Arch Virol (2012) 157:397–400
DOI 10.1007/s00705-011-1179-5

ANNOTATED SEQUENCE RECORD

Complete genome sequence of a banana bract mosaic virus isolate infecting the French plantain cv. Nendran in India

V. Balasubramanian · R. Selvarajan

Received: 30 June 2011 / Accepted: 16 November 2011 / Published online: 2 December 2011

Abstract The first complete genome sequence of an Indian isolate (TRY) of Banana bract mosaic virus (BBrMV) was determined following virus RNA extraction from the French plantain cv. Nendran (AAB). The complete genome was 9711 nucleotides excluding the poly(A) tail and had a genome organization similar to that of a Philippine (PHI) isolate characterized earlier. When compared to BBrMV-PHI, the complete genome sequence of BBrMV-TRY was 94% identical at the nucleotide level and its ten mature proteins had amino acid sequence identities ranging from 88 to 98%. Phylogenetic analysis suggests that the BBrMV-TRY isolate is closely related to the BBrMV-PHI isolate.

Banana bract mosaic virus (BBrMV), a member of the genus *Potyvirus*, family *Potyviridae*, is the causal agent of bract mosaic disease (BBrMD) [13], which causes serious yield losses in banana and plantain in India [9, 10] and the Philippines [6]. This disease was first reported in the Philippines in 1979 [3] and subsequently has been reported from several banana-growing countries [4–6, 13]. In India, BBrMD was first reported in 1966 in plantain cv. Nendran as Kokkan disease of unknown etiology in Kerala by Samraj et al. [7]. Later the casual agent of Kokkan disease was authentically confirmed as BBrMV [4, 10]. BBrMD has now been recorded from several banana growing states in India viz., Kerala, Tamil Nadu, Karnataka and Andhra Pradesh [4, 9, 10] and has been identified as a disease of national importance in India.

BBrMV has flexuous filamentous particles (660-760 × 12 nm) with a single stranded positive sense RNA genome. The complete genome sequence of a BBrMV isolate from the Philippines (BBrMV-PHI) is 9711 nucleotides long excluding the 3′ terminal poly(A) tail [2]. This virus is transmitted by several aphid species in non-persistent manner [3]. Partial and complete coat protein sequences have been determined for some Indian isolates [4, 5], but a complete genome sequence is lacking. Here, we report the complete genome sequence of an isolate of BBrMV infecting the French plantain cultivar Nendran (AAB), which is widely grown in Tamil Nadu and Kerala states of India. This Indian isolate is designated BBrMV-TRY and compared with other isolates.

The BBrMV-TRY isolate originated from a symptomatic plantain plant that was taken from the experimental fields of the National Research Centre for Banana (NRCB), Tiruchirapalli, and then maintained under insect-proof glasshouse conditions. The complete genome of the BBrMV-TRY isolate was generated from nine overlapping fragments (nucleotide positions of each fragments are as follows: 1-1115; 951-2486; 2026-3686; 3651-5585; 5449-5744; 5586-6314; 6305-7043; 7001-8603; 8599-9711) using primers based on the nucleotide sequence of the BBrMV-PHI isolate (DQ851496). Total RNA from 100 mg of infected leaf tissues was isolated using an RNeasy Plant Mini Kit (QIAGEN Inc. Valencia, CA, USA) according to the manufacturer's instructions. First strand complementary DNA (cDNA) was synthesized using a RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas INC, Maryland, USA) with an oligo(dT) primer according to the manufacturer's instructions. The 20 µl reaction mixture was incubated at 65°C for 5 min and 42°C for 60 min and

V. Balasubramanian · R. Selvarajan (✉)
Molecular Virology Lab, Division of Crop Protection,
National Research Centre for Banana, Thogamalai road,
Thayanur Post, Tiruchirapalli 620102,
Tamil Nadu, India
e-mail: selvarajanr@gmail.com

subsequently incubated at 70°C for 10 min to inactivate the RT enzyme. PCR was done using Taq DNA polymerase (Sigma-Aldrich, USA) with 10× PCR buffer. The reaction volume of 50 µl contained 4.0 µl of cDNA, 1 µl of each primer (each 20 mM), 4.0 µl dNTPs (10 mM) and 2.5 U of enzyme. PCR amplification was performed in a Mastercycler gradient PCR machine (Eppendorf, Germany). Thermocycling parameters consisted of a single cycle of 94°C for 2 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 46-55°C for 1 min, elongation at 72°C for 2 min and a final elongation at 72°C for 10 min. The 5′-terminal sequence was amplified using a primer designed from 5′ end of the BBrMV- PHI isolate. The amplified RT-PCR products were resolved by electrophoresis through 1.5% agarose gels, and DNA was purified from the gel bands using a MinElute Gel Extraction Kit (QIAGEN Inc. Valencia, CA, USA). The purified DNA was cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA) according to the manufacturer's instructions and introduced by transformation into the competent *Escherichia coli* strain DH5α using standard molecular biology methods [8]. Plasmid DNA was isolated from an overnight culture using a GenElute Plasmid Miniprep Kit (Sigma-Aldrich, USA). The recombinant clones containing inserts were confirmed by restriction digestion with *Eco*RI. Two clones from each RT-PCR product were sequenced in both directions using an ABI PRISM BigDye Terminator Kit at Chromous Biotech Pvt Ltd, Bangalore, and the sequences were aligned using CLUSTAL W [14]. Sequence identities were calculated from the ''Sequence Identity Matrix'' using Bioedit program version 7.05 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The putative cleavage sites on the BBrMV polyprotein and conserved domains of the putative functional proteins were determined by comparison of its polyprotein sequence with those of other representative potyviruses [1]. Phylogenetic trees were generated by the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA) software program version 4.0 [12]. The BBrMV-TRY sequence determined here has been deposited in the NCBI GenBank database under accession no. HM 131454.

The complete genome of the BBrMV-TRY isolate from India is 9711 nucleotides (nt) long excluding the 3′ poly (A) tail. The 5′ untranslated region (UTR) has a G + C content of 42.2% and A + T content of 57.8%. The 5′UTR is assumed to be 128 nt and the 3′UTR (without the poly (A) tail) is 208 nt. The 5′UTR of BBrMV-TRY shared high sequence identity with that of BBrMV-PHI (98%), while the 3′UTR sequence shared 94% identity with the 3′ UTR of BBrMV-PHI. The genome consists of a single large open reading frame (ORF) of 9378 nucleotides. Similar to the BBrMV-PHI isolate, a pyrimidine residue (C) was present at the -3 position rather than a purine. However, the fact that the first few amino acids translated from this putative start codon were highly conserved when compared to many other potyviruses suggests that the first in-frame ATG triplet in this viral genome is the correct initiation codon. The AUG start codon was located at nucleotide positions 128-130, and the termination codon (UAG) at positions 9504-9506, translating into a polyprotein of 3125 amino acids with a molecular mass (Mr) of 354.43 kDa. Nine putative protease cleavage sites were identified in the BBrMV-TRY polyprotein. The serine protease catalytic residues H-X8-E-X30-GWSG are perfectly conserved in the C-terminal part of the P1 protein. The resulting P1 protein is 329 aa long and shares about 88% aa sequence identity with that of the BBrMV-PHI isolate. The cleavage site at the C-terminus of P1 occurs at the dipeptide Y/S. The HC-Pro cleavage site at the C-terminal G/G dipeptide is part of the conserved motif of the potyvirus amino acid sequence YLVG/G. The remaining seven protease recognition sites are putatively cleaved by the NIa-Pro at dipeptides Q/S, Q/N, Q/N, E/G, E/G, Q/H and Q/S, which are also found in other potyviruses. The putative cleavage sites of BBrMV-TRY are very similar to BBrMV-PHI [2].

Consistent with other potyviruses, the polyprotein of BBrMV-TRY is predicted to give rise to ten putative mature proteins. The amino acid sequence positions of each predicted protein are as follows: P1,1-329; HC-Pro, 330-786; P3, 787-1133; 6K1, 1134-1185; CI, 1186-1819; 6K2, 1820-1872; VPg, 1873-2062; NIa-Pro, 2063-2305; NIb, 2306-2825 and CP, 2826-3125. These proteins contain almost all of the conserved motifs, such as H-8X-E-30X-GWSG in the P1 protein, KITC, present as RISC, and PTK, present as PSA, in the HC-Pro region for aphid transmission, FRNK in the HC-Pro region for symptomatology and gene silencing, AVGSGKST in the CI region for helicase function, NMYG in the VPg for viral RNA attachment function, GDD in the NIb for replicase activity, and DAG in the CP region for aphid transmission [11, 15]. Most of these motifs are highly conserved in comparison with other reported potyviruses, with the exception that the T and K residues of the PTK motif in the HC-Pro-coding region of BBrMV were replaced by S and A, respectively. These replacements were unexpected because this motif is identical in all potyviruses with fully sequenced genomes. Similarly, the K and T residues of the KITC motif in HC-Pro are replaced with R and S. As this change is also observed in the BBrMV-PHI isolate, the significance of this change in this motif on aphid transmission is not known. The sequence was verified, however, by PCR using specific primers to amplify across this region [2]. In the VPg protein, the N residue of the NMYG motif of the BBrMV-PHI isolate was replaced by an S residue in BBrMV-TRY. The coat protein gene potentially codes for a protein of 33.4 kDa.

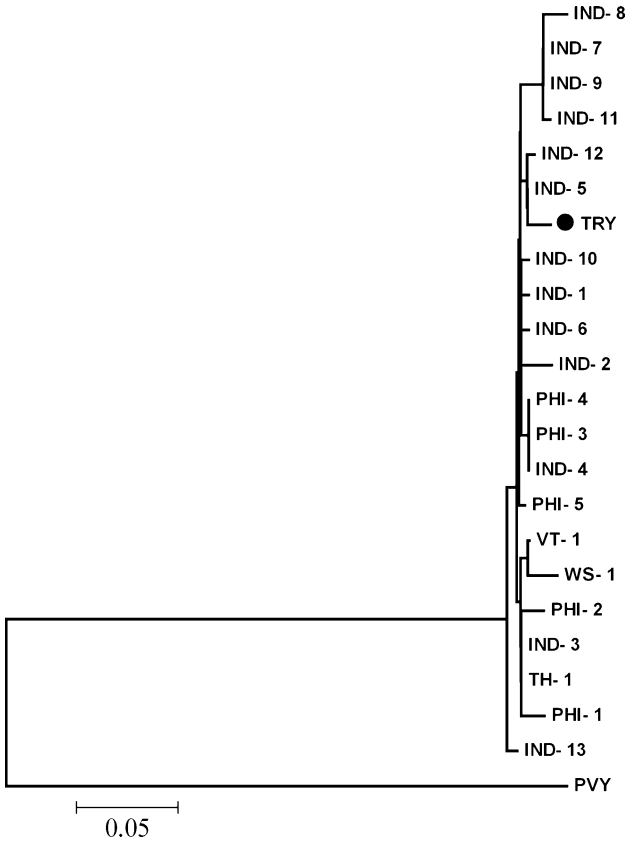

Fig. 1 Neighbor-joining tree based on the deduced amino acid sequences of the BBrMV-TRY (HM131454) coat protein with other BBrMV isolates. Bootstrap analysis was applied using 1000 replicates. The scale bar represents a genetic distance of 0.05. The phylogenetic tree was generated using MEGA4 and the neighbor-joining algorithm. The distances computed using the Poisson correction method were derived from a multiple alignment using ClustalW. The abbreviations of the names of the isolates included in the analysis and their corresponding accession numbers (in parentheses) are as follows. *PHI-1*, Philippines (AFO71590); *PHI-2*, Philippines (AFO71585); *PHI-3*, Philippines (AFO71586); *PHI-4*, Philippines (EU414267); *PHI-5*, Philippines (DQ851496); *WS-1*, WesternSamoa (AFO71587); *VT-1*, Vietnam (AFO71588); *TH-1*, Thailand (AFO71589); *IND-1*, India, Coimbatore (AFO71582); *IND-2*, India, Coimbatore (AFO71583); *IND-3*, India, Tiruchchirappalli (AFO71584); *IND-4*, India (EU699770); *IND-5*, India, Tiruchchirappalli (EU009210); *IND-6*, India, Coimbatore (AY494979); *IND-7*, India, Andhra Pradesh (AY953427); *IND-8*, India, Andhra Pradesh (HM348778); *IND-9*, India, Andhra Pradesh (HM348779); *IND-10*, India, Andhra Pradesh (HM348780); *IND-11*, India, Andhra Pradesh (HM348781); *IND-12*, India, Andhra Pradesh (HM348782); *IND-13*, India, Karnataka (EF654655). The tree was rooted to potato virus Y (PVY; NC001616)

BBrMV-TRY shared the highest polyprotein amino acid sequence identity with the BBrMV-PHI isolate (96%). For the individual proteins, the two geographically distinct BBrMV isolates shared the following amino acid sequence identities: P1, 88%; HC-Pro, 98%; P3, 95%; 6K1, 98%; CI, 98%; 6K2, 96%; VPg, 97%; NIa, 98%; NIb, 97%; and CP, 98%. The nucleotide sequences of the complete genomes of the two isolates were 94% identical, and the CPs were 95% identical at the nucleotide level. Phylogenetic analysis for the amino acid sequences of coat protein gene was performed for 21 isolates of BBrMV, using PVY as an outgroup (Fig. 1). The consensus tree from the bootstrap analysis confirmed that the BBrMV-TRY isolate was closely related to isolates IND 5 and IND 12 and clustered with most of the BBrMV isolates, including the BBrMV-PHI isolate. The IND 2 isolate from the Coimbatore region of Tamil Nadu state was distinctly placed compared to other BBrMV isolates. This study provides the first report of the complete nucleotide sequence of BBrMV from India. This information will assist in the development of molecular diagnostic tests and effective disease control strategies.

Acknowledgments The authors are thankful to the Director of the National Research Centre for Banana (NRCB), Tiruchirapalli, T.N (India), for providing the necessary facilities. The Indian Council of Agriculture Research (ICAR) Project No.16 (20/2004-Hort.II) under the network project on "Diagnosis of emerging plant viruses" is duly acknowledged for financial support.

References

1. Adams MJ, Antoniw JF, Beaudoin F (2005) Overview and analysis of the polyprotein cleavage sites in the family *Potyviridae*. Mol Plant Pathol 6:471–487
2. Ha C, Coombs S, Revil PA, Harding RM, Vu M, Dale JL (2008) Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses. Arch Virol 153:25–36
3. Magnaye LV, Espino RRC (1990) Note: Banana bract mosaic, a new disease of banana I. Symptomatology. Philippine Agric 73:55–59
4. Rodoni BC, Ahlawat YS, Varma A, Dale JL, Harding RM (1997) Identification and characterization of banana bract mosaic virus in India. Plant Dis 81:669–672
5. Rodoni BC, Dale JL, Harding RM (1999) Characterization and expression of the coat protein-coding region of banana bract mosaic potyvirus, development of diagnostic assays and detection of the virus in banana plants from five countries in Southeast Asia. Arch Virol 144:1725–1737
6. Roperos NI, Magnaye LV (1991) Status of banana diseases in Asia and the Pacific. In: Valmayor R (ed) International network for the improvement of banana and plantain. Montpellier, France
7. Samraj J, Menon MR, Christudas SP, Satyarajan PK (1966) Kokkan, a new disease of banana (*Musa paradisiaca* Linn.). Agric Res J Kerala 4:116
8. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
9. Selvarajan R, Balasubramanian V (2008) Banana viruses. In: Rao GP (ed) Characterization diagnosis & management of plant viruses. Studium Press, Houston, pp 109–124
10. Singh SJ, Selvarajan R, Singh HP (2000) Detection of bract mosaic virus (kokkan disease) by electron microscopy and serology. In: Singh HP (eds) Banana-improvement, production and utilization. Proceedings of Conference in AIPUB, Tiruchirappali, India, pp 381–383
11. Shukla DD, Ward CR, Brunt AA (1994) The *Potyviridae*. CAB International, Wallingford

12. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
13. Thomas JE, Geering ADW, Gambley CF, Kessling AF, White M (1997) Purification, properties and diagnosis of banana bract mosaic potyvirus and its distinction from abaca mosaic potyvirus. Phytopathology 87:698–705
14. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties, and weight matrix choice. Nucleic Acids Res 22:4673–4680
15. Urcuqui-Inchima S, Haenni AL, Bernardi F (2001) Potyvirus proteins: a wealth of functions. Virus Res 74:157–175