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Early Detection of Banana Bunchy Top Virus in India Using Polymerase Chain Reaction

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A polymerase chain reaction (PCR) assay was used to detect the Indian isolate of banana bunchy top virus at early stages of infection in banana suckers before symptom expression. The viral DNA was detected from a single viruliferous banana aphid *(Pentalonia nigronervosa)* at 1.0 kb. The six plant species viz., *Zingiber officinale, Colocasia esculenta, Catheranthus roseus, Canna indica, Hedychium coronarium* and *Alphinium* sp. upon inoculation with BBTV showed negative results in PCR assay. Using PCR assay the BBTV could be detected in meristem tip cultured banana plants before symptom expression. In field condition the meristem tip cultured banana plants expressed BBTV symptom 45 days after planting.

Keywords: BBTV, DNA, PCR, transmission, 1.0 kb.

Banana bunchy top disease (BBTD) caused by banana bunchy top virus (BBTV) is the most devastating disease of banana in many banana growing areas in Asia, Africa and the South Pacific (Dale, 1987) and the area affected by this disease has been expanding. BBTD was recently reported in China (Hu and Sun, 1990), Hawaii (Lai, 1990) and Pakistan (Khalid and Soomro, 1993). The most important symptoms of BBTD is the occurrence of prominent dark green streaks of variable lengths in the petioles, midribs and veins. These streaking and dotting appeared like a "Morse code" pattern (Magee, 1940). As a result of cytopathological changes in the phloem tissues, the successive leaves become progressively dwarfed, they develop marginal chlorosis or yellowing and further become upright, crowded and bunched at the apex of the plant giving a "rosette" or "bunchy top" appearance (Iskra, 1990).

The virus is transmitted by the banana aphid (*Pentalonia nigronervosa* Coq) in a persistent manner (Magee, 1940; Hu et al., 1996). The virus has spread from Fiji and caused devastating problems in many countries including Fiji (1927), Tongo and Samoa (1967), Vietnam (1961) and Guam (1982) (Dale, 1987). In India, the disease was confirmed in 1943 in the Travancore state (Now Kerala). The disease has also spread to Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Bihar, West Bengal, Assam and Uttar Pradesh (Varghese, 1945; Singh, 1979). In Tamil Nadu, the BBTV has almost destroyed the cultivation of choice variety, hill banana syn. *Sirumalai / Vellavazhai* cultivation in lower Palani hills.

BBTV is an isometric particle with a diameter ranging from 18 to 22 nm with a single coat protein about Mr 20,500 (Harding et al., 1991; Thomas and Dietzgen, 1991). Although initially the nature of nucleic acid associated with BBTV was confusing (Wu,

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1994), the recent information's revealed that they are circular single-stranded DNAs with multiple DNAs in the genome (Burns et al., 1993), at least six DNA components, each of about 1.0 kb (Thomas and Dietzgen, 1991; Chu et al., 1993). Karan et al. (1994) has demonstrated the two groups of BBTV isolates, the Asian group including isolates from Philippines, Taiwan and Vietnam and the South Pacific group which includes isolates from Australia, Burundi, Egypt, Fiji, India, Tonga and Western Samoa.

Several studies on varietal reaction to BBTV infection has been done over last three decades. Capoor (1967) found that all the 45 cultivars grown in India were susceptible to BBTV. Mohan and Lakshmanan (1987) reported that AA and AAA genomes were severely affected by BBTV. Jose (1981) has listed cultivars which show resistant and tolerant reactions.

In addition to banana, natural hosts of banana aphid include *C. esculenta, G. officinale,* ornamental bananas *(Musa coccinea* and *M. velutina),* heliconias (*Heliconia* spp.) and bird of paradise *(Strelitzia reginae).* BBTV was detected serologically in white ginger and Canna lily (Su, 1993). It is important to determine that the above plant species are hosts of BBTV.

Currently the recommended strategy for control of BBTV is to identify virus infected plants as early as possible, removing the diseased plants and replanting with virus-free banana plants (Dale, 1987). Vector indexing is also an important strategy for the control of BBTV. In order to make this approach successful, it is necessary to have a sensitive, rapid and reliable indexing assay for the detection of BBTV.

Various immunological methods have been successfully used to detect BBTV (Wu and Su, 1990; Hu et al., 1993). Polymerase chain reaction has been used in numerous studies for the rapid, sensitive and reliable detection of badnaviruses (Lockhart and Olszewski, 1993). Recently PCR based method have been developed to detect BBTV (Xie and Hu, 1995).

In this paper, polymerase chain reaction was used to detect the presence of BBTV in individual aphid and banana suckers.

Materials and Methods

BBTV and banana aphid sources

BBTV infected banana samples and viruliferous banana aphids *(P. nigronervosa)* were collected from an infected *Sirumalai* (ABB) banana from the lower Palani hills of Tamil Nadu, where BBTV is endemic. The infected banana plants had characteristic symptoms of BBTV infection. Healthy banana plants and BBTV free banana aphids were collected from the well-maintained banana garden in Coimbatore district of Tamil Nadu, where BBTV has never been detected.

Transmission studies of BBTV to other plants

To determine the other potential plant species as a host of BBTV, transmission studies were conducted by inoculating viruliferous aphids. The following plant species

were selected based on the aphid colonization in those plants. Twenty-five viruliferous aphids were transferred to other plant species viz., *Zingiber officinale, Colocasia esculenta, Catheranthus roseus, Canna indica, Hedychium coronarium* and *Alphinium* sp. separately and allowed one-week inoculation access period. The plants were evaluated 5 months after inoculation. The newly emerged leaves were tested by PCR for the presence of BBTV. The experiment was repeated three times.

Screening banana varieties against BBTV

The banana plants in each variety were inoculated with 20 viruliferous aphids for an inoculation access period of 48 h after which the aphids were killed by spraying monocrotophos (0.1%). The inoculated plants were kept in insect proof glasshouse for symptom expression. Each variety was replicated three times. The varieties were selected based on their table purpose.

Virus detection assays

(i) ELISA

The DAS-ELISA was performed to detect the presence of BBTV as per Clark and Adams (1977) using polyclonal antiserum (produced against Indian BBTV and also obtained from J. E. Thomas, Australia). The antiserum produced in rabbit against BBTV was used at 1 : 1000 dilution. Alkaline phosphatase conjugated goat antirabbit IgG (Sigma) was used as enzyme conjugate at 1 : 5000 dilution and *p*-Nitrophenyl Phosphatase (PNP) was used as substrate. Absorbance was read at 405 nm.

(ii) POLYMERASE CHAIN REACTION (PCR)

The polymerase chain reaction assay was carried out as described previously (Xie and Hu, 1995) with minor modifications (Hu et al., 1996). The leaf or aphid samples were ground in 500 µl extraction buffer containing 100 mM Tris-HCl (pH 8.0), 1.4 M Na Cl, 20 mM EDTA, 2% Hexadecyltri-methylammonium bromide (CTAB) and 0.2% Mercaptoethanol. Total DNA was recovered by precipitation with ethanol and dissolved in 100 µl TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). One µl of the sample was used for PCR reaction. PCR was conducted in a 50 µl reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1% Triton X-100, 0.2 mM each dNTP, 2.0 mM $MgCl_2$, 50 pmol of each primer Primer A: 5'-GGCGAATTCTATAAATAGACCTCCC-3', Primer B: 5' CGGAGCGTGCGCTGTAAA-3' and 2.5 units of Taq DNA polymerase (Genei). The primer pair designed from the putative replicase region of component one DNA of BBTV. Initial denaturation at 94 °C for 4 min was followed by 40 cycles consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 2 min and extension at 72 °C for 3 min with final extension at 72 °C for 10 min. Amplified PCR products were analyzed by electrophoresis in 1.5% agarose gel in 1X TBE buffer (Sambrook et al., 1989) and the gel was viewed in an UV transilluminator (Fotodyne 3440). The amplified PCR product was compared with 100 bp DNA ladder.

Results

Using PCR assay BBTV was detected from the field infected banana leaves and viruliferous aphids at 1.0 kb level. The amplification of viral DNA by PCR at 1.0 kb level revealed that the primers were able to amplify the component one DNA of BBTV genome *(Fig. 1).* It is possible to detect the BBTV in single viruliferous aphid, which will be more useful to study the epidemiology of the disease *(Fig. 2).*

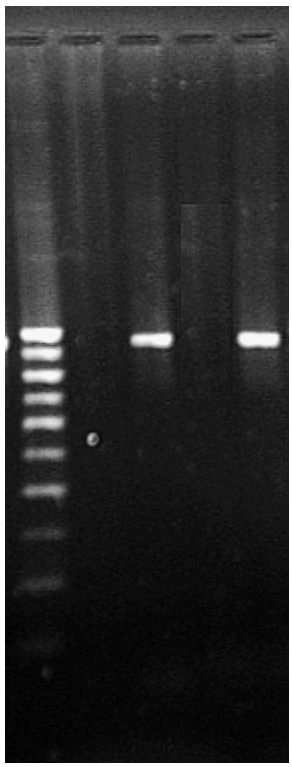

Fig. 1. Detection of BBTV by PCR

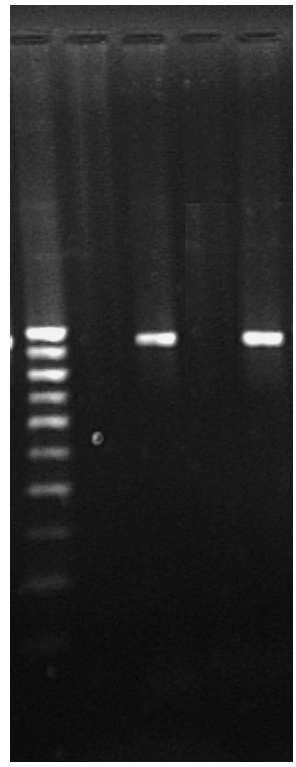

Fig. 2. Detection of Banana bunchy top virus in aphids by PCR

Comparative techniques for BBTV indexing

Thirteen plant samples were tested for presence of BBTV by PCR and ELISA. The PCR generated the 1.0 kb amplification product of BBTV from 12 out of 13 samples. However ELISA detected the BBTV from 7 out of 13 samples tested *(Table 1)* based on their colour intensity measured at 405 nm was more than three times higher than the control.

Table 1

Comparative detection of BBTV by symptom, PCR and ELISA

Variety	Symptoms	PCR	ELISA
Sirumalai	Dark green streaks on the mid-rib	+ve	+ve
Sirumalai	Reduced leaf lamina	+ve	+ve
Sirumalai	Symptomless	+ve	–ve
Ney Poovan	Dark green streaks on the mid-rib	+ve	+ve
Ney Poovan	Reduced leaf lamina	+ve	+ve
Ney Poovan	Symptomless	–ve	–ve
Karpooravalli	Symptomless	+ve	–ve
Robusta	Reduced leaf lamina	+ve	+ve
Robusta	Symptomless	–ve	–ve
Robusta	Dark green streaks on the mid-rib	+ve	+ve
Tissue culture plants Dwarf Cavendish (2 months old) Sample 1	Symptomless	+ve	–ve
Sample 2	Symptomless	+ve	–ve
Sample 3	Symptomless	+ve	–ve

Table 2

Screening banana varieties against banana bunchy top virus

S. No.	Variety	Genome	No. of plants infected out of three	Per cent transmission (Visual observation)	PCR test results*
1	Vennettu Kunnan	AB	1	33.3	+ve
2	Valia Kunnan	AB	1	33.3	+ve
3	Duga Mungar	ABB	2	66.6	+ve
4	Alshi	ABB	3	100	+ve
5	Kuri Bontha	ABB	1	33.3	+ve
6	Kanchi Kela	ABB	1	33.3	+ve
7	Nalla Bontha	ABB	1	33.3	+ve
8	Lambi	ABB	1	33.3	+ve
9	Barsain	ABB	1	33.3	+ve
10	Karpoora Valli	ABB	1	33.3	+ve
11	Ney Poovan	AB	1	33.3	+ve
12	Bankel	ABB	2	66.6	+ve

*Result of three plants tested

Screening banana varieties against BBTV

The results of visual observation showed that eleven varieties were susceptible to BBTV out of 12 varieties tested. The BBTV transmission varied from 33 to 100 per cent according to the varietieal phenology. In PCR assay, all the varieties tested were susceptible to BBTV except Vennattu Kunnan *(Table 2).*

Infection of BBTV to other plant species

The BBTV could not be detected from five months after aphid inoculated plants viz., *G. officinale, C. esculanta, C. indica, C. roseus, H. coronarium* and *Alphinium* sp. (five individuals in each plant species) *(Fig. 3).* The positive control banana plants inoculated by BBTV produced typical BBTV symptoms within 45 days and had positive reaction for BBTV by PCR assay. The results from aphid transmission studies showed that *G. officinale, C. esculanta, C. indica, C. roseus, H. coronarium* and *Alphinium* sp. are probably non hosts of BBTV.

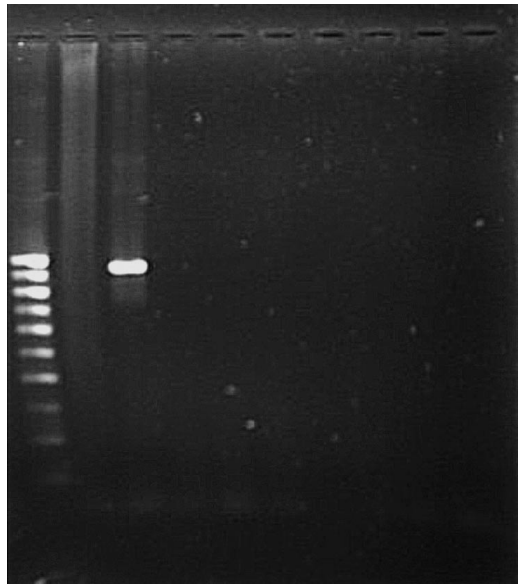

Fig. 3. Detection of BBTV in other host by PCR

Discussion

It has been reported that the BBTV genome consists of at least six circular ssDNA components with a size ranging from 1000 to 1200 nucleotides (Chu et al., 1993; Xie and Hu, 1995). The amplified PCR products in our study was 1.0 kb. Thomas and Dietzgen

(1991) described that BBTV has at least six ssDNA components, each of about 1.0 kb in size. The BBTV was detected at 1.0 kb in size by PCR in BBTV infected banana and viruliferous aphids (Hu et al., 1996). It is a very useful technique to detect BBTV at early stages of infection before symptom expression which may help to revive the hill banana cultivation in lower Palani hills of Tamil Nadu.

The aphid transmission studies of BBTV showed that the aphids were able to colonize these plant species but unable to transmit BBTV. The host range of BBTV is limited to plants in the Musaceae (Dale, 1987). Magee (1940) was unable to transmit BBTV to *Canna* sp. and *Solanum tuberosum.* Ram and Summanwar (1984) reported that *C. esculenta* as a host of BBTV, but transmission of BBTV to *C. esculenta* has not been confirmed (Dale, 1987). In the present study using PCR assay the BBTV has not been detected in *C. esculenta.*

Thirteen field samples tested by ELISA and PCR for comparison, the PCR assay is more useful for detection of BBTV (12 out of 13) compared to ELISA (7 out of 13). PCR based methods are more sensitive than ELISA based methods (Rowhani et al., 1995).

All assays compared in this study (dot blot, FiPSA and ELISA data not shown) are sensitive and specific for the detection of BBTV in diseased banana plant samples. PCR is by far the most sensitive and reliable (Hu et al., 1996). In the present investigation it was not possible to detect BBTV from other plant species viz., *G. officinale, C. esculenta, C. indica, C. roseus, H. coronarium* and *Alphinium* sp. Through PCR assay, BBTV could be detected from the equivalent of 80 ηg banana leaf tissue and also from individual aphid. The results demonstrated the usefulness of PCR in studies of epidemiology and detection of BBTV.

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