



Next generation sequencing reveals the first presence of Okra enation leaf curl virus and its Betasatellite in diseased Okra plants in Iraq

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<https://doi.org/10.59658/jkas.v12i3.4293>

Received: July 25, 2025	Abstract Okra Enation Leaf Curl Virus (OELCuV) is a monopartite <i>Begomovirus</i> often associated with betasatellites and alphasatellites. OELCuV has been reported in different countries. However, it had not yet been reported from Iraq. Transcriptome sequencing data from Karbala fields grown with viral-infected okra were investigated for viruses and their satellites via de novo assembly of the unmapped reads to the okra genomes. Predicted nucleotides and proteins from these assembled unmapped reads aligned OELCuV and its betasatellite sequences with 97.9% and 96.3% identity, respectively. The Phylogenetic analysis confirmed the species identity as Okra Enation Leaf Curl Virus and Okra enation leaf curl betasatellite by gathering them in separated clades with the identical isolates and strains. This report documents for the first time the earliest OELCuV and its betasatellite infection of okra plants in Iraq. Keywords: Next generation sequencing, Okra enation leaf curl virus and its betasatellite, okra.
Accepted: Aug. 18, 2025	
Published: Sep. 15, 2025	

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is a major vegetable crop grown in the tropics and warmer parts of the temperate zone. It is an excellent source of dietary fiber and minerals, particularly folates as well as vitamins A, B, C, and K; iron, calcium, manganese, and magnesium; and powerful antioxidants such as xanthin and lutein compounds [1].

Significant constraints to the production of okra are due to biotic stress. The Okra Enation Leaf Curl Virus (OELCuV) is a member of the *Begomovirus* genus that infects okra plants primarily through whiteflies *Bemisia tabaci*, with high pathogenicity for Okra Enation Leaf Curl Disease (OELCuD). The symptoms include enations on leaf surfaces and stunted growth of plant hosts with a reduction in the yields as well as the quality of the crops. Molecular analysis revealed OELCuV as a single-stranded DNA genome approximately 2.6 to around 2.7 kb long with betasatellite molecules often associated with members belonging to the *Begomovirus* group [2,3].

The betasatellite associated with OELCuV has been found to have greater nucleotide sequence identity with previously characterized betasatellites, which may assist the pathogenesis of Okra Enation Leaf Curl Virus and further enhance disease symptom

severity. This proves that the higher nucleotide sequence identity between OELCuB and other known betasatellites can probably help in the pathogenesis of OELCuV and further increase the severity of its disease symptoms. The association of this betasatellite with pathogenicity on okra means it enhances the severity of pathogenicity in terms of more severe symptoms, showing an interaction between the virus and its satellites [4, 5].

In terms of genetic diversity, many strains of OELCuV and associated betasatellites have been described from different geographical regions, which proves the evolutionary nature of these pathogens under environmental pressure and agricultural practices [6,7]. Further, phylogenetic analysis of viral genome sequences has reported that betasatellites are highly variable, indicating possibilities for crop management difficulties due to adaptability, in addition to the altitudinous potential for horizontal gene transfer between related species [4, 8]. Much research has been carried out on OELCuV and its satellites from different regions of the world, with special reference to India and Sri Lanka, where this particular virus creates a great menace in okra cultivation [6, 9]. However, it has not been reported from Iraq. Hence, this study sought to assess the viral diversity associated with the okra plant through Next-Generation Sequencing.

Materials and Methods

Plant Sampling and RNA Extraction

Symptomatic okra leaf samples exhibiting classic viral infection signs—including foliar mottling, interveinal chlorosis, deformation and necrosis, leaf size reduction, vein thickening, and curling (Figure 1)—were observed in multiple okra fields. These samples were randomly collected from fields located in Karbala Province, Iraq (Latitude: 32°36'52" N; Longitude: 44°1'27" E). Total RNA was extracted from these diseased tissues using the Axen™ Total RNA Extraction Kit (Macrogen, South Korea), following the manufacturer's standard protocol [10].



Figure (1): Various okra leaves showing viral-like symptoms

RNA Sequencing and Bioinformatics Analysis

The extracted genomic RNA was used to produce cDNA for constructing a paired-end Illumina shotgun library utilizing the TruSeq Nano DNA Library Prep Kit (Illumina, San Diego, CA, USA). Sequencing was performed at Macrogen Inc. (Seoul, South Korea) via the Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads [11, 12].

Raw sequence reads were first aligned to the okra reference genome using Bowtie2 (v2.4.5). Reads that did not align were subjected to de novo assembly using SPAdes (v3.15.4). The resulting contigs were queried against the NCBI GenBank plant virus database (<https://www.ncbi.nlm.nih.gov/genome/viruses/>) via both BLASTn and BLASTp algorithms [12, 13].

To confirm the presence of viral agents, raw sequencing reads were mapped to reference genomes of Okra Enation Leaf Curl Virus (OELCuV) (GenBank Accession No. OL580747.1) and its associated betasatellite (OELCuB) (Accession No. KU296214.1) using Geneious Prime® (v2022.1.1).

Open reading frames (ORFs) of both OELCuV and OELCuB were predicted and annotated through BLASTp analysis against the NCBI non-redundant protein database. Comparative sequence analysis with related viral isolates was conducted using ClustalW alignment integrated in MEGA software (v10.1.5). A phylogenetic tree was constructed using the neighbor-joining (NJ) method under bootstrap analysis (1,000 replicates) to assess evolutionary relationships [14, 15].

Results and Discussion

Okra enation leaf curl virus

Results of the bioinformatics analysis (Fig. 2) revealed the presence of several overlapping contigs showing significant similarity to the genome of Okra leaf curl virus, which is currently classified as Okra enation leaf curl virus (OELCuV), based on the latest taxonomy by the International Committee on Taxonomy of Viruses [16]. This virus belongs to the genus *Begomovirus* within the family Geminiviridae.

The identification was further confirmed through mapping of raw sequence reads, which revealed a 96.9% genome coverage and 97.9% sequence identity with the reference genome of OELCuV (Fig. 25). De novo assembly of these virus-like reads showed a genomic structure identical to that of the reference virus, consisting of six open reading frames (ORFs) encoding six proteins: Pre-coat protein (gene AV2), Coat protein (gene AV1), Replication enhancer protein (gene AC3), Transcription activator protein (gene AC2), Replicase (gene AC1), and AC4 protein (gene AC4).

The virus isolate under investigation was designated as Okra enation leaf curl virus isolate Okra/Iraq-1 and has been submitted to the GenBank database; the accession number is currently pending.

A comprehensive review of the scientific literature and plant virus databases confirmed that this is the first report of OELCuV infecting okra fields in Iraq.

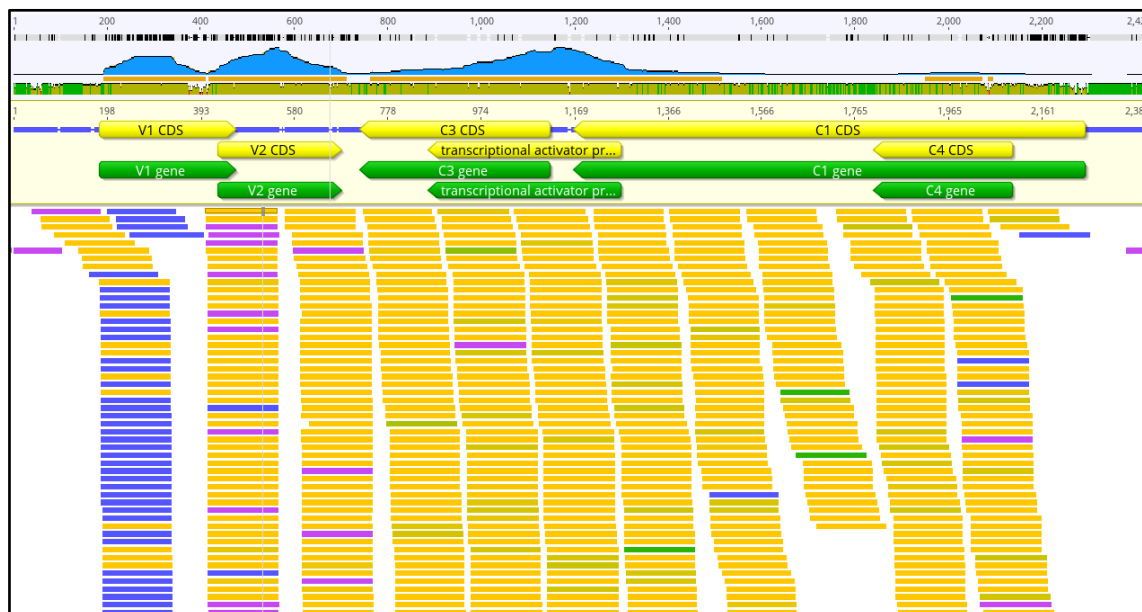


Figure (2): Raw sequencing reads matching the genome of the OELCuV isolate Okra/Iraq-1. Yellow bars represent the locations of coding regions, while green bars indicate the positions of corresponding genes in the reference genome. Equidistant colored bars (orange, blue, purple, and green) represent individual reads aligned to the viral genome.

Furthermore, the BLASTn similarity analysis (>97%) and phylogenetic assessment of the Iraqi isolate OELCuV isolate Okra/Iraq-1 confirmed its close genetic relationship with several corresponding global isolates and strains, particularly those reported from Egypt, Oman, and Mali (Accession Nos. MW015091.1, NC028985.1, and EU024120.1). These isolates clustered within the same phylogenetic clade, indicating a high degree of sequence similarity, likely due to their descent from a common ancestral origin (Fig. 3).

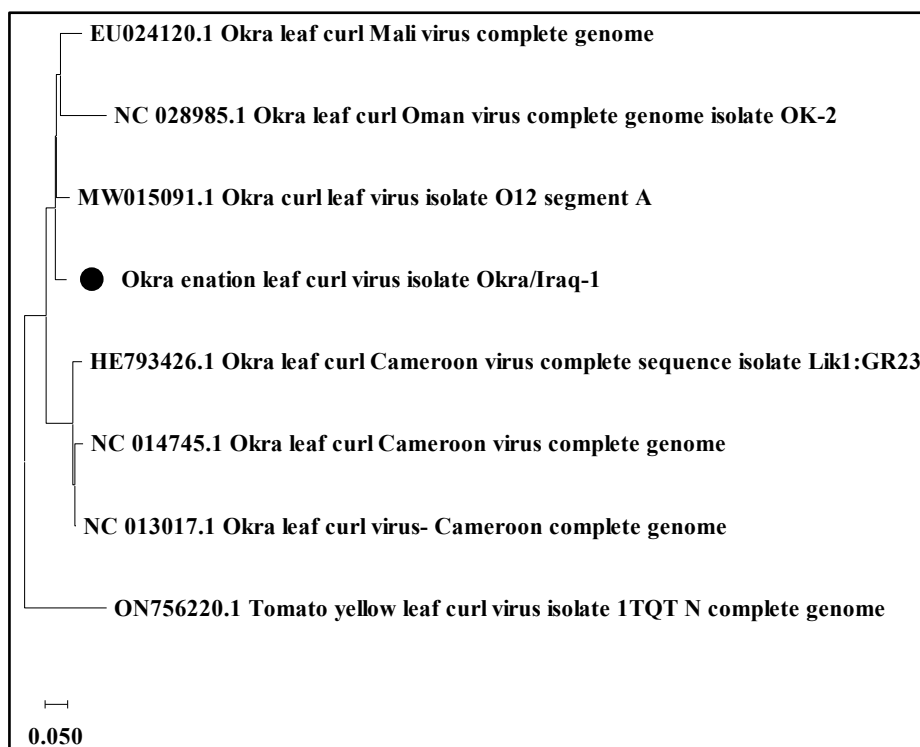


Figure (3): Phylogenetic relationship between the Iraqi isolate Okra leaf curl virus isolate Okra/Iraq-1 (indicated by a black dot) and its global counterparts of Okra leaf curl virus, based on the former nomenclature of the same virus.

Okra enation leaf curl betasatellite

The results also revealed the presence of the viral satellite (OELCB), which belongs to the genus *Betasatellite* within the family *Tolecusatellitidae*, in infected plants from both genetic backgrounds. Multiple overlapping contigs were identified that showed significant similarity to the reference genome of this viral satellite in both genotypes. The raw sequencing reads covered 35.9% of the whole genome of the betasatellite, with a 96.3% sequence identity (Fig. 4).

The isolate under investigation was designated as Okra enation leaf curl betasatellite isolate Okra/Iraq-1. A thorough review of available literature and virus sequence repositories found no prior documented record of this betasatellite, which is commonly associated with Okra enation leaf curl virus, in okra plants in Iraq. Therefore, this documentation likely represents the first report of this betasatellite in okra cultivation in the country.

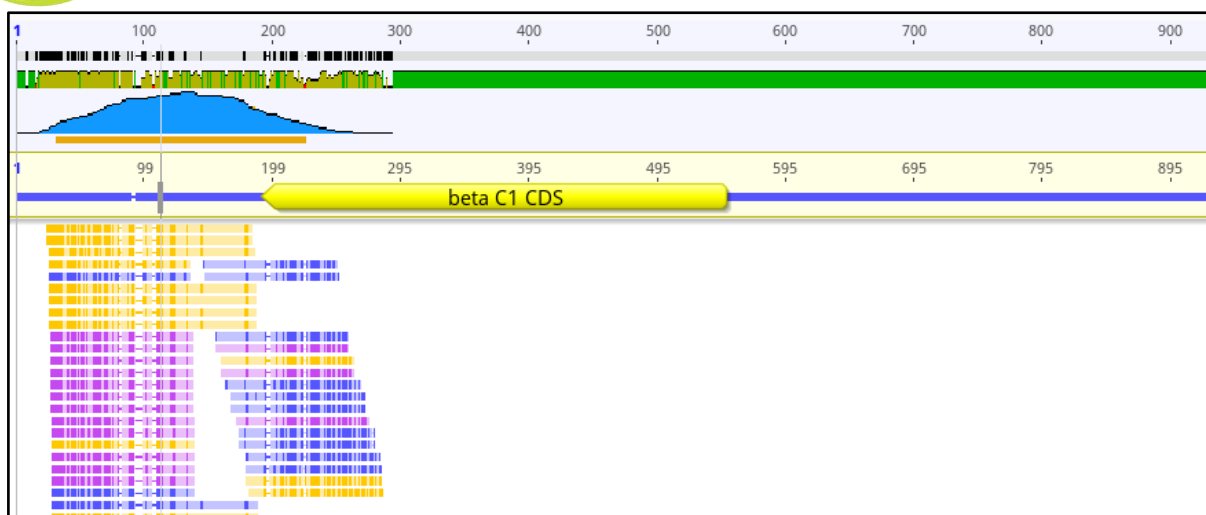


Figure (4): Raw sequencing reads matching the reference genome of the viral satellite OELCB isolate Okra/Iraq-1. The green bar represents the whole genome of the betasatellite, while the equidistant purple, yellow, and blue bars represent the reads obtained from infected okra plants that aligned with the satellite genome.

Additionally, the BLASTn similarity and phylogenetic analyses (Fig. 5) of the obtained betasatellite sequence confirmed its classification within the same species, as indicated by the high sequence identity (>97%). The isolate clustered within a single phylogenetic branch along with several other isolates of the same betasatellite, particularly the Indian isolates KU296213 and OR262356.

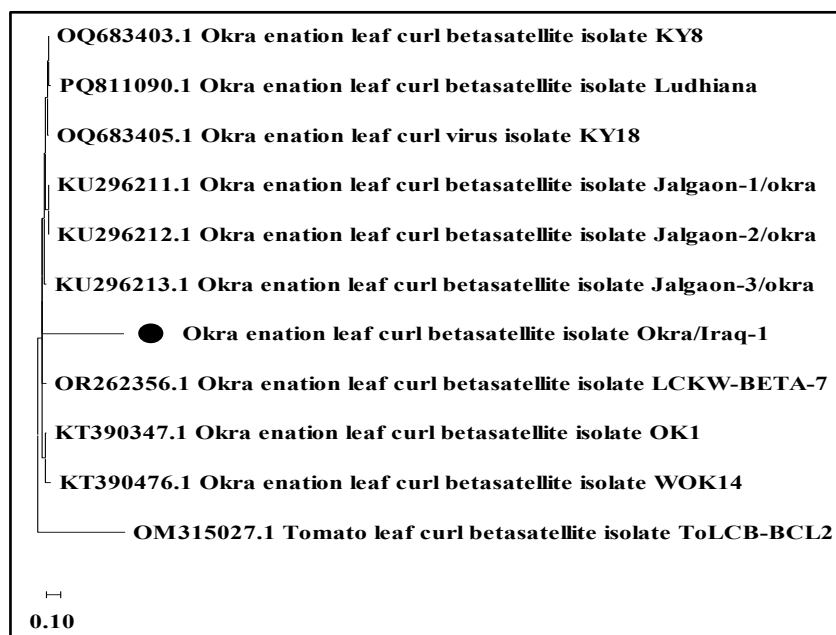


Figure (5): Phylogenetic relationship between the Iraqi isolate of the viral satellite OELCB isolate Okra/Iraq-1 (indicated by a black dot) and other global strains and isolates belonging to the same betasatellite.

The present study provides the first molecular evidence of (OELCuV) and its associated betasatellite infecting okra plants in Iraq, marking a significant extension of the known geographic distribution of this begomoviral complex. OELCuV is a monopartite member of the genus *Begomovirus* (family Geminiviridae), commonly associated with betasatellites, which play critical roles in symptom expression and pathogenicity [17].

In this study, transcriptome sequencing and de novo assembly enabled the detection of OELCuV and its betasatellite from infected okra plants grown in Karbala Governorate, Iraq. The high nucleotide identity 97.9% of the assembled viral sequence with the OELCuV reference genome, along with 96.3% identity for the betasatellite sequence, strongly supports their classification within the same viral species complex, in line with the criteria established by the International Committee on Taxonomy of Viruses [16].

The phylogenetic analysis placed the identified isolates within well-supported clades that include other OELCuV isolates from countries such as India, Egypt, Oman, and Mali, confirming their close evolutionary relationships. Similar geographic clustering has been reported in begomovirus studies from South Asia and Africa, suggesting the movement of viruses via infected plant materials or insect vectors like *Bemisia tabaci* [18, 19].

The detection of OELCuV in Iraq raises important agricultural and phytosanitary concerns, particularly given the economic importance of okra in the region. *Begomoviruses*, especially those associated with betasatellites, are known to induce severe symptoms such as leaf curling, yellowing, and stunted growth, leading to significant yield losses [20]. Moreover, the co-infection with betasatellites such as OELCB often exacerbates disease symptoms by modulating host defense responses and enhancing viral replication [21].

The use of RNA-seq data for viral detection in this study highlights the power of next-generation sequencing (NGS) approaches in plant virology. Unlike traditional PCR-based methods, RNA-seq allows for unbiased detection of both known and novel viral sequences, especially from non-target reads that are often discarded in genomic studies [22].

The absence of previous reports of OELCuV in Iraq may reflect limited surveillance or underdiagnosis, emphasizing the need for comprehensive monitoring of viral pathogens in key crops. This is especially critical in light of the increasing globalization of trade and climate change, which are known to facilitate the emergence and spread of plant viruses into new territories [23, 24].

This study represents the first confirmed report of OELCuV and its associated betasatellite infecting okra plants in Iraq. These findings contribute to a broader understanding of the epidemiology and molecular diversity of begomoviruses in the Middle East. Further studies are needed to evaluate the incidence, vector transmission, and potential impact of OELCuV on local crop production, as well as to develop integrated disease management strategies.



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