

Non-radioactive detection of banana bunchy top nanovirus

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ABSTRACT

*In this study, molecular methods of polymerase chain reaction (PCR) and chemiluminescence were found to be successful for the accurate detection of banana bunchy top nanovirus (BBTV). In case of PCR, a specific DNA region (about 1000 bp) of the viral genome belonging to BBTV-DNA-1 segment was easily detected and visualized by agarose gel electrophoresis. A number of 96 treatments for optimizing the use of PCR for BBTV detection were applied using six dilutions of purified virus preparation, four concentrations of each primer and Taq DNA polymerase enzyme. The recommended condition for virus detection was found to be 20-30 pmoles and 1 U per 50-reaction from the primers and the enzyme, respectively. Moreover, six DNA concentrations extracted from the virus-infected banana plant were used in which 125 ng DNA was found to be the most effective. This DNA concentration was used for the detection of the BBTV in 3 parts of the apical meristems obtained from virus-infected plants micropropagated via tissue culture approach. Results showed that 10, 50 and 80 % of the upper, middle and bottom parts were infected, respectively. In addition, four dilutions from DNA extracted from the viruliferous banana aphid (*Pentalonia nigronervosa* Coq) were used and the data showed that the virus was detected in as low as 10^{-1} and 10^{-2} dilutions. In case of chemiluminescence detection, the virus was easily detected in either DNA extracted from BBTV-infected or symptomless banana plants up to 10^{-3} dilution using a cold DNA probe labeled with digoxigenin. Therefore, it is suitable for the detection of viral infection at initial stage, where the virus titers in the plant was rather low. The results also confirmed the use of PCR technology for BBTV detection as well as the use of the upper part of the apical meristems for micropropagation and production of virus-free banana plants.*

Key words: Non-radioactive detection, Dot blot, chemiluminescent DNA probe, PCR, micropropagation, viruliferous banana aphid, DNA extraction.

INTRODUCTION

Banana bunchy top nanovirus (BBTV) is an isometric virus particle (18-20 nm), consists of at least six components of

circular ssDNA, each about 1 kb (Burns *et al.*, 1995). The virus has a single protein with M_r of about 20-21 kDa (Sadik and Gad El-Karim, 1997), and naturally transmitted by a banana aphid (*Pentalonia nigronervosa*, Hu *et al.*,