



Association of a monopartite begomovirus and associated betasatellite with yellow vein disease of a weed host, *Senna italica* Mill. In Oman

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Abstract *Senna italica* Mill. plants exhibiting yellowing and stunting symptoms typical of begomovirus infection were collected in Oman. Molecular characterization using begomovirus and betasatellite primers in polymerase chain reaction followed by rolling circle amplification, cloning and analysis of sequences revealed the *S. italica* plants were infected by an isolate of Chilli leaf curl virus and tomato leaf curl betasatellite. The study describes the etiology of a yellow vein disease, identified for the first time, affecting a common weed in Oman.

Keywords Begomovirus · Chilli leaf curl virus · Tomato leaf curl betasatellite · *Senna italica*

Geminiviridae is a family of plant pathogens consisting of a circular single stranded DNA (ssDNA) genome. Based on variability in their genome organization, insect vectors and host range, *Geminiviridae* is divided into nine different genera [6]. Among all genera, *Begomovirus* is the largest genus (consist of nearly 424 species) which is transmitted by a whitefly (*Bemisia tabaci*; Family: *Aleyrodidae*) insect. The genome of begomoviruses is either bipartite, consisting of two (DNA A and DNA B) genomic components or monopartite, which comprising of a single (DNA A) ~ 2.7 kb component. The DNA A component of begomoviruses consists of genes that encode four six ORFs (Rep, TrAp, RE_n and C4) in the complementary-sense and CP and the V2 in the virion-sense. Most of the monopartite

begomoviruses are also associated with betasatellites (family *Tolecusatellitidae*, genus *Betasatellite*) [1]. Betasatellites are circular, ssDNA molecules of ~ 1350 nt in size which depends on a helper virus (the main virus DNA genome) for replication, movement, and transmission between plants.

A weed (*Senna italica* Mill.) showing begomovirus-like symptoms (yellowing and stunting-Fig. 1b) near an infected tomato field in Oman (coordinates 26.1644° N 56.2426° E) were collected in 2018. Total nucleic acid was extracted from five infected and two healthy plants by modified Cetyl trimethylammonium bromide (CTAB) method as described earlier. Initially core coat protein region of begomovirus (~ 0.7 kb) was detected from three samples using polymerase chain reaction (PCR) with TYLCD-356 (5'-ATCATTTCACKCCCGYCTCGA-3') and TYLCD-1044 (5'-GCRTGMGTACABGCCATA TACA-3') begomovirus primers [5]. Subsequently, complete three full-length begomovirus (~ 2.7 Kb) clones (Sen4, Sen6 and Sen8), one from each symptomatic plant, was produced with *Bam*HI endonuclease restriction in rolling circle amplification (RCA). The sequences obtained from the clones were 2762 (Sen4), 2763 (Sen6) and 2764 (Sen8) nucleotide (nt) long and submitted in the GenBank under acc. no. MT188559-, MT188561). The genome organization of the clones were typical of previously reported begomoviruses, representing two genes on the virion sense as CP and V2 and four (Rep, TrAp, RE_n and C4) on the complementary sense. SDT analysis showed that the three full-length sequences have greater than 99% nt sequence identity match, confirming them to be isolates of a single begomovirus species. Pairwise sequence analysis with SDT and selected reference sequences from the GenBank, showed that the three isolates have a maximum 97.3–99.8% nt identity match with a strain of Chilli leaf

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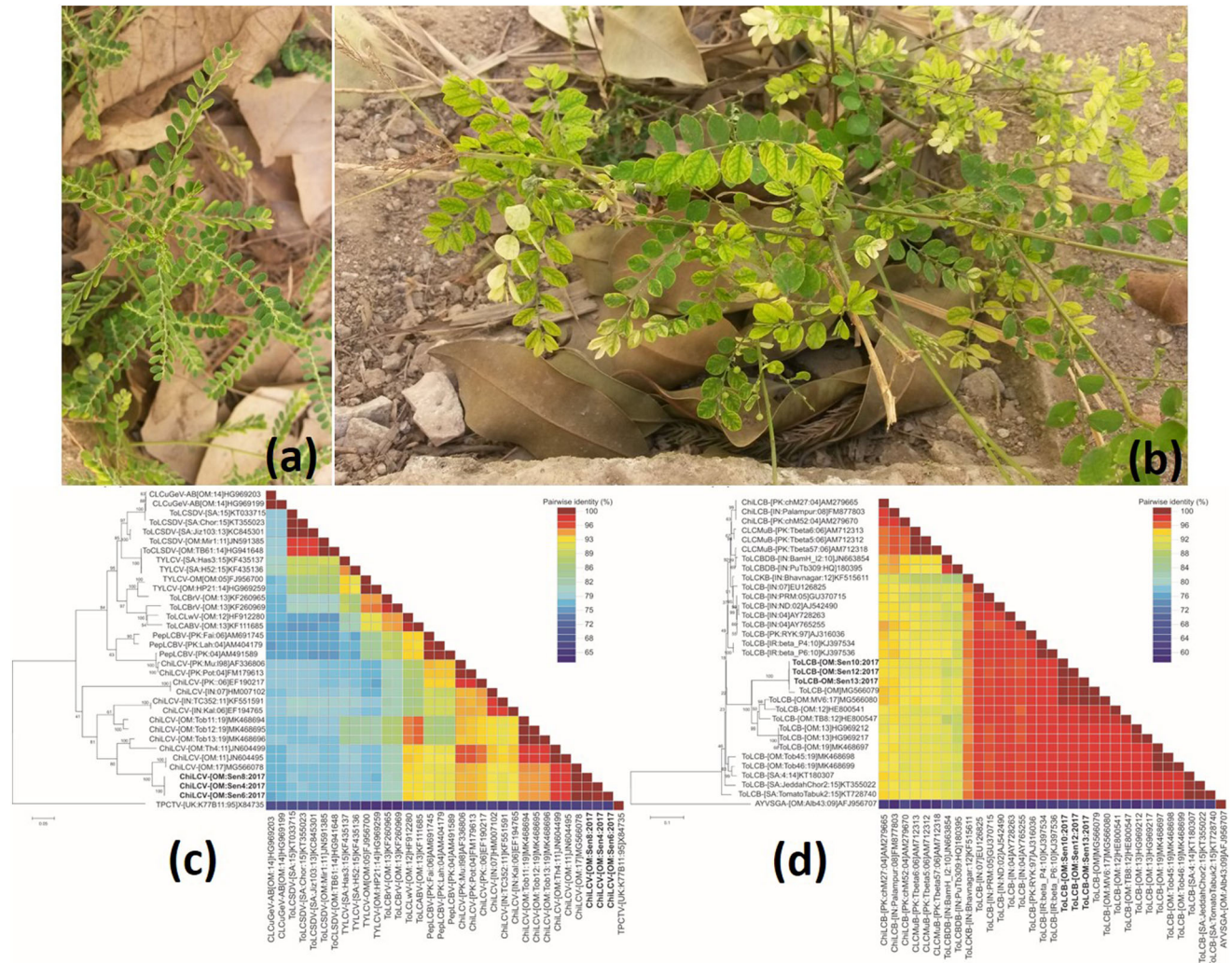


Fig. 1 Comparison of healthy and diseased *Senna italica* healthy plant, a plant with leaf yellowing and stunting symptoms (b).

curl virus (ChiLCV: GenBank acc. no. MG566078). This was further supported by a phylogenetic analysis based on the complete sequences of ChiLCV obtained from *S. italica* and selected reference sequences from the GenBank. These isolates along with isolates of ChiLCV previously identified from Oman cluster distinctly from other ChiLCV isolates reported from the Indian sub-continent, with the sequences from *S. italica* being most closely related to the ChiLCV isolates (Fig. 1c). Based on the results of this study, it appears that the host range of ChiLCV has expanded in recent years to include wild or weedy plants such as *S. italica*.

Three full-length betasatellite clones (Sen10, Sen12 and Sen13) one from each of the three symptomatic *S. italica* plants were produced by PCR using Sat101(5'-GTAGG-TACCACTACGCTACGCAGCAGCC-3')/Sat102 (5'-AG TGGTACCTACCCTCCCAGGGGTACACAC-3') betasatellite primers [3]. The obtained sequences of betasatellite clones are 1366 (Sen10), 1367 (Sen11) and 1368 (Sen12) nt

long and are accessible in the GenBank s under the acc. no.MT188562-MT188564. Sequence analysis indicated that these clones have genome organization analogous to the reported betasatellites, with a single conserved ORF in the complementary sense (known as β C1) (coordinates 191–547 nt) which translate a product of 118 amino acids, a sequence region rich in adenine (coordinates 722–968 nt), and a sequence well conserved among all betasatellites known as the satellite conserved region. SDT analysis showed that the betasatellites isolated from *S. italica* have a nucleotide sequence identity match between 98.4 and 99.3% with each other. Pairwise sequence analysis using SDT, and selected tomato leaf curl betasatellite (ToLCB) sequences from the databases, showed the ToLCB from *S. italica* have the highest levels of sequence identity match (99.6%) to one specific isolate of ToLCB, MG566079. Overall, the sequences from *S. italica* showed higher levels of sequence identity match to isolates of ToLCB originating from the Arabian Peninsula than to isolates either from Iran or the Indian sub-

continent (India and Pakistan). This is supported by phylogenetic analysis which shows isolates identified from *S. italica* clustering with ToLCB isolates from the Arabian Peninsula and forming a clade separate from other ToLCB isolates (Fig. 1d), with the closest other clade being that of isolates from Iran. Hence, it indicates that the ToLCB characterized from this study identified to be the isolate of previously reported ToLCB. Only two betasatellites, ToLCB and Okra leaf curl betasatellite (OLCB) are found in Oman [2]. Among the two, ToLCB is the most prevalent and is closely associated with begomoviruses identified from the country. The role of ToLCB was investigated in an earlier study which showed that it worked as a pathogenicity determinant as well as suppressor for posttranscriptional gene silencing. It is obvious that if ToLCB remains persistent in the field, ultimately it would affect the crop production of the country [4].

The presence of both begomovirus and betasatellite in different host plants is due to the polyphagous nature of their whitefly vector. Eradication and elimination of alternate host plants that act as reservoirs for different begomoviruses and associated DNA satellites during early stages of the crop is very essential.

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