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# *Paracoccus burnerae* (HOMOPTERA; PLANOCOCCIDAE) AS A VECTOR OF *Banana streak virus*

Muturi S M[1, 2*], Wachira F N[3], Karanja L S[4], Wambulwa M C[2] and Macharia E[4]

[1]Department of Biological Sciences, Meru University of Science &Technology, P.O Box 972, Meru, Kenya.
[2]Department of Biochemistry and Molecular Biology, Egerton University, P. O Box 536, Egerton, Kenya.
[3]Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), P. O. Box 765, Entebbe, Uganda.
[4]Kenya Agricultural Research Institute (KARI), P. O. Private Bag, Njoro, Kenya.

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**ABSTRACT**

The *Banana streak virus* (*BSV*) is a causative agent of the banana streak disease (BSD) which causes considerable damage to banana production in tropical countries. The virus is vectored by several mealy bug species. However, the competence of the oleander mealy bug (*Paracoccus burnerae*), in the transmission of *BSV* is unknown. Rolling Circle Amplification (RCA) technique was used to select both diseased and healthy plantlets for transmission experiments. RCA was conducted on viruliferous instars of *P. burnerae* and virus-inoculated plantlet DNA samples. The results revealed that *P. burnerae* is a vector of *BSV*. However, during hot conditions (24-30ºC), the insect was unable to acquire and transmit *BSV*. Under cool conditions (9-20ºC), a minimum of 6 h of feeding time was necessary for *P. burnerae* instars to become viruliferous. These results indicate that *P. burnerae* is a vector of *BSV* and transmission efficiency depends on the ambient temperature and the feeding time.

* Corresponding author

E-mail: samuelmuturi37@yahoo.com (Muturi S M)

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## 1 Introduction

The *Banana streak virus* (*BSV*), the causative agent of viral leaf streak disease of banana (*Musa* spp.), occurs in most banana-growing regions worldwide (Fargette et al., 2006). Disease symptoms are highly variable and can include chlorotic and necrotic streaking along the leaf lamina, distortion of leaves and petioles, stem cracking, abnormal bunch development and death of the growing point (Dahal et al., 2000). In some cases newly emerging leaves remain symptomless. Yield losses of 6 to 15% have been associated with streak disease (Dahal et al., 2000; Daniells et al., 2001). These values are influenced by the cultivar, the virus species, and environmental conditions.

*BSV* is a member of the genus *Badnavirus* (family Caulimoviridae) and has a genome comprising non-covalently closed, double-stranded DNA of approximately 7.2 to 7.8 kbp. Integrated *Badnavirus* sequences are known to occur within the banana genome (Geering et al., 2005). For *BSV* in particular, two types of integrated sequences are known to occur. The first type includes the majority of banana endogenous pararetrovirus and incomplete virus genome which is incapable of causing infections. The second type, known as endogenous activatable *BSV*s, consists of the entire genome of characterized episomal *BSV*s, which are multiple noncontiguous regions of the virus DNA combined with host-genomic sequences. Under certain stress conditions, particularly tissue culture and hybridization, recombination events occur in the integrated sequences, allowing the reconstituted viral genome to be activated and there by resulting in episomal infections (Ndowora et al., 1999; Grigoras et al., 2009; Cote et al., 2010). Although incomplete integrants have been found in both A and B genomes derived from wild progenitors of domesticated banana, *M. acuminata* and *M. balbisiana*, respectively, the activatable *BSV*s have only been detected in the B genome of the various banana accessions (Geering et al., 2000; Geering et al., 2001; Gayral et al., 2008).

*BSV* is highly variable at both the genomic and serological level (Harper et al., 1998; Geering et al., 2000; Harper et al., 2002; Geering et al., 2005; Harper et al., 2005; Lheureux et al., 2007), a characteristic which presents challenges for the development of both polymerase chain reaction (PCR) and antibody-based diagnostic tests. The presence of integrated *Badnavirus* sequences further complicates disease diagnosis using PCR-based approaches due to false positives arising from integrated sequences (Harper et al., 1999; Provost et al., 2006; Iskra-Caruana et al., 2009). To circumvent the detection of integrated DNA, immuno-capture (IC)-PCR is used as the "gold standard" for *BSV* indexing. However, this method has limitations such as the inability of the antiserum to capture all *BSV* isolates (Harper et al., 2002); the use of several primer sets are unlikely to detect the entire *BSV* sequence diversity; and the presence of contaminating, carryover nucleic acid remaining in capture tubes can lead to false positives. As such, an alternative sequence-independent diagnostic test called Rolling Circle Amplification (RCA), was developed by James et al., (2011). This technique not only enables the specific detection of the circular *Badnavirus* genome but also the discrimination between episomal and integrated viral DNA. The RCA is also very sensitive to very low titer of DNA, as little as 1 pg of template nucleic acids (Reagin et al., 2003; Johne et al., 2009).

*BSV* is acquired and spread by several mealybug species (Kubiriba, 2001; Kubiriba,2005; Meyer, 2006, Meyer et al.,2008) with *Planococcus citri* (Cox, 1989; Watson et al., 1995) and *P. ficus* (Cox,1989) having the highest transmission efficiency, 100% and 80% respectively and *Dymicoccus brevipes* (Williams & Granara de Willink, 1992) having the lowest transmission efficiency of 20% (Meyer, 2006). The variation in transmission efficiency amongst mealybug species is attributed to differences in receptors of the vector that interact with the capsid protein of the virus during acquisition access feeding. Depending on the mode of transmission, feeding time affects the transmission efficiency and mode of transmission of plant viruses (Raccah & Ferere, 2009). There are nineteen species of mealybugs that have been reported worldwide delete, but only five have been used in the greenhouse experiments as vectors (Kubiriba et al., 2001; Kubiriba, 2005; Meyer, 2006; Meyer et al., 2008;). Previous studies have revealed that *D. brevipes* (Williams & Granara de Willink, 1992), *P. citri* (Cox, 1989; Watson et al., 1995), *P. ficus* (Cox, 1989), *Saccharicoccus sacchari* (Williams & Granara de Willink, 1992) are potential *BSV* vectors (Kubiriba, 2001; Meyer, 2006). *BSV* acquisition feeding time by mealybug vectors range from 5 min to12 h as reported by various researchers (Su-Hong-ji, 1998; Kubiriba et al., 2001; Kubiriba, 2005). In this study however, we collected a new *BSV* vector (*P. burnerae*) from a banana plantation and used it in greenhouse experiments to establish its vector competence, ambient temperatures and its acquisition feeding time for *BSV* using both IC-PCR and RCA techniques.

## 2 Materials and Methods

### 2.1 Screening of virus-source plants and receptor plants

*BSV*-infected banana plantlets were obtained from infected banana germplasm materials at Kenya Agricultural Research Institute (KARI), Njoro greenhouse and *BSV*-banana plantlets were obtained from healthy germplasm at KARI, Njoro tissue culture laboratory. The *BSV* status of all plantlets was confirmed by immuno-capture-PCR (IC-PCR) using the standard protocol described by Harper et al. (2002) and modified by Karanja, 2008; Karanja et al. (2009), and by rolling circle amplification (RCA) as described by James et al. (2011) The two techniques were used to overcome the high heterogeneity of *BSV* at both the genomic and serological level (Harper et al., 1998; Geering, et al., 2000; Harper et al., 2002; Harper et al., 2005; Geering et al., 2005; Lheureux et al.,

2007), and to detect low viral titer in inoculated plantlet tissues by RCA (Reagin et al., 2003; Johne et al., 2009 ) . Each of these banana plantlets selected were established in a 2 kg polythene bag and used at 4-leaf stage.

2.2 Collection of mealy bugs

Mealy bugs were collected under the pseudostem sheaths and on the roots of the infected banana plants as described by Kubiriba et al., (2001). Vector collections were done in infected banana fields at KARI, Kisii Research centre. The mealy bug specimens were identified to the species level based primarily on adult female morphological features. After collection, the insects were reared on pumpkin fruits placed in black cages which provided the dark conditions, and an ambient temperature of 9-20ºC necessary for their optimal development. The advantages of using pumpkin fruits include the fact that fruit is not a *BSV* host and mealy bugs are easy to remove from hard skinned pumpkins for inoculation experiments, thus facilitating the determination of the acquisition feeding time. To prevent contamination by crawling insects, the cage was placed on a pan containing soapy water. Since mealy bugs have a life cycle of 4-6 weeks, the mealybug colonies were reared for four months to achieve the necessary number of specimens for the tests.

2.3 Transmission trail experiments during hot (24-30ºC) and cool (9-20ºC) conditions

*Paracoccus burnerae* from the rearing cages (200 insects) were starved overnight in a bowl covered with a 50nm x 50nm pore size net. Before acquisition feeding, five *P. burnerae* nymphs (first instars) were sampled randomly and screened for *BSV* using RCA. The starved mealybug nymphs (200 insects) were allowed to feed on *BSV*- infected banana plants in the greenhouse. The nymphs (6-10) were then sampled after 5min, 35min, 1h, 2h, 3h, 4h, 5h, 6h, and 7h of feeding on *BSV*-infected banana plants (acquisition feeding and placed on healthy banana plants for 96h to transmit *BSV* (inoculation feeding). Experimental design: 9 time intervals x 3 replicates x 4 banana inoculation cages. At each time interval, 5$^{th}$ second instar nymphs feeding on infected banana plants were randomly picked and tested for the presence of *BSV* using RCA. Both the acquisition and inoculation feeding trials were done in black clip cage made of plastic material, labeled for each time interval tested. As with the rearing cages, these cages were also placed on a pan containing soapy water. The soapy water provides a clean acquisition and inoculation environment by preventing crawling insects from contaminating the cages. A *BSV*-free plant of the same cultivar [Cavendish variety] unexposed to viruliferous *P. burnerae* nymphs was used as the negative control. After 96h, 3 inoculated leaves of each banana in the inoculation cage were sampled for BSV detection by RCA.

2.4 Deoxyribonucleic acid isolation from plant tissues

Total DNA was isolated using a modification of the cetyltrimethylammonium bromide (CTAB) protocol described by Gawel & Jarret, (1991) and modified by James et al. (2011). Fresh (0.4g) or dried (0.04g) leaf tissue was ground in 3ml of extraction buffer (100mM Tris-HCl, pH 8.0; 50mM EDTA; 1.4 M NaCl; 80mM $Na_2SO_3$; 2% PVP-10; and 2% cetyltrimethylammonium bromide) using a mortar and pestle. Samples were incubated at 65ºC for 15 min and then centrifuged for 5 min at 18,000×g. The supernatant was subsequently mixed with an equal volume of chloroform: isoamylalcohol (24:1) and the mixture centrifuged at 18,000×g for 5 min. After a second chloroform extraction, the supernatant was mixed with an equal volume of isopropanol and incubated at room temperature for 5 min. Nucleic acids were pelleted by centrifugation as described above and the pellets were washed with 70% ethanol, air dried, and resuspended in 50µl of sterile distilled water. The DNA was left at 4ºC overnight to fully dissolve and stored at -80 ºC afterward.

2.5 Immuno-capture Polymerase Chain Reaction (IC-PCR)

To prepare sample for IC-PCR, sap from *BSV*- infected and *BSV*-free banana leaves were extracted according to the protocol described by Harper et al. (2002) with the following modification. One gram of fresh leaf sample was ground in 5ml of *BSV* extraction buffer [phosphate buffered saline (PBS) + polyvinylpyrrolidone (PVP)]. Before carrying out the IC-PCR on the samples, optimization was done for the antibody and antigen by standardizing the concentration to1:1000, 5: 1000 and 10:1000 tissue: carbonate coating buffer. The virus was concentrated in 0.1M sodium chloride containing 4% polyethylene glycol, and stirred for 2h at room temperature. The virus was precipitated at 16,000×g for 20 min in a microfuge. Thin-walled propylene microfuge tubes were coated with polyclonal antibodies against 32 *sugarcane bacilliform viruses* (*SCBV*s) and *BSV* Mysore isolate PMX2RC (Ndowora & Lockart, 2000). The tubes were then washed three times with 100µl of PBS-Tween-20 to remove the unwanted salts that may prevent antibody-antigen interactions. Washing for three times, led to uncoating of the tubes with the antibodies and formation of primer dimmers. Sap extract (100µl) of the sample leaves was added to each tube and the tubes were incubated at 37ºC for 3 h. The tubes were again washed twice with PBS-T, once with sterile distilled water (SDW), and then dried briefly before carrying out PCR directly in the tubes.

2.6 Rolling circle amplification (RCA, Templiphi)

The full length *Banana streak virus* genome was amplified using a TempliPhi$^{TM}$ Kit (GE Healthcare, Buckinghamshire, United Kingdom) according to James et al. (2011). The Templiphi kit contained sample buffer, reaction buffer (salt and dNTPS) and enzyme mix (*Phi29* DNA Polymerase + random primers in 50% glycerol).Two master mixes were

prepared. For master mix 1, 5µL of TempliPhi sample buffer was mixed with 1µL of the isolated sample and 1µl of a 50µM stock solution (4.16pmol/µL of each primer) of TempliPhi degenerate primers as described by several researchers (James et al., 2011; Wambulwa et al., 2012; Wambulwa et al., 2013). This mix was then heated at 95ºC for 3 min to denature the double-stranded DNA followed by cooling to room temperature or 4ºC. Master mix 2 was prepared by mixing 5µL of TempliPhi reaction buffer and 0.2µL of TempliPhi enzyme mix. Five microlitres of the master mix 2 (TempliPhi premix) was transferred to the cooled, denatured sample (master mix 1), and incubated at 30ºC for 18h. After the incubation period, the *Phi*29 DNA polymerase was heat inactivated at 65ºC for 10 min. The samples were then cooled and stored at 4ºC.

2.7 Restriction enzyme digestion and gel electrophoresis

Ten microlitres of the TempliPhi reaction product from each of the mealybug specimens and plant tissues sampled were incubated separately with the restriction enzyme *Kpn*I (Gibco BRL, Eggenstein) for 2h. A 20µL aliquot of the digested TempliPhi product was mixed with 2µL of 5×gel loading dye (Biolabs) and gel electrophoresed for 20 min at 100 V on a 1% SYBR Safe-stained agarose gel using 1×TAE as the running buffers. The gel was visualized under ultra violet (UV) light with Gel Doc (Bio-RAD) software (USA). Internal standards (positive controls) for *BSV* isolates generated using *Kpn*I (New England Biolabs) were used to identify the isolate(s) present in each sample based on published isolates (James et al., 2011; Wambulwa et al., 2013). The relationships between isolate types were determined using the restriction fragment data. The data was presented as tables and figures representing the success and failure in *BSV* transmission by *P. burnerae*. Data generated from this study was compared with the standard characteristics as described by Raccah & Fereres, (2009).

## 3 Results and Discussion

3.1 Screening of virus source plants and virus free plants

Chirume, a triple A-genome containing cultivar, tested positive for *BSV* but Cavendish, also a triple A-genome containing cultivar, tested negative for *BSV* with both IC-PCR (Table 1, Fig. 1) and RCA (Table 1, Fig. 2) techniques. Triple A-genome containing cultivars lack the endogenous activatable *BSV* sequences that can cause episomal infection under certain stress conditions (Ndowora et al., 1999; Dallot, et al., 2001; Cote et al., 2010), thus the positive detection of *BSV* sequences in the Chirume cultivar was due to the presence of the virus rather than activatable integrants in the B-genome of the banana cultivar. The negative results for the Cavendish cultivar indicated that these were free from *BSV* sequences (both from endogenous activatable integrants and the virus itself).

The virus-source plants that exhibited very pronounced symptoms gave positive results with both the IC-PCR and RCA techniques (Table 1, Fig. 1 and 2) which is indicative of the presence of episomal viral particles that were captured in the first step of the combined serological and DNA based method in IC-PCR. In the immune-capture step, only the viral particles (antigens) are bound by the polyclonal antibodies and not the viral nucleic acids. Mealybugs species usually pick the episomal DNA particles and not naked nucleic acids (Meyer et al., 2006; Meyer et al., 2008).

Table 1 Detection of *BSV* in virus source plants, Chirume cultivar + (3/3), and virus-free plants, Cavendish cultivar - (6/6).

| Cultivar | Immunocapture PCR | | | Rolling Circle Amplification | | |
|---|---|---|---|---|---|---|
| | Rep. 1 | Rep. 2 | Rep. 3 | Rep. 1 | Rep. 2 | Rep. 3 |
| **Virus source plant (Chirume)** | + | + | + | + | + | + |
| **Virus free plants** | | | | | | |
| Cavendish | – | – | – | – | – | – |
| Cavendish | – | – | – | – | – | – |
| Cavendish | – | – | – | – | – | – |
| Cavendish | – | – | – | – | – | – |
| Cavendish | – | – | – | – | – | – |
| Cavendish | – | – | – | – | – | – |
| **Controls** | | | | | | |
| Positive (cultivar Mysore) | + | + | + | + | + | + |
| Negative (SDW) | – | – | – | – | – | – |

Rep: replications; SDW: Sterilized distilled water.

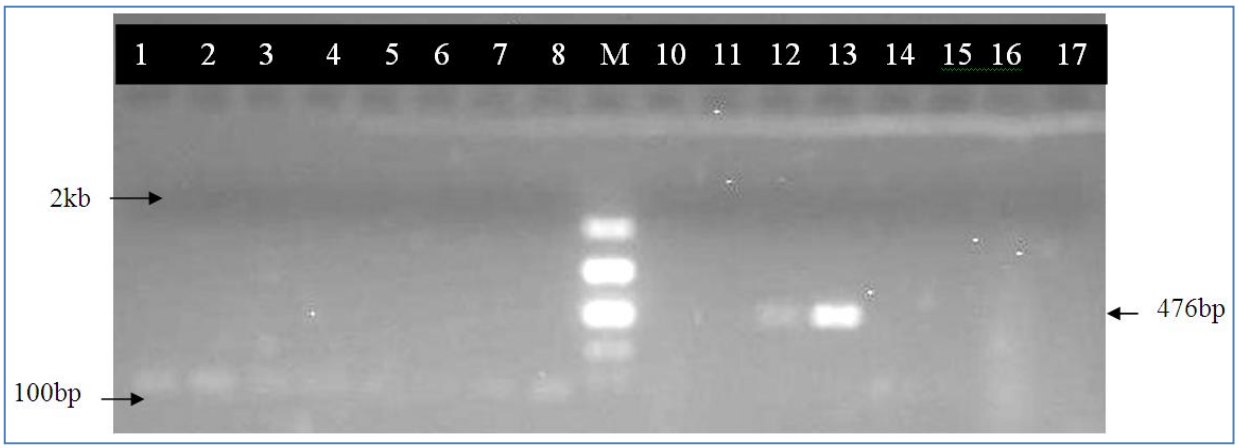


Figure 1 Immunocapture-PCR products from screening virus-source and virus-plants (healthy) before their use in acquisition and transmission experiments. Lanes 1-8, 10, 11, 14, 15, 16 and 17 are virus-free Cavendish cultivar plants; Lanes 12 and 13 are virus-source Chirume cultivar plants and Lane M is the Molecular marker (Easyladder, Bioline).

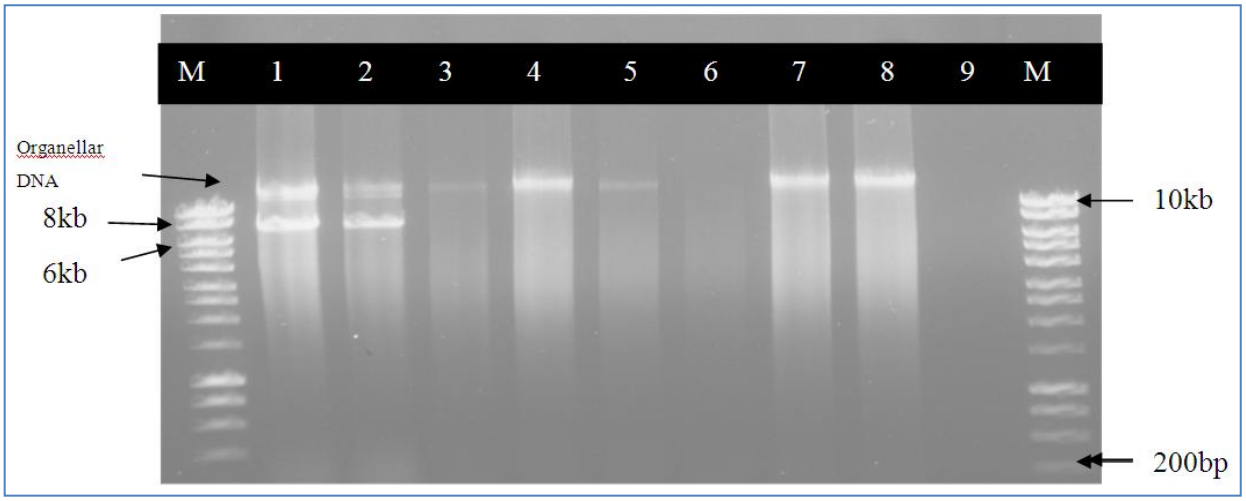


Figure 2 Rolling Circle Amplification products from screening virus-source and virus-free (healthy) plants before their use in acquisition and transmission experiments. Lanes 1-2 are virus-source Chirume cultivar plants; Lanes 3-8 are virus-free Cavendish cultivar plants; Lane 9 is the negative control (Sterilized distilled water) and Lane M is the molecular marker (Hyperladder $^{TM}$1, Bioline).

Reports from other studies have shown that naked nucleic acids of viruses that are transmitted semi-persistently and persistently, can not cause any infection to the host plants if mechanically inoculated in the host plant (Raccah & Ferere, 2009). Karanja et al. (2008) and Karanja (2009) reported that *BSV* could not be mechanically transmitted from diseased to healthy plants, making it impossible to amplify BSV integrated sequences in the PCR step. The healthy test plants in this study tested negative for BSV with the IC-PCR technique indicating that the plants had no episomal viral capsid particles.

The RCA from the virus-source and the virus-free plants confirmed that the former were infected with *BSV* the latter were not (Fig. 2). The RCA technique only amplifies the circular DNA of *BSV*, not the linear DNA of the plant (James et al., 2011). While it is plausible that the plant mitochondria and chloroplast circular DNAs were also amplified, these cannot be confounded with *BSV* since their genome sizes are always larger than that of *BSV*. The RCA products of these plants organells genome would always be above the hyperladder (Fig. 2) (James et al., 2011).

3.2 Transmission trials during hot (24-30ºC) and cool (9-20ºC) conditions

The results revealed that *P. burnerae* acquired *BSV* after feeding on infected plants for a minimum of 6 h (Tables 2 and 3, Fig. 3 and 4). Cool conditions were also more favourable to acquisition of the virus (Table 3, Fig 4). Virus acquisition only took place when mealybugs fed on the virus-source plant under dark condition of 9-20ºC or 8-13ºC. The results suggested that in addition to the reported mealy-bug species, *P. burnerae* is also a potential vector of this virus to the banana plants.

In this study, it was found that P. burnerae can transmit BSV and that the acquisition feeding time was 6 h. The oleander

mealybug (*P. burnerae*) has been shown to transmit ensete streak disease (ESD) in Kenya but not banana viruses, *BSV*, *BBTV*, or *CMV* (Ben-Dov, 1994; Williams and Matile-Ferrero, 2000). *Paracoccus burnerae* nymphs were able to acquire the virus and transmit it to healthy banana plants after a minimum of 6 h of feeding time on infected plants. This implies that the feeding time has a significant effect on the successful transmission of *BSV*. The efficiency of transmission by increased with prolonged feeding time.

This is consistent with studies carried out on *cauliflower mosaic virus* which belong to the same genus as *BSV* (Caulimovirus). Palacios et al. (2002) reported that semi-persistent transmitted viruses require more feeding time for effective transmission as opposed to non-persistent mode of transmission. The acquisition feeding time in non-persistent mode of transmission takes seconds to minutes during the time of probing as the vector searches for food.

The long acquisition feeding time for the semi-persistent mode of transmission is associated with viruses that are found in the phloem and *BSV* has been reported to be found in the phloem of the banana plants (Walkey, 1991). Hence, brief probing of the mealybugs on the infected banana plants does not lead to successful acquisition of the virus.

Table 2Transmission of *BSV* during hot conditions (24-30ºC) for different feeding periods, after 4 days of inoculation

| | No. of mealy bugs | Feeding period | Detection of BSV by RCA in exposed plants | | |
|---|---|---|---|---|---|
| | | | Rep.1 | Rep.2 | Rep. 3 |
| Chirume Cultivar | 10 | 30 min | - | - | - |
| Chirume Cultivar | 10 | 1h | - | - | - |
| Chirume Cultivar | 9 | 2h | - | - | - |
| Chirume Cultivar | 7 | 3h | - | - | - |
| Chirume Cultivar | 8 | 4h | - | - | - |
| Chirume Cultivar | 6 | 5h | - | - | - |
| Chirume Cultivar | 8 | 6h | - | - | - |
| Chirume Cultivar | 9 | 7h | - | - | - |
| Chirume Cultivar | 7 | 12h (8-13ºC) | + | + | + |
| **Negative (HC & SDW)** | N/A | N/A | - | - | - |
| **Positive (Mys)** | N/A | N/A | + | + | + |

No.- Number; Rep-Replicate; HC-Healthy plants; Mys-Mysore; SDW-Sterilized distilled water; N/A-Positive and Negative controls not fed on by viruliferous mealybugs**.**

Table 3 Transmission of *BSV* during cool days (9-20ºC) for different feeding periods, after four days of inoculation.

| Source of transmission | No. of mealybugs | Feeding period | Detection of BSV by RCA (Templiphi ) | | |
|---|---|---|---|---|---|
| | | | Rep.1 | Rep.2 | Rep. 3 |
| Chirume Cultivar | 9 | 30min | - | - | - |
| Chirume Cultivar | 7 | 1h | - | - | - |
| Chirume Cultivar | 10 | 2h | - | - | - |
| Chirume Cultivar | 6 | 3h | - | - | - |
| Chirume Cultivar | 9 | 4h | - | - | - |
| Chirume Cultivar | 9 | 5h | - | - | - |
| Chirume Cultivar | 5 | 6h | + | + | + |
| Chirume Cultivar | 6 | 7h | + | + | + |
| Chirume Cultivar | 7 | 12h (8-13ºC) | + | + | + |
| **Negative (HC & SDW)** | N/A | N/A | - | - | - |
| **Positive (Mys)** | N/A | N/A | + | + | + |

No.- Number; Rep-Replicate; HC-Healthy plants; Mys-Mysore; SDW-Sterilized distilled water; N/A-Positive and Negative controls not fed on by viruliferous mealybugs.

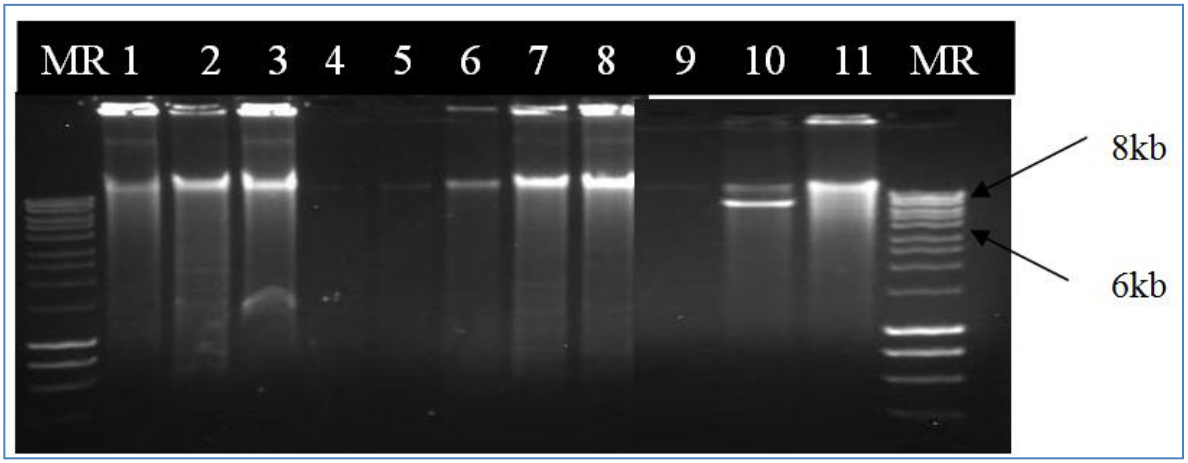


Figure 3 RCA products of DNA from plants inoculated with *BSV* by viruliferous mealybugs during hot days (24-30ºC). Lanes 1-8 are plant samples for feeding periods of 30min, 1h, 2h, 3h, 4h, 5h, 6h, 7h, respectively; Lane 9 is the negative control (Sterilized distilled water); Lane 10 is the positive control (Mysore); Lane 11 is a virus-free plant; and Lane MR- is the Molecular marker (Hyperladder ™ 1, Bioline). Arrow shows an RCA product corresponding to positive control.

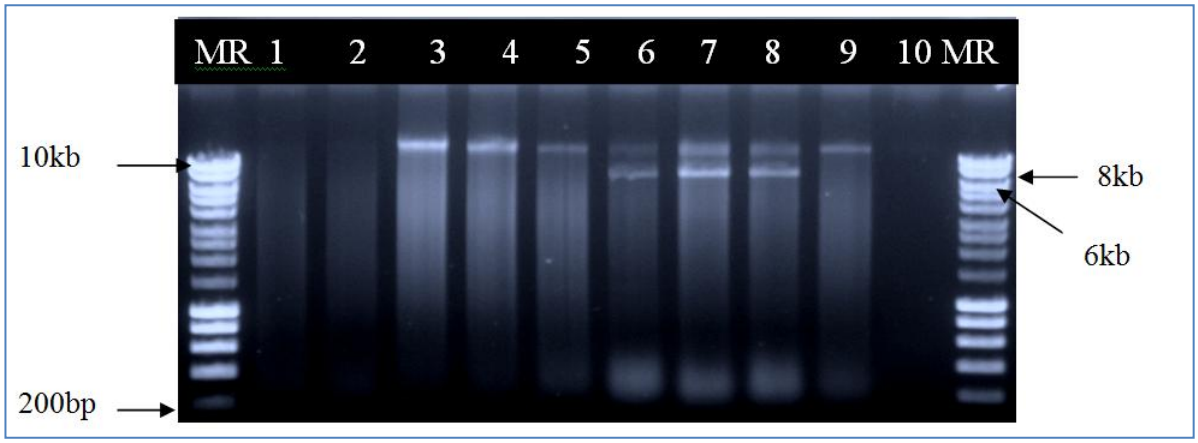


Figure 4 RCA products of DNA from plant samples fed on by viruliferous mealybug during cool days (9-20ºC)., Lanes 1-5 are plant samples for feeding periods of 1h, 2h, 3h, 4h, 5h, respectively; Lanes 6-7 are plant samples for feeding periods of 6h and 7h; Lane 8 is the positive control (Mysore); Lane 9 is virus-free plant; Lane 10 is the negative control (Sterilized distilled water); and Lane MR is the Molecular marker (Hyperladder™ 1, Bioline). Arrow shows an RCA product corresponding to infected material and positive control.

The rate of *BSV* acquisition does not depend on the number of intracellular punctures produced by the vector, but it increases sharply after phloem ingestion as confirmed by Palacios et al. (2002).

These findings are consistent with the model of "sequential acquisition" of the various components of the *cauliflower mosaic virus* transmissible complex as reported by Drucker et al. (2002), where certain proteins produced by the host or another virus in case of co-infections are acquired in specific inclusion bodies before virion complexes acquisition.

The acquisition access feeding period of *BSV* for other mealybug species ranges from 5 min to 24 h as confirmed by Su-Hong-ji (1998) and Kubiriba (2005). Kubiriba (2005) used three species of mealybugs (excluding *P. burnerae*) to conclude that mealybugs can acquire *BSV* within a minimum of 5 min. However, a study carried out in Taiwan by Su-Hong-ji (1998) suggested that, *P. citri* can only acquire *BSV* after 24 h. The difference in acquisition access feeding period determined in this study and those determined in other studies is probably associated with the vector species used, and more so with environmental conditions. The acquisition time of *BSV* by *P. citri* was greatly influenced by environmental factors in Taiwan as confirmed by Su-Hong-ji (1998). Acquisition of *BSV* was more successful during the winter season than during summer season for two *P.citri* biotypes. The influence of the environmental factors on the acquisition time has been corroborated by this study. In this study, in Kenya, *P. burnerae* was unable to acquire the virus during hot days (24-30ºC); the acquisition was only successful during cool days (9-20ºC) or at night (8-13ºC). Temperature affects both mealybug acquisition and inoculation feeding. Mealybugs seem to only be able to make short or brief probes during hot conditions (unfavorable). During these probes the mealybugs stylets do not reach the phloem, where the *BSV* is found, thus the vector fails to acquire the virus. A prolonged probing period during mealybug

feeding seems to be a requirement for *BSV* acquisition. The prolonged feeding period can only occur when the environmental conditions are favorable for vector feeding, cool conditions in the case of *P. burnerae*.

The variation in the acquisition feeding time and may be the inoculation feeding time from one country to another may be related to different environmental conditions that condition mealybug survival. Mealybugs require moist and dark conditions for their survival. The inoculation time of *BSV* by mealybugs was reported to be at peak after 72 h of inoculation access feeding as confirmed by Kubiriba et al. (2001& 2005). No information on the reason for this delay of the virus in the vector gut during inoculation feeding has been revealed, but it can be theorized that such delay could be due to the interaction of the vector with the *BSV* capsid coat protein during transmission process and the time taken by the vector to make prolonged probes.

**4 Conclusions**

The mealy-bug, *P. burnerae* is one of the vectors of banana streak disease with an acquisition feeding time of 6 h during cool days (9-20ºC). Both feeding time and the prevailing environmental conditions affect the acquisition feeding time. Acquisition rate increases with longer feeding time, which is characteristic of the semi-persistent mode of transmission. Cool conditions also promote *BSV* acquisition by *P. burnerae*.

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