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ORIGINAL ARTICLE

Genetic variability of Cotton leaf curl betasatellite in Northern India

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Abstract Cotton is an important crop and its production is affected by various disease pathogens. Monopartite begomovirus associated betasatellites cause Cotton leaf curl disease (CLCuD) in Northern India. In order to access the occurrence and genetic variability of Cotton leaf curl betasatellites, an extensive field survey was conducted in states of Rajasthan, Punjab and Haryana. We selected the betasatellite sequence for analysis as they are reported as important for disease severity and sequence variability. Based on the field observations, the disease incidence ranged from 30% to 80% during the survey. Full genome and DNA β were amplified from various samples while no amplicon was obtained in some samples. The nucleotide sequence homology ranged from 90.0% to 98.7% with Cotton leaf curl virus (CLCuV), 55.2–55.5% with Bhendi yellow vein mosaic virus, 55.8% with Okra leaf curl virus and 51.70% with Tomato leaf curl virus isolates. The lowest similarity (47.8%) was found in CLCuV-Sudan isolate. Phylogenetic analysis showed that analyzed isolates formed a close cluster with various CLCuV isolates reported earlier. The analysis results

Abbreviations: CLCuD, Cotton leaf curl disease; CLCuV, Cotton leaf curl virus; PCR, polymerase chain reaction; SCR, satellite conserved region

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show sequence variation in Cotton leaf curl betasatellite which could be the result of recombination. The results obtained by genome amplification and sequence variability indicate that some new variants are circulating and causing leaf curl disease in Rajasthan, Punjab and Haryana.

1. Introduction

Cotton is an important crop and cotton production is seriously hampered by CLCuD in India and Pakistan (Sattar et al., 2013). The incidence of CLCuD has been reported in almost all the growing belt in North India (Rishi and Chauhan, 1994; Briddon et al., 2001; Sharma and Rishi, 2003; Zaffalon et al., 2011; Rajagopalan et al., 2012). India is an important producer of cotton in the world. In 1990, this disease was observed as an epidemic with approximately 30–40% estimated loss in Multan, Pakistan (Zhou et al., 1998; Briddon and Markham, 2000; Asad et al., 2003). During 1997–98 a sudden increase in CLCuD was reported in Northern India. The variability of CLCuV and betasatellite molecule in Northern India and other regions has been published earlier in various reports (Sanz et al., 1999; Briddon et al., 2001, 2003; Kirthi et al., 2004; Nawaz-ul-Rehman et al., 2012; Sohail et al., 2014). The characteristic symptoms included leaf curling; vein thickening followed by cup formation under the leaves. Interestingly, DNA β sequences of Cotton leaf curl Gezira virus from various geographical locations were found to be very similar. Recently it has been reported that, only one DNA β molecule can interact with four distinct CLCuV and produce typical symptoms (Mansoor et al., 2003a,b). Most of the begomoviruses identified in the Old World were found to be monopartite while the New World begomoviruses have bipartite genomes. Recently, a native monopartite begomovirus infecting cotton has been identified (Melgarejo et al., 2013; Sanchez-Campos et al., 2013). In the Old World most of the monopartite begomovirus were associated with betasatellites (Briddon and Mansoor, 2008; Briddon et al., 2012).

Betasatellites have small (~1.4 kb), circular, ssDNA genome and their replication and movement fully depend upon a helper virus (Briddon et al., 2003; Mansoor et al., 2003a,b; Leke et al., 2013). The sequences of betasatellites have three major features – a single βC1 gene, adenine rich sequence region and satellite conserved region containing stem-loop structure, which is known for the origin of replication in geminiviruses (Hanley-Bowdoin et al., 1999; Briddon, 2003; Briddon et al., 2003). The function of βC1 gene is mediated by a typical encoded protein. The βC1 gene is known as a pathogenicity determinant, post-transcriptional gene silencing suppressor and mediates virus movement (Cui et al., 2005; Kon et al., 2007; Qazi et al., 2007; Saeed et al., 2007; Amin et al., 2011; Iqbal et al., 2012). The study presented here has analyzed the sequences of Cotton leaf curl betasatellites recently isolated from North India and has identified specific sequence variations among samples collected from three states.

2. Materials and methods

2.1. Field survey and sample collection

Field survey was conducted during the cotton cropping season from 2008 to 2011 in the major cotton fields of Rajasthan, Punjab and Haryana. Virus infected samples were collected from cotton plants with typical symptoms such as leaf curling, enation and stunting of plant.

2.2. PCR amplification and cloning of betasatellites

Total genomic DNA was isolated using the Cetyl trimethyl ammonium bromide method (Doyle and Doyle, 1990) and polymerase chain reaction (PCR) was conducted by using about 100 ng template DNA and 10 Pico moles of forward and reverse primers. Virus infection was confirmed by PCR using specific coat protein gene forward and reverse primers (CPF-AATTATGTCGAAGCGAGCTGC and CPR-TAATATCAATTCGTTACAGAG). Betasatellites were amplified by specific DNA β primers (βF-GGTACCACTACGCTACGCAGCAGCC and βR-GGTACCTACCCTCCCAGGGGTACAC) designed from the beginning and end of the viral genome from the published sequences. During PCR, *Taq* DNA polymerase (2.5 units) (MBI Fermentas, USA) 5 µl of 10× buffer, 1 µl of 10 mM dNTPs and 1 µl (10 Pico moles) of forward and reverse primers were used. The final volume was made up to 50 µl using sterile distilled water. The PCR amplified fragments of DNA β were gel eluted and purified by using a QIA quick Gel Extraction Kit (Qiagen, USA) and cloned into pGEMT-easy vector. The positive clones were identified by colony PCR and restriction enzyme digestions.

2.3. Sequence and phylogenetic analyses

The sequencing of clones was performed in DNA sequencer (ABI Prism, Perkin Elmer) at JK AgriGenetics Ltd, Hyderabad. The obtained sequences were analyzed using Bioedit software (version 5.0.9). The full sequence DNA β and associated betasatellites were initially selected for homolog using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST and the sequence showed better scores selected for genetic variability study.

3. Results

3.1. Field survey and sample collection

During the field survey, disease incidence was recorded up to 30–80% in different fields of major cotton growing areas of