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Detection of Banana Bunchy Top Nanovirus using Polymerase Chain Reaction in Different Egyptian Banana Cultivars

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ABSTRACT

In this study, polymerase chain reaction (PCR) was used for detection of banana bunchy top nanovirus (BBTV) in different Egyptian banana cultivars, i.e., Baradica, Grand Nain and Maghraby. Using two specific primers designed based on the nucleotide sequence of BBTV-Eg-DNA-2.2 (component 2), a fragment with a size of about 1056 bp was amplified from the DNA extracts obtained from BBTV-infected banana plants. This fragment did not amplified from the DNA extracts of the healthy banana plants, which was used as a negative control. A concentration of 125 ng DNA was used as a template for PCR amplification. These results showed that the PCR technique is a suitable technology for detection of BBTV infection as an initial stage, where the virus titer in the plant was still low. This is due to the successful detection of the virus in the symptomsless banana plants.

Key Words: Banana; BBTV; Polymerase chain reaction; Detection

INTRODUCTION

A number of molecular methods, such as polymerase chain reaction (PCR), have become available for studying the plant viruses (Levy & Hadidi, 1991; Hu *et al*., 1993; Sadik *et al.*, 1997; Harding *et al.*, 2000) and other microorganisms (Abdel-Fattah *et al.*, 2003).

Banana bunchy top nanovirus (BBTV) is the most serious virus affecting banana in Egypt as well as the world over (Dale, 1987; Othman *et al*., 1996). BBTV particles are isometric (18-20nm). The virion consists of at least six components of circular ssDNA, each about 1kb (Burns *et al*., 1995), transmitted by *Pentalonia nigronervosa* (Hu *et al*., 1996), and a phloem-limited virus (Dale, 1987; Sadik *et al*., 1999). The virus used to occur in low concentrations in banana-infected plants (Harding *et al*., 1991; 2000). Therefore, the early detection of BBTV, in the symptomless young plants, plays an important role in its control (Harding *et al*., 2000).

For BBTV detection several methods were successfully used, such as double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) (Othman *et al.*, 1996) and dot-blot immunoassay (DBIA) (Greeing & Thomas, 1996). The PCR was successfully used for detecting several plant viruses (Wetzel *et al*., 1992, Hadidi *et al*., 1993) and BBTV (Xie *et al.*, 1994; Harding *et al.*, 2000). Thomas *et al*. (1995) recommended that BBTV be readily transmitted through tissue culture in banana cv. Ladyfinger and Cavendish cv. Williams. Therefore, this study was designed to use the PCR technology for detecting the BBTV in naturally symptomless and virus-infected banana plants of different Egyptian cultivars.

MATERIALS AND METHODS

Source of plant samples. BBTV-infected as well as symptomless banana samples, stored at –20°C, from three different banana cultivars, i.e., Baradica, Gand Nain and Maghraby were obtained from Laboratory of Virology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Extraction of total nucleic acids. The total nucleic acids (TNAs) were extracted from healthy or BBTV-infected banana midrib tissues based on the method of Harding *et al.* (2000). The DNAs were purified and their concentrations were determined as recommended by Abdel-Hamid *et al*. (2003). The DNA concentration was adjusted to 125 ng/μL and 1 μL was used as a template for PCR detection.

PCR detection. In this experiment, the PCR was conducted on a volume of 50 μL as described by Harding *et al.* (2000). Two specific primers named $\mathbf{P_1}$, 5'GGT CCC CTT TAA GAT TCC TTT CTT CGT CGC3' and P_2, 5'ACT CAA AAT ACA GCT GTC ATT GAA TTA TTA3', designed based on the nucleotide sequence of BBTV-DNA-Eg-2.2 (Sadik, 1994) were kindly provided by Dr. A.S. Sadik, Ain Shams University, Cairo, Egypt. The PCR amplification was performed in a Perkin-Elmer/DNA Thermal Cycler 480 (Norwalk, CT) for 35 cycles after initial denaturation for 4 min at 95°C. Each cycle consisted of denaturation at 94°C for 45 sec, annealing temperature at 54°C for 60 sec and extension at 72°C for 60 sec. The final primer extension cycle was extended to 10 min.

DNA electrophoresis. The amplified products were resolved by electrophoresis in a 1.2% agarose at 80 volt, for 1 h with TAE buffer (Sambrook *et al*., 1989). DNA was visualized by staining gel in ethidium bromide (0.5 μg/mL) and photographed under UV light.

RESULTS AND DISCUSSION

PCR is one of the most sensitive molecular methods for pathogen detection (Mullis & Faloona, 1987). In this technique, the process uses a series of hot, cold, and warm cycles. Hot cycles split DNA into single strands. Cold cycles allow the primers to attach. During warm cycles, the enzyme makes a copy of each piece of primed DNA. If a virus is present, PCR will produce a large quantity of certain piece of DNA.

The importance of PCR lies in its ability to amplify specific DNA from as short as 50 bp to over 10000 bp in length, more than a millionfold in a few hours (Wetzel *et al*., 1991). Several investigators described the development of PCR primer pairs for essentially all groups of viral pathogens (Wetzel *et al*., 1992; Hadidi *et al*., 1993; Podleckis *et al*., 1993; Shamloul *et al*., 1995; Sadik *et al*., 2001). In this study, a number of 63 samples of three different banana cultivars, i.e., Baradica, Grand Nain and Maghraby, 21 for each cultivar, were collected. Eight, nine and seven samples exhibiting no external symptoms were used with the three applied cultivars, respectively. The DNAs were extracted and 125 ng from each preparation were used as a template for PCR detection of BBTV using two specific primers belonging to the nucleotide sequence of BBTV-Eg-DNA-2.2 as described by Sadik (1994).

The BBTV was successfully detected *via* PCR in all the virus-infected samples represented 100% (Table I). On the other hand, 3, 3 and 2 samples represented 37.5, 33.33, and 28.57% from Baradica, Grand Nain and Maghraby banana cultivars, respectively, were found to be PCR positive. These results paid attention to the early detection of BBTV in particular in the symptomless banana plant materials produced *via* tissue culture. Results also showed that the virus infection was higher in the case of Baradica banana cultivar followed by Grand Nain and Maghraby cultivars. Results are illustrated in Figs 1, 2 and 3.

Sadik *et al.* (1999) reported that the PCR was a useful technique in detecting the BBTV in different purification steps. Harding *et al.* (2000) used the 16 oligonucleotides as primers for amplification of a fragment with a size of about 1000 bp from the BBTV-DNA-1 as reported by Harding *et al*. (1993).

Table I. BBTV detection in different banana cultivars (Baradica, Grand Nain, and Maghraby) *via* PCR using two specific primers belonging to BBTV-Eg-DNA-2.2.

Banana cultivars	Symptoms	No. of tested samples	PCR detection No.	%
Baradica	Infected	13	13	100.00
	Symptomless	8	3	37. 50
Grand Nain	Infected	12	12	100.00
	Symptomless	9	3	33.33
Maghraby	Infected	14	14	100.00
	Symptomless	7	2	28.57

Fig. 1. 1.2% Agarose gel in TAE buffer stained with ethidium bromide shows PCR detection of BBTV in symptomless (Lane 1, 2 & 3) and naturally virus-infected (Lane 4, 5, 6 & 7) banana samples from Baradica banana cultivar. A PCR fragment with a size of about 1056 bp was amplified. N: Negative control (PCR mixture without DNA), P: Positive control and M: Lamda DNA/*Hind* III Marker (Promega).

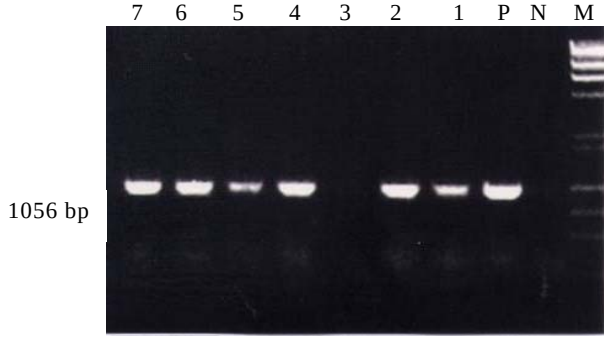


Fig. 2. 1.2% Agarose gel in TAE buffer stained with ethidium bromide shows PCR detection of BBTV in symptomless (Lane 1, 2, 3 & 7) and naturally virus-infected (Lane 4, 5, 6 & 8) banana samples from Grand Nain banana cultivar. A PCR fragment with a size of about 1056 bp was amplified. N: Negative control (PCR mixture without DNA), P: Positive control and M: Lamda DNA/*Hind* III Marker (Promega).

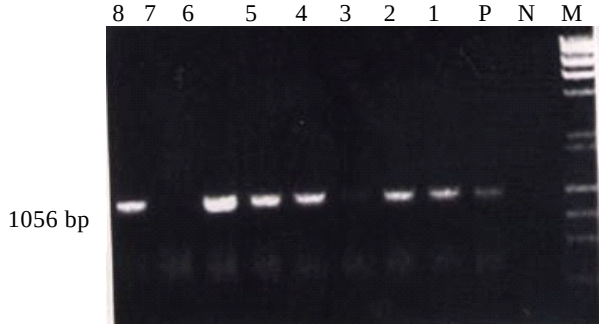
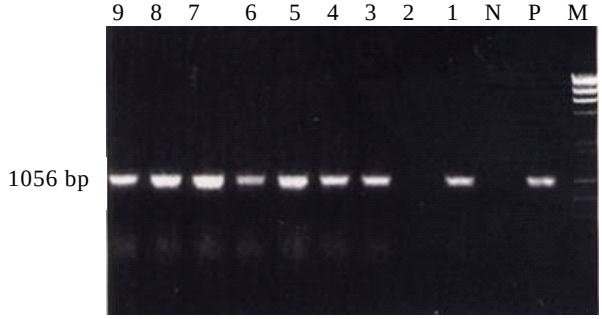


Fig. 3. 1.2% Agarose gel in TAE buffer stained with ethidium bromide shows PCR detection of BBTV in symptomless (Lane 1, 2 & 3) and naturally virus-infected (Lane 4, 5, 6, 7, 8 & 9) banana samples from Maghraby banana cultivar. A PCR fragment with a size of about 1056 bp was amplified. N: Negative control (PCR mixture without DNA), P: Positive control and M: Lamda DNA/*Hind* III Marker (Promega).



To conclude, the PCR was able to detect BBTV by targeting their genetic material in virus-infected banana as well as symptomless samples due to higher sensitivity of this method than ELISA. This conclusion is in full agreement with that reported by Wetzel *et al.* (1991) and Sadik *et al.* (1997).

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