Identification of Different Strains of Banana Streak Virus in Sri Lanka

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ABSTRACT

Banana streak disease caused by banana streak virus is a recently described disease, which occurs in most banana producing countries. It can adversely affect on yield and fruit quality of banana. In Sri Lanka, banana streak virus infection is most common in the *Embul* variety.

Banana streak virus is a variable virus with different strains. Isolates of the virus differ in severity of symptoms produced, which mainly include chlorotic and necrotic streaks on leaf lamina. The high degree of genomic and serological heterogeneity among banana streak virus isolates has led to problems in developing reliable indexing methods for virus identification and disease control. Integration of segments of viral genome into *Musa* genome and possible activation of the virus during tissue culture may limit the usefulness of *in vitro* propagation of banana. Integration of viral sequence is also a hindrance to international exchange of *Musa* germplasm. Hence, this study was conducted to identify different strains of banana streak virus present in Sri Lanka. Viral DNA isolated from different banana varieties infected with banana streak virus was subjected to polymerase chain reaction with four specific primer pairs *viz,* Mys F1/R1, RD F1/R1, GF F1/R1, and Cav F1/R1 specific for BSV-Mys, BSV-RD, BSV-GF, and BSV-Cav virus isolates, respectively. Only BSV-Mys strain was detected in infected *Embul, Pulathisi* and *Kolikuttu* varieties. BSV-Mys, BSV-RD, and BSV-GF strains were detected in infected *Seenikesel* variety. Only BSV-GF strain was detected in infected Cavendish variety. BSV-Cav strain was not detected from any of the varieties studied.

KEYWORDS: Banana Streak Virus, Polymerase Chain Reaction, Strains.

INTRODUCTION

Banana (*Musa* spp.) is the most widely cultivated and consumed fruit in Sri Lanka. It is also an attractive perennial fruit crop for farmers due to its high economic gains throughout the year. Currently, diseases caused by four major viruses are primarily responsible for poor banana cultivation in Sri Lanka including Banana Bunchy-top Virus (BBTV), Cucumber Mosaic Virus (CMV), Banana Streak Virus (BSV) and Banana Bract Mosaic Virus (BBrMV).

Banana streak virus (BSV), the causal agent of banana streak disease, is considered to be the most frequently occurring virus of *Musa* in the world and has been associated with yield loss (Agindotan *et.al.*, 2003). In Sri Lanka, its presence was first confirmed by Thomas *et al* in 1997.

BSV is a member of the family Caulimoviridae, and genus *Badnavirus*. It contains a circular double stranded DNA genome of 7.4 kb in size (Lockhart and Olszewski, 1993).

A high degree of heterogeneity exists among isolates of BSV and they differ serologically and genomically (Lockhart and Olszewski, 1993; Geering *et al.*, 2000), making diagnosis difficult. Variability of symptoms produced is also associated with the strain differences. Most characteristic symptom of this disease is chlorotic streaks on the leaf lamina, which become necrotic with the time. However, BSV infection may go unnoticed, since expression of foliar symptoms is periodic, streaks are not evident on all leaves, and emerging leaves may not show symptoms. Stunting of the infected plants, distortion of the fruits with thinner peel on small bunches, heart rot of the pseudostem and plant death have also been noted (Geering *et al.*, 2000).

Integration of segments of viral genome into B genome of *Musa* (LaFleur *et al.*, 1996) which can be activated in certain genotypes of *Musa* by stress including *in vitro* propagation, breeding, and other stress factors has limited the development of tissue culture of improved banana and plantain hybrids (Ndowora *et al.*, 1999; Haper *et al.*, 1999). Thus, the principle source of BSV transmission is through vegetative propagation including suckers and tissue cultured plantlets derived from infected sources. In addition, disease transmission by mealy bug species (*Planococcus citri*) has also been reported (Lockhart and Jones, 2000).

At present, the rate of the spread of BSV in Sri Lanka is not at a high level. Nevertheless, this disease is more prevalent in tissue culture propagated banana. Recently, BSV infection has been recorded from a plantation with 1000 plants in Aswatta and from a cultivation with 750 plants at Kanampalla (DOA / WP, 2004). It has been revealed that widely cultivated local variety of *Embul* (AAB, Mysore) is more susceptible to BSV. *Embul* has the highest demand for cultivation and it is now being micropropagated widely (Hirimburegama *et al.*, 2004). Extensive use of a susceptible variety *i.e. Embul* through tissue culture may pose a potential risk of wide spread of the BSV in future.

As the complexity of BSV infection has compounded with the genomic and serological heterogeneity of BSV isolates, there is an urgent requirement of studying genetic diversity among BSV isolates in Sri Lanka. Through these studies, it is hoped to improve diagnostic tests for better virus indexing procedures and to have better understanding of the epidemiology of the virus. Awareness of the genetic diversity of BSV would also provide basis for the development of specific methods to detect the presence of integrated sequence of BSV in *Musa* spp. Development of a such method is important in

confirming disease free materials for tissue culture, from tissue culture and for local and international trade especially by confirming the absence of the integrated form of BSV.

Four distinct isolates of BSV, namely BSV-RD, BSV-Cav, BSV-Mys, and BSV-GF, have been identified based on polymerase chain reaction (PCR) and by DNA hybridization assay in previous studies (Lockhart and Olszewski, 1993, Geering *et al.*, 2000). Complete genome of one BSV isolate from Nigeria (BSV- Onne) recently has also been sequenced (Geering *et al.*, 2000).

This study was conducted with the objective of identifying the presence of four distinct strains of BSV (BSV-RD, BSV-Cav, BSV-Mys, BSV-GF) in Sri Lanka based on polymerase chain reaction with four BSV strain - specific primer pairs.

MATERIALS AND METHODS

This study was conducted at the laboratory of Plant Virus Indexing Centre, Gabadawatta, Homagama, from January to June 2005.

Collection of Samples

Leaf samples from seven varieties of banana (conventionally propagated and tissue cultured) with chlorotic and necrotic streak symptoms suggestive of BSV infection, were collected from home gardens, small and large scale plantations in wet and intermediate zones of Sri Lanka. Collected samples included *Embul*, *Pulathisi*, *Seenikesel*, *Kolikuttu*, Cavendish, Ash plantain (*Alukesel*) and *Prasada* varieties. These samples were either immediately used or preserved at -20°C for later investigations.

Extraction of Viral DNA

Extraction of viral DNA was done according to Su, (1999). A leaf lamina tissue of 0.25g, with prominent chlorotic streaks was extracted with 1350 µl of DNA extraction buffer (100 mM Tris-HCl, 100 mM EDTA, 250 mM NaCl, pH 8.0) and 150 µl of 10% Sarkosyl (N-Lauroyl sarcosine). The tissue suspension was stirred and transferred to a 1.5 ml eppendorf tube and incubated at 55°C for 1h in a water bath. Tubes were then centrifuged at 6000 rpm for 5 minutes. Afterwards, 400 µl of supernatant was saved and incubated for 10 min. at 65°C after addition of 50 µl of 5 M NaCl and 50 µl of 10% CTAB (Cetyltrimethylammonium bromide) in 0.5 M NaCl mixture. Then, the mixture of 250 µl of chloroform / isoamyl alcohol (24:1) was added and mixed thoroughly and spun at 11000 rpm for 5 minutes. Four hundred microlitres of supernatant was collected and a mixture of 300 µl of phenol / chloroform / isoamyl alcohol (25:24:1) was added and mixed thoroughly. Tubes were spun at 11000 rpm for 5 minutes. A supernatant of 300 µl was saved and 0.6 volume of isopropanol was added to precipitate the nucleic acid.

Thereafter, tubes were incubated at -20°C for overnight and then spun at 12000 rpm for 15 minutes. Pellets were washed with 70% ethanol to remove CTAB residuals then briefly dried and finally resuspended in 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Amplification of Viral DNA by Polymerase Chain Reaction (PCR)

BSV DNA was amplified with four primer pairs, RD-F1/R1, Cav-F1/R1, Mys-F1/R1, and GF-F1/R1, which were specific for BSV-RD, BSV-Cav, BSV-Mys, and BSV-GF, viral DNA respectively (Geering *et al.*, 2000) (Table 1).

PCR was carried out in 25 µl volume containing 10x PCR buffer 2.3 µl, Magnesium chloride 25 mM ($MgCl_2$) 1.5 µl, dNTPs 10 mM (deoxynucleoside triphosphates – mixture of dATP, dGTP, dCTP, dTTP) 0.5 µl, primer (F1 and R1) 2 µl from each, Taq DNA polymerase (5u/µl) 0.5 µl, DNA template (1:30 dilution of original extracted sample) 2.0 µl, and deionized water 14.2 µl.

Thermal cycler parameters for Mys-F1/R1, RD-F1/R1, and GF-F1/R1, primers were one cycle at 94°C for 30 sec., followed by 35 cycles at 94°C for 10 sec., 64°C for 30 sec. and 72°C for 1 minute. For the Cav-F1/R1 primer, amplification condition was one cycle at 94°C for 30 sec., 35 cycles at 94°C for 10 sec., 60°C for 30 sec. and 72°C for 1 minute. After amplification, tubes were soaked at 4°C for 2 hr before electrophoresis. Negative PCR controls included healthy samples from cv. *Embul* and cv. *Seenikesel*.

Table 1. Sequences of primers specific to four different BSV strains and predicted sizes of Polymerase Chain Reaction (PCR) products

Primer	Primer sequence	Size of the product (bp)	BSV strain
RD-F1 RD-R1	5'-ATCTGAAGGTGTGTTGATCAATGC-3' 5'-GCTCACTCCGCATCTTATCAGTC-3'	522	BSV-RD
Cav-F1 Cav-R1	5'-AGGATTGGATGTGAAGTTTGAGC-3' 5'-ACCAATAATGCAAGGGACGC-3'	782	BSV-Cav
Mys-F1 Mys-R1	5'-TAAAAGCACAGCTCAGAACAAACC-3' 5'-CTCCGTGATTTCTTCGTGGTC-3'	589	BSV-Mys
GF-F1 GF-R1	5'-ACGAACTATCACGACTTGTTCAAGC-3' 5'-TCGGTGGAATAGTCCTGAGTCTTC-3'	476	BSV-GF

Analysis of PCR Products

Amplified PCR products were analyzed by gel electrophoresis on a 1% agarose gel containing 0.05 µg/ml ethidium bromide. After electrophoresis, the gel was photographed under ultra violet light. The DNA molecular maker φx-174 RF DNA -Hae III, was used as the size maker.

Size of the amplified products was recorded by comparing the position of the amplified bands with DNA molecular maker. Then, it was compared with the expected size of the PCR product specific for each of the BSV strains (Table 1).

RESULTS

The number of samples tested in each variety and the results of the PCR are given in Table 2. Using Mys F1/R1 primer pair it was possible to amplify about 589 bp DNA fragment from BSV isolates of *Embul* (AAB group, Mysore sub group) from 10 samples, *Pulathisi* (AABB group) from 2 samples, *Kolikuttu* (AAB group) from 1 sample and *Seenikesel* (ABB group) from 1 sample (Table 2). The amplified products of these samples were comparable with the expected size of the PCR product for Mys F1/R1 primer pair (Plate 1). Therefore, it confirmed the presence of BSV-Mys strain in these samples (Table 2). Mys F1/R1, RD F1/R1, and GF F1/R1, primer pairs were able to amplify relevant genomic portions from BSV DNA extract of *Seenikesel* (ABB) by giving the expected size of the amplified DNA fragments of 589 bp, 522 bp and 476 bp, respectively (Plate 1 and Plate 2). Hence, the three BSV strains namely BSV-Mys, BSV-RD, and BSV-GF, were detected in single sample of *Seenikesel* (ABB) (Table 2). About 476 bp DNA fragment from BSV DNA extract of Cavendish (AAA group) was able to amplify by GF F1/R1 primer pair (Plate 2), and the presence of BSV-GF strain was confirmed in that sample (Table 2).

None of the 49 samples tested produced an amplified band with Cav F1/R1 primer pair (Table 2). Hence, BSV-Cav strain was not detected in this investigation. Samples from Ash Plantain (ABB group) and *Prasada* were not able to produce an amplified fragment from any of the four specific primer pairs (Table 2) indicating the absence of the four BSV strains in these samples. No DNA was amplified from the corresponding healthy plants used as negative PCR controls (Plate 1 and Plate 2).

Table 2. Detection of BSV strains from BSV infected different banana varieties by Polymerase Chain Reaction (PCR) with specific primer pairs

Variety	No. of samples tested	No. of samples amplified	Samples amplified primer pairs				Detected BSV strains
			Mys F1/R1	RD F1/R1	GF F1/R1	Cav F1/R1	
Embul (AAB, Mysore)	18	10	+	—	—	—	BSV-Mys
Kolikuttu (AAB)	17	01	+	—	—	—	BSV-Mys
Pulathisi (AABB)	07	02	+	—	—	—	BSV-Mys
Cavendish (AAA)	03	01	—	—	+	—	BSV-GF
Ashplantain (ABB)	02	0	—	—	—	—	Not detected
Seenikesel (ABB)	01	01	+	+	+	—	BSV- Mys BSV-RD BSV-GF
Prasada	01	0	—	—	—	—	Not detected

— *Negative (No amplification)* + *Positive (amplification was resulted)*

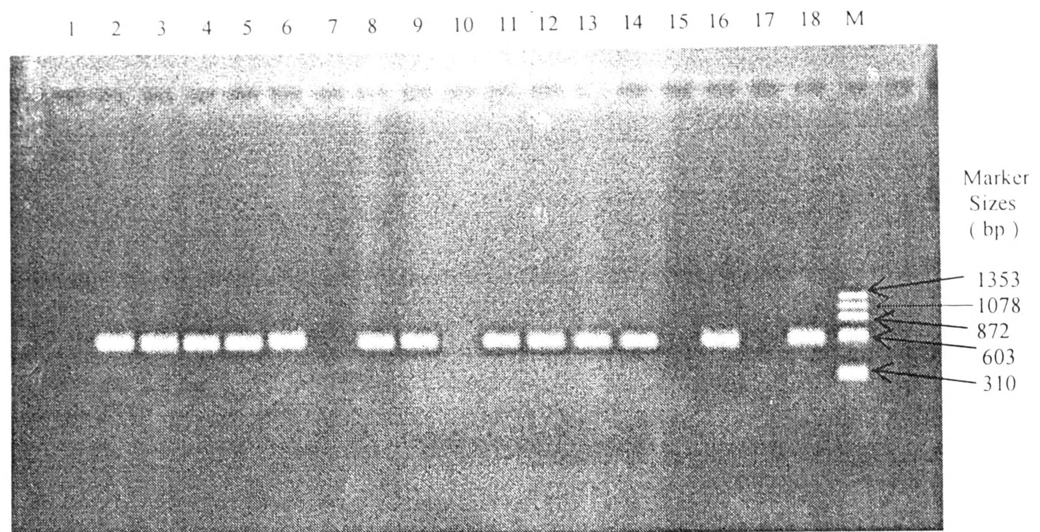

Plate 1. PCR amplification of 589 bp fragment from BSV DNA extracts of different banana varieties using Mys F1/R1 primer pair.

Lane 1 – Healthy sample amplified by Mys F1/R1 primer pair, Lane 2 and 4 – amplified 589 bp fragment from BSV infected cv. Pulathisi, Lane 3, 5, 8, 9, 11-13, 16 and 18 – amplified 589 bp fragment from BSV infected cv. Embul, Lane 6 – amplified 589 bp fragment from BSV infected cv. Seenikesel, Lane 14 – amplified 589 bp fragment from BSV injected cv. Kolikuttu, Lane M – DNA marker (φx – 174 RF DNA – Hae III).

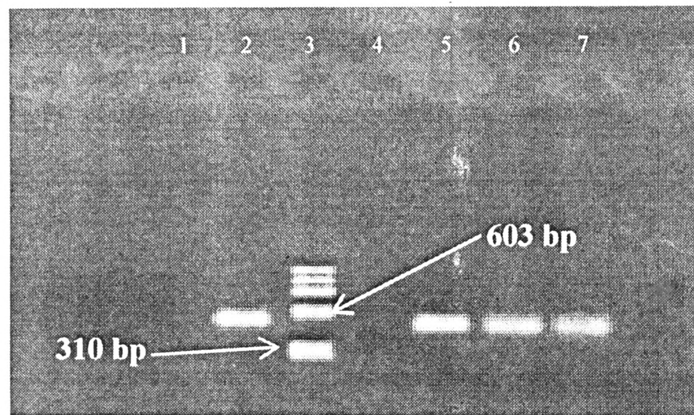

Plate 2. PCR amplification of 522 bp fragment and 476 bp fragment from BSV DNA extracts of different banana varieties using RD F1/R1 and GF F1/R1 primer pairs respectively.

Lane 1 - Healthy sample amplified by RD F1/R1 primer pair, Lane 2 – 522 bp fragment from BSV infected cv. Seenikesel amplified by RD F1/R1 primer pair, Lane 3 – DNA size marker (φx – 174 RF DNA – Hae III), Lane 4 – Healthy sample amplified by GF F1/R1 primer pair, Lane 5 – 476 bp fragment from BSV infected cv. Cavendish amplified by GF F1/R1 primer pair. Lane 6 and 7 – 476 bp fragment from BSV infected cv. Seenikesel amplified by GF F1/R1 Primer pair.

DISCUSSION

Visual identification of BSV infection is more complicated than the other viral infections in banana because symptoms can be variable and probably depend on strain differences, banana cultivars, vector activity, and environmental conditions (Geering *et al.*, 2000). In addition, expression of foliar symptoms is periodic, streaks are not evident on all leaves, and emerging leaves may not show symptoms (Geering *et al.*, 2000). Moreover, BSV infected plants may remain symptomless. BSV symptoms often confused with those of cucumber mosaic virus (CMV). Due to such reasons, collection of BSV infected banana plants was difficult in this investigation. Therefore, most of the collected samples were from banana plants suggestive of BSV. This may be a reason for the loss of amplification in most of the samples. In addition, there might be uncharacterized BSV strains in collected samples of which genomic portions could not be amplified by primer pairs used in this investigation.

In the present study, leaves with prominent chlorotic streaks were taken for the extraction of viral DNA since those places are rich with viable multiplying viruses than in necrotic places. Even though miniprep virus purification method is mostly practiced for the viral DNA extraction, Dassanayake (1999) has reported its limitations in applying for BSV DNA extraction from field samples. The method used in this study (Su, 1999), was proved as efficient, simple and successful method for BSV DNA extraction for PCR analysis.

In this study, the PCR analysis with specific primer pairs was used to differentiate strains of BSV. PCR based methods has many advantages over the serological detection methods as the serological

detection of BSV is complicated by the occurrence of a wide degree of serological diversity among virus isolates (Lockhart and Olszewski, 1993). Moreover, serological methods are not capable to differentiate the strains of BSV.

Present results suggested that diseased plants of *Embul, Pulathisi, Kolikuttu*, and Cavendish had single infection while in *Seenikesel* infection has been occurred as mixed infection. This may provide an opportunity to test the presence of a correlation between strain and the symptoms produced. Furthermore, it is possible to test whether any chances exist to enhance the severity of disease if the infection has been caused by mixed strains. However, isolates cannot be clearly categorized as mild or severe since it has been revealed that, one BSV isolate that produces mild symptoms in one banana cultivar may cause lethal systemic necrosis in another cultivar (Lockhart, 2000).

In this study, it was possible to detect the presence of three different strains of BSV in Sri Lanka while BSV-Cav strain was not detected in any of the collected samples. However, for the confirmation of the absence of the BSV-Cav strain in Sri Lanka it is required to conduct further studies by collecting samples island wide.

CONCLUSIONS

In Sri Lanka, presence of BSV-Mys, BSV-RD, and BSV-GF, strains was confirmed in this investigation. Out of the strains, only BSV-Mys strain was detected in BSV infected banana cvs. *Embul, Pulathisi*, and *Kolikuttu*, while only BSV-GF strain was detected in BSV infected cv. Cavendish. BSV-Mys, BSV-RD, and BSV-GF, strains were detected in BSV infected cv. *Seenikesel*. Results of this investigation could be utilized to improve diagnostic tests of BSV for better virus indexing procedures and to have better understanding of the epidemiology of the virus. Moreover, in present study it was possible to confirm the suitability of PCR technology together with Mys F1/R1, RD F1/R1, and GF F1/R1, specific primers for strain level detection of BSV. Therefore, there is a potential to apply this method in quarantine purposes to enhance the sensitivity of BSV detection. Furthermore, the viral DNA extraction protocol developed by Prof. Su, used in this study was proved successful and can be adopted for the extraction of BSV DNA from field samples for PCR based diagnosis of viral diseases of banana.

Further studies should be carried out to detect the integrated sequences of BSV in *Musa* spp. and the possibility of activation of integrated sequences through the application of tissue culture. Further, awareness of these integrated BSV sequences in *Musa* genome could help in the rescue of infected genotypes through recombinant DNA technology.

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