# Molecular cloning and expression of recombinant coat protein gene of banana bunchy top virus in *E. coli* and its use in the production of diagnostic antibodies

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## ABSTRACT

*Banana bunchy top virus is a multicomponent virus, comprising of at least six integral components, with a circular single-stranded DNA genome. In this study, specific rabbit polyclonal antibodies against bacterially expressed coat protein of Banana Bunchy Top virus (BBTV, genus Nanovirus) were produced using a recombinant DNA approach. The BBTV capsid protein (CP) gene located on component 3 was cloned in an expression vector pQE-30 (Qiagen). Expression of the CP with an N-terminal hexahistidine tag in Escherichia coli M15 cells was induced by adding isopropyl-3-D-1-thiogalactoside (IPTG) to a final concentration of 1 mM. About 13 mg of bacterially expressed CP was purified from 1 litre of bacterial liquid culture using a Ni-NTA resin column (Qiagen). The expressed CP which migrated as a protein of approximately 21 kDa in sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) was identified by its strong reaction with polyclonal antibodies produced against BBTV purified particles (BBTV-AS) and Ni-NTA-AP conjugate in Western blots. Expressed and purified CP (SDS-PAGE 21 kDa band) was injected into a white rabbit, using two subcutaneous and three intramuscular injections at weekly intervals. The antiserum produced was evaluated for BBTV and FBNYV detection in Western blot and dot blot immunoassays (DBIA). The antiserum raised against the expressed CP (BBTV-$AS_{6xHis}$) gave strong BBTV-specific DBIA reactions and very weak background reactions with non-infected tissues, similar to those produced by polyclonal antibodies raised against BBTV purified virion (BBTV-AS). Furthermore, (BBTV-$AS_{6xHis}$) polyclonal antibody reacted specifically with both the denatured recombinant protein and the disrupted BBTV virus particle as well as FBNYV purified particles in DBIA. These results showed that the (BBTV-$AS_{6xHis}$) polyclonal antibody is useful for the detection of BBTV in infected tissues by dot blot tests.*

**Key words:** *BBTV-6x-His-tagged-fusion protein, prokaryotic expression vector, pQE-30, Polyclonal antisera, nickel affinity chromatography, western blotting, recombinant protein, PCR, DBIA.*

## INTRODUCTION

Banana *(Musa* spp.) are grown in about 121 countries (F.A.O., 2001) providing a major source of carbohydrates for over 400 million people in tropical countries (Swennen *et al.*, 1995). Over 102 million metric tonnes are produced yearly (F.A.O., 2001). Banana also provides a major source of income for smallholders (Nweke *et al.*, 1988).