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Diversity of alphasatellites associated with cotton leaf curl disease in Pakistan

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ABSTRACT

BackgroundCotton is a major cash crop of Pakistan and its production is mainly hindered by cotton leaf curl disease (CLCuD). This disease is caused by monopartite begomovirus associated with two satellites named as betasatellite and alphasatellite. Betasatellites are true satellites entirely dependent on helper begomoviruses and are symptom determinants which are essentially required for the typical symptoms of the disease. Alphasatellites are self-replicating circular ssDNA molecules which are associated with CLCuD complex. The role of alphasatellite is not fully understood.

ResultCotton samples showing typical CLCuD symptoms were collected from areas across central Punjab, Pakistan during year 2011–12. All samples contained alphasatellites. Mixed-infection of alphasatellites associated with CLCuD complex was documented. Few samples showed the presence of more than one species of alphasatellite. A total of 45 alphasatellites were cloned and sequenced. The size of these alphasatellite ranges from 1362 to 1378 bp. All alphasatellites showed three conserved features i.e. 1) A stem-loop structure with a nonanucleotide (TAGTATTAC) sequence (2) An ORF encoding a Rep protein of about 36.6 kDa, having up to 315 amino acids (3) An A-rich region of ~200 nt. Based on BLAST results we have found six distinct species of alphasatellites namely; *Gossypium darwinii* symptomless alphasatellite (GDarSLA), Guar leaf curl alphasatellite (GrLCuA), Okra leaf curl alphasatellite (OLCuA), Tomato leaf curl Pakistan alphasatellite (ToLCPKA), Cotton leaf curl Multan alphasatellite (CLCuMA), and Cotton leaf curl Burewala alphasatellite (CLCuBuA). This was also confirmed by phylogenetic analysis. By considering the species cut-off limit for alphasatellites (83%) the isolates fall into 5 species. But the percentage identity between some CLCuBuA and CLCuMA was 83.3, so they are proposed to be considered as two different species.

ConclusionOur data shows that at least six species of alphasatellites are found associated with cotton leaf curl disease in Pakistan. Field samples are often associated with multiple species and one sample was found associated with three distinct alphasatellites in a single plant under field conditions. Infection of multiple alphasatellite and their probable role in CLCuD are discussed.

1. Background

Cotton leaf curl disease (CLCuD), a severe constraint on cotton production in the Indian subcontinent is characterized by leaf curling and dark green veins that often develop into leaf like out growth on underside of leaf called enations. Infected plants show

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stunted growth that result in loss in cotton production. This disease was first reported from Sudan and Egypt in 1930s. The disease was first recorded in 1967 from Multan in Pakistan but similar symptoms were recorded much earlier on tobacco and some other plants. It assumed epidemic proportions in Punjab province of Pakistan in early 1990′s (Briddon, 2003; Mansoor et al., 2008).

CLCuD is caused by member of family *Geminiviridae*. The geminiviruses are a family of plant-infecting phytopathogens with ssDNA genomes encapsidated in twin icosahedral, geminate capsids (Fauquet et al., 2003). They have a wide range of hosts and are diverse in their genome architecture. They are known to cause diseases in economically important crops such as cotton, cassava, tomato, maize and wheat in tropical and subtropical regions around the globe (Harrison & Robinson, 1999; Moffat, 1999; Mansoor et al., 2003; Mansoor et al., 2006).

Earlier based on the type of vector, host range and genome organization, the family *Geminiviridae* was divided into four genera, *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus* (Fauquet et al., 2003). Recently three new genera have also been proposed and agreed to be included in the family *Geminivirdae* which are *Becurtovirus*, *Turncurtovirus* and *Eragrovirus* which make a total the seven genera of geminiviruses (Adams et al., 2013). CLCuD is caused by members of the genus *Begomovirus* which is a diverse group of geminiviruses, transmitted by the whitefly *Bemisia tabaci* (Hanley-Bowdoin et al., 1999). Begomoviruses have either bipartite or monopartite genomes. Most begomoviruses consists of two genomic components referred to as DNA-A and DNA-B (Navot et al., 1991). Both of these components are essential for virus proliferation. However monopartite begomoviruses have only single genomic component that resembles DNA-A which is sufficient to cause disease (Stanley et al., 2005). Monopartite begomoviruses, which are native to the Old world (OW) are frequently found to be associated with satellite molecules of ~1.4 kb in length. These are known as betasatellites and alphasatellites (Mansoor et al., 1999; Saunders et al., 2000). The betasatellites are class of ss DNA satellites which have been identified in recent past (Briddon & Stanley, 2006). In most of the cases these satellites are required by their helper begomoviruses to develop symptoms in hosts from which they have been identified and isolated (Saunders et al., 2000; Briddon et al., 2001; Jose & Usha, 2003). Betasatellite encodes a single open reading frame (ORF) in complementary-sense strand (known as βC1 gene) which is a symptom/pathogenicity determinant, a suppressor of post transcriptional gene silencing (PTGS) , enhance viral genome levels in plants and also involved in virus movement in plants (Jose & Usha, 2003; Amin et al., 2011; Saunders et al., 2004; Qazi et al., 2007; Saeed et al., 2005).

Alphasatellites (~1380 bp) are self-replicating circular ssDNA molecules which are reliant on helper virus for encapsidation and transmission. Earlier they were termed as DNA 1 (Mansoor et al., 1999; Briddon et al., 2004). Alphasatellites are a mystery in plant virology. Their precise function and occurrence with begomovirus complexes yet needs to be elucidated. A few reports suggest that they have a role in suppression of PTGS (host defence) and symptom attenuation (Idris et al., 2011; Nawaz-Ul-Rehman et al., 2010; Wu & Zhou, 2005). Alphasatellites are thought to have evolved from nanoviruses. They possess three conserved regions: (1) A stem-loop structure with a nonanucleotide (TAGTATT/AC) sequence similar to the members of family *Nanoviridae*. This region has ori, where Rep cleaves DNA to start the rolling circle replication (RCR) process, (2) An ORF encoding a Rep protein of about 36.6 kDa, having up to 315 amino acids, (3) An A-rich region of ~200 nt, considered as a stuffer sequence (Briddon et al., 2004).

Alphasatellites are found to be associated with many begomovirus/betasatellite complexes, such as those causing CLCuD and okra leaf curl disease (Mansoor et al., 2003; Mansoor et al., 2006). Since the emergence of a resistance breaking species of begomovirus *Cotton leaf curl Burewala virus* (CLCuBuV), many studies have been made on etiology of the disease (Amin et al., 2006; Amrao et al., 2010). In the period of 2009–10 alphasatellites started to appear with CLCuD complexes. In this study the sequence diversity of alphasatellite components was assessed in cotton, covering the central Punjab region of Pakistan. Many of the plant samples were found with the presence of more than one type of alphasatellite. Here we report a single cotton plant with the presence of three different species off alphasatellites in field conditions. The role of alphasatellite in the disease and implications of multiple species in a single plant is discussed.

2. Results

Leaves from infected cotton plants showing typical symptoms of CLCuD (Fig. 1) were collected from different regions of central Punjab, Pakistan. During 2011, the incidence of disease was high and cotton plants showed moderate to severe symptoms, such as; vein thickening, vein darkening, upward and downward curling and in some cases enations on veins were observed. Origin of samples, symptoms, severity and incidence of disease are mentioned in Table 1. The locations from where samples were collected are indicated in Fig. 2.

2.1. Alphasatellite cloning, amplification and sequencing

Alphasatellites were amplified using universal primers DNA1 01 & DNA1 02 (Bull et al., 2003) from extracted DNA samples. The majority of the samples showed amplification of a product of ~1.4 kb. Representative molecules were cloned in T/A cloning vector, confirmed by restriction digestion analysis and sent for sequencing. A total of 45 full length clones were obtained in this study, which were sequenced in their entirety by removing all ambiguities. These sequences were submitted to database and are available in EMBL, DDJB and GenBank nucleotide sequence database. The accession No. of these sequences along with annotations is shown in Table 2. The sequence study revealed that all the 45 alphasatellites were in range of 1362–1378 bp length and had three conserved regions; a conserved hairpin structure with a nononucleotide sequence, an ORF and an A-rich region. The nonanucleotide sequence was found to be highly conserved with no mismatch (Fig. 3). The stem region showed some variance