Development of recombinant coat protein for immunodetection of *Cucumber mosaic virus* (Banana isolate)

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**Introduction**

Banana (*Musa* spp.) is one of the most important fruit crops in India, especially in southern regions. Cultivation of tissue culture banana is very popular among banana growers in Kerala. Viruses infecting banana can have a direct effect on production, up to 40-100 per cent yield reduction. Infectious chlorosis caused by *Cucumber mosaic virus* (CMV) in banana is cosmopolitan. This is an emerging viral disease in Kerala. Virus indexing is an inevitable practice to ensure quality of tissue culture plants. Recombinant coat protein based antiserum is adopted now a days for serological indexing of plant viruses. For this, recombinant CMV coat protein is essential.

**Objective**

To produce recombinant coat protein through expression vector and immunodetection of *Cucumber mosaic virus* infecting banana in Kerala.

**Methodology**

- Amplification of CMV coat protein gene by RT-PCR using CMV coat protein (CP) specific primer
- Sequencing and *in silico* analysis of pGEM- T/CMV- CP
- Designed CMV-CP specific primer for gene expression in pRSET-C
- Amplification of coat protein for expression analysis
- Cloning of coat protein gene to expression vector pRSET- C
- Isopropyl β-D-1-thiogalactopyranoside Induction of CMV-CP gene
- Expression of recombinant coat protein of CMV and purification using Ni-NTA column (Gualti et al., 2016)
- Evaluation of recombinant protein with polyclonal antiserum using DAC-ELISA and Western blotting

**Symptoms of CMV on banana leaves**

![Symptoms of CMV on banana leaves](Image)

**Results**

1. Development of molecular clones of *Cucumber mosaic virus* coat protein gene

   - Total RNA isolated from infected leaves
   - Ligation of purified PCR product with pGEM- T vector
   - PCR amplification of CMV-CP gene
   - Confirmation of pGEM- T/CMV- CP cloning
   - Blue and White screening
   - Molecular cloning
   - Molecular sequencing
   - Confirmation of CMV-CP based on molecular weight
   - Molecular clones of CMV-CP gene in pGEM-T vector

2. Development of expression clones of *Cucumber mosaic virus* coat protein gene

   - Selected pRSET-C expression vector
   - Designed CMV-CP specific primer for expression analysis
   - PCR product purification using commercially available kit
   - Standardised the annealing temperature
   - Amplified CMV-CP gene using designated primer and Pfu DNA polymerase
   - Molecular sequencing
   - Amplified CMV- CP recombinant based on molecular weight
   - Selected pRSET-C: CMV-CP recombinant
   - Ligated purified CMV- CP amplicom with pRSET-C vector

3. Production of recombinant coat protein of CMV

   - Cell B/L21 colonies in LB broth
   - Induced the culture with IPTG
   - Disolved the protein in Tris- NaCl (pH 6.0) buffer
   - SDS-PAGE of purified protein
   - NTA column purification

**Reference:**