Nucleotide Sequences of Two Circular Single-Stranded DNAs Associated with Banana Bunchy Top Virus

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ABSTRACT

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Two circular single-stranded DNAs designated DNA I and II of the banana bunchy top virus (BBTV) genome were cloned and sequenced. The sequence of BBTV DNA I consisted of 1,106 nucleotides and contained four open reading frames (ORFs) potentially encoding proteins with molecular masses ranging from 5.4 to 33.18 kDa and one putative stem-loop structure of 29 nucleotides. The sequence of BBTV DNA II had 1,096 nucleotides and contained four ORFs encoding putative proteins with sizes ranging from 5.6 to 32.77 kDa and one putative stem-loop structure of 32 nucleotides. The largest ORF (V2) in DNA II encoded a putative replicase with the nucleotide triphosphate-binding motif (GGEGKS). DNAs I and II had a 54.3% identity in total sequence and two conserved regions with more than 80% sequence identity. The two BBTV DNA sequences showed no close relationship with geminivirus DNA sequences but some similarity with the coconut foliar decay virus (CFDV) DNA sequence (about 39% identity). DNAs I and II showed considerable similarity with BBTV component 1, with 50.3 and 52.9% identity, respectively. The loop sequences of putative stem-loop structures in both BBTV DNAs were highly homologous and also had considerable similarity with those of geminivirus, CFDV DNAs, and BBTV component 1.

Banana bunchy top disease (BBTD) is the most important virus disease of banana (*Musa* spp.) in the Eastern Hemisphere (21). Recently the disease was reported for the first time in China (12), Hawaii (14), and Pakistan (13).

BBTD was considered to be caused by a possible member of the luteovirus group, based on disease characteristics, transmission in a persistent manner by aphids, and induction of phloem damage in infected plants (16). Purification of banana bunchy top virus (BBTV) had not succeeded until 1987, when liquid nitrogen was used to freeze the diseased tissues prior to pulverization and solvent extraction, and the extract was subjected to overnight stirring at a low temperature followed by low/high-speed centrifugation (25). This procedure was successfully applied to an isolate of BBTV from diseased banana in Australia (11,22) and Pakistan (13). The available reports demonstrate that BBTV is an isometric particle with a diameter of 18–20 nm. However, the reported nucleic acids associated with BBTV differ from each other and include single-stranded (ss) RNA (25), ssDNA and ssRNA (22), and ssDNA (10). Recently two circular single-stranded DNAs (cssDNA), each about 1.1 kb, were associated with BBTV, and the partial nucleotide sequences of these two cssDNA, designated BBTV cssDNA I and BBTV cssDNA II, were determined (24).

Previously reported plant viruses with cssDNA consist of the geminiviruses (1,7,9), coconut foliar decay virus (CFDV) (18), and subterranean clover stunt virus (SCSV) (3,4). The geminiviruses have been classified into three subgroups based on host range, insect vector, and number of DNA components (6). The first subgroup contains viruses with one DNA component that infect monocotyledonous plants via leafhopper transmission. The second subgroup consists of viruses with bipartite genomes that infect dicotyledonous plants via whitefly transmission. The third subgroup has characteristics of the other two groups, including monopartite genomes, leafhopper transmission, and infection of dicotyledonous plants. BBTV is disseminated by aphids among *Musa* species that are monocotyledonous plants and is composed of at least two DNA components (24). Although, the size of BBTV particles resembles the half-geminate particle of geminiviruses, the size of BBTV DNA (1.1 kb) is smaller than that of geminiviruses (2.7 kb). CFDV contains one 1.29-kb cssDNA (18), whereas SCSV has several ~0.85- 0.88-kb cssDNAs (3–5). Recently, one DNA component of the BBTV genome (BBTV-C1) was cloned and sequenced (11). This component is a cssDNA and contains 1,111 nucleotides with one large open reading frame (ORF) encoding a putative replicase.

In this paper, we report the complete nucleotide sequence of the two cssDNAs and compare both of them with cssDNAs of other plant viruses.

MATERIALS AND METHODS

Virus isolation and DNA extraction. Banana bunchy top virus was isolated from infected banana tissues collected from banana plantations in southern Taiwan following the methods described by Wu and Su (25). Encapsidated DNA was isolated from purified BBTV by methods described previously (24). The total DNA of banana tissues was isolated following the modified procedures of Draper and Scott (8). One gram of BBTV-infected or healthy banana tissue was ground to a powder after dipping in liquid nitrogen and was mixed with 15 ml of extraction buffer (0.1 M Tris-HCl, 0.5 M NaCl, 0.05 M EDTA, and 0.01 M 2-mercaptoethanol, pH 8.0) and 2 ml of 10% sodium dodecyl sulfate (SDS). The mixture was incubated at 65 C for 12 min before the addition of 5 ml of 5 M potassium acetate, incubated in ice for 30 min, and clarified by centrifugation at 12,000 rpm for 30 min. The nucleic acid was precipitated from the supernatant with isopropanol and resuspended in Tris-EDTA buffer (50 mM Tris-HCl and 10 mM EDTA, pH 8.0). DNA was further purified by digesting the suspension with RNase A (15 μg/ml) and proteinase K (140 μg/ml) at 37 C for 90 min. The mixture was extracted with phenol-chloroform and chloroform, precipitated with ethanol, and resuspended in 50 μl of sterile distilled water.

Polymerase chain reaction (PCR) and DNA cloning of PCR products. Complementary DNAs of BBTV were synthesized,

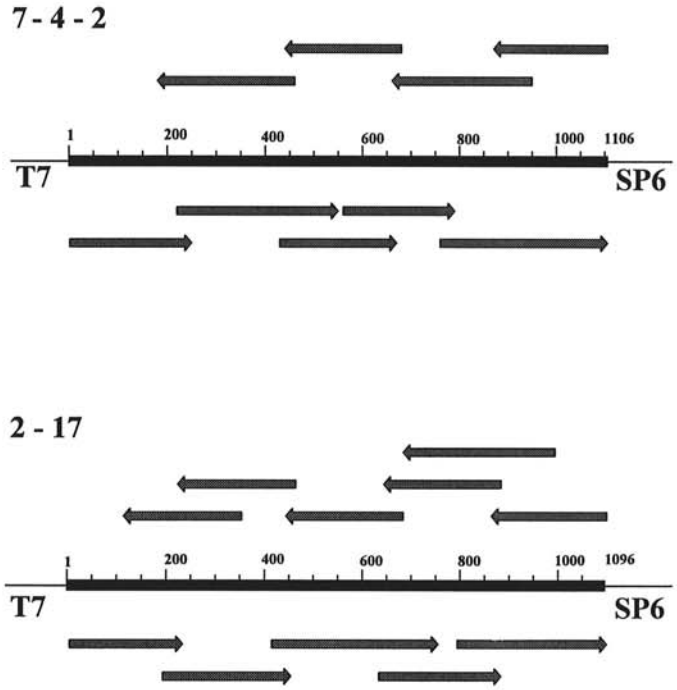

Fig. 1. Sequencing strategy of banana bunchy top virus (BBTV) DNA I (clone 7-4-2) and DNA II (clone 2-17). Thicker black line represents the BBTV DNA, and thinner black line represents the plasmid vector. The direction and extent of sequencing are indicated by arrows.

digested with mung bean nuclease, and ligated into pGEM 7zf(+). The recombinant plasmids were transformed into *Escherichia coli* strain JM109. After the nucleotide sequences of BBTV cDNA clones were determined (24), two pairs of PCR primers were designed. PCR products were obtained using encapsidated DNA of BBTV as template and two pairs of primers each in opposite directions designed according to the partial nucleotide sequence of the two BBTV cssDNAs (24). The BB-1, BB-3 pair consisted of CATGGTCTATCGAGGCAAG and GCAGATTCAATTGACGGA, respectively, and the 2-2, 2-3 pair consisted of ACCACCGGAGTACCCAGTTC and TCCTGGTTCGAAGAAGCGCA, respectively.

PCR was performed under the same conditions as those previously described (24). The products were purified by agarose gel electrophoresis and eluted from the gel using GENECLEAN II (Bio 101, Inc., La Jolla, CA). Purified DNA fragments were treated further with mung bean nuclease (Boehringer Mannheim Biochemicals, Indianapolis, IN) and ligated into plasmid pGEM 7zf(−) (Promega, Madison, WI), which was blunt-ended by digestion with *Sma*I. The recombinant plasmid was transformed into cells of *E. coli* strain DH5α, and the potential recombinant clones were identified by screening on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) substrate (15,23). BBTV clones were confirmed by PCR (17) with the BB-1, BB-3 primer pair for BBTV cssDNA I and the 2-2, 2-3 primer pair for BBTV cssDNA II (24) and by Southern hybridization (20) with digoxigenin (dig)-labeled BBTV-DNA probe prepared following the methods described previously (24).

Sequence analysis. Base sequences of BBTV-DNA clones were determined by the dideoxynucleotide chain termination method

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1     TATATAAACC GAGGTGGCTT AGTATTACCC ACCTCGGAAC ACTACCTCTG   50
51    AACGCCTGGA GATGTCCAGT CCCTCTCTTA AGTGGTGCTT CACTCTGAAT  100
101   TACTCCTCCG CGGCAGAGAG AGAAAACTTT CTCTCTCTTC TGAAGGAGGA  150
151   GGATGTTCAC TACGCTGTCG TCGGCGACGA AGTCGCTCCG GCCACCGGCC  200
201   AGAAGCACCT CCAGGGATAT CTATCCCTGA AAAAGAGAAT CCGCCTCGGC  250
251   GGATTGAAGA AGAAGTATGG TTCCCGTGCT CACTGGGAGA TTGCCAGAGG  300
301   AACGGACGAA GAGAATTCGA AGTACTGTTC AAAAGAAACC CTAATTCTCG  350
351   AATTAGGGTT TCCTGTTGTT AATGGTTCTA ATAAAAGGAA AATATCGGAG  400
401   ATGGTTGCTC GTTCTCCTGA TCGCATGAAA ATTGAACAGC CTGAGATATT  450
451   TCACAGATAT CAATCTGTGA ATAAGTTAAA AAAATTCAAG GAGGAGTTCG  500
501   TTCATCCTTG CCTCGATAGA CCATGGCAGA TTCAATTGAC GGAGGCAATT  550
551   GACGAGGAAC CCGATGATCG AAGCATCATC TGGGTCTATG GTCCTTATGG  600
601   TAATGAGGGT AAATCAACAT ATGCGAAGTC ACTAATCAAG AAGGATTGGT  650
651   TCTACACCAG GGGTGGGAAG AAGGAGAATA TCTTATTCTC CTATGTGGAC  700
701   GAAGGATCTG ACAAGCATAT AGTATTTGAT ATTCCTCGTT GTAATCAGGA  750
751   TTATTTAAAT TATGATGTAA TAGAGGCATT AAAGGATAGG GTTATAGAGA  800
801   GTACTAAATA CAAACCCATA AAGATAGTTG AATTAGGTAA AATACATGTA  850
851   ATCGTCATGG CGAATTTCAT GCCTGACTTC TGTAAAATCT CCGAAGATCG  900
901   AATAAAAATC ATTTATTGCT GAAGAACACT CTATCACGGG GACACGCTAT  950
951   GACAATCGTA CGCTAAAAAT CATTATAATT AATATTTGAA TTATGGGCCG 1000
1001  CAGGCCCATT AAGGATGTTC CGGCCCATTA ATACGGGCCT TCGGCCCGTT 1050
1051  ACGCTGAAGT TGCGCTGAAG CTTCCTTCGG AAGATACCTG GGCGACCTCT 1100
1101  GAACGC                                                1106
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Fig. 2. Nucleotide sequence of banana bunchy top virus (BBTV) DNA I (clone 7-4-2) containing 1,106 nucleotides represents a full-length BBTV-DNA synthesized by polymerase chain reaction. Arrows (← →) indicate the stem of the stem-loop structure. Underlines (_) indicate the starting position of the conserved regions. GENBANK accession L32166.

(19), using the United States Biochemical Corporation Sequenase Version 2.0 DNA Sequencing Kit (Cleveland). SP6 and T7 primers were purchased from Promega, and other synthetic oligonucleotide primers (16–22 nucleotides) were synthesized by Kwai Shin Company (Taiwan) using Cruachem DNA synthesizer PS 250. Comparative analysis of BBTV sequences was done with the MacMolly computer-software package (Soft Gene Gmbh, Berlin, Germany) and the PC/GENE sequence analysis package (Intelligenetics, Inc., Mountain View, CA)

Determination of the virion-sense strand. After the nucleotide sequences of the two DNAs from BBTV were determined and two conserved regions in BBTV-DNA were found, two oligonucleotides (C1: TGGTGCTTCACTCTGAATTACTCCTCCGCG; and C2, the complementary sequence of C1: CGCGGAGGAGTAATTCAGAGTGAAGCACCA) were designed and synthesized based on 30 nucleotides in the starting position of the conserved region (position 83-112 on DNA I). The two oligonucleotides were labeled by dig with the DNA 3′ end-labeling kit (Boehringer). Encapsidated DNA of BBTV, PCR products obtained using encapsidated DNA of BBTV as template and BB-1, BB-3 as primers, and total DNA from banana tissues were electrophoresed in 1.2% agarose and transferred to Hybond-N membrane (Promega) followed by Southern hybridization (20) with dig-labeled C1 or C2 probes. The hybridization was conducted at 68 C for 15 h in buffer containing 5× SSC (1× SSC is 0.15 M sodium chloride and 0.015 sodium citrate, pH 7.0), 1% blocking reagent (Boehringer), 1% SDS, and DNA probe.

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BBTV DNA II (Clone 2-17/ Clone 2)

2-17 TATATAAGGA GGAGCGGCTA GTATTACCCG CTCCTCCTCG CACTTCCTCC  50
2    ---------- ------C--- ---------- ---------- -CT-------

2-17 TCGCACCTGA CGTCATCATT ATGTCCTCTT TTAAATGGTG CTTCACTCTG  100
2    ----C----- ---------- ---------- ---------- ----------

2-17 AATTATTCCT CCGCAGCGGA GCGAGAAGAC TTTCTCGCTC TTCTGAAGGA  150
2    ---------- ---------- ---------- ---------- ----------

2-17 GGAGGATGTT CACTACTCTG TCGTCGGCGA CGAAGTCGCT CCGGCCACCG  200
2    ---A--GT-A A-T---G--- ---------- ---------- ---AG-----

2-17 GCCAGAAGCA CCTCCAGGGA TATCTATCCC TGAAAAAATC AATTCGCCTC  250
2    -T-G------ ---------- ---------- ----G----- T---AAG--T

2-17 GGCGGATTGA AAAAGAAGTA TGGCTCCCGT GCTCACTGGG AGATTGCGAA  300
2    --T------- -G----G--- CTCT--GAAG ---------- ---GG----G

2-17 AGGAAGTGAC GAACAGAATC GCAGATACTG TTCGAAGGAA ACCCTAGTTC  350
2    -----C---T ---------- ---------- ---------- ----*****-

2-17 TTGAACTGGG TACTCCGGTG GTTCCTGGTT CGAAGAAGCG CAAGCTTCTC  400
2    ---------- ---------- ---------- ---------- ----------

2-17 GATAGATTCA GAGAGAGCCC TGAGGAATTG AAGATGGACG ATCCATCCAA  450
2    ---------- ---------- ---A------ ---------- ----------

2-17 GTATCGCAGA TGCTTGGCAG TGGAATCAAT TAAAGATGCC AGAATTAATT  500
2    ---------- ---------- ---------- ---------- ----A-----

2-17 CCGAATGGGT TCACGAACTA AAAGAATGGC AAAATAAATT AATTCAACAC  550
2    ---------- ---------- ---------- ---------- ----------

2-17 ATCGAAGGTG TTCCTGATGA TCGAAGTATC ATCTGGGTAT ACGGTCCCAA  600
2    ---------- ---------- ---------- ---------- -------TGC

2-17 CGGAGGCGAA GGAAAGTCAA CCTTCGCAAG ATATCTATCA TTAAAACCCG  650
2    ---------- ---------- ---------- ---------- --------T-

2-17 GATGGGGATA TATCAACGGT GGAAAGACGT CGGATATGAT GCACATCATA  700
2    ---------- ---------- ---------- ---------- ----------

2-17 ACGATGGATC CTGATAATCA TTGGATTATT GATATCCCCA GAAGTCATTC  750
2    ---------- ---------- ---------- ---------- ----------

2-17 AGATTATCTG AATTATGGCG TTATAGAACA AATTAAGAAT AGAGTTTTAA  800
2    ---------- ---------- ---------- ---------- ----------

2-17 TAAATACAAA ATACGAACCA TGTGTGATTA GAAAAGATGG ACAAAATGTC  850
2    ---------- ---------- ---------- ---------- ----------

2-17 CATGTAATTG TTATGGCAAA TGTGTTGCCT GATTATTGTA AAATTTCAGA  900
2    ---------- ---------- ---------- ---------- ----------

2-17 AGATAGAATA AAAATAATTA ATTGTTGAGA AAGGAAACTT CCTCCGCAAG  950
2    ---------- ---------- ---------- ---------- ----------

2-17 CAATCAAAAA GCACGTGGAC CCCACACGGT AGCTTGCAGA ACACGCTATC  1000
2    ---------- ---------- ---------- ---------- ----------

2-17 ATTAAATGCA TCAGAAAATC ATTATAATTA ATAAATCTCT TATTGGGCCG  1050
2    ---------- ---------- ---------- ---------- ----------

2-17 CAGGCCCATG TAAGGCCCAT TACTTAATGG GCCGACCTCC TCGCAC      1096
2    ---------- ---------- ---------- ---------- -----C-
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Fig. 3. Nucleotide sequence of banana bunchy top virus (BBTV) DNA II. Clone 2-17, containing 1,096 nucleotides, represents a full-length BBTV-DNA synthesized by polymerase chain reaction. Clone 2, containing 1,091 nucleotide nucleotides, represents a possible partial clone of BBTV-DNA. Stars (★★★★★) indicate the five nucleotides absent in clone 2. Arrows (←→) indicate the stem of the stem-loop structure. Underlines (__) indicate the starting position of the conserved region. GENBANK accession L32167.

RESULTS

Five clones representing two cDNAs of BBTV were obtained. Nucleotide sequences of clones 7-4-2 and 2-17, representing the full-length clones of BBTV DNAs I and II, respectively, were derived from data obtained by the strategy shown in Figure 1. Clone 7-4-2 contained 1,106 nucleotides as shown in Figure 2. There were some nucleotide sequence differences (20 mismatches) between BBTV-cDNA clone 1, which contained 287 nucleotides (24), and PCR clone 7-4-2. Whether the variation indicated by the 8% sequence differences was due to the fidelity of PCR or caused by the DNA variation in the BBTV genome is unknown.

Clone 2-17 contained 1,096 nucleotides as shown in Figure 3. In comparison with BBTV-cDNA clone 2 (24), which consisted of 1,091 nucleotides (Fig. 3), PCR clone 2-17 consisted of five more nucleotides (from position 345 to 349 of clone 2-17) that

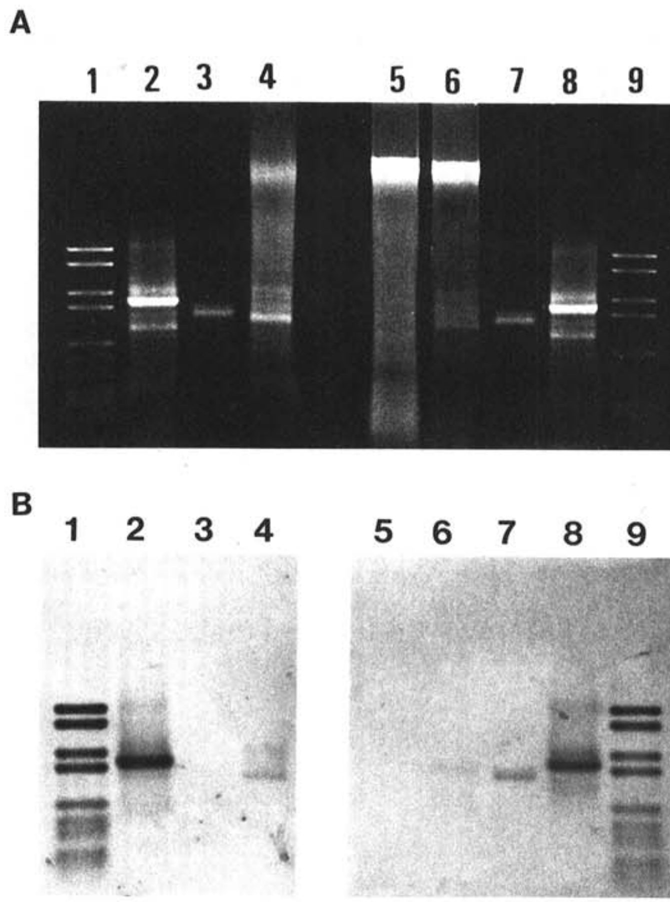
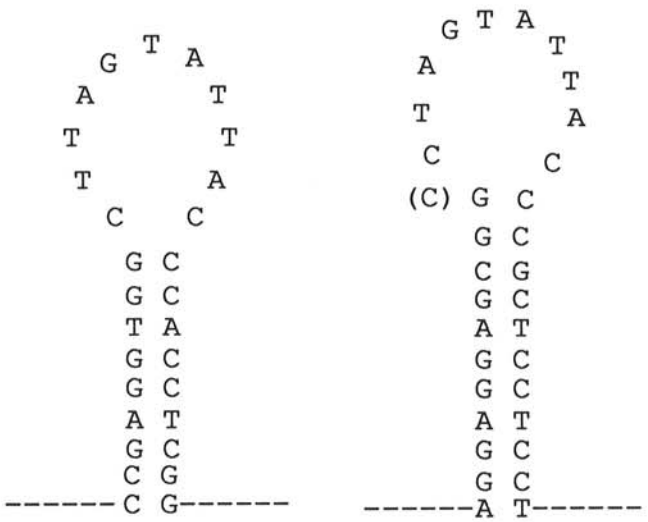


Fig. 4. Determination of sequence orientation of banana bunchy top virus (BBTV) single-stranded DNA: **A,** Encapsidated BBTV-DNA (lanes 3 and 7), total DNA extracted from BBTV-infected tissues (lanes 4 and 6), healthy tissues (lane 5), polymerase chain reaction products obtained from primer pair BB-1, BB-3 (lanes 2 and 8), and DNA size markers labeled with digoxigenin (lanes 1 and 9) separated on 1.2% agarose gel and visualized with ethidium bromide staining. **B,** After photographing, the DNAs were transferred to nylon membrane and hybridized with probe C1 (lanes 1–4) and probe C2 (lanes 5–9), respectively.

were absent in BBTV-cDNA clone 2. PCR clone 2-17 had many nucleotide sequence differences (4.5%) in comparison with BBTV-cDNA clone 2, especially between nucleotides 154 and 310 (with 21% difference). Another PCR clone (2-10) at this region (from 154 to 310 nucleotides) had only three nucleotide differences in comparison with clone 2-17 (data not shown). Based on the low mutation rate between two PCR products of clones 2-10 and 2-17 and the considerable difference (21%) between clones 2-17 and 2 in this region (from 154 to 310 nucleotides), the difference was not considered to be caused by infidelity of PCR. Clone 2 may represent another DNA of BBTV. Whether this is correct remains to be investigated.

To determine which sequence orientation of BBTV ssDNA was encapsidated within virions, BBTV-encapsidated DNA, total DNA from diseased and healthy banana tissues, and PCR products obtained from primer pair BB-1, BB-3 were hybridized with C1 and C2 probes, respectively. Results showed that the C2 probe hybridized encapsidated DNA, but the C1 probe did not, although the C1 probe hybridized the PCR product from the BB-1,BB-3 primer pair and total DNA from diseased banana tissues as the C2 probe did (Fig. 4). In addition, the C1 probe was not hybridized to a control extract from healthy plants. These results also confirmed that the sequence presented in Figures 2 and 3 was the orientation present within virions.

Both BBTV DNAs I and II were circular based on the results shown previously (24), and each had one putative stem-loop structure. In DNA I, the sequence CCGAGGTGG was inversely repeated (Fig. 2, ← →) and was predicted to form a stable stem structure (Fig. 5) with a loop sequence of CTTAGTATTAC. The stem sequence (AGGAGGAGCGG) in DNA II was different from that (CCGAGGTGG) in DNA I, but the two putative stem loop structures had very high homology with only one base difference in loop sequence (Fig. 5). The nucleotide 1 position was determined based on their putative stem-loop structures in both DNAs. The two BBTV DNAs had two conserved regions (CR) with more



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DNAI (clone 7-4-2):--CTTAGTATTAC--
DNAII (clone 2-17):---CTAGTATTAC--
          clone 2:--CCTAGTATTACC-
  GEMINIVIRUSES:----TAATATTAC--
          CFDV(+):----TAATACTAG--
              (-):---CTAGTATTA---
          BBTV-C1:----TATTATTAC--
```

Fig. 5. Organization of the stem-loop structure in banana bunchy top virus (BBTV) DNA I (left) and DNA II (right, including clones 2-17 and 2). The loop sequences of the BBTV DNAs were compared with those of geminivirus coconut foliar decay virus and BBTV-C1.

than 80% sequence identity. The first CR (CR-1), which consisted of 208 nucleotides, was located between positions 83 and 290 of BBTV DNA I (clone 7-4-2) and between positions 86 and 293 of BBTV DNA II (clone 2-17). In CR-1, the sequence identity between clone 7-4-2 and clone 2 was 82%, whereas that between clone 7-4-2 and clone 2-17 was 92%. The second CR (CR-2), which contained 78 nucleotides, was located between positions 845 to 922 of BBTV DNA I (clone 7-4-2) and between positions 851 to 928 of BBTV DNA II (clone 2-17). They had 82% sequence identity.

Putative ORFs were identified in both BBTV DNA I (clone 7-4-2) and DNA II (clone 2-17), in both virion- (V) and complementary-sense (C) strands. ORFs with the potential to encode proteins with a molecular mass greater than 5 kDa in BBTV DNAs I and II are shown in Figure 6A and Table 1 and Figure 6B and Table 2, respectively. In BBTV DNA I, there was one ORF in virion-sense (V1) and three in the complementary-sense

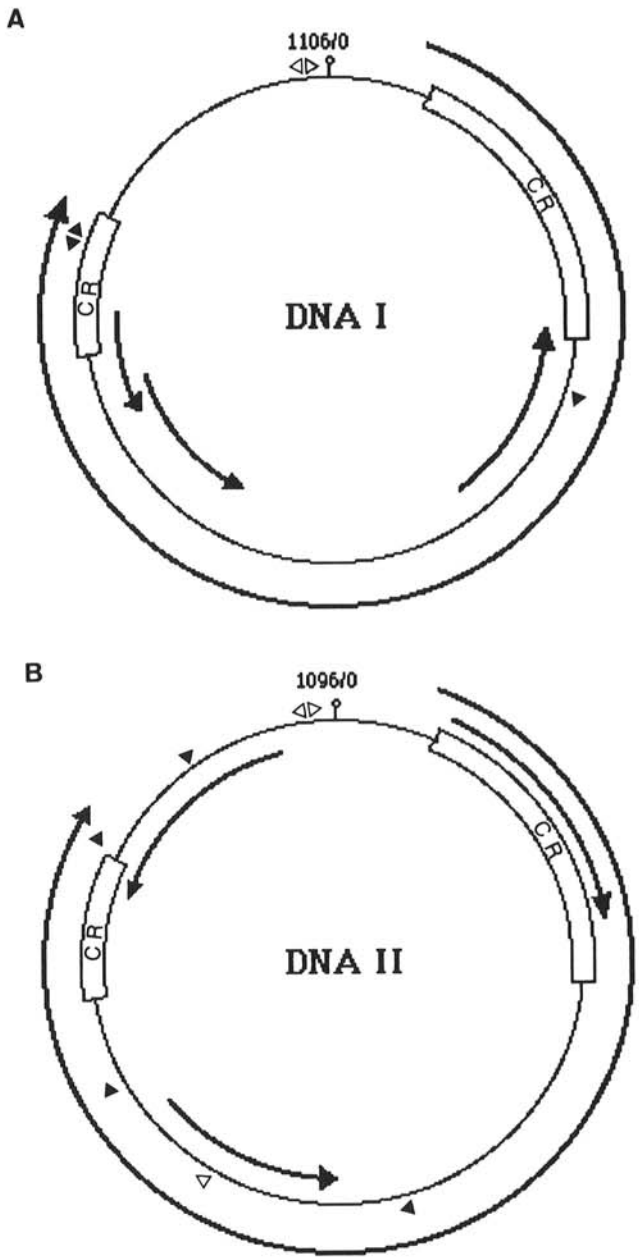

Fig. 6. Proposed genome organization of banana bunchy top virus (BBTV) **A,** DNA I and **B,** DNA II. Open reading frames on the virion-sense (clockwise) strand and complementary-sense (anticlockwise) strand are displayed by arrows. The positions of the stem-loop structures (○), conserved region (CR), potential TATA boxes (△), and poly (A) signals (▲) also are marked.

(C1, C2, and C3) encoding putative viral proteins with sizes ranging from 5.4 to 33.18 kDa. The largest ORF, V1, had 858 nucleotides (starting from 62 to 920) that coded for a 33.18-kDa protein (286 amino acids). The predicted amino acid sequence is shown in Figure 7A. One TATA box potential promoter element (TATATAA) was located at the 5′ side of the putative stem-loop structure (from 1 to 7 nucleotides) in BBTV DNA I. Two poly (A) signals (AATAAA) were present in positions 380–385 and 901–906 (Figs. 2 and 6A).

In BBTV DNA II, there were two ORFs in the virion-sense (V1 and V2) and two in the complementary-sense (C1 and C2) encoding putative viral proteins with sizes ranging from 5.6 to 32.77 kDa. The largest ORF, V2, had 855 nucleotides (starting from 71 to 926) that coded for a 32.77-kDa protein (285 amino acids). The predicted amino acid sequence is shown in Figure 7B. One TATA box potential promoter element (TATATAA) was located at the 5′ side of the putative stem-loop structure (from 1 to 7 nucleotides), and one closely related sequence element (GATATATC) was located at position 657–664. Four poly (A) addition signals (AATAAA) were present in positions 533–538, 799–804, 907–912, and 1,030–1,035 (Figs. 3 and 6B).

Alignment of these two sequences with published sequences

TABLE 1. Open reading frames (ORF) of banana bunchy top virus DNA I

ORF[a]	Nucleotide Start	Nucleotide Stop	Protein M_r[b]
V1	62	920	33,184
C1	779	638	5,733
C2	912	762	5,535
C3	426	282	5,407

[a]V and C indicate virion- and complementary-sense ORFs, respectively.
[b]Size of putative translation products.

TABLE 2. Open reading frames (ORF) of banana bunchy top virus DNA II

ORF[a]	Nucleotide Start	Nucleotide Stop	Protein M_r[b]
V1	85	307	8,357
V2	71	926	32,777
C1	1,059	885	6,550
C2	699	546	5,703

[a]V and C indicate virion- and complementary-sense ORFs, respectively.
[b]Size of putative translation products.

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A:      DNAI-V1 (286 AA)

  1  MSSPSLKWCF TLNYSSAAER ENFLSLLKEE DVHYAVVGDE VAPATGQKHL QGYLSLKKRI
 61  RLGGLKKKYG SRAHWEIARG TDEENSKYCS KETLILELGF PVVNGSNKRK ISEMVARSPD
121  RMKIEQPEIF HRYQSVNKLK KFKEEFVHPC LDSPWQIQLT EAIDEEPDDR SIIWVYGPYG
181  NEGKSTYAKS LIKKDWFYTR GGKKENILFS YVDEGSDKHI VFDIPRCNQD YLNYDVIEAL
241  KDRVIESTKY KPIKIVELGK IHVIVMANFM PDFCKISEDR IKIIYC

B:      DNAII-V2(D2)/BBTV-Component 1(C1)(285/286 AA)
     1
D2   MSSFKWCFTL NYSSAAERED FLALLKEEDV HYSVVGDEVA PATGQKHLGG YLSLKK..SI
C1   MARYVVCWMF TINNPTTLPV MRDEIKYKVY QVDRGQEGTR HVQGYVEMKR RSSLKQMRGF
     61
D2   RLGG.L.KKK YGSRAHWEIA K.GSDEQNRR YC...SKETL VLELGTPVVP GSKKRKLLDR
C1   FPGAHLEKRK .GSQEEARSY CMKEDTRIEG PEEFGS.FKL SCNDNLFDV. IQDMRETHKR
     121
D2   FRE.SPEELK MDDPSKYRRC LAV..ESIKD ARINSEWVHE LKEWQNKLIQ HIEGVPDDRS
C1   PLEYLYDCPN TFDRSKDTLY R.VQAEMNKT KAMNS.WRTS FSAWTSEVEN VMAQ.PCHRR
     181
D2   IIWVYGPNGG EGKSTFARYL SLKPGWGYIN GGKTSDMMHI ITMDPDNHWI IDIPRSHSDY
C1   IIWVYGPNGG EGKTTYAKHL MKTRNAFYSP GGKSLDICRL YNYE.DIV.I FDIPRCKEDY
     241
D2   LNYGVIEQIK NRVLINTKYE PCVIRKDGQN VHVIVMANVL PDYCKI.SED RIKIINC
C1   LNYGLLEEFK NGIIQSGKYE P.VL.KIVEY VEVIVMANFL PKEG.IFSED RIKLVSC
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Fig. 7. The predicted amino acid sequences of **A,** open reading frame (ORF)-V1 in DNA I and **B,** ORF-V2 in DNA II. The sequence of DNA II ORF-V2 (D2) was compared with that of banana bunchy top virus (BBTV)-C1 ORF-V1 (C1). Underlines (_) indicate the identical amino acid, and the box represents the putative NTP-binding motif.

of geminiviruses and CFDV DNA using the PC/GENE sequence analysis package (Intelligenetics) showed no close sequence relationship between DNA I or II and geminiviruses (<25% identity) but did show some similarity between BBTV DNA II and the reported sequences of CFDV DNA (about 39% identity). Alignment of DNAs I and II with BBTV-C1 showed considerable similarity between DNA I and BBTV-C1 (50.3% identity) and between DNA II and BBTV-C1 (52.9% identity). However, the conserved regions (CR-1 and CR-2) that appeared in DNAs I and II did not exist in BBTV-C1. In geminiviruses, the conserved region started at the base of the stem of the putative stem-loop structure, whereas in BBTV DNA the conserved region did not start from the stem of the putative stem-loop structure but from position 86. However, the nucleotide sequences of the loop in both BBTV DNAs (CTTAGTATTAC and CTAGTATTAC) do have some similarity with that of geminivirus (TAATATTAC), CFDV ((+)TAATACTAG/(−)CTAGTATTA), and BBTV-C1 (CTTATTATTAC).

The predicted amino acid sequences of ORF-V1 in DNA I and ORF-V2 in DNA II were compared with ORF-V1 in BBTV-C1. DNA II-V2 had some amino acids identical to BBTV-C1-V1 and consisted of an NTP-binding motif G(GE)GKS (starting at position 178) that is proposed to be associated with virus replicase (Fig. 7B).

DISCUSSION

The virions of BBTV consisted of at least two cssDNAs, designated BBTV ssDNAs I and II, of about 1.1 kb (24). In this study, BBTV DNAs I and II contained 1,106 and 1,096 nucleotides, respectively. The two BBTV DNAs had two conserved regions with more than 80% sequence identity and a putative stem-loop structure each. These stem-loop structures had a very high sequence homology. There were eight ORFs with the potential to encode proteins with molecular masses greater than 5 kDa in BBTV DNAs I and II, but the sizes of these proteins (Tables 1 and 2) were not close to that of the BBTV coat protein, which was estimated at 21 kDa by Wu and Su (25), 20.5 kDa by Thomas and Dietzgen (22), and 20.1 kDa by Harding et al (10). BBTV DNA I had an ORF (DNA I-V1) encoding a large 33.18-kDa putative viral protein. Its function needs to be further investigated. DNA II, however, had an ORF (DNA II-V2) encoding a putative replicase of 32.77 kDa with an NTP-binding motif (GGEGKS).

The sequences of BBTV DNAs I and II differed from that recently reported by Harding et al (11). Their BBTV DNA (component 1) contained 1,111 nucleotides with sequence identities of 50.3 and 52.9% to that of DNAs I and II here, respectively, and did not have a sequence region showing high homology with the two conserved regions (CR1 and CR2) of BBTV DNAs I and II. Its loop sequences (CTTATTATTAC) of the putative stem-loop structure also differed from that of BBTV DNAs I (CTTAGTATTAC) and II (CTAGTATTAC). Furthermore, the larger putative viral proteins encoded from these three different BBTV DNAs were different, although the DNA II-V2 also encoded a putative replicase with an NTP-binding motif as BBTV-C1-V1. Recently Burns et al (2) reported that the BBTV genome was composed of at least six cssDNA components. In our laboratory, we also have obtained six DNAs associated with the BBTV genome (*unpublished data*). Four of them have a conserved region and the sequence of high homology, while the other two are quite different from them and each other. All these DNAs probably represent different components of the viral genome. However, they also may represent the different viruses causing the bunchy top disease.

Among other plant viruses containing cssDNAs, only geminivirus (1,9) and CFDV (18) have had their viral DNA sequenced. BBTV DNAs are smaller than geminivirus DNAs (1.1 versus 2.6 kb). These two viruses also have little similarity in nucleotide sequence (<25% identity). However, both viruses contain genomic DNAs that each have a stem-loop structure with highly similar loop sequences and conserved regions in nucleotide sequence. BBTV DNAs seem to have a closer relationship with CFDV DNA in loop sequence and size (1.1 versus 1.3 kb). These two viruses have about 39% sequence identity. BBTV and SCSV also have similar genome structures (5). In SCSV, the genome contained three or four species of cssDNA each of about 850–880 nucleotides (5). It is unknown at this time whether CFDV, SCSV, and BBTV belong to the same new virus group.

ADDENDUM

After the submission of this paper, a paper appeared in which Yeh et al (26) reported a sequence of DNA of BBTV. Their BBTV-C2 seems to be related to DNA I of this report. Positions 1–934 and 1,039–1,106 of DNA I and positions 20–952 and 1,047–19 of BBTV-C2 have high homology. There were only 15 unmatched nucleotides among 1,002 nucleotides in these regions. DNA I contained additional three-nucleotide inserts at positions 241, 242, and 301, whereas BBTV-C2 contained additional two-nucleotide inserts at positions 63 and 400. However, DNA I contained a region at position 935–1,038 highly different from that of BBTV-C2 at position 953–1,046. DNA I and BBTV-C2 are, therefore, considered to be different DNAs associated with BBTV.

LITERATURE CITED

1. Abouzid, A. M., Polston, J. E., and Hiebert, E. 1992. The nucleotide sequence of tomato virus, a new geminivirus isolated from tomatoes in Florida. J. Gen. Virol. 73:3225-3229.
2. Burns, T. M., Harding, R. M., Hafner, G., Beetham, P., and Dale, J. L. 1993. Single-stranded DNA genome organization of banana bunchy top virus. 9th Int. Congr. Virol. Abstr. Page 98.
3. Chu, P. W. G., and Helm, K. 1988. Novel virus-like particles containing circular single-stranded DNAs associated with subterranean clover stunt disease. Virology 167:38-49.
4. Chu, P. W. G., Keese, P., Qiu, B. S., Waterhouse, P. M., and Gerlach, W. L. 1990. Novel ssDNA genome organization of a new plant virus. 8th Int. Congr. Virol. Abstr. Page 125.
5. Chu, P. W. G., Keese, P., Qiu, B. S., Waterhouse, P. M., and Gerlach, W. L. 1993. Putative full-length clones of the genomic DNA segments of subterranean clover stunt virus and identification of the segment coding for the viral coat protein. Virus Res. 27:161-171.
6. Davies, J. W., and Stanley, J. 1989. Geminivirus genes and vectors. Trends Genet. 5:77-81.
7. Donson, J., Accotto, G. P., Boulton, M., Mullineaux, P. M., and Davies, J. W. 1987. The nucleotide sequence of a geminivirus from *Digitaria sanguinalis*. Virology 161:160-169.
8. Draper, J., and Scott, R. 1988. The isolation of plant nucleic acid. Pages 199-236 in: Plant Genetic Transformation and Gene Expression—A Laboratory Manual. J. Draper, R. Scott, P. Armitage, and R. Walden, eds. Blackwell Scientific Publisher, Oxford.
9. Dry, I. B., Rigden, J. E., Krake, L. R., Mullineaux, P. M., and Rezaian, M. A. 1993. Nucleotide sequence and genome organization of tomato leaf curl geminivirus. J. Gen. Virol. 74:147-151.
10. Harding, R. M., Burns, T. M., and Dale, J. L. 1991. Virus-like particles associated with banana bunchy top disease contain small single-stranded DNA. J. Gen. Virol. 72:225-230.
11. Harding, R. M., Burns, T. M., Hafner, G., Dietzgen, R. G., and Dale, J. L. 1993. Nucleotide sequence of one component of banana bunchy top virus genome contains a putative replicase gene. J. Gen. Virol. 74:323-328.
12. Hu, X. L., and Sun, Z. H. 1990. Major diseases of banana in China. 3rd Int. Conf. Plant Prot. Tropics. Malaysian Plant Protection Society, Kuala Lumpur. Page 194.
13. Khalid, S., Soomro, M. H., and Stover, R. H. 1993. First report of banana bunchy top virus in Pakistan. Plant Dis. 77:101.
14. Lai, P. Y. 1990. Eradication of banana bunchy top disease in Hawaii. 3rd Int. Conf. Plant Prot. Tropics. Malaysian Plant Protection Society, Kuala Lumpur. Page 194.
15. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Matthew, R. E. F. 1982. Classification and nomenclature of viruses. Intervirology 17:1-200.
17. Mullis, K., and Faloona, F. A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. 155:335-350.
18. Rohde, W., Randles, J. W., Langridge, P., and Hanold, D. 1990.

Nucleotide sequence of a circular single-stranded DNA associated with coconut foliar decay virus. Virology 176:648-651.
19. Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.
20. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.
21. Stover, R. H. 1972. Banana, Plantain and Abaca Diseases. Commonwealth Mycological Institute, Kew, England.
22. Thomas, J. E., and Dietzgen, R. G. 1991. Purification, characterization and serological detection of virus-like particles associated with banana bunchy top disease in Australia. J. Gen. Virol. 72:217-224.
23. Vieira, J., and Messing, J. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259-268.
24. Wu, R. Y. Two circular single stranded DNAs associated with banana bunchy top virus. J. Phytopathol. In press.
25. Wu, R. Y., and Su, H. J. 1990. Purification and characterization of banana bunchy top virus. J. Phytopathol. 128:153-160.
26. Yeh, H. H., Su, H. J., and Chao, Y. C. 1994. Genome characterization and identification of viral-associated dsDNA component of banana bunchy top virus. Virology 198:645-652.