

Identification and molecular characterization of cotton leaf curl Gezira betasatellite and two distinct begomoviruses infecting papaya trees in the Kingdom of Saudi Arabia

Khadim Hussain^{1,2*}, Zaheer Khalid¹, Mahmoud A. Amer^{1,3}, Muhammad Zaman¹, Muhammad Amir¹, Ibrahim M Al-Shahwan¹, Adel M. Zakri⁴, Mohammed A. Al-Saleh¹

¹ Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia.

² Department of Bioinformatics and Biotechnology, Government College University Faisalabad Pakistan.

³ Viruses and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt

⁴ Department of Plant Production, College of Food and Agriculture Science, King Saud University, Riyadh, Saudi Arabia

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ABSTRACT

Begomovirus is the largest genus in the family *Geminiviridae* and constitutes more than 445 virus species. Begomoviruses are characterized by single-stranded circular genomes with monopartite or bipartite components and transmitted by whitefly (*Bemisia tabaci*). Begomoviruses cause severe diseases in many economically important crops throughout the world. Typical symptoms of a begomovirus infection including severe leaf curling, vein thickening, vein darkening and reduced leaf size were observed in papaya plants in the Dammam district of the Eastern Province of Saudi Arabia during the growing season in 2022. A total of 10 samples were collected, and total genomic DNA was isolated from naturally infected papaya tree samples and subjected to PCR amplification using universal diagnostic primers for begomoviruses and associated satellites. Three PCR-amplified genomic components of begomoviruses and betasatellite namely P61Begomo (645 bp), P62Begomo (341 bp) and P62Beta (563 bp) were sent for Sanger DNA sequencing to MacroGen Inc. These partial viral genome sequences were submitted to Genbank database and accession numbers ON206051, ON206052 and ON206050 were assigned to P61Begomo, P62Begomo and P62Beta respectively. Phylogenetic analysis and pairwise nucleotide sequence identity studies identified P61Begomo as Tomato yellow leaf curl virus, P62Begomo as DNA A component of a bipartite begomovirus Watermelon chlorotic stunt virus and P62Beta as begomovirus associated betasatellite; Cotton leaf curl Gezira betasatellite. To the best of our knowledge, this is the first report of a begomovirus complex infecting papaya (*Carica papaya*) in the Kingdom of Saudi Arabia.

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Introduction

The family *Geminiviridae* includes viruses with single-stranded circular DNA genomes ranging from 2.5-5.2 kb in size and encapsidated in twinned quasi-icosahedral (geminate) capsids (1, 2). This family of plant viruses is comprised of 520 virus species which are distributed among 14 genera, categorized based on their genome sequence, genome organization, host range and insect vectors (<https://talk.ictvonline.org/taxonomy/>) (1, 3). Geminiviruses are destructive plant pathogens infecting a wide range of monocotyledonous as well as dicotyledonous plants and are transmitted by a variety of insect vectors (4). Geminiviruses have a near-global distribution in tropical and subtropical regions that threatens various crops and causes significant economic losses (1).

The genus *Begomovirus* (family *Geminiviridae*) is the largest genus of plant viruses with 445 species (<https://talk.ictvonline.org/taxonomy/>) (1). Viruses belonging to this genus are transmitted from an infected plant to healthy plant hosts by whitefly (*Bemisia tabaci*) and they may consist of monopartite or bipartite genome structures. Monopartite genomes contain a single genomic component while bipartite genomes comprise two components

DNA-A and DNA-B which share a region of high nucleotide sequence identity known as the common region (CR). The CR is approximately 200 nucleotides (nts) long and includes the origin of replication (5, 6).

Papaya (*Carica papaya*), a major tropical, sweet, large, and herbaceous food crop, belongs to the order Brassicales (family: *Caricaceae*) and is cultivated throughout tropical regions of the world. However, its commercial potential is not achieved due to many insect pests and pathogens including bacteria, fungi, and viruses. Among viral diseases, papaya leaf curl disease (PaLCD) caused by a complex of begomovirus species is a major damaging factor, which affects the overall production of papaya (7). PaLCD caused by a geminivirus was first reported by Thomas and Krishnaswamy in 1939 (8) and, subsequently, the causative virus was further confirmed by (9). Since then, the number of begomovirus species infecting papaya crops has been increasing. Begomovirus-infected papaya plants show typical symptoms of begomovirus infection, including severe leaf curling, leaf yellowing, vein swelling and leaf distortion. 15 different begomovirus species have been reported to infect papaya (10). In Saudi Arabia, papaya is commercially cultivated in the Dammam and Qatif districts of the Eastern province. In our present study mo-

* Corresponding author. Email: khadim787@gmail.com, malsaleh@ksu.edu.sa

lecular characterization of a novel begomovirus complex infecting *Carica papaya* in Saudi Arabia has been described. To the best of our knowledge this is the first report of mixed begomovirus infection in papaya in Saudi Arabia.

Materials and Methods

During the year 2021, different regions of Saudi Arabia were visited and papaya trees showing typical begomovirus symptoms including server leaf curling, leaf distortion and vein swelling symptoms (Figure 1B) were collected from Dammam and Qatif districts of the Eastern province of Saudi Arabia. The total genomic DNA of symptomatic leaves was extracted using the modified CTAB method as described in (11). The DNA was subjected to PCR amplification with the different diagnostic primers available in the plant virology laboratory, Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia (Table 1). The positive PCR products were amplified from papaya samples with universal diagnostic primers BegomoI and BegomoII and the universal primers Beta01 and Beta02 (12-14). The amplicons were sent to Macrogen Inc. Korea for Sanger sequencing of both strands. The partial genome sequences of different components of begomoviruses and betasatellite were cleaned and assembled into a single consensus viral sequence using Lasergene (DNA-Star Inc., Madison, WI, USA) and submitted to the GenBank database. Potential genes in the sequences of the begomovirus and associated betasatellite sequences were determined using the online NCBI tool ORFfinder (<https://www.ncbi.nlm.nih.gov/orffinder/>).

Closely related sequences were retrieved from public databases using BLAST (15) and multiple sequence alignments were performed using Clustal W, as implemented by MEGA 7.0 (16). Pairwise sequence comparisons were done using MegAlign, as implemented by Lasergene and Sequence Demarcation Tool (SDT) (17). Phylogenetic analysis was conducted by constructing a phylogenetic tree using the neighbor-joining algorithm as implemented by MEGA 7.0 with 1,000 bootstrap replicates. Abbreviations of begomovirus and their satellite names are shown in the ICTV taxonomy.

Results

Complete Sanger sequencing of PCR-amplified viral genomic components on both strands through their entirety revealed that the amplified product size of the isolate P61Begomo was 645 bp long, encompassing the N-terminal of CP and covering N-terminals of REn and TrAP (nucleotide coordinate 779-1423), the isolate P62Begomo was 341 bp long, encompassing N-terminals of REn and TrAP

(nucleotide coordinate 1079-1419) and isolate P62Beta was 563 bp long, encompassing A-rich region and satellite conserved region (SCR) of betasatellite (nucleotide coordinate 794-1356). Conversely, attempts to amplify the DNA B component of a begomovirus with the DNA B primers (Anwar et al. 2020) were unsuccessful in all samples. The partial viral genome sequences were submitted to the Genbank database and accession numbers ON206051, ON206052 and ON206050 were assigned to P61Begomo, P62Begomo and P62Beta respectively. The isolate P61Begomo nucleotide sequence (ON206051) was confirmed as a partial genome of *Tomato yellow leaf curl virus* (TYLCV), the isolate P62Begomo nucleotide sequence (ON206052) was confirmed as DNA A component of a bipartite begomovirus *Watermelon chlorotic stunt virus* (WmCSV) and the isolate P62Beta nucleotide sequence (ON206050) was confirmed as a partial genome component of begomovirus associated betasatellite; Cotton leaf curl Gezira betasatellite (CLCuGB). Phylogenetic analysis of isolate P61Begomo (ON206051) with other isolates of begomovirus sequences retrieved from databases showed that our isolate was grouped with the isolates of TYLCV from different regions. Pairwise nucleotide sequence identity analysis revealed that isolate P61Begomo (ON206051) showed the highest nucleotide sequence identity of 89.6 % with TYLCV isolate AZ23-1 (MN397780) which has been isolated from tomatoes in Saudi Arabia. The nucleotide sequence identity with all other isolates of TYLCV ranged between 85.7-88.3%. The isolate P61Begomo (ON206051) showed 49.1 and 52.9% nucleotide sequence identity with the same genomic regions of two isolates of Papaya leaf curl virus (NC_004147 and NC_014707) isolated from Papaya (Figure 2 A & B). Phylogenetic studies of the isolate P62Begomo (ON206052) with other begomovirus sequences retrieved from databases showed that our isolate made clade with different isolates of WmCSV, a bipartite begomovirus isolated from Watermelon, Zucchini, Cucumber etc. Pairwise nucleotide sequence identity exhibited the highest nucleotide sequence identity of 87.7% with two isolates of WmCSV (KC876038 and

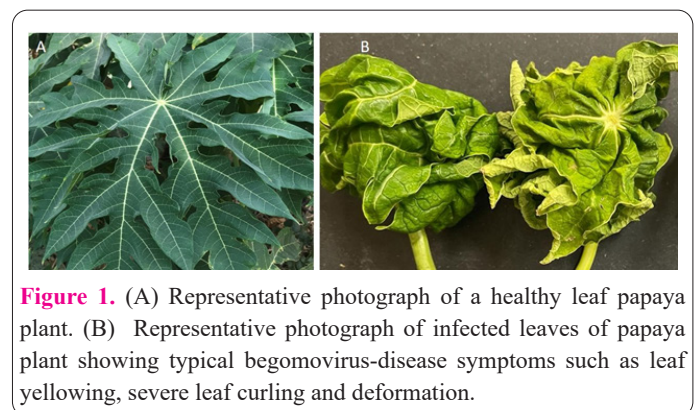


Figure 1. (A) Representative photograph of a healthy leaf papaya plant. (B) Representative photograph of infected leaves of papaya plant showing typical begomovirus-disease symptoms such as leaf yellowing, severe leaf curling and deformation.

Table 1. List of primers used for the amplification of virus genome components from collected papaya samples.

Primer Name	Primer sequence	Amplification length	Primer length
Begomo I	CCGTGCTGCTGCCCCATTGTCCGCGTCAC	1.1 kb	30
Begomo II	CTGCCACAACCATGGATTCACGCACAGGG		29
DNA-B F	GGTACCCGTAACGATCTTGAACATATGTCCC	2.7 kb	30
DNA-B R	GGTACCCTATCTGGCTATAGGTCCGAACG		29
β 01	GGTACCACTACGCTACGCAG CAGCC	1.4 kb	25
β 02	GGTACCCTACCCTCCAGGGG TACA		24

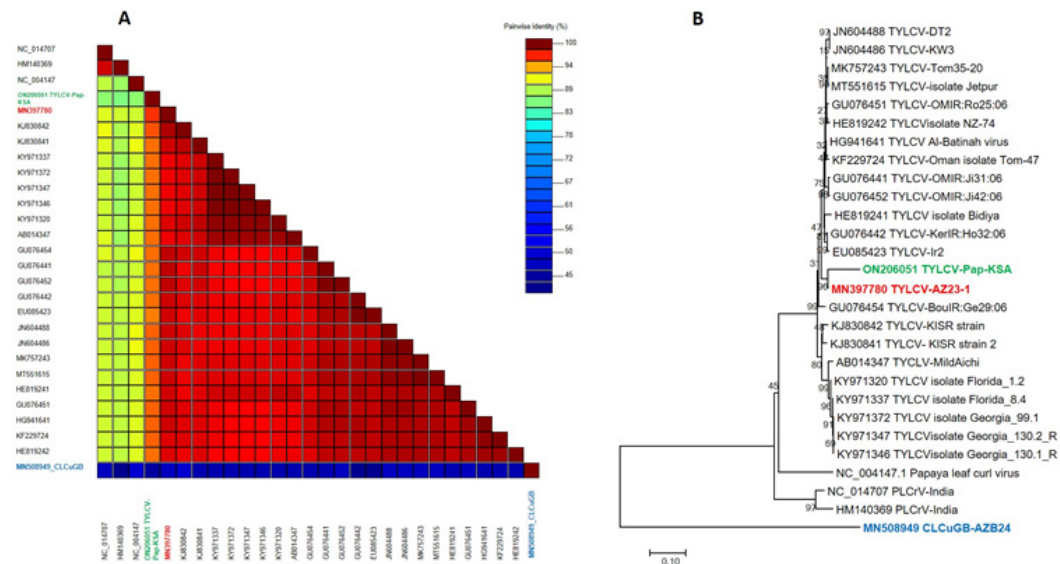


Figure 2. (A) Distance matrix illustrating the pairwise nucleotide identity percentage of TYLCV-Pap-KSA (ON206051) as compared with other most similar sequences. (B) Neighbor-joining phylogenetic tree based on the alignment of TYLCV-Pap-KSA (ON206051) sequence with closely related TYLCV sequences. A closely grouped isolate of TYLCV is highlighted with red font color. An unrelated sequence of betasatellite molecule was used as an outgroup and highlighted in blue font color.

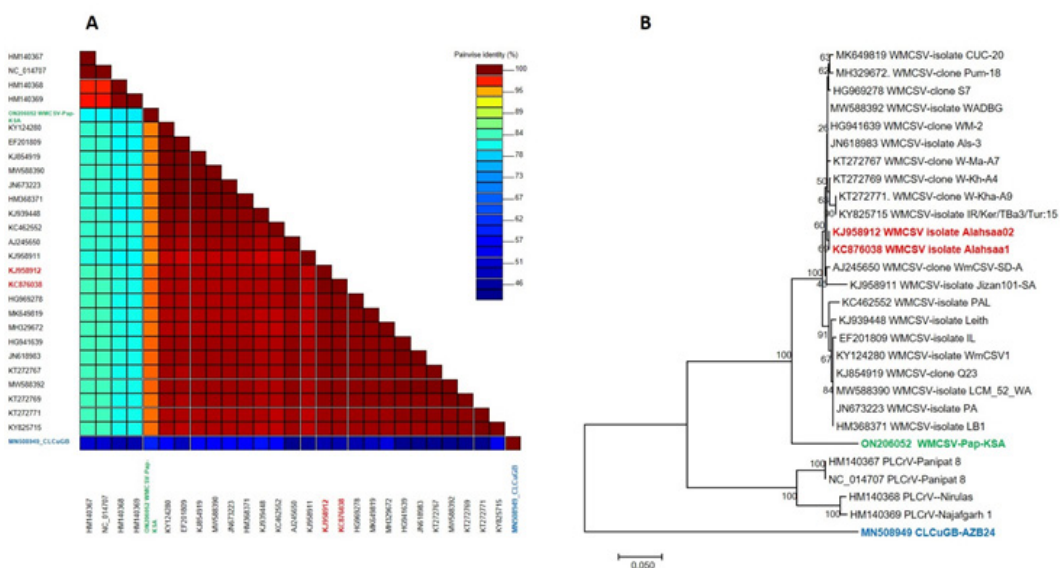


Figure 3. (A) Distance matrix illustrating the pairwise nucleotide identity percentage of WmCSV-Pap-KSA (ON206052) as compared with other most similar sequences. (B) Neighbor-joining phylogenetic tree based on the alignment of WmCSV-Pap-KSA (ON206052) sequence with closely related WmCSV sequences. A closely grouped isolates of WmCSV is highlighted with red font color. An unrelated sequence of betasatellite molecule was used as an outgroup and highlighted in blue font color.

KJ958912) isolated from Zucchini. All other isolates of WmCSV showed 84.5-87.1% nucleotide identity with our isolate (Figure 3 A & B). Phylogenetic analysis of betasatellite isolate P62Beta (ON206050) with other isolates of betasatellite sequences retrieved from databases indicated that our isolate was grouped with different isolates of cotton leaf curl Gezira betasatellite (CLCuGB) isolated from tomato and okra from Saudi Arabia, UAE and Oman. Pairwise nucleotide sequence identity showed our isolate has the highest nucleotide sequence identity of 97.2% with CLCuGB (MN508949) isolated from a tomato in Saudi Arabia. Sequence identity with all other isolates of CLCuGB ranged from 86.9-92.9%. The nucleotide sequence identity of our isolate with Papaya leaf curl betasatellite

(MF683837) was as low as 38.9% sequence identity (Figure 4 A & B). Abbreviated names of virus species were retrieved from ICTV (<http://www.ictvonline.org/virus-taxonomy.asp>) and their sequences were retrieved from online database GenBank.

Discussion

The results of recent studies indicate the emergence of diverse begomoviruses in economically important crops in the Arabian Peninsula, generally, and particularly in Saudi Arabia. These studies reported infection of different begomoviruses in crop plants from Saudi Arabia, Oman, UAE and Yemen (18-23). There are numerous factors including

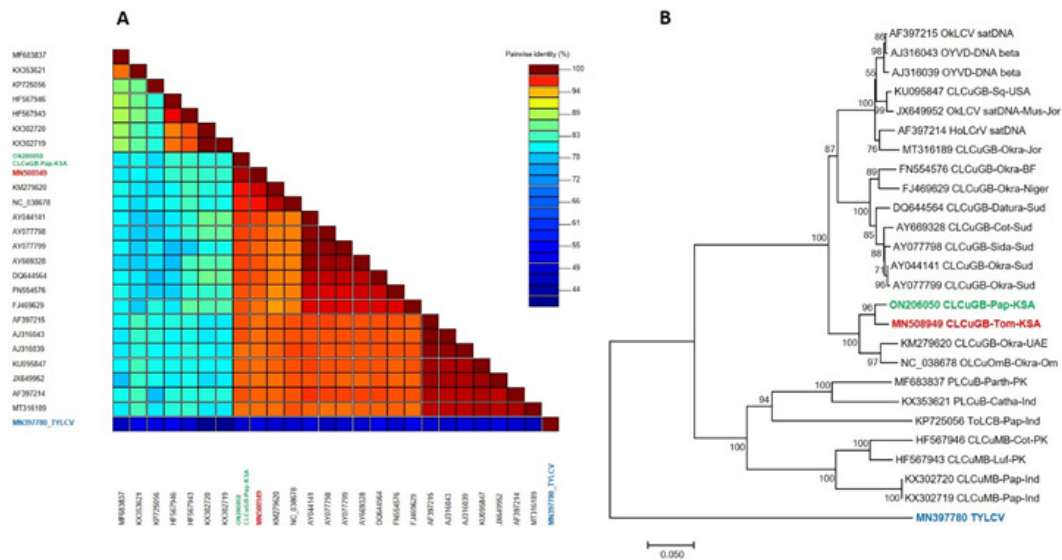


Figure 4. (A) Distance matrix illustrating the pairwise nucleotide identity percentage of CLCuGB-Pap-KSA (ON206050) as compared with other most similar sequences. (B) Neighbor-joining phylogenetic tree based on the alignment of CLCuGB-Pap-KSA (ON206050) sequence with closely related CLCuGB sequences. A closely grouped isolate of CLCuGB is highlighted with red font color. An unrelated sequence of the TYLCV genome was used as an outgroup and highlighted in blue font color

rapid mutation, recombination, pseudorecombination, vector population, climate change and alternate hosts that may be responsible for this emergence of diverse strains and species of begomoviruses. Papaya leaf curl disease is caused by begomoviruses and is a serious threat to the production and quality of papaya fruits. This disease is associated with diverse species of begomoviruses; a few examples are *Papaya yellow leaf curl virus* (PaYLCV) (24), *Papaya leaf curl virus* (PaLCuV) (25), *Papaya leaf crumple virus* (PaLCrV), *Chilli leaf curl virus* (ChiLCV), *Duranta leaf curl virus* (DLCV), *Papaya severe leaf curl virus*, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl virus* (ToLCV), *Cotton leaf curl Multan virus* (CLCuMuV), *Tomato leaf curl Gujarat virus* (ToLCGV), *Croton yellow vein mosaic virus* (CYVMV), *Pedilanthus leaf curl virus* (PeLCV), *Ageratum enation virus* (AEV), *Cotton leaf curl Burewala virus* (CLCuBuV) (10), *Tomato leaf curl Bangladesh virus* (ToLCBV) (26), *Ageratum yellow vein virus* (AYVV) (27), AYVV-China (28) and CLCuGV (21). These diverse strains also extend their host range in different plant species. The mixed infection of plant viruses is a common phenomenon. In our present study, we report the mixed infection of a monopartite begomovirus TYLCV and a bipartite begomovirus WmCSV associated with a betasatellite Cotton leaf curl Gezira betasatellite infecting papaya in Saudi Arabia. To the best of our knowledge, this is the first report of a monopartite/bipartite begomovirus-betasatellite complex infecting papaya. Previously Tomato yellow leaf curl virus has been reported infecting papaya associated with tomato yellow leaf curl betasatellite (20) or in mix infection with RNA viruses (29). Previously begomoviruses have also been reported in mixed infection in Solanaceous and Fabaceous crops (30), Pepper (31), tomato (32), *Jatropha gossypifolia* (33). In papaya tree mixed viral infections of DNA and RNA viruses have been reported in Southern Texas USA which includes the infection of *Lettuce chlorosis virus* (genus Crinivirus), *Papaya ring spot virus* (genus Potyvirus) and *Tomato yellow leaf curl virus-IL* (genus Begomovirus) (29). WmCSV was first identified in watermelon in Yemen

(34). In Saudi Arabia, WmCSV was reported firstly infecting watermelon in 2014 (18). Natural hosts of WmCSV are mainly members of the family Cucurbitaceae including watermelon (*Citrullus lanatus*), melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) and Solanaceae including tomato (*Solanum lycopersicum*) and *Datura innoxia* (19, 35). WmCSV has been reported as mixed infection with *Tomato leaf curl Palampur virus* (ToLCPaV) infecting watermelon. Both are bipartite begomoviruses and complement the genomic components of each other causing pseudorecombination (36). Results in this study are reporting, for the first time, the infection of WmCSV in Papaya (family *Caricaceae*) and the mixed infection of WsCSV with TYLCV in association with CLCuGVB. Betasatellite is sub genomic component associated with begomoviruses that relies on helper begomovirus for its genome replication and encapsidation. CLCuGVB was first reported in association with CLCuGV infecting cotton, okra and *Sida alba* in Sudan (37). Later on, this combo of CLCuGV and CLCuGVB was found to be associated with okra leaf curl disease in Niger (38), Jordan (39) and Burkina Faso (40). Betasatellite molecules are promiscuous in their dependence on helper begomoviruses and are transreplicated by diverse species of begomoviruses (41) (42) (22). Pseudorecombination or reassortment of genomic components is a common phenomenon in begomoviruses and our results present a novel complex of begomovirus causing leaf curl disease in Papaya in Saudi Arabia. Further studies are needed to elucidate the complete genome sequences of TYLCV, WmCSV DNA A and CLCuGB for recombination analysis and their competency for transreplication of betasatellite. Infectivity analysis needed to understand the mechanism of disease etiology.

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Conflict of Interests

The authors declare no conflict of interest.

Author contributions

Conceived the project and designed the studies: Khadim Hussain and Mohammed A. Al-Saleh. Sample collection and execution of experiments in the lab: Mahmoud A Amer, Zaheer Khalid, Muhammad Zaman and Muhammad Amir. Analysis of data and drafting of the manuscript: Khadim Hussain. Critical revision of the manuscript for important intellectual content: Mohammed A. Al-Saleh, Ibrahim M Al-Shahwan, Adel M. Zakri. All authors read and approved the final version of the manuscript.

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