



Original Research Article

Use of Polymerase Chain Reaction for the Detection of Banana Bunchy Top Virus Infection in Three Successive Generation of Banana

Jai Godheja^{1*}, Sudhir K Shekhar¹, DR Modi¹ and T Chatterjee²

¹Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, India

²Department of Biotechnology, Raipur Institute of Technology, Raipur, India

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Abstract: Banana is one of the most important fruit in India. Viral infection causes a great loss in Banana production with a ratio of about 20-30% loss and occasionally reaching 50-80%. The most indigenous banana are infected by BBTB; Banana bunchy top virus, a complex circular single-stranded DNA virus with multiple genomic components. On mature plants infected with BBTB, they appear to be "bunched" at the top of the plant, new leaves emerge with difficulty, are narrower than normal, the symptom for which this disease is named. For the early detection of BBTB, PCR technique was used for the plant samples. Suckers were used for isolating genomic DNA using sodium sulphite method. DNA was quantified (400 ng/μl) and then PCR was used for detecting BBTB by using BBTB specific primers. The agarose gel was loaded with ladder DNA, followed by sample DNA. Among 3 DNA samples all showed amplification by specific primers of BBTB. During the course of my practical it has been clear that the third generation plant of banana was also infected by BBTB. Therefore it is very important to detect the presence of these virus early in infected plant and proper eradication of infectious plant, before it passes to another healthy plants by its vector *Pentagonia nigronervosa*.

Key Words: Banana, BBTB, sucker

Introduction

The word banana is derived from the Arabic word for finger. There are more than 300 kinds of banana but only a few are commercially important. Banana is the man's oldest and most valued fruit crop. It is prized for its nutritive value with high carbohydrates (22.2%), fiber (0.84%) and protein (1.1%) with less fat (0.2%) and water (75.7%). The World production of banana is about 95 million tons and most of the production is consumed locally. In India, it's per hectare yield is also the highest (30 - 35 MT/ha) of all the fruits. Total area under banana is 3.70 lakh ha, next to Mango. Among the States, Maharashtra contributes the maximum area of about 90,000 ha. In Maharashtra, area under banana is concentrated in Jalgaon district with nearly 60% area in that district.

Banana is an important fruit crop in India. Four major viral pathogens viz., bunchy top virus, streak virus, bract mosaic virus and cucumber mosaic virus are known to cause significant yield loss in India and spread vertically through tissue culture plants. Viral diseases threaten banana production with a ratio of about 20-30% and occasionally reach 50-80%. Banana bunchy top viral disease is

most severe on banana. It is very destructive viral disease in many countries including India. It causes severe losses because infected plants produce no fruit. It also affects some ornamentals such as *Canna sp.* BBTB, is transmitted by aphids in the persistent manner.

BBTB is the causal agent of banana bunchy top disease (BBTD) (Dale, 1987), and is classified as a member of nanoviruses based on its molecular characterization (Dugdale *et al.*, 1998 and Harding *et al.*, 2000). BBTB has only recently been isolated and characterized (Wu and Su, 1990; Wu and Su, 1991; Harding *et al.*, 1991). Virus diseases are serious, as insect vector are abundant and there are many alternative hosts, (M.K.Dhanya *et al.*, 2007). Bananas infected by BBTB show dash-like streaks as the first observable symptoms. As the disease progresses the leaf blades become narrow, and plants show symptoms such as stunting and bunched leaves. The fruits, if any, are malformed. Finally, the disease (Banana bunchy Top Disease; BBTD) results in plant death. Because of this direct influence on productivity, BBTB is considered to be the

***Corresponding Author:**

Jai Godheja,
Research Scholar,
Department of Biotechnology,
Babasaheb Bhimrao Ambedkar University,
Lucknow, India.

most economically destructive disease of banana. The disease is widespread in Asia.

BBTV is a phloem-limited virus found in a very low concentration in virus-infected plants, which is a major obstacle in the production of specific polyclonal antibodies (PABs) for its detection. Dale (1987) recommended a strategy for controlling BBTV based on identifying virus-infected plants as early as possible followed by removing the diseased plants and replanting virus-free banana plants. Banana bunchy top is transmitted in a persistent manner only by banana aphid (*Pentalonia nigronervosa*) (Fig. 1A), its sole biological vector (Lava Kumar, 2009) and through vegetative propagation, but not by artificial manual inoculation.



Figure 1A: Wing less Banana Aphid (*Pentalonia nigronervosa*)

The banana aphid, which acquires the virus after at least four (but usually about 18) hours of feeding on an infected plant. The aphid can retain the virus through its adult life, for a period of 15–20 days. During this time, the aphid can transmit the virus to a healthy banana plant by feeding on it, possibly for as little as 15 minutes but more typically for about two hours. Disease symptoms usually appear about a month after infection. Controlling aphids is essential to reducing the spread of BBTV. Aphid populations may increase during the spring or during warm, dry weather, and they can often be found on suckers. To minimize the spread of BBTV, all infected plants and their mats must be destroyed with an approved herbicide. Destroying infected plants with Roundup Ultra Max can help reduce the spread of BBTV.

Bananas and plantains are vegetatively propagated crops, and as such, virus contamination of planting material is a

significant issue. Once infected, a plant and its progeny will remain infected thereafter. Although tissue culture is an effective means of freeing planting materials of most pests and diseases, viruses are usually very difficult to eliminate. However, even though some viruses have only been recently been discovered or characterized, functional detection methods are available for all of them. Good summaries of the characteristics, symptoms, and accepted indexing methods for most of the above viruses can be found in (Diekmann and Putter, 1996). While symptomatology is the easiest of the detection methods, it is not always reliable. In some cases, classic symptoms may be displayed allowing fairly certain identification of the virus involved. However, mixed infections are a frequent occurrence and in these cases symptoms are not always clear. Symptoms caused by different viruses can be similar and infected plants may even be symptomless.

Virus indexing is an important step to assure the mother plants free of viruses before mass multiplication and distribution. PCR is widely used for virus testing for many of the plant viruses. Various forms of serological assays are currently available for all seven of the banana viruses discussed. When suitable antisera are available, the most sensitive and efficient practical assay for banana virus indexing is enzyme-linked immunosorbent assay (ELISA). Specific polyclonal and monoclonal antibodies are available for BBTV, BSV, CMV, BBrMV (Diekmann and Putter, 1996) and the banana potyvirus.

Material and Methods

Source of material

The leaf samples of *Musa paradisiaca* were collected from different origin (Fig 1B, 1C and 1D). First two generation samples were collected from the commercial field of Rajnandgaon and the third generation samples were collected from commercial field of banana from Nanadanvan, Raipur. The leaves samples of all the three generation were collected and were respectively diagnosed for BBTV infection. The leaves were selected on the basis of the characteristic symptoms of Banana Bunchy Top Virus (BBTV), on mature plants infected with BBTV, new leaves emerge with difficulty, are narrower than normal, are wavy rather than flat, and have yellow (chlorotic) leaf

margins. They appear to be “bunched” at the top of the plant, the symptom for which this disease is named. Severely infected banana plants usually will not fruit, but if fruit is produced, the banana hands and fingers are likely to be distorted and twisted.



Figure 1B: First Generation Plants



Figure 1C: Second Generation Plants



Figure 1D: Third generation Plants

The samples were selected on the basis of the characteristic symptoms of Banana Bunchy Top Virus (BBTV), on mature plants infected with BBTV, new leaves emerge with difficulty, are narrower than normal, are wavy rather than flat, and have yellow (chlorotic) leaf margins. They appear to be “bunched” at the top of the plant, the symptom for which this disease is so named.

Severely infected banana plants usually do not fruit, but if fruiting occurs, the banana hands and fingers are likely to be distorted and twisted. Then 200mg weighed plant materials (Leaves) were grinded in mortar and pestle using liquid nitrogen to a fine powder. The sample was transferred to a presterile eppendorf's tubes.

DNA isolation

There are two successive methods for isolation of Plant Genomic DNA but we had worked on Sodium Sulphite method (V.K. Baranwal, S. Muzumder, 2003). The PCR method was carried out with ready to use chemicals from “Genie, Bangalore” and the BBTV specific reverse and forward primer were made available by IARI, New Delhi. Separation of Amplified DNA was done through gel electrophoresis and the gel was visualized on UV transilluminator and photographed by using digital camera.

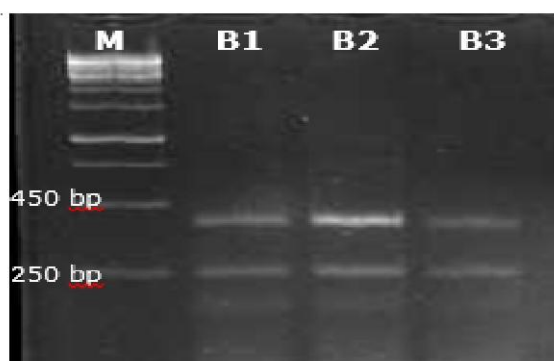
DNA Quantification

The isolated DNAs were loaded in the well of 0.8 % Agarose gel along with known standards and electrophoresis was done under for 1^{1/2} hour. The quantity of DNA was estimated using quantitative method. Almost uniform amount of DNA was extracted from each sample. The quantity was estimated to be ~400 ng/μl. The quantified DNA was used for PCR assay by BBTV specific primers.

PCR assay by BBTV specific Primers

An investigation was made to access the occurrence of Banana Bunchy Top Virus in the samples collected from Mother Orchid, Commercial Field. Concentrated samples of DNA isolated from infected plants were used for PCR assay and a set of BBTV specific primers were used to detect integrated viral DNA in Banana genomic DNA in the collected samples. PCR was conducted in the volume of 0.8μl agarose gel electrophoresis is then used for separation of amplified DNA in influence of electric field. The agarose gel was loaded with 1 kb + ladder in 1st Lane, followed by and lane 2-4 carry 3 samples of DNA isolated from respective samples of banana (Fig. 1E).

Figure 1E: The Electrophoretic pattern of the samples showing all the samples infected with BBTv



M: Ladder 1 Kb

B1: Banana Sample from Rajnandgaon field

B2: Banana Sample from Rajnandgaon field

B3; banana Samples from Nanadanvan Field

Detection of BBTv Infection using BBTv specific primers.

The presence of BBTv infection was detected by using BBTv specific primers (450 to 250 bp).

Table 1A: BBTv specific primers

Primer	Forward primer	Reverse Primer	Location
BBTv (~250 amplicon)	TAGATCCATGG TCAGACAAGAA	ATAAAGCTTTCAAA CATGATATGT	206±229
BBTv (~450 Internal control)	GATCTATTGAA GCTGTG	CTAACTTCCATGTCT CT	426±452

Result

The presence of the bands indicates that the selected sample was infected by viral genome. The result was that that all three samples collected from Rajnandgoan and Nandanvan showed the presence of BBTv virus. As shown in fig 5 size of amplified DNA is approximately 450- 200bp when compared with ladder. Among 3 DNA samples of Banana, lane 2, 3 & 4 showed amplification by specific primers of BBTv s. The PCR assay was conducted with one positive and one negative control.

On the basis of PCR assay performed by BBTv specific primer, sample 2, 3 & 4 of banana showed integrated BBTv DNA. In the present investigation it was observed that virus was detected in sample form plant belongs to farmer's field & mother orchid.

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