



# Association of cotton leaf curl Gezira virus with tomato leaf curl betasatellite infecting *Carica papaya* in Iran

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## Abstract

Papaya leaves exhibiting severe leaf curling and stunting symptoms were tested for the presence of *begomovirus* infection. The putative full-length cotton leaf curl Gezira virus (CLCuGV) and tomato leaf curl betasatellite (ToLCB) molecules were amplified, sequenced and analyzed. This is the first study providing the identification of CLCuGeV and ToLCB infecting papaya in Iran.

**Keywords** *Geminiviridae*: *begomovirus* · Cotton leaf curl gezira virus · Tomato leaf curl betasatellite · *Carica papaya*

Plant infecting viruses of the family *Geminiviridae* are consisting of circular, single-stranded DNA (ssDNA) molecules. Geminiviruses are currently divided into nine genera based on insect vector, genome organization and sequence similarities (Zerbini et al. 2017). Begomoviruses have genomes of either a DNA-A (monopartite) or DNA-A and DNA-B (bipartite). The genomes of monopartite begomoviruses are homologous to the DNA-A components of bipartite begomoviruses encodes Rep, TrAp, RE<sub>n</sub> and AC4 protein products in the complementary-sense whereas, CP and AV2 in the virion-sense. The DNA-B component encodes nuclear shuttle protein (NSP) and movement protein (MP). Most monopartite begomoviruses reported from Asia, Europe, Middle East and Australia have been shown to be associated with betasatellite, which have no or little sequence identity with the helper virus (Briddon and Stanley 2006). The βC1 protein of betasatellites have been shown to be involved in symptom determinant, overcoming RNA silencing-based host defenses and movement function. In many cases betasatellites enhance the symptoms of their helper viruses to plants raising viral DNA levels and extending host range (Briddon et al. 2000;

Saunders et al. 2000). Papaya (*Carica papaya*) belonging to the *Caricaceae* family is a dicot woody plant, native to Mexico, America (Central and South) but now adapted in nearly all tropical regions. Different *begomovirus* species have been reported to infect papaya in Pakistan (Nadeem et al. 1997), Taiwan (Chang et al. 2003), China (Shen et al. 2014), India (Saxena et al. 1998, Sing-Pant et al. 2012; Varun and Saxena 2018; Nehra et al. 2019), Nepal (Shahid et al. 2013), Iran (Bananej et al. 2016), Bangladesh (Hamim et al. 2019) and Oman (Khan et al. 2014). Similarly, association with betasatellites has also been reported in papaya leaf curl disease (PLCD), for example, papaya leaf curl betasatellite, croton yellow vein mosaic betasatellite (Singh-Pant et al. 2012) and tomato leaf curl Java betasatellite (Shahid et al. 2014). During a survey in Feb-2010, leaf samples of four papaya plants exhibiting yellowing and leaf curling symptoms typical of *begomovirus* infection (Fig. 1) and two apparently healthy were collected from two farms approximately seven kilometers apart in Bahu-Kalat, Baluchestan province, Iran. The DNA extracts were obtained using a CTAB method (Porebski et al. 1997). Initial detection of *begomovirus* was confirmed for all samples using PCR with a set of diagnostic primers AC1048/AV494 amplifying ~550nt of the coat protein gene (Wyatt and Brown 1996). Rolling circle amplification (RCA) using φ29 polymerase (GE Healthcare Limited, UK) was employed and full-length *begomovirus* (~2.7 kb) was obtained after digestion of RCA products with *Bam*HI restriction enzyme. The sequences of two potentially full-length clones, of 2,763 and 2,765 nt, were obtained from papaya plants, respectively which were deposited in GenBank (acc.no. MN328257-58)

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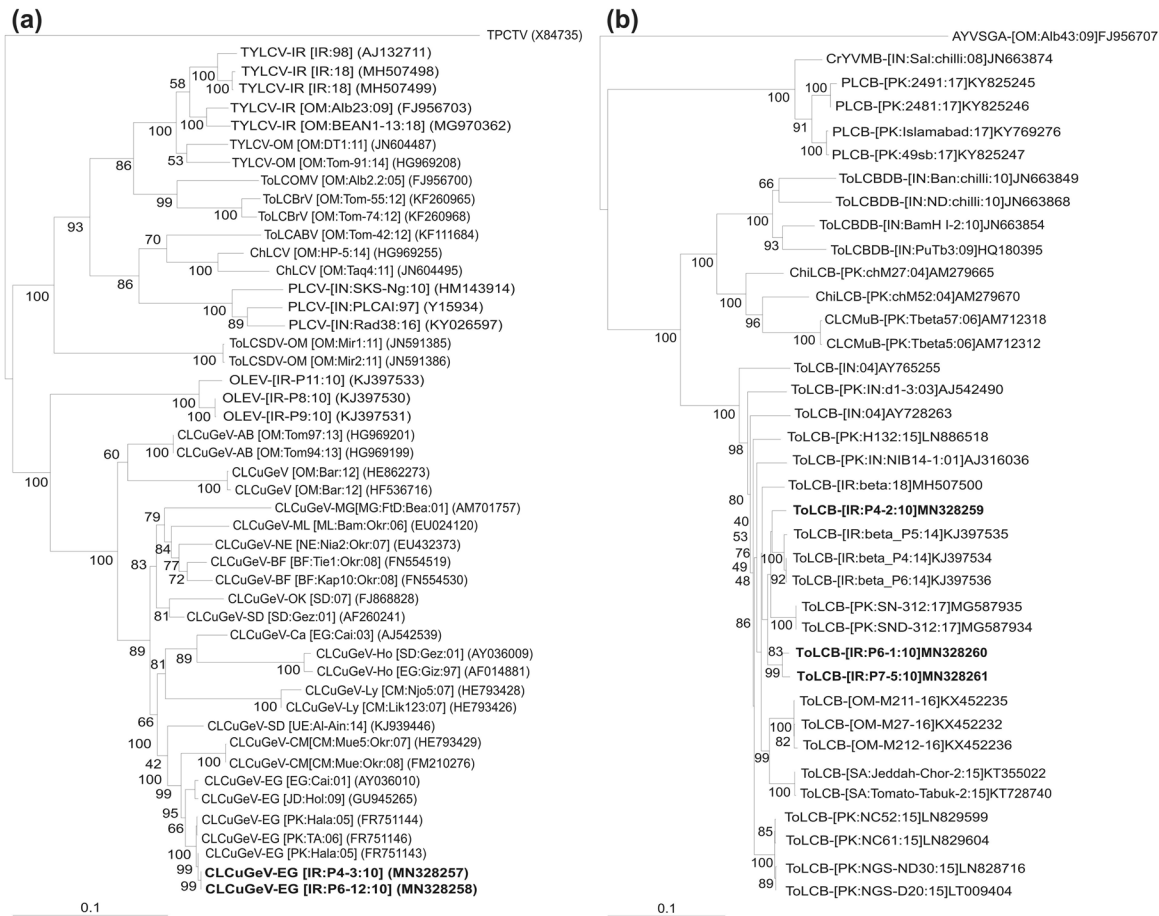
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**Fig. 1** *Carica papaya* plant showing severe leaf curling and stunting symptoms infected by cotton leaf curl Gezira virus and associated tomato leaf curl betasatellite



(Supplementary table 1). Pairwise nt comparisons using the SDT v1.2 (Muhire et al. 2014) indicated that these sequences exhibited 99.5% nucleotide (nt) identity with each other and have the highest levels of identity (99.8%) to an isolated of the "Egypt" strain of cotton leaf curl Gezira virus (CLCuGeV-EG[PK:Sin:NT7:05]; FR751143) identified in cotton leaf curl disease affected cotton in Pakistan (Tahir et al. 2011). In Phylogenetic analysis virus sequences grouped with CLCuGV (Egyptian strains) (Fig. 2a). According to the applicable species segregation criteria (Brown et al. 2015), the virus isolates identified from papaya is the isolates of Egypt strain of CLCuGeV. The associated betasatellite (~1.4 kb) was

obtained using abutting primers SAT101/SAT102 (Shahid et al. 2017) and sequences are available in GenBank (acc no. MN328259-61). Analysis of the betasatellite showed them to have a structure typical of betasatellites, consisting of a satellite conserved region, an A-rich sequence, and a single conserved ORF in the complementary-sense (the  $\beta$ C1 gene; Briddon et al. 2003). Blast and STD analysis showed that these sequences share 95.6 to 97.6% nt identity with each other and showed highest 95.3% nt identity with ToLCB reported from chilli (MG587934-5) from Pakistan. Based on the species demarcation criteria set for betasatellites (Adams et al. 2017), the betasatellites clones are isolates of



**Fig. 2** Phylogenetic dendrograms were produced using Mega7 based upon alignments of the complete nucleotide sequences of the CLCuGeV (a) and ToLCB (b). Vertical branches are arbitrary, horizontal branches are proportional to calculate mutation distances. Values at nodes indicate percentage bootstrap values (1000 repli-

cates). The virus and betasatellite trees were arbitrarily rooted on the sequence of Tomato pseudo-curly top virus (TPCTV) and Ageratum yellow vein Singapore alphasatellite (AYVSGA) respectively. The sequences originating from *Carica papaya* are indicated by bold text in each case

ToLCB. This conclusion was supported by a phylogenetic tree showing the sequences isolated from papaya to cluster with previously characterized ToLCB (Fig. 2b). We identified a *begomovirus* associated with a betasatellite molecule infecting papaya. Normally, CLCuGeV is known to infect malvaceous plant as cotton and okra (Tahir et al. 2011). Apparently the *begomoviruses* originated from Middle east and sub-continent are restricted due to geographical barriers. However, there is strong evidence for spread of *begomoviruses* to westwards. Tomato leaf curl Palampur virus and tomato leaf curl New Dehli virus reported from sub-continent and has spread to Iran and Spain, respectively (Juárez et al. 2014; Zaidi et al. 2017). Surprisingly, CLCuGeV along with alphasatellite and a defective betasatellite complex has been reported recently to infect okra in Southern Texas, USA (Villegas et al. 2019). To date only one earlier study has shown that Okra enation leaf curl virus has been identified from papaya plant. The results of this study show the first association of CLCuGeV with

ToLCB in PLCD and further studies will be required to assess pathogenicity as well as geographic incidence of CLCuGeV and ToLCB in Iran.

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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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