dry areas appeared more stunted and displayed more signs of chlorosis than those taken from higher rainfall areas. A 1300 bp PCR amplicon, characteristic of potyviruses (Fig. 2), was produced in only two cultivars suspected to be virus-infected, 'C-104' and 'CBS 49'. Although all three cultivars were present in both dry and intermediate rainfall zones, there was no positive amplification of virus fragments derived from cDNA of 'Carolina Lee' samples (Table 2). A total of 7 positively amplified SPFMV virus fragments by PCR were observed from the twenty-five samples analysed (Table 2). Analysis of the consensus sequences of the amplified fragments showed that the sequence was derived from SPFMV. This virus was therefore present throughout all rainfall zones in the island on cultivar 'C-104' and in both intermediate and dry zones on cultivar 'CBS 49' (Table 2). Sequence results of the amplified products indicated 96% identity with an SPFMV isolate (GenBank accession No. AY523547), and 100% identity with isolate KNG59b polyprotein gene (FJ795751.1), for both 'CBS 49' and 'C-104'. No PCR amplicons of the expected sizes for SPCSV, SPVG and SPV2 were obtained in the tested samples.

Viruses detected by small RNA sequencing and assembly Deep sequencing of the three libraries representing cDNA from samples of cultivars 'C-104', 'CBS 49' and 'CBS 32' showed the presence of several viruses capable of infecting sweet potato including, potyviruses, badnaviruses and mastreviruses. In each library, most reads were 24 nt long, with GAF 318–43 comprising the largest number of reads (929,987), followed by GAF 318-41 (878,153) and GAF 318-42 (550,482), and ranged in size from 18 to 25 nts. Virus contigs detected by both VirusDetect software v1.5 and

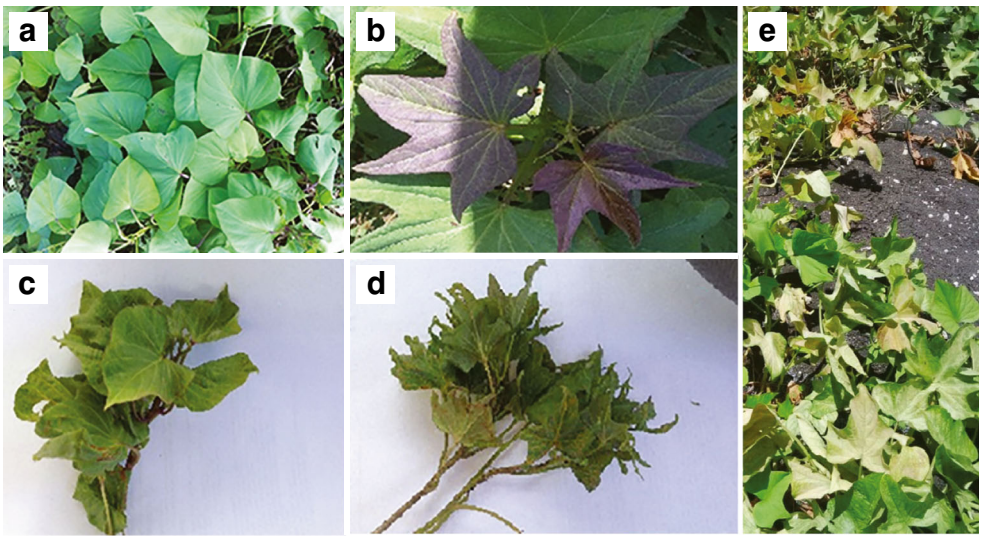

Fig. 1 Contrasting virus symptoms from suspected SPVD-like infected sweet potato in intermediate (1500–1600 mm of precipitation) (**a** and **b**) and dry (1100–1500 mm of precipitation) (**c**, **d** and **e**) rainfall zones in Barbados. **a** and **c**, cultivar C-104 with mild and chlorosis to severe leaf distortion, respectively. **b** and **d**, cultivar CBS 49 with mosaic and leaf distortion, respectively. E, cultivar CBS 32 with leaf curling and mild yellow mosaic symptoms

Table 2 Geographic location and detection of SPFMV by PCR in sweet potato cultivars sampled from three rainfall zones in Barbados (December 2013–February 2014)

Parish	Rainfall zone	Fields	Cultivars			
			C 104	CBS 49	Carolina Lee	CBS 32
St. Phillip	Dry[a]	Armag	+[d]	+	nd[f]	nd
	Dry	Groves	–	-[e]	–	nd
	Dry	Foursquare	–	–	–	nd
Christchurch	Dry	Graeme Hall	–	–	–	nd
	Dry	Hannays	+	+	–	nd
St. Michael	Intermediate[b]	Drax Hall	–	–	–	nd
St. Peter	Intermediate	Colleton	+	+	nd	nd
St. George	Intermediate	Buttals	–	–	nd	nd
St. Thomas	Wet[c]	Fisherpond	+	–	nd	nd
	Wet	Nicholls	–	–	nd	nd

[a] low rainfall 1100–1500 mm (dry zone); [b] intermediate rainfall 1500–1600 mm (intermediate zone); [c] high rainfall 1600–1900 mm (wet zone); [d] positive PCR result; [e] negative PCR result, f- PCR not conducted due to lack of available variety; [d] positive PCR result, [e] negative PCR result, [f] PCR not conducted due to lack of available variety

BLASTn in Geneious 11.02 included potyviruses (SPFMV), begomoviruses (SPLCV, *Ipomoea yellow vein virus* IYVV), mastreviruses (SPSMV-1), and badnaviruses (SPPV-A, SPPV-B, and SPPV-C). Each library was then mapped to the most frequently occurring contigs in Geneious (11.02), representing four known references (Fig. 3A1-C3). Summaries of viruses detected in these libraries are presented in the supplementary Tables S1 to S3, along with their corresponding contig lengths and depths of coverage.

Caulimoviridae composed of badnaviruses represented the largest number of contigs representing sRNAs targeting viruses in GAF 318-41, −42 and − 43 (supplementary Tables S1-S3). Interestingly, sRNAs targeting SPFMV representing *Potyviridae* had the lowest number of contigs of the three main virus families represented in each library (Fig. 3). No begomoviruses were detected in cultivar CBS 32 represented by cDNA library GAF 318-43.

In each library, the sequences of two unclassified mastreviruses (GenBank accession Nos. FJ569045 and FJ560946) with 90–94% coverage to mastreviruses were identified (supplementary Tables S1 to S3). An amplified 840 bp PCR fragment from a mastrevirus was confirmed by PCR and DNA sequencing on infected sweet potato leaves taken from C-104 (supplementary Fig. S1B). Similarly, PCR confirmation of a 302 bp amplicon also from a C-104 leaf sample, confirmed the presence of badnaviruses. Contig 1 from GAF 318-41 corresponding to sample C-104 shared 94% similarity to the complete genome of sweet potato badnavirus B (FJ560944), and the contig 18 shared 99% similarity to sweet potato symptomless mastrevirus 1 movement protein V1 gene (FJ569045) (Fig. 3). Begomoviruses were also confirmed by amplification of a 912 bp fragment of DNA from leaf samples of CBS 49 using primers SPG1 and SPG2 (Supplementary Fig. S1C).

Generally, in the analysed leaf samples, the most frequently assembled contigs were represented by strains of begomoviruses (56%), specifically by SPLCV found in GAF 318-41 and GAF 318-42, followed by those derived from badnaviruses (29%) (Fig. 4). Potyviruses, although found in each library, represented only (10%) of the viruses represented in the virome, with SPFMV occurring most frequently in cultivar CBS 32 represented by the cDNA library GAF 318-43 (Fig. 4). Contigs representing SPCSV strains were not found in the tested samples (Fig. 4). The complete genomes of sweet potato badnavirus A and B and segment A of SPLCV were present in all three libraries in addition to the partial genome segment of sweet potato badnavirus C.

Discussion

The current study confirmed the presence of SPFMV by standard PCR and sRSA in samples of sweet potato showing SPVD-like symptoms in Barbados, as previously reported by James et al. (2003). It is interesting that more severe and different virus symptoms were observed in dry areas on the island, when compared with those in intermediate and wet zones. Moreover, only cultivars ‘C-104’ and ‘CBS 49’ showed positive PCR results for potyviruses in all three rainfall zones. ‘C-104’ and other cultivars such as ‘Carolina Lee’ with similar characteristics (red-skinned with white flesh) are traditionally grown throughout the island and have a long history of cultivation. The ‘C-104’ cultivar is apparently highly susceptible to viruses in Barbados, and in the cDNA library that was sequenced, contigs matched to all three families of virus represented in the study. This was in stark contrast to cultivar ‘CBS 32’ which was the cultivar that replaced ‘Carolina Lee’ for deep sequencing because of the absence

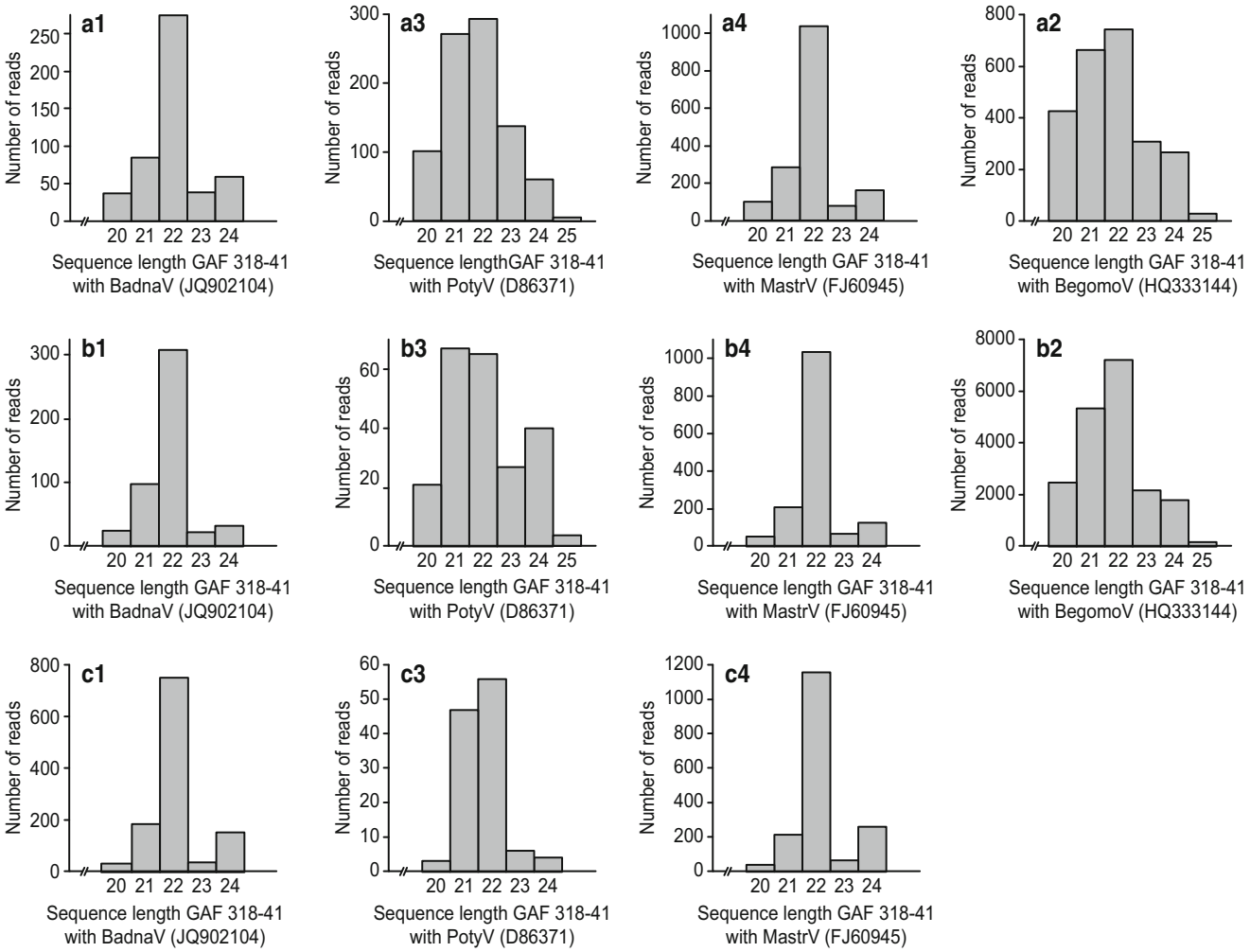

Fig. 3 Bar graphs depicting the amount and size of small RNAs mapped to each of the sweet potato viruses detected in infected sweet potato leaves represented by cDNA libraries GAF 318-41 (A1-A4), GAF 318-42 (B1-B4) and GAF 318-43 (C1-C3) corresponding to cultivars C-104, CBS 49, and CBS 32, respectively, from Barbados. Reference sequences used for mapping are indicated between brackets below each graph. The y- axis indicates the number of reads mapped to each reference, and x-axis indicates the length of each read

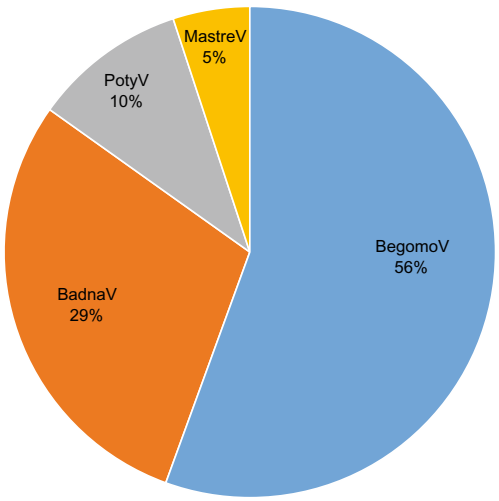

Fig. 4 Abundance of detected virus contigs of small interfering RNAs in the sweet potato leaf virome in Barbados from three cDNA libraries of cultivars C-104, CBS 49, and CBS 32

of a positive PCR result in the study. While we cannot confirm by sRSA whether the PCR result from 'Carolina Lee' was a false negative, this cultivar had similar RNA concentrations to all other extracted leaf samples.

'CBS 32' had a distinct absence of begomovirus sequences that matched to the virus contigs in the cDNA library GAF 318–43. Moreover, the symptoms of 'CBS 32' were leaf curling and slight yellow mosaic in contrast to the leaf distortion observed in 'C-104' and 'CBS 49'. Notably, samples of 'CBS 32' selected for deep sequencing were also obtained in the dry rainfall zone, as were samples of 'C-104' and 'CBS 49'. Sweet potato cultivation in Barbados is characterized by the steady replacement of rounded white or cream-fleshed types such as 'Carolina Lee' with elongated orange-fleshed cultivars such as 'CBS 49' and 'CBS 32'. This may be one of several reasons for the differences in the sweet potato leaf virome in 'CBS 32' (a recently introduced cultivar) when compared with that of 'C-104' and 'CBS 49' on the island. Therefore,

further examination of the effect of begomovirus infection in 'CBS 32' in Barbados is required.

A 28% frequency of SPFMV occurred among diseased samples tested with standard PCR using POT1/POT2 primers on the cultivars 'C-104' and 'CBS 49', while SPFMV was detected by sRSA (10% of the contigs assembled matched to the virus) in these cultivars and 'CBS 32'. The increased number of hits to SPFMV observed by standard PCR may reflect that more plants were sampled than leaves selected for the three cultivars for sRSA. However, combined, both methods showed that SPFMV was consistently present in all three cultivars in virus-infected sweet potato leaves in Barbados. Most viruses represented by these samples were DNA viruses, which are usually symptomless in single infection. The presence of begomoviruses, badnaviruses, and mastrevirus detected by sRSA in samples from Barbados was not surprising, since these viruses are widely distributed worldwide (Lotrakul et al. 1998; Lozano et al. 2009; Hassan et al. 2016). A limit to this study was that completely asymptomatic sweet potato leaves were not used because its purpose was to examine diseased leaves displaying a suspected range of SPVD-like symptoms in Barbados. Note also that SPFMV and begomoviruses may be symptomless in some cultivars (Cuellar et al. 2015; Gutiérrez et al. 2003; James et al. 2003; Tairo et al. 2005; Untiveros et al. 2008) and therefore asymptomatic sweet potato leaves should be examined by PCR in the future.

Extensively reported, the crinivirus SPCSV occurs in a low titre in synergistic reactions with other viruses, particularly SPFMV and begomoviruses, which are also known as sweepoviruses (Cuellar et al. 2015; Gibson and Kreuze 2015; Tugume et al. 2016). SPVD is usually diagnosed or confirmed when typical disease symptoms (Gutiérrez et al. 2003) are matched by the detection of both SPFMV and SPCSV in sweet potato samples (Gutiérrez et al. 2003; Clark et al. 2012; Gibson and Kreuze 2015). The outcome of this standard practice was not realized in the current study. Both the previous study by James et al. (2003) using NCM-ELISA and the current studies (PCR and sRSA) report a very low virus titre or the absence of SPCSV in virus-like symptoms on sweet potato in Barbados. Hence, the synergistic consequence of SPFMV and SPCSV infections may not be the cause of the SPVD-like symptoms observed in Barbados. Moreover, symptoms of leaf narrowing (in lobate) and fan-shaped (in non-lobate) leaves are typically observed in SPVD (Gibson et al. 1998; Gutiérrez et al. 2003; Untiveros et al. 2007). These symptoms were not observed in Barbados on non-lobulated shaped leaves of cultivars such as 'C-104', which indicated that SPCSV might not be involved in sweet potato disease caused by viruses in Barbados. Furthermore, Kreuze et al. (2009) report that approximately 100,000 reads are required for the detection of SPCSV, while many fewer reads (approx. 50,000) are necessary for *Mastrevirus* and *Potyvirus* detection by sRSA. In the current study, although reads exceeded 100,000 in the three libraries, SPCSV was not detected by sRSA, which is considered a universal, robust, and reliable method for plant virus detection (Kashif et al. 2012), including of unknown viruses even in extremely low virus titre (Kreuze et al. 2009). Unknown contigs were further exhaustively checked manually in BLAST and in Geneious 11.02 software, but there were no signs of a new unknown virus.

The data presented here strongly suggested that other virus associations were present in the leaf virome. Cuellar et al. (2015) reported that infected sweet potato from St. Vincent may also harbour begomoviruses and mixed virus infections. When the trade in root crops between neighbouring islands, the growth of similar cultivars and the proximity of these islands are considered, these factors may contribute to similar virus associations in commercial sweet potato cultivars grown throughout the region. Hence, the results of sRSA indicated a major proliferation of two other virus families (*Geminiviridae* and *Caulimoviridae*) representing three different virus species: SPSMV-1 (genus *Mastrevirus*), SPLCV (genus *Begomovirus*) and SPPV (genus *Badnavirus*), in addition to SPFMV (genus *Potyvirus*). Essentially, these results suggested that the SPVD-like virus symptoms observed in Barbados were a complex of the two main virus families found (61% *Geminiviridae,* comprising begomoviruses and mastreviruses, and 29% *Caulimoviridae,* comprising badnaviruses) (Fig. 3 A1-C3) or were caused by severe strains of these viruses as single entities or in mixed infections. Infections with begomoviruses are usually symptomless and in complex with SPFMV may result in very severe infection and yield loss in sweet potato fields (Wasswa et al. 2011; Cuellar et al. 2015; Hassan et al. 2016). In addition, Cuellar et al. (2015) also showed that mixed begomovirus and SPCSV infection displays variable symptoms and produces very low titres of SPCSV and very high titres of the begomoviruses. Moreover, the pattern of amount and size distribution of the small RNAs presented here is like that of the mixed synergistic infections with begomoviruses and SPCSV from St. Vincent (Cuellar et al. 2015) and to that for SPFMV with SPCSV (Kreuze et al. 2009). Since begomoviruses are now reported in each sweet potato growing region globally, including the centres of origin (Lotrakul et al. 1998; Lozano et al. 2009; Trenado et al. 2011; Albuquerque et al. 2012; Gibson and Kreuze 2015; Tugume et al. 2016), their presence in the sweet potato leaf virome of local and commercial cultivars in Barbados is not surprising.

As a single group, begomoviruses dominated the detected viruses in sweet potato in Barbados, followed by badnaviruses and potyviruses. Additionally, a few mastreviruses were found (Figs. 3 and 4, supplementary Tables S1-S3). Mastreviruses have also been previously reported in sweet potato in Tanzania and Honduras (Kashif et al. 2012; Mbanzibwa et al. 2011) and

two of the reported viruses share 97% coverage with FJ60945 and FJ60944 in the GenBank database. These viruses were found by sRSA in all three libraries and confirmed by sequencing of PCR amplified products. Therefore, several begomoviruses are likely part of the sweet potato virus complex in Barbados.

Viruses which are asymptomatic in sweet potato may be common in the Caribbean region and therefore pose a severe challenge to the maintenance of clean planting material by farmers. Therefore, an in-depth understanding of these viruses will assist in the adoption of relevant disease management strategies that are increasingly important for the local and regional sweet potato industry.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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