Detection of Sugarcane Bacilliform Virus Using the Polymerase Chain Reaction

Kathryn S. Braithwaite, Natasha M. Egeskov, and **Grant R. Smith,** David North Plant Research Centre, Bureau of Sugar Experiment Stations, Indooroopilly, Queensland 4068, Australia

ABSTRACT

Braithwaite, K. S., Egeskov, N. M. and Smith, G. R. 1995. Detection of sugarcane bacilliform virus using the polymerase chain reaction. Plant Dis. 79:792-796.

A sensitive assay based on the polymerase chain reaction (PCR) has been developed to detect sugarcane bacilliform badnavirus (SCBV) in diseased sugarcane plants. Two sets of primer pairs, which prime the synthesis of DNA from the putative aspartic protease and reverse transcriptase coding regions, were evaluated. Best results were obtained with the primer pair SCBVF5 and SCBVR5, which primes the synthesis of a 221-bp virus-specific fragment from total nucleic acid extracts of diseased sugarcane plants. The viral origin of the PCR products was confirmed by Southern hybridization with a 1,602-bp fragment from the cloned SCBV genome. This diagnostic test was used to detect SCBV infection of noble canes (*Saccharum officinarum*), commercial hybrids, clones within the "*Saccharum* complex" including *S. robustum, S. spontaneum, S. barberi, Erianthus arundinaceus,* and *E. ravennae*, as well as banana infected with banana streak virus. This sensitive screening test has been used to show that SCBV is widely distributed throughout *Saccharum* and related germ plasm.

Sugarcane bacilliform badnavirus (SCBV) is a recently recognized pathogen of sugarcane (11). The virus is widely distributed throughout the major geographical regions in which sugarcane is grown, infects both noble canes (*Saccharum officinarum* L.) and commercial cultivars (*Saccharum* L. interspecific hybrids) (1), and is suspected to be the cause of yield losses in variety Mex57-473 (11). Symptoms of infection are variable and may include flecks or freckles on the leaves, or diagnostic symptoms may be absent. The major vector is the pink sugarcane mealybug *Saccharicoccus sacchari* (Cockerelle) (12).

Serological and transmission studies indicate that SCBV is closely related to banana streak virus (BSV) (9). SCBV can be transmitted by *S. sacchari* from infected sugarcane to banana with SCBV-infected banana developing symptoms indistinguishable from those described for BSV-infected banana (12). BSV can be mechanically transmitted to sugarcane, although no foliar symptoms are observed, and the Moroccan strain of BSV is serologically indistinguishable from some isolates of SCBV (11). Therefore, SCBV and BSV can be considered as strains of the same virus (12).

SCBV and BSV are both members of the badnavirus group (10). Virus particles in this group are bacilliform in shape and contain a double-stranded DNA genome. The genome of one strain of SCBV has been cloned and sequenced (3). The largest open reading frame is capable of encoding a protein with regions of similarity to the RNA-binding domains, aspartic proteases, and replicases of retroelements. The presence of these coding regions plus a $tRNA^{met}$ binding site suggests that SCBV, like Commelina yellow mottle virus (CoYMV), the type member of the badnavirus group, is a pararetrovirus (15). The genomes of two other badnaviruses, rice tungro bacilliform virus (RTBV) (7, 16) and cacao swollen shoot virus (CSSV) (6) have also been cloned and sequenced.

Until recently, the most reliable method of diagnosing SCBV-infected sugarcane was by immune electron microscopy (IEM) (2). However, IEM is slow, tedious, and lacks sufficient sensitivity to detect the virus in plants with low viral titer. Diagnosis by symptomatology is unreliable because the symptoms of infection are variable or absent, while diagnosis using indicator plants is unreliable because the virus is difficult to transmit mechanically. Mauritian workers reported that enzyme-linked immunosorbent assay (ELISA) was also unreliable for detection of SCBV, except in samples from heavily infected noble canes (2). ELISA appears to be sensitive to viral strain variation, as more reliable results were obtained after a BSV antiserum was substituted for the Moroccan SCBV antiserum (1). A rapid, reliable, sensitive test was required to screen sugarcane germ plasm in quarantine for SCBV infection and to determine the field distribution of SCBV in commercial canes. Due to the limitations of IEM, ELISA, and symptomatology for diagnosis of SCBV infection, a detection system based on the polymerase chain reaction (PCR) (18) was developed.

MATERIALS AND METHODS

Primers. Two primer pairs (Table 1) were used in this study. The primers, SCBVF1, SCBVF5, and SCBVR5, were chosen by aligning the conserved amino acid sequences within the aspartic protease and reverse transcriptase coding regions of SCBV (3), CoYMV (15), RTBV (7,16) and CSSV (6). The published nucleotide sequence of SCBV (3) was then used to select 24-mer sequences from within these regions. SCBVR1 was chosen from an upstream region of the aspartic protease coding region to give a matching GC ratio with SCBVF1, and did not coincide with a conserved amino acid region.

Plant material. Commercial sugarcane and noble canes composed the majority of sugarcane germ plasm screened for SCBV infection. In this study, several close relatives of sugarcane that are members of the "*Saccharum* complex" (5), were also tested (Table 2). The banana cultivars Mysore and Prata were kindly provided for this study by K. G. Pegg and J. E. Thomas, Queensland Department of Primary Industries (QDPI), Australia. The banana cultivar Mysore was originally shown to be infected with BSV in Australia by J. E. Thomas using IEM.

A preparation of SCBV virions, purified from the noble cane IJ76-465 by the method of Lockhart and Autrey (11), was kindly supplied by V. G. Hardy and D. S. Teakle, The University of Queensland, Australia and was used in PCR reactions.

Nucleic acid preparations. Total nucleic acid extracts (TNAE) were prepared from infected leaves using a method modified from Smith et al. (21). Approximately 50 mg of leaf was frozen and ground in liquid nitrogen. An aliquot of

Table 1. Primers developed for polymerase chain reaction amplification of SCBV from infected sugarcane

Primer pair	Position	Sequence	Region amplified	Product size
SCBVF1	4,782	5′-GAT ACA GGT GCA ACA AGG TCC TGT	Aspartic	425 bp
SCBVR1	5,207	5′-CTC CTT CAT CTC CTC AAG AAG CCT	Protease	
SCBVF5	5,589	5′-TCA AAG TTT GAT TTG AAG AGC GGG	Reverse	221 bp
SCBVR5	5,810	5′-CTC CGA GAA AAC CAA TAT GTC ATC	Transcriptase	

Corresponding author: G. R. Smith, DNPRC, BSES, PO Box 86, Indooroopilly, Qld 4068 Australia. E-mail: Grant.Smith@mailbox.uq.oz.au

Accepted for publication 11 April 1995.

STE buffer (0.5 ml), containing 50 mg per ml polyvinylpolypyrrolidone (STE = NaCl 100 mM, Tris-Cl 50 mM, EDTA 1 mM, pH 7.0), followed by 60 µl of 10% sodium dodecyl sulfate (SDS), was added and, when thawed, transferred to a 1.5-ml microfuge tube containing 0.5 ml phenol/chloroform/isoamyl alcohol (25:24:1) and 0.1 volume 3 M sodium acetate (pH 5.2). The sample was mixed by inverting the tube several times and centrifuged at 10,000 *g* at 4°C for 10 min. The aqueous supernatant was re-extracted with an equal volume of phenol/chloroform/isoamyl alcohol and 0.1 volume sodium acetate as described above. Highly colored samples, presumably due to a high phenolic content, were extracted a third time with phenol/chloroform/isoamyl alcohol. Nucleic acids were precipitated from the final aqueous phase with 0.1 volume 3 M sodium acetate and 2 volumes ethanol and centrifugation at 15,000 *g* at 4°C for 20 min. The pellet was washed with cold 75% ethanol, dried and resuspended in sterile deionized water. Concentration of the nucleic acid extract was determined by measuring the absorbance at 260 nm. Yields of 10 to 30 µg of nucleic acid were obtained using this method. Two template dilutions (50 ng and 25 ng) were prepared for each sample using sterile deionized water.

Plasmids. The plasmid pSCBV20, containing the cloned genome of one isolate of SCBV (3), was kindly supplied by N. E. Olszewski, University of Minnesota, St. Paul. The probe for Southern hybridizations was prepared by restricting pSCBV20 with *Pst*I and *Sph*I. The 1,602-bp fragment encompassing the proposed aspartic protease and reverse transcriptase coding regions was purified from a 1% agarose gel using a Geneclean kit (Bio 101 Inc.) and subcloned into pUC19 to produce the plasmid pPS1602. Cells of *E. coli* strain DH5α were transformed with the plasmids and the plasmid DNA prepared using the alkaline lysis method described by Sambrook et al. (19).

PCR conditions. The PCR solution (25 µl) contained buffer supplied by the manufacturer (Biotech Int.), 200 µM each dATP, dCTP, dGTP and dTTP, 4 mM $MgCl_2$ (unless otherwise stated), and 1 unit *Taq* DNA polymerase (Biotech Int.). Template DNA was boiled with the primers (final concentration each 275 nM) for five min, quenched on ice, centrifuged briefly, then added to the PCR solution. The PCR solution was overlaid with mineral oil.

PCR conditions were 94°C for 7 min; three cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min; then 37 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; followed by a 72°C extension for 7 min. During optimization, the annealing temperature was varied for some experiments. Reactions were cycled in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Norwalk, Conn.). Plasmid pSCBV20 was included in the PCR experiments as a positive control, although this template was not boiled and quenched with the primers. In some experiments an aliquot of purified SCBV virions was added to the PCR solution and then boiled with the primers. Three negative controls were used: (a) an extraction control (a total nucleic acid extraction prepared without plant material); (b) a PCR control (all PCR solutions including primers, but without template), and (c) a boiled PCR control (primers boiled separately as described above, but without template).

PCR products were separated by electrophoresis through 1.5% agarose (Bio-Rad Laboratories, Richmond, Calif.) in TAE (Tris-acetate 40 mM, EDTA 1 mM, pH 8.0), stained with ethidium bromide and photographed. φX174/*Hae*III or λ/*Bst*EII digests were included on the gels as molecular weight markers.

Southern hybridizations. After electrophoresis, PCR products were transferred to positively charged nylon membranes (Boehringer Mannheim) by alkaline transfer for 3 h in 100 mM NaOH (20). Plasmid pPS1602 was used as the hybridization probe. The plasmid was linearized with the restriction enzyme *Bgl*II and labeled with digoxigenin according to the manufacturer's instructions (Boehringer Mannheim). Hybridizations were performed at 65°C and the bound probe was detected with the DIG luminescent detection kit (Boehringer Mannheim) according to the manufacturer's instructions. Results were recorded on Kodak X-Omat film.

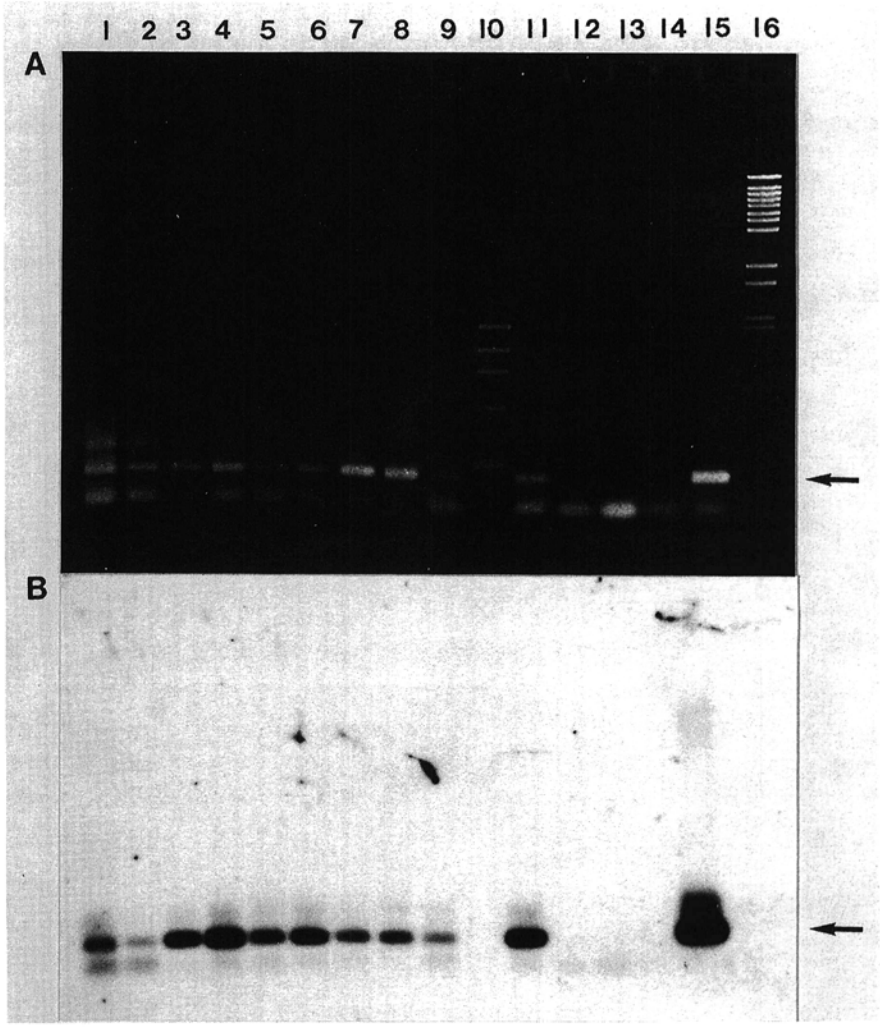

Fig. 1. Detection of sugarcane bacilliform virus (SCBV) by polymerase chain reaction (PCR) amplification. The primer pair SCBVF5/SCBVR5 was used to prime synthesis of a 221-bp product (arrowed) from total nucleic acid extracts of commercial sugarcane (lane 1: Q120, lane 2: Q138), noble canes (lane 3: IJ76-468, lane 4: EK28, lane 5: IJ76-321), members of the "*Saccharum* complex" (lanes 6: *S. robustum* IJ76-445, lane 7: *S. spontaneum* IK76-6, lane 8: *Erianthus arundinaceus* IJ76-407) and banana cv. Mysore (lane 9). An equivalent sized product was amplified from SCBV virions (lane 11) and the cloned SCBV genome (lane 15). No visible products were detected in the three negative controls (lane 12: extraction control [total nucleic acid extract without plant material], lane 13: PCR control [PCR reaction without template, primers not boiled], lane 14: boiled PCR control [PCR reaction without template, primers boiled]). Lane 10: φX174/*Hae*III markers, lane 16: λ/*Bst*EII markers. **(A)** ethidium bromide–stained gel. **(B)** Southern blot after hybridization with DIG-labeled subcloned fragment of the virus genome.

Restriction analysis of PCR products. SCBV-specific DNA amplified from selected plant samples was further characterized by restriction analysis. After completion of the PCR amplification, 15-µl aliquots were removed and digested with the endonuclease *Alu*I (Boehringer Mannheim) for 1 h. Restriction products were separated through 12% polyacrylamide gels for 1 h at 200V and the gels stained with ethidium bromide. A ϕX174/*Hin*fI digest was used as the molecular weight marker.

RESULTS

A number of primer pairs were initially selected from the published sequence of Bouhida et al. (3) to prime the synthesis of different regions of the SCBV genome. Two primer pairs, SCBVF1/SCBVR1 and SCBVF5/SCBVR5, consistently primed the amplification of products, and were further evaluated. SCBVF1/SCBVR1 primes the synthesis of a 425-bp fragment from the aspartic protease coding region of SCBV (data not shown). However, the primer pair SCBVF5/SCBVR5, which primes the synthesis of a 221-bp fragment from the reverse transcriptase coding region, generates a more intense and consistent amplification product. Using this primer pair, the expected sized DNA fragment of 221 bp was amplified from SCBV-infected sugarcane leaves (Fig. 1A). An equivalent-sized band was also amplified when either the cloned viral genome or an aliquot of purified SCBV virions was added to the PCR solution. Southern hybridizations verified the viral origin of the amplified 221-bp product (Fig. 1B).

Table 2. *Saccharum* germ plasm shown to be infected with SCBV by polymerase chain reaction amplification using the primers SCBVF5 and SCBVR5

Clone tested	Botanical name
IJ76-445	*S. robustum* Brandes & Jesw. ex Grassl
IM76-229	*S. robustum*
IK76-6	*S. spontaneum* L.
Agoule	*S. barberi* Jesw.
Rakhra 1382	*S. barberi*
IJ76-407	*Erianthus arundinaceus* (Retz.) Jesw.
US67-8-1	*E. ravennae* (L.) P. Beauv.

Southern hybridizations. Hybridizations using a digoxigenin-labeled, cloned fragment from one SCBV isolate indicated an apparent low homology between the probe and the amplification products. Under high stringency washing conditions (0.1X SSC, 0.1% SDS, 68°C; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), the probe only hybridized to the positive control (pSCBV20, data not shown). To detect hybridization with PCR products from sugarcane or banana extracts, or the virion solution, it was necessary to use moderate stringency washes (0.2X SSC, 0.1% SDS, 42°C) (Fig. 1B). Even under moderate wash conditions, the PCR products amplified from some clones that appeared intense on ethidium bromide stained gels (for example lanes 7 and 8), produced only faint hybridization signals.

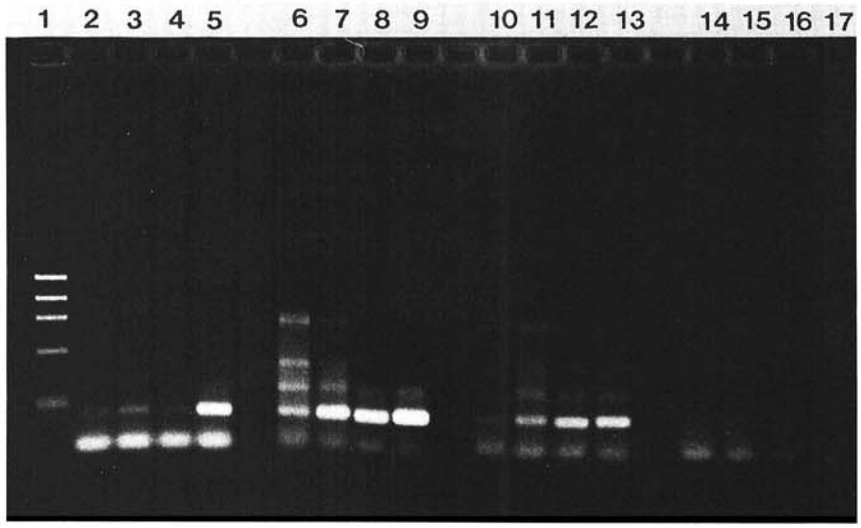

Fig. 2. The effect of template boiling and annealing temperature. Template preparation and polymerase chain reaction (PCR) annealing temperature were altered to determine the optimal conditions for amplification of sugarcane bacilliform virus (SCBV)–specific DNA from total nucleic acid extracts with the primers SCBVF5/SCBVR5. Lanes 2 through 5 represent an annealing temperature of 50°C for three cycles then 55°C for 37 cycles. The template and primers were added directly to the PCR tube without boiling. Lanes 6 through 9 represent the same annealing conditions, but the template was boiled and quenched with the primers. In lanes 10 through 13, the template was boiled and the annealing temperature was 55°C for 40 cycles. In lanes 14 through 17, the template was boiled and the annealing temperature for 40 cycles was 60°C. Lane 1: ϕX174/*Hae*III markers; lanes 2, 6, 10, 14: noble cane Ajax; lanes 3, 7, 11, 15: noble cane MQ38-2288; lanes 4, 8, 12, 16: noble cane NG57-99; lanes 5, 9, 13, 17: SCBV virions.

Banana and the "*Saccharum* complex." SCBV was detected in most of the noble canes tested and in many commercial hybrids. In addition, SCBV was also detected in some closely related plant species within the "*Saccharum* complex" (Table 2) including *S. robustum, S. spontaneum,* and *E. arundinaceus* (Fig. 1).

A band of 221 bp could also be detected in the banana cultivar Mysore showing streak symptoms characteristic of BSV infection (Fig. 1). The amplified product hybridized to the cloned SCBV probe and the hybridization signal was detected after moderate stringency washing conditions. No product was amplified from nucleic acid extracts of banana cultivar Prata that was not infected with BSV (data not shown). Therefore the conditions described above may also be applicable to diagnosis of BSV-infected banana.

PCR conditions. A number of components of the PCR system were varied to determine the optimal conditions for amplification of SCBV from TNAE. Boiling the template DNA with the primers, followed by quenching on ice, increased the sensitivity of detection for most sugarcane samples (Fig. 2). This procedure was not necessary for amplification from solutions containing either the cloned virus genome or purified SCBV virions.

Although the theoretical annealing temperature (T_m–5°C) for SCBVF5 and SCBVR5 is 63°C, a lower annealing temperature was used in the initial cycles of the PCR program. This often led to nonspecific amplification products being visible on gels. However, only a single PCR product was detected after Southern hybridization (Fig. 1B). Performing all 40 cycles with an annealing temperature of 55°C led to a slight decrease in intensity of the bands observed on agarose gels (Fig. 2). An annealing temperature of 60°C greatly reduced the intensity of the amplified products from the sugarcane samples tested (Fig. 2).

Altering the $MgCl_2$ concentration between 4 mM and 2 mM had little effect on the observed intensity of amplification products (data not shown). A concentration of 1 mM resulted in inconsistent amplification of the 221-bp DNA fragment.

TNAE template amounts ranging from 200 ng to 5 ng (in a 25-µl PCR reaction) were suitable for amplification of the diagnostic DNA fragment (data not shown). TNAE amounts of 50 or 25 ng gave the most consistent results for a range of sugarcane samples and are now used for routine screening of sugarcane germ plasm. Dilutions of the cloned viral genome (pSCBV20) ranging from 1 ng to 1 ag (10^{-9} – 10^{-18} g) were used to determine the sensitivity of the PCR test for SCBV detection. In these experiments, plasmid template and primers were not boiled and quenched together. A template concentration of 10 fg (10×10^{-15} g) in a 25-µl PCR

reaction gave an amplified product able to be detected when 10-µl samples were separated by electrophoresis and stained with ethidium bromide (data not shown).

Restriction analysis. Restriction fragment length polymorphisms (RFLPs) within the amplified DNA products were revealed after digestion with *Alu*I and separation in polyacrylamide gels. Two *Alu*I sites (at positions 5,675 and 5,775) occur within the SCBVF5/SCBVR5 amplified fragment of pSCBV20 (3). The expected *Alu*I restriction pattern was observed after digestion of the PCR product amplified from Q120 and pSCBV20 (Fig. 3). However, different patterns were observed after digestion of the PCR products amplified from banana, *S. officinarum* accessions, or SCBV virions purified from the noble cane IJ76-465. The DNA fragments amplified from *S. robustum* and *E. arundinaceus* did not contain any *Alu*I restriction sites.

DISCUSSION

Sugarcane bacilliform badnavirus was detected in nucleic acid extracts from diseased sugarcane by PCR amplification of a diagnostic 221-bp region from the viral genome. The amplification of equivalent-sized DNA products from solutions containing either purified SCBV virions or the cloned viral genome, in addition to positive Southern hybridization data, confirms the viral origin of the 221-bp amplified product. The widespread occurrence of SCBV in *Saccharum* germ plasm and lack of definitive symptoms precluded designating a true negative sugarcane control for the PCR test. However, no viral specific DNA was detected in samples from banana cultivar Prata that was not infected with BSV, and this result combined with the PCR controls provides evidence that the test only detects viral specific DNA. This PCR-hybridization test detected banana streak virus in diseased banana cultivar Mysore, providing further evidence for the concept that BSV and SCBV are strains of the same virus (12).

The primers SCBVF5 and SCBVR5 were selected from regions that appeared to be highly conserved between badnaviruses and caulimoviruses based on alignment of the amino acid sequence within the reverse transcriptase coding regions of SCBV, CSSV, RTBV, CoYMV, cauliflower mosaic virus (CaMV), carnation etched ring virus (CERV), figwort mosaic virus (FMV) and soybean chlorotic mottle virus (SbCMV) (3,6,7,15,16). Amino acid sequences of reverse transcriptases of retroviruses and hepadnaviruses of animals and humans have also been found to be highly conserved (14). Although the reverse transcriptase amino acid sequence between the badnaviruses and caulimoviruses is conserved, there is variation in the nucleotide sequence between the individual viruses, and it is uncertain whether the primer pair SCBVF5/SCBVR5 would necessarily amplify this region from other badnaviruses. Targeting these apparently conserved regions for annealing sites, basing the primer sequences on the known SCBV nucleotide sequence, and using a PCR amplification protocol with a low initial annealing temperature were considered to be the best strategies to detect all SCBV isolates in *Saccharum* germ plasm.

The variable hybridization between the probe and the PCR products suggests variation within the amplified region. While the primer binding sites are based on apparently conserved regions, there may be less homology in the DNA sequence between the priming sites. Differences in intensity between ethidium bromide–stained bands and hybridization signals have also been observed when DNA from sugarcane strains of maize streak geminivirus (MSV) was amplified using a PCR-based detection system and hybridized with a MSV probe (17). Similarly, differences in the hybridization signals between PCR fragments amplified from BSV isolates have been recently reported (13). The presence of RFLPs in the region between the SCBVF5 and SCBVR5 annealing sites is further evidence that there is variation in the DNA sequence between SCBV isolates, but to date only one isolate of SCBV has been cloned and sequenced. Whether this DNA variation has biological significance is yet to be established, although antigenic variation between SCBV isolates, presumably due to differences in the virion coat protein, has also been reported (2,13).

SCBV was detected in noble canes, commercial hybrids, and members of the "*Saccharum* complex." demonstrating the widespread distribution of this virus. Surveys carried out worldwide using IEM or ELISA have shown that a high proportion (90 to 100%) of noble canes are infected with SCBV (1,2,4). Noble canes often contain high numbers of particles and infection may be associated with flecking symptoms. However, commercial hybrid canes with mild or nonexistent symptoms were thought to contain little or no virus (2,8). Because of low viral titer and lack of reliable symptoms in many clones, a sensitive detection test was required. The PCR-based test described here detected SCBV in commercial canes, *Erianthus* species, and other *Saccharum* species. Because the test is more sensitive than IEM or ELISA, SCBV is now known to be more widespread than previously thought. Similarly, a PCR-based test recently developed for RTBV detected the virus in inoculated seedlings that tested negative by ELISA and did not show symptoms (22).

The PCR primers SCBVF5/SCBVR5 and conditions described here provide a specific, sensitive method for diagnosing SCBV-infected sugarcane. This method is currently being applied to screening and surveying *Saccharum* germ plasm in quarantine and in the field to ascertain the full extent and distribution of SCBV infection. The testing of other primer sequences is in progress and, in combination with further restriction analyses, may eventually be used to identify and differentiate isolates of SCBV.

ACKNOWLEDGMENTS

We wish to thank Warren Owens, Nils Berding, and Rob Magarey for providing sugarcane material, Greg Taylor for advice on running polyacrylamide gels, and David Teakle for comments on the manuscript. The financial and logistical support of BSES is gratefully acknowledged.

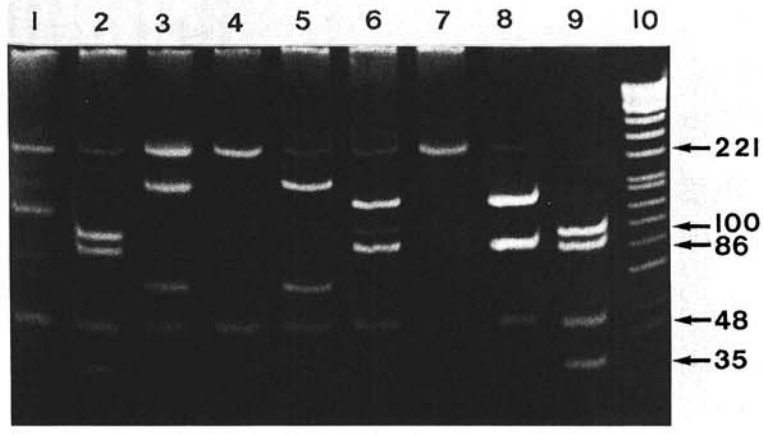

Fig. 3. Restriction fragment length polymorphisms present in sugarcane bacilliform virus (SCBV) isolates. Aliquots of polymerase chain reaction mixes after amplification were digested with *Alu*I and separated on 12% polyacrylamide gels followed by ethidium bromide staining. Lane 1: banana cv. Mysore, lane 2: commercial cane Q120, lane 3: noble cane IJ76-468, lane 4: *Saccharum robustum* IM76-229, lane 5: noble cane MQ38-2288, lane 6: noble cane EK28, lane 7: *Erianthus arundinaceus* IJ76-407, lane 8: SCBV virions purified from IJ76-465, lane 9: cloned SCBV genome, pSCBV20; lane 10: ϕX174/*Hin*fI markers. The full length amplified product from pSCBV20 is 221 bp and three restriction fragments of 35 bp, 86 bp and 100 bp are obtained after digestion. Primer-dimers of 48 bp and faint nonspecific amplification products are also visible.

LITERATURE CITED

1. Autrey, L. J. C., Boolell, S., Lockhart, B. E. L., Jones, P., and Nadif, A. The distribution of sugar cane bacilliform virus in various geographical regions. Proc. Int. Soc. Sugar Cane Technol. (In press.)
2. Autrey, L. J. C., Saumtally, S., Dookun, A., and Boolell, S. 1990. Occurrence of sugarcane bacilliform virus in Mauritius. Proc. S. Afric. Sugar Technol. Assoc. 64: 34-39.
3. Bouhida, M., Lockhart, B. E. L., and Olszewski, N. E. 1993. An analysis of the complete sequence of a sugarcane bacilliform virus genome infectious to banana and rice. J. Gen. Virol. 74:15-22.
4. Comstock, J. C., and Lockhart, B. E. 1990. Widespread occurrence of sugarcane bacilliform virus in U.S. sugarcane germ plasm collections. Plant Dis. 74: 530.
5. Daniels, J., and Roach, B. T. 1987. Taxonomy and evolution. Pages 7-84 in: Sugarcane Improvement Through Breeding. D. J. Heinz, ed. Elsevier, Amsterdam.
6. Hagen, L. S., Jacquemond, M., Lepingle, A., Lot, H., and Tepfer, M. 1993. Nucleotide sequence and genomic organization of cacao swollen shoot virus. Virology 196:619-628.
7. Hay, J. M., Jones, M. C., Blakebrough, M. L., Dasgupta, I., Davis, J. W., and Hull, R. 1991. An analysis of the sequence of an infectious clone of rice tungro bacilliform virus, a plant pararetrovirus. Nucleic Acids Res. 19:2615-2621.
8. Irey, M. S., Baucum, L. E., and Lockhart, B. E. 1992. Occurrence of sugarcane bacilliform virus in a Florida variety collection. J. Am. Soc. Sugar Cane Technol. 12:16-21.
9. Lockhart, B. E. L. 1986. Purification and serology of a bacilliform virus associated with banana streak disease. Phytopathology 76: 995-999.
10. Lockhart, B. E. L. 1990. Evidence for a double-stranded circular DNA genome in a second group of plant viruses. Phytopathology 80:127-131.
11. Lockhart, B. E. L., and Autrey, L. J. C. 1988. Occurrence in sugarcane of a bacilliform virus related serologically to banana streak virus. Plant Dis. 72:230-233.
12. Lockhart, B. E., and Autrey, L. J. C. 1991. Mealy bug transmission of sugar cane bacilliform and sugar cane clostero-like viruses. Proc. ISSCT Sugar Cane Pathol. Workshop, 3rd. Mauritius Sugar Industry Research Institute. 17.
13. Lockhart, B. E. L., and Olszewski, N. E. 1993. Serological and genomic heterogeneity of banana streak badnavirus: implications for virus detection in *Musa* germplasm. Pages 105-113 in: Breeding Banana and Plantain for Resistance to Diseases and Pests. J. Ganry, ed. CIRAD/INIBAP, Montpellier, France.
14. Mack, D. H., and Sninsky, J. J. 1988. A sensitive method for the identification of uncharacterized viruses related to known virus groups: Hepadnavirus model system. Proc. Natl. Acad. Sci. USA 85:6977-6981.
15. Medberry, S. L., Lockhart, B. E. L., and Olszewski, N. E. 1990. Properties of *Commelina* yellow mottle virus's complete DNA sequence, genomic discontinuities and transcript suggest that it is a pararetrovirus. Nucleic Acids Res. 18:5505-5513.
16. Qu, R., Bhattacharyya, M., Laco, G. S., De Kochko, A., Subba Rao, B. L., Kaniewska, M. B., Elmer, J. S., Rochester, D. E., Smith, C. E., and Beachy, R. N. 1991. Characterization of the genome of rice tungro bacilliform virus: Comparison with *Commelina* yellow mottle virus and caulimoviruses. Virology 185:354-364.
17. Rybicki, E. P., and Hughes, F. L. 1990. Detection and typing of maize streak virus and other distantly related geminiviruses of grasses by polymerase chain reaction amplification of a conserved viral sequence. J. Gen. Virol. 71:2519-2526.
18. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.
19. Sambrook, J., Fritsch, E.F., and Maniatis, T. A. 1989. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. Smith, G. R., Clarke, M. L., Van de Velde, R., and Dale, J. L. 1994. Chemiluminescent detection of Fiji disease virus with biotinylated DNA probes. Arch. Virol. 136:325-334.
21. Smith, G. R., Van de Velde, R., and Dale, J. L. 1992. PCR amplification of a specific double-stranded RNA region of Fiji disease virus from diseased sugarcane. J. Virol. Meth. 39: 237-246.
22. Takahashi, Y., Tiongco, E. R., Cabauatan, P. Q., Koganezawa, H., Hibino, H., and Omura, T. 1993. Detection of rice tungro bacilliform virus by polymerase chain reaction for assessing mild infection of plants and viruliferous vector leafhoppers. Phytopathology 83:655-659.