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## Research Article

# Molecular characterization of banana bunchy top virus movement protein encoding DNA-M component isolated from hill banana and Grand Naine

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### Abstract

Genome of Banana bunchy top virus (BBTV) which causes the bunchy top disease in banana is transmitted by banana black aphid (*Pentalonia nigronervosa*). BBTV genome comprises six circular, single stranded DNA components, each coding for a single protein. Movement protein is encoded by the DNA-M component of BBTV, which plays a major role in the virus movement, causing systemic infection in banana. We report the isolation of BBTV and complete sequencing of DNA-M from 5 samples of Hill Banana and 2 samples of Grand Naine cultivated in Tamil Nadu by using the designed abutting primers in the conserved region. Bioinformatic analysis revealed two near identical BBTV DNA-M sequences among the five samples in Hill Banana. BBTV DNA-M isolated from both the samples of Grand Naine were identical. Multiple sequence alignment of the isolated movement protein with other sequences deposited in GenBank showed very high levels of sequence conservation. Further, phylogenetic analysis was done with DNA-M to determine evolutionary relationship with other isolates of BBTV available in India. Characterization of the movement protein of BBTV is very significant in the context of its role as a suppressor of gene silencing in banana.

### Key words

DNA isolation, PCR amplification, movement protein, sequence analysis, Hill Banana, Grand Naine

### Introduction

Banana bunchy top disease (BBTD) is a serious disease of banana crop, threatening majority of the banana cultivation in India. The disease was first reported in Fiji during 1889 and Taiwan, Egypt and Australia in 1890, 1901, 1913 respectively (Fahny, 1927; Wardlaw, 1961). Later, it was introduced to Sri Lanka in 1913 and then to Southern parts of India in 1940. This later on spread to remaining parts of the country (Wardlaw, 1961), and the world, causing devastating problems in banana growing countries like Fiji, Egypt, Philippines, Taiwan and China (Dales, 1987). BBTD spreads through vegetative propagules such as suckers, corm and tissue cultured planting material. Bunchy top disease of banana is caused by *Banana bunchy top virus* (BBTV). BBTV - a multi-component, circular single-stranded DNA virus belonging to Nanoviridae family of the genus Babuvirus (Harding *et al.*, 1991, Burns *et al.*, 1994; Hu *et al.*, 2007; Amin *et al.*, 2008) is transmitted by the banana aphid, *Pentalonia nigronervosa* in a persistent and circulative manner (Magee, 1927). BBTV genome consists of at least six circular single-stranded DNA components each of ~1.1 Kb (Burns *et al.*, 1995). They include DNA -R (encoding a rolling-circle replication initiator protein, Rep), DNA-S (encoding the coat protein, CP), DNA-M (encoding the movement protein, MP), DNA-C (encoding the cell-cycle link protein, Clink), DNA-N (encoding

the nuclear shuttle protein, NSP) and DNA-U3 (the function of a potentially encoded protein remains unknown) (Burns *et al.*, 1995; Amin *et al.*, 2008; Visnoi *et al.*, 2009; Selvarajan *et al.*, 2010; Wickramaarachchi *et al.*, 2016; Baldodiya *et al.*, 2019). Except the DNA-R component, all other components encode single ORF in virion sense and DNA-R has an additional small internal ORF, function of which is still unknown. There are two groups of BBTV isolates (Karan *et al.*, 1995), the South Pacific group (isolates from Australia, Burundi, Egypt, Fiji, India, Tonga and Western Samoa) and the Asian group (Vietnam, Philippines and Taiwan), based on sequence analysis of BBTV DNA-1, 3 and 6 (Wanitchakorn *et al.*, 2000). All BBTV genome components share a common conserved region (CR-M), a stem-loop common region (CR-SL), a potential TATA box, a polyadenylation signal associated with each ORF (Burns *et al.*, 1995; Beetham *et al.*, 1997; Banerjee *et al.*, 2014).

Hill Bananas, which are known for their special flavour and long shelf life, are unique to the state of Tamil Nadu. They are perennial in nature and often cultivated along with coffee and pepper in a multitier system. Two ecotypes (AAB) namely, Virupakshi and Sirumalai are grown in regions at a height of 2000 to 5000 feet with well distributed

annual rainfall of 1250-1500 mm in the lower Palani hill, Sirumalai and Kolli hills. BBTV is the primary cause for reduction in the area of Hill Banana cultivation from 18,000 ha in 1970s to a mere 2,000 ha at present. Grand Naine (AAA), being a popular banana variety occupies a large area of cultivation in Tamil Nadu. The present study was taken up to understand the genetic diversity of movement protein of BBTV genome in Tamil Nadu.

### Materials and Methods

BBTV infected leaf samples of Hill Banana were collected from Palani region and Grand Naine samples from Coimbatore region of Tamil Nadu. Genomic DNA was isolated from BBTV infected leaf tissues using CTAB method (Doyle and Doyle, 1990). About 100 mg of leaf bits were weighed and homogenized with CTAB buffer [10 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2 % CTAB, 0.1 % (v/v)  $\beta$ -Mercapthoethanol and 2 (v/w) % PVP] to isolate the total DNA. The DNA was precipitated with ice cold iso-propanol and washed with 70 % ethanol to remove the salts. The pellet obtained was air dried and dissolved with 100  $\mu$ l of sterile water and stored at -20 °C for further analysis. The DNA samples were quantified using Nanodrop spectrophotometer ND-1000. The DNA samples were resolved on a 1.0 % agarose gel.

BBTV movement protein specific forward primer (5' TGACCAGAAGACGTGTATGGA 3') and reverse primer (5' GGTATTCTACCAAATACCTCACGA 3') were designed in the conserved region by obtaining sequence available from GenBank, NCBI database using Primer 3 online software (<http://bioinfo.ut.ee/primer3-0.4.0/>). DNA-M of BBTV genome was amplified with PCR conditions with initial denaturation at 94 °C for 5 min followed by 35 cycles: Denaturation at 94 °C for 1 min, 51 °C annealing for 45 sec, extension at 72 °C for 1 min final extension was given at 72 °C for 5 min. PCR reactions were performed in Thermal Cycler (Eppendorf, Germany) in a final volume of 20  $\mu$ l. The PCR reaction mixture contained 1  $\mu$ l of genomic DNA (50 ng/ $\mu$ l), 2.0  $\mu$ l of 10X PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl and 1.5 mM MgCl<sub>2</sub>), 0.5  $\mu$ l of 100 mM dNTPs, 1.0  $\mu$ l each of 10  $\mu$ M respective forward and reverse primers, 0.3  $\mu$ l of 1 unit Taq DNA polymerase (TaKaRa Bio USA, Inc.) and 14.2  $\mu$ l of sterile distilled water. The PCR products were resolved on 1 % Agarose gel and electrophoresed at 70 V for 1 h. The amplified products were visualized on UV transilluminator and documented in gel documentation system (Syngene, UK).

The PCR amplicons of BBTV DNA-M were purified using gel extraction kit (Biobasic Canada, Inc.) following the manufacturer's instructions. Purified PCR product was sequenced with the forward and reverse primer. Further, two additional primers were designed and were employed to sequence the ends of the PCR amplicon (Agrigenomes Pvt. Ltd., Kochin, India).

DNA sequence reads of the BBTV DNA-M were assembled using the online contig assembly software (<http://doua.prabi.fr/software/cap3>). After DNA sequence assembly, ExPASy protein translation tool (<https://web.expasy.org/translate/>) was employed to find the movement protein ORF. Translated movement protein sequences were compared with other deposited BBTV movement protein sequences.

The phylogenetic analysis of MP (movement protein) gene of BBTV genome component was performed to understand the genetic grouping of BBTV isolates among the BBTV isolates of India and outside of the India. The sequence obtained were analysed by assembling and aligning using Bioedit software (<https://bioedit.software.informer.com/7.2/>). The nucleotide sequences of BBTV MP gene were compared with sequences available in GenBank, NCBI Database. The phylogenetic tree of MP gene of BBTV isolates was constructed by aligned nucleotide sequences using the neighbouring joining and bootstrap phylogeny of MEGA7 software (<https://www.megasoftware.net>).

### Results and Discussion

Genomic DNA samples were isolated from BBTV infected Hill Banana and Grand Naine banana cultivated in Tamil Nadu. We designed abutting primers to amplify ~1.1 kb full length BBTV DNA-M by using high conserved region of known BBTV isolates deposited in NCBI (Fig. 1). Using the primers pairs, PCR amplification of full length BBTV DNA-M was observed in 5 samples of hill banana and 2 samples of Grand Naine (Fig. 2). Similar results were also reported by Wickramaarachchi *et al.* (2016) with respect to PCR amplification of DNA-M component of Sri Lankan isolate and by Baldodiya *et al.* (2019) in Jahaji Indian banana cultivar popularly grown in Assam.

PCR amplified products were then sequenced to unravel the DNA sequence. Trimmed DNA sequence reads of forward primer, reverse primer and two additional primers were assembled by CAP3 contig assembly programme available in

BioEdit software. Complete DNA sequence was obtained and through *in silico* analysis identified the presence of large ORF in the virion sense strand. Translated ORF codes for BBTV movement protein using ExPasy protein translator tool. The detailed features of BBTV DNA-M component are given in Table 1. Movement protein sequence was predicted to code for 117 amino acids protein when compared with other BBTV movement protein sequences from Indian isolates available in GenBank, NCBI (Fig. 3). The sequences obtained after analysis were compared with DNA-M sequences available in NCBI using blast search and showed very less variation among the DNA-M sequences of Hill Banana and Grand Naine comparing other sequences of DNA-M. Similar finding was also reported by earlier workers (Amin *et al.*, 2008, Vishnoi *et al.*, 2009, Wickramaarachchi *et al.*, 2016 and Baldodiya *et al.*, 2019). Multiple sequence alignment among the movement protein sequence shows that it is highly conserved among the Indian isolates (Fig. 4). This is in agreement with previous reports (Vishnoi *et al.*, 2009; Selvarajan *et al.*, 2010 and Banerjee *et al.*, 2014).

The phylogenetic analysis of isolated MP gene sequences was performed to predict the evolutionary relationship with other BBTV MP gene sequences available in NCBI. This analysis showed close relationship between the BBTV DNA-M isolates of Hill Banana -1 and Grand Naine -1 by forming a single cluster. Besides, they were found to have close proximity to the Hill Banana isolates of Tamil Nadu (EU190971). BBTV DNA-M of Hill Banana-2 isolate was found to be closely related to BBTV DNA-M of banana cv. Robusta, India (KM607238) and Chennai BBTV M isolate (KJ513018), forming a single cluster (Fig. 5). The present study revealed that among the five samples of Hill Banana from Tamil Nadu, all four isolates are similar except sample 2 which showed variation with few nucleotides in conserved region. Whereas, the two Grand Naine samples collected from Coimbatore showed no variation and are likely to be very similar. The phylogenetic analysis was carried out using two samples of Hill Banana, which showed variation and one Grand Naine sample. The result revealed that Hill Banana sample-1 and Grand sample was closely related to each other with maximum similarities and also found to be closely similar to previous sequence of Hill Banana deposited in NCBI (Accession number EU190971).

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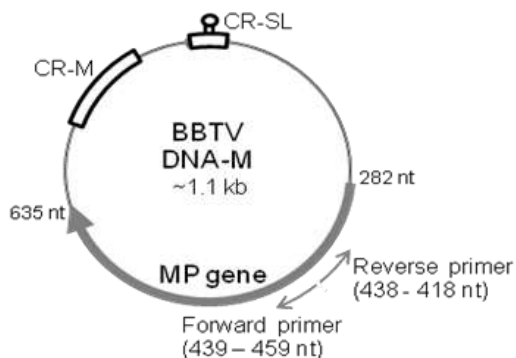
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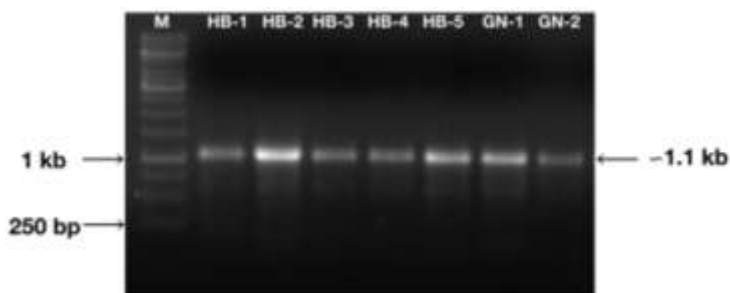


**Table 1. Features of the DNA-M component of Hill Banana and Grand Naine**

<b>Cultivar</b>	<b>Accession numbers</b>	<b>Size (nt)</b>	<b>Predicted CDS size (location)</b>	<b>Predicted protein (s) (aa/kDa)</b>	<b>Predicted molecular mass of encoded protein (s) kDa</b>	<b>Geographic origin of isolate</b>
Hill Banana-01	MN011923	1046	354 (282-635)	117	13.83	Thadiyankudisai
Hill Banana-02	MN011924	1046	354 (282 -635)	117	13.83	Lower Palani
Grand Naine	MN011925	1046	354 (282-635)	117	13.83	Coimbatore



**Fig. 1.** Genome organization of BBTV DNA-M component consists of major common region (CR- M), stem-loop common region (CR-SL), ORF (movement protein gene), TATA box and polyadenylation signal sequences.



**Fig. 2.** PCR amplification of DNA-M using abutting primers; Lane M- 1 kb ladder, HB-1 to 5 (Hill Banana samples) and GN-1 to GN-2 (Grand Naine samples).

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10      20      30      40      50      60      70      80      90
DNA M  AGCGCTGGGCTTATTATTACCCCCAGCGCTCAGGACGGGACATCAAGTGCCTTCAACAAATGCACGTGAGTGATATAAGGGACATAACGG
      Stem loop region
100     110     120     130     140     150     160     170     180
DNA M  GTTCAGATAACGGTATCTTTGGTTTAGAATATAACGTCACGCTGTAAGCGAGAGGCCACGTGACTAAGTCAAATGTATTGAATAAACATT
190     200     210     220     230     240     250     260     270
DNA M  TGAAGTCCGGTAGCTTCCGAAGGAAGTTAGGATGCTTCGTGGCGAAGCAAACCAATTTATATATGCTTAGGCTTCCGGCCATAAATAG
      TATA box
280     290     300     310     320     330     340     350     360
DNA M  GACCCGTGCTAAATGGCATTAAACAACAGAGCGGGTTAAACTATTCTTTGAATGGTTTTTGTCTTTGCAGCAATATTTATTGGATTACAA
      H A L T T E R V K L F F E W F L F F A A I F I A I T
370     380     390     400     410     420     430     440     450
DNA M  TATTATATATATTGTGGTTTGGCTCTTTGAGGTACCGAAGTATAATTAAGCAGCTCGTGAGGTATTTGGTAGAATACCTGACCAGAGAC
      I L Y I L L V L L F E V P K Y I K Q L V R Y L V E Y L T R R
460     470     480     490     500     510     520     530     540
DNA M  GTGTATGGATGCAGAGAACGCAGTTATCGGAGGCAACTGGAGACGTAGAGTTTCGGCAGAGGTATTGTGGAAACAGACGGGATCAACAC
      R V W M Q R T Q L S E A T G D V E F G R G I V E D R R D Q Q
550     560     570     580     590     600     610     620     630
DNA M  CGGCTGTCATACCACAGGCATCTCAGGTATCCCTCTCAACAATTAGAAAGGATGATCAAGGAAGACGAGGAACGTCGGACCTATGT
      F A V I P Q A S Q V I P S Q Q I R R D D Q G R R G N V G P M
640     650     660     670     680     690     700     710     720
DNA M  TTTAATACACGGTATTGTAATATATGAAATATAAATGTGGATTGATGATAAGGTACATACATATATGATGATAAGAAACATATTG
      F *
730     740     750     760     770     780     790     800     810
DNA M  TAATATGTGAATTGTAAACGAGTGTGAATGTATACAACATACACACTATGAAATACAAAGACGCTATGACAAATGTATGGGTATCTG
820     830     840     850     860     870     880     890     900
DNA M  ATTAGGTATCCTAACGATCTAGGGCCGAAGGCCGTGAGCAATATGGCTCGAAATAATGTTTAACAAACAATATACATGATACGGATAG
910     920     930     940     950     960     970     980     990
DNA M  TTGAATACATAAACAACTAGGTATACAATACAACAACCTGTTGTAAGAAAATAAAAAAATAAGAAGGAGATATATTGTGTTGGATAAG
1000    1010    1020    1030
DNA M  CTTGGCAACCACCACTTTAGTGGTGGGTGAGATGTCGCCGAGTTAGTG
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**Fig. 3.** The nucleotide sequence of the BBTV DNA-M full length isolated from Hill Banana. Amino acid sequence of the translated ORF of BBTV DNA-M is given. This nucleotide sequence was submitted in the GenBank under the accession numbers MN011923, MN011924, MN011925



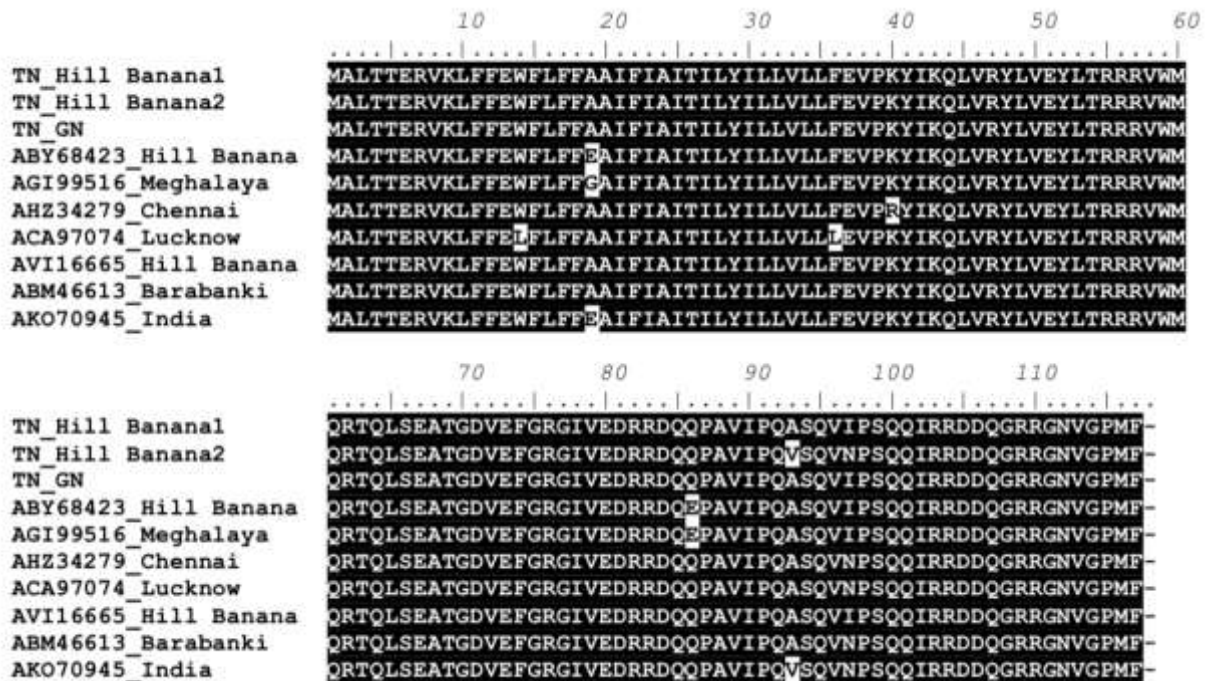


Fig. 4. Multiple sequence alignment of translated BBTV movement protein with other geographical isolates from India. Amino acid sequence variations are highlighted.

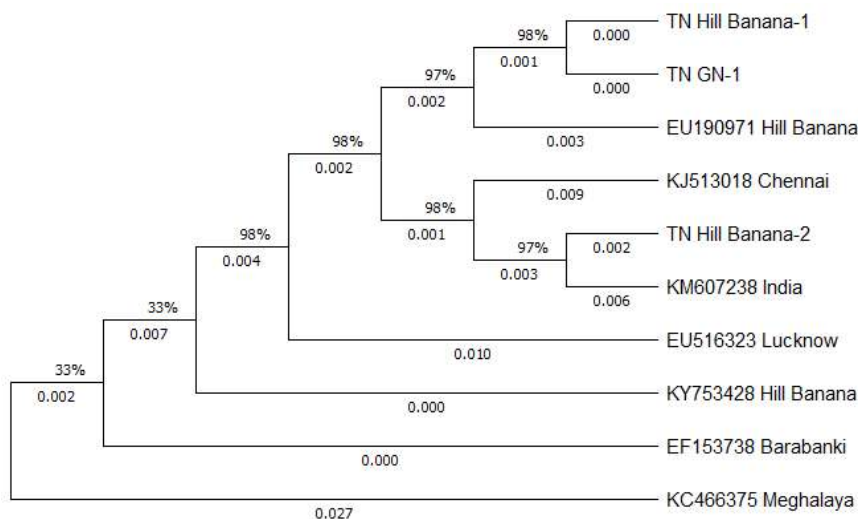


Fig. 5. Neighbour-joining phylogenetic dendrogram of BBTV DNA-M component of Hill Banana and Grand Naine samples and other BBTV isolates of India and others country sequences available in NCBI GenBank.



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