Annotated sequence First report of Soybean chlorotic blotch virus infecting Okra (Abelmoschus esculentus) in Cameroon

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Research Article

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Abstract

Leaves from okra (*Abelmoschus esculentus*) plants exhibiting unusual viral disease symptoms were tested for the presence of begomovirus infection. The putative full-length *Soybean chlorotic blotch virus* (SbCBV) components A and B molecules, respectively were amplified, sequenced, and analyzed. This is the first study identifying SbCBV, a bipartite begomovirus infecting okra in Cameroon and the centra Central African region.

Full Text

Okra leaf curl disease (OLCD) remains a major production constraint of okra in West and Central Africa and has been reported in Burkina Faso, Cameroon, Chad, Ghana, Mali, Nigeria and the Ivory Coast [1-4]. Throughout western Africa, from where okra (*Abelmoschus esculentus*; family Malvaceae) is believed to have originated, OLCD is the most damaging viral disease of okra. Previously, the causal agent was identified as a begomovirus based on serological and electron microscopic evidence [1-2, 5]. Of late, cotton leaf curl Gezira virus (CLCuGeV) has been associated with OLCD in Burkina Faso, Cameroon, Niger and Sudan [4, 6-10], Okra leaf curl Cameroon virus (OLCuCMV) has so far been associated with OLCD only in Cameroon [4], and okra yellow crinkle virus (OYCrV) has been associated with the disease in Cameroon and Mali [3, 4, 11]. All three; CLCuGeV, OLCuCMV and OYCrV have been isolated with various associated betasatellites and/or alphasatellites [3, 4, 7-10, 12]. With the identification of OLCuCMV and new alphasatelites in Burkina Faso and Cameroon [12], the observation of unusual symptoms on okra (Fig 1), the importance of deep sequencing in pathogens identification and discovery and a heavy infestation with whiteflies; the insect vector for Begomoviruses, the objective of this study was to identify and characterize the suspect begomovirus (es) and the associated satDNAs, if present associated with the unusual OLCD observed in Ekona , in Fako Division, in Southwestern Cameroon.

Leaf samples were collected from three symptomatic and three asymptomatic okra plants from Ekona ($4^{o}13\ 49$ N and $9^{o}20\ 12$ E), a village in the leeward side of mount Cameroon, Fako Division in Southwestern Cameroon. The leaves were pressed onto FTA Classic Cards (Whatman International Ltd, Maidstone, UK) as previously described in Leke et al. [13]; Owor et al. [14]. The viral DNA was recovered from the FTA classic cards following the method described in Leke et al. [13]; Owor et al. [14]; Ndunguru et al. [15]. The viral circular, ssDNA was enriched in each of the samples by rolling cycle amplification (RCA) (GE Healthcare Bio-Science, Piscataway, NJ). The RCA products from the symptomatic as well as asymptomatic plants were pooled to constitute OEK13 for the symptomatic plans and OEK_C13 for the asymptomatic plants. The two samples were subjected to Illumina sequencing (MisEquation 2 × 150-bp configuration). The sequences were assembled using UGENE ver 33.0 [16]. The putative full-length begomovirus sequences were 2,671-bp for DNA-A and 2,649-bp for DNA-B.

The BLASTn [17] and pairwise SDT [18] analyses of the full-length begomovirus sequences from Ekona, Cameroon (OEK13) revealed their closest match at 98.7% for the DNA-A (KT444615) component to the DNA-A component of *Soybean chlorotic blotch virus* (SbCBV), identified from Soybean in Nigeria [19] in

West Africa and cassava in Cameroon [20] in Central Africa. Similar analyses for the DNA-B (KT44416) component revealed a similar relatedness at 98.4% to the DNA-B component of SbCBV identified from cassava in Cameroon and Togo [20]. As expected, the genome sequences from okra in Ekona, Southwestern Cameroon was observed to have a bipartite begomovirus genome organization. Thus, DNA-A contained the open reading frames (ORFs) AV1 and AV2 in the virion sense strand, and AC1, AC2, AC3, and AC4 in the complimentary sense strand while DNA-B contained BV1 in the virion sense strand and BC1 in the complimentary sense strand. No alphasatellite or betasatellite genome and other virus-specific contigs were present in the Illumina reads, perhaps because bipartite begomoviruses have not been shown to associate these satelllites. Based on the Illumina-derived viral sequences, the endonucleases with unique restriction sites *Pst*I in DNA-A and *Hind*III in DNA-B were used to digest the RCA products of each of six samples (three symptomatic and three asymptomatic), which yielded ~2.8-bp fragments from each of the symptomatic plants and none from asymptomatic plants. The fragments were cloned into similarly digested pGEM-3Zf (+/-) plasmid vector (Promega) and sequenced bidirectionally by primer walking. The Sanger- and Illumina derived okra sequences were of equal length and exhibited 100% sequence identity for both DNA-A and DNA-B components. According to the recommendations of the International Committee on Taxonomy of Viruses [21] and the close phylogenetic relatedness of OEK13 with previously described SbCBV isolates (**Fig. 2**), the unusual symptoms observed on okra in Ekona, is associated with SbCBV. We reported for the first-time cassava being infected by SbCBV in Cameroon and Togo [20]. The identification of SBCBV from cassava and now from okra, could be directly linked to climate change that favors the adaptation of insect vectors to new endemic plant hosts. This therefore necessitates an urgent need for further surveys to determine the distribution and spread of these emerging begomoviruses in West and Central Africa.

Declarations

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

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Figures

Figure 1

Okra (*Abelmoschus esculentus*) plant from Southwestern Cameroon exhibiting viral disease symptoms, typical of begomovirus infection

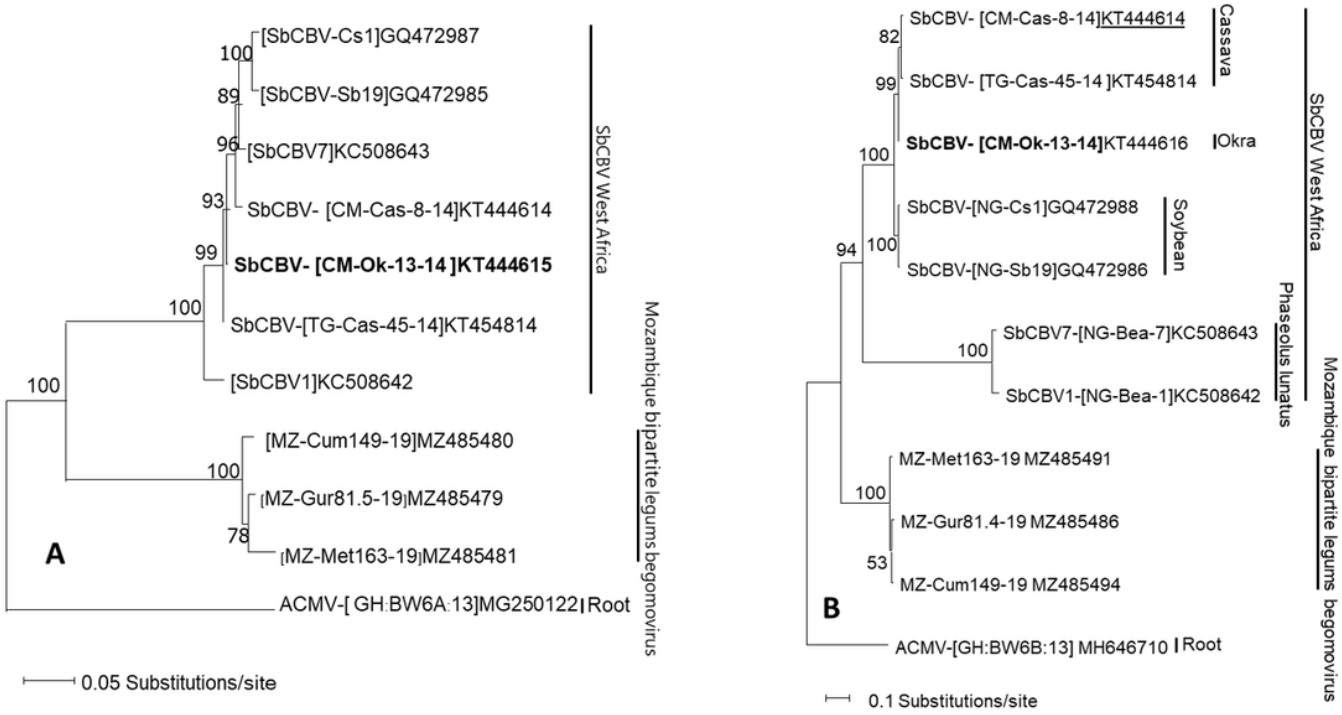

Figure 2

Phylogenetic relationships between the DNA-A and B components of *Soybean chlorotic blotch virus* (SbCBV) and related begomoviruses (abbreviations according to Brown *et al.*, 2015). Trees constructed using the neighbour-joining algorithm in MEGA X. SbCBV DNA-A (A) and DNA-B (B) sequences identified in this study are shown in bold. Horizontal lines are proportional to the number of nucleotide substitutions per site. Bootstrap values of ≥70% are

shown at the major nodes. GenBank Accession Nos. and isolate names for each virus are indicated.