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TRANSMISSION OF EPISOMAL *BANANA STREAK VIRUS* BY MEALYBUGS OF DIFFERENT HOST PLANTS

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ABSTRACT

The B genome of *Musa* cultivars contain several infectious endogenous sequences of Banana streak virus (BSV) that constrains bunch growth and harvest. BSV can be easily transmitted into the banana plant by mealybugs (*Pseudococcidae*) which are known to subsist on banana and other plantain. Several species of mealybugs from different host plants were collected to assess the efficiency to transmit BSV. The study was conducted at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños. The results showed that episomal BSV can be transmitted to uninfected banana not only by mealybugs of *Musa* sp. (*Pseudococcus elisae* and *Dysmicoccus brevipes*) but also by mealybugs of *Manilkara zapota* (unreported), *Anonna muricata*, *Ananas comosus* (*Dysmicoccus brevipes*) and *Nephelium napaceum*. The mealybugs from pineapple and rambutan had the highest transmission efficiency (100%). The inoculated test plants exhibited typical BSV symptoms at 115 and 267 days post-inoculation when mealybugs from pineapple and rambutan were used respectively. Multiplex immunocapture PCR assay detected four episomal BSV species from the test plants either singly or in mixed infections. Single BSMYV infection elicited the most severe symptoms. Samples that yielded negative results to species identification suggest the presence of new species of BSV in the Philippines

Key words: *Musa, Pseudococcidae,* banana streak disease, immunocapture, virus screening

INTRODUCTION

Banana streak disease (BSD) is the most widely distributed among the viral diseases infecting banana and plantain worldwide. The disease was first reported in 1974 in banana fields in Southern Morocco and has been then reported in Africa, Asia, Central and South America, and Oceania (Lockhart and Jones 2000). Many spontaneous disease outbreak have been reported over the years but with no confirmed epidemic worldwide apart from the reported 1996 Uganda epidemic in East Africa, where BSV was classified endemic (Tushmereirwe et al. 1996; Iskra-Caruana et al. 2014).

BSD symptoms are characterized by yellow to white, broken or continuous chlorotic streaks and spindle-shaped lesions. These chlorotic lesions progressively turn necrotic on the leaves and may produce black streak appearance in older leaves. The symptoms are erratically distributed on the plant and are not shown on all leaves. Additionally, pseudostem-splitting, reduction of plant height, fruit malformation and size reduction, delay in bunch emergence and maturation and plant collapse were observed (Lockhart 1986; Natsuaki and Furuya 2007). The causal agent of BSD was initially referred to as *Banana streak virus* (BSV). However, at present, the International Committee on Taxonomy of

Viruses (ICTV), has recognized nine distinct BSD-causing viruses, all belonging to the genus Badnavirus of the family Caulimoviridae. These viruses were referred to as BSV species (BSV's) which were distinguished based on sequence information, genetic and serological analyses (Lockhart 1986; Geering et al. 2000; Jones 2000; Harper et al. 2004 and 2005; Geering et al. 2005a and b; Geering and Parry 2011; Lheureux et al. 2007; Bhat et al. 2016).

BSV is a plant pararetrovirus with circular dsDNA which is approximately 7.2 to 7.8 kbp long and encodes reverse transcriptase (RT) for replication. It is encapsidated into bacilliform particles which are not only transmitted horizontally but also vertically as homologous integrated BSV sequence within the *M. balbisiana* genome (LaFleur et al. 1996; Harper et al. 1999; Ndowora et al. 1999; Lheureux et al. 2003; Gayral et al. 2008; Chabannes et al. 2013; Iskra-Caruana et al. 2014). These endogenous BSV sequences (eBSVs) are partial and rearranged sequences with various number of copies which are integral into the banana genome as a result of illegitimate recombination between the virus and the plant genome possibly during repair of breaks in plant DNA (Natsuaki and Furuya 2007, Gayral et al. 2008, Liu et al. 2012, Iskra-Caruana et al. 2014). Viral replication can occur without introgression into the banana genome (Natsuaki and Furuya 2007). Under known stress conditions, such as the use of *in vitro* tissue culture or interspecific breeding, eBSVs can escape spontaneously from the banana genome and reconstitute *de novo* infectious particles or episomal BSV. Consequently, activated eBSVs or episomal BSV sequences causes the Banana streak disease (Ndowora et al. 1999; Dallot et al. 2001; Lheureux et al. 2003; Gayral et al. 2008; Cote et al. 2010; Iskra-Caruana 2010; Chabannes et al. 2013; Chabannes and Iskra-Caruana 2013).

The majority of plant viruses are vector-borne. Seventy six of plant viruses are transmitted by arthropods, nematodes and fungi (Chi-Wei et al. 2010). Banana streak virus is a mealybug-transmitted member of the pararetrovirus genus *Badnavirus*. Although BSV can be readily transmitted experimentally to banana by mealybugs, attempts to transmit the virus by mechanical inoculation have failed and this was attributed to the very high levels of phenolic compounds, latex and other inhibitory substances present in bananas (Lockhart 1986 and 1995).

Several mealybug species known to transmit BSV in the field were reported according to the geographic location of the mealybug populations. *Planoccocus musa* was reported in BSV-infected fields of Nigeria while *Dysmicoccus* spp. was reported in West Africa and South America. Earlier experimental transmission of episomal BSV was done using *Dysmicoccus brevipes*, *Planococcus citri*, *Planococcus ficus* and *Pseudoccocus longispinus*. Out of which, only *P. longispinus* was the only vector reported that does not transmit BSV (Meyer 2006). Therefore it is necessary to identify all mealybug species that are possible vectors and efficient transmitters of the virus in order to apply effective management strategies for the disease.

In the Philippines, many of our bananas (*Musa sp)* are planted along other crops such as: rambutan (*Nephelium napaceum)*, chico (*Manilkara zapota*), guyabano (*Anonna muricata)*, papaya (*Carica papaya*), pineapple (*Ananas comosus)* and others. Several mealybug species have already been identified. *Pseudococcus elisae* and *Dysmicoccus brevipes* on banana, *Dysmicoccus brevipes* is also present in papaya and pineapple, while *Planococcus lilacinus* was recorded on guyabano and rambutan (Lit and Calilung 1994 a and b). Subsequent studies reported *Pseudococcus lepelleyi* and *Dysmicoccus neobrevipes* on guyabano and *Cataenococcus hispidus* on rambutan (Lit 1997).

This study was conducted to evaluate the transmittability of BSV to banana by mealybugs of other plant species: *A. muricata*, *C. papaya*, *A. comosus*, and *N. napaceum*, assess the relationship between the mealybugs from other plant species and BSV symptom severity, and identify the BSV species transmitted.

MATERIALS AND METHODS

Source of BSV infected plants. Banana plants showing typical symptoms of BSV were collected from Laguna (Bay, Cavinti, Majayjay, Pasong Kipot), Batangas (Tanauan), Quezon (Lucban, Lucena, Pagbilao) and Davao del Sur (Hagonoy) (Fig. 1), and were kept in insect-proof cages. Collected plants or corms were individually planted in a 24-liter plastic pails containing sterile soil.

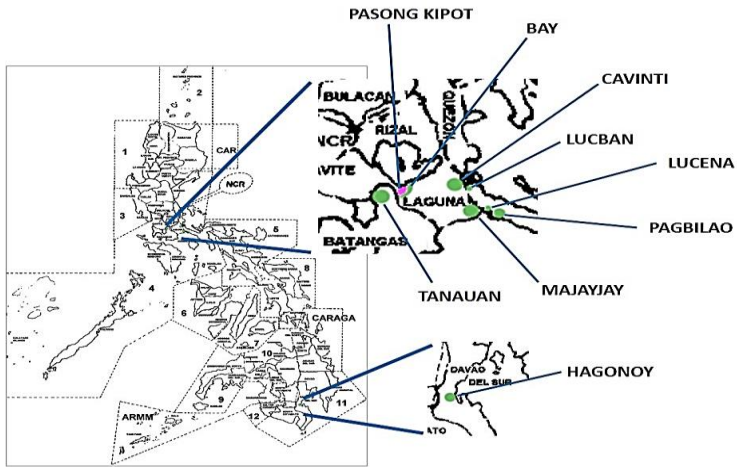

Fig. 1. Geographical representation of the collection sites for symptomatic banana samples (www.fao.org).

Crude sap extraction. The sap extraction method and immunocapture procedure was conducted following the method published by Thomas (2008) and Geering et al. (2000) with some modifications. Sample collection was done by cutting approximately 0.5 gram of banana leaf samples from the second youngest fully expanded leaf of the plant. For sap extraction, samples were individually placed in plastic collection bags containing 4 ml of sap extraction buffer (0.05 M Tris-HCl, 2.5% skim milk, 0.5% sodium sulfite). The collected samples were properly labelled and temporarily stored in a cooler with ice packs and were immediately brought to the laboratory for homogenization. Otherwise, samples were stored in freezer (0 to -5 °C). One ml of homogenized samples were individually transferred onto 1.5 ml Eppendorf tubes, tightly capped and spun for five minutes at 10,000 rpm using Centrifuge 5424 (Eppendorf, Germany).

Detection of Endogenous BSV. In this study, all polymerase chain reaction (PCR) assays were done twice. *Musa* virus indexing procedures by Su (1999), Geering et al. (2000) and Thomas (2008) were modified and optimized. Endogenous BSV was detected using a pair of primers BSV F1 (5' – CAACTCAAGAGCCTAGTATGC – 3') and BSV R2 (5' – TACCTCCGACCGTATTTCCAG – 3') (Su 1999) with an expected product size of 220 bp (Geering et al. 2005a and b). A total reaction mix volume of 15 ųl [1X PCR buffer (Invitrogen), 1. 5 mM $MgCl_2$ (Invitrogen), 0.20 mM dNTPs, 0.10 ųM of each primer pair, 1.2 units of Taq polymerase (Vivantis) and 2 ųl of sap template] was assayed in Mycycler (Bio-Rad Laboratories Inc., USA) with the following PCR conditions: 1 cycle of denaturation at 94°C for 4 min, annealing at 50°C for 1 min, extension at 72°C for 2 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min; and 1 cycle of final extension of 10 min at 72°C.

Detection of Episomal BSV. Immunocapture Polymerase Reaction (IC-PCR) was used to detect episomal BSV. The viral protein was captured by a mixture of *Sugarcane Bacilliform Virus* (SCBV) antibody (AGDIA Co.) and carbonate coating buffer with a recommended dilution of 1:200 (Thomas

2008). Fifty (50) µl of the SCBV-carbonate coating buffer mixture were pipetted onto sterile 0.2 ml microcentrifuge tubes, incubated overnight at 4 °C, and discarded. Fifty (50) µl of the antigen (samples) were then loaded onto the tubes individually and incubated for 3-4 hours at 37 °C. Afterwards, the tubes were washed thrice with 1X PBS-T buffer, allowed to stand for three minutes every time and a final wash of distilled water. Episomal BSV infected *Musa* were then used as donor plants and positive controls for subsequent activities.

Rearing of transmission vector. Mealybugs used in this study were obtained from banana (*Musa paradisiaca* Linn.), chico (*Manilkara zapota*), guyabano (*Anonna muricata*), papaya (*Carica papaya*), pineapple (*Ananas comosus*), and rambutan (*Nephelium napaceum*) (Fig. 2). The mealybugs from these plants were reared separately on detached immature squash fruits. The other mealybug species were directly introduced into the banana seedling.

Fig. 2. Mealybugs used as the vector of banana streak virus (BSV).

BSV Transmission set-ups. Endogenous sequences can be found in both *M. acuminata* (A genome) and *M. balbisiana* (B genome) banana. However, episomal sequences were found to be associated only to B genome *Musa* (Ndowora et al. 1999; Geering et al. 2000; Lheureux et al. 2003; Gayral et al. 2008). Hence, in this study, B genome *Musa* tissue culture-derived banana plants were used. Available tissue culture materials were potted in 3x3x5 inch plastic bags with sterile soil mixed with coir dust and kept in the greenhouse for 2 months to acclimatize the plantlets before the virus transmission procedure. All plants were maintained in the greenhouse of the Institute of Plant Breeding (IPB, UPLB). The transmission set-up consisted of six treatments: (i) transmission from episomal BSV infected banana using *Musa* mealybugs to B genome tissue culture-derived recipient plants; (ii) transmission using *M. zapota* mealybugs; (iii) transmission using *A. muricata* mealybugs; (iv) transmission using *C. papaya* mealybugs; (v) transmission using *A. comosus* mealybugs; and (vi) transmission using *N. napaceum* mealybugs. The B genome tissue culture-derived *Musa* that served as recipient plants were obtained from the *M. balbisiana in vitro* collection of the institute.

Acquisition and inoculation period. Transmissions using the mealybugs from the different host plants were done separately in the insect-proof screenhouse of IPB-UPLB. Adult mealybugs were starved for 4 hours and were placed on the leaves of the infected field samples (donor plants) previously tested for the presence of episomal BSV using IC-PCR. Each species of mealybugs were allowed to feed on the BSV infected *Musa* plants (donor) for 3 days. After the acquisition period, 20 mealybugs from each plantain were removed from the donor plant and were transferred to tissue culture-derived B genome *Musa* plantlets (potted-out approximately 2 months prior to experiments).

Disease assessment. The inoculated plants were closely observed for the appearance of the characteristic symptoms of BSV infection such as chlorotic streaks (Natsuaki and Furuya 2007). The days starting from insect transmission to the appearance of symptoms were recorded. All banana plant samples were checked for virus activation (episomal BSV infection) at three months and six months period after inoculation using IC-PCR. Disease severity rating was done on the youngest fully expanded leaf of each sample and was quantified using a modified scale of 0 to 5 (Karanja et al. 2013;

Wambulwa et al. 2013), where 0 is no symptoms, 1 is localized flecks, 2 is scattered discontinuous streaks, 3 is continuous streaks covering moderate portion of lamina, 4 is continuous chlorotic streaks, and 5 is necrotic streaks.

BSV strain identification using Multiplex PCR (Mp-PCR). Strain identification of samples tested positive for episomal BSV was accomplished using the same crude sap extraction method and PCR cocktail concentration except for the final concentration of 0.40 µM BSV strain-specific primer mix (Table 1) used and the use of a multiplex touchdown condition for the assay. The multiplex touchdown PCR assay condition is as follows: 1 cycle of initial denaturation at 94°C for 30 s; followed by 2 cycles of denaturation at 94°C for 20 s, annealing at 64°C for 30 s, extension at 72°C for 1 min; 2 cycles of denaturation at 94°C for 20 s, annealing at 62°C for 30 s, extension at 72°C for 1 min; 10 cycles of denaturation at 94°C for 20 s, annealing at 60°C for 30 s, extension at 72°C for 1 min; 25 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s, extension at 72°C for 1 min and 1 cycle of final extension of 3 min at 72°C. Multiplex IC-PCR was done following the same multiplex touchdown conditions mentioned with the application of the previously mentioned immunocapture method for BSV (Le Provost et al. 2006).

Table 1. BSV Strain-specific primers used to distinguished BSV sequences.

Target Virus	Name of Primer	Primer Sequences	Product Size
BSOLV*	RD-F1	5'-ATCTGAAGGTGTGTTGATCAATGC-3'	522
	RD-R1	5'-GCTCACTCCGCATCTTATCAGTC-3'	
BSGFV*	GF-F1	5'-ACGAACTATCACGACTTGTTCAAGC-3'	476
	GF-R1	5'-TCGGTGGAATAGTCCTGAGTCTTC-3'	
BSMYV*	Mys-F1	5'-TAAAAGCACAGCTCAGAACAAACC-3'	589
	Mys-R1	5'-CTCCGTGATTTCTTCGTGGTC-3'	
BSIMV**	914-F1	5'-TGCCAACGAATACTACATCAAC-3'	384
	914-R1	5'-CACCCAGACTTTTCTTTCTAGC-3'	

*Geering et al. 2000, **Chabannes et al. 2013

Visualization of PCR Products. An electrophoregram of 5µl PCR products was achieved by gel electrophoresis using 1.5% w/v Agarose (Vivantis) in 0.5X TAE (Tris-Acetate-EDTA) buffer, stained in GelRedTM solution and visualized using the GelDoc XR+ documentation system (Bio-Rad Laboratories Inc.). The amplicons were quantified through the comparison of their product sizes against the 1 Kb plus DNA marker (Invitrogen, USA).

Data analysis. Analysis of variance (ANOVA) was done using GraphPad Instat 3.10 to determine the relationships among mealybug type/vector source, symptom expression and episomal BSV infection.

RESULTS AND DISCUSSION

Presence of Episomal BSV on selected materials. The 43 plants collected from eight locations showed chlorotic and necrotic streaks (Fig. 3). Among these, only 30 plants were found to be episomal BSV positive (Table 2). Episomal BSV infected *Musa* were then used as donor plants and positive controls for this experiment. Interestingly, severe symptoms were observed in plants grown in areas with high elevation and low temperature. Infected plants exhibited symptoms from the leaves, petiole, down to the pseudostem. More samples could have been assayed if collections were not only focused on plants that showed typical symptoms of the disease on leaves. Symptoms of BSV infection were observed to be erratically distributed on the plant and may not be present on all leaves. Additionally, there have been reports of alternating symptomatic and asymptomatic stages in infected

plants even though the virus was detected at all stages (Dahal et al. 1998; Dahal et al. 2000a and b; Lockhart and Jones 2000; Harper et al. 2002).

Table 2. Summary of survey and collection of source plants screened using IC-PCR.

Place of Collection		Symptomatic plants	eBSV positive
Batangas	Tanauan	3	1
Davao del Sur	Hagonoy	1	1
Laguna	Bay	9	2
	Cavinti	7	6
	Majayjay	4	3
	Pasong Kipot	12	10
Quezon	Lucena	4	4
	Pagbilao	3	3
TOTAL		43	30

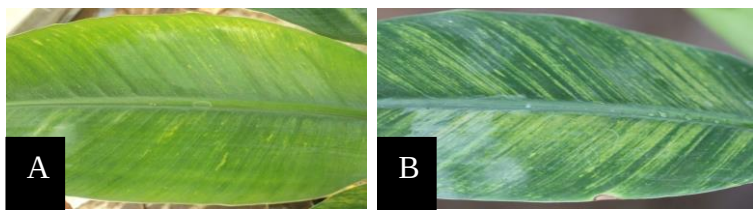

Fig. 3. Mild (A) and severe (B) chlorotic streaks on leaves of *Musa* sp. infected with Banana Streak Virus.

Mealybug transmission of *Banana streak virus*. Mealybug from banana, chico, guyabano, pineapple and rambutan transmitted eBSV to healthy *Musa* plants (Table 3). Chlorotic streaks which turn necrotic vary from 100 and 267 days after mealybug transmission. The earliest infection occurred in plants inoculated with mealybugs of banana, followed by plants inoculated with pineapple mealybugs. Plants inoculated with the virus using mealybugs from rambutan and chico exhibited typical BSV symptoms after 267 and 219 days after transmission, respectively. The results of the transmission studies show that episomal BSV can be transmitted by mealybugs from banana (*Musa* sp.), chico (*Manilkara zapota*), guyabano (*Anonna muricata*), pineapple (*Ananas comosus*), and rambutan (*Nephelium napaceum*) to episomal BSV-free *Musa balbisiana*. This suggests that the spread of activated BSV can be facilitated by mealybugs from these crops that are planted around banana growing areas. Therefore it is necessary to identify all mealybug species that are possible BSV vectors and further assess their efficiency to transmit the virus. Similar results have been reported on the grapevine leaf roll virus (GLRV) which can be transmitted by several species of mealybugs including soft-scale insects. Nine species of mealybugs were reported vectors of GLRaV (Lemaguet et al. 2012).

Table 3. Mean symptom expression (based on scale 0 to 5) for banana samples infected with episomal BSV.

Mealybug Host	% of Plants infected***		Mean**	Days to symptom expression
	3 *	6 *		
Musa balbisiana	55.2	79.3	1.48±1.527	100
Manilkara zapota	83.3	83.3	1±1.637	219
Anonna muricata	60.0	60.0	1.4±1.95	185
Carica papaya	0	0	0±0	0
Ananas comosus	75.0	100	2.83±0.619	115
Nephelium napaceum	0	100	2.17±1.211	267

*months after inoculation **0 to 5 symptom expression scale (Karanja et al. 2013).
***confirmed using IC-PCR assay.

The specific identity of the BSV vector collected from banana, guyabano and rambutan needs to be confirmed because of the presence of two (banana and rambutan) and three (guyabano) mealybug species in one plant species. However, *D. brevipe*s (on pineapple) and the mealybug species from chico, are possible vectors of the banana streak virus since no other species are present/recorded on the plants. The mealybug species has not been identified yet, however, the buff mealybug *Nipacoccus nipae* reported on coconut and palms was also observed on chico and banana (Caasi-Lit et al. 2009). Nineteen species of mealybug belonging to 13 genera are known to occur on *Musaceae* (Watson and Kubiriba 2005). Experimental transmission of BSVs also has been demonstrated with the pink pineapple mealybug *Dysmicoccus brevipes* (Cockerell) by Kubiriba and co-workers (2001). Other species reported to transmit BSV are Dysmicoccus spp. in West Africa and South America, *Planococcus mu*sa in Nigeria, *Ferrisia virgata* (striped mealybug) in India (Selvarajan et al. 2006), and *Paracoccus burnerae* (Muturi et al. 2013) in South Africa.

Symptom severity and mealybug of other plant host. Symptom severity of the youngest fully expanded leaf of each sample was scored on a 0 to 5 modified scale (Karanja et al. 2013; Wambulwa et al. 2013). The results shown in Table 3 indicate that the symptoms were more severe in *Musa* infected using *A. comosus* mealybugs (with highest symptom expression mean of 2.83 ± 0.619 and 100% episomal BSV infection at 6 months after inoculation). The proportion of the samples infected with episomal BSV was consistent for six months using *M. zapota* and *A. muricata* mealybugs. *Manilkara zapota* mealybug infected samples expressed type 1 symptoms at 83.3% infected samples compared to *A. muricata* mealybug infected samples which expressed type 2 symptoms at a lower percentage of 60%. The mean symptom expression for samples inoculated with *N. napaceum* mealybugs followed that of *A. comosus* mealybugs at 2.17±1.211, also with 100% infection six months post inoculation. *Musa* inoculated using *C. papaya* mealybugs were negative to episomal BSV. Analysis of variance (One-way ANOVA) with regards to symptom expression showed that BSV inoculation using the six mealybug species differ significantly ($P<0.05$). The higher proportion of infected plants in the sixth month shows that virus concentration increases with plant growth. Thus, early detection and elimination of infected plants are important in mitigating the disease.

Results of this study also showed that some correlation exists between the mealybug used in the inoculation of BSV and the degree of the symptoms observed. However, since previous studies have shown that many factors influence BSV symptom expression, such as temperature (Dahal et al. 1998) and other environmental conditions (Mobambo et al. 1996; Daniells et al. 2001), the study can only be considered as a glimpse into the complex vector-virus-plant relationship.

BSV species identification. Species identification was done on all episomal BSV positive plants. Only 9 out of the 48 plants inoculated using mealybugs of banana showed positive results. Four episomal BSV species were detected as mixed infections in 7 plants, 4 with BSGF-MYV, 2 with BSMYV-IMV and 1 with BSOL-IMV (Table 4). While single infections of BSGFV and BSMYV were detected in two different plants.

Table 4. Species identification of episomal banana streak viruses in plants inoculated with BSV using mealybugs from different plants.

Mealybugs Source	Multiplex IC-PCR						
	BSGFV	**BSIMV**	**BSMYV**	**BSOLV**	**BSGF-MYV**	**BSOL-IMV**	**BSMY-IMV**
Banana	1	0	1	0	4	1	2
Chico	0	0	0	0	0	0	0
Guyabano	0	0	0	0	0	0	0
Pineapple	0	0	0	0	0	0	0
Rambutan	0	0	0	0	0	0	0

The BSMYV infected test plant was observed to have the most severe symptoms in the study at category 4 (continuous and conspicuous chlorotic streaks) followed by the BSVGFV infected sample with category 3 symptoms (continuous streaks covering moderate portion of the lamina) according to Wambulwa et al. 2013. Symptom severity remain high in plants with mixed infections of BSMY-IMV that either fall under category 2 (scattered discontinuous streaks) or in between categories 2 and 3. While the BSVGF-MYV infected plants fall under category 3.

More severe symptoms were observed in samples with mixed infections, such that the more the number of isolates, the more severe the symptoms (Karanja et al. 2008). This is, however, in contrast with the observations made in this study. The more severe symptoms were found on single infections of BSMYV and BSGFV, and an exemption of BSGF-MYV. The severe symptoms caused by the Mysore isolate can be attributed to the more immunogenic epitopes of BSMYV compared to other isolates (Wambulwa et al. 2013). This contradiction on observed severity of symptoms between single and mixed infections calls for further research.

Multiplex-IC-PCR was not able to identify the BSV species infecting the remaining episomal BSV positive plants inoculated using mealybugs from chico, guyabano, pineapple, and rambutan (Fig. 4), this suggests that new BSV species may be present in the Philippines.

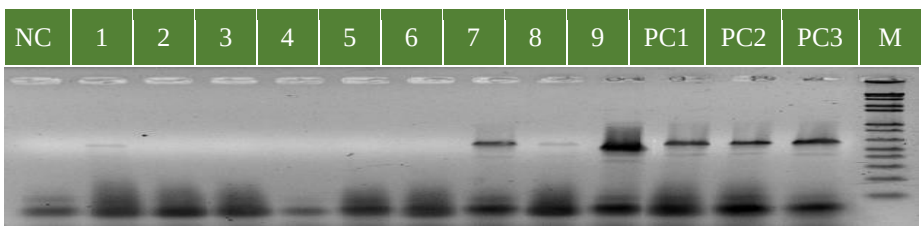

Fig. 4. Agarose gel (0.5%) electrophoresis of products from Multiplex IC- PCR assay using primer mix of BSGFV (476 bp), BSIMV (384 bp), BSMYV (589 bp), and BSOLV (522 bp) primers. Lane NC – negative check, Lane 1-3 – B2-B4 (6 MAT*****), Lane 4-6 – G1-G3 (3 MAT), Lane 7-9 – B15, B17, B18 (3 MAT), Lane PC1-PC3 – positive check (known infected plant), and Lane M – molecular weight marker (1 kb plus ladder). *****MAT **–** months after transmission.

CONCLUSION

Episomal BSV can be transmitted to uninfected banana by mealybugs of *Musa* sp. (*Pseudococcus elisae* and *Dysmicoccus brevipes*), *Manilkara zapota* (unreported), *Anonna muricata*, *Ananas comosus* (*Dysmicoccus brevipes*) and *Nephelium napaceum*. Virus transmission efficiency and mean symptom severity was notably highest using the vectors from the last two crops. Mealybugs play a major role in the natural spread of the virus. Since bananas in the Philippines are also planted alongside the aforementioned crops, management strategies should therefore be addressed at reducing the population of these mealybug species from other crops.

Additionally, four episomal BSV species were detected in this study either in single or mixed infections. Single infections were observed to elicit more severe symptoms in contrast to a previous report and needs further verification. Several samples that showed negative results to species identification suggests the presence of a new species of BSV in the Philippines. It is imperative therefore, to evaluate bananas from other areas for presence of BSV to avoid further spread of the disease. Host resistance to this virus and to the vectors of BSV is one of the most effective control measure for both pests. In addition, removal of infected banana plants is one of the most practical and appropriate means to control the disease.

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