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First Report of Tomato Leaf Curl Kerala Virus Associated With Yellow Vein Net Disease of *Alternanthera tenella* From Central India

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Alternanthera tenella (family Amaranthaceae) originates from the tropics and sub-tropics, and is distributed in Africa, China, India, Sri Lanka and the USA. It is used as a folk medicine, having anti-inflammatory and diuretic properties. In March 2022, yellow vein net symptoms were observed on *A. tenella* growing in a nursery in Bhopal, Central India (Figure 1). Following the methodology of Srivastava et al. (1977), the disease was transmitted by whiteflies (*Bemisia tabaci*) from naturally infected plants to healthy seedlings of *A. tenella*, *Capsicum annum Nicotiana tabacum* and *Solanum lycopersicum* (each 5/5), with symptoms manifesting 30 days post-infection. The agent was identified as a begomovirus using coat protein



FIGURE 1 | *Alternanthera tenella* showing yellow vein net symptoms (A) compared to a healthy plant (B) observed at Bhopal, Madhya Pradesh, Central India.

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TABLE 1 | Sequence identities of the begomovirus isolate from *A. tenella* (PP700231) with other begomovirus isolates

Accession no.	Virus	Host	Country	Percentage identity (%)
MZ648028	ToLCKeV-[IN:AGB:Fenugreek:2022]	*Trigonella foenum-graecum*	India	98.05
EU910140	ToLCKeV-[IN:ND:Tomato:2008]	*Solanum lycopersicum*	India	97.22
KF551575	ToLCKeV-[IN:ND:Tomato:2013]	*Solanum lycopersicum*	India	96.29
KX827601	ToLCKeV-[PK:FSD:Glycine:2018]	*Glycine max*	Pakistan	95.64
NC_011135	ToLCKeV-[IN:ND:Tomato:2018]	*Solanum lycopersicum*	India	95.46
EU910141	ToLCKeV-[IN:ND:Tomato:2008]	*Solanum lycopersicum*	India	95.46
OQ128344	ToLCKeV-[IN:KT:Beet:2023]	*Beta vulgaris*	India	93.38
KX665543	ToLCKeV-[PK:PJ:Brassica:2018]	*Brassica rapa*	Pakistan	93.16
KY926895	ToLCKeV-[PK:FSB:Populus:2019]	*Populus alba*	Pakistan	92.94
LT795117	PeLCuV-[PK:SKP:Brassica:2017]	*Brassica rapa*	Pakistan	92.45
MN020537	ToLCKV-[IN:BA:Tomato:2020]	*Solanum lycopersicum*	India	91.64
MN885491	CLCKoV-[PK:FSB:Glycine:2021]	*Glycine max*	Pakistan	90.32
KC952005	ToLCGanV-[IN:GT:Tomato:2014]	*Solanum lycopersicum*	India	90.22

Abbreviations: CLCKoV, cotton leaf curl Kokhran virus; PeLCV, Pedilanthus leaf curl virus; ToLCGaV, tomato leaf curl Gandhinagar virus; ToLCKeV, tomato leaf curl Kerla virus; ToLCKV, tomato leaf curl Karnataka virus.

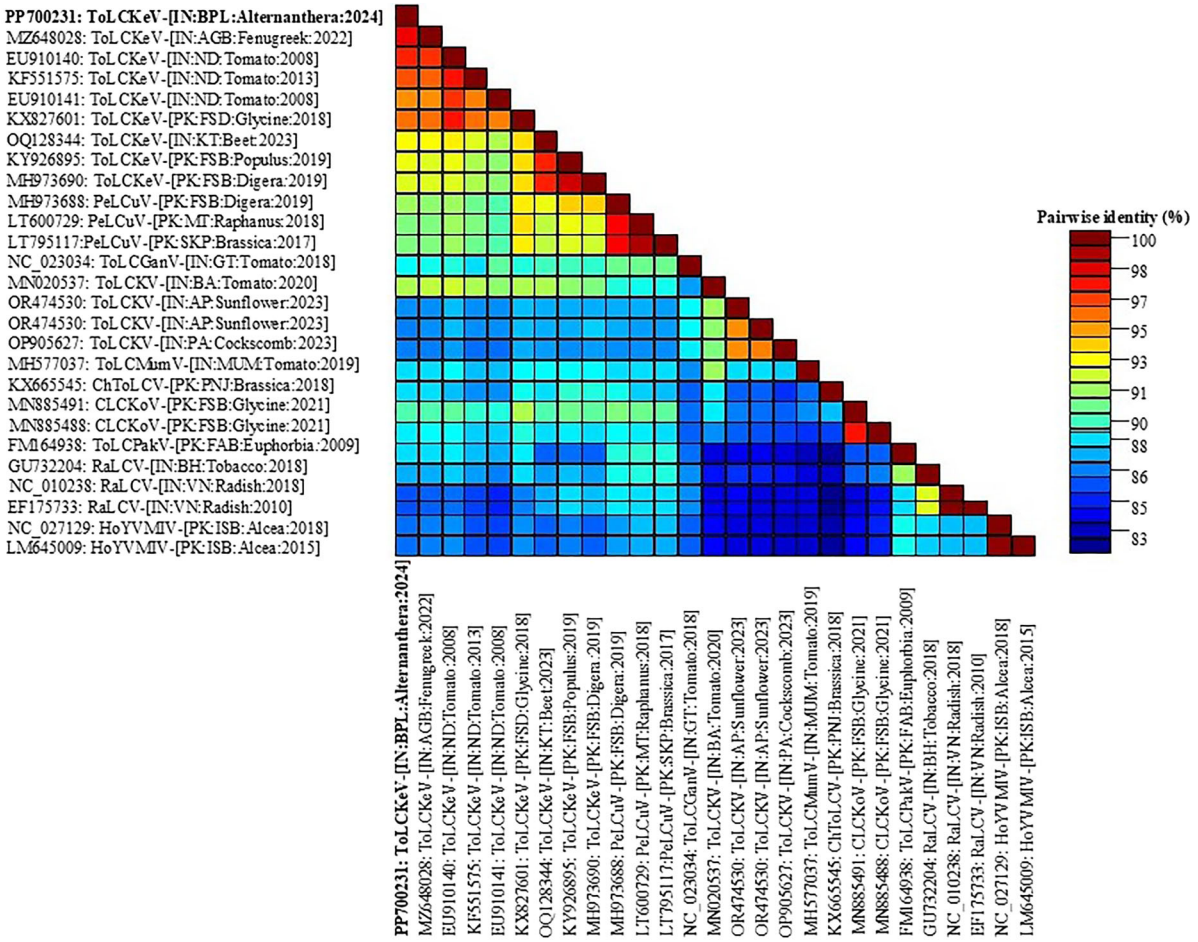


FIGURE 2 | The DNA-A genome of (PP700231) of the virus isolated from *A. tenella* showed the highest nucleotide pairwise identity scores with other begomovirus isolates using the species demarcation tool. ChToLCV, cherry tomato leaf curl virus; CoLCKoV, cotton leaf curl Kokhran virus; HoYVMIV, hollyhock yellow vein mosaic Islamabad virus; PeLCV, Pedilanthus leaf curl virus; RaLCV, radish leaf curl virus; ToLCGaV, tomato leaf curl Gandhinagar virus; ToLCKeV, tomato leaf curl Kerala virus; ToLCKV, tomato leaf curl Karnataka virus; ToLCMuV, tomato leaf curl Mumbai virus; ToLCPakV, tomato leaf curl Pakistan virus.

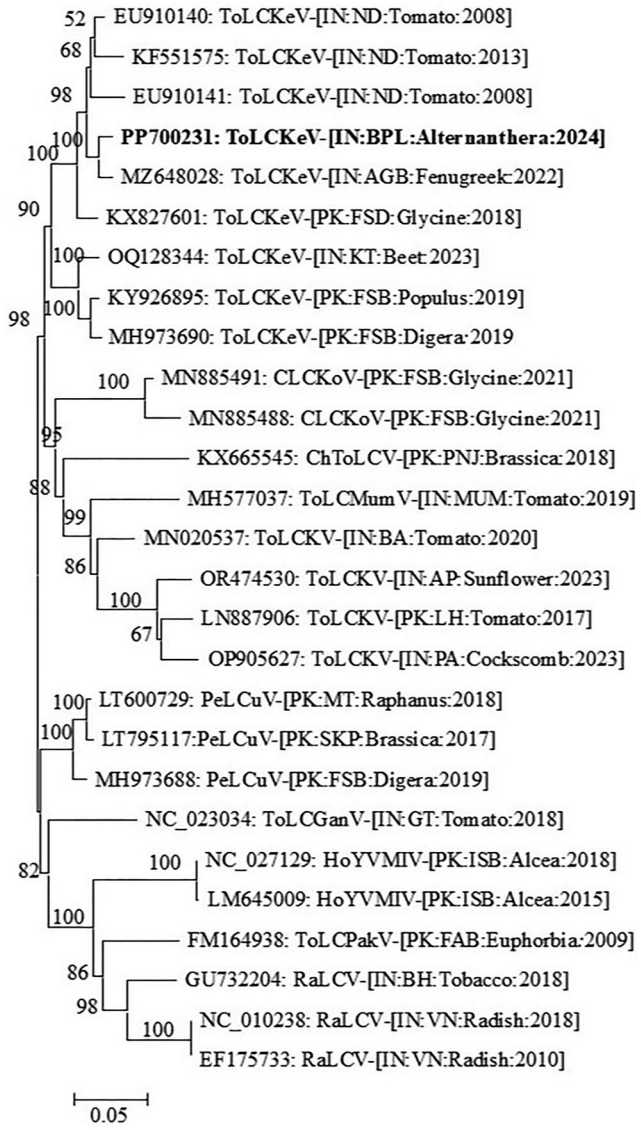


FIGURE 3 | Phylogenetic analysis of the complete viral DNA-A genome (PP700231) of *A. tenella* showing close relationships with isolates of ToLCKeV and other begomovirus isolates. Analysed using the MEGA (v. 11.0) with 1000 replicates bootstrapping and a dendrogram was generated with the neighbour-joining method and viewed by the NJ plot program. ChToLCV, cherry tomato leaf curl virus; CoLCKoV, cotton leaf curl Kokhran virus; HoYVMIV, hollyhock yellow vein mosaic Islamabad virus; PeLCV, Pedilanthus leaf curl virus; RaLCV, radish leaf curl virus; ToLCGaV, tomato leaf curl Gandhinagar virus; ToLCKeV, tomato leaf curl Kerala virus; ToLCKV, tomato leaf curl Karnataka virus; ToLCMuV, tomato leaf curl Mumbai virus; ToLCPakV, tomato leaf curl Pakistan virus.

gene-specific PCR primers (5′-ATGGCGAAGCGACCAG-3′ and 5′-TTAATTTGTGACCGAATCAT-3′).

For molecular characterisation, total DNA was extracted from leaves of five diseased and one asymptomatic plant. A begomovirus was amplified from diseased samples (but not the asymptomatic sample) by PCR using three sets of begomovirus-specific primers: CPIT-I/CPIT-T, PALIv 1978/ PARIc 496 and PALIv 722/ PALIc 1960 (Rojas et al. 1993; Singh 2005). The expected PCR amplicons (c. 0.8, 1.2 and 1.2 kb, respectively) were sequenced (Barcode Bioscience Pvt. Ltd., India), and the consensus nucleotide sequence of the complete DNA-A genome (2772 nt) was submitted to GenBank (Accession No. PP700231). However, no amplicons were amplified from diseased samples by PCR targeting the DNA-B and the DNA-β satellite molecule, suggesting that the virus is monopartite.

BLASTn analysis of the DNA-A genome (PP700231) of the begomovirus isolate from *A. tenella* revealed the highest nucleotide sequence identity (92.94%–98.05%) with isolates of tomato leaf curl Kerala virus (ToLCKeV; MZ648028, EU910140 and EU910141) from a range of hosts in India and Pakistan (Table 1). The pairwise identity scores were determined using the species demarcation tool. The isolate from this study shared the highest nucleotide sequence identity (93%–98%) with isolates of ToLCKeV (Figure 2). Phylogenetic analysis of the complete viral DNA-A genome (PP700231) of *A. tenella* was done through MEGA v11.0. The genome had close phylogenetic relationships with isolates of ToLCKeV (MZ648028 and EU910141) and also shared distinct relationships with other begomovirus isolates reported globally (Figure 3). Based on the sequence identity and the phylogenetic relationships, the virus isolated from *A. tenella* was identified and characterised as an isolate of ToLCKeV.

Previously, ToLCKeV associated with yellow mosaic disease was reported in tomato (Pandey et al. 2010) and *Beta vulgaris* (Lakshminarayana Reddy et al. 2024) from India. ToLCKeV has also been reported recently associated with papaya with leaf curl disease from India (Priyanka Akhter and Saxena 2024). To the best of our knowledge, this is the first report of ToLCKeV associated with yellow vein net disease on *A. tenella* from India as well as globally. Whiteflies (*B. tabaci*) observed in the affected nursery are likely to transmit ToLCKeV, and this risk should be considered for future disease surveillance programmes.

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