

First Full Length Genome Sequence of Bean Common Mosaic Necrosis Virus (BCMNV) Isolated from Common Bean in Western Kenya

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Abstract: Bean common mosaic necrosis virus (BCMNV) is one of the most common and most destructive viruses of common bean and can cause a yield loss as high as 100%. The common bean (*Phaseolus vulgaris* L) is an important legume crop for food and cash in Kenya. In Kenya, there is inadequate documentation on the strains of the virus infecting common bean. This information is crucial in devising control measures. This study therefore, sought to characterize BCMNV isolates from western Kenya. Leafy samples showing virus-like symptoms were collected and analysed by Enzyme linked immunosorbent Assay (ELISA) and or next generation sequencing (NGS). Extraction of total RNA from ELISA positive samples was done using RNeasy Plant Mini Kit and NGS carried out following Illumina protocol to determine diversity of the virus. NGS data was trimmed and the sequence reads assembled into contigs, which were analyzed against virus sequence database. Phylogenetic analyses and comparisons were performed using MEGA7 program. The first complete genome sequence of Bean common mosaic virus (BCMNV) is reported from a Kenyan isolate. NGS technology revealed full-length sequence of BCMNV from an isolate BG 12 from Bungoma County with a genome of 9584 nt in length. Phylogenetic analysis of full-length sequences available through the Genbank clustered the isolate with the Tanzanian isolate strain TN-1 and two USA isolates, TN1a and NL-3K.

Keywords: BCMNV, Genome, Sequence, Kenya

1. Introduction

Bean common mosaic necrosis virus (BCMNV) belongs to the Family *Potyviridae*, genus *Potyvirus*, which is the largest of the eight genera currently assigned to the family by the International Committee on Taxonomy of Viruses [1]. The virus infects legumes in many parts of the world and causes significant yield losses of up to 100% [2]. BCMNV is seed-borne and its spread in the field by aphids in a non-persistent manner. BCMNV is monopartite, single stranded positive-sense RNA virus with flexuous rod-shaped

virions that are 750 nm in length with a diameter of 11–13 nm [3]. The virion RNA molecule is approximately 10 kb long with a 3' terminal polyA tail [4]. The genome has the 5' end covalently linked to a virus-encoded protein (VPg) and the 3' end polyadenylated. An important property of potyviruses is that 10 out of 11 proteins that they encode are derived from a single polypeptide precursor [5]. The other protein, P3N-PIPO, is separately synthesized from a small ORF [6]. Discovery of BCMNV began in 1917 when its close member BCMV was identified and named variously as Bean virus 1, Bean mosaic virus, and *Phaseolus* virus 1 [7]. Until 1943, it was presumed that BCMV strains were identical pathogenically since the

originally identified BCMV isolate was lost [8]. Differential symptoms on ten bean cultivars identified the distinct pathogenic groups of BCMV in 1943. The results were utilised in taxonomy classifying the strains into the seven pathogenic groups (I–VII). BCMV strains were later categorized to A and B serotypes based on differential responses of various bean cultivars to infection, coat protein (CP) serology and proteolytic digests of CPs analysis [9]. Berger *et al.* (1997) [10] later reclassified serotypes A and B as BCMNV and BCMV respectively. BCMNV has five identified strains i.e. TN-1, NL-8, NL-3, NL-3K and NL-5 [11]. Strains of BCMV are many including very distinct viruses, such as *Azuki bean mosaic virus* (AzMV), *Blackeye cowpea mosaic virus* (BICMV), *Peanut stripe virus* (PStV) and *Dendrobium mosaic virus* (DeMV) [12]. By 2015, there were 22 BCMV full length genome sequences and 9 BCMNV full length genome sequences available through NCBI currently [13]. 3' UTR sequence data and CP genes have been used to group BCMV isolates [14]. BCMNV is transmitted by seeds up to 83% in *Phaseolus vulgaris* L and by several aphid species [15]. Aphid species including; Bean aphid (*Aphis fabae*), Cowpea aphid (*Aphis craccivora*), Pea aphid (*Acyrtosiphon pisum*), Green peach aphid (*Myzus persicae*) and Potato aphid (*Macrosiphum euphorbiae*) are known to transmit the virus [16]. Seed transmission occurs irregularly and may majorly depend on age of the plant at the time of infection, virus strain and bean variety. Aphid transmission occurs non-persistently and spread the virus on short distances as compared to seed transmission that may spread viruses around the world [17]. Infected plants are produced even at low seed transmission and at most suitable time for vector transmission may result in spreading the virus in the field very fast [18]. Systemically infected plants prior to flowering may produce infected seeds and this may account for the higher transmission rates [19]. Seed transmission is minimal or may not occur if the infection occurs after flowering [20]. *Phaseolus* species form the natural hosts of BCMNV mainly restricted to *P. vulgaris* [21]. However, the virus has been isolated naturally from other leguminous species including *Phaseolus lunatus*, *Phaseolus acutifolius*, *Arachis hypogaea*, *Cajanus cajan*, *Bauhinia purpurea*, *Centrosema pubescens*, *Chenopodium quinoa*, *Crotalaria juncea*, *Crotalaria incana*, *Crotalaria spectabilis*, *Cucumis sativus*, *Lupinus angustifolius*, *Glycine max*, *Lablab purpureus*, *Lupinus albus*, *Lupinus luteus*, *Macroptilium lathyroides*, *Macroptilium atropurpureum*, *Medicago sativa*, *Melilotus alba*, *Trifolium incarnatum*, *Pisum sativum*, *Vicia sativa*, *Vigna subterranea*, *Rhynchosia minima*, *Vigna unguiculata*, *Vigna vexillata*, *Sesbania herbacea*, *Trifolium pretense*, *Trifolium repens*, *Trifolium subterraneum*, *Trifolium hybridum*, *Vicia villosa* and *Vigna radiata*.

2. Materials and Methods

During the short rain season in September to October 2016 and the long rain season in April to June 2017, surveys were conducted in six agro ecological zones that are in major

common bean growing counties of Western Kenya. Symptomatic and asymptomatic leaf samples showing virus-like symptoms of mosaic, leaf distortion, downward curling, mottling, vein necrosis, local lesions, stunting or a combination of these were collected. Serological and molecular analysis.

Triple Antibody Sandwich (TAS) ELISA was conducted following manufacturers (DSMZ, Germany) protocol. 65 out of 240 of the samples collected, tested positive for BCMNV. Eleven TAS ELISA positive samples were further analysed by next generation sequencing.

Total RNA was extracted from semi-purified virions using All Prep RNA Mini Kit (Qiagen RNAeasy) according to the manufacturers' recommendation. Before starting, 10 µl Mercaptoethanol was added to Buffer RLT or RLC. 44 ml ethanol was added to concentrated Buffer RPE. 100 mg ca. plant material (3 cm x 3 cm) was ground in a sample bag in liquid N₂ and 450–1000 µl RLT or RLC added and grinded again. 450 µl of this lysate was transferred to a Qias shredder and centrifuged 2' at full speed. The flow-through was transferred to a new tube without disturbing the cell-debris pellet. 0.5 volume (225 µl) abs. ETOH was added and mixed by pipetting. The sample including any precipitate that may have formed (650 µl) was transferred to an RNeasy spin column, Centrifuged 15s at 10000 rpm and the flow-through discarded. 700 µl buffer RWE was added and centrifuged 15 s at 10000 rpm to wash the spin column membrane. The flow-through was discarded and the collection tube reused. 500 µl buffer RPE was added and centrifuged 15s at 10000 rpm and the flow-through discarded. 500 µl buffer RPE was added again and centrifuged 2 min at 10000 rpm, the flow-through then discarded. The spin column was placed in a new 1.5 ml tube and 50 µl RNase free water directly added to the membrane then Centrifuged 1 min at 10000 rpm. Final eluate contains purified RNA. RNA isolated from host plants was used in molecular analysis. RNA was quantified and applied in RNA Stable (Biomatrix). A spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure total RNA concentration and purity. To check the integrity of the total RNA, we used the Biological analyzer Agilent 2100 (Agilent, Santa Clara, CA, USA). Eleven mRNA-seq libraries were prepared at the BeCA-ILRI using the Illumina TruSeq Stranded mRNA Library preparation kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. Libraries were pooled for multiplexed sequencing using an Illumina MiSeq System to generate single end (SE) reads of 50 nt. Trimmed reads were used for de novo assembly and contigs aligned to the viral genomes database (<ftp://ftp.ncbi.nih.gov/genomes/Viruses/all.fna.tar.gz/>, downloaded on October 2017) using CLC Genomics Workbench 10.1.2. The assembled contigs were subjected to a BLASTn search against the GenBank database [22]. Complete and partial BCMNV sequences used for comparison and phylogenetic analyses were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic analyses and comparisons were performed using the MEGA v. 7 [23] and Dna SP v. 5 programs [24].

3. Results and Discussion

The BCMNV genome of isolate BCMNV KG12 has 9627 nucleotides. The sequence has been deposited in DNA Data Bank of Japan (DDBJ) database with accession number (LC493096.1) The Kenyan isolate of BCMNV is similar to that of other potyviruses [25]. During BLASTn search, The BCMNV KG12 isolate sequence was 94 - 99% identical to 11 BCMNV complete genomes obtained from the Genbank and therefore show a close evolutionary relationship. The comparison of this Kenyan isolate with eleven complete genomes from the GenBank shows a small variation of less than 10%.

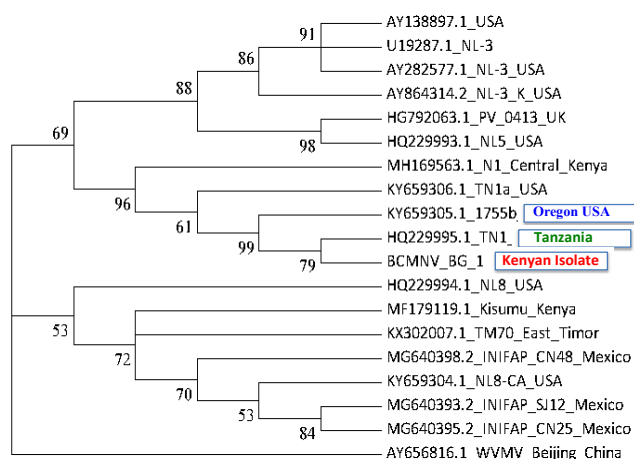


Figure 1. A maximum likelihood phylogenetic tree of BCMNV isolate BCMNV BG2. Generated using Mega 7 (Tamura-Nei default settings) from the alignment of seventeen full-length genomic sequences for BCMNV and an outgroup *Wisteria vein mosaic virus* (WVMV).

Phylogenetic analysis of BCMNV BG 12 isolate nucleotide sequences conducted using MEGA7 and the maximum likelihood algorithm to investigate the relationship between the isolate and 18 other BCMNV sequences in the GenBank as of October 2018 (Figure 1) placed BCMNV BG 12 isolate alongside an isolate from Oregon and that from Tanzania. This is an indication that the three isolates are closely related and all belong to strain TN-1 (Tanzania). This can only be possible if the Oregon isolate was either taken from East Africa or the East African ones came from there most likely in bean or other legume seeds for planting. The coverage of sequence using the conventional methods such as PCR succeeded by Sanger sequencing is relatively low and the technique is time consuming while NGS approaches give out numerous reads that are large enough to detect the target virus's quasi-species. Moreover, prior knowledge of the virus sequence is not to reveal the sequence of a virus or viroid, which hitherto may have not been known. Hence, several studies associated with virus and viroid quasi-species, prefer to use diverse NGS techniques instead of those based on the conventional PCR [26].

4. Conclusion

Symptoms of BCMNV are indistinguishable from other

viruses in the field especially in western Kenya. Molecular detection for BCMNV being (+) ssRNA genome requires extraction of total RNA. The detection of BCMNV from samples together with information on bean varieties is important to define actions necessary to manage the virus in western Kenya. The application of next-generation sequencing forms a very efficient method in detection of plant viruses. Identification of viral RNA genomes in infected plant material by NGS is possible through the extraction and sequencing of total RNA or double stranded RNA. Diversity of BCMNV is less varied even with the geographical distance. This knowledge of BCMNV strain spectrum in Kenya is an important pre-requisite in exploitation of disease management through host resistance, which is the only durable and economic method of managing Bean common mosaic disease.

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