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NEW DISEASE REPORT

First report of Tomato leaf curl Kerala virus infecting Carica papaya in India

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Papaya (*Carica papaya*) is a tropical fruit widely grown and consumed for its medicinal and pharmacological qualities. Papaya is frequently infected with plant viruses which leads to a reduction in yield and nutritional value. A survey was conducted in 2021 in the Lucknow district of Uttar Pradesh, India, and leaves from diseased papaya plants showing symptoms of leaf curl disease were collected from five different fields. The diseased papaya plants had deformed leaves with severe upward and downward curling, thickened and twisted petioles, and thickened veins with a leathery appearance (Figure 1). The symptoms on the plants and the presence of whiteflies suggested a begomoviral infection.

Tootal genomic DNA was extracted from five leaves using the CTAB method (Murray & Thompson, 1980) and PCR was performed using coat protein(CP)-specific primers (AV494/AC1048) designed from the core region of the CP gene of DNA-A of geminiviruses (Wyatt & Brown 1996). A product of the expected size (c. 560 bp) was amplified from three of the samples. The amplified product was purified and sequenced (Genbank Accession No. PP072296). A BLAST analysis revealed that the two of the sequences shared 99% nucleotide sequence identity with *Tomato leaf curl Kerala virus* (ToLCKeV) (MH973690.1); the third shared identity with *Chilli leaf curl virus*. No amplification was found when samples were screened for the presence of DNA-B using the DNA-B specific degenerate primer pair (PBL1v2040/PCRc1) (Rojas et al., 1993) suggesting that the virus is monopartite. We were also unable to amplify any associated satellite molecules in the samples using alpha (DNA101/DNA102) or beta satellite-specific primers (β01/β02) (Bull et al., 2003).

MEGA 11 software was used for multiple alignment using ClustalW and phylogenetic study using the neighbour-joining method (Tamura & Nei 1993; Tamura et al., 2021). A total of 34 sequences of 560 bp from the core CP region of different begomoviruses were retrieved from GenBank and a phylogenetic tree was constructed using the neighbour-joining method (with 1,000 bootstrap replicates) based on the shared sequence identity among the isolates (Table 1). The phylogenetic tree showed that the current isolate is closely related to isolates of ToLCKeV from Faisalabad, Pakistan (MH973690.1) and Karnataka, India (OQ128343.1) identified in *Digera muricata* and beetroot, respectively (Figure 2), and shared 95–99% identity with other ToLCKeV isolates. The results obtained were only from the 560 bp sequence, complete genome analysis may provide a different inference.

To the best of our knowledge, this is the first report of ToLCKeV associated with leaf curl disease on papaya. This study provides evidence for expanding the host range of ToLCKeV. However, it is not possible to prove conclusively that the observed symptoms were caused by ToLCKeV, other pests and pathogens may have a role in the aetiology of the disease. The frequent occurrence of different begomovirus species on papaya is a matter of serious concern. This study provides information to aid the development of host resistance strategies as well as in studies of viral genome and evolution.

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FIGURE 1 Papaya leaf samples showing leaf curl disease symptoms observed in Lucknow district of Uttar Pradesh, India

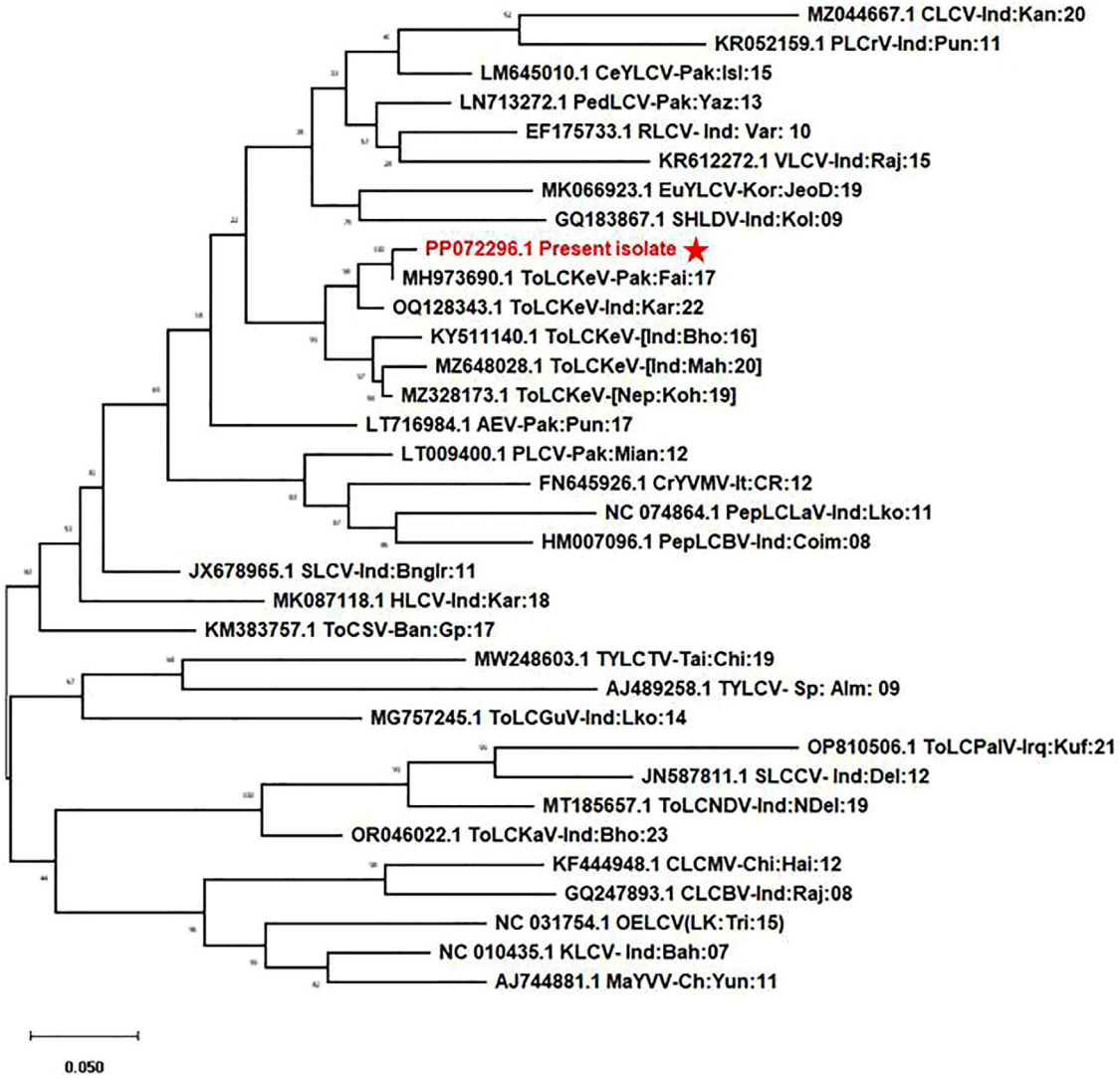

FIGURE 2 Phylogenetic tree constructed using ClustalW programme showing the relationship between the present isolate and related begomovirus species. The current papaya isolate (indicated in red) showed close homology with *Tomato leaf curl Kerala virus* and a relationship with other begomoviruses including *Ageratum enation virus* (AEV), *Papaya leaf curl virus* (PLCV) and *Sunn hemp leaf distortion virus* (SHLDV)

TABLE 1 Begomovirus DNA-A sequences used for phylogenetic analysis

Virus	Acronyms	GenBank Accession No.
Tomato leaf curl Kerala virus-Papaya-[India:Lucknow:2021]	ToLCKeV-P-[Ind:Lko:21]	PP072296.1
Tomato leaf curl Kerala virus-[India:Karnataka:2022]	ToLCKeV-[Ind:Kar:22]	OQ128343.1
Tomato leaf curl Karnataka virus-[India:Bhopal:2023]	ToLCKaV-[Ind:Bho:23]	OR046022.1
Tomato leaf curl New Delhi virus-[India:New Delhi:2019]	ToLCNDV-[Ind:NDel:19]	MT185657.1
Papaya leaf curl virus-[Pakistan:Mianwali:2012]	PLCV-[Pak:Mian:12]	LT009400.1
Tomato leaf curl Palampur virus-[Iraq:Kufa:2021]	ToLCPalV-[Irq:Kuf:21]	OP810506.1
Chilli leaf curl virus-[India:Kanpur:2020]	CLCV-[Ind:Kan:20]	MZ044667.1
Kenaf leaf curl virus-[India:Bahraich:2007]	KLCV-[Ind:Bah:07]	NC_010435.1
Tomato leaf curl Gujarat virus-[India:Lucknow:2014]	ToLCGuV-[Ind:Lko:14]	MG757245.1
Cotton leaf curl Multan virus-[China:Hainan:2012]	CLCMV-[Chi:Hai:12]	KF444948.1
Okra enation leaf curl virus-[Sri Lanka:Trincomalee:2015]	OELCV(LK:Tri:15)	NC_031754.1
Pepper leaf curl Lahore virus-[India:Lucknow:2011]	PepLCLaV-[Ind:Lko:11]	NC_074864.1
Tomato leaf curl Kerala virus-[Pakistan:Faisalabad:2017]	ToLCKeV-[Pak:Fai:17]	MH973690.1
Cotton leaf curl Burewala virus-[India:Rajasthan:2008]	CLCBV-[Ind:Raj:08]	GQ247893.1
Tomato yellow leaf curl Thailand virus-[Taiwan:Chiayi:2019]	TYLCTV-[Tai:Chi:19]	MW248603.1
Hollyhock leaf curl virus-[India:Karnataka:2018]	HLCV-[Ind:Kar:18]	MK087118.1
Pedilanthus leaf curl virus-[Pakistan:Yazman:2013]	PedLCV-[Pak:Yaz:13]	LN713272.1
Tomato yellow leaf curl virus-[Spain:Almeria:2002]	TYLCV-[Sp:Alm:09]	AJ489258.1
Radish leaf curl virus-[India:Varanasi:2007]	RLCV-[Ind:Var:10]	EF175733.1
Cestrum yellow leaf curling virus-[Pakistan:Islamabad:2013]	CeYLCV-[Pak:Isl:15]	LM645010.1
Malvastrum yellow vein virus-[China:Yunnan:2011]	MaYVV-[Ch:Yun:11]	AJ744881.1
Euphorbia leaf curl virus-[Korea:Jeollabuk-Do:2019]	EuYLCV-[Kor:JeoD:19]	MK066923.1
Sunflower leaf curl virus-[India:Karnataka:2011]	SLCV-[Ind:Bnglr:11]	JX678965.1
Ageratum enation virus-[Pakistan:Lahore:2017]	AEV-[Pak:Pun:17]	LT716984.1
Sunn hemp leaf distortion virus-[India:Kolkata:2009]	SHLDV-[Ind:Kol:09]	GQ183867.1
Pepper leaf curl Bangladesh virus-[India:Coimbatore:2008]	PepLCBV-[Ind:Coim:08]	HM007096.1
Tobacco curly shoot virus-[Bangladesh:Gazipur: 2017]	ToCSV-[Ban:Gp:17]	KM383757.1
Croton yellow vein mosaic virus-[Italy:Ca'Tron di Roncade:2012]	CrYVMV-[It:CR:12]	FN645926.1
Vinca leaf curl virus 15-[India:Rajasthan:2015]	VLCV-[Ind:Raj:15]	KR612272.1
Squash leaf curl China virus-[India:Delhi:2012]	SLCCV-[Ind:Del:12]	JN587811.1
Papaya leaf crumple virus-[India:Punjab:2011]	PLCrV-[Ind:Pun:11]	KR052159.1
Tomato leaf curl Kerala virus-[India:Bhopal:2016]	ToLCKeV-[Ind:Bho:16]	KY511140.1
Tomato leaf curl Kerala virus-[India:Maharashtra:2020]	ToLCKeV-[Ind:Mah:20]	MZ648028.1
Tomato leaf curl Kerala virus-[Nepal:Koholpura:2019]	ToLCKeV-[Nep:Koh:19]	MZ328173.1

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