New Disease Reports

Association of *Cotton leaf curl Burewala virus* and its satellite molecules with leaf distortion symptoms of cotton in India

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Cotton is one of the most important cash crops grown in India and Pakistan. During 2009-2010, leaf distortion symptoms (Fig. 1a,b) were observed on about 15-20 % of the cotton plants growing at CSIR-NBRI, Lucknow, India. A population of whiteflies (*Bemisia tabaci*) was noticed in the vicinity. Based on the typical symptoms and the presence of whiteflies, begomovirus infection was suspected. Therefore, transmission of the disease was attempted through whiteflies from affected plants to healthy cotton seedlings and resulted in similar symptoms 23-25 days post inoculation.

PCR was performed using total DNA isolated from leaf samples of plants with symptoms employing DNA A-specific primer pairs: F1For (5'-TTAAGAAAAGACCAGTCGGAGGG-3') and F1Rev (5'-CATTTCCATCCGAAC ATTCAGGG-3'); and F2For (5'-TTGACATCTGAGCTTGATTTAGC-3') and F2Rev (5'-TAACCTTCCGAATCTGGACGACCT-3'). PCR products from all the infected leaf samples showed the expected sizes of approximately 1200 bp (for F1 For/F1 Rev primers) and approximately 1700 bp (for F2 For/F2 Rev primers), confirming the begomovirus infection. PCR amplicons were sequenced, and sequence data assembled and combined to the full-length sequence of a DNA-A fragment consisting of 2,758 nucleotides (GenBank Accession No. HM461866). The sequence shared highest identity (99%) with several isolates of *Cotton leaf curl Burewala virus* (CLCuBV; AM774295, FN645932, HM461863). A phylogenetic tree including the nucleotide sequences of several begomoviruses suggested its close relationship with CLCuBV reported from Pakistan (Fig. 2).

An amplicon of approximately 1400 bp was amplified from the DNA of symptom-bearing plant samples using alphasatellite DNA-specific primers (Bull *et al*., 2003). The underlying 1396 bp sequence of alphasatellite DNA (HQ343234) shared 85% identity and close relationship with alphasatellite DNA associated with *Cotton leaf curl Shahdadpur virus* (CLCuShV; Amrao *et al*., 2010) (Fig. 3). Furthermore, a β satellite DNA of approximately 1300 bp was also amplified using β satellite DNA-specific primers (Kumar *et al*., 2010). The β satellite molecule (HM140826) shared highest 97% identity with a variant of Cotton leaf curl Multan β satellite DNA (CLCuMB) reported from India (AY744380) and 92% with Cotton leaf curl Burewala betasatellite (FN658722). As the species demarcation cut-off value for β satellite molecules currently is 78% (Briddon *et al*., 2008), the β satellite associated with the disease appeared to be an isolate of CLCuMB. Based on highest sequence identity and closest phylogenetic relationships, the leaf distortion symptoms of cotton were considered to be associated with CLCuBV, β satellite and alphasatellite DNA. CLCuBV has been originally reported from Burewala territory in Pakistan as a variant of *Cotton leaf curl Multan virus* (Mahmood *et al.*, 2003) which induced leaf curl symptoms. Further studies suggested that CLCuBV is a recombinant which consists of sequences derived from *Cotton leaf curl Multan virus* and *Cotton leaf curl Kokhran virus*. The β satellite has also been found associated with the cotton leaf curl disease (Amin *et al*., 2006; Amrao *et al*., 2010). We report here the association of CLCuBV, β satellite and alphasatellite DNA with leaf distortion symptoms of cotton in India.

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Figure 1

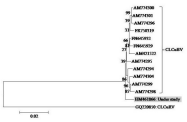

Figure 2

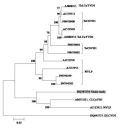

Figure 3

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