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## Molecular characterization of cotton infecting *Begomovirus(es)* and DNA satellites associated with cotton leaf curl disease in Pakistan

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Cotton leaf curl disease (CLCuD) has been a considerable encumbrance to the cotton production worldwide including Pakistan. The disease has been caused by a complex of *Begomoviruses* in association with cotton leaf curl Multan *alpha/beta-satellite*. Previously, 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. In this study, symptomatic cotton samples were collected from the cotton belt in Punjab province. Total nucleic acid from leaf tissues was extracted and subjected to PCR. Thirty full-length genomic components were cloned and sequenced, corresponding to *Begomovirus* and associated DNA *alpha*- and *beta-satellite*. Further, a partial dimeric (0.63 mer) DNA-A, (0.55 mer) *beta*- and (0.9 mer) *alphasatellite* clones were constructed in a binary vector and used in *Agrobacterium*-mediated inoculation of *Nicotiana benthamiana*, *N. tabacum*, *Lycopersicon 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 as 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.

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