*Trans. Natl. Acad. Sci. Tech. Philippines 21: 287-291 (1999).*
*ISSN 0115-8848*

# MOLECULAR CLONING OF THE PHILIPPINE ISOLATE OF BANANA BUNCHY TOP VIRUS (BBTV)

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## ABSTRACT

Coding sequences of three single-stranded DNA (ssDNA) components from the Philippine isolate of banana bunchy top virus (BBTV) were cloned. These three viral genomic components encode a replicase protein, a coat protein and movement protein. A polymerase chain reaction (PCR) method was utilized to amplify specific ss DNA sequences using total nucleic acid extracts from banana leaf samples. Using plant transformation vector pBI121, constructs were made containing each of these three viral genes.

***Key words:*** Banana, Banana bunchy top virus (BBTV), molecular cloning, polymerase chain reaction (PCR)

## INTRODUCTION

Banana bunchy top disease, caused by banana bunchy top virus (BBTV), is the most devastating viral disease of bananas in many banana producing areas, including Asia, Africa and the South Pacific (Dale, 1987). The virus causes stunting and significant reduction in yield. Bananas infected at the early stage of growth do not produce fruit. BBTV, an 18-20 nm isometric virus, has a multicomponent genome of at least six circular single stranded DNA (Harding et al., 1991; Thomas and Dietzgen, 1991; Wu and Su, 1990).

Attempts to control BBTV include use of disease-free planting materials and eradication of infected plants. There is no known genetic resistance to BBTV

among cultivated genotypes of banana. Most commercially important cultivars are sterile and parthenogenetic and not amenable to traditional breeding strategies. The use of coat protein and other mutated viral genes for development of resistant transgenic plants can be applied to banana-BBTV system.

Components of BBTV genome have been cloned in Australia, Taiwan and Hawaii (Burns et al., 1995; Harding et al., 1993; Wu et al., 1994; Xie and Hu, 1995; Yeh et al., 1994). In this paper, we report the cloning of coding regions of three components of the Philippines BBTV isolate. These constructs will be used for banana plant transformation.

## MATERIALS AND METHODS

Primers were designed and synthesized based on published sequences of the coding regions of the three BBTV DNA components. (Burns et al., 1995). For the BBTV component 1 (BBTV-Rep), which encode the replicase protein, two oligonucleotide primers, RL and RR were designed. RL is located just downstream of the stem-loop region and upstream of the coding region. It extends from nucleotide 77 to 94 with seven extra nucleotides GGC GAA T added at the 5' end to produce an EcoRI site. RL is derived from the viral sense orientation. RR is located downstream of the coding region from nucleotide 1019 to 1002 in the complementary orientation.

RL: 5'-GGC GAA TTC TAT AAA TAG ACC TCC C-3'
RR: 5'-CGG AGC GTG CGC TGT AAA-3'

For the BBTV component 3 (BBTV-CP), which encode the coat protein, two oligonucleotide primers, CPL and CPR were designed. CPL is located upstream of the coding region. It extends from nucleotide 228 to 247 with eight extra nucleotides CAT CCGA CC added at the 5' end to produce an NcoI site. CPL is derived from the viral sense orientation. CPR is located downstream of the coding region from nucleotide 799 to 780 in the complementary orientation with nine extra nucleotides.

CPL: 5'-CAT CGA CCA TGG CTA CGT ATC CGA AGA A-3'
CPR: 5'-CTC TCC ATG GCG TGT TGT ATG TTA TTT GG-3'

For the BBTV component 4 (BBTV-MP), which encode the movement protein, two oligonucleotide primers, MPL and MPR were designed. MPL is located upstream of the coding region. It extends from nucleotide 279 to 298 with eight extra nucleotides CAT CGA CC added at the 5' end to produce an NcoI site. MPL is derived from the viral sense orientation. RR is located downstream of the coding region from nucleotide 640 to 621 in complementary orientation with ten extra nucleotides.

MPL: 5'-CAT CCG CCA TGG CAT TAA CAA CAG AGC G-3'
MPR: 5'-CTC TCC ATG GAC CGT GTA TTA GAA CAT AGG-3'

The above primers were used in PCR (polymerase chain reaction) to amplify coding sequences of the three BBTV components. Total nucleic acid extracted was used as templates. Samples of total nucleic acid were produced from Philippine isolate of BBTV-infected and healthy leaf lamina and midribs following procedures developed by Xie and Hu (1995). PCR was conducted under the following conditions: 94°C 5 min; 30 cycles of 94°C 1 min, 58°C 1 min, 72°C 3 min; and a final 72°C 10 min.

For BBTV-Rep, the amplified product was treated with Klenow fragment, digested by EcoRI and then ligated into pBluescript ks. To make constructs containing the replicase gene in plant transformation vector pBI121, the PCR fragment in pBluescript ks was subcloned into pBI 525 and then into pBI121.

For BBTV-CP and BBTV-MP, the amplified products were treated with Klenow fragment, digested by NcoI and then ligated into NcoI linearised pBI525. to make constructs containing the desired genes, the gene fragment in pBI525 was digested with HindIII and EcoRI and the ligated into pBI121. Cloning and transformation of Escherichia coli employed standard procedures (Sambrook et al., 1989).

## RESULTS AND DISCUSSION

The result of typical amplification of BBTV components from extracts of infected plant is shown in Fig. 1 (BBTV component 1); Fig. 2 (BBTV component

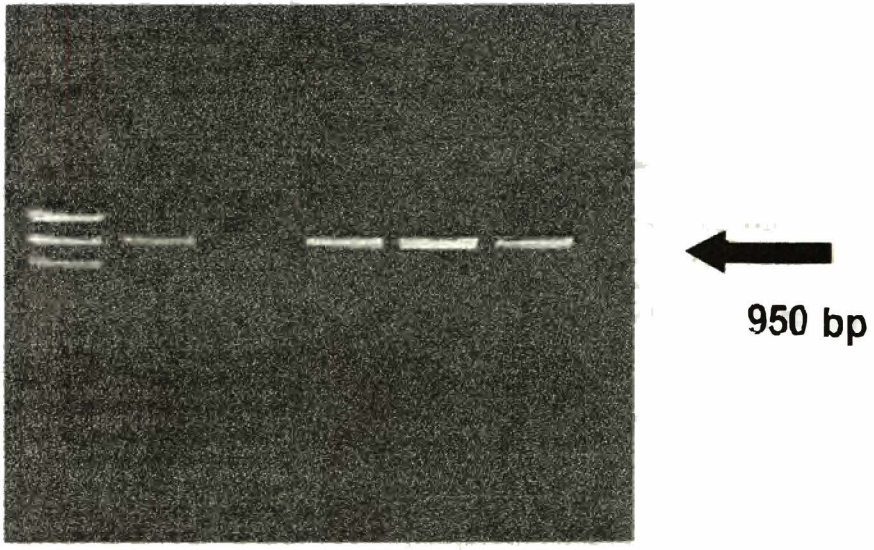


Figure 1. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer RL and RR. Lane 1: DNA size marker Lane 3: healthy control. Lane 4-6: DNA extracted from BBTV-infected leaf.

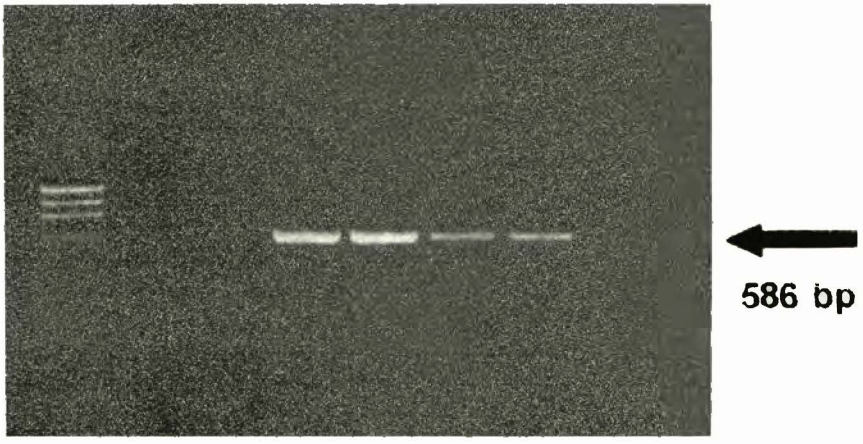


Figure 2. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer CPL and CPR. Lane 1: DNA size marker Lane 3: healthy control, Lane 4-7: DNA extracted from BBTV-infected leaf.

3); Fig. 3 (BBTV component 4). A band of approximately 950 bp was amplified using RL and RR primers, 586 bp for CPL and CPR primers and 380 bp for MPL and MPR primers. Bands were amplified in all reactions containing target DNA, primers and enzyme. No specific product was produced in the absence of either primers or target DNA, nor from the reactions which contained total nucleic acid extracted from healthy leaf.

The PCR products were cloned and transformants were analyzed by mini-alkaline lysis method. Inserts from potential recombinants were excised from the purified plasmids. The sizes of DNA inserts for the three BBTV components were obtained as expected. A total of six clones for BBTV-Rep, five for BBTV-CP, and three for BBTV-MP were chosen for further analysis and characterization. These gene constructs will be utilized for banana plant transformation work.

In this paper, the cloning experiments relied on primers designed based on published sequences of BBTV. PCR was shown to be a useful method in producing BBTV clones form infected tissue samples. The PCR method can be used for small circular DNA viruses provided that some sequence data is available.

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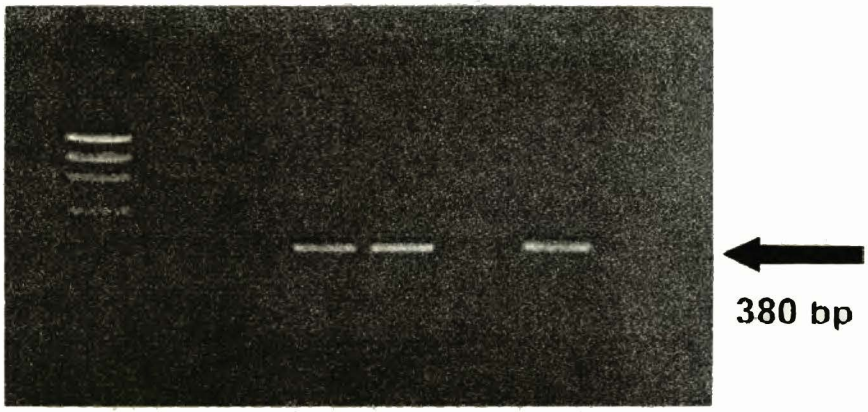


Figure 3. Agarose gel electrophoresis of PCR products derived from banana leaf extracts using primer MPL and MPR. Lane 1: DNA size marker Lane 3: healthy control, Lane 4-7: DNA extracted from BBTV-infected leaf.

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