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SHORT COMMUNICATION

Complete genome sequencing of banana bract mosaic virus isolate infecting cardamom revealed its closeness to banana infecting isolate from India

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Abstract The complete genome of banana bract mosaic virus (BBrMV), a Potyvirus belonging to the family Potyviridae causing chlorotic streak disease of cardamom (*Elettaria cardamomum*) in India was determined for the first time from a naturally infected cardamom var. Njallani Green Gold through reverse transcription PCR using nine sets of primers designed to different overlapping regions of the genome. The complete genome has 9708 nucleotides excluding poly (A) tail and has the genome organization similar to that of BBrMV isolates infecting banana and flowering ginger (*Alpinia purpurata*). The virus has a single open reading frame of 9372 nucleotides that encodes for a polypeptide of 3124 amino acids which is later cleaved into ten matured proteins. The length and arrangements of different proteins in BBrMV-Cardamom was similar to other BBrMV isolates except for the P1 protein that showed a single amino acid deletion. Comparison with three available complete genome sequences revealed that, BBrMV-Cardamom isolate is more closer to BBrMV-Banana isolate from India (BBrMV-TRY) (96.7% identity) than to BBrMV-Banana isolate from Philippines and flowering ginger isolates from USA (94.5%). Analysis of polyprotein and their individual proteins also showed close identity of BBrMV-Cardamom and BBrMV-TRY. The phylogenetic analysis also suggested that BBrMV-Cardamom isolate is closely related to other BBrMV isolates.

Keywords Banana bract mosaic virus · Cardamom · Complete genome · Sequence analysis · Phylogenetic analysis

Banana bract mosaic virus (BBrMV) (genus: *Potyvirus*; family: Potyviridae) was first reported from banana in the Philippines during 1979. Till date BBrMV infecting banana has been reported only in a few banana growing countries of Africa and Asia such as Costa Rica, Columbia, Ecuador, India, Philippines, Sri Lanka, Western Samoa, Thailand and Vietnam [2, 4, 6, 8, 10, 13]. The banana plant infected with BBrMV is characterized by mosaic symptoms in bracts, bunch distortion, streaks on the petiole and pseudostem. It is spread through aphids and vegetative planting material. Till recently, BBrMV was known to infect only *Musa* spp. In the year 2010, BBrMV causing mosaic streaking, cupping and browning of flowers of flowering ginger (*Alpinia purpurata*) (Fam: Anigiberaceae) in Hawaii, USA was reported [15]. Subsequently in 2012 our lab reported infection of cardamom (*Elettaria cardamomum*) with BBrMV that cause chlorotic streak disease for the first time [12]. Cardamom, a member of the Zingiberaceae is one of the economically important spice crops mainly grown in India, Sri Lanka, Papua New Guinea, Tanzania and Guatemala [9]. The cardamom of commerce is the dried fruit (capsules) of the cardamom plant. The chlorotic streak incited by BBrMV in cardamom is characterized by intravenous streaks along the veins and midrib, mottling on the pseudostem and petioles, loosening of leaf sheath and stunting of plants [12].

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BBrMV is a flexuous rod shaped virus with 700–850 nm in length and 12–15 nm in width. It has a single stranded RNA genome of 9711–9713 bases excluding the poly (A) tail at the 3′ end. The genome has untranslated region (UTR) both at 5′ and 3′ end and a single open reading frame (ORF) coding for a large polypeptide that is co-translationally processed into 10 matured proteins. So far, complete genome sequence of only three isolates of BBrMV (two isolates infecting banana, one each from Philippines and India [1, 5] and one isolate infecting flowering ginger in Hawaii, USA [16] are available. Till date only coat protein (CP) gene sequence of BBrMV infecting cardamom is available [12]. Here, we report the complete genome sequence of an isolate of BBrMV infecting cardamom (BBrMV-Cardamom) in India.

Cardamom var. Njallani Green Gold naturally infected with BBrMV showing typical symptoms collected from Wayanad District, Kerala, India was used for the study. Total RNA was extracted from 100 mg of leaf tissue using the method described earlier [12]. The complete genome of the BBrMV was amplified through RT-PCR using nine sets of primers designed based on multiple sequence alignment of all three available complete BBrMV sequences that generated overlapping sequences at the ends (Supplementary Table 1). RT-PCR products were cloned into pTZ57R/T (Fermentas, USA) and selected clones were sequenced twice from both directions at the automated sequencing facility available at AgriGenome, Kochi, Kerala, India. The sequences were assembled using SEQAID [7] and open reading frames (ORFs) were identified using by ORF finder [www.ncbi.nlm.nih.gov/projects/gorf]. Sequences of complete genome of all three available BBrMV isolates and partial genome sequence of BBrMV isolates were retrieved from NCBI database and used for analysis. The analyses were carried out using the complete genome in the form of nucleotide and translated amino acid sequences. Sequences were aligned using clustalX [14], sequence identity percentage was calculated using Bioedit. Phylogenetic analysis was performed using Bayesian algorithm in MrBayes [11] with a bootstrap analysis of 10,000 replicates.

RT-PCR gave expected products in different primer pairs used for amplification (Supplementary Fig. 1). The sequences were assembled, analysed and ORFs were predicted and submitted to GenBank which is available as accession number MG758140. The complete genome of BBrMV cardamom isolate (designated as BBrMV-Cardamom) comprised of 9708 nucleotides (nt) excluding poly (A) tail with a GC content of 41.2%. The genome consists of a single ORF of 9372 nt potentially coding for a large polyprotein of 3124 amino acids with a MW 354.323 kDa and untranslated regions both at 5′ (128 nt) and 3′ (208 nt) ends (Fig. 1a). The complete nt and amino acid sequence of the polyprotein of BBrMV-Cardamom shared maximum identity of 96.7 and 97.2% respectively, with BBrMV isolate infecting banana from India (designated as BBrMV-TRY) (GenBank accession number HM131454) [1] followed by BBrMV isolate infecting flowering ginger (*Alpinia purpurata*) in Hawaii, USA (94.5 and 96.4%) (designated as BBrMV-Ginger) (GenBank accession number KT456531) [16] and BBrMV isolate infecting banana in Philippines (94.5 and 96.3%) (designated as BBrMV-PHI) (GenBank accession number DQ851496) [5]. The 5′ end of BBrMV-Cardamom is assumed to be of 128 nt while 3′ UTR is 208 nt. The A/T rich (60.9%) 5′ UTR of BBrMV-Cardamom isolate shared highest identity of 94.5% with BBrMV-TRY and BBrMV-PHI while 3′ UTR of BBrMV-Cardamom (with AT content of 61.5%) shared highest identity (96.5%) with BBrMV-PHI followed by BBrMV-Ginger (95.1%) and BBrMV-TRY (94.1%). Like other BBrMV isolates, BBrMV-Cardamom also contained two potybox-like blocks-potybox 'a' and potybox 'b' in its 5′ UTR. However, a single 'A' residue in BBrMV-Cardamom, BBrMV-TRY and BBrMV-PHI is replaced by a 'G' residue in BBrMV-Ginger [16]. The initiation codon 'ATG' is similar in all BBrMV isolates including BBrMV-Cardamom while termination codon 'UAA' is similar to BBrMV-Ginger [16] while it is 'UAG' in BBrMV-TRY and BBrMV-PHI [1, 5].

The ORF encodes a polypeptide consisting of a viral P1 protein (328 amino acids) (from 129 to 1112 nt), helper component protein (HC-Pro) (457 amino acids) (from 1113 to 2483 nt), P3 protein (347 amino acids) (from 2484 to 3524 nt), 6K1 protein (52 amino acids) (from 3525 to 3680 nt), cylindrical inclusion protein (CI) (634 amino acids) (from 3681 to 5582 nt), 6K2 protein (53 amino acids) (from 5583 to 5741 nt), VPg protein (190 amino acids) (from 5742 to 6311 nt), nuclear inclusion protein a (NIa) (243 amino acids) (from 6312 to 7040 nt), nuclear inclusion protein b (NIb) (520 amino acids) (from 7041 to 8600 nt) and coat protein (CP) (300 amino acids) (from 8601 to 9500 nt) (Fig. 1a). The + 2 ORF coding for *pipo* (Pretty Interesting Potyviridae ORF [3] is located at nt position from 2948 to 3194 in the BBrMV-Cardamom (Fig. 1a). All the nine cleavage sites of the putative polyprotein of BBrMV-Cardamom (Y/S, G/G, Q/S, Q/N, Q/N, E/N, E/G, Q/H and Q/S) were similar to other three BBrMV isolates. Many of the potyviral proteins have multiple roles like, P1 is a protease involved in virus-host interactions; HC-Pro is required for aphid transmission, replication and also acts as a viral RNA silencing suppressor besides acting as a protease; P3 and 6K1are involved in replication; PIPO is required for cell-to-cell movement; CI is a RNA helicase involved in cell-to-cell movement and replication; 6K2 is involved in membrane modifications for formation of VRC-like structures; VPg interacts with eukaryotic initiation factor 4E which is essential to viral infectivity and

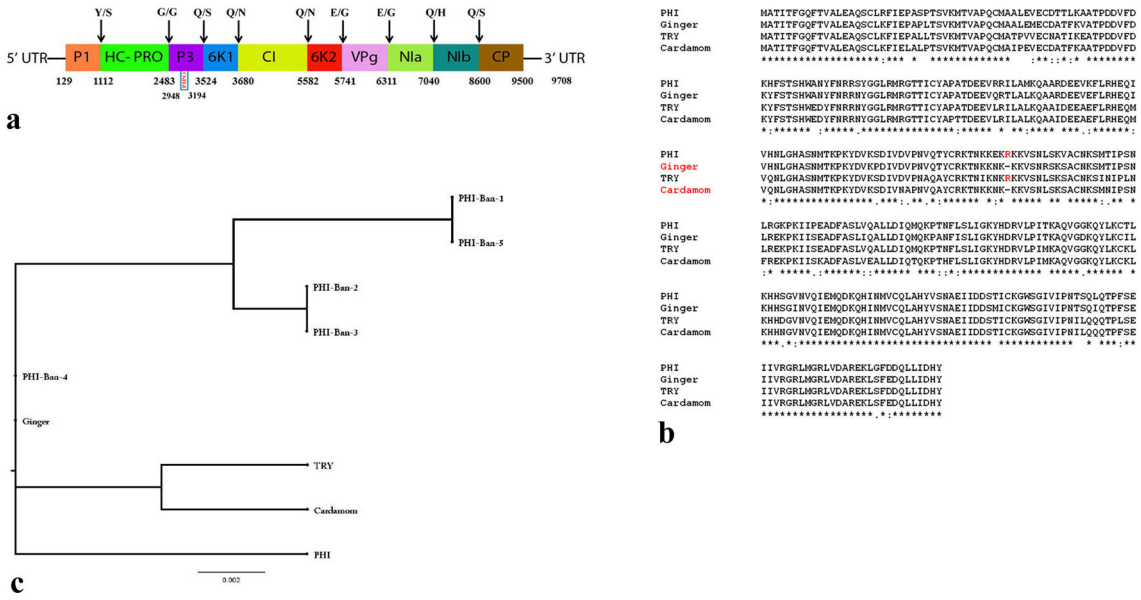

Fig. 1 Genome map and analysis of banana bract mosaic virus infecting cardamom (BBrMV-Cardamom). **a** The position and dipeptide motif of the cleavage sites are indicated. Expression of Pretty Interesting Potyviridae ORF (PIPO) in the + 2 frame is also shown. The number shows the start position of each of the proteins. **b** Multiple sequence alignment of the amino acid sequences of the P1 protein of all four BBrMV isolates showing a deletion of one amino acid in the BBrMV-Cardamom and BBrMV-Ginger. **c** Phylogenetic tree based on the predicted amino acid sequences of BBrMV-Cardamom cylindrical inclusion protein with corresponding regions of other BBrMV isolates. The designations of names of isolates included and their GenBank accession numbers: PHI-Ban-1 (Philippines-Banana; JN791676), PHI-Ban-2 (Philippines-Banana; JN791675), PHI-Ban-3 (Philippines-Banana; JN791674), PHI-Ban-4 (Philippines-Banana; JN791673), PHI-Ban-5 (Philippines-Banana; JN791672), PHI (Philippines-Banana; DQ851496), TRY (India-Banana; HM131454), Ginger (USA-flowering ginger; KT456531), Cardamom (India-Cardamom; MG758140, This study)

cell-to-cell movement; NIa is a protease, interacts with VPg and required for infectivity; NIb is the viral replicase, forms crystalline inclusion bodies; CP is involved in encapsidation and cell-to-cell movement [1, 3, 5, 16].

When nt sequences of all the 10 proteins were compared individually, the BBrMV-Cardamom showed maximum identity with BBrMV-TRY followed by BBrMV-Ginger and BBrMV-PHI. Similarly, when amino acid sequences of all individual proteins including PIPO were compared, BBrMV-Cardamom showed highest identity with BBrMV-TRY followed by BBrMV-Ginger and BBrMV-PHI except for the 6K2 protein where BBrMV-Cardamom shared maximum identity with BBrMV-Ginger and BBrMV-PHI (Table 1). Comparison also revealed closeness of BBrMV-Ginger with BBrMV-PHI for nt and amino acid sequences of all 11 proteins (Table 1). Comparison of amino acid sequences of all 10 proteins and the PIPO among all four available complete BBrMV genomes showed 6K1 protein as the highly conserved while the P1 protein as the least conserved proteins (Table 1). Compared to banana infecting BBrMV isolates (BBrMV-TRY and BBrMV-PHI), the P1 protein showed one amino acid deletion at the same location in non-banana infecting BBrMV isolates (BBrMV-Cardamom and BBrMV-Ginger), the significance of this deletion is not known (Fig. 1b). As reported in other BBrMV and potyvirus isolates, BBrMV-Cardamom also

Table 1 Pair wise per cent identities in the amino acid sequences of the polyprotein and each of the individual proteins of BBrMV-Cardamom and other BBrMV isolates

BBrMV isolates	Polyprotein	P1	HC-Pro	P3	6K1	CI	6K2	VPg	NIa	NIb	CP	PIPO
Cardamom-TRY	97.2	93.0	98.9	97.6	100	98.7	96.2	96.3	98.7	97.1	96.3	98.7
Cardamom-Ginger	96.4	88.1	98.4	97.6	100	98.2	100	95.7	98.3	96.3	94.4	97.5
Cardamom-PHI	96.3	87.5	98.2	97.1	98.0	97.9	100	96.8	97.9	96.3	94.6	96.2
Ginger-PHI	97.5	90.8	98.0	96.5	98.0	99.0	100	98.9	98.7	98	97.5	98.7
TRY-PHI	96.4	88.1	98.0	95.3	98.0	98.2	96.2	97.3	98.3	96.5	95.4	95.0
Ginger-TRY	96.5	88.1	98.2	95.3	100	98.5	96.2	96.3	98.7	96.5	95.2	96.2

contained several functional motifs in its polyprotein such as H-X_8-E-X_{30}-CWSG (in the P1 protein), GYCY-X_{71}-H, RISC, PSA, FRNK, ERNK, IGR and CCC (in the HC-Pro protein), AVGSGKST (in the CI protein), NMYG (in the VPg protein), H-X_{34}-D-X_{67}-GDCG-X_{14}-H (in the NIa protein), GDD (in the NIb protein) and DAG, QMKAA (in the CP) [1, 5, 16].

Phylogenetic analysis of the complete genome both by using nt and amino acid sequences of all four available BBrMV isolates showed closeness of BBrMV-Cardamom with BBrMV-TRY while, BBrMV-Ginger was grouped with BBrMV-PHI. It is reported that sequence of the CI gene reflects the complete genome of potyviruses and hence can be used for identification and differentiation of genera and species [5]. Thus, phylogenetic analysis based on all nine available CI sequence was done which also grouped BBrMV-Cardamom with BBrMV-TRY (Fig. 1c). Further, phylogenetic tree based selected 44 coat protein amino acid sequences (Supplementary Table 2) also grouped BBrMV-Cardamom with BBrMV isolates from India (Supplementary Fig. 2). Analysis showed high variability among Indian population of BBrMV compared to BBrMV populations from South East Asian countries.

In conclusion, this study provides first report of the complete genome sequence of BBrMV infecting cardamom and its comparison with other three BBrMV isolates infecting banana and flowering ginger.

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