

Banana streak virus from India and its detection by polymerase chain reaction

Anita K Cherian², V K Baranwal^{1*}, V G Malathi¹, R P Pant¹ and Y S Ahlawat¹

¹Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India

²Department of Plant Pathology, Banana Research Station, Kerala Agricultural University, Kannara, Thrussur 680 652, India

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Banana streak virus (BSV) is an important pathogen of bananas and plantains (*Musa* spp.). The authors cloned and sequenced part of the genomes of two isolates of BSV from Kerala, BSV-K1 and BSV-K3. Both the clones contained a sequence covering a part of open reading frame III. The multiple sequence alignment of amino acids showed that both the isolates had a very high degree of identity with each other and clustered with Nigerian isolates of BSV (BSV-Onne) but not with Australian isolate of BSV (BSV-Mys). The relationship of these two isolates with other badnaviruses and *Rice tungro bacilliform virus* was also analysed. BSV was detected by PCR amplification in samples, which were symptomatic but negative in electron microscopy.

Keywords: *Banana streak virus*, banana, PCR detection, badnavirus

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Introduction

Banana streak disease, reported from Africa, Europe, Latin America, Caribbean and Asia-Pacific Region either on the basis of symptoms or serology and electron microscopy, is caused by the pathogen, *Banana streak virus* (BSV)^{1,2}. BSV has bacilliform-shaped particles (approx. 30×130-150 nm) and a circular ds DNA genome (approx. 7.4 kb)^{2,3}. The virus has been transmitted only to plants within family *Musaceae* using mealybug vectors². Infection can cause delay in fruit bunch emergence, harvest and can reduce bunch weight also^{2,4}. Banana Research station (BRS), Kannara, Kerala, maintains one of the richest germplasm collections, which includes many exotic varieties along with local accessions. Symptoms of BSV, such as chlorotic streaks and black streaks on leaves of banana (Fig. 1), were observed on two accessions, BRS-1 (AAB) and Mysore Poovan (AAB).

BSV detection based on symptoms, which vary considerably⁵, is often confusing because of similarity of symptoms with those of *cucumber mosaic virus*. Serologically, BSV is moderately immunogenic and individual antiserum often failed to detect some BSV isolates⁶. Hence, Polymerase Chain Reaction (PCR)

using the primers generally used from the conserved domain of virus genome would be more useful in quick and reliable detection of BSV. In this study, using gradient annealing temperature of primers having different T_m values, an attempt has been made to optimize the PCR for detection of BSV isolates from two symptomatic banana cultivars, BRS-1 and Mysore Poovan. Based on this information, PCR amplified fragment was cloned and sequenced for the two isolates of BSV from India.

Materials and Methods

Sources of Samples

BSV isolates, BSV-K1 and BSV-K3, were collected from naturally infected *Musa* spp., BRS-1 (AAB) and Mysore Poovan (AAB) respectively from the fields of BRS, Kannara, Kerala.

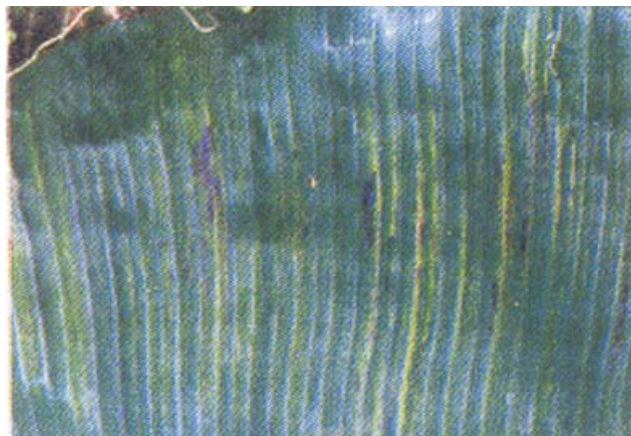
Leaf Dip Electron Microscopy

Symptomatic leaf tissue (3 mm diam) from two cultivars was crushed over a clean glass slide in 0.01 M phosphate buffer (pH 6.5). Homogenate (10 µl) was placed on a parafilm and EM carbon coated grid (film side downward) was floated on the surface of the droplet for 10 min. The grid was then removed and washed with double distilled water. Drops (2-4) of 2% uranyl acetate (UA) were added on the filmed surface of the grid. Excess stain was removed and the grid was left to dry and then viewed under electron microscope [(Model)- Jeol 100 ex II].

*Author for correspondence:

Tel: 91-11-25843474; Fax: 91-11-25841311

E-mail: vbaranwal2001@yahoo.com



DNA Isolation

DNA was isolated from 100 µg of young symptomatic leaves of diseased plant showing no virus particles or virus particles under electron microscope using DNeasy plant mini kit (Qiagen, GmbH, Hilden, Germany) by the protocol provided by them. The symptomatic leaves, which showed no virus particles (EM-) or those with virus particles (EM+) and healthy leaves, were used for the isolation of DNA.

Primers and PCR

Specific primers were manually designed from the sequence data of the Onne of isolate BSV (GenBank accession No. AJ 002234) from the region corresponding to the conserved domain of reverse transcriptase and RNase H (sense primer BSV5466 5'-AGAGTGGGTTTCATCAAGTAGC and anti sense primer BSV 6196 5'-GAATTTCCCGCTCGCATAAG). The primer number indicates the position on the BSV genome. PCR amplification was performed in 50 µl reaction mixtures using 1 µM of primer, 200 µM each of dNTPs, 0.05 unit/µl of Taq DNA polymerase (Fermentas, Lithuania) and 1 × reaction buffer, 1.5 mM of MgCl₂ and 5 µl of DNA template either from symptomatic or non-symptomatic (healthy) banana plants. Using the gradient function of thermocycler (Biometra model T-Gradient Thermoblock, Germany), a temperature gradient of annealing temperature of 53-63°C (53, 53.9, 56.2, 57.4, 58.6, 59.8, 60.9, 62 and 63°C) was set. The other parameters of PCR amplification were: one cycle of 94°C denaturation for 4 min, 30 cycles each at 94°C for 30 s, gradient temp (53-63°C) for 30 sec and 72°C for 30 sec followed by one cycle of final extension for 10 min. From completed PCR reaction mixture, 15 µl

was analysed by electrophoresis on 1% agarose gel containing ethidium bromide and viewed under transilluminator.

Cloning and Sequencing of Viral DNA

The amplified DNA was excised and eluted from the gel using QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany). The purified PCR product was ligated into pGEM-T Easy vector (Promega, Madison, USA). Competent *Escherichia coli* (strain DH 5 α) were transformed by standard molecular biology procedures⁷. Recombinant clones were identified by colony PCR as well as restriction endonuclease and selected clones were sequenced at the automated DNA sequencing facility, Department of Biochemistry, Delhi University, South Campus, Delhi, India. Sequence data were compiled by Seq. Aid vers. 3.6(1). Sequences were obtained from GenBank having following accession numbers: BSV-Onne, AJ002234; BSV-Mys, AF214005; *Citrus yellow mosaic virus* (CYMV), AF 347695; *Commelina yellow mottle virus* (ComYMV), X52938; *Cacao swollen shoot virus* (CSSV), NC-001547; *Sugarcane bacilliform virus* (SCBV), NC-003031; and *Rice tungro bacilliform virus* (RTBV), NC_00194. Sequences were aligned using CLUSTAL W and the sequence identity matrix for pair wise combination of aligned sequences was calculated with Bio Edit Sequence Alignment Editor⁸. Trees were created using neighbour-joining. Robustness of the tree was determined by bootstrap sampling of multiple sequence alignment (1000 sets) and a consensus tree was generated with treecon for Window (ver 1.3b) package.

Results

Electron Microscopy

The leaf dip preparation of infected leaf of banana showed typical bacilliform particles, which indicated that the streak like symptoms in banana at BRS, Kannara are associated with BSV. However, out of 6 leaf dip preparations from different symptomatic plants of two cultivars (3 from each sample), only two samples of BRS-1 showed the presence of bacilliform particles under electron microscope (Fig. 2).

PCR

Using gradient temperature in one block and one experiment, intensity of amplification was found maximum at 58.6°C compared to other temperature range (53-63°C). Based on this information amplicons



Fig. 2—Bacilliform virus particles associated with Banana streak disease of banana BRS-1 as seen in the leaf dip preparation under electron microscope

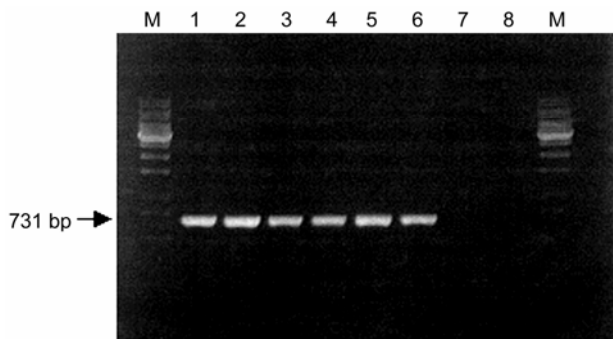


Fig.3—PCR amplification from EM positive and EM negative samples of banana streak disease plant. M-1Kb gene ladder; 1 & 2 Amplicon of DNA template from EM positive diseased banana sample BRS-1; 3 & 4 Amplicon of DNA template from EM negative diseased banana sample BRS-1; 5 & 6 Amplicon of DNA template from EM negative diseased banana sample Mysore Poovan; 7 & 8 No amplicon from DNA template from EM negative healthy banana sample BRS-1, No amplicon from DNA template from EM negative healthy banana sample Mysore Poovan

of approx. 730 bp were obtained from EM positive sample of BRS-1 as well as EM negative samples of both the cultivars of banana showing symptoms of BSV. No PCR product was obtained from DNA extracted from EM negative healthy banana leaves (Fig. 3).

Sequence Analyses

Sequence analyses of the clones of amplicons showed that both BSV isolates were 731 bp long. Sequence of both the BSV isolates had similarities with other badnaviruses particularly with BSV isolates retrieved in database searches. The sequence of BSV-K3 from banana cv Mysore Poovan was identical to BSV-Onne, the Nigerian isolate of BSV, sharing identity of 99%. However, the isolate BSV-K1 from banana cv BRS-1 was less identical with BSV-Onne, sharing similarity of 97%. However, both these isolates and BSV Onne were very distinct from BSV-Mys isolate reported from Australia as all these three isolates shared a homology of 75% only with BSV-Mys in the region of RT and RNase H domains in ORF III polyprotein (Table 1).

The sequence obtained by reverse primer was reverse complemented and combined with that obtained with forward primer to get a continuous sequence in viral sense and the 731 nucleotides were translated to 243 amino acids. The sequences of both BSV isolates have been deposited in the GenBank (Accn. nos, AY101188 & AY101189). The alignment of amino acid sequences revealed that BSV isolates, K1 and K3, showed more than 98 % similarity in the region indicating that the region is highly conserved and differed with BSV-Mys isolate from Australia. On comparing the predicted amino acid sequence of

Table 1— Amino acid sequence identity of pair wise combination of different strains of *Banana streak virus* and other badnaviruses for a reverse transcriptase and ribonuclease H domain of open reading frame (ORF) III polyprotein

	% Amino acid sequence identity								
	BSV-M	BSV-O	BSV-K1	BSV-K3	ComYMV	CSSV	CYMV	SCBV	RTBV
BSV-M	100	75.2	75.4	75.8	65.1	61.4	67.6	63.5	43.0
BSV-O	---	100	97.9	99.5	65.1	63.9	67.6	63.1	44.2
BSV-K1	---	---	100	98.3	64.7	63.5	67.2	62.7	44.2
BSV-K3	---	---	---	100	65.1	63.9	67.6	63.1	44.2
ComYM	---	---	---	---	100	58.6	62.7	63.9	41.3
CSSV	---	---	---	---	---	100	69.2	59.4	44.6
CYMV	---	---	---	---	---	100	60.4	43.4	---
SCBV	---	---	---	---	---	---	---	100	44.2
RTBV	---	---	---	---	---	---	---	---	100

The badnaviruses are *Banana streak virus* (BSV) isolates -Onne, -K1, -K3 and -Mys, respectively), *Commelina yellow mottle virus* (ComYMV), *Cacao swollen shoot virus* (CSSV), *Citrus yellow mosaic virus* (CYMV), *Sugarcane bacilliform virus* (SCBV) and *Rice tungro bacilliform virus* (RTBV).

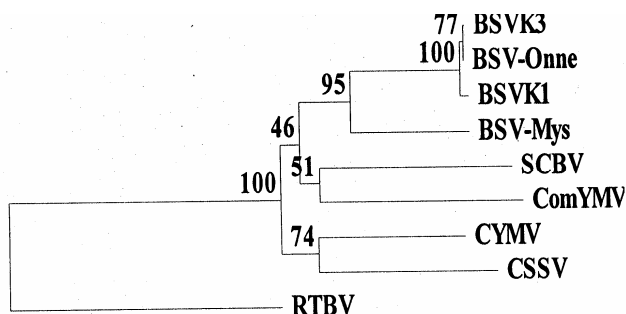


Fig. 4—Phylogenetic tree depicting the relationships of the various badnaviruses [rice tungro bacilliform virus (RTBV) used as root], based on the alignment of the reverse transcriptase and ribonuclease H domain of the ORF III polyprotein. The badnaviruses are *Banana streak virus* (BSV) isolates -Onne, -K1, -K3 and -Mys respectively), *Commelina yellow mottle virus* (ComYMV), *Cacao swollen shoot virus* (CSSV), *Citrus yellow mosaic virus* (CYMV) and *Sugarcane bacilliform virus* (SCBV). Horizontal branch length is proportional to genetic distance.

two BSV isolates with that of other badnaviruses, RTBV was found very distinct badnavirus. The phylogenetic tree (Fig. 4) based on putative amino acid alignment of reverse transcriptase and RNase H gene showed that the two BSV isolates, K1 and K3, clustered along the Nigerian and Australian isolates and all of them together occupied distinct branch well separated from SCBV and ComYMV. However, BSV isolates were more closely related to SCBV and ComYMV, as they all branch separately from CSSV and CYMV.

Discussion

Variation in BSV isolates, K1 and K3, based on partial genome sequence, has been shown⁹. The variation in nucleotide sequence of the two isolates was not more than 3% and was very closely related to Nigerian isolate, BSV-Onne. This is the first report of BSV from two banana cultivars. Though Immunosorbent electron microscopy of viral mini preps, using a multi-strain antiserum to BSV is considered as most reliable method for detection of BSV strains¹⁰, the limited facility of electron microscopy and complex virus purification and immunization steps in production of antibodies makes immunosorbent electron microscopy less preferable for routine detection. Therefore, PCR based detection of BSV would be more useful. Though, there is evidence that genomic sequences of BSV are integrated into *Musa* genome¹¹ and can be amplified in PCR amplification. But in the present study, bacilliform particles of BSV were seen in two of the

symptomatic samples, which indicated that the amplification of viral genomes was from the virus particles and not from the integrated host genome. PCR using primers from conserved domain of genomes of badnaviruses has been used in numerous studies for the rapid, sensitive and reliable detection of different badnaviruses^{6,12-18}. Present study indicated that primers from highly conserved region of BSV could be used for detection of BSV more reliably than electron microscopy and serology.

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