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# Serological and Sero-molecular Studies on Banana Bunchy Top Disease and in Different Parts of Virus-infected Banana Plant

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## ABSTRACT

Banana bunchy top disease (BBTD) caused by banana bunchy top nanovirus (BBTV), is the most economically important virus disease affecting banana crop. Two different methods, i.e., indirect-enzyme linked immunosorbent assay (I-ELISA) and immunocapture-polymerase chain reaction (IC-PCR) were used for studying the presence of BBTV in virus-infected and symptomless banana plants. In addition, the presence of such virus in different parts of a banana plant was also studied. IC-PCR technology was found to be more sensitive than I-ELISA for BBTV detection. Results showed that some samples which gave negative ELISA values were positive *via* IC-PCR. It was also found that BBTV was detected in roots, midrib, corm and leaves. As a PCR fragment with a size of about 1056 bp was amplified. The use of the monoclonal antibodies specific to the Australian BBTV isolate confirmed the geographical relationship between the Egyptian BBTV isolate and that of Australia based on the sequencing analysis.

**Key Words:** BBTD; I-ELISA; IC-PCR; Detection; Roots; Stem; Pseudostem; Leaves

## INTRODUCTION

Banana bunchy top disease (BBTD) is considered the most serious disease in banana (*Musa spp.*) worldwide (El-Afifi, 1984; Dale, 1987; Khalid *et al*., 1993 and Hu *et al*., 1993). The enzyme-linked immunosorbent assay (ELISA) was found to be effective for BBTV detection in field-infected bananas in Australia (Dietzgen & Thomas, 1991), Pakistan (Khalid *et al.,* 1993) and Egypt (Othman *et al*., 1996). The virus was successfully detected in BBTV-infected and symptomless banana plants.

The polymerase chain reaction (PCR) technique was reported as a useful method for BBTV detection in the total nucleic acid extracts from healthy and BBTV-infected banana plants (Harding *et al.,* 2000). Wetzel *et al.* (1992) reported that the immunocapture-PCR (IC-PCR) technique is a combination of ELISA and PCR techniques that allows for the detection of low concentrations of viruses in oxidizing plant extracts. Shamloul *et al*. (1995) utilized three pairs of primers for immunocapture-PCR (IC-PCR) and PCR for detection of BBTV in BBTV-infected banana leaves or tissue culture or viruliferous aphids (*Pentalonia nigronervosa*).

Symptomless infected plants may cause serious problems to banana growers, as they could act as a good source for spreading the virus *via* banana aphid (*P. nigronervosa*). Therefore, detection of BBTV in symptomless bananas is considered the ultimate goal of this study using monoclonal antibodies specific to the Australian BBTV isolate *via* I-ELISA and IC-PCR.

## MATERIALS AND METHODS

**Source of samples.** Nine samples of the cv. Maghraby banana plants classified as 7, 1 and 1 symptomless, virus-infected and healthy samples, respectively, and previously subjected to PCR detection by Soweha (2003, unpublished data) were used.

**Source of antiserum specific to BBTV.** Monoclonal antibodies (MAbs) specific to the Australian BBTV isolate produced by Prof. Dr. John Thomas, Department of Primary Industry (DPI), Queensland Univeristy, Brisbane, Australia and kindly provided by Dr. Atef S. Sadik, Laboratory of Virology, Faculty of Agriculture, Ain Shams Univeristy, Cairo, Egypt were used.

**I- ELISA detection.** According to the method of Devergne *et al.* (1981) the BBTV was detected in the applied samples using 0.1 g of midrib tissue. It is important to mention that a dilution of $10^{-1}$ of extracted sap was used. The ELISA values were determined after 20 min at $A_{405}$ nm using an ELISA reader. Sample which gave double folds of the ELISA values of the healthy (-ve control) was considered as a positive as recommended by Clark and Adams (1977) and Othman *et al.* (1996).

**IC-PCR detection.** In this experiment the method given by Shamloul *et al*. (1995) was carried out. A microtiter plate was coated with MAbs specific to BBTV and incubated overnight 4$^{o}$C and the plate was washed three times with PBS-Tween 20. The detected samples in addition to negative control (healthy sample) were added to the IgG-coated wells and left for 3 hrs at 37$^{o}$C then washed three times as mentioned above. To each well, 10 $\mu$l of 1 %

Triton X-100 preheated at 65°C for 5 min were added to each well, then the plate was vortexed for 1 min. The resulting mixture *ca* 12 $\mu$l was transferred to 500 $\mu$l IC-PCR tube to be used as a DNA template for virus amplification as reported earlier (Soweha, unpublished data).

**DNA electrophoresis.** The amplified products were analysed on a 1.2% agarose gel electrophoresis in Tris-Borate-EDTA (TBE) buffer and detected by staining with ethidium bromide (Sambrook *et al.*, 1989).

**BBTV detection in different parts.** The BBTV was detected in different parts, i.e., roots, midrib, corm and leaves of BBTV-infected plant *via* IC-PCR as mentioned above.

## RESULTS AND DISCUSSION

Serological (ELISA) (Clark & Adams, 1977 and Othman *et al*., 1996) and sero-molecular (IC-PCR) (Nolasco *et al.,* 1993; Martinez-Herrera *et al.,* 1994; Weston *et al.*, 1994) techniques have been developed to detect a considerable number of economically important plant viruses. For BBTV, based on the nucleotide sequence of BBTV-DNA-1 such technique was reoprted by Shamloul *et al*. (1995) using six oligonucleotides and Sadik *et al*. (1997) using two oligonucleotides. In addition Shamloul *et al*. (1995) showed that the PCR fragments (439, 446 & 476 bp) were hybridized with a cRNA probe of cloned BBTV-DNA in Southern blot and dot blot hybridizations.

Data in Table I showed that in the case of virus-infected samples, three techniques were equal in detection of BBTV. On the other hand, sample 5 that gave PCR positive and recorded as a negative sample (0.349) using the I-ELISA technique was found to be positive using the IC-PCR technique. This result proved that PCR and IC-PCR were more sensitive than ELISA in virus detection. Xie *et al*. (1994) also confirmed that PCR was 8000 more sensitive than ELISA in BBTV detection. In the case of IC-PCR, four (samples 1, 2, 4 & 5) out of the seven samples exhibited no external symptoms were found to be positive. This was due to the fact that a fragment with a size of 1056 bp was amplified from their infectious saps. Two out of the four samples, i.e., number 2 (0.350) and number 4 (0.348) which gave negative results with both ELISA and PCR techniques were positive *via* IC-PCR. This could be explained by immunocapturing the virions by the specific antibody-bound viral particles and releasing the viral genome, the template for PCR (Wetzel *et al.*, 1992).

Results in Table II showed that the IC-PCR technique was successfully confirmed the presence of BBTV in the all tested banana parts i.e., roots, midrib, corm and leaves. Thomas and Dietzgen (1991) detected the BBTV in midrib and leaf lamina tissues. Wu and Su (1992) reported that the distribution of BBTV in different parts of diseased plant in Taiwan using the ELISA test.They found that BBTV was detected in leaf, midrib and petiole but in the roots and rhizome. On the other hand, Sadik (1994) revealed confirmed the presence of BBTV in roots, midrib, corm, pseudostem leaves and meristem using a DNA probe labeled with $^{32}$P *via* Southern blot and dot blot hybridization techniques.

**Table I. Detection of BBTV in symptomless Maghraby banana samples *via* I-ELISA and IC-PCR techniques**

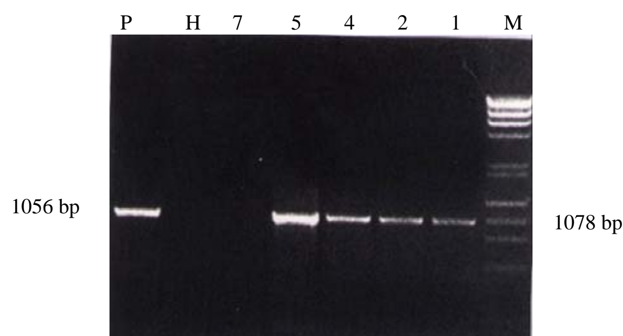
| Banana samples | PCR detection* | I-ELISA detection Value | I-ELISA detection Result | IC-PCR |
|---|---|---|---|---|
| Symptomless | | | | |
| 1 | + | 0.657 | + | + |
| 2 | - | 0.350 | - | + |
| 3 | - | 0.221 | - | - |
| 4 | - | 0.348 | - | + |
| 5 | + | 0.349 | - | + |
| 6 | - | 0.239 | - | - |
| 7 | - | 0.218 | - | - |
| Virus-infected (+ ve control) | + | 1.723 | + | + |
| Healthy (-ve control) | - | 0.176 | - | - |

*: As reported by Soweha (2003, unpublished data).

**Table II. Detection of BBTV in different parts of Maghraby banana plant infected with BBTD**

| Plant parts | IC-PCR detection of BBTV | | | |
|---|---|---|---|---|
| | Roots | Midrib | Corm | Leaves |
| | + | + | + | + |

**Fig. 1. Agarose gel (1.2%) in TBE buffer stained with ethidium bromide shows IC-PCR detection of BBTV in symptomless (1, 2, 4, 5 & 7) and virus-infected (P: positive control) Maghraby banana samples. A PCR fragment with a size of about 1056 bp was amplified. H: Healthy sample (negative control) and M: Lamda DNA/*Hind* III Marker (Promega).**

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