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RAPID COMMUNICATION

First identification of tomato leaf curl Palampur virus in Oman: detection and characterization

Muhammad Shafiq Shahid*, Abdullah M. Al-Sadi

Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khod, Oman

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*Corresponding address:
mshahid@squ.edu.om

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Abstract

The complete genome sequence of tomato leaf curl Palampur virus (ToLCPlV), that infects a papaya (*Carica papaya*) plant, was determined. The virus genome was composed of 2,756 and 2,719 nucleotides (nt) in length, encoding all proteins required for replication, encapsidation and movement with the genome features typical of a bipartite begomovirus. Pairwise identity, derived using the Sequence Demarcation Tool (SDT), identified that the virus DNA A and DNA B shared maximum sequence identity 98–99% corresponding to the DNA A of ToLCPlV ([IR: Jir-T65X:08] JF501720) and 96–98% to the cognate partner DNA B of ToLCPlV ([IR: Jir1:T55P:07] FJ660423), respectively. The evolutionary relation using phylogenetic dendrograms of DNA A and DNA B genome components were clustered with ToLCPlV genomes of DNA A and DNA B of Iranian isolates. This study provides the first evidence of a bipartite ToLCPlV infecting papaya in the Sultanate of Oman and also indicates the requirement for more surveillance of this virus in Oman, as ToLCPlV is a major threat to tomato and other vegetable crops in South Asia (India and Pakistan) and in Iran.

Keywords: bipartite begomovirus, diversity, papaya, ToLCPlV

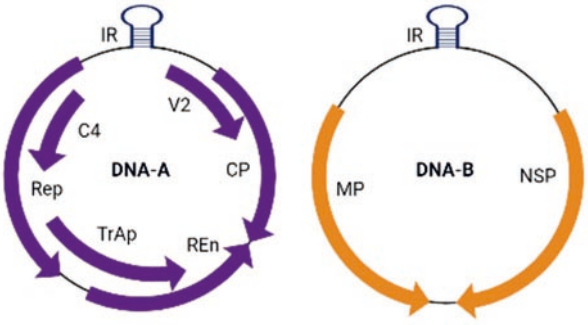
Single-stranded DNA (ssDNA) viruses belong to the family of monophyletic groups of viruses recognized as *Geminiviridae*. These phytopathogens cause severe losses to both monocotyledonous and dicotyledonous crops (Rojas *et al.* 2005). Subject to the genome structure, type of transmitting vector and host assortment, the family includes 520 virus species that are divided into 14 genera. Among them the begomovirus (BGV) genus consists of 445 recognized virus species, distributed globally through a complex of cryptic (with 44 known) whitefly (*Bemisia tabaci*) species and causes massive economic losses to crops (Walker *et al.* 2021). They can infect dicotyledonous including economically important host plants. The viruses of the BGV genus are further distributed into monopartite (containing a single DNA A) or bipartite (DNA A and DNA B), whereas monopartite BGVs are mainly accompanied with DNA satellites. Monopartite BGVs primarily occur in the Old World (OW), namely, Australia, Asia, the Middle East, Africa and Europe, whereas bipartite BGVs are mainly found in America, known as the New World (NW) (Idris *et al.* 2011; Shafiq *et al.* 2021). In bipartite BGVs, the DNA A component is homologous to the monopartite BGV genome which encodes replication associated protein (Rep), replication enhancer protein (Ren), transactivator protein (Trap), a symptom determinant protein (C4), coat protein (CP) and pre-coat protein (V2) which is lacking in the NW BGVs. The DNA B of bipartite BGV encodes proteins for cell-to-cell movement as movement protein (MP) and for long distance movement, identified as nuclear shuttle protein (NSP). Both cognate DNA A and DNA B genome share a generic region located in the intergenic region (IR), with an adequate level of similarity to allow the Rep protein of DNA A to replicate cognate DNA components (Hanley-Bowdoin *et al.* 1999). Tomato leaf curl Palampur virus (ToLCPlV) is an emerging bipartite begomovirus that is widespread in Iran, Pakistan and India. It infects several important crops including bitter gourd, cucurbits, cucumber,

melon, muskmelon, tomato, and watermelon (Heydarnejad *et al.* 2009; Ali *et al.* 2010; Namrata *et al.* 2010; Esmaeili *et al.* 2015).

In 2018, as a part of a BGV survey of papaya (*Carica papaya*) plants displaying severe symptoms of leaf curling, vein thickening, downward leaf cupping and stunted growth typical of BGV phenotypes were collected (symptomatic $n = 3$ and non-symptomatic $n = 2$) from two locations from Khasab in Oman (26.1654°N 56.2426°E) (Fig. 1). The viral genome material was extracted from papaya leaves using the CTAB method Doyle (1987), with slight modifications. Initial BEG detection was identified by PCR with a thermal cycler C1000 TouchTm (Bio-Rad, USA) using AV494 (viral strand) and AC1048 (complementary strand) CP based degenerate primers (Wyatt and Brown 1996), which amplified approximately 550 bp. One µg of purified, total nucleic acid preparations was added to each tube containing PCR mix and a total reaction volume of 50 µl. The PCR master mix contained 150 µM dNTPs, 2.5 Mm $MgCl_2$, 1.25 units of Taq DNA polymerase, and 20 pmol of each primer. PCR was carried out in a thermocycler with 35 cycles, each consisting of 1 min at 93°C (denaturation), 20 s at 58°C (annealing), and 30 s at 72°C (extension) and 10 min at 72°C for final extension. The amplified fragment was analyzed by sequencing and BLAST (https://blast.ncbi.nlm.nih.gov). The results confirmed that they were associated with tomato leaf curl Palampur virus (ToLCPlV). To recover the complete genome of the ToLCPlV infected papaya, the extracted DNA was used in rolling circle amplification (RCA) employing TempliPhi 100 Amplification Kit (GE Healthcare, USA), and yielded high molecular weight concatemer products. RCA was carried out in a total volume of 20 µl with 150–250 ng DNA, 10 mM dNTPs, 100 µM n-hexamer primer plus reaction buffer and 10 units of phi29 polymerase. The RCA conditions were 95°C for 3 min, addition of enzyme followed by incubation at 30°C for 18–20 h and completed by inactivating the enzyme at 65°C for 10 min. The RCA amplified product was digested with *Nde*I/*Kpn*I restriction enzyme, the digestion fragment was approximately 2.7 kb which was detected on 0.75% agarose gel. The digested fragment was purified and cloned into pGEM-T Easy vector systems (Thermo Fisher Scientific, USA) at the compatible restriction sites. At least two full-length clones (DNA A and DNA B) from each plant were confirmed and sequenced completely through the Sanger sequencing method by Macrogen Inc. (South Korea). Sequences were assembled and manipulated using Lasergene package (DNAStar Inc., Madison, WI, USA).

The complete DNA A genome of bipartite BGV isolates (PV14-1, PV15-3 and PV16-4) were determined to be 2,756 nucleotides (nt) in length (GenBank acc. no. MZ423187–MZ423189) (Table 1). The DNA A components had a genome structure typical of bipartite BGV discovered from the OW, including four ORFs encoding for Rep (367 kDa protein), TrAp (139 kDa protein), REn (136 kDa protein) and AC4 (58 kDa protein) in the complementary sense and two ORFs encoding for CP (256 kDa protein) and MP (115 kDa protein) in the virion strand (Fig. 2 and Table 1). The highest nucleotide identity (98–99%) was associated with the isolates of ToLCPlV reported from Iran ([IR: Jir8:T58P:08] FJ660431) (Heydarnejad *et al.* 2013), followed by isolates from Pakistan (96–98%) and India (92–97%) using the Sequence Demarcation Tool (SDT 1.2) (Fig. 3) (Muhire *et al.* 2014). According to the ICTV criteria set at 94 and 91% for demarcation of new strains and species the virus isolates identified here from papaya were the new isolates of ToLCPlV (Brown *et al.* 2015). None of the recombinant event was identified for ToLCPlV in RDP 4.1 program using available algorithms (viz. RDP, GENECONV, BootScan,

Fig. 1. Papaya plant naturally infected by tomato leaf curl Palampur virus displaying leaf curling and downward leaf cupping symptoms



Tomato leaf curl palampur virus (ToLCPlV)

Fig. 2. Genome of tomato leaf curl Palampur virus: DNA A and DNA B

Table 1. Features of tomato leaf curl Palampur virus (ToLCPalV) isolated from field infected papaya plants

DNA A									DNA B				
isolate	acc. no.	size [nt]	position of genes (coordinates)/no. of amino acids [predicted coding capacity in kDa]						isolate	acc. no.	size [nt]	position of genes (coordinates)/ no. of amino acids [predicted coding capacity in kDa]	
			CP	V2	Rep	TrAP	REn	C4				BV1	BC1
PV14-1	MZ423187	2,756	280–1050/ 256 (28.42)	120–467/ 115 (12.77)	1499–2602/ 367 (40.74)	1177–1596/ 139 (15.43)	1047–1457/ 136 (15.1)	2269–2445/ 58 (6.44)	PV14-6	MZ423190	2,719	426–1232/ 268 (29.75)	1298–2143/ 281 (31.19)
PV15-3	MZ423188	2,756	280–1050/ 256 (28.42)	120–467/ 115 (12.77)	1499–2602/ 367 (40.74)	1177–1596/ 139 (15.43)	1047–1457/ 136 (15.1)	2269–2445/ 58 (6.44)	PV15-5	MZ423191	2,719	426–1232/ 268 (29.75)	1298–2143/ 281 (31.19)
PV16-4	MZ423189	2,756	280–1050/ 256 (28.42)	120–467/ 115 (12.77)	1499–2602/ 367 (40.74)	1177–1596/ 139 (15.43)	1047–1457/ 136 (15.1)	2269–2445/ 58 (6.44)	PV16-8	MZ423192	2,719	426–1232/ 268 (29.75)	1298–2143/ 281 (31.19)

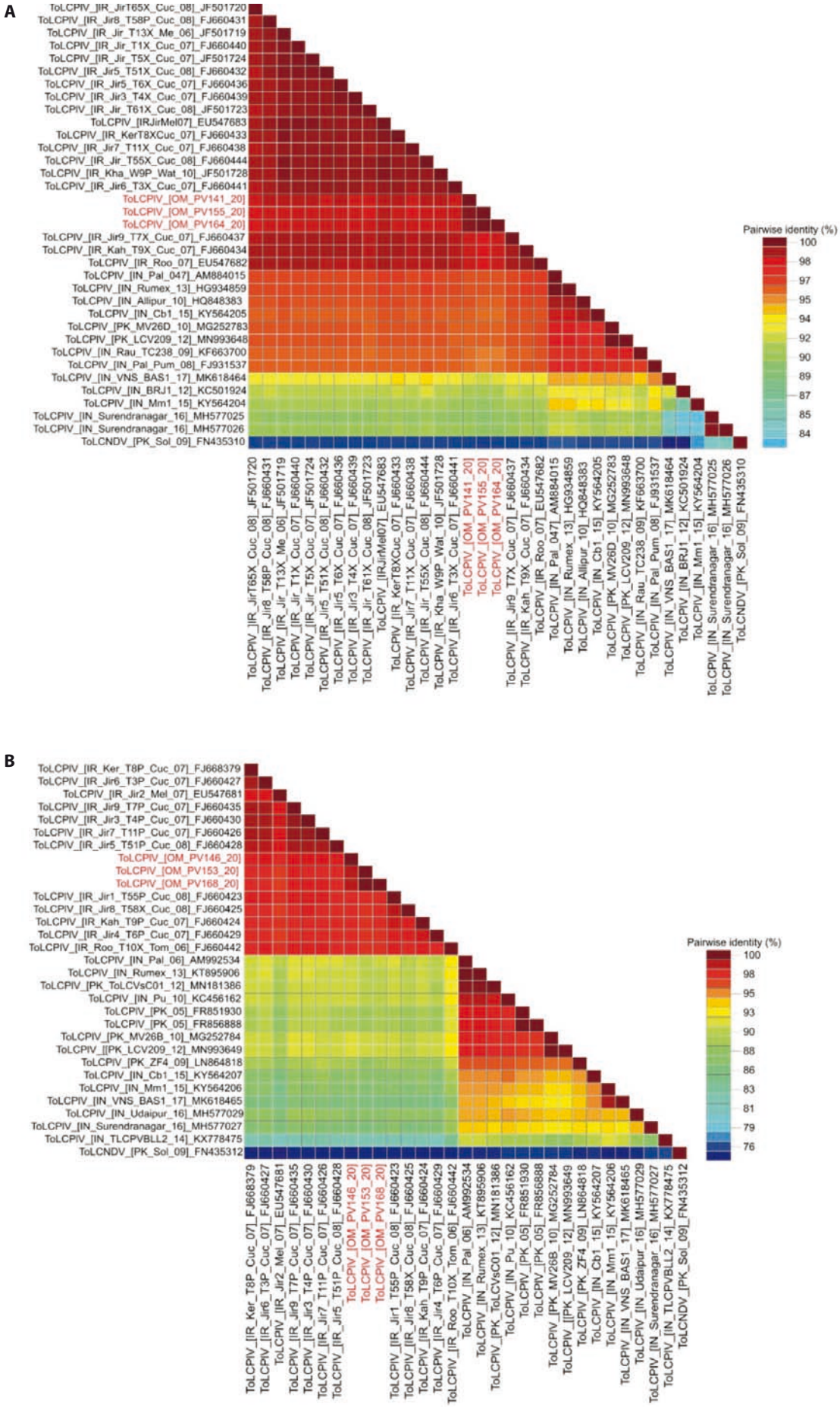


Fig. 3. Pairwise sequence analysis using MUSCLE alignment in the species demarcation tool (SDTv1.2) with sequences of DNA A (A) and DNA B (B)

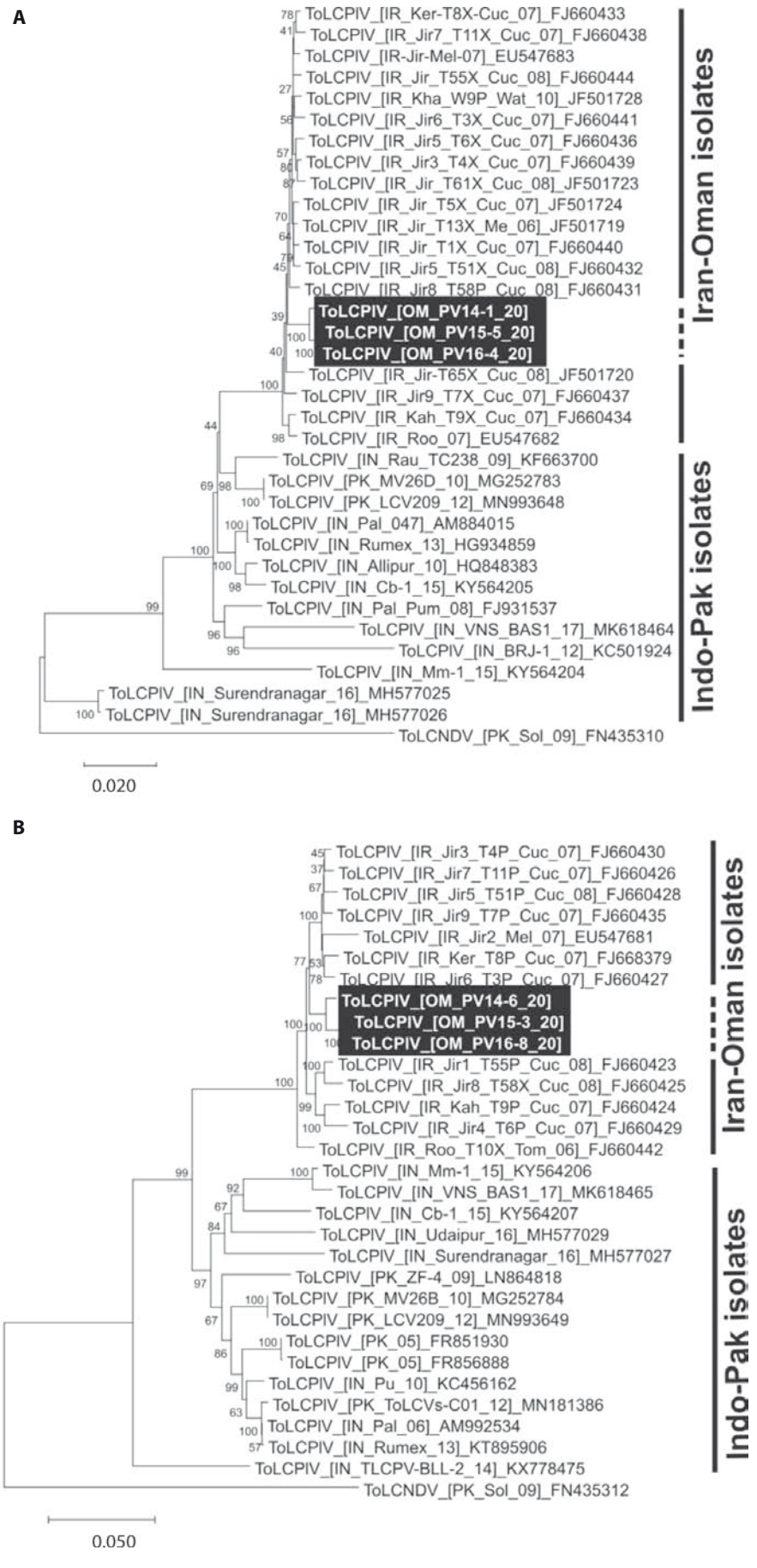


Fig. 4. Phylogenetic dendrograms based on complete nucleotide sequences of bipartite begomovirus genome were constructed in MEGAX software with Neighbor joining (NJ) algorithm and with best fit kimura-2 parameter and 1000 bootstrap value. Both trees were arbitrarily rooted on the distinct sequences of tomato leaf curl New Delhi virus DNA A (A) and DNA B (B), respectively

MaxChi, SiScan, Chimaera and 3SEQ) (Martin *et al.* 2015). The evolutionary relationships of DNA A sequences of ToLCPlV indicated a degree of geographical clustering among DNA A of ToLCPlV isolates. The phylogenetic dendrogram based on complete nucleotide sequences of bipartite begomovirus genome were constructed in MEGAX software with Neighbor joining (NJ) algorithm and with best fit kimura-2 parameter and 1,000 bootstrap. The trees were arbitrarily rooted on the distinct sequences of tomato leaf curl New Delhi virus DNA A and DNA B, respectively. The results of the phylogenetic trees showed that ToLCPlV DNA A isolates of this study were closely related to the DNA A of ToLCPlV Iranian isolates. The geographical clustering reflected that the Omani and Iranian isolates evolved at a similar time and are closer to each other than what has been reported from India and Pakistan (Fig. 4). Three DNA B isolates (PV14-6, PV15-5 and PV16-8) identified were 2,719 nt in length (accession numbers MZ423190–MZ423192, respectively) (Table 1). The cognate DNA B components had a genome arrangement characteristic of DNA B molecules typical in all bipartite BGV genomes, and were comprised of a NSP (31 kDa protein) and MP (29 kDa protein). SDT analysis revealed that DNA B nucleotide sequence identity was 96–98% with Iran isolate ToLCPlV ([IR: Jir6:T3P:07] FJ660427) (Fig. 3D). In phylogenetic analysis, the DNA B of ToLCPlV from the Oman group with cognate DNA B of Iranian ToLCPlV isolates were distant from Indian or Pakistani isolates (Fig. 4). Papaya belongs to the family Caricaceae. Due to its dietary benefits and demand, papaya fruit production has significantly increased over the last few decades in different geographical countries. Meanwhile, different BGVs such as okra enation leaf curl virus (OELCuV), cotton leaf curl Gezira virus (CLCuGeV), papaya leaf curl virus (PaLCV), papaya leaf curl China virus (PaLCCNV), ageratum yellow vein virus (AYVV), tomato leaf curl Bangladesh virus (ToLCBaV), and radish leaf curl virus (RaLCV) have been found to infect this fruit plant in different countries (Chang *et al.* 2003; Shahid *et al.* 2013; Shen *et al.* 2014; Bananej *et al.* 2016; Tang *et al.* 2018; Hamim *et al.* 2019; Nehra *et al.* 2019; Bananej *et al.* 2021). Although a cotton infecting monopartite BGV (CLCuGeV) has been identified which infects papaya plants in Oman (Khan *et al.* 2012), none of the bipartite BGV has been reported to infect papaya plants in the country. This is the first study showing bipartite BGV infecting papaya plants in Oman. The highest nt identity and close clustering of ToLCPlV isolates with Iranian isolates indicates that the introduction of ToLCPlV into Khasab happened quite recently, possibly through agriculture trade between both countries. ToLCPlV has been reported to infect different crop species, resulting in heavy crop losses in Iran which is very close to the Khasab area. Additionally, Iran and Oman have frequent agriculture trade through the Khasab port. It is possible that ToLCPlV was transfered to Oman via trade of infected materials (vegetables, ornamental or fruit plants). Nevertheless, such theories need to be verified at the genome level. Due to limited agricultural land, papaya plants are grown close to vegetable crops in Oman, where intercropping of different crops particularly tomato (a primary host for ToLCPlV) is routine in farmers' fields. It is feared that the whitefly vector may transmit the virus to the tomato plants. To prevent a very likely outbreak of ToLCPlV epidemics in tomato fields, we suggest discouraging the planting of crops near papaya plants. If the cultivation of different crops in neighboring fields is unavoidable, then the cultivation approach, planting distance, and scheduling should be thoroughly analyzed to efficiently control ToLCPlV.

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