Diseases Caused by Viruses

First Report of *Papaya leaf curl virus* Associated with Leaf Curl Disease in *Cestrum nocturnum* in Pakistan

Aamir Lal,¹ Eui-Joon Kil,^{1,2,†} Kainat Rauf,³ Muhammad Ali,^{3,†} and Sukchan Lee^{1,†}

¹ Department of Integrative Biotechnology, Sungkyunkwan University, Suwon, Korea

² Department of Plant Medicals, Andong National University, Andong, Korea

³ Department of Life Sciences, School of Science, University of Management and Technology, Lahore, Pakistan

Funding: Funding was provided by Animal and Plant Quarantine Agency (Z-1543086-2017-21-01). Plant Dis. 104:3089, 2020; published online as <https://doi.org/10.1094/PDIS-12-19-2681-PDN>. Accepted for publication 11 May 2020.

Night-blooming jasmine (*Cestrum nocturnum*; family *Solanaceae*) is one of the ornamental and medicinal plants grown in tropical and subtropical regions (Shaista and Amrita 2016). *Papaya leaf curl virus* (PaLCuV) (genus *Begomovirus*, family *Geminiviridae*) is a bipartite circular ssDNA molecule that causes severe disease in papaya (Byun et al. 2016) and other solanaceous crops such as tobacco and tomato (Varun et al. 2017). In June 2018, during a routine survey to record begomoviruses other than cotton-infecting viruses in Pakistan, leaf curl and leaf roll disease symptoms were observed in three jasmine plants in a house garden in Lahore (31°N, 74° E), near Sheikhpura, Punjab province, Pakistan. In addition, low infestations of whiteflies were observed on symptomatic plants. Leaf samples from all three symptomatic plants were collected. Initially, total nucleic acid was extracted from a symptomatic plant using the CTAB method (Doyle and Doyle 1990). Polymerase chain reaction (PCR) amplification using universal primers Beg-F/Beg-R (Lal et al. 2020) resulted in the amplification of ~1 kb

DNA fragment. The amplicon was cloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced by a commercial sequencing service (Macrogen, Seoul, Korea). The BLASTn analysis revealed 98% nucleotide identity with an isolate of PaLCuV (KY978407). Based on the sequence obtained, new primers PaL11-F (5'-ACGCCATTCTCTGCTT GAGC-3') and PaL12-R (5'-GCGTGTGTTATCTGGGAGATTCA-3') were designed to obtain the full-length DNA-A of PaLCuV. PaLCuV DNA-A of approximately 2.8 kb was successfully amplified through PCR, cloned, and sequenced (GenBank accession no. MN839534), which also showed 97% sequence identity with PaLCuV (KY978407). The remaining *C. nocturnum* samples were also investigated through PCR amplification and found infected with PaLCuV. Furthermore, Southern blot hybridization was carried out in which the DNA extracted from one sample was resolved in agarose gels and transferred onto nylon membranes, followed by the hybridization with a [³²P] dCTP-labeled full-length clone of PaLCuV (Kil et al. 2016). The pattern of hybridization of single- and double-stranded DNA bands of different conformations was observed, thus confirming the presence of begomovirus, and sequencing results confirmed it as PaLCuV. To our knowledge, this is the first report of PaLCuV associated with leaf curl disease of *C. nocturnum* from Pakistan. *C. nocturnum* can be a reservoir for PaLCuV that could infect papaya crops in Pakistan. The *Solanaceae* family (to which *C. nocturnum* belongs) contains many economically important crops (grown in Pakistan) that could also be susceptible to the PaLCuV isolate reported in this note.

References:

- Byun, H.-S., et al. 2016. Plant Dis. 100:1958.
- Doyle, J. J., and Doyle, J. L. 1990. Focus 12:13.
- Kil, E.-J., et al. 2016. Sci. Rep. 6:19013.
- Lal, A., et al. 2020. Plant Dis. 104:2034.
- Shaista, A., and Amrita, P. 2016. J. Med. Pl. Stud. 4:13.
- Varun, P., et al. 2017. Protoplasma 254:2055.

The author(s) declare no conflict of interest.

Keywords: *Papaya leaf curl virus*, *Cestrum nocturnum*, begomovirus, geminivirus, Solanaceae

[†]Indicates the corresponding author.

E-mail: viruskil@anu.ac.kr (E.-J.K.) and ali4982@gmail.com (M.A.) and cell4u@skku.edu (S.L.)