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EFFECTIVE DETECTION OF BANANA BUNCHY TOP NANOVIRUS USING DNA PROBES

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ABSTRACT

According to DNA sequences of components one and four of BBTV isolate, that were isolated from AGERI cultivated area, Egypt, two pairs of primers were designed to amplify BBTV replicase (BBTV-*rep*) and movement protein (BBTV-*mv*) genes. The amplified fragments were cloned into pGEM-T easy vector and two new plasmids were created, i.e., pBBTV-Rep and BBTV-MV and transformed into *Escherichia coli*. Results showed that PCR products with a size of about 1.1 Kbp, which represented BBTV-*rep* and BBTV-*mv,* were successfully amplified. In case of BBTV-*mv*, a number of 17 clones were subjected to minipreparation followed by restriction endonuclease analysis. Results showed that 9 (1-5, 12-14 and 16) out of the 17 clones were found to be recombinant and 1.1 Kbp fragments were released. For Southern blotting analysis, the previous gel was southern blotted onto a membrane and the same 9 clones gave signals when the membrane was hybridized with ^{32}P-labeled-PCR probe of BBTV-*mv* amplified from DNA of BBTV-infected plants. On the other hand, similar probe was prepared from pBBTV-MV plasmid and used for virus detection in BBTV-infected plants. On the other hand, a PCR product amplified from the pBBTV-Rep plasmid was labeled with ^{32}P and used as a probe for detection of BBTV in virus-infected plants as well as the healthy as control. Findings of this study could be concluded in developing a rapid, effective and sensitive detection of BBTV in nucleic acids of either PCR amplified from BBTV-infected plants or ^{32}P-DNA-PCR labeled probe *via* Southern blot hybridization

INTRODUCTION

Banana bunchy top disease (BBTD) caused by banana bunchy top virus (BBTV), which is a major constraint to banana crop (Sun *et al*., 2001; Mansoor *et al*., 2005). BBTD is a disastrous disease in bananas, and spreading all over the world. Virus-free plantlets are used in banana production to prevent BBTD; therefore, it is very important to establish specific, quick and sensitive method to detect BBTV (Sun *et al*., 2001).

Early detection of plant viruses is the main way to control virus-induced diseases, accordingly, sensitive detection systems are essential. Serological methods based on the protein components of viral particles have been commonly used in plant virus detection (Clark *et al*., 1986, Van Regenmortel and Dubs, 1993), nucleic acid hybridization has proved to be a very reliable and sensitive technique in plant virus disease diagnosis (Matthews, 1991 and Hull and Al-Hakim, 1993).

Hsu *et al*. (2000) has used a sensitive method to detect bamboo mosaic virus (BaMV) and its associated satellite RNA (satBaMV) by ^{32}P- and digoxigenin (Dig)-

labelled probes synthesized from cDNA clones of BaMV genomic (L probe) and satBaMV (S probe) RNA. Both the ^{32}P- and Dig-labelled L and S probes could detect as little as 490 pg of BaMV viral RNA by slot and dot-blot hybridization. In infected leaf extracts, ^{32}P-labelled L and S probes detected virus at 25-fold higher dilutions than Dig-labelled probes, which were also successfully used to detect BaMV infection in plants derived from meristem-tip culture.

Recently, many methods have become available for virus detection at an early stage of infection either in meristems or in plants. In addition to the immunological assays (Lommel *et al*., 1982; Lin *et al*., 1990), nucleic acid hybridization and reverse transcriptase (RT-PCR) are effective methods for virus detection (Seal and Coater, 1998 James *et al*., 1999). Karan *et al*., (1997) demonstrated using of either component specific PCR primers or BBTV DNA-2, 3, 4, 5 and 6 as probes that were present in all isolates tested from seven countries.

In this study, DNA probes of BBTV-DNA-1 and BBTV-DNA-4 labeled with ^{32}P were used for virus detection as a sensitive method for BBTV detection in either recombinant clones or BBTV-infected plants.

MATERIALS AND METHODS

DNA extraction: DNA was extracted from BBTV-infected banana plants cv. Maghrabi according to the method of Harding *et al*., (2000).

PCR amplification: The purified DNA was used as template for PCR amplification of BBTV-DNA-1 and BBTV-DNA-4 components by using two specific primers pairs as shown in Table-1 according to the nucleotide sequences and PCR programs of Harding *et al.,* (1993) and Burns *et al*., (1995) respectively.

Table-1: Primer pairs used for PCR amplification of two DNA probes belonging to BBTV-DNA-1 and BBTV-DNA-4 components

Primers	Nucleotide sequences (5'---------- 3')	Size (nt)
DNA-1		
R-1	CTG CGC CAT GAT ATT CTC CAC CTC T	25
F-1	CCA TGT CAT CGG AGA ATA ATT TGG G	25
DNA-4		
R-4	ACC TGA GAT ACA TGTGGT ATG ACA G	25
F-4	AAT CCC TTC TCA ACC AAA TAG AAG G	25

PCR purification, cloning and bacterial transformation: A High Pure PCR Purification kit (Roche) was used for purification of the PCR products, then ligated into pGEM-T easy vector (Promega) using T4 DNA ligase (Roche) and transformed into *E. coli* JM109 based on the method of Harding *et al*., (1993).

Probe labeling: Based on the method of Harding *et al.,* (1991), an oligo-labelling kit (Bresatec) was used for preparing DNA insert probe-labeled with α ^{32}P-dCTP.

Southern blotting and hybridization: On electrophoresis, DNA was blotted onto Hybond-N probe membranes according to the method given by Harding *et al*., (1991) using 10 x SSC as a transfer buffer (Sambrook *et al*., 1989).

All membranes were hybridized with ^{32}P-labeled probe(s). The membranes were then exposed to X-ray film to develop an autoradiographic image post 1 hour. The DNAs from healthy were used as a control.

RESULTS AND DISCUSSION

ELISA is now popularly used to detect BBTV (Sun *el al.*, 2001). The sensitivity of this method is not sufficient, and pseudo-positive results often occur. Burns *et al*., (1995) identified a short stretch of hydrophobic residues in the N terminus of

the predicted gene product of BBTV DNA-4 and suggested that it may be a viral movement protein (MP). Similar hydrophobic regions have been identified in the predicted gene products of faba bean necrotic yellow virus (FBNYV) component 4 (C4) and sugarcane streak virus (SCSV) component 1 (C1) (Boulton *et al*., 1993 and Katul *et al*., 1997).

In this study, according to DNA sequences of components one and four of BBTV isolate, that were isolated from AGERI cultivated area, Egypt, two pairs of primers were designed to amplify BBTV replicase (BBTV-*rep*) and movement protein (BBTV-*mv*) genes. Results in Figure-1 showed that a PCR product with a size of about 1.1 Kbp belonging to BBTV-DNA-4 component was amplified. Nega-tive controls which represent PCR of DNA extracts of healthy banana plants showed that no PCR fragments were amplified. The amplified fragments were cloned into pGEM-T easy vector and two new plasmids were created, i.e., pBBTV-Rep and BBTV-MV (Figure 2) and then transformed into *E. coli*.

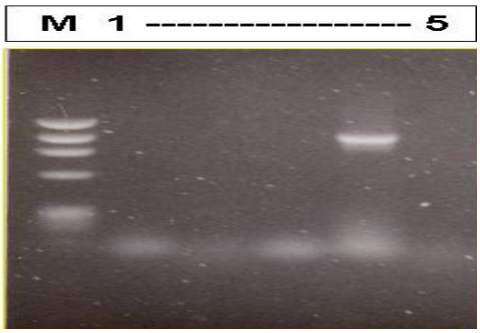

Figure-1: Agarose gel (1.2%) stained with ethidum bromide shows PCR isolation of BBTV-*mv* gene. A PCR product with a size of about 1.1 Kbp was amplified (Lane 4). Lane 1, negative control; Lanes 2 & 3 and 4 & 5 represent PCR of DNA extracts of healthy and BBTV-infected banana plants, respectively. M: DNA marker.

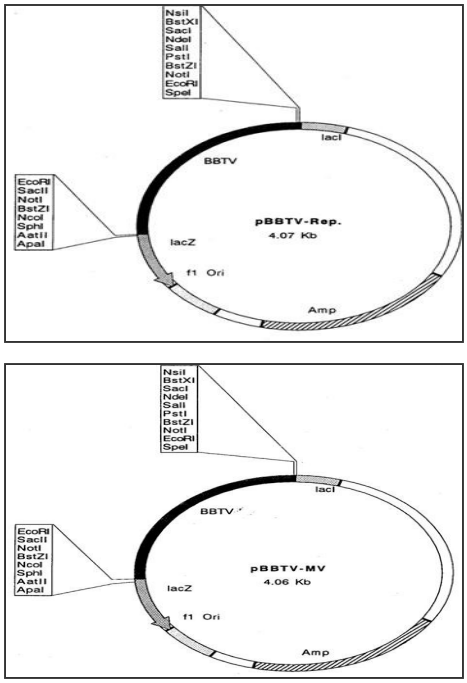

Figure-2. Construction of BBTV-rep and BBTV-*mv* genes into pGEMT-easy vector creating two new plasmids called pBBTV-Rep and pBBTV-MV.

In case of BBTV-*mv*, a number of 17 clones of *E. coli* were subjected to minipreparation followed by restriction endonuclease analysis. Results in Figure-3 showed that 9 (1-5, 12-14 and 16) out of the 17 clones were found to be recombinant and 1.1 Kbp fragments were released. The same gel was Southern blotted onto a membrane and results showed that the same 9 clones gave signals when the membrane was hybridized with ^{32}P-labeled-PCR probe of BBTV-*mv* amplified from DNA of BBTV-infected plants Figure-4.

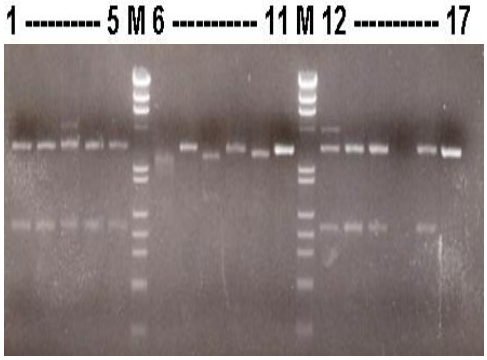

Figure-3: Upper: Agarose gel (1.2%) stained with ethidum bromide shows restriction endonuclase analysis of DNA plasmids of 17 recombinant clones of *E. coli*. Fragments with a size of about 1.1 Kbp were released from 9 recombinant clones (1-5, 12-14 & 16).

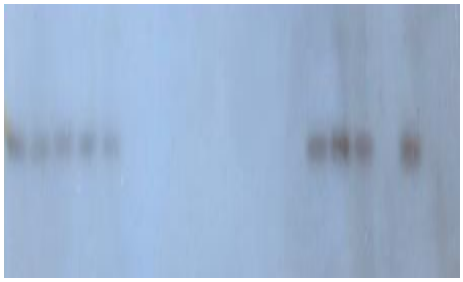

Figure-4: Southern blot analysis of 17 recombinant clones of *E. coli* on restriction endonuclase analysis. Note, 9 clones gave signals when the membrane was hybridized with ^{32}P-labeled-PCR probe of BBTV-*mv* amplified from DNA of BBTV-infected plants

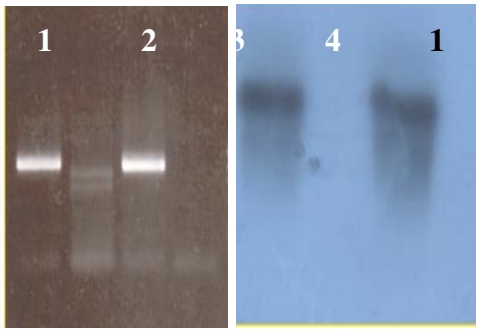

Figure-5: Upper: Agarose gel (1.2%) stained with ethidum bromide shows PCR fragments a size of about 1.1 Kbp of BBTV-*mv* gene amplified from BBTV-infected plants (Lanes 1 & 3) and healthy plant (Lane 2). Lane 4, negative control. Lower: Southern blot analysis of the upper gel using a ^{32}P-labeled-PCR probe of BBTV-*mv* amplified from pBBTV-MV plasmid.

In addition, a PCR product belonging to BBTV-*rep* gene was amplified from BBTV-infected plant and labeled with ^{32}P and used as a probe for detection of BBTV. Result in Figure-6 showed that restriction endonuclease analysis of DNA plasmid (pBBTV-Rep) of some clones of *E. coli*. Fragments with a size of about 1.1 kbp were released. On Southern blotting hybridization with the prepared probe, a strong autoradiographic image with 4 recombinant clones as well as the positive control (pBBTV-MV plasmid) was observed. Similar results were obtained by Harding *et al*. (2000).

As a conclusion, a sensitive detection of BBTV in nucleic acids of banana plants either by PCR using specific primers or by Southern blot analysis. The detection method applied in this investigation could be used as a rapid and effective method for early detection of BBTV and consider as an important step in virus control.

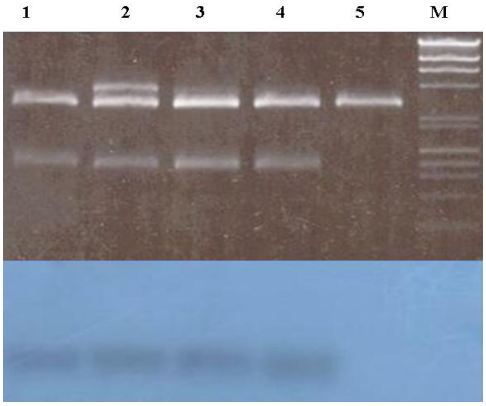

Figure-6: Upper: Agarose gel (1.2%) stained with ethidum bromide shows restriction endonuclase analysis of DNA plasmids of 4 recombinant clones of *E. coli* (Lanes 1-4). Lane 5: undigested pBBTV-Rep plasmid. Fragments with a size of about 1.1 Kbp were released. Lower: Southern blot analysis of the upper gel using a ^{32}P-DNA-PCR labeled probe amplified from BBTV-infected plants

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