Development of Molecular Diagnostic Tool for Cotton Leaf Curl Disease (CLCuD) Using Betasatellite Based Molecular Marker

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Cotton Leaf Curl Disease (CLCuD) is caused by viruses that belong to genus, begomovirus. Five different species of viruses interact with one Betasatellite, responsible for symptom development, Cotton Leaf Curl Multan Betasatellite(CLCMulB). Instead of designing primers for multiple species of viruses(as reported in previous studies),detection on the basis of Betasatellite proved more convenient. This study describes the design and validation of PCR primers undertaken to facilitate molecular detection of CLCuD using betasatellite based marker. DNA from symptomatic cotton leaves from Pakistan was extracted using CTAB method. Different variants of CLCMulB were downloaded from NCBI GenBank and using MEGA-X software primer pairs which targeted conserved region of sequences in alignment were designed for testing. Expected product size was 401 bp. PCR was carried out to amplify the conserved region of CLCMulB. Afterwards Gel Electrophoresis was done to confirm PCR product and quality was checked by running 1% agarose gel. Sequences obtained were assembled using Bio Edit and DNA Star software. All sequences showed homology at 99-100% with CLCMulB sequences available in GenBank. Phylogenetic analysis (using Maximum Likelihood method) revealed that CLCMulB reported in study falls in same clade as previously reported CLCMulB from subcontinent. The designed primers for amplification of CLCMulB successfully amplified three different strains of CLCMulB, so study reports an efficient and reliable diagnostic test for early detection of CLCuD. This disease can spread to uninfected cotton growing regions via alternate host of begomoviruses. Screening plants prior to import or export using this diagnostic tool will greatly reduce likelihood of CLCuD introductions throughout the world.

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