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**BENEFIT**

The protocols developed on virus detection in banana allows the possibility of providing banana growers of virus-free planting materials.

**CONCLUSION**

Virus detections in banana, such as Banana Bunchy Top Virus, Banana Streak Virus and Banana Bract Mosaic Virus had been successful using PCR technique. The techniques on PCR and RT-PCR may eventually, offer diagnostic services to the general public.

A cost-efficient protocol in the detection of Banana Bunchy Top Virus simultaneous with Banana Streak Virus was formulated. This can result in much savings in terms of time and resources.

**INFORMATION BULLETIN**

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**DETECTION OF PLANT VIRUSES IN BANANA USING Polymerase Chain Reaction (PCR)**

**Source of Technology**

Project: ‘Detection of Plant Viruses in Banana and Garlic’

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Disease symptoms:

- Spindle-shaped lesions on the leaves
- Red streaking of the pseudostem

Lane 1: MW Marker
Lane 2: Uninfected Tissue
Lane 3: BBrMV-infected Tissue
Lane 4: BBrMV-infected Tissue

PCR amplified the product using Forward primer for BBrMV which generated 324 bp in size.
is the newest technique to detect plant viruses, which is more accurate than ELISA. Virus detection protocol for banana using this technique was developed for three major plant viruses in banana.

**INTRODUCTION**

Banana is a very important fruit crop in the Philippines. It ranked second, in terms of area, volume and value of production, next to coconut (BAS, 2006). It is also the second leading export crop in the country.

Banana is propagated vegetatively through suckers and in vitro. Like any other crop, it can be easily infected with viruses through mechanical transmission of insects. Most farmers do not renew their planting materials and they are not aware that their plants are already infected with virus diseases. As a consequence, yield reduction is observed. To date, degeneration of planting materials through the accumulation of viruses is ongoing. Continuous spread of diseases is observed not only in the Philippines, but also in other countries.

Detection and identification of plant viruses in banana is important in addressing the problem. Symptoms of virus infection in banana are frequently inadequate to conclude to a positive identification. Hence, the biological activity of a certain virus in banana must be understood and identified.

The application of molecular techniques gives a large ‘tool-bag’ for detection and diagnosis of plant viruses. A scientific technique through Polymerase Chain Reaction (PCR) can be used. This technique amplifies a single piece of DNA and generates into thousands and millions of copies of a particular DNA sequence. Using this method is more accurate than any other techniques of detections.

**OBJECTIVES**

To establish a protocol in the detection of banana bunchy top virus (BBTV), banana streak virus (BSV) and banana bract mosaic virus (BBrMV) using PCR technique;

To formulate a cost-efficient protocol in the simultaneous detection of BBTV and BSV.

**METHODOLOGY**

**Collection of leaf samples**

Leaf samples of banana Lakatan and Cardava banana were collected from plants showing symptoms of BBTV, BSV and BBrMV from banana plants growing in the campus of MMSU, Batac City.

**Nucleic acid extraction**

1. Homogenize the leaf midrib by grinding with liquid nitrogen, adding 3 ml of CTAB;
2. Incubate the mixture at 65°C for 45 minutes then, centrifuge;
3. Clarify the supernatant with phenol, precipitate with isopropanol and resuspend in quality water.

**PCR and analysis of products**

1. Perform PCR for BBTV and BSV using 50µl of reaction mixture;
2. Perform RT-PCR for BBrMV using 20µl for reverse transcription and 30µl of reaction mixture for polymerase chain reaction analysis;
3. Analyze the products by electrophoresis using 1.4% agarose gels;
4. Stain the gel with ethidium bromide, visualize and analyze by Doc-HR LS Image Acquisition Software.

**RESEARCH HIGHLIGHTS**

**INTRODUCTION**

Virus diseases in banana reduce yield from 40 to 100%.

Yellow striate mosaic, broken lines and diamond-shaped patterns along the leaf veins; reduced plant growth, vigor, bunch size and yield; and fruit distortion

**SIMULTANEOUS PCR DETECTION OF BBTV AND BSV**

**PCR DETECTION OF BBTV**

Disease symptoms:
- Internode shortening with a bunchy appearance at the top of the plant

Lane 1: Molecular Weight Marker
Lane 2: BBTV
Lane 3: BBTV & BSV
Lane 4: BSV

**PCR DETECTION OF BSV**

Disease symptoms:
- ~221 bp

Lane 1: Molecular Weight Marker
Lane 2: BSV

**SIMULTANEOUS PCR DETECTION OF BBTV AND BSV**

**PCR amplified the product using primer pair for BBTV which generated ~1100 bp.**

**PCR amplified the product using primer pair for BSV which generated a 221 bp.**

**PCR amplified the product using primer pair for BBSV which generated a 221 bp.**