Molecular characterization and infectivity studies of an isolate of *Cotton leaf curl Kokhran virus-Layyah*

Malik Nawaz Shuja[1, 2]* and Muhammad Tahir[1]

[1] Dept. of Plant Biotechnology, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan.

[2] Dept. of Microbiology, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan.

Abstract

Cotton leaf curl disease (CLCuD) has been a considerable encumbrance to the cotton production worldwide including Pakistan. The disease is caused by various dis-tinct viruses of the genus Begomovirus (family Geminivir-idae) in association with a disease-specific betasatellite –cotton leaf curl Multan alpha- and beta-satellite (CLCuMA and CLCuMB). Since 2001, when resistance to CLCuD in cotton was broken, only one virus was consistently identified in cotton exhibiting CLCuD symptoms in Punjab province (Pakistan) – cotton leaf curl Burewala virus (CLCuBuV). An analysis of all CLCuBuV isolates available in the databases showed these to represent only a single strain, based on currently applicable criteria. In this study, we reported a new strain of cotton infecting begomovirus from District Layyah, a probable recombinant of *Cotton leaf curl Kokhran virus* and *Cotton leaf curl Multan virus* – the two predominant species attributed to CLCuD in Pakistan. A partial dimeric (0.63 mer) DNA-A, (0.55 mer) beta- and (0.9 mer) alpha-satellite clones were constructed in a binary vector and used in *Agrobacterium*-mediated inoculation of *Nicothiana benthamiana, N. tabacum, Lycopercicum esculentum, cucurbita* and *Cucumis sativus.* Leaf curling, deformation and yellowing like symptoms were observed on inoculated plants. Following PCR amplification, the viral DNA as well sub-genomic components were detected in newly emerging non-inoculated leaves. Both molecular and biological studies indicate that this new isolate is a virulent strain of CLCuD and has the potential to aggravate symptomology.

Keywords: Begomovirus; Betasatellite; Cotton leaf curl disease; Recombinant

***Corresponding Author: maliknshuja@gmail.com**

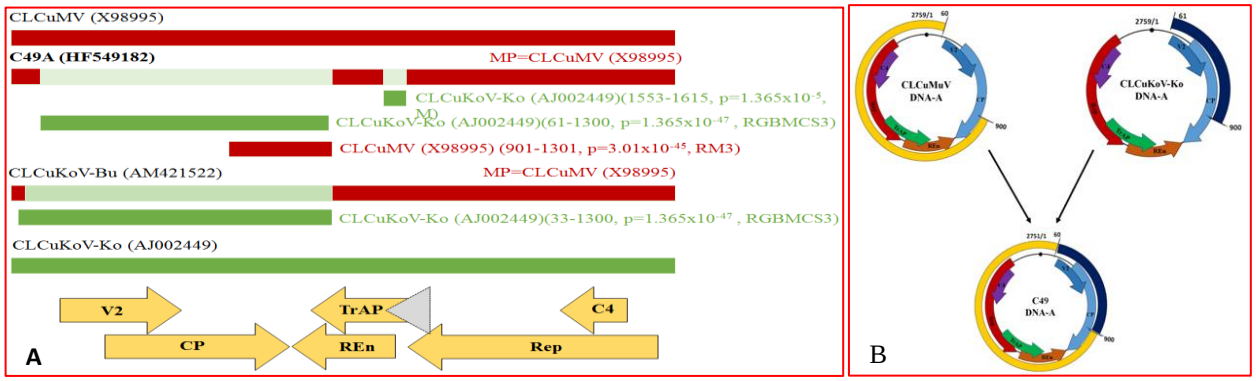


Figure 1. RDP analysis and map of an isolate C49A. (A). RDP analysis shows the major parent (MP) and minor parent for each recombinant sequences are indicated with breakpoint position (nucleotide coordinates of the 5' and 3' breakpoints) and highest p-value. **(B).** Map of an isolate C49 DNA A showing the sequences originating from *Cotton leaf curl Multan virus* (CLCuMuV) and *Cotton leaf curl Kokhran virus* - strain Kokhran (CLCuKoV-Ko). The fragments are colored as Yellow, originating from CLCuMuV, and Dark Blue, originating from CLCuKoA-Ko.

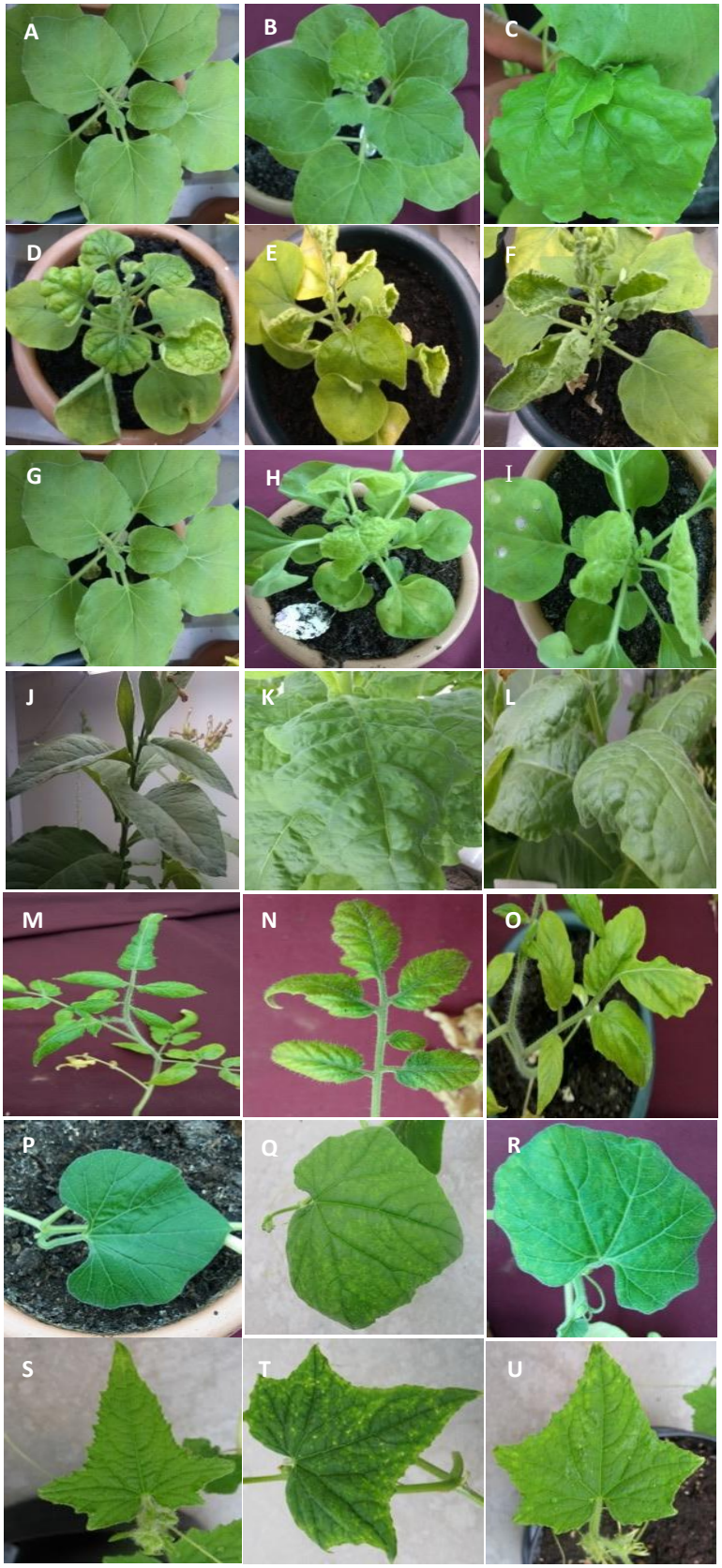


Figure 2. Infectivity analysis of isolate C49 and C28 (CLCuBuV-as a control), *Nicotiana benthamiana, Nicotiana tabacum, Lycopercicum esculentum, cucurbita* and *Cucumis sativus* plant were agro-inoculated with C-49A (C, J, M, P and S), C49A and C49β (D, E, H, K, N, Q, and T), C49A, C49β and C49α (F, I, L, O, R and U), C28A (G),C28A, and C28β (H) C28A,C28β and C28α (I), mock plant (B) and control healthy plant (A).

Publications:

1. Shuja et al., (2018). First report of Bhendi yellow vein mosaic virus and cognate Croton yellow vein mosaic betasatellite and Cotton leaf curl Multan alphasatellite in symptomatic *Abelmoschus esculentus* in Pakistan. Disease Notes; The American Phytopathological Society (Under review)
2. Shuja et al., (2018). First report of Catharanthus yellow mosaic virus and Pedilanthus leaf curl virus infecting *Duranta errecta* L. in Pakistan. Disease Notes; The American Phytopathological Society (Under review)
3. Shuja et al., (2018). Molecular characterization of cotton infecting begomoviruses and DNA satellites associated with Cotton leaf curl disease in Pakistan. Acta Virologica (Submitted)
4. Muhammad Ali, Sidra Anwar, Malik Nawaz Shuja, Rajiv Kumar Tripathi and Jaswinder Singh (2018).The genus Luteovirus from infection to disease. European Journal of Plant Pathology. DOI:10.1007/s10658-018-1425-8
5. Shuja et al., (2017). Occurrence of a recombinant alphasatellite carrying sequences derived from the helper virus in cotton leaf curl disease affected cotton Tropical Plant Pathology. 42(5): 397-402. DOI 10.1007/s40858-017-0161-5
6. Shuja et al., (2015). Detection and molecular characterization of cotton infecting begomoviruses from Pakistan. 6th Congress of European Microbiologists, June 7-11, 2015, Maastricht, The Netherland
7. Shuja et al., (2014). Identification of distinct of Cotton leaf curl Burewala virus. Archives of Virology.150 (10)-2787-90
8. Shuja et al., (2011). Maize breeding for marginal lands- Physiological and molecular approach to decipher response and selection of maize recombinant inbred lines (RILs) under water deficit at early growth stage. African Journal of. Biotechnology. . 10 (18)-3521-3527.
9. Shuja et al. (2010). Evaluating the response of Wheat Genotypes to Clipping. International. Journal of Agriculture and Biology.12:111-114
10. Ali, M., M.N. Shuja, M. Zahoor and I. Qadri (2010). Phytic acid- how far have we come? African Journal of. Biotechnology. 9 (11)- 1551-1554