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NEW DISEASE REPORT

First report of a begomovirus infecting *Cordyline fruticosa*

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Cordyline fruticosa (family *Asparagaceae*) is an ornamental plant, native to Southeast Asia and the Western Pacific region but grown in many regions of the world. It is a palm-like evergreen shrub with a strong, usually unbranched stem, and three main varieties: tricolour (green with red, pink and cream), red edge (green with red streaks) and plain green. The species has been reported to be infected with members of the *Closterovirus* and *Emaravirus* genera.

A survey was conducted in November 2019 in the Alawalpur region of Jalandhar district in Punjab, India, and young leaves of five *C. fruticosa* plants showing chlorotic spots were collected from different fields in close vicinity (Figure 1). The presence of whiteflies and the symptoms suggested a begomovirus infection. Genomic DNA from the leaf samples was extracted and subjected to PCR using begomovirus-specific degenerate primers according to Wyatt & Brown (1996). Amplicons of c. 570 bp were obtained from all the leaf samples. The amplicons were gel purified and the eluted products were sequenced by Eurofins Genomics India (Bangalore, India). Analysis showed that all five sequences were identical, and a BLAST search showed that these sequences were most similar to the DNA-A component of *Tomato leaf curl New Delhi virus* (ToLCNDV). To amplify the complete DNA-A of the isolate, PCR primers were designed (TLCV-AF 5′-GAGGTCAGCAATCTGCCAAC-3′ and TLCV-AR 5′-CTCTAGCTGATCTGCCATCG-3′) and the reaction was done using 2.5μl DNA sample, 18.2 μl water, 0.5 μl each of forward and reverse primer, 0.5 μl 100 mM dNTPs, 2.5 μl 10× *Taq* buffer and 0.3 μl (1.5 units) *Taq* DNA polymerase (Genei, India). PCR was done with an initial



FIGURE 1 Symptomatic leaves of *Cordyline fruticosa* plant

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TABLE 1 Begomovirus DNA-A sequences used for phylogenetic analysis

Virus	Accession No.	Abbreviation
Tomato leaf curl New Delhi virus- [India: Punjab: 2019]	MW269629	ToLCNDV- [Ind: Pun:19]
Tomato leaf curl New Delhi virus- [India: Punjab:2019]	MT185657	ToLCNDV- [Ind: Pun: 19]
Bitter gourd yellow vein virus- [India: Kanpur: 2014]	KM190927	BYVV- [Ind: Kan:14]
Squash leaf curl China virus- [India: Delhi: 2012]	JN587811	SLCCV- [Ind: Del:12]
Tomato leaf curl Karnataka virus- [India: Karnataka: 2019]	KF551585	ToLCKV- [Ind: Kar:19]
Melon yellow mosaic virus- [Thailand: 2019]	MH665365	MeYMV- [Th:19]
Bhendi yellow vein Haryana virus- [India: Haryana: 2019]	FJ561298	BhYVHV- [Ind: Har:19]
Pumpkin yellow mosaic Malaysia virus- [Malaysia: Negeri Sambilan: 2007]	EF197941	PYMMV- [Mal: NeS: 07]
Melon leaf curl virus- [Pakistan: Punjab: 2010]	AM494976	MeLCV- [Pak: Pun: 10]
Loofa yellow mosaic virus- [VietNam: 2003]	AF509739	LYMV- [VNam: 03]
Chilli leaf curl virus- [India: Amritsar: 2009]	GU136803	ChLCV- [Ind: Asr: 09]
Papaya leaf curl virus- [India: New Delhi: 2009]	DQ629103	PaLCV- [Ind: NDel: 09]
Syndrella yellow vein clearing virus- [India: 2012]	KX363443	SyYVCV- [Ind:12]
Ageratum enation virus- [India: Lucknow: 2012]	JQ911765	AgEV- [Ind: Luck:12]
Papaya leaf crumple virus- [India: Rajasthan: 2016]	KP698312	PaLCrV- [Ind: Raj:16]
Hollyhock leaf curl virus- [India: Karnataka: 2019]	MK087118	HoLCV- [Ind: Kar:19]
Pedilanthus leaf curl virus- [Pakistan: Faisalabad: 2016]	LN713272	PeLCV- [Pak: Fai:16]
Emilia sonchifolia yellow vein Thailand virus- [Thailand: 2017]	KY373213	ESYVTV- [Th: 17]
Cestrum yellow leaf curling virus- [Pakistan: Islamabad: 2015]	LM645010	CeYLCV- [Pak: Isl: 15]
Malvastrum yellow vein virus- [China: Yunnan: 2011]	AJ744881	MaYVV- [Ch: Yun: 11]
Radish leaf curl virus- [India: Varanasi: 2010]	EF175733	RLCV- [Ind: Var: 10]
Crassocephalum yellow vein virus- [China: Yunnan: 2019]	MK622789	CrYVV- [Ch: Yun: 19]
Coccinia mosaic Tamil Nadu virus- [India: Tamiln Nadu: 2014]	KM244719	CoMTV- [Ind: Tn :14]
Kenaf leaf curl virus- [China: Yunnan: 2010]	HM448898	KeLCV- [Ch: Yun:10]
Cotton leaf curl Bangalore virus- [India: Bangalore: 2005]	GU112003	CLCBV- [Ind: Bglr: 05]
Euphorbia yellow leaf curl virus- [Pakistan: Punjab: 2018]	KT159766	EuYLCV- [Pak: Pun: 18]
Tomato yellow leaf curl virus- [Spain: Almeria: 2009]	AJ489258	TYLCV- [Sp: Alm: 09]

denaturation at 94°C for 3 minutes, 30 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 40 seconds and extension at 72°C for 3 minutes 15 seconds, and final extension at 72°C for 7 minutes. Amplicons of c. 2.7 kb were obtained from the five samples and were cloned and sequenced. A representative sequence was deposited in GenBank (Accession No. MW269629) and BLAST analysis showed it to be most closely related to an isolate of ToLCNDV from bitter gourd (MT185657) with 99.51% identity. The MEGA X software was used for multiple alignment and phylogenetic analysis (Kumar et al., 2018). A total of 27 sequences (Table 1) belonging to different begomovirus species were selected for phylogenetic and recombination analysis. Pairwise alignment of the nucleotide sequences was performed using ClustalW and a phylogenetic tree was constructed using the neighbour-joining method (with 1000 bootstrap replicates) in MEGA X (Saitou & Nei 1987; Tamura et al. 2004). The sequence from the present study showed a close relationship with the ToLCNDV bitter gourd isolate (MT185657) and *Bitter gourd yellow vein virus* [India: Kanpur: 2014] (KM190927; Figure 2). Only a 1440 nt long sequence was used for this phylogenetic analysis and analysis of the complete genome may give a different conclusion. To the best of our knowledge this is the worldwide, first report of any begomovirus infecting *C. fruticosa*.

The geographical range of ToLCNDV is rapidly increasing (Zaidi et al., 2017) and this report adds another species to its expanding host range. However, there is not sufficient evidence to prove that ToLCNDV causes the described symptoms. Cordyline plants are grown in gardens for ornamental purposes and infected plants may serve as a virus reservoir for other hosts. This will need to be considered in developing management strategies to control this virus.

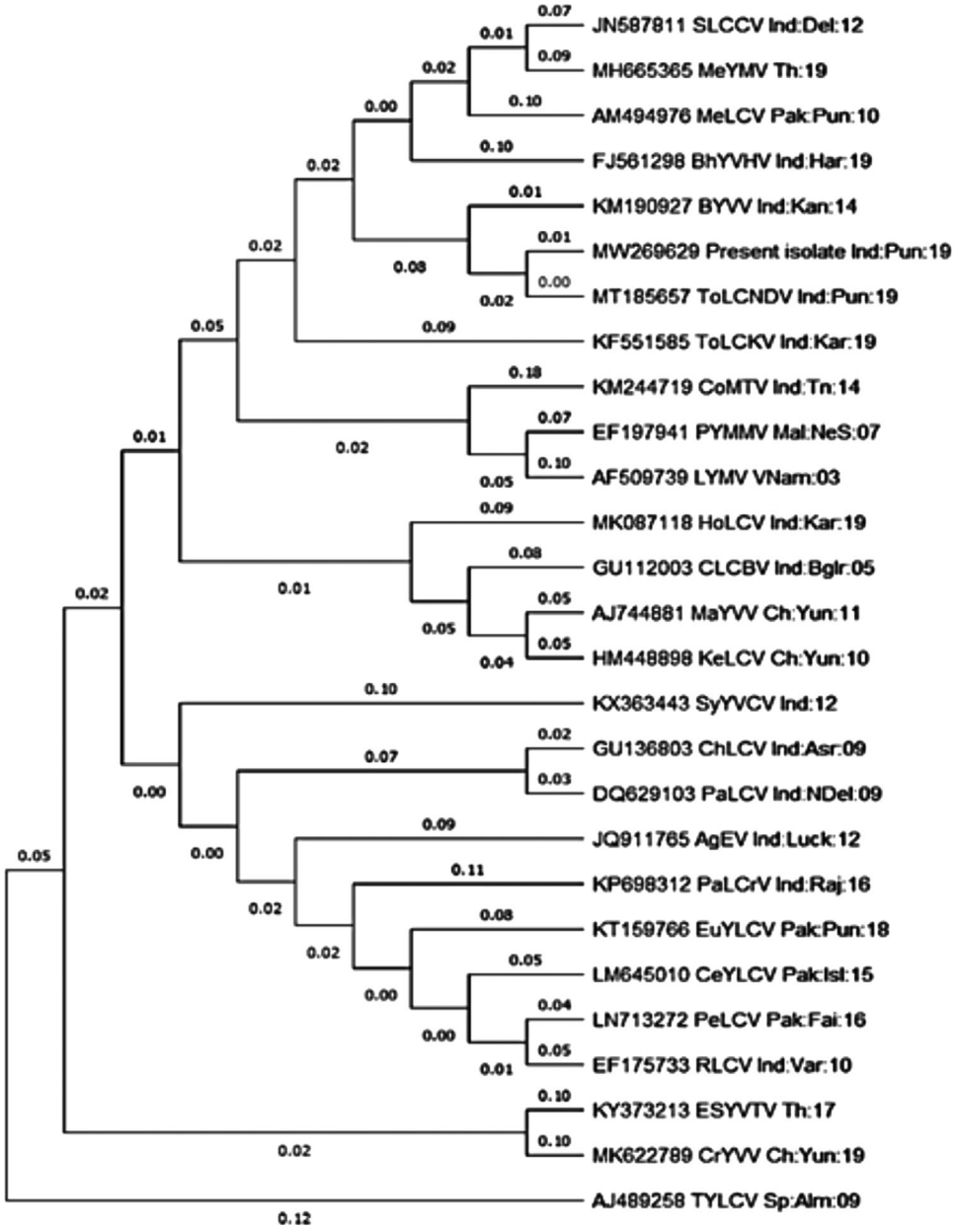


FIGURE 2 Phylogenetic tree constructed using ClustalW programme showing the relationship between the present isolate and related begomovirus species. The isolate from *Cordyline fruticosa* shows close homology with *Tomato leaf curl New Delhi virus. Tomato yellow leaf curl virus* (Almeria, Spain; AJ489258) was used as an outgroup

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